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TLR4-Induced Inflammation Is a Key Promoter of Tumor Growth, Vascularization, and Metastasis

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Abstract

Toll-like receptor-4 (TLR4) is a powerful pathway best known for inducing inflammation in response to bacteria-produced lipopolysaccharide. TLR4 is also activated by endogenous ligands produced by host-damaged cells and a chemo-drug paclitaxel. Under normal conditions, TLR4 is expressed mainly in macrophages and, at a lower level, in epithelial, endothelial, and stromal cells. Activated TLR4 significantly increases inflammatory cytokines and enhances cell proliferation, migration, invasion, and survival. While these functions in normal cells are essential for host defense and tissue repair, TLR4 overexpression in malignant cells promotes tumor growth and metastasis. This is because pro-oncogenic effects of activated TLR4 in tumor cells are amplified by similar event in TLR4-positive tumor-associated cells including endothelial cells and their mobilized progenitors. The collective activation of multiple cell types within the tumor promotes chemoresistance and metastasis. Here, we summarize the current knowledge of the TLR4 pathway and its functional outcomes in normal and tumor cells. We also discuss its underappreciated role in supporting tumor progression through vascular activation and recruitment of endothelial progenitors. The review considers several open questions regarding the impact of TLR4-mediated pro- and antitumor effects, structural requirements for recognition of the TLR4 complex, and a potential contribution of chemotherapy to tumor spread.

Keywords: Toll-like receptor-4, paclitaxel chemotherapy, tumor growth, tumor blood and lymphatic vessels, metastasis

1. Introduction

Human Toll-like receptor-4 (TLR4) was discovered in 1997 through a bioinformatics approach based on the homology of similar Toll protein found in *Drosophila* [1, 2]. *Drosophila* Toll protein-mediated protection against fungus infection was the first clue suggesting the role of mammalian TLR4 in activation of the immune system. The complex of TLR4 and its co-receptors is the primary sensor and responder to lipopolysaccharides (LPS), the major constituents of membranes of Gram-negative bacteria [3]. Based on exquisite sensitivity to LPS and high level of TLR4 expression in macrophages, it was initially thought that the main function of this protein is restricted to an inflammatory response aimed at eradication of microbial pathogens.

Subsequent studies, however, discovered intricate interactions of TLR4 with multitude of other molecules besides LPS suggesting a much broader role in homeostasis, tissue repair, and longevity in addition to the immune defense. These functions of TLR4 directly relate to cancer initiation, progression, and response to therapy.

2. Basic structure of TLR4 gene and protein

Human TLR4 gene contains four exons, and it is located in the long arm of chromosome 9 [4]. TLR4 is Type-1 transmembrane protein belonging to the family of Toll-like receptors consisting of 10 members in humans and 12 proteins in mice [5]. TLR4 protein is composed of an extracellular domain, a C-terminal leucine-rich repeat (LRR) domain, and a single transmembrane sequence connected to an intracellular Toll/IL-1 receptor (TIR) region that conveys signaling [5]. LRR and TIR domains are responsible for ligand recognition and signal transduction, respectively [6].

3. TLR4 expression in normal organs

The main cell types that express TLR4 under normal conditions are cells of innate immunity including monocytes [7], macrophages [8], neutrophils [9], and dendritic cells [10]. In addition, TLR4 is also found in T cells [11] and B lymphocytes [12] albeit at lower level. Osteoclasts, macrophage-like cells in the bone marrow, also express TLR4 [13]. Embryonic stem cells [14] as well as adult hematopoietic-myeloid [15], lymphoid [15], mesenchymal [16], blood vascular endothelial [17], and lymphatic endothelial [18] progenitors all express TLR4 and signaling components of this pathway. Among non-hematopoietic cells, TLR4 is expressed in blood vascular [19] and lymphatic [20] endothelia as well as at low concentration in fibroblasts [21], keratinocytes [22], and epithelial cells of most normal organs including the colon [23], intestine [24], ovary [25], kidney [26], and lungs [27]. Professional phagocytes and their myeloid precursors express the greatest amount of TLR4 and exhibit the most pronounced response to its ligands [28]. The level and composition of TLR4 signal-transducing molecules vary among different cell types, particularly between myeloid and epithelial cells [29]. However, all positive cells share the basic traits of this pathway such as responsiveness to LPS reflected by activation of transcription factors of the NF- κ B family and production of inflammatory cytokines. During inflammation, TLR4-expressing normal cells significantly increase secretion of inflammatory mediators [25, 30] due to co-upregulated TLR4 [31], its intracellular adapters [32], and co-receptors [10] which enhances cooperation among the pathway's components. Likewise, malignant TLR4-positive cells express much higher levels of inflammatory proteins as compared with their normal counterparts [33, 34].

4. Physiological functions of TLR4 in normal organs

In order to understand the full impact of TLR4 on cancer progression, it is useful to be familiar with its physiological role in normal organs. The main function of TLR4 in normal cells is restoration of tissue homeostasis perturbed by either pathogens or sterile injury. Response to pathogens is dictated by its ability to recognize and respond to LPS [3] and other microbial [35] and non-bacterial [36] lipids. The bacterial lipid-containing products are collectively called pattern-associated molecular patterns (PAMPs). Recognition of PAMPs triggers upregulation of inflammatory proteins that mediate proliferation [37], chemotactic migration [38], and survival

[39] of innate immunity cells, all of which is necessary for their division, mobilization to infected tissue, and cytoprotection against bacterial toxins and stresses of inflamed environment. LPS also induces differentiation of myeloid progenitors resulting in rapid maturation of dendritic cells and their superior antigen presentation to T lymphocytes necessary for eradication of invading pathogens [40].

Another broad panel of TLR4-activating molecules are called damage-associated molecular patterns or DAMPs. These molecules are produced by severely damaged or dying cells [41, 42]. The TLR4 responsiveness to this class of activators creates regenerative environment that helps to repair the injured tissue by replacing its lost or damaged components. This function of TLR4 is equally critical for long-term survival of the entire organism as the ability to generate pathogen-destroying cells. Therefore, in this situation TLR4 activates local and bone marrow (BM)-derived myeloid (BMDM) progenitors wired to differentiate the required cell types that replenish the damaged tissue. Not surprisingly, TLR4-expressing macrophages derived from BMDM progenitors dominate the late stages of wound healing acting to restore post-injury homeostasis, including rebuilding of functional vasculature [43]. In parallel to systemic effect, local epithelial and stromal cells activated by TLR4 produce copious amounts of chemotactic factors to recruit these progenitors while also being induced to divide, migrate, and re-populate the wounded area.

Given the TLR4 role in homeostasis, it is not surprising that it activates and induces differentiation of BM-derived progenitors from myeloid and lymphoid lineages [15], mesenchymal stem cells [16], blood vascular endothelial progenitors [17], lymphatic endothelial progenitors [18], and local tissue epithelial stem cells [14]. Chemokines recruiting these progenitors are produced by local stroma and epithelium as demonstrated by TLR4-dependent upregulation of a variety of cytokines in inflamed fibroblasts [42], hepatic stellate cells [44], as well as intestinal [45], alveolar [27], ovarian [25], and renal [26] epithelial cells. In addition to production of chemokines, TLR4 activation of epithelial cells increases their division [46] and survival [47] similarly to its effects on immune and hematopoietic cells described above. Tissue infiltration by immune cells and BM progenitors is facilitated by increased vascular permeability [48, 49] and upregulation of cell adhesion proteins that facilitate leukocyte-endothelial interactions [50]. These two major events permitting recruitment of BM-regenerating cells are induced by TLR4 activation of blood vascular endothelium [51] and vessel-supporting pericytes [52]. Lastly, TLR4 activation of lymphatic endothelial cells [20, 53] is essential for collection of tissue-mobilized macrophages from the inflamed tissue followed by their transportation to locoregional lymph nodes. Lymphatic-mediated removal of macrophages and dendritic cells from the affected site helps in resolving the inflammation and mounting the adaptive immune response through antigen presentation to T cells in the local nodes. Simultaneous activation of TLR4 in epithelium, stroma, endothelium, BM, and immune organs ensures tissue recovery in a coordinated and timely manner. TLR4 plays a central role in this process by coordinating epithelial cytokine release and activation, production of new cells in the BM, their recruitment to injured tissue, and resolution of residual inflammation that collectively promote tissue repair [54, 47]. While all these processes are essential for homeostasis, similar TLR4 activities in the context of cancer result in devastating outcomes including protection of tumor cells against anticancer drugs and their improved ability to generate and invade newly formed vessels.

5. TLR4 expression in malignant tumors

Although TLR4 expression was initially thought to be restricted to hematopoietic cells, subsequent studies discovered functional TLR4 in a variety of normal epithelial

Tumor type	No. patients	Detection method	Comments	P-value	Ref
Various solid tumors	1,294	Meta analysis	Data from 15 independent studies show high correlation between TLR4 expression and poor disease-free and overall survival	0.001 and 0.05	[81]
Breast	665	RT-PCR	Significantly shorter survival of patients with TLR4 polymorphisms	0.006	[82]
Breast	50	IHC	High TLR4 expression in tumor cells significantly correlated with lymph node metastasis	0.022	[76]
Breast	74	IHC	High TLR4 expression in infiltrating mononuclear cells highly correlated with lymphatic metastasis and recurrence	0.0001	[79]
Breast	120	IHC	Elevated TLR4 expression was strongly associated with tumor size and lymphatic metastasis	0.05	[83]
Prostate	133	ELISA	High TLR4 expression correlated with elevated PSA after ablation, an event defined as biochemical recurrence	0.05	[84]
Ovarian	109	RT-PCR and IHC	High expression of TLR4 significantly correlated with worse of overall and disease-free survival	0.01	[85]
Non-small cell lung cancer	126	IHC	Elevated TLR4 expression was strongly associated with TNM stage and lymphatic metastasis	0.001	[86]
Ovarian	57	IHC	TLR4 was detected in 46.3% of cases. Co-expression of TLR4 and its adaptor MyD88 was highly associated with advanced tumor stage	0.05	[87]
Colorectal	108	IHC	High expression of TLR4 strongly correlated with shorter overall survival	0.0001	[77]
Colon	53	IHC	High expression of TLR4 strongly correlated with disease progression	0.05	[73]
Hepatic	106	IHC	TLR4 was expressed in 86% of tumor specimens and significantly correlated with tumor size	0.01	[64]
Pancreatic	30	RT-PCR and IHC	TLR4 was expressed in 69.2% of tumor specimens and significantly correlated with tumor size, vascular invasion and lymphatic metastasis	0.001	[72]
Esophageal	87	RT-PCR and IHC	TLR4 expression in mononuclear cells was detected in 48.3% of specimens and significantly correlated with lymphatic metastasis	0.05	[88]

Tumor type	No. patients	Detection method	Comments	P-value	Ref
Melanoma	35	IHC	TLR4 expression in mononuclear cells significantly correlated with shorter relapse-free survival	0.001	[80]

Table 1.
Correlation of TLR4 expression with tumor growth, metastasis and poor patient survival in clinical human cancers.

cells [25, 26]. Malignant transformation amplifies TLR4 expression while typically preserving its functionality [55]. In fact, constitutive expression of epithelial TLR4 might contribute to development of cancer if persistently activated by pathogenic and environmental ligands which leads to vicious cycles of self-propagating inflammation. For instance, TLR4 interaction with *H. pylori* products in normal gastric epithelium might lead to chronic gastritis and progression into gastric intestinal metaplasia [56]. Analysis of cell lines from other organs also consistently showed substantial upregulation of TLR4 in tumor cells compared with their normal counterparts. Protein, mRNA, and functional activity of the TLR4 pathway have been demonstrated in tumor lines derived from many human epithelial lines including breast [33, 57], ovarian [58, 59], lung [60], prostate [61, 62], head and neck [63], hepatic [64], gastric [65], and pancreatic [66] cancers. It is also expressed in melanoma [67], glioblastoma [68], and various lines derived from hematopoietic malignancies [69]. Mouse tumor cell lines from a variety of tissues replicate these findings [70, 71].

TLR4 expression is not only limited to cultured tumor cell lines but is also detected in malignant cells in clinical human cancers. Moreover, the majority of clinical studies showed that TLR4 expression strongly correlates with poor prognosis due to increased tumor size [72], stage [73], loss of differentiation [74], chemoresistance [73, 75], venous invasion [72], lymph node metastasis [72, 75–78], recurrence [79], and shorter patient survival [77, 80] (**Table 1**). A recently published meta-analysis examining data from 15 independent encompassing analyses of different tumors from 1294 patients found strong associations (P-values 0.001–0.05) between TLR4 expression and reduced overall and disease-free survival [81]. Collectively, these studies demonstrate high expression of TLR4 in a variety of human solid tumors suggesting it plays a prominent role in inflammation-fueled cancer progression and metastasis.

6. Signal transduction and intracellular pathways induced by TLR4

Given the emerging evidence for the TLR4 pro-tumorigenic role (**Table 1**), it is important to understand the basic steps in this pathway, its positive and negative molecular regulators, as well as biochemical requirements for intracellular signal transduction in both normal myeloid and malignant epithelial cells. The best understanding of this pathway is derived from the studies of macrophages activated by LPS. Initiation of a signal cascade is mediated through recruitment of adapters interacting with the TIR domain of TLR4. Upon ligand-induced oligomerization of TLR4, the TIR domain recruits myeloid differentiation factor-88 (MyD88) that forms heterodimers with Mal (MyD88 adapter-like) partner, a protein specific for TLR4 pathway [89]. Upon binding to TIR, MyD88-Mal complex recruits members of the IL-1R associated kinase (IRAK) family, IRAK1 and IRAK4 [90]. Once phosphorylated, IRAK1/4 dissociates from the complex and recruits TNF receptor associate factor-6 (TRAF6), an E3 ubiquitin ligase and a critical mediator of

TLR2 and TLR4 signaling [91]. Polyubiquitination mediated by TRAF6 activates MAP3 kinases and TGF-beta associated kinase (TAK1) [92]. MAP3K activation leads to stimulation of p38/JNK pathways, whereas TAK1 together with TAB1/2 kinases phosphorylates the inhibitor of cytosolic NF- κ B complex leading to release and nuclear translocation of NF- κ B p65/p50 heterodimers [92]. This initiates the canonical NF- κ B cascade resulting in transcription of a broad panel of inflammatory and pro-survival genes. This is amplified by genes transcribed by AP-1, CREB, and Sp1, factors activated through the parallel p38/JNK cascade.

Independently of MyD88 pathway, viruses and some TLR4 ligands also activate TIR domain-containing adapter inducing interferon-beta protein (TRIF) that is associated with TRIF-related adapter molecule (TRAM). TRIF also activates NF- κ B; however, the expression level and the composition of the downstream transcribed genes are distinct from the MyD88-dependent activation. For instance, TRIF specifically activates IRF3 factor that induces robust transcription of antiviral interferons necessary to fight viral infections as opposed to classic MyD88-NF- κ B targets such as TNF α and IL-6 that are predominantly upregulated after exposure to bacterial LPS.

