2017 NextGen Genomics, Biology, Bioinformatics and Technologies (NGBT) Conference

2nd – 4th October 2017, Bhubaneswar, Odisha, INDIA

The objective of this NGBT conference is to promote application of NextGen sequencing and genomics technologies for basic and translational science in all areas of biology including human genetics, drug discovery, clinical medicine, biomarkers, diagnostics, animal, plant and agricultural sciences.
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SGRF is a not-for-profit foundation set up to promote research, education and community health. At SGRF, our objective is to pursue research on a broad canvass covering medical, agricultural (both plants and animals) and environmental issues; but our projects are focused and designed to achieve specific goals. We study the genomics of the color phenotype in Purple puttu, a colored rice; we offer free diagnostic tests for retinoblastoma in children; we work on the development of biological control agents against insect pests and addressing the various wild life epidemics that plague the region. Much of the sequence information obtained in these studies will be made available to the not-for-profit research community. For instance, the entire genome of a South Asian Indian female that our sister organization SciGenom and collaborators determined is freely available to researchers who request access. Our other activities in academics include organizing and supporting conferences, workshops and seminars as well as providing scholarships and excellence in science/teaching awards. “NextGen Genomics, Biology, Bioinformatics and Technologies” (NGBT) Conference is one such conference series being organized by SGRF every year.

NGBT Conference 2017 is the seventh edition of our international conference series during 2-4 October, 2017 at the Mayfair Convention, Bhubaneswar.

The conference is being organized by SGRF Conferences, the education wing of SciGenom Research Foundation (SGRF), a not-for-profit research and educational organization, along with several co-hosts, viz., Toronto Recombinant Antibody Centre (TRAC), Centre for the Commercialization of Antibodies and Biologics (CCAB) - both from Canada, Institute of Bioinformatics (IOB), Bangalore, Kalinga Institute of Industrial Technology University (KIIT), and National Institute of Science Education & Research - both from Bhubaneswar.

The highly interdisciplinary flavor of the meeting is evident by the decorated list of highly accomplished speakers with varied expertise from around the globe. Apart from discussing the cutting edge NGS technologies and their applications, there were focused sessions on plant, animal, microbial and medical genomics. During these three days program, there were fifteen keynote lectures from some of the thought leaders in the community. To encourage, educate and inspire the next generation of scholars, we have offered forty four full scholarships and ninety nine partial scholarships for young researchers to participate in the conference. We also have selected four projects for in-kind support under the genomics grant programme for young researchers; another seventeen projects have been short listed for possible support. Further, we also offered special prizes for poster presentations based on review by a panel of experts. As before, the young students and post-docs were also given a chance to pitch their research finding for the poster awards in one minute stage presentations in front of an international panel of experts.

We hope that the attendees were benefitted by interactions with peers as well as leaders and made use of this opportunity to network.

If you have any suggestions for the next year’s conference based on your experience in this one, please do let us know.
Members of the NGBT 2017 Organizing Committee

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NGBT 2017 Scientific Programme

Monday, October 02, 2017 - Day 1

Concurrent Session I [Central Hall]

08:30 – Registration begins
09:15-10.00 – Conference Inauguration & Host Introductions

Concurrent Session II [Crystal Hall - 1]

Concurrent Session III [Crystal Hall - 2]

Session 01 - Keynote lectures [Central Hall]

Chair: Dr. Sekar Seshagiri

10:15-10:45 - Keynote lecture 01 - Dr. Partha Majumder, Dr. Andrew Peterson & Dr. Jeff Wall, NIBMG, India, UCSF & Genentech, USA - Genome Asia Consortium "Human Genetic Variation in Asia and implications for medical genetics"
10:45-11:15 - Keynote lecture 02 - Dr. Thangaraj K, CCMB, India - Burden of recessive diseases in South Asia: population and genomic perspectives

11:15-11:30 – Break

Session 02 – Single Cell/Cancer Genomics [Central Hall]

Chair: Dr. Harsha Gowda

11:30-11:50 - Talk 1 - Dr. Zora Modrusan, Genentech Inc., USA - Single cell RNA sequencing advancements support efficient transcriptional profiling of thousands of cells from diverse sample types
11:50-12:10 - Talk 2 - Dr. Jyotsna Batra, APCRC, Australia - Prostate cancer in the post-GWAS era
12:10-12:30 - Talk 3 - Dr. G Kumaresan, Madurai Kamaraj University, India - Integrative functional genomics guided approaches for the development of next-generation sub-stratification of tumors and personalized therapeutics

Session 03 – Stem Cells/Development/Gene Expression [Crystal Hall - 1]

Chair: Dr. Andrew Peterson

11:30-11:50 - Talk 4 - Dr. Anoop Vadakan Cherian, Universities of Giessen and Marburg Lung Centre, Germany - An endothelial cell front leads alveolar septation in the mouse lung
11:50-12:10 - Talk 5 – Dr. Shrinivasrao Mane, Elanco, USA - Impaired Adaptive Thermogenesis in Mice Lacking Glycerol-3-Phosphate Acyltransferase 4
12:10-12:30 -Talk 6 - Dr. Jay Gopalakrishnan, University of Cologne, Germany - Mechanisms of neural stem cells homeostasis in 3D human brain organoids
Session 04 – Genomics in Agriculture [Crystal Hall - 2]

Chair: Dr. Pradeep Marri

11:30-11:50 - Talk 7 - Dr. Lambodhar Behera, CRRI, India - Genomic approaches to decipher low light tolerance at vegetative stage in rice

11:50-12:10 - Talk 8 - Dr. Mukesh Jain, JNU, India - Exploring molecular signatures associated with seed development and seed size determination in crop plants

12:10-12:30 - Talk 9 - Dr. VB Reddy, AgriGenome, India - Functional Genomic Analysis of Abiotic and Biotic Stress Tolerance in Pearl Millet

12:30-14:00 - Lunch & Exhibitor visit - Art Exhibition [Central/Crystal/Basement]

Session 05 – Author Speaker [Central Hall]

Chair: Dr. Sekar Seshagiri

14:00-14:45 - Author speaker #1 – Mr. Pranay Lal - A Deep Natural History of the Indian subcontinent

14:45-15:00 - Break

Session 06 - Disease/Clinical/Medical Genomics I [Central Hall]

Chair: Dr. Arkasubhra Ghosh

15:00-15:20 - Talk 10 - Dr. Chirantan Bose, MedGenome, India - Molecular Advances in Hemato-Oncology in the light of new WHO Classification for Hematolymphoid Malignancies

15:20-15:40 - Talk 11 - Dr. Padma Srikanth, Sri Ramachandra University, India - Translational utility of NGS in clinical medicine in a tertiary care center in South India

15:40-16:00 - Talk 12 - Dr. Nic Waddell, QIMR Berghofer, Australia. Cancer genomics and its clinical potential

Session 07 – Metagenomics [Crystal Hall - 1]

Chair: Dr. Dhinoth Kumar

15:00-15:20 - Talk 13 - Dr. Chandan Badapanda, Xcelris Labs Ltd, India - Using QIIME to analyze 16S rRNA genes and ITS sequences from Microbial Communities


15:40-16:00 - Talk 15 - Dr. Aundy Kumar, IARI, India - Metagenomic analysis of pomegranate phyllosphere microbiome succession in relation to bacterial blight

Session 08 - Genome & Proteome [Crystal Hall - 2]

Chair: Dr. Shrinivasrao Mane

15:00-15:20 - Talk 16 - Dr. Harsha Gowda, IOB, India - Delineating molecular alterations associated with tobacco induced cellular transformation using proteogenomics approach

15:20-15:40 - Talk 17 - Dr. Jonathan Gershoni, Tel Aviv University, Israel - Profiling the IgOme - the repertoire of antibodies in polyclonal serum
15:40-16:00 - Talk 18 - Dr. M.G. Madanan, Regional Medical Research Centre (ICMR), Andaman & Nicobar, India - An in silico analysis reveals pathogen specific outer membrane proteins of Leptospira

16:00-16:15 - Break

Session 09 - Disease/Clinical/Medical Genomics II [Central Hall]

Chair: Dr. TS Sridhar

16:15-16:35 - Talk 19 - Dr. Vidya Veldore, MedGenome, India - Applications of NGS panels in MRD and Risk Stratification in Hematological Malignancies: MedGenome's Experience

16:35-16:55 - Talk 20 - Dr. Govind Babu, Kidwai Mem. Inst. of Onc., India - Integration of next generation sequencing into clinical practice for detecting clinically relevant mutations in cell free DNA of patients with metastatic/locally advanced non small cell lung cancer

Session 10 - Genome sequencing/Conservation Biology [Crystal Hall - 1]

Chair: Dr. Arun Zachariah

16:15-16:35 - Talk 21 - Dr. Uma Ramakrishnan, NCBS, India - A genomic perspective on tiger conservation

16:35-16:55 - Talk 22 - Dr. Mrinalini, NUS, Singapore - Genome sequencing of a sexually dimorphic snake, the Temple Pitviper

Session 11 - CRISPR Editing/Microbial Genomics [Crystal Hall - 2]

Chair: Dr. Boney Kuriakose

16:15-16:35 - Talk 23 - Dr. Sruthy Maria Augustine, Institute of Molecular Biotechnology, Germany - CRISPR-Cas9 System for Plant Genome Editing

16:35-16:55 - Talk 24 - Dr. Ram Nageena Singh, AgriGenome, India - Unexplored microbial treasures of Himalaya: Rich Microbial Gene pool of India

16:55-17:10 - Break

Session 12 - Keynote Lectures [Central Hall]

Chair: Dr. Sekar Seshagiri

17:10-17:40 - Keynote lecture 03 - Dr. Stephen Turner, PacBio, USA - SMRT Sequencing: Takes You to a New Realm of Genome, Epigenome and Transcriptome Analysis

Session 13 - Keynote Lectures [Central Hall]

17:40-18:20 - Author Speaker #2 - Shri Jairam Ramesh - "Environment and Economy"

18:20-18:30 - Q &A

Session 14 - Posters [Basement]

18:30-19:30 - Poster Viewing # 1-155

18:30-19:30 - Book signing and author speaker reception

Sponsored Events
Tuesday, October 03, 2017 - Day 2

Concurrent Session I [Central Hall]
08:30 - Registration begins

Concurrent Session II [Crystal Hall - 1]

Concurrent Session III [Crystal Hall - 2]

09:00-09:50 - Session 15 - Poster competition presentations (Chair: Dr. Harsha Gowda)

10:00-20:00 - Session 16 - Art Exhibition, Vendor Exhibits

Session 17 - Keynote lectures [Central Hall]

Chair: Dr. Sekar Seshagiri

10:00-10:30 - Keynote lecture 04 - Dr. Joseph Beechem, Nanostring, USA - Next Generation Optical Barcodes for Amplification-free, Library-free, Single-Molecule Next-Generation Sequencing (Hyb&Seq) and high-plex spatially resolved imaging (Digital Spatial Profiling)

10:30-11:00 - Keynote lecture 05 - Dr. Pramod K Srivastava, Uni. of Connecticut, USA - Basics of personalized immunotherapy: what is a good antigen?

11:00-11:10 - 2016 SGRF Genome Grant winner talk (05B) - Dr Arun Shastry, DART, India - Matrilineal Inheritance of casual variants in Duchenne Muscular Dystrophy (DMD) in Multi-Generational Families

11:10-11:30 - Break

Session 18 - Immunology/Cancer immunology/Therapy [Central Hall]

Chair: Dr. Amitabha Chaudhuri

11:30-11:50 - Talk 25 - Dr. Sunil K Raghav, Institute of Life Sciences, India - Integrative genomics identifies NCoR1 as a master repressor of tolerogenic program in dendritic cells

11:50-12:10 - Talk 26 - Dr. Bhawna Gupta, KIIT, India - Understanding autoimmunity: unconventional modalities for better therapies

12:10-12:30 - Talk 27 - Dr. Arati Khanna Gupta, MedGenome, India - Familial Colorectal cancers: focus on identification of peptides that could serve as cancer vaccines

Session 19 - Biomarkers/Chromatin Structure/Cancer Signaling [Crystal Hall - 1]

Chair: Dr. Mallika Singh

11:30-11:50 - Talk 28 - Dr. Debrabrata Mukhopadhyay, Mayo Clinic College of Medicine and Science, USA - Shedding microparticles: From biomarkers discovery and beyond

11:50-12:10 - Talk 29 - Dr. Sriharsa Pradhan, NEB, USA - High resolution open chromatin profiling of cancer cells

12:10-12:30 - Talk 30 - Dr. Senthil Kumar Nachimuthu, Mizoram University, India - Novel mitochondrial DNA mutations associated with gastric tumorigenesis in Mizo population, Northeast India
Session 20 - Agricultural Genomics [Crystal Hall - 2]

Chair: Dr. Arjula Reddy

11:30-11:50 - Talk 31 - Dr. Sanjib Kumar Panda, Assam Unii., India - RNA Seq reveals transcriptional regulation in Rice for Aluminum tolerance

11:50-12:10 - Talk 32 - Dr. Hiroyuki Koyama, Gifu University, Japan - Fishing of aluminum tolerance genes by genomewide approaches

12:10-12:30 - Talk 33 - Dr. Lakshminarayana R Vemireddy, Institute of Frontier Technology, India - Exploration and exploitation of novel dwarf genes in rice (Oryza sativa L.)

12:30-14:00 - Lunch & Exhibitor visit - Art Exhibition [Central/Crystal/Basement]

Session 21 - Keynote lectures [Central Hall]

Chairs: Dr. Pardeep Mari & Dr. Sekar Seshagiri

14:00-14:25 - Keynote lecture 06 - Dr. Rajeev Varshney, ICRISAT, Hyderabad, India - Integrating-omics in breeding for accelerating genetic gains in legume crops

14:25-14:50 - Keynote lecture 07 - Dr. Rod A Wing, Arizona Genomics Institute, USA - Harnessing 15MY of Oryza evolutionary history to help solve the 10-billion people question

14:50-15:00 - Break

Session 22 - Targeted Therapy/Immunotherapy [Central Hall]

Chair: Dr. Abhijith Chowdhury

15:00-15:20 - Talk 34 - Dr. Amitabha Chaudhuri, MedGenome, USA - Defining the Onco-Immunogenic identity of tumors to predict survival: A meta-analysis of TCGA data

15:20-15:40 - Talk 35 - Dr. Kumar Prabhash, TMH, India - Targeted therapy and drug repurposing - Indian context

15:40-16:00 - Talk 36 - Dr. Mamun Al Mahtab, Bangabandhu Sheikh Mujib Medical Uni., Bangladesh - Immune therapy for Hepatitis B virus related chronic liver diseases

Session 23 - Biomarkers/Drug Discovery [Crystal Hall - 1]

Chair: Dr. Ramchand CN

15:00-15:20 - Talk 37 - Dr. Kini Manjunatha, NUS, Singapore - Natriuretic peptide analogs with vasodilatory or renal activities: personalized care of heart failure patients

15:20-15:40 - Talk 38 - Dr. Frederic Fellouse, University of Toronto, Canada - Synthetic antibodies for serotype-specific Dengue diagnostics

15:40-16:00 - Talk 39 - Dr. B Sesikeran, NIN, India - Path to approval of novel biologics in India

Session 24 - Animal/Agricultural/Microbial/Viral/ Genomics [Crystal Hall - 2]

Chair: Dr. R Chandra Babu

15:00-15:20 - Talk 40 - Dr. Nazir A Ganai - Sher-e-Kashmir University of Agricultural Sciences and Technology, India - OMICS of Pashmina fiber development
15:20-15:40 - Talk 41 - Dr. Raj Joshi, Uni. of Alberta, Canada - Delineating the complex small RNA networks in the interaction between Allium sativum L. and the basal rot fungus Fusarium oxysporum f. sp. cepae

15:40-16:00 - Talk 42 - Dr. Babu Azariah, TRAI, India - Development, formulation and pilot scale production of an NPV (Nucleo Polyhedro Virus) a biocide for management of the looper Hyposidra talaca infesting tea in North East India

16:00-16:10 - Break

Session 25 - DNA/RNA in Disease [Central Hall]

Chair: Dr. Bhawna Gupta

16:10-16:30 - Talk 43 - Dr. Suvro Chatterjee, Anna Uni., India - Profiling the patterns of miRNAs and transcriptome of human vasculature under micro-gravity: A template study using space science for solving health problems on the Earth

16:30-16:50 - Talk 44 - Priyadarshini Pande, Genes2Me, India - Cell free cancer DNA - An ideal monitoring tool for disease progression

Session 26 - Ancient Genomics to Ayur Genomics [Crystal Hall - 1]

Chair: Dr. Harsha Gowda

16:10-16:30 - Talk 45 - Dr. Maanasa Raghavan, Uni. of Cambridge, UK. In search of our molecular past: Reconstructing human population histories using ancient DNA

16:30-16:50 - Talk 46 - Dr. Mitali Mukerji, IGIB, India - Delineation of human genetic individuality for precision medicine through Ayurgenomics: insights from exomes of extreme Prakriti types

Session 27 - Precision Breeding & Bio-farming [Crystal Hall - 2]

Chair: Dr. George Thomas

16:10-16:30 - Talk 47 - Dr. Karunakaran Maruthachalam, Pioneer, India - Robot-assisted High Throughput Genotyping to Speed up the Crop Breeding Process

16:30-16:50 - Talk 48 - Dr N. Subramonian, Sugarcane Breeding Inst., India - Sugarcane as a platform for the production of high value protein molecules : a promising strategy for molecular farming

16:50-17:00 - Break

Session 28 - Keynote Lectures [Central Hall]

Chairs: Dr. Sekar Seshagiri & Dr. Sachdev Sidhu

17:00-17:25 - Keynote lecture 08 - Dr. Vish Nene, ILRI, Kenya - Improving ruminant vaccine design by focusing on bovine antibody and CD8 T-cell antigen-specific responses

17:25-17:50 - Keynote lecture 09 - Dr. Sachdev Sidhu, Uni of Toronto, Canada - Systems Biologics: The Next Therapeutic Revolution

Session 29 - Posters [Basement]

17:50-18:50 - Poster Viewing # 155-310
Entertainment & Dinner
19:00-20:30 – Entertainment
20:30-21:30 - Dinner

Wednesday, October 04, 2017 - Day 3

Concurrent Session I [Central Hall]
08:30 - Registration begins

Concurrent Session II [Crystal Hall - 1]

Concurrent Session III [Crystal Hall - 2]
09:00-09:50 - Session 30 - Poster competition presentations [Chair: Dr Harsha Gowda]

10:00-20:00 - Session 31 - Art Exhibition, Vendor Exhibits and Poster Display

Session 32 - Keynote Lectures [Central Hall]
Chair: Dr. Sekar Seshagiri
10:00-10:30 - Keynote lecture 10 - Dr. Janina Jeff, Illumina, USA - Genotyping Screening Arrays: An Economic Solution to Improve Precision Medicine Research and Power Genomic Discoveries Across the World
10:30-11:00 - Keynote lecture 11 - Dr. Stephane Angers, Uni of Toronto, Canada - Leveraging genome-wide CRISPR screens and synthetic lethal interactions for novel cancer therapeutics
11:00-11:10 - Journal Presentation (11B) - Mr. Joseph Tryble, JoVE, India - A Novel Solution to Science’s Biggest Problem: Ending the Reproducibility Crisis with Video

11:10-11:30 – Break

Session 33 - Clinical Genomics/Biomarkers [Central Hall]
Chair: Dr. Debasmita P. Alone
11:30-11:50 - Talk 49 - Dr. Ramprasad VL, MedGenome, India - Cell free DNA in Prenatal testing and clinical Oncology
11:50-12:10 - Talk 50 - Dr. TS Sridhar, St. Johns Research Institute, Bangalore, India - Tumour stroma interactions promote metastatic and chemoresistant behaviors in breast cancer
12:10-12:30 - Talk 51 - Dr. Vijayalakshmi Ramshankaar, Cancer Institute (W.I.A.), India - Molecular portrait of oral cancers – South Indian study

Session 34 – Bioinformatics [Crystal Hall - 1]
Chair: Dr. Ravi Gupta
11:30-11:50 - Talk 52 - Dr. Eric Stawiski, Genentech Inc, USA - A Framework for Interpreting Medically Relevant Variants in Asian Cohorts
11:50-12:10 - Talk 53 - Dr. Dinesh Velayutham, AgriGenome, India - A comparative study of RNA-Seq analysis pipelines to assess speed, specificity and sensitivity

12:10-12:30 - Talk 54 - Mr. Henry Wang, Qiagen, Taiwan - From Sample to Insight – QIAGEN Bioinformatics Solution

Session 35 - Animal Genomics/Biology [Crystal Hall - 2]

Chair: Dr. Pradeep Marri

11:30-11:50 - Talk 55 - Dr. Jayakumar Sivalingam, National Bureau of Animal Genetic Resources, India - ddRAD – A Recent Approach for Marker Discovery and Diversity Study in Livestock Species

11:50-12:10 - Talk 56 – Dr. NH Mohan - National Research Centre on Pig, India - Dynamics of peripheral blood mononuclear cell transcriptome with respect to season and breed in pigs: Implications on animal adaptability

12:10-12:30 - Talk 57 - Dr. Sukanta Mondal, ICAR- National Institute of Animal Nutrition and Physiology (NIANP), India - Genomic approaches / strategies for minimizing early embryonic loss

12:30-14:00 - Lunch & Exhibitor visit - Art Exhibition [Central/Crystal/Basement]

Session 36 - Keynote lectures [Central Hall]

Chairs: Dr. George Thomas & Dr. Sekar Seshagiri

14:00-14:25 - Keynote lecture 12 - Dr. Charlie Boone, Uni of Toronto, Canada - A global genetic interaction network maps a wiring diagram of cellular function

14:25-14:50 - Keynote lecture 13 - Dr. Andrey Shaw, Genentech, USA - Approaches to using Single Cell Transcriptome Profiling to understand the structure and function of the kidney glomerulus

14:50-15:00 - Break

Session 37 - Short Talks - Disease & Developmental Biology [Central Hall]

Chair: Dr. Sameer Phalke

15:00-15:10 - Talk 58 – Ms. Devi Santhosh, Uni of Wisconsin, USA - Role of Dynamic GPCR Signaling During Germinal Matrix Blood Vessel Development

15:10-15:20 - Talk 59 - Mr. Nambram Somendro Singh, Uni of Delhi, India - Assessment of antibiotic resistance genes and integrons in commensal Escherichia coli from the Indian urban waste water: Implications and significance for public health

15:20-15:30 - Talk 60 - Mr. Prajish Iyer, ACTREC Tata memorial Hospital, India - Diversity of Somatic Alterations and Salmonella Infection in Gastric Cancer by Whole Exome Sequencing

15:30-15:40 - Talk 61 - Mr. Manas Dikhit, RMRI Patna, India - Computational screening of Six Antigens for potential MHC class II restricted epitopes and evaluating its CD4+ T-Cell Responsiveness against Visceral Leishmaniasis

15:40-15:50 - Talk 62 - Ms. Chitra G, VIT, India - Efficiency of ubiquitous chromatin opening elements in driving the expression of human CD18 within self-inactivating lentiviral vectors for gene therapy applications

Session 38 - Short Talks - Plant Genomics [Crystal Hall - 1]

Chair: Dr. Harshoa Gowda

15:00-15:10 - Talk 63 - Dr. Gunjan Sirohi, GGSIPU, India - High-throughput sequencing of small RNAs and analysis of differentially expressed microRNAs associated with Brassinosteroid signaling in Arabidopsis thaliana


15:30-15:40 - Talk 66 - Ms. Prasidhee V, SRM Uni, India - Sequencing, de novo assembly, functional annotation and analysis of Cardiospermum halicacabum L. leaf transcriptome using the Illumina platform

15:40-15:50 - Talk 67 - Ms. Reshma Patil, Shivaji University, India - Comparative transcriptome analysis of flowers of polygamodioecious Kokum; Garcinia indica

Session 39 - Short Talks - Plant/Animal/Human Biology [Crystal Hall - 2]

Chair: Dr. Uma Ramakrishnan

15:00-15:10 - Talk 68 - Mr. Adil Lateef, SRM Uni, India - De novo assembly and analysis of Solanum trilobatum L. leaf transcriptome using Next Generation Sequencing Technology

15:10-15:20 - Talk 69 - Mr. Anjan Kumar Pradhan, Orissa University Of Agriculture and Technology, India - Identification of genetic variant in buffalo genome using ddrad sequence

15:20-15:30 - Talk 70 - Mr. Sankaranarayanan Srinivasan, Sree Sastha Institute of Engineering and Technology, India - Generation and usage of a genetically engineered Virophage with RTase for the treatment of Ebola

15:30-15:40 - Talk 71 - Ms. Ipsita Mohanty, Regional Medical Research Centre (ICMR), India - Effect of Wolbachia on Dengue infection in Endemic districts of Odisha

15:40-15:50 - Talk 72 - Ms. Nipa Basak, CCMB, India - Epigenetic signatures of high altitude adaptation in Tibetan population

15:50-16:00 - Break

Session 40 - Disease/Vector Biology [Central Hall]

Chair: Dr. Mitali Mukerji

16:00-16:20 - Talk 73 - Dr. Rajnikant Dixit, National Institute of Malaria Research, India - Decoding Genetic power of mosquito-microbe-parasite interactions and malaria transmission in Indian malarial vectors

16:20-16:40 - Talk 74 - Dr. Ranjana Pathania, IIT Roorkee, India - Acinetobacter baumannii- Exploring the non-coding RNA landscape to understand its physiology and multiple drug resistance

16:40-17:00 - Talk 75 - Dr. Subbiah Ramasamy, Temple University, USA - Xenobiotics induced glucose dyshomeostasis

Session 41 - Genome and Gene Expression [Crystal Hall - 1]

Chair: Dr. Arati Khanna Gupta

16:00-16:20 - Talk 76 - Dr. Mukund Ramakrishnan, IISER Berhampur, India - Control of Clock Gene Expression through Facultative Heterochromatin

16:20-16:40 - Talk 77 - Dr. Swarkar Sharma, J&K Uni., India - High Mitogenomes Diversity in Jammu and Kashmir Defies that Ancient Human Migrations in India were of Males Exclusively

16:40-16:50 - Talk 78 - Ms. Indu Sharma, Shri Mata Vaishno Devi University, India - Y chromosome haplogroup distribution in different ethnic groups of Jammu and Kashmir, India

16:50-17:00 - Talk 79 - Ms. Esha Bandyopadhyay, SRM Uni, India - Ancient genomics in India: Clarifying the maternal origins of 160-year-old human remains
Session 42 – Genomics [Crystal Hall - 2]

Chair: Dr. Sriharsa Pradhan

16:00-16:20 - Talk 80 - Dr. Avid Hussain, Agilent, India - Agilent SureSelect XT HS: A rapid NGS Assay for FFPE samples with 10 ng input DNA

16:20-16:40 - Talk 81 - Dr. Juggnu Jain, Sapien Biosciences and Sarrum Sciences, India - Mining and mapping of Indian breast cancer genome using a database of >7500 breast cancer cases collated over 10 years from 5 geographically distinct tertiary care centers can reveal novel prognostic markers and drug targets

16:40-17:00 - Talk 82 - Dr. Priyadarshini Pande, Genes2Me, India - Whole genome microarray analysis leads to improvement in diagnostic yield in prenatal genetic testing

17:00-17:10 - Break

Session 43 - Awards and Closing Remarks [Central Hall]

Chairs: Dr. Sekar Seshagiri & Dr. George Thomas

17:10-17:50 - Poster Awards, Genome Grants Awards, & close/wrap-up
Category: Animal genomics

Ancient genomics in India: Clarifying the maternal origins of 160-year-old human remains

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Abstract

Sequencing DNA from archaeological remains has opened up new possibilities for furthering our understanding of the origins and evolutionary history of modern humans [1]. However, most ancient DNA (aDNA) studies, thus far, have focused on ancient samples obtained from permafrozen and temperate regions, where preservation conditions are better suited for long-term DNA survival. Consequently, this has left a void in aDNA research in tropical regions such as South Asia. The primary aims of the present study were to (a) test the feasibility of extracting DNA from historical samples (~160 years old) from northern India, and (b) correlate obtained mitochondrial DNA (mtDNA) signatures with geographical origins of the individuals, as reported in historical records. A total of 30 molars were subjected to DNA extractions and Illumina indexed library preparation. All laboratory work was performed following strict aDNA standards in the clean laboratory at the Centre for Cellular and Molecular Biology, Hyderabad. Complete mtDNA genomes were targeted from all 30 samples following the DNA hybridization method outlined in Maricic et al., 2010 [2]. Captured libraries were sequenced on the Illumina HiSeq 2500 platform (100 bp paired-end mode) at MedGenome Inc., Bangalore. Obtained sequences were trimmed for residual adapters using AdapterRemoval and mapped to the revised Cambridge Reference Sequence (rCRS) using BWA. HaploGrep2 [3] was used to assign mtDNA haplogroups to each sample. We successfully obtained endogenous mtDNA sequences from all 30 samples, as confirmed by typical aDNA damage (cytosine deamination on the ends of DNA molecules). Coverage and depth of sequencing were in the range of 91-99.5% and 6X-371X, respectively. To ascertain the maternal origins of the individuals, mtDNA haplogroups of our samples were compared to a database compiled from published mtDNA sequences from modern South Asian individuals. Based on this, we were able to confirm northern Indian origins for the studied individuals, although further fine-scale resolution will only be available with a more comprehensive comparative dataset. This pilot study shows the feasibility of obtaining aDNA from India and its immense value in understanding the population history of humans over time.

References

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Citation: Bandyopadhyay, E., Sehrawat, J.S., Rai, N. and Raghavan, M. Ancient genomics in India: Clarifying the maternal origins of 160-year-old human remains [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 13, https://doi.org/10.24870/cjb.2017-a1
Category: Animal genomics

A genome-wide map of circular RNAs in adult zebrafish

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Abstract

Circular RNAs are a new addition to the growing list of diverse species of RNAs that are formed by covalent linked 3' and 5' end forming a closed loop structure. Circular RNAs are characteristically resistant to exonuclease treatment and are relatively stable to linear transcripts. Circular RNAs are formed by alternate splicing mechanism but do not follow the canonical order of exons. Backsplice junctions are unique to circRNAs. CircRNAs are shown to possess potential to act as miRNA sponges and control transcription of mRNAs. CircRNAs are also reported as biomarkers for the disease like Alzheimer's, Parkinson's and cancer. A huge number of circRNA transcripts have been identified in model organisms including C.elegans, mouse, Drosophila as well as human. But there are no circular RNAs reported in zebrafish that is a very good model to study developmental stages, cardiovascular and blood-related disorders. In order to use zebrafish as a model organism and study the role of circRNAs in disease, we have used in-house generated RNA-sequencing data for five tissues including blood, brain, muscle, gills and heart. We discarded the reads mapped contiguously and full length over reference genome and identified back-splice junctions for putative circRNA transcripts. We identified a total of 3428 circRNA junctions out of which 78% were tissue specific. We validated 22 selected candidates for 5 tissues based on literature significance. We quantitatively analysed 5 tissue-enriched candidates using Real-time PCR. We also observed that major proportion of circRNAs is originating from protein coding loci. These circRNAs could be used to further study their role in hematopoietic and cardiovascular diseases.

Category: Animal genomics

**Genome –wide variation and demographic history of small cats with a focus on Felis species**

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**Abstract**

Majority of the 38 known cat species are classified as small and they inhabit five of the seven continents. They survive in a vast range of habitats but still 12 out of the 18 threatened felids are small cats. However, there has not been enough progress in the field of small cat research as they generally get overshadowed by the charismatic big cats. Here we attempt to create a resource for small cat research especially of the genus Felis which has six species out of which two are classified as vulnerable by IUCN and at least one more is at risk. We collected tissue samples of four Felis chaus (Jungle cat) from central India and used available whole genome sequences of nine individuals from four other Felis species, two individuals of Prionailurus bengalensis and an Otocolobus manul. These whole genome sequences were filtered and aligned with the already published domestic cat (Felis catus) genome assembly. Felids are closely related species and reads from all species in our study aligned with the domestic cat genome with a rate of at least 93%. We estimated the existing genomic variation by calculating heterozygous SNP encounter rate. So far, it seems that all wild cats have more genetic variation than Felis catus species. This can be attributed to the inbreeding in these cats. Among the wild cats, Felis silvestris seems to have the highest level of genetic variation. To understand the reasons behind the distribution of genetic variation in small cats, we estimated the demographic histories of each of the species using PSMC. This method can only detect demographic changes more than 1000 generations ago. We observe that roughly all species share a parallel history in terms of population increase. The most interesting and important feature might be that all wild small cat population sizes increased exponentially around twenty thousand years ago as opposed to domestic cat and big cats which declined around this time. Another interesting feature of the demographic history is all the small cats seem to have recovered from the effects of Toba Volcano eruption which had triggered a glacial maximum leading a decline in big cat population. Thus it seems the partitioning of genetic variation has happened less than ten thousand years ago owing to anthropogenic activities?

**Citation:** Khan, A., Vinekar, R., Thatte, P. and Ramakrishnan, U. Genome –wide variation and demographic history of small cats with a focus on Felis species [Abstract]. In: Abstracts of the NGHT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 15. https://doi.org/10.24870/cjb.2017-a3
Molecular Cloning and In-Silico Analysis of A WGS derived genomic contig of a putative Angiotensinogen from the Teleost Sebastes Schlegelii

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Abstract

Angiotensinogen (AGT) is the major substrate in the Renin-Angiotensinogen system (RAS), the primary hormonal signaling cascade ascribed primarily towards body fluid and blood pressure regulation, with peripheral albeit salient pro-inflammatory immune roles[1]. A WGS derived genomic DNA contig sequence with a presumed angiotensinogen gene (3802 bp with a 1383 bp, 6-exon coding region) was acquired from Sebastes schlegelii (Rock Fish) and subjected to extensive computer-assisted sequence analysis. The polypeptide derived via sequence based prediction tools defined a length of 460 amino acids, with a molecular mass of 51.3 kDa. Furthermore, RFAgt revealed a signal peptide incorporating approximately 19-residues upstream the putative angiotensinogen I signature motif (²⁰NRVYVHPFYL²⁹), with the peptide cleavage site residing between ¹⁹Ala-Asp²⁰, indicating its secretory nature. RFAgt also demonstrated a Serpin domain (between residues 9-458) with conserved sequence motif (⁴³¹LSINRPFFFSV⁴⁴¹), implicating a sequence-specific non-inhibitory role[1]. Sequence homology and genetic distance based phylogenetic analysis (augmented by 1000-iteration bootstrap analysis) revealed that RFAgt is evolutionary proximate to the AGT’s of Oplegnathus fasciatus, Larimichthys crocea and Rhabdosargus sarba. Validation of the In-silico predicted ORF conducted via PCR amplification using sequence specific primers (F-5’ATG CGG TCG CCT CTT CTA GC-3’ and R-5’- TTA CAG TGT AGG ATT GAT GAT CTT GCC-3’), and subsequent visualization via Gel-electrophoresis revealed a concomitant band at 1383 bp. Consecutively, upon purification, an attempt was made to ligate the product into a pGEM®-T Easy vector (size 3015 bp). The experimental component will further expound on the Tissue-specific expression analysis with anticipated highest expression in the liver and a challenge (injury/infection) based expression study with a potential upregulation of RFAgt expression during physiological stress expected [1].

References


Category: Animal genomics

A study on climatic adaptation of dipteran mitochondrial protein coding genes

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Abstract

Diptera, the true flies are frequently found in nature and their habitat is found all over the world including Antarctica and Polar Regions. The number of documented species for order diptera is quite high and thought to be 14% of the total animal present in the earth [1]. Most of the study in diptera has focused on the taxa of economic and medical importance, such as the fruit flies Ceratitis capitata and Bactrocera spp. (Tephritidae), which are serious agricultural pests; the blowflies (Calliphoridae) and oestrid flies (Oestridae), which can cause myiasis; the anophelies mosquitoes (Culicidae), are the vectors of malaria; and leaf-miners (Agromyzidae), vegetable and horticultural pests [2]. Insect mitochondrion consists of 13 protein coding genes, 22 tRNAs and 2 rRNAs, are the remnant portion of alpha-proteobacteria is responsible for simultaneous function of energy production and thermoregulation of the cell through the bi-genomic system thus different adaptability in different climatic condition might have compensated by complementary changes is the both genomes [3,4]. In this study we have collected complete mitochondrial genome and occurrence data of one hundred thirteen such dipteran insects from different databases and literature survey. Our understanding of the genetic basis of climatic adaptation in diptera is limited to the basic information on the occurrence location of those species and mito genetic factors underlying changes in conspicuous phenotypes. To examine this hypothesis, we have taken an approach of Nucleotide substitution analysis for 13 protein coding genes of mitochondrial DNA individually and combined by different software for monophyletic group as well as paraphyletic group of dipteran species. Moreover, we have also calculated codon adaptation index for all dipteran mitochondrial protein coding genes. Following this work, we have classified our sample organisms according to their location data from GBIF (https://www.gbif.org). Finally, result suggests that dipteran insects from different regions are gone through distinct selection process and even our outcome also indicate that dipteran mitochondrial genes from different climatic condition shows differential efficacy in their translation process.

References


Citation: Kabiraj, D. and Bora, U. A study on climatic adaptation of dipteran mitochondrial protein coding genes [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 17. https://doi.org/10.24870/cjb.2017-a5
This abstract has been withdrawn by the author
SNP genotyping to monitor wild tigers for conservation

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Abstract

Tigers have experienced dramatic range contraction in the recent past and currently occupy only 7% of their historical range [1]. Genetic tools can be used effectively to monitor wild species of conservation concern such as tigers. Such approaches allow us to identify individuals, reconstruct relatedness between them, and monitor connectivity between populations. Microsatellite markers are currently used across many laboratories to study tiger population and conservation genetics. However, non-invasive samples such as feces continue to present challenges with high error rates and low amplification success for such microsatellite loci [2]. In this study, we developed a panel of Single Nucleotide Polymorphism (SNP) markers and experimental pipeline for use with fecal samples from wild tigers. Multiplex PCR followed by Illumina sequencing of pooled, barcoded samples allowed fast implementation of these protocols. A total of 339 SNPs were targeted and amplified in short fragments of 40 base pairs. All samples were run in triplicate to investigate error among replicates. In the first run the protocol was tested using captive tiger fecal samples of different ancestry and a varying target DNA concentration. Following this, non-invasive samples (fecal, saliva and shed hair) collected from multiple field sites across India were tested. Results revealed that samples with very low initial concentration of target DNA (<1ng) had high genotyping success. The observed probability of identity (the probability of obtaining the same genotype for two different individuals) was found to be very low (PID = 2.4E-85, PID sibs = 4.4E-44). Replicate genotypes of the same sample were highly similar with rare occurrence of mismatches. No differences were observed in success rate for the different ancestries. Application of the protocol to fecal samples collected from wild tigers showed that success was independent of sample type. Initial concentration of template DNA seems to govern success, and generally samples with 0.02 ng/µl or higher showed high genotyping success. Replicate genotypes were still highly consistent. In summary, we demonstrate the utility of a highly multiplexed SNP genotyping protocol from non-invasively collected tiger samples. We suggest that such protocols will help in generating data faster, cheaper, while also being compatible across labs.

References


Identification of genetic variant in buffalo genome using ddRAD sequence

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Abstract

*Bubalus bubalis* (water buffalo) is an agro-economically important livestock species due to its multipurpose use in India and other Asian countries. Among the total of 13 recognized breeds of water buffalo, majority are milch breeds in India and some of them have been listed on a state-level conservation plan by the Ministry of Agriculture, Government of India. As buffalo milk occupies the highest share in Indian dairy sector, the future improvement in traits of economic importance is dependent on genetic variation present within and between the breeds. Even though they have an important role in Indian agricultural economy, most of the breeds have not been exploited for their full genetic potential. Molecular markers like single nucleotide polymorphisms (SNPs) can play a significant role in livestock improvement through conventional breeding programmes. The aim of this study is to identify single nucleotide polymorphisms (SNPs) from buffalo genome using ddRAD sequencing through STACKS pipeline. Here we have used double digest restriction-associated DNA sequencing (ddRAD) for identification and annotation of genetic variant from three traits of buffalo such as Milk yield, Lactation period, Age at first calving. The Stacks pipeline is used to create genetic maps and conduct population analysis. It assembles loci from an individual’s sequence reads by using a reference sequence. The total SNPs found in buffalo for three important traits Milk yield, Lactation period and Age at first calving are 25802, 9218 and 17914, respectively. The total genotypes, genotype frequencies and genotype map have been calculated in the population. For computation of population genetic measures (The inbreeding coefficient of an individual) $F_{IS}$ and (An estimate of nucleotide diversity) $\pi$ within populations and (A measure of population differentiation) $F_{ST}$ between populations has been found.

Category: Animal genomics

Identification of molecular markers in *Labeo rohita* towards better carbohydrate utilization

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**Abstract**

The contribution of aquaculture products in providing nutritional and food security to human is increasing expeditiously with the increase in animal protein demand. Feed cost contributes more than 60% of the cost of aquaculture production. Henceforth, formulation of cheap fish feed is one of the greatest challenges in aquaculture industry. Carbohydrates are the cheap source of dietary energy. So their level of utilization in fish is an exciting area in research for decreasing the fish feed cost. Molecular markers such as microsatellite and single nucleotide polymorphism (SNP) are used for genetic mapping, quantitative trait loci identification and genome-wide association studies in several aquaculture species. In this experiment, SNPs and microsatellite markers linked to carbohydrate utilization in *Labeo rohita* were identified. Liver tissue samples of *Labeo rohita* and *Labeo bata* were collected from individuals fed with a customized diet with 40% carbohydrate for a period of 21 days. RNA was extracted and cDNA library was prepared and sequenced on Illumina NextSeq 500 platform. 7.5 GB of data was generated from each species. Assembly of rohu data resulted in 70,225 contigs, out of which 6284 microsatellite markers were identified. Among which, 3838, 1817, 488, 132 and 9 were di-, tri-, tetra-, penta- and hexa-repeats, respectively. Primer modelling was successful for 4190 sequences. Similarly, 2, 14, 071 SNPs were identified using CLC bio v7.0.4 and utilizing Illumina reads obtained from *Labeo bata*. This study can be helpful in efficient use of carbohydrate in *Labeo* species for decreasing feed cost globally.

**References**


Enabling rapid and efficient selection of Next generation sequencing algorithms to align sequences to predict differentially expressed genes in cancer

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Abstract

Next-generation sequencing (NGS) has revolutionized technology in genomics; enabling entire genome or sampling of transcriptomes to be sequenced more efficiently than ever before. There are several applications on sequencing include molecular diagnostics to identify the disease condition based on genetic variants and also used in agrigenomics for crop improvement. Although, there are several limitations to use NGS in genomic research include the development of bioinformatics pipelines and algorithms to retrieve, analyze and store large genomic data. Analysis of genomic data typically require bioinformatics pipelines of substantial levels to analyze large data is typically require computing power is most cost effective. While NGS technologies have significant implications on clinical and agricultural data for optimization and availability of appropriate methodologies and tools to analyze, visualize and interpret NGS data are still in their infancy. With an objective of this study was used ovarian cancer sequencing data to analyze clinical variant to predict clinical biomarkers. Here, with focus on implementation of NGS data analysis algorithms, advantages and challenges of implementing NGS.

Citation: Patil, M.L., Madagi, S.B. and Prashantha, C.N. Enabling rapid and efficient selection of Next generation sequencing algorithms to align sequences to predict differentially expressed genes in cancer [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 22. https://doi.org/10.24870/cjb.2017-a10
Category: Bioinformatics

Molecular Docking and Molecular Dynamics Simulation studies of DHFR inhibitors in Plasmodium falciparum

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Abstract

Malaria, caused by Plasmodium falciparum is a very common disease that causes 2.5 million deaths worldwide. This makes designing of lead molecules for malaria very exigent. DHFR has been known to be one of the major targets of antimalarial drug therapy which functions as a fundamental cofactor in the synthesis of histidine and methionine as well as purine nucleotides. Inhibition of this DHFR blocks the reduction of Dihydrofolate (DHF) to Tetrahydrofolate (THF) and hence prevents the synthesis of DNA, resulting in death of Plasmodium falciparum. Pyrimethamine is a Diaminopyrimidine that inhibits pfDHFR (Plasmodium falciparum DHFR) at a concentration that is 1000 times less than that required to inhibit the mammalian DHFR. Virtual screening is performed to find Pyrimethamine analogs from PubChem database. Docking studies are performed on DHFR (PDB ID: 3QGT) with Pyrimethamine and its 193 derivatives and the differences in their binding modes are investigated. The binding score suggests 53 derivatives to be more potent than Pyrimethamine which has a score of -24.7 showing interaction with Ile14, Asp54 and Ile164. The compound with best binding score (-35) showed interaction with Ile14, Cys15, Asp54, Phe58, Ser108, Ser111, Ile164 and Tyr170. The compounds are screened based on hydrogen bonding, π-π interactions, halogen bonding and orientation within the binding site with high binding score using Maestro (v.11.0.014, Schrodinger). The best screened compound is selected for Molecular Dynamic Simulation analysis up to 20ns using Desmond (v.4.8, Schrodinger) which represents a good starting point for further in vivo experimentation and can probably serve as an ideal lead compound for the treatment of Malaria.

Category: Bioinformatics

Text-Mining Applications for Creation of Biofilm Literature Database

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Abstract

The massive information hidden in the biomedical field, in the form of publications is growing exponentially therefore it is not possible for researchers and practitioners to keep themselves updated with all the developments in any specific field. Manual effort to transform unstructured text into structured is a laborious process. Automatic techniques for relation extraction provide a solution to the problem. Text Mining is one such technique defined as “the discovery by computer of new, previously unknown information, by automatically extracting and relating information from different written resources, to reveal otherwise ‘hidden’ meanings’. The current study is focused on biofilm literature where biofilms are dense, highly hydrated cell clusters that are irreversibly attached to a substratum, to an interface or to each other, and are embedded in a self-produced gelatinous matrix composed of extracellular polymeric substances. Biofilm research has become a very important field, due to their high mechanical resilience and resistance to antibiotic treatment, they constitute a significant problem in both industry and health care.

So in the present research published corpora of 34306 documents for biofilm was collected from PubMed database along with non-indexed resources like books, conferences, newspaper articles, etc. and these were divided into five categories i.e. classification, growth and development, physiology, drug effects and radiation effects. These five categories were further individually divided into three parts i.e. Journal Title, Abstract Title, and Abstract Text to make indexing highly specific. Text-processing was done using the software Rapid Miner_v5.3, which tokenizes the entire text into words and provides the frequency of each word within the document. The obtained words were normalized using Remove Stop and Stem Word command of Rapid Miner_v5.3 which removes the stopping and stemming words. The obtained words were stored in MS-Excel 2007 and were sorted in decreasing order of frequency using Sort & Filter command of MS-Excel 2007. The words are visualization through networks obtained by Cytoscape_v2.7.0. Now the words obtained were highly specific for biofilms, generating a controlled biofilm vocabulary and this vocabulary could be used for indexing articles for biofilm (similar to MeSH database which indexes articles for PubMed). The obtained keywords information was stored in the relational database which is locally hosted using the WAMP_v2.4 (Windows, Apache, MySQL, PHP) server. The available biofilm vocabulary will be significant for researchers studying biofilm literature, making their search easy and efficient.

Citation: Gupta, K. and Kumar, A. Text-Mining Applications for Creation of Biofilm Literature Database [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 24. https://doi.org/10.24870/cjb.2017-a12
Molecular Docking and Molecular Dynamic studies of Phytocompounds with HIF-1α, HIF-2α, and SREBP1c to Explore its Inhibitory Effect on Metabolic disorders and in Cancer

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Abstract

Hypoxia-inducible factors (HIFs) are important components of the cellular oxygen-signaling pathway. In response to low oxygen tensions, HIFs facilitate both oxygen delivery and adaptation to oxygen deprivation by regulating the expression of genes that are involved in glucose uptake and metabolism, angiogenesis, erythropoiesis, cell proliferation, and apoptosis. Hence HIFs role in the regulation of different cancers is crucial. Moreover, these proteins also play a role in the hepatic lipid metabolism. SREBP1c is a transcriptional factor and as well as key regulator of lipid metabolism through different signaling pathways. Hence, our study focuses to study the association between different inhibitory ligands with these key proteins. In order to investigate the binding mechanism of five phytocompounds, Curcumin, Digoxin, Epigallocatechin gallate (EGCG), Epigallocatechin (EGC) and Gallocatechin gallate (GCG) with drug targeted receptors viz., HIF-1α (PDB ID:5LA9), SREBP1c (PDB ID:1AM9) and HIF-2α (PDB ID:2A24) molecular docking and molecular dynamics simulation were performed. The best score among above compounds, on the basis of hydrogen bonding while docking by FlexX software, curcumin showed best score among all phytocompounds to HIF-1α (-20.72) and HIF-2α (-11.76), also for SREBP1c protein though Curcumin showed good score (-12.23) but EGC had an superiority, because the complex had more hydrogen and aromatic hydrogen bond and it also has an interaction with cytosine (DC26) residue from DNA and has score -12.03. Three independent molecular dynamics simulations (20ns) runs indicated general stability of curcumin in binding pocket of HIF-1α, HIF-2 α and EGC in SREBP1c as well as the tendency to form hydrogen bonds with water molecules in HIF-1α and SREBP1c also EGC form hydrogen bond with cytosine in SREBP1c. These results enhance further in vitro and in vivo experimentation and can probably serve as an ideal molecule for cancer treatment and metabolic disorders.

