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TOPIC HIGHLIGHT

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Toll-like receptor signaling in colorectal cancer: Carcinogenesis to cancer therapy

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Abstract

Toll-like receptors (TLRs) are germ line encoded innate immune sensors that recognize conserved microbial structures and host alarmins, and signal expression of major histocompatibility complex proteins, costimulatory molecules, and inflammatory mediators by macrophages, neutrophils, dendritic cells, and other cell types. These protein receptors are characterized by their ability to respond to invading pathogens promptly by recognizing particular TLR ligands, including flagellin and lipopolysaccharide of bacteria, nucleic acids derived from viruses, and zymosan of fungi. There are 2 major TLR pathways; one is mediated by myeloid differentiation factor 88 (MYD88) adaptor proteins, and the other is independent of MYD88. The MYD88-dependent pathway involves early-phase activation of nuclear factor of kappa light polypeptide gene enhancer in B-cells 1 (NF-κB1) and all the TLRs, except TLR3, have been shown to activate this pathway. TLR3 and TLR4 act *via* MYD88-independent pathways with delayed activation of NF-κB signaling. TLRs play a vital role in activating immune responses. TLRs have been shown to mediate inflammatory responses and maintain epithelial barrier homeostasis, and are highly likely to be involved in the activation of a number of pathways following cancer therapy. Colorectal cancer (CRC) is one of the most common cancers, and accounts for almost half a million deaths annually worldwide. Inflammation is considered a risk factor for many common malignancies including cancers of the colorectum. The key molecules involved in inflammation-driven carcinogenesis include TLRs. As sensors of cell death and tissue remodeling, TLRs may have a universal role in cancer; stimulation of TLRs to activate the innate immune system has been a legitimate therapeutic strategy for some years. TLRs 3/4/7/8/9 are all validated targets for cancer therapy, and a number of companies are developing agonists and vaccine adjuvants. On the other hand, antagonists may favor inhibition of signaling responsible for autoimmune responses. In this paper, we review TLR signaling in CRC from carcinogenesis to cancer therapy.

Key words: Toll-like receptor; Colorectal cancer; Carcinogenesis; Prognosis; Cancer therapy

Core tip: Toll-like receptors (TLRs) are innate immune

sensors which can recognize inflammatory mediators. TLRs have been shown to mediate inflammatory response and maintain epithelial barrier homeostasis. Inflammation is a risk factor for many cancers including colorectal cancer (CRC). The key molecules involved in inflammation-driven carcinogenesis include TLRs. In this paper, we reviewed TLR signaling in CRC from carcinogenesis to cancer therapy.

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INTRODUCTION

Toll-like receptor biology

Toll-like receptors (TLRs) are a family of evolutionally conserved pattern recognition receptors (PRRs)[1-3]. TLRs are included in the type I transmembrane glycoprotein receptor family with N-terminal ligand-recognition, transmembrane, and intracellular C-terminal signaling domains[4]. Currently, 13 TLRs have been identified in humans and mice, and equivalent forms of many of these have been found in other mammalian species[5]. TLRs recognize a wide range of microbial moieties, and engagement by their respective ligand(s) triggers activation of intracellular signaling cascades leading to the induction of genes involved in antimicrobial host defence, such as those encoding proinflammatory cytokines and chemokines[6,7].

TLR signaling has been investigated extensively in recent years. There are two important TLR pathways: one is dependent on myeloid differentiation factor 88 (MYD88) adaptor proteins and the other is independent of MYD88. All TLRs commonly use MYD88 as the downstream adapter protein except TLR3. After activation with their individual ligands, TLRs recruit MYD88, leading to subsequent activation of downstream factors, including nuclear factor κB (NF-κB), mitogen-associated protein kinase (MAPK), and interferon (IFN) regulatory factors[8,9]. TLR signaling activates transcription factors, and generates cytokines as well as chemokines via intracellular pathways (Figure 1). TLR2 and TLR4 combine with their respective ligands to form dimeric complexes. The configuration is then changed and 5 specific adapters within cells are recruited, including MYD88, TIR domain-containing adaptor protein (TIRAP)/MYD88 adaptor-like (Mal), TIR domain-containing adaptor-inducing IFNβ (TRIF), TRIF-related adaptor molecule (TRAM), as well as sterile α and armadillo motif-containing protein (SARM)[4]. Immune cell expressing TLRs play important roles in immune responses against invading pathogens. TLRs recognize conserved pathogen-associated molecular patterns (PAMPs) expressed on a wide array of microbes, as well as danger-associated molecular patterns (DAMPs) released from stressed or dying cells[10].

