Review paper

Inflammation and cancer – double face of Toll-like receptors

ALEKSANDRA DĄBROWSKA[1], ROBERT SŁOTWIŃSKI[1,2], SYLWIA KĘDZIORA[1]

[1]Department of Immunology and Nutrition, Medical University of Warsaw, Warsaw, Poland
[2]Department of Surgical Research and Transplantology, Medical Research Center, Polish Academy of Sciences, Warsaw, Poland

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.*

(Centr Eur J Immunol 2012; 37 (1): 67-70)

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]

Neoplasm	Inflammatory disease	Aetiological factor
Gastric adenocarcinoma, MALT	Gastritis, ulcers	*Helicobacter pylori*
Hepatocellular carcinoma	Hepatitis	Hepatitis B and C virus
Burkitt's lymphoma, non-Hodgkin's lymphoma	Mononucleosis	Epstein-Barr virus
Bladder carcinoma	Bladder inflammation	Schistosomiasis
Cervical carcinoma	Chronic cervicitis	Human papillomavirus
Gall bladder cancer	Chronic cystitis	Opisthorchis viverrini
Colorectal cancer	Inflammatory bowel disease	Intestinal pathogens

Correspondence: Aleksandra Dąbrowska, Department of Immunology and Nutrition, Medical University of Warsaw, Pawińskiego 3, 02-106 Warsaw, Poland, phone +48 22 572 02 47, fax +48 22 572 02 46, e-mail: aleksandra_dabrowska@op.pl

tified on human cells (TLR 1-11) and 13 on mouse (TLR 1-13). They are expressed both by cells involved in innate immune response and cells involved in adaptive immunity [4-6]. Upon stimulation with appropriate ligand TLRs undergo conformational changes which launch cascade of reactions eventually leading to production of proinflammatory cytokines such as interferon α (IFN-α), tumor necrosis factor α (TNF-α), interleukins – IL-1, IL-6, IL-4, IL-8, IL-12, granulocyte colony stimulating factor (G-CSF), granulocyte-macrophage colony stimulating factor (GM-CSF) and chemokines: CCL2, CCL3 and CCL4 [7].

The central point of the inflammatory response is the activation of nuclear factor κB (NFκB), leading to the production of proinflammatory cytokines, adhesion molecules and growth factors [8]. By promoting cell proliferation and inhibition of apoptosis, activation of NFκB may promote progression of cancer cells. This is supported by the fact that the constitutive expression of NFκB was observed in many cancers, including breast, colon, pancreas, thyroid, urinary bladder and skin [9]. In addition, cancer cells characterized by a constant activation of NFκB proteins are resistant to chemotherapy, while blockage of NFκB increases their sensitivity to drugs [10]. As potent regulators of transcription factor NFκB activity, TLRs may play 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. In addition, the 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 this receptor 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 leaded 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 activation 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 throughTLR2 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 anticancer 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.

Acknowledgments

This work was supported by grants no. NN 402 306836 and NN 401 103340 founded by the Ministry of Science and Higher Education, Poland.

References

1. Balkwill F, Mantovani A (2001): Inflammation and cancer: back to Virchow? Lancet 357: 539-545.
2. Coussens LM, Werb Z (2002): Inflammation and cancer. Nature 420: 860-867.
3. Vagefi PA, Longo WE (2005): Colorectal cancer in patients with inflammatory bowel disease. Clin Colorectal Cancer 4: 313-319.

