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# Potential markers for early diagnostics of Colorectal cancer and Inflammatory bowel disease in humans : intestinal microorganisms and immune system (teammates or rivals)

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## Abstract

Chronic inflammation of the bowel is the characteristic of Inflammatory bowel diseases including Ulcerative colitis and Crohn's disease. The incidence of Inflammatory bowel disease (IBD) in Western Europe is 14/100,000 of inhabitants. Duration and severity of inflammation increases the risk of Colorectal cancer (CRC) development by about 60% in IBD patients. CRC is the 3rd most common type of tumor in Western population. Every year, more than one million new cases are diagnosed with more than 600,000 deaths.

The early detection of CRC, originating from any part of the colon-rectum, is desirable because it can be cured surgically if diagnosed timely. Identification of new early markers for IBD and CRC is very important.

This review deals with the searching and identification of possibly new early markers for the above mentioned pathologies. We have focused on intestinal microorganisms (changes in qualitative and quantitative composition of microbiome, selection of candidate microorganisms) and immune system markers (cytokines, chemokines, nuclear factor ϰB, Toll-like receptors and Receptor for Advanced Glycation End Products).

**Keywords**: Colorectal cancer, Inflammatory bowel disease, Immune markers, Cytokines, Microorganisms, *Escherichia coli*, *Fusobacterium nucleatum*

## Introduction

### Inflammatory bowel diseases

Chronic inflammation of the bowel is the characteristic of Inflammatory bowel diseases (IBD) including Ulcerative colitis (UC) and Crohn's disease (CD). UC is limited to the colon with major involvement of mucosa, while CD can affect any segment of the gastrointestinal tract from the mouth to the anus with "skip lesions" as a characteristic feature and inflammation is trans-mural. A genetic predisposition for IBD was noted, and patients with this condition are more prone to the development of malignancy [1-3].

The incidence of IBD in Western Europe is 14/100,000 of inhabitants [4]. Duration and severity of inflammation increases the risk of Colorectal cancer (CRC) development by about 60% in IBD patients [5]. Chronic inflammation represents the appropriate microenvironment for tumor development via stimulation of immune cells [6].

### Colorectal cancer

Colorectal cancer (CRC) is the 3rd most common type of tumor in Western population. Every year, more than one million new cases are diagnosed with more than 600,000 deaths [7-9]. CRC is a serious problem for public health and is a significant socioeconomic burden [7]. When the tumor is confined to the colon (stages I and II), surgical therapy could be successful with 5 years survival rate in more than 80%

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cases. But in the case of metastasis, the prognosis is still poor [8].

The early detection of CRC, originating from any part of the colon-rectum, is desirable because CRC can be cured surgically if diagnosis is done in a timely fashion. However, the several attempts to define early markers of active carcinogenesis have not produced suitable results for definitive and practical applications so far [10].

**Aetiology of Colorectal cancer**

Aetiology of CRC is multifactorial. Wide spectrum of risk factors is associated with CRC development – e.g. genes coding the tumor suppressors (adenomatous polyposis coli, β-catenin and p53), IBD, high consumption of red meat, obesity, diabetes, smoking, hard drinks, etc. In the past few years, predisposition factor has been mentioned frequently due to which the qualitative and quantitative composition of intestinal microbiome has also garnered much attention [7, 11].

This review is mainly focused on the interconnection between chronic inflammation of the bowel and subsequent development of colitic cancer.

## Intestinal microbiome - the link for CRC development?

Colon harbors different kinds of microorganisms in amount $10^{13} – 10^{14}$ with more than $3\times10^{6}$ genes (100-fold more genes in comparison with human genome) collectively called intestinal microbiome. Microbiome plays an important role in the maintenance of host homeostasis by synthesis of vitamin K, production of different nutrients, influencing mucosal immune system, defence against translocation and outgrowth of undesirable pathogenic microorganisms [12, 13]. The composition of intestinal microbiome is very heterogeneous with more than 1000 different microbial species. The microbial burden in large intestine is 12-fold higher compared to small intestine. Interestingly, there is 12-fold higher risk of CRC development in colon compared to small intestine [14]. It has been proved that composition of intestinal microbiome is relatively stable during time, but dietary changes, IBD, ongoing infection, probiotic and antimicrobials usage, etc. can significantly influence qualitative and quantitative composition of microbiome [5]. These changes, so called intestinal dysbiosis, have been considered to be promoting factor for IBD and subsequent CRC development [13]. Some bacterial strains can be the ”drivers“ contributing to CRC development. Some of them can be only the ”passengers“ using tumor mass as carbon source and probably affecting tumor growth and progression. Mechanisms of microbial action may include alteration of balance between pro- and anti-inflammatory signals, and direct effect of bacterial enterotoxins on mucosal cells and intracellular pathways [14].