TLR in disease and cancer

TLRs play a major role in microbe-host interactions and innate immunity[11]. TLRs are very important in early innate immune defense mechanisms by activating canonical and non-canonical pathways of inflammation. Because TLRs are primary sensors of PAMPs, DAMPs, and stress signals associated with allergen exposure, genetic variations in the TLR genes may influence the incidence, severity, and outcome of allergic diseases[2]. Several single-nucleotide polymorphisms within the TLR genes are associated with altered susceptibility to infectious, allergic, and inflammatory diseases as well as cancers[2].

More and more evidence suggests that malfunction of TLR signaling contributes significantly to the development of autoimmune connective tissue diseases[3], tuberculosis[9], severe acute pancreatitis[12], necrotizing enterocolitis[13], atherosclerosis[14], alcohol-induced liver disease and non-alcoholic steatohepatitis[15]. TLR signaling plays a role in regulating injury responses of chronically injured precancerous organs and promoting malignant cell survival[16]. The TLR/MYD88 pathway is essential for microbiota-induced development of colitis-associated cancer, and it was demonstrated that the severity of chronic colitis directly correlates with colorectal tumor development and that bacterial-induced inflammation drives progression from adenoma to invasive carcinoma[17]. TLRs and MYD88 signaling have been shown to be associated with hepatic inflammation and hepatomitogen expression which is important for hepatocarcinogenesis, suggesting that a better understanding of TLR signaling pathways may help to clarify the mechanisms of tumorigenesis, and provide new therapeutic targets for hepatocellular carcinoma[15]. Researchers have found that TLR9 initiates a cascade of immune responses: expression of TLR9 promotes angiogenesis and cancer progression, and reduces survival, so an understanding of how TLR9 boosts angiogenesis may help refine the development of anticancer agent[18,19]. Table 1 showed TLR functions in disease and cancer.

TLR in therapy

As the evidence for the involvement of TLRs in multiple immune diseases has increased, more and more research has shown that TLRs could be a therapeutic target for inflammatory diseases. TLR2 could be a useful therapeutic target for the development of antagonists given the range of diseases that are associated with this receptor[20]. The humanized version of OPN-305 entered phase I clinical trials for the treatment of inflammatory autoimmune diseases[20-22]. Small synthetic compounds, acting as TLR3 agonists and/or TLR2/TLR4, TLR7/9 and MYD88 antagonists may favor the inhibition of signaling responsible for autoimmune responses in multiple sclerosis and experimental autoimmune encephalitis[20].

Various TLR agonists have been considered for multiple clinical applications, including cancer immunotherapy, and the TLR7 agonist imiquimod is approved for topical

