

Tumor-Associated Macrophages (TAMs): A Critical Activator In Ovarian Cancer Metastasis

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Abstract: Tumor-associated macrophages (TAMs) that appear in every stage of cancer progression are usually tumor-promoting cells and are present abundantly in the tumor-associated microenvironment. In ovarian cancer, the overall and intratumoral M1/M2 ratio is a relatively efficient TAM parameter for predicting the prognosis of patients, especially for serous tissue type cancer. TAMs exhibit immunological checkpoint modulators, such as the B7 family and programmed death-ligand 1 (PD-L1), and play a key role in the development, metastasis and invasion of ovarian cancer, but the underlying mechanism is barely understood. Ovarian cancer is a severe gynecological malignancy with high mortality. Ovarian cancer-associated death can primarily be attributed to cancer metastasis. The majority of patients are diagnosed with wide dissemination in the peritoneum and omentum, limiting the effectiveness of surgery and chemotherapy. In addition, unlike other well-documented cancers, metastasis through vasculature is not a usual dissemination pathway in ovarian cancer. This review sheds light on TAMs and the main process and mechanism of ovarian cancer metastasis.

Keywords: ovarian cancer, tumor associated macrophages, classical activated macrophage, alternative activated macrophage, transcoelomic metastasis, hematogenous metastasis

Introduction

Ovarian cancer is the most deadly female reproductive system malignancy and the fifth leading cause of death in women with cancer.¹ Ovarian carcinoma cannot be defined as a single disease as they are composed of five histological subtypes including epithelial, serous, endometrioid, clear cell and mucinous cancer.²⁻⁴ The high lethality of ovarian cancer is associated with the lack of warning symptoms at an early stage leading to diagnosis at advanced stages (FIGO stage III or IV)⁵ Moreover, screening tests for ovarian cancer are not sensitive. Screening for ovarian cancer possesses several obstacles like shortage of specific detection markers and high false-positive rates for morbidity.³ The mortality rate of ovarian cancer patients did not differ significantly between screened and unscreened women.⁶

Monocyte-macrophage cell lineage are essential inflammatory components of the ecological tumor niche and strongly influence disease progression.⁷⁻¹⁰ In hepatocellular carcinoma, tumor-associated macrophages (TAMs) secrete IL-6 to enhance CD44⁺ cancer stem cells' activity and benefit tumor progression dependent on Signal Transducer And Activator Of Transcription 3 (STAT3) signaling.¹¹ TAMs promote tumor progression to varying degrees: by cultivating cancer stem cells, supporting genetic instability, promoting metastasis and domestication of protective

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adaptive immunity.^{12,13} Owing to expression of programmed death 1 (PD-1) ligands PD-L1, cytotoxic T lymphocyte-associated antigen-4 (CTLA-4) ligands B7-1 and B7-H4, TAM-focused therapeutic strategies are pivotal in immunotherapy such as immune checkpoint therapy.¹⁴⁻¹⁹

TAMs are intimately related to ovarian cancer metastasis.^{20,21} Metastasis-associated macrophages (MAM), a distinct phenotype of macrophages, are required for metastatic extravasation.²² Migrating influx of tumor cells with TAM moves at a higher speed in a more direct way.^{23,24} This process is achieved through self-reinforcement paracrine loops, including colony-stimulating factor 1 (CSF1) produced by cancer cells, hepatocyte growth factor (HGF) released from endothelial cells and epidermal growth factor (EGF) produced by TAM.^{25,26} TAM is located in the center of the spheroid and secretes EGF, which upregulates intercellular cell adhesion molecule-1 (ICAM-1) on tumor cells and α M β 2 integrin on TAM to promote the binding between tumor cells and TAM, thereby contributing to the formation of spheroids which emerge in the early phase of transcoelomic metastasis.²⁷

Origin Of TAMs

As we all know, TAMs, the most abundant immune-related stromal cells in the tumor microenvironment, are vital orchestrators in tumor progression.²⁸ Meanwhile, they also played an indispensable role in tumor development, metastasis, invasion and angiogenesis.^{29,30} So what is the process of TAM transformation?

