

The Role of tumor-associated macrophage in tumor progression

Koji Fukuda¹, Aya Kobayashi¹, Kounosuke Watabe¹

¹Department of Medical Microbiology, Immunology and Cell Biology, Southern Illinois University, School of Medicine, 751 N Rutledge St. PO Box 19626, Springfield, IL 62779-9626, USA

TABLE OF CONTENTS

1. Abstract
2. Introduction
3. Monocyte recruitment and M1/M2 polarization
 - 3.1. Monocyte recruitment
 - 3.2 Differential functions of M1 and M2 macrophages
 - 3.3. TAM resemble M2 macrophage
4. How does TAM stimulate tumor progression?
 - 4.1. TAM promote both angiogenesis and lymphangiogenesis
 - 4.2. TAM suppress anti-tumor immune responses
 - 4.3. TAM promote invasion and metastasis of tumor
5. The role of TAM in tumor stem cells
6. Clinical implication
7. Conclusions and future directions
8. Acknowledgement
9. References

1. ABSTRACT

The tumor progression is not only regulated by metastasis promoting and suppressing genes in cancer cells but it is also strongly influenced by the interaction between cancer cells and the stromal cells. An abundance of inflammatory mediators and leukocytes has been known to promote cancer metastasis, and tumor associated macrophages (TAM) are the key players in the link between inflammation and cancer. TAM are derived from peripheral blood monocytes that are recruited into the tumor by inflammatory chemokines. Upon activation by cancer cells, TAM gain the ability of pro-tumoral functions including expression of various growth factors, promotion of angiogenesis and suppression of adaptive immunity, and many of these factors also play critical roles in cancer metastasis. In this review, we will summarize the recent information about the function of TAM in the inflammatory micro-environment of solid tumors and discuss the potential targets for future therapeutic approaches.

2. INTRODUCTION

Tumor progression is a complex multi-step process including transformation, tumor cell growth, invasion and metastasis. The latter two steps are the most critical steps in determining the aggressive phenotype of human cancers, and they are the major obstacles for the successful treatment of cancer patients (1). However, the progression of tumor is not considered as an independent process of tumor cell per se, and it is rather strongly influenced by the nature of the surrounding non-malignant cells, as previously shown using oncogene-driven tumor in transgenic mice (2). Thus, it is becoming clear that different types of growth factors and cytokines released from the cells of tumor microenvironment and signal pathways induced by cell to cell interactions play an important role in the tumorigenesis and metastasis. The tumor microenvironment includes many resident cell types, such as fibroblasts, endothelial and immune-competent cells and they all interact and promote cancer cell growth.

A specialized group of macrophages called tumor associated macrophages, TAM, has recently gained considerable attention due to evidence that shows their pivotal roles in the growth and invasion of the tumor cells by providing a unique tumor microenvironment. The presence of TAM has long been considered as evidence of a host response against the growing tumor (3, 4). Macrophages have the ability to kill tumor cells *in vitro* when they are appropriately stimulated by TH1 cytokines such as lipopolysaccharides (LPS) and interferon (IFN- γ). However, bacterial stimuli and TH1 cytokines that induce M1 type polarization are usually not present in tumor. Instead, differentiating macrophages at the tumor site are likely to encounter factors that frequently polarize them to M2 type macrophages, which are activated by various interleukins such as IL-4, IL-10, and/or IL-13 (22, 28). M2 macrophages that infiltrated into the tumor sites are significantly correlated with the promotion of tumor growth and metastatic development in an animal model (5). Thus, it is generally accepted that TAM have an M2 phenotype (6), which has the essential role in stimulating tumor progression and invasion. The strategic location of TAM also suggests that these cells are important regulators of anti-tumor immunity. Therefore, characterization of TAM phenotype is crucial for the understanding of tumor-derived signals and identification of the molecular mechanisms that might be amenable to therapeutic intervention. This review will focus on the function of TAM and other myeloid-derived tumor-infiltrating cells as pivotal players in the tumor microenvironment, in the hope that a better understanding of their roles in tumor progression will lead to development of efficient anticancer therapies.

3. MONOCYTE RECRUITMENT AND M1/M2 POLARIZATION

3.1. Monocyte recruitment

Blood monocytes are not fully differentiated cells and are susceptible to various environmental stimuli. CCL2 is the main regulator of monocyte recruitment from the circulation and for differentiation to macrophage at the tumor sites. Interestingly, CCL2 secreted from monocytes was reported to increase brain endothelial permeability by rearrangement of intracellular actin and alteration of tight junction assembly (7), which may promote monocytes recruitment into the brain. The role of CCL2 in macrophage accumulation at the tumor sites is supported by the evidence that levels of tumor-derived CCL2 correlates with the level of TAM in several types of adenocarcinoma, including ovarian, breast and pancreas (8, 9). Recent study demonstrated that expression of small interfering RNA to CCL2 in fibroblasts significantly suppressed liver metastasis without affecting primary tumor growth, cell proliferation or TAM recruitment (10). Moreover, TAM themselves also produce CCL2 and recruit the monocytes into the tumor sites, suggesting that anti-CCL2 antibodies combined with other drugs may serve as an effective anti-tumor strategy (11). In addition to CCL2, CCL5, CCL7, CXCL1, CXCL8, and CXCL12 are also known to be involved in monocytes recruitment (12).

The attraction of circulating monocytes to the tumor site is not only controlled by chemokines, but also by

other growth factors such as vascular endothelial growth factor (VEGF), platelet derived growth factor (PDGF), transforming growth factor (TGF β), and macrophage colony stimulating factor (M-CSF). M-CSF is the main regulator of polarization of monocytes/macrophages and promotion of macrophage survival and differentiation (primarily M-CSF) (13). At the tumor site, depletion of M-CSF significantly suppresses the infiltration of macrophages, which is correlated with a significant delay in tumor progression (14). On the other hand, overexpression of M-CSF by tumor cells was shown to dramatically increase macrophage differentiation, which was correlated with promotion of tumor growth. M-CSF overexpression is also commonly observed among tumors of the reproductive system, such as ovary and uterine (15, 16, 17). Thus, in response to multiple chemokines and growth factors including CCL2, M-CSF, PDGF, TGF, VEGF β that are secreted from tumor cells, monocytes are recruited into tumor sites followed by polarization into “classic” M1 and “alternative” M2 macrophage, which is also called TAM (Figure 1).

3.2. Differential functions of M1 and M2 macrophages

Macrophages undergo activation in response to diverse signals including microbial products and cytokines. Classically activated M1 macrophages are induced by immune stimuli such as interferon- γ (IFN- γ), in concert with bacterial lipopolysaccharide (LPS) or cytokines (tumor necrosis factor α (TNF α) and GM-CSF). The M1 activation is mediated by multiple signal transduction pathways such as signal transducer and activator of transcription (STAT), nuclear factor kappa-light-chain enhancer of activated B cells (NF κ B), and mitogen-activated protein kinases (MAPK) (18). Generally, M1 macrophages are characterized by its high capacity of presenting antigens, high IL-12 and IL-23 production and consequent activation of polarized type I T cell responses (19). Moreover, they have cytotoxic ability toward tumor cells as well as toward cells that have ingested intracellular micro-organisms, by releasing high levels of toxic intermediates such as nitric oxide (NO), reactive oxygen intermediates (ROI), TNF and MHC class II molecules (20, 21).