Citation: Yadav, R.S., Lokhande, K.B., Reddy, V.D. and Swamy, K.V. Molecular Docking and Molecular Dynamic studies of Phytocompounds with HIF-1α, HIF-2α, and SREBP1c to Explore its Inhibitory Effect on Metabolic disorders and in Cancer [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 25. https://doi.org/10.24870/cjb.2017-a13
Category: Bioinformatics

High throughput virtual screening to identify a novel inhibitor against Pyrazinamide resistant tuberculosis

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Abstract

In recent years multi drug resistance tuberculosis (TB) has become a serious health problem globally. The emergence of multi-drug resistant mycobacterium strains has made most of the convention drugs ineffective. Therefore development of new therapeutic strategies such as finding of novel and more efficient inhibitors against drug resistant mutant proteins are required. In this study, we have analyzed the mechanism of mutations responsible for resistance against first-line anti-tuberculosis pyrazinamide pro-drug. First, pyrazinamide (pro-drug), activated Pyrazinamide (drug) and its isoforms were analyzed for their binding affinity against mutant forms of PncA (Pyrazinamidase) at the ligand binding cavity. It was observed that due to the mutations, after conversion of pro-drug to drug, the strong binding of PncA reduces the release of activated form of Pyrazinamide to inhibit other virulent proteins. So in order to discover a novel Drug molecule against mutant PncA, high throughput virtual screening was performed at the same cavity with the 826 drugs like antituberculant compounds derived from ChEMBL database. The predicted lead molecule was found with having suitable affinity and bond interactions in both wild and mutant PncA protein. For the further confirmation, the lead compound was compared against some frequently occurring mutations individually. In all mutated forms, the lead molecule was found more efficient than the activated Pyrazinamide. Hence we believe that this molecule may act as a novel drug to improve the therapy of pyrazinamide resistant tuberculosis.

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Citation: Jagadeb, M. and Sonawane, A. High throughput virtual screening to identify a novel inhibitor against Pyrazinamide resistant tuberculosis [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 26. https://doi.org/10.24870/cjb.2017-a14
Targeted Molecular Dynamics to determine Focal Adhesion Targeting Domain Folding Intermediates

Pallavi Mohanty and Sonika Bhatnagar

Abstract

The Focal adhesion kinase (FAT) domain of Focal Adhesion Kinase is a four helical bundle known for conformational plasticity. FAT adopts two distinctly different conformations i.e., close (cFAT) and arm-exchanged (aeFAT) states under native conditions [1]. The slow transition from cFAT to aeFAT is likely to proceed through an open intermediate state that allows YENV motif to attain β-turn conformation and phosphorylation of Y925 by Src kinases [2]. The two end states of FAT are known to interact with Paxillin and are responsible for maintaining steady state in Heart while intermediate conformation interacts with Grb2-SH2 leading to Pathological Cardiac Hypertrophy (PAH) [2]. 10ns Targeted Molecular Dynamics (TMD) was done between c- and aeFAT in order to explore the conformational transition and to capture pathologically relevant oFAT. Cluster and dynamic cross correlation analysis (DCCA) of TMD generated trajectory was done and the selected FAT intermediate was docked with Grb2-SH2 using HADDOCK v2.2 docking followed by molecular dynamics. Conservation analysis of FAT-Grb2 binding site was done using CONSURF [3]. A Pharmacophore FAT-Grb2 complex was generated using SPARKv1.2 and submitted for Virtual screening using BLAZE v4. Drug likeliness and ADMET properties were calculated using MOLINSPIRATION tool. TMD reveals six clusters and DCCA showed positively and negatively correlated region along the transition pathway. Intermediates with competence for Grb2 interaction were docked with Grb2 and best binding complex was further refined. MMPBSA binding energy calculations revealed the best binding pose where the phosphorylated YENV motif of Human FAT interacted with a charged and hydrophobic pocket of Grb2. The conservation analysis showed that the charged pocket was more conserved in comparison with the hydrophobic pocket, hence providing useful insights on binding and specificity determining residues in Grb2. Virtual screening using the pharmacophore yielded 3829 hits out of ~10 lakh ligand library. Further ADMET refinement and AUTODOCK batch docking identified five high affinity inhibitors for disruption of the oFAT-Grb2 interface. Pharmacological modulation of the FAT-Grb2 interaction will help in the development of selective inhibitors against PAH.

References


Citation: Mohanty, P. and Bhatnagar, S. Targeted Molecular Dynamics to determine Focal Adhesion Targeting Domain Folding Intermediates [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 27. https://doi.org/10.24870/cjb.2017-a15
Category: Bioinformatics

Drug targets and lipid biomarkers of hyperlipidemia associated diseases

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Abstract

Hyperlipidemia (HL) is characterized by elevated level of plasma lipids affecting regular functioning of lipid-protein interactions leading to disease. The traditional lipid biomarkers utilized for detection of individuals with HL and Cardiovascular Diseases (CVD) risk, sometimes fail to predict future CVD events therefore, establishing need of novel lipid biomarkers for CVD detection. It was attempted to predict the lipid biomarkers using systems biology and by a meta-analysis of lipidomics studies. A lipid-protein-protein interaction network (LPPIN) was built by incorporating differentially expressed genes in fatty liver of obese subjects obtained from Gene Expression Omnibus, with the interacting lipids obtained from STITCH database. The protein interactions were derived using PathwayLinker and lipid interactions were acquired from STITCH 4.0. For identification of novel lipid biomarkers comparative analysis of normolipidemic, hyperlipidemic and CVD lipid profile was conducted. Cholesterol, diacylglycerol, phosphatidylinositol-bis-phosphate and inositol-triphosphate were central to LPPIN, therefore had the largest systemic effect. The choke point analysis identified proteins having maximum interactions with network lipids, malfunctioning of which could lead to HL associated diseases. Cluster analysis recognized CVD, cancer, Alzheimer's disease and type-2-diabetes to be linked with HL. Lipids associated with the disease clusters consisted of triacylglycerol, cholesterol, oleic acid, linoleic acid, arachidonic acid, palmitate, inositol triphosphate, inositol-1,4-bisphosphate and phosphatidinositol-4-phosphate. Approved HL drug targets like Proprotein Convertase Subtilisin Kexin Type 9 and Niemann-Pick C1-Like 1 may be repurposed for treatment of hyperlipidemia associated diseases. Using a combination of gene prioritization and bridging centrality, Coactosin-like binding protein 1, Vasodilator stimulated phosphoprotein and Hedgehog acyltransferase were identified as a potential drug target for CVD, cancer and Alzheimer's, respectively. A comparative lipidomics analysis revealed that palmitoyl-lysophosphatidylcholine level was decreased while plasma level of free fatty acids and ceramides were elevated in HL and CVD. Both HL and CVD were associated with changes in lipid composition. HL was associated with increased level of saturated diacylglycerol, triacylglycerol and phospholipids while CVD was associated with increase in small chain fatty acids with low double bond content in triacylglycerol, cholesteryl ester and sphingomyelin. Our work highlights new drug targets and biomarkers for hyperlipidemia.

Category: Bioinformatics

Systems-level organization of non-alcoholic fatty liver disease progression network

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Abstract

Non-Alcoholic Fatty Liver Disease (NAFLD) is a hepatic metabolic disorder that is commonly associated with sedentary lifestyle and high fat diets. NAFLD is prevalent in individuals with obesity, insulin resistance and Type 2 Diabetes (T2D). The clinical spectrum of NAFLD ranges from simple steatosis to Non-Alcoholic Steatohepatitis (NASH) with fibrosis, which can progress to cirrhosis and hepatocellular carcinoma. The pathogenesis of NAFLD is complex, involving crosstalk between multiple organs, cell-types, and environmental and genetic factors. Dysfunction of White Adipose Tissue (WAT) plays a central role in the development of NAFLD and other metabolic disorders. WAT is an active endocrine organ that regulates whole-body energy homeostasis, lipid metabolism, insulin sensitivity and food intake by secreting biologically active molecules (lipokines, adipokines and cytokines). WAT dynamically reacts to nutrient excess or deprivation by remodelling the number (called hyperplasia) and/or size (called hypertrophy) of adipocytes to store fat or supply nutrients to other tissues by lipolysis, respectively. Adipose tissue remodelling is also accompanied by changes in the composition or function of stromal vascular cells and ECM. The major objective of our study was to identify and characterize the metabolic and signaling modules associated with the progression of NAFLD in the VAT. We performed Weighted Gene Co-expression Network Analysis (WGCNA) to organize microarray data obtained from the VAT of patients at different stages of NAFLD into functional modules. In order to obtain insights into the metabolism and its regulation at the genome scale, a co-expression network of metabolic genes in the Human Metabolic Network (HMR2) was constructed and compared with the co-expression network constructed based on all the varying genes. We also used the prior network information on adipocyte metabolism (GEM) to verify and extract reporter metabolites. Our analysis revealed the coordination of metabolism and inflammation in NAFLD patients. We found that genes of arachidonic acid, sphingolipid and glycosphingolipid metabolism were upregulated and co-expressed with genes of proinflammatory signaling pathways and hypoxia in NASH/NASH with fibrosis. These metabolic alterations might play a role in sustaining VAT inflammation. Further, the inflammation related genes were also co-expressed with genes involved in the ECM degradation. We interlink these cellular processes to obtain a systems-level understanding of NAFLD.

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Category: Bioinformatics

Comparative Genome analysis of *Plasmodium sp.* and identification of unique signature with Next Generation Sequencing Technology

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Abstract

Malaria is a malignant disease which is growing all over the world and its causative agent. *Plasmodium* species easily develops resistant to commonly used antimalarial drugs easily. These empower different strains of *Plasmodium* e.g. *Plasmodium falciparum* and *Plasmodium vivax* to infect humans with malaria. To get the deeper molecular insights, next generation sequencing data were used for further analysis as it has shifted the paradigm of genomics to address biological questions with high confidence and in timely manner. The short reads for above mentioned parasites were retrieved from SRA (Sequence read archive) and *de novo* assembly was performed. Several novel genes along with known genes were predicted from assembled contigs, Functional annotation followed by gene ontology and pathway analysis. Comparison between species gave structural and functional diversity of the specific genes responsible for disease condition which further can be studied for disease biology.

Category: Bioinformatics

Meaningful interpretation by reanalyzing the publicly available dataset: A case study of *Salvia miltiorrhiza*

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Abstract

MicroRNAs are a newly discovered class of non-protein small RNAs with 22-24 nucleotides and evolutionary conserved post-transcriptional regulatory RNAs, which shows an enormous role in several biological and metabolic process. Plant derived miRNA entered into the body fluid and regulated the expression of host mRNA. *Salvia miltiorrhiza* is an important medicinal plant known for its potent cardiovascular and anticancer activity and hence, it was selected to identify the cross kingdom regulatory mechanism between medicinal plant and human. In this study, total 8 highly stable putative novel miRNA were predicted from the publically available ESTs of *Salvia miltiorrhiza*, out of which 2 miRNA were found to be regulating 32 target genes in human. Functional annotation, gene ontology and network analysis were carried out based on their significance and find out the association with the prominent disease like cancer and cardiac diseases. The network analysis showed the some important network protein like SOCS2, CD274, STAT3, TRAF3, and CXCL2 with their associated pathways. The predicted miRNA have a significant potential role in cytokine, Apoptosis and EGFR receptor signalling pathways and its biological regulation. It may be further validated using in-vivo experiment for broader picture into their miRNA epigenetic mechanism and action.

References


Category: Bioinformatics

SHIVGAMI: Simplifying the titanic blastx process using aVailable GAthering of coMputational unIts

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Abstract

Assembling novel genomes from scratch is a never ending process unless and until the homo sapiens cover all the living organisms! On top of that, this denovo approach is employed by RNASeq and Metagenomics analysis. Functional identification of the scaffolds or transcripts from such drafted assemblies is a substantial step routinely employs a well-known BlastX program which facilitates a user to search DNA query against NCBI-Protein (NR:~120Gb) database. In spite of having multicore-processing option, blastX is an elongated process for the bulk of lengthy Query inputs. Tremendous efforts are constantly being applied to solve this problem by increasing computational power, GPU-Based computing, Cloud computing and Hadoop based approach which ultimately requires gigantic cost in terms of money and processing. To address this issue, here we have come up with SHIVGAMI, which automates the entire process using perl and shell scripts, which divide, distribute and process the input FASTA sequences as per the CPU-cores availability amongst the computational units individually. Linux operating system, NR database and blastX program installations are prerequisites for each system. The beauty of this stand-alone automation program SHIVGAMI is it requires the LAN connection exactly twice: During ‘query distribution’ and at the time of ‘process completion’. In initial phase, it divides the fasta sequences according to the individual computer’s core-capability. Then it will evenly distribute all the data along with small automation scripts which will run the blastX process to the respective computational unit and send back the results file to the master computer. The master computer finally combines and compiles the files into a single result. This simple automation converts a computer lab into a GRID without investment of any software, hardware and man-power. In short, SHIVGAMI is a time and cost savior tool for all users starting from commercial firm to a common man, utilises the “Little Drops of Water make a Mighty Ocean” concept without any requirement of parallel processing. The automation and compilation of SHIVGAMI is under process and will be freely available shortly to the users.

References


**Citation:** Mangukia, N., Patel, M. and Rawal, R. SHIVGAMI : Simplifying tHe titanIc blastx process using aVailable GAthering of coMputational unIts [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Pages 32-33. https://doi.org/10.24870/cjb.2017-a20
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Screening of phytochemicals from selected plants with antifungal properties against RXLR effector protein Avr3a11 in Phytophthora capsici

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Abstract

Phytophthora Capsici is a fungal plant pathogen which causes significant damage to broad range of commercial & medicinally valuable plants like black pepper, tomato, watermelon, etc. Chemical compounds like fungicides are commonly used against Phytophthora infections. Prolonged inhalation of fungicides by humans, leads to neural & visual disturbances & lung infections. They can also permanently silence or reprogram normal genes that last for several generations & are very harmful to the environment too. An alternative to chemical control of fungal pathogens is by introducing phytochemicals, which are potentially active against Phytophthora capsici.

The study involves computational screening of phytochemicals with antifungal activity of plants against Avr3a11 in P. Capsici. Avr3a11 is an RXLR effector protein which functions as a virulence factor when recognised by plant immune receptors. The functional domain in Avr3a11 interacts with Resistance (R) proteins of the plant thereby triggering ETI (Effector Triggered Immunity) in plants. The phytochemicals from Turmeric, Garlic and Neem were used as ligand molecules. The 3D structure of Avr3a11 was retrieved from PDB (PDB id: 3ZR8) & the ligand structures collected from PubChem. Molecular docking was carried out in Discovery studio package to assess the binding energy of the phytochemicals with Avr3a11 in its functional domain. The phytochemical Alliin from garlic showed significant binding interactions with the target-Avr3a11 compared to the commonly used fungicides, indicating that Alliin can act as a potential inhibitor of Avr3a11. An in vitro assay of the plant extracts on phytoththora capsici also gives a validation of the docking study. This study provides insight into the potential use of phytochemicals to effectively combat the Phytophthora infections in plants.

References


Citation: Rani, J.R., Aswathy, T.R., Kumar, M.S., Nair, A.S. and Soniya, E.V. SHIVGAMI : Screening of phytochemicals from selected plants with antifungal properties against RXLR effector protein Avr3a11in Phytophthora capsici [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 34. https://doi.org/10.24870/cjb.2017-a21
Category: Bioinformatics

**sigFeature: an R-package for significant feature selection using SVM-RFE & t-statistic**

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Abstract

Depending on the sub-site of the primary tumour, up to thirty percent of the patients with clinical and radiological node negative HNSCC may have occult metastases. Therefore, currently, up to seventy percent patients with node negative neck disease receive unnecessary therapy to ensure a minority who are truly at risk [1]. The treatment of HNSCC involves surgery, radiotherapy or multimodality therapy like surgery together with adjuvant radiotherapy or chemo radiotherapy. HNSCC is typically considered as a homogeneous tumour group, i.e., histopathologically identical, but they are often genetically disparate and exhibit variable biological behaviour and response to treatment between and within anatomical sub-sites [2]. Currently, treatment decisions for patients with HNSCC are still based on clinical, radiological and pathologic parameters. No molecular markers are used for treatment decision, except in ongoing research protocols. To identify those patients who are truly at risk, a novel feature selection method has been introduced based on expressional genomic data in this study. In data mining, feature selection is an extremely dynamic field of research for classification in the field of machine learning technology. The aim of feature selection is to select a small subset of a feature from a larger pool, rendering not only a good performance of classification but also biologically meaningful insights. Filter methods e.g. the support vector machine recursive feature elimination (SVM-RFE) is recognised as one of the most effective methods. The RFE-SVM algorithm is a greedy method that only hopes to find the best possible combination for classification without considering the differentially significant feature between the classes. To overcome this limitation of SVM-RFE, our proposed approach which is based on RFE-SVM and t-statistic is to find out differentially significant features along with the good performance of classification. The experimental results which we obtained after analysing six publicly available micro array datasets are very promising and show the contribution in feature selection in machine learning technology. The main conclusion is that the selected features are differentially significant between the classes and able to produce good classification accuracy which will help further downstream analysis for strengthening the biological aspect.

References


Category: Bioinformatics

Visualization of plant Regulomics Network in Graph using Neo4j

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Abstract

Plants have highly interacted with the environment through phenotypically. The phenotype of plants controls via epigenome by DNA methylation, histone modification, small RNAs like (miRNAs/siRNAs), transcription factors and repetitive elements. These all regulatory factors work together in various stress conditions of plants for the protection or survival. The regulators perform their function in the module of a mechanism at TGS or PTGS. Some regulators may be activated or deactivated according to their action of the mechanism. Such type of interaction briefly called Regulomics which control or regulate the genotype through epigenomics. In, various types of interaction have been considering for the case studies. Most important interactions related to small RNAs and DNA methylation for their expressional silencing of key genes in various pathways. The possible interaction of miR-Gene, miR-TF, TF-miR, TF-Gene and Gene-Gene is mostly found in the complex network of plants. Need to provide a dynamic, interactive, systematic and versatile visualization technique to provide a comprehensive platform to view such complex interaction graph networks for plants. Graph based interaction networks visualization available for regulomics contains various interactions. For these Neo4j have been emerging NoSQL based graph tool to visualize versatile networks in graphical as well as user defined formats. We provide the graph based big-data networks in various plants like Thale cress, Soyabean, Maize, Rice, Populus, medicago, tomato, etc. Each plants network has more than ~30,000 nodes and their edges or relationships. It has been very fast and friendly for users. This facility provides vital impact for plant sciences in the area of network interaction in future.

References

[2] Neo4j v3.2.2 (https://neo4j.com/)

Citation: Panzade, G. and Shankar, R. Visualization of plant Regulomics Network in Graph using Neo4j [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 36. \url{https://doi.org/10.24870/cjb.2017-a23}
Category: Bioinformatics

**Codon based co-occurrence network motifs in human mitochondria**

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**Abstract**

The nucleotide polymorphism in human mitochondrial genome (mtDNA) told by codon position bias plays an indispensable role in human population dispersion and expansion. Herein, we constructed genome-wide nucleotide co-occurrence networks using a massive data consisting of five different geographical regions and around 3000 samples for each region. We developed a powerful network model to describe complex mitochondrial evolutionary patterns between codon and non-codon positions. It was interesting to report a different evolution of Asian genomes than those of the rest which is divulged by network motifs. We found evidence that mtDNA undergoes substantial amounts of adaptive evolution, a finding which was supported by a number of previous studies. The dominance of higher order motifs indicated the importance of long-range nucleotide co-occurrence in genomic diversity. Most notably, codon motifs apparently underpinned the preferences among codon positions for co-evolution which is probably highly biased during the origin of the genetic code. Our analyses manifested that codon position co-evolution is very well conserved across human sub-populations and independently maintained within human sub-populations implying the selective role of evolutionary processes on codon position co-evolution. Ergo, this study provided a framework to investigate cooperative genomic interactions which are critical in underlying complex mitochondrial evolution.

**References**

In silico identification and characterization of differential expressed genes (DEGs) associated with grain and panicle number in rice

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Abstract

Grain number is an important trait for yield in rice. Several genes have been identified controlling grain and panicle number, which has direct or indirect effect on yield. Some genes play a key role for panicle formation and number of panicles per plant. The number of panicles per plant is directly regulating the grain number per plant. In present study, in silico approach was adopted for identifying differentially expressed genes associated with grain number and panicle number. Further, pathway enrichment analysis of these genes performed. The microarray data, GSE51616, downloaded from the GEO database originally submitted by Wang et al. (2014). Young leaves in vegetative stage (35-days old) and developing panicles (0.1cm) from field-grown OX-Ghd7HJ19 transgenic and wild-type plants with two biological replicates were used to isolate RNA for chip analysis. Background correction and normalization of raw microarray data was carried out using the Robust Multichip Averaging (RMA) method of affy packages of R (v. 3.1.3). The linear regression model package, limma was utilized to classify chips into two groups. The Bayes method (Benjamini and Hochberg) was used to correct for multiple testing. Adjusted P-value < 0.01 and |logFC| > 2 was used as a cut-off to identify differentially expressed genes. We identified 393 differentially expressed genes, which mainly belongs to either Phosphatidylethanolamine-binding protein family or DUF3778 domain family. Proteins of these two families regulate formation of high grain number as well as panicle number. These genes like Ghd7, Ehd1, Dep1, Os10g0463400, Os03g0752800, Os03g0215400, Os06g0157700 and Os06g0157500 function to integrate the dynamic environmental inputs with phase transition, architecture regulation, and stress response to maximize the reproductive success of the rice plant. Thus, these genes can act as potential target protein for increasing grain and panicle number in rice plant, which will lead to increase in yield of rice grain.

Citation: Gouda, G., Gupta, M.K., Vadde, R., Donde, R., Kumar, J., Nayak, S., Behera, L. and Mohapatra, T. In silico identification and characterization of differential expressed genes (DEGs) associated with grain and panicle number in rice [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 38. https://doi.org/10.24870/cjb.2017-a25
Category: Bioinformatics

Insights into the mode of recognition of DIII of dengue E protein with GRP78: A molecular dynamics approach

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Abstract

Dengue virus (DENV), a single stranded RNA positive-strand virus belongs to Flaviviridae causes Dengue in several parts of South-East Asia. During infection, dengue virus proteins interact with host cellular constituents, thus promotes the remodeling of the cell to facilitate virus production. Recent studies have shown that DENV E protein interact the cellular chaperone GRP78. GRP78 that plays a dual role in virus life cycle i.e., virus entry and virus replication, is a novel host factor that could be a potential therapeutic target. Currently, the three-dimensional interaction between GRP78 and DENV E protein remains largely unknown. It is assumed that DENV E protein interacts with the C-terminus of GRP78, and the C-terminus of GRP78 is believed to be the predominant protein interacting domain, while the N-terminus is believed to contain regulatory domains that mediate C-terminal binding. Although the exact E protein domain mediating binding to GRP78 is not known, it has been proposed that GRP78 and DENV E protein interact through the immunoglobulin like structure in the DENV E protein that resides in domain III (DIII). So, the present study was undertaken to unravel molecular basis of GRP78 and DENV E protein interaction through molecular modeling, protein-protein docking and Molecular dynamics simulations. The three-dimensional structures of DIII of E protein from DENVI was modelled and docked against crystal structure of GRP78 (PDB ID: 3LDL) using ClusPro. The top ranked pose from ClusPro was again refined using HADDOCK. Molecular dynamics simulation was performed to understand mode of recognition and dynamics stability of the refined DIII-GRP78 complex in aqueous solution for 10 ns. The critical residues i.e., Thr303/Lys46, Lys295/Lys152 and Lys399/Asn239 identified in this study are indispensable for DIII mediated interaction of dengue virus with host protein GRP78. The results from this study is expected shed deep insights into the crucial host factors that could be targeted to cripple virus infection and ultimately lead to development of effective anti-viral therapy for DENV in near future.

Category: Bioinformatics

Homology modelling and docking studies on Neuraminidase enzyme as a natural product target for combating influenza

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Abstract

Influenza remains to be dreadful with yearly epidemics and sudden pandemic outbreaks causing significant mortality, even in nations with the most advanced health care systems. Thus, there has been a long-standing interest to develop effective and safe antiviral agents to treat infected individuals. Attempt to identify suitable molecular targets as antiviral compounds have focused recently on the influenza virus neuraminidase (NA), a key enzyme in viral replication [1]. In this research, virtual screening was done on a total of 600 natural compounds from 22 ethno medicinal Indian herbs for activity against neuraminidase enzyme exploiting representative protein conformations selected from molecular dynamics simulations. Neuraminidase enzyme sequences from different existing strains available on National Center of Biotechnology Information [2] (NCBI) protein database were aligned using Clustal W [3] and CLC workbench 10 [4] to find the conserved residues. Neuraminidase protein sequence from H1N1 strain available on NCBI was used to structure 3D target model predicted against dataset from Protein data bank using modeller [5]. The target model was validated on different parameter at SAVES Server [6]. Using this target model a pharmacophore model was developed using ligand based strategy exploiting the three known inhibitors. The docking parameters were validated by redocking Zanamivir to its co-complex 2009 H1N1 NA crystal structure (PDB ID: 3TI5) generating best pose with a RMSD value of 0.7543 Å. This model was then used for in silico analysis of a library of natural compounds from 22 ethno medicinal Indian herbs known to have antiviral activity taken downloaded from PubChem database and selected on the basis of drug likeliness. All the compounds were docked in the binding pocket of neuraminidase. Top compounds having binding affinity better than or comparable to the control drug Zanamivir were selected and analysed for their ADME and toxicity. Their binding pattern in the 150 loop was studied along with their interaction with the active site conserved residues. Electrostatic interactions were the main driving force in the binding affinity of the potential inhibitors. These hit compounds will provide direction for further in- vivo and in -vitro validation and may play an important role in finding novel Neuraminidase inhibitors against influenza.

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Citation: Singh, N. and Chandra, R. Homology modelling and docking studies on Neuraminidase enzyme as a natural product target for combating influenza [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 40, https://doi.org/10.24870/cjb.2017-a27
Category: Bioinformatics

Determination of data analysis pipeline for detection of Thap-9 binding sites in human genome

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Abstract

Transposons are ubiquitously expressed DNA sequences that can move from one position within the genome to another, resulting in alteration of gene expression, mutations and generation of genome diversity. Human Thap-9 protein is homologous to Drosophila P-element transposase. Previous studies have reported that Human Thap-9 shows excision and integration activity in human cells. Over the past decade, the realm of biotechnology has witnessed an upsurge of computational tools available for analysing ChIP-seq data. ChIP-seq (Chromatin immunoprecipitation-sequencing) has proven to be a versatile tool for detecting protein-DNA interactions. Since its advent, several modifications of this technique have come into existence. ChIP-exo is a modification of conventional ChIP-seq, which uses lambda exonucleases to digest protruding ends of DNA in protein-DNA complexes followed by next generation sequencing. This technique gives better resolution of binding sites (20 bp-90 bp), reduces noise and can detect weaker protein-DNA interactions. We are trying to develop a data analysis pipeline for finding Thap-9 binding sites in human genome using ChIP-exo generated reads. For this purpose, we have compared the outputs of different computational tools for various steps in the analysis and have used different freely available online tools for alignment (Bowtie2 and BWA), peak calling (MACS, MACE and GEM) and visualization of the binding sites (MEME and HOMER).

References


Citation: Sharma, V. and Majumdar, S. Determination of data analysis pipeline for detection of Thap-9 binding sites in human genome [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 41. https://doi.org/10.24870/cjb.2017-a28
Category: Bioinformatics

Is ecological source important in phylogenomics analysis: a pilot study involving 17 Drosophila species using multilocus approach

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Abstract

For over a century, the fruit fly Drosophila has accomplished itself as a resourceful experimental model organism in the field of biological research. It has a long history of being scrutinized by biologists for evolutionary studies either through cytogenetic, phylogenetic or comparative genomics analysis. Next Generation Sequencing (NGS) technologies are also bringing a revolutionary shift in phylogenetic exploration as now whole genome can be utilized for the analysis. The goal of the present study is to examine the robustness of the molecular markers which are frequently been employed in resolving the phylogeny of Drosophila genus. Ten protein coding nuclear loci were utilized to infer the phylogenetic relationships across 17 taxonomically known species including four Indian Drosophila and one Zaprionus species as outgroup in the present study, including Adh, Amy-p, Cypc, Gld, Gpdh, IARS, Marf, per and tim and Xdh. The DNA sequences of selected nuclear genes in four Indian Drosophila and Zaprionus species were retrieved from whole genome sequences (WGS) generated by us through Next Generation Sequencing Technology on Illumina platform. The selected genes were predicted using Augustus as gene prediction program. Neighbour joining, Maximum likelihood and Bayesian phylogenetic methods were employed in order to reconstruct and compare the evolutionary history. Our phylogenetic trees reconstructed using Adh, Amy, Gld, Gpdh, Xdh shows results which were in concordance with previous studies as Indian species were placed closer with their respective group/subgroups members. However, phylogenies obtained using Marf, Cypc, IARS, per and tim genes showed that Indian species were forming a separate clade rather than occupying their own taxonomical position, thereby, confirming their close evolutionary relationship. This could be due to the ecological factors that are bringing remarkable variation in the sequence of these marker genes. So, the present study reveals that ecological origin of the study species should be contemplated while ascertaining its phylogenetic positions.

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Category: Bioinformatics

**Finding commonality between the pattern of histone modifications across normal and cancer cell types dictated by DNA sequence features**

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**Abstract**

**Introduction:** Histone modification, a covalent post-translational modification of histone proteins, influences gene expression by altering chromatin structure giving rise to key characteristics to various cell types. While interaction of histone proteins with DNA is not known to be dependent on DNA sequence features, histone modifications are highly regulated in certain genomic regions. This specificity is required in normal cells to prevent genome instability, chromosome segregation defects and to maintain cellular homeostasis and is altered in cancer. Identifying [1] if specific sequence features are associated with certain histone modifications and [2] if these associations are consistently disrupted in transformed cells, will establish links between the genotype and epigenotype giving us valuable predictability about histone code. In this study, we have analyzed ChIP-seq data of different histone modifications in human across primary and immortalized cell lines from ENCODE database.

**Experiments and key results findings:** We have identified unique as well as common genomic regions that carry histone marks commonly in primary and immortal cell lines. Additionally, motif finding analysis indeed shows certain modifications are associated with crucial genes required for cell survival and function; some uniquely in transformed cell lines. The regions with consistently different histone marks are currently being studied to check whether they are associated with certain cytosine methylation profile too. Our results suggest a genotypic predisposition for epigenotype.

**References**


**Citation:** Datta, S., Patel, M., Patel, D. and Singh, U. Finding commonality between the pattern of histone modifications across normal and cancer cell types dictated by DNA sequence features [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India. Can J biotech, Volume 1, Special Issue, Page 43. [https://doi.org/10.24870/cjb.2017-a30](https://doi.org/10.24870/cjb.2017-a30)
Category: Bioinformatics

Osteoporosis Gene Interactome: A comprehensive *in silico* analysis

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Abstract

Today it is a challenge for clinicians and researchers to understand the comprehensive relationship between molecular and physical functioning of genes towards the progression of disease. Although genomic advancements have produced significant data to identify the genes involved in many complex diseases but a loop exists since a single gene is not attributable to a particular concept. In our work, we tried to find key neighbours involved in osteoporosis by analysing osteoporosis disease module (interactome) using both experimental and clinical methodologies which may also contain mechanisms that are collective with other disease modules. Our idea was strengthened by the findings of previous GWAS p-value studies wherein the level of gene expression was different in both diseased as well as normal conditions. We thus, constructed a gene-gene and protein-protein interaction network for 104 genes linked with 173 genetic variants (single nucleotide polymorphisms) that revealed significant hub proteins which might be fundamentally linked to disease pathogenesis. We further performed gene ontology and functional enrichment analysis followed by KEGG pathway analysis to analyze and validate the role of these genes for their pathophysiological and functional activities. Our analysis revealed the polymorphism in SOST and LRP5 genes as significant conservative SNPs which might have a substantial role in the onset of osteoporosis and its development.

Citation: Singh, B. and Hasija, Y. Osteoporosis Gene Interactome: A comprehensive *in silico* analysis [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 44. https://doi.org/10.24870/cjb.2017-a31
An integrated genomic analysis on miRNAs and SNPs associated with vitiligo to reveal potential drug candidates

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Abstract

Vitiligo is characterized by the occurrence of depigmented patches on the skin caused as a result of progressive loss of functional melanocytes. It is a polygenic disorder entailing both genetic and non-genetic factors intricately and as such is not yet clearly understood. Hence, a comprehensive understanding of the entire spectrum of disease susceptibility and pathogenesis remains a challenge. Emerging evidence over the decades underlines the cardinal role of miRNAs in disease development and having a significant role in melanocyte development and survival. Also, identifying the susceptible genes and their variants that influence disease onset is fundamental to unravel the rationale for disease susceptibility. We, therefore, applied a systems biology approach to identify potential miRNAs and their target genes to construct a miRNA-target gene network revealing essential miRNAs that might be significantly related to vitiligo. We further investigated the susceptible genetic variants associated with vitiligo and prioritized a few proteins in our protein-protein interaction network as significant hub proteins. Network-based polypharmacological studies of such hub proteins are helpful in analysing a large set of disease-associated proteins which might initiate better diagnosis with the feasibility of personalized treatment for vitiligo patients in the future. Our polypharmacogenomic systems analysis highlighted novel drug candidates and drug repositioning candidates for vitiligo. Furthermore, we investigated the pathogenic effect of the plausible single nucleotide polymorphisms (SNPs) using computational platforms and carried out preliminary protein modelling to implicate the role of SNPs in disease pathogenesis. Thus, our analysis unveiled significant findings which may provide an insight of the mechanisms of vitiligo development and progression, thereby, driving the way towards improved therapeutic and diagnostic interventions for vitiligo management.

Citation: Rahman, R. and Hasija, Y. An integrated genomic analysis on miRNAs and SNPs associated with vitiligo to reveal potential drug candidates [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 45. https://doi.org/10.24870/cjb.2017-a32
Category: Bioinformatics

**Fundamental principles governing sporulation efficiency: A network theory approach**

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**Abstract**

Integrating network theory approaches over time-resolved genome-wide gene expression data, we proposed a network-based framework, which considered intricate dynamic regulatory relationships of transcription factors and target genes, for assessing the molecular underpinnings underlying extreme phenotypic differences between two strains of the yeast, *Saccharomyces cerevisiae*. Using network attributes which have demonstrated tremendous success in understanding and predicting behaviors in a wide range of complex biological and social systems, we identified factors and candidate genes that acted as crucial regulators of sporulation in the highly sporulating SK1 strain. We then carried out independent network-based investigations of S288c gene expression profiles and identified the molecular events that occur in SK1 strain but fail to occur in S288c strain, which eventually lead to low sporulation efficiency of S288c. Results suggested that late appearance of known early sporulation regulators and a delay in crosstalk between functional modules can be construed as the prime reasons behind low sporulation efficiency of the S288c. Revelation of meiosis-associated genes for SK1 and mitotic genes for S288c through weak ties analysis and late appearance of hierarchical modularity were further indications of delay in regulatory activities essential to initiate sporulation in S288c. Our results demonstrate the potential of this framework in identifying candidate nodes contributing to phenotypic diversity of developmental processes in natural populations.

**References**


**Citation:** Sarkar, C., Gupta, S., Sinha, H. and Jalan, S. Fundamental principles governing sporulation efficiency: A network theory approach [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 46. [https://doi.org/10.24870/cjb.2017-a33](https://doi.org/10.24870/cjb.2017-a33)
Category: Bioinformatics

An insight into structural and functional characteristics of 3-hydroxy 3-methyl glutarylCoA reductase from Ocimum species

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Abstract

Secondary metabolites, the biological compounds secreted by plants as an aid to support their growth and development under stress conditions or as a part of their defense mechanism, now hold equal importance for mankind who employs it immensely for medication, flavorings, aroma, etc. Wide applicability of these compounds instigates one to understand the biosynthesis, structure and regulation of these bioactive molecules. Terpenoids form the largest group of secondary metabolites which comprise of a wide range of structurally and functionally distinct metabolites synthesized either via mevalonate pathway or non-mevalonate pathway. Targeting a key regulatory enzyme of this pathway, modulation of which would alter the carbon flux would be beneficial to enhance our knowledge about the above issue. For this the transcriptome (from SRA) of different Ocimum species was mined out for important pathway genes using various bioinformatics approaches. Amongst them 3-hydroxy 3-methyl glutaryl CoA reductase (HMGR) was selected which is the rate limiting enzyme in mevalonate pathway which controls the conversion of HMG-CoA to mevalonic acid. Isolation, cloning, protein expression, purification, etc. would be discussed in detail in the meeting. Full length protein was also characterized through bioinformatics tools to study its structure, properties, conserved domains, etc. Increase in secondary metabolite production by alteration of HMGR pool along with transcript modulation studies in planta revealed that HMGR gene governs the biosynthesis of secondary metabolites. Transcriptome mapping of different HMGR homologs which on comparison within member of same genus revealed its divergent nature which could account to its multifunctional role in different plants. Besides, providing a deep insight about the enzyme function combination of such molecular, transgenic and bioinformatics tools would help to develop strategies to engineer the HMGR mediated flux and also valuable metabolites in plants.

References


Citation: Bansal, S. and Sangwan, N.S. An insight into structural and functional characteristics of 3-hydroxy 3-methyl glutarylCoA reductase from Ocimum species [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 47. https://doi.org/10.24870/cjb.2017-a34
Category: Bioinformatics

Characterization of novel Phophatase from the genome of *Genlisea aurea* – An *in silico* approach

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Abstract

Insectivorous plants use enzymes to digest their prey. These plants found in the tropical areas like forest of east India. Mostly insectivorous plants produce their own digestive enzymes to digest their captured insects and small animals diverge from protozoa to invertebrates. The plants need extreme sunlight and rainwater to sustain. These plants consume insects to suck the nutrients from the pray since the plant grows in nutrient less soil especially in nitrogen and potassium. The studies have shown the digestive enzyme from the plants has the proficiency to fight against the various diseases in human like Cancer, Diarrhea, Cholera, Hepatitis, Digestive process related diseases also the phytochemicals found in the insectivorous plants shows resistance against the various metabolic targets of numerous human diseases. Our study has collected 810 putative digestive enzymes with blast hit and domain search; we have characterized the full enzymes using partial sequence as a template and predicted the function. The structure modelling has done for the phosphatase enzymes using I- Tesser server. Our future study includes *in vitro* identification of digestive enzymes in Genlisea aurea and its further application in degrading the waste materials.

Category: Bioinformatics

Bioinformatics Database Tools in Analysis of Genetics of Neurodevelopmental Disorders

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Abstract

Bioinformatics tools are recently used in various sectors of biology. Many questions regarding Neurodevelopmental disorder which arises as a major health issue recently can be solved by using various bioinformatics databases. Schizophrenia is such a mental disorder which is now arises as a major threat in young age people because it is mostly seen in case of people during their late adolescence or early adulthood period. Databases like DISGENET, GWAS, PHARMGKB, and DRUGBANK have huge repository of genes associated with schizophrenia. We found a lot of genes are being associated with schizophrenia, but approximately 200 genes are found to be present in any of these databases. After further screening out process 20 genes are found to be highly associated with each other and are also a common genes in many other diseases also. It is also found that they all are serves as a common targeting gene in many antipsychotic drugs. After analysis of various biological properties, molecular function it is found that these 20 genes are mostly involved in biological regulation process and are having receptor activity. They are belonging mainly to receptor protein class. Among these 20 genes CYP2C9, CYP3A4, DRD2, HTR1A, HTR2A are shown to be a main targeting genes of most of the antipsychotic drugs and are associated with more than 40% diseases. The basic findings of the present study enumerated that a suitable combined drug can be design by targeting these genes which can be used for the better treatment of schizophrenia.

References


Category: Bioinformatics

**In silico** design of PHA synthase and its validation by PHAs producing bacterial isolates

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Abstract

Biopolymers are important alternatives to the petroleum-based plastics due to environment friendly manufacturing processes, biodegradability and biocompatibility. Therefore use of novel biopolymers such as polylactide, polysaccharides, aliphatic polyesters and polyhydroxyalkanoates (PHAs) is of interest. PHAs are biodegradable polyesters of hydroxyalkanoates (HA) produced from renewable resources by using microorganisms as intracellular carbon and energy storage compounds. Even though PHAs are promising candidate for biodegradable polymers, however, the production cost limits their application on an industrial scale. Therefore an attempt was made to model different PHAs synthases which are the key enzyme in the biosynthesis of Polyhydroxyalkanoates as the structural information of this enzyme is in dark veil. Then molecular docking of class I PHA Synthase from *Ralstonia Eutrophia* was done to study the PHA synthase activity. As there are lots of strain which needs to explore for the production of PHA. This investigation leads to find out the most industrial applicable microbes. Few bacterial isolates from soil sample were screened for production of PHA followed by the validation of the enzymatic activity and its product characterization to understand its structural properties.

Citation: Sahoo, S., Mohapatra, S., Rath, S.N., Balabantray, S. and Hazra, S. *In silico* design of PHA synthase and its validation by PHAs producing bacterial isolates [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 50. https://doi.org/10.24870/cjb.2017-a37
Category: Bioinformatics

Role of HYL1, a dsRNA binding partner of DCL1, in selecting a unique cryptic motif in plant miRNAs

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Abstract

MicroRNAs (miRNAs) are small ~21nt long endogenous non-coding RNAs that are involved in post-transcriptional silencing of target mRNAs. miRNAs are processed from precursors having a stem-loop structure that are recognized and cleaved by Dicer-like1 (DCL1) with the help of dsRNA binding protein Hyponastic leaves1 (HYL1) and a zinc finger protein named Serrate (SE). Unlike animal miRNAs where miRNA foldbacks are mostly uniform, plant miRNA foldbacks are very diverse in length and structure. Processing mechanism and the nature of RNA motifs in animal pre-miRNAs are comparatively well studied (Ha and Kim, 2014) than plant miRNAs. There have been few efforts to understand the molecular signatures that affect accuracy and efficiency of miRNA processing. Bulges in the lower stem has been shown influence precision and efficiency of processing (Song et al., 2010; Werner et al., 2010; Mateos et al., 2010) and the loop length negatively influences miRNA abundance (Jagtap et al., 2014).

Through computational analysis, we find that there is a unique cryptic motif present in plant miRNAs. This cryptic motif is conserved among diverse plant groups, suggesting a conserved mechanism that selects the motif. We hypothesize that HYL1 could be responsible for observed selection for cryptic motif in miRNAs. hyl1 mutants are seen to lack precise precursor processing ability and accumulate miRNAs at much lower levels. We have identified amino acid residues in HYL1 that are likely to mediate selection of specific cryptic motif in miRNA precursors and help in precise cleavage by DCL1. As expected, these residues are present only in HYL1, but not among other RNA binding protein partners of DCL1 and other DCL proteins that produce small RNAs without a cryptic motif. Our results pave way for understanding a previously unknown determinant of plant miRNA biogenesis.

Citation: Narjala, A., Nair, A.K., Ramesh, A. and Shivaprasad, P.V. Role of HYL1, a dsRNA binding partner of DCL1, in selecting a unique cryptic motif in plant miRNAs [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 51. https://doi.org/10.24870/cjbi.2017-a38
Category: Bioinformatics

Computational attempts for synthesis of potent antibacterial sulfamethoxazole-monocyclic terpenes conjugates

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Abstract

To develop 6 conjugate agents of the moribund antibiotic sulfamethoxazole (SMZ) joined to 6 individual monoterpenes, followed by protocols of medicinal chemistry as potent antibacterials, against multidrug resistant (MDR) human gruesome pathogenic bacteria. Antibacterial activities of the proposed conjugates were ascertained by the prediction of activity spectra of substances (PASS) program. Drug-likeness parameters and toxicity profiles of conjugates were standardized with the Lipinski rule of five, using cheminformatic tools, Molsoft, molinspiration, OSIRIS and ProTox. Antibacterial activities of individual chemicals and conjugates were examined by targeting the bacterial folic acid biosynthesis enzyme, dihydropteroate synthases (DHPSSs) of bacteria, Bacillus anthracis, Escherichia coli, Staphylococcus aureus, Streptococcus pneumoniae and Mycobacterium tuberculosis, with 3D structures of DHPSSs from protein data bank. According to the PASS program, biological spectral values of conjugate-2, conjugate-5 and conjugate-6 were ascertained effective with ‘probably active’ or ‘Pa’ value > 0.5, for anti-infective and anti-tuberculocic activities. Using molecular docking against 5 cited bacterial DHPSSs, effective docking scores of 6 monoterpenes in the specified decreasing order (kcal/mol): −9.72 (eugenol against B. anthracis), −9.61 (eugenol against S. pneumoniae), −9.42 (safrol against B. anthracis), −9.39 (thymol, against M. tuberculosis), −9.34 (myristicin, against S. pneumoniae) and −9.29 (thymol, against B. anthracis); whereas the lowest docking score of SMZ was -8.46 kcal/mol against S. aureus DHP. Similarly, effective docking scores of conjugates were as specified (kcal/mol.): −10.80 (conjugate-4 consisting SMZ + safrol, against M. tuberculosis), −10.78 (conjugate-5 consisting SMZ + thymol, against M. tuberculosis), −10.60 (conjugate-5 against B. anthracis), −10.26 (conjugate-2 consisting SMZ + eugenol, against M. tuberculosis), −10.25 (conjugate-5, against S. aureus) and −10.19 (conjugate-2 against S. pneumoniae). Conjugates-2 and -5 were the most effective antibacterials based on Lipinski rule of five with lethal doses 3471 and 3500 mg/kg, respectively and toxicity class levels. Conjugate-2 and conjugate-5 were more effective than individual monoterpenes and SMZ, against pathogenic bacteria. Synthesis, characterization and in vitro antibacterial study with acute toxicity testing for Wister rat model of the conjugate-5 could land at success in the recorded computational trial and it could be promoted for synthesis in the control of MDR bacteria.

Category: Bioinformatics

A study on nitrogen fixation related proteins

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Abstract

Nitrogen fixation is the process of conversion of free nitrogen (molecular and elemental) into nitrogenous compounds and to make it available for plants absorption. The main objective of this work is to collect nitrogen fixation related genes from the public databases and to discriminate nitrogen fixation genes from non-nitrogen fixation genes through computational approach. The nitrogen fixation genes were collected from IMG/M database and the non-nitrogen fixation genes were retrieved randomly from UniProt online server. To classify both nitrogen fixation and non-nitrogen fixation gene, Support Vector Machine (SVM) was used and a model was developed. The SVM predicted with 0.9886364 sensitivity and 0.9090909 specificity from total dataset which means 98% and 90% of true positive and true negative results. These SVM result indicates that the predicted model is very good. This model would be helpful for understanding the role of genes involved in nitrogen fixation and the scientist who were worked in this area.

Category: Bioinformatics

Automated tool for virtual screening and pharmacology-based pathway prediction & analysis

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Abstract

The virtual screening is an effective tool for the lead identification in drug discovery. However, there are limited numbers of crystal structures available as compared to the number of biological sequences which makes (Structure Based Drug Discovery) SBDD a difficult choice. The current tool is an attempt to automate the protein structure modelling and automatic virtual screening followed by pharmacology-based prediction and analysis. Starting from sequence(s), this tool automates protein structure modelling, binding site identification, automated docking, ligand preparation, post docking analysis and identification of hits in the biological pathways that can be modulated by a group of ligands. This automation helps in the characterization of ligands selectivity and action of ligands on a complex biological molecular network as well as on individual receptor. The judicious combination of the ligands binding different receptors can be used to inhibit selective biological pathways in a disease. This tool also allows the user to systemically investigate network-dependent effects of a drug or drug candidate.

