Links between Toll-like receptor 4 and breast cancer

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Toll-like receptors (TLRs) have generated an extraordinary amount of interest in cancer research since the last decade. TLRs are a family of pattern recognition receptors that is involved in the host defense against microbial infections. It is well known that the activation of TLRs leads to the production of biological factors that drive inflammatory responses and activate the adaptive immune system. More recently, TLR-mediated signaling pathways have been shown to support tumor cell growth in vitro and in vivo. In this review, we describe recently emerged links between TLR4 and breast cancer oncogenesis, and future perspectives for the targeting of TLR4 in breast cancer therapy.

Introduction

Despite the advances in multimodal adjuvant and neoadjuvant therapies, the management of advanced breast cancer remains a significant problem. In western societies, breast cancer is the most common malignant cancer in women, being responsible for 90% of fatalities due to metastasis to distant sites including the lungs, bone, liver and brain.1 This has increased the necessity for new therapeutic modalities that treat the local and systemic components of the disease, in particular for patients who do not respond to conventional treatments.

Inflammation is a physiological process involved in tissue repair, remodeling and immune protection. During tissue injury and the subsequent process of wound healing, the inflammatory process mediated by immune cells not only contains potential invasion by pathogens, but also promotes cell proliferation and neovascularization. At a closer look, malignant lesions share a large amount of inflammatory cells and many other characteristics with chronically inflamed tissues.2 Several studies have provided strong evidence indicating that bacterial- and viral-induced inflammatory processes can mediate oncogenesis.3 Chronic infection and inflammation are considered two of the most prominent epigenetic and environmental factors contributing to oncogenesis and tumor progression.4 In experimental models, the surgical removal of tumors is followed by an exacerbated growth of dormant metastasis, especially after administration of lipopolysaccharide (LPS).5 It has also been shown that mice inoculated with 4T1 cancer cells via the tail vain manifested increased numbers of lung metastases after the administration of LPS.6

Most of the reports on Toll-like receptors (TLRs) have focused on their expression pattern and function in cells of the immune system. Recently, however, TLR expression and function in cancer cells and the links of TLRs with oncogenesis and tumor progression has generated a considerable amount of interest.3 Still, how TLRs and cancer interrelate remains very controversial and contradicting data can be found in the literature. Thus, on one hand, TLRs appear to suppress cancer progression in many models.7–13 On the other hand, TLRs have been reported to enhance cancer progression.14–19

The current literature implicates TLRs in general and TLR4 in particular in many cancer types. So far, however, data linking TLRs to breast cancer area are very limited and failed to recognize whether the effect TLR4 on breast tumorigenesis originate from cancer cell-intrinsic or immune-mediated effects. Most importantly, the current literature does not give a useful guide for future modulation of TLR4 for breast cancer management.

Overview of TLRs

Until recently, innate immunity was considered to rely on non-specific responses mediated by the phagocytic activity of macrophages and neutrophils. TLRs were first implicated in immune responses when mutations in Drosophila toll receptor were found to result in a high susceptibility to fungal infections and in the defective production of antifungal peptides. Further studies have shown that there is a specific receptor interacting with fungi.20 Subsequently, a human homolog of Toll (hToll) was identified and characterized for its ability to induce the production of inflammatory cytokines and the expression of co-stimulatory molecules.21 This was followed by the remarkable discovery that LPS-hyporesponsive mice bear a mutation in the gene coding for their hToll homolog.22

To date, 10 members of TLR family have been identified in humans and 13 in mice. After their discovery, several genetic studies have revealed their respective ligands. TLR2 in conjuction with either TLR1 or TLR6 recognizes various bacterial components including peptidoglycan, lipopeptides and lipoproteins of gram-positive bacteria and mycoplasma.23,24 TLR3 recognizes...
double-stranded RNA (dsRNA) as produced by replicating viruses. The LPS from gram-negative bacteria is recognized by TLR4. TLR5 recognizes bacterial flagellin. TLR7 recognizes synthetic imidazoquinoline-like molecules, guanosine analogs such as loxoribine, single-stranded RNA (ssRNA) species from type I human immunodeficiency virus (HIV-1), vesicular stomatitis virus (VSV) and influenza virus, and some small interferons (IFNs), as well as other chemokines and cytokines (MHC)-peptide complexes, co-stimulatory molecules, type I interferons (IFNs), IL-1 receptor family members signal through MYD88. TLR3 signals through TRIF, while TLR4 can signal via both the MYD88 and the TRIF pathways. TLR binding leads to activation of nuclear factor κB (NFκB), mitogen-activated protein kinases (MAPKs), including c-JUN N-terminal kinases (JNKs), p38 and extracellular signal-regulated kinases (ERKs), and IFN-regulatory factor 3, 5 and 7 (IRF3, IRF5 and IRF7) signaling pathways. These signals are essential for both innate and adaptive immune responses.

