Should a Toll-like receptor 4 (TLR-4) agonist or antagonist be designed to treat cancer? TLR-4: its expression and effects in the ten most common cancers

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Abstract: Toll-like receptor 4 (TLR-4) is well known for its host innate immunity. Despite the fact that TLR-4 activation confers antitumor responses; emerging evidence suggests that TLR-4 is associated with tumor development and progression. It is now clear that overactivation of TLR-4, through various immune mediators, may cause immune response dysfunction, resulting in tumorigenesis. Different cancers could have different extents of TLR-4 involvement during tumorigenesis or tumor progression. In this review, we focus on infection- and inflammation-related TLR-4 activation in noncancer and cancer cells, as well as on the current evidence about the role of TLR-4 in ten of the most common cancers, viz, head and neck cancer, lung cancer, gastrointestinal cancer, liver cancer, pancreatic cancer, skin cancer, breast cancer, ovarian cancer, cervical cancer, and prostate cancer.

Keywords: drug design, cancer treatment, myeloid differentiation factor 2, MD-2, tumor progression, pathogen-associated molecular patterns, PAMPs

Introduction

Toll-like receptors (TLRs) are a recently discovered family of pattern recognition receptors that show homology with the *Drosophila* Toll protein and the human interleukin (IL)-1 receptor family. The first member of the TLR family to be identified was a *Drosophila* protein[1] implicated in dorsoventral patterning during embryonal development. TLRs are evolutionarily conserved proteins characterized by an extracellular leucine-rich repeat (LRR) domain, the transmembrane domain, and the cytoplasmic intracellular Toll/IL-1 receptor-like (TIR) domain. LRRs, found in both cytoplasmic and transmembrane proteins, play a vital role in ligand recognition and signal transduction. There are 12 TLRs, in which ten human isoforms of TLRs (TLR-1 to 10) have been identified. The LRR, which is deputed to recognition of the ligand, is composed of 19–25 tandem repeats of 24–29 amino acids, folded in strands and in helices that are linked by loops.[2] The transmembrane domain and TIR domain are highly conserved among the TLRs.[3]

TLR-4 structure

Recently, TLR-4 was the first identified TLR whose crystal structure (Protein Data Bank [PDB] ID: 3FXI) was solved (Figure 1),[4] leading to the derivation of computational simulation models that predict the mechanism of its interaction with its cognate ligands. The TIR domain, which shares homology with the IL-1 receptor (IL-1R),

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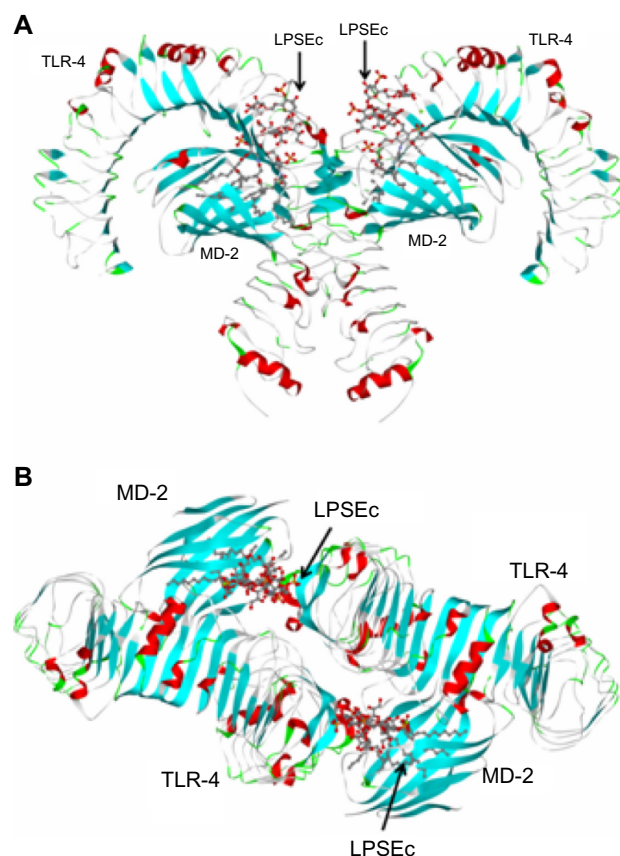Fax +60 38 656 7229
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Figure 1 Structure of the TLR-4/MD-2/LPSEc complex.
Notes: (**A**) Side view of the symmetrical dimer of TLR-4/MD-2/LPSEc complex. (**B**) Top view of the complex. LPSEc folded in between TLR-4 and MD-2 and formed a homodimer complex. The TLR-4/MD-2 complex will initiate signal transduction that is essential for the activation of TLR-4. The images were captured in Vida 4.2.1 (Open Eye Scientific Software, Santa Fe, NM, USA) software; using the human TLR-4/MD-2 crystal structure, with LPSEc cocrystalized (PDB ID: 3FXI).
Abbreviations: LPSEc, lipopolysaccharide from *Escherichia coli*; MD-2, myeloid differentiation protein-2; PDB, Protein Data Bank; TLR-4, Toll-like receptor 4.

is responsible for the propagation of the signal within the cell, through interaction with a complex signaling cascade. Human TLR-4 is located on chromosome 9q32-q33 and contains four exons.[5]

TLR-4 signaling pathway

TLR-4 regulates the inflammatory responses against gram-negative bacteria, as shown in Figure 2. Lipopolysaccharide (LPS) is the major component of the outer membrane of gram-negative bacteria and triggers TLR-4 signaling.[6,7] TLR-4 requires myeloid differentiation factor 2 (MD-2) for its activation.[8] LPS will first bind to the LPS-binding protein (LBP) and then to the cluster of differentiation (CD)-14 before binding to TLR-4/MD-2 complex. The role of CD-14 is to enhance the sensitivity of the TLR-4/MD-2 complex.[9,10] The binding of LPS to the TLR-4/MD-2 complex triggers conformational changes in the structure of the TLR-4/MD-2 complex,

leading to TLR-4/MD-2 homodimerization (activation) and resulting in the production of proinflammatory cytokines, through the myeloid differentiation primary response protein 88 (MyD88)-dependent pathway, and the production of type 1 interferons, through a MyD88-independent pathway (via the interaction of TIR domains with adaptor molecules).[11]

