Toll-Like Receptors (TLRs): The Role in Tumor Progression

D.V. Shcheblyakov, D.Y. Logunov*, A.I. Tukhvatulin, M.M. Shmarov, B.S. Naroditsky, A.L. Gintsburg

Gamaleya Research Institute of Epidemiology and Microbiology, Russian Academy of Medical Sciences

*E-mail: ldenisy@yahoo.com

Received 28.08.2010

ABSTRACT **Toll-like receptors (TLRs) are major components of the innate immune system that recognize the conserved molecular structures of pathogens (pathogen-associated molecular patterns; PAMPs). TLRs are found in many different cell types, ranging from epithelial to immunocompetent cells. TLR binding triggers the expression of several adapter proteins and downstream kinases, leading to the induction of key pro-inflammatory mediators. This results in the activation of both the innate immune response (elevated expression of antiapoptotic proteins, proinflammatory cytokines, and antibacterial proteins), as well as the adaptive immune response (maturation of the dendritic cells, antigen presentation, etc.). In consequence of their ability to enhance the specific and nonspecific immune reactions of an organism, TLR agonists are widely used in the therapy of infectious diseases and, as adjuvants, in the therapy of malignant neoplasia. However, to date, TLRs have had the opposite effects on tumor progression. On the one hand, TLR ligands can suppress tumor growth. On the other hand, TLR agonists can promote the survival of malignant cells and increase their resistance to chemotherapy. The purpose of this review is to summarize the available data on the effects of TLRs and their agonists on tumor progression, as well as the mechanisms underlying the differences in the effects of TLRs on tumor growth.**

KEYWORDS **toll-like receptors, agonists of innate immune receptors, tumor progression, innate immune response, inflammation**

ABBREVIATIONS **TLR – toll-like receptor, LPS – lipopolysaccharide, NF-kB – nuclear transcription factor kB, PRR – pattern recognition receptor, PAMP – pathogen-associated molecular pattern, DAMP – damage-associated molecular pattern, IRF – interferon regulatory factor, ss- and dsRNA – single-stranded and double-stranded ribonucleic acid, TNF-α – tumor necrosis factor α, IL – interleukin, IFN – interferon, NK-cells – natural killers, siRNA – small interfering RNA, TGF – transforming growth factor**

INTRODUCTION

According to the modern concept, inflammation is one of the main causes behind the appearance and progression of a tumor [1, 2]. The causative mechanism of this phenomenon is not well understood, although some key events at the site of the inflammation, required for the appearance and progression of a tumor, are known.

1) The cells localized on the site of the inflammation sustain a high activity of the transcription factor NF-kB [3], which is responsible for the expression of anti-inflammatory cytokines, many of them (GROα,β,γ, IL-8, MIP-3α) exhibiting a tumor-stimulating action [4, 5]. Moreover, NF-kB is regarded as a major antiapoptotic factor, as it activates the expression of a series of genes that encode antiapoptotic proteins, such as IAP, Bcl-2, Bcl-X, etc. These proteins elevate the cells' resistance to various stress agents associated with inflammation [6, 7].

2) An inflammation is accompanied by the induction of the oxidative stress that causes the appearance and accumulation of mutations and genome rearrangements in cells [8].

3) Many anti-inflammatory cytokines (GROα/CXCL1, GROβ/CXCL2, GROγ/CXCL3, IL-8/CXCL8, MIP-3a, and IL-1) and growth factors (TGF-β1, PDGF, bFGF, TGF-α, IGF-I, IGF-II) that enhance the migration of stromal (fibroblasts) and epithelial cells, followed by their proliferation, are secreted at late stages of an inflammation [9]. In a chronic inflammation, processes of reparation and alteration often occur simultaneously, which enforces the cells to proliferate under hypoxia and genotoxic stress conditions, thus increasing the risk of mutations.

The most common and well-studied cause of inflammation is microbial invasion; the process by which the pathogen can affect, in one way or another, the host cell's homeostasis.

One of these mechanisms is the interaction of the pathogen's highly conserved molecular domains with pattern-recognizing receptors (PRR – RIG-I-like receptors, Nod-like receptors, C-type lectins, Toll-like receptors (TLRs), etc.) localized on the surface or intracellular membranes of eukaryotic cells [10].

Thanks to their capability to bind with various bacterial ligands, PRRs play a crucial role in the development of an inflammation, by initiating the development of the innate immune response through increasing the expression of some antiapoptotic proteins, anti-inflammatory

cytokines, and antibacterial proteins. They also stimulate the acquired immune response by inducing the maturation of dendrocytes, presentation of the captured antigen, and differentiation of naive T-helpers [11].

Therefore, studies focusing on the role of PRRs in the induction of tumorigenesis and stimulation of tumor progression in the course of bacterial infection are a pressing priority.

In this review, we focused our attention on the role of TLRs in the development of inflammatory processes and attempted to gauge their interrelationship with tumor progression.

The data accumulated to date suggest an association between TLRs and tumor growth. However, these data are contradictory; both the tumor-stimulating [12, 13] and tumor-suppressing [14, 15] effects of TRLs have been reported.

As such, we have sought to summarize the available data and brood over the possible mechanisms that underpin the variations in the observed effects of TLRs on tumor growth.

THE ROLE OF TLRs

TLRs function as members of the PRR family that mediates specific recognition of conserved Pathogen-Associated Molecular Patterns (PAMPs). TLRs activate the innate immune system by binding with PAMP and largely determine the development of the adaptive immune response [16, 17]. The most conserved role of TLRs is the activation of the antimicrobial immune response in both the skin and mucosa of respiratory, gastrointestinal, and urogenital tracts.

TLRs recognize microbial molecules, which results in the development of inflammatory reactions caused by the activation of the NF-kB regulating expression of anti-inflammatory cytokines (TNF-α, IL-1, IL-6, etc.) and chemokines (MCP-1, MCP-3, GM-CSF, etc.).

