



# The Role of Osteopontin in Tumor Progression Through Tumor-Associated Macrophages

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Osteopontin (OPN) is a multifunctional phosphorylated protein. It is widely involved in solid tumor progression, such as intensification of macrophage recruitment, inhibition of T-cell activity, aggravation of tumor interstitial fibrosis, promotion of tumor metastasis, chemotherapy resistance, and angiogenesis. Most of these pathologies are affected by tumor-associated macrophages (TAMs), an important component of the tumor microenvironment (TME). TAMs have been extensively characterized, including their subsets, phenotypes, activation status, and functions, and are considered a promising therapeutic target for cancer treatment. This review focuses on the interaction between OPN and TAMs in mediating tumor progression. We discuss the strategies for targeting OPN and TAMs to treat cancer and factors that may affect the therapeutic outcomes of blocking OPN or depleting TAMs. We also discuss the role of cancer cell- vs. TAM-derived OPN in tumorigenesis, the mechanisms of how OPN affects TAM recruitment and polarization, and why OPN could mediate anti-tumor and pro-tumor effects, as well as previously reported discrepancies.

**Keywords:** osteopontin, tumor associated macrophage, tumor microenvironment, tumor progression, immune regulation

## INTRODUCTION

Osteopontin (OPN) is encoded by the secreted phosphoprotein 1 (SPP1) gene. OPN is named for its role as a bridge between cells and hydroxyapatite through the function of Arg-Gly-Asp (RGD) and polyaspartic acid motifs (1). It has also been shown that activated T cells express high levels of OPN in the early stage, which is therefore named the early T-lymphocyte activation-1 (Eta-1) protein in the view of immunology (2).

The expression of OPN is negatively correlated with the prognosis of patients with colorectal, head, and neck cancers (3, 4), and it has been identified as a biomarker for tumor progression in prostate cancer (5), non-clear cell renal cell carcinoma (6), hepatocellular carcinoma (HCC) (7), and non-small cell lung cancer (NSCLC) (8). OPN can promote the malignant progression of various cancers by regulating tumor angiogenesis (9), distant metastasis (10–12), maintenance of a stem-like phenotype (13), tumor stromal fibrosis (14), activation of cell proliferation pathways (15, 16), medical treatment resistance (17), and interference with immune function (18–20).

The tumor microenvironment (TME) contains multiple cells that play a crucial role in cancer pathogenesis. There are immune cells, fibroblasts, extracellular matrix, and related cytokines harbored in the TME. In contrast, tumor-associated macrophages (TAMs) are the main component of TME and are considered promising targets for the diagnosis and treatment of cancer. TAMs interact with cancer-associated fibroblasts (CAFs) and other immune components to facilitate the development and progression of cancers.

It was reported that OPN is expressed in activated TAMs and OPN plays an essential role in TAM function during tumorigenesis and tumor progression. But the underlying mechanisms of the OPN in the regulation of TAMs have not been thoroughly investigated. This review summarizes the recent studies of OPN and TAMs and discusses the potential mechanisms for the function of OPN on TAMs.

## OPN AND ITS RECEPTORS

OPN protein, which is produced by tumor cells, endothelial cells, smooth muscle cells, fibroblasts, and immune cells, is extensively modified after translation. The molecular weight of OPN varies from 44 to 75 kd, depending on the living organ species and cell types (21). The structure, regulation, physiological, and pathological effects of OPN have been well summarized in the recently published reviews, especially for age-related nonalcoholic fatty liver disease, chronic liver disease, cardiac fibrosis, pulmonary fibrosis, and multiple sclerosis (7, 22–25).

The structure of OPN consists of the RGD sequence, SVVYGLR sequence, thrombin cleavage site, matrix metalloproteinase (MMP) site, calcium and heparin binding domains (**Figure 1**) (26, 27). Human OPN can form five different isoforms: OPN-a (full length), OPN-b (lacks exon 5), OPN-c (lacks exon 4), OPN-4 (also termed OPN-d, lacks exon 4 and 5), and OPN-5 (the longest isoform, with an extra exon located between canonical exons 3 and 4) (7, 28, 29). The elevated level of OPN-a suggests a poor clinical prognosis in gastric cancer (30). OPN-b resists tumor cell apoptosis in glioma (31). OPN-c is not present in normal breast tissue but is highly expressed in breast cancer and promotes tumor progression

independent of traditional prognostic molecules, such as ER, PR, and HER2, as a marker of breast cancer progression (32, 33). OPN-4 and OPN-5 are expressed in esophageal adenocarcinomas and distinct cancer cell lines (34). OPN-5 is expressed higher than OPN-b and OPN-c in normal skin (29). However, the mechanism of OPN-4 or OPN-5 in regulating tumor progression is not fully investigated.