7. Role of co-receptors in activation of TLR4

One persistent misconception in the cancer research literature is that TLR4 directly binds LPS and other activating ligands. However, it has been firmly established that TLR4, as a single protein, has very low affinity to LPS. Triggering the TLR4 pathway by LPS in monocytes and macrophages requires at least four co-receptors: myeloid differentiation protein-2 (MD2), CD14, and CD11b bound to CD18 (a complex of integrins known as Mac-1). Secreted MD2 binds to and stabilizes extracellular portion of TLR4 which creates a highly specific site for LPS recognition [93]. Intracellular MD2 also aids cytosolic trafficking of TLR4 from Golgi to the plasma membrane [94]. Physical interaction among MD2, LPS, and TLR4 has been shown in multiple elegant studies employing a combination of high-resolution structural analyses, phenotype comparison in genetically modified mice, and other rigorous approaches [95] that show a critical role of MD2 in LPS recognition by TLR4 in either mouse or human cells.

Likewise, the essential role of CD14 has been shown by physical binding of CD14 to lipid A portion of LPS using crystal structure analyses [35] and resonance energy transfer [96] as well as by hypo-responsiveness to LPS in mice lacking CD14 [97] or in the presence of CD14-blocking antibodies [7]. The function of CD14 is to accept a monomer of LPS from a blood-circulating LPS-binding protein and transfer it to a pre-made pocket in the MD2/TLR4 complex [98]. The absence of CD14 from TLR4-/MD2-positive cells results in a 1000-fold reduced sensitivity to LPS compared with cells expressing this protein [99]. The involvement of CD11b/CD18 in reactivity to LPS is somewhat less certain although some studies using a point mutation identified a specific LPS-binding site in CD18 integrin [100]. Additional studies showed lack of cytokine production in Mac-1-deficient mice [101] and other deficiencies in TLR4 signaling upon treatment with anti-CD11b or anti-CD18 antibodies in vivo [9]. While some studies suggested that optimal signaling requires LPS engagement with TLR4/MD2 complex and Mac-1, others found lesser requirements for CD11b/CD18 [102]. This issue, therefore, remains unresolved as well as the mechanisms of TLR4 signal transduction induced by various endogenous ligands dissimilar from LPS. Importantly, very few structural studies were performed in TLR4-expressing normal or tumor epithelial cells that might have entirely different requirements for TLR4 co-receptors and signal transduction than macrophages. Tumor epithelial cells are unlikely

to express myeloid-specific CD14 and CD11b and might not uniformly express MyD88, MD2, and other TLR4 pathway essential proteins. How exactly TLR4 transmits the multitude of functional effects in the epithelial cell context is largely unknown.

8. LPS is the most rigorously confirmed TLR4 agonist

The best-studied ligands of TLR4 are various LPS molecules expressed in Gram-negative bacteria. The basic structure of LPS includes a lipid A component with 4–6 fatty acyl chains, an oligosaccharide core, and covalently attached O-antigen polysaccharide side chain [103]. The presence of phosphates in the lipid A and oligosaccharides confers the highly negative charge to LPS enabling its reactivity with cationic bactericidal host molecules such as beta-defensins [104]. CD14 binds the lipid A portion of LPS leaving acyl chains exposed to an LPS-binding site on the TLR4/MD2 complex. The presence of six acyl chains (as opposed to five or four) confers the best potency in activating TLR4 [105], presumably because the four acyl chains perfectly fit the hydrophobic cavity in MD2 [106] and the other two chains support the complex and promote its oligomerization [107]. This structural requirement illustrates the exquisite specificity of the binding site in the TLR4/MD2 complex to LPS raising reservations about the potential suitability of this complex for binding alternative ligands [95]. A functional and some structural evidence exists demonstrating TLR4-dependent cell activation triggered by lipid-like molecules (e.g., lipoteichoic acid [108], minimally oxidized LDL [109], and free saturated fatty acid palmitate [110]) whose binding might be supported by a hydrophobic site on CD14. Among other proposed TLR4-activating ligands, some structural evidence exists for a chemotherapeutic drug paclitaxel (PXL) [95, 101] and endogenous ligands HMGB1 [111] and S100A8 [112]. At the present, mechanisms by which other functional TLR4 ligands induce signal transduction are largely unknown.

9. Chemotherapeutic drug paclitaxel as a TLR4 ligand and a promoter of tumor growth and metastasis

For cancer researchers, a TLR4 stimulating effect of PXL is of special significance. This is because PXL and the entire class of its derivatives known as taxanes are widely used as anticancer cytotoxic drugs against a variety of human malignancies [113]. Resistance, however, occurs frequently [114], and the underlying mechanisms are poorly understood. PXL, the active component of all taxanes, exerts its cytotoxic action via binding to microtubules leading to their over-stabilization which results in apoptotic death of dividing cells [115]. The pro-survival effects of activated TLR4 pathway can explain many attributes associated with PXL chemoresistance including activation of NF- κ B [116], phosphorylation of Bcl-2 and Bcl-XL [117], and resultant evasion of apoptosis [33, 58] in spite of continuous therapy.

Despite somewhat limited direct evidence confirming physical interactions of PXL with the TLR4 complex, there is overwhelming functional evidence for PXL activation of the TLR4 pathway in malignant human and mouse cells (**Table 2**). The early studies demonstrated that wild-type (WT) mice with functional TLR4 injected with PXL upregulated nearly an identical panel of inflammatory cytokines as those injected with LPS [3]. That was in sharp contrast to myeloid cells from TLR4-deficient mice that failed to respond to either molecule [118]. Mice lacking MyD88, the major intracellular adapter of TLR4, also did not respond to LPS

Tumor type	Specie	Study type	Taxane-mediated effects	Ref
Breast and ovarian	Human	In vitro	PXL induced degradation of IkappaB alpha followed by activation of NF-κB and MAPK	[126]
Renal	Human	In vitro	PXL activated ERK1/2 and induced Bcl-2 and EGFR	[127]
Breast	Human	In vitro	Only PXL-responsive tumor lines showed marked increase in AP-1 and NF-κB binding to IL-8 promoter	[128]
Breast	Human	In vitro & In vivo	Curcumin, an inhibitor of NF-κB, blocked PXL-dependent upregulation of pro-survival, proliferative and angiogenic proteins including MMP9 & VEGF-A	[129]
Prostate	Human	In vitro	Docetaxel increased tumor cell survival by activating PI3K/AKT pathway and TLR4 silencing reduced this effect	[61]
Breast	Human	Tumor xenografts in mice	PXL therapy induced NF-κB, Bcl-2, and VEGF-A, and anti-VEGF-A antibody blocked this effect	[130]
Various	Human	In vitro	In a cell-based screening of 1,280 compounds, PXL was identified as one of the most potent inducers of invasion	[131]
Non-small cell lung carcinoma	Human	Clinical study	Pre-operative taxane therapy increased COX2, PGE2 and microvessel density in patients	[132]
Breast	Human	In vitro & models in vivo	PXL upregulated cytokines mainly in TLR4-positive tumor lines; an anti-TLR4 antibody blocked this effect	[33]
Breast	Human	Clinical study	Taxane chemotherapy increased circulating angiogenic factors and endothelial cell progenitors in patients	[133]
Breast	Human & Mouse	Tumor models in mice	Taxane chemotherapy increased circulating CSF1 and tumor homing of BM pro-vascular myeloid cells	[134]
Breast	Human	Clinical study	Neoadjuvant taxane therapy increased angiogenesis in patients, possibly through VEGF-A and Notch pathways	[135]
Breast	Human	Tumor xenografts in mice	TLR4-positive tumors responded to PXL by increasing cytokines, homing of myeloid pro-vascular cells, lymphangiogenesis, and metastasis to lymph nodes	[136]
Breast	Human & Mouse	In vitro	PXL induced differentiation of lymphatic endothelial progenitors in TLR4-positive BM myeloid precursors	[18]
Breast	Human & Mouse	Tumor models in vivo	Docetaxel increased tumor lymphatic formation and metastasis to lymph nodes	[137]

Table 2.
Pro-oncogenic Effects of Paclitaxel and Docetaxel (selected list)

or PXL [119]. Treatment with PXL faithfully reproduced all known events in the LPS-inducing cascade including dual activation of MyD88 and TRIF pathways [58, 119–121]; phosphorylation of IRAK1 and IRAK4 [58, 122]; NF-κB activation

[33, 118, 122]; signaling through PI3K/pAKT [61, 121, 123] and activation of MAP kinases [119], p38 [124], JNK [123], ERK1/2 [33, 125], AP-1, and STAT3 [116]; and transcription of inflammatory genes and pro-survival factors [33, 118].

PXL-induced upregulation of inflammatory, prometotic, and pro-survival genes is evidenced by transcriptional analyses of drug-treated human cancer lines from breast [33, 122, 136], ovarian [58, 121], prostate [61], lung [116, 138], and melanoma [139] origins. Upregulation of these genes is evidenced as early as 6 hours posttreatment [138] which eliminates the possibility that this is a secondary effect resulting from cell death. Inhibition of IRAK1, an intracellular kinase whose phosphorylation is required for TLR4-dependent activation of NF- κ B [90], in breast cancer lines suppressed an inflammatory response to PXL concomitant with decreased chemoresistance in vivo [122]. Correlation with chemoresistance was also recorded in an independent study that employed two different models of isogenic TLR4-negative and TLR4-positive lines [33, 136]. Mediation of PXL signaling through TLR4 is also suggested by studies with TAK-242 (also known as CLI-095), a TLR4-specific inhibitor that blocks interactions between the TIR domain and intracellular adapters [140]. Breast carcinoma cells pre-treated with TAK-242 reduced PXL-induced expression of inflammatory cytokines by nearly 90% [33]. Multiple studies showed that PXL effects on tumor epithelial or immune cells are largely TLR4-dependent since silencing TLR4 by siRNAs or blocking its actions by specific antibodies or sequence-based peptide inhibitors significantly reduced both NF- κ B activation and subsequent molecular (e.g., cytokine upregulation) and cellular (e.g., division and migration) activities [33, 61, 121].

While the notion of PXL sharing the TLR4 pathway with LPS is generally accepted, the molecular basis for physical TLR4 recognition by PXL is still a matter of debate, particularly in the context of nonmyeloid cells. CD14 was reported to play a minimal role in transmission of PXL signals [141], and it is typically absent in normal and tumor epithelial cells. PXL was reported to bind to CD18 [101], but the partner for the Mac-1 complex, CD11b, is typically absent from non-hematopoietic cells such as tumor cells from epithelial malignancies. The requirement for MD2 turned out to be particularly contentious as several studies reported an exclusive specificity of mouse but not human MD2 in supporting PXL-induced signaling [142, 143]. The implication would be that PXL affects only cells from the mouse specie, but this contradicts extensive evidence demonstrating functional LPS-mimetic effects of PXL on human tumor cells (**Table 2**) and normal monocytes [18]. One group found that PXL does bind to human TLR4/MD2 complex but acts as an antagonist [143]. However, this is inconsistent with increased migration, invasion, and other activities of human tumor cells [62, 144] as well as with direct stimulating effects on multiple signaling pathways [122]. It should be noted that two studies that identified PXL as an inhibitor of TLR4 were performed in HEK-293 cells, a human embryonic kidney line immortalized by adenovirus A5 which caused substantial deviations from the epithelial phenotype including expression of neuron-specific genes [145]. HEK-293 cell line was very useful for identification of structural components of the TLR4 complex but might not necessarily reflect the biology of human tumor cells due to differences in organ origin (many responders are derived from tissues other than kidney), stemness status (embryonic vs. adult), and unique genetic changes caused by virus. The latter could lead to significant differences in expression of TLR4 co-receptors and other regulatory proteins. In general, the majority of structural studies with PXL have been performed with either HEK-293 or macrophages. It therefore remains to be established whether the same mechanisms control PXL interactions with the TLR4 complex in human epithelial neoplastic cells.

10. Endogenous ligands of TLR4 released from damaged and necrotic cells

The class of endogenous ligands comprises more than 30 molecules that share no structural resemblance within the members of the group and either LPS or PXL. The list of proposed endogenous TLR4 ligands includes a variety of structurally dissimilar molecules ranging from high-mobility group box-1 (HMGB1) [146] to S100A8 [27, 147] and fibronectin [148]. Alternative names for endogenous ligands are DAMPs mentioned above [41], danger factors [42], and alarmins [149]. The current concept suggests that DAMPs normally segregated into cytosol or intracellular compartments are secreted upon exposure to inflammatory cytokines [146] or passively released from damaged or necrotic cells [71]. Soluble DAMPs then activate receptors of innate immunity such as TLR2, TLR4, and RAGE that are charged not only with protection against pathogens but also with restoration of homeostasis [41]. This is an appealing concept that fits well with well-established activation of TLR4 during sterile injury [150] and remodeling of wounded tissue or tumor [151]. It might also explain activation of TLR4 in cancers developed at sterile anatomic sites and, particularly, during cytotoxic therapy that produces massive cell death.

DAMP-mediated activation of the TLR4 pathway in tumor cells has been shown by numerous independent studies using genetically modified mice with absent or mutated TLR4 [152], blocking anti-TLR4 antibodies [153], specific inhibitors [152], and gain- of-function or loss-of-function approaches [153]. For instance, anti-TLR4 antibody reduced HMGB1-induced proliferation of mouse lung carcinoma cells [154]. HMGB1-dependent recruitment of c-Kit⁺ progenitors [155], angiogenesis [156], and lymphangiogenesis [157] was significantly reduced in TLR4 ^{-/-} non-tumor-bearing mice suggesting similar outcomes in the presence of tumor. HMGB1-induced angiogenesis was also shown in study with UV-damaged keratinocytes in which the released factor increased inflammation, cell proliferation, and migration of melanoma cell toward the endothelial monolayer [144]. All effects were reduced in TLR4- and MyD88-deficient mice as well as in the presence of TAK-242, a specific peptide inhibitor of TLR4 signal transduction [144]. Consistent with this finding, HMGB1 released from necrotic skin cells enhanced inflammation and recruitment of BM myeloid cells and promoted tumor formation, all of which were reduced in TLR4 null mice [158]. Overexpression of HMGB1 in hepatic carcinoma correlated with tumor invasion, and knockdown of this protein suppressed metastasis [159]. Chemotherapy that produces massive necrosis and hence release of HMGB1 and other pro-inflammatory intracellular factors was shown to enhance tumor relapse and metastasis in a model of colon cancer [71]. A variety of tumor- and metastasis-promoting effects have also been reported for other endogenous TLR4 ligands including S100A8/A9 [152], SAA3 [147], hyaluronic acid [153], heat-shock proteins [68, 160], and peroxiredoxin-1 [161].

While the existing functional evidence strongly supports DAMP activation of the TLR4 pathway, the question remains whether these factors are truly ligands for TLR4 or ancillary molecules that stabilize its membrane complex or potentiate intracellular signaling. This uncertainty stems from structural dissimilarity between LPS and many TLR4-activating molecules; a potential risk by LPS contamination of recombinant factors produced in Gram-negative bacteria [162]; and lack of clear structural evidence for physical interaction with TLR4 or MD2 proteins for some ligands [95]. That said, binding of mammalian cell-produced, endotoxin-free SAA3 and S100A8 to TLR4/MD2 complex was shown by surface plasmon resonance [163], and binding of HMGB1 to the same complex was demonstrated by a point mutation in Cys106 that severely reduced the HMGB1 capacity to activate TLR4 [111]. Determination of other ligands as binding partners, amplifiers [164], or assistants might need to be resolved in the future studies.