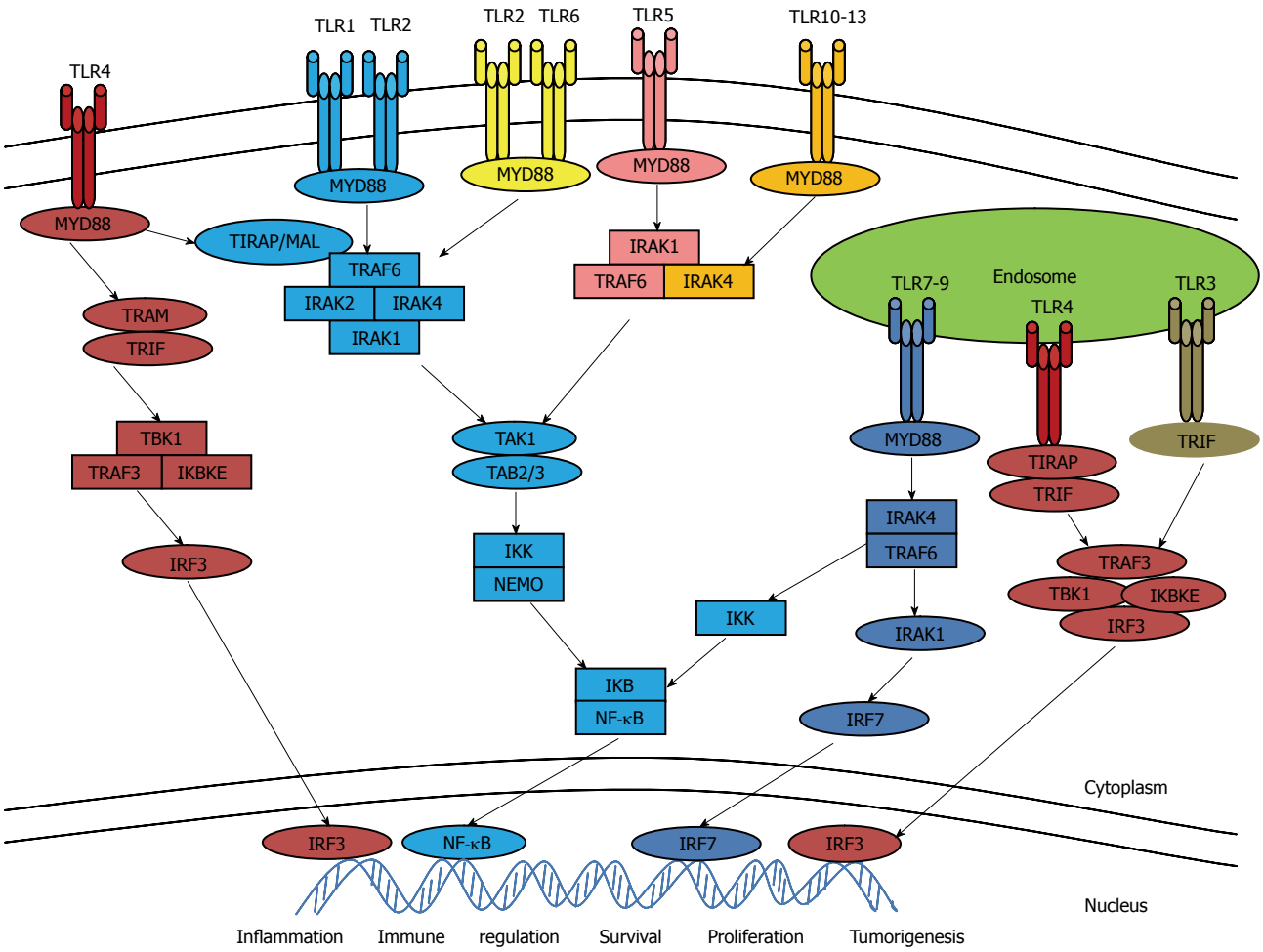

Figure 1 Toll-like receptor signaling pathways. There are 2 major toll-like receptor (TLR) pathways: One is mediated by myeloid differentiation factor 88 (MYD88) adaptor proteins, and the other is independent of MYD88. With the exception of TLR3, all other TLRs commonly use MYD88 as the downstream adapter protein. Activated TLRs recruit MYD88, leading to subsequent activation of the downstream targets. TLR2 and TLR4 combine with their respective ligands to form dimeric complexes and change their configuration, and then recruit specific adapters within cells, including MYD88, TIR domain-containing adaptor protein (TIRAP)/MYD88 adaptor-like (Mal), TIR domain-containing adaptor-inducing interferon (IFN)-β (TRIF) and TRIF-related adaptor molecule (TRAM). The subsequent activation of the IKK complex, consisting of TBK1/TRAF3/IKKε, NEMO/IKBKE, induces phosphorylation of IKBKE, leading to the activation of transcription factors. TLRs bind bacterial and viral PAMPs, leading to activation of proinflammatory and anti-viral signaling pathways including NF-κB1 and IFN regulatory factor-3 (IRF3) and 7 (IRF7), then activation of transcription factors and production of inflammatory cytokines, leading to inflammation, immune regulation, survival, proliferation and tumorigenesis.

Table 1 Toll-like receptor functions in disease and cancer

TLR	Disease	Function
TLRs	Autoimmune disease	Microbe-host interactions and innate immunity
TLRs	Infectious disease	Activating canonical and non-canonical pathways of inflammation
TLRs	Cancer (CRC, HCC, *etc*)	Carcinogenesis
TLRs	Allergic diseases	Primary sensors of PAMPs, DAMPs and stress signals associated with allergen exposure
TLRs	Tuberculosis	Recognition of Mycobacterium tuberculosis
TLRs	Systemic inflammatory response syndrome	Development of syndrome
TLR2/TLR4	Atherosclerosis	Development of disease
TLR4	NEC	Development of intestinal barrier failure
TLR4	Alcohol-induced liver injury	Activation of Kupffer cells
TLR9	NASH	Development of disease
TLR9	Cancer	Angiogenesis

TLR: Toll-like receptor; CRC: Colorectal cancer; HCC: Hepatocellular carcinoma; PAMPs: Pathogen-associated molecular patterns; DAMPs: Danger associated molecular patterns; NEC: Necrotizing enterocolitis; NASH: Non-alcoholic steatohepatitis.

therapy of basal cell carcinoma[23]. Most TLR-targeted therapeutics are intercellular nucleic acid-derived immunoregulatory sequences. such as TLR3, TLR7, TLR8, and TLR9. Agents can also target cell surface TLRs, including TLR2 and TLR4. These therapeutics may be used in oncology, immune disease, and infectious disease[1].

