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Identification of Drug Target Properties and its validation on *Helicobacter pylori*

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Abstract

An analysis of 472 proteins taken from the drug target database and considered as bacterial drug targets has been carried out. A number of sequential properties viz. length, molecular weight, hydrophobicity, cellular localization, transmembrane helices, glycosylation and signal peptide were determined. Based on these properties, a range was set for each property and it was considered that a protein can be a drug target if that protein property comes in the same range. To validate, the method was applied to *Helicobacter pylori* having 1602 proteins. The properties were calculated for proteins from *H. pylori* and the range was applied to find the drug target. After analysis of the whole proteome, 5 proteins have been found to have all the properties in the range. The results were cross checked and it has been found that the resultant proteins are also drug targets for other pathogens. It indicated that the sequential properties of successful target help in finding the new drug target for the pathogen.

Keywords: Drug targets, *Helicobacter pylori*, Hydrophobicity, Sequence, Glycosylation, Transmembrane, Peptide, Pathogen

Introduction

Drug target is the macromolecule that interacts with the drug used to treat a disease. Most of the drug targets are proteins or nucleic acids (DNA or RNA). The protein drug targets mainly include enzymes, receptors, transporter proteins and ion channels proteins. The drug after interaction with the drug target either inhibits its activity or changes its conformation resulting in change in the function(s) of the target. The dysfunction of the target cures the disease either by killing or halting the growth of the pathogen. Therefore, drug target is considered to be much important to cure a disease [1]. Drug targets are not the disease causing but are considered as disease modifying molecules. Drug targets are classified into different classes and subclasses based on the activity. For example, oxidoreductase and ligases for enzymes, ligand gated ion channels and G protein coupled receptor for receptors, voltage gated ion channels and solute carriers for transporter proteins and many other classes/subclasses have been considered to be the potent drug targets [2].

For any drug discovery, drug target identification is a very crucial step and must be accurate as the whole process of drug discovery depends on the drug target [3]. There is a need for identification of drug targets for many arising diseases and also for the pathogens which acquire resistance to the current treatment. There are varieties of *in silico* methods that are used by many scientists to find the drug target. These methods find the drug target using the genome of the pathogen, metabolic pathways, virulence factors, etc. [4]. Barh et al. [5] predicted the drug targets for *Cornebacterium pseudotuberculosis* using genome analysis. Chordia et al. [6] determined the drug targets for *Listeria monocytogenes* using interactome analysis. Yadav et al. [7] showed the targetable virulence factors for pneumonia using bioinformatics tools. The other workers predicted drug targets for *Helicobacter pylori* using various *in silico* approaches [8, 9]. Here, we have developed a new method for identification of drug target that is based on the already available drug targets. This method is designed considering a view that identifying new targets based on already reported drug targets has higher chances of success.

For a drug target, there must be certain properties that make it different from other proteins or molecules. These properties include molecular weight, sequence length, secondary structure (α-helix and β-sheet contents), transmembrane helices, hydrophobicity, sub-cellular localization, signal peptidase and glycosylation sites. In the present study, all these properties were calculated using available tools and were analyzed for all the reported drug targets. Based on the analysis, a criterion (range) was set for each property that must be present in a protein to be a drug target. Any protein having all the set properties can be considered as the drug target. The method developed has been applied on the proteins of *H. pylori* to find the potent drug targets. The *H. pylori*, also named as *Campylobacter pylori*, is a Gram-negative, microaerophilic bacterium which is colonized in the stomach. It is an infectious agent responsible for peptic ulcers, gastritis and gastric carcinoma [10]. The currently used drugs reduce the pain but do not fight against the *H. pylori*. Recently, Megraud et al. reported the development of antibiotic resistance of *H. pylori* against many antibiotics like clarithromycin, levofloxacin and many more [11]. Therefore, there is a need of the drug target specific for *H. pylori* to discover a drug that can fight against *H. pylori* to cure the disease.

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There are variety of databases that have the repository of bacterial drug targets viz. DrugBank, Therapeutic Target Database (TTD), Potential Drug Target Database (PDTD) and many more. In the present study, for protein properties, 472 bacterial drug targets were taken from TTD and PDTD. Thereafter, on the basis of protein property analyses, drug targets have been identified for *H. pylori* and validated.

