The role of Toll-like receptors in ovarian cancer

Vlad Gata1,2, Ignat Florin Laurentiu1,2
1Faculty of Medicine, "Iuliu Hatieganu" University of Medicine and Pharmacy, Cluj-Napoca, Romania; 2"Ion Chiricuta" Institute of Oncology, Cluj-Napoca, Romania

Summary

Ovarian cancer (OC) is the leading cause of death in gynecological malignancies in western countries. Chemoresistance represents a major issue and a better understanding of the interactions that take place within the tumor microenvironment is needed, such as the connection between inflammation and cancer. Toll-like receptors (TLRs) are part of the basic mechanisms that are involved in the activation of the innate immune system, with extensive activation of various transcription factors, that subsequently lead to an adaptive immune response, making them key players that modulate the inflammatory response and tumor dynamics. In the same time, activation of TLRs on OC cells can lead to a different type of response that favors an aggressive phenotype and tumor progression. Herein, we review the recent evidence towards the role of TLRs in OC and the therapeutic strategies that have already commenced.

Key words: chemoresistance, ovarian cancer, toll-like receptor, tumor microenvironment

Introduction

Ovarian cancer (OC) is the second most frequent gynecological cancer, and in terms of mortality, it ranks first among women in Western countries [1]. Even if more than 60% of patients are diagnosed at advanced disease stages, over the past two decades there have been no significant changes in overall survival [2,3]. About 80% of patients obtain a complete response following primary treatment, which consists of radical surgery and platinum-based chemotherapy [4], but the great majority will have recurrences and will develop resistance, which will finally lead to death. In the management of platinum-resistant or platinum-refractory OC there is no standardized sequence of optimal use of various chemotherapy drugs [5], although in recent years there has been a surge in the development of innovative agents [6,7].

Taking into account the protracted course of this disease, where the physician represents for the patients the most important point of contact, explaining why treatments often fail, additional research is needed to provide a better understanding of this disease [8].

Angionenesis-targeted therapies have been validated in clinical trials, but their impact on disease-free interval has been so far modest, without prolongation of overall survival [9]. Some of the reasons why targeting angiogenesis in OC still requires further investigations might be linked to the presence of OC stem cells [10-12], various players within the tumor microenvironment [13-15] or OC cells [16].

One of the greatest current problems is the incomplete understanding of molecular chemoresistance mechanisms, which affect a considerable
proportion of patients [17]. There has been recent evidence of interactions occurring between the tumor microenvironment and tumor cells, which can directly influence response to treatment [18,19]. In this respect, there is a real need to understand the connection between inflammation and cancer, as well as the key events that lead to the appearance of an inflammatory response and its role in tumor progression.

In this review we have outlined the most important data regarding TLRs in OC. We reviewed Medline indexed articles using the following key words: ovarian cancer, toll-like receptor, chemoresistance. Each relevant article was screened for further relevant publications.

Over the past decade, progress has been made in understanding the way inflammatory cells actively participate in this process by cytokine secretion [20]. In this tumor microenvironment, a number of inflammatory cells have been identified, among which neutrophils, eosinophils, dendritic cells, lymphocytes and tumor-associated macrophages [21]. Initially, it was speculated that these inflammatory cells have a suppressive role; subsequently it was demonstrated that they play a dual role and might also be involved in tumor progression and the decrease of immunovigilance [22,23].

Although OC was initially considered a poorly immunogenic tumor, recently there has been evidence supporting the hypothesis according to which disease progression is due to quantitative and qualitative changes in some immunosuppressive leukocyte subpopulations [24,25].

One of the basic mechanisms involved in the activation of the innate immune system is represented by TLRs [26]. Immune response in antifungal defense was evidenced for the first time about 20 years ago in Drosophila experiments [27].

TLRs belong to the family of type I transmembrane receptors, which specifically recognize pathogen-associated molecular patterns (PAMPs). These can be carbohydrate, lipid, protein or nucleic acid structures expressed by different pathogens such as bacteria, fungi, viruses or parasites [28]. In addition to recognizing exogenous structures, TLRs can also recognize endogenous structures [29], frequently cell debris resulting from cell death [30]. So far, 10 TLRs expressed in a wide range of tissues have been identified in humans [31], and their expression has been confirmed in both normal and tumor ovarian tissue [32].

Intracellular signaling subsequent to TLR activation can occur by two different pathways, MyD88-dependent or independent. The MyD88-dependent pathway leads to NF-kB activation and proinflammatory cytokine production, and the MyD88-independent signaling pathway finally results in interferon type I production [33]. TLRs can be considered an important link between innate and adaptive immunity, because with their activation in macrophages and dendritic cells, an inflammatory response occurs which leads to an extensive activation of various transcription factors, including NF-kB [34], and subsequent cytokine secretion will lead to the recruitment and activation of cells involved in adaptive immune response.

TLR activation in dendritic cells (DC) induces an alteration of the inflammatory profile in the tumor microenvironment, by an increase in interferon type I secretion, tumor antigen processing capacity, and overexpression of co-stimulating cells for an immunostimulating response [25,35]. In addition, there is evidence suggesting that TLR activation can modulate immune response by direct action on CD4+ and CD8+ T cells, as well as by suppression of regulatory T (Treg) cell activity [36,37].

