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REVIEW

Roles of Toll-like Receptors in Cancer: A Double-edged Sword for Defense and Offense

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Toll-like receptors (TLRs) belong to a class of pattern-recognition receptors that play an important role in host defense against pathogens by recognizing a wide variety of pathogen-associated molecular patterns (PAMPs). Besides driving inflammatory responses, TLRs also regulate cell proliferation and survival by expanding useful immune cells and integrating inflammatory responses and tissue repair processes. TLR signaling, which is centrally involved in the initiation of both innate and adaptive immune responses, has been thought to be restricted to immune cells. However, recent studies have shown that functional TLRs are expressed not only on immune cells, but also on cancer cells, thus implicating a role of TLRs in tumor biology. Increasing bodies of evidence have suggested that TLRs act as a double-edged sword in cancer cells because uncontrolled TLR signaling provides a microenvironment that is necessary for tumor cells to proliferate and evade the immune response. Alternatively, TLRs can induce an antitumor immune response in order to inhibit tumor progression. In this review, we summarize the dual roles of TLRs in tumor cells and, more importantly, delve into the therapeutic potential of TLRs in the context of tumorigenesis.

Key words: Toll-like receptor, Antitumor, Tumorigenesis, Inflammation, Tumor immunotherapy, Proinflammatory cytokines

INTRODUCTION

Innate immunity represents the first line of defense against invading microbial pathogens. Innate immunity is ontogenetically older than adaptive immunity; however, the innate recognition of pathogens is the first step in inducing adaptive immunity. In vertebrates, innate and adaptive immunity are overlapping and intervening. One major difference in the biology of the two systems is that several responses of innate immune recognition are encoded in the germline DNA, which is in contrast to adaptive immune responses for which these responses do not require any gene rearrangements (Janeway and Medzhitov, 2002). Innate immune responses are triggered by bacteria, viruses, protozoa, and fungi, which are non-self molecules, and involve the nonspecific activation of neutrophils, monocytes, macrophages, dendritic cells (DCs), natural killer (NK) cells and complements. Innate immunity to microbial pathogens relies on the specific host-receptor detection of pathogen- and danger-derived molecular signatures, such as pathogen-associated molecular patterns (PAMPs) and danger-associated molecular patterns (DAMPs, also known as damage-associated molecular patterns) (Geddes et al., 2009). The foot soldiers of the innate immune system, namely DCs and macrophages, express a number of germline-encoded pattern recognition receptors (PRRs) that specifically recognize and ingest pattern recognition molecules (PAMPs and DAMPs) and release cytokines that attract secondary, active and defensive cells from the blood. The PRRs include members of nucleotide oligomerization domain proteins, which contain leucine-rich repeats (NLRs), retinoic acid-inducing gene (RIG)-like helicases (RLHs), and toll-like receptors (TLRs)

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(Akira et al., 2006). As our understanding of innate immunity has developed, our interest in applying this knowledge to clinical problems has also increased. Most of these translational efforts have been centered on TLRs, which are members of one of the largest and most well-studied family of PRRs (Basith et al., 2011). Moreover, the awarding of the prestigious Nobel Prize in Physiology or Medicine (2011) to Bruce Beutler and Jules Hoffmann for their discoveries concerning the activation of innate immunity underscores the timeliness and significance of this research.

TLRs are type I transmembrane glycoproteins that are characterized by the presence of an extracellular domain (ectodomain; ECD) that contains leucine-rich repeats (LRRs), which are primarily responsible for mediating ligand recognition, a single transmembrane helix, and an intracellular Toll-like/interleukin-1 (IL-1) receptor (TIR) domain that is responsible for downstream signaling (Manavalan et al., 2011). To date, 10 and 12 functional TLRs have been identified in humans and mice, respectively. TLR1-9 are conserved in both species; however, mouse TLR10 is not functional because of a retrovirus insertion, and TLR11-13 have been lost from the human genome (Kawai and Akira, 2010). TLRs play a crucial role in host defense against invading pathogens by recognizing pattern recognition molecules. However, the most potent role of TLRs in host defense is regulating the innate and adaptive immune responses of epithelial cells, which act as the first line of defense at mucosal sites, such as the skin and respiratory, genitourinary and gastrointestinal tracts. In addition, TLRs play an important role in mediating leukocyte recruitment to infected tissues and in the uptake of microorganisms by phagocytic cells (Picker and Butcher, 1992; Blander and Medzhitov, 2004). The activation of antigen-presenting cells (APCs), such as DCs, and the stimulation of both T- and B-cell-mediated immune responses are partly due to the ligation of TLRs (Schnare et al., 2001).

Since first being described in the fruit fly, *Drosophila melanogaster*, the TLR family of PRRs has become a major component in innate immunity, innate-adaptive crosstalk, infectious diseases and inflammatory conditions. Additionally, TLRs play a pivotal role in maintaining tissue homeostasis by regulating tissue repair and regeneration. TLR signaling has been shown to regulate apoptosis through the expression of antiapoptotic proteins or inhibitors of apoptosis (Ioannou and Voulgarelis, 2010). Emerging evidences now implicate TLRs in inflammation-associated cancers. However, this concept of innate immunity leading to malignancy dates back over 100 years. Dr. William Coley demonstrated in the late 19th century that crude microbial extracts (killed *Streptococcus pneumonia* and *Serratia marcescans*, commonly known as Coley's toxin), when administered repeatedly, could promote an antitumor response in different types of cancers (Coley, 1894). That approach is still used today in the form of *Bacillus* Calmette-Guerin (BCG), which is used for the treatment of bladder cancer (Bassi, 2002). It is highly likely that these agents trigger an innate immune response through multiple TLRs in order to promote direct tumoricidal activity and a platform for the development of antitumor immunity.

An association between the development of cancer and inflammation has long been appreciated (Balkwill and Mantovani, 2001). In 1858, Rudolf Virchow noticed that cancer often developed at sites of chronic inflammation (Chen et al., 2008). The inflammatory response orchestrates host defenses to microbial infection and mediates tissue repair and regeneration, which may occur due to infectious or noninfectious tissue damage. Under normal conditions, inflammation is tightly controlled in order to restrict cell proliferation until infections are resolved and tissue repair is completed. However, these inflammatory responses can also promote tumorigenesis through multiple means, including promoting the antiapoptotic effects of nuclear factor-κB (NF-κB; a transcription factor that is commonly engaged in inflammatory conditions), inducing oxidative damage to DNA, and inducing the tissue repair response (Balkwill and Mantovani, 2001; Coussens and Werb, 2002; Rakoff-Nahoum and Medzhitov, 2009). Over the past several years, numerous cancers have been shown to be associated with local chronic inflammation (chronic ulcerative colitis and Crohn's disease with colon cancer, chronic *Helicobacter pylori* infections with gastric cancer, chronic bronchitis with lung cancer, chronic pancreatitis with pancreatic cancer, papillomavirus infection with cervical cancer, and chronic cholecystitis with gall bladder cancer) (Balkwill and Mantovani, 2001; Balkwill and Coussens, 2004).

TLRs appear to provide signals that are necessary for the resolution of inflammation but also play crucial roles in cancer. Therefore, TLRs function as double-edged swords, with both pro- and antitumor consequences. However, the exact mechanisms by which TLRs interact with tumor cells and how these cells are able to escape immunological eradication have only recently started to unravel. Therefore, understanding the roles of TLRs in tumor biology may pave the way for the discovery of novel therapeutic targets in cancer therapy. Here, we review the roles of TLRs in tumorigenesis and discuss the possibility of TLRs as targets for tumor therapy. We hope to provide new insights into the pathogenesis and progression of cancer.

TOLL-LIKE RECEPTOR SIGNALING

TLRs are expressed on sentinel cells of the immune system, including macrophages and DCs, which are the key sensors of pathogen invasion (Miyake, 2007). The initial step in signal transduction involves the dimerization of two receptor chains, which is induced by the binding of a specific ligand. Alternatively, in the case of TLR7, 8 and 9, the receptor may be present in the cell as a preformed, yet inactive dimer, and ligand binding may cause reorientation of the TIR domains (Zhu et al., 2009; Basith et al., 2011). In either case, the TLR-TIR domain interaction serves as a nucleating act for the recruitment of downstream signaling adapter proteins. Myeloid differentiation primary response gene 88 (MyD88), MyD88 adaptor-like (Mal; also known as TIR domain-containing adapter protein, TIRAP), TIR domain-containing adaptor inducing interferon (IFN)-β (TRIF; also known as TIR domain-containing adapter molecule 1, TICAM1), TRIF-related adaptor molecule (TRAM; also known as TICAM-2), and sterile α- and armadillo motif-containing protein (SARM) are the five adaptor proteins that contain TIR domains, which function in TLR signaling (O'Neill et al., 2003; Basith et al., 2011).