Methods

Data collection

For bacterial drug property analysis, drug targets were taken from TTD and PDTD databases. The datasets consisted of 285 bacterial targets from TTD and 187 bacterial targets from PDTD. A total of 472 targets were taken for prediction and analyses of the properties. These data were collected by using the combination of search terms viz. bacteria drug target, bacterial drug target and bacteria in both the databases. Thereafter, redundant results were removed and rest of the drug targets were used for the analyses. TTD can be accessed at http://bidd.nus.edu.sg/group/cjttd/ [12] and PDTD can be accessed at www.dddc.ac.cn/pdtd/ [13].

Prediction of drug properties

Sequence properties of all these 472 drug targets were identified using different tools. The ExPasy ProtParam was used for the identification of sequence length, molecular weight and hydrophobicity [14]. The secondary structure was predicted using CFSSP (Chou and Fasman Secondary Structure Prediction) server [15]. The transmembrane helices contents were determined using TMpred [16], whereas sub-cellular localization was predicted using PSORTb [17]. The signal peptidase was predicted using Phobius [18] and glycosylation was predicted using GlycoEP tool [19].

Analyses of the drug properties

After prediction, all the properties of the drug targets were analyzed. A cut-off score (range) was selected for all the properties of the drug targets. Cut off score was taken based on the average and mode of the scores for each property.

Validation on *Helicobacter pylori*

The proteome of *H. pylori* 2017 was downloaded from NCBI (National Center for Biotechnology Information) ftp (file transfer protocol) site [20]. The *H. pylori* has a total of 1602 proteins in its proteome. The above mentioned properties were calculated for all the proteins of *H. pylori*. Thereafter, proteins that have all the properties in the given range were selected as drug targets. The entire methodology used has been outlined in Fig. 1.

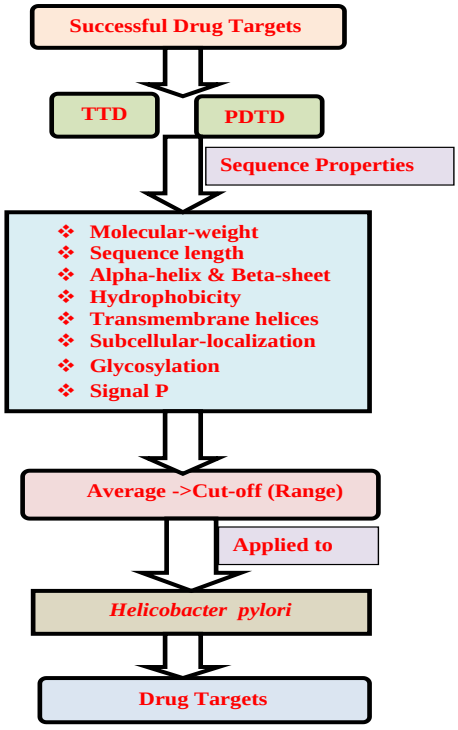

Fig. 1: Flowchart depicting the entire methodology.

Results and Discussion

The dataset of drug targets used in the study was downloaded from TTD and PDTD databases. The whole dataset contains 472 proteins and all of which are the drug targets for bacterial pathogens. These two databases were used since both have the collection of drug targets which are published in various scientific journals. Instead of searching each drug target in literature, the entire dataset from the databanks were taken that saved the time and the chances of any error were reduced.

The entire dataset of 472 proteins was used for analyses of various sequential properties viz. sequence length, molecular weight, hydrophobicity, glycosylation, transmembrane helices, sub-cellular localization and signal peptidase. These properties were selected as these are some of the characteristics that screen the protein to be a drug target. It has been reported that a protein can be a drug target if it is essential for the pathogen's survival [21]. In the present study, search was made for the set of characteristics that makes a protein a drug target. Therefore, for characterization of the drug target, the dataset of drug target from the drug target databank was taken.

Each protein in the dataset was taken and its properties were calculated using various tools. Firstly, the sequence length of the drug target was determined using the ExPasy ProtParam tool. In general, proteins of less than 100 amino acids length (mini-proteins) are less likely to represent essential genes [22, 23] and therefore were considered insignificant as drug targets. Sequence length shows the primary structure of the protein, and it forms the backbone of the protein structure and the structure forms the functional unit of the protein. Therefore, sequence length is a very important characteristic of a protein. The average sequence length of proteins in the drug target dataset was found to be 516.80. Considering it, in the present study, the range of sequence length to be a drug target was set to be 400 to 600.