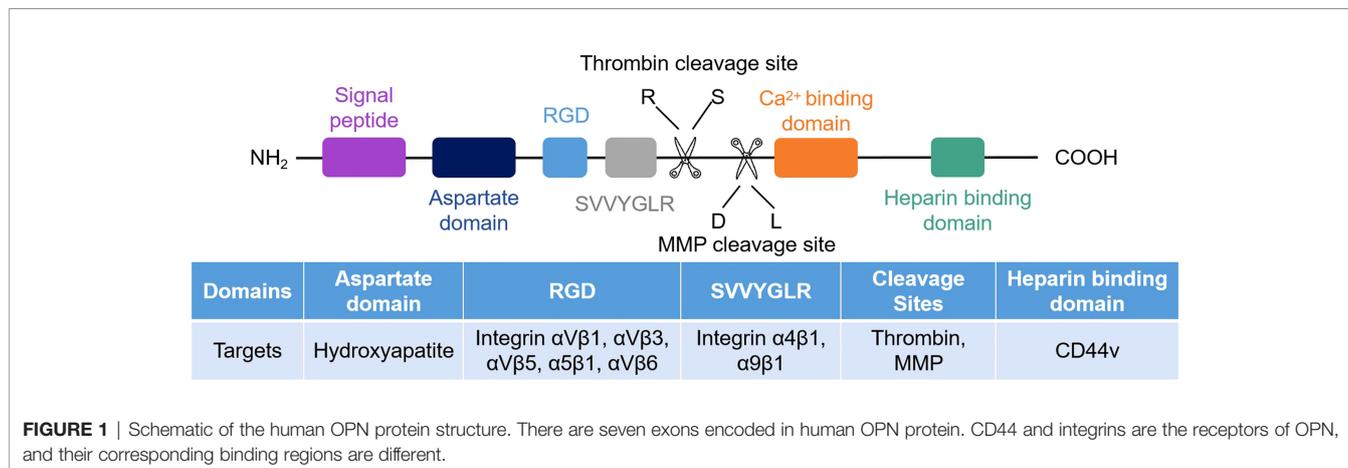
The functions of OPN also vary with different receptors. CD44 and partial integrin proteins (integrins  $\alpha V\beta 1$ ,  $\alpha V\beta 3$ ,  $\alpha V\beta 5$ ,  $\alpha V\beta 6$ ,  $\alpha 4\beta 1$ ,  $\alpha 5\beta 1$ , and  $\alpha 9\beta 1$ ) are known OPN receptors (23). By binding to these receptors, OPN triggers various signaling pathways and regulates tumor progression (**Figure 2**).

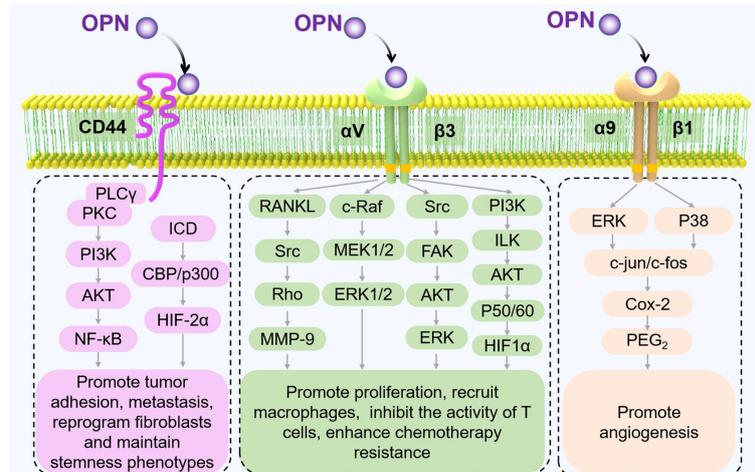
CD44 proteins, which form a multifunctional family of single-chain transmembrane glycoproteins, play an essential role in tumor progression and metastasis (41). CD44 isoforms, CD44v6 and v10, are engaged in the interaction of OPN. OPN which was secreted by tumor-associated cells, increases the expression of CD44v6 in colorectal cancer stem cells (CR-CSCs) by activating the PI3K/AKT pathway, thereby promoting the migration and metastasis of CR-CSCs (42). In malignant pleural mesothelioma, OPN transfection significantly increases the adhesion of tumor cells to hyaluronic acid (HA), which acts as a barrier to drugs, resulting in drug resistance of tumor cells to NVB, VP-16, and gemcitabine (GEM) (43).

