**The critical role of macrophages in ovarian cancer treatment**

Yiran Wang 1, Mingyi Wang 1\*

1. Department of Obstetrics and Gynecology, General Hospital of Western Theater Command of Chinese People’s Liberation Army, Chengdu 610083, China

Corresponding Author:

Prof. Mingyi Wang, Department of Obstetrics and Gynecology, General Hospital of Western Theater Command of Chinese People’s Liberation Army, Chengdu 610083, China. E-mail: wangmingyiog@163.com

**Abstract:** The occurrence of and poor prognosis associated with ovarian cancer (OC) pose a serious threat to the health of middle-aged and elderly women. Thus, there is an urgent need to understand the pathogenesis of OC and establish effective therapeutic measures. The OC microenvironment is thought to facilitate malignancy, as well as close relationships among several types of cells. Macrophages are known to be present in the OC microenvironment. They are usually the M1 pro-inflammatory or M2 anti-inflammatory subtype and contribute to the microenvironment via cytokine secretion. The poor prognosis associated with OC is closely related to the negative regulation of M2 macrophage polarization, which contributes to the immune escape of tumor cells and maintains the malignant growth and distant metastasis of OC cells. In this review, we have focused on the involvement of macrophages in OC during the aging process and the macrophage-based therapeutic strategies for OC.

**1. Introduction**

Ovarian cancer (OC) is the most lethal type of malignant tumor of the female reproductive system [[1](#_ENREF_1" \o "Siegel, 2022 #19)]. The malignant progression of OC is associated with multiple elements, including gene mutation, ovulation frequency, an abnormal host immune response, oncogene activation and silencing of tumor suppressor genes, and interaction of tumor cells with growth factors and cytokines in the tumor-associated microenvironment [[2](#_ENREF_2" \o "Berek, 1994 #7), [3](#_ENREF_3" \o "Drakes, 2018 #8)]. Patients with OC have lower progression-free survival (PFS) and overall survival (OS) rates due to the lack of effective methods for early diagnosis. Cytoreductive surgery (CRS) and chemotherapy with paclitaxel/platinum are the most common therapeutic treatments; however, 70% of OC patients are at risk of recurrence and chemotherapy resistance [[4](#_ENREF_4" \o "Hanker, 2012 #9)]. Among the many cytological mechanisms involved in the malignant progression of OC, macrophage polarization is widely considered an important component.

Macrophages are a class of natural immune cells with a variety of physiological functions [[5](#_ENREF_5" \o "Hirayama, 2017 #10)]. Upon stimulation, macrophages can be polarized into the M1 and M2 phenotypes. The characteristics of several subtypes of macrophages are displayed in Table 1 [[6-20](#_ENREF_6" \o "Hourani, 2021 #11)]. The tumor-associated macrophages (TAMs) found in malignant tumor microenvironments are typically M2 macrophages, and these regulate tumor growth, migration, and angiogenesis by producing a large number of cytokines, growth factors, extracellular matrix remodeling molecules, and other molecules [[21](#_ENREF_6" \o "Hourani, 2021 #11)]. Previous studies have found that M2 macrophage polarization is closely correlated with the malignant progression of colon cancer [[22](#_ENREF_7" \o "Funada, 2003 #12)], prostate cancer [[23](#_ENREF_8" \o "Larionova, 2020 #13)], liver cancer [24], thyroid cancer [25], craniocerebral tumors [26], pancreatic cancer [[27](#_ENREF_12" \o "Kurahara, 2011 #17)], and other tumors. Therefore, regulating the activity and phenotype conversion of macrophages [2[8-29](#_ENREF_12" \o "Kurahara, 2011 #17)] is a potential therapeutic strategy for OC that could improve the poor prognosis associated with OC. This review focuses on the role macrophage polarization plays in OC during aging and treatment strategies based on macrophage modulation.

**2. The role of macrophages in the poor prognosis associated with ovarian cancer (OC)**

OC has the highest mortality rate of all the malignant tumors of the female reproductive system. Macrophages play important roles in the OC microenvironment; they affect the host’s ability to defend against microbes, viruses, and parasites, as well as against tumor cells. OC TAMs are generally the M2 phenotype, contributing to the occurrence, development, distant metastasis, and angiogenesis of malignant tumors and hence the poor prognosis associated with OC [30]. It has been demonstrated that large proportions of CD163+ M2 macrophages are present in epithelial OCs and are related to poor prognosis [31]. In addition, a high M1 to M2 macrophage ratio in OC tissue is associated with early diagnosis and long survival of tumor patients [32]; and the reverse has also been shown [33].