Citation: Kumar, S., Satpati, S. and Dixit, A. Automated tool for virtual screening and pharmacology-based pathway prediction & analysis [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 54. https://doi.org/10.24870/cjb.2017-a41
Comparison of the modulation of FGFR signalling by thalidomide and its analogs lenalidomide and pomalidomide

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Abstract

Thalidomide, a powerful teratogen, re-emerged as a wonder drug for its teratogenic, anti-angiogenic and anti-tumor properties. Being FDA approved for Multiple Myeloma along with the analogs lenalidomide and pomalidomide is currently being tested in more than 2000 clinical trials for a range of conditions including solid tumors and inflammatory disorders. Fibroblast growth factor receptors (FGFRs) play key roles in embryonic development and cancer. There are indications that thalidomide might be linked to FGFR biology, however no experimental evidence is available till now.

To understand the effects of thalidomide and its analogs, lenalidomide and pomalidomide, we utilized \textit{in silico} predictive tools, kinome profiling, transcriptome and phosphoproteome tools to study the modulation of FGFR signalling in endothelium. Genecodis and Enrichr with the differentially expressed genes were used to obtain the Gene Ontology, Transcription factor, Pathway and miRNA enrichments. The association of the drug with FGFR signalling was investigated at various levels. Protein- chemical network tool, STITCH and Pocketome predicted strong association of thalidomide with FGFR2. At gene expression level, FGFR1 and FGFR2 were found to be affected under the three drug treatments in \textit{in vitro} and \textit{in vivo} models. Kinomescan results suggest the binding of thalidomide with high affinity to a mutant FGFR3 (G697C) and FGFR2. To validate this, we checked the activity of FGFR2 kinase under the three drug treatments and found that they affected the kinase activity in a dose-dependent manner with pomalidomide having lowest $IC_{50}$ value. Blind docking using Autodock revealed the possible binding sites and interestingly all the three analogs were predicted to bind to Lys517 of FGFR2. Lys517 is one of the ATP binding sites, suggesting that possibly analogs interfere with the ATP binding. Taken together, FGFRs could be potential targets of thalidomide and its analogs and the modulation of FGFRs by thalidomide partially explain the teratogenic and anti-tumor properties of the drug. Thus through different platforms, the mechanisms of drugs could be understood in a better way. This in turn will aid in modifying the drug structures resulting in the development of new analogs with more efficacies and reduced undesired effects.

Citation: Sundaresan, L., Kumar, P. and Chatterjee, S. Comparison of the modulation of FGFR signalling by thalidomide and its analogs lenalidomide and pomalidomide [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 55. https://doi.org/10.24870/cjb.2017-a42
Screening of novel inhibitors targeting Human Papillomavirus 16 E6/AP/P53 ternary complex towards development of therapeutic strategies against HPV-mediated oncogenesis

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Abstract

Cervical cancer is the fourth most common cancer in women worldwide. It is well known that high-risk HPV is the main etiological agent for this infectious viral carcinoma. Human papillomaviruses are small (50 nm) double-stranded DNA viruses composed of a genome of 8 kilobase pair, enclosed inside a non-enveloped capsid protein. The genome includes three portions: (a) early genes (E1, E2, E4, E5, E6, E7) those regulate the vegetative and productive phase of viral life cycle; (b) late genes (L1, L2) which encode the capsid protein and (c) a noncoding regulatory region called long control region (LCR) involved in the regulation of viral replication and transcription. The HPV oncoproteins E6 and E7 recognize numerous host proteins, in large part by hijacking cellular domain-motif interaction networks. E6 and E7 oncoproteins disrupt cell cycle checkpoint control by inhibiting CDKs inhibitors (P21, P27) and degrading P53. In the process of E6 mediated degradation, E6 binds to a short leucine (L)-rich LxxLL consensus sequence within the cellular ubiquitin ligase E6AP3. Subsequently, the E6/E6AP heterodimer recruits and degrades p53. The LxxLL peptide of E6AP is sufficient to render E6 liable to interact with p53 ‘core’ (DNA binding) domain of p53 required for the interaction with E6/E6AP\textsuperscript{9–11}. In the present study, we explored specific novel inhibitors targeting three different druggable pocket i.e., E6-binding cleft, LxxLL pocket of AP and the p53-binding cleft of E6/E6AP/p53 ternary complex using AutoDock tool. A total of five novel compounds with higher binding energy were identified as potential competitive inhibitors against HPV16 E6/AP/P53 ternary complex. The combinatorial strategies targeting these druggable pockets are expected to open up better avenues for the development of therapeutic strategies against HPV-mediated oncogenesis in near future.

Citation: Senapati, R., Dehury, B. and Dwibedi, B. Screening of novel inhibitors targeting Human Papillomavirus 16 E6/AP/P53 ternary complex towards development of therapeutic strategies against HPV-mediated oncogenesis [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 56. \url{https://doi.org/10.24870/cjb.2017-a43}
Category: Cancer Genomics

Mutational profiling of KRAS and its association with non-small cell lung carcinoma in Indian Kashmiri population

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Abstract

Lung cancer represents commonest cancer worldwide, with a high mortality rate as the disease becomes clinically apparent at advanced stages. The most common molecular alterations observed in NSCLC lie in the mutations of KRAS. These mutations occur in 15–30% of NSCLC and are more frequent in adenocarcinoma. We have screened prospectively all newly diagnosed patients with NSCLC (n=70) for the ability to be analysed for KRAS mutations. Seventy, blood samples of Non-small cell lung cancer were collected from Department of Hematology, Sher-i- Kashmir Institute of Medical Sciences (SKIMS). Blood DNA was extracted from these cases. DNA sequencing was performed on all the samples for detection KRAS codon 12, 13 activating mutations. Mutation status was compared with patient clinicopathological characteristics. The prevalence of KRAS mutation rate in NSCLC in the Kashmiri population was 30%. The significant association was seen between KRAS gene mutation and histological types of lung cancer. The higher frequency was seen in adenocarcinoma (ADC) (28.84%) than squamous cell carcinoma (SCC) (6%). The difference was statistically significant (OR=0.81, 95% CI=0.257-2.588, p < 0.01). Among the different stages, the higher frequency of KRAS (exon 2) mutation was reported in NSCLC patients in advanced stage (38.09%) than the early stages (17.85%). The difference was statistically significant (OR=0.353, 95% CI=0.112-1.116, p<0.05). A statistically significant difference was reported between smokers and non-smokers with respect to the KRAS (exon 2) mutation (OR=4.899, 95%CI=1.273-18.77, p < 0.01). The significantly higher frequency of this mutation was reported in NSCLC patients (29.16%) with metastasis (OR=0.941 95% CI=0.319-2.775, p < 0.03). KRAS (exon 2) mutation is a common molecular alteration in NSCLC and occurs most predominantly on codon 12, 13, characterizing 30% of the total mutations found in Kashmiri population. These mutations are significantly associated with clinicopathological characteristics of patients.

Exome sequencing reveals novel oncogenic mutations in early-onset sporadic rectal cancer

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Abstract
Colorectal cancer (CRC) is an aging related disease with 72% of newly diagnosed cases aged 65 years or older (Cancer Registration Statistics, 2012). Past few decades of research from the West have identified aberrantly activated canonical Wnt/β-catenin signaling and microsatellite instability (MSI) as the major pathways driving CRC tumorigenesis. In India however majority of cases appear to belong to a younger age group with preponderance of rectal cancer. Molecular genetic screening of early-onset sporadic rectal cancer (EOSRC) performed earlier from our laboratory identified a significant proportion of EOSRC to be driven neither by aberrant Wnt signaling nor MSI. We performed whole exome sequencing of 27 tumor/normal pairs obtained from surgically resected rectal adenocarcinomas (microsatellite stable with no aberrant Wnt signaling) using the Illumina HiSeq 2500 platform. We identified recurrently mutated genes in EOSRC including known tumor suppressors (TSG) and oncogenes such as TP53, APC, KRAS, SMAD4 and PIK3CA. More importantly, we discovered mutations in uncharacterized TSGs and oncogenes such as ARID2, FAT3, FAT4, ZEB2 and TRPC7. These included putative oncogenic mutations in the zinc finger domains of ZEB2, a DNA-binding transcriptional repressor that promotes epithelial to mesenchymal transition in tumors. Functional work is underway to characterize the effect of these mutations on CRC tumor progression. APC mutations detected in this study were present in major population of cells, yet were not driving β-catenin to the nucleus. This observation suggests β-catenin degradation independent tumor suppressor function of APC, which is being validated.

Category: Cancer Genomics

Delineating miRNA profile induced by chewing tobacco in oral keratinocytes

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Abstract

The major established etiologic risk factor for oral cancer is tobacco (chewed, smoked and snuffed forms). Chewing form of tobacco is predominantly used in India making it the leading cause of oral cancer. Despite being one of the leading causes of oral cancer, the molecular alterations induced by chewing tobacco remains largely unclear. Carcinogenic effect of chewing tobacco is through chronic and not acute exposure. To understand the molecular alterations induced by chewing tobacco, we developed a cell line model where non-neoplastic oral keratinocytes were chronically exposed to chewing tobacco for a period of 6 months. This resulted in increased cellular proliferation and invasive ability of normal oral keratinocytes. Using this cellular model we studied the differential expression of miRNAs associated with chewing tobacco and the altered signaling pathways through which the aberrantly expressed miRNAs affect tumorigenesis. miRNA sequencing was carried out using Illumina HiSeq 2500 platform which resulted in the identification of 427 annotated miRNAs of which 10 were significantly dysregulated (≥ 4 fold; p-value ≤ 0.05) in tobacco exposed cells compared to untreated parental cells. To study the altered signaling in oral keratinocytes chronically exposed to chewing tobacco, we employed quantitative proteomics to characterize the dysregulated proteins. Integration of miRNA sequencing data with proteomic data resulted in identification of 36 proven protein targets which (≥1.5 fold; p-value ≤ 0.05) showed expression correlation with the 10 significantly dysregulated miRNAs. Pathway analysis of the dysregulated targets revealed enrichment of interferon signaling and mRNA processing related pathways in the chewing tobacco exposed cells. In addition, we also identified 6 novel miRNA in oral keratinocytes chronically exposed to chewing tobacco extract. Our study provides a framework to understand the oncogenic transformation induced by chromic tobacco exposure in normal oral keratinocytes.

Category: Cancer Genomics

Comprehensive analysis of long non-coding RNAs in early stage breast cancer

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Abstract

Breast cancer is one of the most common cancers in India as well as worldwide. Among Indian females, the age adjusted incidence rate of breast cancer is as high as 25.8 per 100,000 women and mortality rate of 12.7 per 100,000 women. Accumulating evidence highlights the potential role of long non-coding RNAs (lncRNAs) in breast cancer development. LncRNAs are emerging as important players in tumorigenesis as they are known to participate in various cancerous processes including proliferation, apoptosis, and invasion. LncRNAs are reported to be dysregulated in a number of cancers, demonstrating both oncogenic and tumor suppressive roles, thus suggesting their aberrant expression may be a substantial contributor in cancer development. However, their mechanism of action is poorly understood. Several studies have been published describing lncRNA expression profile of various breast cancer subtypes in different stages of cancer; however, their aberrant expression has not been systematically investigated in early stages. We have carried out expression profiling using deep sequencing of total RNAs to identify lncRNA expression profile in ductal carcinoma in situ and early stage breast cancers. We identified 103 differentially expressed (DE) lncRNAs of which 21 lncRNAs showed progressive pattern based on transcripts per million (TPM) in unmatched normal, adjacent normal, ductal carcinoma in situ, and stage 1 breast cancers. We identified several novel lncRNAs including RP11-295M3.4, LINC01614, RP11-527N22.1, RP11-126H7.4 and RAMP-AS1 that have not been reported in breast cancer before. These findings provide insights into lncRNA expression landscape in early stage breast cancers.

Integrated multi-omics analysis reveals potential mechanisms of acquired resistance to erlotinib in head and neck cancer cells

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Abstract

Epidermal growth factor receptor (EGFR) is overexpressed in 90% of head and neck squamous cell carcinomas (HNSCC). However, most clinical trials with tyrosine kinase inhibitors (TKIs) have shown modest response rates due to development of acquired resistance. We performed whole exome sequencing (WES) of an isogenic pair of erlotinib-sensitive (SCC-S) and resistant (SCC-R) HNSCC cell lines to elucidate the molecular mechanisms that govern acquired resistance to erlotinib. Exome sequencing resulted in identification of 148 non-synonymous single nucleotide variants (SNVs) in 139 genes and copy number alterations (CNA) (≥2 fold) affecting 339 genes in SCC-R cells compared to SCC-S cells. Comparison of SNVs from SCC-R against post-translational modification databases resulted in identification of loss of ubiquitinylation site at p.K57E in dual specificity mitogen-activated protein kinase kinase 1 (MAP2K1) which was validated using in-house high-throughput proteomic data. Substitution mutation K57N in MAP2K1 is shown to result in its constitutive activation and subsequent gefitinib resistance in lung adenocarcinoma. We also identified a well-known driver mutation p.G13R in Harvey rat sarcoma viral oncogene homolog (HRAS) (AIIF: 20.41%). In addition, we also observed CNA in other genes of this pathway including RAC-beta serine/threonine-protein kinase (AKT2), glycogen synthase kinase-3 alpha (GSK3A), Rho guanine nucleotide exchange factor 1 (ARHGEF1) amongst others. Corresponding protein expression changes of these genes were also observed in proteomics data. Quantitative phosphoproteomics revealed hyperphosphorylation of other proteins involved in MAPK pathway such as serine/threonine-protein kinase B-raf (BRAF), MAP2K2, mitogen-activated protein kinases such as MAPK1 and MAPK3. Integrative multi-omic analysis revealed constitutive activation of key intermediates of MAPK pathway in SCC-R cells compared to SCC-S cells which may be essential in the development of acquired resistance to erlotinib in these cells. We hypothesize that combinatorial treatment regime involving inhibition of putative targets such as MAP2K1 with erlotinib therapy may aid in tackling acquired erlotinib resistance in HNSCC patients.

Category: Cancer Genomics

Identification of genes responsible for anti-VEGF resistance in tumor cells

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Abstract

Angiogenesis is the process of formation of new blood vessels from pre-existing vessels, which plays a key role in physiological as well as pathological conditions. It is a tightly regulated process involving the interplay of a number of pro and anti-angiogenic factors. Dysregulation of the balance between these factors lead to excess angiogenesis or inhibition of angiogenesis contributing to pathological conditions such as cancer, inflammation, atherosclerosis, tumor growth & rheumatoid arthritis. Vascular endothelial growth factor (VEGF) is an endothelial cell specific growth factor which is a critical mediator in angiogenesis and targeting VEGF signaling is considered a key therapeutic approach for blocking angiogenesis in anti-VEGF therapy. But recently it has been noted that certain tumors develop resistance to anti-VEGF therapy and develop capillaries by some alternative mechanisms. This may be due to the activation of other pathways which have a proper connection with the downstream signaling of VEGF mediated angiogenesis. To shed light on the mechanisms and mediators of resistance to anti-angiogenic therapy, we analysed a set of microarray expression data showing resistance to antiVEGF therapy from databases and differentially expressed genes were identified. A total of 31 dataset were considered for the study, out of it one data set was used for the present study. The dataset contained 4 test and control samples, each having 34182 genes, out of which 796 genes were differentially expressed. Among the differentially expressed genes, 63 genes were 2 fold up regulated and 60 genes were 2 fold downregulated in both the sets. And these genes were classified based on the molecular function, cellular behavior and biological process. The results provide valuable biological insights into how tumors form resistance to anti-therapy.

References


Identification of differentially expressed snoRNAs in Ovarian Cancer from RNA-Seq data

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Abstract

Non-coding RNAs (ncRNAs) are a large class of important regulatory molecules involved in many physiological and cellular processes. Small nucleolar RNAs (snoRNAs), a subset of the small ncRNAs, are molecules located in the cell nucleolus. Even though, they are one of the most ancient and evolutionary conserved ncRNAs, they are gaining more prominence and attention in the recent years only. The classical function of snoRNAs is to act as guide RNAs of rRNAs and nucleolytic processing of the rRNA transcripts. However several scientific evidences have indicated that other than the classical functions, they are involved in multiple functions such as metabolic stress regulation, modulation of alternative splicing, controlling cell behavior, etc. and the dysregulation of snoRNAs could contribute to carcinogenesis. Even though many independent works have been carried out, to examine the role of snoRNAs in several human diseases including cancer, specific projects to study the cumulative role of snoRNAs in a disease is limited. The advent of high throughput and deep sequencing technologies has opened up new avenues for carrying out such studies. This study focusses on the utilization of snoRNAs as potential biomarkers characteristic to ovarian cancer based on a RNA-Seq data. We have downloaded a transcriptome data, [PRJNA209481] from the NCBI BioProject, pertaining to human ovarian cancer cell lines. Downstream analysis was done by Tuxedo pipeline of RNA-Seq data analysis. The differential expression analysis by Cuffdiff threw a total of 847 differentially expressed genes, of which we found, 71 snoRNAs to be up regulated and 34 snoRNAs as down regulated. Many of the previously reported snoRNAs having a role in tumorigenesis in a variety of cancers are also seen to be dysregulated in this study. Further investigation is underway to analyze the effect of differentially expressed snoRNAs in ovarian cancer and their potential as biomarkers.

References


A multi-omic analysis to characterize cigarette smoke induced molecular alterations in esophageal cells

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Abstract

Esophageal squamous cell carcinoma (ESCC) is one of the most common cancers with high mortality rate. Smoking is one of the established risk factors of ESCC. However, there is limited data on molecular alterations associated with cigarette smoke exposure in esophageal cells. Understanding the effects of cigarette smoke on esophageal squamous epithelial cells at a molecular level would lead to a better understanding of the pathobiology of ESCC which has implications for identification of early biomarkers and therapeutic targets. To investigate the effect of cigarette smoke exposure, we developed a cell line model where Het1A cells (non-neoplastic human esophageal epithelial cells) were chronically treated with cigarette smoke condensate (CSC) for 2 months, 4 months, 6 months and 8 months. We carried out comparative proteomic, phosphoproteomic and whole exome sequencing analyses on CSC treated and untreated cells. Increased cell proliferation, invasion and anchorage independent growth of Het1A cells was observed after chronic exposure to cigarette smoke. Using quantitative proteomic and phosphoproteomic analyses, we identified 35 proteins and 118 phosphoproteins that showed differential expression. Bioinformatics analysis of differentially expressed proteins and phosphoproteins showed enrichment of molecules involved in DNA damage response pathway. To further understand the mutational burden associated with cigarette smoke, we did whole exome sequencing of CSC treated and untreated cells which also revealed mutations and copy number alterations in genes associated with DNA damage response. By correlating WES, proteomic and phosphoproteomic results, we observed potential loss of function in HMGN2 and MED1 that were reported as potential tumor suppressors and are known to play important role in DNA damage response. We also observed decreased expression of HMGN2 in tissue section of ESCC. Overexpression of HMGN2 and MED1 lead to decreased proliferative and invasive ability of CSC treated cells. These findings suggest that cigarette smoke affects genes and proteins associated with DNA damage response pathways which might play a vital role in development of ESCC.

Comparative Study of Transcriptomic profiling and Functional enrichment in Ovarian Cancer Cell lines

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Abstract

High-throughput cDNA sequencing (RNA-seq) has emerged as a sophisticated tool for transcriptomic studies, especially for identifying differentially expressed genes (DEGs) and measuring the transcripts between different sample groups or conditions. There are several pipelines and tools available for performing the task, but still there is no general consent for the protocol to be used for the analysis. In this comparative study, transcriptomic profiling of Ovarian cancer cell lines data sets were carried out by using two different pipelines- ‘Tuxedo’ protocol (Tophat, Cufflinks-Cuffdiff, CummerBund) and ‘new Tuxedo’ protocol (HISAT, StringTie, Desq2) were used for estimating the transcript abundancies and for analysing differential expression. ‘New Tuxedo’ protocol was found to be fast and efficient than ‘Tuxedo’ protocol and the run time on an 8 GB RAM PC was ~ 2 hr and ~ 6 days, respectively. A total of 613 and 371 DEGs were obtained by using ‘Tuxedo’ and ‘New Tuxedo’ pipeline, respectively. Functional profiling was performed, by a comparative study of high throughput functional enrichment tools (clueGO, DAVID, EnRichr, FunRich, gProfiler, GSEA, PANTHER and webGestalt) to get the functions and pathways of most enriched genes involved in ovarian cancer cell lines. The common biological pathways and Gene Ontology (GO) terms were extracted with common genes from all the tools to get most enriched genes with the GO functional terms. Thus, the characterization of biological pathway and GO processes (Biological processes and Molecular Function) of most enriched gene sets involved in ovarian cancer cell lines were obtained.

References


Citation: Tripathi, N., Sunitha, P. and Nair, A.S. Comparative Study of Transcriptomic profiling and Functional enrichment in Ovarian Cancer Cell lines [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 65. https://doi.org/10.24870/cjb.2017-a52
Category: Cancer Genomics

**HOXA9 and SOX1 – a promising DNA methylation based diagnostic biomarker for epithelial ovarian cancer**

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**Abstract**

Epigenetic alterations play a major role in cancer. Transcriptional silencing by CpG island hypermethylation is a potential mechanism for the inactivation of tumor related genes. Aberrant DNA methylation patterns might be used as a biomarker for diagnosis and management of cancer patients. Ovarian cancer is characterized by few early symptoms, presentation of the disease at late stage and resulting poor survival. At present, no single epigenetic biomarker is able to accurately detect early ovarian cancer in either tissue or body fluid. Analysis of the methylation status of multiple genes simultaneously in a blood based assay may provide a more sensitive and specific method for the molecular classification and diagnosis of ovarian cancer. To develop a potential, DNA methylation based screening assay for early diagnosis of ovarian cancer, we quantitatively assessed the promoter methylation of HOXA9 and SOX1 gene in 54 ovarian cancer and 18 non neoplastic ovarian specimens by means of a high throughput quantitative, real time PCR based technique (MethyLight). We identified DNA methylation of HOXA9 and SOX1 to be the best discriminator between cancer and non-neoplastic tissue. The gene methylation achieved 93.47% and 78.26% sensitivity for HOXA9 and SOX1, respectively when analyzed in singleplex assay. However the sensitivity increased to 95.92% in the multiplex assay when either or both of the HOXA9 and SOX1 gene promoters showed methylation thereby indicating that these genes appear to have great potential to be evaluated for their methylation level in cell-free DNA or serum DNA as a non-invasive diagnostic marker the early diagnosis of ovarian cancer.

**Citation:** Singh, A. and Sachan, M. HOXA9 and SOX1 – a promising DNA methylation based diagnostic biomarker for epithelial ovarian cancer [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 66. [https://doi.org/10.24870/cjb.2017-a53](https://doi.org/10.24870/cjb.2017-a53)
Category: Cancer Genomics

**Mutational landscape of cytokine genes across major tumour types identifies new targets**

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**Abstract**

**Introduction**: Components of immune system communicate extensively in tumour micro environment. Normally, immune system engages with tumours to inhibit its further progression. Simultaneously, cancer cells learn cues derived from immune system to its own growth advantage. Cytokines are cell signaling messengers that affect disease pathogenesis, non-specific response to infection, specific response to antigen, etc. A battery of cytokines are produced in the tumour microenvironment, when released in response to infections and inflammations, can function to inhibit tumour development and progression. Cancer cells also release cytokines that promote growth, extenuate apoptosis and perform invasion and metastasis.

**Hypothesis**: Alterations in cytokine signaling genes might help tumour to misguide immune system. The aim of the study is to identify such genomic alterations in cytokine genes that may drive major human cancers.

**Methods**: We did extensive literature survey to prepare a list of known cytokine genes (n=776) which were validated in multiple databases. To know the baseline DNA variation in cytokine genes, we analyzed DNA variations in healthy human population from the 1000 Genome project dataset. Somatic mutational landscape for cytokine genes were analyzed in 32 different human cancer types (TCGA data). Significantly mutated genes were detected using MutSig2CV and Oncodrive FM analysis. Genes found significant in both analysis were tabulated. Standard statistical and bioinformatics analysis were done further to identify putative driver events.

**Result**: We detected 9 significantly mutated cytokine genes across major tumor types. *EDN1* was found to be most significantly mutated, in multiple tumour types; apart from genes like *CDH1, B2M, HLA-B, IL4, TRIM22, TGFBI, GDF1* and *CRABP2*.

**Discussion**: Our systematic survey of somatic mutations in cytokine genes, in major tumour types, identified novel genes targets such as *EDN1* gene. *EDN1* is a chemokine, also a potent vasoconstrictor. *EDN1* signaling modifies tumour microenvironment by regulating contribution of cells around tumor stroma through both autocrine and paracrine mechanisms, by promoting tumour cell proliferation, neovascularization, etc. Other significantly mutated genes are associated with antigen presentation, cell proliferation and chemoattraction. Rational combination therapy with current inhibitors to disrupt these signaling networks in tumor microenvironment, may improve clinical outcomes in patients.

Identification of the bimodal transcriptional regulation of SP1 transcription factor in sub-sets of gastric cancer

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Abstract

Stomach cancer is the second leading cause of cancer death and fourth most common cancer in the world. Deregulated transcription programs and signaling pathways are the major driving forces involved in the initiation and progression of cancer. Identification of the dysregulated transcription programs and genes from the transcriptome of gastric tumors would pave the way for i) the identification of major dysregulations involved in gastric cancer, ii) stratification of tumors, and iii) development of targeted therapies. Specificity protein (SP1) is a transcription factor and aberrant expression of SP1 is known to confer proliferative and metastatic advantage to tumor cells. The role of SP1 mediated expression in gastric cancer was investigated in the genome-wide mRNA profile of gastric cancer cell line upon SP1 silencing. Gene-set based cumulative expression analysis of the available SP1 regulated gene-sets revealed the involvement of certain SP1 target genes in a sub-set of gastric tumors while the another group of genes in another sub-set. This shows the bi-modal involvement of different SP1 regulated genes in different sub-types of gastric tumors. The expression of a set of SP1 genes were found positively correlated with the oncogenic signatures of MYC, E2F and mTOR signaling with their elevated expression in intestinal type tumors. Another category of SP1 genes, which are up-regulated upon SP1 silencing were found expressed in diffuse type tumors along with the activated VEGFA, OCT4, and TGF-β signaling. These results reveal that SP1 transcriptionally activates the genes involved in intestinal type and represses the genes involved in diffuse type gastric tumors. This shows the dual role of SP1 regulated transcription in sub-sets of gastric tumors and warrant further investigation in diagnostic and therapeutic contexts.

Category: Cancer Genomics

**Delineation of HIF1α mediated transcription program and the oncogenic signaling pathways in gastric tumors**

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**Abstract**

HIF1α is a transcription factor activated under hypoxic condition in many cancer types and has been implicated in cancer cell proliferation, invasion and energy metabolism. Towards understanding the role of HIF1α mediated transcription in gastric cancer, the HIF1α gene signatures established to date were analyzed for their expression across the mRNA profiles of gastric tumors. HIF1α regulated genes were identified to involve and associated with the signaling pathways and processes such as integrin signaling, Wnt, EGF, FGF, VEGFA, PI3K, TGFβ and NFkB signaling pathways. The HIF1α genes were identified to play a significant role in energy metabolism including glycolysis, drug resistance due to epithelial to mesenchymal transition and cancer cell survival. In gastric tumors, the HIF1α regulated genes were observed to express in diffuse, poorly differentiated and stage-3 tumors. The analyses reveal i) activation of HIF1 in a sub-set of gastric tumors, ii) the pathways associated with the HIF1 activation in gastric tumors, and iii) genes involved in HIF1α mediated transcription in gastric cancer. We are further investigating the drugs that would best suit for this sub-set of tumors with activated HIF1α.

**References**


**Citation:** Rathinam, D. and Ganesan, K. Delineation of HIF1α mediated transcription program and the oncogenic signaling pathways in gastric tumors [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 69. https://doi.org/10.24870/cjb.2017-a56
Category: Cancer Genomics

Molecular alterations associated with chronic exposure to cigarette smoke and chewing tobacco in normal oral keratinocytes

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Abstract

Tobacco usage is a known risk factor associated with development of oral cancer. It is mainly consumed in two different forms (smoking and chewing) that vary in their composition and methods of intake. Despite being the leading cause of oral cancer, molecular alterations induced by tobacco are poorly understood. To investigate the adverse effects of cigarette smoke/chewing tobacco exposure in oral keratinocytes, we developed two cellular models where normal oral keratinocytes were chronically exposed to cigarette smoke and chewing tobacco for a period of 8 months. Cellular assays reveal that OKF6/TERT1 cells acquire an oncogenic phenotype after chronic exposure to cigarette smoke/chewing tobacco. We employed both whole exome sequencing (WES) and quantitative proteomics approaches to investigate the molecular alterations in oral keratinocytes (OKF6/TERT1) chronically exposed to smoke and chewing tobacco. Exome sequencing revealed a much higher rate of C>A transversions in smoke exposed cells in conjunction with previous studies. In contrast, C>G transversions were observed to be higher in chewing tobacco exposed cells. Diverse mutations in both treated cells further highlight the distinct effects of each exposure. Distinct proteomic alterations were observed in smoke and chewing tobacco exposed cells compared to parental cells. In addition, we observe enrichment of different signaling cascades in transformed oral cells upon chronic exposure to either cigarette smoke or chewing tobacco. Current analysis defines a clear distinction in the molecular dysregulation in oral cells in response to different tobacco-based insults. Future studies are needed to validate some of the genetic and proteomic alterations unique to each form of tobacco exposure. This study can serve as a reference for fundamental damage on oral cells as a consequence of exposure to different forms of tobacco.

Category: Cancer Genomics

Potential role of TIGAR in OSCC: tumorigenesis and survival

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Abstract

Despite the improvement of treatment modalities, OSCC remains a prevalent disease in India and 50% of OSCC patients die within 5 years of disease detection. One of the major reasons for treatment failure is imbalances of metabolic profile in cancer. TIGAR (TP53-induced glycolysis and apoptosis regulator), is a p53-inducible protein that functions as fructose-2, 6-bisphosphatase and fructose-1, 6-bisphosphatase, reducing the glycolytic rate and promoting the Pentose Phosphate Pathway. Consistent with increased activation of the PPP, cells expressing TIGAR have higher NADPH levels and a concomitantly enhanced ability to regulate levels of cellular ROS and thus reduce oxidative stress. Apart from the metabolic function of TIGAR, it has another role in the survival of cancer. Recently found that TIGAR is upregulated in some cancer, the main focus of our proposed study is to find out the potential role of TIGAR in tumorigenesis and survival of OSCC. For this we are using OSCC cell line as well as primary OSCC sample, some our recent finding suggesting that TIGAR is upregulated in OSCC cell line as well as patient sample compared their respective control, and also found that TIGAR knockdown decreases cell viability and drug sensitivity. Our main focus is how TIGAR regulated OSCC cell survival and what mechanism behind this, for this we will use RNA-Seq in TIGAR KD cell and TIGAR OE cell and found that how many pathway or which gene is affecting when targeting TIGAR. So overall our findings prove that TIGAR has some role in the survival of OSCC.

Citation: Shriwas, O., Prasad, P. and Dash, R. Potential role of TIGAR in OSCC: tumorigenesis and survival [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 71.
https://doi.org/10.24870/cjb.2017-a58
Identification of Biomarkers for Stage Prediction in Papillary Renal Cell Carcinoma

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Abstract

Papillary Renal Cell Carcinoma (PRCC) is a heterogeneous disease accounting for 10%-15% of renal cell carcinomas. A comprehensive analysis is required to find the genes that are responsible for the stage progression in PRCC. The advent of next generation sequencing techniques (NGS) has produced a lot of high throughput data from patients that can be analyzed to address this problem. The low sample size, noise and high dimensionality of the data though enhances the complexity, requiring the use of sophisticated methods. In our study we propose a machine learning pipeline fulfilling a two-fold objective: 1) To find suitable genes that could serve as potential biomarkers for stage progression in PRCC. 2) To build a classifier using the above biomarkers that can predict the stage of a given patient. The RNA-Seq data of PRCC was taken and divided into training set (80%) and test set (20%). Different groupings of training data were created and on each group different feature selection algorithms. The features (genes) extracted were then combined based on voting. The selected features from each feature selection algorithm were then used to train the classifiers on the training data. The performance of the model on the test data was evaluated using various measures. To further check the quality of the genes a 10 fold cross validation was performed on microarray cohort of PRCC. The selected genes we get are robust with overlap among the features derived from the various feature selection algorithms. The best of the classifiers trained above gave an accuracy > 86 % and area under Receiver Operating Curve (AUC) > 0.8. The 10 fold cross validation on microarray data using the above features yields best accuracy > 85 % and AUC > 0.84 enhancing our confidence on our gene sets. The feature sets which we get could be further investigated for identifying their role in stage progression. Further our pipeline could be used for analyzing other cancer data sets.

Deciphering the Diversity of Somatic Alterations and Salmonella Infection in Gallbladder Cancer by Whole Exome Sequencing

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Abstract

Introduction: Gallbladder cancer is relatively a rare lethal malignancy with dismal prognosis. While in India there is high incidence (3.9-8.6/1,00,000) with majority of patients having advanced disease. Recent developments in next generation sequencing technologies have enabled the discovery of new molecular therapeutic targets in many human cancers.

Objectives: Interrogate the landscape of somatic alterations in Indian gall bladder cancer using whole exome sequencing technology.

Material and Methods: We interrogated the coding region of 27(10 paired and 7 unpaired) Indian gall bladder cancer samples using whole exome sequencing at an average coverage of 100X and above. We further validated the findings using an additional set of 27 FFPE samples.

Results: Using a bioinformatics filtering approach, we identify a total of 5060 somatic variants found across 17 tumors consisted of 3239 missense, 1449 silent, 131 nonsense, 135 indels and 106 splice site mutations The average mutation rate considering the paired tumors is about 7.7 mutations/mb. We found TP53 (35.2%), ERBB2 (17.6%), SF3B1 (17.6%), ATM (17.6%) and AKA11 (17.6%) mutations in more than two samples by exome sequencing analysis. Furthermore, we examined our exome sequencing data for identifying Salmonella sequences as well as presence of 143 HPV types using computation subtraction based on HPVDetector. Based on our evaluation we found association of typhoidal Salmonella strains in 11 of 26 gall bladder cancer samples and non-typhoidal Salmonella species in 12 of 26 samples, 6 samples were co-infected with both.

Conclusions: The profiling of somatic alterations and identification of non typhoidal Salmonella traces may aid in changing the current treatment paradigm of gall bladder cancer.

Integrated Genomic Analysis of Early Stage Tongue Tumors Revealed Recurrent Transcript Fusions

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Abstract

Introduction: Tongue cancer is the most predominant form of oral cancer in developed countries with varying incidence in developing countries. In India, tongue cancer accounts for 21\% of head and neck squamous cell carcinoma (HNSCC) and known to display occult node metastasis during early stages of disease. The major etiological factors associated with tongue cancer includes tobacco related products, alcohol and human papilloma virus (HPV) infections.

Objective: Portrait of genomic aberrations underlying the genome of tobacco/nut chewing HPV-negative early stage tongue tumors.

Materials and Methods: Whole transcriptome sequencing of 17 HPV-negative early stage tongue squamous cell carcinoma (TSCC) tumors and 4 HNSCC cell lines. Validation of findings in an additional set of 44 paired HPV-negative early stage TSCC tumor samples.

Results: Using bioinformatics approaches, we present the first glance of a portrait of 242 tumor specific transcript fusions, followed by exhaustive validation of 12 candidate fusion transcripts across 44 paired HPV-negative early TSCC tumor samples and 4 HNSCC cell lines. Comparative analysis of our data with various fusion databases revealed 48 previously described transcript fusions in various cancer types. We have identified and validated novel somatic recurrent fusion transcripts in tumor samples. Here, we present a comprehensive landscape of transcript fusions underlying the genome of HPV-negative early stage tongue tumors.

Conclusions: Characterization of the recurrent transcript fusions described here could serve as attractive candidates to facilitate in diagnosis of HPV-negative early stage TSCC patients.

Category: Cancer Genomics

Domain-restricted mutation analysis to identify novel driver events in human cancer

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Abstract

Analysis of mutational spectra across various cancer types has given valuable insights into tumorigenesis. Different approaches have been used to identify novel drivers from the set of somatic mutations, including the methods which use sequence conservation, geometric localization and pathway information. Recent computational methods suggest use of protein domain information for analysis and understanding of the functional consequence of non-synonymous mutations. Similarly, evidence suggests recurrence at specific position in proteins is robust indicators of its functional impact. Building on this, we performed a systematic analysis of TCGA exome derived somatic mutations across 6089 PFAM domains and significantly mutated domains were identified using randomization approach. Multiple alignment of individual domain allowed us to prioritize for conserved residues mutated at analogous positions across different proteins in a statistically disciplined manner. In addition to the known frequently mutated genes, this analysis independently identifies low frequency Meprin and TRAF-Homology (MATH) domain in Speckle Type BTB/POZ (SPOP) protein, in prostate adenocarcinoma. Results from this analysis will help generate hypotheses about the downstream molecular mechanism resulting in cancer phenotypes.

References


Citation: Desai, S., Chandrani, P. and Dutt, A. Domain-restricted mutation analysis to identify novel driver events in human cancer [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 75.\texttt{https://doi.org/10.24870/cjb.2017-a62}
Category: Cancer Genomics

Elucidating the mechanisms of resistance to tyrosine kinase inhibitors in lung cancer patients

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Abstract

Introduction: Lung tumors with mutations in epidermal growth factor receptor (EGFR) gene represent a clinically distinct subtype of lung cancer and are observed at a frequency of 23% among Indian patients. The standard practice for treatment of EGFR mutated lung cancer patients includes tyrosine kinase inhibitors (TKIs) erlotinib and gefitinib. Although initial clinical responses are observed, resistance to TKIs develops within year from the start of treatment. In about fifty percent of cases, the resistance is caused due to a secondary T790M mutation in the EGFR gene. Additionally, MET amplification and histological transformation of tumors are known to confer TKI resistance in a small subset of patients. Nonetheless, there is an unmet need to elucidate novel ways by which lung tumors acquire resistance to EGFR targeting TKIs.

Objectives: To delineate novel mechanisms of acquired resistance to EGFR-TKIs by characterizing the differential profile of drug sensitive and resistant state among lung tumors using integrated genomics approaches.

Material and Methods: A retrospective collection of FFPE DNA samples (n=45) from tumors at baseline and rebiopsy along with paired blood sample was done for a total of 15 EGFR mutated lung cancer patients. Only tumor samples which were negative for EGFR T790M (as confirmed by orthologous technologies) were selected in the study with anticipation that such samples would be enriched novel resistance mechanisms. Whole exome sequencing at an average coverage of 100X was performed for these samples.

Results: The whole exome data was analyzed using an in-house developed pipeline. Of all the known resistance mutations, we identified EGFR T790M mutation in five out of fifteen patients. Other than T790M we expect to identify novel resistance causing mutations from the analysis of ten patients with unknown resistance mechanisms. Whole exome sequencing at an average coverage of 100X was performed for these samples.

Genetic effect of monoamine oxidase B (MAOB) gene on ASD associated behavior phenotypes

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Abstract

Autism spectrum disorder (ASD) is a male predominance, behaviorally defined neurodevelopmental disorder which is characterized by impairment in social communication and restricted and repetitive activities. Abnormalities in serotoninergic function play a major role in ASD pathophysiology. Monoamine oxidases, encoded by two X-chromosomal genes MAOA and MAOB regulate the serotonergic function by the degradation of serotonin and other biological amines. Therefore, the objective of present study is to investigate genetic correlation of MAOB markers with the severity of specific behavioral traits as scored by Childhood Autism Rating Scale (CARS) has been examined as quantitative trait (QT) analysis using IBM-SPSS program. A total of 225 ASD patients (190 male and 35 female) were recruited after psychometric evaluation done by DSM-IV-TR/DSM-5 criteria and assessment by CARS. Genotyping carried by PCR/RFLP/sequencing methods, and population were found in Hardy-Weinberg equilibrium. The outcome of the QT analysis indicating the increased score in overall CARS were associated with G and C allele of MAOB marker rs3027449 (p-value: 0.03) and rs1040399 (p-value: 0.01), respectively in male ASD children. In addition to this, major alleles of studied polymorphisms of gene were found to be statistically associated with the higher impairment in social communication domain only in male ASD children. Overall outcome of the study suggests likely involvement of MAOB with ASD in a gender-specific manner with the severity in behavior phenotypes. Considering the cumulative impact of these markers in regulating the severity of the behavioral symptoms of ASD, it is likely that MAOB gene is associated with the disorder.

Category: Clinical Genomics

**Gene-Specific-Candidate-Driven Study to decipher Genetic Predisposition to Rotavirus Infection**

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**Abstract**

Recent report of WHO shows 113000 children in India succumb to death due to Rotavirus diarrhea. Lack of knowledge about pathogenesis of virus has led to lack of therapy for severely infected patients. Previous studies have found that, animal rotavirus requires sialyl glycan moieties on cell surface for pathogenesis. Present study states that human rotaviruses also follows same path and this specificity of virus leads to host genetic predisposition for the infection as well as the disease. Two hundred children less than 5 years of age clinically suspected of viral diarrhea were screened for rotavirus infection. EDTA blood was processed for analyzing DNA sequences of various fucosyltransferase genes. Lewis antigens which are secretory form of ABO Histo Blood Group Antigens were correlated with the genotype of patient. Genetics of HBGA secretion, particularly, basis of Le\(^b\) expression manifested by fucosyltransferase-2 enzyme was studied in healthy individuals and was compared in cases of rotavirus positive and negative diarrhea. Positive clinical isolates with various genotypes were purified from stool samples and gene for VP4 - surface spike protein was sequenced. Using Bioinformatics interphase, three dimensional protein structures were modeled and their functional domains were analyzed. All these modeled proteins were docked with Le\(^b\) HBGA (Lewis-b Histo Blood Group Antigens) using molecular docking software. In present study, to investigate possible association of the rotavirus with host genome, we screened highly suspected genes involved in expression of glycoproteins on enterocytes. This study performed for prevalent Indian strains of rotaviruses provides possible evidence that, VP8 domain of VP4 spike protein utilizes Le\(^b\) surface antigen for attachment and entry to enterocytes in the intestine. The FUT2 and FUT3 gene has been found to show significant association with the rotavirus infection hence can serve as a biomarker for genetic predisposition to Rotavirus diarrhea. Knowledge of molecular biology of the Rotavirus pathogenesis may open up new paths for vaccines and therapy. Data presented here is first of its kind which deciphers Host-Rotavirus interaction by parallel experiments of epidemiological study and In Silico study.

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Category: Clinical Genomics

MACF1 gene variant rs2296172 is associated with type 2 diabetes susceptibility in the Bania population group of Punjab - India

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Abstract

Microtubule Actin Cross linking Factor 1 (MACF1) gene variant rs2296172 has been associated with Type 2 Diabetes (T2D). However, this variant has never been evaluated as such in Indian populations. We replicated this variant in pooled population of Northwest India and specifically in an endogamous caste group, Bania of Punjab, India. We genotyped variant rs2296172 by Taqman allele discrimination assay in 651 T2D patients and in 568 healthy controls from Northwest India. The association of the SNP with T2D was evaluated by case - control association study design. The SNP rs2296172 of MACF1 was found to be significantly associated with T2D with p value = 0.009 in Northwest Indian population but allelic distribution was observed to be deviated from Hardy-Weinberg equilibrium (HWE). Assuming population stratification the most plausible cause, we further evaluated the samples belonging to Bania caste group from Punjab, India. We observed significant association of this SNP with T2D with OR = 1.71 (1.03-2.83) at 95%CI, (p =0.03) and sample set following HWE. MACF1 variant rs2296172 was found to be associated with T2D in endogamous ethnic population group (Bania) of Punjab, India. Deviation from Hardy-Weinberg equilibrium in the pooled population group from Northwest India, underlines that Indian population sub structure exists and may have implications in association studies. Thus, ideal case - control association study design in Indian populations is to evaluate endogamous population groups rather than the conventional practice of pooling samples based on geography or linguistic affinities only.

Category: Clinical Genomics

Incidental and clinically actionable genetic variants in 1005 whole exomes and genomes from Qatar

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Abstract

Next generation sequencing (NGS) technologies such as whole genome and whole exome sequencing has enabled accurate diagnosis of genetic diseases through identification of variations at the genome wide level. While many large populations have been adequately covered in global sequencing efforts little is known on the genomic architecture of populations from Middle East, and South Asia and Africa. Incidental findings and their prevalence in populations have been extensively studied in populations of Caucasian descent. The recent emphasis on genomics and availability of genome-scale datasets in public domain for ethnic population in the Middle East prompted us to estimate the prevalence of incidental findings for this population. In this study, we used whole genome and exome data for a total 1005 non-related healthy individuals from Qatar population dataset which contained 20,930,177 variants. Systematic analysis of the variants in 59 genes recommended by the American College of Medical Genetics and Genomics for reporting of incidental findings revealed a total of 2 pathogenic and 2 likely pathogenic variants. Our analysis suggests the prevalence of incidental variants in population-scale datasets is approx. 0.6%, much lower than those reported for global populations. Our study underlines the essentiality to study population-scale genomes from ethnic groups to understand systematic differences in genetic variants associated with disease predisposition.

Targeted genome-wide DNA methylation profiling of ovarian granulosa cells from women with PCOS

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Abstract

Polycystic ovary syndrome (PCOS) is a complex endocrinopathy of obscure pathophysiologic origins, globally affecting 6-15% of women of childbearing age. Emerging evidence on repercussions of environmental insults and changing lifestyles on fecundity and reproductive health have necessitated the study of tissue-specific epigenetic alterations in PCOS development. In semblance to follicular and oocyte defects observed in PCOS ovaries, targeted bisulfite sequencing was performed to generate the methylome signatures of ovarian granulosa cells (GCs) obtained from age-BMI matched women with PCOS (n=3) and healthy, regularly menstruating controls (n=3) using next generation sequencing approach. Paired end sequencing of samples was carried out on Illumina HiSeq 2500® platform and data were analyzed using the Bismark tool. Methylation levels of a few selected genes relevant to ovarian function were further validated in GCs obtained from 10 controls and 10 women with PCOS by pyrosequencing. Relative transcript levels of these genes were assessed by q-RT PCR using Taqman assays. In the methylome analysis, a total of 6486 CpG sites representing 3840 genes associated with pathways such as Wnt signaling, G-protein receptor signaling, angiogenesis, chemokine and cytokine mediated inflammation and integrin signaling showed differential methylation in PCOS. Of these, a total of 2977 CpG sites representing 2063 genes were identified as hypomethylated while 3509 CpG sites in 1777 genes were found to be hypermethylated. Additionally, differential methylation was also noted in several non-coding RNAs regulating vital ovarian functions and which are reported to be dysregulated in PCOS. This data provides compelling evidence in support of epigenetic alterations as etiopathogenic factors associated with ovarian dysfunction in PCOS.

Acknowledgements

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This abstract has been withdrawn by the author
Next-generation sequencing-based molecular diagnosis of chronic non-spherocytic hemolysis in erythrocytic enzymopathies

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Abstract

Mutations in genes encoding red blood cell enzymes are often inherited in an autosomal recessive manner and can lead to chronic nonspherocytic hemolytic anemia (CNSHA) in homozygotes and compound heterozygotes. Usual clinical manifestations include jaundice, cholelithiasis and splenomegaly with normocytic normochromic hemolysis. Phenotypes range from fully-compensated hemolysis (without anemia) to transfusion-dependent states. Definitive diagnosis requires biochemical testing of enzyme levels, which for rarer enzymes are often difficult and not easily available. Molecular diagnosis using a gene-by-gene approach is expensive, time-consuming and cumbersome. Targeted resequencing can expedite the molecular diagnosis in cases where the hemolysis remains unexplained after routine laboratory tests. Ten patients with clinical and laboratory evidence suggestive of hemolytic anemia, but negative family history, were enrolled. Various biochemical and molecular tests were used to exclude glucose-6-phosphate dehydrogenase (G6PD) deficiency, thalassemias, hemoglobinopathies, autoimmune hemolysis, hereditary spherocytosis and pyruvate kinase (PKLR) deficiency. Common G6PD and PKLR variants were excluded by molecular tests. DNA Libraries were prepared using TruSight One™ panel and sequenced on MiSeq™ Sequencing System. MiSeq Reporter™ and VariantStudio™ v2.1 were used for analysis, classification, and reporting of genomic variants reporting genomic variants. All 10 patients’ diagnoses were resolved by targeted resequencing: two had G6PD deficiency, two had glucose-6-phosphate isomerase (GPI) deficiency and six unexpectedly had pyruvate kinase deficiency despite pyruvate kinase enzyme activity assays previously being normal in all. All the mutations were predicted deleterious by PolyPhen, SIFT, Provean, mutpred and Mutationtaster software. The mutations were validated in parents and/or siblings (where available) to establish the mode of inheritance. Our data demonstrates the high clinical utility of next-generation sequencing for molecular diagnosis of CNSHA due to red cell enzymopathies. This is important as a molecular diagnosis aids genetic counselling and future antenatal diagnosis, and also streamlines management, avoiding unnecessary further investigations. Our results also caution that pyruvate kinase deficiency may be missed by conventional biochemical testing approaches.

