

Ovarian cancer and inflammation. Part 1. Pro-inflammatory cytokines.

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ABSTRACT

Ovarian cancer is the most threatening cause of death among gynecologic malignancies and represents the fifth leading cause of death from all cancers for women. Research reveals that ovarian cancer patients exhibit significant immune responses against the tumor. In this review of the current literature chiefly the interaction of ovarian cancer tumor cells and the immune system is

discussed. There is increasingly growing evidence that pro-inflammatory cytokines are involved in intricate complex of mechanisms responsible for tumorigenesis, and delicate balance between pro- and anti-inflammatory cytokines is critical for the antitumor host immune response.

Keywords: Ovarian cancer, inflammation, cytokines

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INTRODUCTION

The most common causes of death diagnosed among gynecologic malignancies is ovarian cancer (OC). In 2012, it was estimated that 238,719 cases were diagnosed and 151,905 women died from this disease worldwide [1]. Ovarian cancer incidence and mortality estimates in Europe from 2012 were 65,538 and 42,704 respectively [2]. In Poland OC is the second most frequent invasive malignancy of the female genital tract with an estimated 3,600 cases diagnosed annually [3].

A number of studies suggest that factors related to the inflammation of the ovarian surface epithelium such as ovulation, endometriosis and pelvic inflammatory diseases are associated with an increased risk for epithelial ovarian cancer (EOC). Inflammatory mediators and several cytokines produced by activated innate immune cells such as tumor necrosis factor (TNF)- α and interleukins have been shown to promote tumor initiation, growth and cancer progression [4]. The most important hypothesis regarding EOC carcinogenesis is the ovulation theory, which relates the risk of OC to incessant ovulation. To support this hypothesis, there is growing interest in the etiological role of the inflammation that accompanies each ovulation [5].

Chronic inflammation triggers cellular events that can promote malignant transformation of cells and carcinogenesis. There are at least two ways in which cytokines can activate the growth of tumor cells. They can enhance tumor growth directly by functioning as growth factors, promoting metastasis by increasing cell adhesiveness and/or amplifying tumor angiogenesis. Several pro-inflammatory cytokines, chemokines, vascular endothelial growth factor (VEGF), matrix metalloproteinase-9 (MMP-9), arachidonate 5-lipoxygenase (5-LOX), and cyclooxygenase-2 (COX-2), play critical role controlling apoptosis, angiogenesis, proliferation, invasion, and metastasis. Cytokines can also be powerful modulators of the immune system, enhancing tumor growth by blocking cell-mediated mechanisms for both identifying and destroying tumor cells [6, 7]. The aim of this article is to summarize current views on the role of TNF- α , IL-1, IL-6, IL-8, IL-12 and IL-18 in chronic inflammation and ovarian carcinogenesis.

Tumor Necrosis Factor (TNF- α)

The relationship between unresolved, chronic inflammation and malignancy is quite well established. TNF- α is one inflammatory mediator that has been implicated in carcinogenesis, due to its participation in chronic inflammatory diseases [8]. TNF- α production in the ovarian cancer microenvironment has been recognized for decades, with tumor-infiltrating macrophages likely to be the

major source [9]. In *ex vivo* studies, exogenous TNF- α stimulated ovarian tumor cells to produce TNF- α and to proliferate [10].

TNF- α that is constitutively expressed in the malignant ovarian surface epithelium generates and sustains an intricate network of other mediators that promote tumor growth and peritoneal spread. As compared to benign lesions several reports showed strong immunohistochemical staining of TNF- α in malignant tissues. Recent study showed that histological grade 1 (G1) tumors were significantly correlated with increased TNF- α expression. In addition, early stages (I and II, FIGO) were associated with elevated TNF- α expression, although this was considered on the borderline of statistical significance [11]. Subjects with elevated tissue expression of TNF receptor-2 (TNF-R2) in EOC correlated with the highest risk of cancer progression, while patients with increased stromal TNF- α content showed lower survival rates [7].