The signaling pathways utilized by TLRs differ, which results in varied cellular responses. For example, all TLRs utilize the MyD88-dependent signaling pathway with the exception of TLR3, which exclusively uses the TRIF pathway, to induce the expression of proinflammatory cytokine genes (Medzhitov et al., 1998). Most TLRs activate the classical/canonical inflammatory signaling pathway through MyD88 (Takeda and Akira, 2004), which, in turn, recruits interleukin-1-receptor associated kinases (IRAKs) and tumor necrosis factor (TNF) receptor-associated factor 6 (TRAF6). TRAF6, in turn, activates transforming growth factor (TGF)-activated kinase 1 (TAK1) that phosphorylates and activates the inhibitor of kappa light polypeptide gene enhancer in B-cells kinase (IKK) complex, culminating in the release and translocation of NF-κB to the nucleus, thereby inducing the synthesis of proinflammatory cytokines, such as TNF-α, IL-6 and IL-1, which are key mediators of inflammatory responses (Kawai and Akira, 2005; Akira et al., 2006; O'Neill, 2006). Conversely, TLR3 and certain signals of TLR4 can signal independently of MyD88 by utilizing the TRIF adaptor protein. This alternative/noncanonical pathway culminates in the activation of TRAF3 and interferon regulatory factor 3 (IRF3), resulting in the secretion of type I IFNs, which are required for an effective antiviral response (Pandey and Agrawal, 2006).

TLR4 is unique among the TLRs in its ability to activate 2 distinct signaling pathways. One pathway is activated by the adaptor proteins, TIRAP and MyD88 (canonical pathway), which leads to the induction of proinflammatory cytokines, and the second pathway is activated by the adaptors, TRIF and TRAM (noncanonical pathway), which leads to the induction of type I IFNs (Fig. 1). Until recently, it had been generally believed that these two signaling pathways were activated simultaneously at the plasma membrane. However, a study from Ruslan Medzhitov's lab showed that the two signaling pathways are induced sequentially and that the TRAM-TRIF pathway is only operational from early endosomes following endocytosis of TLR4 (Kagan et al., 2008). Additionally, TLR stimulation leads to the activation of other signaling pathways, such as JNKs, MAPKs, p38, ERKs and IRFs (IRF3, 5, and 7) (Lee and Kim, 2007). These signals are crucial for the orchestration of innate and adaptive immune responses, inflammation and tissue repair in the host (Fig. 1).

EXOGENOUS AND ENDOGENOUS TLR LIGANDS

The TLR family can be largely divided into 2 subgroups, extracellular and intracellular, depending on their cellular localization. TLR1, 2, 5, 6 and 10 (extracellular TLRs), which are largely localized on the cell surface, recognize PAMPs. Conversely, TLR3, 7, 8 and 9 (intracellular TLRs) are localized in intracellular organelles, such as endosomal/lysosomal compartments and the endoplasmic reticulum. The subcellular localization of TLR4 is unique because it is localized to both the plasma membrane and endosomal vesicles (Kagan et al., 2008). Based on their amino-acid sequences and genomic structures, TLR1, 2, 6 and 10 are closely related and constitute the TLR2 subfamily. TLR1 and 6 form heterodimers with TLR2. Similarly, TLR7, 8 and 9 constitute the TLR9 subfamily (Takeda et al., 2003). Among the TLRs, the ligand (lipopolysaccharide, LPS) of TLR4 was first identified through genetic studies (Lemaitre et al., 1996). Since then, TLRs have been shown to recognize and mediate signaling for a wide variety of microbial pathogens. Lipopeptides or lipoproteins are recognized by TLR2 in complex with TLR1 or 6, while viral double-stranded RNA (dsRNA) is recognized by TLR3, flagellin is recognized by TLR5, single-stranded RNA (ssRNA) is recognized by TLR7 and 8, and host- or pathogen-derived DNA is recognized by TLR9. Other extracellular components, such as MD-2 and CD14, are also critical for the recruitment of TLR4 ligands.

In addition to the known pathogen/microbial-derived ligands, TLRs also recognize endogenous and synthetic