TLR-4 plays an important role in innate immunity as the first line of host defense. TLR-4 is expressed in normal epithelial cells, immune cells, and in cancer cells.[12] Most human cells express a low level of TLR-4 and high levels of TLR-antagonist proteins, such as Toll-interacting protein (TOLLIP), which prevent the overexpression of TLR.[13–16] The normal immune and epithelial cells present in the skin, digestive, respiratory, and reproductive systems activate the host's immune systems pathways through pathogen-associated molecular patterns (PAMPs) and danger associated molecular patterns (DAMPs). TLR-4 responds to the various invading exogenous pathogens through PAMPs and recognizes the endogenous ligands from necrotic cells through DAMPs (Figure 3).[17] Although innate immunity appears to be a nonspecific response, it could differentiate "self" molecules and pathogens through the pattern recognition receptors on TLR-4.[18]

Despite the promising innate immune responses from TLR-4, there is growing evidence that TLR-4 activation appears to act as a double-edged sword in cancers, ie, TLR-4 activation has been linked to both cancer inhibition and growth. Given the links of infection and inflammation with cancer, further evaluation of the role of TLR-4 in cancer is warranted. The detailed biological relationships between TLR-4 and cancers are still poorly understood. Therefore, in this review article, we gather the knowledge of TLR-4 activation in infection and inflammation related cancers. Also, we surveyed TLR-4 expression and its effects in various cancers (Table 1), viz, head and neck cancer, lung cancer, gastrointestinal cancer, liver cancer, pancreatic cancer, skin cancer, breast cancer, ovarian cancer, cervical cancer, and prostate cancer. Ultimately we wish to establish the therapeutic indication for whether TLR-4 agonist or antagonist is required in treating cancers. The details of the role of TLR-4 as one of the key mediators in innate immunity responses[19–21] and of the brief relationship between TLRs and inflammatory diseases,[22–25] TLR-4 and infections,[26,27] and potential TLR-4 ligands[28,29] are not the focus of this review paper, as these topics have been covered by other authors.

TLR-4 in infection and its relation to cancer

The recognition of PAMPs by TLR-4 during microorganism invasion occurs at the plasma membrane, endosomes,
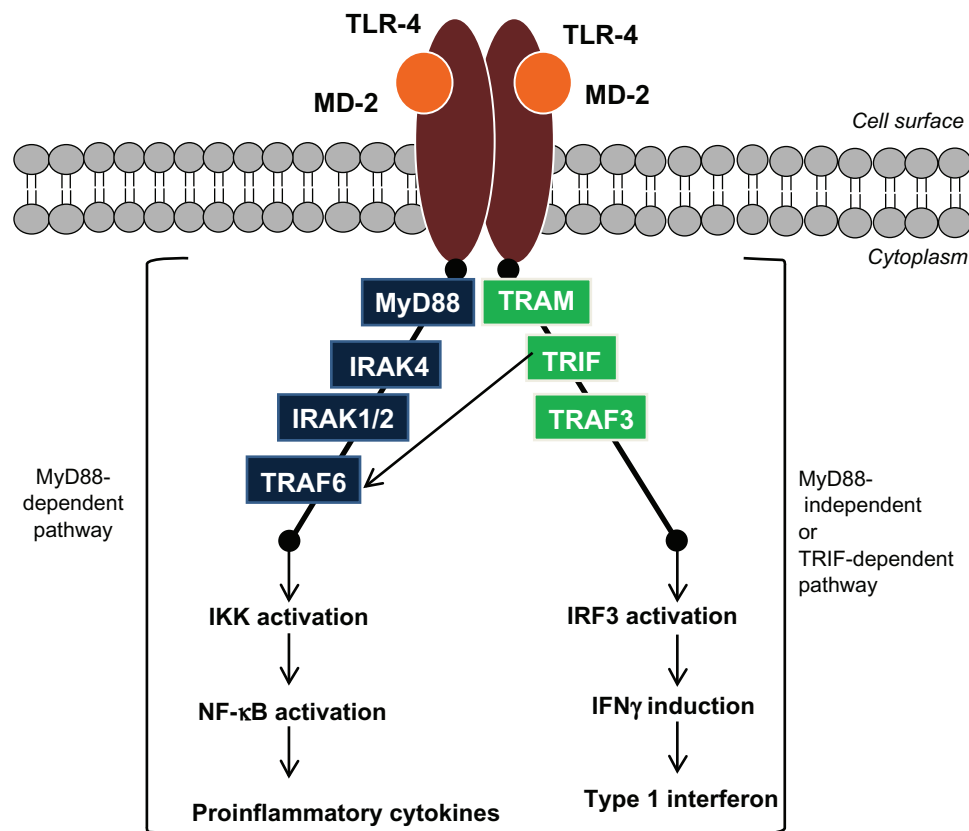
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Figure 2 Schematic illustration of the TLR-4 signaling pathway.
Notes: TLR-4/MD-2 complex homodimerization activates the MyD88-dependent pathway and TRIF-dependent pathway. In the MyD88-dependent pathway, MyD88 recruits IRAK4, IRAK1/2, and TRAF6 to activate IKK and leads to NF-κB activation; in the TRIF-dependent (or MyD88-independent pathway), TRAM/TRIF activates TRAF3 and leads to IRF3 activation and induction of IFNγ.
Abbreviations: IFN, interferon; IKK, inhibitory kappa B alpha kinase; IL-1R, interleukin 1 receptor; IRAK, interleukin-1 receptor-associated kinase; IRF3, interferon regulatory factor 3; MD-2, myeloid differentiation protein-2; MyD88, myeloid differentiation protein 88; NF-κB, nuclear factor-kappaB; TLR-4, Toll-like receptor 4; TRAF, tumor necrosis factor receptor-associated factor; TRAM, TRIF-related adapter protein; TRIF, Toll/IL-1R-domain containing adapter-inducing IFN-β.

lysosomes, and endolysosomes.[30] The innate immunity usually enhances epithelial proliferation, wound healing, and the acute inflammatory responses.[31] In normal host homeostasis, host immune cells activate various antitumor activities to prevent noncarcinogenic cells from evolving into carcinogenic cells. In acute pathogenic infection, CD8^{+} T-cells are activated and in turn, induce natural killer cells activation. The activated CD8^{+} T-cells eliminate any tumor through tumor-associated antigen-specific immunity, while the activated natural killer cells stimulate dendritic cells (DCs) to modulate adaptive antitumor immunity. However, studies have suggested that in a tumor microenvironment, the antitumor activities of the infiltrating immune cells were downregulated, due to the activated TLR-4 present in cancer cells.[32–36]

A recent human study reported that *Helicobacter pylori*, one of the common pathogenic gram-negative bacteria, induced a high expression of TLR-4 in normal gastric mucosa at the initial exposure. The authors suggested that early exposure to LPS in the gastric mucosa reduces the expression of

TOLLIP and leads to the production of proinflammatory cytokines. The overexpression of TLR-4 and the chronic production of these inflammatory cytokines contributes to aberrant transcription of caudal type homeobox 2 (CDX-2), phenotypic change to intestinal metaplasia, and a lower TOLLIP expression in the cells. The progressive increase in the levels of CDX-2 activates pro-oncogenic intracellular pathways and may lead to cancer.[16]