Integrins play a central role in the interaction with receptors that are involved in cell adhesion and signal transduction. Numerous studies have demonstrated that integrins have multiple functions in tumorigenesis (44). Combined with integrins, particularly  $\alpha V\beta 3$  and  $\alpha 9\beta 1$ , OPN could mediate cell–cell and cell–ECM interactions and promote tumor progression (45, 46).

**Integrin  $\alpha V\beta 1$ .** Integrin  $\alpha V\beta 1$  is highly expressed in mesenchymal cells (MSCs). Further studies have revealed that the expression of C/EBP $\alpha$  and C/EBP $\beta$ , which play an important role in promoting adipogenic differentiation, is upregulated in the absence of OPN or the blockade of integrin  $\alpha V\beta 1$ . Therefore, OPN maintains a balance between normal adipogenesis and osteogenesis of MSCs by inhibiting C/EBP activation through integrin  $\alpha V\beta 1$  (47, 48).

**Integrin  $\alpha V\beta 3$ .** In non-small-cell lung cancer, OPN promotes inhibitor resistance of acquired epidermal growth factor receptor





**FIGURE 2** | Receptors of OPN and their signaling pathways in tumor progression. The different signaling pathways between OPN and its major receptors, CD44 and integrins, are shown to mediate pathological processes in TME (17, 35–40).

tyrosine kinase (EGFR-TKI) by upregulating integrin  $\alpha V\beta 3$  expression and activating downstream FAK/AKT and ERK signaling pathways that promote tumor cell proliferation (17).

**Integrin  $\alpha V\beta 6$ .** Integrin  $\alpha V\beta 6$  binds with OPN on the surface of the porcine Tr cell line (pTr2) to promote the adhesion of pTr2 cells and cationic dependence. Similar to pTr2 cells, porcine uterine epithelial cells (pUE) also bind with OPN through integrin  $\alpha V\beta 3$  expressed on their surfaces. OPN regulates trophoblast ectodermal cell migration and epithelial cell adhesion by binding with integrin  $\alpha V\beta 6$  or  $\alpha V\beta 3$  respectively on their surfaces (49).

**Integrin  $\alpha 4\beta 1$ .** In rheumatoid arthritis and alcoholic hepatitis models, monocytes and neutrophils recruited by cleaved OPN highly express integrin  $\alpha 4\beta 1$  on inflammatory sites by binding with the exposed SLAYGLR motif. In line with this finding, OPN-mediated migration of monocytes and neutrophils is almost entirely inhibited by antibodies against the SLAYGLR motif (M5 antibody) (50, 51). In multiple sclerosis models, OPN increases phosphorylation of IKK $\beta$  and activation of the NF- $\kappa$ B pathway in target cells by binding to integrin  $\alpha 4\beta 1$  expressed on the surface of target cells (52).

**Integrin  $\alpha 5\beta 1$ .** The presence of the divalent cation Mn $^{2+}$  and/or phorboster TPA significantly increases the activation of  $\alpha 5\beta 1$ , which is required for the binding of integrin  $\alpha 5\beta 1$  to OPN by RGD motifs (53).

**Integrin  $\alpha 9\beta 1$ .** The results from melanoma B16F10 mouse model studies revealed that OPN significantly increased the infiltration of CD31 $^{+}$  cells and cyclooxygenase subtype 2 (COX-2) positive macrophages in tumor cells. OPN was found to activate the ERK and P38 signaling pathways by binding with integrin  $\alpha 9\beta 1$ , leading to the expression of COX-2, a key rate-limiting enzyme that regulates prostaglandin synthesis in macrophages (9).