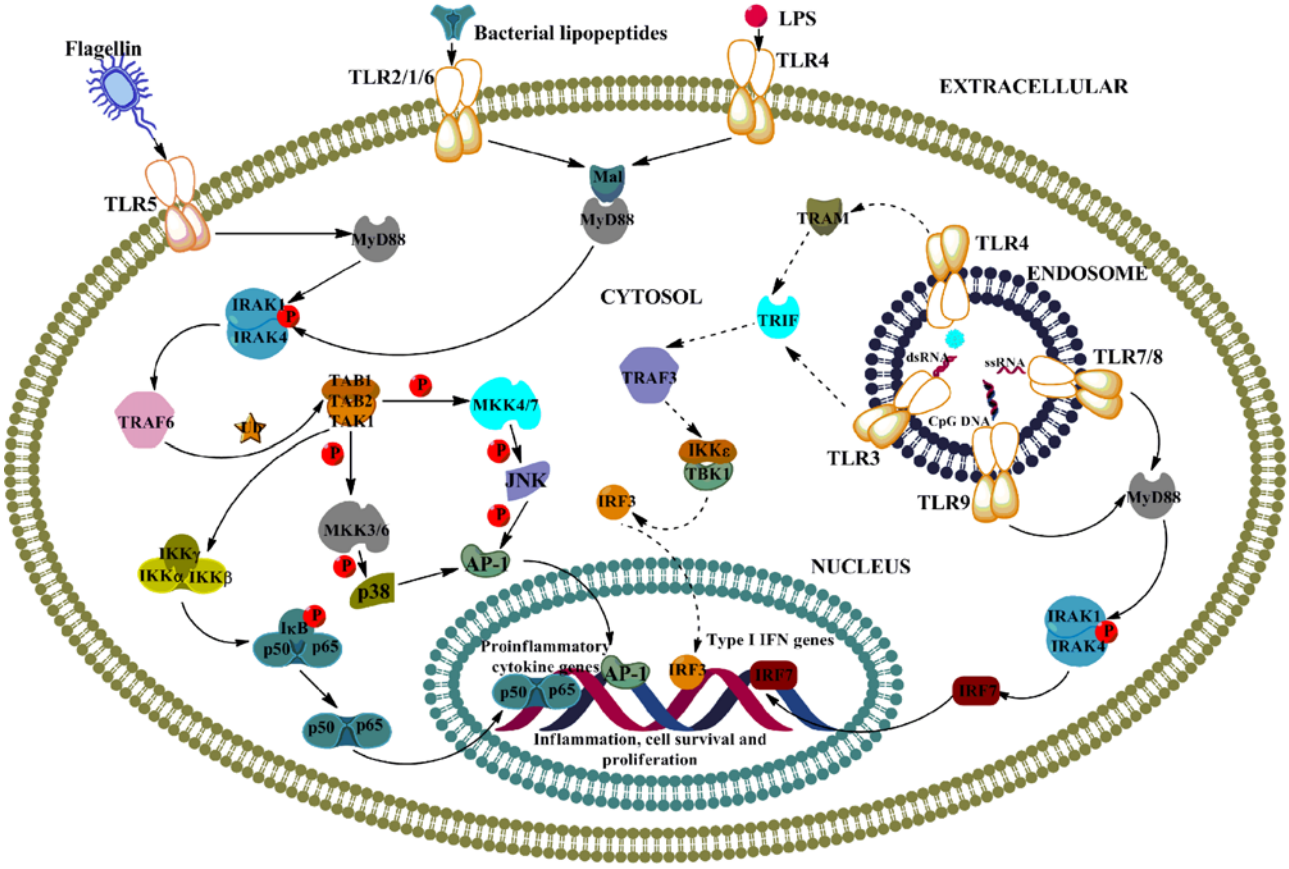

Fig. 1. General Toll-like receptor (TLR) signaling pathway. TLRs localize to different subcellular compartments according to the molecular nature of their respective ligands. Following ligation with their respective ligands, TLRs recruit downstream adapter proteins, such as Mal, MyD88, TRAM and TRIF. TLR signaling is mediated by at least 2 distinct signaling pathways: MyD88-dependent and TRIF-dependent signaling pathways. MyD88-dependent signaling pathway activates IRAK4, TRAF6, and IKK complexes. These sequentially activate signaling pathways of p38, JNK, and NF-κB, leading to a series of specific cellular responses, such as cell survival, proliferation and inflammation. The TRIF-dependent signaling pathway activates TRAF3 and the IKKε/TBK1 complex. TLR3, 7, 8 and 9 activate IRF3 and IRF7, which mediate the production of type I IFNs and other antiviral immune responses. General MyD88-dependent signaling (canonical pathway) is shown by the line arrow. MyD88-independent signaling (noncanonical/TRIF pathway) is shown by the dashed arrow. Mal: MyD88-adapter like, MyD88: Myeloid differentiation primary-response protein 88, IRAK: Interleukin-1-receptor associated kinase, TRAM: TRIF-related adaptor molecule, TRIF: TIR domain-containing adaptor inducing interferon-β; TRAF: Tumor necrosis factor receptor-associated factor, TAB: Transforming growth factor-β activated kinase 1/MAP3K7 binding protein, TAK: Transforming growth factor-activated kinase, IKK: inhibitor of kappa light polypeptide gene enhancer in B-cells kinase, MKK: Mitogen-activated protein kinase kinase, IRF: interferon regulatory factor, AP-1: Activator protein 1.

ligands, which are capable of inducing the release of proinflammatory cytokines from the monocyte-macrophage system (Beg, 2002; Zhang and Schluesener, 2006). In contrast, few studies have implicated endogenous TLR ligands in tumor pathogenesis. Furthermore, the surveillance model proposed by Johnson et al. suggests that the immune system recognizes not only endogenous and exogenous molecules, but also the degradation products of endogenous macromolecules, such as heparan sulfate and polysaccharide fragments of hyaluronan (Johnson et al., 2003). The signals that are mediated by the degradation of macromolecules are present during development and in conditions of infection and tissue injury. These signals activate TLRs and initiate protective inflammatory responses and the repair of damaged tissues. Endogenous TLR ligands, which are often referred to as alarmins, serve as early warning signals to the host immune system. It has also been reported that dying tumor cells release endogenous TLR ligands that are involved in tumor progression. As these host-derived nonmicrobial molecules are released following tissue injury and cell death and are able to elicit similar responses as PAMPs, they have been collectively categorized as DAMPs. Both PAMPs and DAMPs initiate immune responses through TLR signals (Ellerman et al., 2007). TLR ligand-mediated

sterile inflammation has been linked to a variety of pathological processes, especially tumorigenesis. Thus, understanding ligand-mediated TLR signaling in tumorigenesis is becoming an important issue for tumor prevention and therapy. The list of ligands for TLRs is shown in Table I.

Table I. Toll-like receptors (TLRs) and their associated ligands

TLR	Ligand		
	Danger-associated molecular patterns (DAMPs; endogenous)	Pathogen-associated molecular patterns (PAMPs; exogenous): Origin	Synthetic analog
TLR1		Lipopeptides: Bacteria and Mycobacteria. Soluble factors: *Neisseria meningitidis.*	Triacyl lipopeptides
TLR2	Heat shock protein 60 (HSP60), HSP70, HSP96, high-mobility group protein B1 (HMGB1), and hyaluronic acid	Lipoprotein/lipopeptides: Gram-positive bacteria, Mycoplasma, Mycobacteria, and Spirochetes. Peptidoglycan: Gram-positive bacteria. Lipoteichoic acid: Gram-positive bacteria. Phenol-soluble modulin: *Staphylococcus epidermidis* Heat-killed bacteria: *Listeria monocytogenes.* Porins: *Neisseria.* Atypical lipopolysaccharide (LPS): *Leptospira interrogans, Porphyromonas gingivalis.* Soluble factors: *Neisseria meningitidis.* Glycolipids: *Treponema maltophilia.* Outer membrane protein A: *Klebsiella pneumonia.* Glycoinositolphospholipids: *Trypanosoma cruzi.* Phospholipomannan: *Candida albicans.* Structural viral proteins: Herpes simplex virus, Cytomegalovirus. Hemagglutinin: Measles virus. Lipoarabinomannan: Mycobacteria. Zymosan: *Saccharomyces.*	Diacyl and triacyl lipopeptides
TLR3	Self double-stranded RNA (dsRNA), mRNA	Viral dsRNA.	Poly(I:C), Poly(I:C_{12}U)
TLR4	HSP22, HSP60, HSP70, HSP96, HMGB1β-defensin 2, extra domain A of fibronectin, hyaluronic acid, heparan sulfate, fibrinogen surfactant-protein A	LPS: Gram-negative bacteria. HSP60: *Chlamydia pneumonia.* Envelope proteins: Respiratory syncytial virus and mouse mammary tumor virus. Fusion protein: syncytial virus. Glycoinositolphospholipids: *Trypanosoma cruzi.* Taxol: Plant product.	Lipid A mimetics (Monophosphoryl lipid A, aminoalkyl glucosamine 4-phosphate), E6020, E5531, E5564
TLR5		Flagellin: Gram-positive or Gram-negative bacteria	Discontinuous 13-amino acid peptide CBLB502
TLR6		Diacyl lipopeptides: Mycoplasma. Lipoteichoic acid: Gram-positive bacteria. Phenol-soluble modulin: *Staphylococcus epidermidis* Zymosan: *Saccharomyces.* Heat-liable soluble factor: Group B *streptococcus.*	Diacyl lipopeptides
TLR7	Endogenous RNA	Viral single-stranded RNA (ssRNA).	Oligonucleotides, Imidazoquinolines (Imiquimod, Resiquimod), Guanosine nucleotides (Loxoribine, Isatoribine), Bropirimine
TLR8	Endogenous RNA	Viral ssRNA.	Imidazoquinolines (Resiquimod)
TLR9	Endogenous DNA	Unmethylated CpG motifs: Bacteria and viruses. Hemozoin: *Plasmodium.*	CpG oligodeoxynucleotides (CpG 7909, CpG 10101, 1018 ISS)
TLR10		Not determined	
TLR11		Profilin-like molecule: *Toxoplasma gondii.*	

EXPRESSION OF TLRS IN TUMOR CELLS/ CELL LINES

TLRs are expressed on the surface of monocytes, macrophages, DCs and epithelial cells or in the cytoplasm of cells from different tissues (Marodi, 2006). However, several studies have shown that TLRs are not only expressed on immune cells, but are also expressed on tumor cells, where they may influence tumor growth and host immune responses (Huang et al., 2005; Chen et al., 2008). The discovery of TLRs in tumor cells has heralded a renaissance of interest among innate immunity and tumor biology researchers. Previous studies have shown that TLRs are expressed by a wide variety of tumor cells/cell lines, including those of both mouse and human (listed in Table II). TLR expression in tumor cells/cell lines appears to promote tumorigenesis by facilitating cell survival and migration in a tumor microenvironment that is characterized by chronic inflammation and PAMPs (Pikarsky et al., 2004).