An animal study supported that TLR-4-deficient mice were protected from colitis-associated tumors.[37] The authors found that the MyD88 pathway of TLR-4 contributed to microbiota-induced colitis-associated cancer. The severity of chronic colitis was directly correlated with its colorectal cancer development.[38] Several other studies have also suggested that MyD88 promoted carcinogenesis in epithelial cells, in several in vivo studies.[39–41]

Bacterial-induced inflammation via TLR-4 can also induce tumor progression.[38] Killeen et al reported that LPS increases the expression of urokinase plasminogen activator
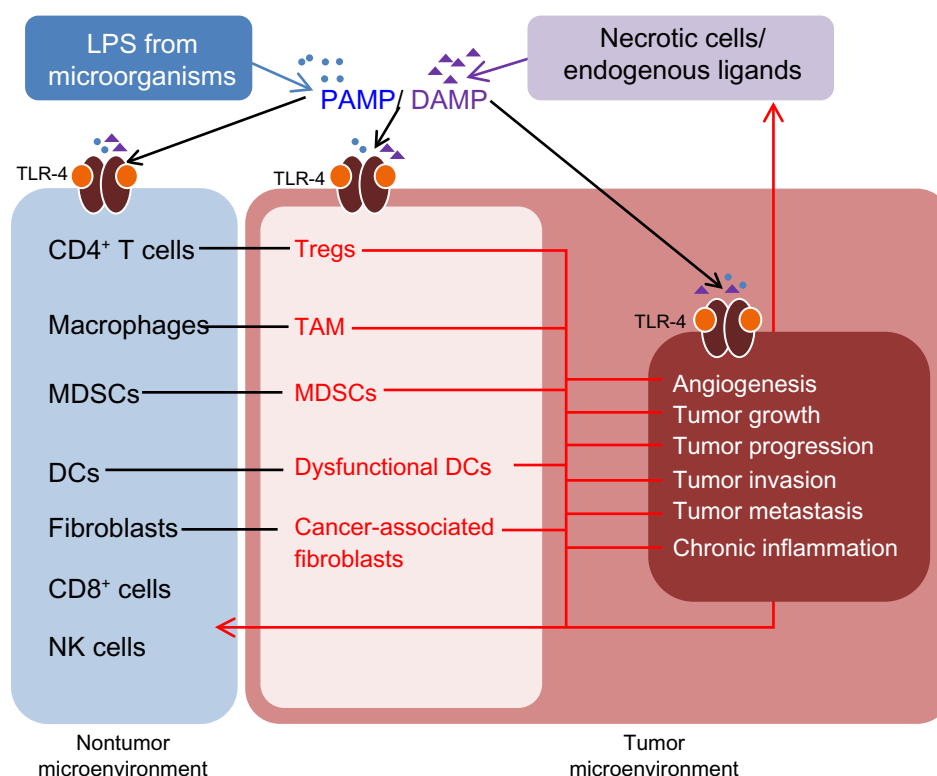
Figure 3 TLR-4 signaling induces the transformation of the nontumor microenvironment to a tumor microenvironment.
Notes: Lipopolysaccharide from *Escherichia coli* produces PAMP while the necrotic cells and other endogenous ligands produce DAMP. Both activate TLR-4 in both nontumor and tumor environments. Prolonged activation of TLR-4 induces CD4+ cells to be transformed into CD4+CD25+Foxp3+ Tregs; macrophages to be transformed into TAM; MDSCs are recruited into tumor microenvironments and activated; dendritic cells will lose antitumor effects; fibroblasts are transformed into cancer associated fibroblasts; anti-tumor effects of CD8+ and NK cells are impaired. In the presence of immunosuppressive cytokines, pro-inflammatory cytokines and chemokines, there is chronic inflammation, tumor growth, progression, angiogenesis, invasion, and tumor metastasis.
Abbreviations: CD, cluster of differentiation; DAMP, danger-associated molecular patterns; MDSCs, myeloid derived suppressor cells; NK, natural killer; PAMP, pathogen-associated molecular patterns; TAM, tumor associated macrophages; TLR-4, Toll-like receptor 4; Tregs, regulatory T cells; DCs, dendritic cells; LPS, lipopolysaccharide.

and urokinase plasminogen activator receptor in colorectal cancer cells.[42] LPS also enhances the colorectal cancer cell adhesion and invasion, but not in the presence of TLR-4 blocking antibody.[42] LPS helps the cancer cells evade immune surveillance via the production of IL-6, IL-12, inducible nitric oxide synthase as well as via the expression of antiapoptotic proteins, such as X-linked inhibitor of apoptosis.[43,44] Studies have also suggested that the chronic activation of TLR-4 from microorganism invasion induces oncogenic potential in the host, through the chronic activation of nuclear factor-kappaB (NF-κB) and cyclooxygenase-2 (COX-2).[31,45,46]

TLR-4 in inflammation and its relation to cancer

The association between cancers and inflammation induced by PAMPs is clearly evident; however, the association between PAMPs and TLR-4 expression in cancers is minimal. This suggests nonpathogenic TLR-4-induced inflammation also plays an important role in cancers. In the noncancerous condition, cell death is usually driven by phagocytosis or apoptosis.

On the other hand, in cancer cells, necrosis-induced cell death is related to the release of DAMP (Figure 3). Certain DAMP, such as high mobility group box-1 protein (HMGB1), could potentially promote cancer progression.[19,47] HMGB1 is a deoxyribonucleic acid (DNA)-binding protein secreted from cells upon cytokine stimulation or necrotic cell death. Cancer cells have been found to have high expression of HMGB1.[48,49] HMGB1 has also been reported to induce cancer cell invasion, migration, and metastasis[50,51] as well as in vitro endothelial cell proliferation and in vivo neovascularization.[52] Anti-HMGB1 antibody was also shown to inhibit angiogenesis.[53] This evidence collectively suggests HMGB1 as an endogenous TLR-4 ligand that induces carcinogenesis.[28,54] In one study, it was found that the levels of these DAMP-derived molecules were high in the tumor microenvironment and that they induced TLR-4-related chronic inflammation, leading to carcinogenesis, cancer progression, and metastasis.[12] Another study highlighted that the TLR-4/MD-2 complex enhanced the formation of regions of hyperpermeability, through upregulation of C-C chemokine receptor type 2

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Table 1 Summary of TLR-4 expression and its effects on various tumors