OPN can also interact with some G protein coupled receptors (GPCRs) (e.g., through  $\beta 2$ -adrenergic receptors (ARs) to regulate

cardiomyocyte fibrosis and bone metabolism). In terms of cardiomyocyte fibrosis, OPN inhibits the expression of cAMP and exchange protein directly activated by cyclic-adenosine monophosphate1 (Epac1), where cAMP is the downstream signaling and major second messenger generated by  $\beta 2$ -ARs, and Epac1 is one of the effectors of cAMP that can impede collagen synthesis (54, 55). In bone metabolism-associated processes, isoproterenol (ISO) stimulates sympathetic nervous system tension and causes bone mass loss in WT mice but does not affect OPN-KO mice. However, neutralized extracellular OPN yields limited improvement in ISO-induced bone loss. The mechanism may have two aspects: 1) OPN is the necessary element of ISO-inducing bone metabolism; and 2) intracellular OPN (iOPN) inhibits GPCRs, inhibiting the production of cAMP generated by  $\beta 2$ -ARs and cAMP-response element transcription in osteoblasts (56).

## TAMs IN TUMOR

Solid tumors are *in vivo* three-dimensional organ-like structures consisting of tumor cells and non-malignant stromal cells. Tumor-associated macrophages (TAMs) are the major components of tumor-infiltrating immune cells (57, 58). TAMs are the only colonies of macrophages present in TME. Macrophages in TME could harbor either an activated M1 or an alternatively activated M2 polarization profile by factors in TME (59). Strictly speaking, TAM is not a macrophage classification, which dictates the opposing effects on plasticity or heterogeneity in TME. Current studies have shown that TAMs consist of tissue-resident macrophages and peripheral blood-derived monocytes (60). TAMs can constantly be subjected to transition between M1 and M2, and different phenotypes of macrophages can co-exist in TME (61, 62). Activated M1-like TAMs are characterized as producing reactive oxygen species/

reactive nitrogen species and pro-inflammatory cytokines (e.g., interleukin-1 $\beta$ , interleukin-6, and tumor necrosis factor- $\alpha$ ) and contributing to the innate immune defense and the role of killing tumor cells. Therefore, activated M1-like TAMs are considered anti-tumor M1 (63). However, M2-like TAMs consist of low efficiency of antigen presentation and promote cancer progression through the production of the immunosuppressive cytokines, such as IL-10 and transforming growth factor- $\beta$  (TGF- $\beta$ ), which have been identified as the main factor of immunosuppression and the marker of poor prognosis in the tumor microenvironment (64). M2-like TAMs could be defined into four subtypes: M2a, M2b, M2c, and M2d (65). Furthermore, the M2d subset plays a role in immune suppression and pro-tumor, which could be activated by growth factors and cytokines in the TME. Most TAM phenotypes were M2-type macrophages, which facilitate tumor growth and metastasis, tissue remodeling, promotion of angiogenesis, and adaptive immune suppression (66).

Triple-negative breast cancer (TNBC) is famous for its high tumor heterogeneity, which may lead to reduced patient response to medical treatment (67). Tumor heterogeneity is not only related to the cancer cells themselves but also to the immune cells infiltrating the TME. RNA sequencing results revealed that M2-type TAMs are the main constituents of TME in TNBC (68). In these TAMs, the expression of TGF- $\beta$ 1, MS4A6A, CD163, IL8, and PLAUR genes were significantly increased, which are closely related to angiogenesis and epithelial–mesenchymal transition (EMT) (67). Meanwhile, other immunosuppressive cells in the TME, such as T-reg cells, indirectly promote the activation of M2-type macrophages to protect tumor cells from cytotoxic killing and inhibit the immune response (69). Furthermore, TAMs could counteract the anti-tumor effect of tumor infiltrating NK and T cells and exert a synergistic promotion effect on immunosuppressive TME with myeloid-derived suppressor cells, tumor-associated DCs, and neutrophils (70).