First report of the mutational and phenotypic spectrum of Hereditary Spherocytosis in Indian patients by targeted resequencing

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Abstract

Hereditary spherocytosis (HS) is a common inherited hemolytic anemia characterized by the presence of microspherocytes. The pathogenesis involves defects in any of the several genes coding for membrane proteins that link the membrane skeleton to the overlying lipid bilayer. Membrane proteins include ankyrin, band 3, β- and α-spectrin and protein 4.2. We studied the molecular spectrum and genotype-phenotype correlation of HS in Indian patients.

Complete blood counts, incubated osmotic fragility test, antiglobulin test, eosin-5’ dye-binding test were done in 76 cases from 50 families to diagnose HS. RBC membrane ghosts were prepared and were analysed on gradient gels (4-12%). Relative quantification of mRNA isolated from enriched reticulocytes was done by qRT-PCR. cDNA sequencing of ANK1, SPTB, SLC4A1 and EPB42 genes were done (ABI3130). TruSight One™ sequencing panel was used for preparing libraries in 11 cases that were sequenced on Miseq™ (Illumina). MiSeq Reporter™ and Variant Studio™ were used for analysis, characterization and annotation of variants. Possible pathogenic variants were validated by Sanger sequencing in cases and family members. G6PD-deficiency, α-thalassemia and UGT1A1 polymorphism were studied as phenotype modifiers.

SDS-PAGE, qRT-PCR and cDNA sequencing were not contributory in deciphering molecular pathologies due to instability of mutated RNA and compensatory protein production by normal allele. NGS uncovered novel pathogenic mutations in ANK1 and SPTB out of which 30% were splice site, 30% were indels and 40% were nonsense mutations in 10 patients. Inheritance was non-dominant in 50% and autosomal dominant in 30% cases. G6PD Mediterranean variant in four HS patients led to greater transfusion requirements. Possible pathogenic variants were validated by Sanger sequencing in cases and family members. G6PD-deficiency, α-thalassemia and UGT1A1 polymorphism were studied as phenotype modifiers.

This first ever study on the molecular spectrum of HS from India revealed predominantly sporadic and dominantly-inherited defects in ANK1 and SPTB in patients. Most of the cases (70%) presented at an early age with jaundice and anemia. Co-inherited G6PD deficiency and Gilbert’s syndrome led to phenotypic variability. NGS provided a sensitive, cost-effective and rapid tool for understanding the molecular pathology of HS patients.

Category: Clinical Genomics

A homozygous $KLF1$ gene mutation presenting as mild Thalassemia Intermedia unraveled by targeted Next Generation Sequencing

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Abstract

The krupple-like factor 1 ($KLF1$) is a crucial transcription factor that is responsible for the proper maturation of the erythroid cells. Recent studies have demonstrated that mutations in $KLF1$ gene may lead to increased fetal hemoglobin ($HbF$) and reduced or borderline hemoglobin A2 ($HbA2$) levels. Increased $HbF$ levels and concomitant $\alpha$-thalassemia are two main modifiers that can ameliorate the clinical and hematological severity of $\beta$-thalassemia. Mutations in $KLF1$ have been found in association with $\beta$-thalassemia. DNA was extracted with QIAmp DNA Blood kit and quantified spectrophotometrically. Gap PCR was used to screen common HPFH deletions and Sanger’s sequencing was done to screen $\beta$-globin ($HBB$) mutations. Libraries were prepared using TruSight One sequencing panel and sequenced on MiSeq Sequencing System. MiSeq Reporter and Variant Studio were used for data analysis. A 56 years male presented with splenomegaly and unconjugated hyperbilirubinemia with normal hematological indices. Hemoglobin high performance liquid chromatography revealed 72.3% $HbF$, 0.5% $HbA_2$ and 25.2% $HbA_0$. Patient was found to be clinically consistent with mild TI. No mutation/s in $HBB$ was found by Sangers sequencing. Hereditary Persistence of Fetal Hemoglobin (HPFH) deletions [HPFH1, HPFH2, HPFH3, Chinese $\delta^\alpha$ deletion, Asian-Indian inversion-deletion] were also found to be negative. Targeted resequencing revealed a novel homozygous probably causative mutation in $KLF1$ [c. 943C>T (p.Arg301Cys)]. This mutation was found to be probably damaging via PolyPhen2 and SIFT. The patient’s son showed 5% $HbF$ with heterozygous mutation. This is the first report from India where a homozygous mutation in $KLF1$ gene is implicated with high $HbF$ in a patient with TI. Thus, mutations which affect the activity of $KLF1$ gene may lead to high level of fetal hemoglobin in patients presenting as TI with no $HBB$ mutations.

Transcriptome profiling reveals novel expression markers that predispose patients to develop post-photorefractive keratectomy corneal haze

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Abstract

Photorefractive keratectomy is an excimer laser [1] based ablation surgery of corneal surface used for correcting refractive errors. Corneal haze is the result of an aggressive wound healing response with an incidence rate [2] of 1.44% post PRK, making it an important health burden. Studies thus far have only focused on molecular alterations post haze development. Since the corneal epithelium is an important mediator of the stromal haze response, we studies its role in predisposing subjects to develop aberrant wound healing response. Corneal epithelium samples collected intra-operatively from clinically healthy patients during PRK. This epithelium from 6 eyes that developed haze postoperatively and 10 eyes of age matched controls without haze were compared. Gene expression microarrays were performed for the mRNA samples followed by ontological analysis of underlying molecular pathways. The identified targets were validated in an independent set of post haze epithelial samples from 3 subjects with PRK induced haze. In vitro studies were done on HCE cells for differential dose of TGFβ for inflammatory markers, corneal structure & fibrosis associated genes and regulators of signal transduction. In addition, loss and gain of function studies was performed using PREX1 as a novel, prototype target. Mean age of groups was 25-28 years. A total of 1100 up and 1700 down regulated genes were revealed by microarray. Alterations in Oxidative stress, ECM-Receptor interactions, Wnt signaling pathway and CXC motif containing chemokines contributes to cellular proliferation and wound healing, which is observed in in vitro model. In cornea novel target PREX1, an oxidative stress gene, when over expressed exhibits faster wound closure in HCE cells with and without TGFβ. Loss of function using PREX1 shRNA shows reduced wound closure. Our study shows that novel genes are involved in pathogenesis of post PRK haze. PREX1 over expression results in faster wound healing and modulating these pathways can prevent haze post PRK in future.

References


Citation: Nimisha, Shetty, R. and Ghosh, A. Transcriptome profiling reveals novel expression markers that predispose patients to develop post-photorefractive keratectomy corneal haze [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 86. https://doi.org/10.24870/cjb.2017-a73
Category: Clinical Genomics

Parental Consanguinity Among Schizophrenia Patients

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Abstract

Some studies have reported parental consanguinity as a risk factor for schizophrenia. These findings need replication in different socio-cultural settings. Hence we studied inbreeding to examine its effect on susceptibility to schizophrenia. A case-control study was conducted among people living in a rural community at Turuvekere (SZ, n = 120; controls, n = 222). The prevalence of consanguinity was estimated from family history data (‘self report’), followed by DNA analysis using SNPs (n = 384) (‘DNA-based’ rates) in order to add substantial reliability to our data. Self reported parental consanguinity was elevated among the patients (SZ: 10.71%, controls: 7.69%). Tests for normality of the DNA based estimates for coefficients of inbreeding ‘f’ showed that ‘f’ was not normally distributed. Mann-Whitney U test showed parental consanguinity rates are significantly elevated among the patients relative to the healthy individuals (p = 0.035). Our data suggest that schizophrenia is associated with higher parental consanguinity. Larger cross-sectional studies are warranted to validate our findings.

References


Citation: Agarwal, V., Thirthalli, J., Kumar, N.C. and Christopher, R. Parental Consanguinity Among Schizophrenia Patients [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 87. https://doi.org/10.24870/cjb.2017-a74
DNA sequence variation and determination of the putative \textit{PvCSP} gene as potential vaccine target for \textit{Plasmodium vivax} malaria in India

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Abstract

Evolutionary genetic studies in genomes offer excellent opportunities to infer population structure and demographic history of species populations. However, such kinds of studies are very limited in malaria. Malaria is highly endemic in India and both of the causative agents of malaria, \textit{Plasmodium vivax} and \textit{Plasmodium falciparum} occur in almost equal proportion. The widespread distribution of \textit{P. vivax} is attributing to socio-economic loss, and thereby increasing the public health concern. Therefore, it is important to understand the genetic features of \textit{P. vivax} population in India. Comparative genomics of \textit{P. falciparum} and \textit{P. vivax} has revealed several syntenic chromosomal segments. One such 200 kb segment has been utilized to design several small DNA fragments from non-coding regions, and tested for ‘putatively neutral’ marker for inference of population structure and demography of \textit{P. vivax}. Utilizing 126 \textit{P. vivax} isolates collected from 10 different widespread geographic locations in India, it was found that two neutral DNA fragments (P10 and P17) showed fairly less nucleotide diversity in all the population samples of \textit{P. vivax}. A sudden drop in diversity in putatively neutral genetic fragments indicates the role of positive natural selection under the hitchhiking model of molecular evolution. Evolutionary genetic studies in the regions surrounding P10 and P17 with functional validation might provide meaningful insights and help identify targets in \textit{P. vivax} in India. The study can further extended to \textit{P. falciparum} as it is syntenic to \textit{P. vivax}. It was found that the neutral fragment P17 is flanked by putative circumsporozoite protein (\textit{PvCSP}) gene. Since, CSP is a major surface protein of the infective stage of malaria parasite; it is believed that the \textit{PvCSP} gene might be under a certain kind of selective pressure. The objective of this study is to obtain new DNA sequence information of the putative \textit{PvCSP} gene in isolates from India and compare it with the estimated diversity of the non-coding DNA fragments located in-and around this gene for inference of natural selection. Effectiveness of putative \textit{PvCSP} gene as a suitable vaccine candidate on the basis of genetic diversity in parasite populations can be evaluated.

Citation: Dash, M., Das, A. and Sinha, A. DNA sequence variation and determination of the putative \textit{PvCSP} gene as potential vaccine target for \textit{Plasmodium vivax} malaria in India [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 88, https://doi.org/10.24870/cjb.2017-a75
Category: Clinical Genomics

**Association of common and rare genetic variants with cognition in schizophrenia**

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**Abstract**

Cognitive dysfunction is one of the core features in schizophrenia (SZ), which is a common neuro-psychiatric disorder affecting ~1% of the population globally. Cognitive impairment is related to social deficits with severity and breadth of these impairments varying across patients. Further, different cognitive domains seem to be dysfunctional in different patients. Severity of cognitive decline depends on the age of onset of SZ, with higher severity in early onset cases and generally manifests before the actual onset of psychosis. Though estimated heritability of cognition is ~50-70% little is known about its genetic basis. Of note, contemporary antipsychotic medication is also not effective in addressing this endophenotype. In this study we recruited SZ patients (n=158) from Dr. RML hospital, New Delhi and assessment of cognition was performed using Hindi version of University of Pennsylvania Computerized Neurocognitive Battery (Penn CNB). Eight selected cognitive domains namely abstraction and mental flexibility, attention, face memory, spatial memory, working memory, spatial ability, sensorimotor and emotional processing known to be impaired among patients with SZ were measured. Whole exome sequencing of the study cohort was performed and data were processed using standard tools and software. To identify variants/genes associated with cognitive domains, we performed two different levels of association testing: 1) linear regression analysis using the common variants (MAF>0.01) and eight different domains of cognition; and 2) gene-level tests for rare variant (MAF<0.01) association using burden tests (CMC, CMC-Wald & Zeggini). We identified 11 common variants associated (P<10⁻⁷) with different cognitive domains, which withstood Bonferroni corrections and rare variant burden analysis identified four genes associated (P<10⁻⁷). Genes identified by these two approaches and their implications for cognitive deficits will be presented.

**Citation:** John, J., Kukshal, P., Bhatia, T., Deshpande, S.N., Nimgaonkar, V.L. and Thelma, B.K.. Association of common and rare genetic variants with cognition in schizophrenia [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 89. https://doi.org/10.24870/cjb.2017-a76
Pseudoexfoliation and Alzheimer’s associated CLU risk variant, rs2279590 lies within an enhancer element and regulates CLU, EPHX2 and PTK2B gene expression

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Abstract

Pseudoexfoliation (PEX) is an age related ocular disorder characterized by deposition of protein aggregates on the surface of anterior eye tissues. Advanced stage of PEX is called as pseudoexfoliation glaucoma (PEXG) which leads to gradual degeneration of optic nerve and loss of vision compared to that of less severe stage called pseudoexfoliation syndrome (PEXS). PEXG is the leading contributor of secondary glaucoma worldwide. It shares similar pathological alterations with Alzheimer’s disease (AD) with characteristic deposition of fibrilar protein aggregates and gradual deterioration of nerves with age. Studies done in the past suggest a prominent genetic factor underlying the pathogenesis of PEX. Here, we examined the role of two genetic variants (rs3087554 and rs2279590) within the gene clusterin (CLU) as risk factor in the pathogenesis of PEX by performing a case-control study in Indian population. Through, bidirectional sequencing and genetic analysis, both of the variants were found to be significantly associated with PEX. Further functional analysis was carried out for the 7th intronic SNP rs2279590 which previously has been picked as a risk factor for AD. In silico analysis suggests rs2279590 resides in an active regulatory region and is an eQTL for CLU gene expression from the data in ENCODE and GTEx project, respectively. Alleles at rs2279590 were shown to differentially regulate CLU expression in lens capsule tissues. Reporter assays show that rs2279590 is within an active enhancer element and 3C assays reveal a promoter-enhancer interaction mediated by CLU promoter and rs2279590 loci. Deletion of 115bp region flanking the rs2279590 variant through CRISPR-Cas9 demonstrated a decreased CLU expression. Molecular assays show that rs2279590 with allele “A” constitutes a transcription factor binding site for heat shock factor-1 (HSF1) but not with allele “G”. After binding, HSF1 abrogates the enhancer effect of the locus as validated by reporter assays. Interestingly, rs2279590 locus also has a widespread enhancer effect on two nearby genes, PTK2B and EPHX2; both of which are risk factors for AD. Together, our study unveils a mechanistic role of the common variant rs2279590 that can affect both PEX and AD by regulating the expression of a specific set of genes.

Targeted next generation sequencing identifies novel mutations in Indian patients with retinal dystrophies

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Abstract

Retinal dystrophies (RD) are a group of inherited ocular disorders of the retina causing blindness in more than 2 million people worldwide. RDs are characterized by clinical variability and progressive vision loss. It is associated with high degree of genetic heterogeneity. In order to correlate RDs clinically and genetically and to develop novel therapeutic approaches, genetic testing is of utmost importance. Prior requisite of a genetic test is genetic counselling. The proband and family members underwent genetic counselling including a detailed family history. Pre-test education was vital to help these families understand the importance of genetic test for the proband and validation of the report by testing the parents/siblings blood samples to confirm the genetic mutation. We performed targeted next-generation sequencing (NGS) in clinically confirmed 21 unrelated patients who showed different forms of RD and validated in their family members using panel comprising 184 genes, which covered previously associated genes with retinal disease. The sequencing analysis revealed a total of 21 different mutations in patients with RDs including Leber’s Congenital Amaurosis, Cone-Rod dystrophy, Retinitis Pigmentosa, Achromatopsia and Stargardt disease. Among these, seven mutations were unreported and fourteen variants were reported. We found five novel mutations with existing spectrum of gene mutations identified in Indian patients with the characteristic features of RDs. The knowledge of the pathogenic gene mutation in the affected family member was used to correlate with the proband’s clinical diagnosis, to screen other family members suspected of having similar symptoms and also for carrier testing. In some cases of retinal dystrophy with overlapping clinical symptoms, the genetic report was used to confirm the RD. Post-test genetic counselling was done to discuss the implications of the genetic mutation on the prognosis and management of the RD.

Category: Clinical Genomics

High-throughput genetic analysis in a cohort of patients with Ocular Developmental Anomalies

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Abstract

Anophthalmia and microphthalmia (A/M) are developmental ocular malformations in which the eye fails to form or is smaller than normal with both genetic and environmental etiology. Microphthalmia is often associated with additional ocular anomalies, most commonly coloboma or cataract [1, 2]. A/M has a combined incidence between 1-3.2 cases per 10,000 live births in Caucasians [3, 4]. The spectrum of genetic abnormalities (chromosomal and molecular) associated with these ocular developmental defects are being investigated in the current study. A detailed pedigree analysis and ophthalmic examination have been documented for the enrolled patients followed by blood collection and DNA extraction. The strategies for genetic analysis included chromosomal analysis by conventional and array based (affymetrix cytoscan HD array) methods, targeted re-sequencing of the candidate genes and whole exome sequencing (WES) in Illumina HiSEQ 2500. WES was done in families excluded for mutations in candidate genes. Twenty four samples (Microphthalmia (M)-5, Anophthalmia (A)-7,Coloboma-2, M&A-1, microphthalmia and coloboma / other ocular features-9) were initially analyzed using conventional Geimsa Trypsin Geimsa banding of which 4 samples revealed gross chromosomal aberrations (deletions in 3q26.3-28, 11p13 (N=2) and 11q23 regions). Targeted re sequencing of candidate genes showed mutations in CHX10, PAX6, FOXE3, ABCB6 and SHH genes in 6 samples. High throughput array based chromosomal analysis revealed aberrations in 4 samples (17q21dup (n=2), 8p11del (n=2)). Overall, genetic alterations in known candidate genes are seen in 50% of the study subjects. Whole exome sequencing was performed in samples that were excluded for mutations in candidate genes and the results are discussed.

References


Category: Clinical Genomics

CMV genotyping using different samples in post renal transplant recipients with CMV disease

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Abstract

CMV is the most common viral infection which occurs in post renal transplant recipients (PTR). There are four different gB genotypes (gB1 to gB4) which exist in CMV. Studies have reported that mixed infection with different genotypes will cause severe clinical manifestations as well as co-infection with other herpesvirus including Epstein-Barr virus (EBV) [1]. CMV can cause compartmentalized disease involving different organs with different genotypes. There are reports in immuno compromised individuals with different genotypes [2, 3]. Institutional ethics committee approval was obtained prior to conduct of the study (IEC-NI/08/DEC/07/46). Whole blood, saliva and urine were collected from PTR. DNA were extracted (Qiagen DNA mini kit) and CMV quantitative PCR targeting ppUL83 gene was performed with CMV R-gene™ using an ABI 7900 Fast real time PCR (SDS Version: 2.4). PTR who had high viral load (>1000 copies/ml) in any three or two samples were included for CMV genotyping PCR targeting gB region (410-bp) [2]. DNA sequencing was performed in ABI 3730 GA platform by Sanger method and sequences were analysed by reference strains. A total of 24 samples were collected from 9 PTR. Among these four PTR had high viral load in all three samples (whole blood, urine & saliva) and those with high viral load (n=5) in 2 samples (Whole blood & urine/saliva) were screened for CMV genotyping. Majority of the strains belonged to genotype B1 and only one PTR was infected with genotype B2 in three samples. In PTR with genotype B1, gastro intestinal infection (GI) was predominantly found in 78% (n=7) followed by graft dysfunction (GDF) in 56% (n=5) of the PTR. PTR who detected with genotype B2 was associated with fever, leukopenia (CMV syndrome), GDF and also found with EBV infection. Co-infection with EBV was observed in 44% (n=4); VZV and HSV type 1 was also observed. Genotypes are associated with the severity of the disease and co-infection with other herpes virus infections. In our study subjects, genotype B1 predominantly noted as reported in western countries. Study on distribution of genotypes among PTR may help to determine the specific strains for vaccine development.

References


Citation: Barani, R., Mani, M., Sarangan, G., Soundararajan, P., Palani, G. and Srikanth, P. CMV genotyping using different samples in post renal transplant recipients with CMV disease [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 93. https://doi.org/10.24870/cjb.2017-a80
Category: Clinical Genomics

Damaging stop gain/loss and frameshift mutations in autism subjects outline impairment in neuronal migration and adhesion pathways

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Abstract

Genetic heterogeneity makes it challenging to identify causal-genes responsible for autism pathogenesis. Till date, research studies report only a handful of high confidence genes for autism. There is a need to identify damaging genomic-variants, predisposing an individual towards autism manifestation. Of special interest is stop gain/loss mutations found in the exome. Such variants are prevalent, having an estimated number of 100-200 occurrences per human-genome. Stop-gains and frameshifts may lead to functional consequences. Based on stringent inclusion-exclusion criteria, the study recruited 150 autism subjects of Indian origin, of which 13 were used for WES. To understand the nature and possible consequences of these variants, we first analyzed their characteristics at the genome-level. Genome-wide analysis of more than 30000 variants provided statistical-significance to identify sequence-specific features for severity and to build a pathogenicity score. This sequence-based pathogenicity score was then applied to the analysis of variants in autism susceptibility. Several damaging stop gain/loss mutations encompassing autism genes CDH5, DDX23, CLDN5, and DPP3 were identified with protein truncations ranging from 20-70%. Loss of function mutations disrupted protein domains involved in various autism related pathways such as neuronal migration, synaptogenesis, and neuronal adhesion. Mutations were identified with previous evidences for neuronal migration and adhesion pathways in Drosophila sp, C. elegans and mice models. Homozygosity mapping analysis to identify risk-homozygous-haplotypes showed evidence of recessive polymorphisms in GIGYF1, SERPINE1, and EPHB6. Recessive alleles were identified across all the samples while polymorphisms in FOLH1, BCKDK, CDH11, and CTCF were specific. Mutations in language-specific genes, GCFC1 and MRPL19 were associated with autism phenome. A novel autism candidate gene CLDN5 that physically interacts with genes involved in various autism pathways was identified. CLDN5 belongs to the leukocyte-transendothelial-migration pathway and elevated in autism cortex, impairing the blood brain barrier leading to compensatory gene expression and protein accumulation. This on-going study identified several damaging mutations specific for autism in Indian population, adding to the growing body of mutational spectrum. Validations through Sanger sequencing and allele specific PCR is being done for the mutations identified.

Category: Clinical Genomics

Unravelling Mitochondrial Dysfunction in Rheumatoid Arthritis patients

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Abstract

Rheumatoid arthritis (RA) is a chronic, inflammatory, autoimmune disease associated with systemic, extra-articular and articular effects, causing permanent disability, early morbidity; making the patient compromised with a worldwide prevalence of 0.8%, commonly effecting women with a rate of 0.7% in India. With improved and developing therapeutics, this disease needs special focus for improved diagnosis and better treatment. The hyperactivity of immune cells is responsible for pathogenesis and progression of the disease. This study unravels the changes in mitochondria of RA patients which may be a potential reason for abnormal functioning of immune cells against self-antigens and occurrence of the disease. In this study we examine the following aspects of mitochondrial functions in the peripheral blood mononuclear cells (PBMCs) of patients and their paired control samples: 1) Change in mitochondrial membrane potential (MMP); 2) mitochondrial mass; 3) mitochondrial superoxide and 4) ATP levels. Patients satisfying the 2010 ACR/EULAR classification criteria for RA diagnosis were enrolled in this study. PBMCs of RA patients and controls were collected by differential gradient centrifugation. MMP, mass and superoxide levels were measured using respective commercially available dye using flow cytometry. ATP levels were measured by lysing equal number of cells from patients and controls using ATP measurement kit. In our case control cohort, we found a significant decrease in MMP (p<0.005) in PBMCs of RA patients where the change in mitochondrial mass was insignificant. The mitochondrial superoxide levels were found to be significantly low (p<0.005) in PBMCs of RA patients where the decrease in total cellular ATP was insignificant. Reduced potential will disturb proper functioning of mitochondria in PBMCs which may affect most important function of mitochondria to produce ATP and various other functions. Results depict dysfunction in basic mitochondrial activities which may be a reason for abrupt functioning of immune cells, leading to autoimmunity in RA patients.

Citation: Khanna, S., Tripathy, A., Padhan, P. and Gupta, B. Unravelling Mitochondrial Dysfunction in Rheumatoid Arthritis patients [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 95. https://doi.org/10.24870/cjb.2017-a82
Category: Clinical Genomics

**Network level analysis of Aging and Alzheimer’s disease**

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**Abstract**

Alzheimer’s disease (AD) is a neurodegenerative disorder affecting the memory and cognitive functions in the aged population. The hallmarks of AD include accumulation of amyloid plaques, and neurofibrillary tangles (NFT) in the brain, and neuroinflammation leading to synaptic dysfunction, alterations in energy metabolism and apoptosis. Although genomic studies on AD have been performed extensively, the molecular mechanism of disease progression is still not clear. One possible reason might be the interaction of age and disease in the progression of AD.

To understand the contribution of aging and disease in the progression of AD, we adopted the network level approach to analyze the transcriptomic data obtained from the human postmortem brain tissues. We have performed gene co-expression network analysis and Knowledge-based (Integrated-PPI) network analysis involving 3 groups: young (<50 years), aged (>70 years) and AD (>70 years with AD). Co-expression network analysis identified modules/processes related to the phenotype (aging and disease). Both aging and disease are associated with increase in inflammation and its related processes involving the activation of microglia and reactive astrocytes. The significant differences in aging and disease are related to cytoskeleton remodelling, loss of synaptic transmission and oxidative phosphorylation. Further, the expression data was integrated with PPI network and various graph theoretical network measures were computed. Using edge betweenness network measure; we identified the significant unstable/active subnetworks and their hub genes. This study identifies the molecular mechanism that protects the aging brain from AD and that makes it susceptible to AD.

**References**


**Citation:** Lanke, V., Moolamalla, S. and Vinod, P.K. Network level analysis of Aging and Alzheimer’s disease [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 96. [https://doi.org/10.24870/cjb.2017-a83](https://doi.org/10.24870/cjb.2017-a83)
Do Mitochondria have hidden answer for RA aetiology?

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Abstract

Background: Rheumatoid arthritis (RA) is a systemic, debilitating and chronic inflammatory disorder affects 1% of the total world population. Due to absence of a defined aetiology and associated side effects of conventional medication has made obtaining permanent cure a challenge. Finding “one for all” cure is difficult due to genetic variations among populations. Deciphering genetic information, difference in regulatory network and epigenetic changes between populations can lead us to design a cure based on the principles of personalized medicine. Mitochondrial dysfunction, being a significant player in many autoimmune diseases, has also been accused for RA. Nucleotide changes can result in amino acid change, deletion or addition in enhancer, transcription factor or translation factor binding sites hampering overall function of mitochondria.

Methods: Mitochondrial DNA from peripheral blood mononuclear cells was accused as primary culprit and its genomic information was assessed through next generation sequencing (NGS) for both healthy controls and RA patients. NGS data were compared with rCRS (revised Cambridge Reference Sequence) and Indian genomic sequences to find out any variations, polymorphisms (SNPs) and heteroplasmies involved. Further, qPCR was performed to check the behaviour of several mitochondrial genes in case of both healthy controls and RA patient samples.

Results: Careful evaluation of NGS data confirmed presence of several SNPs in mitochondrial genes especially involved in OXPHOS system. Several subunits of NADH dehydrogenase and ATP synthase were found to be altered in case of RA. Similarly, expression profiles of mitochondrial genes were found to be different in RA samples when compared with healthy controls.

Conclusion: Initial investigation into mitochondrial genome confirms our suspicion of involvement of mitochondrial dysfunction in RA.

Category: Clinical Genomics

Mutations in Hepatitis B virus polymerase gene/partial surface gene among Chronic HBV carriers as markers for anti-viral drug resistance and escape mutants

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Abstract

Resistance to anti-viral drugs is a global problem in the treatment of HBV. Around 350 million people are infected with HBV worldwide. In India there are 50 million chronic HBV carriers [1]. HBV is double stranded DNA virus, it replicates by reverse transcription process. The error rate of HBV reverse transcriptase is of 4.6x10⁻⁵/nucleotide/site/year [1]. This results in the emergence of mutations in HBV genome. There are 10 genotypes and 4 subtypes [2, 4]. The treatment and disease progression is genotype specific [3]. The objective of the study was to identify mutations in HBV pol gene and HBs gene and their impact on disease and diagnosis. Individuals positive for HBsAg by ELISA with HBV viral load more than 2000 IU/ml were recruited in the study (n=32). Blood samples were collected from 32 individuals after obtaining written informed consent. DNA was extracted from the plasma samples (Qiagen, Hilden, Germany). Conventional PCR targeting reverse transcriptase and surface gene (partial) was performed [4]. DNA sequencing (1300 bp) was performed on ABI 3730 GA platform (Applied Biosystems, USA). The sequences were analyzed for drug resistance using HBV geno2pheno drug resistance tool [1, 6] (http://hbv.gen2pheno.org/). Mean age of the study subjects was 46.8 ± 14.1. Males (n=22) were predominant than female (n=10). The median ALT level was 45 U/L. HBe Antigen was found to be positive in 65% (n=21) and negative in 35% (n=11). Genotype D (68.7%) was most predominant followed by genotype A (18.7%) and genotype C (15.6%). The rtL180M and rtM204V lamivudine, entecavir and telbivudine refractory mutation was noticed in one individual. Compensatory mutation rt169V was found in one individual. Several minor mutations were detected in which 5 participants belonged to genotype D had substitutions in p gene hotspots including rt169, rt173, rt180, rt184, rt202, rt204, rt236, rt250. Recently, there are changes in the treatment of chronic HBV disease. However, emergence of mutations in HBV is increasingly documented. Understanding the viral mutations and their associations with clinical presentations will assist in the customized patient care.

References


Category: Clinical Genomics

A comparative study between infectious and systemic inflammation

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Abstract

Activation of innate immune system may occur as a result of either external (mostly infection-mediated inflammation) or internal factors (systemic inflammation). Distinct stimuli act on the immune cells to induce diverse pathways leading to characteristic gene expressions in these cases. Bacterial inflammation, caused primarily by its lipopolysaccharides (LPS), conceives an array of diseases including intestinal bowel disease (IBD), ulcerative colitis and sepsis. In contrast, release of pro-inflammatory cytokines such as IL-6 or TNF-\(\alpha\) leads to chronic inflammatory diseases, for example, rheumatoid arthritis (RA), juvenile idiopathic arthritis, Castleman’s disease, etc. It is important to understand the signatures of infectious and systemic gene expression for better designing of treatment regime against inflammatory diseases. To understand the distinctive pattern of gene expression between infectious inflammation and systemic inflammation, THP-1 macrophages were treated individually with LPS (100 ng/mL), IL-6 (50 ng/mL) or TNF-\(\alpha\) (10 ng/mL) and global transcriptomic analysis was performed using Agilent’s human 8x15K array. The common set of differentially expressed genes in IL-6 and TNF-\(\alpha\)-treated cohorts were compared with LPS-treated cohorts. Our analysis revealed that 2743 and 150 genes contributed to LPS-mediated inflammation and systemic inflammation with respect to untreated samples, respectively (fold change \(\geq 1.5\)). 868 commonly expressed genes contributed to systemic inflammation with respect to LPS-mediated inflammation. Among these commonly expressed genes, only 68 genes were observed to contribute to both types of inflammation, suggesting their importance in activation of diverse pathways in LPS-mediated and systemic inflammation. A detailed functional annotation of these genes revealed that EGR1, JUN, NF-\(\kappa\)B, REL, STAT-1 and BCL-3 are important transcription factors (TFs) for distinctive signatures between these two types of inflammation. In addition, these TFs were found to be involved in innate immune response. Further investigation into the gene expression dataset from rheumatoid arthritis patients (treated with anti-TNF-\(\alpha\) antibody) revealed 24 genes which are present within these 68 genes having an inverse mode of expression. This observation suggests the importance of these 24 genes for designing novel therapeutic targets.

Resolving the conflict of mating versus blood feeding: exploring role of *quick-to-court* gene in the mosquito *Anopheles culicifacies*

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Abstract

Mosquitoes are the deadliest animal in the world. Mosquitoes transmit several vector borne disease (VBDs) such as malaria, dengue, chikungunya, zika fever, yellow fever and responsible for a loss of millions of lives annually. Though, suppression of mosquito population by means of chemical insecticides plays a crucial role in controlling vector population. However, fast emergence of insecticide resistance limits the efforts and demanding to design alternative molecular tools to fight against these VBDs. One of the potential strategies may include interfering complex feeding and/or mating behavioural properties. Compared to female mosquito male mosquito have an indirect effect in disease transmission and thus least studied. Males induce several post-mating behavioural changes in females, including the induction of host seeking and blood feeding behavior. Although, a successful mating events are guided by non-genetic circadian rhythm, but how genetic factors manages the sequential events of swarm formation, suitable mate finding and aerial coupling remains poorly investigated. While understanding the complex feeding behaviour of adult *An. culicifacies* female mosquito, we identified and analyzed a unique transcript (383 bp) from the olfactory system of the blood-fed mosquito, encoding the *’quick to court’* (QTC) protein. It is a homolog of *Drosophila* coiled-coil QTC (Q9VMU5) protein and shown to play an important role in driving the male courtship behaviour. A comprehensive *in silico* analysis predicted a 1536 bp long transcript encoding 511 AA long protein in the mosquito genome. Age dependent and sex specific transcriptional profiling revealed that both male female mosquitoes attain the specific age of adulteration on 5-7 days. Circadian clock dependent *Ac-qtc* profiling indicated that late evening natural dysregulation of *Ac-qtc* by unknown mechanism may promote the successful insemination event during active copulation. Together, our findings provide first molecular evidence that *Ac-QTC* proteins may have dual mode of action in the regulation of cluster of mosquito olfactory genes that are linked to mating success and/or blood feeding in adult female mosquitoes. A sex specific and circadian rhythm dependent comparative RNAseq analysis of neuro-olfactory and reproductive organs may facilitate to identify key molecular factors, regulating complex events of mating behavior in the mosquitoes.

Differential effects of genetic - and diet - induced obesity on fertility, spermatogenesis and sperm epigenome in adult male rats

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Abstract

Obesity is a global health issue affecting millions of people of different age groups. The incidence of male obesity induced infertility is rising in couples undergoing ARTs suggesting that obesity is an established risk factor for male infertility. Recent studies demonstrate that paternal diet induced obesity could induce epigenetic disturbances in offspring. Obesity is a multifactorial disorder with predominantly genetic or environmental causes. No studies have compared the effect of genetic and diet induced obesity on male reproduction. The present study aims to delineate effects of obesity on male fertility, spermatogenesis and sperm epigenome using two rat models: genetically induced obese (GIO) – WNIN/OB and diet induced obese (DIO) – High fat diet. Body weights were similar in both groups, but, differential effects on hormonal profiles were observed. Fertility assessment showed decreased litter size mainly due to increased pre- and post-implantation loss in DIO group. However, GIO group were infertile due to decrease in libido. We observed a decrease in sperm counts in GIO group but not in DIO group despite the body weights being similar in both the groups. Flow cytometry and cell type specific marker expression studies in testis revealed that both DIO and GIO affect mitosis and differentiation process by increasing spermatogonial proliferation. In DIO group, no effect was observed on meiosis whereas in GIO group, we observed an effect on meiosis. Spermiogenesis process was affected in both the groups. In order to study the effect of genetic and diet induced obesity on different aspects of spermatogenesis, we performed qRT-PCR to study expression of genes involved in spermatocyte progression, spermiogenesis process, reproductive hormone receptors and leptin signaling in testis. Since epigenetic mechanisms are susceptible to environmental and genetic changes, we analyzed the methylation status of Igf2-H19 DMR in spermatozoa of both the groups by pyrosequencing and observed hypomethylation in GIO group; however, no changes observed in DIO group. Differential effects were observed in both DIO and GIO group. Our study demonstrates the differences in the effects of genetic and diet induced obesity on the male germ line suggesting that human obesity induced subfertility/infertility could be a combination of both environmental and genetic factors.

Category: Functional Genomics

Hydrogen sulfide modulates basal metabolic circuitry – A transcriptome sequencing assisted insight

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Abstract

Hydrogen Sulfide (H$_2$S), the third gasomessenger to be discovered after nitric oxide (NO) and carbon monoxide (CO), is known for its distinctive health promoting effects on various organ systems, including cardiovascular system. This molecule has now qualified as an authentic mediator of specific cellular signal transduction pathways [1, 2]. The mechanistic insight into its cytoprotective role however remains incompletely understood. To understand the molecular circuitry regulated by H$_2$S augmentation, we utilized an exogenous donor of H$_2$S, Sodium hydrogen sulfide (NaHS), and performed unbiased global transcriptome sequencing (Illumina) in cardiac cells (H9c2 cardiomyoblasts). These experiments yielded differential transcriptome of the cells with varying levels of H$_2$S (with or without NaHS treatment for 6 hrs). We subjected this dataset to multiple pathway mining tools, including gene ontology analysis, functional annotation clustering and co-expression network analysis, to infer biological themes hidden as concerted differential gene expression signatures [3]. We, interestingly, observed common biological processes in different analysis strategies, suggesting authentic, conserved nature of cellular response to H$_2$S. Biological networks, largely associated with metabolic/ redox processes were recognized; within three gross themes steroid/ isoprenoid biosynthesis, oxidoreductase coenzyme metabolism (representing pentose phosphate pathway, PPP) and glutathione metabolism. Glucose-6-phosphate dehydrogenase (G6PD) rate-limiting enzyme within PPP stood as the highest degree node in majority of these networks. Also, genes related to oxidative stress and redox signaling were enriched. Interestingly, these pathways appear to be centrally linked by nicotinamide nucleotide cofactor (NADPH) homeostasis. We further supported this proposition at functional level by performing various enzyme activities besides recording cellular NADP/NADPH and GSH levels in established cellular as well as rat model system. In summary, our data suggested profound influence of H$_2$S on integrated cellular metabolic circuitry modulating redox homeostasis in cardiac cells.

References


Citation: Chhabra, A., Bhargava, K. and Sharma, M. Hydrogen sulfide modulates basal metabolic circuitry – A transcriptome sequencing assisted insight [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 103. https://doi.org/10.24870/cjb.2017-a89
Category: Functional Genomics

Inflammation induced insulin resistance is associated with DNA methylation changes in vascular endothelial cells

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Abstract

Vascular insulin resistance manifests in decreased production of nitric oxide subsequently leading to vasoconstriction and atherosclerosis. Inflammatory molecules such as IL-6 are known to induce insulin resistance in vascular endothelial cells. Epigenetic mechanisms including promoter DNA methylation have been demonstrated in development and progression of metabolic disorders and atherosclerosis. However, precise and underlying epigenetic mechanisms governing vascular insulin resistance are not known. Human endothelial cells treated with a) IL-6 and insulin together, b) pretreated with IL-6 and c) hyperinsulinemic conditions induced vascular insulin resistance leading to decreased Akt/eNOS activation and subsequently stabilized STAT3 phosphorylation. In 3D spheroid and matrigel assays, IL-6 abrogated insulin effects on angiogenesis. IL-6 induced insulin resistance was associated with down regulation of DNMT1 and DNMT3B, but not DNMT3A and resulted in decreased enzyme activity leading to global DNA hypomethylation. Protein levels of DNMT1 and DNMT3B were inversely correlated with S-phase of cell cycle. CpG microarray analysis revealed hypomethylation of promoters associated with 199 genes and promoter hypermethylation of 98 genes. Further, methylation status of promoters of genes associated with insulin signaling and angiogenesis such as RPS6KA2, PI3KR2, FoxD3, Exoc7, MAP3K8, ITPKB, EPHA6, IGF1R and FOXC2 were validated by bisulfite sequencing. Differentially methylated CpG sites of four out of five hypomethylated genes harboured putative binding site for HMGB1, a potent inflammatory mediator, suggesting HMGB1 might serve as epigenetic switch facilitating a pro-inflammatory milieu in response to IL-6 in endothelial cells. Our data indicates causal link between IL-6 induced DNMT1 changes and altered gene expression involved in insulin signaling and angiogenesis.

Citation: Balakrishnan, A., Satyamoorthy, K. and Joshi, M.B. Inflammation induced insulin resistance is associated with DNA methylation changes in vascular endothelial cells [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 104. https://doi.org/10.24870/cjb.2017-a90
How cyanobacterial signalling system behaves in different media component with time? – a Genomics and Transcriptomics approach

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Abstract

In this study, we are aiming at deciphering the physiological and molecular mechanisms behind cyanobacterial IQ system i.e. the putative signalling component proteins [1] in Mastigocladus laminosus using a reverse genetics approach. Polymorphic Mastigocladus laminosus belongs to one of the least studied but most evolved family of cyanobacteria, the Stigonemataceae. Its unique properties to withstand extreme environmental conditions and economic value make it a suitable candidate for genomics and transcriptomics studies. Whole genome sequencing of M. laminosus was carried out using Illumina MiSeq. Good quality paired end and mate pair library data were assembled into a draft genome. Annotation showed that a major portion of genes are part of several critical pathways such as two component regulatory system, ABC transporters, etc. We set up pilot experiments for finding suitable time points for checking the fatty acid production differential in strains growing in nitrogen supplemented and nitrogen depleted media. In both the conditions, between 0th day and 12th day the fatty acid production difference was the maximum. Taking the cue from this, we performed the transcriptome experiment. Results show that among the differentially expressed genes, the signalling genes of two-component systems are the predominant class. Chemotaxis family two component hybrid kinases are found in a cluster within the genome. Also several other non-ribosomal peptide synthetases /polyketide synthetases classes were the second most predominant class showing a promise of becoming a source of novel secondary metabolite production. Several heat shock protein coding genes have been identified. Phosphate and molybdate ABC transporter expression were upregulated with respect to time but not affected from the presence or absence of nitrogen in media. Our future work will include characterizing the finer details of the significant genes through pathway analysis and exploring the metabolome. From preliminary analysis of the genome and transcriptome of M. laminosus, it is evident that cyanobacteria have evolved to sense unfavourable condition by their huge interactive network of regulatory components and survived by producing compounds that are mostly unexplored yet from the earliest period of this planet.

References


Ameliorating potential of Equisetum arvense against the Cyclophosphamide induced genotoxic damage in mice

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Abstract

Medicinal plants have always been on the vanguard whether regarding the treatment of a number of ailments or even cancer. It has been suggested that the use of antimutagens/anticarcinogens in everyday life can be the most effective way to avert human cancer and genetic diseases. Equisetum arvense, commonly known as the field horsetail or common horsetail (Sehetband or Brahmgund locally in Kashmir), is a very common, bushy perennial herb native to the northern hemisphere and rich in secondary metabolites. In the present study, we evaluated the potential of the plant E. arvense against the cytotoxic and mutagenic effects induced by cyclophosphamide (chemotherapeutic agent) in the bone marrow cells of mice using the Chromosome assay (CA) and Mitotic index (MI) in vivo as the biomarkers. E. arvense was subjected to extraction with hexane, ethanol and aqueous solvents. Screening for antimutagenic activity was carried out using albino mice as the model organism. Toxicological study was performed following 3 protocols: pre-treatment, simultaneous treatment and post-treatment with the three extracts of the plant and the mutagen. In order to find out the phytocomponents responsible for showing the highest antimutagenic activity, phytochemical analysis was also carried out using GC-MS. The present study was focused on evaluating the mutagenic/antimutagenic potential of the plant Equisetum arvense which exhibited potent antimutagenic activity against the cyclophosphamide induced mutations. Chromosomal aberrations and mitotic index were used as biomarkers to assess the mutations. In the present study mice treated with CPA showed significant increase in aberrant metaphases, CAs (including and excluding gaps), while decreased cellular proliferation rate (MI) compared to the control group. The plant extracts were not cytotoxic or mutagenic to the animal. The highest antimutagenic activity (98%) was shown by the ethanolic extract. The analysis of the effect of extracts on the cytotoxicity induced by CPA showed a significant improvement. The efficacy of present chemotherapeutics has been limited by its toxicity and for the cells developing resistance against the therapy. Because of its ability to prevent chromosomal damage, E. arvense is likely to open an interesting field concerning its possible use in clinical applications, most importantly in cancer as a chemopreventive agent or even as a coadjuvant to chemotherapy to reduce the side effects associated with it.

Category: Functional Genomics

Classification of enhancer promoter interaction pairs based on expression patterns and distances involved in disease manifestation in human

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Abstract

Enhancers—the non-coding regions of genomes—are responsible for regulation of transcription of interacting genes. In different cell lines different regions act as enhancers. Enhancer-promoter interaction (EPI) models suggest that enhancer helps in the assembly of transcription factors along with RNA polymerase II and interacts with promoters to increase the expression of corresponding genes. During transcription, enhancer itself undergoes transcription giving rise to small RNAs, known as enhancer RNA. Presence of Transcription Start Sites (TSS) in annotated enhancer regions is also defined as active enhancers. Three different human cell-lines namely, Gm12878, K562 and H1-hesc which are normal, cancerous and stem cell-lines respectively were studied. K-medoids algorithm was used to segregate EPI in all the cell-lines. Three clusters were derived on the basis of expression of enhancer, expression of their interacting promoters and distance between the two. Statistical t-test analysis showed that all clusters were different from each other. Cluster-1 (expression of enhancer Mean (eeMean) =59.12, Median (eeMedian) =12.09) and cluster-2 (eeMean=1799.9, eeMedian=1468) differ from each other on the basis of enhancer’s expression. Cluster-2 (distance mean=20521, eeMean=1799.9; distance median=7984, eeMedian=1468.5) was different from cluster-3 (distance mean=180798, distance median=162626) on the basis of distance and the expression of TSS at enhancer. Finally cluster-1 (distance mean=18030, distance median=6966) and cluster-3 (distance mean=180798, distance median=162626) differ from each other on the basis of distance. RNAseq analysis showed 7 upregulated genes in K562 compared to Gm12878. Further, EPI distributions of MYC, RAD23B and Insulin like growth factors showed similar pattern in K562 and H1hesc, and they were present in cluster-1. Whereas EPI of MDN1, CDKN1C, and eukaryotic translation elongation factor2 in K562 were present in cluster-1 and EPI of H1hesc were present in cluster-1 and cluster-3. EPI of Erythrocyte membrane protein were segregated into cluster-1 and cluster-3 for both K562 and H1hesc, whereas all of these interactions were absent in Gm12878. Overall these results suggest that enhancer activities are mainly responsible for carcinogenesis in K562 cell-lines otherwise absent in normal cell-lines.

Gene expression program of regeneration in *Eisenia fetida*: a transcriptomics study

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**Abstract**

Annelids form a connecting link between segmented and non-segmented organisms. In other words, phylogenetically, the segmented body pattern starts from Annelida, a phylum that consists of thousands of species, including marine worms, freshwater leeches and earthworms that inhabit deep layers of soil to environmental niches in forests and cultivated land. We are using *Eisenia fetida* (Indian isolate) as a top dwelling, vermicomposting worm due to its ability to regenerate its posterior after damage, injury or complete removal. On average, *Eisenia fetida* has 100-110 segments. We separated the anterior (upto 55-60\textsuperscript{th} segment) and posterior of the worm, and allowed it to regenerate. In this model, only the posterior could be regenerated after injury. We isolated RNA from the regenerated tissue and the immediate adjacent old tissue at 15 days, 20 days and 30 days during regeneration. We carried out transcriptome sequencing and analysis. With the aim of identifying specific factors which promote nerve regeneration, we have annotated the differentially expressed genes. In all organisms which possess a segmented body, the expression pattern of the Hox cluster is conserved. Hox gene expression, a conserved developmental phenomenon in establishment of body plan has been studied by comparative genomics of other annelids like the marine worm *Capitella telleta*, the leech *Helobdella robusta*. We have used a combination of high-throughput sequencing based techniques and validation through cell and molecular biology to identify key aspects of the gene expression program of regeneration in this worm. Besides the transcriptome, we have also done whole genome sequencing, miRnome and metagenome sequencing of this terrestrial annelid.

