JBUON 2017; 22(5): 1092-1096
ISSN: 1107-0625, online ISSN: 2241-6293 • www.jbuon.com
E-mail: editorial_office@jbuon.com

REVIEW ARTICLE

The role of Toll-like receptors in ovarian cancer

Vlad Gata[1,2], Ignat Florin Laurentiu[1,2]

[1]Faculty 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 chemotherapeutic 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

Correspondence to: Dr. Vlad Gata. "Ion Chiricuta" Institute of Oncology, 34-36 Republicii Str, 400015 Cluj-Napoca, Cluj, Romania.
Tel: +40 745311776, E-mail: gatavlad@yahoo.com
Received: 11/07/2017; Accepted: 23/07/2017

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 naive 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 inductor 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 mollecular 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-NFkB 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 duplicitous effects of TLRs in OC in order to maximize clinical results.

Acknowledgements

We would like to thank the University of Medicine and Pharmacy "Iuliu Hatieganu" Cluj-Napoca for the internal research grant no. 4945/16/08.03.2016.

Conflict of interests

The authors declare no confict of interests.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2016. CA Cancer J Clin 2016;66:7-30.
2. du Bois A, Reuss A, Pujade-Lauraine E, Harter P, Ray-Coquard I, Pfisterer J. Role of surgical outcome as prognostic factor in advanced epithelial OC: a combined exploratory analysis of 3 prospectively randomized phase 3 multicenter trials: by the Arbeitsgemeinschaft Gynaekologische Onkologie Studiengruppe Ovarialkarzinom (AGO-OVAR) and the Groupe d'Investigateurs Nationaux Pour les Etudes des Cancers de l'Ovaire (GINECO). Cancer 2009;115:1234-44.
3. Vergote I, Trope CG, Amant F et al. Neoadjuvant chemotherapy or primary surgery in stage IIIC or IV OC. N Engl J Med 2010;363:943-53.
4. Vlad C, Kubelac P, Alexandru I, Achimas-Cadariu P. The role of primary debulking in advanced OC patients. JBUON 2016;21:1320.
5. Oronsky B, Ray CM, Spira AI, Trepel JB, Carter CA, Cottrill HM. A brief review of the management of platinum-resistant-platinum-refractory OC. Med Oncol 2017;34:103.
6. Gherman C, Braicu OL, Zanoaga O et al. Caffeic acid phenethyl ester activates pro-apoptotic and epithelial-mesenchymal transition-related genes in OC cells A2780 and A2780cis. Mol Cell Biochem 2016;413: 189-98.
7. Diab Y, Muallem MZ. Targeted Therapy in OC. A Comprehensive Systematic Review of Literature. Anticancer Res 2017;37:2809-15.
8. Achimas-Cadariu P, Iancu M, Kubelac P, et al. Expectations and perspectives of OC patients about cancer management in Romania. The international NOGGO-ENGOT trial: EXPRESSION III. Eur J Cancer Care (Engl) 2016;0:1-8.
9. Shaw D, Clamp A, Jayson GC. Angiogenesis as a target for the treatment of OC. Curr Opin Oncol 2013;25:558-65.
10. Onisim A, Iancu M, Vlad C et al. Expression of Nestin and CD133 in serous ovarian carcinoma. JBUON 2016;21:1168-75.
11. Onisim A, Achimas-Cadariu A, Vlad C, Kubelac P, Achimas-Cadariu P. Current insights into the association of Nestin with tumor angiogenesis. JBUON 2015;20: 699-706.
12. Markowska A, Sajdak S, Markowska J, Huczynski A. Angiogenesis and cancer stem cells: New perspectives on therapy of OC. Eur J Med Chem 2017 (in press).