In contrast, IL-4 and IL-13 promote alternatively activated M2 macrophages. M2 is a generic name covering different forms of macrophage activation other than M1, including cells exposed to IL-4, IL-13, immune complexes, IL-10, and glucocorticoid hormones (22) (23). IL-4, IL-13 and glucocorticoid hormones attenuate the IL-1 system by promoting expression of the decoy receptor IL-1RII. Moreover, IL-4 and IL-13 is known to induce the IL-1 receptor antagonist (IL-1ra) and inhibit IL-1 (26). While M1 macrophages express high level of pro-inflammatory cytokines (IL-1, TNF, IL-6 and IL-23), M2 macrophages are characterized by their low level of production and by poor antigen presenting capacity (26). This is reflected in their general properties: IL-12 low, IL-10 high. This phenotype decreases the inflammatory responses and adaptive Th2 immunity, actively scavenge debris, and induce angiogenesis, tissue remodeling, and lack of tumoricidal capabilities (26). M2 macrophages are

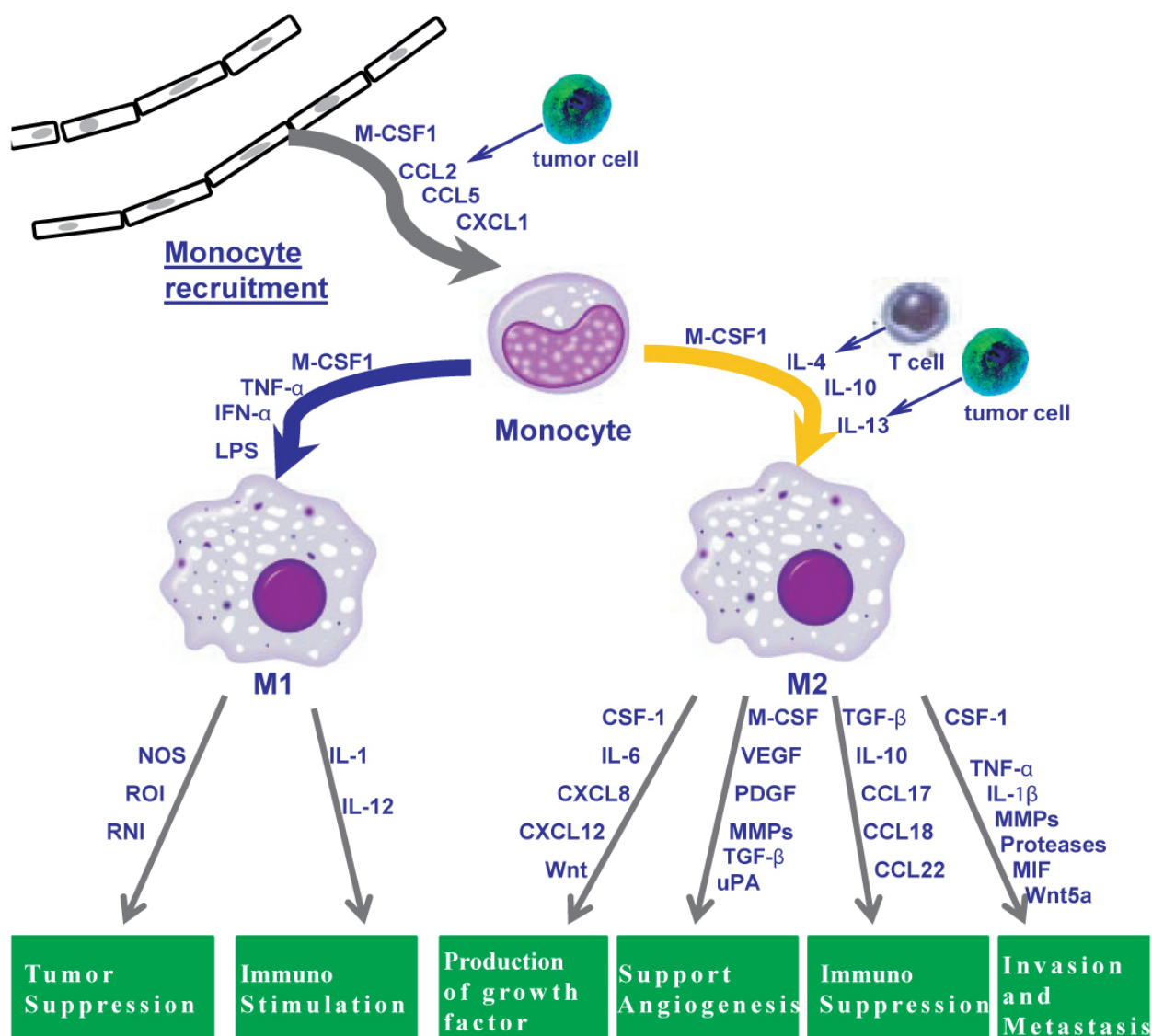


Figure 1. M1, M2 polarization and function of macrophage

generally considered to be more heterogeneous than M1 cells and, to reflect these differences, they are further subdivided into M2a, M2b, and M2c cells. M2a (induced by exposure to IL-4 or IL-13) and M2b (induced by stimulation with immune complexes, and agonists of Toll-like receptors (TLRs) or the IL-1 receptor antagonist (IL-1ra)) macrophages exert immunoregulatory functions and drive Th2 responses (27), whereas M2c cells (generated by stimulation with IL-10) play a predominant role in the suppression of immune responses and tissue remodeling (28). Furthermore, in line with their tissue repairing function, M2 cells have high levels of scavenger, mannose and galactose-type receptors (28). Interestingly, M2 macrophages express high level of arginase and the consequent generation of ornithine and polyamines, while M1 macrophages express inducible nitric oxide synthase (iNOS; NOS2) that produce nitric oxide. This metabolic switch occurs preferentially during the activation of the

M2a and M2c polarization programs (26). Moreover, other factors including IL-6, TGF β , and prostaglandin E2 (PGE2) are also able to influence macrophage polarization toward M2 profile (29). Thus, M2 macrophages are oriented to the promotion of tissue remodeling and angiogenesis, regulation of immune responses, as well as promotion of tumor growth, while M1 macrophages are considered to be potent effector cells that kill micro-organisms and tumor cells and produce pro-inflammatory cytokines. TAM display similar characteristics to M2-polarized macrophages, which will be discussed later in more detail. However, the M1/M2 paradigm is not an entirely adequate to describe TAM characters. Recent evidence shows that their phenotype is plastic and varies between tumor types, stages of tumor development, and their location within the tumor microenvironment (i.e. their responses to local signals) (30). Therefore, further clarifications of M1/M2

polarization in different stages and location of tumors are required.

3.3. TAM resemble M2 macrophage

In most but not all (31) tumors investigated, TAM have an M2-like phenotype. How macrophages are activated and contribute to the pro-tumor function in tumor microenvironment is an intriguing question. It was previously shown that tumor cell products such as extracellular matrix components, IL-10, and CSF-1 stimulated macrophages and set them in M2 macrophages that have the capacity to promote cancer (32). In a mammary carcinoma model, CD4⁺ T cells were shown to induce metastasis by causing M2 activation via IL-4 stimulation (33). In ovarian cancer, it has been reported that TAM produced low levels of NO (34) and only a minority of macrophages localized at the periphery scored positive for iNOS (35). Moreover, in contrast to M1 polarized macrophages, TAM have been identified to be poor producers of reactive oxygen intermediates (ROIs), which is consistent with the hypothesis that these cells represent a skewed M2 population (35).

Activation of NF- κ B promotes the transcription of several pro-inflammatory genes. In mouse models, TAM displayed defective NF- κ B activation in response to the M1 polarizing signal with LPS (36). This explains why TAM produce the low levels of inflammatory cytokines such as IL-12, IL-1b, TNF α , and IL-6. Other evidence also suggests that p50 homodimers, negative regulators of NF- κ B, are abundant in TAM and are responsible for its defective activation (37). Furthermore, TAM from p50 ^{-/-} tumor-bearing mice produced cytokines characteristic to M1 macrophages, and their splenocytes produced high levels of Th1 cytokines (e.g. IFN- γ), which were correlated with a delay in tumor growth (37). Therefore, restoration of NF- κ B activity or inhibition of p50/NF κ B activity in TAM has a possibility to restore M1 phenotype, which may provide therapeutic benefit by driving anti-tumor activities.

