**The critical role of Macrophages** **in the treatment for Ovarian Cancer**

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**Abstract:** Nowadays, the occurrence and poor progression of ovarian cancer (OC) make the serious threat to the health of middle-aged and elderly women, so it is extremely urgent to get hang of its pathogenesis and take effective therapeutic measures. The OC microenvironment is thought to be involved in numerous malignant biological behaviors and close relationship among several kinds of cells. Macrophages participate in the composition of OC microenvironment, which are usually classified as M1 pro-inflammatory subtype or M2 anti-inflammatory subtype due to its ability of secreting different effective cytokines. The poor progression of OC is closely relevant to the negatively regulation of M2 macrophages polarization, which contribute to the immune escape of tumor cells, and maintain the malignant growth and distant metastasis of OC by secreting several immunological factors. In this review, we have focused on the involvement of macrophages in OC during ageing process and the macrophages-based treatment therapeutic strategies for OC.

**1. Introduction**

Ovarian cancer (OC) is the most lethal malignant tumor of female reproductive system [[1](#_ENREF_1)]. The malignant progression of OC is associated with multiple elements, including abnormal host immune response, ovulation frequency, oncogene activation and silence of tumor suppressor gene, gene mutation, and interaction with growth factors and cytokines in tumor associated microenvironment, etc [[2](#_ENREF_2), [3](#_ENREF_3)]. Patients with OC have lower progression-free survival (PFS) and overall survival (OS) due to lack of effective methods for early diagnosis. The cytoreductive surgery (CRS) and chemotherapy with paclitaxel/platinum are the relevant basic therapies, while 70% of OC patients are still at risk of recurrence and chemotherapy resistance [[4](#_ENREF_4)]. Among many cytological mechanisms involved in the malignant progression of OC, macrophage polarization has been widely concerned as an important component.

Macrophages are a class of natural immune cells with a variety of physiological functions [[5](#_ENREF_5)]. Upon individual stimulation, macrophages could polarize into M1 and M2 phenotypes. Then the differences among several subtypes of macrophages have been displayed in Table 1 [[6-20](#_ENREF_6)]. In the microenvironment of malignant tumors, the tumor associate macrophages (TAMs) are considered as M2 macrophages, which regulate the tumor growth, migration and angiogenesis by producing a large number of growth factors, extracellular matrix remodeling molecules and cytokines, etc [[21](#_ENREF_6)]. According to the relevant reports, M2 macrophage polarization is closely correlated with the malignant progression of colon cancer [[22](#_ENREF_7)], prostate cancer [[23](#_ENREF_8)], liver cancer [24], thyroid cancer [25], craniocerebral tumor [26], pancreatic cancer [[27](#_ENREF_12)] and other tumors. Therefore, it is a potential therapeutic strategy to prevent the poor progression of OC by regulating the activity and phenotype conversion of macrophages [2[8-29](#_ENREF_12)]. This review has focused on the role of macrophage polarization in OC during ageing and the treatment strategies by modulating macrophages.

**2. The role of macrophages in the poor progression of ovarian cancer**

OC performs the highest mortality rate in malignant tumors of female reproductive system. Macrophages play important roles in the OC microenvironment, which not only affect host's defense against microbes, viruses and parasites, but also against tumor cells. TAMs belong to M2 macrophages, which are associated with the poor prognosis of tumors and play an important role in the occurrence and development, distant metastasis and angiogenesis of malignant tumors [30]. It has been demonstrated that the large number of CD163+ M2 macrophages is associated with poor prognosis of epithelial ovarian cancer [31]. In addition, the high M1/M2 ratio in ovarian tumor tissue is associated with early prognosis and long survival of tumor patients [32], otherwise, patients have poor prognosis and reduced survival [33].