TLRs have been implicated in the transcriptional and posttranslational regulation (proteolytic cleavage and secretion) of antimicrobial factors, such as defensins α and β, phospholipase A2, lysozyme, and so on. [18]. TLRs intensify the phagocytosis of microorganisms and optimize their inactivation by regulating the release of peroxy radicals and nitric oxide [19, 20].

It is known that TLRs localized on the surface of endothelial cells indirectly support the migration of leucocytes into the inflammation focus by stimulating the expression of leucocyte adhesion molecules E-selectin and ICAM-1 [21].

The TLR stimulation directly leads to the elevation of interferon (IFN)-α/β production by both stromal and hematopoietic cells, which is important for the organism to defend itself against certain viral and bacterial infections [22]. Moreover, as was recently discovered, TLRs – via the activation of a series of factors, such as FADD, caspase 8, and protein kinase R (PKR) or the stimulation of IFN-α/β expression – can induce the development of apoptosis, an important mechanism of tissue protection against microbial pathogens [23].

TLRs play a crucial role in the regulation of the adaptive immune response. In particular, the TLR-dependent activation of professional antigen-presenting dendritic cells is determinative in several essential processes providing the development of the adaptive immune response, such as the activation of mature T-cells, processing and presentation of microbial antigens, elevation of the expression of the costimulatory molecules (CD80, CD86) required for the activation of naive CD4+-T-cells, and suppression of regulatory T-cells via IL-6 production [24]. The TLR-dependent activation is also important for B-cell proliferation and maturation during the infection process [25].

Thus, TLRs play an important role in initiating the development of the inflammatory process (activation of innate immune reactions) in response to the introduction of various pathogens (including protozoa, fungi, bacteria, and viruses). Moreover, according to the modern view, pathogen recognition by TLRs is the midpoint

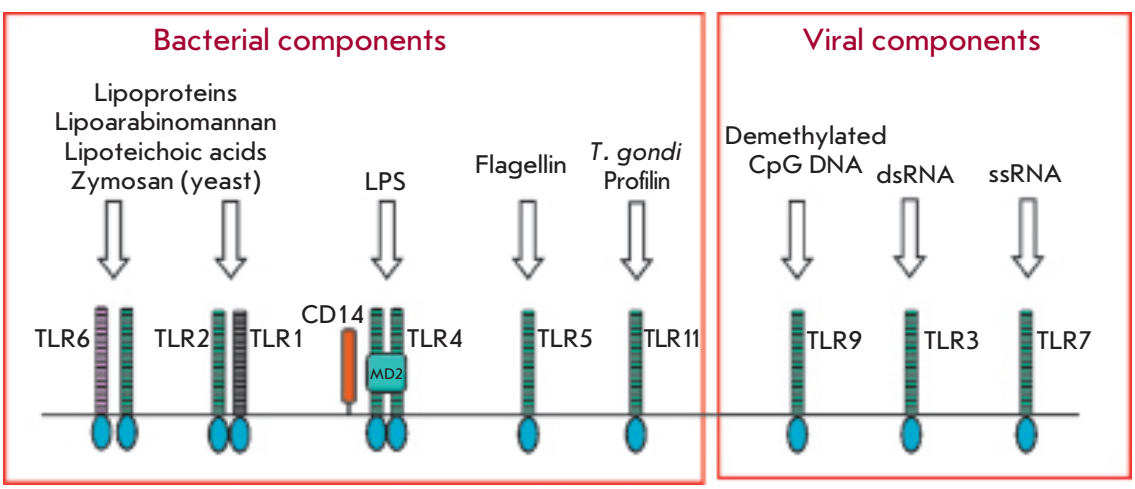

Fig. 1. Toll-like receptors and their ligands.

in the development of the adaptive immune response, which is the second line of defense [11]. TLRs also participate in the functioning of the normal gastrointestinal system and are implicated in the pathogenesis of autoimmune diseases (systemic lupus erythematosus), arthritis, atherosclerosis, and certain other disorders [26, 27]. Recent data indicate that TLRs can either activate antitumor immunity [28, 29] or, on the contrary, stimulate tumor progression [30, 31].

TLR STRUCTURE, EXPRESSION IN DIFFERENT CELL TYPES, AND SPECIFICITY TO VARIOUS MOLECULAR STRUCTURES (PAMP AND DAMP)

Based on their structure, TLRs are members of the family of IL-1 receptors (IL-1R). They are transmembrane proteins that are expressed on the cell's surface and in subcellular compartments (particularly endosomes). TLR localization depends on the type of the recognized ligand. For example, the TLRs 1, 2, 4, 5, and 6, which bind with structural bacterial components, are localized on the cell's surface, while the TLRs 3, 7, 8, and 9, primarily recognizing virus-associated nucleic acids (dsRNA, ssRNA, and DNA), are localized in endosomes, where they interact with the ligands that appear after the deproteination of virions [16].

The TLR's structure includes the N-terminal leucine-rich repeat (LRR) responsible for ligand binding, one transmembrane domain, and the C-terminal cytosolic signaling domain (homologous to the intracellular domain of IL-1R) [32].

TLRs are expressed in most types of cells in the human body, including non-hematopoietic epithelial and endothelial cells. The number of simultaneously expressed TLRs and their combination are specific for each cell type; the largest number is observed in cells of hematopoietic origin, such as macrophages, neutrophils, and dendritic cells (Table 1) [33].

To date, 13 different TLRs have been identified in mammals: 10 in humans and 12 in mice. The TLRs 1 through 9 are conserved in humans and mice; however, some difference exists. The gene encoding TLR10 is only found in humans, whereas the TLR11 gene is present in both species, but possesses functionality in mouse only [34].

The hallmark of TLRs that distinguishes them from the receptors of acquired immunity (T- and B-cell receptors) is their capability to recognize not just unique epitopes, but also the evolutionary-conserved pathogen-associated molecular patterns (PAMP), which are widely distributed in all taxa of microorganisms and viruses, regardless of their pathogenicity.