11. Cellular and molecular consequences of TLR4 activation in the context of cancer

TLR4 is a powerful pathway that simultaneously enhances several key cell functions including differentiation, proliferation, migration, invasion, and survival. On molecular level, the hallmark of TLR4 stimulation is strong upregulation of inflammatory cytokines that act as autocrine and paracrine activators of the first cell responders to TLR4 ligands and nearby stroma, respectively, as well as systemic alert signals for immune organs. In tumors, parallel TLR4 activation of neoplastic and immune cells often results in “double-edge sword” effects [165] due to conflicting functional implications for each cell type. An antitumor effect of TLR4 activation of dendritic cells (DC) is evident by enhanced maturation [166], migration [167], improved antigen presentation [168], better activation of cytotoxic T cells [168], and increased tumor cell death [169]. However, induction of similar pro-mitotic, survival, and migratory functions in malignant and tumor-associated cells has a pro-tumorigenic effect. TLR4 agonists are often proposed to be used clinically for enhancing antitumor therapy [170]; however, it is worth considering both local and systemic consequences of TLR4 activation. The effects on host immune and hematopoietic cells are not straightforward as improved DC functions are counterbalanced by increased generation of myeloid-derived suppressor cells [171] that inhibit antitumor responses. TLR4 activation of BM immature myeloid cells leads to generation of provascular progenitors [17, 18] that increase tumor vessel formation and metastasis [136, 172]. Lung recruitment of the host BM-derived myeloid cells by TLR4 ligands SAA3 in combination with S100A8/A9 has been shown to create a pulmonary pre-metastatic niche, thus ensuring successful establishment of tumor lesions in distant organs [147]. Likewise, TLR4 activation of T cells may enhance their immunosuppressive effects [173] rather than anticancer activities. In our studies performed in tumor-bearing immunocompetent and immunodeficient mice, we often observe a transiently reduced growth upon injection of TLR4-differentiated myeloid progenitors. However, tumor growth resumes at later stages along with substantial increase in lymph node metastasis which correlates with injection of myeloid-derived progenitors [18]. These studies suggest that development of either TLR4-suppressing or TLR4-activating therapeutic strategies should take into account the impact of modulators on all TLR4-positive cells at both tumor and systemic organs.

12. Direct functional effects of TLR4 on tumor cells

Most solid tumors that originated from epithelial, neuronal, and skin cells do not exhibit the same level of responsiveness to LPS as myeloid cells. Nevertheless, functionally, TLR4 activation of tumor cells largely reproduces the known effects in cells of innate immunity. On molecular level, this includes MyD88-dependent activation of NF- κ B [33, 71, 174], MAPK [75], PI3K/AKT [61, 175], ERK1/2 [176], c-Jun [69], p38 [75, 176, 177], and other pathways that collectively upregulate inflammatory cytokines [174, 178], metalloproteases [179], and pro-survival factors [59, 180]. Among multiple LPS-induced cytokines in cancer cells are particularly important angiogenic factor VEGF-A [55, 60, 181], immunosuppressive TGF-beta [60, 182], and a variety of chemokines recruiting BM-derived myeloid cells [34, 178] that promote tumor progression through their own mechanisms. On a cellular level, TLR4 activation increases tumor cell proliferation [69, 177, 183], migration [64, 175], invasion [159], and survival [58, 176, 184] that collectively results in resistance to therapy [58, 61, 69, 136, 183]. Some studies also reported

increased stemness due to expansion of cancer stem cells [153, 183]. Additional effects include induction of epithelial-mesenchymal transition (EMT) [66, 180] and evasion of immunosurveillance [183], both of which might reflect TLR4-activated macrophage properties enabling tissue repair and resilience against pathogen-produced toxins. Undoubtedly, combination of these effects profoundly impacts tumor growth, chemoresistance, and metastasis.

While it is not possible to discuss all relevant reports due to very extensive literature on the subject, it is worthwhile to highlight several general points. First, the majority of reports showed a significant increase in oncogenic and metastatic potential of tumor cells treated with TLR4 ligands, whereas a minority described an opposite effect [185]. This suggests that in most situations, although both pro- and antitumor effects are induced by TLR4 signaling, the former might be prevailing over the latter. Second, the pro-oncogenic effects have been observed across the entire tumor source spectrum including breast [136, 174, 175], ovarian [58, 121], prostate [61, 62, 184], lung [55, 70], pancreatic [186], colon [176, 180], colorectal [75, 78], and hepatocellular [159, 177] carcinoma lines as well as glioma [183], myeloma [69], and melanoma [144] cells. This indicates a widespread role of TLR4 signaling in human solid cancers which should be considered by clinical therapeutic strategies. Third, TLR4 pathway activated by PXL [33, 58, 62], LPS [175, 184], or other ligands [154] equally affects mouse [178] and human [33, 58] cancer cells. This point is important not only because of the widespread use of mice for modeling human cancers but also because of a lingering debate whether PXL-dependent activation is restricted to mouse cells [142, 187]. Evidence from multiple studies demonstrating PXL-dependent activation of human tumor cells (**Table 2**; reviewed in [188]) indicates that this is not a case. Lastly, mediation through the TLR4 protein by PXL and endogenous ligands has been shown by many approaches including RNA interference [61, 62], anti-TLR4 blocking antibodies [33, 189], TLR4-specific inhibitors [33, 144], as well as use of TLR4^{-/-} mice [30, 144, 158] and isogenic tumor lines with differential TLR4 expression [33]. Although the studies of alternative TLR4 ligands would still benefit from stronger structural evidence for direct recognition of the TLR4 complex, the combined functional evidence derived from multiple independent studies cannot be dismissed. The currently available data show mainly a pro-tumorigenic impact of TLR4 expressed in human and mouse cancer cells resulting from exposure to LPS or TLR4 alternative ligands.

13. Importance of TLR4-induced autocrine loops in normal myeloid cell physiology and tumor pathology

One important function of the TLR4 pathway in macrophages during pathogenic invasion is to amplify the signaling to hasten proliferation and survival of resident macrophages as well as recruitment of BM-derived myeloid cells to the septic site. Macrophages effectively achieve this goal by establishing autocrine loops through coincided upregulation of secreted cytokines and corresponding membrane-inserted receptors. This positive reinforcement ensures sustained expression of downstream targets necessary for prolonged survival, resilience, and heightened activities of immune cells. For instance, LPS-upregulated IL-1 and IL-18 are co-expressed with their receptors that signal through the MyD88 pathway in addition to TLR4 [190] which doubles the outcomes of the combined signaling [191]. LPS-induced co-expression of IL-8, TNF alpha, and other cytokines with their receptors was reported to amplify macrophage functions by enhancing activation of NF- κ B [39] and STAT1 [192]. This pattern is mimicked by TLR4-positive tumor cells

activated by either LPS [178] or PXL [33]. In our study with TLR4-expressing breast carcinoma lines, we observed that PXL-induced transcription of multiple cytokines was coordinated with upregulation of matching receptors evident by parallel elevation of CXCL2/CXCR2, CCL20/CCR6, and CSF1/CSF1R pairs [33]. Similarly to positive reinforcement in activated macrophages, signaling through these additional pathways in tumor cells significantly increased expression of pro-survival proteins such as pAKT and ERK1/2 [33]. These findings highlight a cytoprotective program naturally induced by TLR4 in macrophages and reproduced by tumor cells that might represent a key mechanism underlying tumor evasion of apoptosis and resistance to therapy.

14. Indirect pro-oncogenic effects of TLR4 mediated by cells in the tumor microenvironment (TME)

TME has profound effects on tumor progression [193, 194]. Some of the pro-oncogenic and pro-metastatic effects of TLR4 are mediated by TME cells such as macrophages, fibroblasts, smooth muscle cells, and pericytes as well as endothelium lining blood and lymphatic vessels. Macrophages are natural responders to TLR4 ligands having the highest expression of this receptor. Under inflammatory conditions, TLR4 is further upregulated by LPS [31] and cytokines [195] through positive feedback loops. Tumor-associated macrophages (TAMs) are well-known promoters of metastasis through secretion of growth-promoting cytokines, proteases [196], and immunosuppressive factors [197]. TAMs are also significant source for lymphangiogenic factors that increase lymphatic density and metastasis [198]. Importantly, TAMs from TLR4-deficient mice have a significantly reduced capacity to activate NF- κ B leading to deficient production of angiogenic and inflammatory factors that promote tumor growth [55].

Cancer-associated fibroblasts (CAFs) are another cell type found in most solid tumors but particularly pronounced in pancreatic [199], colorectal [200], and breast [201] cancers. Inflamed human and mouse fibroblasts express TLR4/MD2 complex and respond to TLR4 ligands [202]. Deletion of TLR4 prevents fibrosis in vivo [202] suggesting that CAF-expressed TLR4 might play a non-redundant role in tumor pathology. This is, indeed, supported by several studies. TLR4 expression in CAFs in human colorectal cancer was associated with high recurrence rate and poor patient survival [200]. CAFs associated with breast cancer were identified as a main source of HMGB1 that activated neighboring TLR4-positive tumor cells [203]. Functional TLR4 was also found to be expressed in another TME component, tumor-recruited mesenchymal stem cells (MSC), which was evidenced by LPS-activated NF- κ B, PI3K and IRF1, and upregulation of downstream cytokines [204]. High level of TLR4 was also detected in human pericytes that are ontogenically related to MSC [52]. When activated by either LPS or HMGB1, pericytes upregulated classic NF- κ B genes including cytokines and cell adhesion molecules (CAMs) such as VCAM-1 and ICAM-1. The latter greatly increase leukocyte adhesion to pericyte monolayer [52] which might play a key role in transmigration of blood-circulating immune cells and hematopoietic progenitors through the vascular barrier and infiltration of the tumor interstitium. Tumor-recruited cells harbor BM-derived myeloid-suppressive cells that promote immuno-evasion [205] as well as provascular progenitors that expand tumor vasculature [206, 207]. Both of these TME populations are known to advance tumor progression. Collectively, these studies illustrate a prominent role of TLR4 signaling in cross-talk of various TME compartments that propagate circuits supporting tumor-associated and malignant cells.

15. Role of TLR4 in tumor angiogenesis and lymphangiogenesis

Although majority of studies have been focused on myeloid cells, it is difficult to overstate the functional impact of the TLR4 pathway on vasculature. Blood vessels are the first responders to circulating septic molecules, and lymphatic vessels, among other functions, help mounting the adaptive immune response by collecting pathogenic antigens from the infected tissue and delivering them to regional lymph nodes. Blood vascular endothelial cells (BEC) and lymphatic endothelial cells (LEC) from human [19, 208] and mouse [50] origins express TLR4 and are naturally equipped to sense and respond to TLR4 ligands [49, 209]. Endothelial cells (EC) from both large [208] and microvessels [19] express TLR4 and accessory molecules for ligand recognition [32] and intracellular signaling [49, 209, 210]. The TLR4 specificity of endothelial response to LPS and other ligands has been documented using mice with deleted receptor [50], ectopic overexpression of nonfunctional protein [19], siRNA [211], and anti-TLR4 monoclonal antibodies [19].

Similarly to myeloid cells, activation of endothelial TLR4 results in NF- κ B-mediated upregulation of inflammatory cytokines [19] that includes prominent expression of angiogenic factors VEGF-A and PDGF-BB [212]. Along with this shared pattern, EC also display a distinct response to TLR4 activation such as high upregulation of transcription factor FOXC2 and induction of DLL4-Notch signaling [210] regulating vascular sprouting [213]. Likewise, CAM upregulation, which is detected in all TLR4-activated cells, is particularly pronounced in endothelium [212]. TRAF6, the key regulator of the TLR4 pathway, also mediates endothelial-specific responses, e.g., disruption of the vascular barrier [48, 49]. The resultant increase in vascular permeability combined with CAM upregulation strongly promotes recruitment of blood-circulating cells [50, 155] many of which (e.g., BM-derived immature myeloid cells) have potent independent effects on generation of tumor vessels [206]. Indeed, microvessel density in clinical human pancreatic cancers was found to be strongly associated with TLR4 expression [211]. A putative endogenous ligand of TLR4, peroxiredoxin-1, was shown to increase tumor growth by promoting tumor vasculature [161]. These studies support the concept that TLR4 activation of tumor endothelium promotes vascular formation through direct induction of angiogenic factors and sprouting and, indirectly, by facilitating transmigration of blood-circulating tumor-promoting immune cells.

Whereas tumor angiogenesis is indispensable for expansion of tumor mass, it might be less relevant to metastasis than lymphatic vessels that have invasion-prone discontinuous basement membrane [214] and are naturally equipped to transport cells to regional lymph nodes [215]. Hematogenous metastasis typically occurs later from the blood vessels in the lymphatic lesions as has recently been shown in several mouse models [216, 217]. Cell trafficking to lymph nodes is mediated exclusively by tumor-associated lymphatic vessels, and increase in density of these vessels is directly associated with metastasis in breast [218] and many other human cancers [188]. Functional TLR4 is highly expressed in LEC as evidenced by substantial upregulation of NF- κ B-dependent chemokines that recruit macrophages from inflamed tissue to the lymphatic vessels leading to the draining nodes [20]. The absence of TLR4 significantly reduces lymphatic vessel formation resulting in increased edema and decreased transport through the lymphatic channels [53]. Paclitaxel, a potent mimetic of LPS, promotes lymphangiogenesis in breast cancer models concomitant with highly elevated lymphatic metastasis and, subsequently, increased recurrence [136]. Collectively, these studies present strong evidence for TLR4-dependent promotion of metastasis through activation of both blood and lymphatic endothelia in the tumor.

16. Indirect provascular effects of TLR4 through differentiation of BM-derived endothelial progenitors and their recruitment to tumors

One often overlooked aspect of systemic TLR4 signaling is its tremendous impact on differentiation and recruitment of BM-derived provascular progenitors. Angiogenesis and lymphangiogenesis are tightly regulated in adult organism and, with few exceptions (e.g., wound healing and a female reproductive cycle), are induced only during sustained unresolved inflammation or cancer. The latter are one and the same because all solid tumors have a persistent inflammatory component. Physiologically, any increase in tissue mass or remodeling is accompanied by expansion of blood and lymphatic vessels necessary to serve the new vascular bed. TLR4 responds to this host default program not only by activating local endothelium but also by inducing differentiation of provascular progenitors from early BM precursors.

In all vertebrates, embryonic stem cells derived from hemogenic endothelium express TLR4-NF- κ B pathway that is necessary and sufficient to create hematopoietic stem/progenitor cells [15]. The latter give rise to both immune and vascular endothelial cells [219, 220]. Adult mesenchymal [16, 204] and hematopoietic stem cells express functional TLR4 as evidenced by LPS-induced self-renewal and their differentiation into appropriate lineages. Both blood vascular [17, 221] and lymphatic endothelial [18] progenitors vigorously respond to LPS [17] and other TLR4 ligands [221] by increasing proliferation, migration, and expression of transcription factors that control their differentiation into endothelial-like cells. When ligands are present systemically (e.g., released from necrotic tumors and during bolus PXL chemotherapy), TLR4-induced differentiation of BM progenitors occurs in parallel with upregulation of TLR4-dependent CCL2, CCL20, and other chemokines that recruit these progenitors to tumor [136, 174, 178, 222]. In experimental models, mobilization of genetically traceable BM-derived provascular progenitors increased tumor angiogenesis [172], whereas recruitment of myeloid-derived lymphatic endothelial progenitors (M-LECP) substantially increased tumor lymphangiogenesis [136] and lymphatic metastasis [18]. Taken together, these studies demonstrate how TLR4-induced differentiation of BM endothelial precursors and upregulation of tumor chemokines act in concert to increase blood and lymphatic networks that ultimately promote metastasis.