2.1 Potential mechanisms involving M2 macrophages that facilitate OC progression

M2 macrophages promote the immune escape of tumor cells by releasing immunosuppressive factors in the OC microenvironment. For example, during the progression of a malignant tumor, macrophages that are stimulated with interleukin (IL)-4, IL-10, and IL-13 polarize into the M2 phenotype and secrete IL-4, IL-5, and IL-6, which in turn induce the progression of angiogenesis, immunosuppression, and matrix remodeling [34]. TAMs regulate tumor-cell migration in the microenvironment by modulating the secretion of and interactions between epithelial growth factor (EGF), tumor necrosis factor alpha (TNF-α), and colony stimulating factor-1 (CSF-1) [35]. In the OC microenvironment, TAMs promote cell invasion by enhancing the activity of c-Jun and NF-κB and the upregulation of SR-A [36,37]. The cytokines and chemokines secreted by OC cells can increase macrophage recruitment and polarization [38]. For example, it has been shown that leukemia inhibitory factor (LIF) and IL-6 secreted by OC cells promote the differentiation of macrophages into the M2 phenotype [39]. In another study, CCL2 released by epithelial OC cells was found to increase the recruitment of macrophages and M2 polarization in the tumor microenvironment through CCL2/MCP-1 signaling [40]. It has also been shown that TNF, CCL22, and CXCL12 secreted by OC cells induce polarization of M0 macrophages into M2 macrophages in the tumor microenvironment [41]. In addition, the expression of the transmembrane protein semaphorin 4D (SEMA4D) was found to be higher in an OC cell line and the cell culture supernatant than in normal human ovarian cells and the cell culture supernatant, and peripheral blood mononuclear cells (PBMCs) were found to tend to differentiate into M2 macrophages when stimulated by recombinant soluble SEMA4D [42]. It is also likely that COX-2 derived from OC stem cells affects M2 macrophage polarization via activation of the JAK and COX-2/PGE2 signaling pathways [43].

2.2 Effects of macrophages on the malignant progression of different forms of OC

Macrophages play different roles in several types of histologically classified OC. TAM infiltration is most common in serous and mucinous OC, and the infiltration of M2 macrophages predicts a poor prognosis for serous OC cases [46]. Serous OC accounts for more than 70% of all epithelial OCs. Ciucci et al. found that patients with low-grade serous OC had less infiltrating CD68+ macrophages and M2 CD163+ macrophages in tumor tissues than patients with high-grade serous OC [47]. These results suggest that the differentiation activity of M2 macrophages is related to the occurrence, development, and metastasis of different types of histologically classified OC [48].

In women, smoking can activate nicotinic receptors, and this activation has been shown to polarize macrophages into the M2 phenotype, thereby increasing the risk of mucinous OC [49]. However, the relationship between smoking, macrophage polarization, and the risk of mucinous OC 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 may inhibit endometriosis in endometrioid carcinoma and clear cell carcinoma of the ovary and thus play a role in alleviating malignant progression [51]. It has also been shown that glypican-3 (GPC3), which is specifically expressed in ovarian clear cell carcinoma, can inhibit ovarian tumor growth in mice by enhancing the proportion of M1 macrophages [52]. Furthermore, B7-H4 is expressed on the surface of OC cells and is associated with the infiltration of T cells and CD14+ macrophages in ovarian clear cell carcinoma but not in serous OC and ovarian endometrioid carcinoma [53].

Despite several studies having shown that M1 macrophages have significant anti-tumor effects, Untack Cho et al.found that M1 macrophages could promote OC cell metastasis by activating the NF-κB signaling pathway [54].

Together, these findings suggest that TAMs play important roles in the development and progression of OC. Hence, the effects of macrophages in different polarization states on the malignant progression of different histological subtypes of OC must be further explored.

