Inflammation and cancer – double face of Toll-like receptors

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Abstract

Toll-like receptors (TLRs) are a group of proteins involved in recognition of highly conserved microbial structures and endogenous molecules released during tissue damage. With ability to trigger up-regulation of proinflammatory cytokines, chemokines TLRs are considered as key components of innate immune response. Growing body of evidence suggest that TLRs are expressed not only by immune cells but also by tumor cells, probably supporting their escape from immune surveillance and increasing their invasiveness. This work review mechanisms underlying contribution of TLRs to inflammation, tumor development and tumor progression.

Key words: Toll-like receptors, inflammation, cancer.

Introduction

In the nineteenth century, after observing leukocytes in the tumor tissue, Rudolf Virchow suggested existence of a link between the presence of inflammation and development of cancer [1]. It is now widely accepted that carcinogenesis may be associated with infection, chronic inflammation or tissue damage. The share of infectious agents in the development and progression of cancer has been demonstrated in several types of cancer (Table 1) [2, 3].

Toll-like receptors and pathogenesis of cancer

Toll-like receptors (TLRs) are a group of proteins involved in recognition of highly conserved microbial structures, pathogen-associated molecular patterns (PAMPs) and endogenous molecules released during tissue damage, danger-associated molecular patterns (DAMPs) [4]. With ability to trigger up-regulation of proinflammatory cytokines, chemokines TLRs are considered as key components of innate immune response. To date, 11 TLRs have been iden-

Table 1. Inflammation-associated cancers according to [2, 3]

<table>
<thead>
<tr>
<th>Neoplasm</th>
<th>Inflammatory disease</th>
<th>Aetiological factor</th>
</tr>
</thead>
<tbody>
<tr>
<td>Gastric adenocarcinoma, MALT</td>
<td>Gastritis, ulcers</td>
<td>Helicobacter pylori</td>
</tr>
<tr>
<td>Hepatocellular carcinoma</td>
<td>Hepatitis</td>
<td>Hepatitis B and C virus</td>
</tr>
<tr>
<td>Burkitt’s lymphoma, non-Hodgkin’s lymphoma</td>
<td>Mononucleosis</td>
<td>Epstein-Barr virus</td>
</tr>
<tr>
<td>Bladder carcinoma</td>
<td>Bladder inflammation</td>
<td>Schistosomiasis</td>
</tr>
<tr>
<td>Cervical carcinoma</td>
<td>Chronic cervicitis</td>
<td>Human papillomavirus</td>
</tr>
<tr>
<td>Gall bladder cancer</td>
<td>Chronic cystitis</td>
<td>Opisthorchis viverrini</td>
</tr>
<tr>
<td>Colorectal cancer</td>
<td>Inflammatory bowel disease</td>
<td>Intestinal pathogens</td>
</tr>
</tbody>
</table>

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Development of cancer appears to be confirmed by the fact [15, 16]. The hypothesis linking inflammation with the progression of hepatocellular carcinoma is supported by the increase in the presence of colon cancer cells. In addition, cancer cells characterized by the constant activation of NF\(\kappa\)B proteins are resistant to chemotherapy, while blockage of NF\(\kappa\)B increases their sensitivity to drugs [10]. As potent regulators of transcription factor NF\(\kappa\)B activity, TLRs may play an important role in the tumor progression.

One of the best examples illustrating the relationship between chronic inflammation and carcinogenesis is the impact of Helicobacter pylori infection on the development of gastric cancer. Analysis of published studies, including a group of 1,228 people revealed that the relative risk of gastric cancer incidence increases sixfold following H. pylori infection [11]. Potential mechanism underlying carcinogenic effect of H. pylori may be chronic activation of TLR4 expressed on gastric epithelial cells by bacterial lipopolysaccharides (LPS). In the course of gastritis expression of TLR4 increases [12]. Helicobacter pylori infection is accompanied by activation of TLR4 on the surface of gastric epithelial cells and increased synthesis of proinflammatory cytokines and chemokines. The consequence is chronic gastritis, which may, through the intestinal metaplasia, lead to gastric cancer [13]. Research conducted by Schmausser et al. showed that TLR4 and TLR5 receptors are expressed also by gastric cancer cells, possibly enabling the contact of tumor cells with H. pylori. In vitro studies demonstrated that H. pylori – derived LPS in mechanism dependent on TLR5 activation, induces expression of IL-8, which stimulates tumor angiogenesis, therefore favoring cancer progression [14].

