Review

Diverse Functions of Macrophages in Different Tumor Microenvironments

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Abstract

Tumor-associated macrophages are a major constituent of malignant tumors and are known to stimulate key steps in tumor progression. In our review in this journal in 2006, we postulated that functionally distinct subsets of these cells exist in different areas within solid tumors. Here, we review the many experimental and clinical studies conducted since then to investigate the function(s), regulation, and clinical signif-

icance of macrophages in these sites. The latter include three sites of cancer cell invasion, tumor nests, the tumor stroma, and areas close to, or distant from, the tumor vasculature. A more complete understanding of macrophage diversity in tumors could lead to the development of more selective therapies to restore the formidable, anticancer functions of these cells. *Cancer Res; 78(19); 5492–503. ©2018 AACR.*

Introduction

Tumor-associated macrophages (TAM) are abundant in most types of malignant tumor and promote tumor angiogenesis, the escape of cancer cells from the tumor into the circulation, and the suppression of antitumor immune mechanisms. They also help circulating cancer cells to extravasate at distant sites like the lungs and then promote their survival and persistent growth into metastatic colonies. An increasing number of studies have also shown that TAMs can either antagonize, augment, or mediate the antitumor effects of cytotoxic agents, tumor irradiation, anti-angiogenic/vascular damaging agents, and checkpoint inhibitors (1–3).

The origin(s) of these cells is currently a topic for debate. Recent studies have shown that macrophages in many steady-state tissues are not derived from circulating monocytes as originally thought, but rather from embryonic macrophages (particularly from the yolk sac) that are laid down in tissues during development. These progenitors persist into adulthood by local proliferation, and thus maintain themselves independently of the adult hematopoietic system. Alternatively, in some adult tissues like the intestines, the major macrophage populations derive form the bone marrow via monocyte recruitment whereas others can be chimeric (4). Initially, TAMs in mouse tumors were also thought to be derived largely from blood monocytes (5), however, recent studies have shown that, in some mouse models of brain and pancreatic cancer, they are derived from both blood monocytes and embryonic macrophages. Moreover, the selective depletion of each of these two TAM subtypes showed that only the latter supported the

growth of established tumors (6, 7). Further studies are required to see if this mixed ontogeny also extends to other tumor types.

TAMs often exhibit an array of activation states. In general, they are skewed away from the "classically" activated, tumoricidal phenotype (sometimes referred to as M1) towards an "alternatively" activated tumor-promoting one (M2). However, like macrophages in many other tissues, TAMs show remarkable functional plasticity and often express markers characteristic of both activation states (2, 8) making such binary definitions inaccurate. In our review in 2006 (9), we proposed that TAM functions might, at least in part, be regulated by their location within tumors. We suggested that they exhibited different functions in least three tumor sites; areas of invasion by cancer cells in early tumor development, the stroma, and hypoxic/necrotic areas. Since then, a considerable number of studies have investigated their functions and regulation in these—and other—sites in mouse tumor models, and examined the clinical significance of these spatially distinct TAM subsets (the latter are shown in Table 1).

In this update, we now outline the progress made in understanding TAM behavior in the following tumor sites: three different areas of cancer cell invasion; areas of high cancer cell density (the so-called tumor "nests"); the perivascular (PV) niche; and poorly vascularized, hypoxic/necrotic tumor areas (Figs. 1 and 2). We also discuss the clinical/therapeutic implications of these TAM subsets.

Invasive Areas

There are at least three main sites where the increased invasive behavior of cancer cells has been detected during tumor progression. First, around preinvasive lesions where the uncontrolled proliferation of newly transformed, neoplastic cells leads to their invasion through the basement membrane into the surrounding normal parenchyma to form a carcinoma. This has been well documented in tissues like the mammary gland where cancer cells invade through the duct or lobule wall to become an invasive carcinoma (Fig. 1). Then, in established tumors, at the "tumor-stroma border (TSB)" between cancer cell nests and the stroma within the tumor mass, and at the "invasive front (IF)" where cancer cells invade into surrounding normal tissues (Fig. 2).

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Table 1. TAMs in different areas of human tumors: correlation with important clinicopathologic features

Tumor type	Invasive front	Tumor nests	Tumor area Stroma	Perivascular	Hypoxic/necrotic area	Reference
Breast	ND	No correlation between TAMs and OS or RFS	High TAMs correlate with reduced OS or RFS	ND	ND	(43)
Breast	ND	High TAMs correlate with reduced RFS	High TAMs correlate with reduced RFS (only in ER/PR$^+$ subtype)	ND	ND	(34)
Breast	ND	High TAMs correlate with increased tumor MVD, but not mitotic index	High TAMs correlate with increased mitotic index	ND	ND	(104)
Breast	ND	ND	ND	ND	High TAMs in poorly vascularized areas correlate with increased tumor MVD and poor RFS and OS	(74)
Breast	ND	ND	ND	High "TMEMs" (including PV TAMs) correlate with distant metastases. No correlation with tumor size, grade or LNM	ND	(105)
Breast	ND	ND	ND	High HIF-2α$^+$ TAMs correlate with increased tumor MVD, grade, and reduced OS but not RFS	ND	(106)
Bladder	ND	ND	High TAMs associate with reduced LNS, and with improved OS in CD163$^+$ tumor	ND	ND	(49)
Endometrial	No correlation with RFS	High TAMs correlate with improved RFS	High TAMs correlate with increased LNM but not with RFS	ND	High TAMs correlate with increased myometrial invasion, higher clinical stage and better RFS	(30)
Gastric	High S100A8/A9$^+$ TAMs at invasive front and stroma correlate with higher histological grade and LNM but not OS	High S100A8/A9$^+$ TAMs correlate with higher histologic grade but not OS	See left	ND	ND	(107)
Gastric	High CD163$^+$ TAMs at invasive front and stroma correlate with higher MVD, more significant LNM and tumor invasion, and reduced OS	High CD163$^+$ TAMs correlate with higher histological grade	See left	ND	ND	(44)
Gastric	ND	High TAMs correlate with higher frequency of tumor cell apoptosis and improved RFS	ND	ND	ND	(31)
Gastric	ND	High TAMs correlate with worse surgical outcome	ND	ND	ND	(108)