The specificity of PAMP recognition is well studied for the majority of TLRs. The ligands of TLR 1–9 and 11 are now known (Fig. 1). The biological role and the specificity of human TLR10 and murine TLR 12 and TLR 13 remain unclear [16].

Table 1. Activation of the transcription factors NF-κB and IRF by different TLRs.

TLR type	Activation of NF-κB	Activation of IRF
TLR2/1/6	+	-
TLR3	+	+ (IRF3)
TLR4	+	+ (IRF3)
TLR5	+	-
TLR7	+	+ (IRF5, 7)
TLR8	+	+(IRF5, 7)
TLR9	+	+(IRF5, 7)

The most common microbial ligands of TLRs are the following:

- bacterial lipopeptides, lipoteichoic acid (LTA), and peptidoglycans; mycobacterial lipoarabinomannan; and zymosan from the fungal cell wall – which bind with TLR2 that forms heterodimers with TLR1, TLR6, and CD14;
- LPS of Gram-negative bacteria; the TLR4 ligand;
- flagellin, a principal component of bacterial flagella that activates TLR5;
- protozoan profilin-like structures binding with TLR11;
- DNA (demethylated CpG-islets) recognized by TLR9;
- dsRNA, the TLR3 ligand;
- ssRNA, the TLR7 and the TLR8 ligand.

It was shown recently that TLRs can be activated by many endogenous molecules, the so-called alarmins (hyaluronic acid, heat shock proteins, etc.), appearing in tissue decay. These heterogeneous in their nature and structure (PAMP and alarmins) substances recognized by TLRs are presently allied to one family called DAMP (Damage Associated Molecular Patterns) [35].

SIGNALING CASCADE ACTIVATED FOLLOWING THE INTERACTION OF TLRS WITH THEIR OWN LIGANDS

Now, after describing the TLR's structure and its actions, we can focus on the processes set in motion after their binding with their own ligands.

The binding of a ligand with TLR initiates a cascade of signals going back to the plasmatic TIR-domains of TLR. The signal from the TIR-domain – via the adapter molecules MyD88 (myeloid differentiation factor 88), TIRAP (TIR domain-containing adapter proteins), TICAM1 (TRIF), TICAM2 (TIR-containing adapter molecule 2) – is transmitted to the corresponding kinases (TAK, IKK, TBK, MAPK, JNKs, p38, ERK, Akt, etc.) that specifically activate the transcription factors (NF-kB, AP-1, and IRF) responsible for the expression of various anti-inflammatory and antimicrobial factors. All TLRs, except for TLR3, transmit the signal to kinases using MyD88.

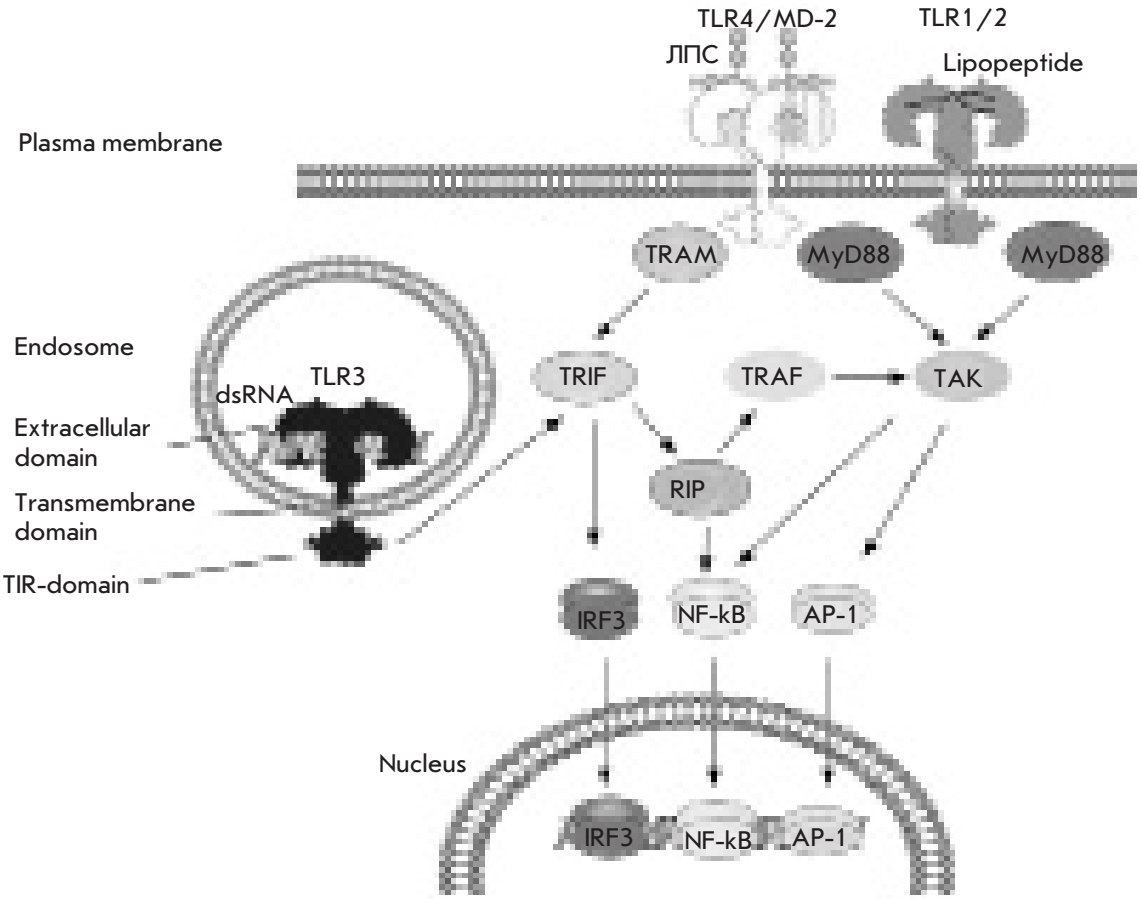

Fig. 2. TLR-dependent signaling pathways.