Transformation From Ly6C⁺ CCR2⁺ Monocytes

By using modern pedigree tracking techniques, the recent understanding of the origin of macrophages has undergone profound changes. Accumulating evidence indicates that circulating Ly6C⁺ CCR2⁺ monocytes are critical progenitors for macrophages.³¹⁻³⁴ Bead labeling and BrdUrd incorporation experiments indicate that all different TAM subpopulations in TS/A tumors originate from Ly6C^{hi} monocytes. Deficiency of chemokine (C-X3-C motif) receptor 1 (CX3CR1) on monocytes leads to TAM recruitment via accumulation of Ly6C^{hi} inflammatory monocytes.³⁵ However, TAM can also originate from erythrocyte progenitor cells (EMP) that develop in the yolk sac of embryos in cancers such as gliomas and pancreatic cancer.^{36,37}

Recruitment Of Monocytes/Macrophages

Monocytes produced from bone marrow hematopoietic stem cells are recruited into tumor tissue and subsequently polarized into TAM.^{38,39} In brain malignancies, brain-dwelling microglia and monocyte-derived macrophages contribute to amplify the TAM pool. In lung cancer, interstitial resident macrophages of embryonic origin together with monocyte-derived (MoD) macrophages contribute to the TAM pool. Interstitial pulmonary macrophages serve as nutritional support for tumor cells, while MoD cells are involved in tumor remodeling and proliferation.³⁸

Recruitment of monocytes contributes to the augmentation of TAM population. In response to chemokines and growth factors secreted by tumor cells and stromal cells contained in TME, peripheral blood mononuclear cells originating from the bone marrow are locally aggregated and polarized to TAM.³⁹ This course is mainly modulated by CSF-1 and chemokines^{40,41} (Figure 1).

Intriguingly, growing information indicates that spleen constitutes an extramedullary reservoir of monocytes. During cancer progression, spleen can significantly amplify pro-tumor TAM response.⁴² According to this, we can infer that TAMs accumulated in tumor area segment originated from the spleen.

Mechanism Of Monocytes Recruitment

Among the broad spectrum of human neoplasms, recruitment mechanisms of monocytes are multifarious. Integration of C-C chemokine receptor type 2 (CCR2) and chemokine C-C motif ligand 2 (CCL2) elicit monocytes to cluster to primary or secondary tumor loci.^{31,43} CCL20, the specific ligand for CCR6, contributes to migration and accumulation of monocytes in vitro and in vivo. So, CCL20-CCR6 can accelerate tumor development via recruitment of monocytes.⁴⁴ Vascular endothelial growth factor A (VEGF-A) is a crucial angiogenic factor while it also acts as an indispensable chemoattractant related to monocytes recruitment. Elevated VEGF-A combined with interleukin-4 (IL-4) and interleukin-10 (IL-10) induce skin carcinogenesis by promoting M2-polarized macrophages in cells and angiogenesis. Besides, extracellular matrix (ECM) components⁴⁵ and hypoxia^{46,47} also promote macrophages to cluster into tumor location.

Properties And Functions Of TAMs Polarization Of TAMs

Macrophages are crucial components of both innate and adaptive immune system and are involved in pathogen