Type of cancer	TLR-4 expression	Effect of TLR-4
Head and neck cancer	↑[70–72]	• Enhances cancer cell growth, NF-κB translocation, activated phosphatidylinositol 3-kinase/Akt pathway, and upregulation of IRAK-4 expression, IL-6 production, IL-8 production, VEGF, and granulocyte macrophage colony-stimulating factor[71]
Lung cancer	↓[75]	• Inhibits lung carcinogenesis[75]
		• Increases immunosuppressive cytokines, production of proangiogenic chemokine, and resistance to apoptosis[35]
Esophageal cancer	↑[35,78]	• Overexpression in esophageal cancers[86]
Gastric cancer	↑[86]	• Increases NF-κB signaling and IL-8 secretion[88]
		• Increases *Helicobacter pylori*-induced gastric tumor cells[90]
		• Increases further interaction with *H. pylori* and induces secretion of gastric carcinoma-promoting factors[90]
		• Increases COX-2 and PGE2[33]
		• Higher risk of gastric cancer in the TLR-4 gene polymorphism[92–100]
Colorectal cancer	↑[87–89]	• Increases COX-2 and PGE2 signaling and early colorectal carcinogenesis, inhibit apoptosis, and promotes angiogenesis[99,100]
		• Increases the generation of reactive oxygen species[168]
		• Blocking of TLR-4 inhibits cancer proliferation[45]
		• Blocking of TLR-4 reverses the tumor-mediated suppression of T-cell proliferation and natural killer cell activity[45]
		• Blocking of TLR-4 prolonged tumor-bearing mice survival[45]
		• Increases colon cancer cell adhesion and invasion, through the urokinase plasminogen activator system and β1 integrin[42,117,118]
		• Higher risk of colorectal cancer with the TLR-4 gene polymorphism[115]
Liver cancer	↑[103,104]	• Increases the liver tumor formation[123]
		• Increases *Nanog* gene expression, which induces liver oncogenesis[123]
		• Stimulates carcinogen-induced liver injury and cancer[42]
Pancreatic cancer	↑[123]	• Poor prognosis with overexpressed TLR-4 patients[126]
		• Blocking of TLR-4 or MyD88 decreased the LPS-dependent increase of cancer invasive ability[6]
Skin cancer	↑[126]	• Increases NF-κB signaling, upregulation of MMP-2, expression, and IL-8 production[130]
		• Increases tumor progression with persistent TLR-4 activation[34]
		• Protects against chemical carcinogen-induced tumor formation[133]
Breast cancer	↑[34]	• Blocking of TLR-4 inhibits cancer proliferation[137]
		• Blocking of TLR-4 reduces IL-6 and IL-8[138]
		• Breast cancer metastasis is associated with activation of TLR-4[138–142]
Ovarian cancer	↑[137]	• Increases cytokine (IL-6, MCP-1, and CXCL1) secretion[36]
		• Increases NF-κB signaling[36]
		• Blocking of TLR-4 improves paclitaxel resistance[36,146,148]
		• Inducible heat shock proteins from ovarian cancer induce neutrophil activation through TLR-4 and cause tumor progression as well as tumor lysis[147]
Cervical cancer	↑[36,146]	• Pathogenic microorganisms induce upregulation of integrin β6, TLR-3, and TLR-4 in cervical cancer cells[152]
Prostate cancer	↑[124,152]	• Increases MyD88-dependent NF-κB signaling[158]
		• Increases VEGF[159]
		• Increases TGF-β1[159]

Notes: ↑ = increased compared with normal cells; ↓ = decreased compared with normal cells.
Abbreviations: Akt, protein kinase B; COX-2, cyclooxygenase-2; IL, interleukin; IRAK-4, interleukin-1 receptor-associated kinase; LPS, lipopolysaccharide; MCP-1, monocyte chemoattractant protein 1; MMP-2, matrix metalloproteinase-2; MyD88, myeloid differentiation protein 88; NF-κB, nuclear factor-kappaB; PGE2, prostaglandin E2; TGF, transforming growth factor; TLR-4, Toll-like receptor 4; VEGF, vascular endothelial growth factor; CXCL1, chemokine C-X-C motif ligand 1.

(CCR2) expression, in inflamed mice and thus increased the rate of lung metastasis.[55] Metastasis was found to be initiated also by serum amyloid, through TLR-4-dependent NF-κB inflammation pathways.[56] All these latest findings clearly suggested the pivotal role of the TLR-4/MD-2 complex in inflammation-associated cancers.

TLR-4 signaling in tumor cells versus immune cells

The hallmarks of inflammation-associated cancers are the presence of inflammatory cells and cytokines in the tumor microenvironments that leads to tumor growth, angiogenesis, tumor invasion, and/or metastasis.[57,58] TLR-4 activation

upregulates the proinflammatory cytokines and chemokines (CCL, CXCL, CCR, CXCR, IL-6, IL-18, tumor necrosis factor [TNF]-α, etc) as well as immunosuppressive cytokine (transforming growth factor [TGF-β]1, IL-10 etc), especially in tumor microenvironment (Figure 3).[12] CD4^{+}, CD25^{+}, and Foxp3^{+} regulatory T cells in the tumor microenvironment secrete IL-10 and TGF-β, which limit the antitumor effect of non-regulatory T-cells.[12,59] In tumors, associated macrophages have been shown to release angiogenic and lymphangiogenic factors that promote metastasis.[12,60] Myeloid-derived suppressor cells recruited to the tumor microenvironment under the influence of IL-1β, IL-6, and prostaglandin (PG)E$_2$ were also shown to impair the antitumor response of the host, through the release of nitric oxide synthase and TGF-β.[12] DCs too, were found to be dysfunctional in the tumor microenvironment, due to the suppressive effects of vascular endothelial growth factor (VEGF), IL-6, IL-1, TGF-β, COX-2 and PGE$_2$.[12,61] Elsewhere, fibroblast was transformed into cancer-associated fibroblast (CAF) in the tumor microenvironment, and TGF-β-induced CAF activation promoted tumor growth and proliferation. TLR-4 activation has also been related to TGF-β signaling–related cancer growth.[12,62–64]

As a result of these findings, these infiltrating immune cells were hypothesized to support cancer progression, angiogenesis, and metastasis.[32–36] All the above suggest that the release of various immunosuppressive cytokines, proinflammatory cytokines, and chemokines during the activation of TLR-4 could contribute to cancer formation in normal epithelial cells, immune cells, and cancer cells.