## Interaction of microorganisms and immune system – role of chronic inflammation in the pathogenesis of CRC

The immune response to microbes starts during the interaction of innate immune system (pattern recognition receptors (PRRs)) with specific conserved microbial components, microbe-associated molecular patterns (MAMPs). PPRs could be also “triggered” by host’s products of metabolism or by substances released from injured tissues bearing damage-associated molecular patterns (DAMPs). The hypothesis that tissue damage has a potential to induce the production of self-reactive T cells and autoimmunity reaction was published as well. After the PRRs-MAMPs interaction, activated innate immune cells mediate the adaptive immune response leading to antigen-specific, protective immune response. It causes serious damage to host’s tissues and inflammation development [15].

IBD results from uncontrolled inflammatory response to indigenous intestinal microbiota in genetically predisposed individuals [16]. The crucial role of gut microbiota in IBD pathogenesis is supported by the following three facts:

-Lesions in IBD patients are most frequently localized in the areas of the highest amount of bacteria. Administration of selective antibiotics or fecal stream diversion create suitable environment for IBD development.
-GIT inflammation is mitigated or is not developed in some IBD animal models in germ free conditions.
-Microbial dysbiosis and the presence of bacteria with more pathogenic characteristic (e.g. adherent-invasive *E. coli*, *Mycobacterium avium* s. *paratuberculosis*) was diagnosed in IBD patients. The intestinal inflammation development (exorbitant immune reaction) can be caused or can be the consequence of microbial dysbiosis or enhanced virulence of the above mentioned microorganisms [15].

Arthur and Jobin (2013) define a new paradigm in their study. They assume that chronic inflammation alone is not sufficient to promote CRC and the presence of specific microorganisms is necessary for the development of pathological process. Composition of intestinal microbiome is altered during the inflammatory process and overgrowth of microorganism with tumor promoting properties is facilitated. Endogenous mediators of inflammation and bacterial-derived mediators stimulate CRC development. Permanent monitoring of composition of intestinal microbiome in healthy population and in CRC patients should be realized in more details. Based on this knowledge, novel and improved therapeutic modalities should be subsequently applied in the treatment of IBD and CRC patients [12].

More detailed information about immune markers involved in IBD and CRC development is provided in the section ‘Immune markers’.

## Changes in composition of intestinal microbiome in patients with IBD and CRC

Composition of intestinal microbiome is changed and diversity is reduced in IBD patients. Lower counts of bifidobacteria, *Bacteroides* and *Firmicutes*, particulary *Clostridium leptum* and *Clostridium coccoides* were observed. On the other hand, higher occurrence of *Peptostreptococcus* sp., actinobacteria and gammaproteobacteria was found in IBD patients in comparison with healthy controls using next-generation sequencing (NGS) [5, 17]. Cultivation methods revealed increased prevalence of adherent-invasive *E. coli*. *Fusobacterium nucleatum* isolates obtained from IBD patients were significantly more invasive and "triggered" pro-inflammatory response in cultured epithelial cells assays in comparison with clinical isolates from healthy controls [5].

In CRC patients, significantly increased amounts of *Fusobacterium* sp., *Enterobacteriaceae* (*E. coli*, *Klebsiella* sp., *Shigella* sp.), *Bacteroides* (*B.*) sp. (*B. vulgatus* and *B. stercoris*), *Campylobacter* sp., *Erysipelotrichaceae*, *Collinsella* sp., *Peptostreptococcus* sp., *Anaerotruncus* sp., *Enterococcus* sp., *Streptococcus* (*Str.*) *bovis* were reported. Lower representation of *Clostridium* cluster IV, such as *Faecalibacterium prausnitzii* and *Rosburia* sp. was detected in the patients [7, 11, 12, 17].