**3. Macrophages can affect ovarian function during aging**

Immune dysregulation associated with aging affects the balance between immune cell subtypes and their relevant functions, resulting in the occurrence and progression of cancer [55]. Several characteristics of the aging process, such as non-infectious low-grade chronic inflammation, contribute considerably to age-related pathological changes [56] and functional decline [59]. It has also been shown that aging stimulates higher expression of a large number of innate immune system macromolecules, cytokines, and multi-protein complexes [57]. In addition to their beneficial roles, IL-1β and IL-18 also contribute to the occurrence and progression of disease during aging [58].

Ovarian aging is a natural process characterized by follicular depletion and a reduction in oocyte quality, which result in loss of ovarian function, cycle irregularity, and eventually infertility and menopause [60]. Ovarian aging can also involve ovarian myocyte inflammation and the gradual development of OC [61]. In cases of OC-related inflammation, it is critical to maintain the balance between the macrophage phenotypes so that it is in favor of protection against OC rather than in favor of malignant progression.

Macrophages have the ability to modulate ovarian function during the aging process due to their roles in follicle growth regulation, tissue remodeling during ovulation, and corpus luteum formation and regression [62]. Interestingly, in cases where there is poor progression of OC, the increased number of macrophages present may indicate the critical role these immune cells play during the aging process [63]. Additionally, M2 macrophages are the main source of inducers in the tumor-associated microenvironment and contribute to the regulation of tumor metastasis, tumor invasion, and other malignant behaviors [64]. Negatively modulating M2 macrophage polarization and reducing the proportion of macrophages could delay OC progression.

**4. Therapeutic strategies for OC that involve modulating macrophages during the aging process**

The occurrence and development of tumors can trigger a series of inflammatory reactions that can serve as therapeutic targets. On the one hand, it has been found that inflammatory microenvironments can promote drug resistance and gene instability in tumor epithelial cells and affect the infiltration and colonization of immune cells, such as macrophages [65,66]. On the other hand, TAMs act as a “bridge,” interacting with tumor cells during the occurrence and development of malignant tumors. At present, there are four therapeutic approaches that target TAMs: inhibition of the growth of TAMs, prevention of the recruitment of macrophages, repolarization of M2-like TAMs into M1 macrophages, and nanoparticle- and liposome-based delivery [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]. Several therapeutic drugs that target TAMs are being investigated 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 surface proteins [70]. Nanoparticles loaded with cisplatin can be endocytosed by TAMs and thus affect tumor cells and play a role in targeted therapy [71]. Histidine-rich glycoprotein (HRG) has been found to regulate the repolarization of M2-like TAMs into M1 macrophages; hence, it could be used to inhibit the proliferation and metastasis of malignant tumors and promote an anti-tumor immune response [72]. Paclitaxel, an anti-tumor drug used in the treatment of OC, can regulate the repolarization of M2 macrophages into M1 macrophages through the TLR4-dependent pathway, thereby inhibiting tumor growth [73]. Studies have also indicated that the relationship between macrophage polarization and OC can be affected by cisplatin. In cisplatin-sensitive tumor cells, macrophages promote the epithelial-mesenchymal transition (EMT) process and EMT-related gene expression, while such effects cannot be found in cisplatin-resistant tumor cells, suggesting that macrophage polarization plays a significant role in malignant tumor progression [74].

Some plant extracts have been found to inhibit tumor growth by altering macrophage polarization. For example, in OC, neferine affects angiogenesis by regulating the polarization of TAMs, thus exerting anti-tumor effects [75]. In other research, deoxyschisandra extracted from berries has been found to inhibit the activity of M2 macrophages, and onionin A has been found to have a cytotoxic effect on OC cells and restrain the activity of M2 macrophages [76, 77]. Thus, these findings indicate that targeting macrophage polarization is an effective strategy for inhibiting the malignant progression of OC.

1. **Discussion and perspective**

OC is considered the most malignant gynecological tumor type because it has atypical clinical symptoms, is difficult to diagnose early, and gradually develops chemotherapy resistance during treatment. Also, there is no effective treatment for high-grade recurrent OC, and aging is a major factor in the occurrence and progression of OC. Therefore, there is an urgent need to understand the molecular mechanisms involved in the malignant progression of OC and to develop effective therapeutic drugs.

The tumor microenvironment is a complex network of cytokines, exosomes secreted by different cells, immune cells, fibroblasts, and mesenchymal stem cells. To maintain a suitable tumor microenvironment, several cytokines are released by different types of immune cells during aging. Hence, the occurrence and development of tumors are largely affected by the innate and adaptive immune responses.