TLR-4 in modulation of angiogenesis and its relation in limiting tumor progression

Despite the fact that activated TLR-4 has procarcinogenic effects, the antitumor effect induced by activated TLR-4 should not be neglected. One study reported that the *Mycobacterium bovis* bacillus Calmette–Guérin cell wall skeleton enhanced the cytotoxic activity of T-cells and macrophages against cancer cells, probably through TLR-4. Bacillus Calmette–Guérin cell wall skeleton induced TNF-α secretion from DCs through TLR-2 and TLR-4 signaling and thus induced the maturation of DCs.[65] Elsewhere, OK-432, a penicillin-killed and lyophilized preparation of *Streptococcus pyogenes* exhibited potent immunotherapeutic effect in cancers.[66] Studies supported that OK-PSA, the molecule isolated from a butanol extract of OK-432, induced anticancer immunity and DC maturation, via TLR-4.[67] It was also found

that OK-PSA-induced cytokine production was inhibited by anti-TLR-4 monoclonal antibody. In other work, the compound isolated from *Aeginetia indica L.* (AILb-A) induced NF-κB activation in NF-κB-dependent reporter cells containing TLR-4 plasmid; AILb-A also did not induce any cytokine production in TLR-4 deficient mice.[67] Finally, synthetic serine-based glycolipid (CCL-34)-activated macrophages were shown to induce cancer cell death through TLR-4 dependent pathways.[68] These findings suggest TLR-4 activators induce antitumor immunity through TLR-4 signaling.

Role of MD-2 in cancer

The expression of MD-2 in most cancer cells has not been well studied. Limited evidence has concluded that MD-2 plays a role in cancer progression only. One study highlighted that MD-2 was overexpressed in highly invasive colorectal cancer cells (SW837), in poorly differentiated, moderately invasive colorectal cancer cells (HT-29), and in well-differentiated but non-invasive colorectal cancer cells (Caco-2).[69] Another study reported that serum amyloid A 3, a major component of acute phase inflammation, binds to MD-2 and activates the MyD88-dependent TLR-4/MD-2 pathway and thus facilitates lung metastasis.[70] Therefore, MD-2 could be related to the degree of differentiation, proliferation, and migration capacity of cancers.

TLR-4 in head and neck cancer

The laryngeal carcinomas predominate in this category.[71] The treatment of head and neck cancers remains a big challenge for oncologists. A study in Poland of 20 laryngeal cancer patients with no distant metastasis (M$_0$=100%) reported TLR-2-, TLR-3-, and TLR-4-expression in the laryngeal carcinoma microenvironment. Although TLR-4 is the least frequently expressed TLR on laryngeal tumors, it is still the most frequently expressed TLR on inflammatory cells in all tumor masses and tumor stroma. This fact suggests the possibility of TLR-4 involvement in the escape of tumors from immune surveillance. The detection of TLR-2, TLR-3, TLR-4, and major histocompatibility complex (MHC) class II antigens in all laryngeal carcinoma suggests the role of TLRs in the activation of adaptive immunity.[72]

TLR-4 was also detected in laryngeal tumor tissue (n=27), oral cavity tumor tissue (n=10), and cancer cells (PCI-1, PCI-13, and PCI-30).[73] Moreover, TLR-4 levels in tumors were correlated with tumor differentiation. TLR-4 was shown to enhance the release of tumor progression mediators, resulting in tumor proliferation and progression.[73,74] A study reported cancer cells express significantly higher levels of TLR-4 and

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NF-κB compared with noncancerous cells. A positive correlation was also established between TLR-4 levels in laryngeal tumor central cells, with tumor front grading.[74] Another study, published in 2012, suggested the increased activity of TLR-4, TNF receptor-associated factor (TRAF) 6, and IL-1 receptor-associated kinase (IRAK) 1 in advanced laryngeal carcinoma, suggesting the TLR-4/MyD88-dependent signaling pathway involvement in laryngeal carcinoma progression.[75]

TLR-4 in lung cancer

Worldwide, lung cancer is the most common cause of cancer-related deaths in men and women. Lung cancer mortality rates have been rising in recent decades. Chronic inflammatory disease, such as chronic obstructive pulmonary disease (COPD), has been identified as a risk factor for lung cancer.[76] Since TLR-4 is also actively involved in the immune response against cancers, researchers have postulated that TLR-4 exerts both a defensive role in normal cells and a negative role in cancer cells. However, the available evidence is still not conclusive on the link between TLR-4 and lung cancer.

A study of functional TLR-4 and mutated TLR-4 in mice found that mice with the former had less lung capillary permeability, less weight loss, leukocyte inflammation, and primary tumor formation. Thus, the authors, Bauer et al, postulated that TLR-4 inhibits lung carcinogenesis by inhibiting tumor progression.[77] The researchers proved TLR-4 activation could protect the lungs from being inflamed during any potential tumorigenesis.[78] Elsewhere, a lower level of TLR-4 in the nasal epithelium of a smoker compared with a nonsmoker was observed, with a profound reduction in patients with severe COPD.[79] This finding suggests the potential role of TLR-4 for airway inflammation and lung cancer progression.

In vitro studies have also found that TLR-4 is constantly expressed and upregulated on human lung cancer cells.[33,80] In one study, the level of TLR-4 was significantly linked with the production of immunosuppressive cytokines, production of proangiogenic chemokine, and with resistance to apoptosis by lung cancer cells.[33] Despite the reported significance of TLR-9 in lung cancer progression,[81–84] a positive correlation ($P<0.05$) of TLR-4, but not TLR-9, with tumor differentiation in lung cancer patients was reported.[80]

TLR-4 in gastrointestinal cancer

The gastrointestinal tract (esophagus, stomach, and small and large intestine) is often exposed to pathogens and carcinogens. Epidemiological studies suggest chronic inflammation, whether pathogen-related or not, increases the risk of gastrointestinal cancer.[31,85]

Globally, there has been an increase in esophageal cancer.[86] Esophageal cancer usually occurs in Barrett's esophagus. The link or relationship between inflammation and tumorigenesis in esophageal cancer remains controversial. Esophageal motility allows pathogen transit from the oral cavity to the stomach, making it difficult to quantify PAMP and bacteria exposure. Single nucleotide polymorphism arrays have suggested that in patients with esophageal squamous cell carcinoma, genetic alterations in TLR-4 (9q32-q33) along with other chromosomal mutations are associated with higher cancer proliferation and metastasis.[87] Immunohistochemistry and reverse transcription polymerase chain reaction of samples from 87 esophageal cancer patients revealed that TLR-4 as well as TLR-3, TLR-7, and TLR-9 are overexpressed in esophageal cancer. The isolated mononuclear inflammatory cells associated with higher lymph node metastasis and invasion were also shown to express a high level of TLR-4.[88] The level of mucosal expression of TLRs in the esophagus has not yet been elucidated.[31]

H. pylori infection has been identified as the root cause of stomach cancer. Gastric inflammation is an invariable finding in patients infected with *H. pylori* and represents the host immune response to the organism. *H. pylori* infection leads to gastric inflammation, characterized histologically by surface epithelial degeneration and infiltration of the gastric mucosa, through acute and chronic inflammatory cells. In *H. pylori* infections, TLR-4 on the gastric epithelial cells regulates the LPS response.[89–91] Gastric biopsy samples have suggested *H. pylori* infections accelerate TLR-4 and MD-2 expression in human gastric epithelial cells,[90] which in turn enables the gastric carcinoma cells to further interact with *H. pylori* and consequently, induce the secretion of gastric carcinoma promoting factors.[92] Elsewhere, anti-TLR-4 antibody inhibited *H. pylori*-induced messenger ribonucleic acid (mRNA) expression of human β-defensin in gastric cancer cells (MKN 45).[93] These results further strengthened the hypothesis that *H. pylori* induce the expression of TLR-4 in gastric epithelial cells.

