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Review

Toll-like receptor signalling and their therapeutic targeting in colorectal cancer

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ABSTRACT

Intestinal homeostasis is dependent on the proper host/microbiota interaction via pattern recognition receptors. Toll-like receptors are a specialised group of membrane receptors which detect pathogen-associated conserved structures. They are present in the intestinal tract and are required for intestinal homeostasis. Dysregulation in the Toll-like receptor signalling can conceivably result in a dysregulated immune response which could contribute to major intestinal pathologies including colorectal cancer. Evidence for the role of microbiota and toll-like receptors in colorectal cancer is emerging. In this report the evidence for the contribution of toll-like receptors to the pathogenesis of colorectal cancer; potential mechanisms affecting toll-like receptor signalling; and their therapeutic targeting in colorectal cancer are reviewed.

Contents

1. Introduction

Human intestinal tract is home to a complex ecosystem of commensal bacteria; the number and composition of which varies in different segments and between individuals. A relatively lower number and fewer species reside in the stomach and upper small intestine due to the specific composition of luminal constituents and the propulsive motion of the region. However, the distal part of the small intestine and colon is habitat to a diverse and densely populated microbiota [1]. Given the special environment of the gastrointestinal tract and the constant exposure to a diversified population of microorganisms, it is advantageous that the intestinal epithelium is tolerant to normal microflora [2]. Toll-like receptors (TLRs) along with other members of pattern-recognition receptors (PRRs) are responsible for sensing the normal flora and mounting an appropriate response. Under normal circumstances, the intestinal tract remains tolerant to

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normal microbiota while in inflammatory bowel disease (IBD) this is not the case. IBD is a chronic relapsing and remitting inflammation of the gastrointestinal tract which is clinically manifested with diarrhoea, abdominal pain, rectal bleeding, and malnutrition. IBD is proposed to be caused by host–microbiota dysbiosis and the consequent dysregulated immune response. The dysbiosis develops at various levels: the composition of commensals shifts towards more pathogenic strains, the epithelial barrier function is impaired due to altered mucin production or increased epithelial permeability. Additionally, the host–microbiota interaction is altered and the genetic variation of the host elicits an abnormal immune response to the commensals [3].

There is a constant interaction between intestinal mucosa and the microbiota via PRRs. TLRs are a group of cell surface or cytoplasmic proteins which detect the conserved structural motifs named pathogen- and damage-associated molecular patterns (PAMPs and DAMPs respectively). To date, ten and 13 TLRs have been identified in human and mouse, respectively. TLR1, TLR2, TLR4, TLR5, and TLR6 reside on the plasma membrane whereas TLR3, TLR7, TLR8, and TLR9 are localised to the endolysosomal compartment. TLRs recognise diverse repertoire of PAMPs including peptidoglycan, lipopeptide and lipoprotein (TLR1/TLR2 and TLR2/TLR6), double-stranded RNA (TLR3), lipopolysaccharide (LPS; TLR4), flagellin (TLR5), single-stranded RNA (TLR7 and TLR8), and bacterial unmethylated CpG island (TLR9). Binding of TLRs to their respective PAMPs results in homo- or heterodimerisation of TLRs; as a consequence of which adaptor proteins including myeloid differentiation factor 88 (MyD88) and TIR domain containing adaptor-inducing interferon-β (TRIF) are recruited. All TLRs, except TLR3, signal via the MyD88-dependent pathway ultimately leading to activation of the transcription factors nuclear factor (NF)-κB and activating protein-1 (AP-1). TLR3 function is exclusively mediated via TRIF-dependent pathway. TLR4 can signal via both MyD88- and TRIF-dependent mechanisms. TRIF-dependent TLR signalling results in activation of interferon-regulatory factor 3 (IRF3) and NF-κB in a MyD88-independent manner. TLR pathway activity culminates in the expression of pro-inflammatory cytokines, type I interferon (IFN), and chemokines [4]. TLRs have been detected in the gastrointestinal tract both in the intestinal epithelial cells (IECs) and stromal components. Under physiologic conditions, all TLRs except TLR10 have been detected in IECs through immunohistochemistry or gene expression analysis. While TLR1, 2, 3, 4, 5, and 9 are expressed in both small and large intestines in mouse and human, TLR 6, 7, and 8 are only detected in human colon and mouse small intestine. Under physiologic conditions, TLR2 and 4 are expressed at a low level whereas, TLR3 is abundantly present in IECs. In addition, lineage-specific and polarised expression of TLRs have also been described [5]. TLRs have specialised function and build a complex network of bacteria recognition properties with distinct roles. Under normal circumstances, only a subset of TLRs is detectably expressed in the intestinal epithelium. However, TLRs are differentially expressed in the intestinal epithelium of IBD patients [6–8] and IBD-associated colorectal cancer (CRC) [9]. TLRs are linked to cancer susceptibility and are implicated in tumourigenesis in several cancers [10]. In addition, TLRs are being targeted in a number of conditions including cancer [11–13]. Chronic inflammation has been long known as a major driving force in the development and progression of colitis-associated cancer (CAC) [14]. The role of inflammation and commensals in CRC is emerging [15–17]. Dysregulated TLR signalling is a mechanism as a consequence of which a pathogenic immune response to normal microbiota can ensue. It is speculated that dysregulated immune response to the microbiota underlies many human pathologies including CRC. TLRs contribution to tumourigenesis and therapeutic interventions in CRC is under rigorous investigation. TLRs contribute to the sporadic and inflammation-induced intestinal tumourigenesis. For example, TLR4 promotes intestinal tumourigenesis [9,18,19] whereas TLR2 protects against colitis-associated cancer [20]. The nature of TLR signalling alterations and its role in intestinal tumourigenesis is still unclear. In addition, the therapeutic implications of TLR alteration in CRC are not understood. Hereby, we review the literature on TLR signalling in CRC and summarise the findings in terms of the contribution of TLRs to pathogenesis, diagnosis, prognosis, and treatment of CRC. In addition, the potential mechanisms through which TLR signalling is altered in CRC are discussed.