A growing number of studies are showing that suppressing and eliminating tumor cells by activating the innate immune system is an effective tumor treatment strategy.

Macrophages are part of the infiltrating immune cell population in the tumor microenvironment and are involved in regulating the malignant progression of OC. In most cases, M1 macrophages have an anti-tumor effect, while M2-like TAMs support immunosuppression and tumor immune escape. Among all the infiltrating immune cells in the tumor microenvironment, TAMs are typically the most abundant cell type. 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 and positively correlated with tumorigenesis. Hence, alteration of the M1 to M2 macrophage ratio is a potential strategy for treating OC and improving the associated prognosis.

**Consent for publication**

Not applicable.

**Declaration of competing interest**

The authors declare that they have no conflict of interest.

**Data Availability**

Not applicable.

**References**

1. Siegel RL, Miller KD, Fuchs HE, Jemal A: **Cancer statistics, 2022**. *CA: a cancer journal for clinicians* 2022, **72**(1):7-33.

2. Berek JS, Martinez-Maza O: **Molecular and biologic factors in the pathogenesis of ovarian cancer**. *The Journal of reproductive medicine* 1994, **39**(4):241-248.

3. Drakes ML, Stiff PJ: **Regulation of Ovarian Cancer Prognosis by Immune Cells in the Tumor Microenvironment**. *Cancers* 2018, **10**(9).

4. Hanker LC, Loibl S, Burchardi N, Pfisterer J, Meier W, Pujade-Lauraine E, Ray-Coquard I, Sehouli J, Harter P, du Bois A *et al*: **The impact of second to sixth line therapy on survival of relapsed ovarian cancer after primary taxane/platinum-based therapy**. *Annals of oncology : official journal of the European Society for Medical Oncology* 2012, **23**(10):2605-2612.

5. Hirayama D, Iida T, Nakase H: **The Phagocytic Function of Macrophage-Enforcing Innate Immunity and Tissue Homeostasis**. *International journal of molecular sciences* 2017, **19**(1).

6. Gambaro SE, Zubiría MG, Portales AE, Rey MA, Rumbo M, Giovambattista A. **M1 macrophage subtypes activation and adipocyte dysfunction worsen during prolonged consumption of a fructose-rich diet.** *The Journal of nutritional biochemistry.* 2018;61:173-182.

7. Stein M, Keshav S, Harris N, Gordon S. **Interleukin 4 potently enhances murine macrophage mannose receptor activity: a marker of alternative immunologic macrophage activation.** *The Journal of experimental medicine*. 1992;176(1):287-292.

8. Mantovani A, Garlanda C, Locati M. **Macrophage diversity and polarization in atherosclerosis: a question of balance.** *Arteriosclerosis, thrombosis, and vascular biology*. 2009;29(10):1419-1423.

9. Mosser DM. **The many faces of macrophage activation.** *Journal of leukocyte biology.* 2003;73(2):209-212.

10. Gordon S, Martinez FO. **Alternative activation of macrophages: mechanism and functions.** *Immunity*. 2010;32(5):593-604.

11. Jetten N, Verbruggen S, Gijbels MJ, Post MJ, De Winther MPJ, Donners MMPC. **Anti-inflammatory M2, but not pro-inflammatory M1 macrophages promote angiogenesis in vivo**. *Angiogenesis*. 2014;17(1):109-118.

12. Sierra-Filardi E, Vega MA, Sánchez-Mateos P, Corbí AL, Puig-Kröger A. **Heme Oxygenase-1 expression in M-CSF-polarized M2 macrophages contributes to LPS-induced IL-10 release.** *Immunobiology*. 2010;215(9-10):788-795.

13. Mosser DM, Edwards JP. **Exploring the full spectrum of macrophage activation.** *Nature reviews Immunology.* 2008;8(12):958-969.

14. Porta C, Rimoldi M, Raes G, Brys L, Ghezzi P, Di Liberto D, Dieli F, Ghisletti S, Natoli G, De Baetselier P, Mantovani A, Sica A. **Tolerance and M2 (alternative) macrophage polarization are related processes orchestrated by p50 nuclear factor kappaB.** *Proceedings of the National Academy of Sciences of the United States of America.* 2009;106(35):14978-14983.