4. HOW DOES TAM STIMULATE TUMOR PROGRESSION?

4.1. TAM promote both angiogenesis and lymphangiogenesis

Angiogenesis is an M2-associated function that represents a pivotal event in tumor growth and progression. In human cancer, accumulation of TAM has been associated with angiogenesis and with the production of many factors including growth factors, angiogenic factors, angiogenesis-modulating enzymes and chemokines. In lung cancer, TAM was shown to promote tumor progression by contributing to stroma formation and to angiogenesis through the secretion of PDGF, in conjunction with TGF β production by cancer cells (22). TAM also secrete another angiogenic factor, thymidine phosphorylase, which *in vitro* promotes endothelial cell migration, and they also release various angiogenesis modulating enzymes including MMP-2, MMP-7, MMP-9, MMP-12, and cyclooxygenase-2 (6, 38, 39). In K14-HPV16 transgenic mice treated with estrogen, MMP-9 secreted from TAM was demonstrated to contribute to angiogenic

development (40). It was also known that MMP-2 expression was increased in several tumors, which was correlated with nodal status and tumor stages (41).

TAM have their tendency to accumulate into necrotic regions of tumors which is characterized by low oxygen tension (42). This localization is regulated by tumor hypoxia, which promotes the expression of HIF-1 dependent genes such as VEGF, CXCL12, and its receptor CXCR4 that control TAM migration in vascular regions (43). Therefore, TAM accumulate preferentially in the lower vascularized regions of tumors with low oxygen tension (44), and hypoxia in these cells promotes a specific pro-angiogenic program. Low oxygen conditions promote expression of HIF-1 and HIF-2 followed by overexpression of pro-angiogenic molecules. The important function of HIF-1 was underlined by the observation that in hypoxic regions, the abrogation of this transcription factor promoted an impaired motility and cytotoxicity of macrophage (45). Hypoxia tightly controls the expression of various chemokines, and HIF-1 especially regulates the expression of CXCL12 and CXCR4 (46). In addition, TAM secrete other chemokines involved in the process of angiogenesis such as CCL2, CCL5, CXCL8, CXCL1, and CXCL13. CXCL5 and CXCL8 were known to be associated with promotion of neovascularization (12).

TAM also promote lymphangiogenesis, a process mediated by a number of factors including VEGF-C and VEGF-D via VEGFR3 (48, 49). For instance, in human cervical cancer, VEGF-C production by TAM was proposed to play a role in peritumoral lymphangiogenesis and subsequent dissemination of cancer cells with formation of lymphatic metastasis (48). Moreover, lymphatic endothelial growth factors released from TAM were shown to be related to peritumoral lymphangiogenesis (48, 49). Recently, it was shown that as a regulator of tumor-associated lymphangiogenesis, metastasis-associated protein 1 (MTA1) promoted lymphangiogenesis in colorectal cancer by mediating the VEGF-C expression (98). Thus, macrophage recruited *in situ* represent an indirect pathway of amplification of angiogenesis and lymphangiogenesis in concert with various molecules directly produced by tumor cells. However, TAM also have the capability to inhibit angiogenesis through the production of the anti-angiogenic protein, plasminogen activator inhibitor type-2 (PAI-2). It was shown, in a mouse model, that TAM stimulated with GM-CSF secreted PAI-2 and this observation correlated with the fact that prostate cancer cells engineered to express high level of GM-CSF completely suppressed tumor progression (50). Furthermore, thrombospondin1 (TSP1) produced by macrophages inhibited migration and proliferation of endothelial cells *in vitro* and neovascularization *in vivo* (51). Therefore, TAM have not only a positive effect, but also negative effect on angiogenesis.

4.2. TAM suppress anti-tumor immune responses

As described above, in general, macrophages are capable of presenting tumor-associated antigens, lysing tumor cells, and stimulating the antitumor functions of T

cells and NK cells. However, TAM in the tumor microenvironment lack these activities, leaving the host without the ability of an effective antitumor immune response. In addition, several lines of experimental evidence have emerged for TAM being potent immunosuppressors. Under the influence of the tumor-derived factors, TAM lose the ability to present tumor-associated antigens and hence to stimulate the anti-tumor functions of T cells and natural killer (NK) cells. Modulation of host immune response to tumors by TAM is accomplished through their increased expression of cytokines, chemokines, and enzymes that influence the function of antigen-presenting cells as well as specific immune effectors such as B and T cells (52).

TAM produce and release several immunosuppressive cytokines, of which IL-10 has been most studied. In addition, TAM produce low level of immunostimulatory cytokines including TNF α , IL-1 and IL-12, mainly due to defective NF- κ B activation, at least in TAM of advanced cancer (22). The defective production of the major immunostimulatory cytokine IL-12 may also be due to the activity of IL-10, produced either by TAM or by the tumor cells. It was previously shown that IL-10, alone or in concert with IL-6, was responsible for the up-regulation of macrophage B7-H4 expression, a molecule involved in the suppression of tumor-associated antigen-specific T cell immunity (54). Moreover, some of these immunosuppressive activities may be regulated by over-activation of transcription factors, Stat3 (56). It should be noted that inhibition of Stat3 resulted in promoting cytotoxicity and antigen-presenting function in activated macrophages and is associated with retardation of tumor growth (56).

Part of the immunosuppressive activity of TAM is exerted indirectly by their secretion of chemokines that preferentially attract T cell subsets devoid of cytotoxic functions. Among chemokines, immunosuppressive activity is tightly regulated by the function of CCL18, which is enhanced by Th2 cytokines: IL-4, IL-13 and IL-10, and recruitment of naive T cells by interacting with an unidentified receptor (57). It is noteworthy that, in the ascitic fluid of human ovarian carcinoma, CCL18 has been identified as the most abundant chemokine (58), and it was also shown that CCL18 was only released from TAM, whereas no release of this cytokine from ovarian carcinoma cells was observed (55, 57). Moreover, it has been reported that two other chemokines, CCL17 and CCL22, are abundantly secreted by TAM (12, 22), and these chemokines interact with the CCR4 receptor that was expressed mostly by Th2 cells and by Treg (61). Therefore, some of the chemokines secreted from TAM actively contribute to suppress anti-tumor immune responses.

4.3. TAM promote invasion and metastasis of tumor

The intense cross-talk between macrophages and neoplastic cells guarantees the continuous process of matrix deposition and remodeling, which facilitates tumor growth and invasion of the surrounding tissues. TAM contribute to tumor progression by promoting invasion of malignant cells and also by making their movement easier through the

release of cytokines, growth factors and proteinases. For example, co-culture of neoplastic cells with macrophages promoted invasiveness of malignant cells through TNF α -dependent MMP induction in macrophages (62, 63). Other evidence comes from the influence of lung macrophages on endothelial cells, which leads to MMP-9 secretion via a VEGFR-1-dependent mechanism (64). In co-culture experiments of macrophages with MCF-7 breast cancer cells, overexpression of MMP in MCF-7 also have been demonstrated (65, 66). Moreover, in *in vitro* model of M2-polarized macrophage derived from peripheral blood monocytes cocultured with BCC cells, TAM promoted the transmigration of BCC cells through the induction of MMP9 released from BCC cells and this MMP9 was induced through the up-regulation of COX-2 and NF- κ B of BCC cells (67). In a mouse melanoma model, IL-1 released from TAM enhanced metastatic development (68). IL-1 is well known to promote the inflammatory genes such as NF- κ B and COX-2. Thus, TAM may contribute to invasion or metastasis of cancer cells by releasing the key factors such as IL-1, which activates their expression of MMP9.