4. Jeong E, Lee JY (2011): Intrinsic and extrinsic regulation of innate immune receptors. Yonsei Med J 52: 379-392.
5. Hedayat M, Takeda K, Rezaei N (2010): Prophylactic and therapeutic implications of toll-like receptor ligands. Med Res Rev [Epub ahead of print].
6. Miggin SM, O'Neill LA (2006): New insights into the regulation of TLR signaling. J Leukoc Biol 80: 220-226.
7. O'Neill LA (2008): The interleukin-1 receptor/Toll-like receptor superfamily: 10 years of progress. Immunol Rev 226: 10-18.
8. Baldwin AS Jr. (1996): The NF-kappa B and I kappa B proteins: new discoveries and insights. Annu Rev Immunol 14: 649-683.
9. Schwartz SA, Hernandez A, Mark Evers B (1999): The role of NF-kappaB/IkappaB proteins in cancer: implications for novel treatment strategies. Surg Oncol 8: 143-153.
10. Cheng Q, Lee HH, Li Y, et al. (2000): Upregulation of Bcl-x an Bfl-1 as a potential mechanism of chemoresistance, which can be overcome by NF-κB inhibition. Oncogene 19: 4936-4940.
11. Helicobacter and Cancer Collaborative Group (2001): Gastric cancer and Helicobacter pylori: a combined analysis of 12 case control studies nested within prospective cohorts. Gut 49: 347-353.
12. Schmausser B, Andrulis M, Endrich S, et al. (2004): Expression and subcellular distribution of Toll-like receptors TLR4, TLR5 and TLR9 by gastric epitheliumin H. pylori infection. Clin Exp Immunol 136: 521-526.
13. Schmausser B, Andrulis M, Endrich S, et al. (2005): Toll-like receptors TLR4, TLR5 and TLR9 on gastric carcinoma cells: an implication for interaction with *Helicobacter pylori*. Int J Med Microbiol 295: 179-185.
14. Smith Jr MF, Mitchell A, Li G, et al. (2003): Toll-like receptor (TLR) 2 and TLR5, but not TLR4, are required for Helicobacter pylori-induced NF-kappa B activation and chemokine expression by epithelial cells. J Biol Chem 278: 552-560.
15. Eaden JA, Abrams KR, Mayberry JF (2001): The risk of colorectal cancer in ulcerative colitis: a meta-analysis. Gut 48: 526-535.
16. Weber A, Boege Y, Reisinger F, Heikenwälder M (2011): Chronic liver inflammation and hepatocellular carcinoma: persistence matters. Swiss Med Wkly 141: w13197.
17. Rothwell PM, Fowkes FG, Belch JF, et al. (2011): Effect of daily aspirin on long-term risk of death due to cancer: analysis of individual patient data from randomised trials. Lancet 377: 31-41.
18. Huang B, Zhao J, Li H, et al. (2005): Toll-like receptors on tumor cells facilitate evasion of immune surveillance. Cancer Res 65: 5009-5014.
19. Zhou M, McFarland-Mancini MM, Funk HM, et al. (2009): Toll-like receptor expression in normal ovary and ovarian tumors. Cancer Immunol Immunother 58: 1375-1385.
20. Wang EL, Qian ZR, Nakasono M, et al. (2010): High expression of Toll-like receptor 4/myeloid differentiation factor 88 signals correlates with poor prognosis in colorectal cancer. Br J Cancer 102: 908-915.
21. Doan HQ, Bowen KA, Jackson LA, Evers BM (2009): Toll-like receptor 4 activation increases Akt phosphorylation in colon cancer cells. Anticancer Res 29: 2473-2478.
22. Ikebe M, Kitaura Y, Nakamura M, et al. (2009): Lipopolysaccharide (LPS) increases the invasive ability of pancreatic cancer cells through the TLR4/MyD88 signaling pathway. J Surg Oncol 100: 725-731.
23. Chochi K, Ichikura T, Kinoshita M, et al. (2008): Helicobacter pylori augments growth of gastric cancers via the lipopolysaccharide – toll like receptor 4 pathway whereas its lipopolysaccharide attenuates antitumor activities of human mononuclear cells. Clin Cancer Res 14: 2909-2917.
24. He W, Liu Q, Wang L, et al. (2007): TLR4 signaling promotes immune escape of human lung cancer cells by inducing immunosuppressive cytokines and apoptosis resistance. Mol Immunol 44: 2850-2859.
25. Curtin JF, Liu N, Candolfi M, et al. (2009): HMGB1 mediates endogenous TLR2 activation and brain tumor regression. PLoS Med 6: e10.
26. Zhang JJ, Wu HS, Wang L, et al. (2010): Expression and significance of TLR4 and HIF-1α in pancreatic ductal adenocarcinoma. World J Gastroenterol 16: 2881-2888.
27. Hua D, Liu MY, Cheng ZD, et al. (2009): Small interfering RNA-directed targeting of Toll-like receptor 4 inhibits human prostate cancer cell invasion, survival, and tumorigenicity. Mol Immunol 46: 2876-2884.
28. Goto Y, Arigami T, Kitago M, et al. (2008): Activation of Toll-like receptors 2, 3, and 4 on human melanoma cells induces inflammatory factors. Mol Cancer Ther 7: 3642-3653.
29. Li H, Han Y, Guo Q, et al. (2009): Cancer-expanded myeloid derived suppressor cells induce anergy of NK cells through membrane-bound TGF-beta 1. J Immunol 182: 240-249.
30. Rodríguez PC, Ochoa AC (2008): Arginine regulation by myeloid derived suppressor cells and tolerance in cancer: mechanisms and therapeutic perspectives. Immunol Rev 222: 180-191.
31. Kim S, Takahashi H, Lin WW, et al. (2009): Carcinoma-produced factors activate myeloid cells through TLR2 to stimulate metastasis. Nature 457: 102-106.
32. Pirinen R, Leinonen T, Böhm J, et al. (2005): Versican in non-small cell lung cancer: relation to hyaluronan, clinicopathologic factors, and prognosis. Hum Pathol 36: 44-50.
33. Wang W, Xu GL, Jia WD, et al. (2009): Ligation of TLR2 by versican: a link between inflammation and metastasis. Arch Med Res 40: 321-323.
34. Wiemann B, Starnes CO (1994): Coley's toxins, tumor necrosis factor and cancer. Pharmacol Ther 64: 529-564.
35. Adams S (2009): Toll-like receptor agonists in cancer therapy. Immunotherapy 1: 949-964.
36. Murata M (2008): Activation of Toll-like receptor 2 by a novel preparation of cell wall skeleton from Mycobacterium bovis BCG Tokyo (SMP-105) sufficiently enhances immune responses against tumors. Cancer Sci 99: 1435-1440.
37. Jackson AM, Alexandroff AB, Kelly RW, et al. (1995): Changes in urinary cytokines and soluble intercellular adhesion molecule-1 (ICAM-1) in bladder cancer patients after bacillus Calmette-Guérin (BCG) immunotherapy. Clin Exp Immunol 99: 369-375.
38. El-Omar EM, Ng MT, Hold GL (2008): Polymorphisms in Toll-like receptor genes and risk of cancer. Oncogene 27: 244-252.
39. Sun J, Wiklund F, Zheng SL, et al. (2005): Sequence variants in Toll-like receptor gene luster (TLR6-TLR1-TLR10) and prostate cancer risk. J Natl Cancer Inst 97: 525-532.
40. Boraska Jelavić T, Barisić M, Drmic Hofman I, et al. (2006): Microsatelite GT polymorphism in the toll-like receptor 2 is associated with colorectal cancer. Clin Genet 70: 156-160.