Numerous reports have demonstrated abnormally high levels of TNF- α in the blood of OC patients. Within groups of patients with the same tumor type, higher levels of TNF- α correlated with more advanced tumor stage and shorter survival time. However, circulating TNF- α is not always detectable in cancer patients and can vary among individual patients over time and the course of disease [12]. It has been shown that plasma levels of TNF- α differ statistically between patients with versus without OC [13, 14]. Charles et al. [15] demonstrated the importance of TNF- α in promoting tumor growth and in the progression of ovarian malignancies. Recently, Kolomeyevskaya et al. [16] have suggested an interaction between ascites TNF- α and IL-6 in driving tumor progression of advanced OC. They did not observed a consistent interaction of these cytokines with clinical variables (e.g., age, stage, histology, and optimal tumor debulking). Additional exploratory analysis, including multivariate models with clinical variables, showed trends to interactions between levels of TNF- α , IL-6, and IL-8. In each of these interactions, high ascites levels of two or all three of these cytokines correlated with reduced progression-free survival.

Interleukin 1 (IL-1)

Interleukin-1 (IL-1) is a pleiotropic cytokine that primarily affects inflammatory and immune responses, and it also regulates other homeostatic functions of the body and has important influence on the pathogenesis of disease. Excessive IL-1 levels have been implicated in the pathogenesis of acute or chronic inflammatory diseases and malignancies. IL-1, like many other cytokines, has fundamental effects on malignant processes. On the one hand, it is involved in carcinogenesis and malignant transformation, tumor

growth, invasion, and metastasis, but on the other hand, it activates innate and specific immune effector mechanisms that limit the growth of the tumor. In the tumor tissue, IL-1 is produced by host cells, such as stromal cells and infiltrating leukocytes, in response to factors secreted by the malignant cells or as part of the inflammatory response that frequently accompanies tumor growth. However, IL-1 can also be generated and secreted by malignant cells, due to inherent alterations in patterns of cytokine gene expression or in response to host-derived cytokines. The IL-1 family consists of agonistic and antagonistic molecules, as well as receptors. The two major agonistic proteins are IL-1 α and IL-1 β . The third important molecule is the IL-1 receptor antagonist (IL-1Ra), which is a physiological inhibitor of preformed IL-1; it binds to IL-1 receptors without transmitting an activation signal [17].

Interleukin-1 is an alert, upstream, pro-inflammatory cytokine that is generated primarily by myeloid cells. IL-1 initiates and propagates inflammation, mainly by inducing a local cytokine network and enhancing inflammatory cell infiltration to affected sites and by augmenting adhesion molecule expression on endothelial cells and leukocytes. Pro-inflammatory mediators were recently shown to play an important role in tumor-mediated angiogenesis, and blocking their function may suppress tumor progression [18]. IL-1 participates in all phases of malignancy, including carcinogenesis (the *de novo* generation of malignant cells), the invasiveness of already existing tumor cells, and patterns of interactions of the malignant cells with the host's immune system. In experimental tumor models and in cancer patients, increased local levels of IL-1 usually correlate with tumor invasiveness and a bad prognosis. In microenvironmental stroma cells, in leukocytes, and also in malignant cells, exogenous IL-1 induces secretion of growth, invasiveness-promoting factors, i.e. MMPs, and angiogenic factors [i.e. VEGF and basic fibroblast growth factor (bFGF) and ELR-positive CXC chemokines, i.e. IL-8 and macrophage chemotactic protein-1 (MCP-1)]. Although not studied in a comparative manner, it is possibly IL-1 β , which is secreted to a larger extent - compared with mainly cell associated IL-1 α active forms - induces the pro-inflammatory molecule cascade at tumor sites that increases their invasiveness [19].

In previous studies, Huleihel et al. [20] demonstrated that cancerous ovarian tissues express and secrete higher levels of IL-1 α , IL-1 β , IL-6, as well as TNF- α compared to normal ovarian tissues. They also suggested that these cytokines may have a role in the pathogenesis of OC. Recently, Trabert et al. [21] were the first to demonstrate an association between elevated circulating IL-1 α and OC risk; however, given that only 18,4% of values

were above the lower limit of detection for this marker, these results should be interpreted with caution. Finally, studies of Colotta et al. [22] have shed a new light on molecular and cellular links connecting inflammation with cancer. Two pathways have been identified and outlined. In the extrinsic pathway, local inflammatory conditions promote malignant transformation of the tissue. In the intrinsic pathway genetic events give rise to neoplasia, initiate the upregulation of inflammatory circuits promoting tumor progression and growth [23].

Interleukin 6 (IL-6)

Interleukin-6 is mainly involved in inflammation by controlling differentiation, proliferation, migration, and apoptosis of target cells. They have, however, additional roles in a variety of other processes such as metabolism, embryonic development and memory consolidation. A dysfunction of the complex regulatory cytokine network might lead to acute and chronic inflammation, autoimmune diseases or neoplastic disorders [24].