## INTERACTIONS BETWEEN OPN AND TAMs

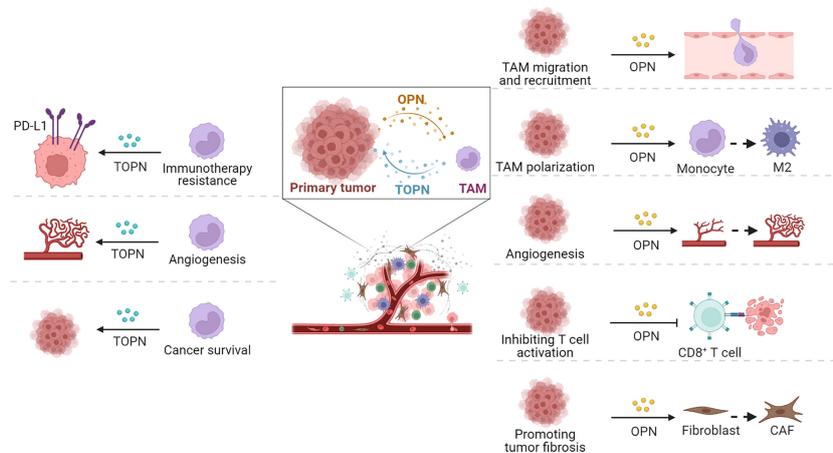
OPN is identified as an immunomodulatory molecule of activated T lymphocytes and is known as early T lymphocyte activation-1 (Eta-1) (71). It functions as a proinflammatory cytokine and chemokine that plays a crucial role in immune cell functions, including T, B, NK, and NKT cells; macrophages; DCs; monocytes; neutrophils; and eosinophils (72–74). OPN, released by tumor cells and TAMs in TME, has been identified as a multifunctional factor in cancer promotion and metastasis in several cancers (21), including breast, stomach, lung, prostate, liver, and colon cancer. It has been revealed that TAMs secrete excess colony-stimulating factor-1 (CSF1) with the help of OPN in hepatocellular carcinoma TME. This process facilitated the recruitment of macrophages and the transformation of the TAMs, which increased the expression of PD-L1 and the immune suppressive microenvironment (18).

The OPN in TAMs is known as TOPN. OPN expressed by myeloid and tumor cells endows tumor immune tolerance by inhibiting CD8 T-cell activation and recruiting inhibitory macrophages (18, 75, 76). Interferon regulatory factor 8 (IRF8) is an important transcription factor in myeloid cells and plays a key role in the development of monocytes and plasmacytoid dendritic cells (77, 78). IRF8 is mostly silent in MDSCs and could directly bind to the SPP1 promoter to inhibit OPN expression (79). In this way, the silencing of IRF8 in MDSCs and tumor cells led to increased expression of OPN. When OPN was overexpressed, it inhibited IFN $\gamma$  production in mouse CD8 T cells, thereby reducing the antitumor activity of CD8 T cells (75). The results reported by Li et al. indicated that OPN secreted by TAMs upregulated PD-L1 expression through the NF- $\kappa$ B/P65 pathway in an NSCLC mouse model, and TOPN was positively correlated with PD-L1 expression in NSCLC patients (19). FAP<sup>+</sup> fibroblasts and SPP1<sup>+</sup> macrophages are the major components of TME in colorectal cancer, which are characterized as major contributors to the desmoplastic tumor structure and immunotherapy resistance against PD-L1 in colorectal cancer (80). Some pieces of evidence have demonstrated that OPN infiltrating macrophages facilitated tumor cell survival and angiogenesis in glioblastoma multiforme (GBM) (81). In TNBC, TOPN released by TAMs can also regulate tumor metastasis. TAMs release cytokines including OPN, CCL7, CCL19, CXCL7, NRG3, HGF, and TGF- $\beta$ 3 and regulate tumor metastasis (82). Understanding the link between TOPN and TAMs in TME and their various functions in tumor progression, angiogenesis, and stromal remodeling may provide a novel target for cancer treatment. Moreover, OPN secreted from tumor cells is one of the crucial drivers of TAMs recruitment and polarization, tumor angiogenesis, and tumor fibrosis promotion, which will be discussed later.

Given the supporting functions of TME for tumor cells, an appropriate TME is important to the development and progression of tumors, except for the malignant characteristics of tumor cells themselves. The TME plays an important role in the recruitment of immunosuppressive cells, education or destruction of normal stromal cells and vascular endothelial cells, and metastasis to distant areas to escape immune surveillance of the host. Numerous studies have confirmed that tumor-associated OPN participates in TAMs migration and recruitment, polarization, tumor fibrosis, tumor angiogenesis, and immune homeostasis (Figure 3).

## OPN PROMOTES TUMOR PROGRESSION BY ACTING ON TAMs

Various cancer models have confirmed that OPN can regulate tumor progression by recruiting macrophages. In tumor tissues, OPN, as a major chemokine, can regulate macrophage migration by interacting with integrin  $\alpha$ V $\beta$ 5, CD44, GPCR, or the CSF1–CSF1R axis. With the accumulation of macrophages in TME, they are educated to become M2-type TAMs, and further



**FIGURE 3** | Functions of OPN and TOPN secreted by tumor cells and macrophages separately in TME. OPN played roles in tumor progression through TAMs (right part) and the effect of macrophage-derived OPN, termed TOPN, functioned in tumor tissue summarized (left part).

promote tumor stromal fibrosis by secreting TGF- $\beta$  or platelet-derived growth factors (PDGFs). Recently, single-cell RNA sequencing (scRNA-seq) verified that the cluster of SPP1<sup>+</sup> TAMs can also be active CAFs. Meanwhile, in the presence of OPN, TAMs promote angiogenesis through JAK/STAT3, NF- $\kappa$ B, and ERK/p38 signaling pathways.