While both LPS and PXL can expand provascular progenitors, the latter is more pertinent to oncology due to widespread use of taxanes in clinical practice. PXL is an active component of all taxanes including widely clinically used docetaxel and nab-paclitaxel. Although counterintuitive, the new concept of chemotherapy-driven metastasis mediated by the TLR4-PXL axis [188, 223, 224] is substantiated by ample evidence from clinical studies. A single dose of PXL to breast cancer (BC) patients doubles the blood level of inflammatory cytokines [225]. An independent study of BC patients before and after PXL therapy confirmed these data and further demonstrated in mice that PXL significantly increases tumor recruitment of BM-derived progenitors [226]. An additional study in BC patients found that PXL increased mobilization of myeloid-derived progenitors by >300% [227]. A separate analysis conducted before and after taxane therapy in BC patients showed a > five-fold increase in tumor-infiltrating myeloid cells and subsequent metastasis [228]. Similar extent of tumor enrichment by CD11b⁺/CD14⁺ myeloid cells concomitant with enhanced metastasis was shown in a large study of patients (N = 699) treated by taxanes [134]. Tumor recruitment of these cells was strongly associated with lymphatic metastasis that doubled in taxane-treated compared with untreated patients [134]. In experimental models, PXL treatment increased angiogenesis and metastatic properties in tumor cells [229]. We recently showed that PXL

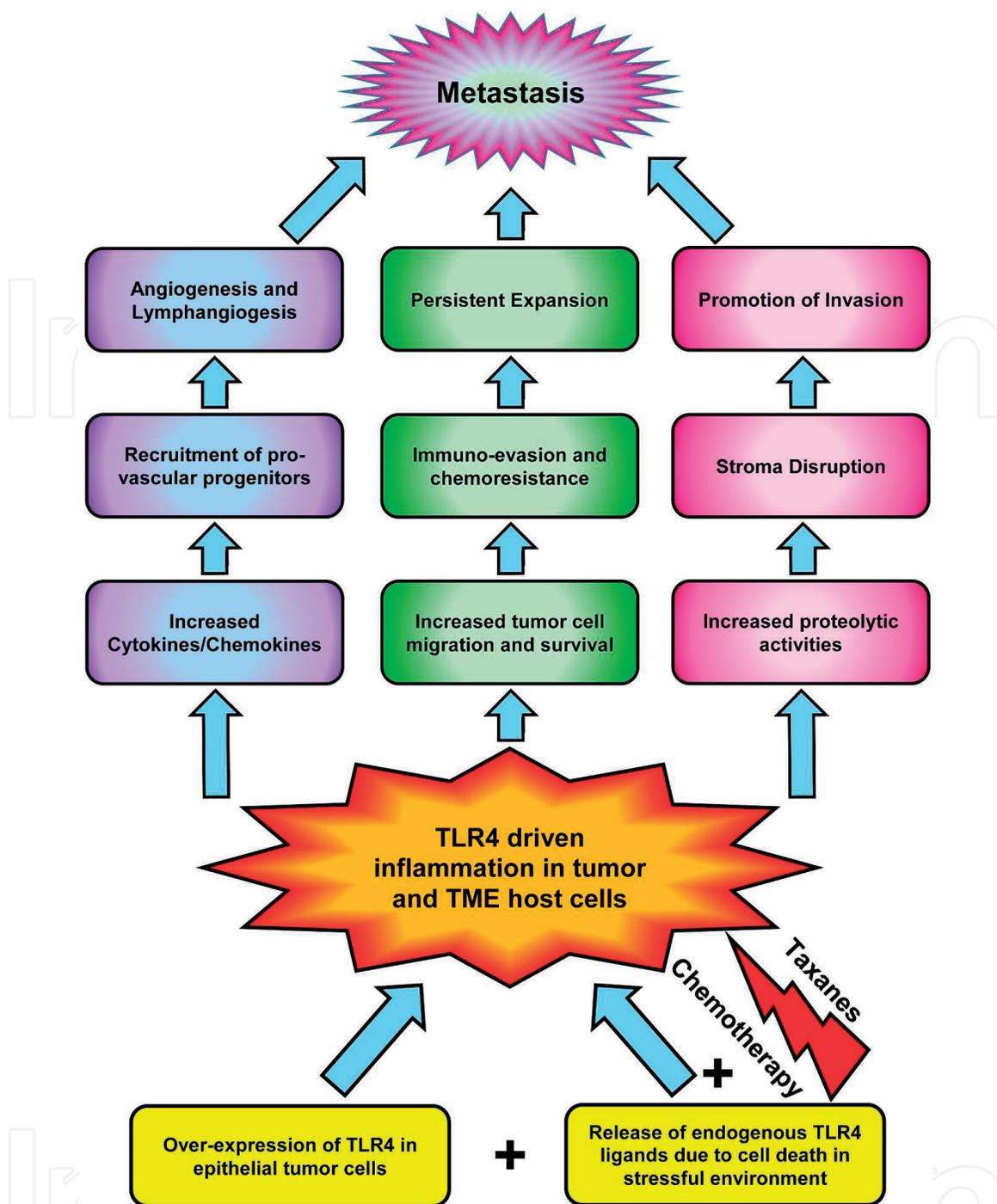


Figure 1.

Activation of TLR4 in tumor cells and tumor microenvironment. TLR4 is overexpressed in malignant cells and in a variety of host cells within the tumor mass including endothelial cells, fibroblasts, pericytes, immune cells, and various progenitors recruited by TLR4-dependent chemokines to tumor inflammatory environment. TLR4 is activated mainly by endogenous ligands released from all expressing cells due to necrotic death induced by pathological surroundings as well as by taxane chemotherapy due to direct activation of the receptor and, indirectly, through increase of endogenous ligands from dead and damaged cells. Following, TLR4 signaling leads to upregulation of inflammatory cytokines, pro-survival and migratory factors, and proteases that disrupt extracellular matrix. This collectively increases not only tumor growth but also its metastatic potential due to enhanced vascular invasion and resistance to cytotoxic therapies. Metastasis is also enhanced because of direct activating effects on tumor endothelium as well as recruitment of provascular progenitors that aid in generation of new vessels.

chemotherapy increased differentiation of BM hematopoietic-myeloid precursors into lymphatic progenitors [18], tumor mobilization of these progenitors, intratumoral lymphangiogenesis, and enhanced metastasis to lymph nodes [18, 136]. Broader recognition of this emerging concept should help in developing novel approaches to combat metastasis such as targeting TLR4-dependent BM differentiation and tumor recruitment of provascular myeloid cells.

17. Summary of TLR4-dependent mechanisms that specifically promote metastasis

Among multitude of pro-oncogenic effects of TLR4, those that specifically affect metastasis relate first and foremost to acquisition of traits normally restricted to inflamed macrophages, that is, enhanced migration, invasion, proliferation, and survival (**Figure 1**). However, this is strongly supplemented by effects on TLR4-expressing cells in the tumor environment from which most relevant are BEC and LEC. Undoubtedly, expansion of angiogenesis and lymphangiogenesis confers direct enhancement of tumor spread through these channels which may occur independently of TLR4 expression in tumor cells. Additionally, the ability to expand BM provascular and stromal progenitors and recruit them to tumor by chemokines—a specific property of inflamed macrophages—substantially supports new vessel and stroma formation. Importantly, the newly acquired properties enabled by the TLR4 signaling are further enhanced by endogenous factors released due to spontaneous necrotic death and chemotherapeutic treatments. Under these conditions, PXL, as an activator of TLR4, takes a special place because of its pro-inflammatory effects through release of endogenous ligands due to tumor cell kill as well through direct action on the TLR4 complex. Ultimately, these processes occur in parallel at tumor and systemic, mainly immune organs, sites. TLR4-positive tumor cells have a pro-metastatic advantage by coordinating generation of new transporting channels and enhanced migratory/invasive potential as well as the enhanced capacity to survive in the circulation and at new sites (**Figure 1**). TLR4-negative tumor cells might have a reduced metastatic potential due to the absence of direct stimulating effects, but they are still benefited from the regenerative tumor environment that is strongly supported by inflammation orchestrated by TLR4. Taking into account a comprehensive systemic view on tumor and chemotherapy effects on TLR4-mediated activities should facilitate the rational development of new anti-metastatic strategies.

Acknowledgements

Preparation of this manuscript was supported by grants from the National Institute of Health (1R01-CA199649), the Team Science Grant (TSG) from the Simmons Cancer Institute at Southern Illinois University, and Illinois William E. McElroy Foundation awarded to Sophia Ran.

Conflict of interest

The authors have no conflicts of interest.

Acronyms and abbreviations

BEC	blood vascular endothelial cell(s)
BM	bone marrow
BMDM	bone marrow-derived myeloid cells
CAFs	cancer-associated fibroblasts
DAMPs	damage-associated molecular patterns
ECP	endothelial cell progenitor(s)
LN(s)	lymph node(s)

LEC	lymphatic endothelial cell(s)
LPS	lipopolysaccharide
LVD	lymphatic vessel density
M-LECP	myeloid-derived lymphatic endothelial cell progenitor(s)
MMP	matrix metalloproteinase(s)
NF- κ B	nuclear factor-kappa B
PAMPs	pathogen-associated molecular patterns
PXL	paclitaxel
TAM(s)	tumor-associated macrophage(s)
TME	tumor microenvironment
VEGF-A	vascular endothelial growth factor-A

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References

- [1] Medzhitov R, Preston-Hurlburt P, Janeway CA Jr. A human homologue of the *Drosophila* Toll protein signals activation of adaptive immunity. *Nature*. 1997;**388**:394-397. DOI: 10.1038/41131
- [2] Kawai T, Akira S. Toll-like receptor downstream signaling. *Arthritis Research and Therapy*. 2005;**7**:12-19. DOI: 10.1186/ar1469
- [3] Poltorak A, He X, Smirnova I, Liu MY, Van HC, Du X, et al. Defective LPS signaling in C3H/HeJ and C57BL/10ScCr mice: Mutations in *Tlr4* gene. *Science*. 1998;**282**:2085-2088
- [4] Rock FL, Hardiman G, Timans JC, Kastelein RA, Bazan JF. A family of human receptors structurally related to *Drosophila* Toll. *Proceedings of the National Academy of Sciences of the United States of America*. 1998;**95**:588-593
- [5] Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: Update on Toll-like receptors. *Nature Immunology*. 2010;**11**:373-384. DOI: 10.1038/ni.1863
- [6] Kaisho T, Akira S. Toll-like receptor function and signaling. *Journal of Allergy and Clinical Immunology*. 2006;**117**:979-987. DOI: 10.1016/j.jaci.2006.02.023
- [7] Kim D, Kim JY. Anti-CD14 antibody reduces LPS responsiveness via TLR4 internalization in human monocytes. *Molecular Immunology*. 2014;**57**:210-215. DOI: 10.1016/j.molimm.2013.09.009
- [8] Akashi S, Shimazu R, Ogata H, Nagai Y, Takeda K, Kimoto M, et al. Cutting edge: Cell surface expression and lipopolysaccharide signaling via the Toll-like receptor 4-MD-2 complex on mouse peritoneal macrophages. *Journal of Immunology*. 2000;**164**:3471-3475. pii: ji_v164n7p3471
- [9] Zhou X, Gao XP, Fan J, Liu Q, Anwar KN, Frey RS, et al. LPS activation of Toll-like receptor 4 signals CD11b/CD18 expression in neutrophils. *American Journal of Physiology. Lung Cellular and Molecular Physiology*. 2005;**288**:L655-L662. DOI: 10.1152/ajplung.00327.2004
- [10] Zanoni I, Ostuni R, Capuano G, Collini M, Caccia M, Ronchi AE, et al. CD14 regulates the dendritic cell life cycle after LPS exposure through NFAT activation. *Nature*. 2009;**460**:264-268. DOI: 10.1038/nature08118
- [11] Komai-Koma M, Gilchrist DS, Xu D. Direct recognition of LPS by human but not murine CD8+ T cells via TLR4 complex. *European Journal of Immunology*. 2009;**39**:1564-1572. DOI: 10.1002/eji.200838866
- [12] Schweighoffer E, Nys J, Vanes L, Smithers N, Tybulewicz VLJ. TLR4 signals in B lymphocytes are transduced via the B cell antigen receptor and SYK. *Journal of Experimental Medicine*. 2017;**214**:1269-1280. DOI: 10.1084/jem.20161117
- [13] Chang EJ, Kim HJ, Ha J, Kim H, Ryu J, Park KH, et al. Hyaluronan inhibits osteoclast differentiation via Toll-like receptor 4. *Journal of Cell Science*. 2007;**120**:166-176. DOI: 10.1242/jcs.03310
- [14] Lee SH, Hong B, Sharabi A, Huang XF, Chen SY. Embryonic stem cells and mammary luminal progenitors directly sense and respond to microbial products. *Stem Cells*. 2009;**27**:1604-1615. DOI: 10.1002/stem.75
- [15] He Q, Zhang C, Wang L, Zhang P, Ma D, Lv J, et al. Inflammatory signaling regulates hematopoietic stem and progenitor cell emergence in

vertebrates. *Blood*. 2015;**125**:1098-1106. DOI: 10.1182/blood-2014-09-601542

[16] He X, Wang H, Jin T, Xu Y, Mei L, Yang J. TLR4 activation promotes bone marrow MSC proliferation and osteogenic differentiation via Wnt3a and Wnt5a signaling. *PLoS One*. 2016;**11**:e0149876. DOI: 10.1371/journal.pone.0149876

[17] He J, Xiao Z, Chen X, Chen M, Fang L, Yang M, et al. The expression of functional Toll-like receptor 4 is associated with proliferation and maintenance of stem cell phenotype in endothelial progenitor cells (EPCs). *Journal of Cellular Biochemistry*. 2010;**111**:179-186. DOI: 10.1002/jcb.22686

[18] Volk-Draper LD, Hall KL, Wilber AC, Ran S. Lymphatic endothelial progenitors originate from plastic myeloid cells activated by Toll-like receptor-4. *PLoS One*. 2017;**12**:e0179257. DOI: 10.1371/journal.pone.0179257

[19] Faure E, Equils O, Sieling PA, Thomas L, Zhang FX, Kirschning CJ, et al. Bacterial lipopolysaccharide activates NF-kappaB through Toll-like receptor 4 (TLR-4) in cultured human dermal endothelial cells. Differential expression of TLR-4 and TLR-2 in endothelial cells. *The Journal of Biological Chemistry*. 2000;**275**:11058-11063

[20] Kang S, Lee SP, Kim KE, Kim HZ, Memet S, Koh GY. Toll-like receptor 4 in lymphatic endothelial cells contributes to LPS-induced lymphangiogenesis by chemotactic recruitment of macrophages. *Blood*. 2009;**113**:2605-2613. DOI: 10.1182/blood-2008-07-166934

[21] Vink A, Schoneveld AH, van der Meer JJ, van Middelaar BJ, Sluijter JP, Smeets MB, et al. In vivo evidence for a role of Toll-like receptor 4 in the development of intimal lesions. *Circulation*. 2002;**106**:1985-1990

[22] Miller LS, Modlin RL. Human keratinocyte Toll-like receptors promote distinct immune responses. *The Journal of Investigative Dermatology*. 2007;**127**:262-263. DOI: 10.1038/sj.jid.5700559

[23] Ohkawara T, Takeda H, Miyashita K, Nishiwaki M, Nakayama T, Taniguchi M, et al. Regulation of Toll-like receptor 4 expression in mouse colon by macrophage migration inhibitory factor. *Histochemistry and Cell Biology*. 2006;**125**:575-582. DOI: 10.1007/s00418-005-0092-y