13. Vlad C, Kubelac P, Onisim A, Irimie A, Achimas-Cadariu P. The role of CDCP1 (CUB domain-containing protein 1) and ADAM12 (a disintegrin and metalloproteinase 12) in OC. JBUON 2015;20:673-79.
14. Vlad C, Kubelac P, Onisim A et al. Expression of CDCP1 and ADAM12 in the OC microenvironment. JBUON 2016;21:973-78.
15. Achimas-Cadariu P, Irimie A, Achimas-Cadariu L, Neagoe I, Buiga R. Could serologic and ultrasonographic indexes be useful for therapeutic decisions in patients with OC? Chirurgia (Bucur) 2009;104:287-93.
16. Kubelac MP, Fetica B, Vlad IC, Fulop A, Popa A, Achimas-Cadariu P. The role of inhibitor of DNA-binding 1 (ID-1) protein and angiogenesis in serous OC. Anticancer Res 2014;34:413-6.
17. Harter P, Heitz F, du Bois A. Surgery for relapsed OC: when should it be offered? Curr Oncol Rep 2012;14:539-43.
18. Suh DH, Kim HS, Kim B, Song YS. Metabolic orchestration between cancer cells and tumor microenvironment as a co-evolutionary source of chemoresistance in OC: a therapeutic implication. Biochem Pharmacol 2014;92:43-54.
19. Junttila MR, de Sauvage FJ. Influence of tumour micro-environment heterogeneity on therapeutic response. Nature 2013;501:346-54.
20. Balkwill F, Coussens LM. Cancer: An inflammatory link. Nature 2004;431:405-6.
21. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? The Lancet 2001;357:539-45.
22. Coussens LM, Werb Z. Inflammation and cancer. Nature 2002;420:860-7.
23. Philip M, Rowley DA, Schreiber H. Inflammation as a tumor promoter in cancer induction. Semin Cancer Biol 2004;14:433-9.
24. Lavoué V, Thédrez A, Levêque J et al. Immunity of human epithelial ovarian carcinoma: the paradigm of immune suppression in cancer. J Transl Med 2013;11:147.
25. Scarlett UK, Rutkowski MR, Rauwerdink AM et al. OC progression is controlled by phenotypic changes in dendritic cells. J Experim Med 2012;209:495-506.
26. O'Neill LA, Golenbock D, Bowie AG. The history of Toll-like receptors - redefining innate immunity. Nat Rev Immunol 2013;13:453-60.
27. Lemaitre B, Nicolas E, Michaut L, Reichhart JM, Hoffmann JA. Pillars article: the dorsoventral regulatory gene cassette spatzle/Toll/cactus controls the potent antifungal response in Drosophila adults. Cell 1996;86:973-83.
28. Takeda K, Kaisho T, Akira S. Toll-like receptors. Annu Rev Immunol 2003;21:335-76.
29. Tsan M. Toll-like receptors, inflammation and cancer. Semin Cancer Biol 2006;16:32-7.
30. Baccala R, Hoebe K, Kono DH, Beutler B, Theofilopoulos AN. TLR-dependent and TLR-independent pathways of type I interferon induction in systemic autoimmunity. Nat Med 2007;13:543-51.
31. Roach JC, Glusman G, Rowen L et al. The evolution of vertebrate Toll-like receptors. Proc Natl Acad Sci USA 2005;102:9577-82.
32. Zhou M, McFarland-Mancini MM, Funk HM, Husseinzadeh N, Mounajjed T, Drew AF. Toll-like receptor expression in normal ovary and ovarian tumors. Cancer Immunol Immunother 2009;58:1375-85.
33. Kawai T, Akira S. The role of pattern-recognition receptors in innate immunity: update on Toll-like receptors. Nat Immunol 2010;11:373-84.
34. Akira S, Takeda K, Kaisho T. Toll-like receptors: critical proteins linking innate and acquired immunity. Nat Immunol 2001;2:675-80.