Activation of the TLR4 pathway may cause chronic inflammation and increase production of reactive oxygen and nitrogen species (ROS/RNS), leading to oxidative and nitrosative stress and TLR-related diseases. This implies that drugs or substances that modify these pathways may prevent or improve TLR-related diseases, for example, anti-lipopolysaccharide (LPS) strategies, aim to neutralize LPS and TLR4/MYD88 antagonists, including eritoran, CyP, EM-163, epigallocatechin-3-gallate, 6-shogaol, cinnamon extract, N-acetylcysteine, melatonin, and molecular hydrogen[24]. Rajput *et al*[25] correlated TLR4 expression with resistance to paclitaxel in either depleted or overexpressed TLR4 protein breast cancer cell lines and found that paclitaxel not only killed tumor cells but also enhanced their survival by activating the TLR4 pathway, suggesting that blocking TLR4 could significantly improve the response to paclitaxel therapy. TLR4 is critical for the airway inflammatory response, and agents targeting TLRs are being actively pursued as novel therapies for the treatment of airway diseases such as asthma[26]. Synthetic oligodeoxynucleotide-expressing CpG motifs (CpG-ODN) are TLR9 agonists that can enhance the antitumor activity of DNA-damaging chemotherapy and radiation therapy in preclinical mouse models, and findings provide evidence that the tumor microenvironment can sensitize cancer cells to DNA-damaging chemotherapy, thereby expanding the benefits of CpG-ODN therapy beyond induction of a strong immune response[27]. TLR9 agonists can exert antitumor effects by blocking angiogenesis; it is likely that TLR-induced IFNs play an important role as IFNα is well known to suppress tumor angiogenesis[16].

TLR IN COLORECTAL CANCER CARCINOGENESIS

General introduction

Colorectal cancer (CRC) is the fourth leading cause of cancer-related death in the world and the third leading cause in the United States[28]. Initiation and progression of malignancies is the result of a series of complex processes that depend upon multiple interactive factors[29]. There are 3 distinct molecular mutagenic pathways, including chromosomal, microsatellite instability, and epigenetic pathway in colon carcinogenesis[11,22].

Inflammation is considered a risk factor for many common malignancies including CRC[29,30]. The key molecules involved in inflammation-driven carcinogenesis include TLRs, NF-κB signaling, pro- and anti-inflammatory cytokines, growth factors, kinase tumor suppressor proteins, cyclooxygenases, and nitric oxide synthases[31]. Pimentel-Nunes *et al*[32] found persistently positive TLR expression and lower expression of TLR inhibitors associated with higher TLR protein levels throughout the spectrum of lesions of colon carcinogenesis[22]. TLR3 may indicate the tendency of normal tissue to form adenoma or CRC[17].

Each receptor in CRC carcinogenesis (in vivo and in vitro)

TLR2: TLR2 is encoded by a DNA sequence that codes 784 amino acids[9]. This type I transmembrane receptor is composed of an extracellular leucine-rich domain, a single transmembrane domain, and a cytoplasmic domain[9]. Colon carcinogenesis is associated with increased expression levels of TLR2 and TLR4. Functional TLR2 and TLR4 polymorphisms significantly alter the risk of CRC. Smoking and obesity may influence the risk of CRC along with these genetic profiles[33]. TLR2 is unique in its requirement to form heterodimers with TLR1 or TLR6 for the initiation of signaling and cellular activation[11]. Tumor cells from TLR2 knockout mice showed less cell death and suppressed senescence[16]. Nihon-Yanagi *et al*[34] suggested that TLR2 activation may also be involved in sporadic colon carcinogenesis in humans.

In CRC , the role of TLR2 is still controversial. One study showed that there were no differences between wild-type and TLR2-deficient mice in CRC[16,35]. However, another study showed increased tumor development and higher interleukin (IL) 6, IL17A and phospho-signal transducer and activator of transcription 3 (STAT3) levels in CRC in TLR2-deficient mice[16,36]. In colitis, TLR2 plays a protective role against the development of colitis-associated cancer[36]. TLR2 plays a key role in Gram-positive bacterial, mycobacterial, fungal, and spirochetal cell wall component recognition, while TLR4 seems to be a key receptor of the Gram-negative component LPS; both TLR2 and TLR4 in cancer patients are implicated in carcinogenesis and antitumor treatment; the lower stress response in laparoscopic colectomy *vs* open colectomy provides an impetus to investigate the long-term results of laparoscopic colectomy *vs* open colectomy for CRC[37]. Some papers also showed that TLR2 and TLR4 were both associated with survival after diagnosis of colon cancer, but not rectal cancer[38].