Several tumor cells/cell lines express single or, more commonly, multiple TLRs (Table II). It is believed that each TLR signal has a particular effect on tumors. Outcomes of TLR stimulation not only depend on receptors, but also vary according to different tumor types. TLR2 expression on human oral cancer cells and dysplastic oral epithelial cells has recently been reported (Ng et al., 2011). Positive TLR2 expression in the tumor microenvironment suggests that immune surveillance is activated against the altered epithelial cells, whereas TLR2 expression by malignant keratinocytes may be indicative of resistance to apoptosis as a prosurvival mechanism. Huang et al. showed that *Listeria monocytogenes* promotes tumor growth through TLR2 (Huang et al., 2007). This effect was mediated by the direct interaction of the tumor cells with bacteria, as demonstrated by increased NF-κB activity. Functional TLR3 expression has been demonstrated on cells

Table II. Expression of TLRs in various tumor cells/cell lines

TLRs expressed	Tumor cells/cell lines	References
TLR1, 7, and 9	Multiple myeloma cells	(Jego et al., 2006)
TLR2	Oral squamous cell carcinoma	(Ng et al., 2011)
TLR2, 3, and 4	Laryngeal carcinoma	(Szczepanski et al., 2007)
TLR2, 4, and 5	Intestinal adenocarcinoma	(Pimentel-Nunes et al., 2011)
TLR2, 3, 4, and 5	Ovarian carcinoma	(Zhou et al., 2009)
TLR3	Human neuroblastoma (NB) cells, breast adenocarcinoma, cervical, hepatocellular, papillary thyroid, nasopharyngeal and lung carcinomas, and murine colon carcinoma	(Salaun et al., 2006, 2011; McCall et al., 2007; Jiang et al., 2008; Yoneda et al., 2008; Zhang et al., 2009a; Chuang et al., 2011; Guo et al., 2012)
TLR3, 4, and 9	Breast and prostate carcinomas	(Gonzalez-Reyes et al., 2010; Gonzalez-Reyes et al., 2011)
TLR3, 4, 7, and 9	Esophageal squamous cell carcinoma	(Sheyhidin et al., 2011)
TLR4	Colon carcinoma, human head and neck squamous cell carcinoma, melanoma cell lines, NB-1 neuroblastoma, lung carcinoma, pancreatic ductal adenocarcinoma, colorectal carcinoma, adrenocortical carcinoma, ovarian cancer cell lines, bladder cancer cells	(Hassan et al., 2006; Molteni et al., 2006; Doan et al., 2009; Qian et al., 2009; Szajnik et al., 2009; Szczepanski et al., 2009; Kanczkowski et al., 2010; Tang et al., 2010; Zhang et al., 2010; Xu et al., 2011)
TLR4 and 9	Lung carcinoma	(Zhang et al., 2009b)
TLR4, 5, and 9	Gastric carcinoma	(Schmausser et al., 2005)
TLR5	Cervical tumor cells, breast cancer cells, colon carcinoma, gastric cancer cells	(Kim et al., 2008; Rhee et al., 2008; Cai et al., 2011; Song et al., 2011)
TLR7 and 8	Lung carcinoma, colorectal carcinoma	(Cherfils-Vicini et al., 2010; Grimm et al., 2010)
TLR9	Lung carcinoma, cervical cancer cells, prostate carcinoma, renal cell carcinoma, breast cancer cells, ovarian cancer cells	(Droemann et al., 2005; Lee et al., 2007; Berger et al., 2010; Ronkainen et al., 2011)
Multiple TLRs	Human breast cancer cell line MDA-MB-231, murine tumor cell lines (MC26, 4T1, RM1, B16, LLC1), human tumor cell lines (HCT15, SW620, MCF7, UACC-62, MDA-MB435)	(Huang et al., 2005; Yang et al., 2010)

of several human cancers, including neuroblastoma, breast adenocarcinoma, and cervical, hepatocellular, papillary thyroid, nasopharyngeal and lung carcinomas. TLR4 expression in human lung cancer cells was demonstrated (He et al., 2007b). TLR4 ligation on tumor cells enhanced the secretion of immunosuppressive cytokines and induced resistance to TNF-α and TNF-related, apoptosis-inducing, ligand (TRAIL)-induced apoptosis. In a study (Huang et al., 2005), the expression of TLR4 in murine tumor cell lines was reported. LPS-induced TLR4 activation in these tumor cell lines facilitated tumor evasion from immune surveillance. Hassan et al. demonstrated that human neuroblastoma (NB)-1 cells expressed the intracellular form of TLR4 but not the cell surface form (Hassan et al., 2006). Although the cells expressed TLR4 and possessed all of the molecules required for LPS response, they were unresponsive to LPS treatment, suggesting the intracellular expression of TLR4 (Hassan et al., 2006). TLR4 is reportedly overexpressed in colorectal cancer cells from patients with colitis and in colorectal cancer cells from a murine model of colitis (Fukata et al., 2007). Kundu et al. showed that primary and immortalized prostate epithelial cells exhibit increased proliferation in response to TLR4 and TLR9 ligands (Kundu et al., 2008). Increased expression levels of TLR5 and TLR9 contribute to cervical carcinogenesis (Lee et al., 2007; Kim et al., 2008). The levels of expression of TLR5 and TLR9 gradually increase during the progression of low-grade cervical intraepithelial neoplasia (CIN) to high-grade CIN and then to invasive cervical squamous cell carcinoma. It has been demonstrated that TLR9 overexpression and stimulation with hypomethylated DNA augment the migratory capacity of cancer cells (Berger et al., 2010). Various levels of TLR9 expression have been demonstrated in tumor specimens from patients with prostate cancer, breast cancer, astrocytoma and glioblastoma (Ilvesaro et al., 2007). Droemann et al. showed the expression of TLR9 in human lung cancer tissues and various tumor cell lines (Droemann et al., 2005).

Few reports have shown the presence of multiple TLRs on established tumor cells/cell lines. Szczepanski et al. showed the expression of TLR2, 3 and 4 in the tumor microenvironment of human laryngeal carcinoma cells (Szczepanski et al., 2007). Similarly, TLR2-5 were expressed in ovarian cancer cell lines (Zhou et al., 2009). TLR4, 5 and 9 were strongly expressed not only by gastric cancer cells, but also by metaplastic and dysplastic gastric epithelial cells from patients with *Helicobacter pylori* gastritis (Schmausser et al., 2005). Multiple TLRs, such as TLR2-4 and 9, were expressed in lung cancer cell lines (He et al., 2007b). Furthermore, Goto et al. showed the expression of multiple TLRs (TLR2-4) in human cutaneous melanoma both *in vivo* and *in vitro* (Goto et al., 2008). These TLR ligations may promote events of metastasis, thus setting the stage for tumor promotion. Human myeloma cells express multiple TLRs, such as TLR1, 7 and 9. Their activation by bacterial ligands promotes tumor growth and evasion from conventional therapies (Jego et al., 2006). One recent study showed that prostate carcinomas with high TLR3, 4 and 9 expression levels exhibit a higher probability of biochemical recurrence (Gonzalez-Reyes et al., 2011). The high expression levels of TLR3, 4 and 9 in breast carcinomas may indicate their association with metastasis (Gonzalez-Reyes et al., 2010). Additionally, TLR4 and 9 expression has been demonstrated in a wide variety of tumor cells, thus promoting the immune escape of cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. Several reports on the expression of single or multiple TLRs in tumor cells/cell lines are shown in Table II.

DOUBLE-EDGED SWORD ROLES OF TLR IN TUMORIGENESIS

TLRs play pivotal roles in the activation of innate immunity against invading pathogens, cytokine production and the development of adaptive immune responses. In contrast to the protective role of TLR against infectious pathogens, several studies suggest that TLRs that are expressed on tumor cells also contribute to tumor progression. The complicated interactions between tumor cells, immune cells, and pattern recognition molecules, such as PAMPs and DAMPs, in the tumor microenvironment (Fig. 2) can promote the progression of tumors (tumorigenic effects), as well as support the inappropriate immune enhancement or antitumor immune tolerance (antitumor effects) through TLR signaling. Thus, activation of TLR in tumor cells acts as a double-edged sword.

TLR as a negative regulator of cancer

Evidence of antitumor effects of microbial products can be dated back to the early 18th century when Deidier (1725) reported that infection in cancer patients could be concomitant with the remission of malignant diseases (Garay et al., 2007). In the 1890s, William B. Coley observed that repeated injections of a mixture of bacterial toxins served as an efficient antitumor therapeutic agent. Although Coley did not know the exact mechanisms of how the bacteria were able to produce this effect, he produced bacterial toxins for cancer treatment. Coley reported the successful use of these bacterial toxins in patients with soft tissue sarcoma,