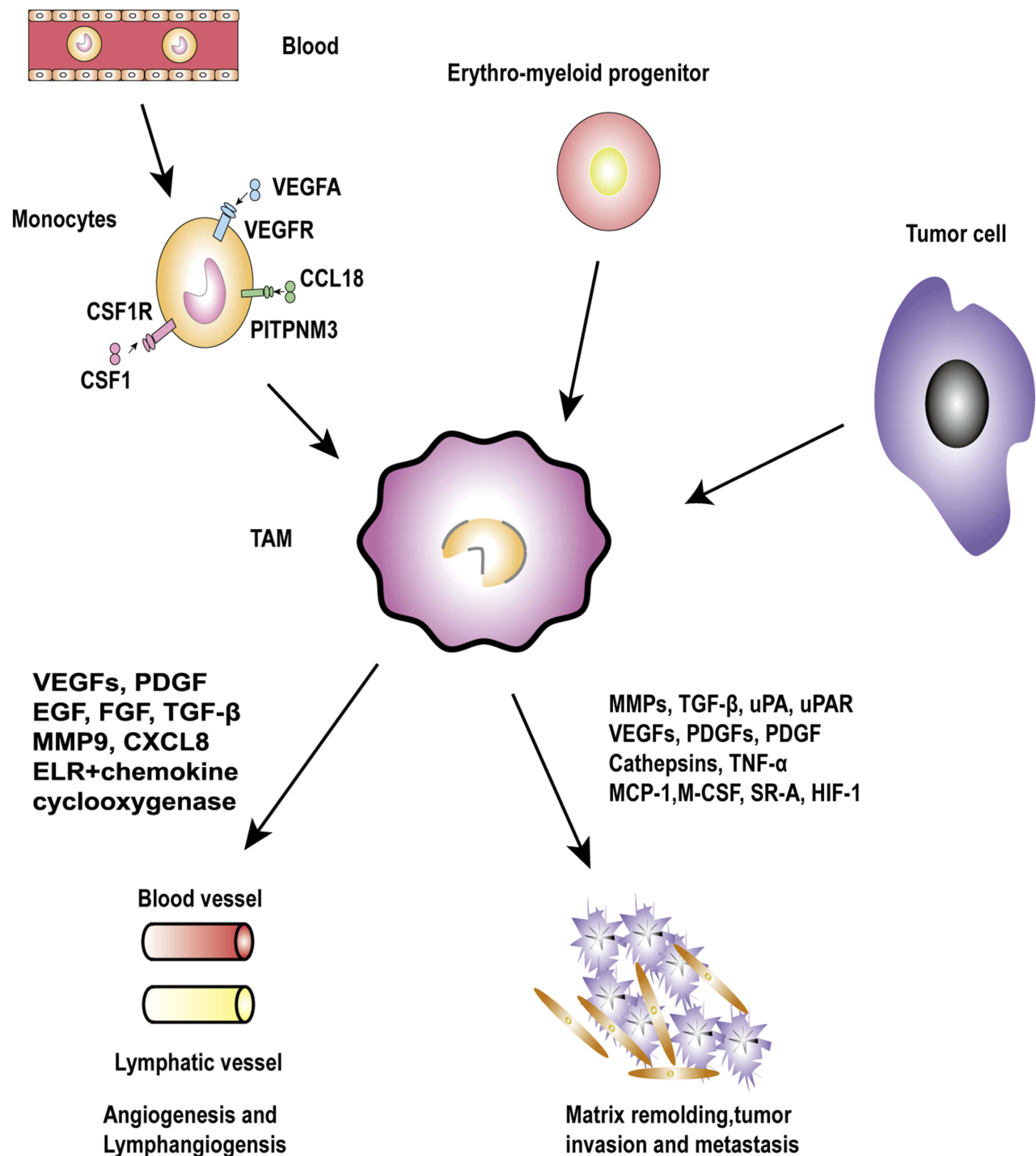


Figure 1 Cytokines and chemokines that influence TAM transformation and function.

Abbreviations: VEGFA, vascular endothelial growth factor A; VEGFR, vascular endothelial growth factor receptor; CCL18, chemokine ligand 18; PITPNM3, Membrane-associated phosphatidylinositol transfer protein; CSF1, colony-stimulating factor; CSF1R, colony-stimulating factor receptor; PDGF, platelet-derived growth factor; EGF, epidermal growth factor; FGF, fibroblast growth factor; TGF-β, transforming growth factor-β; MMP9, matrix metalloproteinase 9; CXCL8, chemokine (C-X-C motif) ligand 8; uPA, urokinase plasminogen activator; uPAR, urokinase plasminogen activator receptor; TNF-α, tumor necrosis factor-α; MCP-1, methylcyclopropene-1; M-CSF, macrophage colony-stimulating factor; SR-A, scavenger receptor A; HIF-1, hypoxia-inducible factor.

response and in-tissue homeostasis.⁴⁸ According to the cytokines that macrophages are exposed to, they can be polarized into two mainstreams, classically activated

macrophage (M1) and alternatively activated macrophage (M2).^{49,50} M1 with IL-12^{high}, IL-23^{high} and IL-10^{low} phenotype is superior in the elimination of microbes and

tumor cells. Conversely, M2 with IL-12^{low}, IL-23^{low}, IL-10^{high} phenotype and mannose receptor and scavenger receptor A are potent effector cells that promote parasite containment, angiogenesis and tissue remodeling, thereby yielding pro-tumoral functions.⁵¹ The notion that the process of M1 polarization is mediated by interferon or lipopolysaccharides (LPS) has been widely recognized during the past several years. IL-4 and IL-13 can directly transform macrophages into M2,^{52,53} whereas IL-13, IL-25 and other cytokines promote the formation of M2 indirectly.^{53–55} Furthermore, M2 consists of three well-defined forms, including M2a (induced by exposure to IL-4, IL-13), M2b (induced by immune complexes, agonists of Toll-like receptors or IL-1R) and M2c (induced by contacting to IL-10 and glucocorticoid hormones).^{56–59}

Macrophages are dynamic cells that can transform into either M1 or M2 with their specific properties. So, polarization does not represent terminal differentiation. When substitution arises in the cytokines contained in the medium, polarized macrophages in a position either revert back to uncommitted M0 or transform into M2.⁶⁰ And inhibition of IκB kinase (IKK) beta activity can result in a shift in TAM from M2 to M1, as evidenced by enhanced expression of IL12, MHCII and iNOS and reduction of IL-4Rα, TNF-α and arginase.^{61,62}