TLR4: TLR4 is composed of 839 amino acids. It is activated by bacterial LPS as well as lipotechoic acid[9]. TLR4 is expressed on human colon cancer cells and is functionally active. It is important in promoting immune escape of human colon cancer cells by inducing immunosuppressive factors as well as apoptosis resistance[31]. The TLR4 signaling pathway has oncogenic effects both *in vitro* and *in vivo*. The increased individual expression of TLR4 and IL6 is a common feature of CRCs and is associated with poor prognosis[39-41]. To demonstrate the role of TLR4 signaling in colon tumorigenesis, Wang *et al*[39] examined the expression of TLR4 and MYD88 in CRC, and suggested that high expression of TLR4 and MYD88 is associated with liver metastasis, and is an independent predictor of poor prognosis in patients with CRC. Their findings also sug-

gest that TLR4/MYD88 signaling contributes to CRC tumorigenesis not only in colitis-associated cancer but also in sporadic CRC[39]. Other studies also showed that TLR4 signaling activates NF-κB through the MYD88 pathway, leading to transcription of pro-inflammatory cytokines as well as many important components of the inflammatory response[42].

TLR4 is overexpressed in mouse and human inflammation-associated CRC, and TLR4-deficient mice are strongly protected against colon carcinogenesis, suggesting that TLR expression on tumor cells promotes tumor progression directly or indirectly[8]. TLR4 expression by stromal fibroblasts is associated with poor prognosis in CRC[43]. The TLR4 variant D299G induces neoplastic progression in Caco-2 intestinal cells and is associated with advanced human colon cancer, implying a novel link between colonic carcinogenesis and aberrant innate immunity[44]. Single TLR4, LY96 (MD-2), and CXC chemokine receptor 7 (CXCR7) expression levels are significantly correlated with human CRC TNM stage, advanced histological grade, tumor size, and lymph node metastasis; furthermore, concomitant expression of TLR4, LY96 and CXCR7 has been shown to be associated with increased potential for carcinoma growth and metastasis in human CRC[29]. Cammarota *et al*[45] found that adenocarcinoma patients (pT1-4) with higher TLR4 expression in the stromal compartment had a significantly increased risk of disease progression, and high TLR4 expression in the tumor microenvironment represents a possible marker of disease progression in colon cancer. Nox enzymes are major sources of endogenous ROS generation in response to inflammatory mediators, including cytokines, growth factors, and hypoxic conditions, all of which are elevated in response to surgical trauma[42,46,47]. It was shown that the LPS-Nox1 redox signaling axis plays a crucial role in facilitation of colon cancer cell adhesion, thus increasing the potential for colon cancer cell metastasis. Nox1 may represent a valuable target to prevent colon cancer metastasis[42].

TLR9: TLR9 recognizes unmethylated CpG motifs in bacterial DNA[9]. TLR9 is expressed mainly in intracellular vesicles such as the endoplasmic reticulum, lysosomes, endosomes and endolysosomes, where they recognize microbial nucleic acids[1]. TLR9 recognizes DNA derived from both DNA bacteria and viruses[1,48]. Several studies have shown that TLR9 engagement on CD4 T cells can enhance their survival and therefore, could potentiate antitumor responses by prolonging T cell survival[10,49]. The role of TLR9 signaling in colonic carcinogenesis remains unclear. It was recently reported that oligodeoxynucleotides targeting TLR9 have opposite effects in modulating DNA repair genes in tumor cells *vs* immune cells, and enhance the biologic effects of chemotherapy. TLR9 expression was decreased in hyperplastic and villous polyps from patients who developed CRC, suggesting a possible protective role of TLR9 expression against malignant transformation in the colorectal mucosa[50]. Table 2 and Figure 2 show the TLRs involved in CRC.

TLR IN CRC PROGNOSIS

It was reported that high expression of the TLR4/ MYD88 signal was correlated with poor prognosis of CRC[51]. In the tumor microenvironment, high TLR4 expression represents a possible marker of disease progression in colon cancer[45]. TLR4 expression in stromal fibroblasts is associated with poor prognosis in CRC, therefore, TLR4 expression in fibroblasts could be a useful prognostic marker in CRC[43]. It has been documented that the deregulated activation of STAT3 and NF-κB is a common feature of gastrointestinal cancers and invariably correlates with poor prognosis; NF-κB and STAT3 are key downstream signal transducers of the TLR families and IL-6 cytokine, respectively; the molecular mechanisms are associated with cross-talk between the IL-6 cytokine family/STAT3 signaling network and the TLR family/NF-κB signaling network, and there is potential benefit in their therapeutic targeting in colorectal and gastric cancers[7]. Genetic variations in TLR2, TLR3 and TLR4 may influence colon cancer development as well as survival after diagnosis with colon cancer[38]. Persistent TLR-specific activation of NF-κB in CRC and particularly in tumor-initiating cells may sustain further tumor growth and progression through perpetuation of signaling from inflammatory and tissue repair mechanisms, with consequent self-renewal of pluripotent tumor cells. TLR7 and TLR8 expression on PROM1 $(CD133)^+$ cells in CRC may play a specific role in tumorigenesis and tumor progression[52].