In a study comparing the adenoma patients with healthy controls, increased abundance of *Firmicutes*, *Bacteroidetes* and *Proteobacteria* was observed in the adenoma group. In resected bowel of patients with adenocarcinoma of colon *Coriobacteria*, *Roseburia*, *Fusobacteria* and *Faecalibacteria* were increased in the tumor site in comparison with the adjacent non-tumor tissue [12]. Kim et al. (2017) published that the presence of *H. pylori* is significantly interconnected with colorectal adenoma and advanced colorectal neoplasm development [18].

*E. coli* (possessing polyketide synthetase (pks) islands) and *Fusobacterium nucleatum* were isolated from clinical samples of CRC patients in the most cases. Their promotive role in tumorigenesis has been proved in experimental models. Some virulence factors, like genotoxin and colibactin in *E. coli* and adhesin FadA in *Fusobacterium nucleatum*, have been suggested to support progression of CRC in experimental models [5, 19]. Moreover, McCoy et al. (2013) published that there is a correlation between production of interleukin (IL)-10 and tumor necrosis factor (TNF)-α and presence of *Fusobacterium* sp. in clinical samples from CRC patients [20]. Conversely, *Lactobacillus acidophilus*, *Lactobacillus* S06 and *Eubacterium aerofaciens* colonization correlated with low risk of CRC in humans [11].

## Immune system related markers

As is shown by many studies, the initial positive inflammatory reaction (acute) induced by alarmins and stress proteins, released by the tissue in which mutated cells start uncontrolled replication, can eliminate the cancer cells. If cancer cells are not completely ablated, after a period of equilibrium between anticancer response and cancer cell replication, the tumor can progress when the acute inflammation shifts toward chronic process. Under this condition, many physiological factors that are normally involved in the regulation of inflammation and tissue repair are made available in the pathological tissue. They are used by cancer to better establish and escape the immune response (neo-angiogenesis, fibrous stroma remodeling, fibrosis, immune tolerance and immune suppression). Some of these factors are transforming growth factor (TGF)-β, IL-10, epidermal growth factor (EGF), vascular endothelial growth factor (VEGF), matrix metalloproteinases (MMPs), collagen, extracellular matrix (ECM) glycans, etc. [21-24]. Moreover, colitis was found to be associated with inflammatory response mediated by T helper (Th)1 and Th17 immune response. Indeed, tumors from patients in initial stage of CRC produce higher levels of IL-17 and IL-23, together with high levels of IL-6. Important role of regulatory cells (e.g. Foxp3+ T cells and dendritic cells) has been emerged recently [11, 12]. Important immune markers are described in more details in subsequent parts of the manuscript.

## Cytokines

### *Interleukin 6*

Elevated IL-6 and IL-6 receptor levels in IBD patients led to the "colonization" of lamina propria of colon with T lymphocytes. The suggested mechanism was the up regulation of anti-apoptotic factors. In blood sera and tumor biopsies of CRC patients, increased IL-6 concentrations were detected [25, 26].

### *Interleukin 10*

IL-10 (anti-inflammatory cytokine) decreases IL-6 release and plays an important role in colitis-associated cancer (CAC) development. Deficiency in IL-10 led to the development of spontaneous colitis in mice. Enhanced inflammation in these mice resulted in increased carcinogenesis after azoxymethane (AOM) injection in comparison with wild-type mice [12]. In patients with IL-10 receptor mutation (abrogation of IL-10 signaling), timely and aggressive tumor progression was observed [25].

### *Interleukin 21*

Elevated IL-21 levels were detected in tumor tissue of patients with CAC [25].

### *Tumor necrosis factor-α*

TNF-α, a key product of Th17 cells with inflammatory properties, has anti-oncogenic effect (controls tumorigenesis by induction of apoptosis in epithelial cells) [11]. The deficiency in TNF-α signaling (application of AOM and dextran sodium sulphate (DSS)) in mouse model resulted in reduction of mucosal harm, infiltration with inflammatory immune cells and cytokine production. Deceleration in tumor progression was also observed [25]. On the other hand, TNF-α induces activation of monocytes and neutrophils (inflammation development) and subsequent production of reactive oxygen species (ROS) with DNA damage potential [11].