Repolarization Of TAMs

As mentioned above, macrophages are functionally plastic. Modulated by molecules in the TME, macrophages can be repolarized from an anti-inflammatory to a pro-inflammatory phenotype. In ovarian cancer ascites, macrophages polarized into M1 phenotype producing less VEGF, CCL18 and MMP9.⁶³ Nowadays, editing macrophage repolarization from M2 to M1 to inhibit cancer progression raises high interest. Bossche et al found that M1-related suppression of mitochondrial oxidative phosphorylation prevents M1 from polarizing into M2. Reducing the production of nitric oxide can inhibit decline of mitochondrial function, thus improving macrophage phenotype repolarization.⁶⁴ Certain microRNA are shown to facilitate repolarization of TAMs. MiR-125b is expressed in macrophages at a higher level than other immune cells and responsible for macrophages repolarization. Amplifying expression of miR-125b has been viewed as a bright method of repolarizing TAMs. Hyaluronic acid-based nanoparticles delivery system of miR-125b has been exploited in transforming miR-125b into TAMs in Neha et al test.⁶⁵ During the course of M1 to M2 polarization, we can see

that the miR-155 levels were strikingly attenuated. On the contrary, the miR-155 levels were elevated in M2 to M1 transformation. Further experiments prove the hypothesis that microRNA-155 did encourage M2 to M1 repolarization.⁶⁶ Present studies demonstrated that miR-146a involved as a negative regulator in the acquisition of pro-inflammatory cytokines. It has been proved that tumor necrosis factor-related apoptosis-inducing ligand (TRAIL) can exert cytotoxic effects on tumor cells by re-educated M2 to an M1 phenotype in a miR-146a-dependent way.⁶⁷ Additionally, activin A is also a key trigger in the macrophage repolarization caused by GM-CSF and impaired the acquisition of M2 phenotype via Smad2-dependent transduction.⁶⁸ In the resolution of inflammation, TAMs repolarization partly relies on the dissociation of P2X7R from caspase-1 activation.⁶⁹ Aside from the molecules, the pathway transduction is also involved in TAMs repolarization. NF-κB signaling is essential in mannose-sensitive hemagglutination pilus strain of *Pseudomonas aeruginosa* (PA-MSHA) mediated-repolarization in macrophages.⁷⁰ Besides, MAPK/ERK pathway is also indispensable in the course of repolarization.⁷¹

Functional Properties Of TAMs

They have other specific functional properties. M1 macrophages exhibit proinflammatory properties as they show elevated expression of IL-1β, IL-6, IL-12 and TNF-α and accompanied with Th1-mediated immune responses, whereas M2 macrophages show anti-inflammatory properties via enhanced expression of anti-inflammatory cytokines.⁷² M1 is superior in cytotoxic and antitumor activity, whereas M2a and M2c are expert in driving type II response and immunoregulatory function; M2c is also associated with suppression of immune response and tissue remodeling.⁵⁶ In inflammation response, lipopolysaccharide (LPS) or interferon-γ activates NO Synthase 2, which can mutate arginine into OH-arginine and subsequently into NO when macrophages contact the Th1-type cytokines. M1 can exert disruption via this kind of mechanism. On the contrary, when macrophages interact with Th2 cytokines, such as IL-4, IL-10 and IL-13, arginase I decompose arginine into urea and ornithine and then metabolized into proline and polyamines. Proline regulates the production of collagen, whereas polyamines mediate cell proliferation. So, the damaged extracellular matrix can be reconstructed through this process.^{73,74} This metabolic conversion occurs preferentially during activation of the M2a and M2c polarization programs.⁵⁶

TAMs In Ovarian Cancer

TAMs are considered to be one of the most abundant invasive immune cells in ovarian cancer patients' tissues and ascites. Studies on the presence of TAMs in ovarian cancer suggest that macrophages tend to the M2 phenotype and express M2 signature markers like CD204, CD206, CD163 and IL-10.²⁰ Intra-islet M1/M2 TAM ratio is a critical factor related to ovarian cancer prognosis because increased M1/M2 ratio presented with improved progression-free survival (PFS).⁷⁵ In Xia et al's meta-analysis, which involved 9 studies including 794 patients, worse PFS was associated with a high density of CD163+ TAMs and a higher ratio of CD163+/CD68+ TAMs in ovarian cancer.⁷⁶ The upregulation genes linked to interferon signaling in TAMs negatively related to ovarian cancer survival and IFN γ -mediated recovery of IL-12 induction in macrophages may be a possible explanation for this phenomenon.⁷⁷ Besides, it is reported that CCL22 produced by TAMs generates chemokine gradient that induces Treg cells moving toward local microenvironment of ovarian cancer, thus raising the percentage of Tregs. Accumulation of Tregs may be a pivotal mechanism of immunosuppression.⁷⁸ During ovarian cancer stem cells and M2 macrophage interaction procedure, the paracrine WNT can be fired up and constitutes a positive feedback loop. This feedback loop likely enhances the aggressive phenotype of macrophages and cancer cells.⁷⁹ Interestingly, compared with other histological subtype, macrophages are more frequently accumulated in serous and mucinous ovarian carcinoma. Meanwhile, low grade serous ovarian cancer was reported to have a lower density of CD68+ macrophages (M1).⁸⁰