### OPN Facilitates the Migration and Recruitment of TAMs

OPN can act as a chemotaxin for macrophages and is involved in the control of macrophages migration and recruitment. It has been well demonstrated both *in vivo* and *in vitro* that integrins, CD44, chemokines, GPCRs are intimately involved in the regulation of macrophage migration, which process can be regulated by OPN.

In hepatocellular carcinoma (HCC), tumor-released OPN can stimulate macrophages to secrete CSF1 through the PI3K-ATK-p65 signaling pathway and then induce infiltration of macrophages. Excessive macrophages play an important role in the recurrence of HCC (18, 83). In GBM patients, high OPN expression is positively correlated with TAMs infiltration and tumor progression and negatively correlated with survival prognosis. In line with the studies in humans, depletion of OPN in mice resulted in reduced TAM infiltration and increased the survival rate of mice with GL261 GBM (76). The mechanism of these phenotypes is associated with integrin  $\alpha$ V $\beta$ 5, which is highly expressed in GBM-infiltrated TAMs (76). Zhu et al. (84) found that OPN and GPCRs are related to the migration of macrophages. Their study indicated that in the presence of N-formyl-methionyl-leucyl-phenylalanine (fMLP), which can activate GPCRs, WT macrophages migrate nearly twice as much as OPN<sup>-/-</sup> macrophages. CD44<sup>-/-</sup> macrophages exhibit the lowest migration rate. With the supplement of exogenous OPN, the migration of OPN<sup>-/-</sup> macrophages can be restored in an OPN dose-dependent manner. However, exogenous OPN did not rescue the impaired chemotaxis of

CD44<sup>-/-</sup> macrophages to fMLP. These results indicate that CD44 is the necessary factor in the GPCRs-mediated migration and that iOPN could modulate the CD44 activity.

However, in the spontaneous tumor model of breast cancer, although OPN is highly expressed in the tumor tissues of transgenic mice, the incidence of spontaneous tumor and tumor volume are independent of the presence of OPN. Unexpectedly, the number of macrophages in tumors of WT and OPN-KO mice has been found to be independent of the OPN genotype (85). The authors speculated that the lack of OPN may lead to compensatory mechanisms that promote tumor progression or the dependence of spontaneous tumor models on OPN, which may be different from orthotopic implanted tumor models. It has been demonstrated in another study that OPN-KO mice showed a reduction of infiltrating macrophages in tumor tissue, while OPN-KO treatment has little effect on infiltrating macrophages in normal tissue (9). The above studies indicated that OPN can be used as a specific factor to regulate the roles of macrophages in the infiltration of tumor tissues.

### OPN Induces and Maintains the Alternative M2 Activation of TAMs

Alternatively, the activated M2 polarization profile of TAMs is considered an indispensable component of TME. Although most literature has confirmed that OPN has a recruitment effect on macrophages, this is inconsistent with the effects of OPN on the TAM polarization (18, 76). OPN originating from tumor cells can induce the monocytes to undergo alternative M2 activation. The percentage of M2 macrophages was significantly increased when the human monocyte cell line U937 was treated with OPN-positive conditioned medium of the human gastric cancer cell line AGS. While a mixture of co-cultured OPN<sup>+</sup> AGS and U937 cells was inoculated into the back skin of nude mice, the xenografts from the mixture showed faster growth and correlated with poorer survival compared with the inoculation

of OPN<sup>+</sup> AGS cells alone (86). In HCC patients, the expression of OPN was positively correlated with the infiltration of TAMs in tumor tissues. By analyzing the numbers of tumor-infiltrating immune cells and profiles of chemically induced liver tumors from WT and OPN-KO mice, OPN derived from host and HCC cells was found to stimulate macrophages to secrete CSF-1 and then activate the CSF1-CSF1R axis of macrophages to promote macrophage chemotaxis and M2-like polarization in HCC cells (18). These studies show that OPN participates in the process of M2-like macrophage polarization and maintains an M2-like macrophage phenotype.