Recent studies indicate also a link between other inflammatory diseases and cancer, such as nonspecific inflammatory bowel disease (Crohn’s disease, ulcerative colitis) and colorectal cancer and a correlation between liver inflammation and the development of hepatocellular carcinoma [15, 16]. The hypothesis linking inflammation with the development of cancer appears to be confirmed by the fact that nonsteroidal anti-inflammatory drugs (NSAIDs) medication can reduce the risk of developing certain types of cancer [17].

### Toll-like receptors and tumor progression

Growing body of evidence suggest that Toll-like receptors are expressed by tumor cells, probably supporting their escape from immune surveillance and increasing their invasiveness [18]. The presence of TLR receptors was demonstrated in ovarian, colon, pancreas, stomach, lung and brain tumors [19-25].

In studies concerning presence of TLR4 receptor in pancreatic cancer cells, Zhang et al. [26] reported not only increased expression of TLR4 in tumor cells, but also the relationship between TLR4 expression level and tumor size and presence of metastases in lymph nodes. As a result, Kaplan-Meier analysis showed shorter survival time of patients whose tumor tissue showed increased expression of TLR4. In author’s opinion it indicates a direct involvement of the TLR4 receptor in tumor growth and metastases formation. One of the possible mechanisms explaining these processes may be TLR4 activation by endogenous ligands released from the cell matrix during tumor growth.

The importance of TLR4 signaling in the process of metastases formation by prostate cancer cells were highlighted by Hua et al. [27]. Their study showed that human prostate cancer cell lines with high metastatic potential (PC3, DU145), exhibit significantly higher expression of TLR4 than cells with low metastatic potential (LNCaP). Therefore authors suggest that the level of TLR4 expression in prostate cancer cells may correlate positively with the presence of metastases. Additionally, silencing of TLR4 gene using siRNA significantly reduces ability of tumor cells to migrate and invade, eventually promoting their apoptosis. Using of the plasmid construct containing TLR4 gene silencing sequence in mouse model of prostate cancer resulted in inhibition of tumor growth, which, as suggested by the researchers, points to the possibility of using gene silencing techniques in therapy of prostate cancer.

Similar results were obtained in study investigating the role of TLR4 signaling in colon tumorigenesis. In a study performed by Wang et al. [20] it was observed that 20% of the tumors samples obtained from patients with colon cancer showed high expression of TLR4 receptor, and 23% of tumors expressed higher level of MyD88 protein as compared with a healthy colon mucosa and polyps. Additionally, high expression of TLR4 and MyD88 correlated with the presence of colon cancer metastases in liver, which worsened the prognosis in these patients.

Ikebe et al. showed that stimulation of human pancreatic cancer cells (Panc-1, ASPCA-1) with LPS increased their ability to form metastases [22]. In vitro, LPS-treated pancreatic cancer cells were characterized by increased invasive ability. The mechanism of this action was dependent...
on activation of the transcription factor NFκB, as a result of TLR4 stimulation. Silencing of TLR4 and MyD88 genes with siRNA abolished described effect of LPS on pancreatic cancer cells, suggesting the possibility of using the TLR4/MyD88 pathway blockade in therapy.