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Table 1. TAMs in different areas of human tumors: correlation with important clinicopathologic features (Cont'd)

Tumor type	Tumor area					Reference
	Invasive front	Tumor nests	Stroma	Perivascular	Hypoxic/necrotic area	
Gastric	High TAMs correlate with fewer liver metastases	ND	ND	ND	ND	(25)
Cervical	High CD163+ TAMs correlate with increased LNM and reduced RFS	High CD163+ TAMs show no correlation with RFS	High CD163+ TAMs show no correlation with RFS	ND	ND	(47)
Cervical	ND	ND	ND	ND	High HIF-2α+ TAMs correlate with reduced DFS and higher risk of local recurrence	(75)
Cervical	No correlation with lymphatic metastasis	No correlation with lymphatic metastasis	High TAMs correlate with strong lymphatic metastasis	ND	ND	(109)
Pancreas	ND	No correlation with OS	High CD163+ (but not CD68+) TAMs correlate with reduced OS	ND	ND	(46)
Bile duct	High TAM density correlates with higher recurrence and reduced OS and RFS	ND	ND	ND	ND	(110)
Bile duct	ND	ND	High CD163+ TAMs correlate with poorer differentiated histology, nodal metastasis. High CD163+ TAMs in stroma together with low number of CD8+ T cells at cancer nests correlates with reduced OS	ND	ND	(111)
Oral	No correlation between the number of CD68+ TAMs and tumor grade	High CD163+ TAMs correlate with higher tumor grade	No correlation between the number of CD68+ TAM and tumor grade	ND	ND	(112)
Oral	ND	No correlation	High TAMs correlate with higher tumor grade, increased LNM and reduced OS and DFS	ND	ND	(45)
Non-small-cell lung cancer (NSCLC)	ND	High CD163+ TAM density correlates with increased LNM (but not OS)	High CD163+ TAM density correlates with increased LNM (but not OS);	ND	ND	(48)
Lung (meta-analysis of 21 studies)	ND	High TAM density correlates with better 3-year OS but not 5-year OS; specifically, high MI(+)-TAMs associate with better 3- and 5-year OS. M2(+)-TAMs was not associated with 3- or 5-year OS	High TAM density correlates with worse 3- and 5-year OS; specifically, high M2-TAMs was associated with reduced 5-year (but not 3-year) OS	ND	ND	(113)
Prostate	ND	High TAMs correlate with poorer tissue differentiation	High TAMs correlate with lower clinical stage	ND	ND	(32)
Esophageal	ND	High CD163+ TAMs correlate with higher clinical stage and increased LNM	High CD163+ TAMs correlate with higher clinical stage and increased LNM	ND	ND	(33)

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Table 1. TAMs in different areas of human tumors: correlation with important clinicopathologic features (Cont'd)

Tumor type	Tumor area					Reference
	Invasive front	Tumor nests	Stroma	Perivascular	Hypoxic/necrotic area	
Esophageal	ND	High TAMs correlate with higher clinical stage, increased LNM and reduced OS	High TAMs correlate with higher clinical stage, increased LNM and reduced OS	ND	ND	(114)
Esophageal	ND	High TAM density correlates with reduced OS	High TAM density correlates with improved OS	ND	ND	(36)
Colorectal	High TAMs correlate with fewer liver metastases and improved OS	ND	ND	ND	ND	(27)
Colon	High TAMs correlate with improved OS	ND	ND	ND	ND	(21)
Liver (hepatocellular carcinoma, HCC)	ND	ND	ND	High CD14$^+$CD16$^+$TIE2$^+$ TAM density correlates with increased MVD	ND	(59)
Melanoma	High TAMs correlate with reduced OS	High CD163$^+$ (not CD68$^+$) TAMs correlate with reduced PFS and OS	High CD163$^+$ TAMs correlate with reduced PFS and OS; high CD68$^+$ TAMs with only PFS	ND	ND	(35)

NOTE: "TAM," CD68$^+$ cell unless otherwise stated; "High TAMs;" "high number of TAMs in a given area. *M1-like: CD68$^+$HLA$^-$DR$^+$ cells; M2-like: CD163$^+$ alone, CD204$^+$ alone, CD68$^+$CD163$^+$, CD68$^+$CD206$^+$, or CD68$^+$IL10$^+$ cells. CD68 was used to immunolabel TAMs in tumor sections unless otherwise stated.