Cytokines and growth factors released from TAM are known to promote metastasis and invasion through up-regulation of various genes in cancer cells. However, this interaction is reciprocal and TAM are also activated by the secretory factors from cancers. Cancer cells express the EGF receptor and release CSF-1, which attracts macrophages and enhances the expression of EGF by macrophages. EGF promotes the expression of CSF-1 by cancer cells, thereby generating a positive feedback loop (69). In breast cancer cells, it was shown that either of these signals was required for cell invasion through activation of tumor-cell motility (70), while inhibition of either pathway blocks cell movement of both cell types. Furthermore, the number of tumor cells entering the bloodstream is dramatically suppressed with reduction of number of macrophages along the vessels or inhibition of EGF signaling (69, 70). Thus, many studies show that cross-talk between TAM and cancer cells through cytokines and growth factors promote the invasion and metastasis of cancer. Note that macrophages isolated from human breast tumors express abundant EGF (72); however, this has yet to be shown in perivascular TAM. Therefore, it remains to be formally established whether this subpopulation of TAM is essential for metastasis.

5. THE ROLE OF TAM IN CANCER STEM CELLS

Tumor relapse and metastasis remain major obstacles for improving overall cancer survival, which may be due at least in part to the existence of cancer stem cells (CSC). CSC are characterized by tumorigenic properties, the ability of self-renewal, forming differentiated progeny, and developing resistance to therapy (73). These cells generally express CD44^{high}/CD24^{low} on the cell surface, and it was shown that CD44^{high}/CD24^{low} surface phenotype could be promoted by epithelial-mesenchymal transition (EMT) in normal human and mouse mammary tissues (74). EMT is a process that allows epithelial cells to separate from their neighbors and migrate to distal regions during embryonic development, and play a fundamental role

Table 1. TAM as a therapeutic target

Therapeutic Strategy	Target	Agent
Recruitment of TAM	CCL5	Met-CCL5
	CCL2	Trabectedin
		Paclitaxel
Angiogenesis, Immune Suppression	MMP	MMP Inhibitors
		Linomide
		Zoledronic Acid
Induction of M1 Polarization	IL-1, TNF	N-3 Fatty Acids
	NF-kB	Omega-3 Fatty Acids
		CpG plus Anti IL-10 Receptor Antibody

during invasion and metastasis of carcinoma cells. It was demonstrated that activated macrophage altered the morphology and gave a mesenchymal phenotype to HepG2 cells(75). Notably, co-culture of macrophage and lung adenocarcinoma cell line induced the expression of E-cadherin in cancer cells (76). Loss of E-cadherin is also a major phenomenon of EMT, which might release cancer cells from the primary locus into distant sites.

The Wnt protein is one of secreted signaling molecule which regulates a wide variety of normal and pathological processes, including embryogenesis, differentiation and carcinogenesis (77). When Wnt is activated, β -catenin is stabilized, enabling it to move to the nucleus, where it binds T cell factor (TCF) followed by induction of the expression of various target genes including leucine-rich repeat-containing G-protein-coupled receptor (Lgr) 5, which are involved in stem cell proliferation (78). There are several reports that TAM released Wnt-ligand that activates Wnt signaling pathway for proliferation and invasion. In an *in vitro* experiment, co-culture of MCF-7 cells and macrophages was shown to up-regulate Wnt 5a in TAM which was also accompanied by activation of *AP-1/c-Jun* in MCF-7, which then stimulated the Wnt signaling pathway in carcinoma cells to promote invasion (79). In gene set analysis, these macrophages were also specifically enriched for molecules involved in Wnt signaling (80). Therefore, Wnt-ligands secreted from TAM might promote invasion of cancer cells through activation of stem cells proliferation by the Wnt/ β -catenin pathway. In addition, it has become apparent that signaling pathways such as Notch and Hedgehog, which are central to the normal and neoplastic development of the central nervous system, are playing important roles in cancer stem cell regulation (81). Thus, TAM might activate phenotype of cancer stem cells through the induction of EMT, suppression of E-cadherin and the expression of protein that related to stem cell proliferation, which might confer migratory and invasive properties. However, the role of TAM in cancer stem cells is yet largely unknown, so that further investigations of detailed functional mechanism are still needed.

6. CLINICAL IMPLICATION

The degree of the cancer progression is greatly affected by its microenvironment, and understanding the mechanism likely leads to an identification of novel targets for anti-cancer therapy. Cancer cell often develops resistance to various types of therapies due to their inherent genomic instability. An alternative approach is to focus on targeting various non-neoplastic cells that are associated

with the tumor microenvironment, such as macrophages, fibroblasts and endothelial cells. In order to target TAM, there are still several obstacles that need to be overcome. Endothelial cells, stromal cells, and inflammatory cells are not malignant, therefore, successful therapy needs to precisely target the cancer components and avoid attacking the surrounding normal cells.

The cytokine profiles of microenvironment and localization of TAM may influence the function of TAM and therefore the prognostic value of TAM. TAM infiltration has been shown to correlate with poor prognosis in carcinomas of the breast, cervix, bladder, prostate, lung, and brain tumors (24, 45, 47, 51, 71). For example, the relation between TAM density and the density of microvessels and the influence of TAM density on prognosis were investigated in a clinical study with 113 pulmonary adenocarcinoma patients (83). In this study, a significant reduction in patient survival rate was showed in tumors with a high TAM density. In prostate carcinoma, the volume density of TAM to a shorter survival time was also noted (84). These observations make TAM a potential diagnostic marker as well as a target for anti-cancer therapy (Table 1).

In monocytes recruitment, CCL5 and CCL2 are the most important signals, and treatment of murine breast cancers with Met-CCL5 (receptor antagonist) was shown to suppress the number of infiltrating macrophages, which was associated with a significantly reduced tumor size (97). Trabectedin (Yondelis), an anti-tumoral drug, has immunomodulatory properties on mononuclear phagocytes. This drug reduced the production of CCL2 and has a selective cytotoxic effect on monocytes and macrophages including TAM (85). Anti-CCL2 antibody also has the potential to suppress the TAM recruitment, and in combination with docetaxel induces a significant tumor regression in prostate cancer models (86, 87). Thus, these chemokines and chemokine receptors are potential targets for therapeutic strategies in controlling inflammatory tumors growth.

After monocytes differentiate to TAM in tumor sites, TAM secrete various factors including growth factors, cytokines and proteinases, which promote tumor angiogenesis, immuno-suppression and metastasis of cancer. Therefore, there are many reports of potentially amenable therapeutic interventions of these factors. In an orthotopic breast tumor model, anti-VEGF-A antibody therapies reduced the development of new blood and lymphatic vessel, followed by decreased incidence of lymphatic and pulmonary metastasis (88). N-3 fatty acids

have been shown to inhibit expression of IL-1 β , and TNF α secreted by monocytes and macrophages (89). Linomide, an anti-angiogenic agent, caused significant reduction of the tumour volume and inhibition of the stimulatory effects of TAM on tumor angiogenesis (90). Various types of MMPs that are secreted by TAM are known to promote the angiogenesis and invasion of cancer cells, and therefore, they are also potential therapeutic targets. In a genetic mouse model, MMP inhibitors, such as biphosphonate zoledronic acid, was shown to suppress MMP-9 secretion by TAM, which suppressed the overall tumor growth (91). Thus, specific inhibitors against secretory factor from TAM may serve as the potential targets for anti-cancer drugs that may suppress tumor progression and metastasis. However, inhibition of only one of TAM's function may not likely to be sufficient to block tumor growth because TAM have various functions to associate with tumor growth, invasion and metastasis. Therefore, it may be necessary to suppress the whole function or reduce the M2 specific activities of TAM.