2.1 Potential mechanism of M2 macrophages in facilitating poor progression of ovarian cancer

M2 macrophages perform the ability in promoting the immune escape of tumor cells by releasing immunosuppressive factors in OC. For example, during tumor malignant progression, macrophages could polarize into M2 phenotypes in the stimulation with IL-4, IL-10, and IL-13, which in turn induce the progression of angiogenesis, immunosuppression, and matrix remodeling by secreting IL-4, IL-5, and IL-6 [34]. TAMs are responsible for regulating tumor cell migration in the microenvironment through modulating the secretion and interaction of epithelial growth factor (EGF), TNF-α and colony stimulating factor-1 (CSF-1) [35]. In OC microenvironment, TAMs promote cell invasion by enhancing the activity of c-Jun and NF-κB, as well as the upregulation of SR-A level [36,37]. The cytokines and chemokines secreted from OC cells can increase the macrophage recruitment and promote macrophage polarization [38]. For example, the leukemia inhibitory factor (LIF) and IL-6 secreted from OC cells promote the differentiation of macrophages into M2 phenotypes [39]. The CCL2 released from epithelial ovarian cancer cells increase the recruitment of macrophages and M2 polarization in the tumor microenvironment through CCL2/MCP-1 signaling [40]. TNF, CCL22 and CXCL12 secreted from OC cells induce the polarization from M0 macrophages to M2 macrophages in tumor microenvironment [41]. In addition, the transmembrane protein semaphorin 4D (SEMA4D) is highly expressed in OC cell line and the supernatant when compared with normal human ovarian cells and the supernatant, and the peripheral blood mononuclear cells (PBMCs) are tend to differentiate into M2 macrophages with when stimulated by recombinant soluble SEMA4D [42]. Likely, the COX-2 derived from ovarian cancer stem cells affects M2 macrophage polarization by the activation of JAK and COX-2/PGE2 signaling pathways [43].

2.2 Effects of macrophages in the malignant progression of different forms of ovarian cancer

Macrophages play different roles in several histological classifications of OC. The infiltration of TAMs is most common in serous and mucinous OC, and the infiltration of M2 macrophages predicts a poor prognosis of serous OC [46]. Serous OC accounts for more than 70% of all epithelial OC. Ciucci *et al.* found that, compared with patients with high-grade serous OC, patients with low-grade serous OC had less infiltrating (CD68+) macrophages and M2 (CD163+) macrophages in tumor tissues [47]. These results suggest that the differentiation activity of M2 macrophages is related to the occurrence, development and metastasis of OC with different histological classifications [48]. For women, smoking can activate nicotinic receptors, which is conducive to the M2 macrophage polarization, thereby increasing the risk of mucinous OC [49]. However, the relationship among smoking, macrophage polarization, and the risk of mucinous ovarian cancer needs to be further investigated. Endometrioid carcinoma and clear cell carcinoma of the ovary are mostly caused by endometriosis [50]. A study suggested that CDC42+ macrophages could inhibit endometriosis in endometrioid and clear cell carcinoma of ovary, and thus play a certain role in alleviating malignant progression of cancer [51]. Glypican-3 (GPC3), specifically expressed in ovarian clear cell carcinoma, is capable of inhibiting ovarian tumor growth in mice by enhancing the proportion of M1 macrophages [52]. B7-H4 is expressed in the surface of OC cells and is associated with the infiltration of T cells and CD14+ macrophages in ovarian clear cell carcinoma, while have no concern with serous OC and ovarian endometrioid carcinoma [53]. Although several studies have shown that M1 macrophages have significant anti-tumor effects, Untack Cho *et al.* found that M1 macrophages could promote OC cell metastasis by activating NF-κB signaling pathway [54]. These above findings suggest that macrophages, as a type of immune cells in the tumor microenvironment, play an important role in the development and prognosis of OC. Currently, the mechanism of macrophages in different polarization states on the malignant progression of OC with different histological subtypes needs to be further explored.