[24] Ungaro R, Fukata M, Hsu D, Hernandez Y, Breglio K, Chen A, et al. A novel Toll-like receptor 4 antagonist antibody ameliorates inflammation but impairs mucosal healing in murine colitis. *American Journal of Physiology. Gastrointestinal and Liver Physiology*. 2009;**296**:G1167-G1179. DOI: 10.1152/ajpgi.90496.2008

[25] Zhou M, Farland-Mancini MM, Funk HM, Husseinzadeh N, Mounajjed T, Drew AF. Toll-like receptor expression in normal ovary and ovarian tumors. *Cancer Immunology, Immunotherapy*. 2009;**58**:1375-1385. DOI: 10.1007/s00262-008-0650-y

[26] Wolfs TG, Buurman WA, van Schadewijk A, de Vries B, Daemen MA, Hiemstra PS, et al. In vivo expression of Toll-like receptor 2 and 4 by renal epithelial cells: IFN-gamma and TNF-alpha mediated up-regulation during inflammation. *Journal of Immunology*. 2002;**168**:1286-1293

[27] Chakraborty D, Zenker S, Rossaint J, Holscher A, Pohlen M, Zarbock A, et al. Alarmin S100A8 activates alveolar epithelial cells in the context of acute lung injury in a TLR4-dependent manner. *Frontiers in Immunology*. 2017;**8**:1493. DOI: 10.3389/fimmu.2017.01493

[28] Zarembek KA, Godowski PJ. Tissue expression of human Toll-like receptors

and differential regulation of Toll-like receptor mRNAs in leukocytes in response to microbes, their products, and cytokines. *Journal of Immunology*. 2002;**168**:554-561

[29] Palha De SC, Blum CM, Sgroe EP, Crespo AM, Kurt RA. Murine mammary carcinoma cells and CD11c(+) dendritic cells elicit distinct responses to lipopolysaccharide and exhibit differential expression of genes required for TLR4 signaling. *Cellular Immunology*. 2010;**266**:67-75. DOI: 10.1016/j.cellimm.2010.08.015

[30] Fukata M, Chen A, Vamadevan AS, Cohen J, Breglio K, Krishnareddy S, et al. Toll-like receptor-4 promotes the development of colitis-associated colorectal tumors. *Gastroenterology*. 2007;**133**:1869-1881. DOI: 10.1053/j.gastro.2007.09.008

[31] Liu YH, Huang D, Li ZJ, Li XH, Wang X, Yang HP, et al. Toll-like receptor-4-dependence of the lipopolysaccharide-mediated inhibition of osteoblast differentiation. *Genetics and Molecular Research*. 2016;**15**:1-11. DOI: 10.4238/gmr.15027191

[32] Wang W, Deng M, Liu X, Ai W, Tang Q, Hu J. TLR4 activation induces nontolerant inflammatory response in endothelial cells. *Inflammation*. 2011;**34**:509-518. DOI: 10.1007/s10753-010-9258-4

[33] Rajput S, Volk-Draper LD, Ran S. TLR4 is a novel determinant of the response to paclitaxel in breast cancer. *Molecular Cancer Therapeutics*. 2013;**12**:1676-1687. DOI: 10.1158/1535-7163.MCT-12-1019

[34] Son DS, Parl AK, Rice VM, Khabele D. Keratinocyte chemoattractant (KC)/human growth-regulated oncogene (GRO) chemokines and pro-inflammatory chemokine networks in mouse and human ovarian epithelial

cancer cells. *Cancer Biology and Therapy*. 2007;**6**:1302-1312

[35] Kim JI, Lee CJ, Jin MS, Lee CH, Paik SG, Lee H, et al. Crystal structure of CD14 and its implications for lipopolysaccharide signaling. *The Journal of Biological Chemistry*. 2005;**280**:11347-11351. DOI: 10.1074/jbc.M414607200

[36] Zhou ZB, Yang B, Li X, Liu H, Lei G. Lysophosphatidic acid promotes expression and activation of matrix metalloproteinase 9 (MMP9) in THP-1 cells via Toll-like receptor 4/nuclear factor-kappaB (TLR4/NF-kappaB) signaling pathway. *Medical Science Monitor*. 2018;**24**:4861-4868. DOI: 10.12659/MSM.906450

[37] Finnin M, Hamilton JA, Moss ST. Characterization of a CSF-induced proliferating subpopulation of human peripheral blood monocytes by surface marker expression and cytokine production. *Journal of Leukocyte Biology*. 1999;**66**:953-960

[38] Fan J, Malik AB. Toll-like receptor-4 (TLR4) signaling augments chemokine-induced neutrophil migration by modulating cell surface expression of chemokine receptors. *Nature Medicine*. 2003;**9**:315-321. DOI: 10.1038/nm832

[39] Lombardo E, varez-Barrientos A, Maroto B, Bosca L, Knaus UG. TLR4-mediated survival of macrophages is MyD88 dependent and requires TNF-alpha autocrine signalling. *Journal of Immunology*. 2007;**178**:3731-3739

[40] Kawai T, Akira S. TLR signaling. *Cell Death and Differentiation*. 2006;**13**:816-825. DOI: 10.1038/sj.cdd.4401850

[41] Sirisinha S. Insight into the mechanisms regulating immune homeostasis in health and disease. *Asian Pac. Journal of Allergy and Clinical Immunology*. 2011;**29**:1-14

- [42] Bhattacharyya S, Varga J. Endogenous ligands of TLR4 promote unresolving tissue fibrosis: Implications for systemic sclerosis and its targeted therapy. *Immunology Letters*. 2018;**195**:9-17. DOI: 10.1016/j.imlet.2017.09.011
- [43] Mantovani A, Biswas SK, Galdiero MR, Sica A, Locati M. Macrophage plasticity and polarization in tissue repair and remodelling. *The Journal of Pathology*. 2013;**229**:176-185. DOI: 10.1002/path.4133
- [44] Ouyang Y, Guo J, Lin C, Lin J, Cao Y, Zhang Y, et al. Transcriptomic analysis of the effects of Toll-like receptor 4 and its ligands on the gene expression network of hepatic stellate cells. *Fibrogenesis and Tissue Repair*. 2016;**9**. DOI: 10.1186/s13069-016-0039-z
- [45] Takahashi K, Sugi Y, Hosono A, Kaminogawa S. Epigenetic regulation of TLR4 gene expression in intestinal epithelial cells for the maintenance of intestinal homeostasis. *Journal of Immunology*. 2009;**183**:6522-6529. DOI: 10.4049/jimmunol.0901271
- [46] Yokota S, Okabayashi T, Rehli M, Fujii N, Amano K. *Helicobacter pylori* lipopolysaccharides upregulate Toll-like receptor 4 expression and proliferation of gastric epithelial cells via the MEK1/2-ERK1/2 mitogen-activated protein kinase pathway. *Infection and Immunity*. 2010;**78**:468-476. DOI: 10.1128/IAI.00903-09
- [47] Jiang D, Liang J, Li Y, Noble PW. The role of Toll-like receptors in non-infectious lung injury. *Cell Research*. 2006;**16**:693-701. DOI: 10.1038/sj.cr7310085
- [48] Wolfson RK, Chiang ET, Garcia JG. HMGB1 induces human lung endothelial cell cytoskeletal rearrangement and barrier disruption. *Microvascular Research*. 2011;**81**:189-197. DOI: 10.1016/j.mvr.2010.11.010
- [49] Liu A, Gong P, Hyun SW, Wang KZ, Cates EA, Perkins D, et al. TRAF6 protein couples Toll-like receptor 4 signaling to Src family kinase activation and opening of paracellular pathway in human lung microvascular endothelia. *The Journal of Biological Chemistry*. 2012;**287**:16132-16145. DOI: 10.1074/jbc.M111.310102
- [50] Andonegui G, Bonder CS, Green F, Mullaly SC, Zbytniuk L, Raharjo E, et al. Endothelium-derived Toll-like receptor-4 is the key molecule in LPS-induced neutrophil sequestration into lungs. *The Journal of Clinical Investigation*. 2003;**111**:1011-1020. DOI: 10.1172/JCI16510
- [51] Harari OA, Alcaide P, Ahl D, Lusinskas FW, Liao JK. Absence of TRAM restricts Toll-like receptor 4 signaling in vascular endothelial cells to the MyD88 pathway. *Circulation Research*. 2006;**98**:1134-1140. DOI: 10.1161/01.RES.0000220105.85182.28
- [52] Guijarro-Munoz I, Compte M, Alvarez-Cienfuegos A, Alvarez-Vallina L, Sanz L. Lipopolysaccharide activates Toll-like receptor 4 (TLR4)-mediated NF-kappaB signaling pathway and proinflammatory response in human pericytes. *The Journal of Biological Chemistry*. 2014;**289**:2457-2468. DOI: 10.1074/jbc.M113.521161
- [53] Zampell J, Elhadad S, Avraham T, Weitman E, Aschen S, Yan A, et al. Toll-like receptor deficiency worsens inflammation and lymphedema after lymphatic injury. *American Journal of Physiology. Cell Physiology*. 2011;**4**:709-719. DOI: 10.1152/ajpcell.00284.2011
- [54] Chao W. Toll-like receptor signaling: A critical modulator of cell survival and ischemic injury in the heart. *American Journal of Physiology. Heart and Circulatory Physiology*.

2009;**296**:H1-H12. DOI: 10.1152/
ajpheart.00995.2008

[55] Lee CH, Wu CL, Shiao AL. Toll-like receptor 4 signaling promotes tumor growth. *Journal of Immunotherapy*. 2010;**33**:73-82. DOI: 10.1097/CJI.0b013e3181b7a0a4

[56] Hishida A, Matsuo K, Goto Y, Hamajima N. Genetic predisposition to *Helicobacter pylori*-induced gastric precancerous conditions. *World Journal of Gastrointestinal Oncology*. 2010;**2**: 369-379. DOI: 10.4251/wjgo.v2.i10.369

[57] Zaks-Zilberman M, Zaks TZ, Vogel SN. Induction of proinflammatory and chemokine genes by lipopolysaccharide and paclitaxel (Taxol) in murine and human breast cancer cell lines. *Cytokine*. 2001;**15**:156-165. DOI: 10.1006/cyto.2001.0935

[58] Szajnik M, Szczepanski MJ, Czystowska M, Elishaev E, Mandapathil M, Nowak-Markwitz E, et al. TLR4 signaling induced by lipopolysaccharide or paclitaxel regulates tumor survival and chemoresistance in ovarian cancer. *Oncogene*. 2009;**28**:4353-4363. DOI: 10.1038/onc.2009.289

[59] Kelly MG, Alvero AB, Chen R, Silasi DA, Abrahams VM, Chan S, et al. TLR-4 signaling promotes tumor growth and paclitaxel chemoresistance in ovarian cancer. *Cancer Research*. 2006;**66**:3859-3868. DOI: 10.1158/0008-5472.CAN-05-3948

[60] He W, Liu Q, Wang L, Chen W, Li N, Cao X. TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. *Molecular Immunology*. 2007;**44**:2850-2859. DOI: 10.1016/j.molimm.2007.01.022

[61] Zhang Y, Wang Y, Yuan J, Qin W, Liu F, Wang F, et al. Toll-like receptor 4 ligation confers chemoresistance to

docetaxel on PC-3 human prostate cancer cells. *Cell Biology and Toxicology*. 2012;**28**:269-277. DOI: 10.1007/s10565-012-9221-2

[62] Hua D, Liu MY, Cheng ZD, Qin XJ, Zhang HM, Chen Y, et al. Small interfering RNA-directed targeting of Toll-like receptor 4 inhibits human prostate cancer cell invasion, survival, and tumorigenicity. *Molecular Immunology*. 2009;**46**:2876-2884. DOI: 10.1016/j.molimm.2009.06.016

[63] Shuyi Y, Feng W, Jing T, Hongzhang H, Haiyan W, Pingping M, et al. Human beta-defensin-3 (hBD-3) upregulated by LPS via epidermal growth factor receptor (EGFR) signaling pathways to enhance lymphatic invasion of oral squamous cell carcinoma. *Oral Surgery, Oral Medicine, Oral Pathology, Oral Radiology, and Endodontics*. 2011;**112**:616-625. DOI: 10.106/j.triplo.2011.02.053

[64] Jing YY, Han ZP, Sun K, Zhang SS, Hou J, Liu Y, et al. Toll-like receptor 4 signaling promotes epithelial-mesenchymal transition in human hepatocellular carcinoma induced by lipopolysaccharide. *BMC Medicine*. 2012;**10**. DOI: 98. DOI: 10.1186/1741-7015-10-98

[65] Basu S, Pathak SK, Chatterjee G, Pathak S, Basu J, Kundu M. *Helicobacter pylori* protein HP0175 Transactivates epidermal growth factor receptor through TLR4 in gastric epithelial cells. *The Journal of Biological Chemistry*. 2008;**283**:32369-32376. DOI: 10.1074/jbc.M805053200

[66] Liu CY, Xu JY, Shi XY, Huang W, Ruan TY, Xie P, et al. M2-polarized tumor-associated macrophages promoted epithelial-mesenchymal transition in pancreatic cancer cells, partially through TLR4/IL-10 signaling pathway. *Laboratory Investigation*. 2013;**93**:844-854. DOI: 10.1038/labinvest.2013.69

- [67] Molteni M, Marabella D, Orlandi C, Rossetti C. Melanoma cell lines are responsive in vitro to lipopolysaccharide and express TLR-4. *Cancer Letters*. 2006;**235**:75-83. DOI: 10.1016/j.canlet.2005.04.006
- [68] Thuringer D, Hammann A, Benikhlef N, Fourmaux E, Bouchot A, Wettstein G, et al. Transactivation of the epidermal growth factor receptor by heat shock protein 90 via Toll-like receptor 4 contributes to the migration of glioblastoma cells. *The Journal of Biological Chemistry*. 2011;**286**:3418-3428. DOI: 10.1074/jbc.M110.154823
- [69] Bao H, Lu P, Li Y, Wang L, Li H, He D, et al. Triggering of Toll-like receptor-4 in human multiple myeloma cells promotes proliferation and alters cell responses to immune and chemotherapy drug attack. *Cancer Biology and Therapy*. 2011;**11**:58-67. DOI: 10.4161/cbt.11.1.13878
- [70] Ieguchi K, Omori T, Komatsu A, Tomita T, Deguchi A, Maru Y. Ephrin-A1 expression induced by S100A8 is mediated by the Toll-like receptor 4. *Biochemical and Biophysical Research Communications*. 2013;**440**:623-629. DOI: 10.1016/j.bbrc.2013.09.119
- [71] Luo Y, Chihara Y, Fujimoto K, Sasahira T, Kuwada M, Fujiwara R, et al. High mobility group box 1 released from necrotic cells enhances regrowth and metastasis of cancer cells that have survived chemotherapy. *European Journal of Cancer*. 2013;**49**:741-751. DOI: 10.1016/j.ejca.2012.09.016
- [72] Zhang JJ, Wu HS, Wang L, Tian Y, Zhang JH, Wu HL. Expression and significance of TLR4 and HIF-1 α in pancreatic ductal adenocarcinoma. *World Journal of Gastroenterology*. 2010;**16**:2881-2888
- [73] Cammarota R, Bertolini V, Pennesi G, Bucci EO, Gottardi O, Garlanda C, et al. The tumor microenvironment of colorectal cancer: Stromal TLR-4 expression as a potential prognostic marker. *Journal of Translational Medicine*. 2010;**8**:112. DOI: 10.1186/1479-5876-8-112
- [74] Hasimu A, Ge L, Li QZ, Zhang RP, Guo X. Expressions of Toll-like receptors 3, 4, 7, and 9 in cervical lesions and their correlation with HPV16 infection in Uighur women. *Chinese Journal of Cancer*. 2011;**30**:344-350
- [75] Hsu RY, Chan CH, Spicer JD, Rousseau MC, Giannias B, Rousseau S, et al. LPS-induced TLR4 signaling in human colorectal cancer cells increases beta1 integrin-mediated cell adhesion and liver metastasis. *Cancer Research*. 2011;**71**:1989-1998. DOI: 10.1158/0008-5472.CAN.2833
- [76] Ehsan N, Murad S, Ashiq T, Mansoor MU, Gul S, Khalid S, et al. Significant correlation of TLR4 expression with the clinicopathological features of invasive ductal carcinoma of the breast. *Tumour Biology*. 2013;**34**:1053-1059. DOI: 10.1007/s13277-013-0645-y
- [77] Wang EL, Qian ZR, Nakasono M, Tanahashi T, Yoshimoto K, Bando Y, et al. High expression of Toll-like receptor 4/myeloid differentiation factor 88 signals correlates with poor prognosis in colorectal cancer. *British Journal of Cancer*. 2010;**102**:908-915. DOI: 10.1038/sj.bjc.6605558
- [78] Xu H, Wu Q, Dang S, Jin M, Xu J, Cheng Y, et al. Alteration of CXCR7 expression mediated by TLR4 promotes tumor cell proliferation and migration in human colorectal carcinoma. *PLoS One*. 2011;**6**:e27399. DOI: 10.1371/journal.pone.0027399
- [79] Gonzalez-Reyes S, Marin L, Gonzalez L, Gonzalez LO, Del Casar JM, Lamelas ML, et al. Study of TLR3, TLR4 and TLR9 in breast carcinomas

and their association with metastasis. *BMC Cancer*. 2010;**10**. DOI: 665. DOI: 10.1186/1471-2407-10-665

