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Review

The role of Toll-like receptor 9 in gynecologic cancer

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ABSTRACT

Toll-like receptor 9 (TLR9) plays a major role in the fight against DNA viruses infections. Despite its antitumor properties, inappropriate activation of TLR9 during chronic inflammation may cause the activation of transcription factors inducing pro-cancerous activities. Thus, the relationship between TLR9 and cancer remains highly confrontational especially in gynecological cancers and cervical cancer induced by viruses. In this review, we focus on the beneficial and detrimental role of TLR9 in gynecological carcinogenesis. TLR9 contributes to tumor regression by inducing cytotoxic T cell response (CTL), reducing the numbers of myeloid-derived suppressor cells (MDSCs), the tumor-associated macrophages (TAMs) and the regulatory T cells (T regs). It can however, also promote tumor progression and invasiveness of cervical tissue. Therefore, the dichotomous role of TLR9 needs to be carefully investigated in the setting of neoplastic disease.

1. Introduction

Chronic inflammation, the seventh hallmark of cancer, is known as the most important epigenetic and environmental factor leading to tumor progression. Human papillomavirus (HPV) infection is among the most relevant example of microbial infection that can predispose to cancer development. Cervical neoplasia occurs when there is a persistent infection by at least one high-risk HPV through the action of the two early viral oncoproteins E6 and E7. *Helicobacter pylori* infection causes chronic gastritis and is the strongest known risk factor for the development of gastric cancer. Thus, manipulating chronic inflammation remains among the best and logical steps toward preventing many types of malignant diseases [1].

Immunological surveillance is an important method by which cancer is controlled in healthy people. Infections by such viruses or bacteria modulate the host immune response allowing the persistence of the pathogen and the progression of the infection. These pathogens interfere with the recruitment, the maturation, and the activation of the cells belonging to innate immunity such the antigen-presenting cells (APC) leading to the modulation of the adaptative immune response by notably disrupting the Th1 and TH2 balance.

The Toll-like receptors (TLRs) play a pivotal role in early innate immune defense mechanisms and in pathogen-host interaction by recognizing conserved pathogen-associated molecular patterns (PAMPs) expressed on a wide array of microbes and by activating pathways of inflammation. A growing number of reports have shown that TLRs could be a therapeutic target for inflammatory diseases [2]. While the importance of TLRs in the inflammatory signals in carcinogenesis is clearly demonstrated [3], the antitumor or pro-tumor role of these receptors remains controversial. Indeed, TLRs activation has been shown to exert an antitumor activity in many cancer models [4] but have been also reported to promote tumor cell survivor [3]. Various TLRs agonists have been investigated for cancer immunotherapy and among those agonists for TLR9. We will here review the beneficial and detrimental role of TLR9 activation in gynecological carcinogenesis.

2. Overview on TLRs

Toll receptor was first discovered in Drosophila in 1996 as an essential pathway to control resistance to fungal and Gram-positive bacterial infections. Soon after in 1997, Janeway and his team have discovered TLR4 and its role in the recognition of LPS and in the subsequent cellular responses in mammals. Since then, a

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growing number of TLRs have been implicated in the innate recognition of the divers PAMPs present on the surface of different pathogens. TLRs are type 1 trans-membrane proteins with an N-terminal ectodomain containing a various numbers of Leucin-Rich-Repeat (LRRs). This domain interacts with microbial components or agonists. The highly conserved C-terminal cytoplasmic domain of TLRs contains domains similar to the one present in the interleukin-1 receptor (IL-1R) intracellular domain. This Toll/IL-1R (TIR) domain is also shared by several downstream adaptors molecules that are then responsible for the initiation of signaling pathways activated downstream of TLRs. Owing to their immune-regulatory role, TLRs represent a very attractive therapeutic target for many adjuvants to treat numerous infectious, autoimmune diseases and cancer. The role of TLRs in the activation of the immune system was a revolutionary finding by the Nobel Prize for medicine awarded in 2011 [5]. However, inappropriate activation of these pathways such as continuous stimulation of TLRs by microbial products can lead to constitutive activation of transcriptional factors acting downstream of these receptors such as Nuclear Factor κ B (NF-κB) and Signal Transducer and Activator of Transcription 3 (STAT3). These two factors cooperate to trigger pro-cancerous activity through the activation of multiple effectors implicated in the induction of pre-neoplastic mutation, the stimulation of angiogenesis or the resistance to apoptosis. Indeed, the expression of the anti-apoptotic proteins, B-cell lymphoma 2 (Bcl-2) and BCL-XL (B-cell lymphoma-extra large Bcl-XL) are promoted by both NF-κB and STAT3 [6]. Thus whereas increasing evidences point towards the critical role of TLR in tumorogenesis and tumor regression, activation of TLRs appears also to play a role in inducing tumor immune evasion [7,8]. These two contrasting scenarios are probably organ, cell or context dependent. It is quite evident that TLRs can exert a pivotal role in many organs. Thus, one should be aware towards any potential therapeutic manipulation of TLRs and further mechanistic investigations of TLRs on specific cell types involved in tumor biology will be required.