Genetic and functional involvement of ZEB1 and FEN1 genes in FECD pathogenesis

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Abstract

Fuchs’ endothelial corneal dystrophy (FECD) is a dominantly inherited complex disorder, clinically manifested as thickened collagenous deposition on corneal Descemet’s membrane with excrescences called guttae [1, 2]. The current study intends to understand the genetic and functional role of FECD candidate genes, ZEB1 (Zinc finger E-Box binding homeodomain) and FEN1 (Flap endonuclease 1). For this, genetic co-segregation of polymorphic variants in these genes with FECD was assessed in a sample Indian population comprising of 76 FECD patients and 180 unrelated age-matched controls. Gene scan through bi-directional sequencing identified novel polymorphic association at rs220057 (OR= 1.92, 95% CI= 1.246-2.96, P= 0.016) in ZEB1. Gel-shift assays confirmed the binding of transcription factor, ZEB1 at the promoter proximal region of a collagen gene, COL8A2 that contains DNaseI hypersensitivity signatures as per ENCODE data.

Genotyping of the polymorphisms in FEN1 (rs174538 and rs4246215) gene indicate a higher risk of developing FECD (OR= 6.50, 95% CI= 1.84-23.01, P= 0.004) for rs4246215/TT carriers. Comet assay analysis from blood leucocytes of study participants also revealed heightened endogenous DNA damage in FECD carriers of rs4246215/TT genotype. These results confirm genetic involvement of ZEB1 and FEN1 genes in Indian FECD cohort and provide insights on their functional roles. ZEB1 binding on COL8A2 promoter proximal region provides initial reports suggesting probable regulatory role of this transcription factor over collagen expression. Our report also suggests a predisposition of FECD patients to increased endogenous DNA damage, which can be reasoned with increased FEN1 expression reported previously in rs4246215/TT lung tissues [3]. Further analysis on downstream effects of ZEB1 regulation and FEN1-coupled DNA denaturation will advance the current knowledge on FECD pathogenesis.

References


Category: Functional Genomics

CGGBP1-CTCF dynamics in regulation of chromosomal interactions

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Abstract

Genome organisation and gene expression is regulated by specific DNA sequences that include “insulator elements”. Insulator proteins, such as CTCF bind to insulator elements to block spreading of silent chromatin in-cis or inhibit interactions between transcriptional enhancers and promoters. By binding to insulators in a methylation-sensitive manner, CTCF establishes and maintains contrasting transcription patterns on either side of the insulator elements [1]. Though details of CTCF-insulator activities have been worked out, mechanisms of regulation of insulator activity by other proteins is unknown. CTCF-binding insulators are retrotransposon-derived, the same elements to which CGGBP1 binds making CGGBP1 a candidate insulator regulator factor [2]. Objective is to explore role of CGGBP1-CTCF dynamics in regulation of insulator activity. 1064Sk skin fibroblasts were grown in presence or absence of CGGBP1 in growth stimulated or starved condition. ChIP-seq was performed to identify CGGBP1-binding DNA sequence motifs [3]. We have observed a strong overlap between binding sites of CTCF and CGGBP1 [4, 5]. CGGBP1 and CTCF seem to share the retrotransposons-derived M1 and M2 motifs. Unlike in quiescent cells, growth factor-stimulation increased CGGBP1 binding to CTCF-CTCF binding sites with decreased CTCF insulator activity. The distance between CGGBP1 M1 and M2 motifs was longer in quiescent cells as compared to growth stimulated cells. Our results suggest that CGGBP1 negatively regulates CTCF insulator activity in normal cells in a growth signal-dependent manner.

References


Category: Functional Genomics

**Genome-wide CpG and non-CpG methylation regulation by CGGBP1**

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**Abstract**

DNA cytosine methylation occurs in all three sequence contexts: CpG and CHG (symmetrically on the two strands) and CHH (asymmetrically on one strand). While mechanisms of CpG methylation regulation are well studied; the non-CpG methylation is beginning to be understood. Cytosine methylation patterns genome-wide are required for gene expression regulation, allele-specific functions of genomic loci, genomic integrity and silencing of repetitive elements. Studying proteins that exhibit cytosine methylation methylation-sensitive DNA-binding can unveil novel cytosine methylation regulatory mechanisms and their consequences. One such recently reported cytosine methylation regulator protein is human CGGBP1 that has CpG and non-CpG cytosines both in its binding sites. CGGBP1 depletion affects CpG methylation at repetitive elements. Normal human foreskin fibroblasts 1064Sk cells were stably transduced with lentivirally expressed CGGBP1 shmiR and control shmiR. Genomic DNA was isolated followed by bisulfite treatment and subjected to deep sequencing (Illumina). Cytosine methylation state in presence and absence of CGGBP1 was ascertained by Bismark. Through genome-wide bisulfite sequencing, we describe the effects of CGGBP1 loss-of-function on CpG, CHG and CHH methylation at a base level resolution. We observed a dynamic bimodal balancing of methylation upon CGGBP1 depletion; as it causes both gain and loss of methylation, with spatial overlap at annotated functional regions and not identifiable with any sequence motifs. However, we observed a clear association of methylation changes with GC skew genome-wide. CGGBP1 depletion causes clustered methylation changes in cis, upstream of GC skew promoters complemented by clustered occurrences of methylation changes in proximity of transcription start sites of known cytosine methylation regulatory genes, altered expression of which can regulate cytosine methylation in trans. CGGBP1 maintains a balance between pro- and anti-cytosine methylation mechanisms independent of the nucleotide sequences, but acts at the higher level of DNA structure, nucleotide composition bias and secondary structure formation ability of the strands. Our findings collectively convey that CGGBP1 is a regulator of cytosine methylation in all sequence contexts, including CHG and CHH, genome-wide through a combination of cis and trans-acting mechanisms.

**References**


Functional Genomic investigation of Peroxisome Proliferator-Activated Receptor Gamma (PPARG) mediated transcription response in gastric cancer

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Abstract

Cancer is a complex and progressive multi-step disorder that results from the transformation of normal cells to malignant derivatives. Several oncogenic signaling pathways are involved in this transformation. PPARG (Peroxisome proliferator-activated receptor gamma) mediated transcription and signaling is involved in few cancers. We have investigated the PPARG in gastric tumors. The objective of the present study was to investigate the PPARG mediated transcriptional response in gastric tumors. Gene-set based and pathway focused gene-set enrichment analysis of available PPARG signatures in gastric tumor mRNA profiles shows that PPARG mediated transcription is highly activated in intestinal sub-type of gastric tumors. Further, we have derived the PPARG associated genes in gastric cancer and their expression was identified for the association with the better survival of the patients. Analysis of the PPARG associated genes reveals their involvement in mitotic cell cycle process, chromosome organization and nuclear division. Towards identifying the association with other oncogenic signaling process, E2F regulated genes were found associated with PPARG mediated transcription. The current results reveal the possible stratification of gastric tumors based on the PPARG gene expression and the possible development of PPARG targeted gastric cancer therapeutics. The identified PPARG regulated genes were identified to be targetable by pioglitazone and rosiglitazone. The identification of PPARG genes also in the normal stomach tissues reveal the possible involvement of these genes in the normal physiology of stomach and needs to be investigated.

Epigenetic signatures of high altitude adaptation in Tibetan population

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Abstract

Genetic adaptations in high-altitude populations which provide them survival benefit/advantage in high altitude have been well documented. However, till date, very limited studies on the epigenetic adaptation in high altitude human population. Therefore, we aimed to study the high altitude adaptation in Tibetan population, with respect to epigenetic adaptation. DNA methylation is one of the major epigenetic marks, role of which has been suspected in a spectrum of gene-environment interaction and biological processes. The most common form of DNA methylation in vertebrates is 5-methylcytosine, mostly observed in CpG context. Recent advancements in the field of DNA sequencing made it possible to analyse genome-wide methylation rapidly with high resolution, however, study of methylation at population level to explore population specific gene-environment interaction is not in long race. Therefore, the present study has been designed to analyse DNA methylation signatures (using whole genome bisulfite sequencing) in Tibetan population (presently inhabiting Karnataka since last 50-60 years) but were native of Ladakh (above 5000 meters) since generations along with Indian populations who are living at low altitude (~10 meters). DNA was isolated from blood, collected from the subjects after informed consent. DNA was converted using bisulfite reagents and whole genome bisulfite sequencing (WGBS) was performed using Illumina-2500 platform (Medgenome Pvt. Ltd.). Analysis of WGBS data was performed using various statistical/bioinformatics tools such as bedtools, Bioconductor and R package to find out methylation sites that are significantly different. We observed 6 differentially methylated regions in Tibetans, highland population, of which, 5 were hypo methylated and one was hypermethylated. The present study reveals differential hypo methylation of CYP2E1 and CRELD1 genes, previously reported to be involved in high altitude adaptation (Simonson et al., 2010; Dong et al., 2014), which would of greater interest. Besides this, we observed novel epigenetic differences in chromosome 7, 11 and 15. Our study, for the first time reveals genome wide level of methylation difference in Tibetan population (native of high altitude since generations) residing in low altitude with other mainland Indians of low altitude which could be important epigenetic markers of natural selection. Comparison with native high altitude Tibetan would make the scenario clearer, which is in the process.

Citation: Basak, N., Nizamuddin, S. and Thangaraj, K. Epigenetic signatures of high altitude adaptation in Tibetan population [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 113.

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Category: Functional Genomics

Role of NPR1 dependent and NPR1 independent genes in response to Salicylic acid

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Abstract

NPR1 (Nonexpressor of pathogenesis-related gene) is a transcription coactivator and central regulator of systemic acquired resistance (SAR) pathway. It controls wide range of pathogenesis related genes involved in various defense responses, acts by sensing SAR signal molecule, Salicylic acid (SA). Mutation in NPR1 results in increased susceptibility to pathogen infection and less expression of pathogenesis related genes. The present study aimed to identify the role of NPR1 in gene expression after the Salicylic acid induction. For this RNA-seq was performed in Arabidopsis thaliana Col-0 and npr1-1 in response to Salicylic acid. RNA-seq analysis revealed a total of 3811 differentially expressed gene in which 2109 genes are up-regulated and 1702 genes are down-regulated. We have divided these genes in 6 categories SA induced (SI), SA repressed (SR), NPR1 dependent SI (ND-SI), NPR1 dependent SR (ND-SR), NPR1 independent SI (NI-SI), NPR1 independent SR (NI-SR). Further, Gene ontology and MapMan pathway analysis of differentially expressed genes suggested variety of biological processes and metabolic pathways that are enriched during SAR defense pathway. These results contribute to shed light on importance of both NPR1-dependent (ND) and NPR1-independent (NI) gene acting downstream to Salicylic acid induction in SAR pathway. The present study aimed to identify the role of NPR1 in gene expression after the Salicylic acid induction.

Citation: Agarwal, N., Agarwal, S., Babita, and Sawant, S.V. Role of NPR1 dependent and NPR1 independent genes in response to Salicylic acid [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 114. https://doi.org/10.24870/cjb.2017-a100
Category: Metagenomics

**Understanding role of genome dynamics in host adaptation of gut commensal, *L. reuteri***

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**Abstract**

*Lactobacillus reuteri* is a gram-positive gut commensal and exhibits noteworthy adaptation to its vertebrate hosts. Host adaptation is often driven by inter-strain genome dynamics like expansion of insertion sequences that lead to acquisition and loss of gene(s) and creation of large dynamic regions. In this regard we carried in-house genome sequencing of large number of *L. reuteri* strains origination from human, chicken, pig and rodents. We further next generation sequence data in understanding invasion and expansion of an IS element in shaping genome of strains belonging to human associated lineage. Finally, we share our experience in high-throughput genomic library preparation and generating high quality sequence data of a very low GC bacterium like *L. reuteri*.

**Citation:** Sharma, S., Kumar, S., Patil, P.P., Midha, S., Korpole, S. and Patil, P.B. Understanding role of genome dynamics in host adaptation of gut commensal, *L. reuteri* [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 115. [https://doi.org/10.24870/cjb.2017-a101](https://doi.org/10.24870/cjb.2017-a101)
Assessment of antibiotic resistance genes and integrons in commensal *Escherichia coli* from the Indian urban waste water: Implications and significance for public health

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Abstract

Antibiotics like β-lactams, quinolones/fluoroquinolones, aminoglycosides and tetracycline constitute the major mainstay of treatment against most infectious diseases including *Escherichia coli*. Indiscriminate use of antibiotics for human and animal well-being has generated an enormous evolutionary pressure on bacteria especially *E.coli*, which has a highly plastic/evolving genome. Though, antibiotic resistance (AR) has been extensively studied in pathogenic *E.coli*, commensal strains have been studied less owing to lesser clinical significance. However, commensal strains pose a serious threat as reservoirs and transmitters of resistance genes to other bacteria. Therefore, the present study was undertaken to investigate the prevalence of resistance genes and integrons in commensal *E.coli* isolated from river Yamuna, Delhi, India, which receives plentiful urban waste water. Eighty three well-characterized *E.coli* strains of phylogroups A and B1 isolated from river Yamuna were investigated. Antimicrobial susceptibilities and minimal inhibitory concentrations (MICs) for β-lactams, aminoglycosides, tetracycline and quinolone/fluoroquinolone were determined by disk diffusion and Etest, according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Production of Extended spectrum β-lactamases (ESBL) and AmpC was investigated. Prevalence of antibiotic-resistance genes for β-lactams (*bla*TEM, *bla*SHV, *bla*CTX-M, *bla*OXA, *bla*CMY-42), aminoglycosides (*rmtA*, *rmtB*, *rmtC*, *armA*, *str*, *aacC2*), tetracycline (*tetA*, *tetR*, *tetM*, *tetW*), and plasmid-mediated quinolone resistance, PMQR (*qnrA*, *qnrB*, *qnrC*, *qnrD*, *qnrS*, *qep*, *aac*) were assessed. Integrons and gene-cassette arrays were characterized. Commensal *E.coli* strains showed a higher resistance to ampicillin (95%), less to cefazolin (45%) and still lesser to tetracycline (15%). About 19% of these strains showed multidrug resistant (three or more classes of antibiotics), of which 15% also produced ESBLs. None of the strains produced AmpC β-lactamases. About 6% of the strains were concurrently fluoroquinolone-resistant and ESBL producers. The *bla*TEM was present in most strains (95%), followed by *bla*CTX-M (15%). Aminoglycoside-resistance genes viz. *str* and *armA* were detected in 6% and 8% strains, respectively; tetracycline-resistance genes *tetA* and *tetR* in 3% and 6% strains, respectively; and PMQR gene *qnrS* in 15% of the strains. Class I integron was detected in 64% of the isolates, of which 7 strains had 3 different variable region gene-cassette arrays. *dfrA* and *aadA* gene families were widespread among the gene-cassettes identified.

Molecular typing of antibiotic resistant bacteria isolated and identified as ESBL producers from polluted water reservoirs

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Abstract

Anthropogenic polluted reservoirs are the mating hub for antibiotic resistant genes and multidrug resistant bacteria (MDR). The rapid emergence of this MDR is the consequence of mutations in the genes as well as the horizontal gene transfer of mobile elements carrying the resistant genes. Current study focuses on isolation and characterization of Extended spectrum β-lactamase (ESBL) producers from diverse water resources of Pune city and to understand the genetic modifications responsible for multidrug resistance using whole genome sequencing (Next Generation sequencing-Illumina sequencing). The identified isolates were Pseudomonas aeruginosa, Escherichia coli, Enterobacter cloacae and Stenotrophomonas maltophilia. Mechanism of resistance developed by all isolates was efflux pump as per the genes (adeL, macA, macB, ros B) identified by Comprehensive Antibiotic Resistance Database. Primary phenotypic detection of isolates as ESBL producers and AmpC hyper producers was supportive as identified genes were resistant to all antibiotics including last resorts like carbapenems, peptide antibiotics. Rapid emergence of antibiotic resistance was seen in one isolate due to presence of additional 19 antibiotic resistant genes (blaI, exo bet lactamase, PDC 9, CMY-83, mec I, etc.). The investigation alarms the deadly pollution of reservoirs due to haphazard use of antibiotics which pressurizes rapid emergence and persistence of MDR.

Citation: Yewale, P.P., Shridhar, A., Hatekar, P.A., Mandal, A., Nawani, N.N. and Jass, J. Molecular typing of antibiotic resistant bacteria isolated and identified as ESBL producers from polluted water reservoirs [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 117. https://doi.org/10.24870/cjb.2017-a103
Category: Metagenomics

Microbial diversity assessment within continuous subsurface sediment core of estuarine region of Mahi river basin, Western India

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Abstract

The Mahi estuary is located at the western fringe of the Gujarat alluvial plain in Mainland Gujarat and presents an interesting geomorphic set up. Subsurface microbial diversity plays a vital role in maintenance of good soil health, because microorganisms are involved in many essential functions such as soil formation, elemental cycles, contaminant degradation, and the maintenance of groundwater quality. In the Indian subcontinent, subsurface microbial processes and the diversity are not well studied. Understanding of subsurface microbial diversity of Mahi river basin will help to understand microbial ecology of the Indian subcontinent. Here we examined depth related bacterial diversity pattern within different strata of a vertical sediment section of estuarine region of Mahi river basin. Sediment core samples were collected by core drilling method from Chokari (~17 m deep) (CRD). Upper ~8 m part of sediment core comprises of an estuarine sequence (Holocene age) which is underlain by fluvial sequence (pleistocene age) that continues further down the core. We selected two samples from depth 61.25 cm and 187.5 cm (CRD 2 and CRD 6, respectively) which lie within estuarine sequence and one sample (CRD 27) from 1000 cm depth which lies within fluvial sequence for microbial diversity analysis by using Illumina based sequencing of V3-V4 region of 16S rRNA gene. Total 642462 reads (~ length 250 bp) were obtained which comprised of 32763 OTUs (Operational Taxonomic Units). Abundant OTUs were affiliated with Actinobacteria, Bacteroidetes, Proteobacteria, Plancomycetes, Firmicutes, Chloroflexi, Cyanobacteria, Acidobacteria, and candidate division TM7 phyla. Actinobacteria, Proteobacteria, Firmicutes, Cyanobacteria and candidate division TM7 decreased significantly as the depth decreases. Within paleosols of late pleistocene age (CRD 27) Bacteroidetes were comprise of 75.32% OTUs while within Holocene samples it comprises of only 19.78% and 1.63% OTUs (CRD 2 and CRD 6, respectively). Taxonomic patterns of OTUs were similar within Holocene samples while pleistocene sediment sample shows different pattern based on pairwise beta-diversity patterns. Conclusively microbial diversity within subsurface sediment core of estuarine region of Mahi river basin are highly diverse indicating availability of different energy source and electron acceptors in microhabitats within the estuarine region of Mahi river basin.

Citation: Shah, A., Maurya, D.M. and Archana, G. Microbial diversity assessment within continuous subsurface sediment core of estuarine region of Mahi river basin, Western India [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 118. https://doi.org/10.24870/cjb.2017-a104
Category: Metagenomics

**Genotypic characterization of multi-drug resistant coliform bacteria: Insights into their mechanisms of antibiotic resistance using Whole Genome Sequencing**

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**Abstract**

Anthropogenically polluted water is a potential reservoir for pathogenic microorganisms and micro-contaminants like antibiotics. Due to the selective pressure of antibiotics, resident bacteria tend to acquire resistance mechanisms through mutations, genome rearrangements and horizontal gene transfer. (Darvinism: Survival of the fittest!). This study aimed to isolate and characterize multi-drug resistant coliform bacteria from natural water bodies of Pune city and to analyse whole genome sequences for identification of genomic alterations possibly responsible for multi-drug resistance (MDR). The isolates were identified by next generation sequencing. Sequence type of isolates was determined by Multilocus sequence typing (MLST). Genes responsible for antibiotic resistance were identified using Comprehensive Antibiotic Resistance Database (CARD). The isolates were found resistant to third and fourth generation cephalosporins and carbapenems which is very alarming as these are the antibiotics of last resort. The mechanisms of resistance developed by isolates were efflux pump mediated drug resistance and β- lactamase production. Mutation rate was found higher when set of genes responsible for efflux pump mediated drug resistance (mdt A, mdt B, mdt C, mex J, mex K, opr N) was analysed. Mutation leading to change in single amino acid (Arg-235 to Lys) was detected in *Pseudomonas aeruginosa* ST- 635 for the gene *bla*<sub>PDC-3</sub>. *Escherichia coli* ST-410 and ST- 617 were found single amino acid variants for the gene *bla*<sub>CMY-47</sub> (Pro-121 to Ser). Mutations observed in CMY-47 and PDC-3 are indicative of rapid evolution of AmpC β- lactamases. Indiscriminate use of antibiotics has resulted into emergence and dissemination of MDR leading to antibiotic- driven adaptive bacterial evolution.

**Citation:** Hatekar, P.A., Kulkarni, S., Yewale, P.P. Mandal, A., Nawani, N.N. and Jass, J. Genotypic characterization of multi-drug resistant coliform bacteria: Insights into their mechanisms of antibiotic resistance using Whole Genome Sequencing [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 119. [https://doi.org/10.24870/cjb.2017-a105](https://doi.org/10.24870/cjb.2017-a105)
Category: Metagenomics

**Effect of rice beer on gut bacteria**

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**Abstract**

The human gut is colonized by trillions of bacteria, called microbiota influences human health and is effected by several host factors. The studies in humans and model organisms have clearly demonstrated that out of several important factors, diet has the most dominant role in regulation of the gut microbiota. Additionally, with an increase in the knowledge on the microbiota, the connections between microbial actions on dietary consumption are being revealed. Consumption of fermented beverages holds a long tradition and accounts for approximately one-third of the human diet globally. In various societies, fermentation has not only been well established as a process for food preservation, human nutrition, traditional medicine and culture but also for the improving the sensorial characteristics, such as texture, flavor and aroma and most importantly for the magnification of the nutritional values. Consumption of rice beer is an essential part of the socio-cultural life of several tribes of North-East India. It is believed to be effective against several ailments such as amebiosis, acidity, vomiting and has health modulating effects including cholesterol reduction and endocrine function. Effect of rice beer was tested on mice model. 17 healthy Swiss albino mice were taken for the study and divided into two groups: control and treated. Rice beer was fed to the treated group once daily and fecal samples were collected. Metagenomic DNA from stool samples was extracted and V6- V8 region of the 16S rDNA gene was amplified, followed by Denaturing Gradient Gel Electrophoresis (DGGE). The DGGE gel was scored using GelCompar II software. Gas Chromatography Mass Spectrometry (GCMS) analysis of stool samples was also carried out. Multidimensional scaling (MDS) plot of the DGGE profiles showed distinct clustering of control and treated groups, indicating the effect of rice beer consumption on gut microbes.

**References**


**Citation:** Bhaskar, B. and Khan, M.R. Effect of rice beer on gut bacteria [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 120. [https://doi.org/10.24870/cjb.2017-a106](https://doi.org/10.24870/cjb.2017-a106)
Category: Metagenomics

Differential effects of whisky brands on human gut microbiome and fecal metabolome

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Abstract

The gut bacteria have significant impact on human physiology and are influenced by dietary habit [1]. Apart from normal diet, alcoholic beverages have also been shown to influence gut microbial makeup. The wine polyphenols have been linked to increase the beneficial bacteria in the gut after 4 weeks of consumption [2]. Consumption of alcoholic beverages for longer period (>10 years) has also been correlated to detrimental gut bacterial dysbiosis [3]. The contrasting effects of alcoholic beverages in these two studies necessitate further research. Globally, 45.7% of alcoholic drinkers are spirit drinkers with India having the highest (71%) [4]. In India whisky is preferred by most of the drinkers and 1400 million liters of whisky was consumed in India in the year 2012 [5]. Till date, no study has been reported to understand the effect of long-term consumption of different types of whisky on gut bacterial profile (GBP). In this purview a pilot study of gut bacterial and metabolite profile was performed between the whisky drinker (n=18) and non-drinker (n=8) along with rice beer drinkers (n=3). PCR-denaturing gradient gel electrophoresis (PCR-DGGE) coupled with next generation sequencing (NGS) analysis on illumina miseq platform revealed decrease in gut bacterial diversity in the drinkers compared to the non-drinkers. The whisky types have differential effects on the GBP. The GBP of whisky type 1 drinkers had higher abundance of Clostridiaceae and Enterobacteriaceae (fold change log 2: 3.33 & 3.1537, respectively; \( p < 0.002 \)) in comparison to the non-drinker group, while the type 2 whisky drinkers had increased abundance of Lactococcus and Streptococcus (fold change log 2: 9.1827 & 4.2986; \( p < 0.002 \)) compared to the non-drinker group. The butyric acid producing genera, Ruminococcaceae was found to be decreased in both the whisky drinking cohorts (fold change log 2: -1.5449 & -2.7327, respectively; \( p < 0.002 \)). Short-chain fatty acids (SCFA), mainly butyric acid, acetic acid and propanoic acid were found to be decreased in both the whisky drinker groups in comparison to the non-drinkers (\( p < 0.05 \)). The differential effects of whisky types with equal alcohol content indicate that constituents of whisky other than the alcohol also influence the gut bacterial composition.

References

Category: Metagenomics

A comparative genomics approach to find out the probiotic effects of *Lactobacillus casei* Lbs2 isolated from healthy gut of Indian population

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**Abstract**

Lactobacillae are gram positive diverse group of species and have association with nutrient rich niches like humans, animals and plants. *Lactobacillus casei* is considered as one of the most competent probiotic throughout the world. Its microbiological feature historically well-established but genomic analysis including comparative genomics is recent. *Lactobacillus casei* Lbs2 strain was isolated from the gut of a healthy north Indian individual and sequenced. We compared the genomes of *Lactobacillus casei* Lbs2 with 8 other complete genomes of the same species e.g.; LC2W, BL23, BDII, W56, 12A, Zhang, LOCK919, ATCC393 using BRIG (Blast Ring Image Generator), Gene enrichment analysis using Fischer Extract test in R. Lbs2 strain has a number of genes including bile tolerance, stress response re-iterating its probiotic stand. Interestingly, genes coding for transposons, co-enzyme transport and metabolisms are enriched in the Indian Genome. Presence of large number of transposons indicates this genome is undergoing expansion and under adaptive selection pressure. When we compared our genome based on Multilocus Sequence Typing (rMLST), we found this strain is closely similar to *Lactobacillus fermentum* rather than other *L. casei* strains. Comparison of Lbs2 strain with other *L. casei* strains indicates ATCC393 (isolated from daily product) is closer than others.

**Citation:** Ghosh, S., Bhowmik, S. and Tripathy, S. A comparative genomics approach to find out the probiotic effects of *Lactobacillus casei* Lbs2 isolated from healthy gut of Indian population [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 122. https://doi.org/10.24870/cjb.2017-a108
Comparative genomics of Westiellopsis prolifica a freshwater cyanobacteria uncovers the prolific and distinctive metabolic potentials

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Abstract

Cyanobacteria are one of the ancient Micro-organisms that originated about 2.5 billion years ago. They are a very rich source for production of various natural compounds that are largely scalable in pharmaceutical and biotechnology industries. The unicellular Cyanobacteria are more ancient than the multicellular forms. In this study, we are exploring the genomes of a multi cellular, heterocystous, true branching Cyanobacteria, Westiellopsis prolifica belonging to order Nostocales. Complete genome is essential to serve as a reference for other sequencing projects and from which we can confirm the presence of various useful metabolic genes which are important for manufacturing pharmaceutical products. Here we report the draft assembly of Westiellopsis prolifica genome of 7.2 Mb with 19 scaffolds and the N50 and largest contig sizes are 2650655 bp and 3476031 bp, respectively. The phylogenomic studies from the literature reveal the closest relative of Westiellopsis prolifica are Fischerella sp. pcc 9431, Fischerella sp. pcc 9939 and Hapalosiphon welwitschii. Our preliminary comparative genomic analysis revealed that the sequence identity with the neighbouring clades were less, although we observed the large set of genes were syntenic and arranged in conserved in clusters. Genome mining on these organisms identified several clusters of NRPS, polyketide biosynthesis, two-component system, heterocyst differentiation genes and Nif genes were conserved in these genomes. We identified 21 clusters of secondary metabolites, which include NRPS and polyketide genes. For extraction of metabolites, we used several organic solvents. These extract contain various metabolic products which can be further exploited for the large scale production by genetic engineering approaches. Our Future work includes checking the RNAseq expressions of these metabolite producing genes.

Citation: Verma, V., Malar C, M. and Tripathy, S. Comparative genomics of Westiellopsis prolifica a freshwater cyanobacteria uncovers the prolific and distinctive metabolic potentials [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 123. https://doi.org/10.24870/cjb.2017-a109
Category: Metagenomics

**De novo** assembly, functional annotation and comparative alignment of whole genome of a halo-tolerant *Exiguobacterium profundum* PHM11 with related genomes

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Abstract

Advances in the next-generation sequencing (NGS) technologies have invigorated the exploration of microbial genomes in retrieving the hidden traits. In this study, high-throughput next generation whole genome sequencing of a halotolerant *E. profundum* PHM11 was performed on Illumina HiSeq paired end sequencing platform and assembled through **de novo** Linux based approaches using Velvet (V 1.2.10.) algorithm package. Quality filtering in **de novo** sequencing produced 72,947,390 reads with total size of 733519536 bp (7335.1 Mb). High quality reads having minimum and maximum contig lengths of 202 and 958471 (~0.95 Mb) bp were further considered for assembly. Final PHM11 genome has a size of ~2.92 Mb comprising 70 contigs, 47.93% G+C content with 761858 (26.08%), 757313 (25.92%), 699924 (23.96%), and 700172 (23.97%) percentages of adenine, thymine, cytosine and guanine nucleotides. Throughout micro-satellite mining of genome showed a total of 3005 SSRs, covering 0.1 of whole PHM11 genome, with relative abundance; 1029, relative density; 10951, and percentages of penta repeats; 65.75, hexa repeats; 28.75, mono repeats; 3.89, and tetra-repeats; 1.59, respectively. Gene networks related to the arrangement of key genes and presence of lysogenic phage DNA were reflected through generating the chromosome map of PHM11 genome. Functional annotations of genome reflected the different protein families, and hidden inherent metabolic pathways providing unusual features. A total of 3033 protein coding genes and 33 non-protein coding genes were identified; out of these only 2316 could be characterized and 737 were reported as hypothetical. Random genes of different metabolic pathways were amplified from its genome and authenticated through their sequencing. Genome-rearrangements in PHM11 could be deciphered through aligning its genome with thirteen other genomes of different *Exiguobacterium* species.

Deciphering the advanced methodology to investigate the survival of A. sphaerica in iron enriched region of Chhattisgarh

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Abstract

Cyanobacteria are among those tremendous microbial community that adds both aesthetic and ethnic value to the nature. They majorly contribute as a primary producer via photosynthesis due to the presence of various photosynthetic pigments (chlorophyll a, carotenoids, and phycobilisomes) and nitrogen fixation. Apart from this, cyanobacteria also work differently from the league because they bear enormous plasticity in their nature and can survive in the most extreme situations such as saline, thermocline environment, metal and heavy metal prone environment, etc. Iron, one of the second most abundant metal, third most limiting nutrient and fourth most abundant trace element that achieves equal position to that of macro element because of its contribution in all the vital life supporting activities such as photosynthesis, nitrogen fixation and electron transport chain mechanism. The availability and unavailability of iron leads to production of ROS within the cell which is directly linked to an oxidative stress via Haber weiss reaction and Fentons mechanism. So to assess the iron induced stress in cyanobacteria, soils from different locations of Chhattisgarh, have been tested for iron concentration by using Atomic Absorbtion Spectrophotometry (AAS). Then, number of cyanobacterial species was isolated from Turkadih, Bilaspur, and Chhattisgarh having highest iron concentration (140 ppm). They were treated with different concentrations of iron (0, 20, 50, 75, 100, 150 and 200 µM FeCl3). Result suggested that only Anabaena sphaerica a filamentous, heterocystous cyanobacterium could survive up to 100 µM FeCl3 (5 times higher concentration as is used for standard growth medium) and rest of the concentrations were found to be lethal for all the cyanobacterial species. The alterations in morphological, physiological and biochemical attributes were assessed and investigated. Further, proteome analysis (2D- Gel Electrophoresis) of A. sphaerica suggested that some unique proteins need proper investigation via MALDI-TOF. Concisely, it can be said that this part of research creates an interest to investigate at higher and advanced level for the most iron tolerant species isolated from the iron enriched region of Chhattisgarh.

References

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Citation: Kunui, K. and Singh, S.S. Deciphering the advanced methodology to investigate the survival of A. sphaerica in iron enriched region of Chhattisgarh [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 125. https://doi.org/10.24870/cjb.2017-a111
Detection of bacterial and viral pathogens in hospitalized children with acute respiratory illness and determination of different socio demographic factors as important cause of the disease in Odisha, India

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Abstract

The paper an attempt has been made to analysis the status of acute respiratory tract infection among children in India. In the present study we aimed to present first time the detection of viruses, bacteria and mix infection of viruses and bacteria in hospitalized children with ARI and also to analyze the influence of socioeconomic status of parent in two divergent geographical settings of Odisha. Hospitalized children with ARI aged <5 were recruited from July 2014 to June 2015. Nasopharyngeal/Oropharyngial swabs were collected for detection of common respiratory viruses by reverse transcriptase chain reaction (RT-PCR). Bacteria were isolated by routine culture methods. Bivitiate analysis including chi square was used as test of significance. The analysis revealed 150 (56%) were detected with ≥1 bacteria, 40 (15%) with ≥1virus, 22 (8.2%) with ≥2 bacteria and 20 (7.4%) with both bacteria and virus. Most frequently detected pathogens were Klebsiella pneumonae (18.3%), Streptococcus pneumonae (12.7%), Parainfluenza A (36.6%) and Influenza- A 18 (30%). Incidences of pathogens were detected more among children <1 year, Gender discrimination in the form of dietary neglect of the female children has also been noted mostly in case of tribal patients. The present study had identified low socioeconomic status, poor housing conditions, illiterate mothers, birth weight, tobacco smoking families and nutritional status as important determinants for ARI. Interventions to improve these modifiable risk factors can significantly reduce the ARI burden among children especially in tribal population.

Citation: Biswal, B., Dwibedi, B. and Kar, S.K. Detection of bacterial and viral pathogens in hospitalized children with acute respiratory illness and determination of different socio demographic factors as important cause of the disease in Odisha, India [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 126. https://doi.org/10.24870/cjb.2017-a112
Prevalence of Staphylococcus aureus associated with Skin and Soft Tissue Infection (SSTI) among septic patients from Bhubaneswar

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Abstract

Staphylococcus aureus is a major gram positive bacterial pathogen that causes a wide spectrum of clinical infections, ranging from localized soft-tissue infections to life-threatening bacteremia and endocarditis. S. aureus can infect tissues when the skin or mucosal barriers have been breached. This can lead to many different types of infections, including boils, carbuncles (a collection of boils) and abscesses. Deeply penetrating S. aureus infections can be severe. The incidence of methicillin resistant S. aureus (MRSA) in India ranges from 30-70%. The present study investigates the detection of S. aureus from pus swabs of hospitalized patients (Capital Hospital, Bhubaneswar) having skin infections and abscesses and its' susceptibility pattern to different antibiotics. Out of 230 samples collected 204 (88.9%) were culture positive for different bacterial pathogens from which S. aureus was 54 (23%). The incidence rate of S. aureus among male and female group studied was 56.3% and 43.7%, respectively. The isolated S. aureus was found to be resistant to most of the antibiotics such as azithromycin, doxycycline, ciprofloxacin, tetracycline, gentamycin, ofloxacin, chloramphenicol, ampicillin and oxacillin. Among the various antibiotics, the isolated S. aureus strains revealed resistant to methicillin (MRSA) and vancomycin (VRSA) were 90.7% and 14.8, respectively. The MRSA strains were confirmed genotypically by amplification of methicillin resistant (mec A) gene. S. aureus identification and its antibiogram profile are highly essential for implementation of treatment and control of the infection in Odisha.

Citation: Mohanty, A. and Pal, B.B. Prevalence of Staphylococcus aureus associated with Skin and Soft Tissue Infection (SSTI) among septic patients from Bhubaneswar [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 127. https://doi.org/10.24870/cjb.2017-a113
Category: Metagenomics

An Insight into the Microbial Community Structure of White Rann of Kachchh: A Study towards Functional Aspects and Taxonomic Profiling

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Abstract

The desert environment owns a distinctive set of microbial communities as compared to other environment across the globe. Saline desert has unique ecosystem containing well adapted microflora exclusive to the niche. In spite of the importance of ecological processes in saline desert ecosystem, diminutive amount of knowledge is known and understood about indigenous microbial community, functional diversity, biotechnologically potential genes, biogeochemical processes and carbon sequestration abilities. The present study describes the microbial community composition and their functional inheritance from the White Rann of Kachchh. The microbial population was predominantly fashioned by bacteria species followed by archaea and eukaryota. The abundance of bacterial species \textit{Salinibacter} (7.4%), \textit{Burkholderia} (4.3%) of phylum Proteobacteria and \textit{Firmicutes}, respectively was evidently observed, while archaeal population abundantly contains \textit{Haloarcula} (14%) and \textit{Natromonas} (8%) of phylum \textit{Euryarchaeota} and \textit{Crenarchaeota}, respectively. The functional capabilities were shaped by primarily by genes involved in amino acid transport and metabolism, carbohydrate transport and metabolism, energy production and conversion. This study revealed that the microbial community has developed mechanisms for carbon fixation, stress response, synthesis of osmoregulant to cope up with fluctuations of high and low osmotic pressure in this saline environment. The diversity indices suggested that this profound study may perhaps be more appropriate for better understanding of ecology, White Rann of Kachchh.

References


Category: Metagenomics

Diversity of cultivable vaginal microbiota in asymptomatic women of reproductive age in Mumbai, India

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Abstract

Microbes in the vaginal microbiota form a mutual relation with its constituent members and its host. In recent years our acquaintance with vaginal microbiota has widened, however, insufficient knowledge is available in Indian scenario. In the present study, the diversity of cultivable vaginal microbiota in asymptomatic women of the reproductive age group from Mumbai was investigated using multiplex PCR and species specific PCR, validated by 16sRNA Sanger sequencing. Vaginal samples taken from 199 women were classified according to Nugent score as normal (n=147), intermediate (n=23) and bacterial vaginosis (n=29) indicating 14.5% asymptomatic BV. Cultivable Lactobacilli were recovered from 97.9% (195) participants. The abundance of vaginal Lactobacilli was reduced in women with BV. Of 147 women, 110 were considered healthy, as 37 women colonized vaginal Candida. The most predominant vaginal Lactobacillus spp. in healthy women were L. iners (70.9%), L. crispatus (26.4%), L. reuteri (20.9%), L. gasseri (18.2%), and L. jensenii (15.5%). Our data demonstrated a profound shift in the prevalent vaginal Lactobacillus spp. when comparing women with healthy and diseased conditions. In women with normal flora colonizing Candida, L. rhamnosus (24.3%) was one of the prevalent Lactobacilli. L. crispatus was identified as a specific species present only in the healthy state. L. iners was found to be the most frequent vaginal Lactobacillus irrespective of the vaginal health. Majority of the women harbored heterogeneous population of Lactobacillus indicating their cumulative effect in maintaining the vaginal niche. Among the single species population, distinct diversity of Lactobacilli were found in women with Normal, Intermediate and BV microflora. Though most frequently identified, L. iners, significantly coexisted with other Lactobacillus spp., suggesting its minimal protective role alone in the vaginal niche. About one third of study population colonized Candida, most of which were Non albicans Candida; whereas BV related genera viz., Gardnerella, Atopobium, Prevotella and Megasphaera was observed at high prevalence even in women with healthy microflora. This recommends the use of in-depth analysis wherein detection of a specific species can be employed as a disease marker. Our study provides an insight into the overall structure of vaginal community that may provide fundamental information for future investigations using metagenomics, in-vitro approaches and metaproteomics.

Citation: Pramanick, R., Parab, S., Mayadeo, N., Warke, H. and Aranha, C. Diversity of cultivable vaginal microbiota in asymptomatic women of reproductive age in Mumbai, India [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 129. https://doi.org/10.24870/cjb.2017-a115
Category: Metagenomics

Marine microbe with potential to adhere and degrade plastic structures

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Abstract

Extensive usages of plastics have led to their accumulation as a contaminant in natural environment worldwide. Plastic is an inert and non-biodegradable material, due to its complex structure and hydrophobic backbone [1]. Conventional methods for reduction of plastic waste such as burning, land-filling release unwanted toxic chemicals to the environment and harming living organism of land as well as the ocean. There is growing interest in development of strategies for the degradation of plastic wastes to clean the environment [2]. Marine bacteria have evolved with the capability to adapt and grow in the diverse environmental conditions [3]. We studied the ability of marine bacteria for destabilization and utilization of different plastic films (LDPE, HDPE, PVC and PET) as a sole source of carbon. An active bacterial strain AIIW2 was selected based on the triphenyl tetrazolium chloride reduction assay, and it was identified as Bacillus species based on 16S rRNA gene sequence. The viability of the strain over the plastic surface was studied and confirmed by bacLight assay with fluorescent probes. Scanning Electron Microscope and Atomic Force Microscope images suggested that bacterial interaction over the plastic surface is causing deterioration and roughness with increasing bacterial incubation time. In Fourier transform infrared spectra of treated plastic film evidenced stretching of the (-CH) alkan rock chain and (-CO) carbonyl region, suggested the oxidative activities of the bacteria. The results revealed that ability of bacterial strain for instigating their colonization over plastic films and deteriorating the polymeric structure in the absence of other carbon sources [4]. Moreover, production of extracellular enzymes such as esterase, laccase, and dehalogenase which are reported to support utilization of plastics was confirmed by plate assays. In concise, our results suggested that the marine bacterial strain AIIW2 have the ability to utilize different plastics and dictates the need for the further studies on the underlying biological process. We planned to explore the genes encoding the enzymes involved in degradation of plastic through whole genome study and metabolic profiling to investigate any phenotypic changes [5]. Establishing microbial resources for the degradation of plastics is an ecofriendly approach which could be useful in reduction of its accumulation.

References


Citation: Kumari, A., Chaudhary, D.R. and Jha, B. Marine microbe with potential to adhere and degrade plastic structures [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 130. https://doi.org/10.24870/cjb.2017-a116
Category: Metagenomics

Comparative genomic analysis of *Mycobacterium immunogenum* strain CD11_6, a new potential vaccine strain against *Mycobacterium tuberculosis*

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Abstract

*Mycobacterium tuberculosis* (*Mt*) infection is a growing challenge to the scientific world due to drug resistance. In this study, we predicted safety, efficacy and the molecular basis for the potential of strain *Mycobacterium immunogenum* (*Mi*) as a vaccine strain against *Mt* that in some preliminary experiments had reduced the bacterial counts of *Mt* in infected organs of mice during immunization studies along with generation of memory CD4 T cells and CD8 T cells. *Mi* strain CD11_6 was an isolate from duodenal mucosa of a celiac disease patient that was characterized and sequenced. Rapid Annotation using Subsystem Technology (RAST) server was used to annotate, and identify the virulence determinant genes and other features that make it a potential vaccine candidate. Genome of *Mt* strain H37Rv and its vaccine strain *Mycobacterium bovis* (*Mb*) AFF2122/97 was also retrieved from genome database of NCBI and compared with *Mi*. Virulence determinant genes of *Mi* were mapped and compared with virulent *Mt* strain H37Rv and strain *Mycobacterium bovis* (*Mb*) AFF2122/97. This comparative analysis revealed that *Mi* is less virulent as compared to *Mb* and *Mt* whereas *Mi* contains comparable number of genes coding for antigenic surface membrane proteins, membrane transport proteins and cytosolic proteins that predicts its probable vaccination attributes against *Mt*. Further, sequence alignment results and exploring predicted proteome of the strain CD11_6 also indicates that it has potential candidate vaccine peptides belonging to membrane proteins of *Mt*.

The study signifies that *Mi* strain CD11_6 has sufficient antigenic repertoire that might have led to activate memory T cells against *Mt* and causing its eradication. Our further work on this line to validate the role of reported surface membrane proteins may help to know about molecular basis for action of *Mi* that will improve the present vaccination strategies against *Mt*.

Category: Metagenomics

Next-Generation Sequencing for Typing and Detection of ESBL and MBL *E. coli* causing UTI

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Abstract

Next-generation sequencing (NGS) has the potential to provide typing results and detect resistance genes in a single assay, thus guiding timely treatment decisions and allowing rapid tracking of transmission of resistant clones. We can be evaluated the performance of a new NGS assay during an outbreak of sequence type 131 (ST131) *Escherichia coli* infections in a teaching hospital. The assay will be performed on 100 extended-spectrum- beta-lactamase (ESBL) *E. coli* isolates collected from UTI during last 5 years. Typing results will be compared to those of amplified fragment length polymorphism (AFLP), whereby we will be visually assessed the agreement of the Bio-Detection phylogenetic tree with clusters defined by AFLP. A microarray will be considered the gold standard for detection of resistance genes. AFLP will be identified a large cluster of different indistinguishable isolates on adjacent departments, indicating clonal spread. The BioDetection phylogenetic tree will be showed that all isolates of this outbreak cluster will be strongly related, while the further arrangement of the tree also largely agreed with other clusters defined by AFLP. With these experiments we will detect the ESBL and MBL strains and the patient can be prescribed the antibiotics accordingly.

Citation: Nayak, N. and Sahu, M.C. Next-Generation Sequencing for Typing and Detection of ESBL and MBL *E. coli* causing UTI [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 132. https://doi.org/10.24870/cjb.2017-a118
Category: Metagenomics

Salinity and macrophyte drive the biogeography of the sedimentary bacterial communities in a brackish water coastal lagoon

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Abstract

Coastal lagoons are represented by steep gradients in physical, chemical, and biological parameters and are regarded as one of the most productive ecosystems in the world. These lagoons are at an intermediate position between the freshwater and marine water systems. Huge amount of influx of organic matter and nutrient load with the freshwater inputs can be seen in such lagoons. The increased influx of organic matter and nutrients fuel in the lagoon increases the chance of eutrophication. The sedimentary microbial communities play an important role in preventing eutrophication by supporting a diverse assemblage of aerobic and anaerobic microbial communities. Considering the importance of sedimentary bacterial communities, numerous studies have investigated their ecological roles and biogeographical patterns in a variety of aquatic ecosystems. Compared to the marine and freshwater ecosystems, estuarine coastal lagoons are highly dynamic, still are poorly understood with respect to their sedimentary communities. Our hypothesis was that bacterial communities would exhibit biogeographical patterns which would be strongly associated with the biotic and abiotic factors. Using Illumina sequencing of the 16S rRNA genes from bulk surface sediments, we investigated the sedimentary bacterial communities, their spatiotemporal distribution, and compared them with the rhizosphere sediment communities of an exotic weed; P. karka and a native seagrass species; H. uninervis in a brackish water estuarine lagoon, Chilika (India). Comparison of bacterial communities with the environmental factor was done using Redundancy analysis. Spatiotemporal patterns in bacterial communities were linked to specific biotic factors (e.g., presence and type of macrophyte) and abiotic factors (e.g., salinity) that drove the community composition. Comparative assessment of communities highlighted bacterial lineages that were responsible for segregating the sediment communities over distinct salinity regimes, seasons, locations, and presence and type of macrophytes. Several bacterial taxa were specific to one of these ecological factors suggesting that species-sorting processes drive specific biogeographical patterns in the bacterial populations. Overall, this study provides a comprehensive understanding of the spatiotemporal dynamics and functionality of sedimentary bacterial communities in a tropical brackish water coastal lagoon and highlighted the role of biotic and abiotic factors in generating the biogeographical patterns in the bacterial communities.

Category: Metagenomics

Genome scale metabolic network reconstruction of *Spirochaeta cellobiosiphila*

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**Abstract**

Substantial rise in the global energy demand is one of the biggest challenges in this century. Environmental pollution due to rapid depletion of the fossil fuel resources and its alarming impact on the climate change and Global Warming have motivated researchers to look for non-petroleum-based sustainable, eco-friendly, renewable, low-cost energy alternatives, such as biofuel. Lignocellulosic biomass is one of the most promising bio-resources with huge potential to contribute to this worldwide energy demand. However, the complex organization of the Cellulose, Hemicellulose and Lignin in the Lignocellulosic biomass requires extensive pre-treatment and enzymatic hydrolysis followed by fermentation, raising overall production cost of biofuel. This encourages researchers to design cost-effective approaches for the production of second generation biofuels. The products from enzymatic hydrolysis of cellulose are mostly glucose monomer or cellobiose unit that are subjected to fermentation. *Spirochaeta* genus is a well-known group of obligate or facultative anaerobes, living primarily on carbohydrate metabolism. *Spirochaeta cellobiosiphila* sp. is a facultative anaerobe under this genus, which uses a variety of monosaccharides and disaccharides as energy sources. However, most rapid growth occurs on cellobiose and fermentation yields significant amount of ethanol, acetate, CO2, H2 and small amounts of formate. It is predicted to be promising microbial machinery for industrial fermentation processes for biofuel production. The metabolic pathways that govern cellobiose metabolism in *Spirochaeta cellobiosiphila* are yet to be explored. The function annotation of the genome sequence of *Spirochaeta cellobiosiphila* is in progress. In this work we aim to map all the metabolic activities for reconstruction of genome-scale metabolic model of *Spirochaeta cellobiosiphila*.