15. Date D, Das R, Narla G, Simon DI, Jain MK, Mahabeleshwar GH. **Kruppel-like transcription factor 6 regulates inflammatory macrophage polarization.** *The Journal of biological chemistry*. 2014;289(15):10318-10329.

16. Gao S, Mao F, Zhang B, Zhang L, Zhang X, Wang M, Yan Y, Yang T, Zhang J, Zhu W, Qian H, Xu W. **Mouse bone marrow-derived mesenchymal stem cells induce macrophage M2 polarization through the nuclear factor-κB and signal transducer and activator of transcription 3 pathways.** *Experimental biology and medicine (Maywood, NJ)*. 2014;239(3):366-375.

17. Lang R, Patel D, Morris JJ, Rutschman RL, Murray PJ. **Shaping gene expression in activated and resting primary macrophages by IL-10.** *Journal of immunology (Baltimore, Md : 1950)*. 2002;169(5):2253-2263.

18. Grinberg S, Hasko G, Wu D, Leibovich SJ. **Suppression of PLCbeta2 by endotoxin plays a role in the adenosine A(2A) receptor-mediated switch of macrophages from an inflammatory to an angiogenic phenotype.** *The American journal of pathology.* 2009;175(6):2439-2453.

19. Ferrante CJ, Pinhal-Enfield G, Elson G, Cronstein BN, Hasko G, Outram S, Leibovich SJ. **The adenosine-dependent angiogenic switch of macrophages to an M2-like phenotype is independent of interleukin-4 receptor alpha (IL-4Rα) signaling.** *Inflammation.* 2013;36(4):921-931.

20. Malyshev I, Malyshev Y. **Current Concept and Update of the Macrophage Plasticity Concept: Intracellular Mechanisms of Reprogramming and M3 Macrophage "Switch" Phenotype.** *BioMed research international*. 2015;2015:341308.

21. Hourani T, Holden JA, Li W, Lenzo JC, Hadjigol S, O'Brien-Simpson NM: **Tumor Associated Macrophages: Origin, Recruitment, Phenotypic Diversity, and Targeting**. *Frontiers in oncology* 2021, **11**:788365.

22. Funada Y, Noguchi T, Kikuchi R, Takeno S, Uchida Y, Gabbert HE: **Prognostic significance of CD8+ T cell and macrophage peritumoral infiltration in colorectal cancer**. *Oncology reports* 2003, **10**(2):309-313.

23. Larionova I, Tuguzbaeva G, Ponomaryova A, Stakheyeva M, Cherdyntseva N, Pavlov V, Choinzonov E, Kzhyshkowska J: **Tumor-Associated Macrophages in Human Breast, Colorectal, Lung, Ovarian and Prostate Cancers**. *Frontiers in oncology* 2020, **10**:566511.

24. Sica A, Invernizzi P, Mantovani A: **Macrophage plasticity and polarization in liver homeostasis and pathology**. *Hepatology* 2014, **59**(5):2034-2042.

25. Ryder M, Ghossein RA, Ricarte-Filho JC, Knauf JA, Fagin JA: **Increased density of tumor-associated macrophages is associated with decreased survival in advanced thyroid cancer**. *Endocrine-related cancer* 2008, **15**(4):1069-1074.

26. Guadagno E, Presta I, Maisano D, Donato A, Pirrone CK, Cardillo G, Corrado SD, Mignogna C, Mancuso T, Donato G *et al*: **Role of Macrophages in Brain Tumor Growth and Progression**. *International journal of molecular sciences* 2018, **19**(4).

27. Kurahara H, Shinchi H, Mataki Y, Maemura K, Noma H, Kubo F, Sakoda M, Ueno S, Natsugoe S, Takao S: **Significance of M2-polarized tumor-associated macrophage in pancreatic cancer**. *The Journal of surgical research* 2011, **167**(2):e211-219.

28. Wang J, Li D, Cang H, Guo B: **Crosstalk between cancer and immune cells: Role of tumor-associated macrophages in the tumor microenvironment**. *Cancer medicine* 2019, **8**(10):4709-4721.

29. Zheng MJ, Li X, Hu YX, Dong H, Gou R, Nie X, Liu Q, Ying-Ying H, Liu JJ, Lin B: **Identification of molecular marker associated with ovarian cancer prognosis using bioinformatics analysis and experiments**. *Journal of cellular physiology* 2019, **234**(7):11023-11036.