3. Role of TLR9 in carcinogenesis

TLR9, also known as CD289 (cluster of differentiation 289) was first cloned and identified in 2000 as a receptor for unmethylated cytosine phosphate guanine CpG-DNA as well as for bacterial DNA [9]. We distinguish three classes of synthetic CpG ODN depending on their nucleotide sequence and length: CpG class A, CpG class B, CpG class C. CpG class A induces high amounts of type I IFNs in plasmacytoid dendritic cells and strongly activate the maturation of antigen-presenting cells (APC), but is a weak B-cell activator. CpG-B strongly induces B-cell proliferation and differentiation but weakly activate type I IFN production and APC maturation.

TLR9 is expressed on intracellular vesicules membranes and recognizes both bacteria and viruses derived-DNA. It plays an important role in fighting Herpes Simplex Virus (HSV1, HSV2), murine cytomegalovirus (MCMV) and Adenovirus infections. The DNA of these viruses can engage TLR9 which results in the secretion of IFNα by plasmacytoid dendritic cells (pDCs) in a MyD88-dependent manner [10–12]. TLR9 can also recognize DNA of *Mycobacterium tuberculosis*, Brucella [13], *Streptococus pneumoniae* [14] Helicobacter [15] and Cryptococcus neoformans [16] bacteria. Hemozoin (HZ), a metabolic product of *Plasmodium* sp. and a Malaria pigment, is also another TLR9 ligand [17].

Upon ligand binding, the TIR domain of TLR9 recruits MyD88 which forms a supramolecular complex with TNF receptor-associated factor 6 (TRAF6), Interleukin-1 receptor-associated kinases 1 and 4 (IRAK1/IRAK4), and Interferon Regulatory Factor 7 (IRF7). Once phosphorylated, IRF7 translocates to the nucleus and induces the expression of type I inteferon and interferon inducible genes. Stimulation of TLR9 with its ligands leads also to the activation of other transcription factors, including nuclear factor κB (NF-κB) and activator protein-1 (AP-1) [18].

TLR9 are expressed on conventional dendritic cells (cDCs) and macrophages. It is also expressed in the healthy human colon, respiratory epithelial cell lines, and gastric epithelium of human stomach [19–21]. TLR9 plays also a pivotal role in the crosstalk between DCs and Natural Killer (NK) cells to achieve the activation of both cells types [22].

The ability of TLR9 to properly activate antigen-presenting cells (APCs) and specially DCs toward the priming of T cells [23], have supported studies of the effectiveness of TLR9 agonist, either as monotherapy or as adjuvants for vaccination. Indeed, CpG ODN acts as strong adjuvants to polarize helper T cell responses toward Th1 phenotype. CpG ODN can increase the radiosensitivity of human glioma cells [24], enhance the antitumor effects of T cell [25], shift immune response towards CD8T cells and decrease the number of CD4CD25 Treg cells [26] in C57BL/6 mice. On the other hand, The stimulation of TLR9 through their ligands induce conventional DCs and macrophages to produce pro-inflammatory cytokines such as TNFα, IL6 and IL12 and to up-regulate surface expression of MHC II and co-stimulatory molecules. TLR9 is also highly expressed on human pDCs, known as "professional type I Interferon producing cells". Type I interferon from TLR9-stimulated pDCs directly activate cytotoxic T cells [23] and helper T cells [27].

However, despite promising preclinical studies involving cervical carcinoma in animal models, clinical trials have been discouraging and no FDA-approved TLR9 agonist is available for tumor therapy. This failure of TLR9 agonists as cancer immunotherapeutics may be explained by the induction of immune suppressive factors, such as IL-10, T regulatory cells (Treg), and PD-L1. Moreover, TLRs may promote tumor formation in certain organs such as the stomach, pancreas and the liver [21,28,29]. This concept remains one of the most relevant scientific discoveries in the field of TLRs and cancer biology during the past decade [3].

Several previous works have shown that TLR9 signaling promotes tumor growth, survival and immune evasion in cervical cancer as well as in many other cancer types, such as gastric, prostatic, breast, lung cancer, glioma, oesophageal and oral squamous cell carcinoma [7,8]. Whether TLR9 promotes or suppresses cancer development remains thus under discussion (Fig. 1), and the polymorphism of TLR9 and the genetic variations of its signaling pathway are among the tracks being explored [30].

4. Cervical cancer and TLR9

Cervical cancer (CC), ranking second to breast cancer, is a public health problem affecting women worldwide and especially in developing countries where Pap smear screening has not been successful to control the disease. The HPV is the etiologic agent of cervical cancer and its persistence a key early event of the infection. Innate immunity, the first line of defense against infection, plays a major role in resistance to infection. TLR9 was demonstrated to be expressed in HPV-positive cervical neoplasia, on human foreskin, vaginal and cervical keratinocytes cell lines bearing episomal or integrated copies of HPV16 and 18 in several CC derived cell lines and in formalin-fixed paraffin-embedded or frozen cervical tissue sections [31]. Our findings [32] and other reports [33–37] using Tunisian, Canadian, Korean, Chinese, and Italian women formalin-fixed and paraffin-embedded tissue specimens, have shown an increasing expression of TLR9 in neoplastic cervical epithelia in the course of progression of the disease. All these reports suggest that TLR9 up-regulation could be beneficial to tumor progression and invasiveness of cervical tissue.