**Citation:** Manna, B. and Ghosh, A. Genome scale metabolic network reconstruction of *Spirochaeta cellobiosiphila* [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 134. [https://doi.org/10.24870/cjb.2017-a120]
Metagenomic analysis of microbial heterogeneity and stress response Mechanisms in Desert


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Abstract

Desert environments have high spatial heterogeneity evidenced by the occurrence of relatively low vegetation as small patches. Given the low vegetation cover, soil microorganisms are the main drivers of ecosystem processes in desert environments and thought to have unique stress-response and metabolic adaptation. Therefore, exploring the microbial spatial heterogeneity and metabolic adaptation is crucial for interpreting ecological patterns in the desert environment. In this study, we have evaluated the spatial heterogeneity of physicochemical parameters, soil microbial diversity and metabolic adaptation of microorganisms at meter scale. Soil samples were collected from two quadrates of 4x4 meter size from a desert environment (Bikaner, Thar Desert, India) which face hot arid climate with very little rainfall and extreme temperatures. Analysis of variance of the physicochemical parameters revealed that calcium and sulphate ions were the most important variables between the quadrates. Microbial diversity was studied using Illumina bar coded sequencing by targeting V3-V4 regions of 16S rDNA. As the results, 702504 high-quality sequence reads, assigned to 173 operational taxonomic units at the species level. The most abundant phyla in both quadrates were Actinobacteria, Proteobacteria, and Firmicutes. At the genus level, Gaiella was most abundant, followed by Streptomyces, Solirubrobacter, Aciditerrimonas, Geminicoccus, Geodermatophilus, Microvirga, and Rubrobacter. Between the quadrates, massive variation was found in the abundance of Aciditerrimonas (iron-reducing, moderately thermophilic), Geminicoccus (phototrophic), and Solirubrobacter. The automated metabolic functional mapping of abundant bacterial community revealed diverse activities, and correlated with physicochemical parameters of quadrates. For instance, relatively large numbers of bacteria were mapped with nitrogen and sulphur mineralizing activities in both quadrates. As the whole, there is a strong correlation between spatial variation of ions, microbial diversity and functional attributes in the studied quadrates, and patchy nature in local scale. Additionally, the relative abundance of Actinobacteria, a phylum with large number of industrially important bacterial species, in both quadrates suggests that the desert environment may be considered for bioprospection of bioactive natural products.

Inhibition of Drp-1 dependent mitochondrial fission augments alcohol-induced cardiotoxicity via dysregulated Akt signaling

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Abstract

Cardiovascular disorders (CVDs) still claim high mortality in spite of advancements in prognosis and treatment strategies. Alcohol is one of the most commonly consumed drugs globally and chronic/binge consumption (BAC 0.08 g/dL in 2 hours) is a risk factor for CVDs. However, the aetiology and pathophysiological mechanisms of alcohol induced cardiotoxicity are still poorly understood. Mitochondria are the prime site for the ATP demands of the heart and also ethanol metabolism. These subcellular organelles depict dynamic fusion and fission events that are vital for structure and functional integrity. While fused mitochondrial improve ATP production and cell survival, increased fragmentation can be the cause or result of apoptosis. In this study, we proposed to analyze the mechanism of mitochondrial fission protein Drp-1-dependent apoptosis during alcohol toxicity. Male Wistar rats (220-250 kg body weight) were given isocaloric sucrose or ethanol for 45 days, orally, via drinking water and intermittent gavage twice a week. Histopathological examination of the heart displayed hypertrophy with mild inflammation. Drp-1 immunoblotting showed over-expression of the protein during ethanol treatment. We next hypothesized that inhibiting Drp-1 could attenuate alcohol-induced cardiotoxicity. Interestingly, silencing Drp-1 with siRNA in-vitro augmented cytotoxicity. Also, crude mitochondrial fraction showed increased Bak aggregation, reduced cytochrome c release but increased SMAC/DIABLO. We analyzed the Akt cell survival signaling and found that PTEN showed over-expression at both transcriptional and translational level with no significant change in total Akt but down-regulation of p-Akt (Ser473). In conclusion, we have shown that inhibition of Drp-1 dependent mitochondrial fission is not cardioprotective against alcohol-induced apoptotic signaling and augments the cytotoxicity. To our knowledge, this study is the first to interlink cell survival AKT signaling as the cause for cytotoxicity during Bax/Bak dependent apoptosis, where inhibition of Drp-1 dependent fission fails to protect.

Citation: Sivakumar, A., Subbiah, R. and Balakrishnan, R. Inhibition of Drp-1 dependent mitochondrial fission augments alcohol-induced cardiotoxicity via dysregulated Akt signaling [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 136. https://doi.org/10.24870/cjb.2017-a122
Counter the Presence of Endotoxin- Reviewing Molecular and Downstream Approach

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Abstract

Endotoxins are major components of the outer membrane of gram negative bacteria. Endotoxin mainly includes lipopolysaccharides (LPS) which consist of O-antigen region, a core oligosaccharide and lipid A. LPS causes various side effects such as septic shock, hyperthermia, hepatic shock, etc. Exposure to endotoxin causes histological variations in inflammatory cells. Research at molecular level has revealed that ATPase activity is inhibited by endotoxin. LPS plays a crucial role in inhibition of mitochondrial functions and cellular transport system. Various attempts are being made for the treatment of deteriorating effects of endotoxins on immune system. These include use of anti-endotoxin antibodies and receptor molecules to block the endotoxin receptors. This work discusses over the aspects of removal of endotoxin by the ways of development of purification techniques and also highlights the research conducted to alter the release and/or affect the structure of endotoxin which may lead to reducing the ill-effects. Taking the molecular approach the effect of endotoxin could be reduced thereby inducing the reaction between small subunits of endotoxin and LPS inhibitors preventing the formation of large stable complexes thereby reducing its effect. Downstream approach is followed in order to remove the endotoxins as contaminant in the biological preparations. This can be achieved by improving the separation system of the biological molecules. Use of active biomolecules in place of synthetic chemicals may prove effective. This paper discusses the molecular and processing aspects to counter endotoxin.

A genomic insight into the origin and dispersal of Austroasiatic speakers in South and Southeast Asia

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Abstract

India and Southeast Asia are home to diverse linguistic groups; the Austroasiatic language group being one of them. The Austroasiatic speakers live in scattered settlements in these regions. What led to such dispersed distribution over this vast geographical space is yet to be resolved. Our work is aimed at reconstructing the migration route of early Austroasiatic settlers and examines their relationship with other linguistic groups. We genotyped 511 unrelated individuals from India and Malaysia out of which 189 were Austroasiatic. The rest belonged to Indo-European, Dravidian, Tibeto-Burman and Austronesian language families. Jarawa and Onge populations from Andaman and Nicobar Islands were also included. Our genotype data was combined with that of 940 individuals from HGDP dataset. We analyzed nearly 0.3 million autosomal SNPs and found that allele frequency correlation between Malaysian Austroasiatics and Indian Tibeto-Burmans was slightly higher ($R^2$= 0.77) than with Indian Austroasiatics ($R^2$= 0.72). Principal Components Analysis revealed that Malaysian Austroasiatic clustered closer to Tibeto-Burman than to Indian Austroasiatic. Similar clustering pattern was obtained by fineSTRUCTURE cluster dendrogram. The ADMIXTURE analysis inferred genetic component that is modal to the Malaysian Austroasiatic, is also significantly higher amongst Tibeto-Burman than Indian Austroasiatic ($P < 2.117e-10$), indicating that genetic distance correlates better with geography than language. Studying segments which were Identity by descent between individuals belonging to two different linguistic groups; i.e. Austroasiatic and Tibeto-Burman, we found Tibeto-Burman sharing larger number of segments with Malaysian Austroasiatic, but overall smaller in size. On the other hand the segments shared between the two Austroasiatic populations (India and Malaysia) are comparatively larger in size ($P= 0.034$) but smaller in number. Our analyses indicate that Malaysian Austroasiatic and Tibeto-Burman initially split from a common ancestor. Then a small group of individuals separated from Malaysian Austroasiatic giving rise to the present day Indian Austroasiatic. Treemix and D-statistics analysis provided evidence for gene flow between Malaysian Austroasiatic and Tibeto-Burman post split. Meanwhile, the southward migration of East Asians resulted in an extensive genetic exchange between East Asians and Tibeto-Burman as was evident in our ADMIXTURE analysis. This subsequent genetic exchange might have shaped the present day language structure.

Category: Miscellaneous

DisFace: A Database of Human Facial Disorders

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Abstract

Face is an integral part of human body by which an individual communicates in the society. Its importance can be highlighted by the fact that a person deprived of face cannot sustain in the living world. In the past few decades, human face has gained attention of several researchers, whether it is related to facial anthropometry, facial disorder, face transplantation or face reconstruction. Several researches have also shown the correlation between neuropsychiatry disorders and human face and also that how face recognition abilities are correlated with these disorders. Currently, several databases exist which contain the facial images of several individuals captured from different sources. The advantage of these databases is that the images in these databases can be used for testing and training purpose. However, in current date no such database exists which would provide not only facial images of individuals; but also the literature concerning the human face, list of several genes controlling human face, list of facial disorders and various tools which work on facial images. Thus, the current research aims at developing a database of human facial disorders using bioinformatics approach. The database will contain information about facial diseases, medications, symptoms, findings, etc. The information will be extracted from several other databases like OMIM, PubChem, Radiopedia, Medline Plus, FDA, etc. and links to them will also be provided. Initially, the diseases specific for human face have been obtained from already created published corpora of literature using text mining approach. Becas tool was used to obtain the specific task. A dataset will be created and stored in the form of database. It will be a database containing cross-referenced index of human facial diseases, medications, symptoms, signs, etc. Thus, a database on human face with complete existing information about human facial disorders will be developed. The novelty of the database lies in the fact that it is the first of its kind. The front end will be developed using HTML (Hyper Text Mark-up Language) and CSS (Cascading Style Sheets). The back end will be developed using PHP (Hypertext Pre-processor). JAVA Script will be used as scripting language and MySQL (Structured Query Language) will be used for database development as it is most widely used RDBMS (Relational Database Management System). XAMPP (X (cross platform), Apache, MySQL, PHP, Perl) open source web application software will be used as the server.

Citation: Kaur, P., Krishan, K. and Sharma, S.K. DisFace: A Database of Human Facial Disorders [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 139. https://doi.org/10.24870/cjb.2017-a125
Altered Expression of Angiogenic Factors in Follicular Fluid of Women with Polycystic Ovary Syndrome (PCOS)

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Abstract

Polycystic ovary syndrome (PCOS) is a common and heterogeneous disorder, affecting women at reproductive age. Follicular growth is arrested in PCOS leading to cysts formation and anovulation. Follicular fluid produced in the growing antral follicles provides the micro-environment for developing oocyte and contains several factors including proteins, steroids, polysaccharides, and metabolites that modulate oocyte developmental competence and ovulation. Our earlier study on comparative proteomics of follicular fluid (FF) has revealed alteration of several angiogenic factors and ECM proteins [1] in PCOS women indicating angiogenesis may be altered in PCOS. Angiogenesis is crucial for follicular growth, selection of dominant follicle, ovulation and further corpus luteum (CL) formation, and these processes are affected in PCOS.

Vascular endothelial growth factor A (VEGFA) and basic fibroblast growth factor (bFGF) are important angiogenic factors. We measured them in FF and serum by ELISA and observed higher level of VEGFA and lower level of bFGF in PCOS compared to control. The ECM proteins, heparin sulfate proteoglycan and fibronectin1 which plays role in angiogenesis were also downregulated in PCOS. The angiogenic capacity of FF from PCOS and Controls were evaluated by tube formation and scratch wound assay using human umbilical vein endothelial cells (HUVECs) and found to be altered in PCOS. Glycosylation is most abundant PTM and many of the angiogenic proteins found in our proteomic study undergo glycosylation and hence we carried out glycoproteomic analysis of FF by enriching glycoproteins using lectins followed by iTRAQ LC-MS/MS analysis. We found glycosylated SERPINA1, an anti-angiogenic protein to be up-regulated in PCOS. This indicates the follicular angiogenesis is altered in PCOS. Further studies are ongoing to gain more knowledge of angiogenic factors that are involved in PCOS pathophysiology and to develop new treatment strategies.

References


Citation: Patil, K., Yelamanchi, S., Gowda, H., Prasad, T.S. and Mukherjee, S. Altered Expression of Angiogenic Factors in Follicular Fluid of Women with Polycystic Ovary Syndrome (PCOS) [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India. Can J biotech, Volume 1, Special Issue, Page 140. https://doi.org/10.24870/cjb.2017-a126
Category: Miscellaneous

Isolation, Characterization, Screening, Formulation and Evaluation of Plant Growth Promoting Rhizobacteria

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Abstract

Plant growth promoting rhizobacteria (PGPR) are bioresources which may be viewed as a novel and potential tool for providing substantial benefits to the agriculture. Soil is the dynamic living matrix and the major source of food security providing various resources of plant growth and maintaining life processes. PGPR are originally defined as root-colonizing bacteria that cause either plant growth promotion or biological control of plant diseases. Chemical fertilizers are used for killing pathogens, increase crop yield but long term use of chemical fertilizers lead to adverse effect to the soil profile and is the reason for decrease in soil productivity, on the other hand PGPR promote plant growth directly by either facilitating resource acquisition (nitrogen, phosphorus and essential minerals) or modulating plant hormone levels, or indirectly by decreasing the inhibitory effects of various pathogens on plant growth and development in the forms of biocontrol agents. PGPR is the indispensable part of rhizosphere biota that when grown in association with the host plants can stimulate the growth of the host. PGPR seemed as successful rhizobacteria in getting established in soil ecosystem due to their high adaptability in a wide variety of environments, faster growth rate and biochemical versatility to metabolize a wide range of natural and xenobiotic compounds. Isolated PGPRs from selective crop rizosphere soil were used for further growth promotion and biocontrol studies in the green house and field. Different studies have been carrying out to develop some new bioformulations and evaluate their efficacy in promoting crop seedlings growth characteristics. Field trials were performed to evaluate selective crops with formulations of several plants PGPR in a production system. The present review highlights the Plant growth promoting rhizobacteria as an alternative of chemical fertilizer for sustainable, environment friendly agriculture.

Detection of glucose level using a novel biodegradable sensor substrate

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Abstract

Diabetes is a metabolic disorder which causes unstable blood sugar level because of improper supply of insulin or due to the body’s poor response to insulin. Genetics and life style choices are a major cause to this chronic disorder. Glucose is marked as one of the best biomarkers for the detection of diabetes. With the increase in diabetic population, there is a huge demand for glucose biosensors. Treatment and control of diabetes is possible with continuous monitoring of blood glucose level. A cost-effective bedside monitoring kit is the immediate solution for the diagnosis of diabetes. The test strips available with the current point of care diagnostics kits are costly and non-biodegradable. This work proposes a biodegradable substrate made of ‘thaliyola’ which portrays the intrinsic value of Indian traditional leaves as base material for diagnostics. This study deals with an enzymatic biosensor where glucose oxidase and horse raddish peroxidase reacts with glucose whose reaction is estimated with help of potassium iodide resulting in a color change. The color variation obtained from different glucose concentrations is captured using imaging equipment for further analysis. Image of size 80 * 80 pixel was selected from the scanned samples, whose mean red component was estimated using scientific computing tool. A consistent trend was observed between the red component intensities and glucose levels taken for the study. The correlation between the concentration of glucose and mean red component proves the use of ‘thaliyola’ as a good substrate material. In future, this opens a possibility to develop cost effective and biodegradable glucose level estimation devices.

References


Wnt and inflammatory pathway mediated differential expression of water channel protein in glaucomatous trabecular meshwork

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Abstract
Aquaporin (AQP) is an integral membrane protein which helps in the transport of water and small solutes [1]. The functions of AQP0 and AQP6 have not been studied in Glaucomatous trabecular meshwork. Intra-ocular Pressure (IOP) is regulated by Wnt signaling but till date no report has been published in patient trabecular meshwork tissue [2]. Effect of GSK3β (a Wnt signaling regulator) on IFNβ, an important immune-modulatory cytokine, has not been studied in Glaucoma. This study explores the cross talk between Wnt signaling and inflammatory pathway in context of water channel proteins’ expression. Trabecular meshwork tissue after glaucoma surgery was collected with prior approval of the Institutional Ethics Committee and written informed consent. AXIN2, IFNβ, IL6, AQP0, AQP6 and AQP9 gene expression analysis was done in trabecular meshwork tissue from glaucoma filtration surgery patients (n=32) and cadaveric controls (n=20). Human Trabecular Meshwork (HTM) cells, cultured in DMEM with 10% FBS and 1% PSA were treated with Wnt activator (AZD2858; GSK3β inhibitor) or inhibitor (XAV939; β-catenin inhibitor) and analysed after 24 hrs for expression of the same genes. In patient TM samples, we observed a downregulation of AQP0, AQP9 (p=0.002) and significant gain in AQP6 (p≤0.03) and IL6 (p=0.04) associated with increased Axin2 (p=0.04) and reduced IFNβ (0.01) when compared to controls. Activation of Wnt pathway (using chemical activator AZD2858) in HTM culture mimics the patient data demonstrating a reduction in AQP0, AQP9 and IFNβ expression, but significantly induced Axin2, AQP6 and IL6. Wnt inhibitor XAV939 reversed the same observations. This is the first study that illustrates the role for water channel proteins’ expression such as AQP0, AQP6 and AQP9 in glaucomatous trabecular meshwork. An inverse correlation was observed between expression of AXIN2 (Up-regulated) and IFNβ (down-regulated) in Glaucomatous trabecular meshwork and HTM cells which may be associated with inhibition of GSK3β. The data reveals interaction between Wnt and IFNβ as a possible mechanism that regulates expression of critical AQPs i.e. AQP 0, 6 and 9 with subsequent loss of hydrostatic and osmotic balance in glaucoma. Our observations suggest feasible role of Wnt inhibitors as therapy for glaucoma.

References

Plant extract AE11 acts as a potent modulator of adipocyte development and function

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Abstract

Use of herbal formulation to modulate adipocyte development and function has been argued as a potent strategy to tackle alarmingly increasing problem of obesity and associated disease like type 2 diabetes and other cardiovascular problems. However, till date no single formulation exists with good efficacy and little side effects. In this study we are investigating the effect of AE11 (6% v/v, nontoxic to 3T3-L1), a plant extract prepared using cold maceration of shade dried leaves of a common flowering plant of Lakhimpur district of Assam, on 3T3-L1 pre-adipocyte differentiation and function. AE11 very efficiently decreased lipid accumulation in differentiating 3T3-L1 cells. To understand the mechanism of such inhibition, we performed gene expression analysis using semi-quantitative PCR for adipogenic master regulator PPARγ1 and PPARγ2. A marked reduction in expression of both of the genes were observed in AE11 treated differentiating 3T3-L1 cells. Western blot analysis confirmed reduction of the two factors at protein level as well. Not surprisingly PPARγ downstream GLUT4, PLN1, FABP4, FAS and LPL mRNA content was also reduced in treated groups. Interestingly mRNA content of the transcription factor GATA2, which is a negative regulator of PPARγ expression and is normally downregulated during adipogenesis, found to be very high in the AE11 treated cells. This raised a possibility of GATA2 mediated downregulation of PPARγ in AE11 treated groups. GATA3 mRNA content was however not different in treated and untreated groups. mRNA of CCAAT enhancer binding protein α (CEBPα) which is a positive regulator of PPARγ expression was decreased by AE11 exposure to 3T3-L1 cells during differentiation. AE11 targeted the expression of another positive regulator of PPARγ expression, SREBP1c. SREBP1c mRNA content was decreased upon AE11 treatment in differentiating 3T3-L1 cells. These preliminary results suggest AE11 is an effective modulator of adipocyte development and function by targeting positive and negative regulators of PPARγ gene expression. The authors thank Department of Biotechnology (DBT) for providing fellowship and funds to carry out the work.

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Modulation of inflammation and autophagy pathways by trehalose containing eye drop formulation in corneal epithelial cells: implications for dry eye disease

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Abstract

Ocular surface inflammation is an immunological perturbation activated in response to various adverse conditions and is a key biomarker to understand the disease pathology and its underlying immunological landscape [1]. The molecular link between inflammation and autophagy, often implicated in disease conditions, is poorly understood. The aim of this study is to understand the regulation of inflammation signaling pathways by using a well-established modulator of autophagy, trehalose (TRE), on desiccation stress-induced inflammation in SV40 immortalized human corneal epithelial cells. To mimic the dry eye condition, HCE cells were exposed to desiccation stress at 80% confluency in a six well tissue culture plate. The medium was completely aspirated and cells were kept for drying at room temperature for 10 min. Fresh medium with TRE was added and incubated for 6 hrs. The regulation of induced inflammatory and autophagic gene expression and protein activation by TRE formulation (1.2%) was studied. Optimal drug treatment concentrations were determined by dose escalation cytotoxicity studies. Gene expression was evaluated by quantitative PCR, while protein expression and functions were tested by immunoblotting and fluorescence imaging (Cyto-ID, Lysotracker Red). TRE formulation was able to rescue the morphological changes due to desiccation stress. Live to dead cell ratio increased upon TRE treatment. TRE treatment reduced inflammation induced gene expression of IL-6 (2%), MCP-1 (33.31%), IL-8 (9.56%), MMP-9 (18.96%), and TNFα (58.16%) in HCE. Active form of p38, p44/42, and p65 protein levels were altered significantly by TRE treatment. LAMP1 and LC3 autophagy protein markers were also altered with desiccation stress and TRE treatment. The data demonstrate that TRE formulation is effective in reducing desiccation stress induced inflammation in HCE. Further increased phosphorylation of p38, p44/42 and elevated levels of LC3 and LAMP1 suggest that induction of autophagy. This could be a protective mechanism of autophagy in the desiccation stress model. All together our data suggest that TRE may have a novel role on reducing inflammation through autophagy in HCE. Therefore, TRE might be a potential therapeutic for ocular surface treatment.

References


Role of Vegetarian Diet in preventing diabetes in population practicing sedentary lifestyle: A case study in Eastern region of India

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Abstract

India in twenty-first century has seen a rapid transformation in dietary convention, with immoderate intake of calorie-rich food along with a sedentary lifestyle. The prevalence of type 2 diabetes (T2D) is quite alarming and observed to be 1.6 to 2 times as high among non-vegetarians (NV) compared to vegetarians. Dietary factors and physical activity are two major factors in T2D predisposition and disease management. Recent studies have shown that physical activity and vegetarian diets improve insulin sensitivity and glycemic control. The current investigation was carried out to observe the effect of diet in two Indian communities practicing sedentary lifestyle through a retrospective cross-sectional study. Depending on the lacto vegetarian diet (LV) and non-vegetarian dietary patterns in individuals, the study population was divided into two groups. Two Indian communities namely Jain and Marwari as LV and Odia as NV those are residing in Bhubaneswar, Odisha were considered for this study. The survey was conducted from January 2015 to April 2015. A total of 403 participants (253 male and 150 female) aged 30-80 years were enrolled in the study. Individuals undergoing medication for any known diseases, such as diabetes mellitus, rheumatoid arthritis, etc. including pregnant women or those with polycystic ovarian syndrome were also excluded from the study. Fasting blood samples were analyzed for blood sugar, glycated hemoglobin (HbA1C), and lipid profile. Body mass index (BMI) and waist circumference (WC) measurements were also recorded. The incidence of T2D was lower in lacto-vegetarian (1.7%) than in NV group (5.3%) despite similar lipid profiles and BMI/WC between these two groups. Fasting blood sugar (FBS) was positively correlated with LDL and VLDL levels and negatively correlated with HDL, only in lacto-vegetarian group. The study ignited that although the sedentary lifestyle and fat-rich diet of the LV group had an effect on individual’s overall lipid profile and BMI/WC, the LV diet practiced has beneficial effects on blood sugar levels/T2D. The study suggests that a shift to an LV diet may be helpful for those leading an inactive lifestyle by compulsion.

Category: Miscellaneous

Host selection, multiple blood feeding behaviour and plasmodium parasite infection of Anopheles vector in Kalahandi district, Odisha, India

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Abstract

Identification of host preference and presence of Plasmodium parasite in field collected mosquito are important parameters to measure for effective vector control of malaria. The objective of this study was to identify mammalian blood meals and Plasmodium parasite from a single mosquito by using multiplex PCR assay. The blood specific primer set for multiplex PCR of Human, Cow, Goat and Buffalo targeting mitochondrial cytochrome b was developed to identify blood meals of field collected mosquitoes. The plasmodium specific primer set for multiplex PCR of P. falciparum, P. vivax, P. malariae, P. ovalae was developed to identify presence of parasite in mosquitoes. 342 female Anopheles mosquito species viz Anopheles culicifacies, Anopheles fluviatilis and Anopheles subpictus were collected for analysis of blood meal and the positive human blood fed mosquito was analyzed for the presence of sporozoites. The overall human blood index was found to be 41%, 17%, 23% in An. culicifacies, An. fluviatilis and An. Subpictus, respectively. 150 of human blood fed mosquito were harbouring Plasmodium parasites, 1.4% of which were confirmed to P. falciparum. In addition to An. Fluviatilis, An. culicifacies were also found positive for malaria parasites. The present study exhibits the human feeding tendency of Anopheles vectors highlighting their malaria parasite transmission potential. The present study may serve as a model for understanding the human host preference of malaria vectors and detection of malaria parasite inside the anopheline vector mosquitoes in order to update their vectorial status for estimating the possible role of these mosquitoes in malaria transmission. The study has used PCR method and suggests that PCR based method should be used in this entire malarious region to correctly report the vectorial position of different malaria vectors.

Effect of *Wolbachia* on Dengue infection in Endemic districts of Odisha

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Abstract

Dengue is the most important arboviral disease posing considerable threat to human and animal health in tropical and subtropical countries. The causative agent for dengue viruses (DENV) are primarily the infectious female *Aedes aegypti* mosquitoes and to a lesser extent its sister taxon infectious female *Aedes albopictus* mosquitoes. Persistent DENV infections play a role in the cycling pattern of dengue outbreaks. Due to lack of proper treatment, strategies for blocking pathogen transmission by mosquito vectors have been proposed as a means of augmenting current control measures to reduce the growing burden of vector-borne diseases. In this scenario, the use of *Wolbachia* has been proposed to reduce dengue transmission. *Wolbachia*, a gram negative endosymbiont bacterium is naturally present in over 20% of all insects including *Aedes albopictus* mosquito. In our study, polymerase chain reaction (PCR) was used to determine the presence of *Wolbachia* from field collected *Ae. albopictus* from various parts of the Odisha using wsp primers. *Ae. albopictus* had *Wolbachia* infection ranging from 65 to 100%. Field collected *Wolbachia* infected mosquitoes were challenged with DENV infection. At seven days following infected blood-feeding, an increase in *Wolbachia* densities was displayed to a greater extent compared to control mosquitoes. Our result indicates that virus-blocking is likely to persist in *Wolbachia*-infected mosquitoes suggesting that *Wolbachia* may serve as a successful biocontrol strategy for reducing dengue transmission in the field.


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Induction of apoptosis by Fe(salen)Cl through caspase-dependent pathway specifically in tumor cells

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Abstract

Iron-based compounds possess the capability of inducing cell death due to their reactivity with oxidant molecules, but their specificity towards cancer cells and the mechanism of action are hitherto less investigated. A Fe(salen)Cl derivative has been synthesized that remains active in monomer form. The efficacy of this compound as an anti-tumor agent has been investigated in mouse and human leukemia cell lines. Fe(salen)Cl induces cell death specifically in tumor cells and not in primary cells. Mouse and human T-cell leukemia cell lines, EL4 and Jurkat cells are found to be susceptible to Fe(salen)Cl and undergo apoptosis, but normal mouse spleen cells and human peripheral blood mononuclear cells (PBMC) remain largely unaffected by Fe(salen)Cl. Fe(salen)Cl treated tumor cells show significantly higher expression level of cytochrome c that might have triggered the cascade of reactions leading to apoptosis in cancer cells. A significant loss of mitochondrial membrane potential upon Fe(salen)Cl treatment suggests that Fe(salen)Cl induces apoptosis by disrupting mitochondrial membrane potential and homeostasis, leading to cytotoxicity. We also established that apoptosis in the Fe(salen)Cl-treated tumor cells is mediated through caspase-dependent pathway. This is the first report demonstrating that Fe(salen)Cl can specifically target the tumor cells, leaving the primary cells least affected, indicating an excellent potential for this compound to emerge as a next-generation anti-tumor drug.

To study role of clinical variables and VKORC1 polymorphism in determination of stability of INR in neurological patients on acenocoumarol

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Abstract

Oral anticoagulation (OAC) is difficult to maintain in therapeutic range and their efficacy may be influenced by number of clinical and genetic variables. The objective of the study is to evaluate the role of VKORC1 polymorphism and correlate with stability of anticoagulation. It is a hospital based study. Patients on OAC were included during 2013-2016. The patients received OAC for cardioembolic stroke, cortical venous sinus thrombosis (CVST) and prevention of deep vein thrombosis (DVT). Demographic, clinical and neurological findings were recorded. Stability of OAC was determined by percentage of international normalized ratio (INR) values in therapeutic range (PINR). PINR >65% were defined as stable and <65% was defined unstable. VKORC1 polymorphism was studied by polymerase chain reaction and was related to daily dose of OAC and stability of INR. 157 patients with median age of 40 years were included. Ninety two patients received OAC for secondary stroke prevention, 62 for CVST and 3 for DVT. Out of 2976 INR reports, 1458 (49%) were in the therapeutic range, 997 (33.1%) were below and 521 (17.5%) above the therapeutic level. Stable INR was obtained in 75 (47.77%) patients only and was improved by drug modification in 3, and dietary adjustment in 12 patients. VKORC1 polymorphism revealed GG in 127 (80.9%), GA in 22 (14%) and AA genotype in 8 (5.1%) patients. Therapeutic range of INR was seen in 49%, below therapeutic range in 31.5% and above in 17.5%. VKORC1 polymorphism was related to mean daily dose of OAC but not to stability of INR.

References


Evaluation of a topical herbal drug for its in-vivo immunological effect on cytokines production and antibacterial activity in bovine subclinical mastitis

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Abstract

Mastitis is an inflammatory disorder caused by microorganisms. Currently antibiotics have been mainstay of mastitis therapy. However, their use is associated with cost issue and human health concern. Some herbs exert beneficial effects on bacterial pathogens through immunomodulation by influencing cytokine production. To assess the effect of herbs on cytokine profile, total bacterial load and somatic cell count in two breeds of cattle harboring subclinical mastitis. The response to treatment was evaluated by enumerating somatic cell count, total bacterial load and studying the expression of different cytokines (IL-6, IL-8, IL-12, IFN-Ɣ and TNF-α). The expression profiles were carried out using real time PCR, by collecting milk on days 0 as well as 5 and 21 post last treatment and data were analyzed using Statistical analysis system software. Pre and post treatment SCC in mastitic quarters statistically did not differ significantly, however, total bacterial load declined significantly from day 0 onwards in both the breeds. Highly significant differences (P < 0.01) were observed in all the cytokines on day 0, 5, and 21 post last treatments in both the breeds. The comparison between crossbred and Gir cattle revealed a significant difference in expression of IL-6 and TNF-α. However, other cytokines exhibited a similar pattern of expression in both breeds, which was non-significant. The topical herbal drug exhibited antibacterial and immunomodulatory activities and thus the work supports its use as an alternative to antibiotics against subclinical udder infection in bovines.

Citation: Kher, M., Bhatt, V., Sheth, N., Kunjadia, A., Nauriyal, D. and Joshi, C. Evaluation of a topical herbal drug for its in-vivo immunological effect on cytokines production and antibacterial activity in bovine subclinical mastitis [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 151. https://doi.org/10.24870/cjb.2017-a137
Assessment of Health problems and Genetic Damage in Residents of Uppal Industrial Area

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Abstract

Uppal and Patancheru are two important industrial zones in twin cities. Since 1980’s many industries such as pharmaceutical drugs, paints, pesticides, steel, plastics, textiles, leather, rubber, etc. were established. Many a time complaints of Industrial Pollution from residents of Uppal industry area were received by the government with a request to kindly take necessary step to prevent industrial pollution in this area. Although very few studies were carried out on people living here and hence an attempt was made to take up a large scale study. Epidemiological, genetic, cytogenetic and molecular studies were carried out in subjects living in Uppal industrial area. For comparison the residents of Ameerpet were selected (control group). 560 people residing in and around Uppal Industrial belt were selected for epidemiological studies and for comparison people living in non-industrial area (Ameerpet) and not exposed to either agricultural or industrial chemicals occupationally were selected (Control group). The results showed an increased frequency of health problems when compared to control group. The prominent health problems were hypertension, diabetes, renal problems, skin problems, gastrointestinal disorders, infections, nervous disorders, muscular pain, respiratory disorders, and weakness/lassitude. Reproductive outcome of 175 couples living in this area was studied. The reproductive outcome was assessed by measuring the incidence of abortions, stillbirths, malformations, prenatal deaths, etc. The results showed a decrease in the fertility (87.33% against 88.88% in control group) and a high incidence of abortions (8.41% against 5.09% in control group). Studies on incidence of chromosomal aberrations and sister chromatid exchanges in 60 people living here were taken up and results shown the increased levels of cytogenetic damage when compared to the controls.

Category: Miscellaneous

Ecology and host-symbiont interactions drives the strain-specific association of Wolbachia with Indian Drosophila host

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Abstract

The discovery of Wolbachia, a gender bender of insect population revolutionized the field of medical biology with the possibility of using Wolbachia infected host to control the spread of major vector borne diseases. Drosophila has long been known as an invertebrate experimental model and the presence of Wolbachia strains in this species makes it unique for the entomologist to explore the different Wolbachia strains present in the Drosophila host and understand host-symbiont interactions. In the light of these developments we obtained whole genome sequences of four different Wolbachia strains using NGS technology based on Illumina platform isolated from two human commensal Indian Drosophila species i.e. Drosophila melanogaster and Drosophila ananassae from Kochi (Southern India) and Ahmedabad (Western India). The strains were identified as wMel to be present in D. Melanogaster and wRi in D. Ananassae based on BLAST analysis. This was the first attempt at obtaining the whole genomes of Indian Wolbachia strains. Further we compared these novel genome assemblies and observed strain-specific sequence similarity in certain gene sequences known for their significant role in strain identification or host symbiont interaction. Upon comparison of the Indian Wolbachia genomes with the earlier sequences one, we also observed India specific sequence similarity in some of the genes. In order to understand better the Wolbachia-host association we also studied the associated bacterial diversity in these two Drosophila species. The distribution of the associated bacterial species was found to be both species as well region dependent. The findings of the current study support that ecology also plays a major role in gene-genome evolution and host preferences. The results of the above study provide us an overview of Wolbachia-host interaction and strain specific nature of this endosymbiont. However, in order to further understand the exact forces at play that makes such an association, deeper study needs to be carried out.

References


Citation: Singhal, K. and Mohanty, S. Ecology and host-symbiont interactions drives the strain-specific association of Wolbachia with Indian Drosophila host [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 153. https://doi.org/10.24870/cjb.2017-a139
Effect of Lead acetate on oxidative stress and antioxidant defence system of *Bacillus subtilis* and plasmid (pBSIISK) isolated from DH5α

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Abstract

Environmental contamination by heavy metals has been one of the major concerns for ecological and public health. Although some heavy metals are required for metabolic processes, but their excessive accumulation in living organisms is always detrimental. High concentration of lead affects all living organisms including soil flora, fauna and microorganisms. Presence of such heavy metals in environment could certainly cause the decrease in the community diversity. This study was aimed to investigate the effect of lead acetate on growth and antioxidant defence system of *Bacillus subtilis* in dose (0, 0.125, 0.25 and 0.5 mM) and time (6, 12, and 24 h) dependent manner, and also assess its deleterious effects on plasmid-pBSIISK isolated from DH5α strain. The results indicate that the cell number was declined significantly with increase in concentration of the heavy metal at different time of their growth phase. Lipid peroxidation (LPx) and reduced glutathione (GSH) levels were significantly enhanced in response to lead acetate, whereas the activities of antioxidant enzymes, superoxide dismutase (SOD) and catalase (CAT) were decreased in presence of lead acetate. Glutathione S-transferase (GST) activity was increased at 6 h and 12 h, but decreased at 24 h in response to lead acetate. *In vitro* study indicates that lead acetate potentially damage the plasmid (pBSIISK) isolated from DH5α strain.

References


Citation: Patri, S., Sahu, S., Parida, B., Baral, B., Prusty, A., Samanta, L. and Jena, S. Effect of Lead acetate on oxidative stress and antioxidant defence system of *Bacillus subtilis* and plasmid (pBSIISK) isolated from DH5α [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 154. [https://doi.org/10.24870/cjb.2017-a140](https://doi.org/10.24870/cjb.2017-a140)
Effect of ethanolic bark extract of the mangrove plant *Xylocarpus granatum* on oxidative stress indices in streptozotocin-induced diabetic mice testis

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Abstract

Pervasiveness of Diabetes Mellitus is advancing all over the world and uncontrolled state of hyperglycemia due to defects in insulin signaling results in severe complications including deleterious effect on male reproductive function where oxidative stress is implicated. The objective of this study is to investigate the effect of different concentration of *Xylocarpus granatum* bark extract (Xg) and compare the effects with commercial drug Glibenclamide (Glb) in testis of streptozotocin (STZ) induced diabetic BALB/c mice as a function of oxidative stress. Mice were divided into 6 groups, viz; Group-I: Control, Group-II: 200 mg Xg/Kg/day body weight, Group-III: STZ (125 mg/kg/day body weight), Group -IV: STZ+Glb (3 mg/kg /day body weight), Group-V: Xg(100 mg/Kg/day body weight), Group-VI: Xg (200 mg/Kg/day body weight) were administered orally for 30 days. The results showed low sperm count with increased lipid peroxidation in testis of diabetic mice than other groups with diminished antioxidant enzymes (Superoxide dismutase and catalase) which was restored by 200 mg/day/kg treatment with Xg extract. On the other hand, though significant alterations in testicular glutathione content and activities of Glutathione Peroxidase/reductase and Glutathione S transferase was observed in response to the treatments; the plant extract didn’t show a linear dose response. Since Glb has a greater risk of cholestatic jaundice and is not recommended to patients with G6PD deficiency, this natural product may be developed into an anti-diabetic drug. However, more linear dose response titrations are necessary to validate the efficacy of the extract.

References


Partial purification and sugarcane bagasse induction of extracellular thermostable Amylase by Bacillus sp. under submerged fermentation

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Abstract

Enzyme with relatively low stability shows an industrial incompetence which creates an urgent need of potential enzymes with high stability in a cost effective way. Thus, thermostable enzymes derived from microorganism are the alternative resources for convenient industrial application. Our present study was aimed for production of thermostable amylase with wide range of pH and temperature stability, and induction of over production of the enzyme by using sugarcane bagasse as the sole carbon source. The strain was an isolate from railway track of Cuttack railway station, Odisha, India. Morphologically the strain was identified as Bacillus species by gram staining, and a potential amylase producer in normal Luria Bertani (LB) broth at 60°C. The optimum extracellular amylase production observed was 0.085 U/ml after 6 hours at 60°C under submerged culture condition. Extracellular amylase was precipitated through ammonium salt fractionation, and partially purified by Sephadex G-50 column chromatography. The partially purified protein under SDS-PAGE was found to be resolved into three distinct bands of molecular weights approximately 108.69 kDa, 78.12 kDa and 65.63 kDa. The amylase enzyme showed wide range of temperature stability till 90°C and pH stability from pH 5 to 8, respectively. However, the optimum stability of amylase was found at 90°C in pH 8. The induction study has been carried out with abundantly available agro-industrial waste product sugarcane bagasse for over production of amylase in four different concentrations (0.5%, 1.0%, 2.0%, and 5.0%). The highest amylase production observed was 0.190 U/ml with 5.0% inducible substrate after 10 hours of submerged culture condition at 60°C. Thus, the strain is found to be a potential producer of extracellular amylase and further studies are needed for the optimization of increased production and induction of other possible enzymes by different inducible substrates.

References


Direct LPS recognition and activation of CD8+T cells via TLR4 in patients with rheumatoid arthritis

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Abstract

Background: Rheumatoid arthritis (RA) is an autoimmune disease characterized by abnormal immune responses to self-antigens. Though the pathogenesis of RA is not yet fully elucidated, it is known to be induced by environmental factors on a genetically susceptible background. Toll-like Receptors (TLRs) have been established to recognize specific patterns of microbial components and lead to systemic immune responses in Rheumatoid arthritis (RA). TLRs are expressed by cells in inflamed joints of RA patients and variety of endogenous TLR ligands is present within those joints. This study suggests that the over expression of TLR4 in CD8+T cells from RA patients may contribute to the abnormal immune activation of pro inflammatory cytokines and enhance the acute inflammation.

Methods: Eighty seven RA patients and 70 healthy donors participated in this study. Clinical variations like disease duration, number of actively inflamed joints, number, and type of bones deformities, CRP, RF, Anti-CCP, ESR (Erythrocyte Sedimentation Rate), and therapeutic interventions were recorded for each patient and DAS 28 scores were calculated with the help of the clinician. We analyzed the expression of TLR4 in transcript level by real-time PCR and protein level by flow cytometry in CD8+T cells of RA patients. Different cytokines level was checked after stimulation of CD8+T cells in TLR4 agonist. We have checked the MAP Kinase – ERK signal transduction in CD8+T cells.

Results: A significant increase of TLR4 in both transcript level and protein level in patients with RA compared to healthy donors. We got a strong positive correlation between TLR4 expression and DAS 28 score. The ROC curve analysis confirmed the significance of TLR4 expression in RA patients. We found that TLR4 ligand responsiveness significantly increased the expression of different inflammatory mediators in purified CD8+T cells of RA patients compared with healthy individuals after in vitro stimulation. Our result showed TLR4 stimulation induces ERK phosphorylation in CD8+T cells.

Conclusion: In summary, our data suggest an increased expression of TLR4 in CD8+T cells play a major role in inflammation of RA patients.

Category: Miscellaneous

Tnf-α negatively regulates Th2 differentiation in humans

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Abstract

TNF-α is a pleotropic cytokine with multiple physiological functions and is mainly produced from monocytes, macrophages, DCs and T-cells. Along with its physiological functions TNF-α is also a therapeutic target in rheumatoid arthritis, cerebral malaria, sepsis, IBD and psoriasis. However its role in T helper cell differentiation and homeostasis in the above diseases is unclear. In this study we show that TNF-α selectively inhibits the differentiation of human Th2 cells without affecting Th1 and Th17. TNF-α decreased the ROS generation there by lowered the Erk1/2 phosphorylation in differentiating naïve Tells there by down modulated the IL4 synthesis. Whereas cells treated with Anti TNF-α has shown completely opposite effect. Consequently increased TNF-α in T2DM and RA may account for the lowered Th2 differentiation and their increased AICD sensitivity thus exacerbating pre-existing inflammation.

Citation: Tagirasa, R. Tnf-α negatively regulates Th2 differentiation in humans [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 158. https://doi.org/10.24870/cjb.2017-a144
Category: Miscellaneous

Identification of flavonoids of Wheatgrass (*Triticum aestivum* L.) at various stages of growth and evaluation of their Antioxidant Activity

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Abstract

Wheatgrass are 6-10 days young plantlet of wheat (*Triticum aestivum* L.). Ethopharmacologically, the wheatgrass is recognized for its anti-aging and other health promoting properties. This study was aimed at identifying the phytochemical composition of wheatgrass (var. Chinese Spring) and evaluating its antioxidant potentials at different stages of growth (5th, 7th, 9th, 11th, 13th, 15th day). Phytochemical components were extracted with methanol and total phenolic contents (TPC) and total flavonoid contents (TFC) of the extracts were evaluated. DPPH (1,1′- diphenyl-2-picrylhydrazyl), bleaching of β-carotene and metal chelating activity was used to assess the antioxidant activity of the extract and further correlated with TPC and TFC. Based on comparison of IC₅₀ data, DPPH radical scavenging activity of the extract was found to be best at 7th day of the growth and equivalent to standard gallic acid. The extract exhibited excellent metal chelating activity and β-carotene bleaching property on 9th day of growth and the IC₅₀ corresponded with standard ascorbic acid and butylated hydroxytoluene (BHT), respectively. Preliminary screening with thin layer chromatography identified the probable flavonoids which could be quercetin, rutin or myricetin and their glucosides. Significant correlation between antioxidant activity with TPC and TFC ascertained that phenolics and flavonoids were the major contributors of antioxidant activity.

Y-shaped morphology in *E. coli* may be linked to peptidoglycan synthesis Pathway

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Abstract

Maintenance of cell shape is a crucial feature among all the kingdoms of life. This is required for performing all the basic functions like sensing, motility, surface attachment and nutrient intake, which is also known to be regulated genetically. *E. coli* is a rod shaped bacteria separated into two parts, the cylindrical central region and a curved or caped polar ends. During cell division bacteria divides at midcell into two identical daughter cells, where the new curved cap form at the midcell. Cell wall and a protein mesh beneath its peptidoglycan layer is known to maintain the rod shaped morphology and MreB is one important protein known to be involved in maintaining the bacterial shape. It has been observed that deletion of MreB results in round shaped bacteria. So, one important question that needs attention is how MreB is involved in maintaining different shapes at poles and mid cell.

The cell shape maintenance is thus probably a coordinated event between pool of proteins and a feedback system gives response to form correct cell shape. We have serendipitously discovered a new Y shaped and X-shaped morphology of *E. coli* cells. The branches to form Y or X shaped phenotypes were observed to be originating from either pole or mid cell regions. When we investigated it further by labelling peptidoglycans and looking at membrane architecture we observed active peptidoglycan in pole regions. Since the cells were not showing any rounded morphology we assume that MreB is intact in the genome and some other pathway is involved in maintaining these unique shapes and thereby also involved in regulating cell shape in *E. coli*. Based on our initial investigation we hypothesize that besides MreB, synthesis of PG and conversion of active form of PG to inactive form is also playing an important role in maintaining cell shape. We aim to perform whole genome sequencing and look at transcriptome level to dissect the pathway for maintaining these unique shapes in bacteria.

Citation: Mallick, S. and Beuria, T.K. Y-shaped morphology in *E. coli* may be linked to peptidoglycan synthesis Pathway [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 160. https://doi.org/10.24870/cjb.2017-a146
NCoR1 is a master repressor of the tolerogenic program in dendritic cells

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Abstract

The therapeutic potential of tolerogenic dendritic cells (DCs) for autoimmune disorders and transplantation has been widely proposed. We show here for the first time that nuclear receptor co-repressor 1 (NCoR1) strongly represses the tolerogenic program in activated DCs despite of its known immunogenic role in macrophages that intriguing the paradigm of immune regulation in this lineage through NCoR1. DC specific conditional NCoR1 knock out (NCoR1DC-\textsuperscript{-/-}) mice also shows tolerogenic behavior as we found in our cell line NCoR1 KD CD8\textsuperscript{a+} DCs and consequently increase in Treg cells \textit{in-vivo} or \textit{in-vitro}. Bacterial and parasitic infection in NCoR1DC-\textsuperscript{-/-} animals enhanced Treg development with a concomitant increase in disease burden. Likewise, adoptive transfer of activated NCoR1 KD DCs in helminth-infected mice increased both Tregs and intestinal worm load which suggest NCoR1 as a direct switch controlling tolerogenic genes in DCs. Next we employed integrative genomics approach to dissect the tolerogenic mechanism of NCoR1 in CD8\textsuperscript{a+} DCs in which we found globally NCoR1 prevent most of the tolerogenic genes from recruiting activating transcription factor RelA which allows these cells to mount pathogen specific immune responses. Interestingly we also found most of these tolerogenic genes were marked by super enhancers which might be super repressed by NCoR1 suggesting NCoR1 as a global co-repressor for tolerogenic program in DCs and highlight novel mechanism of tolerogenicity in DCs via NCoR1 which can be flourished in different autoimmune disease pathogenesis.