30. Goswami KK, Ghosh T, Ghosh S, Sarkar M, Bose A, Baral R: **Tumor promoting role of anti-tumor macrophages in tumor microenvironment**. *Cellular immunology* 2017, **316**:1-10.

31. Zhang L, Conejo-Garcia JR, Katsaros D, Gimotty PA, Massobrio M, Regnani G, Makrigiannakis A, Gray H, Schlienger K, Liebman MN *et al*: **Intratumoral T cells, recurrence, and survival in epithelial ovarian cancer**. *The New England journal of medicine* 2003, **348**(3):203-213.

32. Zhang M, He Y, Sun X, Li Q, Wang W, Zhao A, Di W: **A high M1/M2 ratio of tumor-associated macrophages is associated with extended survival in ovarian cancer patients**. *Journal of ovarian research* 2014, **7**:19.

33. Yin M, Shen J, Yu S, Fei J, Zhu X, Zhao J, Zhai L, Sadhukhan A, Zhou J: **Tumor-Associated Macrophages (TAMs): A Critical Activator In Ovarian Cancer Metastasis**. *OncoTargets and therapy* 2019, **12**:8687-8699.

34. Gordon S, Martinez FO: **Alternative activation of macrophages: mechanism and functions**. *Immunity* 2010, **32**(5):593-604.

35. Goswami S, Sahai E, Wyckoff JB, Cammer M, Cox D, Pixley FJ, Stanley ER, Segall JE, Condeelis JS: **Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop**. *Cancer research* 2005, **65**(12):5278-5283.

36. Hagemann T, Wilson J, Kulbe H, Li NF, Leinster DA, Charles K, Klemm F, Pukrop T, Binder C, Balkwill FR: **Macrophages induce invasiveness of epithelial cancer cells via NF-kappa B and JNK**. *Journal of immunology* 2005, **175**(2):1197-1205.

37. Nowak M, Klink M: **The Role of Tumor-Associated Macrophages in the Progression and Chemoresistance of Ovarian Cancer**. *Cells* 2020, **9**(5).

38. Kulbe H, Thompson R, Wilson JL, Robinson S, Hagemann T, Fatah R, Gould D, Ayhan A, Balkwill F: **The inflammatory cytokine tumor necrosis factor-alpha generates an autocrine tumor-promoting network in epithelial ovarian cancer cells**. *Cancer research* 2007, **67**(2):585-592.

39. Browning L, Patel MR, Horvath EB, Tawara K, Jorcyk CL: **IL-6 and ovarian cancer: inflammatory cytokines in promotion of metastasis**. *Cancer management and research* 2018, **10**:6685-6693.

40. Negus RP, Stamp GW, Relf MG, Burke F, Malik ST, Bernasconi S, Allavena P, Sozzani S, Mantovani A, Balkwill FR: **The detection and localization of monocyte chemoattractant protein-1 (MCP-1) in human ovarian cancer**. *The Journal of clinical investigation* 1995, **95**(5):2391-2396.

41. Hagemann T, Wilson J, Burke F, Kulbe H, Li NF, Pluddemann A, Charles K, Gordon S, Balkwill FR: **Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype**. *Journal of immunology* 2006, **176**(8):5023-5032.

42. Chen Y, Zhang L, Lv R, Zhang WQ: **Overexpression of Semaphorin4D indicates poor prognosis and prompts monocyte differentiation toward M2 macrophages in epithelial ovarian cancer**. *Asian Pacific journal of cancer prevention : APJCP* 2013, **14**(10):5883-5890.

43. Zhang Q, Cai DJ, Li B: **Ovarian cancer stem-like cells elicit the polarization of M2 macrophages**. *Molecular medicine reports* 2015, **11**(6):4685-4693.

44. Mills CD, Lenz LL, Harris RA: **A Breakthrough: Macrophage-Directed Cancer Immunotherapy**. *Cancer research* 2016, **76**(3):513-516.

45. Wang H, Yung MMH, Ngan HYS, Chan KKL, Chan DW: **The Impact of the Tumor Microenvironment on Macrophage Polarization in Cancer Metastatic Progression**. *International journal of molecular sciences* 2021, **22**(12).