TLR IN CRC THERAPY

Agonists

TLR agonists play a fundamental role in activating innate and adaptive immune responses[10]. TLR agonists are currently under investigation as vaccine adjuvants in anticancer therapies for their ability to activate immune cells and promote inflammation[10]. A growing body of evidence indicates that TLRs are expressed or can be induced on various cell types, including T cells and tumor cells[8,10].

Current available synthetic TLR2 ligands are based on cell wall constituents of (potential) pathogens, and adjuvant research could possibly benefit from elucidating the variations in the LPS make-up of probiotic strains. With regard to the indispensable role of pattern recognition receptors (PRRs) in facilitating microbe-induced TLR2 function, determination of specific PRRs involved in the recognition of probiotic strains would aid research on the mechanism of action of probiotics. In addition, because microbial manipulation of PRR-TLR crosstalk is used by pathogens to subvert appropriate immune responses, determination of the specific PRRs involved could lead to new therapeutic approaches[11]. The TLR2/4 agonists S100A9 and HMGB1 have been touted as potential biomarkers for CRC, as they are upregulated significantly in

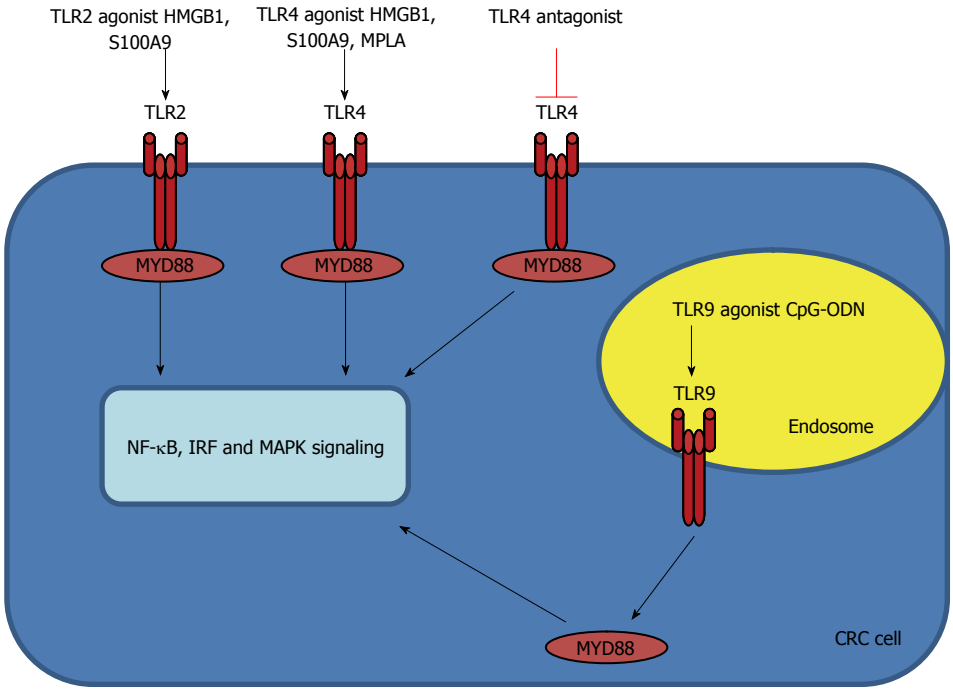

Figure 2 Toll-like receptors in colorectal cancer and therapeutics. Toll-like receptor (TLR) agonists play a fundamental role in activating innate and adaptive immune responses. The TLR2 and TLR4 agonists HMGB1 and S100A9 have been proposed as potential biomarkers for colorectal cancer (CRC). The TLR4 agonist monophosphoryl lipid (MPL) A is approved for use in several vaccines as an adjuvant. TLR9 agonist, commonly referred to as CpG-ODN, has been added to the arsenal of anti-cancer drugs as monotherapy or in combination with chemotherapy, radiotherapy and other immunotherapeutic approaches. Activated TLR2, TLR4 and TLR9 recruit MYD88, with activation of NF-κB, IRF, and MAPK signaling, leads to inflammation, immune regulation, survival, proliferation and tumorigenesis. MAPK: Mitogen-associated protein kinase; IRF: Interferon regulatory factor; NF-κB: Nuclear factor (NF)-κB.