[80] Eiro N, Ovies C, Fernandez-Garcia B, Alvarez-Cuesta CC, Gonzalez L, Gonzalez LO, et al. Expression of TLR3, 4, 7 and 9 in cutaneous malignant melanoma: Relationship with clinicopathological characteristics and prognosis. *Archives of Dermatological Research*. 2013;**305**:59-67. DOI: 10.1007/s00403-012-1300-y

[81] Hao B, Chen Z, Bi B, Yu M, Yao S, Feng Y, et al. Role of TLR4 as a prognostic factor for survival in various cancers: A meta-analysis. *Oncotarget*. 2018;**9**:13088-13099. DOI: 10.18632/oncotarget.24178

[82] Yang CX, Li CY, Feng W. Toll-like receptor 4 genetic variants and prognosis of breast cancer. *Tissue Antigens*. 2013;**81**:221-226. DOI: 10.1111/tan.12096

[83] Wang X, Yu X, Wang Q, Lu Y, Chen H. Expression and clinical significance of SATB1 and TLR4 in breast cancer. *Oncology Letters*. 2017;**14**:3611-3615. DOI: 10.3892/ol.2017.6571

[84] Gonzalez-Reyes S, Fernandez JM, Gonzalez LO, Aguirre A, Suarez A, Gonzalez JM, et al. Study of TLR3, TLR4, and TLR9 in prostate carcinomas and their association with biochemical recurrence. *Cancer Immunology, Immunotherapy*. 2010;**60**:217-226. DOI: 10.1007/s00262-010-0931-0

[85] Zhu Y, Huang JM, Zhang GN, Zha X, Deng BF. Prognostic significance of MyD88 expression by human epithelial ovarian carcinoma cells. *Journal of Translational Medicine*. 2012;**10**. DOI: 77. DOI: 10.1186/1479-5876-10-77

[86] Wang K, Wang J, Wei F, Zhao N, Yang F, Ren X. Expression of TLR4 in non-small cell lung cancer is associated with PD-L1 and poor prognosis in

patients receiving Pulmonectomy. *Frontiers in Immunology*. 2017;**8**:456. DOI: 10.3389/fimmu.2017.00456

[87] Kim KH, Jo MS, Suh DS, Yoon MS, Shin DH, Lee JH, et al. Expression and significance of the TLR4/MyD88 signaling pathway in ovarian epithelial cancers. *World Journal of Surgical Oncology*. 2012;**10**:193. DOI: 10.1186/1477-7819-10-193

[88] Sheyhidin I, Nabi G, Hasim A, Zhang RP, Ainiwaer J, Ma H, et al. Overexpression of TLR3, TLR4, TLR7 and TLR9 in esophageal squamous cell carcinoma. *World Journal of Gastroenterology*. 2011;**17**:3745-3751. DOI: 10.3748/wjg.v17.i32.3745

[89] Fitzgerald KA, Palsson-McDermott EM, Bowie AG, Jefferies CA, Mansell AS, Brady G, et al. Mal (MyD88-adaptor-like) is required for Toll-like receptor-4 signal transduction. *Nature*. 2001;**413**:78-83. DOI: 10.1038/35092578

[90] Cheng Z, Taylor B, Ourthiague DR, Hoffmann A. Distinct single-cell signaling characteristics are conferred by the MyD88 and TRIF pathways during TLR4 activation. *Science Signaling*. 2015;**8**. DOI: ra69. DOI: 10.1126/scisignal.aaa5208

[91] Verstak B, Nagpal K, Bottomley SP, Golenbock DT, Hertzog PJ, Mansell A. MyD88 adapter-like (Mal)/TIRAP interaction with TRAF6 is critical for. *The Journal of Biological Chemistry*. 2009;**284**:24192-24203. DOI: 10.1074/jbc.M109.023044

[92] Hamidi A, von B, V, Hamidi R, Winssinger N, Barluenga S, Heldin CH, Landstrom M. Polyubiquitination of transforming growth factor beta (TGFbeta)-associated kinase 1 mediates nuclear factor-kappaB activation in response to different inflammatory stimuli. *The Journal of Biological Chemistry* 2012; **287**: 123-133. DOI: 10.1074/jbc.M111.285122

- [93] Shimazu R, Akashi S, Ogata H, Nagai Y, Fukudome K, Miyake K, et al. MD-2, a molecule that confers lipopolysaccharide responsiveness on Toll-like receptor 4. *Journal of Experimental Medicine*. 1999;**189**:1777-1782
- [94] Latz E, Visintin A, Lien E, Fitzgerald KA, Monks BG, Kurt-Jones EA, et al. Lipopolysaccharide rapidly traffics to and from the Golgi apparatus with the Toll-like receptor 4-MD-2-CD14 complex in a process that is distinct from the initiation of signal transduction. *The Journal of Biological Chemistry*. 2002;**277**:47834-47843. DOI: 10.1074/jbc.M207873200
- [95] Mancek-Keber M, Jerala R. Postulates for validating TLR4 agonists. *European Journal of Immunology*. 2015;**45**:356-370. DOI: 10.1002/eji.201444462
- [96] Jiang Q, Akashi S, Miyake K, Petty HR. Lipopolysaccharide induces physical proximity between CD14 and Toll-like receptor 4 (TLR4) prior to nuclear translocation of NF-kappa B. *Journal of Immunology*. 2000;**165**:3541-3544
- [97] Moore KJ, Andersson LP, Ingalls RR, Monks BG, Li R, Arnaout MA, et al. Divergent response to LPS and bacteria in CD14-deficient murine macrophages. *Journal of Immunology*. 2000;**165**:4272-4280
- [98] Hailman E, Lichenstein HS, Wurfel MM, Miller DS, Johnson DA, Kelley M, et al. Lipopolysaccharide (LPS)-binding protein accelerates the binding of LPS to CD14. *Journal of Experimental Medicine*. 1994;**179**:269-277
- [99] Golenbock DT, Liu Y, Millham FH, Freeman MW, Zoeller RA. Surface expression of human CD14 in Chinese hamster ovary fibroblasts imparts macrophage-like responsiveness to bacterial endotoxin. *The Journal of Biological Chemistry*. 1993;**268**:22055-22059
- [100] Wong KF, Luk JM, Cheng RH, Klickstein LB, Fan ST. Characterization of two novel LPS-binding sites in leukocyte integrin betaA domain. *The FASEB Journal*. 2007;**21**:3231-3239. DOI: 10.1096/fj.06-7579com
- [101] Bhat N, Perera PY, Carboni JM, Blanco J, Golenbock DT, Mayadas TN, et al. Use of a photoactivatable taxol analogue to identify unique cellular targets in murine macrophages: Identification of murine CD18 as a major taxol-binding protein and a role for Mac-1 in taxol-induced gene expression. *Journal of Immunology*. 1999;**162**:7335-7342
- [102] Han C, Jin J, Xu S, Liu H, Li N, Cao X. Integrin CD11b negatively regulates TLR-triggered inflammatory responses by activating Syk and promoting degradation of MyD88 and TRIF via Cbl-b. *Nature Immunology*. 2010;**11**:734-742. DOI: 10.1038/ni.1908
- [103] Dixon DR, Darveau RP. Lipopolysaccharide heterogeneity: Innate host responses to bacterial modification of lipid a structure. *Journal of Dental Research*. 2005;**84**:584-595. DOI: 10.1777/154405910508400702
- [104] Fruitwala S, El-Naccache DW, Chang TL. Multifaceted immune functions of human defensins and underlying mechanisms. *Seminars in Cell and Developmental Biology*. 2018;**88**:163-172. DOI: 10.1016/j.semcdb.2018.02.023
- [105] Teghanemt A, Zhang D, Levis EN, Weiss JP, Gioannini TL. Molecular basis of reduced potency of underacylated endotoxins. *Journal of Immunology*. 2005;**175**:4669-4676
- [106] Ohto U, Fukase K, Miyake K, Satow Y. Crystal structures of human MD-2 and its complex with antiendotoxic lipid

IVa. Science. 2007;**316**:1632-1634. DOI: 10.1126/science.1139111

2007;**13**:1042-1049. DOI: 10.1038/nm1638

[107] Saitoh S, Akashi S, Yamada T, Tanimura N, Matsumoto F, Fukase K, et al. Ligand-dependent Toll-like receptor 4 (TLR4)-oligomerization is directly linked with TLR4-signaling. *Journal of Endotoxin Research*. 2004;**10**:257-260. DOI: 10.1179/096805104225005904

[113] Schutz FA, Buzaid AC, Sartor O. Taxanes in the management of metastatic castration-resistant prostate cancer: Efficacy and management of toxicity. *Critical Reviews in Oncology/Hematology*. 2014;**91**:248-256. DOI: 10.1016/j.critrevonc.2014.02.003

[108] Cleveland MG, Gorham JD, Murphy TL, Tuomanen E, Murphy KM. Lipoteichoic acid preparations of gram-positive bacteria induce interleukin-12 through a CD14-dependent pathway. *Infection and Immunity*. 1996;**64**:1906-1912

[114] McGrogan BT, Gilmartin B, Carney DN, McCann A. Taxanes, microtubules and chemoresistant breast cancer. *Biochimica et Biophysica Acta*. 2008;**1785**:96-132. DOI: 10.106/j.bbcan.2007.10.004

[109] Miller YI, Choi SH, Wiesner P, Bae YS. The SYK side of TLR4: Signalling mechanisms in response to LPS and minimally oxidized LDL. *British Journal of Pharmacology*. 2012;**167**:990-999. DOI: 10.1111/j.1476-5381.2012.02097.x

[115] Torres K, Horwitz SB. Mechanisms of Taxol-induced cell death are concentration dependent. *Cancer Research*. 1998;**58**:3620-3626

[110] Kochumon S, Wilson A, Chandy B, Shenouda S, Tuomilehto J, Sindhu S, et al. Palmitate activates CCL4 expression in human monocytic cells via TLR4/MyD88 dependent activation of NF-kappaB/MAPK/PI3K signaling systems. *Cellular Physiology and Biochemistry*. 2018;**46**:953-964. DOI: 10.1159/000488824

[116] Collins TS, Lee LF, Ting JP. Paclitaxel up-regulates interleukin-8 synthesis in human lung carcinoma through an NF-kappaB- and AP-1-dependent mechanism. *Cancer Immunology, Immunotherapy*. 2000;**49**:78-84

[111] Yang H, Hreggvidsdottir HS, Palmblad K, Wang H, Ochani M, Li J, et al. A critical cysteine is required for HMGB1 binding to Toll-like receptor 4 and activation of macrophage cytokine release. *Proceedings of the National Academy of Sciences of the United States of America*. 2010;**107**:11942-11947. DOI: 10.1073/pnas.1003893107

[117] Poruchynsky MS, Wang EE, Rudin CM, Blagosklonny MV, Fojo T. Bcl-xL is phosphorylated in malignant cells following microtubule disruption. *Cancer Research*. 1998;**58**:3331-3338

[112] Vogl T, Tenbrock K, Ludwig S, Leukert N, Ehrhardt C, van Zoelen MA, et al. Mrp8 and Mrp14 are endogenous activators of Toll-like receptor 4, promoting lethal, endotoxin-induced shock. *Nature Medicine*.