Cytokines and chemokines produced by tumor cells following the activation of TLRs, may promote cancer development and invasiveness. Goto et al. [28] showed that stimulation of TLRs present on the surface of melanoma cells with adequate ligands led to production of proinflammatory factors: cytokines IL-1, IL-6, TNF-α, G-CSF, COX-2 and chemokines CCL2 and CXCL2. At the same time it came to production of IL-10, which exhibited immunosuppressive activity. The presence of these mediators in the tumor microenvironment may promote tumor growth and also influence the activity of immune cells. Chemokine CCL2 and IL-10 affect the induction of Treg cells, which by the secretion of IL-10 and TGF-β inhibit the antitumor activity of other immune cells [29]. Interleukin 1 and IL-6 recruit to the tumor environment myeloid-derived suppressor cells (MDSC) which by the secretion of arginase and nitric oxide synthase suppress activity of cells involved in antitumor defense [30].

In vitro study demonstrated that TLR4 is expressed by human lung cancer cells [24]. It was also observed that stimulation of TLR4 leads to the production of immunosuppressive TGF-β by the tumor cells as well as pro-angiogenic factors – VEGF and IL-8. In addition, stimulation of TLR4 with appropriate ligand induced lung cancer cells resistance to TNF-α induced apoptosis.

Another of the mechanisms favoring tumor metastases is the activation of myeloid cells infiltrating the tumor through stimulation of TLR2 and its co-receptors TLR6 and CD14 by versican [31]. Belonging to a group of extracellular proteoglycans versican, accumulates both in the tumor stroma and in tumor cells [32]. Versican is involved in adhesion and migration of cancer cells and in the process of angiogenesis. Studies performed in vitro on cell lines, showed that stimulation of TLR2 present on myeloid cells by versican triggered production of proinflammatory cytokines, including TNF-α, which favored the formation of tumor metastases. Activation of fibroblasts and endothelial cells through TLR2 stimulation increased the secretion of IL-8, which stimulates angiogenesis and formation of metastases [33].

### Toll-like receptor agonists in cancer therapy

Despite numerous evidence of carcinogenic effect of stimulation of TLRs, it appears that stimulation of some TLR with appropriate agonists may have therapeutic effects. Probably activation of TLRs is responsible for the therapeutic effect of Coley’s toxin – mixture of killed bacteria *Streptococcus pyogenes* and *Serratia marcescens* – which was observed after administration to the patients with unresectable sarcoma [34]. Stimulation of TLRs with appropriate agonists leads to activation of dendritic cells presenting tumor antigens and activation of T cells. At the moment, two TLR agonists are registered in the monotherapy of cancer in the United States – bacillus Calmette-Guerin (BCG) and imiquimod [35]. By the stimulation of TLR2 and TLR4, BCG [36] causes local secretion of cytokines with anti-cancer activity and leads to the emergence of specific immune responses [37]. Since the positive effects were reported in clinical trials with BCG, it is approved in immunotherapy of bladder cancer.

Imiquimod is a TLR7 agonist registered by the Food and Drug Administration (FDA) in treatment of basal cell skin carcinoma and actinic keratosis. Therapeutic potential of imiquimod is associated with activation of immune cells involved in effective antitumor immune response – B lymphocytes, plasma cells, natural killer (NK) cells and Langerhans cells. Moreover, it has been demonstrated that imiquimod induces apoptosis of cancer cells [35].

### Conclusions

Through the activation of the transcription factor NFκB and induction of synthesis of cytokines, TLRs are a powerful weapon that runs in response to bacterial infection. In the face of recent reports, TLRs regulate also many physiological processes, including tissue repair and apoptosis. It is known that there is a link between TLR gene polymorphisms and the incidence of several cancers, including gastric, prostate and colon [38-40]. Despite many studies, the role of TLRs in the process of carcinogenesis remains unknown. Activation of TLRs resulting in the production of particular cytokines and chemokines, may promote escape of tumor cells from the immune surveillance, cause their resistance to chemotherapeutic agents, and thus promote tumor progression. On the other hand, the local activation of TLRs with suitable ligands may have a relevant therapeutic effect in treating of certain cancers. Therefore, there is a need of further studies on the mechanisms regulating signal transduction of TLRs and their effect on the process of development and progression of cancer cells.

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### References