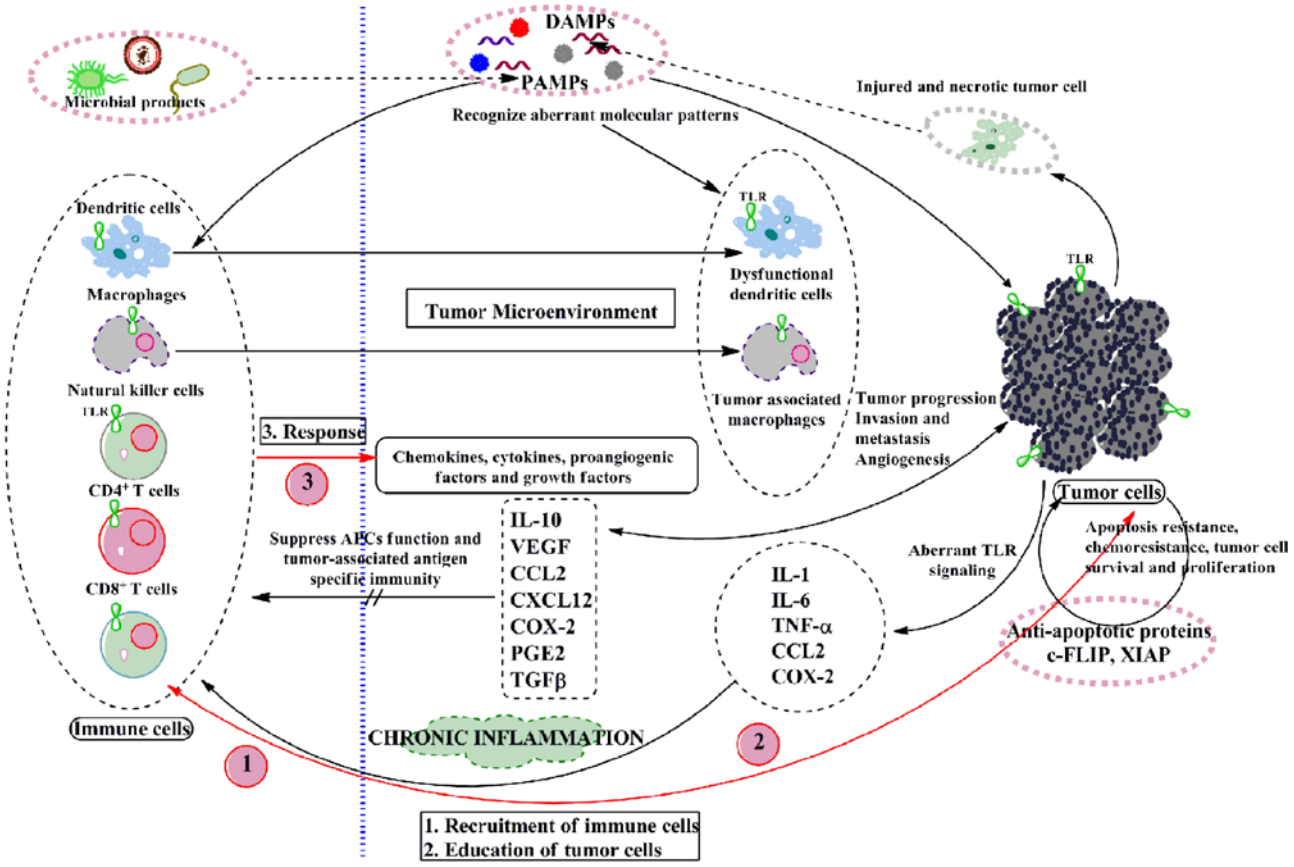

Fig. 2. Interplay between TLRs, inflammation and tumorigenesis. TLRs are expressed on many types of immune cells, tumor cells, tumor stromal cells, and infiltrating immune cells. TLR signals contribute to carcinogenesis in the tumor microenvironment. During cancer progression in the setting of chronic inflammation, TLR ligands activate the TLRs that are expressed in tumor cells. TLRs that are expressed on immune cells and tumor cells are activated by pathogen-associated molecular patterns (PAMPs; derived from microbes) and danger-associated molecular patterns (DAMPs; derived from injured and necrotic cancer cells). These activated cells release cytokines and chemokines that are important components of the tumor microenvironment. Furthermore, cytokine-activated infiltrating immune cells can subsequently induce further cytokines and impair the function of antigen-presenting cells (APCs) and tumor-associated antigen (TAA)-specific immunity, thereby resulting in tumor immunotolerance. Moreover, direct promotions of tumor-cell survival and inflammation-induced chemoresistance have been linked to the hyperactivation of NF-κB in tumor cells, which induces the upregulation of antiapoptotic proteins, such as cellular FLICE-inhibitory protein (c-FLIP) and X-linked inhibitor of apoptosis (XIAP). The subsequent activation of TLRs in cancer cells and the ensuing signaling cascade with the production of cytokines and chemokines may promote cancer cell survival and chemoresistance and, therefore, tumor progression. Tumor-immune cell interaction is shown in three stages: 1) Recruitment - cancer cells recruit immune cells to the tumor microenvironment through the production of chemokines; 2) Education - tumor cells polarize immune cells toward tumor-supporting cells through the secretion of cytokines that regulate immune cells differentiation. Additionally, tumor cells can educate immune cells to produce the type of cytokines that will facilitate tumor growth and metastasis, as well as acquire immune tolerance; 3) Response - differentiated immune cells promote tumor growth and immune tolerance through the production of growth hormones, growth factors, chemokines and cytokines in the tumor microenvironment. These three stages are shown by red arrows.

laying the foundation for utilizing synthetic PAMPs in cancer therapy. In 1943, Shear and Turner discovered that LPS, which was the "hemorrhage-producing fraction" of Coley's toxin, accounted for its antitumor effects (Garay et al., 2007). Polly Matzinger proposed a danger model showing that bacterial mixtures were activators of immune responses (Matzinger, 1994, 2002). A measure of vindication came when Bruce Beutler and colleagues (Poltorak et al., 1998) demonstrated that the receptor identified by Janeway and Medzhitov (Medzhitov et al., 1997), now known as TLR4, acts by detecting distinctive molecular patterns (of LPS) present in the outer membranes of bacteria. In turn, these receptors activate inflammatory compounds that can kill tumor cells.

Coley's notion of the antitumor activity of bacterial

extracts has been revisited and explored by many researchers, which has led to the findings that bacterial components, such as bacterial endo/exotoxins, lipoteichoic acid, and bacterial DNA, have strong antitumor activities through direct tumoricidal effects as well as indirect effects including enhanced innate immune activation (Ishii et al., 2003). We now understand that microbe-derived therapeutics work by stimulating TLR signaling and activating both innate and adaptive immune responses to enhance tumor immunotherapy. OK-432, which is a penicillin-killed and lyophilized preparation of a low-virulence strain of *Streptococcus pyogenes* (group A), is a TLR4 agonist, and it has been successfully used as an immunotherapeutic agent in many types of malignancies, including head, neck, cervical, and gastric cancer and oral squamous cell carcinoma (Okamoto et al., 1967, 2003; Kikkawa et al., 1993; Maehara et al., 1994; Sato et al., 1997). Another bacterial strain, *Mycobacterium bovis* BCG, which acts as a TLR2/4 agonist, is effective against superficial bladder tumors (Morales et al., 1976).

TLR2 signaling protects mice from tumor development in colitis-associated colorectal cancer (CAC) (Lowe et al., 2010). TLR2-deficient mice developed significantly more and larger colorectal tumors than wild-type controls. Furthermore, in the intestinal microenvironment within aberrant crypt foci, significantly higher levels of IL-6 and IL-17A, concomitant with increased phospho-STAT3, were observed, suggesting that TLR2 plays a protective role against the development of CAC. However, in a two-stage chemical carcinogenesis mouse model of inflammation-mediated lung cancer, the presence of functional TLR4 was shown to inhibit lung tumorigenesis, indicating a protective role of TLR4 in this cancer model (Bauer et al., 2005).

Triggering of TLR3 on human tumor cells with poly (I:C) inhibits tumor cell proliferation and triggers apoptosis (Matijevic et al., 2009; Nomi et al., 2010). High doses of poly(I:C) have been shown to induce apoptosis and directly kill tumor and ancillary cells, such as vascular endothelium, in the tumor microenvironment (Salaun et al., 2006). The TLR agonist that is most extensively studied due to its antitumor potential is the TLR9 activator, CpG, which is being studied for the treatment of breast cancer, colorectal cancer, lung cancer, melanoma, glioblastoma, and some lymphomas and leukemias (Krieg, 2008). CpG-containing oligodeoxynucleotide (CpG ODN) agonists directly induce the activation and maturation of DCs, enhance the differentiation of B cells into antibody-secreting plasma cells, and promote the development of antitumor T-cell responses (Hanten et al., 2008). For example, in a murine model of human ovarian cancer, the intraperitoneal administration of CpG ODN produced a strong antitumor effect (De Cesare et al., 2008). Brignole et al. showed that CpG ODN stimulation inhibits the proliferation of TLR9-expressing NB cells, induces caspase-dependent apoptotic cell death, and significantly prolongs the survival of mice bearing NB tumor xenografts (Brignole et al., 2010). Wang et al. showed that TLR9 stimulation of lung cancer cells sensitized tumor cells to apoptosis, leading to the arrest of tumor growth (Wang et al., 2006). Furthermore, when agonists, such as poly(I:C)- or imiquimod-treated tumor cells, are co-cultured with γδ T cells, activation of TLR3 or 7 on tumor cells enhances the cytotoxic activity of γδ T cells of cancer patients and tumor cell lysis by human γδ T cells (Shojaei et al., 2009). However, it should be remembered that not all TLR agonists and not all TLR signaling pathways lead to clinically relevant antitumor activity.