***Transforming growth factor-β***

TGF-β reduces tumor progression based on IL-6 production. TGF-β and IL-10 can serve as "protection" against IBD and CAC development. In transgenic (TGF-β receptor deficient) mice, significantly increased incidence of tumors was observed compared with wild-type mice [25].

## Chemokines

Chemokines release from different cell types in colon is stimulated by TNF-α secretion. Increased production of monocyte chemoattractant protein (MCP)-1 (C-C motif ligand (CCL) 2, activating and chemotactic properties for macrophages) was detected in the mucosa of patients with IBD. Enhanced CCL2 expression was measured in the colon of mice treated with AOM [25].

## Nuclear factor-κB

Activation of nuclear factor (NF) - κB cellular pathway and subsequent cytokines production is crucial for tumor growth and progression [17, 27]. NF-κB release is associated with inflammation development, cell proliferation and survival as well. NF-κB secretion in cytoplasm is controlled by the production of specific inhibitor. Release of this inhibitor is regulated via phosphorylation of IκB kinase (IKK-β) complex. IKK-β mediates inflammation and subsequent tumor development in mouse CAC model (IKK-β absent in intestinal epithelial or in myeloid cells). NOD-like receptor (NLR) production (NLRP12) reduces NF-κB release. $NLRP12^{-/-}$ mice are increasingly susceptible to colitis and CAC development [25].

## Receptors

***Toll-like receptors***

The absence of MyD88 (TLR adaptor) has the significant impact on tumor quantity and size in intestinal tumorigenesis $Apc^{Min/+}$ mouse model (mice with CAC). Moreover, deficiency of bacteria in this model resulted in decreased incidence of tumor or dysplasia in these mice [25, 28].

***Toll-IL-1R8***

Toll-IL-1R8 (TIR8, IL-1 receptor family) inhibits NF-κB secretion via IL-1R complex and TLRs. In mouse model (TIR8 deficient mice, DSS application), significant weight loss, bleeding into intestine, enhanced CAC development and increased mortality was demonstrated [25].

***Receptor for Advanced Glycation End Products***

Receptor for Advanced Glycation End Products (RAGE) is a multi-ligand receptor. Beside advanced glycation products (AGEs), derivatives of glucose produced under ketone hypoxia or cell stress conditions, S100/calgranulin, amyloid beta peptide, beta-sheet fibrils and high-mobility group box-1 protein (HMGB-1) are released [29, 30]. Interestingly, the important mentioned ligand HMGB-1 is also a ligand for Toll-like receptor (TLR)-4 involved in some inflammatory responses [31]. Signaling through RAGE induces intracellular intermediaries including NF-κB, Mitogen-activated protein kinases (MAPK), PI3K/Akt, Rho GTPases, Jak/STAT and Src family kinases [32]. The increased expression of RAGE, as well as its frequent relation with HMGB-1 expression under condition of tissue stress, makes these two molecules suitable as putative new markers for early diagnosis of mucosa alterations together with the stroma architecture imaging. Their evaluation integrated with lysil-oxidase (LOX)-2, TGF-β and myeloid differentiation primary response gene 88 (MyD88) is suitable to create a scale of risk from inflammation to cancer and to allow more precise monitoring of patients by simple biopsy analysis [33, 34].

## Conclusion

Inflammatory bowel disease and Colorectal cancer are the serious health issues with increasing incidence worldwide. For this reason it is imperative to search and identify new early markers for both the pathologies. Therapy of CRC is effective if the diagnosis is determined timely. Presence of specific strains of microorganism (*E. coli* (possessing polyketide synthetase islands) and *Fusobacterium nucleatum*) in tumor tissue of CRC patients could be potentially one of these early markers. Set-up of early immune markers for CRC development and progression could be subsequent: elevated levels of IL-6, IL-17, IL-21, IL-23, NF-kB, MCP-1, RAGEs, HMGB-1 and decreased levels of IL-10, TGF-β, Toll-IL-1R8, etc. in tumor tissue and blood of CRC patients.

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## Conflict of Interest

No conflict of interest exists.

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