[118] Perera PY, Qureshi N, Vogel SN. Paclitaxel (Taxol)-induced NF-kappaB translocation in murine macrophages. *Infection and Immunity*. 1996;**64**:878-884

[119] Byrd-Leifer CA, Block EF, Takeda K, Akira S, Ding A. The role of MyD88 and TLR4 in the LPS-mimetic activity of Taxol. *European Journal of Immunology*. 2001;**31**:2448-2457. DOI: 10.1002/1521-4141(200108)31:8<2448::AID-IMMU2448qt:3.0.CO;2-N

[120] Silasi DA, Alvero AB, Illuzzi J, Kelly M, Chen R, Fu HH, et al. MyD88

predicts chemoresistance to paclitaxel in epithelial ovarian cancer. *The Yale Journal of Biology and Medicine*. 2006;**79**:153-163

[121] Wang AC, Su QB, Wu FX, Zhang XL, Liu PS. Role of TLR4 for paclitaxel chemotherapy in human epithelial ovarian cancer cells. *European Journal of Clinical Investigation*. 2009;**39**:157-164. DOI: 10.1111/j.1365-2362.2008.02070.x

[122] Wee ZN, Yatim SM, Kohlbauer VK, Feng M, Goh JY, Bao Y, et al. IRAK1 is a therapeutic target that drives breast cancer metastasis and resistance to paclitaxel. *Nature Communications*. 2015;**6**:8746. DOI: 10.1038/ncomms9746

[123] Le XF, Hittelman WN, Liu J, McWatters A, Li C, Mills GB, et al. Paclitaxel induces inactivation of p70 S6 kinase and phosphorylation of Thr421 and Ser424 via multiple signaling pathways in mitosis. *Oncogene*. 2003;**22**:484-497. DOI: 10.1038/sj.onc.1206175

[124] Subbaramaiah K, Hart JC, Norton L, Dannenberg AJ. Microtubule-interfering agents stimulate the transcription of cyclooxygenase-2. Evidence for involvement of ERK1/2 AND p38 mitogen-activated protein kinase pathways. *Journal of Biological Chemistry*. 2000;**275**:14838-14845. pii: 275/20/14838

[125] Wang Y, Qu Y, Niu XL, Sun WJ, Zhang XL, Li LZ. Autocrine production of interleukin-8 confers cisplatin and paclitaxel resistance in ovarian cancer cells. *Cytokine*. 2011;**56**:365-375. DOI: 10.1016/j.cyto.2011.06.005

[126] Huang Y, Fan W. IkappaB kinase activation is involved in regulation of paclitaxel-induced apoptosis in human tumor cell lines. *Molecular Pharmacology*. 2002;**61**:105-113

[127] Sumitomo M, Asano T, Asakuma J, Asano T, Horiguchi A, Hayakawa M. ZD1839 modulates paclitaxel response in renal cancer by blocking paclitaxel-induced activation of the epidermal growth factor receptor-extracellular signal-regulated kinase pathway. *Clinical Cancer Research*. 2004;**10**:794-801

[128] Lee LF, Haskill JS, Mukaida N, Matsushima K, Ting JP. Identification of tumor-specific paclitaxel (Taxol)-responsive regulatory elements in the interleukin-8 promoter. *Molecular and Cellular Biology*. 1997;**17**:5097-5105

[129] Kang HJ, Lee SH, Price JE, Kim LS. Curcumin suppresses the paclitaxel-induced nuclear factor-kappaB in breast cancer cells and potentiates the growth inhibitory effect of paclitaxel in a breast cancer nude mice model. *The Breast Journal*. 2009;**15**:223-229. DOI: 10.1111/j.1524-4741.2009.00709.x

[130] Volk LD, Flister MJ, Chihade D, Desai N, Trieu V, Ran S. Synergy of nab-paclitaxel and bevacizumab in eradicating large orthotopic breast tumors and preexisting metastases. *Neoplasia*. 2011;**13**:327-338

[131] Quintavalle M, Elia L, Price JH, Heynen-Genel S, Courtneidge SA. A cell-based high-content screening assay reveals activators and inhibitors of cancer cell invasion. *Science Signaling*. 2011;**4**:ra49. DOI: 10.1126/scisignal.2002032

[132] Altorki NK, Port JL, Zhang F, Golijanin D, Thaler HT, Duffield-Lillico AJ, et al. Chemotherapy induces the expression of cyclooxygenase-2 in non-small cell lung cancer. *Clinical Cancer Research*. 2005;**11**:4191-4197. DOI: 10.1158/1078-0432.CCR-05-0108

[133] Furstemberger G, von Moos R, Lucas R, Thurlimann B, Senn HJ, Hamacher J, et al. Circulating endothelial cells and angiogenic

serum factors during neoadjuvant chemotherapy of primary breast cancer. *British Journal of Cancer*. 2006;**94**:524-531. DOI: 10.1038/sj.bjc.6602952

[134] DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. *Cancer Discovery*. 2011;**1**:54-67. DOI: 10.1158/2159-8274.CD-10-0028

[135] Zhang P, He D, Chen Z, Pan Q, Du F, Zang X, et al. Chemotherapy enhances tumor vascularization via notch signaling-mediated formation of tumor-derived endothelium in breast cancer. *Biochemical Pharmacology*. 2016;**118**:18-30. DOI: 10.1016/j.bcp.2016.08.008

[136] Volk-Draper L, Hall K, Griggs C, Rajput S, Kohio P, DeNardo D, et al. Paclitaxel therapy promotes breast cancer metastasis in a TLR4-dependent manner. *Cancer Research*. 2014;**74**:5421-5434. DOI: 10.1158/0008-5472.CAN-14-0067

[137] Harris AR, Perez MJ, Munson JM. Docetaxel facilitates lymphatic-tumor crosstalk to promote lymphangiogenesis and cancer progression. *BMC Cancer*. 2018;**18**:718. DOI: 10.1186/s12885-018-4619-8

[138] Taxman DJ, MacKeigan JP, Clements C, Bergstralh DT, Ting JP. Transcriptional profiling of targets for combination therapy of lung carcinoma with paclitaxel and mitogen-activated protein/extracellular signal-regulated kinase kinase inhibitor. *Cancer Research*. 2003;**63**:5095-5104

[139] Wu J, Guan M, Wong PF, Yu H, Dong J, Xu J. Icariside II potentiates paclitaxel-induced apoptosis in human melanoma A375 cells by inhibiting TLR4 signaling pathway. *Food and Chemical Toxicology*. 2012;**50**:3019-3024. DOI: 10.1016/j.fct.2012.06.027

[140] Matsunaga N, Tsuchimori N, Matsumoto T, Ii M. TAK-242 (resatorvid), a small-molecule inhibitor of Toll-like receptor (TLR) 4 signaling, binds selectively to TLR4 and interferes with interactions between TLR4 and its adaptor molecules. *Molecular Pharmacology*. 2011;**79**:34-41. DOI: 10.1124/mol.110.068064

[141] Kirikae F, Kirikae T, Qureshi N, Takayama K, Morrison DC, Nakano M. CD14 is not involved in Rhodobacter sphaeroides diphosphoryl lipid A inhibition of tumor necrosis factor alpha and nitric oxide induction by taxol in murine macrophages. *Infection and Immunity*. 1995;**63**:486-497

[142] Kawasaki K, Akashi S, Shimazu R, Yoshida T, Miyake K, Nishijima M. Involvement of TLR4/MD-2 complex in species-specific lipopolysaccharide-mimetic signal transduction by Taxol. *Journal of Endotoxin Research*. 2001;**7**:232-236

[143] Resman N, Gradisar H, Vasl J, Keber MM, Pristovsek P, Jerala R. Taxanes inhibit human TLR4 signaling by binding to MD-2. *FEBS Letters*. 2008;**582**:3929-3934. DOI: 10.106/j.febslet.2008.10.037

[144] Bald T, Quast T, Landsberg J, Rogava M, Glodde N, Lopez-Ramos D, et al. Ultraviolet-radiation-induced inflammation promotes angiogenesis and metastasis in melanoma. *Nature*. 2014;**507**:109-113. DOI: 10.1038/nature13111

[145] Shaw G, Morse S, Ararat M, Graham FL. Preferential transformation of human neuronal cells by human adenoviruses and the origin of HEK 293 cells. *The FASEB Journal*. 2002;**16**:869-871. DOI: 10.1096/fj.01-0995fje

[146] Sims GP, Rowe DC, Rietdijk ST, Herbst R, Coyle AJ. HMGB1 and RAGE in inflammation and Cancer. *Annual Review of Immunology*.

- 2010;**28**:367-388. DOI: 10.1146/annurev.immunol.021908.132603
- [147] Hiratsuka S, Watanabe A, Sakurai Y, Kashi-Takamura S, Ishibashi S, Miyake K, et al. The S100A8-serum amyloid A3-TLR4 paracrine cascade establishes a pre-metastatic phase. *Nature Cell Biology*. 2008;**10**:1349-1355. DOI: 10.1038/ncb1794
- [148] Okamura Y, Watari M, Jerud ES, Young DW, Ishizaka ST, Rose J, et al. The extra domain A of fibronectin activates Toll-like receptor 4. *The Journal of Biological Chemistry*. 2001;**276**:10229-10233. DOI: 10.1074/jbc.M100099200
- [149] Wei F, Yang D, Tewary P, Li Y, Li S, Chen X, et al. The alarmin HMGN1 contributes to anti-tumor immunity and is a potent immunoadjuvant. *Cancer Research*. 2014;**74**:5989-5998. DOI: 10.1158/0008-5472.CAN-13-2042
- [150] Cohen MJ, Brohi K, Calfee CS, Rahn P, Chesebro BB, Christiaans SC, et al. Early release of high mobility group nuclear protein 1 after severe trauma in humans: Role of injury severity and tissue hypoperfusion. *Critical Care*. 2009;**13**:R174. DOI: 10.1186/cc8152
- [151] Kelsh RM, McKeown-Longo PJ. Topographical changes in extracellular matrix: Activation of TLR4 signaling and solid tumor progression. *Trends in Cancer Research*. 2013;**9**:1-13
- [152] Kallberg E, Vogl T, Liberg D, Olsson A, Bjork P, Wikstrom P, et al. S100A9 interaction with TLR4 promotes tumor growth. *PLoS One*. 2012;**7**:e34207. DOI: 10.1371/journal.pone.0034207
- [153] Ferrandez E, Gutierrez O, Segundo DS, Fernandez-Luna JL. NFkappaB activation in differentiating glioblastoma stem-like cells is promoted by hyaluronic acid signaling through TLR4. *Scientific Reports*. 2018;**8**:6341. DOI: 10.1038/s41598-018-24444-6
- [154] Xu X, Zhu H, Wang T, Sun Y, Ni P, Liu Y, et al. Exogenous high-mobility group box 1 inhibits apoptosis and promotes the proliferation of Lewis cells via RAGE/TLR4-dependent signal pathways. *Scandinavian Journal of Immunology*. 2014;**79**:386-394. DOI: 10.1111/sji.12174
- [155] Furlani D, Donndorf P, Westien I, Ugurlucan M, Pittermann E, Wang W, et al. HMGB-1 induces c-kit+ cell microvascular rolling and adhesion via both Toll-like receptor-2 and Toll-like receptor-4 of endothelial cells. *Journal of Cellular and Molecular Medicine*. 2012;**16**:1094-1105. DOI: 10.1111/j.1582-4934.2011.01381.x
- [156] Lin Q, Yang XP, Fang D, Ren X, Zhou H, Fang J, et al. High-mobility group box-1 mediates Toll-like receptor 4-dependent angiogenesis. *Arteriosclerosis, Thrombosis, and Vascular Biology*. 2011;**31**:1024-1032. DOI: 10.1161/ATVBAHA.111.224048
- [157] Han L, Zhang M, Wang M, Jia J, Zhao M, Fan Y, et al. High mobility group Box-1 promotes inflammation-induced lymphangiogenesis via Toll-like receptor 4-dependent signalling pathway. *PLoS One*. 2016;**11**:e0154187. DOI: 10.1371/journal.pone.0154187
- [158] Mittal D, Saccheri F, Venereau E, Pusterla T, Bianchi ME, Rescigno M. TLR4-mediated skin carcinogenesis is dependent on immune and radioresistant cells. *The EMBO Journal*. 2010;**29**:2242-2252. DOI: 10.1038/emboj.2010.94
- [159] Yan W, Chang Y, Liang X, Cardinal JS, Huang H, Thorne SH, et al. High-mobility group box 1 activates caspase-1 and promotes hepatocellular carcinoma invasiveness and metastases. *Hepatology*. 2012;**55**:1863-1875. DOI: 10.1002/hep.25572

- [160] Galluzzi L, Buque A, Kepp O, Zitvogel L, Kroemer G. Immunogenic cell death in cancer and infectious disease. *Nature Reviews. Immunology*. 2017;**17**:97-111. DOI: 10.1038/nri.2016.107
- [161] Riddell JR, Bshara W, Moser MT, Sperry JA, Foster BA, Gollnick SO. Peroxiredoxin 1 controls prostate cancer growth through Toll-like receptor 4-dependent regulation of tumor vasculature. *Cancer Research*. 2011;**71**:1637-1646. DOI: 10.1158/0008-5472.CAN-10-3674
- [162] Tsan MF, Gao B. Endogenous ligands of Toll-like receptors. *Journal of Leukocyte Biology*. 2004;**76**:514-519. DOI: 10.1189/jlb.0304127
- [163] Maru Y, Tomita T, Deguchi A, Ieguchi K, Takita M, Tsukahara F, et al. Drug targeting based on a new concept—targeting against TLR4 as an example. *Endocrine, Metabolic & Immune Disorders—Drug Targets*. 2015;**15**:83-87
- [164] Ehrchen JM, Sunderkotter C, Foell D, Vogl T, Roth J. The endogenous Toll-like receptor 4 agonist S100A8/S100A9 (calprotectin) as innate amplifier of infection, autoimmunity, and cancer. *Journal of Leukocyte Biology*. 2009;**86**:557-566. DOI: 10.1189/jlb.1008647
- [165] Basith S, Manavalan B, Yoo TH, Kim SG, Choi S. Roles of Toll-like receptors in cancer: A double-edged sword for defense and offense. *Archives of Pharmacal Research*. 2012;**35**:1297-1316.1. DOI: 10.1007/s12272-012-0802-7
- [166] Kim JY, Kim YJ, Kim JS, Ryu HS, Lee HK, Kang JS, et al. Adjuvant effect of a natural TLR4 ligand on dendritic cell-based cancer immunotherapy. *Cancer Letters*. 2011;**313**:226-234. DOI: 10.1016/j.canlet.2011.09.009
- [167] Grobner S, Lukowski R, Autenrieth IB, Ruth P. Lipopolysaccharide induces cell volume increase and migration of dendritic cells. *Microbiology and Immunology*. 2013;**8**:61-67. DOI: 10.1111/1348-0421.12116
- [168] Apetoh L, Ghiringhelli F, Tesniere A, Obeid M, Ortiz C, Criollo A, et al. Toll-like receptor 4-dependent contribution of the immune system to anticancer chemotherapy and radiotherapy. *Nature Medicine*. 2007;**13**:1050-1059. DOI: 10.1038/nm1622
- [169] Matsuzawa A, Saegusa K, Noguchi T, Sadamitsu C, Nishitoh H, Nagai S, et al. ROS-dependent activation of the TRAF6-ASK1-p38 pathway is selectively required for TLR4-mediated innate immunity. *Nature Immunology*. 2005;**6**:587-592. DOI: 10.1038/ni1200
- [170] Wolska A, Lech-Maranda E, Robak T. Toll-like receptors and their role in carcinogenesis and anti-tumor treatment. *Cellular and Molecular Biology Letters*. 2009;**14**:248-272. DOI: 10.2478/s11658-008-0048-z
- [171] Chen J, Sun B, Zhao X, Liang D, Liu J, Huang Y, et al. Monophosphoryl lipid a induces bone marrow precursor cells to differentiate into myeloid-derived suppressor cells. *Molecular Medicine Reports*. 2013;**8**:1074-1078. DOI: 10.3892/mmr.2013.1653
- [172] Roodhart JM, He H, Daenen LG, Monvoisin A, Barber CL, van AM, et al. Notch1 regulates angio-supportive bone marrow-derived cells in mice: Relevance to chemoresistance. *Blood*. 2013;**122**:143-153. DOI: 10.1182/blood-2012-11-459347
- [173] Zhu XM, Yao YM, Liang HP, Xu CT, Dong N, Yu Y, et al. High mobility group box-1 protein regulate immunosuppression of regulatory T cells through Toll-like receptor 4. *Cytokine*. 2011;**54**:296-304. DOI: 10.1016/j.cyto.2011.02.017