Based on the report of TLR-4 signaling in colonic COX-2 expression and PGE$_2$ production, Fukata et al suggested that there is a high correlation between TLR-4 signaling and the expression of COX-2 as well as PGE$_2$ in *H. pylori*-associated gastric cancers.[94] The expression of NF-κB in association with COX-2 and TNF-α was postulated to be mediated through TLR-4, but not through TLR-2 or -9, based on the findings from previous research, in which

guinea pig gastric epithelial cells were preincubated with *H. pylori* LPS.[89]

Patients with poorly differentiated gastric adenocarcinomas have been shown to have Thr35 Ala polymorphism in the LRR of TLR-4, but not TLR-2, TLR-6, or TLR-9.[95] This finding supports the hypothesis that TLR-4 gene polymorphism is related to poorly-differentiated gastric adenocarcinoma. In addition, a significantly higher risk of gastric carcinoma has been found with TLR-4 Ala896Gly polymorphism.[96] A case-control study of 171 Italian gastric cancer patients and 151 controls reported TLR-4 Thr399Ile polymorphism, but not TLR-4 Asp299Gly polymorphism, is linked with increased risk of gastric cancer (P=0.023, hazard ratio [HR] =3.62). Further, an increased risk of intestinal gastric cancer (P=0.006, HR=5.38), but not diffuse gastric cancer (P=0.612, HR=1.85) was reported in carriers of TLR-4 Thr399Ile allele.[97] Similar results were reported by repeated studies on the role of TLR-4 Asp299Gly/Thr399Ile single nucleotide polymorphism in relation to gastric carcinogenesis.[98,99] However, another case-control study carried out in Mexico reported neither TLR-4 Asp299Gly polymorphism (P=0.82) nor TLR-4 Thr399Ile polymorphism (P=0.2) was associated with significant incidence of gastric cancer.[100]

TLR-4 in colorectal cancer can be a double-edged sword, enhancing the host anticancer immunity and promoting tumor growth at the same time. In acute intestinal mucosa injury, in response to LPS, TLR-4 expression induces COX-2 expression, which leads to wound healing. Thus, mice that lack MyD88 or TLR-4 signaling have been shown to have a reduced healing ability after an acute injury. However, in chronic intestinal inflammation, TLR-4 induces COX-2 and PGE$_2$ production, which may result in early colorectal carcinogenesis, inhibition of apoptosis, and increase of angiogenesis.[94,101] Thus, blocking of TLR-4 signaling can prevent colon cancer cell (MC26 cells) proliferation and reverse tumor-mediated suppression of T-cell proliferation. Just as in other cancers, chronic inflammation in intestinal epithelial cells is closely related to the incidence rate of colorectal cancer. Healthy intestinal epithelial cells constitutively express TLR-3 and TLR-5, where TLR-2 and TLR-4 are lower in quantity.[102] However, altered expression of TLRs is observed in chronically inflamed intestinal epithelial cells.[101–103] TLR-4 expression is significantly upregulated in inflammatory bowel disease (IBD), but expression of TLR-2 and TLR-5 remains unchanged.[102] A significant induction of TLR-2 and TLR-4 expression has been observed on the submucosa of inflamed intestinal epithelial cells.[104] Also, a

significant increase in TLR-4 expression was identified in the colon cancer cells.[105,106] Patients with higher levels of TLR-4 in colon tumor stroma were shown to have an earlier relapse (14 months) compared with those who had lower expression (40 months).[105] This clearly suggests the role of TLR-4 in colorectal tumorigenesis and progression.

Patients suffering with IBD do have increased risk of cancer (0.5%–1% yearly).[31] The impact of TLR-4 polymorphism on IBD is controversial. No association was reported with TLR-4 Asp299Gly and Thr399Ile polymorphism, in a group of IBD patients from Southern Italy,[107] New Zealand,[108] Germany, and Hungary.[109] However, some studies have suggested a significant link between TLR-4 Asp299Gly polymorphism and either ulcerative colitis or Crohn's disease or both.[110–115] In addition, a meta-analysis reported in 2010, showed a significant association between TLR-4 (Asp299Gly and Thr399Ile) polymorphism with ulcerative colitis (odds ratio [OR] =1.08, 95% confidence interval [CI]: 1.08–1.51), Crohn's disease (OR: 1.29, 95% CI: 1.08–1.54), and IBD (OR: 1.25, 95% CI: 1.06–1.48).[116] As well, TLR-4 Asp299Gly polymorphism was significantly higher in colorectal patients compared with normal healthy adults (P=0.0269).[117] Also, studies report that LPS enhanced colorectal cancer cell adhesion and invasion, through TLR-4- and NF-κB-dependent activation of the urokinase plasminogen activator system and beta-1 integrin, which ultimately leads to tumor progression.[64,118,119] In vivo data suggests TLR-4 inhibition has prolonged the survival rate of tumor-bearing mice (BALB/c).[43] Stimulation of the TLR-4/MD-2 complex by LPS can activate phosphoinositide-3-kinase (PI3 K) signaling and thus promotes the adhesiveness and metastatic capacity of colorectal cancer cells.[120] All these findings have consolidated the role of TLR-4 in colorectal cancer progression.

TLR-4 in liver cancer

Dysregulated innate immunity is an integral component in liver disease. Chronic liver diseases, such as alcoholic liver cirrhosis, have also been established as the major cause of liver cancer.[121] Many liver cells (Kupffer cells, hepatocytes, stellate cells, biliary epithelium, and sinusoidal endothelium) constantly express TLR-4.[122] The levels of TLR-4 have been found to be upregulated in hepatitis B− and hepatitis C−infected cells[123,124] and further upregulated in alcohol-induced liver damage and tumors.[125,126] On the other hand, diethylnitrosamine, a carcinogen, was shown to stimulate TLR-4 signaling in mice, resulting in an increase in the size and number of tumors, while the size

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and number of tumors were found be reduced in MyD88 deficient mice.[40]

TLR-4 in pancreatic cancer

It is very difficult to detect pancreatic ductal adenocarcinoma at early stage because of its anatomic location and insidious nature. LPS could be a triggering factor in the initiation and progression of pancreatitis and pancreatic cancer.[7,127] This suggests the possible role of TLR-4 in pancreatic cancer since LPS is a well-established agonist of TLR-4.