TLR as a positive regulator of cancer

In 2000, Hanahan and Weinberg proposed a model to define the six properties a tumor acquires (Hanahan and Weinberg, 2000). However, increasing evidence suggests that a seventh feature, inflammation, should also be included in this list (Mantovani et al., 2008; Kim et al., 2009). Several studies have postulated that the development of cancer has been associated with microbial infection, injury, inflammation and tissue repair. Moreover, the expression or upregulation of TLRs in tumor cells/cell lines may directly or indirectly contribute to tumorigenesis in numerous organs. Engagement of TLRs in tumor cells through ligands and their subsequent signaling cascades involving cytokine and chemokine production can promote tumor invasion, tumor cell survival (apoptosis resistance), chemoresistance, tumor progression and metastasis.

Tumor cells are under constant surveillance by immune cells for attack. Hence, tumor cells have to overcome such immune surveillance in order to progress. TLRs expressed on tumor cells can upregulate the NF-κB signaling cascade and produce proinflammatory cytokines, chemokines and antiapoptotic proteins that contribute to tumorigenesis and tumor cell proliferation. Additionally, TLRs can recruit more immune cells to enhance immunity in the tumor microenvironment. These devised tumor cells release further proangiogenic factors and growth factors, which enhance the resistance of tumor cells to cytotoxic lymphocyte attack, thereby leading to immune evasion (Fig. 2). The primary way tumor cells evade immune attack is that the tumor cells have devised multiple strategies, such as the secretion of transforming growth factor-β (TGF-β) and vascular endothelial growth factor (VEGF), the upregulation and downregulation of certain surface molecules, and

the shedding of some surface molecules in soluble form (Huang et al., 2008), thus mimicking a normal immune cell for their own proliferation and survival. Hence, the immune cells are not able to discriminate the tumor cells as foreign and are, therefore, not subjected to attack, thereby leading to immune evasion.

TLR ligand administration has been shown to enhance the growth of adoptively transferred tumor cell lines by acting on host cells. TLR4-dependent signaling in the recipient is required for LPS-induced tumor growth. The suggested mechanism involves a host-dependent increase in the circulating levels of TNF that lead to the upregulation of NF-κB-regulated antiapoptotic factors, such as Bcl-XL, cIAP1, and cIAP2, in the tumor cells (Luo et al., 2004). In addition, several studies have demonstrated that the administration of TLR ligands may have protumorigenic effects due to actions on both tumor cells and accessory cells in tumor microenvironments. Huang et al. showed that the intratumoral administration of *Listeria monocytogenes* to a hepatocellular carcinoma cell line accelerates tumor growth through TLR2 signaling (Huang et al., 2007). LPS ligation to TLR4 has been demonstrated to enhance tumor metastasis in a murine model by acting directly on tumor cells, resulting in NF-κB-mediated, β1 integrin-dependent increased *in vitro* tumor endothelial cell adhesion, tumor extracellular matrix adhesion and invasion (Wang et al., 2003). Kundu et al. showed that immortalized prostate epithelial cells express both TLR4 and 9 and exhibit enhanced tumor proliferation when cultured in the presence of their respective ligands (LPS and CpG ODN) due to lower susceptibility to TNF-α-induced apoptosis (Kundu et al., 2008). Triggering of TLR7 and 8, which are expressed by human lung cancer cells, induces cell survival and chemoresistance. TLR7 or 8 ligations with their respective agonists led to activated NF-κB, an upregulated expression of the antiapoptotic protein Bcl-2, increased tumor cell survival and chemoresistance (Cherfils-Vicini et al., 2010). Cervical cancer occurs in a multi-step process, which involves the transformation of normal cervical epithelium to preneoplastic CIN and which will eventually lead to invasive cervical cancer. Two findings suggest that TLR5 and 9 may play a significant role in the tumor progression of cervical neoplasia and may represent a useful marker for the malignant transformation of cervical squamous cells (Lee et al., 2007; Kim et al., 2008).

Recent studies have shown that TLRs are involved in tumor cell apoptosis resistance and chemoresistance. TLR4, which is an important TLR, is present in tumors, such as those in ovarian cancer, prostate cancer and colorectal cancer (Kelly et al., 2006; Hua et al., 2009; Killeen et al., 2009). The activation of TLR4 that is expressed on tumor cells may promote tumor growth and apoptosis resistance. Kelly et al. showed that the activation of TLR4 signaling promotes tumor growth and chemoresistance in epithelial ovarian cancer cells (Kelly et al., 2006). The expression of antiapoptotic proteins, such as the X-linked inhibitor of apoptosis (XIAP) and pAKT, following TLR4 ligation is associated with tumor growth and chemoresistance in ovarian cancer cells. Furthermore, Jego et al. reported that the triggering of TLR7 and 9 induces tumor cell growth and prevents chemotherapy-induced apoptosis in myeloma cells (Jego et al., 2006). These effects are mediated by the induction of the autocrine secretion of IL-6. Thus, the myeloma cells take advantage of infection to spread out and escape the usual therapies. Additionally, He et al. showed that the inhibition of tumor cell apoptosis by TLR4 signaling on human lung cancer cells promotes the immune escape of tumor cells by inducing immunosuppressive cytokines (He et al., 2007b).

Tumor cell evasion may be facilitated by the secretion of a number of factors, such as proteinases, inhibitory cytokines, inflammatory factors, and other small molecules (i.e., nitric oxide). Huang et al. showed that the triggering of TLR4 with LPS in tumor cell lines produces proinflammatory factors, including nitric oxide, IL-6 and IL-12, mimicking the inflammatory cell environment (Huang et al., 2005). These factors result in the resistance of tumor cell lines to cytotoxic T lymphocytes and NK cell attack and evasion from immune surveillance. Another report (Szczepanski et al., 2009) demonstrated that the triggering of TLR4 expressed in human head and neck squamous cell carcinoma promotes tumor development by the increased production of IL-6, IL-8, VEGF and granulocyte macrophage colony-stimulating factor and promotes the immune escape of tumor cells.

Few studies have shown that TLR activation by tumor cells results in adherence to the extracellular matrix and endothelial cells, promoting tumor invasion and metastasis. Two earlier studies (Harmey et al., 2002; Wang et al., 2003) showed that LPS promotes tumor invasion through the TLR4-mediated NF-κB pathway, resulting in the upregulation of inducible nitric oxide synthase (iNOS), matrix metalloproteinase 2 (MMP2), and the β1 integrin subunit. Merrell et al. showed that TLR9 was associated with tumor invasiveness (Merrell et al., 2006). The stimulation of TLR9-expressing breast cancer cells with CpG ODNs dramatically increased their *in vitro* invasion by increasing the activity of MMP13 without affecting MMP8. Another *in vitro* study showed that TLR9 agonists can stimulate prostate cancer invasion by increasing MMP13 activity (Ilvesaro et al., 2007). The specific ligand activation of TLR2, 3

and 4 on human melanoma cells was shown to induce cell migration and promote events of metastasis (Goto et al., 2008). Additionally, there is an increasing body of evidence showing that TLR variants are linked to cancer risk. Previous studies have reported that a TLR4 polymorphism is associated with prostate cancer risk (Zheng et al., 2004; Chen et al., 2005). Subsequently, TLR1, 6 and 10 polymorphisms are also linked to prostate cancer risk (Sun et al., 2005). Moreover, the risk of gastric carcinoma is increased by a functional polymorphism of TLR4 (Hold et al., 2007). Sequence variants of TLR3, 4 and 10 are associated with nasopharyngeal carcinoma risk (Song et al., 2006; Zhou et al., 2006; He et al., 2007a). Sequence variants of TLR2 increase the risk of gastric cancer. Additionally, a TLR2 polymorphism is associated with an increased risk of follicular lymphoma. Few lymphoma subtypes are associated with single-nucleotide polymorphisms (SNPs) in TLR1, 2, 4, 5 and 9. A specific example of SNPs with cancer risk is *H. pylori* infection and the induction of gastric cancer. Recent reports have identified that defective signaling through TLR4 may result in an exaggerated inflammatory reaction with severe tissue destruction in D299G people (El-Omar et al., 2008; Kutikhin, 2011).