However, Wei et al. (76) have indicated that OPN maintained the genetic characteristics and phenotype of M2 TAMs but did not induce TAM polarization (76). In this study, the healthy donors were treated with various concentrations of recombinant OPN protein. After that, the representative markers of M2-like macrophages were examined. Interestingly, the markers of M2-like macrophages did not respond to the treatment with recombinant OPN protein, and the number of M2-like macrophages did not significantly change (76).

Tissue macrophages have long been thought to develop from monocytes that enter tissues after circulating in the blood. However, with the development of molecular technology and the establishment of new animal models, this concept is increasingly questioned (87, 88). Studies on mice and humans have shown that macrophages in tissues can be divided into tissue-resident macrophages (TRMs) and blood-derived macrophages according to their sources and physiological characteristics, and the proportions of blood-derived macrophages and TRMs in different organs are different. Microglia in the brain are derived from primitive macrophages in the embryonic yolk sac, and erythrocytic myeloid progenitor cells (EMPs) in the yolk sac are the primary source of Kupffer cells (89–92). In tumor tissues, macrophages are derived not only from peripheral blood but also from a group of TRMs that are involved in the formation of TAMs. In the above experiments, macrophages were considered a single population without considering different sources of macrophages. Not only is the polarization effect of OPN on the blood source and TRM unclear, but also the polarization effect of OPN on TRM has different origins. Although lacking experimental evidence, the reported different effects of OPN on TAM polarization between different mouse models may be possible due to the difference in TRMs.

It has been reported that PD-L1 is downregulated in OPN deficient macrophages and the markers of M1-like macrophages exist predominately rather than M2-like macrophages (93). Meanwhile, OPN in HCC promoted PD-L1 expression in macrophages by activating the CSF1-CSF1R pathway. The combination of anti-PD-L1 antibody and CSF1R inhibitor could promote the infiltration of CD8<sup>+</sup>T cells and reduce the location of TAMs, which are beneficial to the HCC therapeutic effect of anti-PD-L1 antibody (18).

## OPN Promote Tumor Fibrosis *via* TAMs

OPN can promote tumor fibrosis through its chemotactic effect on macrophages and activation of cancer-associated fibroblasts

(CAFs) in TME (14, 94–96). TAMs and CAFs can cross-talk in the TME: CAFs can secrete chemokines to attract monocytes into the tumor microenvironment and differentiate into TAMs; TAMs can promote fibroblast activation by secreting TGF- $\beta$  or promote fibroblast proliferation by secreting PDGFs (97). Single-cell RNA sequencing (scRNA-seq) analysis from colorectal cancer patients showed that SPP1<sup>+</sup> TAMs expressing syndecan-2 (SDC2) were more likely to interact with CAFs expressing MMP-2 through the combination of SDC2 and MMP-2 to promote the activation of CAFs and tumor tissue fibrosis. Notably, SPP1<sup>+</sup> TAMs were resistant to CSF1R blockade in a mouse model, and high infiltration of SPP1<sup>+</sup> TAMs in colon cancer patients had a poor prognosis (96).

Although TAMs have a significant feature of promoting fibrosis (such as collagen synthesis and deposition, etc.), studies on hepatic fibrosis disease models have found that macrophages play different or opposite roles in different stages of fibrosis progression (98, 99). In the case of inflammatory injury, clearance of macrophages helps alleviate the accumulation of abnormal collagen in the injured liver. Unexpectedly, during the recovery phase, macrophages promoted matrix degradation and absorption. After macrophage depletion, the proportion of Sirius red-staining positive collagen matrix in the liver increases from 1% to more than 3% (98). In a CCL4-induced hepatic fibrosis mouse model, the M1-type macrophages not only had a therapeutic effect on liver fibrosis by increasing the apoptosis of hepatic stellate cells but also recruited more endogenous anti-fibrosis macrophages into the liver by producing chemokines CCL2 and CCL3 (99). Since TAMs are highly heterogenic (100), OPN may play different roles in tumor fibrosis depending on the composition of TAMs in the tumor.

## OPN Promotes Tumor Angiogenesis Through TAMs

Angiogenesis plays a vital role in promoting malignant tumor growth, diffusion, and metastasis. Numerous studies have indicated that TAMs are the crucial factors in regulating tumor angiogenesis (60, 101). TAMs can secrete pro-angiogenic growth factors and release MMPs to promote the degradation of extracellular matrix around blood vessels and facilitate the extension of tumor blood vessels (102).