Table 2 Toll-like receptors role in colorectal cancer

TLR	Carcinogenesis	Prognosis	Treatment
TLR2	Controversial role in mouse model; protective against development of CRC in colitis	Associated with survival after diagnosis of colon cancer	HMGB1, S100A9
TLR4	Oncogenic effects *in vitro* and *in vivo*	Poor progression	HMGB1, S100A9, MPLA
TLR9	Remain unclear; possible protection against malignant transformation in colorectal mucosa		CpG-ODN

CRC: Colorectal cancer; TLR: Toll Like receptor.

CRC and have been shown to be regulated by STAT3, which is hyperactivated in approximately 90% of colorectal tumor biopsies[41,53,54]. The TLR4 agonist monophosphoryl lipid A is approved for use in several vaccines as an adjuvant[1,51].

Rosa *et al*[55] established a *KRAS* mutated CRC model and showed that an immunomodulatory oligonucleotide sequence in combination with cetuximab had an antitumor effect. This is probably based on the alteration of MAPK phosphorylation, resulting in structural and functional changes in the relationship between epidermal growth factor receptor (EGFR) and TLR9[50,55]. Mutation of the *KRAS* gene has a critical role in colon cancer and may cause resistance to anti-EGFR therapy, which is the reason why panitumumab and cetuximab therapy do not show a positive effect on the control of proliferation and metastasis in *KRAS*-mutated colon cancer; this kind of biological therapy could only be useful in the case of patients carrying the wild-type *KRAS* gene; estrogen receptors may take part in colorectal carcinogenesis, and interaction between TLR9 and estrogen receptors may have further therapeutic importance in CRC, and TLR9 agonist therapy has been tested clinically on the colon[50]. The TLR9 agonist, which is commonly referred to as CpG-ODN, has been added to the arsenal of anti-cancer drugs as monotherapy, or in combination with chemotherapy, radiotherapy, and other immunotherapeutic approaches, as they increase antigen presentation and boost anti-tumor B and T cell responses[56]. TLR9 agonists were reported to show TP53-independent activity within human CRC cells, inhibit their proliferation, promote apoptosis, and improve anti-cancer effects of radiotherapy and chemotherapy[17]. One therapeutic advantage of the use of TLR9 agonists in this tumor model could be to sensitize tumors to the toxic effects of radiation treatment[10,57]. Combined administration of a TLR9 agonist and an

Table 3 Summary of therapy in colorectal cancer

Compound	Target (agonist)	Indications	Drug class or trade	Clinical phase
BCG[10,71,72]	TLR2/4	CRC	Synthetic ssRNA	Phase Ⅰ
MPL[10,72]	TLR4	CRC	Synthetic ssRNA	Phase Ⅰ
CBLB502[1,23,71]	TLR5	Colon cancer	Flagellin	Phase Ⅰ
Imiquimod (Aldara)[10,71,72]	TLR7	CRC	Small molecule ssRNA	Phase Ⅰ / Ⅱ / Ⅲ
IMO2055[1,10]	TLR9	CRC	CpG oligonucleotide	Phase Ⅰ / Ⅱ
MGN1703[16]	TLR9	CRC	dSLIM	Phase Ⅱ

IL-10 antagonist is one of the candidates for cancer treatment[8]. Recently, it was shown that TLR ligands may be critical for dendritic cell (DC) activation, and combined TLR activation can lead to better DC maturation status, and also induce more effective antitumor immune responses against colon cancer, showing that it may be a potential strategy to develop more powerful DC cancer vaccines[58].

It was reported that specific small molecule inhibitors of phosphatidylinositol-4,5-bisphosphate 3-kinase, catalytic subunit alpha (PIK3CA) reduce immunosuppression to increase the proinflammatory effects of TLR ligands that support antitumor immunity. Multiple strategies to inhibit PIK3CA in DC led to IL-10 and transforming growth factor-β1 suppression but did affect IL12 or IL1B induction by the TLR5 ligand flagellin[59].

Antagonist

TLR4 plays an important role in innate immunity as the first line of host defense. Most human cells express high levels of TLR antagonist proteins and a low level of TLR4. Tumor progression involves TLR4-mediated irregular and uninhibited production of proinflammatory cytokines, immunosuppressive cytokines as well as chemokines; suggesting that the discovery of TLR4 antagonists may be an ideal strategy to treat tumors. TLR4 antagonists were found to pose a risk of compromising host immunity in other studies, so that it is a scientific dilemma whether a TLR4 agonist or antagonist should be targeted as treatment for cancer[60]. The TLR4/LY96 antagonist antibody inhibited colitis-associated neoplasia in a mouse model, and it was shown that TLR regulation can affect the outcome of both acute colitis and its consequences, *i.e.*, cancer. Targeting TLR4 and other TLR's may ultimately play a role in prevention or treatment of colitis-associated cancer[61].