46. Kawamura K, Komohara Y, Takaishi K, Katabuchi H, Takeya M: **Detection of M2 macrophages and colony-stimulating factor 1 expression in serous and mucinous ovarian epithelial tumors**. *Pathology international* 2009, **59**(5):300-305.

47. Ciucci A, Zannoni GF, Buttarelli M, Martinelli E, Mascilini F, Petrillo M, Ferrandina G, Scambia G, Gallo D: **Ovarian low and high grade serous carcinomas: hidden divergent features in the tumor microenvironment**. *Oncotarget* 2016, **7**(42):68033-68043.

48. Colvin EK: **Tumor-associated macrophages contribute to tumor progression in ovarian cancer**. *Frontiers in oncology* 2014, **4**:137.

49. Yang DC, Chen CH: **Cigarette Smoking-Mediated Macrophage Reprogramming: Mechanistic Insights and Therapeutic Implications**. *Journal of nature and science* 2018, **4**(11).

50. LaGrenade A, Silverberg SG: **Ovarian tumors associated with atypical endometriosis**. *Human pathology* 1988, **19**(9):1080-1084.

51. Canet B, Pons C, Espinosa I, Prat J: **CDC42-positive macrophages may prevent malignant transformation of ovarian endometriosis**. *Human pathology* 2012, **43**(5):720-725.

52. Luo C, Shibata K, Suzuki S, Kajiyama H, Senga T, Koya Y, Daimon M, Yamashita M, Kikkawa F: **GPC3 expression in mouse ovarian cancer induces GPC3specific T cell-mediated immune response through M1 macrophages and suppresses tumor growth**. *Oncology reports* 2014, **32**(3):913-921.

53. Kryczek I, Zou L, Rodriguez P, Zhu G, Wei S, Mottram P, Brumlik M, Cheng P, Curiel T, Myers L *et al*: **B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma**. *The Journal of experimental medicine* 2006, **203**(4):871-881.

54. Cho U, Kim B, Kim S, Han Y, Song YS: **Pro-inflammatory M1 macrophage enhances metastatic potential of ovarian cancer cells through NF-kappaB activation**. *Molecular carcinogenesis* 2018, **57**(2):235-242.

55.  Ostuni R, Kratochvill F, Murray PJ, Natoli G: **Macrophages and cancer: from mechanisms to therapeutic implications.** *Trends Immunol*. (2015), 36:229–39.

56. Franceschi, C. & Campisi, J: **Chronic infammation (infammaging) and its potential contribution to age-associated diseases**. *J. Gerontol. Ser. A Biol. Sci. Med. Sci*. (2014), 69: S4–S9.

57.  Broekmans, F. J., Soules, M. R. & Fauser, B. C: **Ovarian aging: mechanisms and clinical consequences.** *Endocr. Rev*. (2009), 30:465–493.

58. Bruunsgaard, H., & Pedersen, B. K: **Age-related inflammatory cytokines and disease.** *Immunology and Allergy Clinics of North America,* (2003), 23(1): 15–39.

59. Zhang, Z., Schlamp, F., Huang, L., Clark, H. & Brayboy, L: **Infammaging is associated with shifed macrophage ontogeny and polarization in the aging mouse ovary.** *Reproduction.* (2020), 159: 325–337.

60. Jabbour, H. N., Sales, K. J., Catalano, R. D. & Norman, J. E: **Infammatory pathways in female reproductive health and disease.** *Reproduction* (2009), 138: 903–919.

61. Weiss, G., Goldsmith, L. T., Taylor, R. N., Bellet, D. & Taylor, H. S: **Infammation in reproductive disorders**. *Reprod. Sci*. (2009),16: 216–229.

62. Wu, R., Van der Hoek, K. H., Ryan, N. K., Norman, R. J. & Robker, R. L: **Macrophage contributions to ovarian function**. *Hum. Reprod.* (2004), Update 10: 119–133.

63. Tingen, C. M. et al: **A macrophage and theca cell-enriched stromal cell population infuences growth and survival of immature murine follicles in vitro.** *Reproduction* (2014), 141: 809–820.

64. Wu R., Van der Hoek K. H., Ryan N. K., Norman R. J., Robker R. L: **Macrophage contributions to ovarian function.***Human Reprod*. (2004), Update 10: 119–133.