Abbreviations: ND, not determined; LNM, lymph node metastases.

In 2006 (9), we reviewed the early evidence for macrophages gathering around ducts in adenomas in the mammary glands of MMTV-PyMT mice and promoting their transition to invasive lesions. At the time, this had been demonstrated by crossing MMTV-PyMT mice with a strain carrying a recessive null mutation in the gene encoding colony-stimulating factor 1 (CSF-1). The resultant macrophage depletion delayed the progression of preinvasive lesions into invasive, metastatic carcinomas while early recruitment of macrophages accelerated progression to malignancy characterized by invasion (10). Other studies had suggested that macrophages might promote invasion of newly transformed cancer cells in preinvasive mammary lesions by releasing the enzymes, cathepsins and matrix metalloproteinases (MMP), as well as the cytokines, EGF, and TNFα. These were thought to then remodel the extracellular matrix, promote disruption of the basement membrane, accelerate the motility of cancer cells, and increase the migration of cancer cells. More recently, a number of experimental studies have confirmed the important role of macrophages in the transition of preinvasive, hyperplastic mammary lesions to early invasive carcinoma. In MMTV-iFGFR1 mice, progression failed to occur when macrophages were depleted in mice bearing hyperplastic lesions (11). Macrophages were also shown to stimulate the progression of preinvasive lesions in a transplantable, p53-null model of early mammary cancer (12). We also showed that the release of VEGFA by macrophages around preneoplastic lesions in MMTV-PyMT mice to be essential for the "angiogenic switch" that occurs when these lesions progress to early carcinomas (13, 14). Another study showed that macrophages around such preinvasive mammary lesions in mice release CXCR2-binding chemokines, CXCL1 and CXCL5, which promote the migration and invasion of neighboring preneoplastic epithelial cells. Here, a subset of macrophages expressing the cell surface proteins, mannose receptor C type 1 (MRC1 or CD206), class A macrophage scavenger receptor (CD204) and MHCII, were recruited to ductal hyperplastic lesions. When these cells were depleted using clodronate liposomes, their progression to invasive tumors was markedly delayed (15).

Finally, a recent study in a Kras^{G12D} model of lung cancer has shown that deregulated oncogenes in cancer cells like Myc trigger the transition of indolent lung adenomas to aggressive adenocarcinomas. This is because changes in Myc stimulated an increase in CCL9 and IL23 expression by lung epithelial cells. CCL9 then stimulated the accumulation of VEGFA$^+$ macrophages (and thus tumor angiogenesis), and their PD-L1-dependent expulsion of T and B cells. In addition, IL23 prompted the exclusion of adaptive T and B cells and cytotoxic NK cells (Fig. 1; ref. 16).

These findings in mice are supported by clinical studies comparing macrophage levels in low-grade versus high-grade human ductal invasive in situ carcinomas (DCIS). Such lesions are thought to develop into invasive carcinomas of the breast. High-grade DCIS lesions (especially those filled with cancer cells and containing a central area of necrosis, namely "comedo DCIS") are more aggressive and have a greater tendency to become invasive than low-grade DCIS. Higher numbers of CD68$^+$ macrophages have been reported in and around high-grade comedo DCIS than low-grade ones (17). Moreover, analysis of gene expression in 40 cases of DCIS showed that genes upregulated by macrophages following their exposure to a key stimulus upregulated in tumors, CSF-1 were more prevalent in high-grade than low-grade lesions (18).
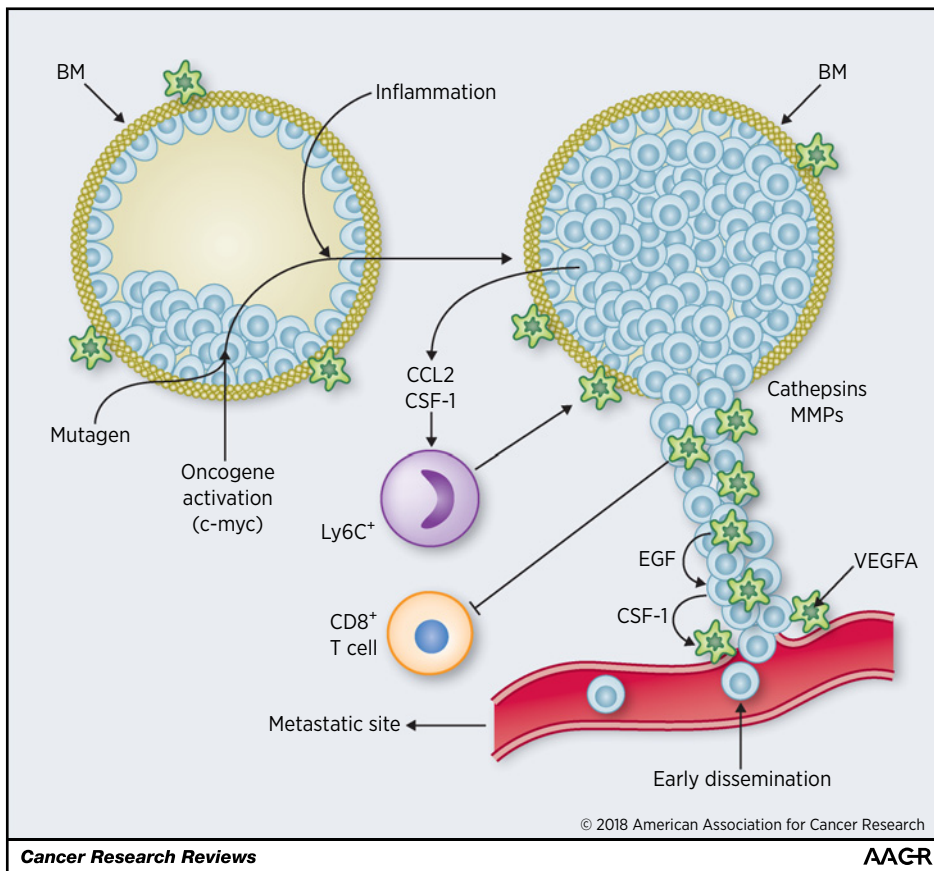
Figure 1.
Macrophages in preinvasive lesions. Epithelial cells within the basement membrane (BM) undergo transformation in response to a number of stimuli. This causes them to release factors that stimulate both the recruitment of Ly6C^{+} monocytes and the migration and/or gene expression of surrounding macrophages. These factors include CSF-1, CCL2, EGF, MMPs, and cathepsins. Cooperation may take place between invading cancer cells (expressing CSF-1) and macrophages (expressing EGF) to facilitate the movement of cancer cells towards neighboring blood vessels. PV VEGFA[+] macrophages then promote the escape of cancer cells into the circulation. Macrophage release of VEGFA also stimulates angiogenesis as preinvasive lesions progress to invasive ones. Invading cancer cells are protected from antitumor immunity by the expression of PD-L1 and IL23 by macrophages in such sites (green, macrophages; blue, epithelial/cancer cells; red, blood vessels; yellow, basement membrane).