It was recently suggested that dynamic changes of the macrophage in tumor might occur during the transition from early neoplastic events to advanced tumor stages (92). These events would drive the M1 toward M2 switch of TAM phenotypes, which is paralleled by the gradual inhibition of NF- κ B during different stages of tumor progression. In this theory, while full activation of NF- κ B in leukocytes would favor M1 inflammation and tumorigenesis, tumor growth and progression may drive inhibition of NF- κ B in infiltrating leukocytes (93). It was shown that defective NF- κ B activation in TAM correlates with impaired expression of NF- κ B-dependent inflammatory functions including expression of cytotoxic mediators and NO and cytokines such as TNF α , IL-1, IL-12 (94). Therefore, restoration of NF- κ B activity is one of potential strategy to restore M1 phenotype and abrogate the whole function of M2 phenotype. For instance, omega-3 fatty acids in fish oil were shown to decrease endotoxin-induced activation of NF- κ B in monocytes and subsequent inflammatory gene expression driven by the NF- κ B transcription factor (95). In a mouse tumor model, the combination of CpG (TLR9 ligand) plus an anti-IL-10 receptor antibody switched infiltrating macrophages from M2 to M1 and triggered the innate immune response debunking, suggesting that this treatment might restore NF- κ B activation and inflammatory functions by TAM (96). In addition to NF- κ B activation, enhancement of the Notch signaling pathway has a possibility to restore M1 phenotype. The DNA-binding protein RBP-J/CBF1 mediates the major transactivation signals of Notch receptors. In a mouse tumor model, forced activation of Notch signaling enhanced their anti-tumor capacity through increase in M1 macrophages, which produced IL12 even in the presence of the M2 inducer, IL4 (53). Moreover, STAT3 signaling in macrophage is well known to regulate immune responses, which is essential for macrophage differentiation toward the M2 phenotype (22). SHIP1 signaling is also known to activate M2 phenotype, and SHIP1deficient mouse displayed a skewed development away from M1 macrophage that have high iNOS levels and produce NO towards M2 macrophages (59). Thus, there are

several signaling pathways that lead the macrophages to differentiate themselves into M2 macrophages. How can we inhibit these signaling pathways in TAM? In order to identify M2-signaling inhibitor, 130 purified compounds from natural products were screened (82). Corosolic acid, contained in several plants such as banana leaves and apples inhibited the expression of CD163, one of the M2 marker, and suppressed the M2 polarization of macrophages and tumor proliferation by inhibition of both STAT3 and NF- κ B activation (60). Corosolic acid was also shown to inhibit the proliferation of glioblastoma cells. Thus, inhibition of macrophage polarization toward the M2 phenotype and restoration of the M1 phenotype could possibly suppress the tumor progression. However, relatively less is known about the exact actions of these drugs that regulate the signaling pathways to abrogate M2 function in TAM. Therefore, additional study about the mechanism of polarization and screening more candidate agents that change M2 to M1 polarization of TAM are needed in the future.

7. CONCLUSIONS AND FUTURE DIRECTION

TAM are the key orchestrators of tumor vascularization, lymphangiogenesis, metastasis, and immunosuppression through various secreting molecules including growth factors, cytokines, and proteinases. The roles of TAM in tumor progression are now clearly recognized and their underlying mechanisms are gradually revealed. TAM facilitate tumor progression and metastatic invasion by promoting angiogenesis, suppressing immune system and even by stimulating the proliferation of cancer stem cells. Therefore, intervening in these pro-tumorigenic functions of TAM is considered to be an effective strategy for cancer therapy. Extensive research is currently underway to develop novel approaches to target recruitment, survival and polarization of TAM. However, tumor microenvironment is complex and consists of various other stromal cells such as endothelial cells, pericytes and fibroblasts, all of which interact with TAM and tumor cells through complex cross-talk signaling. Therefore, to further clarify the role of TAM in tumor progression, it is critical to understand the entire hierarchy of the tumor environment using a valid animal model. Furthermore, these microenvironments are expected to be varying in different organs, and clarifying key players in each organ is another important step to elucidate the organ-specific metastasis. Of particular interest is the role of TAM in cancer stem cells because this type of cells play crucial roles in tumor progression and they are also considered to be responsible for chemo- and radio-therapy resistance. Elucidating the molecular mechanism of interplay between TAM and cancer stem cell may lead to development of new therapeutic strategy for drug-resistant cancer. Another important aspect of cancer progression is a recurrent disease which is the most daunting news to cancer patients. The current cancer stem theory predicts that recurrent tumor arises from cancer stem cells that have been dormant for a long time. How the dormant stem cells are reactivated and grow again after many years is largely unknown; however, tumor microenvironment such as TAM is likely to play crucial roles. Although the research in clarifying the mechanism of dormancy and recurrence has been just

begun, underlining molecular pathways and its relations to TAM are gradually revealing. Targeting TAM for cancer therapy in actual clinical setting is still at its infant stage; however, there are several drugs that partially block the function of TAM and some of them are even already in clinical trials. Further understanding of detailed functional mechanisms and the pathological roles of TAM in tumor microenvironment are expected to facilitate development of novel approaches for cancer therapy.

8. ACKNOWLEDGEMENT

This work was supported by National Institute of Health (R01CA124650 to KW) and Department of Defense (PC101369 to KW and BC096982 to AK).

9. REFERENCES

1. PS Steeg: Metastasis suppressors alter the signal transduction. *Nat Rev Cancer* 3, 55-63 (2003)
2. EY Lin, AV Nguyen, RG Russell, JW Pollard: Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 193, 727-740 (2001)
3. L Bingle, NJ Brown, CE Lewis: The role of tumour-associated macrophages in tumour progression: implications for new anticancer therapies. *J Pathol* 196, 254-265 (2002)
4. F Balkwill, KA Charles, A Mantovani: Smoldering and polarized inflammation in the initiation and promotion of malignant disease. *Cancer Cell* 7, 211-217 (2005)
5. EY Lin, AV Nguyen, RG Russell, JW Pollard: Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 193, 727-740 (2001)
6. JW Pollard: Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 4, 71-78 (2004)
7. SM Stamatovic, RF Keep, SL Kunkel, AV Andjelkovic: Potential role of MCP-1 in endothelial cell tight junction 'opening': signaling via Rho and Rho kinase. *J Cell Sci* 116, 4615-4628 (2003)
8. B Bottazzi, N Polentarutti, R Acero, A Balsari, D Boraschi, P Ghezzi, M Salmona, A Mantovani: Regulation of the macrophage content of neoplasms by chemoattractants. *Science* 220, 210-212 (1983)
9. RP Negus, GW Stamp, MG Relf, F Burke, ST Malik, S Bernasconi, P Allavena, S Sozzani, A Mantovani, FR Balkwill: The detection and localization of monocyte chemoattractant protein-1 (MCP-1) in human ovarian cancer. *J. Clin. Invest* 95, 2391-2396 (1995)
10. SL Hembruff, I Jokar, LYang, N Cheng: Loss of transforming growth factor-beta signaling in mammary fibroblasts enhances CCL2 secretion to promote

mammary tumor progression through macrophage-dependent and -independent mechanisms. *Neoplasia* 12, 425-433 (2010)