Metastasis Of Ovarian Cancer

Ovarian cancer metastasis can proceed through several distinct pathways, including transcoelomic, hematogenous and lymphogenous.⁸¹ However, unlike other well-documented cancers which spread mainly via hematogenous route, transcoelomic pathway is the most predominant route in ovarian cancer.⁸² During this process, adipocytes facilitate ovarian cancer metastasis and support tumor growth.^{83,84}

Transcoelomic Metastasis Process Leave Primary Tumor

The first step of transcoelomic metastatic cascade is to leave the primary tumor site. Before cancer cells detach from the

primary site and initiate the metastatic journey, they experience an epithelial-to-mesenchymal transition (EMT). During EMT, intercellular adhesion of the cell to cell and attachment between an epithelial cell and basement membrane can be loosened.^{85,86} Besides, resistance to anoikis also facilitates the development of activating yes-associated protein 1 (YAP1) pathway which promotes anoikis inhibition and metastasis development. Norepinephrine and epinephrine prevent ovarian cancer cells from anoikis.^{87,88} Inhibitor of c-Met and VEGFR-2, Foretinib (GSK1363089), can enhance anoikis and suppress ovarian cancer metastasis.⁸⁹

Dissemination Within The Peritoneal Cavity

Once cancer cells depart from the primary site, they survive in ascites and evade immunological surveillance in the form of a single cell, aggregates or spheroids. Meanwhile, the physiological movement of ascites promotes exfoliated cancer cells to disseminate within the peritoneal cavity.⁹⁰ In ovarian cancer, ascites is a symptom of advanced stage and represent poor prognosis. Evidence confirmed that malignant ascites generates a circumstance that promotes transcoelomic metastasis of ovarian cancer. Additionally, ascites also facilitates cancer cell escape from immunological surveillance.⁹¹ Ovarian cancer cells in ascites secrete exosome containing CD95 ligand (CD95L) which can induce CD95 positive immune cell apoptosis.⁹² Feki et al identified that cancer cells preferentially colonized in the milk spots located on the subdiaphragmatic surface⁹³ because tissues in the milk spots exhibit enhanced direct migration capability.⁹⁴ It has been widely accepted that VEGF factors contribute to ascites accumulation by increasing vascular peritoneal permeability.⁹⁵⁻⁹⁷

Connect To Peritoneal Mesothelium

After transportation through ascites, carcinoma cells set about undergoing peritoneal implantation. It is reported that attachment of disseminated cancer cells to peritoneal mesothelium can be mediated by CXCL12-CCR4 combination.⁹⁸ And, once cancer cells adhere to the mesothelium to anchor to the metastatic site, matrix metalloproteinase-2 (MMP-2) transcription level is upregulated. Following this, MMP-2 cleaves fibronectin (FN) and vitronectin (Vn) into smaller sections, so that the junction of cancer cells to the small fragments and their receptors $\alpha_5\beta_1$, $\alpha_v\beta_3$ integrin can be tightened.⁹⁹ In addition, cancer cells can exert various methods to enhance the connection with peritoneal mesothelium such as binding to hyaluronan emerging in mesothelial cells via CD44,¹⁰⁰ integrating

with the basement membrane composed of laminin, fibronectin and I, IV collagen¹⁰¹ by integrins. Interestingly, previously described studies discovered that spheroids disseminate into several single cells when they intrude the mesothelium.^{102,103} Additionally, ovarian cancer cells themselves struggle to enhance migration ability by secreting exosomes enriched by CD44. Mesothelial cells absorb these exosomes, inducing upregulation of MMP-9^{3,13} level which can enhance ovarian cancer cells invasion.^{104,105}

Destroy The Mesothelial Lining And Erode Submesothelial Parenchymal Tissues

When ovarian cancer connects to mesothelial monolayer in order to accomplish implantation, they need to destroy the mesothelial lining and erode submesothelial parenchymal tissues.^{106,107} Noticeably, $\alpha_5\beta_1$ integrins combine with myosin contractility within spheroids and destroy mesothelial lining. At an early stage of implantation, adhesion harmoniously collaborates with proteolysis, thus greatly promoting tumor establishment process. As we all know, tumor growth depends on the formation of new blood vessels. When shedding ovarian cancer cells reach a certain size, diffusion alone cannot supply enough nutrients. So, angiogenesis response emerges in metastatic sites.¹⁰⁸ It is now apparent that VEGF is a crucial element involved in the pluripotent activity of angiogenesis.¹⁰⁹ Of note, tumor-associated stromal cells also support shedding cancer cell growth by promoting angiogenesis.¹¹⁰ For example, angiopoietin receptors TIE2 positive TAMs occur with high microvascular density,¹¹¹ neutrophils facilitate VEGFA, BV8 and Mmp9 transcription in order to mediate pro-angiogenic functions by STAT3^{112–116} and TAMs produce Semaphorin-4D (Sema4D) to contribute to proper vessel maturation.¹¹⁷ Besides, the expression of regulated in development and DNA damage response 1 (REDD1) is upregulated in hypoxic TAMs, mammalian target of rapamycin (mTOR) inhibition is mediated by REDD1 leading to suppression of glycolysis in TAMs, thus inducing abnormal vessel formation.^{81,82,118–122}