### TLR4 Signaling

Mammalian TLRs consist of an extracellular domain containing leucine-rich repeats, which are responsible for ligand binding, a transmembrane region and a cytoplasmic Toll/interleukin-1 receptor (TIR) domain, which is required for intracellular signaling. These subcellular compartments where TLRs localize depends on the component which TLRs interact with. Thus, TLRs that recognize lipid and protein ligands (i.e., TLR1, TLR2, TLR4, TLR5 and TLR6) are expressed on the plasma membrane, whereas TLRs that detect nucleic acids (i.e., TLR3, TLR7 and TLR9) are localized to endolysosomal compartments.

TLR-elicited intracellular signaling is archived through one of four adaptor proteins: myeloid differentiation factor 88 (MYD88), TIR-domain-containing adaptor inducing IFNβ (TRIF), TIR domain-containing adaptor protein (TIRAP) and TRIF-related adaptor molecule (TRAM). All TLRs (except for TLR3) and IL-1 receptor family members signal through MYD88. TLR7 and TLR9 are responsible for the recognition of imidazoquinoline and ssRNA. TLR9 is responsible for the recognition of bacterial and viral deoxyxycytidine-phosphate-deoxyguanylate (CpG) DNA motifs and the malaria-associated pigment hemoglobin.

Mouse TLR11 recognizes yet unknown components of uropathogenic bacteria as well as a profilin-like molecule of the protozoan parasite *Toxoplasma gondii*.

In addition to microbial ligands, several endogenous ligands have been reported to stimulate TLRs. These include the heat-shock 60 kDa protein (HSP60) and HSP70, oligosaccharides of hyaluronan, high mobility group box 1 (HMGB1), surfactant protein A, various products of the extracellular matrix such as fibronectin, heparan sulfate, biglycan, fibrinogen, hyaluronan breakdown fragments, endoplasmic reticulum stress proteins B8 (HSPB8), α-crystallin A chain and uric acid crystals.

When TLRs on immature dendritic cells (DCs) interact with their ligands, a program of maturation is initiated, inducing the migration of DCs to lymphoid organs and culminating in the enhanced expression of major histocompatibility complex (MHC)-peptide complexes, co-stimulatory molecules, type I interferons (IFNs), as well as other chemokines and cytokines that are necessary for T-cell activation. TLRs can also stimulate the cellular machinery that mediates antigen processing and presentation. As a result, the proteolysis in endosomes/lysosomes as well as membrane transport and fusion reactions are boosted, and antigen presentation on both MHC Class I and Class II molecules is enhanced. By triggering the maturation of DCs, enabling them to activate T cells, TLRs are considered as an important link between the adaptive and innate immune systems.

![TLR4 signaling through MYD88-dependent and TRIF-dependent pathways.](image-url)
IRF3, together with NFκB, activates the transcription of various target genes including genes coding for type I IFNs.67 The induction of IFNs and IFN-inducible genes are critical for antiviral and antibacterial responses (Fig. 3).68

TLR4 and Cancer

William Coley (1862–1939) observed that repeated injections of a mixture of bacterial toxins purified from the gram-positive bacterium \textit{Streptococcus pneumoniae} and the gram-negative bacterium \textit{Serratia marcescens} served as an efficient antitumor therapeutic agent. This was the first evidence that infection itself may mediate antitumor effects.7 It was later discovered by Shear and Turner that LPS was the component of Coley’s toxin that accounts for its antineoplastic effects.8 As LPS is the ligand for TLR4, these observations indicate that Coley’s toxin may activate TLR4. Since then, other microbe-derived therapeutics with anti tumor activity