2. Toll-like receptors gene expression pattern

It is known that cancer arises as a consequence of accumulation of genomic alteration. Thus, the COSMIC database was searched for somatic mutations in the *TLRs* or *MyD88* in CRC. However, no mutation has been reported in CRC [21]. Subsequently, the cancer genome atlas was queried for TLRs in colorectal cancer. The expression ratio of *TLR1-10* ranges between 52 and 66% in colon cancer. However, more interestingly, methylation ratio is significantly high in CRC where data is available.

Nine studies have investigated the expression pattern of TLRs in normal mucosa, adenoma, and carcinoma. Collectively, TLR2, 4, 7, 8, 9, and 10 expressions increase in parallel with adenoma–carcinoma progression [22–26]. *TLR*4 and *MyD88* expressions increase in CRC progression. Hence, higher expression levels are detected in tumour cells in carcinoma and liver metastasis compared to normal mucosa and adenoma [23,24]. TLR4 expression is predominantly observed in epithelial and stromal (including the immune cells) compartments. Nevertheless, the increasing trend of TLR4 expression is found in all tumour compartments i.e. epithelial, endothelial, and stromal compartments [23]. TLR4 expression is generally higher in CRC compared to normal adjacent mucosa. However, the level of TLR4 expression varies between tumours with some tumours having an overall low TLR4 expression [27]. In another study, TLR2, 3, 4, and 5 are not expressed at mRNA level in colon polyps whereas TLR7 and 9 are significantly upregulated. The levels of expression of TLR7 and 9 are markedly reduced in cancerous hyperplastic polyps whereas there is not a significant difference in other histologic subtypes i.e. adenomatous and serrated polyps. While TLR9 is significantly altered in cancerous villous adenoma polyps, TLR7 is reduced in tubulovillous polyps [26]. Higher level of *TLR*4 mucosal expression is also reported in IBD-associated cancer. The level of TLR4 expression increases in parallel with increasing degree of dysplasia with the highest tissue expression observed in CAC. TLR4 expressing cells are most commonly detected at the base of the crypts in normal controls and lamina propria in the inflamed tissue whilst they are significantly increased in transformed cells in cancer [9,19]. The tissue expression of *TLR*7, 8, 9, and 10 is found to be increased in tumour cells in CRC patients. The expression level positively correlates with the tumour staging. In addition, tumour-initiating $CD133^+$ cells derived from CRC have a significantly higher amount of TLR expression which is more pronounced in late stages [25]. *TLR*2 is more prominently expressed in CRC compared to normal mucosa [22] and in the invasive front compared to the tumour centre [28]. TLR pathway was studied at gene expression level in mucin-depleted foci (MDF) in 1,2-dimethylhydrazine (DMH) treated male F344 rats. MDFs are suggested to be CRC precancerous lesions and commonly harbour APC mutation. TLR2, 3, 6, and 7 were found to be significantly different in MDFs vs. normal mucosa albeit at a very low level [29].

Of note, cell-specific expression of TLRs appears to be important in CRC progression. In normal mucosa, TLR4 expression is predominantly observed in the stromal cells and in the stem cell compartment at the base of the crypts. Whereas in CRC, the expression is more widespread in malignant epithelium, endothelial, and stromal compartments [9,19,23]. TLR4 is demonstrated to be expressed in $Lgr5^+$ intestinal stem cells (ISCs); abrogation of which yields stem cells unresponsive to lipopolysaccharide (LPS) treatment in vitro [30]. Expression of other TLRs in ISCs has not been reported to-date. Even though, $CD133^+$ tumour-initiating cells derived from CRC have a