Hematogenous Metastasis

Characteristic Of Hematogenous Metastasis

It is widely accepted that the mechanism of ovarian cancer metastasis is usually by passive transportation through ascites, while hematogenous metastasis associated with distant metastasis is of limited importance.¹²¹ However, emerging evidence indicates that high grade serous ovarian cancer arising from fallopian tube preferentially spread to

ovaries by hematogenous pathway.¹²³ Furthermore, clinical treatment methods such as inferior vena cava filter placement can increase the risk of hematogenous metastasis.¹²⁴ This phenomenon can be attributed to IVC filter-mediated activation of platelets and proinflammatory response. Moreover, circulating tumor cells (CTCs), which are exfoliated tumor cells from primary sites that have disseminated into peripheral blood, have long been seen as an effective indicator for hematogenous metastasis in various solid tumors as well as in ovarian cancer.^{125–127} So, we should pay sufficient attention to this uncommon pathway of ovarian cancer metastasis.

Mechanism Of Hematogenous Metastasis

The mechanism of hematogenous metastasis is complex. It has been reported that inhibition of CCR4 can suppress EMT transition and decrease CTCs which are related to hematogenous metastasis. This process is associated with downregulated levels of Src and ERKs.¹²⁸ Besides, p90RSK is intimately connected to hematogenous metastasis. p90RSK activates YB-1 to sustain a pro-adhesive circuit including $\alpha_5\beta_1$ integrin, fibronectin and TGF- β 1. However, silencing RSK1/RSK2 can diminish pro-adhesive circuit components' translation. So, knockdown RSK1/RSK2 can also impair hematogenous metastasis of ovarian cancer.¹²⁹ Interestingly, omentum is commonly involved in transcoelomic metastasis, but it has been proved that cancer cells can spread to omentum hematogenously too. Strong expression of ErbB3 in CTCs induces this kind of hematogenous metastasis via ErbB3/NRG1 axis¹³⁰ (Figure 2).

TAMs In Cancer Metastasis

In present study, we consider that infiltration of TAMs in the invasive frontier is associated with metastasis and reversing the polarization of TAMs from M2 to M1 can impair metastasis.^{131–133} However, the underlying mechanism of affecting tumor metastasis through TAMs is still under investigation.

Signal Transduction In TAMs Contribute To Metastasis

TAMs have a wide variety of signal transduction patterns, and there is evidence that their activity is proportional to TAM's tumor-promoting function. In Qian et al's research, we can see that FLT1 signaling in TAMs is essential for metastasis process. FLT1 regulates a range of inflammatory response genes like CSF-1 and FLT1 loss-of-function experiment reduces tumor metastatic efficiency.¹³⁴ In xenograft

cancer mouse models, PDGF-BB-SOX7 axially upregulated IL-33 to promote metastasis by acting on TAMs.¹³⁵ Meanwhile, inhibition of SOCS3 (suppressor of STAT3 signaling) by TAMs prevents cancer metastasis by modifying the macrophage phase and inhibition of the NF- κ B in TAMs can attenuate tumor metastasis by polarizing TAMs to an anti-tumor phenotype.^{136,137} In a mouse model of orthotopic 4T1 mammary cancer, we can conclude that Stat6 pathway in TAMs facilitates metastasis with the aid of protumorigenic and prometastatic function of macrophages.¹³⁸

Molecular Mechanism Of TAMs In Cancer Metastasis

Abundant shreds of evidence reveal that some molecular substances contained in TAMs play a promoting role in the process of tumor metastasis. Cytochrome P450 (CYP) 4A

released by TAMs infiltration is positively related to pre-metastatic niche formation accompanied with tendency to M1 polarization.¹³⁹ In Nielsen et al's experiment, it is apparent that granulins secretion by TAMs transforms resident hepatic stellate cells (hStCs) into pericyte-secreting myofibroblasts, forming a microenvironment that supports the growth of metastatic tumors;¹⁴⁰ Caveolin-1 (Cav1) has been reported to have dual effects of promoting and inhibiting tumor growth. Deletion of Cav1 in macrophages promotes lung metastasis by increasing angiogenesis;¹⁴¹ Release of sphingosine-1-phosphate (S1P) by apoptotic tumor cells stimulates TAMs to secrete Lipocalin 2 to promote metastasis.¹⁴² In renal cell carcinoma model treated with IL-2/anti-CD40 immunotherapy, the expression of TAM-dependent NO in the tumor microenvironment is an