The molecular weight of a protein is considered to be fundamental for its biochemical characterization. Sometimes, apparent molecular weight may be different due to electrostatic/hydrophobic interactions between the protein and the constituents of the solvent. Proteins have different functions which include enzyme catalysis, metabolic regulation, binding and transport of small molecules, gene regulation,

immunological defence and cell structure. From the drug target dataset, the average molecular weight was determined to be 57407.01. Therefore, in the present study, the range of molecular weight of a protein to be a drug target was set to be 45000 to 65000.

The hydrophobicity of a protein depends on the presence of hydrophobic residues in it. It has an important role in the protein folding and aggregation [24]. The hydrophobicity helps the protein in proper folding which in turn helps in proper functioning of the protein. So hydrophobicity of the protein is an important characteristic of the protein sequence. Hydrophobicity was calculated using Kyte and Doolite Index [25]. From the drug target dataset, the average hydrophobicity was found to be -0.299. Therefore, in the present study, the range of hydrophobicity in a protein to be a drug target was set to be -0.150 to -0.350.

The structure of a protein depends on its secondary structure. The percentage of the α- helices and β-sheets in the protein are important for the secondary structure of a protein. Secondary structure has an important role in protein structure, folding and its interaction [26]. From the drug target dataset, the average of α- helices was found to be 69.73 and of β- sheet was 53.69. Therefore, the range of α- helices for a protein to be a drug target was set to be 60 to 80 and for β-sheets to be 40 to 60.

Glycosylation is critical for a wide range of biological processes, including cell attachment to the extracellular matrix and protein-ligand interactions in the cell. It has been reported that about two-third entries of proteins in SWISS-PROT are glycosylated that may be either N-linked carbohydrate units, O- linked or both N- and O-linked [27]. In the present study, it is found that proteins of the drug target dataset used are either potential glycosylated or non-glycosylated. It indicated that these proteins are not proper glycoproteins.

Sub-cellular localization is important to elucidate the function of a protein [28]. The localization decides the function of the protein in that location. Since the present study is done on the bacterial proteins and bacteria do not have a distinct nucleus and other organelles, it is considered that localization is cytoplasm, cytoplasmic membrane and extracellular membranes. In our drug target dataset, we have found that 71% proteins showed their localization in the cytoplasm as shown in Fig. 2. Therefore, it was considered that preferred localization for drug target is cytoplasm.

Presence of signal peptide in a protein helps in its transportation to its location. It is reported that signal peptide is a N-terminal peptide that directs the protein across the membrane of the endoplasmic reticulum in eukaryotes and across the plasma membrane in prokaryotes [29]. Here, for the drug targets, very less frequency for the presence of the signal peptide was found as shown in Fig. 3. This may be due to the reason that localization of the protein for the drug target has been found mainly as cytoplasm and not a membrane location.

It has been reported that the presence of the transmembrane helix in the sequence indicated that it is the sequence of the flanking region or putative helical region. In the present study, an average of 3 transmembranes in the sequence (both inside to outside and outside to inside) has been found. The predicted properties of the drug targets and the ranges required for a protein to be a drug target are shown in Table 1.

Identified properties of the drug targets were validated by applying these properties on the proteins of *H. pylori* to find the drug targets. For the study, proteome of *H. pylori* was used which has 1602 proteins. For each of the proteins, the above properties were calculated using the same tools. Using the range of values as described in the Table 1, proteins were identified having all the values in the same range as obtained from the drug target datasets.

From the entire proteome of the *H. pylori*, five proteins have been found with the values in between the range obtained using the drug target datasets. Table 2 shows the results for the identified drug targets in *H. pylori*. The results indicated that urease α-subunit, phosphopyruvate hydrolase/enolase, CTP (cytidine triphosphate) synthase, protease DO (High temperature requirement protease A enzyme, EC. 3.4.21.107) and UDP-N-acetylmuramylalanyl-D-glutamate-2,6-diaminopimelate ligase are the potential drug targets.

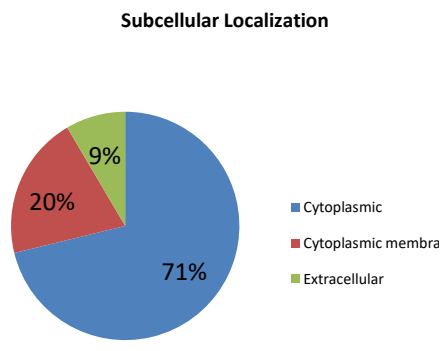

Fig. 2: The distribution of Subcellular localization in drug target dataset.