11. MP Colombo, A Mantovani: Targeting myelomonocytic cells to revert inflammation-dependent cancer promotion. *Cancer Res* 65, 9113-9116 (2005)
12. F Balkwill: Cancer and the chemokine network. *Nat Rev Cancer* 4, 540-550 (2004)
13. P Allavena, A Sica, G Solinas, C Porta, A Mantovani: The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol* 66, 1-9 (2008)
14. S Aharinejad, P Paulus, M Sioud, M Hofmann, K Zins, R Schäfer, ER Stanley, D Abraham: Colony-stimulating factor-1 blockade by antisense oligonucleotides and small interfering RNAs suppresses growth of human mammary tumor xenografts in mice. *Cancer Res* 64, 5378-5384 (2004)
15. EY Lin, AV Nguyen, RG Russell, JW Pollard: Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 193, 727-740 (2001)
16. A Nowicki, J Szenajch, G Ostrowska, A Wojtowicz, K Wojtowicz, AA Kruszewski, M Maruszynski, SL Aukerman, W Wiktor-Jedrzejczak: Impaired tumor growth in colony-stimulating factor 1 (CSF-1)-deficient, macrophage-deficient op/op mouse: evidence for a role of CSF-1-dependent macrophages in formation of tumor stroma. *Int J Cancer* 65, 112-119 (1996)
17. S Aharinejad, D Abraham, P Paulus, H Abri, M Hofmann, K Grossschmidt, R Schäfer, ER Stanley, R Hofbauer: Colony-stimulating factor-1 antisense treatment suppresses growth of human tumor xenografts in mice. *Cancer Res* 62, 5317-5324 (2002)
18. JJ O'Shea, PJ Murray: Cytokine signaling modules in inflammatory responses. *Immunity* 28, 477-487 (2008)
19. FA Verreck, T de Boer, DM Langenberg, MA Hoeve, M Kramer, E Vaisberg, R Kastelein, A Kolk, R de Waal-Malefyt, TH Ottenhoff: Human IL-23-producing type 1 macrophages promote but IL-10-producing type 2 macrophages subvert immunity to (myco) bacteria. *Proc Natl Acad Sci USA* 101, 4560-4565 (2004)
20. K Hiraoka, M Zenmyo, K Watari, H Iguchi, A Fotovati, YN Kimura, F Hosoi, T Shoda, K Nagata, H Osada, M Ono, M Kuwano: Inhibition of bone and muscle metastases of lung cancer cells by a decrease in the number of monocytes/macrophages. *Cancer Sci* 99, 1595-1602 (2008)
21. SM Zeisberger, B Odermatt, C Marty, AH Zehnder-Fjällman, K Ballmer-Hofer, RA Schwendener: Clodronate-liposome-mediated depletion of tumour-

associated macrophages: a new and highly effective antiangiogenic therapy approach. *Br J Cancer* 95, 272–281 (2006)

22. A Mantovani, S Sozzani, M Locati, P Allavena, A Sica: Macrophage polarization: tumor-associated macrophages as a paradigm for polarized M2 mononuclear phagocytes. *Trends Immunol* 23, 549–555 (2002)

23. S Gordon: Alternative activation of macrophages. *Nat Rev Immunol* 3, 23–35 (2003)

24. RH Himes, PR Burton, RN Kersey, GB Pierson: Brain tubulin polymerization in the absence of "microtubule-associated proteins". *Proc Natl Acad Sci U S A* 73, 4397–4399 (1976)

25. IF Lissbrant, P Stattin, P Wikstrom, JE Damber, L Egevad, A Bergh: Tumor associated macrophages in human prostate cancer: relation to clinicopathological variables and survival. *Int J Oncol* 17, 445–451 (2000)

26. A Mantovani, A Sica, S Sozzani, P Allavena, A Vecchi, M Locati: The chemokine system in diverse forms of macrophage activation and polarization. *Trends Immunol* 25, 677–86. (2004)

27. AA Filardy, DR Pires, MP Nunes, CM Takiya, CG Freire-de-Lima, FL Ribeiro-Gomes, GA DosReis: Proinflammatory clearance of apoptotic neutrophils induces an IL-12(low)IL-10(high) regulatory phenotype in macrophages. *J Immunol* 185, 2044–2050 (2010)

28. A Mantovani, A Sica, M Locati: Macrophage polarization comes of age. *Immunity* 23, 344–346 (2005)

29. P llavena, A Sica, G Solinas, C Porta, A Mantovani,: The inflammatory micro-environment in tumor progression: the role of tumor-associated macrophages. *Crit Rev Oncol Hematol* 66, 1–9 (2008)

30. SK Biswas, L Gangi, S Paul, T Schioppa, A Saccani, M Sironi, B Bottazzi, A Doni, B Vincenzo, F Pasqualini, L Vago, M Nebuloni, A Mantovani, A Sica: A distinct and unique transcriptional program expressed by tumor-associated macrophages (defective NF-kappaB and enhanced IRF-3/STAT1 activation). *Blood* 107, 2112–2122 (2006)

31. M Torroella-Kouri, R Silvera, D Rodriguez, R Caso, A Shatry, S Opiela, D Ilkovitch, RA Schwendener, V Iragavarapu-Charyulu, Y Cardentey, N Strbo, DM Lopez: Identification of a subpopulation of macrophages in mammary tumor-bearing mice that are neither M1 nor M2 and are less differentiated. *Cancer Res* 69, 4800–4809 (2009)

32. T Hagemann, J Wilson, F Burke, H Kulbe, NF Li, A Plüddemann, K Charles, S Gordon, FR Balkwill,: Ovarian cancer cells polarize macrophages toward a tumor-associated phenotype. *J Immunol* 176, 5023–5032 (2006)

33. DG DeNardo, JB Barreto, P Andreu, L Vasquez, D Tawfik, N Kolhatkar, LM Coussens: CD4(+) T cells regulate pulmonary metastasis of mammary carcinomas by enhancing protumor properties of macrophages. *Cancer Cell* 16, 91–102 (2009)

34. MR Dinapoli, CL Calderon, DM Lopez: The altered tumoricidal capacity of macrophages isolated from tumor-bearing mice is related to reduce expression of the inducible nitric oxide synthase gene. *J Exp Med* 183, 1323–1329 (1996)

35. AH Klimp, H Hollema, C Kempinga, AG van der Zee, EG de Vries, T Daemen: Expression of cyclooxygenase-2 and inducible nitric oxide synthase in human ovarian tumors and tumor-associated macrophages. *Cancer Res* 61, 7305–7359 (2001)

36. A Sica, A Saccani, B Bottazzi, N Polentarutti, A Vecchi, J van Damme, A Mantovani: Autocrine production of IL-10 mediates defective IL-12 production and NF-kappa B activation in tumor-associated macrophages. *J Immunol* 164, 762–767 (2000)

37. A Saccani, T Schioppa, C Porta, SK Biswas, M Nebuloni, L Vago, B Bottazzi, MP Colombo, A Mantovani, A Sica: p50 nuclear factor-kappaB overexpression in tumor-associated macrophages inhibits M1 inflammatory responses and antitumor resistance. *Cancer Res* 66, 11432–11440 (2006)

38. DG DeNardo, M Johansson, LM Coussens: Immune cells as mediators of solid tumor metastasis. *Cancer Metastasis Rev* 27, 11–18 (2008)

39. A Mantovani, P Allavena, A Sica, F Balkwill: Cancer-related inflammation. *Nature* 454, 436–444 (2008)

40. E Giraudo, M Inoue, D Hanahan: An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. *J Clin Invest* 114, 623–633 (2004)

41. T Krecicki, M Zalesska-Krecicka, M Jelen, T Szkudlarek, M Horobiowska: Expression of type IV collagen and matrix metalloproteinase-2 (type IV collagenase) in relation to nodal status in laryngeal cancer. *Clin Otolaryngol* 26, 469–472 (2001)

42. C Lewis, C Murdoch: Macrophage responses to hypoxia: implications for tumor progression and anti-cancer therapies. *Am J Pathol* 167, 627–635 (2005)