IL-6 is one of the major immunoregulatory cytokines present in the OC microenvironment. Both ovarian cancer cells and associated macrophages produce IL-6, and high serum levels of IL-6 are known to be related to specific immune and metabolic alterations that finally lead to cancer *cachexia*, one of the main causes of death in OC patients. IL-6 has been demonstrated to be involved in the autocrine growth of OC cells most likely by increasing their capacity to secrete MMP-9 [25], thus promoting angiogenesis. Elevated serum IL-6 levels have been detected in patients with systemic cancers as compared to healthy controls or patients with benign diseases. IL-6 has been proposed as a malignancy predictor, with sensitivity and specificity of about 60-70% and 58-90%, respectively [26]. However, there are limited studies available that might be used to define cut-off values for IL-6 as a diagnostic tool.

Likewise, there were strong positive associations between serum IL-6 concentrations and tumor size, tumor stage, or disease progression in patients with OC [27]. Yigit et al. [28] noted a positive correlation between IL-6 concentration in ascites and residual disease after debulking. As compared to primary, advanced disease IL-6 levels were higher at recurrence. In another study, IL-6 levels in ascites correlated significantly with ascites volume and initial tumor size, but not with survival [29]. In contrast, Lane et al. [30] reported that ascites IL-6 levels predicted shorter progression-free survival in patients with ovarian cancer. In addition to its local effects in the tumor microenvironment, tumor-derived IL-6 can stimulate paraneoplastic thrombocytosis in patients with advanced OC that, in turn, is associated with

poor prognosis [31]. In fact, IL-6 plays an important role in the development of ascites as well as the spread of OC through its induction of tumor angiogenesis, thus leading to rapid progression and short survival [32]. Coward et al. [33] demonstrated that IL-6 is part of this cytokine network in OC cells and that IL-6 antagonists may exert therapeutic activity in OC patients by inhibiting of the tumor-promoting cytokine network. It has been demonstrated that IL-6 plasma levels are significantly increased in OC patients relative to patients with benign conditions [34] and that increased IL-6 levels are seen in early stage OC [13]. Median IL-6 levels were higher in patients with early OC than patients with benign adnexal masses, and even higher difference in IL-6 levels in subjects with advanced OC compared to patients with early OC has been observed. However, due to significant overlap between IL-6 levels in the three cohorts, it is believed that plasma IL-6 is not reliable as a stand-alone biomarker for OC. Clendenen et al. [35] reported that women in the highest quartile of IL-6 level versus women in the lowest quartile had a 70% increased risk of OC; however, no associations were observed for TNF- α or its soluble receptors.

Interleukin 8 (IL-8)

Interleukin-8 (IL-8) is a pro-inflammatory chemokine that is basically a chemoattractant and activator of neutrophils during an immune response [36]. The levels of IL-8 are elevated in ovarian cyst fluid, ascites, serum, and tumor tissue from patients with OC [37, 38]. The source of the IL-8 found in ascites has not been well defined. These pro-inflammatory cytokines are involved in different pathophysiological processes including carcinogenesis. Overexpression of IL-8 in OC cells increases anchorage-independent growth, proliferation, angiogenic potential, adhesion and invasion. These effects are decreased on depletion of endogenous IL-8 expression by transfecting IL-8-overexpressing SKOV-3 cells with plasmid encoding for antisense IL-8 [39].

Raised serum concentrations of IL-8 were associated with tumor size, depth of infiltration, or increasing stage of disease in OC [27]. Merritt et al. [40] examined a cohort of OC patients and found that high levels of IL-8 were related to increased disease-specific mortality. In OC-associated ascites, elevated IL-8 levels may lead to neovascularization of tumor implants and decreased IL-17 levels may reduce anti-tumor immune response [41]. Collectively, these data provide the rationale for targeting IL-8 as a therapeutic approach in OC. Wang et al. [39] concluded that IL-8 secreted by OC cells may contribute to malignant behavior of these cells via inducing intracellular molecular signaling. IL-8-stimulated cell proliferation may be associated with alteration of cell cycle progression

and activation of PI3K/Akt and Raf/MEK/ERK, whereas IL-8-potentiated OC cell invasion may be associated with both, increased activity and expression of MMP-2 and MMP-9. Therefore, modulation of IL-8 expression or its related signaling pathway may be a promising strategy for controlling the progression and metastasis of OC.