- [174] Egunsola AT, Zawislak CL, Akuffo AA, Chalmers SA, Ewer JC, Vail CM, et al. Growth, metastasis, and expression of CCL2 and CCL5 by murine mammary carcinomas are dependent upon Myd88. *Cellular Immunology*. 2012;**272**:220-229. DOI: 10.1016/j.cellimm.2011.10.008
- [175] Li J, Yin J, Shen W, Gao R, Liu Y, Chen Y, et al. TLR4 promotes breast Cancer metastasis via Akt/GSK3beta/beta-catenin pathway upon LPS stimulation. *The Anatomical Record (Hoboken.)*. 2017;**300**:1219-1229. DOI: 10.1002/ar.23590
- [176] Tang X, Zhu Y. TLR4 signaling promotes immune escape of human colon cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. *Oncology Research*. 2012;**20**:15-24
- [177] Wang L, Zhu R, Huang Z, Li H, Zhu H. Lipopolysaccharide-induced Toll-like receptor 4 signaling in cancer cells promotes cell survival and proliferation in hepatocellular carcinoma. *Digestive Diseases and Sciences*. 2013;**58**:2223-2236. DOI: 10.1007/s10620-013-2745-3
- [178] Wang L, Liu Q, Sun Q, Zhang C, Chen T, Cao X. TLR4 signaling in cancer cells promotes chemoattraction of immature dendritic cells via autocrine CCL20. *Biochemical and Biophysical Research Communications*. 2008;**366**:852-856. DOI: 10.1016/j.bbrc.2007.12.030
- [179] Yang H, Wang B, Wang T, Xu L, He C, Wen H, et al. Toll-like receptor 4 prompts human breast cancer cells invasiveness via lipopolysaccharide stimulation and is overexpressed in patients with lymph node metastasis. *PLoS One*. 2014;**9**:e109980. DOI: 10.1371/journal.pone.0109980
- [180] Chung YH, Kim D. Enhanced TLR4 expression on colon cancer cells after chemotherapy promotes cell survival and epithelial-Mesenchymal transition through phosphorylation of GSK3beta. *Anticancer Research*. 2016;**36**:3383-3394
- [181] Pei Z, Li H, Guo Y, Jin Y, Lin D. Sodium selenite inhibits the expression of VEGF, TGFbeta(1) and IL-6 induced by LPS in human PC3 cells via TLR4-NF-(K)B signaling blockage. *International Immunopharmacology*. 2010;**10**:50-56. DOI: 10.1016/j.intimp.2009.09.020
- [182] Li C, Li H, Jiang K, Li J, Gai X. TLR4 signaling pathway in mouse Lewis lung cancer cells promotes the expression of TGF-beta1 and IL-10 and tumor cells migration. *Biomedical Materials and Engineering*. 2014;**24**: 869-875. DOI: 10.3233/BME-130879
- [183] Che F, Yin J, Quan Y, Xie X, Heng X, Du Y, et al. TLR4 interaction with LPS in glioma CD133+ cancer stem cells induces cell proliferation, resistance to chemotherapy and evasion from cytotoxic T lymphocyte-induced cytotoxicity. *Oncotarget*. 2017;**8**:53495-53507. DOI: 10.18632/oncotarget.18586
- [184] Jain S, Suklabaidya S, Das B, Raghav SK, Batra SK, Senapati S. TLR4 activation by lipopolysaccharide confers survival advantage to growth factor deprived prostate cancer cells. *The Prostate*. 2015;**75**:1020-1033. DOI: 10.1002/pros.22983
- [185] Andreani V, Gatti G, Simonella L, Rivero V, Maccioni M. Activation of Toll-like receptor 4 on tumor cells in vitro inhibits subsequent tumor growth in vivo. *Cancer Research*. 2007;**67**:10519-10527. DOI: 10.1158/0008-5472.CAN-07-0079
- [186] Liu J, Xu D, Wang Q, Zheng D, Jiang X, Xu L. LPS induced miR-181a promotes pancreatic cancer cell migration via targeting PTEN and MAP2K4. *Digestive Diseases and*

Sciences. 2014;**59**:1452-1460. DOI: 10.1007/s10620-014-3049-y

[187] Zimmer SM, Liu J, Clayton JL, Stephens DS, Snyder JP. Paclitaxel binding to human and murine MD-2. *The Journal of Biological Chemistry*. 2008;**283**:27916-27926. DOI: 10.1074/jbc.M802826200

[188] Ran S. The role of TLR4 in chemotherapy-driven metastasis. *Cancer Research*. 2015;**75**:2405-2410. DOI: 10.1158/0008-5472.CAN-14-3525

[189] Yang H, Zhou H, Feng P, Zhou X, Wen H, Xie X, et al. Reduced expression of Toll-like receptor 4 inhibits human breast cancer cells proliferation and inflammatory cytokines secretion. *Journal of Experimental and Clinical Cancer Research*. 2010;**29**:92. DOI: 10.1186/1756-9966-29-92

[190] Ohnishi H, Tochio H, Kato Z, Kawamoto N, Kimura T, Kubota K, et al. TRAM is involved in IL-18 signaling and functions as a sorting adaptor for MyD88. *PLoS One*. 2012;**7**:e38423. DOI: 10.1371/journal.pone.0038423

[191] Desbien AL, Dubois CN, Reed SJ, Bailor HR, Liang H, Carter D, et al. IL-18 and subcapsular lymph node macrophages are essential for enhanced B cell responses with TLR4 agonist adjuvants. *Journal of Immunology*. 2016;**197**:4351-4359. DOI: 10.4049/jimmunol.1600993

[192] Luu K, Greenhill CJ, Majoros A, Decker T, Jenkins BJ, Mansell A. STAT1 plays a role in TLR signal transduction and inflammatory responses. *Immunology and Cell Biology*. 2014;**92**:761-769. DOI: 10.1038/icb.2014.51

[193] Pietras K, Ostman A. Hallmarks of cancer: Interactions with the tumor stroma. *Experimental Cell Research*. 2010;**316**:1324-1331. DOI: 10.1016/j.yexcr.2010.02.045

[194] Quail DF, Joyce JA. Micro-environmental regulation of tumor progression and metastasis. *Nature Medicine*. 2013;**19**:1423-1437. DOI: 10.1038/nm.3394

[195] Guzzo C, Ayer A, Basta S, Banfield BW, Gee K. IL-27 enhances LPS-induced proinflammatory cytokine production via upregulation of TLR4 expression and signaling in human monocytes. *Journal of Immunology*. 2012;**188**:864-873. DOI: 10.4049/jimmunol.1101912

[196] Liguori M, Solinas G, Germano G, Mantovani A, Allavena P. Tumor-associated macrophages as incessant builders and destroyers of the cancer stroma. *Cancers (Basel)*. 2011;**3**:3740-3761. DOI: 10.3390/cancers3043740

[197] Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancers (Basel)*. 2014;**6**:1670-1690. DOI: 10.3390/cancers6031670

[198] Schoppmann SF, Fenzl A, Nagy K, Unger S, Bayer G, Geleff S, et al. VEGF-C expressing tumor-associated macrophages in lymph node positive breast cancer: Impact on lymphangiogenesis and survival. *Surgery*. 2006;**139**:839-846. DOI: 10.1016/j.surg.2005.12.008

[199] Ijichi H, Chytil A, Gorska AE, Aakre ME, Bierie B, Tada M, et al. Inhibiting Cxcr2 disrupts tumor-stromal interactions and improves survival in a mouse model of pancreatic ductal adenocarcinoma. *The Journal of Clinical Investigation*. 2011;**121**:4106-4117. DOI: 10.1172/JCI42754

[200] Eiro N, Gonzalez L, Gonzalez LO, Fernandez-Garcia B, Andicoechea A, Barbon E, et al. Toll-like receptor-4 expression by stromal fibroblasts is associated with poor prognosis in colorectal cancer. *Journal of*

Immunotherapy. 2013;**36**:342-349. DOI: 10.1097/CJI.0b013e31829d85e6

[201] Radisky ES, Radisky DC. Stromal induction of breast cancer: Inflammation and invasion. *Reviews in Endocrine and Metabolic Disorders*. 2007;**8**:279-287. DOI: 10.1007/s11154-007-9037-1

[202] Bhattacharyya S, Wang W, Qin W, Cheng K, Coulup S, Chavez S, et al. TLR4-dependent fibroblast activation drives persistent organ fibrosis in skin and lung. *JCI Insight*. 2018;**3**:1-17. DOI: 10.1172/jci.insight.98850

[203] Zhao XL, Lin Y, Jiang J, Tang Z, Yang S, Lu L, et al. HMGB1 released by autophagic cancer-associated fibroblasts maintains the stemness of luminal breast cancer cells. *The Journal of Pathology*. 2017;**243**:376-389. DOI: 10.1002/path.4958

[204] Kim SH, Das A, Chai JC, Binas B, Choi MR, Park KS, et al. Transcriptome sequencing wide functional analysis of human mesenchymal stem cells in response to TLR4 ligand. *Scientific Reports*. 2016;**6**:30311. DOI: 10.1038/srep30311

[205] Eruslanov E, Stoffs T, Kim WJ, Daurkin I, Gilbert SM, Su LM, et al. Expansion of CCR8(+) inflammatory myeloid cells in cancer patients with urothelial and renal carcinomas. *Clinical Cancer Research*. 2013;**19**:1670-1680. DOI: 10.1158/1078-0432.CCR-12-2091

[206] Ahn GO, Brown JM. Role of endothelial progenitors and other bone marrow-derived cells in the development of the tumor vasculature. *Angiogenesis*. 2009;**12**:159-164. DOI: 10.1007/s10456-009-9135-7

[207] Ran S, Wilber A. Novel role of immature myeloid cells in formation of new lymphatic vessels associated with inflammation and tumors. *Journal of*

Leukocyte Biology. 2017;**102**:253-263. DOI: 10.1189/jlb.1MR1016-434RR

[208] Hijiya N, Miyake K, Akashi S, Matsuura K, Higuchi Y, Yamamoto S. Possible involvement of Toll-like receptor 4 in endothelial cell activation of larger vessels in response to lipopolysaccharide. *Pathobiology*. 2002;**70**:18-25. DOI: 10.1159/000066000

[209] Zabini D, Crnkovic S, Xu H, Tscherner M, Ghanim B, Klepetko W, et al. High-mobility group box-1 induces vascular remodelling processes via c-Jun activation. *Journal of Cellular and Molecular Medicine*. 2015;**19**:1151-1161. DOI: 10.1111/jcmm.12519

[210] Xia S, Menden HL, Korfhagen TR, Kume T, Sampath V. Endothelial immune activation programmes cell-fate decisions and angiogenesis by inducing angiogenesis regulator DLL4 through TLR4-ERK-FOXC2 signalling. *The Journal of Physiology*. 2018;**596**:1397-1417. DOI: 10.1113/JP275453

[211] Sun Y, Wu C, Ma J, Yang Y, Man X, Wu H, et al. Toll-like receptor 4 promotes angiogenesis in pancreatic cancer via PI3K/AKT signaling. *Experimental Cell Research*. 2016;**347**:274-282. DOI: 10.1016/j.yexcr.2016.07.009

[212] Ma B, Dohle E, Li M, Kirkpatrick CJ. TLR4 stimulation by LPS enhances angiogenesis in a co-culture system consisting of primary human osteoblasts and outgrowth endothelial cells. *Journal of Tissue Engineering and Regenerative Medicine*. 2017;**11**:1779-1791. DOI: 10.1002/term.2075

[213] Hellstrom M, Phng LK, Hofmann JJ, Wallgard E, Coultas L, Lindblom P, et al. Dll4 signalling through Notch1 regulates formation of tip cells during angiogenesis. *Nature*. 2007;**445**:776-780. DOI: 10.1038/nature05571

- [214] Zhu Y, Maric J, Nilsson M, Brannstrom M, Janson PO, Sundfeldt K. Formation and barrier function of tight junctions in human ovarian surface epithelium. *Biology of Reproduction*. 2004;**71**:53-59. DOI: 10.1095/biolreprod.103.022913
- [215] Betterman KL, Harvey NL. The lymphatic vasculature: Development and role in shaping immunity. *Immunological Reviews*. 2016;**271**: 276-292. DOI: 10.1111/imr.12413
- [216] Keklikoglou I, Cianciaruso C, Guc E, Squadrito ML, Spring LM, Tazzyman S, et al. Chemotherapy elicits pro-metastatic extracellular vesicles in breast cancer models. *Nature Cell Biology*. 2018;**21**:190-202. DOI: 10.1038/s41556-018-0256-3
- [217] Brown M, Assen FP, Leithner A, Abe J, Schachner H, Asfour G, et al. Lymph node blood vessels provide exit routes for metastatic tumor cell dissemination in mice. *Science*. 2018;**359**:1408-1411. DOI: 10.1126/science.aal3662
- [218] Ran S, Volk L, Hall K, Flister MJ. Lymphangiogenesis and lymphatic metastasis in breast cancer. *Pathophysiology*. 2009;**17**:229-251. DOI: 10.1016/j.pathophys.2009.11.003
- [219] Lee LK, Ghorbanian Y, Wang W, Wang Y, Kim YJ, Weissman IL, et al. LYVE1 marks the divergence of yolk sac definitive hemogenic endothelium from the primitive erythroid lineage. *Cell Reports*. 2016;**17**:2286-2298. DOI: 10.1016/j.celrep.2016.10.080
- [220] Bailey AS, Willenbring H, Jiang S, Anderson DA, Schroeder DA, Wong MH, et al. Myeloid lineage progenitors give rise to vascular endothelium. *Proceedings of the National Academy of Sciences of the United States of America*. 2006;**103**:13156-13161. DOI: 10.1073/pnas.0604203103
- [221] Nakamura Y, Suzuki S, Shimizu T, Miyata M, Shishido T, Ikeda K, et al. High mobility group box 1 promotes angiogenesis from bone marrow-derived endothelial progenitor cells after myocardial infarction. *Journal of Atherosclerosis and Thrombosis*. 2015;**122**:570-581. DOI: 10.5551/jat.27235
- [222] Fukata M, Hernandez Y, Conduah D, Cohen J, Chen A, Breglio K, et al. Innate immune signaling by Toll-like receptor-4 (TLR4) shapes the inflammatory microenvironment in colitis-associated tumors. *Inflammatory Bowel Diseases*. 2009;**15**:997-1006. DOI: 10.1002/jbd.20880
- [223] Voloshin T, Voest EE, Shaked Y. The host immunological response to cancer therapy: An emerging concept in tumor biology. *Experimental Cell Research*. 2013;**319**:1687-1695. DOI: 10.1016/j.yexcr.2013.03.007
- [224] Daenen LG, Houthuijzen JM, Cirkel GA, Roodhart JM, Shaked Y, Voest EE. Treatment-induced host-mediated mechanisms reducing the efficacy of antitumor therapies. *Oncogene*. 2014;**33**:1341-1347. DOI: 10.1038/onc.2013.94
- [225] Pusztai L, Mendoza TR, Reuben JM, Martinez MM, Willey JS, Lara J, et al. Changes in plasma levels of inflammatory cytokines in response to paclitaxel chemotherapy. *Cytokine*. 2004;**25**:94-102
- [226] Liu G, Chen Y, Qi F, Jia L, Lu XA, He T, et al. Specific chemotherapeutic agents induce metastatic behaviour through stromal- and tumour-derived cytokine and angiogenic factor signalling. *The Journal of Pathology*. 2015;**237**:190-202. DOI: 10.1002/path.4564
- [227] Roodhart JM, Langenberg MH, Vermaat JS, Lolkema MP, Baars A, Giles RH, et al. Late release of circulating endothelial cells and endothelial

progenitor cells after chemotherapy predicts response and survival in cancer patients. *Neoplasia*. 2010;**12**:87-94

[228] Ruffell B, Au A, Rugo HS, Esserman LJ, Hwang ES, Coussens LM. Leukocyte composition of human breast cancer. *Proceedings of the National Academy of Sciences of the United States of America*. 2012;**109**:2796-2801. DOI: 10.1073/pnas.1104303108

[229] Gings-Velitski S, Loven D, Benayoun L, Munster M, Bril R, Voloshin T, et al. Host response to short-term, single-agent chemotherapy induces matrix metalloproteinase-9 expression and accelerates metastasis in mice. *Cancer Research*. 2011;**71**: 6986-6996. DOI: 10.1158/0008-5472.CAN-11-0629

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