**3. The promising relationship between macrophage polarization during ageing**

Ageing-mediated immune dysregulation contributes to the normal dynamic balance of different immunological cell subtypes and relevant functions, resulting in the occurrence and poor progression of cancer [55]. Ageing process displays the several characteristics such as Non-infectious chronic, low-grade inflammation and so on, which also performs large amounts of age-related pathological changes [56]. It is illustrated that a large number of macromolecules, cytokines, and multi-protein complexes employed by the innate immune system upon ageing stimulation [57]. In addition to their beneficial roles, IL-1β and IL-18 also result in the occurrence and poor progression of diseases during ageing [58]. Accumulating evidence suggests that low grade chronic inflammation during ageing could attribute to age-related functional decline [59]. Ovarian ageing is a natural process characterized by follicular depletion and a reduction in oocyte quality, resulting in loss of ovarian function, cycle irregularity and eventually infertility and menopause [60]. Ovarian ageing is characterized by ovarian myocyte inflammation and the gradual development of ovarian cancer [61]. When ovarian cancer related inflammation is under consideration, immune activation is critical for malignant progression or protection of ovarian cancer by maintaining dynamic balance of macrophages polarization.

Macrophages act as crucial cells in innate immune system, which could modulate the normal ovarian function during ageing process, with roles in regulating follicle growth, tissue remodeling at ovulation and formation and regression of corpus luteum [62]. Interestingly, when we make poor progression of ovarian cancer into consideration, the number of macrophages increases may indicate the critical role of these immune cells during ageing process [63]. Additionally, M2 subtypes of macrophages are the main source of inducers in the tumor associated microenvironment and paly critical role in the regulation of tumor metastasis, invasion and any other malignant behaviours [64]. Negatively modulating M2 macrophages polarization and reducing percentage of macrophages could be delaying ovarian cancer progression.

**4. Therapeutic strategies for ovarian cancer by modulating macrophages during ageing process**

The occurrence and development of tumors can trigger a series of related inflammatory reactions and serve as therapeutic target for the development of potential measures. Inflammatory microenvironment is able to promote drug resistance and gene instability of tumor epithelial cells, and also affect the colonization and infiltration of immune cells, such as macrophages [65,66]. Many studies have indicated that TAMs play critical roles of "bridge" to interact with tumor cells during the occurrence and development of malignant tumors. At present, there are four therapeutic approaches by targeting TAMs: inhibition of the growth of TAMs, prevention of the recruitment of macrophages, repolarization of M2-like TAMs into M1 macrophages, and delivery through nanoparticle and liposomal system delivery mode [67]. Studies have shown that human recombinant antibody single-chain variable fragments (scFv) can be used to prevent the binding of mesothelin and macrophages, thus inhibiting the polarization of M0 macrophages into TAMs [68]. Currently, some therapeutic drugs targeting TAMs are still under exploration or have been used in clinical practice. For example, trabectedin can interfere with the survival of TAMs [69], and alemtuzumab reduces the number and activity of TAMs by targeting the surface proteins [70]. Nanoparticles loaded with cisplatin can be endocytosis by TAMs, thus affecting tumor cells and playing a role in targeted therapy [71]. Histidine-rich glycoprotein (HRG) has the ability to regulate the repolarization of M2-like TAMs to M1 macrophages, thereby inhibiting tumor malignant proliferation and metastasis, and promoting the anti-tumor immune response [72]. Paclitaxel, an anti-tumor drug used in the treatment of OC, can regulate the repolarization of M2 macrophage to M1 macrophage through TLR4-dependent pathway, thereby inhibiting the tumor growth [73]. In addition, studies have indicated that the relationship between macrophage polarization and OC can be affected by cisplatin. In cisplatin-sensitive tumor cells, macrophages elevate epithelial-mesenchymal transition (EMT) process and EMT-related gene expressions, while such effects cannot be found in cisplatin-resistant tumor cells, suggesting the significant role of macrophage polarization in tumor malignant progression [74].