Interleukin 12 (IL-12)

Immunomodulatory properties associated with IL-12 include T-lymphocyte and natural killer (NK) cell proliferation and cytotoxic activation, and secretion of interferon (IFN)- γ with subsequent tumor inhibition. Besides, strongly anti-angiogenic, mediated through IFN- γ IL-12 function has been reported [42]. As an anti-angiogenic factor, IL-12 offers the possibility of regulating multiple pathways of inherent anti-tumor/anti-cancer agent activity. Specifically, for OC, it has been shown that a significant correlation exists between a low Th1/Th2 ratio defined as IFN- γ .IL-12p40/IL-6 expression and poor survival for advanced stage OC patients [43] and that higher levels of IL-12 in serum and ascites were correlated with patients having no evidence of disease during follow-up and second-look laparotomy [44]. As it has been shown IL-12 is able to mediate disease eradication in primary tumors as well as eradication of metastasis following surgical removal of primary tumors [45]. Additionally, using a murine model of minimal residual disease, IL-12 was shown to eradicate lymphoma in a transplantation setting without affecting lympho-hematopoietic recovery [46]. Thus anti-tumor potentially therapeutic activity of IL-12 has again been documented, this time by data from mouse models. Importantly, IL-12 exerts anti-tumor effects and mediates tumor regression in models of early, intermediate and late stage disease, illustrating the efficacy of IL-12 anti-cancer therapy even in the setting of advanced disease [47]. These studies provided rationale for the use of this cytokine in cancer therapy and several clinical approaches to utilize IL-12 in cancer therapy even in situations where tumor metastases have already occurred.

IL-12 is a potent mediator of anti-tumor immunity. The exact mechanisms of IL-12 mediated anti-tumor effects continue to warrant further investigation. While translation to the clinical setting has been hampered by toxicity and modest anti-tumor efficacy, localized delivery of IL-12 directly into the tumor may prove to be a successful clinical approach in limited numbers of examined tumors. However, it is perhaps in the setting of adoptive T cell immunotherapy utilizing IL-12 secreting, tumor specific T cells, where the full anti-tumor benefit of IL-12 therapy will be realized with tumor targeted, locally secreted cytokine. This approach will avert systemic toxicity while providing the necessary rise in the activity of

the endogenous immune system to fully eradicate tumor. IL-12 remains a unique and promising cytokine with marked anti-tumor activity and warrants continued rigorous investigation in both the preclinical and clinical settings in order to realize the full anti-tumor potential of this reagent [48].

Interleukin 18 (IL-18)

Interleukin 18 (IL-18), formerly known as IFN- γ inducing factor (IGIF), is a pleiotropic, pro-inflammatory cytokine with potent dual effects on tumor development and progression. On the one hand, IL-18 induces T helper type 1 (Th1) immune response, which is generally regarded as the immune reaction that acts against malignant tumors. On the other hand, IL-18 promotes T helper type 2 (Th2) immune responses that may inhibit recognition of cancer cells by immune cells, increase the adhesion molecules, induce production of angiogenic factors, and promote a pro-metastatic microenvironment [49]. Studies done to determine the prognostic potential of serum IL-18 levels in ovarian carcinoma patients failed to demonstrate a correlation with either stage or histology and it is clear that IL-18 alone cannot be used as a specific marker of OC [50]. IL-18 has been shown to be a multifunctional cytokine that has a critical role in ovarian physiologic function, inflammation, and immune response to cancer. Irrespective of its biological activity, IL-18 concentration significantly increases in the blood of OC patients. Its production is a pathophysiologic feature of cancer connecting inflammatory and immune responses to cancer progression. However, the regulatory pathways for IL-18 production by both ovarian carcinoma cells and tumor-induced host cells and its mechanisms of action remain to be determined [51].

CONCLUSIONS

Although many cytokines contribute to carcinogenesis, their pro- or anti-tumoral roles depend on the balance of these different inflammatory mediators and the stage of tumor development. For this reason, studying the role of these mediators in various tumors or stages of development is essential for designing new personalized treatments using these potential prognostic and therapeutic targets. While progress has been made in the understanding of the mechanisms of cytokines in the ovarian tumorigenic process, establishing a relationship between cytokines concentration and disease progression, survival, and response to therapy still remains a major challenge. Finally, recent oncological advancements point to immunotherapy as a one of the promising novel therapeutic strategies under investigation in OC.

Conflicts of interest

The authors declare no conflicts of interest.

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