43. KL Talks, H Turley, KC Gatter, PH Maxwell, CW Pugh, PJ Ratcliffe, AL Harris: The expression and distribution of the hypoxia-inducible factors HIF-1alpha and HIF-2alpha in normal human tissues, cancers, and tumor-associated macrophages. *Am J Pathol* 157: 411–421 (2000)

44. C Murdoch, A Giannoudis, CE Lewis: Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* 104, 2224–2234 (2004)

45. T Cramer, Y Yamanishi, BE Clausen, I Förster, R Pawlinski, N Mackman, VH Haase, R Jaenisch, M Corr, V Nizet, GS Firestein, HP Gerber, N Ferrara, RS Johnson: HIF-1 α is essential for myeloid cell-mediated inflammation. *Cell* 112, 645–657 (2003)
46. RE Bachelder, MA Wendt, AM Mercurio: Vascular endothelial growth factor promotes breast carcinoma invasion in an autocrine manner by regulating the chemokine receptor CXCR4. *Cancer Res* 62, 7203–7206 (2002)
47. T Hanada, M Nakagawa, A Emoto, T Nomura, N Nasu, Y Nomura: Prognostic value of tumor-associated macrophage count in human bladder cancer. *Int J Urol* 7, 263–269 (2000)
48. SF Schoppmann, P Birner, J Stöckl, R Kalt, R Ullrich, C Caucig, E Kriehuber, K Nagy, K Alitalo, D Kerjaschki: Tumor-associated macrophages express lymphatic endothelial growth factors and are related to peritumoral lymphangiogenesis. *Am J Pathol* 161, 947–956 (2002)
49. RC Ji: Lymphatic endothelial cells, tumor lymphangiogenesis and metastasis: New insights into intratumoral and peritumoral lymphatics. *Cancer Metastasis Rev* 25, 677–694 (2006)
50. G Dranoff, E Jaffee, A Lazenby, P Golumbek, H Levitsky, K Brose, V Jackson, H Hamada, D Pardoll, RC Mulligan: Vaccination with irradiated tumor cells engineered to secrete murine granulocyte-macrophage colony-stimulating factor stimulates potent, specific, and long-lasting anti-tumor immunity. *Proc Natl Acad Sci U S A* 90, 3539–3543 (1993)
51. HB Salvesen, LA Akslen: Significance of tumour-associated macrophages, vascular endothelial growth factor and thrombospondin-1 expression for tumour angiogenesis and prognosis in endometrial carcinomas. *Int J Cancer* 84, 538–543 (1999)
52. G Trinchieri, F Gerosa: Immunoregulation by interleukin-12. *J Leukoc Biol* 59, 505–511 (1996)
53. T Tsao, S Komblau, S Safe, J C Watt, V Ruvo, W Chen, Y Qiu, K R Coombes, Z Ju, M Abdelrahim, W Schober, X Ling, D Kardassis, C Meyer, A Schimmer, H Kantarjian, M Andreeff, M Konopleva: Role of peroxisome proliferator-activated receptor- γ and its coactivator DRIP205 in cellular responses to CDDO (RTA-401) in acute myelogenous leukemia. *Cancer Res* 70, 4949–4960 (2010)
54. I Kryczek, L Zou, P Rodriguez, G Zhu, S Wei, P Mottram, M Brumlik, P Cheng, T Curiel, L Myers, A Lackner, X Alvarez, A Ochoa, L Chen, W Zou: B7-H4 expression identifies a novel suppressive macrophage population in human ovarian carcinoma. *J Exp Med* 203, 871–881 (2006)
55. E Schutyser, S Struyf, P Proost, G Opdenakker, G Laureys, B Verhasselt, L Peperstraete, I Van de Putte, A Saccani, P Allavena, A Mantovani, J Van Damme: Identification of biologically active chemokine isoforms from ascitic fluid and elevated levels of CCL18/pulmonary and activation-regulated chemokine in ovarian carcinoma. *J Biol Chem* 277, 24584–24593 (2002)
56. Z Sun, Z Yao, S Liu, H Tang, X Yan: An oligonucleotide decoy for Stat3 activates the immune response of macrophages to breast cancer. *Immunobiology* 211, 199–209 (2006)
57. A Toscani, RV Mettus, R Coupland, H Simpkins, J Litvin, J Orth, KS Hatton, and EP: Arrest of spermatogenesis and defective breast development in mice lacking A-myb. *Nature* 387, 713–717 (1997)
58. E Schutyser, S Struyf, P Proost, G Opdenakker, G Laureys, B Verhasselt, L Peperstraete, I Van de Putte, A Saccani, P Allavena, A Mantovani, J Van Damme: Identification of biologically active chemokine isoforms from ascitic fluid and elevated levels of CCL18/pulmonary and activation-regulated chemokine in ovarian carcinoma. *J Biol Chem* 277, 24584–24593 (2002)
59. M J Rauh, LM Sly, J Kalesnikoff, MR Hughes, LP Cao, V Lam, G Krystal: The role of SHIP1 in macrophage programming and activation. *Biochem Soc Trans* 32, 785–788 (2004)
60. SC Robinson, KA Scott, JL Wilson, RG Thompson, AE Proudfoot, FR Balkwill: A chemokine receptor antagonist inhibits experimental breast tumor growth. *Cancer Res* 63, 8360–8365 (2003)
61. R Bonecchi, G Bianchi, PP Bordignon, D D'Ambrosio, R Lang, A Borsatti, S Sozzani, P Allavena, PA Gray, A Mantovani, F Sinigaglia: Differential expression of chemokine receptors and chemotactic responsiveness of type 1 T helper cells (Th1s) and Th2s. *J Exp Med* 187, 129–134 (1998)
62. T Hagemann, SC Robinson, M Schulz, L Trümper, FR Balkwill, C Binder: Enhanced invasiveness of breast cancer cell lines upon co-cultivation with macrophages is due to TNF- α dependent up-regulation of matrix metalloproteases. *Carcinogenesis* 25, 1543–1549 (2004)
63. B Mytar, M Wołoszyn, R Szatanek, M Baj-Krzyworzeka, M Siedlar, I Ruggiero, J Wieckiewicz, M Zembala : Tumor cell-induced deactivation of human monocytes. *J Leukoc Biol* 74, 1094–1101 (2003)
64. S Hiratsuka, K Nakamura, S Iwai, M Murakami, T Itoh, H Kijima, JM Shipley, RM Senior, M Shibuya: MMP9 induction by vascular endothelial growth factor receptor-1 is involved in lung-specific metastasis. *Cancer Cell* 2, 289–300 (2002)
65. T Hagemann, J Wilson, H Kulbe, NF Li, DA Leinster, K Charles, F Klemm, T Pukrop, C Binder, FR Balkwill: Macrophages induce invasiveness of epithelial cancer cells via NF- κ B and JNK. *J Immunol* 175, 1197–1205 (2005)