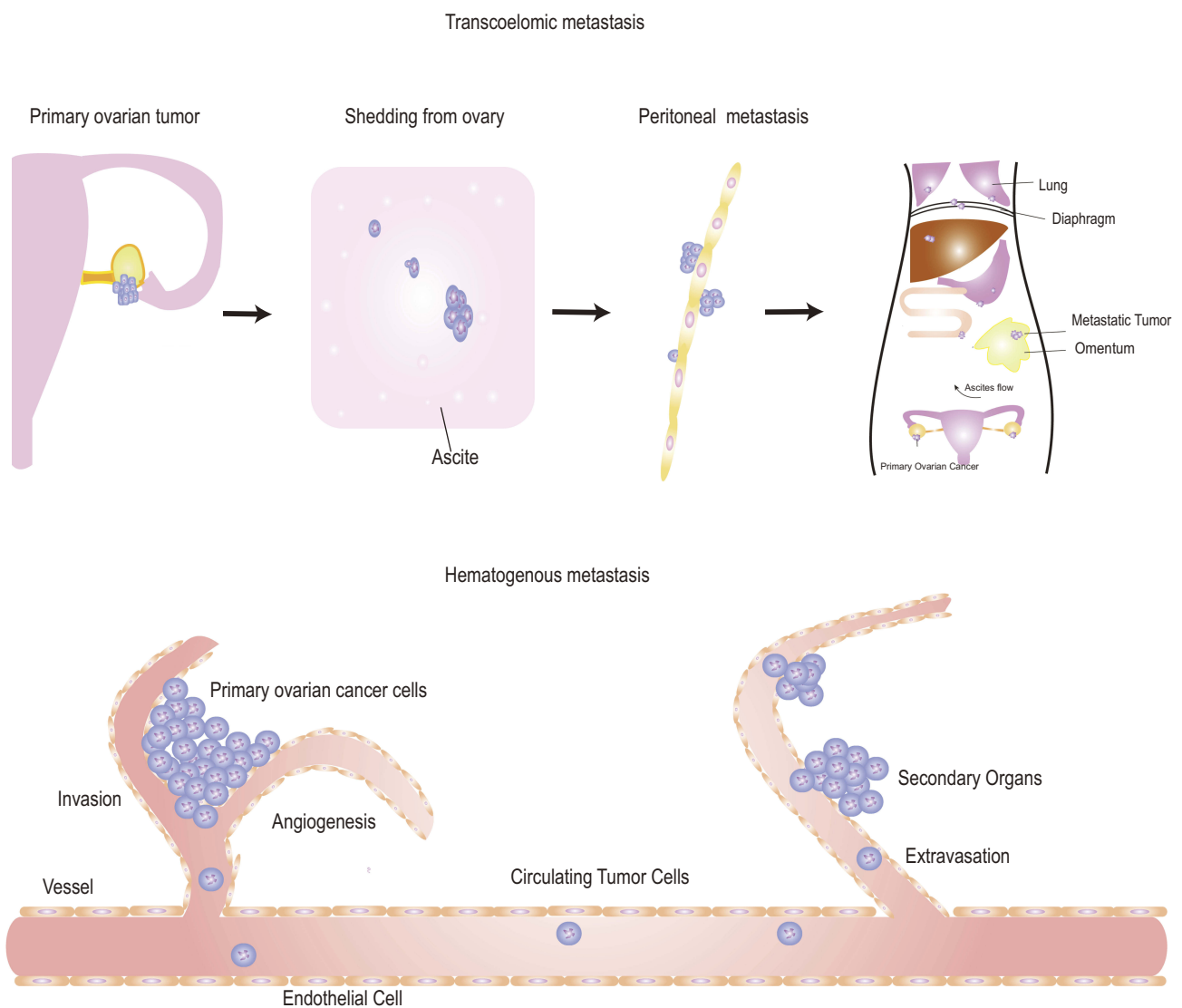


Figure 2 The process of transcoelomic and hematogenous metastasis of ovarian cancer.

important molecular readout, which is essential for regulating MMP activity and adhesion molecule expression, and is the basis for inhibiting the metastatic process;¹⁴³ CCL18 from TAMs acts on its receptor, PITPNM3, to promote breast cancer metastasis.¹⁴⁴