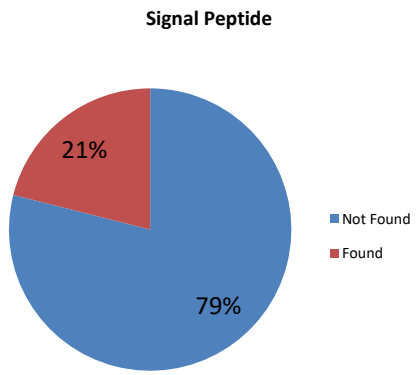

Fig. 3: Presence of signal peptide in drug target dataset.

Table 1: Range set for each of the property of bacterial drug target.

Properties		Average	Range set for drug targets
Molecular weight		57407.01	45000 - 65000
Sequence length		516.80	400 - 600
Hydrophobicity		-0.299	-0.150 to -0.350
Secondary structure	**Alpha-helix**	69.73	60 - 80
	Beta-sheet	53.69	40 - 60
Subcellular-localization	**Cytoplasmic**	336	Cytoplasmic
	Cytoplasmic-membrane	96	
	Extracellular	40	
Transmembrane	**Inside to outside**	3.72	2-4
	Outside to inside	3.64	2-4
Signal Peptide		**0**= 360 **Y**= 96	**0**
Glycosylation	**N-linked**		Potential Glycosylated
	O-linked		Potential Glycosylated

Table 2: Results for the identified drug targets in *H.pylori*.

Target Name / **Properties**		**Urease alpha subunit**	**Phosphopyruvate hydrolase/enolase**	**CTP synthase**	**Protease DO**	**UDP-N-acetylmuramoylalanyl-D-glutamate--2,6-diaminopimelate ligase**
NCBI Accession No		YP_005783048.1	YP_005783135.1	YP_005783328.1	YP_005783415.1	YP_005784448.1
Molecular weight		61713.7	46599.9	56669.4	51721.6	50585.2
Sequence length		569	426	499	476	447
Hydrophobicity		-0.317	-0.165	-0.278	-0.247	-0.259
Alpha-Helix		73.3	74.2	79.6	74.8	80.3
Beta-sheet		56.9	54.5	45.1	40.8	45.4
Transmembrane	Inside to outside	3	2	2	3	2
	Outside to Inside	3	2	3	3	2
Sub cellular-localization		Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic	Cytoplasmic
Signal Peptide		0	0	0	0	0
Glycosylation	**N-linked**	Potential-Glycosylated	Potential-Glycosylated	Potential-Glycosylated	Potential-Glycosylated	Potential-Glycosylated
	O-linked	Potential-Glycosylated	Potential-Glycosylated	Potential-Glycosylated	Potential-Glycosylated	Potential-Glycosylated

Our results are validated since these predicted drug targets have also been reported as drug targets for the other pathogens. Kaplan [30] reported urease α- subunit as a drug target in *Mycobacterium tuberculosis*. The phosphopyruvate hydrolase/enolase has been reported as a drug target for Leishmaniasis [31]. Yoshida et al. [32] found the inhibitor for CTP synthase for respiratory tract infections. Docherty et al. [33] reported proteases as drug target in *Escherichia coli*. The Protease Do has also been reported to be an essential drug target for *E. coli* [34]. UDP-N-acetylmuramoylalanyl-D-glutamate-2,6-diaminopimelate ligase has been reported as a drug target for leprosy [35].

Therefore, it may be said that the targets found for *H. pylori* are valid. This indicated that the correct properties of the drug targets have been identified in the present study and this methodology may be applied to find the drug targets in any pathogen.

Conclusion

Identification of number of properties in drug targets enables us to set the range for the values. Our results indicated that a potent drug target has a sequence length of 400-600 amino acids, molecular weight in the range of 45000 to 65000 daltons, hydrophobicity in the range of minus (-) 0.150 to -0.350, cytoplasmic localization, secondary structure content of 60-80 for α-helix and 40-60 for β-sheet, 2-4 transmembrane helices, no signal peptide and must be potentially glycosylated. These values were applied to *H. pylori* that gave the validated results. Therefore, it can be said that it is an easy and fast process to find the drug targets for any bacterial pathogen. This will fasten the drug discovery procedure.

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Conflict of Interest

We declare that 'No conflict of interest exists'.

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