The TLR3 transmits the signal via TICAM1, and TLR4 – via both MyD88 and TICAM1 (Fig. 2).

Activation of one or the other factor depends on the type of TLR that is transmitting the signal. In particular, almost all TLRs (TLR2 and its co-receptors TLR1 and TLR6, as well as TLR4–9 and TLR11) bind with their own ligands to activate NF-kB, one of the main factors regulating the expression of anti-inflammatory cytokines, such as IL-1, -6, -8, etc. Signal transduction via TLR3, 4, 7–9 leads to the activation of another family of anti-inflammatory transcription factors, IRF. Signals transmitted via TLR3 or TLR4 lead to the activation of IRF3, which regulates the expression of IFN-β and is regarded as a crucial component of antiviral immunity. Signal transduction via TLR7–9 results in the activation of IRF-5 and IRF-7, as well as the expression of IFN-α, which is of critical importance for the antiviral defense. Signaling via TLR2 or TLR5 does not result in the activation of IRF family factors [36].

Thus, the interaction of the particular TLR with its ligand triggers a signaling cascade that leads to the expression of a specific gene pattern (cytokines, antimicrobial factors, etc.).

However, many details of the triggering of TLR-dependent signaling and its downstream effects still remain unclear. To the best of our knowledge, there is no reported data characterizing the overall transcriptome and proteome alterations occurring in response to the activation of distinct TLRs.

Table 2. TLRs in clinical trials.

Cancer	TLR
Non-small cell lung carcinoma, late stage	TLR9
Melanoma, stage IV	TLR7
Melanoma, stage IIIb/c or IV	TLR9
Incompletely re-sectable pancreatic cancer	TLR2/6
Recidivism of non-Hodgkin's lymphoma	TLR9
Recidivism of glioblastoma	TLR9
Chronic lymphocytic leukemia	TLR7

Table 3. Effect of TLRs on tumor growth and development.

Tumor-stimulating activity	TLR	Antitumor activity	TLR
Stimulation of angiogenesis	2, 9	Suppression of angiogenesis	7, 9
Stimulation of proliferation	3, 4	Development of apoptosis	3, 4, 7, 9
Chemoresistance	4	Elevation of chemosensitivity	2, 4, 7
Activation of regulatory T-cells (T_{reg})	4, 5	Inhibition of T_{reg}, antigen presentation	4, 5, 7, 8, 9
		Cytotoxicity	9

TLRS AND TUMORS

Fundamentally different effects of TLRs on tumors have been reported to date. On one hand, TLRs (and their ligands) can suppress tumor growth, on the other hand, they can stimulate tumor progression and influence tumor resistance to chemotherapy. In order to explain these contradictory data, we shall consider both cases in detail.

ANTITUMOR ACTIVITY OF TLRS

Currently, many TLR agonists are in clinical trials as antitumor agents (Table 2). Particularly, both natural (ssRNA) and synthetic (imiquimod) agonists of TLR7 and TLR8 have demonstrated high activity against chronic lymphocytic leukemia and tumors of the skin [37]. The TLR9 ligand CpG can suppress the growth of lymphomas and tumors of the brain, kidney, and skin [28]. The TLR3 ligand poly(IC) possesses a proapoptotic effect on both tumor and surrounding cells (for example, endothelial cells) [38].

It has been reported that the TLR4 agonists LPS from Gram-negative bacteria and OK-432 (picibanil; a lyophilized preparation of a low-virulence strain of *Streptococcus pyogenes*, inactivated by heating with penicillin G) possess high antitumor activity, when administered intratumorally. However, both LPS and OK-432 could not suppress tumor growth upon systemic administration [39]. At present, OK-432 is being tested at the second stage of clinical trials as medication against colorectal and lung cancer. It has also been shown that OM-174, a chemical agonist of TLR2/4, can inhibit the progression of melanoma and increase the survival rate of animals in experiments when introduced together with cyclophosphamide [40]. These experiments have shown that TLR2/4 agonists induce the secretion of TNF-α and expression of inducible NO-synthase. NO is known to induce apoptosis in tumor cells resistant to chemotherapy, thus prolonging the lifespan of mice. Another known antitumor preparation of microbial genesis is BCG (Bacillus Calmette-Guérin), which activates TLR-dependent reactions (TLR2, 4, 9). This preparation has been successfully used in the therapy of urinary bladder tumors for over 30 years [41].

Of note, various TLR agonists are presently in clinical trials as potential medications against tumors of different geneses (Table 2).

One of the main mechanisms underlying the antitumor activity of TLRs is their capability to activate the development of a tumor-specific immune response. The activation of TLRs

1) stimulates (directly or indirectly) the migration of NK-cells, cytotoxic T-cells, and type I T-helpers into the tumor, which causes the lysis of tumor cells via secretion of various effectors (perforin and IFN-γ) [42]; and

2) results in the secretion of type I IFNs (IFN-α, β) [43].

Another possible mechanism underlying the antitumor effect of TLRs is the TLR-dependent transition of tumor-stimulating macrophages (M2 type) into the tumor-suppressing type M1. Type M2 macrophages are characterized by the expression of cytokines, such as TGF-β and IL-10 ---components required for tissue repair and remodeling. TGF-β stimulates tumor cell proliferation, while IL-10 directs the development of the immune response to Th2, thus blocking the development of the cellular antitumor immune response. Alternatively, type M1 macrophages express IL-1, -6, -12, TNF-α, and IFN-γ, thereby stimulating the development of the cellular antitumor (Th1) immune response [44].

TUMOR-STIMULATING ACTIVITY OF TLRs

Chronic infections and inflammations are the most important factors known to stimulate the development of a malignant neoplasm. In particular, stomach cancer can be associated with a chronic inflammation induced by a pathogen; namely, *Helicobacter pylori*, and a chronic inflammation of the digestive tract is often associated with colorectal cancer [45]. Moreover, in some cases the use of nonsteroidal anti-inflammatory drugs can decrease the risk of malignant neoplasm development [46].