Significant expression of TLR-4 (P=0.002) was detected in a study of pancreatic ductal adenocarcinoma as compared with adjacent normal tissues.[128] In this study, there was no correlation found between the levels of TLR-4 and age, gender, location and differentiation of tumor; however, TLR-4 levels were correlated with tumor size, lymph node involvement, venous invasion, and pathological stage. Positive correlations were also observed between TLR-4 and hypoxia-inducible transcription factor-1α (HIF-1α). The expression of NF-κB phosphorylated p65 was also higher in the tumor cells. The patients with overexpressed TLR-4 or overexpressed HIF-1α had a significantly shorter survival period than did the patients with normal expression (P=0.011 and P=0.005, respectively). Longer survival (P=0.014) was noted among patients with neither TLR-4 nor HIF-1α overexpressed compared with patients who had both TLR-4 and HIF-1α overexpressed.[130] A separate in vitro study on human pancreatic cancer cells (Panc-1 and AsPC-1) revealed that TLR-4 was responsible for the invasive ability of cancer cells, mostly due to TLR-4-dependent NF-κB activation.[6] These results demonstrated the importance of TLR-4 in pancreatic cancer proliferation.

TLR-4 in skin cancer

Not only is skin the largest organ in our human body, it also protects us from pathogen invasion through its innate and adaptive immunity. A study has drawn attention to the role of TLRs in atopic dermatitis, psoriasis, acne vulgaris, and skin infection and has suggested the potential of TLRs as a therapeutic target to treat skin cancer.[129] Melanoma cells have a significantly higher amount of TLR-4 levels.[130–133] An in vitro analysis showed the consistent expression of TLR-4 on 13 out of 15 established human metastatic melanoma cells (ME1, ME2, ME5, ME7, ME8, ME9, ME16, ME17, ME18, ME19, ME20, ME21, and ME22) tested. The expression of TLR-2 and -3 was also detected in some, but not in all. TLR-1,-5,-6,-7,-8,-9, and -10 were either absent or weakly expressed. The TLRs, including TLR-4, were upregulated

in melanoma,[32] and it was noted that the coadministration of paclitaxel and icariside II (isolated from *Herba Epimedii*) enhanced apoptosis and decreased the levels of IL-8 and VEGF in human melanoma A375 cells, through the inhibition of TLR-4/MyD88 signaling.[134] A group of German scientists found that TLR-4 was involved in melanoma responses to hyaluronic acid-induced tumor invasion and metastasis.[132]

Another study suggested that the chemical carcinogen 7,12-dimenthylbenz(a)anthracene (DMBA) is associated with endotoxin hypersensitivity mediated through TLR-4-triggered T-cell activation, resulting in cell-mediated immunity. In that study, TLR-4-deficient mice were found to have more tumors compared with normal mice.[135] Another study showed that melanoma inhibits macrophage activation by suppressing TLR-4 signaling.[136] An interesting theory, proposed by Sanchez-Perez et al, is that the intentional generation of an autoimmune response by normal cells could generate a potential antitumor response against tumors of the same type cells. This would explain their finding that the heat shock protein (Hsp)70, a potent immune adjuvant acting through TLR-4 activation, kills inflammatory melanocytes.[131] Thus, TLR-4 activation could be beneficial, at the initial stage, to control melanoma progression. However, the persistent activation of TLR-4 may be harmful to the host.

TLR-4 in breast cancer

Breast cancer is one of the most common cancers affecting women worldwide. The current available therapy has yet to meet the desired outcomes. Two research teams have suggested the role of TLR-2[137] and TLR-9[138] in breast cancer proliferation. In 2010, Yang et al reported that ten TLRs were expressed in MDA-MB-231 cells, the estrogen receptor–negative human breast cancer cells. Among all the TLRs, TLR-4 expression was the highest and was five times higher than TLR-3 expression (the least expressed among the remaining TLR-1 to TLR-10). Functional analyses of ribonucleic acid interference (RNAi) against TLR-4 revealed this successfully inhibited the growth and proliferation of MDA-MB-231 cells and resulted in a significant (P<0.05) reduction of inflammatory cytokines.[139]

In other work, 4T1 (spontaneously metastasizing mammary adenocarcinoma) cells challenged with lipopolysaccharide induced tumor growth and metastasis, by increasing angiogenesis, vascular permeability, and tumor invasion.[140,141] An immunohistochemical study on clinical carcinomas showed a significant association of high TLR-4 expression with local cancer proliferation and lymph node metastasis.[142]

A total of 74 breast carcinomas were collected from patients to study the clinical relevance of TLRs in breast cancer. Tumors with high TLR-4 expression, but not TLR-9 expression, in mononuclear cells were found to have a higher probability of metastasis.[143] In metastatic breast cancer cells, activated TLR-4 regulates the expression of mediators that promote cancer adhesion and invasion. In addition to this, TLR-4 signaling increases microRNA 21 (miR-21) in breast cancer cells, through NF-κB.[144]

Incidents of relapses have been shown to be high in breast cancer patients harboring the TLR-4 Asp299Gly polymorphism who were treated with anthracycline-based chemotherapeutic drugs.[145] This polymorphism also confers to an increased risk of breast cancer progression.[146] However, the levels of the TLR-4 Ala896Gly allele in breast cancer patients were not found to be significantly different from those levels in normal healthy Caucasian women.[147] These studies suggest TLR-4 involvement in breast cancer progression. Future studies are warranted, in developing novel therapeutic approaches targeting TLR-4 against breast cancer.