Some plant extracts display the function to inhibit tumor growth by the alteration of macrophage polarization. For example, in OC, neferine affects angiogenesis by regulating the polarization of TAMs, thus exerting anti-tumor effects[75]. The deoxyschisandra extracted from berries inhibit the activity of M2 macrophage, and onionin A not only enhances the cytotoxic effect of OC cells, but also restrain the activity of M2 macrophages [76,77]. Thus, the above data indicated that the strategy targeting macrophage polarization is an effective way to inhibit the malignant progression of OC.

1. Discussion and perspective

OC is considered as the most malignant tumor in gynecological system because of its atypical clinical symptoms and difficulty in early diagnosis, and gradually developing chemotherapy resistance during treatment. In particular, there is no effective treatment for high-grade recurrent OC. Therefore, it is urgent to effectively understand the molecular mechanism of malignant progression of OC and develop related therapeutic drugs. Ageing also acts as the main element to contribute to the occurrence and poor progression of OC. In order to maintain the suitable tumor microenvironment, several cytokines have been releases by several immune cells during ageing. The tumor microenvironment is a complex network of cytokines, exosomes secreted by different cells, immune cells, fibroblasts, and mesenchymal stem cells. The occurrence and development of tumors are largely affected by the innate and adaptive immune responses. At present, the suppression and elimination of tumor cells by activating the innate immune system has a good tumor treatment effect. Macrophages, as a component of the infiltrating immune cells in the tumor microenvironment, are involved in regulating the malignant progression of OC. In most cases, M1 macrophages have the effect of anti-tumor immunity, while M2-like TAMs are involved in immunosuppression and tumor immune escape. Among all the infiltrating immune cells, TAMs were more abundant. By initiating fibrosis, TAMs regulate the tumor microenvironment, thereby inhibiting immune defense and facilitating angiogenesis. In various tumor types, the number of M2 macrophages in tumors is negatively correlated with patient survival, but positively correlated with tumorigenesis. The alteration of M1/M2 ratio is considered as a potential strategy for the treatment and improvement of the prognosis for OC.

**Consent for publication**

Not applicable.

**Declaration of competing interest**

The authors declare that they have no conflict of interest.

**Data Availability**

Not applicable.

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Table 1. The differences among several subtypes of macrophages.

|  |  |  |  |  |
| --- | --- | --- | --- | --- |
|  | Inducers | Cell expression markers | Cytokine and chemokine  production | References |
| M1a | IFNc, LPS, TNF | Common markers: CD80, CD86, CD68, MHC-II, IL-1R, TLR-2, TLR-4, iNOS, IL10, IL-12;  M1a subtype without CD206 or MGL-1, M1b subtype with CD206 and MGL-1 | TNF-α, IL-1β, IL-6, IL-12, IL-23, IL-27, CXCL9, CXCL10, CXCL11, CXCL16, CCL5, Arg-2, iNOS, ROS | (5-10) |
| M1b |
| M2a | IL-4, IL-13 | MR, AMACI, MHCII, ArgI, IL-1Ra, IL-1R II, FIZZ1, Ym1/2 | TGFβ, IL-10, IL-Ra, CCL17, CCL22, CCL18, CCL22, CCL24 | (11-15) |
| M2b | IC, TLR, IL-1R ligands, IL-1β | MR, MHCII, CD86 | IL-1β, IL-6, TNFα, IL-10, IL-12, CCL1 | (9, 13) |
| M2c | IL-10, Glucocorticoids,  TGFβ | MR, CD163, TLR-1, TLR-8, ArgI | IL-10, TGFβ, CCL16, CCL18,  CXCL13 | (16, 17) |
| M2d | TLR+A2R ligands, adenosine receptor  ligands | VEGF, TNFα, IL-12, IL-10 | IL-10, IL-12, VEGF, TNFα | (18, 19) |
| M3 |  | Arg1, Chi3l3, Ccr2, Cx3cr1, Ccr1, Ccr9; without CD11c or CD206 |  | (20) |