TLRs are key players in the system of innate immunity in animals, including humans; they participate in the mechanisms of inflammatory response when the cells come into contact with various pathogens. The role of TLRs in the development and progression of vari-

ous tumors is being studied in detail currently. Several mechanisms have been proposed to explain TLR implication in the stimulation of tumor formation and development (Table 3).

NF-κB is one of the major factors that provide the interrelationship between chronic inflammation and tumor formation [47]. This factor is constitutively activated in more than 90% of human tumors, such as acute and chronic myeloid leukemia, prostate cancer, multiple myeloma, hepatocarcinoma, etc. [48, 49]. In relation to this, the agents that are capable of activating NF-κB can be directly implicated in tumor development and progression. The interaction of pathogens with TLRs on the cell's surface is known to result in the activation of NF-κB and expression of NF-κB –dependent genes, thus determining the participation of TLRs in the stimulation of carcinogenesis. The activation of NF-κB leads to an increase in the level of IL-1, IL-2, IL-6, IL-10, and TNF-α cytokine production, migration of immune cells to the inflammation's focus due to the increased production of chemokines, "maintenance"of chronic inflammation, increase in the production of anti-apoptotic factors, etc. These indicated properties can promote tumor survival and progression due to the inhibition of apoptosis and cytotoxicity, and induction of angiogenesis [50].

An elevated level of TLRs has been reported in various tumor cells, and the frequency of induced tumor formation is decreased in *TLR* knockout mice [67]. Moreover, the increase in TLR expression on prostate cancer or head and neck tumor cell surfaces can stimulate their proliferation [51].

Huang and associates [31] have demonstrated that *Listeria monocytogenes* has a direct tumor-stimulating effect associated with its ability to activate the TLR2-dependent signaling pathways in ovary cancer cells. Moreover, the TLR2-dependent activation of NF-κB caused by *L. monocytogenes* results in an enhanced resistance of tumor cells to chemotherapeuticals [31]. The interrelationship between TLR2 and tumor progression has been confirmed in another independent study, in which Karin *et al*, [67] proved this receptor's key role in lung cancer metastasis formation. Metastasis and progression of tumors are essentially retarded in *TLR2* knockout mice, compared with wild-type mice. The key role in lung cancer progression belongs to myeloid cells expressing TNF-α in response to their stimulation by versican (proteoglycan of extracellular matrix, ligand for TLR2, the level of which is elevated in many tumor cell types). In our research, we also studied the role of TLR2 in tumor progression. In particular, infection with mycoplasma (*Mycoplasma arginini*) or the addition of its structural components (LAMP) to the cells expressing TLR2 resulted in the suppression of apoptosis and acceleration of tumor growth *in vivo* [52, 53]. Thus, TLRs have a tumor-stimulating effect that is mediated by myeloid cells [54].

Similar data was obtained for TLR4, another member of the TLR family. Systemic (intravenous) injection of LPS (the ligand of this receptor) stimulated breast adenocarcinoma cell migration, increased their invasion, and stimulated angiogenesis in tumors as well [30]. Similar results were obtained on another model – colorectal adenocarcinoma; LPS increased the survival of tumor cells, stimulated their proliferation, and, when injected intraperitoneally, enhanced metastasis [55]. Moreover, Huang, *et al.* have demonstrated that tumor cells expressing TLR4 cause a substantially more aggressive development of a disease, reducing the lifespan of mice in comparison with mice of isogenic line with TLR4 inactivated by specific siRNA. The obtained data has made possible the supposition that endogenous ligands (heat shock proteins, β-defensines, and endogenous LPS thrown from the gut) can influence the progression of TLR4-positive tumors, partially akin to the tumor-stimulating effects of TLR2 and its endogenous ligand versican [56].

However, data illustrating the tumor-stimulating effect of TLRs have been obtained not only for TLR2 and TLR4; elevated expression of TLR5 and TLR9 on the cervical epithelial cell's surface can be associated with cervical cancer progression [57] as well. A high level of TLR9 expression was observed in clinical samples of lung cancer and tumor cell lines. In these cells, TLR9 stimulation by specific agonists resulted in increased production of tumor-associated cytokines [58]. The TLR9 level is also elevated on the surface of human prostate cancer cells [59]. The treatment of such cells with the TLR9 ligands CpG-oligodeoxynucleotide (ODN-CpG) or bacterial DNA elevated the invasion of tumor cells. The elevation of tumor cell invasion via TLR9 activation can be regarded as a new mechanism by which chronic infections can stimulate prostate cancer cell growth.

However, not only various infectious agents and their structural components can stimulate carcinogenesis via interaction with the TLRs. DAMP, the nuclear and cytoplasmic proteins of necrotic cells, are known to serve as TRL ligands. DAMP released from damaged cells can be recognized by various TLRs on the surface of immune cells with subsequent activation of TLR-dependent signals resulting in the suppression of the antitumor immune response and, as a consequence, in the stimulation of tumor progression [60].

The molecules that potentially possess a tumor stimulating effect include heat shock proteins (HSP60, 70), ATP and uric acid, the Ca^{2+}-modulating protein family (S100), the protein HMGB1 and nucleic acids, whose DNA-binding protein HMGB1 is the most well-studied.

HMGB1 released from damaged cells activates the immune system via interaction with TLRs. In cell cultures, HMGB1 was shown to stimulate melanoma, as well as breast, colon, pancreas, and prostate cancer cell growth. HMGB1 can activate TLR2 and TLR4 on the surface of tumor and immune cells and, as a result, induce tumor progression and metastasis [61].