A number of intravital imaging studies have demonstrated the abundance and characteristics of TAMs in the TSBs of MMTV-PyMT tumors. At least two TAM subsets were present: motile, MRC1^{−} and less motile, MRC1^{+} ones (19, 20). Interestingly, high numbers of CD68^{+} TAMs in the TSBs of human colon carcinomas correlate with better overall survival than those with lower numbers (Table 1; ref. 21). However, their MRC1 status was not investigated in this clinical study—they were labeled with an antibody for the pan macrophage marker, CD68—so, it remains to be seen whether these two MRC1 subsets were present, and if one or both contributed to the improved prognosis.

It is noteworthy that antibodies against CD68 continue to be used widely to immunolabel TAMs in such human tumors (Table 1). However, as with many antibodies supposedly labeling individual cell types, those for human CD68 sometimes label cells other than TAMs. For example, a qualitative, immunostaining study reported that some CD68^{+} cells in human breast tumors fail to express detectable CSF-1 receptor (CSF-1R) or CD45, or markers for epithelial cells, endothelial cells, or mural cells (i.e., vascular smooth muscle cells, pericytes, or fibroblasts; ref. 22). The identity of these CD68^{+} cells, whether they exist in other tumor types, and, indeed, if they label with other CD68 antibodies, is not known.

When it comes to the IF of tumors, TAMs in these regions of mouse RIP1-Tag2 pancreatic tumors have been shown to enhance the invasive potential of cancer cells via their expression of cathepsin B and S, two enzymes regulated by IL4 released by cancer cells and tumor-infiltrating T cells (23). Further, CD4^{+} T cells in MMTV-PyMT tumors have been shown to increase the invasiveness of cancer cells via their release of IL4, which then stimulates TAMs to express EGF release (24).