66. T Pukrop, F Klemm, T Hagemann, D Gradl, M Schulz, S Siemes, L Trümper, C Binder: Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proc Natl Acad Sci USA* 103, 5454–5459 (2006)
67. JW Tjiu, JS Chen, CT Shun, SJ Lin, YH Liao, CY Chu, TF Tsai, HC Chiu, YS Dai, H noue, P Yang, ML Kuo, SH Jee: Tumor-associated macrophage-induced invasion and angiogenesis of human basal cell carcinoma cells by cyclooxygenase-2 induction. *J Invest Dermatol* 129, 1016–1025 (2009)
68. R Giavazzi, A Garofalo, MR Bani, M Abbate, P Ghezzi, D Boraschi, A Mantovani, E Dejana: Interleukin 1-induced augmentation of experimental metastases from a human melanoma in nude mice. *Cancer Res* 50, 4771–4775 (1990)
69. S Goswami, E Sahai, JB Wyckoff, M Cammer, D Cox, FJ Pixley, ER Stanley, JE Segall, JS Condeelis: Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. *Cancer Res* 65, 5278–5283 (2005)
70. J Wyckoff, W Wang, EY Lin, Y Wang, F Pixley, ER Stanley, T Graf, JW Pollard, J Segall, J Condeelis: A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* 64, 7022–7029 (2004)
71. J Fujimoto, H Sakaguchi, I Aoki, T Tamaya: Clinical implications of expression of interleukin 8 related to angiogenesis in uterine cervical cancers. *Cancer Res* 60, 2632–2635 (2000)
72. S Tsutsui, K Yasuda, K Suzuki, K Tahara, H Higashi, S Era: Macrophage infiltration and its prognostic implications in breast cancer: the relationship with VEGF expression and microvessel density. *Oncol Rep* 14, 425–431 (2005)
73. LE Ailles, IL Weissman: Cancer stem cells in solid tumors. *Curr Opin Biotechnol* 18, 460–466 (2007)
74. SA Mani, W Guo, MJ Liao, EN Eaton, A Ayyanan, AY Zhou, M Brooks, F Reinhard, CC Zhang, M Shipitsin, LL Campbell, K Polyak, C Briskin, J Yang, RA Weinberg: The epithelial-mesenchymal transition generates cells with properties of stem cells. *Cell* 133, 704–715 (2008)
75. CY Lin, CJ Lin, KH Chen, JC Wu, SH Huang, SM Wang: Macrophage activation increases the invasive properties of hepatoma cells by destabilization of the adherens junction. *FEBS Lett* 580, 3042–3050 (2006)
76. JY Shih, A Yuan, JJ Chen, PC Yan: Tumor-Associated Macrophage: Its Role in Cancer Invasion and Metastasis. *J Cancer* 2, 101–106 (2006)
77. M Katoh, M Katoh: WNT Signaling Pathway and Stem Cell Signaling Network. *Clin Cancer Res* 13, 4042–4045 (2007)
78. N Barker, H Clevers: Leucine-rich repeat-containing G-protein-coupled receptors as markers of adult stem cells. *Gastroenterology* 138, 1681–1696 (2010)
79. T Pukrop, F Klemm, T Hagemann, D Gradl, M Schulz, S Siemes, L Trümper, C Binder: Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proc Natl Acad Sci USA* 103, 5454–5459 (2006)
80. LS Ojalvo, CA Whittaker, JS Condeelis, JW Pollard, : Gene expression analysis of macrophages that facilitate tumor invasion supports a role for Wnt-signaling in mediating their activity in primary mammary tumors. *J Immunol* 184, 702–712 (2010)
81. N Takebe, PJ Harris, RQ Warren, SP Ivy: Targeting cancer stem cells by inhibiting Wnt, Notch, and Hedgehog pathways. *Nat Rev Clin Oncol* 8, 97–106 (2011)
82. Y Fujiwara, Y Komohara, T Ikeda, M Takeya: Corosolic acid inhibits glioblastoma cell proliferation by suppressing the activation of signal transducer and activator of transcription-3 and nuclear factor-kappa B in tumor cells and tumor-associated macrophages. *Cancer Sci* 102, 206–211 (2011)
83. I Takanami, K Takeuchi, S Kodaira: Tumor-associated macrophage infiltration in pulmonary adenocarcinoma: association with angiogenesis and poor prognosis. *Oncology* 57, 138–142 (1999)
84. KM Kerr, SK Johnson, G King, MM Kennedy, J Weir, R Jeffrey: Partial regression in primary carcinoma of the lung: does it occur? *Histopathology* 33, 55–63 (1998)
85. C Sessa, F De Braud, A Perotti, J Bauer, G Curigliano, C Noberasco, F Zanaboni, L Gianni, S Marsoni, J Jimeno, M D'Incalci, E Dall'ò, N Colombo: Trabectedin for women with ovarian carcinoma after treatment with platinum and taxanes fails. *J Clin Oncol* 23, 1867–1874 (2005)
86. P Allavena, M Signorelli, M Chieppa, E Erba, G Bianchi, F Marchesi, CO Olimpico, C Bonardi, A Garbi, A Lissoni, F de Braud, J Jimeno, M D'Incalci: Anti-inflammatory properties of the novel antitumor agent yondelis (trabectedin): inhibition of macrophage differentiation and cytokine production. *Cancer Res* 65, 2964–2971 (2005)
87. S Rozel, CJ Galbán, K Nicolay, KC Lee, S Sud, C Neeley, LA Snyder, TL Chenevert, A Rehemtulla, BD Ross, KJ Pienta: Synergy between anti-CCL2 and docetaxel as determined by DW-MRI in a metastatic bone cancer model. *J Cell Biochem* 107, 58–64 (2009)
88. B Whitehurst, MJ Flister, J Bagaitkar, L Volk, CM Bivens, B Pickett, E Castro-Rivera, RA Brekken, RD Gerard, S Ran: Anti-VEGF-A therapy reduces lymphatic vessel density and expression of VEGFR-3 in an orthotopic breast tumor mode. *Int J Cancer* 121, 2181–2191 (2007)
89. PC Calder: Polyunsaturated fatty acids and inflammation. *Prostaglandins Leukot Essent Fatty Acids* 75, 197–202 (2006)

90. LM Wahl, HK Kleinman, : Tumor-associated macrophages as targets for cancer therapy. *J Natl Cancer Inst* 90, 1583–1584 (1998)
91. E Giraudo, M Inoue, D Hanahan: An amino-bisphosphonate targets MMP-9-expressing macrophages and angiogenesis to impair cervical carcinogenesis. *J Clin Invest* 114, 623–633 (2004)
92. A Sica, V Bronte: Altered macrophage differentiation and immune dysfunction in tumor development. *Clin Invest* 117, 1155–1166 (2007)
93. A Mantovani, A Sica: Macrophages, innate immunity and cancer: balance, tolerance, and diversity. *Curr Opin Immunol* 22, 231-237 (2010)
94. A Mantovani, P Allavena, A Sica, F Balkwill: Cancer-related inflammation. *Nature* 24, 436-444 (2008)
95. TE Novak, TA Babcock, DH Jho, WS Helton, NJ Espat: NF-kappa B inhibition by omega -3 fatty acids modulates LPS-stimulated macrophage TNF-alpha transcription. *Am J Physiol Lung Cell Mol Physiol* 284, 84-89 (2003)
96. C Guiducci, AP Vicari, S Sangaletti, G Trinchieri, MP Colombo: Redirecting *in vivo* elicited tumor infiltrating macrophages and dendritic cells towards tumor rejection. *Cancer Res* 65, 3437–3446 (2005)
97. SC Robinson, KA Scott, JL Wilson, RG Thompson, AE Proudfoot, FR Balkwill: A chemokine receptor antagonist inhibits experimental breast tumor growth. *Cancer Res* 63, 8360–8365 (2003)
98. B Du, ZY Yang, XY Zhong, M Fang, YR Yan, GL Qi, YL Pan: Metastasis-associated protein1 induces VEGF-C and facilitates lymphangiogenesis in colorectal cancer. *World J Gastroenterol* 17, 1219-1226 (2011)

Key Words: Tumor associated macrophage, Cytokines, Inflammation, Cancer, Monocytes, Cancer stem cell, Review

Send correspondence to: Kounosuke Watabe, Department of Medical Microbiology/Immunology and Cell Biology, School of Medicine, Southern Illinois University, 825 N. Rutledge Springfield IL 62702, Tel: 217-545-3969, Fax: 217-545-3227, E-mail: kwatabe@siumed.edu

<http://www.bioscience.org/current/vol4S.htm>