Elevated expression of DAMP proteins, particularly S100 family members, is shown in melanoma cells; these proteins can stimulate the growth of melanoma cells and lymphocytes, peripheral blood lymphocytes, and act as an autocrine tumor growth factor. The S100A4 protein, being a TLR ligand, stimulates the metastasis of breast cancer cells, and its elevated expression is an indicator of a poor prognosis. In spite of the interrelationship between S100A4 and metastasis, the protein can be expressed in macrophages, lymphocytes, and fibroblasts. Recent studies have shown that the S100A8 and S100A9 proteins produced by primary tumor can activate serum amyloid A (SAA) 3 in lung tissues, thereby creating conditions for the formation of a metastatic niche. SAA3 is a ligand for TLR4 on the surface of lung endothelial cells and macrophages. TLR4 activation facilitates the migration of tumor cells from the primary focus into the lung tissue via the creation of a microenvironment favorable to tumor growth. Thus, the suppression of the S100–TLR4-signaling pathway effectively counteracts the formation of metastasis in the lung [62].

In summing up the described effects, one can come to the conclusion that TLR can, on the one hand, directly or indirectly participate in tumor progression, and, on the other hand, increase tumor cell resistance to proapoptotic agents.

The data presented illustrate a complex mechanism showing the tumor-stimulating effect of TLRs and their ligands, which is to be studied in detail. However, despite the complexity of the issue, one can highlight some key points that determine the tumor stimulating effect of TLRs:

1) TLR interaction with its own ligands induces the activation of the NF-κB transcription factor and, as a consequence, an increase in the production of various pro-inflammatory cytokines (IL-6, MCP-1, MIF, GRO-α, etc.), as well as a number of anti-apoptotic proteins, which facilitates a direct or indirect tumor-stimulating effect;

2) TLR-dependent activation of myeloid cells and their progenitors is apparently the determining factor in the formation of metastasis. A series of independent works have demonstrated that myeloid cells migrating from the bone marrow (in response to endogenous stimulation) into tissues play a key role in the formation of metastasis niches [30, 54]. Since endogenous (versican, fibronectin, etc.) and exogenous (microbial origin) TLR ligands are known to be capable of stimulating myeloid cells and their progenitors and increasing the metastatic potential of the tumor, it is very likely that an interrelationship exists between the TLR-dependent activation of myeloid cells and their subsequent involvement in metastasis;

3) TLR activation can stimulate angiogenesis via angiogenic factors, such as IL-8, vascular endothelial growth factor (VEGF), and matrix metalloproteinases (MMP), as well as enhance the adhesive and invasive activity of tumor cells, in line with the increase in the permeability of blood vessels.

TOLL-LIKE RECEPTORS IN TUMOR THERAPY

Antitumor therapy based on the delivery of TLR ligands into the foci of tumor growth seems promising, because TLR agonists can induce an antitumor immune response by regulating the function in immune cells localized in the microenvironment of the tumor. The imiquimod containing the TLR7 agonist may represent an example of such therapy. This drug is used against actinic keratosis and basal cell carcinoma. The possibility of using this drug as an adjuvant in the therapy of melanoma is also under study [63, 64]. Yet another TLR7 agonist, 852A, is used in tumor therapy. Presently, the possibility of using 852A against chronic lymphoid leukosis and other solid tumors is under consideration [65]. ODN-CpG, a TLR9 agonist, induces the activation and maturation of dendrite cells and stimulates the development of the T-cellular antitumor response. Clinical trials of TLR9 agonists are presently under way in the context of their safety and efficacy in the therapy of breast, colorectal, and lung cancers, melanoma, glioblastoma, etc. [28]. Macrophage-activating lipopeptide-2 (MALP-2), a TLR2/6 agonist, has shown encouraging results in the therapy of pancreatic cancer; the intratumoral injection of MALP-2 with gemcitabine during laparotomia substantially increased the lifespan of patients with incompletely re-sectable cancer (from 9 to 17 months) [66]. The described examples demonstrating the effective use of TLR agonists in tumor therapy are evidence of their potential, as well as the reasonableness of further studies directed toward the development of antitumor pharmaceuticals with a similar mechanism of action.

However, as mentioned above, many tumor cells can express TLRs on their surface, and the direct interaction of these cells with TLR ligands can enhance the progression of the tumor and make it less sensitive to chemotherapy. Thus, it is likely that TLR agonists permanently circulating in an organism (pathogenic microorganisms that can overcome the immune barrier; LPS of bacteria comprising normal gut microflora,

which can be thrown into the bloodstream; and own endigenous ligands) can directly or indirectly favor the advancement of tumor progression.

In this regard, a promising avenue for the therapy of malignant tumors is an approach directed toward the suppression of TLR-dependent signaling pathways. The employment of NF-κB inhibitors can be distinguished as a known approach that is already used in the therapy of malignant tumors.

The constitutive activation of NF-κB is observed in tumors, such as Hodgkin's lymphoma (Hodgkin's disease), acute lymphoblastic leucosis, multiple myeloma, breast, colon, lung, ovary, and prostate cancers, various lymphomas, liver cancer, and melanoma, amongst others [48, 49].

The following pharmaceutical groups are used for the suppression of NF-κB activity: nonsteroidal anti-inflammatory agents; substances-inhibiting IKK and COX-2 activities; natural and bioavailable inhibitors of IKKβ, such as flavonoids, prostaglandins, BMS-345541, PS1145, SC-514, and SPC839; as well as proteasome inhibitors that inhibit NF-κB activity by preventing IkB degradation, such as bortezomib (PS-341), irinotekan, gemcitabine, and other drugs widely used in the therapy of tumors of the colon, small intestine, stomach, pancreas, etc. [67].

Since NF-κB is a key component of the TLR-dependent signaling pathway, the use of its inhibitors seems promising for the suppression of TLR-dependent stimulation of tumor growth.

In our opinion, other promising targets are TLRs themselves. Since TLR2 and TLR4 (receptors implicated in the stimulation of tumor growth) are expressed on the cell's surface, specific molecules (antibodies, chemical inhibitors) suppressing their functional activity seem usable. Antibodies that inhibit TLR activities are currently available; however, data on their clinical use have not been reported yet.