TLR-4 in ovarian cancer

A proinflammatory environment in the ovary, such as with ovarian endometriosis, predisposes women to ovarian cancer. Epithelial ovarian cancer cells have been found to express TLR-4, but not the adjacent nondysplasia cells.[34,148] It has been found that ovarian tumors derived from the surface epithelium of normal ovaries express TLR-4, which leads to NF-κB inhibitor (IkB) degradation and the activation of NF-κB for its proinflammatory responses. The MyD88 pathway, a downstream signal of TLR-4, is reported to be essential for LPS-induced ovarian tumor growth.[34] The tumor growth and survival could be due to the interaction between the inducible HspA1 A from ovarian cancer cells with TLR-4 expressed on the neutrophil surface. These neutrophils enhance the production of reactive oxygen species and induce tumor progression as well as tumor lysis.[149]

Paclitaxel has been the drug of choice for the treatment of ovarian cancer. A recent study has proven the association of MyD88 expression with paclitaxel resistance, and paclitaxel-induced proinflammatory cytokine release.[34] Paclitaxel resistance is probably due to the activation of the protein kinase B (Akt) survival pathways and the expression of the antiapoptotic protein X-linked inhibitor of apoptosis protein (XIAP) following TLR-4 ligation.[34,150] The tumors have been found to be resistant to paclitaxel, a TLR-4 ligand, but not to carboplatin.[34] Another laboratory finding has suggested that five ovarian cancer (OVCAR3,

SKOV3, AD10, A2780, CP70) cells with TLR-4 ligation induced IL-1 receptor-associated kinase (IRAK)-4 activation, c-Jun phosphorylation, NF-κB activation, and IL-8, IL-6, VEGF, and monocyte chemotactic protein-1 (MCP)-1 production, all of which promote tumor survival and chemoresistance.[148] These findings suggest that further research on the role of TLR-4 in relation to management and chemotherapy for ovarian cancer should be carried out.

TLR-4 in cervical cancer

The female reproductive tract is constantly exposed to pathogens and carcinogens. Cervical cancer is one of the most common cancers closely linked to infection and inflammation. Human papillomavirus (HPV) has been identified as the leading cause of cervical cancer. HPV cervical infection results in cervical morphological lesions ranging from normal to invasive cancer.[151] The presence of other infectious agents, such as bacteria, protozoa, and viruses, in the female genital tract induce the inflammation and cancer.[152] A multi-center case-control study revealed that *Chlamydia trachomatis* infection induced the cervical cancer.[153] Also, pathogenic *Escherichia coli* and *Pseudomonas aeruginosa* infection, but not the nonpathogenic *Lactobacillus reuteri* infection, caused the upregulation of TLR-4 in cervical cancer cells.[154] In human cervical cancer (HeLa) cells, TLR-4 was found to be the highest expressed TLR, more than 100 times higher compared with the other TLRs.[126] This observation provides proof of the linkage between TLR-4 and the progression of cervical cancer.

Unlike other studies linking higher levels of TLR-4 with cancer, it was found that TLR-4 is downregulated in cervical intraepithelial neoplasia patients compared with healthy women. TLR-4 expression was found to decrease as the histopathologic grade of cervical intraepithelial neoplasia increased. TLR-4 expression was found to be inversely proportional ($P<0.001$) with the expression of p16INK4A, a marker of high-risk HPV infection.[155,156] There are limited studies that conclude on the role of TLR-4 in cervical cancer progression; thus, the TLR-4 Thr399Ile polymorphism ($P=0.044$, OR=2.51, 95% CI: 1.03−6.12) was, again, found to be significantly associated with early stages of cervical cancers among North Indian women.[157] Therefore, further research is needed to fully understand the role of TLR-4 in cervical cancer.

TLR-4 in prostate cancer

Prostate cancer is one of the most common causes of morbidity and mortality in men. Prostate epithelial cells are

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actively involved in inflammatory processes.[158] Higher levels of proinflammatory cytokines, produced through TLR-3, -4, and -9 downstream signaling pathways, were observed in the prostate tissues of cancer patients. In this study, TLR-3 -4 and -9 were highly expressed in prostate cancer tissues but not in benign tissues. Only TLR-3 levels, and not TLR-4 or TLR-9 levels, were found to have statistical significance (P=0.016) with levels of preoperative serum prostate-specific antigen, in prostate cancer patients.[159]

However, in vitro studies have shown the expression of higher levels of TLR-4 on human prostate adenocarcinoma (DU-145) cells and its activation, leading to NF-κB and proinflammatory cytokine production through the MyD88-dependent pathway.[160] Also, TLR-4 activation was found to increase the proangiogenic factor (VEGF) and immunosuppressive cytokine (TGF-β1) secretion in human prostate adenocarcinoma (PC3) cells.[161] Further, a knockdown of TLR-4 in PC3 cells resulted in the reduction of tumor cell migration and invasion.[162] These results support the negative impact of TLR-4 upregulation in prostate cancer.

In addition, single nucleotide polymorphism in the TLR-4 gene is suspected to be associated with the risk of prostate carcinoma.[163] The sequence variant (11381 G/C, also known as rs11536889) in the 3′-untranslated region of the TLR-4 gene was found to be higher in patients with prostate carcinoma, in studies conducted on 1,383 Swedish patients[164] and 157 Korean patients.[165] In one study, a significantly higher risk of prostate cancer (OR: 1.26; 95% CI: 1.01–1.57) was detected among men who had a single nucleotide polymorphism of TLR-4 (GC or CC) compared with the wild-type genotype (GG).[164] However, other studies found no association of prostate cancer (OR: 1.01; 95% CI: 0.79–1.29) with this rs11536889 sequence variant of TLR-4;[166,167] one of these, a study involving 700 prostate cancer patients found that homozygosity of the variant alleles of these eight single-nucleotide polymorphisms of TLR-4 including rs2149356 were found to have a lower risk of prostate cancer.[166] A case-control study of 506 incident advanced prostate cancer patients found two single nucleotide polymorphisms of TLR-4 (rs10759932 and rs2149356) were associated with a higher cancer risk.[167] Nevertheless, Lindstrom et al have suggested that the association of TLR-4 with prostate cancer risk is a chance finding and that to ascertain the relationship, large sample sizes are needed.[168] The inconsistent findings of the association of genetic polymorphism of TLRs with cancer progression clearly supports the need for further investigation in this field.

Conclusion

The activation of TLR-4 is required for host defense against gram-negative bacteria. However, TLR-4 activation may be a double-edged sword, with both antitumor and protumor responses. The general expression of TLR-4 by all the tumor cells, suggesting TLR-4 signaling, may be continually activated and contribute to tumor initiation, progression, and also invasion. Tumor progression involves TLR-4-mediated irregular and uninhibited production of proinflammatory cytokines, chemokines, and also immunosuppressive cytokines; suggesting that the discovery of TLR-4 antagonists might be an ideal strategy to treat cancer. However, TLR-4 antagonists could pose the risk of the compromise of host immunity. Hence, it is a scientific dilemma whether a TLR-4 agonist or antagonist should be targeted for the treatment of cancer. Further studies need to be carried out to fully elucidate the effects of TLR-4 agonists and antagonists in various cancers.

Acknowledgments

Disclosure

The authors report no conflicts of interest in this work.

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