Together, these experimental data accord well with a previous finding that TAMs in the IF of human gastric tumors express the matrix-degrading enzyme, MMP9, and the receptor for the serine protease, urokinase-type plasminogen activator (uPA; which cleaves pro-uPA into its active form; ref. 25). Interestingly, TAMs along the IF of primary human colon carcinomas express CD80 and CD86 (costimulatory signals necessary for T-cell activation), suggesting that they may have the potential to help stimulate antitumor immunity in this type of cancer (26). This could explain the observation that high CD68^{+} TAM levels in the IF of human colorectal tumors correlate with a higher relapse-free survival (RFS; ref. 27; Table 1). However, various TAM subsets may be present in the IF of tumors with some appearing to be immunosuppressive. For example, TAMs in the IF of human hepatocellular carcinomas (HCC) express higher levels of the immunosuppressive, negative checkpoint regulator, PD-L1, than those in neighboring cancer nests, and have been linked to poor survival (28). Furthermore, semaphorin 4D (SEMA4D, CD100), a cytokine upregulated in the IF of Colon26 mouse colon tumors, has been shown to stimulate the number of TAMs expressing the immunosuppressive cytokine IL10 in the IF, and thus suppress the number of activated CD8^{+} T cells in this location. Antibody blockade of SEMA4D suppressed the number of these TAMs at the IF and increased the treatment efficacy of checkpoint inhibitors anti-PD-1 and anti-CTLA4 (Fig. 2; ref. 29).
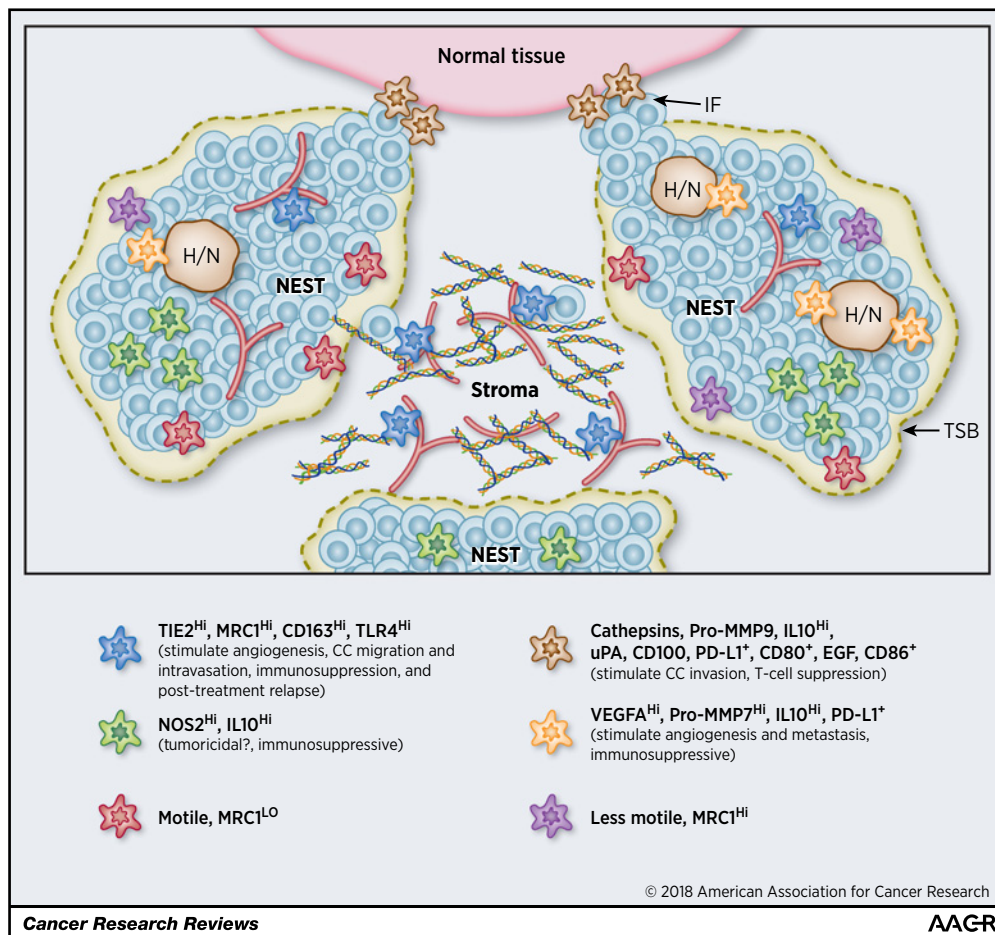
Figure 2.
The phenotype of TAMs in different compartments within established primary tumors. A small subcompartment within a tumor is shown consisting of three tumor "nests" (areas of high cancer cell density) containing hypoxic/necrotic (H/N) areas; the tumor–stroma border (TSB) at the edge of tumor nests (olive dashed line); the stroma (which in most solid tumors is highly vascularized; red); and an invasive front (IF) between this part of the tumor mass and surrounding nonmalignant tissue (pink). Bottom, cell surface markers, enzymes, and cytokines expressed by TAMs in these different regions. The main functions of the various TAM subsets have also been listed.

Cancer Nests

The possible function(s) of TAMs in close proximity to cancer cells in tumor "nests" appears to vary with tumor type. For example, TAMs expressing NOS2, an enzyme linked to the cytotoxic potential of TAMs (via its production of nitric oxide), are seen in intimate contact with cancer cells in some human prostate tumors (30), and high numbers of nest TAMs correlate with an improved prognosis in endometrial cancer (31), and a reduced recurrence in gastric cancer (Table 1; ref. 32). However, high nest TAMs also correlate with reduced overall and RFS in malignant melanomas, as well as breast and esophageal tumors (Table 1; refs. 33–36). TAMs in the nests of human HCCs preferentially express IL10 and recruit immunosuppressive FoxP3$^+$ Treg cells (28), although their number has yet to be shown to be associated with outcome in this disease (Fig. 2).

Interestingly, TAMs have been shown to express the inhibitory receptor signal regulatory protein alpha (SIRPα) at cell surface, which binds to the transmembrane protein, CD47, on cancer cells. When this occurs, it suppresses the ability of TAMs to detect and phagocytose cancer cells. Various studies have shown that blocking CD47 interrupts this "don't-eat-me" signal and triggers cancer destruction by TAMs in mouse tumors, and high CD47 expression is associated with poor prognosis of bladder cancer, acute myeloid leukemia, non-Hodgkin's lymphoma, and breast cancer (37, 38). In this way, cancer cells escape surveillance by TAMs. This would be highly relevant in tumor nests where cancer cells come into close contact with TAMs. It would be interesting to see whether the aforementioned links between high nest TAM numbers and a poor prognosis correlate with the expression of SIRPα and CD47 by TAMs and cancer cells respectively in these sites.