CONCLUSION

TLRs are members of the PRR family. The actions associated with their activation go beyond reactions of the innate immune response. Participation in dendritic cell activation, regulation of specific T- and B-cellular immune responses, elevation of IFN expression, etc., provide for the TLR's implication both in the development of effective innate and adaptive immune reactions in response to the penetration of various pathogens into the organism, and in supporting tissue homeostasis. The reported data in abundant research is evidence that it is possible to use TLR ligands as adjuvants for the immune therapy of malignant neoplasms. It is known, however, that TLR activation on the surface of tumor cells can result in enhanced progression of tumors of various origins.

Such a difference in effects primarily depends on the ligand type. As shown in Table 1, TLR can be divided into two groups: inducing and noninducing IFN production. As a rule, the addition of TLR3, 4, 7, 8, and 9 agonists that activate IRE considerably suppresses tumor growth. However, there is currently no data indicating any anti-tumor effect of the agonists of TLR2, which, unlike the listed receptors (TLR3, 4, 7, 8, 9), cannot activate the production of IFN type I. The way they are introduced is yet another feature leading to differences in the effects of TLR agonists on tumors. Intratumoral introduction of TLR3, 4, 7, 8, 9 ligands induces tumor cell death and, in most cases, a decrease in the tumor's size. The most probable explanation for the anti-tumor activity of these TLRs is their capability to (*A*) induce local expression of IFN types I and II, which are known to induce tumor cells death, and (*B*) activating cell immunity in response to the interaction with ligand. The death of tumor cells, their phagocytosis, and subsequent presentation of tumor-specific antigens cause additional stimulation of specific anti-tumor immunity. However, a series of works [30, 55, 56] have demonstrated that a systemic introduction of TLR4 ligands is often associated with the stimulation of tumor growth. In our opinion, this difference is related to the fact that intratumoral injection of TLR4 ligand (LPS), compared with its systemic introduction, results in substantially higher IFN accumulation directly in the tumor. Since IFNs are short-distance effector proteins acting at sufficiently high concentrations, their production outside the tumor (at systemic introduction) does not result in tumor cell death and, hence, in the development of anti-tumor immunity. Inflammatory cytokines and chemokines can play a dual role after a local or systemic introduction of LPS: they either favor the development of anti-tumor immunity after intratumoral injection of LPS, or positively influence the growth of the tumor, resistance of its cells, and metastasis potential after the systemic introduction of LPS in the absence of targets for the immune system.

Thus, the available data suggest that TLR agonists have a dual effect on tumor growth. This dual TLR effect is indicative of the more complex functional role of TLRs in tumor biology. Such a role by TLRs is obviously beyond the frame of a simple NF-κB activation. The influence of various TLR ligands on tumors must be studied taking into account multiple factors, including the TLR expression level, type of tissue from which the tumor originates, the tumor's microenvironment, and many others. The systemic study of TLR's role and functions in tumor cells can contribute substantially to the development of new antitumor pharmaceuticals with a TLR-dependent mechanism of activity. ●