TLR IN CLINICAL TRIALS

The developmental process for TLR-targeting products in cancer has not been altogether straightforward, and two of the earliest TLR pioneers have had disappointing results. However, there are a number of promising second-generation products currently in development, and targeting of TLR9 for metastatic CRC in clinical phase Ⅱ / Ⅲ trials is being performed by Mologen company[45]. Various TLR agonists are currently under investigation in clinical trials for their ability to orchestrate antitumor immunity[10]. Pollinex Quattro (Allergy Therapeutics Ltd., Worthing, UK) is a vaccine that contains a monophosphoryl lipid adjuvant to stimulate TLR4, combined with ragweed pollen extract for the treatment of seasonal allergic rhinitis[1,62]. Following positive results in phase Ⅲ trials, Allergy Therapeutics have submitted Pollinex Quattro for regulatory approval in Europe[1]. TLR9 is a key determinant of the innate immune responses in both sterile and infectious injury. Specific TLR9 antagonism reduces tissue damage in a wide range of pathologies, and has been delivered by modification of nucleic acids, a recognized ligand for TLR9, and a novel small-molecule enantiomeric analogue of traditional morphinans which has specific TLR9 antagonist properties and reduces sterile inflammation-induced organ damage[52]. Some of the TLR-based therapeutics under evaluation in CRC are shown in Figure 2 and Table 3.

CONCLUSION

TLRs are very interesting receptors and are highly important in the field of adjuvant, pathogen, and probiotic research. TLRs constitute a link between adaptive (specific) and innate (non-specific) immunity, contributing to the capacity of our immune system to efficiently combat pathogens. They also enable immune cells to discriminate between self and nonself antigens[17]. TLRs are connected to the cell signaling machinery *via* intracellular adaptor molecules, and stimulation of the TLR/IL1R signaling pathway activates the major inflammatory transcription factor NF-κB1 by allowing its nuclear translocation[63]. Predictably, MYD88 was shown to play a role in tumorigenesis *via* TLR and IL1 proinflammatory mechanisms[63-65]. TLR-mediated signaling can promote tumor growth, and using a TLR agonist or antagonist in combination with an antigen isolated from tumors may increase the effect of vaccination and evoke specific innate immunity against a tumor[6]. TLR stimulation results in NF-κB1 activation, a key modulator in driving inflammation to cancer and mitogen-activated protein kinases that have been shown to recruit mitotic and prostaglandin endoperoxide synthase 2 (PTGS2)-induced pathways in carcinogenesis[66].

CRC is a major cause of cancer-associated morbidity and mortality worldwide, and is the third most common cancer in men and women; in addition, CRC is the third leading cause of cancer-related deaths, and the incidence of this disease is increasing[67,68]. The role of TLRs in

CRC pathology has not been fully elucidated. Bacterial infection stimulates the TLR/MYD88 pathway in tumor tissues, which leads to the induction of PTGS2 in stromal cells, including macrophages, and induction of the PTGS2/PGE(2) pathway in tumor stroma is important for the development and maintenance of an inflammatory microenvironment in gastrointestinal tumors[69]. Persistent TLR-specific activation of NF-κB in CRC, and particularly in tumor-initiating cells, may thus sustain further tumor growth and progression through perpetuation of signaling in inflammatory and tissue repair mechanisms, with consequent self-renewal of pluripotent tumor cells; activation through self-ligands or viral RNA fragments may maintain this inflammatory process, suggesting a key role in cancer progression[66]. Chronic activation of TLRs expressed by tumor cells from CRC and pluripotent PROM1 $(CD133)^+$ colon cancer initiating cells may sustain inflammation responses, mediate resistance to apoptosis, and promote further tumor progression. Therefore, targeting of TLR signaling may be a potential mechanism to abrogate this inflammation-mediated effect in tumor progression[66]. The pathways that are downstream of TLRs and culminate in proliferation and recruitment of inflammatory cells during injury can be usurped to support cancer development[70].

Although much effort has been put forward to determine TLR ligand requirements and receptor activity, many questions remain. However, there are reasons to be optimistic that TLRs represent strong candidates for cancer targeting. Drug candidates are being developed to target CRC or act as vaccine adjuvants. We hope that they can be safely used systemically and have the power to transform chemotherapeutic interventions in CRC in the near future.

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