The Underlying Mechanism Of TAMs In Promoting Ovarian Cancer Metastasis

Abundant studies indicate that TAMs are frequently identified in patients with ovarian cancer and progression of ovarian cancer is accompanied by an increase in TAMs in the surrounding ascites. However, the in-depth mechanism of TAMs in promoting ovarian cancer metastasis are under exploring. In a severe combined immunodeficient and a syngeneic immunocompetent mouse model, the prometastatic effect of inflammation can be detected. Deletion of macrophages alone, resulting in decreasing of vascular endothelial growth factor, presenting inhibition of ascites formation and peritoneal metastasis.¹⁴⁵ In Kaitlin et al's research, experimental validation proves that secretomes derived from macrophages promoting MMP-9 mediated spheroid spreading by activating JAK2/STAT3 signaling.¹⁴⁶ Liu et al found that upregulation of lipid leads to decrease in M1/M2 ratio, thus facilitates ovarian cancer adhere to lipid-loaded mesothelial cell.¹⁴⁷ In an M2 co-cultured system, it is obvious to see that ovarian cancer cells present stronger migration ability with increased concentration of epidermal growth factor (EGF). However, these effects can be reversed by inhibitor of EGF and overexpression of lncRNA inhibiting metastasis (LIMT).¹⁴⁸ In the process of ovarian cancer metastasis, we can see that P-selectin is overexpressed in mesothelial cell surface, resulting in an increased rolling under ascites flow and adhesion between cancer cells and mesothelial cell. The overexpression of P-selectin is mediated by macrophage inflammatory protein-1 β (MIP-1 β) secreted by macrophages via CCR5/PI3K signaling.¹⁴⁹ The characteristics of the peritoneum of ovarian cancer include massive infiltration of macrophages and high expression of coagulation factors FXII. After treatment of FXII, macrophages exhibit strong tendency to M2 phenotype. Matrigel results proved that the metastasis of ovarian cancer cells was strengthened when infiltrated in medium from FXII-stimulated macrophages.¹⁵⁰

Conclusion

TAMs are involved in various aspects of ovarian cancer treatment like radiotherapy, chemotherapy and

immunotherapy.^{151–153} Macrophage subpopulations with identifiable markers are attractive therapeutic targets for standard and immune therapy. TAM-targeted approaches consist of TAM depletion, inhibition of TAM recruitment and reprogramming of TAMs.^{10,12} Targeting CSF1–CSF1R axis can be an effective method to deplete TAMs.^{154–156} Chemokines have long been associated with macrophage accumulation in tumors.^{42,56} Antibody-specific inhibition of CCL2 greatly reduces tumor development and inhibit invasion process in different experimental models.^{157,158} In conclusion, TAMs significantly influence the pathophysiological process of metastasis.^{10,19,21} Editing M2 phenotype repolarize to M1 is a promising strategy for cancer therapy. For instance, intraperitoneal paclitaxel in combination with MicroRNA-125b can consolidate the anti-tumor efficacy of paclitaxel as seen by impeding formation of ascites and cut down VEGF levels.¹⁵⁹ In mouse models of several adenocarcinomas, like colon cancer, breast cancer and sarcoma, CSF-1R antibody antagonists combined with CD40 agonistic antibody drive repolarization from M2 to a tumoricidal phenotype and prolong survival.¹⁶⁰ Similarly, bacteria-mediated macrophage repolarization is also applicable in cancer therapy. It was found that the combination of heat-killed *Mycobacterium indicus pranii* (Mw) and agonistic GITR antibody (DTA-1) is effective for advanced tumor therapy. Mw aims at repolarizing macrophages synergized with DTA-1 aims at impairing the acquisition of intratumor regulatory T cell create antitumor atmosphere.¹⁶¹ So, identifying the underlying mechanism of TAM in ovarian cancer metastasis is pivotal in inhibiting the spread of ovarian cancer cells into the peritoneum, omentum and vasculature, thus improving the five-year survival rate and reducing mortality. Additionally, it has been elucidated that TAMs play causative role in ovarian cancer metastasis. Removing peritoneal macrophages, rather than other immune cells resident in TME, abrogated tumor progression as shown by peritoneal metastasis.¹⁶² As mentioned earlier, macrophages are involved in various aspects of ovarian cancer progression. Macrophage infiltration is a marker of poor prognosis in ovarian carcinoma, but there is no direct evidence or literature supporting the presence of macrophages as a diagnostic marker for ovarian cancer metastasis. In consequence, we need to explore macrophage-specific biomarker in metastatic ovarian carcinoma. It helps us to clinically screen patients with a strong tendency to metastasize and intervene in advance. It is also of great clinical significance in improving the prognosis of ovarian cancer patients.

Disclosure

The authors report no conflicts of interest in this work.

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