REFERENCES

1. Coussens L.M., Werb Z. // Nature. 2002. V. 420(6917). P. 860–867.
2. Mantovani A., Allavena P., Sica A., Balkwill F. // Nature. 2008. V. 454(7203). P. 436–444.
3. Okamoto T. // Endocr. Metab. Immune Disord. Drug. Targets. 2006. V. 6(4). P. 359–372
4. Thomas H.G., Han J.H., Balentien E., et al. // Methods Enzymol. 1991. V. 198. P. 373–383.
5. Kleeff J., Kusama T., Rossi D.L. // Int. J. Cancer. 1999. V. 81. P. 650–657.
6. Deveraux Q.L., Schendel S.L., Reed J.C. // Cardiol. Clin. 2001. V. 19(1). P. 57–74.
7. Dong Z., Wang J.Z., Yu F., Venkatachalam M.A. // Am. J. Pathol. 2003. V. 163(2). P. 663–671.
8. Bartsch H., Nair J. // Langenbecks Arch. Surg. 2006. V. 391(5). P. 499–510.
9. Moser B., Willimann K. // Ann. Rheum. Dis. 2004. V. 63 Suppl 2. P. 84–89.
10. Rasmussen S.B., Reinert L.S., Paludan S.R. // APMIS. 2009. V. 117(5–6). P. 323–337.
11. Palm N.W., Medzhitov R. // Immunol. Rev. 2009. V. 227(1). P. 221–233.
12. Zeromski J., Mozer-Lisewska I., Kaczmarek M. // Cancer Microenviron. 2008. V. 1(1). P. 37–42.
13. Jego G., Bataille R., Geffroy-Luseau A., et al. // Leukemia. 2006. V. 20(6). P. 1130–1137.
14. Seya T., Akazawa T., Uehori J., et al. // Anticancer Res. 2003. V. 23(6a). P. 4369–4376.
15. Grauer O.M., Molling J.W., Bennink E., et al. // J. Immunol. 2008. V. 15. P. 6720–6729.
16. Medzhitov R. // Nat. Rev. Immunol. 2001. V. 1(2). P. 135–145.
17. Pasare C., Medzhitov R. // Nature. 2005. V. 438(7066). P. 364–368.
18. Zasloff M. // Nature. 2002. V. 415(6870). P. 389–395.
19. Doyle S.E., O'Connell R.M., Miranda G.A., et al. // J. Exp. Med. 2004. V. 5. P. 81–90.
20. Werling D., Hope J.C., Howard C.J., Jungi T.W. // Immunology. 2004. V. 111(1). P. 41–52.
21. Nijhuis M.M., Pasterkamp G., Sluis N.I., et al. // J. Vasc. Res. 2007. V. 44(3). P. 214–222.
22. Kaisho T., Akira S. // J. Allergy Clin. Immunol. 2006. V. 117(5). P. 979–988.
23. Salaun B., Romero P., Lebecque S. // Eur. J. Immunol. 2007. V. 37(12). P. 3311–3318.
24. Iwasaki A., Medzhitov R. // Nat. Immunol. 2004. V. 5(10). P. 987–995.
25. Pasare C., Medzhitov R. // Adv. Exp. Med. Biol. 2005. V. 560. P. 11–18.
26. Li M., Zhou Y., Feng G., Su S.B. // Curr. Mol. Med. 2009. V. 9(3). P. 365–374.
27. Curtiss L.K., Tobias P.S. // J. Lipid. Res. 2009. V. 50 Suppl. P. 340–345.
28. Krieg A.M. // J. Clin. Invest. 2007. V. 117. P. 1184–1194.
29. Chicoine M.R., Zahner M., Won E.K. // Neurosurgery. 2007. V. 60. P. 372–381.
30. Harmey J.H., Bucana C.D., Lu W. // Int. J. Cancer. 2002. V. 101. P. 415–422.
31. Huang B., Zhao J., Shen S. // Cancer. Res. 2007. V. 67. P. 4346–4352.
32. Means T.K., Golenbock D.T., Fenton M.J. // Life Sci. 2000. V. 8. P. 241–258.
33. Diebold S.S. // Handb. Exp. Pharmacol. 2009. V. 188. P. 3–30.
34. West A.P., Koblansky A.A., Ghosh S.// Annu Rev. Cell. Dev. Biol. 2006. V. 22. P. 409–437.
35. Zhang Z., Schluesener H.J. // Cell Mol. Life Sci. 2006. V. 63(24). P. 2901–2907.
36. O'Neill L.A., Bowie A.G. // Nat. Rev. Immunol. 2007. V. 7(5). P. 353–364.
37. Stockfleth E., Trefzer U., Garcia-Bartels C. // Br. J. Dermatol. 2003. V. 149 (Suppl. 66). P. 53–56.
38. Nogueras S., Merino A., Ojeda R. // Heart. Circ. Physiol. 2008. V. 294. P. 708–713.
39. Okamoto M., Oshikawa T., Tano T., et al. // J. Immunother. 2006. V. 29. P. 78–86.
40. D'Agostini C., Pica F., Febbraro G., et al. // Int. Immunopharmacol. 2005. V. 5(7–8). P. 1205–1212.
41. Morales A., Eidinger D., Bruce A.W. // J. Urol. 1976. V. 116. P. 180–183.
42. Street S.E., Cretney E., Smyth M.J. // Blood. 2001. V. 1; 97(1). P. 192–197.
43. Swann J.B., Hayakawa Y., Zerafa N., et al. // J. Immunol. 2007. V. 15;178(12). P. 7540–7549.
44. Martinez F.O., Sica A., Mantovani A., et al. // Front. Biosci. 2008. V. 13. P. 453–461.
45. Balkwill F., Coussens L.M. // Nature. 2004. V. 431. P. 405–406.
46. Robak P., Smolewski P., Robak T. // Leuk. Lymphoma. 2008. V. 49. P. 1452–1462.
47. Pikarsky E., Porat R.M., Stein I., et al. // Nature. 2004. V. 431. P. 461–466.
48. Palayoor S.T., Youmell M.Y., Calderwood S.K., et al. // Oncogene. 1999. V. 18. P. 7389–7394.
49. Griffin J.D. // Blood. 2001. V. 98. P. 2291.
50. Philip M., Rowley D.A., Schreiber H. // Semin. Cancer Biol. 2004. V. 14. P. 433–439.
51. Swann J.B., Vesely M.D., Silva A., et al. // Proc. Natl. Acad. Sci. USA. 2008. V. 105. P. 652–656.
52. Shcheblyakov D.V., Logunov D.Yu, Zubkova O.V., et al. // Mol. Genetika, mikrobiol. i virusol. (Mol. Genet. Microbiol. Virol.). 2008. № 4. P. 163–167.
53. Logunov D., Scheblyakov D., Zubkova O., et al. // Oncogene. 2008. V. 27. P. 4521–4531.
54. Kim S., Takahashi H., Lin W.W., et al. // Nature. 2009. V. 457. P. 102–106.
55. Luo J.L., Maeda S., Hsu L.C., et al. // Cancer Cell. 2004. V. 6. P. 297–305.
56. Huang B., Zhao J., Li H. // Cancer Res. 2005. V. 65. P. 5009–5014.
57. Kim W.Y., Lee J.W., Choi J.J., et al. // Int. J. Gynecol. Cancer. 2008. V. 18. P. 300–305.
58. Droemann D., Albrecht D., Gerdes J., et al. // Respir. Res. 2005. V. 6. P. 1–6.
59. Ilvesaro J.M., Merrell M.A., Swain T.M., et al. // Prostate. 2007. V. 67. P. 774–781.
60. Lotze M.T., Zeh H.J., Rubartelli A., et al. // Immunol. Rev. 2007. V. 220. P. 60–81.
61. Ellerman J.E., Brown C.K., Vera M., et al. // Clin. Cancer Res. 2008. V. 13. P. 2836–2848.
62. Cabezon T., Celis J.E., Skibshoj I., et al. // Int. J. Cancer. 2007. V. 121. P. 1433–1444
63. Adams S., O'Neill D.W., Nonaka D., et al. // J. Immunol. 2008. V. 181. P. 776–784.
64. Spaner D.E., Miller R.L., Mena J., et al. // Leuk. Lymphoma. 2005. V. 46. P. 935–939.
65. Dudek A.Z., Yunis C., Harrison L.I., et al. // Clin. Cancer Res. 2007. V. 13. P. 7119–7125.
66. Schmidt J., Welsch T., Jäger D., et al. // Br. J. Cancer. 2007. V. 97. P. 598–604.
67. Karin M., Yamamoto Y., Wang Q.M. // Nat. Rev. Drug. 2004. V. 3. P. 17–26.