A few studies have reported that OPN can promote tumor-associated angiogenesis by regulating macrophages with different phenotypes. Immunohistochemistry results of tumor tissue indicated that GBM-related macrophages express metalloprotease-disintegrin 8 (ADAM8), which is associated with invasive and poor prognosis (103–105). It has been reported that the supernatant of ADAM8 overexpressed macrophages can induce human umbilical vein endothelial cells (HUVEC) to form more tube-like structures than the ADAM8 deficient group. However, ADAM8 has no correlation with the polarization of macrophages (106). Nevertheless, the expression of OPN is reduced in ADAM8-deficient macrophages (105). Finally, they found that OPN regulates the angiogenesis of ADAM8-deficient macrophages through JAK/STAT3 and NF- $\kappa$ B signaling pathways (105). In

melanoma, the expression of COX-2 in macrophages and the angiogenesis capacity of HUVEC cells were enhanced through an ERK/p38-dependent pathway which was regulated by the OPN secreted by tumor cells (9).

OPN can also promote tumor angiogenesis in a TAM-independent manner. OPN could promote endothelial cell proliferation and activate tumor cells to secrete VEGF. OPN residues in tumors bind to CD44 and integrin receptors to mediate NF- $\kappa$ B, PI3K/Akt, VEGF, uPA, and MMPs to promote endothelial cell proliferation (107, 108). Moreover, OPN regulates the proliferation and growth of muscle-derived angiogenic progenitor cells (MDPCs) through the PI3K/Akt pathway (109). In the breast cancer mouse model, exogenous and tumor-derived OPN can promote VEGF expression and tumor angiogenesis by activating the Brk/NF- $\kappa$ B/ATF-4 signaling pathway (110). However, in the neuroblastoma mouse model, OPN promotes intratumor angiogenesis by stimulating vascular endothelial cell migration (111).

## CONCLUSIONS AND PERSPECTIVE

OPN, as a secreted protein, has complicated biological functions and plays an important role in the regulation of tumorigenesis, anti-tumor immunity, and modulation of TME. The effects of OPN on immune regulation have been confirmed in diverse diseases, such as inflammatory and autoimmune disease models. It is not surprising that the neutralizing antibodies of OPN have been proven to alleviate various inflammatory-mediated diseases, such as osteoporosis, hepatitis, and arthritis. Furthermore, some monoclonal antibodies to OPN have been used in therapy strategies in the context of cancer. For example, anti-OPN antibodies retard the growth and reduce metastasis of breast cancer in mice (112). Unfortunately, the detailed mechanisms of OPN function in TME have not been fully developed. Simply neutralizing or completely depleting their activities is unlikely to be an optimal or effective approach (113–115).

Recently, the potential of programmed macrophage subsets has been explored, while OPN participating in the redefinition of TAMs subpopulations and functions in the steady state would be a promising tumor immune treatment strategy. However, we

believe that the following issues should be considered when developing OPN or TAM-targeted strategies: Firstly, OPN is a multifunctional factor that plays a cell-specific role in inflammation, immunity, and tissue repair, and it has various variants with different activities (116). In the TME, macrophages are the major constituent cell population, but not the only one. The depletion of TAMs may impair the antitumor effect of TME or compensatory stimulation of the proliferation of other cells with immunosuppressive function (i.e., MDSC) and aggravate tumor progression. Secondly, some reports suggest that OPN exhibits anti-tumor characteristics under certain circumstances: 1) OPN deficiency in squamous cell carcinoma mouse models leads to accelerated tumor growth (117). In intrahepatic cholangiocarcinoma, the high expression level of OPN in tumors indicates better overall survival and decreased lymph node metastasis (118), 2) OPN-deficient macrophages exhibit impaired antitumor cytotoxicity (117), and 3) stromal-derived OPN enhances NK cell infiltration into the prostate tumor in the genetically modified mice (119). Thirdly, both OPN and TAMs are phenotypically and functionally heterogeneous (120, 121), and there are still many gaps in our understanding of the effects of OPN on TAMs. Therefore, further in-depth studies are warranted to understand the underlying mechanisms of OPN and TAMs in tumorigenesis and tumor progression, which may offer new hope for future cancer treatments.

## AUTHOR CONTRIBUTIONS

YT, Y-GY, and WL conceptualized the study. YT and LZ performed the literature search, data analysis and drafted the paper. Y-GY and WL critically revised the work. All authors listed have made a substantial, direct, and intellectual contribution to the work and approved it for publication.

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