In contrast to TLR signaling in tumor cells, several studies have also shown that TLR deficiency in tumor cells/cell lines may lead to tumor regression. TLR4 signaling is responsible for cyclooxygenase-2 (COX-2) induction, prostaglandin E2 (PGE2) production, and epidermal growth factor receptor (EGFR) phosphorylation, which promote the development of colitis-associated colorectal tumors (Fukata et al., 2007). The expression of mucosal PGE2 is decreased in TLR4-deficient mice, and TLR4 deficiency protects mice from colitis-associated neoplasia (Hernandez et al., 2010). However, the exogenous administration of PGE2 in TLR4-deficient mice increases colitis-associated tumor incidence and tumor size. Fukata et al. postulated that the targeted inhibition of TLR4 may be effective in preventing the development of colon cancer in inflammatory bowel disease (Fukata et al., 2007). Functional analyses revealed that the abrogation of TLR4 expression in human breast tumor cell lines (Hua et al., 2009) and prostate tumor cell lines (Yang et al., 2010) inhibits tumor invasion and proliferation and induces apoptotic cell death.

In summary, all of these findings imply that inflammatory conditions observed in the tumor microenvironment may not only originate from the immune cells, but also from the tumor cells. Moreover, tumor cells can educate immune cells to produce the type of cytokines that will facilitate tumor growth and metastasis as well as acquire immune tolerance. Thus, the inhibition of the protumor microenvironment and/or rescue of the immune cells from a protumor to an antitumor response may represent a new strategy for tumor biology and tumor immunotherapy.

TLR AGONISTS IN TUMOR IMMUNOTHERAPY

The induction of antitumor immunity through therapeutic vaccinations or the administration of immune-stimulating agents is a promising approach in the treatment of cancer. Due to the importance of TLR signaling in tumorigenesis, TLR agonists have been identified as possible agents in tumor immunotherapy. Harnessing the powerful immunostimulatory properties of TLR agonists has great potential in the development of active immunotherapy against cancer. TLR-agonist-linked tumor immunotherapy is still in a nascent state but growing rapidly in the area of common human malignancies. Several TLR agonists have been demonstrated to produce antitumor effects against established tumors/tumor cell lines in both mice and humans (Table III). A significant number of successful preclinical studies in mice have resulted in a number of efficacy trials in humans.

TLR2, 3 and 4 agonists

The TLR2 agonist, SMP-105, which consists of cell-wall skeleton components, such as mycolic acids and peptidoglycans from *Mycobacterium bovis* (BCG Tokyo), has been approved for the treatment of bladder cancer (Simons et al., 2008). After being treated with the compound, TLR2-knockout mice show impairments of TNF-α and IL-6 production, as well as reduced tumor growth, thereby showing strong adjuvant and antitumor activities. OM-174, another chemically defined TLR2/4 agonist, reduces tumor progression and prolongs survival in B16 melanoma mice (D'Agostini et al., 2005) through the induction of TNF-α secretion and iNOS. OM-174 is a diphosphorylated glucosamine disaccharide bearing 3 fatty acid chains. This compound reduces tumor growth, increases IFN-γ production, and prolongs the survival of mice (D'Agostini et al., 2005). Additionally, it has a good safety profile for its use in humans, and it is currently under development by OM Pharma (now a part of Vifor Pharma Ltd.) as a cancer immunotherapeutic agent (Phase Ib clinical trials). Several clinical trials have reported that the injection of dsRNA (a TLR3 agonist) is associated with survival in cancer patients as functional TLR3 is expressed in breast cancer cells and very highly expressed in both primary and metastatic clear-cell renal and breast carcinoma cells (Salaun et al., 2006). IPH 3102, another high-molecular-mass synthetic dsRNA (TLR3-specific agonist) acts as a potent

Table III. Clinical development status of TLR agonists for cancer therapy

Agonist	Targeting TLR	Cancer	Clinical status	References
SMP-105	TLR2	Bladder cancer	Preclinical	(Murata, 2008; Uenishi et al., 2009)
OM-174	TLR2/4	Cancer	Phase 1b	(D'Agostini et al., 2005; Garay et al., 2007)
BCG	TLR 2/4/9	Bladder cancer	Approved	
IPH 3102	TLR3	Breast cancer, melanoma, and other cancers	Preclinical	(Levenga et al., 2010)
LPS	TLR4	Ductal carcinoma Non-Hodgkin's lymphoma Malignant melanoma	Phase I/II	(Czerniecki et al., 2007)
MPLA + Cervarix	TLR4	Cervical cancer	Approved	(D'Souza et al., 2007)
Stimuvax	TLR4	Lung cancer, breast cancer, prostate cancer, and colorectal cancer	Phase IIIbor IV	(Butts et al., 2005, 2011)
CBLB502	TLR5	Head and neck cancer	Phase I/II	(Burdelya et al., 2008)
Imiquimod	TLR7	Skin cancer Breast cancer	Approved Phase II	(Scheel et al., 2006)
852A	TLR7	Malignant melanoma	Phase II	(Dummer et al., 2008)
VTX-2337	TLR8	Advanced solid tumors	Phase 1a	(Lu et al., 2012)
IMO-2055 + Avastin and Tarceva	TLR9	Lung cancer	Phase I	(Goodchild et al., 2009)
ISS1018 + Rituxan	TLR9	Non-Hodgkin's lymphoma	Phase II	(Krieg, 2008)
CPG-7909 + Herceptin	TLR9	Breast cancer	Phase II	(NCT00043394)
MGN-1703	TLR9	Colorectal cancer	Phase II-III	(Kochling et al., 2008)
MGN-1706	TLR9	Prostate cancer	Phase II	
Agatolimod + Trastuzumab	TLR9	Breast cancer	Phase II	(Dorn and Kippenberger, 2008)

immunostimulator *in vivo* in mice. IPH 3102 activates NF-κB signaling and type 1 IFN response *in vitro* and destroys melanoma and breast cancer cells (Panter et al., 2009). This RNA-based drug candidate is being developed by Innate Pharma for the treatment of breast cancer and as a vaccine adjuvant. It is currently in the preclinical validation development phase.

Monophosphoryl lipid A (MPLA), a TLR4 agonist, is an immunomodulatory product that is less toxic than LPS and that specifically activates the TRAM/TRIF (MyD88-independent) pathway in TLR4 signaling, leading to the induction of IFN-β and the regulation of CD80/86, which is a key aspect of adjuvancy (Mata-Haro et al., 2007). MPLA is used in GlaxoSmithKline's Cervarix, which is a cervical cancer vaccine. This vaccine is used to prevent early stage precancerous lesions, Pap smear abnormalities, and cervical cancer that are caused by human papillomavirus types 16 and 18 (D'Souza et al., 2007). It has recently been approved by the Food and Drug Administration (FDA) for the treatment of cervical cancer. Stimuvax, a BLP25 liposome vaccine, is an innovative cancer vaccine that is designed to induce an immune response to cancer cells expressing MUC1, a protein antigen that is widely expressed in common cancers (Butts et al., 2011). Stimuvax is thought to work by stimulating the body's immune system to identify and destroy MUC1-expressing cancer cells. Stimuvax is being developed by Merck and is currently undergoing Phase IIIb or IV clinical trials.

TLR5, 7, 8 and 9 agonists

CBLB502 (Burdelya et al., 2008) is a bioengineered derivative of a microbial protein, flagellin, that potentially reduces injury from acute stresses, such as radiation and chemotherapy, by mobilizing several natural cell-protective mechanisms, including the inhibition of programmed cell death (apoptosis), the reduction of oxidative damage, and the induction of regeneration-promoting cytokines. This suggests that TLR5 agonists may be valuable as adjuvants for cancer radiotherapy. CBLB502 is currently being developed by Cleveland BioLabs, Inc. under the FDA's Animal Efficacy Rule.