Analysis of ground water and soil samples from severely arsenic affected blocks of Murshidabad district

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Abstract

Contamination of groundwater and soil by arsenic is a serious threat to existence of mankind on the globe. Arsenic contaminates soil and groundwater by natural biogeochemical cycles. However, due to anthropogenic activities like indiscriminate use of arsenic in disinfectants, weedicides, medicines and fertilizers, arsenic toxicity is a severe environmental issue, both at national and global level. U.S. Environmental Protection Agency and World Health Organization prescribed the permissible limit of arsenic in drinking water to be 10 µg/l. Exposure to arsenic at higher levels over a considerable period of time leads to skin lesions and cancer, disorders of cardiovascular, respiratory, gastrointestinal, hepatic and renal systems. Murshidabad is one of the severely arsenic affected districts of West Bengal. We have analyzed soil and groundwater samples from some of the highly arsenic affected blocks of Murshidabad district. Both the soil and groundwater samples have an alkaline pH, a characteristic of the presence of arsenic in the tested samples. Unfortunately, the socio-economic conditions of these villages force the residents to use groundwater as the source of drinking water. Presence of considerably high amount of total dissolved solids in water samples make them further unfit for consumption. High amount of phosphate and iron present in some of the water samples takes a toll on the detoxification and excretory system of the body, if those water samples are consumed on a regular manner. Contamination of soil by the aforesaid contaminants results in biomagnification of these pollutants in the food chain. We could also isolate certain potentially arsenic resistant bacteria from the contaminated soil and water samples. At the next level we have surveyed an arsenic affected village to analyze the clinical manifestation of arsenic poisoning. In this village subjects developed rampant skin lesions throughout the body due to exposure to arsenic contaminated groundwater. Also, the disorders of various physiological systems could be observed in the subjects leading to death of the subject in extreme cases. Children as young as 13 years are also the victims of arsenic toxicity. Further research for bioremediation and inhibition of biomagnification of arsenic is the need of the hour to combat the menace of arsenic toxicity.

References


Citation: Biswas, M., Basu, A. and Mandal, D. Analysis of ground water and soil samples from severely arsenic affected blocks of Murshidabad district [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 162. https://doi.org/10.24870/cjb.2017-a148
Category: Miscellaneous

**Kaempferol attenuates COX-2 expression in IL-6-induced macrophages and carrageenan-induced mouse paw edema by targeting STAT3 and NF-kB**

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**Abstract**

Dietary polyphenols are reported to possess varied pharmacological activities, viz. antioxidant, anti-inflammatory, anti-cancer, anti-allergic actions. Here, we report the efficacy of Kaempferol (kae) to attenuate expression of IL-6 induced cyclooxygenase-2 (COX-2), an inducible isoform of cyclooxygenase enzyme family that catalyzes synthesis of inflammatory mediators, prostanoids and prostaglandins. IL-6 is a pleiotropic cytokine involved in both acute and chronic inflammation. Our results showed that kae attenuated COX-2 expression at both mRNA and protein level in IL-6-induced THP1 macrophages. This attenuation of COX-2 expression by kae involved dose-dependent inhibition of phosphorylation of STAT3 (Tyr 705) and NF-kB p65 (Ser 536) leading to their deactivation and reduced nuclear localization in THP-1 macrophages. Moreover, kae modulates COX-2 expression as well as STAT3 and NF-kB activation in carrageenan-induced mouse paw edema model. RT-PCR and western blot analysis from paw tissues were harvested after kae injection (i.p) followed by carrageenan-treatment in sub-plantar region of right hind paw. Results showed that kae attenuated COX-2 expression and STAT3 and NF-kB activation in carrageenan-induced mouse paw edema, suggesting that inhibition of both IL-6-STAT3-COX-2 and IL-6-NFkB-COX-2 axes by kae might be stimulus-independent. To understand binding affinity of kae with NF-kB and STAT3, docking analysis was performed using Patchdock server. From our findings, we observed strong binding affinity and transient interaction in both NF-kB/kae and STAT3/kae complexes. We noticed negative atomic contact energy and greater interface area for both the complexes. Selected complexes obtained from Patchdock were refined using Firedock online server which also suggested similar negative binding energy profile. It is plausible that kae attenuates COX-2 expression by directly binding to both STAT3 and NF-kB proteins and inhibiting their activation and nuclear translocation.

Role of Dynamic GPCR Signaling During Germinal Matrix Blood Vessel Development

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Abstract

During brain development, blood vessels create a complex network to meet the metabolic demands of neural cells. While G-protein coupled receptor (GPCR) signaling is known to be critical in numerous physiological processes throughout the body, their role in neurovascular signaling is unclear. Our lab has found that in neural progenitors (NPCs) of the germinal matrix (GM), the GPCR S1P receptor 1 (S1PR1) activates endothelial TGFβ signaling through transcriptional regulation of the TGFβ activator, integrin β8. Using chemogenetics, we are investigating the significance of temporal dynamics of this pathway in GM blood vessel maturation. By activating the GPCR pathway through Designer Receptors Activated by Designer Drugs (DREADD) we will first determine the effects of varying doses of the DREADD activator, CNO, on integrin β8 gene expression and pathway activity using cultured primary NPCs. Then we will activate the DREADDs at specific embryonic stages to examine its effects on vessel growth, maturation, and integrity in vivo. These results will likely demonstrate the critical role of dynamic GPCR signaling in NPCs for proper GM vessel development. Similar experiments in a mouse model of Intraventricular Hemorrhage (IVH), a human disease prevalent in premature infants, may also provide clinically relevant information for phenotypic rescue and medical intervention.

Citation: Santhosh, D. and Huang, Z. Role of Dynamic GPCR Signaling During Germinal Matrix Blood Vessel Development [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 164. https://doi.org/10.24870/cjb.2017-a150
Assessment on Reproductive Performance and Hormonal Studies in Rural Women Beedi Rollers in Jagitial District of Telangana State

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Abstract

Beedi manufacturing is the second largest industry in India. It provides employment to millions of women mostly from the poor socioeconomic class. In North region of Telangana, beedi rolling is a major occupation for illiterate women in many villages. It may affect due to the inhalation of unfiltered tobacco dust and volatile and toxic components of tobacco. Biomonitoring of women beedi rollers and their reproductive performance assessment is necessary to take prevention/control the reproduction failure and carcinogen effect on cervical system. Continuous exposed to unfiltered tobacco dust may have systemic effect and lead to many disorders including hormone defects and reproductive health problems. Although studies have been carried out on beedi industry workers and tobacco smoke exposed people at national and international level, no such studies were carried out on women beedi rollers living in rural areas in Telangana State. Hence, this investigation is attempted to understand the study is find to association with hormonal levels and reproductive outcome in rural women beedi rollers of reproductive age in North Telangana. Statistical analysis was done for the obtained results to find the significance between the two groups for the reproductive outcome and Hormonal Studies. Total 50 women (married who are exposed minimum 6-10 years to the unfiltered tobacco dust) beedi rollers in the age group of 25 to 45 years from villages of Jagitial district were enrolled for this study. 50 equal numbers of women in the same age group belonging to the same socio economic status and not exposed occupationally to chemical and physical agents was selected for comparison (control group). Estroidal, Progesterone the T3, T4 and TSH levels were measured found significantly T3, T4 levels were low in the beedi rollers, compared the controls. TSH levels were found to be higher in the beedi rollers. Estroidal and progesterone levels were obtained non-significant. Reproductive outcome in both the groups indicated adverse reproductive outcome in the beedi rollers when compared to the controls. An increase in the frequency of premature births, neonatal deaths, still births and uterine problems and a decrease in the fertility rate and frequency of live births were observed in women beedi rollers when compared to controls. The overall results showed adverse reproductive outcome and imbalance of thyroid hormone profile was found in rural women beedi rollers. These effects in women might be due to occupational exposure to tobacco dust in the work environment. There is a need to be concentrate with more sample size on reproductive outcome and hormonal related diseases in women beedi rollers who work with tobacco from time to time and in order to generate data on various health and reproductive issues. Awareness programmes on safety measures may be taken up for these illiterate women beedi rollers to prevent/control these effects.

Association between interferon beta levels and neuro-retinal degeneration in primary angle closure glaucoma

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Abstract

Introduction: Glaucoma is one of the major causes of irreversible blindness worldwide. The vision loss here is related to elevated intraocular pressure (IOP) mediated degeneration of the optic nerve. Obstruction to aqueous humour outflow is the main reason for increased IOP and the two major types of glaucoma based on this include primary open angle glaucoma (POAG) and primary angle closure glaucoma (PACG). Current strategies are directed against reducing IOP, but progression of neurodegeneration continues despite managing IOP in some patients. This suggests the existence of and the need to explore additional mechanisms that may contribute to disease progression. Interferon beta (IFNβ), an immunomodulator has been used quite effectively in the management of neurodegenerative conditions, hence its role in glaucoma is worthy of investigation.

Objective: To study the relationship between the endogenous expression of IFNβ in the ocular tissues with reference to the severity of PACG.

Methods: Iris, trabecular meshwork (TM) and aqueous humour (AH) were obtained surgically during trabeculectomy from PACG (n=34) patients operated as part of standard of care. Severity of glaucoma was based on Visual Field Index on Humphrey visual fields 24-2 program. Further categories based on type of medication use were also done. RNA extracted from iris and TM was used to determine the expression of IFNβ by quantitative-PCR and IFNβ in AH was measured using ELISA. Institutional ethics committee approval was obtained for the study.

Results: Normalized IFNβ gene expression in iris was lower in severe (0.0557±0.01) compared to mild/moderate (0.1197±0.04) cases. Likewise, its expression in TM was reduced in severe (0.0745±0.06) compared to mild/moderate (0.1945±0.11) cases. IFNβ levels in aqueous humour was lower in severe (149±36 pg/µg of total protein) compared to mild/moderate (439±13 pg/µg of total protein) cases. Anti-glaucoma medications with or without prostaglandin analogs did not alter the trend of lower expression of IFNβ in severe compared to mild/moderate cases in iris and TM.

Conclusion: Decreased levels of IFNβ observed in severe cases suggest that it can predispose to the neurodegeneration in glaucoma. However, its causal role and relevance in theragnostics are yet to be explored.

Category: Miscellaneous

**Generation and usage of a genetically engineered Virophage with RTase for the treatment of Ebola**

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**Abstract**

Ebola virus is a deadly virus that causes Hemorrhagic fever and death in infected individuals. There has been no proper treatment agent for this deadly virus. This novel approach involves treatment and curing of Ebola viral infection by using a genetically engineered Virus (Virophage) that would inhibit its propagation inside the cell after its entry by competing with its expression as well as replication and by reverse transcribing the genetic material of Ebola. The main idea involves the generation of a new Virophage with RTase in it but lacking the RNA dependent RNA polymerase along with VP24 and VP30 genes such that it is replication defective and competes with the actual Ebola’s RNA dependent RNA polymerase resulting in inhibition of its replication. Since the virophage is spliced with the RTase gene, the replication of the virophage’s negative sense RNA using the Ebola’s polymerase would cause the expression of the RTase which would non-specifically reverse transcribe all the available RNAs (both positive and negative sense ssRNA) in the cytosol resulting in the production of the complementary DNA of Ebola as well as the available virophage. Since the Ebola’s polymerase is RNA dependent, it couldn’t produce RNA from the reverse transcribed DNA and there is lesser possibility of the cDNA of Ebola to be integrated into the host’s chromosome. This is because the filoviridae family lacks the integrase enzyme unlike the Retroviridae. As a result, the Ebola’s replication as well as expression in the host is inhibited.

**Experimental design**

**STEP1:** Generation of a virophage that has its genome similar to that of the actual Ebola having RTase but lacking genes of VP24, 35 so as to elicit immunity simultaneously during the inhibition of the virus.

**STEP2:** In-vitro testing for inhibition of ebola’s life cycle.

**STEP3:** In-vivo testing in rats for effective inhibition of Ebola and development of resistance to it.

**Supporting Details**

There has been a report of Ebola survivor, a 48 year old patient (yet Ebola RNA found after 565 days of recovery from EBV using RT PCR in the patient’s semen, although no active viral particles were observed) with previous HIV infection [5].

**References**


**Citation:** Sankaranarayanan, S. and Ansel Vishal, L. Generation and usage of a genetically engineered Virophage with RTase for the treatment of Ebola [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 167. [https://doi.org/10.24870/cjb.2017-a153](https://doi.org/10.24870/cjb.2017-a153)
Category: Molecular Genetics

Y chromosome haplogroup distribution in different ethnic groups of Jammu and Kashmir, India

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Abstract

India is a country with 4,635 different population groups [1]. Jammu and Kashmir is located on the crossroads of Eurasia, bound by China and Tibet from North-East, Afghanistan and Pakistan from North-West. Diversity in Indian population is anticipated as a result of multiple waves of migration and gene flow that occurred in the past [2]. Y chromosomal variations have been documented as markers to represent deep rooted lineage haplogroups and many of these exist among Indian populations, indicating the early habitation of humans in the Indian subcontinent [3]. To find out the distribution of Y chromosome haplogroup in J&K, we genotyped 133 markers of non-recombining region of Y chromosome (NRY) in 384 males of J&K. Genotyping was done by Agena Massarray Platform. Our analysis showed distribution allocated the studied samples into thirteen major haplogroups R, H, J, P, L, K, IJK, C, F, Q, E, G and O highlighting the genetic diversity in the region.

References


Category: Molecular Genetics

**RNAi mediated down regulation of BADH2 gene for expression of 2-acetyl-1-pyrroline in non-scented indica rice IR-64 (Oryza sativa L.)**

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Abstract

Fragrance of rice is one of the most valued grain quality character thus, fetches much higher market price. 2-Acetyl-1-Pyrroline (2AP) is major aroma compound found in all parts of plant except root in scented rice. Classical and molecular genetics analyses revealed that a single recessive gene betaine aldehyde dehydrogenase 2 (Osbadh2) is responsible for expression of 2AP in scented rice. Present study was aimed at inducing expression of 2AP in non-scented indica rice cultivar IR-64 by silencing OsBADH2 via RNAi technique. The regeneration protocol for IR 64 was optimised, 2, 4-D (MS + 2 mg/L) for callus induction, BAP (2.5 mg/L) for shoot induction and half MS supplemented with 0.1 mg/L NAA was found optimum for rooting. A vector pBSK was used for construction of RNAi cassette and pRI101-ON as a binary vector. Agrobacterium (GV3101) mediated transformation was done using embryogenic calli of IR-64 and transgenic calli were selected on MS medium containing kanamycin (250 mg/L). Gas chromatography analyses showed significant amount of 2AP (0.05 ppm) production in RNAi callus. The content of precursors, proline and methylglyoxal were not varied but GABA content was found to be reduced in RNAi callus (5.1±0.03 µg/g) than control (6.4±0.05 µg/g). Further transgenic calli showed 7 fold reduction in expression of BADH2 transcript. The transgenic calli have been regenerated and transformed plants are under observation for further transcriptome analysis and 2AP quantification in seeds. The study demonstrated that RNAi approach could be successfully used for imparting pleasant aroma character in non-scented rice cultivars.

References


Category: Molecular Genetics

Understanding the plant–microbe interaction molecular mechanisms for better exploitation of bio-control agents to enhance sustainable agricultural practices

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Abstract

Trichoderma spp. are well-known bio-control agents which promote the plant growth and suppress the pathogen infection. The beneficial effects are attributed to the production of phytohormones, antibiotics, siderophores and secondary metabolites (SM). Trichodermin and Harzianum A, SMs have documented anti-fungal activities as well. Tri5 gene encodes for trichodiene synthase (TS) contains a terpene fold and involved at the initial step of the biosynthetic pathway of these molecules. Furthermore, domain analysis of proteins from diverse organisms showed that the terpene fold has functional diversity with diverse applications in agriculture, medicine and applied biotechnology. These proteins can be classified into single and multi-domains based on their structures. It was observed that multi-domain proteins carry additional helices which may regulate the catalytic efficiency. Further, activity enhancing mutations with potentially higher catalytic activities were screened. In an offshoot to the above work, we have analyzed binding of Trichodermin with the 25S rRNA that constitutes the petidyltransferase centre (PTC). The trichodermin resistance protein (60S ribosomal protein L3) was reported to overcome the inhibitory effects of trichothecene compounds. Normal mode analysis and MD of trichodermin resistance protein and 25S consisting of PTC showed that the W-finger region of the protein may move towards 25S rRNA and may block the binding pocket of the trichodermin. These results may lead to develop strategies for higher TS activity and the mechanism of action of these molecules involved in plant-microbe interactions. These may be further exploited for enhancing the efficiency of these biotechnological agents used in sustainable agriculture.

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Ectopic Expression of Leishmanial DNA Polymerase β in *Escherichia coli* Confers Survival Advantage against Ultraviolet Radiation

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Abstract

*Leishmania donovani* encounters oxidative environment in the host macrophage and expected to have robust DNA repair mechanisms. Base Excision Repair (BER), a predominant repair pathway in *L. donovani* remains unexplored. Presence of mitochondria in eukaryotes has been projected as a symbiotic relationship since long and the role of DNA polymerase β in repair of mitochondrial DNA has gained importance in recent past. We ectopically expressed Leishmania DNA polymerase β (*Ld*Polβ) under inducible promoter in *E. coli* and found it is biologically active in vitro by using pUC19 as substrate. Further we checked its effect on sensitivity of *E. coli* to UV rays. We find that heterologous *Ld*Polβ slows down the growth of *E. coli* and surprisingly, could protect it from lethal effects of UV to a large extent. Co-expression of leishmania DNA Ligase IIIα (*Ld*LigIIIα) has a synergistic effect on survival advantage offered by *Ld*Polβ. Survival advantage given *Ld*Polβ in *E. coli* is reconfirmed by FACS analysis. Our observations indicate that *Ld*Polβ is crucial for handling ROS induced toxicity inside the mitochondria of the parasite and for its survival inside host macrophage. This studied may lead to explore for finding of the importance of *Ld*Polβ in survival against DNA damaging agents in *L. donovani* and its role in pathogenesis of leishmaniasis, it would help to discover new target and development of newer drug against Leishmaniasis.

Category: Molecular Genetics

**Involvement of mitochondrial intrinsic pathway in rhSP-D (recombinant human Surfactant Protein D) induced apoptosis of prostate cancer cells**

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**Abstract**

Surfactant protein D (SP-D), an innate immune molecule, has an indispensable role in host defense and regulation of inflammation. We reported a novel anti-cancer role of a recombinant fragment of human SP-D (rhSP-D) in leukemic and breast tumor cell lines. A recent study revealed correlation of SP-D expression in Prostate cancer tissues with increased Gleason score and tumor volume. In the present study, we elucidated the role of rhSP-D in prostate cancer using LNCaP (androgen dependent), PC3 (androgen independent) cell lines and primary prostate cancer cells. In accordance with our previous finding, rhSP-D induced apoptosis in LNCaP and PC3 cell lines in a time and dose dependent manner. Isolated primary prostate cancer epithelial cells from explant cultures of tissue biopsies of prostate cancer patients were characterised for the presence of Cytokeratin (epithelial cell), CD10 (negative) and CD164 (positive) markers at protein and transcript level. Anti-prostate tumor effect of rhSP-D was established in the isolated primary prostate cancer epithelial cells. Importantly, primary normal prostate epithelial cells treated with similar concentrations of rhSP-D showed no adverse effect on viability. rhSP-D upregulated phospho p53 and transcripts of Bax and reduced Bcl2 transcripts, suggesting p53 mediated apoptosis in LNCaP cells. rhSP-D induced apoptosis in PC3 cells by lowering phospho ERK1/2 levels and increased BAD transcripts, a distinct mechanism of programmed cell death. Increased release of cytochrome c upon rhSP-D confirmed the activation of mitochondrial intrinsic apoptotic pathway in both the cell types. rhSP-D treatment downregulated transcripts of Bcl2 while upregulated PUMA transcripts, suggesting p53 mediated apoptosis primary prostate cancer cells. Also, positive TUNEL assay confirmed induction of apoptosis by rhSP-D in cancer tissue biopsies. Collectively, our findings reveal an integral role of SP-D in immune surveillance against prostate cancer mediated by two distinct mitochondrial apoptotic mechanisms.

**References**


**Citation:** Thakur, G., Prakash, G., Murthy, V., Sable, N., Menon, S., Bakshi, G., Kishore, U. and Madan, T. Involvement of mitochondrial intrinsic pathway in rhSP-D (recombinant human Surfactant Protein D) induced apoptosis of prostate cancer cells [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 172. [https://doi.org/10.24870/cjb.2017-a158](https://doi.org/10.24870/cjb.2017-a158)
Category: Molecular Genetics

**Efficiency of ubiquitous chromatin opening elements in driving the expression of human CD18 within self-inactivating lentiviral vectors for gene therapy applications**

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**Abstract**

Patients with leukocyte adhesion deficiency type 1 (LAD1) suffer from recurrent bacterial infections due to mutations in the common β₂ integrin subunit (CD18/ITGB2 gene). Treatment options include long-term administration of antibiotics, repeated granulocyte infusions, allogeneic bone marrow or stem cell transplantations, all of which have considerable limitations. Gene therapy could bring about a potential permanent cure for LAD1. We tested different fragment of the ubiquitous chromatin opening element (UCOE) from the human HNRPA2B1-CBX3 locus for their efficiency in driving the expression of human CD18 gene. Twelve new self-inactivating (SIN) lentiviral vectors were constructed, 10 of which incorporating various fragments of the UCOE, two others containing the long and short fragments of the elongation factor 1 alpha promoter (EF1αL, 1169 bp; EF1αS 248 bp) and a murine stem cell virus (MSCV) promoter within the context of the same lentiviral vector. These vectors were tested in vitro for the expression of human CD18 on the surface of CD34⁺ hematopoietic stem cells (HSCs) isolated from both moderate and severe LAD1 patients. Among the promoters tested in moderate patient’s CD34⁺ HSCs, 3'631 bp, 3'652 bp, 3'1262 bp, A2UCOE and EF1αS resulted in higher percentage of CD18⁺ cells (11.4% to 15.1% at MOI 10; 12.7% to 16.5% at MOI 100), comparable to the expression driven by the MSCV promoter (15.2% at MOI 10; 16.1% MOI 100). The 5'655 bp, 5'723 bp, 5'1296 bp, 2598 bp and EF1αL promoters resulted in comparatively lower levels of CD18 expression (10.4% to 11.1% at MOI 10; 5.7% to 10.9% at MOI 100). All the 3’ promoter fragments of the UCOE were further tested in a severe LAD1 patient’s CD34⁺ HSCs, (1.6% to 5% at MOI 10 and 1% to 3.4% at MOI 100). Results obtained from this study would be useful in examining the human CD18 expression on murine LAD1 CD34⁺ HSCs in vitro followed by ex vivo studies to demonstrate the phenotypic correction of LAD1 in a murine model. Efforts are underway to compare the efficiency of gene correction using this conventional gene therapy approach with CRISPR-mediated gene correction of CD18 in human LAD1 CD34⁺ HSCs in vitro.

**Citation:** Gopinath, C., Chodisetty, S., Verma, I.M. and Nelson, E.J.R. Efficiency of ubiquitous chromatin opening elements in driving the expression of human CD18 within self-inactivating lentiviral vectors for gene therapy applications [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 173. [https://doi.org/10.24870/cjb.2017-a159](https://doi.org/10.24870/cjb.2017-a159)
Category: Molecular Genetics

Impact of deletion of a catabolite repressor Mig1 on hyphal morphology and cellulase expression in *Penicillium funiculosum* NCIM1228

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Abstract

Carbon catabolite repression (CCR) is a regulatory mechanism which negatively regulates genes of for ancillary carbon source utilization. It is mediated by Mig1 orthologues, which are Zn finger transcriptional repressors. We studied the effect of CCR disruption in *Penicillium funiculosum* NCIM1228, a hypercellulolytic ascomycete. Upon phylogenetic analysis of fungal genomes, Mig1 presence across all taxa of kingdom fungi revealed its conserved role in catabolite repression. Also it was found to constitute distinct clade from industrially important cellulase producing fungi like *Trichoderma reesei* and *Aspergillus sp.* It shared the clade with other highly evolved fungi *T. cellulolyticus* and *P. marneffei* and represented more recent radiations of evolutionary conserved catabolite repressor Mig1. Genotypic analysis showed that NCIM1228 harbors a truncated yet functional allele of Mig1. Mig1 orthologue of NCIM1228 has a non-sense mutation at 134th amino acid position, making a large C-terminal portion of Mig1 (415aa) dispensable for carbon repression. NCIM1228 was grown in presence of allyl alcohol to check the phenotypic effect. NCIM1228 showed sensitivity to allyl alcohol as compared to *Penicillium funiculosum* (Pf). Deleting active Zn finger domain made NCIM1228 completely sensitive to allyl alcohol. Surprisingly, the deletion showed small and compact colonies with compromised filamentous proliferation while the dry mycelial weight didn’t change when grown on 0.5% glucose supplemented with 2% avicel. Using microscopy, we unraveled that *PfΔMig1* showed reduced aerial hyphae and profuse branching pattern in terminal hyphae resulting in compact colonies. We further observed more than two-fold (7.6 FPU/ml) higher FPU in production media than NCIM1228 under similar condition.

References


Category: Molecular Genetics

Arginylation regulates adipose tissue development and function via modulating PPARγ expression

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Abstract

Arginylation, a poorly understood post translational modification mediated by ATE1 known to regulate protein activity and stability [1]. Unconditional deletion of Ate1 results in embryonic lethality and postnatal whole body deletion of Ate1 in mouse results in adipose tissue dysfunction i.e. loss of visceral fat, exhibit higher metabolic rate and resistant to diet induced obesity [2]. Adipose tissue dysregulation leads to various life threatening diseases like obesity, diabetes, cardiovascular defects and cancer [3, 4]. Current study is undertaken to understand the role of protein arginylation in adipose tissue development and function. Our initial investigation showed an increase in ATE1 protein expression with progression of adipogenesis in 3t3L1 preadipocyte differentiation. Further, treatment of these cells with ATE1 inhibitors, tannic (10 µM) acid and merbromin (75 µM) suppressed lipid accumulation in 3t3l1 cell significantly. Gene expression analysis shows inhibition of peroxisome proliferator activated receptor gamma (PPARγ) 1 and PPARγ2 expression in 3t3l1 cells differentiated in presence of ATE1 inhibitors. PPARγ is a key transcription factor of adipogenesis and plays crucial role in induction of various adipogenic genes which contributes to lipid formation. The mRNA level of PPARγ associated genes glucose transporter 4 (GLUT4), fatty acid binding protein 4 (FABP4) and perilipin (pln1) were found to be downregulated by ATE1 inhibitors, hence results into decrease in lipid accumulation. As PPARγ found to be a target for ATE1 inhibitors, PPARγ1 was overexpressed in Ate1 knockout (KOγ1) and wild type mouse embryonic fibroblast (MEF) cells. Expression of PPARγ1 was found to be significantly low in KOγ1 as compared to its wild type counterpart at transcript level. Interestingly inhibition of PPARγ1 expression in absence of arginylation becomes more profound at protein level. Low level of PPARγ1 impedes adipogenesis when KOγ1 cells were induced to differentiation with poor induction of GLUT4, lipoprotein lipase and FABP4. Current study provides a new protein arginylation dependent adipogenic pathway which promotes adipogenesis by promoting PPARγ.

References


Citation: Singh, A. and Saha, S. Arginylation regulates adipose tissue development and function via modulating PPARγ expression [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 175. https://doi.org/10.24870/cjb.2017-a161
Category: Molecular Genetics

Applications of Recombinant DNA Technology in treating ADA deficiency

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Abstract

What Is Recombinant DNA Technology
RDT or Generic Engineering is a process of manipulation of genes to produce an altered organism. The generic material of one organism is introduced into the genome of another organism. Mistake in the sequencing of genes can produce generic defects, it also arises due to errors in DNA replication often leading to genetic drift.

ADA deficiency
It is a kind of genetic disorder which is caused by the mutation in Adenosine Deaminase enzyme. It causes accumulation of metabolic substances thus affecting the immune system causing Severe Combined Deficiency (SCID). SCID arises from a variety of molecular defects including defects in Y chain, various signaling molecules like JAK-3 and IL-7 receptor. Absence of ADA results in accumulation of deoxyadenosine in the intracellular compartments.

Gene Therapy
This technique involved introduction of altered drugs to treat an infection. The DNA is administered and it reaches the damaged cell and disrupts the protein. The mutated gene that causes the disease is replaced with a healthy gene. It is said to be one of the promising techniques to treat infections.

Gene Therapy approach to treat ADA
1. Use of Retroviral Vectors- Such vectors were made from a retrovirus and were created by replacing harmful retroviral genes with normal ADA genes. These vectors were mixed with T cells which were extracted from a person’s blood and grown in culture dishes. Then the retroviral vectors entered the T cells and implanted the normal ADA gene into T cell chromosome.
2. Q PCR determination of vector copy number-Q PCR was performed with primers and probe to amplify MND-ADA vector. The TAMRA probe was used to detect MND-ADA and GCsapM-ADA vector. All reactions were performed on 7900 sequence detector system.

A lot of research work is going to develop permanent cure for ADA deficiency. Early diagnosis and treatment of active infection to treat SCID is essential. Unlike T cells which live for a few months, stem cells live throughout the patient hence they need lifetime supply of ADA.

Citation: Padhi, A. Applications of Recombinant DNA Technology in treating ADA deficiency [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 176. https://doi.org/10.24870/cjb.2017-a162
Category: Molecular Genetics

Ube3a deficiency inhibits amyloid plaque formation in APPswe/PS1δE9 mouse model of Alzheimer’s disease

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Abstract

Alzheimer’s disease (AD) is a neurodegenerative disorder characterized by progressive decline in memory and cognitive function. Pathological hallmark of AD includes aberrant aggregation of amyloid beta (Aβ) peptide, which is produced upon sequential cleavage of amyloid precursor protein (APP) by β- and γ-secretases. On the contrary, α-secretase cleaves APP within the Aβ sequence and thereby prevents Aβ generation. Here, we investigated the role of ubiquitin ligase Ube3a (involved in synaptic function and plasticity) in the pathogenesis of AD using APPswe/PS1δE9 transgenic mouse model and first noticed that soluble pool of Ube3a was age dependently decreased in AD mouse in comparison with wild type controls. To further explore the role of Ube3a in AD patho-mechanism, we generated brain Ube3a-deficient AD mice that exhibited accelerated cognitive and motor deficits compared to AD mice. Interestingly, these Ube3a-deficient AD mice were excessively obese from their age of 12 months and having shorter lifespan. Biochemical analysis revealed that the Ube3a-deficient AD mice had significantly reduced level of Aβ generation and amyloid plaque formation in their brain compared to age-matched AD mice and this effect could be due to the increased activity of α-secretase, ADAM10 (a disintegrin and metalloproteinase-10) that shift the proteolysis of APP towards non-amyloidogenic pathway. These findings suggest that aberrant function of Ube3a could influence the progression of AD and restoring normal level of Ube3a might be beneficial for AD.

References


Validation of NFBD1 as a novel molecular target in cervical cancer therapy utilising advanced molecular approaches

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Abstract

Cervical cancer is the second most frequent cancer and the most common cause of death in women in developing countries, with HPV-16/18 infection being one of its major characteristics. Other than vaccination, chemotherapy is the most commonly used mode of treatment. However, recurrence of cancer after such treatment remains a major concern. Therefore, we aim to identify a novel molecular target to be used in combination therapy with chemo/radio-therapy. In this regard, Nuclear factor binding domain 1 (NFBD1), a mediator protein, majorly involved in the ATM kinase dependent DNA damage repair pathway can be an important target as it is involved in the activation, retention of ATM kinase and its interacting partners at the site of double stranded DNA breaks. Additionally, higher NFBD1 expression in cervical cancer tissues in comparison to other cancer tissues indicates its oncogenic potential.

Here, we studied changes in NFBD1 expression in response to drug treatment, UV/gamma irradiation in HeLa cell line. Our results indicated increased NFBD1 expression both at mRNA and protein level as studied by SYBR-green assay and western blotting, respectively. Interestingly, we observed increased sensitivity of HeLa cells to PARP inhibitor suggesting that PARP inhibitors can be considered for effective cervical cancer treatment. Further, we performed time- and dose-dependent inhibition assay to study changes in NFBD1 expression with increase in time, inhibitor concentration and UV/gamma irradiation intensity. To understand the significance of this increase in NFBD1 expression, HeLa cells were transfected with NFBD1 shRNA and analysed for response to genotoxic stress through cytotoxicity and annexin V/propidium iodide based apoptosis assay. Our results showed increased programmed cell death in cervical cancer cells knocked down for NFBD1 expression in comparison to un-transfected cells in response to drug treatment.

In summary, changes in NFBD1 expression are of significance in cervical cancer prognosis and treatment. It would be exciting to study NFBD1 expression at different stages of cancer progression as it could be an important marker in cervical cancer prognosis. Along with that, our results clearly indicate that it can be an important molecular target for sensitization of cancer cells to chemo/ or radio- therapy.

References


Category: Molecular Genetics

EMT factor Zeb1 depletion in dendritic cells enhances Helminth clearance in mice by increasing Th2 cell differentiation

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Abstract

Dendritic cells are professional antigen presenting cells that act as bridging link between innate and adaptive immune system. They are equipped with pathogen recognition receptors (PRR) to identify the pathogen associated molecular pattern (PAMPS) on any antigen. DCs elicit an immune response through polarizing T cells towards various subtypes like Th1, Th2 & Tregs. Though DC-T cell interaction has been widely studied, but how this single DC molecule amalgamate various transcriptional signals for translating the message to the T cells and induce diverse immunological responses still needs to be unraveled. Therefore to identify the role of transcription factor in immune programming we have targeted the largest member of TFs family, Zinc Finger Transcription Factors (ZF-TFs). Among various ZF-TFs we have narrowed our study to three interesting candidates Zeb1, Zeb2 and Zbtb10 based on their expression in DCs from an unpublished microarray data. Here in this study we have tried to understand the role of Zeb1, master regulator of EMT program in orchestrating DC responses. Zeb1 links the epithelial – mesenchymal transition and has been widely studied molecule in cancer biology. Except for the fact that it act as transcriptional repressor and represses IL2 gene promoter no other reports are available in immune biology, thereby rendering it a perfect candidate to be used for detailed characterization in dendritic cells. In our study, we found that Zeb1 depleted CD8α+DCs shows an increase in co-stimulatory marker like CD80 & CD86 whereas there is a decrease in MHC class I & II molecule. Thereafter at transcript & protein level we found decrease in pro-inflammatory & anti-inflammatory cytokine like IL6 & IL10 respectively, the bioactive form of IL12 i.e. IL12p70 which polarizes T cells towards Th1 response showed a significant decrease in bio-plex when compared with control CD8α+DCs. The regulatory markers which develop regulatory T cells like Pdl1, IL27 also showed decreasing trend in zeb1 depleted DCs. Thereafter we speculated that Zeb1 perturbed DCs might be involved in default Th2 program. So, we looked into T-cell polarization by co-culture & MLR experiments which showed an increase in GATA3+ T cells, a signature transcription factor for Th2 subtype along with higher levels of IL4, IL5 and IL13 cytokines. To evaluate the in-vivo function of Zeb1 knockdown (KD) cells we developed Helminth Polygyrus (H.Poly) disease model in mice, there we assessed for the worm load in intestine and egg count in the feces which showed a marked decrease in worm count and egg count in Zeb1 KD adoptive transfer mice as compared to control mice. The T cell response was examined through the draining lymph node (mesenteric lymph node) where we found significant increase in GATA3+ T cells along with IL5 and IL13; this suggested that Zeb1 KD DCs polarize the T cells towards Th2 response which results in clearance of H. polygyrus in mice.

Citation: Smita, S. and Raghav, S.K. EMT factor Zeb1 depletion in dendritic cells enhances Helminth clearance in mice by increasing Th2 cell differentiation [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 179. https://doi.org/10.24870/cjb.2017-a165
Category: Molecular Genetics

miRNA-mRNA integrative expression mapping during mouse embryonic stem cell to Neuron progenitor differentiation

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Abstract

Genome-wide transcriptome profiling of mammalian cells has given more insight into complexity of RNA world. Vast majority of RNAs are falling under non-coding category, among which ~22 nucleotides RNA is known as microRNA. It regulates gene expression by repressing translationally or cleaving mRNAs. 6-8 nucleotides region at 5' end of miRNAs bind to 3' UTR region of mRNA called ‘seed region’. This binding is either completely or incompletely complementary. Complete complementarity associates with RNA Induced Silencing Complex (RISC) that able to degrade mRNA with the help of Argonaute protein (Ago). But, incomplete complementarity is believed to be in a type of poised state that can be removed during requirement of that particular protein synthesis. miRNA translational repression by miRNA leads to subsequent mRNA destabilization. These dynamics of miRNA and mRNA integrative expression was further studied in embryonic stem cell to Neuron Progenitor differentiation system. Upon retinoic acid treatment to mouse embryonic stem cell line (R1), without LIF and 2i inhibitors that maintain undifferentiating state in ESC, cells were differentiated to neuronal cell lineage. To study, the dynamic changes in expression during differentiation we performed both microRNA and mRNA sequencing separately in these two states with biologically duplicated samples. Interestingly, we came up with 82 differentially regulated miRNAs, 9 lncRNAs and 4336 mRNA genes during this phage. Along with these 31 novel miRNAs were identified, among them 17 were identified that specific to ESC and 14 were in NP. Interaction maps of miRNA-mRNA was confirmed the down regulation of targeted mRNAs due to upregulated miRNAs during differentiation. KEGG pathways analysis of these genes showed downregulation of signaling pathways regulating pluripotency of stem cells (by miR-466m-3p, miR-466k, miR-1198 and miR344e) and upregulation of axon guidance and Neurotrophin signaling pathways that involves in differentiation of neuronal cells. These studies reveal genome wide miRNAs and mRNAs interaction map in ESC to NP differentiation. That will help in understanding of controlled gene expression patterns during neuronal differentiation due to miRNAs and IncRNAs parallel expressions.

Citation: Nayak, R., Uppada, V. and Kurukuti, S. miRNA-mRNA integrative expression mapping during mouse embryonic stem cell to Neuron progenitor differentiation [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 180. https://doi.org/10.24870/cjb.2017-a166
Category: Molecular Genetics

Spatiotemporal Dynamics of 3D Genome Architecture and Gene Expression during Lactogenic Differentiation of Murine Mammary Epithelial Cells

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Abstract

Orchestration of differential gene expression program during cellular development and differentiation is tightly coordinated in each and every cell type. Cell-type specific gene expression is primarily regulated by the spatial arrangement of genome within the 3-dimensional space of the cell nucleus. Recent evidences suggest that interphase chromosome territories are non-randomly organized in a cell-type specific manner and their neighbourhood are interlinked with cell-type specific gene expression patterns. In this study we made an attempt to study chromosomal dynamics and gene expression in the context of mammary epithelial cells lactogenic differentiation under the influence of lactogenic hormones. We derived genome-wide chromosome conformation capture HiC based chromosome interactions and gene expression by RNA-seq in undifferentiated murine mammary epithelial cells and hydrocortisone and Prolactin hormone treated cells. We found that chromosome territories are non-randomly organized in HC11 cells undifferentiated as well as differentiated cells-types. There seems to be increased and decreased interactions within and in between chromosomes upon differentiation. We derived A & B compartments within each chromosome and their relationship with gene expression. We also studied topologically associated domain reorganization during differentiation which indicates that majority of the TADs are highly conserved and minor or less TADs are highly reorganized during signalling. From these studies, we concluded that differentiation signals promote subtle shift of chromosomal territories neighbourhood but promotes extensive reorganization within or between chromosomes.

This abstract has been withdrawn by the author
Clinical Medicine and Curative Treatment for Diabetes Mellitus

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Abstract

Diabetes is one of the most severe diseases that spread to mankind rapidly in the year 2015, where it is increased to 30%. It is a chronic disease, when it comes it will stand for a lifetime. The major problem in this disease is it affects the body’s ability to use the energy found in food of an affected person. There are three types of diabetes which are TYPE 1, TYPE 2 and GESTATIONAL DIABETES. Normally, human body breaks down the sugars and carbohydrates consumed into a special sugar called glucose. Glucose acts as a fuel in the cells in body. But the cells need insulin, a hormone, in bloodstream in order to take in the glucose and use it for energy. With diabetes mellitus, your body doesn’t make enough insulin, it can’t use the insulin or it produces over excess or a combination of both. High levels of blood glucose can damage the tiny blood vessel in kidneys, heart, eyes or nervous system. So that diabetes is considered as a severe harmful problem that affect the whole cycle of our body and especially if it is left untreated it can eventually cause heart disease, stroke, kidney disease, nerve damage and also even can cause blindness.

According to latest technology and facts there is a possible way to give a complete cure for this unconditional disease. Use of Nano-robots from a sprouted seeds or germinated seed of a Synsepalum dulcificum plant will take as a sample that has the main function of the antidote which has the ability to induce and control the production rate of insulin in pancreas. It has the new form of Nano-robots that are present naturally in it which is in understudies. A possible way of research through the samples will provide a clear solution with Nano-robots that will cure Diabetes. Creation of Nano-robots with onivyde in combination with the samples prepared and Nano-robot is designed with the nano-electric biosensor.

References


Citation: Divya, J.R. Clinical Medicine and Curative Treatment for Diabetes Mellitus [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 183. [https://doi.org/10.24870/cjb.2017-a169](https://doi.org/10.24870/cjb.2017-a169)
Over-expression of the splice variant of CONSTANS enhances the \textit{in vitro} synthesis of silver nanoparticles

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Abstract

Eco-friendly biosynthetic approach for silver nanoparticles production using plant extracts is an exciting advancement in biotechnology and has been successfully attempted in more than 41 plant species. However, an established model plant system for unravelling the biochemical pathways of silver nanoparticle (AgNPs) production is lacking. Here we have shown in \textit{Arabidopsis thaliana} a genetic model plant and in its misexpressing lines of splice variant CONSTANS (COβ) for the silver nanoparticle biosynthesis \textit{in vitro}. Employing the biochemical, spectroscopic, Transmission Electron Microscopy (TEM), Raman spectroscopy, Nuclear Magnetic Resonance (NMR) and powder x-rays diffraction (Powder XRD) methods and using selected mutants and over-expressing line of \textit{Arabidopsis thaliana} involved in sugar homeostasis. Additionally, a comparative analysis of AgNPs synthesis using different transgenic lines of \textit{Arabidopsis} was explored. Here we have shown that plant extract of COβ and \textit{gi-100} (mutant line of \textit{GIGANTEA}) showed the highest potential of nanoparticle production as comparable to Col-0 and over-expressing line of \textit{GIGANTEA} (35SGi). Silver nanoparticles production in the \textit{Arabidopsis} not only opens up a possibility of using molecular genetics tool to understand the biochemical pathways, but also could address the mechanism behind different shapes of AgNPs produced using plant extracts.

Citation: Kumar, A. and Panigrahi, K.C.S. Over-expression of the splice variant of CONSTANS enhances the \textit{in vitro} synthesis of silver nanoparticles [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 184. \url{https://doi.org/10.24870/cjb.2017-a170}
Category: Plant Genomics

Does epigenome is influenced by allopolyplloidisation during the evolution of \textit{Gossypium hirsutum} \\

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Abstract

In ecological and evolutionary time scale, Darwinian selection acts on each nucleotide to shape the whole genome. Beside nucleotide diversity, epigenetic modifications seem to be conserved in intra and inter-species during evolution. \textit{Gossypium hirsutum} (A\textsubscript{1}D\textsubscript{1}) is an allotetraploid crop plant which was evolved from morphologically different diploid ancestors by polyploidisation event expected to occur around 1 million year ago. Its progenitors are \textit{Gossypium arboreum} (A) and \textit{Gossypium ramondii} (D) and they are still available for study. We are keen to understand whether the epigenome structure of A- and D-subgenomes are conserved or influenced by polyploidisation? To understand the influence of polyploidisation on the epigenome, it is important to determine conserved DNA elements which are under purifying selection during evolution. We aligned whole genome sequences of \textit{Gossypium hirsutum}, \textit{Gossypium arboreum}, \textit{Gossypium ramondii} and \textit{T. cacao} by LASTZ aligner taking \textit{T. cacao} as a reference genome. Our analysis revealed conserved DNA elements and their distribution in the A/D sub-genomes. The portion of the A/D sub-genome under purifying selection is also determined. Our future study on DNA methylome of \textit{Gossypium arboreum} (A) and \textit{Gossypium ramondii} (D) and AD-genome of \textit{Gossypium hirsutum} and their correlation with conserved DNA elements will reveal influence of polyploidisation on epigenome.

Citation: Prasad, P. and Sawant, S.V. Does epigenome is influenced by allopolyplloidisation during the evolution of \textit{Gossypium hirsutum} [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 185. https://doi.org/10.24870/cjb.2017-a171
Category: Plant Genomics

**De novo assembly and analysis of Solanum trilobatum L. leaf transcriptome using next generation sequencing technology**

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**Abstract**

RNA Sequencing based *de novo* assembly is a well-developed approach in understanding transcriptomes of non-model plants with limited genomic information. RNA-Seq is a cost-effective tool, offers much data with better coverage and sufficient sequence depth for *de novo* assembly of transcriptomes. In past few years, there has been an increase in utilising RNA-Seq for discovery and identification of functional genes involved in the biosynthesis of active compounds in non-model plants. In this study, we analysed the transcriptome of *Solanum trilobatum* L. leaf using high throughput next generation sequencing. *S. trilobatum* is one of the important medicinal plants belonging to family Solanaceae and commonly available in South India. The studies conducted so far, to understand its therapeutic potential, have yielded positive results. Its extract is used to treat conditions like chronic bronchitis and tuberculosis. It is also reported to have anti-oxidative, hepatoprotective, anti-inflammatory, anti-microbial, anti-tumour activities. The total RNA from *S. trilobatum* leaf was isolated and sequenced using Illumina Hiseq 2500 platform with paired end chemistry. In total, 136,220,612 high quality sequence reads were obtained. The raw reads were pre-processed and assembled into 144,580 assembled transcripts using Trinity- a *de novo* assembler and clustering of transcripts was done using CD-HIT resulting 128,934 unigenes. The unigenes were extensively evaluated and annotated with various databases to identify pathways and genes responsible for biosynthesis of medicinal compounds. Based on similarity search with known proteins 60,097 (46.61% of all unigenes), 35,141 (27.25%), 30,427 (23.60%) and 61,986 (48.07%) had homologs in nr, Pfam, GO and UniProt databases respectively. The comparison against the KEGG database mapped 14,490 (11.23%) unigenes to 138 pathways, where flavonoid biosynthesis pathway was identified to be the highly represented. The expression levels of the transcripts were quantified using RSEM and Reverse Transcription PCR (RT-PCR) of few genes were performed to validate the transcriptome assembly. The SSRs and transcription factors, which could help for the molecular breeding, were also identified. This is the first report of complete transcriptome analysis in *S. trilobatum*. The genomic resources generated will serve as foundation to understand molecular basis of medicinal properties of *S. trilobatum* in further studies.

**Citation:** Adil, L. and Natarajan, P. *De novo* assembly and analysis of Solanum trilobatum L. leaf transcriptome using next generation sequencing technology [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 186. [https://doi.org/10.24870/cjb.2017-a172](https://doi.org/10.24870/cjb.2017-a172)
Category: Plant Genomics

Towards enhancement of yield by molecular stacking of yield contributing genes in rice (Oryza sativa L.)

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Abstract

Rice (Oryza sativa L.) is a staple food for over half of the global population. Rice yield is mainly determined by three complex traits i.e., number of panicles per plant, number of grains per panicle and grain weight which are governed by many genes with minute effect called quantitative trait loci (QTL). As of now, more than 30 QTLs governing yield and its component traits have been cloned and molecularly characterized. Stacking of harmonious QTLs/genes into a single elite variety proved to show higher yields. To this end, in the present study, two strategies have been contemplated to raise the yield ceiling in rice. In the first strategy, the validated known yield genes would be pyramided into a single elite variety by marker-assisted backcross breeding. For this, the validation of majority of the yield gene-specific markers from known high yielding varieties has been completed. In all, 17 markers showed polymorphism with the recurrent parent MTU1010. Using these polymorphic markers as foreground markers, the BC₁F₁ plants obtained from MTU1010/MTU3626 (Donor for DEP1, GW5 and GW8) and MTU1010/Swarna (Donor for GS5 and qSS7) were confirmed for the presence of the yield genes. Later, the confirmed BC₁F₁ plants will be intercrossed to pyramid the yield genes. In the second strategy, the candidate genes for the yield component traits would be mapped and then pyramided into a single elite variety. To this end, the rice varieties MTU3626 and NLR33892 have been chosen as donors for grain weight and grain number, respectively and BPT5204, a fine grain variety has been chosen as recurrent variety. The F₂ populations of the crosses BPT5204/MTU3626 and BPT5204/NLR33892 are being grown in the field. The DNA from 20 plants with extreme phenotype for the targeted traits would be bulk sequenced along with the parents for rapid detection of QTLs using QTL-seq method. Later, the major QTLs would be introgressed into BPT5204 and evaluated for its yield enhancement.