Stroma

In this prominent area of most solid tumors, cancer cells are often sparse or absent. Rather, it consists of a complex network of macromolecules in the extracellular matrix (ECM) including collagen fibrils, laminin, fibronectin, tenascin C, and hyaluronic acid (HA). It is often populated by various nonmalignant cell populations including fibroblasts, endothelial cells, pericytes,

lymphocytes, and myeloid cells (39). A number of studies have shown that ECM components (and/or their proteolytic products), such as fibronectin, laminin-10, versican (a chondroitin sulfate proteoglycan), and HA fragments, regulate the phenotype of macrophages (40). Moreover, Pinto and colleagues (41) showed recently that decellularized ECM isolated from human colorectal tumors stimulates macrophages to express a relatively anti-inflammatory phenotype with increased expression of IL10, TGFβ, and decreased C-C chemokine receptor type 7 (CCR7), TNFα, and IL6 in vitro. Also, stromal TAMs with higher chemokine (C-C motif) ligand 18 (CCL18) production associates with increased metastasis and reduced survival in patients with breast cancer (42). This agrees with a number of studies showing a correlation between high numbers of stromal TAMs in breast, esophageal, gastric, pancreatic, oral, and skin tumors and poor overall survival and/or RFS (Table 1; refs. 34, 35, 43–46). However, this may depend on tumor type as there is no such correlation in endometrial, cervical, and lung cancer (30, 47, 48), and in bladder cancer, it even correlates with reduced lymph node metastasis and improved survival (Table 1; ref. 49).

In addition to the effects of a complex array of components in the "matrisome" of the stroma (i.e., the core ECM proteins including collagens, fibronectins, laminins, proteoglycans, growth factors, chemokines and cytokines, and ECM-remodeling enzymes), the biophysical properties of the stroma also regulate the functions of TAMs. The architecture and stiffness of the ECM have been shown previously to regulate cell behavior (40), and increased substrate stiffness upregulates the expression of various proinflammatory genes by macrophages in vitro by activating TLR4 signaling pathways in these cells (50). Possible effects of matrix rigidity on macrophages in the premetastatic niche have also been reported as the cross-linking of collagens and elastins induced by the enzyme, lysyl oxidase (LOX), modifies the recruitment, invasion, and retention of myeloid cells (51). In an interesting, recent study, high levels of 22 common matrisome constituents (termed the "matrix score") positively correlated with both tumor stiffness and TAM infiltration in ovarian metastases, although it remains to be seen whether the last two are causally linked (52). To add to this complex picture, it should be noted that different areas of stroma within a given tumor may differ in their chemical and biophysical properties and so regulate TAMs differently (Fig. 2).

Interestingly, macrophages in some tissues appear to play an important role in collagen remodeling. Proteolyzed fibrillar collagen recruits macrophages during postpartum mammary involution in rats (53) and macrophages have been shown to facilitate collagen fibrillogenesis in developing mammary glands in mice (54). Given that fibrillar collagen is abundant in stroma of tumors, studies are now warranted to see if this two-way interaction occurs there, and what effects this has, if any, on tumor progression and response to various treatments.

Perivascular Niche

A subset of TAMs lie close to, or on, the abluminal surface of blood vessels in mouse and human tumors (55). These PV cells often express high levels of the M2-associated markers, TIE2 (a major receptor for angiopoietins), MRC1 and CD163, and play a key role in stimulating tumor angiogenesis, metastasis, and relapse after frontline treatments for cancer (56). Because of their relatively high expression of TIE2, these cells were initially

termed "TIE2-expressing monocytes/macrophages (TEM)." When coinjected into mice with mouse mammary cancer cells, the resultant tumors were more vascularized than those generated with cancer cells alone or cancer cells with TIE2$^-$ monocytes (57). Interestingly, the frequency of TEMs has also been shown to positively correlate with microvessel density (MVD) in some human tumor types (Table 1; refs. 58, 59).

Genetic deletion of PV TIE2$^+$ TAMs or the pharmacologic blockade of the main TIE2 ligand upregulated by the tumor vasculature, angiopoietin 2 (AGPT2), demonstrated the importance of this TAM subset in tumor angiogenesis and growth in various mouse models of cancer (60). The subsequent gene expression profiling of TEMs isolated from mouse tumors revealed their higher expression of a number of tumor-promoting genes including Mmp9, Vegfa, Cxcl12, Tlr4, and Nrp1, than TIE2$^-$ TAMs from the same tumors (61).

Intravital imaging studies have shown that some PV TIE2$^+$VEGFA$^+$ TAMs interact closely with both endothelial cells and cancer cells expressing the invasive isoform of actin binding protein mammalian enabled (MENA). These cell trios have been termed the "tumor microenvironments of metastasis" (TMEM) as they are sites of increased intravasation of cancer cells into the blood. PV TAMs in TMEMs upregulate VEGFA and increase the permeability of neighboring blood vessels (62). Their role in promoting metastasis is supported by the finding that high TMEM frequency correlates with increased risk of distant metastasis in patients with ER$^+$HER2$^-$ breast cancer (63). Interestingly, a recent study has shown that TMEMs containing TIE2$^+$VEGFA$^+$ PV TAMs are also present in premalignant lesions in a mouse model of HER2$^+$ breast cancer and promote the early dissemination of cancer cells (Fig. 1; ref. 64).