35. Scarlett UK, Cubillos-Ruiz JR, Nesbeth YC et al. In situ Stimulation of CD40 and Toll-like Receptor 3 Transforms OC-Infiltrating Dendritic Cells from Immunosuppressive to Immunostimulatory Cells. Cancer Res 2009;69:7329-37.
36. Crellin NK, Garcia RV, Hadisfar O, Allan SE, Steiner TS, Levings MK. Human CD4+ T Cells Express TLR5 and Its Ligand Flagellin Enhances the Suppressive Capacity and Expression of FOXP3 in CD4+CD25+ T Regulatory Cells. J Immunol 2005;175:8051-9.
37. Tabiasco J, Devevre E, Rufer N et al. Human Effector CD8+ T Lymphocytes Express TLR3 as a Functional Coreceptor. J Immunol 2006;177:8708-13.
38. Adams M, Navabi H, Croston D et al. The rationale for combined chemo/immunotherapy using a Toll-like receptor 3 (TLR3) agonist and tumour-derived exosomes in advanced OC. Vaccine 2005;23:2374-8.
39. Navabi H, Jasani B, Reece A et al. A clinical grade poly I:C-analogue (Ampligen®) promotes optimal DC maturation and Th1-type T cell responses of healthy donors and cancer patients in vitro. Vaccine 2009;27:107-15.
40. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. Cancer Res 2006;66:605-12.
41. Bellora F, Castriconi R, Dondero A et al. TLR activation of tumor-associated macrophages from OC patients triggers cytolytic activity of NK cells. Eur J Immunol 2014;44:1814-22.
42. Geller MA, Cooley S, Argenta PA et al. Toll-like receptor-7 agonist administered subcutaneously in a prolonged dosing schedule in heavily pretreated recurrent breast, ovarian, and cervix cancers. Cancer Immunol Immunother 2010;59:1877-84.
43. Monk BJ, Facciabene A, Brady WE et al. Integrative Development of a TLR8 Agonist for OC Chemoimmunotherapy. Clin Cancer Res 2017;23:1955-66.
44. Monk BJ, Brady MF, Aghajanian C et al. A phase 2, randomized, double-blind, placebo-controlled study of chemo-immunotherapy combination using motolimod with pegylated liposomal doxorubicin in recurrent or persistent OC: a Gynecologic Oncology Group partners study. Ann Oncol 2017;28:996-1004.
45. De Cesare M, Sfondrini L, Campiglio M et al. Ascites Regression and Survival Increase in Mice Bearing Advanced-stage Human Ovarian Carcinomas and Repeatedly Treated Intraperitoneally With CpG-ODN. J Immunother 2010;33:8-15.
46. Chuang C-M, Monie A, Wu A, Mao C-P, Hung C-F. Treatment with LL-37 Peptide Enhances Antitumor Effects Induced by CpG Oligodeoxynucleotides Against OC. Hum Gene Ther 2009;20:303-13.
47. Sato Y, Goto Y, Narita N, Hoon DS. Cancer Cells Expressing Toll-like Receptors and the Tumor Microenvironment. Cancer Microenviron 2009;2 (Suppl 1):205-14.
48. Li Z, Block MS, Vierkant RA et al. The inflammatory microenvironment in epithelial OC: a role for TLR4 and MyD88 and related proteins. Tumour Biol 2016;37:13279-86.
49. Luo XZ, He QZ, Wang K. Expression of Toll-like receptor 4 in ovarian serous adenocarcinoma and correlation with clinical stage and pathological grade. Int J Clin Exp Med 2015;8:14323-7.
50. Wang C, Winterhoff BJ, Kalli KR et al. Expression signature distinguishing two tumour transcriptome classes associated with progression-free survival among rare histological types of epithelial OC. Br J Cancer 2016;114:1412-20.
51. Kim KH, Xie Y, Tytler EM, Woessner R, Mor G, Alvero AB. KSP inhibitor ARRY-520 as a substitute for Paclitaxel in Type I OC cells. J Transl Med 2009;7:63.
52. Jones SK, Lizzio V, Merkel OM. Folate Receptor Targeted Delivery of siRNA and Paclitaxel to OC Cells via Folate Conjugated Triblock Copolymer to Overcome TLR4 Driven Chemotherapy Resistance. Biomacromolecules 2016;17:76-87.
53. Haricharan S, Brown P. TLR4 has a TP53-dependent dual role in regulating breast cancer cell growth. Proc Natl Acad Sci U S A 2015;112:E3216-25.
54. Kandoth C, McLellan MD, Vandin F et al. Mutational landscape and significance across 12 major cancer types. Nature 2013;502:333-9.