65. Chen D, Zhang X, Li Z, Zhu B: **Metabolic regulatory crosstalk between tumor microenvironment and tumor-associated macrophages**. *Theranostics* 2021, **11**(3):1016-1030.

66. Dijkgraaf EM, Heusinkveld M, Tummers B, Vogelpoel LT, Goedemans R, Jha V, Nortier JW, Welters MJ, Kroep JR, van der Burg SH: **Chemotherapy alters monocyte differentiation to favor generation of cancer-supporting M2 macrophages in the tumor microenvironment**. *Cancer research* 2013, **73**(8):2480-2492.

67. Zheng X, Turkowski K, Mora J, Brune B, Seeger W, Weigert A, Savai R: **Redirecting tumor-associated macrophages to become tumoricidal effectors as a novel strategy for cancer therapy**. *Oncotarget* 2017, **8**(29):48436-48452.

68. Dangaj D, Abbott KL, Mookerjee A, Zhao A, Kirby PS, Sandaltzopoulos R, Powell DJ, Jr., Lamaziere A, Siegel DL, Wolf C *et al*: **Mannose receptor (MR) engagement by mesothelin GPI anchor polarizes tumor-associated macrophages and is blocked by anti-MR human recombinant antibody**. *PloS one* 2011, **6**(12):e28386.

69. Germano G, Frapolli R, Belgiovine C, Anselmo A, Pesce S, Liguori M, Erba E, Uboldi S, Zucchetti M, Pasqualini F *et al*: **Role of macrophage targeting in the antitumor activity of trabectedin**. *Cancer cell* 2013, **23**(2):249-262.

70. Pulaski HL, Spahlinger G, Silva IA, McLean K, Kueck AS, Reynolds RK, Coukos G, Conejo-Garcia JR, Buckanovich RJ: **Identifying alemtuzumab as an anti-myeloid cell antiangiogenic therapy for the treatment of ovarian cancer**. *Journal of translational medicine* 2009, **7**:49.

71. Alizadeh D, Zhang L, Hwang J, Schluep T, Badie B: **Tumor-associated macrophages are predominant carriers of cyclodextrin-based nanoparticles into gliomas**. *Nanomedicine : nanotechnology, biology, and medicine* 2010, **6**(2):382-390.

72. Rolny C, Mazzone M, Tugues S, Laoui D, Johansson I, Coulon C, Squadrito ML, Segura I, Li X, Knevels E *et al*: **HRG inhibits tumor growth and metastasis by inducing macrophage polarization and vessel normalization through downregulation of PlGF**. *Cancer cell* 2011, **19**(1):31-44.

73. Wanderley CW, Colon DF, Luiz JPM, Oliveira FF, Viacava PR, Leite CA, Pereira JA, Silva CM, Silva CR, Silva RL *et al*: **Paclitaxel Reduces Tumor Growth by Reprogramming Tumor-Associated Macrophages to an M1 Profile in a TLR4-Dependent Manner**. *Cancer research* 2018, **78**(20):5891-5900.

74. Kan T, Wang W, Ip PP, Zhou S, Wong AS, Wang X, Yang M: **Single-cell EMT-related transcriptional analysis revealed intra-cluster heterogeneity of tumor cell clusters in epithelial ovarian cancer ascites**. *Oncogene* 2020, **39**(21):4227-4240.

75. Zhang Q, Li Y, Miao C, Wang Y, Xu Y, Dong R, Zhang Z, Griffin BB, Yuan C, Yan S *et al*: **Anti-angiogenesis effect of Neferine via regulating autophagy and polarization of tumor-associated macrophages in high-grade serous ovarian carcinoma**. *Cancer letters* 2018, **432**:144-155.

76. Tan HY, Wang N, Man K, Tsao SW, Che CM, Feng Y: **Autophagy-induced RelB/p52 activation mediates tumour-associated macrophage repolarisation and suppression of hepatocellular carcinoma by natural compound baicalin**. *Cell death & disease* 2015, **6**:e1942.

77. Tsuboki J, Fujiwara Y, Horlad H, Shiraishi D, Nohara T, Tayama S, Motohara T, Saito Y, Ikeda T, Takaishi K *et al*: **Onionin A inhibits ovarian cancer progression by suppressing cancer cell proliferation and the protumour function of macrophages**. *Scientific reports* 2016, **6**:29588.

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) |