Figure 2. MYD88-dependent pathway.
chemoresistance of epithelial ovarian cancer cells.\textsuperscript{77} In human prostate cancer cell lines, TLR4 expression levels correlated positively with metastatic potential.\textsuperscript{14} Similar results were obtained in the setting of head and neck squamous cell carcinoma, as the intensity of TLR4 expression correlates with tumor grade.\textsuperscript{73} Finally, C3H/HeJ mice with a functional mutation of the \textit{Tlr4} gene developed more aggressive skin cancers than their wild-type counterparts in response to 7,12-dimethylbenz[a]anthracene (DMBA).\textsuperscript{74}

**TLR4 and Breast Cancer**

The relationship between TLR4 and breast cancer has been studied from different points of view, yielding several interesting findings. At the cellular level, the migration, invasion, and angiogenic attitude of breast cancer cells at secondary sites increase after the systemic administration of LPS.\textsuperscript{9} The intraperitoneal injection of LPS into BALB/c mice bearing 4T1 cell-derived metastatic breast adenocarcinomas promoted angiogenesis both in vivo and in vitro.\textsuperscript{18} Moreover, the activation of TLR4 on metastatic breast cancer cells has been reported to regulate the expression of integrin $\alpha_v\beta_3$, TPM1 and maspin, and hence to promote the $\alpha_v\beta_3$-mediated adhesion and invasiveness of cancer cells.\textsuperscript{75} Finally, TLR4 signaling appears to increase the expression of miR-21 in breast cancer cells by activating NFκB. Therefore, breast cancer cells may acquire a high metastatic potential upon the TLR4-elicited activation of NFκB.\textsuperscript{75} In the breast tumor microenvironment, $\sim$20% of mononuclear inflammatory cells express TLR4, and the expression levels of TLR3, TLR4 and TLR9 have been proposed as indicators of tumor aggressiveness.\textsuperscript{76}

In the human breast cancer cell line MDA-MB-231, TLR4 was found to be expressed at higher levels than any other TLR. The knockdown of TLR4 resulted in a dramatic reduction of the viability of these cells as well as of IL-6 and IL-8 secretion. This study demonstrated that the knockdown of TLR4 may actively inhibit the survival and proliferation of breast cancer cells.\textsuperscript{77}

In the setting of adjuvant therapies, it has shown that tumor cell death as triggered by chemotherapy or radiotherapy initiates an immune response that contributes to therapeutic success. In this context, the interaction of HMGB1 released from dying tumor cells with TLR4 on DCs is required for the cross-presentation of tumor antigens and the activation of tumor specific cytotoxic T-cell responses.\textsuperscript{78}

Polymorphisms in the gene that encodes TLR4 have recently attracted great interest. Indeed, breast cancer patients harboring the loss-of-function Asp299Gly polymorphism of TLR4 relapse earlier upon anthracycline-based chemotherapy as compared with patients carrying functional TLR4.\textsuperscript{78} When 261 patients and 480 health individuals were investigated for the allelic frequencies of two polymorphisms causing amino acid substitutions in TLR4 (i.e., Asp299Gly and Thr399Ile), it was found that the Asp299Gly polymorphism may confer an increased susceptibility to breast cancer development.\textsuperscript{79}

Recently, we observed a strong link between TLR4 expression on both immune and neoplastic cells and the development
of breast cancer. Our data support the concept that TLR4 plays a significant role in breast cancer progression and metastasis. In particular, we have demonstrated that, on one hand, TLR4 prevents cancer progression and metastasis by operating within the host immune system, whereas, on the other hand, it promoting tumor metabolism when expressed by cancer cells.20

Conclusions

Multiple links between TLR4 and breast cancer have been identified. In particular, it has been shown that TLR4 plays important roles in the migration, invasiveness, and angiogenic potential of cancer cells at primary site or metastatic locations. TLR4 expression is abundantly expressed by both cancer cells and immune cells in the tumor microenvironment. TLR4 has been shown to exert a key role in the presentation of antigens from cancer cells succumbing to chemotherapy and radiotherapy. Moreover, TLR4 polymorphisms may influence the susceptibility of individuals to breast cancer development and/or recurrence. Finally, targeting TLR4 in breast cancer cells has been shown to reduce their metastatic potential. Taken together, these observations suggest that TLR4 is a critical player in breast cancer that warrants further investigation for the development of novel therapeutic modalities.

Disclosure of Potential Conflicts of Interest

The authors declare they have no competing interests or other interests that might be perceived to influence the contents of this paper.

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