References


Category: Plant Genomics

**Transcriptome profiling of floral development in *Dendrocalamus hamiltonii* uncovers floral transition mechanism in bamboos**

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**Abstract**

Bamboos are the giant woody grasses belonging to family Poaceae. The plants have versatile utilities including rural and industrial applications. Bamboos are indeed major players in the economies of many Asian countries. They support an international trade worth more than US$ 2.5 billion per year and this is expected to increase further. Bamboos have long juvenile phase and unique flowering behavior - the major deterrents to their conservation and propagation. Most genera/species have a long intermast period of juvenile stage that varies from 40-120 years after which bamboos flower gregariously, irrespective of geographical locations. The entire culms of all clonal individuals from a single mother, flower simultaneously and die *en masse*. This results in huge loss of valuable germplasm, and is a continuous but unpredictable threat to all standing populations of bamboos. Besides gregarious flowering, some culms of bamboos flower sporadically, set a few seeds annually and die. Although the process of flowering in bamboos demands attention, it is poorly understood. Therefore, an attempt was made to elucidate the molecular mechanism of floral transition in *Dendrocalamus hamiltonii*, a multipurpose bamboo native to Himalayan region using a transcriptomic approach in an *in vitro* system. The Illumina paired-end sequencing was conducted, and a total of 37862456, 35040478 and 35017513 reads were obtained after filtering by RNA-seq of the vegetative, about-to-flower and flowering stages. These were assembled into 191575 transcripts with mean length of 1005.68 bp. A total of 98,782 unigenes were annotated in the NCBI non-redundant protein database and 86,665 in the Swiss-Prot database. Also, 73,802 annotated unigenes were allocated to gene ontology (GO) categories. In them most of the unigenes were categorized into biological process followed by molecular function and cellular component. By searching against the Kyoto Encyclopedia of Genes and Genomes Pathway database (KEGG), 7,222 unigenes were assigned to 372 KEGG pathways and in addition 28905 simple sequence repeats (SSRs) were identified. In total 7439 differentially expressed floral specifiers representing the three floral developmental stages were identified.

**Citation:** Thapa, P., Bhattacharya, A. and Sood, A. Transcriptome profiling of floral development in *Dendrocalamus hamiltonii* uncovers floral transition mechanism in bamboos [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 188. [https://doi.org/10.24870/cjb.2017-a174](https://doi.org/10.24870/cjb.2017-a174)
Category: Plant Genomics

DNA barcoding of endangered medicinal plant
*Cayratia pedata*

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Abstract

Acknowledging the effectiveness of plants and their products in the treatment of diseases, the WHO recognizes that medicinal plants play an important role in the low-cost primary healthcare of about 80% of world’s population in developing countries including India. Plant and other natural products are gaining popularity as an alternative and system of medicine all over the world. *Cayratia pedata* is an indigenous endangered medicinal herb of south India belonging to the family *Vitaceae*. Traditionally, the leaves of this plant have been used as a dietary ingredient in the treatment of ulcers and diarrhoea. In Ayurveda the extract from *Cayratia pedata* is used to prepare formulations prescribed to treat microbial infections, ulcers, inflammations and arthritis. We have identified this plant to be a good source of phytochemicals like alkaloids, tannins, phenolic compounds, flavonoids and terpenoids. Correct identification of any medicinal plant is an absolute requirement in order to avoid errors in collection of the plants used for the formulations whose effectiveness depends on the natural products contained in them. DNA barcoding is a reliable tool in scientifically identifying medicinal plants. The current study explains how DNA barcode analysis of the plant *Cayratia pedata* helps in the proper identification based on nucleotide diversity of short DNA segments. DNA from the leaves of the plant was extracted and the chloroplast gene rbcL was amplified by PCR and sequenced. The sequence was subjected to a BLAST analysis to compare it with that of other species and a phylogenetic tree was constructed. The results confirmed that the plant belonged to the family *Vitaceae*. DNA bar-code analysis is a powerful technique for the identification, vouching and registration of medicinal plants especially when there is high species diversity. This helps in collecting the precise species that has the maximum yield of the active principles needed by the unskilled user as well as the pharmaceutical industry.

References


Category: Plant Genomics

**Sequencing, De novo Assembly, Functional Annotation and Analysis of *Cardiospermum halicacabum* L. Leaf Transcriptome Using Illumina Platform**

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**Abstract**

*Cardiospermum halicacabum*, widely known as balloon plant is an affiliate of the family Sapindaceae. It has significant medicinal properties and is traditionally used in the treatment of numerous diseases including arthritis, edema, psoriasis, asthma, etc. In animal studies, it was found to be effective to treat Alzheimer’s, Parkinson’s, cancer and diabetes. The genomic resources for this plant are highly limited and which in turn limits its medicinal value and pharmacological usage. The present study is aimed to characterize the leaf transcriptome of *C. halicacabum* using high throughput sequencing and advanced genomic tools. To the best of our knowledge, this is the first report on the transcriptome of *C. halicacabum*. De novo transcriptome sequencing was performed using Illumina NextSeq 500 platform. We have identified 40,750 unigenes with an average length of 715 bp, among which 4581 are full-length genes. BLAST against Plant Non Redundant database provided annotation for 13525 genes. Kyoto Encyclopedia of Genes and Genomes database deduced 1228 enzymes with 9688 transcripts involved in 140 pathways. Gene Ontology analysis categorized 17422 unigenes under biological process, 17795 in cellular components and 27274 in molecular function. MISA tool has identified 2802 SSR motifs in this plant. There were 414 transcription factors belonging to 46 transcription factor families. We have ascertained that the flavonoids, steroids and carotenoids synthesized by this plant are responsible for their activity. The enzymes involved in the biosynthesis of these secondary metabolites were validated using Reverse transcription PCR. Our study paves the way to comprehend the medicinal properties of *C. halicacabum* making it easy for further study and in the development of new drugs.

**Citation:** Prasidhee, V. and Natarajan, P. Sequencing, De novo Assembly, Functional Annotation and Analysis of *Cardiospermum halicacabum* L. Leaf Transcriptome Using Illumina Platform [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 190. https://doi.org/10.24870/cjb.2017-a176
Category: Plant Genomics

Computational identification of miRNA and their expressed targets from *Carica papaya*

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**Abstract**

Next Generation Sequencing (NGS) exhibited rapid developments through its high speed sequence annotation and assembly tasks which has changed the perspectives of genome era. NGS brings the great power to make several new biological observations and discoveries from genomes and helped in the growth of allied areas including Metagenomics, De novo sequencing, Amplicon Analysis, Transcriptomics and Small RNA profiling. *Carica papaya* small RNA sequencing helps in the understanding the role of known miRNA and identification of novel miRNA. Functional genomics often begins with studying the gene expression patterns and identification of potential targets that relies on the assumption of small RNA and the mRNA (target) sequence complementarity. The identification of Known miRNA sequences are searched through against the current miRBase version and sequences with less than 2 mismatches with known miRNAs in miRBase was considered and 1724 known miRNA depicted. Computational algorithms predict miRNA targets on the basis of the presumed mode of miRNA–mRNA interactions and also depend on the conservation of their binding sites. The mirDeep prediction software is used to predict new miRNAs where 11 novel miRNA are estimated. In addition, the functional studies of small RNAs can rely on conventional genetic strategies. Intriguingly, the advent of fast and cost effective NGS platforms in the recent past has enabled the proliferation of new ideas regarding the application of characteristic gene structures to different species.

**References**


**Citation:** Jha, N., Patel, M., Mangukia, N., Patel, S. and Rawal, R.M. Computational identification of miRNA and their expressed targets from *Carica papaya* [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 191. [https://doi.org/10.24870/cjb.2017-a177](https://doi.org/10.24870/cjb.2017-a177)
De novo transcriptome analysis of pneumatophores (modified roots) in the true mangrove species Avicennia marina and identification of the genes related to root gas exchange

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Abstract

Mangroves plants which grow in estuaries naturally tolerate extreme conditions of high salinity (90 ppt) and high light intensity. Avicennia marina is a true mangrove tree species with physiological adaptations like modified root system (pneumatophores) and salt excretion glands in leaves as its one of the unique features to consider. The pneumatophores are a special type of roots with negative geotropism that project above the water surface or the level of flooded soils [1]. In contact with air these roots develop lenticels, which improve gas exchange between roots and environment [2]. In swamps and wetlands the presence of pneumatophores facilitates oxygen diffusion through the tissues, maintaining levels adequate for cellular respiration [3]. Objective of this study was to perform the whole transcriptome analysis of pneumatophore tissue of A. marina by Illumina sequencing and to identify putative genes involved in process of root gas exchange. We generated 19.73 million of paired-end reads and assembled into 86,856 unigenes with an average length of 772 bp. Further, annotation, tissue specific gene expression and genes related to root gas exchange will be presented.

References


Category: Plant Genomics

**De novo** assembly of transcriptome and draft chloroplast genome from RNAseq data of *Bacopa monnieri* L. (Bramhi)

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**Abstract**

Medicinal plant naming services have recorded at least 28,187 plant species as being of medicinal use. Only 16% (4,478) of these plants have been cited in medicinal regulatory publications and even a lesser proportion of it has ready-to-use transcript sequence information available in public data bases. *Bacopa monnieri* L. or Bramhi is a widely used medicinal herb mentioned in ancient ayurvedic scripts as a part of medhya rasayanas (brain rejuvenating neutraceuticals). In spite of being an extensively studied plant, it has very little genetic resources in public databases, thereby limiting extensive molecular studies based on genetics. In this study we sequenced the whole transcriptome of *B. monnieri* L. using Illumina Hiseq 2500 producing ~78 million high quality reads, followed by the **de novo** assembly generating a transcriptome size of ~88Mb and 111,290 clustered unigene transcripts. Plant non-redundant database, pfam and uniprot database were used as reference databases and a total of 59,260 (53.25%) transcripts were annotated based on similarity searches. Pathway mapping of the unigenes using Kyoto encyclopedia of genes and genomes revealed 14,816 transcripts involved in 143 pathways. The triterpenoid and sesquiterpenoid biosynthesis pathway was selected to validate the assembled transcripts as the bioactive compounds in *B. monnieri* L. are reported to be triterpenoids. Simple sequence repeat (SSR) analysis excluding mono-nucleotide repeats showed the presence of 10,556 SSR’s in a total of 8892 transcripts. An attempt was made to assemble the draft chloroplast genome from the assembled transcriptome data and ~65% of chloroplast genome has been assembled and in progress. The draft chloroplast genome will also help in shedding light towards the evolution of *B. monnieri* L. The current study will provide information about key enzymes involved in various biosynthetic pathways and also a resource for comparative genomic and transcriptomic studies in future.

**Citation:** Prabhudas, S.K. and Natarajan, P. De novo assembly of transcriptome and draft chloroplast genome from RNAseq data of *Bacopa monnieri* L. (Bramhi) [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 193. https://doi.org/10.24870/cjb.2017-a179
Category: Plant Genomics

Identification of Groundnut miRNA and their targets

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Abstract

MicroRNA (miRNA) are ~22nt small non-coding RNA molecules which play an important role in post-transcriptional gene regulation in both plants and animals. As the miRNAs are highly conserved among species, comparative genomics based homology search has played a key role in identifying new miRNAs in different species whose genomes are not yet sequenced. Arachis hypogaea (groundnut or peanut) is one such legume crop and being grown in more than 100 countries, ranks third worldwide among oilseeds produced. In India, it is the second largest in terms of production but stands first in terms of area of cultivation. Identifying miRNAs and their targets can be helpful in crop improvement. In the present study, we tried to identify new conserved miRNA from the 205442 ESTs through blast search, using previously known plant miRNAs. The non-protein coding sequences with homology showing no more than 3 mismatches were folded back to stem-loop structure. These were subsequently passed through strict filtration criteria to obtain new miRNAs belonging to different miRNA families, as well as their targets.

References


Citation: Ram, M.K., Mukherjee, K. and Pandey, D.M. Identification of Groundnut miRNA and their targets [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 194. https://doi.org/10.24870/cjb.2017-a180
Category: Plant Genomics

**Marker Assisted Introgression of drought tolerance QTLs into popular high yielding varieties of rice**

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**Abstract**

Rice is the major staple cereal of India cultivated in about 44 million hectares area. In recent years, the increased occurrence and severity of drought stress has led to a yield decline in rice. This calls for development of varieties with higher yield potential combining drought tolerance. Even though many QTLs have been identified for various drought-related traits in rice; there are only few efforts to introgress them to develop improved breeding lines. Hence, the present research work aimed to transfer three QTL regions viz. \(qDTY1.1\), \(qDTY2.2\) and \(qDTY4.1\) from DrtIR64 through marker-assisted breeding with the strategy of improving the grain yield of popular high yielding varieties of rice (MTU1010 and NLR34449) under reproductive stage drought stress. The parents (Drt1IR64, MTU1010 and NLR34449) were screened with flanking markers of the targeted QTLs. Among all, RM551 (\(qDTY4.1\)) showed clear polymorphism between drought tolerant (DrtIR64) and susceptible lines (MTU1010 and NLR34449). The flanking markers of \(qDTY2.2\) (RM555, RM279 and RM492) showed polymorphism of DrtIR64 with MTU1010 and NLR34449. Using these polymorphic markers as foreground markers, the BC\(_1\)F\(_1\) plants obtained from MTU1010/DrtIR64 (Donor for \(qDTY1.1\), \(qDTY2.2\) and \(qDTY4.1\)) and NLR34449/DrtIR64 were confirmed for the presence of the targeted genes. Later, the confirmed BC\(_1\)F\(_1\) plants of two crosses will be backcrossed with recipient parents. Screening of the parents is being carried out at molecular level employing SSR markers that spanned the total genome, for identification of polymorphic markers. These will be used as background markers to test the recovery of recurrent genome content in the BC\(_2\)F\(_2\) progeny.

**Citation:** Jeevula, B.N., Eswar, G.R., Gopalakrishna, K., Swarajyalakshmi, B.N., Suresh, E.N., Yaminii, B.R., Bharghavi, M.M. and Vemireddy, L.R. Marker Assisted Introgression of drought tolerance QTLs into popular high yielding varieties of rice [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 195. [https://doi.org/10.24870/cjb.2017-a181](https://doi.org/10.24870/cjb.2017-a181)
Response of *Populus deltoides* Bartr. ex Marsh genes under elevated CO2 through Next-Generation Sequencing (NGS)

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Abstract

The impact of climate change has attracted considerable attention globally. Atmospheric Carbon Dioxide (CO₂) is expected to increase to 900 µmol mol⁻¹ from present level of 400 µmol mol⁻¹ by the end of 21st century. CO₂ is a greenhouse gas that leads climate change have significantly affected structure and function of the terrestrial ecosystem, global carbon, water balance, and also crop productivity. These responses of the plant appear by altering gene expression pattern of different genes involved in anabolic and catabolic processes.

We have conducted a study to see the response of genes to elevated CO₂ inside open top chambers on *Populus deltoides*. One-month-old ramets were exposed for 180 days to treatment (CO₂ 800 µmol mol⁻¹) and control (CO₂ ~400 µmol mol⁻¹). After completion of treatment, leaf tissues were outsourced to Sci-genome for transcriptome sequencing.

This study demonstrated, higher (1754) number of transcript expression in treatment (119,306) compared to control (121,060). Differential gene expression analysis shown 1951 transcripts were down regulated while 2603 transcripts up regulated and 159,982 transcripts have no significance in treatment.

Our results show that plants growing in an environment where atmospheric CO₂ is higher may alter plant adaptation, productivity, vegetation and ecosystem health by changing; the first, number of genes and second, altering gene expression patterns. Such behavior may be a good indicator of developing adaptation strategies of the plant.

Citation: Yadav, S.K., Barthwal, S. and Ginwal, H.S. Response of *Populus deltoides* Bartr. ex Marsh genes under elevated CO2 through Next-Generation Sequencing (NGS) [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 196. https://doi.org/10.24870/cjb.2017-a182
Category: Plant Genomics

Transcriptome profiling and identification of differentially expressed transcripts in response to mid season drought in groundnut *Arachis hypogeae* L

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Abstract

Drought is one of the major constraints in groundnut and its inheritance is governed by many genes with small effects operating in a coordinated manner. To this end, a total of eleven genotypes viz., ICGV 07070, ICGV 07132, TCQS 1398, TCQS 1073, TCQS 1157, TCQS 1173, MLTG 4, Narayani, Tirupati 1, Kadiri 6 and Kadiri 9 were screened in pot culture for moisture stress tolerance. Among the eleven genotypes, TCQS 1157 and MLTG 4 genotypes were more tolerant and Narayani and Kadiri 6 were highly sensitive to moisture stress. To unravel the molecular mechanisms conditioning drought tolerance, transcriptome was profiled cDNA-RAPD in these contrasting genotypes submitted to midseason moisture stress (50-80 DAS) and compared with respective well-watered control in field conditions. cDNA-RAPD profiles were developed with 35 RAPD markers in three regimes of moisture stress i.e., 60, 70 and 80 DAS. The transcript profile data revealed that drought stress induces the expression of many drought responsive transcripts and prolonged moisture stress has enormous impact on gene expression pattern. In the 35 cDNA-RAPD profiles analysed, a total of 823 transcripts were differentially expressed of which 523 transcripts exhibited qualitative difference (switched on: 263 transcripts, switched off: 260 transcripts) and 300 transcripts displayed quantitative differences (up regulated: 122 transcripts, down regulated: 178 transcripts). In the initial stages of moisture stress (10 to 20 days) many novel transcripts were activated along with modulation of gene expression (both up and down regulation) in comparison with well-watered control genotypes. The novel transcripts triggered by moisture stress will play a major role in stress perception, signal transduction, and synthesis of regulators and different compounds associated with drought tolerance mechanism. Under prolonged drought stress (30 days), the expression of novel transcripts were reduced by 30% and the up and down regulated transcripts was increased by 15%. Two moisture stress responsive transcripts were identified with OPA2 (750 bp) and OPA4 (450) markers. They were expressed only under drought stress in resistant genotypes (TCQS1157 and MLTG4) and absent susceptible genotypes and also in well-watered control of all the four genotypes three moisture stress regimes. Further characterization of these transcripts by sequencing will greatly help in understanding nature of the genes and the mechanism by which groundnut plants respond to drought stress. After validation, these two markers can be routinely employed for selection of drought tolerant germplasm lines.

The impact of natural selection on gene associated with panicle number formation in *Oryza sativa*

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Abstract

Panicle number is directly associated with grain number in rice. As the panicle number increases, it affects the total yield of rice. We examined the evolution of genes associated with panicle number formation in *Oryza sativa*. Intramural program written in JAVA script and fastPHASE software used for the generation of genotype and haplotype file of SNPs of 11 individual genes associated with panicle number formation utilizing VCF file obtained from RiceCAP project (USDA/CSREES http://www.uark.edu/ua/ricecap/). Tests for natural selection executed on these genes using the Haplotype data. Tajima’s D and Fu Li’s D* analysis were performed using DNASP v4.0. Rates of non-synonymous Vs synonymous changes were calculated according to the dN/dS algorithm of Nei and Gojobori. dN/dS calculation compared with the ancestral (*Oryza meridionalis*) sequence individually showed that out of 11, almost all genes responsible for grain number formation, Os01g0746400, Os03g0203200, Os03g0706500, Os04g0550600, Os06g0127800, Os06g0154200, Os06g0610350, Os06g0660200, Os08g0162100 and Os11g0528700 are negatively selected throughout evolution. Although Tajima’s D was not found significant, the negative value for 8 genes, Os01g0746400, Os03g0123300, Os03g0706500, Os06g0127800, Os06g0610350, Os06g0660200, Os08g0162100 and Os11g0528700 indicated that low frequency variants are more in number than high frequency variants. For Fu Li’s D*, the significantly negative values in most of the genes, Os01g0746400, Os03g0123300, Os03g0706500, Os06g0127800, Os06g0610350, Os08g0162100 and Os11g0528700 indicated that the high frequency variants detected through Tajima’s D are predominantly singletons. Thus, result from dN/dS, Tajima’s D and Fu Li indicated that negative (purifying) selection acts on genes responsible for panicle number formation. These results will be useful for further investigation on how the genes associated with panicle number and how purifying selection result in stabilizing selection through the purging of deleterious variations that arise.

Identification and characterization of salt-responsive novel miRNAs and their targets in *O. sativa*

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Abstract

Salinity is an inevitable environmental constraint leading to devastated crop productivity. Rice, being a glycophyte, is highly susceptible to salt stress specifically during early vegetative and late reproductive stages. To cope up, the plant has evolved a considerable degree of developmental plasticity so that the potential stress impacts are minimized. One such mechanism is driven by a class of endogenously expressed small RNAs, miRNAs, which have emerged as ubiquitous post-transcriptional gene regulatory molecules. Sequenced genome coupled with high throughput sequencing significantly advances our ability to unravel miRNA-guided stress tolerance mechanisms. Computational analysis revealed hundreds of miRNAs and their potential targets in different plant conditions. A total of eight conserved, and nine novel miRNAs were tested for their expression profiles in non-stressed and stressed conditions. All of these were found to be differentially expressing in different tissues and varied concentrations. Their presence was also checked in a distant wild relative (*O. coarctata*) and a halophyte (*S. maritima*). Targets of respective novel miRNAs were identified and the credibility was duly checked. Target prediction revealed several proteins directly or indirectly involved in imparting salt tolerance to the plant. Our study demonstrates genotype-specific miRNA regulation under salinity stress and evidence for their role in mediating expression of target genes for abiotic stress response. It can also be contemplated in developing transgenic crop cultivar which has increased salt stress tolerance.

Citation: Parmar, S. Identification and characterization of salt-responsive novel miRNAs and their targets in *O. sativa* [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 199. https://doi.org/10.24870/cjb.2017-a185
Category: Plant Genomics

Allele Mining and Allelic Diversity of Genes Governing Grain Size Related Traits in Rice (*Oryza sativa* L.)

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Abstract

Mining elite alleles for grain size is one of the key aspects for the improvement of cultivated rice to suit diverse global consumer preferences. Thus with the goal of identification of novel and superior alleles from the genes governing the grain size related traits by exploring the natural variability present in the rice germplasm, the present study was conducted. 124 rice genotypes were evaluated for different grain traits such as grain length (GL), grain width (GW), grain length to width ratio (GL/GW), and 1000-grain weight (TGW). The germplasm of 124 rice genotypes presented substantial variation for grain size traits. Significant correlations were detected among the grain size traits. All the four traits exhibited normal distribution in the germplasm indicating quantitative inheritance of these traits. In total, 32 molecular markers comprising of 8 grain size gene-specific markers and 24 SSR markers covering all 12 chromosomes were used in this study and all markers showed polymorphism and produced a total of 86 alleles among the 124 rice varieties. Number of alleles ranged from 2 to 4 with an average of 2.68 alleles per locus. The mean polymorphism information content (PIC) value was 0.34. Analysis revealed 124 genotypes could be made into two groups, A and B. The group A exclusively includes the extra-long grain length basmati genotypes. However, the group B again divided into two groups i.e., B1 and B2. The group B1 includes mostly long grain genotypes. The group B2 comprised of all classes of grain length and size genotypes. Based on the population structure Q matrix data the 124 accessions are divided into four clusters/subpopulations, viz., from POP1 to POP4. POP1 subpopulation was grouped under extra-long grain type, POP2 was grouped under long grain type, POP3 and POP4 includes all the four grain size classes. Eight marker-trait associations were identified by screening 124 genotypes with grain size specific primers for GL, GW, L/B and TGW traits. One GS3 gene-specific marker, GS3RGS1 was found to be associated with GL, GW, L/B and TGW traits with their PVE as 15.2%, 16.9%, 10.3% and 7.8%, respectively. Earlier results also reported that the GS3 is the major gene governing the grain length and minor gene for grain width. Similarly, one SSR marker, RM505 was showed association with GL, GW, L/B and TGW traits with their PVE as 4.4%, 2.6%, 1.9% and 3.8%, respectively. The present investigation reinforces the fact that grain size is a complex trait regulated by many genes located on different chromosomes. However, the gene specific markers, for GL, GW, TGW and L/B traits, such as GS3RGS1 and RM505 have potential to be used as foreground markers in marker-assisted breeding. Mining of complete gene sequences and other genes governing grain size traits is warranted further investigation adding some more germplasm.

Category: Plant Genomics

A transcriptomic study on cold stress in two Indian rice varieties using RNA-Seq analysis

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Abstract

Cold weather negatively affects the growth of seedling, which ultimately decrees the production of crops. Studies were done in the context of cold stress related to rice (Oryza Sativa) production, which is an important staple crop taken as food by half of the human population worldwide. It's always been a challenge to tackle out the problems related to such stress conditions. Rice is a model organism for monocots, finding out the molecular markers can help improve different crop varieties against cold stress. Advancement in High throughput techniques such as RNA-Seq, gives us an opportunity to revisit all the aspects of previous studies and improve them in more depth. Here we chose rice at seedling stage of both cold tolerant and susceptible genotype for our transcriptome level study under normal temperature, cold stress, and recovery condition. In our experiment, Genome wide expression profile of both the genotypes at all three different conditions was studied. We detected a total of 3217 and 485 common regulated differentially expressed genes (DEGs) during cold stress and recovery condition respectively. Followed by their gene ontology (GO) enrichment analysis for different functions they involved. By combining co-expression study and cluster analysis, we suggested few of the genes which may be highly responsible for cold stress and not reported before. These results expand the opportunities to explore cold stress and their recovery for crop plants with more detail in future.

Citation: Sahu, S.K. and Tiwary, B.K. A transcriptomic study on cold stress in two Indian rice varieties using RNA-Seq analysis [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 201. https://doi.org/10.24870/cjb.2017-a187
Category: Plant Genomics

Gene expression and sequence analysis of known yield genes in high yielding varieties of rice (*Oryza sativa* L.)

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Abstract

The rice yield is governed mainly by three major traits viz. number of tillers per plant, grain number per panicle, and grain size which are directly associated with rice grain productivity. Rice yield is a complexly inherited trait governed by many genes/QTLs. As of now more than 34 genes have been molecularly cloned and characterized using the donors only from China and Japan (Xu et al., 2016). However, the expression of these genes and sequence variation need to be validated before directly using them in rice breeding programmes. In the present investigation, an attempt has been made to validate the gene expression and sequence variation of the important cloned genes governing yield traits in known donor varieties from India. In the current study, 21 *indica* rice genotypes have been selected. Among them 15 were high yielding and 6 were low yielding. The phenotypic data of the important yield and its component traits such as plant height, number of tillers per plant, panicle length, number of grains per panicle, biological yield, harvest index, etc. have been recorded. The analysis of variance (ANOVA) for the 21 rice genotypes for all the agronomic traits revealed highly significant differences among the entries for all the characters except for grains per panicle, biological yield, harvest index, etc. have been recorded. The samples of flag leaf and young panicle tissues were collected for RNA isolation and the isolated RNA was converted into cDNA. Semi qRT PCR gene expression analysis in the selected rice genotypes revealed differential expression of all yield genes. The *Gna* gene expression with low or nil gene expression such as NLR33892, Ranjit, BPT2601, BPT5204 and Rasi can be used as donors for *Gna* gene introgression into low yielding varieties. The gene *OsSPL14* from high grain number varieties with high gene expression can be used as donor varieties viz. MTU1064, Dee-geo-woo-gen, Rasi, BPT5204, and MTU1010 for *OsSPL14* gene from Indian rice germplasm. The high *GFI* gene expression varieties with high grain weight such as BPT 2678 and Basmati370 are the potential donors for grain weight/grain filling trait. The high grain weight varieties with high *GW8* expression such as MTU3626, IR-8, Dee-geo-woo-gen, MTU1010, MTU1001, and INRC10192 can be used as donors from Indian rice germplasm. The *Ghd7* high gene expression was observed in both high grain number genotypes such as NLR33892, BPT2678, Taichung Native-1, MTU1121, Ranjit and low grain number genotypes IR-8, MTU3626, MTU1001, MTU1064, Tetep, NLR34449 in flag leaf. In case of young panicles, high expression was recorded in high grain number genotypes such as NLR33892, Ravi003, BPT2678, MTU1121, Ranjit and in low grain number genotypes such as IR-8, MTU3626, MTU1001, MTU1064, Tetep, and NLR34449. The high gene expression was observed in both flag leaf and young panicles in both early flowering varieties and late flowering varieties with few exceptions. The high gene expression was observed in both flag leaf and young panicles in both tall plants and dwarf plants. The high grain number, tall plant and late flowering varieties with high *Ghd7* expression such as NLR33892, BPT 2678 and Ranjit can be used as donors from Indian rice germplasm. In the present investigation, besides gene expression analysis, the DNA sequence variation of most differentially expressed yield genes such as *Ghd7*, *DEP1* and *Gna* have also been analyzed in all high yielding and low yielding varieties. The overlapping primers covering the entire gene length including 1000 base pair upstream have been designed and sequenced. The whole genome DNA sequence of *Ghd7* gene (3918 base pair) was resequenced in all 21 rice genotypes. In all 21 rice genotypes, a total of 104 SNPs and 141 indels were detected. It was found that certain nucleotide variations are unique to high grain number varieties such as MTU1121, MTU1010, and Dee-Geo-Woo-Gen. However, there are no nucleotide variations which are common to their respective either low or high grain number varieties INRC10192, Tetep, and MTU1001. There are no nucleotide variations found specific to either tall or semidwarf varieties MTU7029 (G/A) at 1426 and MTU1001 (T/C) at 1425 base pair positions of the gene. There were no nucleotide variations specific to either early or late flowering varieties. The whole genome DNA sequence of *DEP1* gene (4363 base pair) was resequenced in all high yielding and low yielding rice genotypes. In all
varieties studied, 99 SNPs and 338 indels were detected in \textit{DEP1} gene. The whole genomic DNA sequence of \textit{Gn1a} gene (6476 base pair) was resequenced in all genotypes. In total, 97 SNPs and 121 indels were detected in the 4837 base pair alignment. Of these, one SNP (MTU1064) and 28 indels were detected in the promoter region. To conclude, an attempt has been made to identify the Indian donor varieties comprising of important yield genes based on consistent gene expression and sequence analysis with reported donors. The shortlisted donors for the yield traits can be used right away in the rice breeding programmes. The marker-assisted stacking of different yield genes from these multiple donors into a single elite variety could pave the way for designing high yield varieties suitable to different ecologies of India.

Deciphering the role of a miRNA in rice domestication

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Abstract

MicroRNAs (miRNAs) are a class of 21 nt non-coding small RNAs (sRNAs) produced from endogenously expressed MIR genes. miRNAs are mostly involved in development and disease resistance. We are interested in identifying key miRNAs that are differentially expressed among wild and cultivated rice species. Analysis of sRNA datasets from two wild species (O. nivara and O. rufipogon) and one cultivated species of rice (O. sativa var. indica Pusa Basmati-1), revealed a surprisingly higher abundance of small RNAs originating from Chromosome 2 in wild rice species. This locus codes for a novel 22 nt miRNA. This novel miRNA was found to be highly abundant in flag leaf of wild species, a tissue that usually provides 70% of energy required for grain filling. This miRNA targets a group of proteins (Os03g0273200, Os01g0827300, Os01g0850700, Os11g0708100 and Os01g0842500) which are involved in secondary metabolite production, although a functional significance of this interaction has not been understood. The expression of these targets also differs across the species. Typical of 22 nt miRNAs, the identified miRNA also triggers a secondary cascade silencing by producing small interfering RNAs (siRNAs) from target mRNAs in O. nivara. These secondary siRNAs are observed only among wild rice species but not in cultivated rice. Currently we are using a range of genetic, biochemical and molecular techniques to understand role of this novel miRNA in domestication of rice.

References


Citation: Chenna, S. and Tirumalai, V. Deciphering the role of a miRNA in rice domestication [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 204. https://doi.org/10.24870/cjb.2017-a189
Category: Plant Genomics

Marker-assisted introgression of drought tolerance from wild ancestors into popular Indian rice varieties using a 7K Infinium SNP array


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Abstract

Recent advances in the area of genomics have led to the development of high throughput genotyping platforms that have immensely contributed to molecular breeding programs. Custom-designed single nucleotide polymorphism (SNP) arrays provide an efficient, cost effective, high throughput genotyping tool for QTL/gene mapping, variety identification, marker-assisted selection, etc. In the current study, two interspecific libraries of Chromosome Segment Substitution Lines (CSSLs) were evaluated under both drought and control conditions to identify lines with superior yield under drought. The CSSL libraries consisted of 48 BC4F3 lines derived from O. sativa cv. Curinga (tropical japonica) x O. rufipogon, and 32 BC4F3 lines derived from O. sativa cv. Curinga (tropical japonica) x O. meridionalis. The phenotypic screening of these 80 CSSLs led to the identification of MER-20 that yielded well under drought stress. This line was backcrossed with popular rice variety of India, Swarna-Sub1 to introgress wild chromosome segments responsible for reproductive stage drought tolerance. During backcrossing, tracking of wild introgressions and monitoring of recurrent parent genome recovery was facilitated by the use of the Cornell 6K and 7K Infinium rice SNP arrays. The 6K and 7K SNP arrays assayed 5275 SNPs and 7099 SNPs, respectively, distributed across the 12 chromosomes. In our populations of (MER-20X Swarna sub1) BC2F1 lines, 1775 SNPs were polymorphic using the 6K array. The percentage of recurrent parent genome in these backcrossed lines ranged from 33-92% and the percentage of wild donor genome ranged from 8-67%. Using genotypic selection, 5% of plants were identified for further marker assisted backcrossing, based on the presence of the target donor (wild) segment and maximum recovery of recurrent parent background. In the next generation, BC3F1 lines were genotyped using the 7K SNP array, which identified 2521 polymorphic SNPs. In the BC3 generation, 60-95% of the recurrent parent genome was recovered and wild segments accounted for 5-40% of the genome. The best 5% progenies were again selected based on genotype, and selected BC3F2 individuals are now being evaluated for yield under drought stress. The use of the 6K and 7K SNP arrays improved the efficiency and accuracy of genotyping and greatly facilitated tracking of recurrent/donor genomes in backcross lines.

Category: Plant Genomics

Analyzing the structural aspects of Isoprenoid biosynthesis pathway proteins in Ocimum species

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Abstract

Generally thought that the extremely diverse array of secondary metabolites observed within Ocimum species defends against a comparable diverse array of biotic pests, pathogens and herbivores encountered around its natural range. Along with defense the diverse array of secondary metabolite also leads to the therapeutic and remedial property which justifies Ocimum as natural medicinal and aromatic casket. Many of the defense compounds, aroma compounds and medicinal derivatives are secondary metabolites isolated from trichome glands, mainly consist of terpenoids as well as phenylpropanoids. Various pathways fabricating these compounds are known viz. mevalonate pathway (MVA), phenylpropanoid pathway and MEP pathways. The enzyme cascade responsible for various secondary metabolites, need to be explored in various aspects. Here we had studied the MVA pathway enzymes in *O. basilicum* and *O. gratissimum* to figure out variations in enzyme structures due to speciation. Hence, in depth analysis of the transcriptome of *O. basilicum* and *O. gratissimum*, varying in qualitative and quantitative aspects of essential oil were carried out. The transcriptome data from NCBI server was assembled using bioinformatic approaches, nr database at NCBI repository used for annotation, which assigned 60% contigs to known functions. Contigs corresponding to Mevalonate pathway enzymes are isolated using perl pipelines developed in our lab, which were further assembled using CLC workbench to remove redundancy and make larger stretch of sequence. Blastx of these larger sequences assigned them function and they are mapped to validated sequences to make full length. Data from both species led us to overall seven enzymes (total 14) of MVA pathway. These enzymes are studied in detail for various physio-chemical properties, stereochemical properties and motif/domain for protein-protein interaction (PPI) study. Homolog models of all enzymes were predicted, against templates from RCSB database. Threading approach is used for enzymes whose homologs are not available in public domain. Structure analysis (energy minimization, Ramachandran plot, stereospecificity and PDB cleaning, Root Mean Square deviation) helped to infer that amongst seven enzymes key gene from MVA pathway showed variation at three sites within active domain. This study opens up new avenue for secondary metabolite pathway prediction and operation analysis, this will help to develop biotechnological logical tools for Ocimum crop improvement.

Citation: Chandra, M. and Sangwan, N.S. Analyzing the structural aspects of Isoprenoid biosynthesis pathway proteins in Ocimum species [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 206. [https://doi.org/10.24870/cjb.2017-a191](https://doi.org/10.24870/cjb.2017-a191)
Category: Plant Genomics

Mining of genes involved in ROS maintenance and metal uptake in *Withania somnifera* (L.) Dunal under heavy metal stress (Cd)

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Abstract

*Withania somnifera* (L.) Dunal (Solanaceae), also known as Ashwagandha or winter cherry, is one of the most reputed Indian medicinal plants of the traditional Indian systems of medicines, is used in more than 100 formulations of Ayurveda, Unani and Sidha since over 3000 years. Ashwagandha has been used in curing of various diseases and possesses many pharmacological activities due to the occurrence of characteristic phytomolecules i.e. modified steroidal lactones called as withanolides. Cadmium (Cd) has been considered as harmful pollutant to the environment due to its high water solubility, mobility and long biological half-life. Cd is a potential threat to the plant as well as humans beings even at very low concentration. Cd has deleterious effect on plant growth and development. Plants induce various biochemical responses including enzymatic and non-enzymatic antioxidants to minimize the heavy metal toxicity. As the plants are incessantly exposed to essential as well as non-essential heavy metals and have capability to uptake both metals from their environment (growing medium), present work was carried out to evaluate the genes involved in ROS homeostasis and heavy metal uptake including profiling of various other biochemical responses of *W. somnifera* grown under glass house conditions supplemented with exogenous gradient concentration Cd. The various enzymatic and non-enzymatic parameters were analyzed related to ROS determination and Cd deposition in tissues. Along with these analyses, several genes were mined out from the transcriptome of *W. somnifera* involved in ROS homeostasis and heavy metal uptake. Conserved regions of various ROS genes were taken for their expression analysis in Cd treated shoots to know their involvement against Cd stress. Various *in silico* and wet lab studies of gene involved in heavy metal uptake were also conducted for its structural and functional validation. This study revealed that the Cd affects substantially various active growth parameters as well as enzymatic and, non-enzymatic antioxidative responses. Results also revealed the suspected genes involved in ROS homeostasis maintenance and the heavy metal uptake in ashwagandha against Cd stress which will be discussed in detail during the presentation.

Citation: Mishra, B. and Sangwan, N.S. Mining of genes involved in ROS maintenance and metal uptake in *Withania somnifera* (L.) Dunal under heavy metal stress (Cd) [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 207. https://doi.org/10.24870/cjb.2017-a192
Category: Plant Genomics

Identification of isoforms of microRNAs in wheat (*Triticum aestivum* L.) and their role in leaf rust pathogenesis

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Abstract

Bread wheat, a type of grass under genus *Triticum* and species *aestivum* covers the largest land area when production of cereal crops is considered. Being an allohexaploid (2n=6x=42; AABBDD), its genome is contributed by three progenitors and is evolutionarily rich. Rust in leaves, caused by *Puccinia triticina*, severely affects grain quality. MicroRNAs are considered as major components of gene silencing and so have deep role to play during stress. Post transcriptional modification of miRNAs which generates isomiRNAs significantly affects target specificity especially when the modification occurs in 5′end.

A total of four small RNA libraries were prepared through next-generation Illumina sequencing techniques from leaves of two wheat Near Isogenic Lines (NILs), HD2329 (susceptible) and HD2329 + LR24 (resistant). Prior to this, one set of the two NILs was mock inoculated and considered as control (with sRNA library code named SM-mi and RM-mi) while other was treated with urediniospores of leaf rust fungus (with sRNA library code named SPI-mi and RPI-mi). Clean reads in all four libraries were previously used for prediction of 559 novel miRNAs and in the current study it was used to detect isoforms of these miRNAs. A total of 237 isoforms were detected for 41 miRNAs. These isoforms included both 5′ and 3′ modifications of miRNAs. There were 27 miRNAs with 5′ modifications and five miRNAs with 3′ modifications while nine miRNAs showed both types of modifications.

Citation: Dutta, S., Kumar, M. and Mukhopadhyay, K. Identification of isoforms of microRNAs in wheat (*Triticum aestivum* L.) and their role in leaf rust pathogenesis [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 208. https://doi.org/10.24870/cjb.2017-a193
Category: Plant Genomics

Analysis of iron and zinc homeostasis in barnyard millet through transcriptome and ionome approach

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Abstract

Iron (Fe) and Zinc (Zn) are the most essential micronutrients needed for the growth and metabolism of higher plants. Fe and Zn has important role as a component of various enzymes that are involved in chlorophyll biosynthesis, photosynthesis and seed development. Plants have evolved with multifaceted Fe and Zn homeostatic mechanisms that regulate its acquisition from the environment and the movement between organelles, cells, tissues, and organs. In addition, Plant establishes a tightly controlled system including metal specific uptake transporters and transcriptional regulators to balance the uptake, utilization and storage of metal ions. Barnyard millet (Echinocloa frumentaceae), one of the minor millets is superior in Fe and Zn content compared to the most widely consumed cereals like rice and wheat. In the present study, ionomic profiling of grains of several barnyard millet accessions revealed that accession ACM-10-145 accumulates high Fe and Zn content (Fe: 14.5 mg/100g; Zn: 2.18 mg/100g). Furthermore, transcriptomic studies are in progress to understand the key factors involved in metal uptake and translocation in barnyard millet. The research outcome could be exploited for biofortification program in cereals.

Citation: Prabha, V.V., Varanavasiappan, S., Raveendran, M. and Jeyakumar, P. Analysis of iron and zinc homeostasis in barnyard millet through transcriptome and ionome approach [Abstract]. In: Abstracts of the NGBT conference; Oct 02-04, 2017; Bhubaneswar, Odisha, India: Can J biotech, Volume 1, Special Issue, Page 209. https://doi.org/10.24870/cjb.2017-a194
Dynamic transcriptome profiling of the floral buds in the dioecious cucurbit Coccinia grandis using RNA-Seq

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Abstract

Angiosperms exhibits diversified sexual systems encompassing bisexual, monoecious and dioecious conditions. Dioecy offers opportunities to explore separately, the male and female systems giving an insight into the evolutionary, developmental and molecular processes of sex expression in plants. Coccinia grandis (Family: Cucurbitaceae) with small genome size and heteromorphic sex chromosomes is often considered a model dioecious plant for sex evolution. However, the information relating to its genetic orientation, physical state and sex determining factors is highly ambiguous and limited. In the present study we have attempted to identify the molecular basis of sex determination in C. grandis through genome wide transcriptome profiling of the floral buds. About 75 million clean reads were generated resulting in 72,479 unigenes for male library and 63,308 unigenes for female library with a mean length of 736 bp. 1410 unigenes were differentially expressed (DEGs) between the male and female buds as identified from the RNA-Seq pattern and qRT-PCR validation. Functional annotation using BLAST2GO and KEGG revealed high enrichment of DEGs in phytohormone biosynthesis, hormone signaling and transduction, transcriptional regulation and methyl transferase activity. Manifold up-regulation of genes phytohormone responsive genes such as ARF6, ACC synthase1, SNRK2 and BRI1-associated receptor kinase 1 (BAK1) suggest that a signaling crosstalk is implicated in the sex determination of this species. Besides, a wide range of transcription factors including zinc fingers, homeodomain leucine zippers and MYBs were recognized as major determinants of male specific expression in the dioecious plant. Additionally, C. grandis transcriptome revealed 48 target genes for many miRNAs sequences with established role in floral development and sex determination. Overall, our study resulted in the identification of a large amount of molecular resources that could be critical to the mechanism of sexual dimorphism in dioecious plants in general and C. grandis in particular.


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Category: Plant Genomics

In silico identification and functional annotation of miRNAs and their targets from EST and GSS of onion (Allium cepa L.)

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Abstract

MicroRNAs are a class of approximately 20-24 nucleotides (nt) endogenous small RNAs that negatively regulate gene expression and play vital roles in multiple biological processes, including plant growth, development and responses to environmental stresses. Onion (Allium cepa L.), also called as “queen of kitchen” is a bulbous vegetable crop cultivated in almost all parts of the world. However, the miRNA repertoire of onion is highly ambiguous. In the present study, we report the computational identification of miRNAs and their targets from expressed sequence tags (ESTs) and genome survey sequences (GSSs) of Allium cepa L as well as functionally annotated the target genes. By following a stringent pipeline, we used 20225 ESTs and 10725 GSS from onion to identify 9 new potential miRNA belonging to 8 different miRNA families (miR172, miR1134, miR1223, miR6219, miR7725, miR8570, miR8703 and miR8752). Under a stringent condition, 26 potential targets were identified for the 8 miRNAs with distinct functions related to growth and development, signal transduction, metabolism, defense and stress responses. Overall, the present finding will make the pathway for understanding of molecular mechanisms of miRNA in onion and understanding their involvement in post-transcriptional gene silencing mechanism towards regulation of stress responses in this economically important plant.

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Allele-specific physical interactions regulate the heterotic traits in hybrids of *Arabidopsis thaliana* ecotypes

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Abstract

Heterosis is an important phenomenon for the breeding in agricultural crops as it influences yield related traits such as biomass yield, seed number and weight, adaptive and reproductive traits. However, the level of heterosis greatly varies for different traits and different genotypes. The present study focuses on identification of physical interactions between alleles and their role in transcriptional regulation in heterotic plants. Here, we used two *Arabidopsis* ecotypes; Col-0 and C24 as parent for crosses. We performed crossing between these ecotypes and screened the F1 hybrids on the basis of different SSR markers. Further, we used Hi-C to capture intra- and inter-chromosomal physical interactions between alleles on genome-wide level. Then, we identified allele-specific chromatin interactions and constructed genome-wide allele-specific contact maps at different resolutions for the entire chromosome. We also performed RNA-seq of hybrids and their parents. RNA-seq analysis identified several differentially expressed genes and non-additively expressed genes in hybrids with respect to their parents. Further, to understand the biological significance of these chromatin interactions, we annotated these interactions and correlated with the transcriptome data. Thus, our study provides alleles-specific chromatin interactions in genome-wide fashion which play a crucial role in regulation of different genes that may be important for heterosis.

Category: Plant Genetics

Developing climate resilient rice through genomics assisted breeding

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Abstract

Rice is one of the major cereal food crops whose production has to be doubled to achieve the projected demand [1] and current yield trends are not sufficient to meet the projected growth in production. Increasing the rice production by 30% during 2030 needs overcoming challenges viz., yield plateau, declining land, water and labor resources and predicted effects of global climate change. Development of high performance rice genotypes with enhanced yield potential and resilience to climate change will help in sustained increase in rice production. Deployment of genomic technologies can accelerate development and delivery of improved germplasm with enhanced resilience and adaptability [2, 3]. In this context, the present study was undertaken with an aim of developing rice genotypes pyramided with QTLs/genes controlling tolerance against various biotic and abiotic stresses viz., bacterial leaf blight (x13, Xa21), blast (Pi9), Gall midge (Gm4), drought (qDTY1.1 qDTY2.1), submergence (Sub1) and salinity (Saltol). CBMAS14065 an elite culture harboring QTLs controlling tolerance against drought, salinity and submergence was crossed with a donor harboring BLB, Blast and Gall midge resistant genes. True F1s were backcrossed with CBMAS14065 and BC1F1 progenies were subjected to foreground selection using markers linked to the target traits. Superior plants (18) of BC1F1 generation were subjected to background selection which revealed 71.42 - 86.90% recurrent parent (CBMAS14065) genome recovery. Selected BC1F1 plants were advanced to BC2F1 generation backcrossing with CBMAS14065. In BC2F1 generation, through foreground selection 6-8 QTL/gene positive plants have been selected and advanced for further evaluation. The superior lines with desired QTLs/genes will be subjected to rigorous phenotypic evaluation against target stresses and advanced further.

References


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