PV TIE2$^+$ TAMs have also been implicated in the relapse of primary mouse tumors after various forms of treatment. They increase in relapsing glioma after local irradiation, and in lung and mammary tumors after chemotherapy. At such times, they express high levels of CXCR4 and are recruited by upregulated CXCL12 in the PV niche (65, 66). Our studies showed that this TAM subset then stimulates revascularization and regrowth of tumor via their release of VEGFA (66). A later study confirmed that TIE2 expression at TAMs is required to induce vascularization after chemotherapy in mice (67). Furthermore, a recent paper has also demonstrated that newly recruited monocytes also migrate around untreated tumors in a CXCR4-dependent manner. Flourescently-labeled monocytes were seen to extravasate into untreated PyMT tumor implants through vessels mainly in tumor nests, where they are then exposed to TGFβ released by cancer cells. This stimulates these new recruits to upregulate their expression of CXCR4 and migrate towards CXCL12-expressing fibroblasts around tumor blood vessels in collagen-rich stromal areas. Once they are adjacent to vessels, the monocytes differentiate into the metastasis-assisting, PV TAMs reported in TMEMs (62, 68).

Finally, in metastatic sites like the lungs, a subset of CCR2$^+$Ly6C$^+$ macrophages promote the extravasation of cancer cells and their formation of metastases (5). These "metastasis-associated macrophages (MAM)" have been shown in mouse tumor models to directly tether vascular cell adhesion molecule-1 (VCAM-1) on cancer cells via their α4-integrins, a process that subsequently increases cancer cell survival at such metastatic sites (69). Furthermore, binding of CCL2 to CCR2 on MAMs stimulates their release of CCL3, which binds to CCR1 on cancer cells and facilitates their retention in the lungs (70). These MAMs

also promote persistent growth of metastatic lesions through VEGFR1 and CSF-1R signaling (71, 72).

Hypoxic/Necrotic Areas

Hypoxia is a hallmark feature in solid tumors and has been linked to increased invasion and metastasis, resistance to therapy, and poor clinical outcome. Hypoxic areas typically have oxygen tensions (pO2 values) below 10 mm Hg and are located more than 150 μm from tumor blood vessels. They form in tumors when the cellular requirement for oxygen outstrips its supply by the poorly organized tumor vasculature. These sites have been identified in tumor sections using hypoxic cell markers, for example, pimonidazole (PIMO), or immunolabeling for the hypoxia-inducible alpha subunit of the transcription factors, HIFs 1 and 2 (73). High numbers of hypoxic TAMs associate with elevated levels of tumor angiogenesis, metastasis, poor RFS, and/or reduced overall survival in breast, endometrial, and cervical cancer (Table 1; Fig. 2; refs. 30, 74, 75).

When TAMs gather in such areas they upregulate HIFs 1 and 2, and various HIF target genes like VEGFA, GLUT1, and MMP7 (76, 77). TAMs are recruited into these sites by chemokines upregulated due to hypoxia, including C-X-C motif chemokine 12 (CXCL12), endothelial cell monocyte-activating polypeptide-II (EMAP-II), endothelin 2, VEGFA, and SEMA3A (78–80). Hypoxic TAMs become immobilized in hypoxic areas by the direct, inhibitory effect of hypoxia on their mobility (81) and their reduced expression of receptors for tumor-derived chemokines CCR2, CCR5, and NRP1 (79).

Hypoxic TAMs promote tumor angiogenesis, immune evasion, and metastasis in various experimental models. For example, they upregulate an array of proangiogenic and immunosuppressive cytokines in hypoxic tumor areas (76, 77, 82, 83), and when their entry into hypoxic tumor areas is impeded by SEMA3A/NRP1 signaling blockade, tumor angiogenesis is markedly reduced, and antitumor immunity restored (80). Hypoxic TAMs are also able to suppress T-cell activation in a number of ways including their upregulation of IL10 and negative checkpoint regulators such as PD-L1 (80). A recent study also showed that macrophages cocultured with hepatoma cells under hypoxic conditions have increased indoleamine 2, 3-dioxygenase (IDO) expression, which suppresses the proliferation of local cytotoxic T cells and expands Treg cells (84).

Although exposure to hypoxia per se fails to skew TAMs towards a tumor-promoting, phenotype (85), some studies have shown that a low pH and lactate (which accumulate in poorly vascularized, hypoxic areas due to the poor vascular supply) act in concert to induce a proangiogenic phenotype in TAMs, which, in turn, restores blood perfusion (85–87). Indeed, lactic acid can stimulate expression of VEGFA by macrophages (87). As mentioned previously, this cytokine is not only proangiogenic in tumors but also capable of stimulating the intravasation of cancer cells. It remains to be seen whether VEGFA released by TAMs in poorly vascularized areas (i.e., away from blood vessels) contributes to the latter phenomenon.