The hypothesis that a TLR3 agonist might be used for the treatment of OC alongside conventional chemotherapy was launched more than a decade ago. According to it, the exogenous activation of TLR3 in DC can induce the activation, proliferation and survival of naïve tumor-specific CD4+ and CD8+ T cells [38]. The use of a synthetic TLR3 agonist poly(I:C12U) demonstrated in vitro its effectiveness in the phenotypic maturation and functional activation of DC in the case of 5 patients with advanced disease stages, at levels comparable to those of a control group formed by 6 healthy voluntary subjects [39].

Macrophages are a versatile cell population abundant in the stromal compartment of many types of cancers, whose action depends on the presence of different stimuli in the tumor microenvironment. The presence of M2 tumor-associated macrophages (TAMs) supports tissue repair and remodeling processes, thus promoting tumor growth [40]. In a study conducted by Bellora et al., the interaction between tumor-associated macrophages (TAMs) and natural killer (NK) cells was analyzed. TAMs derived from the ascitic fluid of patients with OC had an M2 phenotype, but with TLR stimulation, their conversion to an M1 phenotype was observed, as well as the release of immunostimulating cytokines and effective NK cytolytic activity [41], thus constituting an anti-tumor immune response.

A phase I study evaluating a TLR7 agonist in the case of patients diagnosed with gynecological tumors, including 10 patients with recurrent OC,
demonstrated the clinical activity of this agent with the activation of the immune system and the maturation of DC, the increased antigen presentation capacity opening the way to other studies on this compound [42].

In addition to the innate immune system activating capacity, TLR8 can also inhibit the function of Tregs. Several phase I trials have so far assessed the activity of VTX-2337, a TLR8 agonist, in the case of patients diagnosed with recurrent OC, which proved to be a potent inducer of immune system activation [43]. Although the results of the phase II trial were negative, prespecified subgroup analysis showed a survival benefit in the case of patients treated with a TLR8 agonist who had adverse reactions at the injection site, as well as based on immune responses in vitro [44].

The effectiveness of TLR9 agonists was studied in various animal models of OC, both as monotherapy [45] and in combination with other immunomodulators [46], with favorable results regarding tumor regression as well as immune system activation with anti-tumor effects.

On the other hand, the expression of TLRs was also evidenced in tumor cells, where their activation may induce tumor progression, the dual role of these receptors in OC being thus evidenced. Damage-associated molecular patterns (DAMPs) derived from necrotic cells represent the source of endogenous stimulation of TLRs, which are present at high levels in the tumor microenvironment, supporting in this way chronic inflammation [47]. In the case of OC, their expression can also be used by tumor cells for increased survival, pro-tumoral inflammatory cytokine production, immune response suppression and, consequently, increased chemoresistance [32]. In an immunohistochemical study performed in more than 500 cases, a higher TLR4 and MyD88 expression level in tumor cells represented an independent prognostic factor for lower overall survival [48], being at the same time correlated with the development of chemoresistance [49].

A study that evaluated the prognostic role of transcriptomic expression signature in the case of rare OC histologies concluded that overexpression of genes involved in TLR is correlated with a significantly shorter disease-free interval [50].

Given what was mentioned above, a number of studies attempted to block these tumor cell receptors. Paclitaxel is one of the cytotoxics used as a first-line treatment for OC. However, it is also a TLR4 agonist, and this effect might potentiate tumor growth in a subgroup of patients whose tumor cells have an activated TLR-4-MyD88-NFκB signaling pathway. Kim et al. demonstrated that in the case of these patients, the use of ARRY-520, an inhibitor of the division spindle without agonist action on TLR4, instead of paclitaxel yields better results and does not induce tumor progression or chemoresistance phenomena, thus representing a viable alternative [51]. Subsequent studies demonstrated that knock down of TLR4 in SKOV-3 cancer cells by targeted delivery of siRNA allowed to restore sensitivity to paclitaxel, with an increase in the number of apoptoses of ovarian tumor cells [52]. Another interesting observation is the fact that in the case of breast cancer, the effect of TLR4 activation is dual, depending on the status of TP53. Thus, in the case of wild-type p53 cancers, activation of TLR4 suppresses cell growth, in contrast to its activation in the case of p53-mutated cancers, where a cell growth effect through a series of proinflammatory cytokines is seen [53]. In the case of OC, p53 protein mutations are found in more than 80% of the cases; thus, it can be speculated that in the majority of OC cases, TLR4 activation will lead to tumor progression [54].

**Conclusion**

In recent years, we have witnessed a surge in scientific papers addressing the role of TLRs in OC and their intimate connection with functions of the immune system. This kind of research has opened a new spectrum that explores various immune-related therapies that interact with the host’s natural antitumor response. While many of these therapies that are in preclinical development or early-phase clinical studies have delivered promising results, rational ways of designing future combinations will require in-depth studies that will also address the observed duplicitious effects of TLRs in OC in order to maximize clinical results.

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**Conflict of interests**

The authors declare no conflict of interests.
References

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