TLR7 agonists (Imidazoquinolines) act as potentially targeted immunogenic death agents for chronic lymphocytic leukemia (CLL) (Spaner et al., 2010). The potential importance of these TLR agonists in the treatment of CLL is suggested by their ability to sensitize tumor cells to cytotoxic agents, and their future probably lies in combination with radiotherapies, chemotherapies, monoclonal antibodies, and cancer vaccines (Yu and Chen, 2008). The small molecule TLR7 agonist, 852A, is structurally related to Imiquimod and is currently being evaluated in a Phase II clinical trial by 3M Pharma (now a part of Medicis Pharmaceutical Corporation) for the treatment of melanoma (Harrison et al., 2007). This compound stimulates DCs to produce multiple cytokines, including IFN-α, both *in vitro* and *in vivo*. Imiquimod (Aldara) is the first approved topically active TLR7/8 agonist. It is used for the treatment of cancer and is efficacious against primary skin tumors and cutaneous metastasis. It exerts numerous antitumor effects, including the activation of NF-κB, the induction of proinflammatory cytokines, and the induction of a Th1 response (Schon and Schon, 2008). BCG and Imiquimod have been approved by the FDA for clinical use as monotherapies. VTX-2337 is a small molecule TLR8 agonist that stimulates myeloid DCs and monocytes and enhances NK cell responses. It is administered subcutaneously on a weekly basis and is currently under Phase 1a clinical development in oncology (ClinicalTrial.gov registry number: NCT00688415).

To date, the most promising and most frequently studied interaction in tumor immunotherapy trials seems to be that of TLR9 and its synthetic agonists. CpG ODNs targeting TLR9 have shown substantial potential as vaccine adjuvants and mono- or combination therapies for the treatment of cancer (Vollmer and Krieg, 2009). Immune modulatory oligonucleotides (IMOs) that stimulate TLR9 signaling are currently being developed. IMO-2055 became Idera Pharmaceuticals' first IMO drug candidate to enter clinical development. This drug had anticancer activity in a mouse model when used as a monotherapy, and its activity was amplified when used in combination with chemotherapeutic agents (Goodchild et al., 2009). In a Phase I trial that evaluated the safety and immunological activity of IMO-2055 alone and in combination with chemotherapy agents, IMO was found to be much more efficacious when used in combination. This compound is currently being analyzed in Phase I clinical trial in patients with non-small cell lung carcinoma, in combination with Avastin and Tarceva (ClinicalTrial.gov registry number: NCT00633529) (Krieg, 2008). Another TLR9 agonist in development by Dynavax Technologies is ISS1018. This IMO is currently being used in clinical trials alone or in combination with antigens to combat non-Hodgkin's lymphoma and other cancers. ISS1018 has demonstrated efficacy in treating follicular lymphoma in combination with Rituxan in a Phase II clinical trial (ClinicalTrial.gov registry number: NCT00251394) and in treating non-Hodgkin's lymphoma (Krieg, 2008).

Mologen AG has developed 2 novel types of TLR9 agonists in the form of the DNA immunomodulator dSLIM (double stem-loop immunomodulator). dSLIM activates the immune system in order to protect against tumor-associated antigens by targeting the TLR9 receptor on certain immune cells (Kochling et al., 2008). As a result of chemotherapy and radiotherapy, tumor-associated antigens are released by cancer cells. The immune system activated by dSLIM is in a position to overcome its fatal tolerance towards cancer cells and tumor-associated antigens and attacks them selectively. The results of a completed Phase 1b study confirm an excellent safety profile for MGN1703. Treatment with the investigational drug was well tolerated and no dose-limiting or serious side effects were identified. This cancer medication, MGN1703, is currently being investigated in a Phase II-III clinical study for the treatment of metastasized colorectal cancer. Another DNA-based immunomodulator, MGN1706, is currently in a Phase II clinical study for the treatment of metastasized, hormone-refractory prostate cancer. Agatolimod is a CpG ODN-based therapy (Dorn and Kippenberger, 2008) that is being developed to treat cutaneous T-cell lymphoma (NCT00091208: Phase I-II), a non-Hodgkin's lymphoma, together with Rituximab and Yttrium Y 90 ibritumomab tiuxetan drugs (NCT00438880: Phase I-II) and metastatic breast cancer, in combination with Trastuzumab (NCT00824733: Phase II). Although it is hard to predict where therapeutic targeting of TLRs in tumor biology will be in the future, promising data exists and late-phase clinical trials are on the horizon.

CONCLUSIONS AND FUTURE PERSPECTIVES

Numerous reports have provided convincing evidence that bacterial- and viral-induced inflammatory processes can mediate tumorigenesis (Coussens and Werb, 2002). In 1863, Rudolf Virchow proposed that chronic inflammation supports tumorigenesis. Since then, accumulating evidence supports this hypothesis, and it is estimated that 20% of all cancer-related deaths are associated with chronic infection and inflammation. The relationship between inflammation and tumorigenesis has recently become widely accepted. Studies suggest that tumor cells acquire many properties that are characteristic of immune cells, allowing them to

communicate and regulate the immune system for their own survival and growth (Kelly et al., 2006; Chen et al., 2007). Moreover, NF-κB activation, which is seen in most tumor cells, plays a key role in tumor initiation, progression, metastasis and chemoresistance by mediating the production of a large variety of proinflammatory cytokines, chemokines, growth factors, collagenases and antiapoptotic proteins (Fig. 2). TLRs are among the major activators of NF-κB and are the front-line receptors that elicit an inflammatory response to microbial infection.

TLR activation serves a dual purpose with both pro- and antitumor responses. TLR activation is required for the host defense against invading microbes, and TLR agonists may be utilized as effective immune adjuvants in tumor- or combined immunotherapy. However, abnormal activation of TLRs in tumor cells might facilitate aberrant cytokine profiles that are associated with immune tolerance, tumor progression, and propagation of the tumor microenvironment. To date, therapeutic targeting of TLRs in cancers using several TLR agonists has proven immensely successful in clinical and advanced preclinical programs. However, the successful clinical development of TLR agonists requires careful selection and study of the agent because activation of some TLRs in tumor cells has been shown to enhance tumor growth and metastasis. From our current review, it is apparent that both TLR4- and TLR9-based immunotherapies have progressed well into clinical development.

The major drawback of utilizing immune-related therapies is that animal models may be insufficient for defining new therapeutic approaches. This has been a matter of debate regarding TLR-targeting drugs due to the differences observed in the expression and function of different TLRs, such as the key differences seen between the activation profiles of human and mouse TLR8 (Gorden et al., 2006). Additionally, it is important to note that animal studies are usually carried out using inbred strains, which have less genetic diversity compared to humans. Moreover, responses to TLR7 agonists result in widely varying inductions of IFN-α and proinflammatory cytokines, such as TNF-α, in humans. Therefore, it is likely that cytokine induction in animal models will not reveal the true range of human responses; hence, there is a large margin of error that must be considered when predicting doses and safety windows for clinical trials (Basith et al., 2011).

Although there is an apparent link between TLRs, inflammation and tumorigenesis, a number of important unanswered questions remain. One major question is: What is the difference between the inflammation that drives tumor progression and the inflammation that drives tumor regression? Another concern is that we still do not know exactly how much TLR signaling contributes to the development of chronic inflammation-associated tumors. Understanding this mechanism will facilitate the development of a new strategy for tumor therapy. Moreover, there is a question as to how much the exogenous and endogenous ligands of TLR contribute to tumorigenesis because both ligands are involved in the activation of TLRs in tumor cells. Another intriguing area of study lies in the induction of entirely different biological effects by the activation of various TLRs in tumor cells (Yu et al., 2012). For example, TLR4 signaling usually promotes tumor growth (Fukata et al., 2007), whereas TLR2 activation protects mice from tumor growth (Lowe et al., 2010). Why does different TLR signaling result in opposite outcomes? We believe that all of these important issues will be a frontier field of TLR study in the near future.

Advanced exploration and a better understanding of the relationship between TLRs and the tumor microenvironment are required to elucidate the mechanisms of tumor metastasis and to develop more effective therapeutic approaches to a wide variety of human cancers. Furthermore, understanding the role of TLRs and other PRRs in tumorigenesis should provide interesting insights into cancer development. TLR implications in tumor biology and other related findings have been fruitful in terms of improving our knowledge of the molecular basis of innate immunity, inflammation, and tumorigenesis and, thus, we can anticipate further discoveries in the coming years.

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