Tumor hypoxia can also modulate TAM functions indirectly by stimulating cancer cells to release high-mobility group box 1 protein (HMGB1) that, in turn, stimulates IL10 production by TAMs. Furthermore, this hypoxia–HMGB1–IL10 axis has been shown to stimulate metastasis in the murine B16 tumor model (88). Hypoxia also induces metabolic changes in TAMs, which

then impact directly on the functions of neighboring cells. For example, hypoxia stimulates their expression of REDD1, an mTOR inhibitor and key modulator of metabolism in response to nutrient availability and energy requirement. The resultant inhibition of mTOR in TAMs strongly reduces their glucose uptake and glycolysis, leaving more glucose for neighboring endothelial cells. This results in a more hyperactive and leaky vascular network and the provision of more escape sites for cancer cells into the circulation. So, it is hardly surprising that this mechanism in primary tumors has been shown to drive the formation of distant metastases (89).

Concluding Remarks

A number of experimental studies in mice have now confirmed the ability of different tumor compartments to differentially regulate the phenotype of TAMs. The importance of this is underscored by clinical reports showing that the number and/or phenotype of TAM in specific tumor areas correlate with RFS and/or survival in human tumors (Table 1).

We are beginning to identify the factors regulating this spatial heterogeneity of TAMs in tumors. As mentioned earlier, genetic changes taking place during early neoplasia can be "sensed" by neighboring macrophages and trigger their tumor-promoting functions. Activation of the oncogene, c-Myc, and mutations in the tumor suppressor gene, p53, in breast epithelial cells are prominent in high-grade DCIS, and regulate the function of macrophages in such in such preinvasive lesions (15, 90–92). Later, in established tumors, nest TAMs are exposed to tumor cell-derived factors, hypoxia, low pH, and high lactate concentration (due to the tumor vasculature being unable support the metabolic needs of rapidly proliferating tumor cells; refs. 93, 94). Alternatively, TAMs in the stroma receive a diverse array of signals, including those released by, or expressed on the surface, of endothelial cells, pericytes, fibroblasts, lymphocytes, other myeloid cells, and ECM constituents. However, it may be oversimplistic to assume that any two similar areas within a tumor (e.g., stromal areas) are identical, and thus regulate TAM behavior in the same way. Furthermore, the phenotype of TAMs in a given area will likely change over time as each site changes within the tumor mass.

It also remains to be seen whether TAMs in different tumor areas are variants of the same monocyte/TAM pool conditioned to perform specific functions in response to local signals, or whether they also have different origins. As described earlier, newly recruited monocytes can migrate from one tumor area to another (e.g., nests to the PV niche; ref. 68) and, in doing so, change their phenotype (68). This finding attests to the plasticity—and potential interconversion—of TAMs in different sites in tumors. However, this does not exclude the possibility that some TAM subsets may be recruited from distinct subsets of circulating monocytes (95) or from the proliferation of a local TAM progenitor pool (96).

Also mentioned earlier, a recent cell fate-mapping study has shown that TAMs in mouse brain tumors are derived from both resident brain macrophages (microglia) and blood monocytes (97). Although they shared a common, tumor-induced gene expression signature, they also exhibited considerable differences in their transcriptional profile, suggesting the retention of certain ontogeny-specific characteristics (6). However, it is not known yet whether this phenomenon is limited to brain tumors, or whether

it contributes to the spatial diversity of TAMs in tumors. We also have much to learn about the development of TAM subsets in different areas of metastatic tumors, as virtually every study on this so far has been in the primary setting.

Evidence is also emerging for the role of TAM subsets in certain tumor areas limiting tumor responses to treatment. For example, irradiation, vascular disrupting agents, and cytotoxic drugs induce the expansion of the PV TAMs, which contributes to tumor angiogenesis and relapse after therapy (69, 70, 98). Hypoxic TAMs have also been implicated in tumor resistance to several anticancer treatments and to promote relapse (83).

The demonstration that TAMs stimulate a number of tumor-promoting mechanisms in mouse tumor models prompted the development of therapeutic approaches to deplete or reprogram them (99). To date, general TAM inhibitors, including those targeting the CSF-1-CSF-1R and the CCL-CCR2 axis, have largely failed to show efficacy in cancer clinical trials as monotherapies (100–102), although they may prove to be effective in combination with other therapeutic agents. Although the CSF-1-R inhibitor PLX3397 have shown significant efficacy in tenosynovial giant cell tumors, treatments have also revealed pathologies resulting from the long-term depletion of all macrophages via this inhibitor (103). Targeting specific TAM subsets in tumors may be a better way forward—in order to deplete or re-educate those that are tumor-promoting, while leaving or increasing those capable of being tumoricidal and/or promoting anti-tumor immunity. Advances in our understanding of how the phenotype of TAM subsets in different tumor areas is influenced by their ontogeny, activation status and complex array of local cues will help to develop such a therapeutic approach. Unraveling the complex array of influences on TAM behavior will likely require a multifaceted approach including cell fate mapping studies, high-dimensional, single-cell analysis techniques, and systems biology/computer modeling. However, this could then lead to personalized approach to the selective targeting of appropriate TAM subsets.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Authors' Contributions

Conception and design: M. Yang, C.E. Lewis
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Administrative, technical, or material support (i.e., reporting or organizing data, constructing databases): M. Yang
Study supervision: M. Yang
Other (this is a review article so it has no data): C.E. Lewis

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