Review Article

Tumor-Associated Macrophage: Its Role in Cancer Invasion and Metastasis

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Cancer metastasis is not exclusively regulated by the deregulation of metastasis-promoting or suppressing genes in cancer cells. The interaction between cancer cells and the stromal cells has been shown recently to promote cancer metastasis. The macrophages within the tumor, referring to as tumour-associated macrophages (TAMs), are the pivotal member of stromal cells. TAMs are derived from peripheral blood monocytes recruited into the tumor. Upon activated by cancer cells, the TAMs can release a vast diversity of growth factors, proteolytic enzymes, cytokines, and inflammatory mediators. Many of these factors are key agents in cancer metastasis. The presence of extensive TAM infiltration has been shown to correlate with cancer metastasis and poor prognosis in a variety of human carcinomas. TAMs promote cancer metastasis through several mechanisms including tumor angiogenesis, tumor growth, and tumor cell migration and invasion. There are complex paracrine-signaling networks between TAMs and cancer cells to activate each other. The colony-stimulating factor 1/epidermal growth factor paracrine loop is well known in regulation of breast cancer cells invasion. TAMs-derived proteases, such as matrix metalloproteinases, urokinase-type plasminogen activator, and cathepsin B can promote cancer cells metastasis. The roles of TAMs in epidermal-mesenchymal transition of cancer cells and resistance to cancer treatment are novel fields of study. On the other hand, some investigations showed that the TAMs may play an important role in anti-tumor activity. The control of TAMs to be pro-metastatic or tumoricidal is an important subject for cancer therapy.

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Introduction

Cancer progression is a complex multi-step process that consists of transformation, tumor growth, invasion and metastasis. Tumor invasion and metastasis are the critical steps in determining the aggressive phenotype of human cancers, the obstacles to the successful treatment and major causes of cancer deaths [1]. The spread of tumor cells from a primary tumor to secondary sites within the body is a complicated process involving the degradation of basement membrane, invasion of stroma, adhesion, angiogenesis, cell proliferation, migration, and anti-apoptosis [2]. Numerous genetic changes and a variety of positive and negative factors may be involved in the molecular basis of metastasis [3]. During cancer progression, several rounds of mutation and selection [4] result in highly invasive ability of some cancer cells. In particular, increased expression of metastasis-promoting genes or decreased expression of metastasis-suppressor genes can provide cancer cells with a selective invasive advantage and lead to the clonal outgrowth of a tumor [5]. Many studies focused on identifying the genes controlling metastasis. Studies of differential gene expression between poorly metastatic cancer cells and highly metastatic cancer cells have really identified genes associated with metastasis [6-10]. However, cancer metastasis is not exclusively regulated by the deregulation of intrinsic genes in cancer cells, but also depends on the stromal compartment to create a more tumor promoting microenvironment.

Solid tumors comprise not only malignant cells, but also extracellular matrix (ECM[2]) and many other non-malignant cell types, including fibroblast, endothelial cells and inflammatory cells such as macrophages, neutrophils, mast cells and lymphocytes. The presence of inflammatory cells in tumors was first described in 1863 and this has led to the concept that inflammatory microenvironment plays a key role to promote tumor development and progression [11,12]. The macrophage is the pivotal member of inflammatory cells

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[2]**Abbreviations:** ECM, extracellular matrix; TAMs, tumor-associated macrophages; NSCLC, non-small cell lung cancer; CSF-1, colony-stimulating factor 1; VEGF, vascular endothelial growth factor; MIF, macrophage migration inhibition factor; PyMT, polyoma virus middle T oncoprotein; TNF-α, tumor-necrosis factor-α; MMPs, matrix metalloproteinases; EGF, epidermal growth factor; EMT, epithelial–mesenchymal transition.

within the tumor stroma. It has now been well understood that the majority of malignant tumors contain numerous macrophages as a major component of the host leukocytic infiltrate. These macrophages are referred to as tumor-associated macrophages (TAMs) and most are derived from peripheral blood monocytes recruited into the tumor mass. In the past decade, TAMs have been extensively studied and proposed as a major contributor to tumor progression [13-16]. Upon activation, the TAMs can release a vast diversity of growth factors, cytokines, inflammatory mediators, and proteolytic enzymes. Many of these factors are key agents in tumor progression. There are several comprehensive reviews about the role of TAMs in cancer progression [17-23]. In this review, we will focus on the role of TAMs in promoting cancer invasion and metastasis.

TAMs: prognostic factor of human cancer

Clinical studies have shown a correlation between the numbers of TAMs and poor prognosis for breast, prostate, ovarian, cervical, endometrial, esophageal, and bladder cancers [24-28]. TAMs are also associated with increased angiogenesis or lymph node metastasis in cancer tissues. These observations accord with the results of animal studies using macrophage-depleted mice to investigate the role of macrophages in tumor progression [16].

The data for lung cancer, gastric cancer and glioma are controversial [14]. We found that TAM density correlated positively with tumoral IL-8 expression and intratumoral microvessel density in non-small cell lung cancer (NSCLC) and TAM level was also associated with short patients' relapse-free survival [29]. But, Toomey *et al.* found no association between macrophage count and outcome in NSCLC [30]. Furthermore, Funada *et al.* reported that peritumoral infiltration of macrophages in colorectal cancer was associated with less lymph node metastasis and good prognosis [31]. These conflicting results may reflect the different methods of assessment used, and in some cases differences in the number, grade, and stage of tumors and the small sample size included in some studies. However, there is a fundamental question about these conflicting clinical observations, i.e. are all TAMs the same? The cytokine profiles of microenvironment and localization of TAMs reside may influence the function of TAMs and thereafter the prognostic value of TAMs. Ohno *et al.* particularly paid attention to counting macrophages within gastric carcinoma stroma and islets, and found that tumor islet–infiltrating macrophages (indicated TAMs which invaded into tumor nest) were associated with better survival [32]. Welsh *et al.* recently evaluated the relationship of tumor islet macrophage and patients' survival in NSCLC, and showed that tumor islet macrophage density and tumor islet/stromal macrophage ratio emerged as favorable independent prognostic indicators in patients with NSCLC. In contrast, increasing stromal macrophage density was an independent predictor for reduced survival [33]. The findings indicate that the exact microanatomic localization of these inflammatory cells is critical in determining the relationship to prognosis. There was some experimental evidence to support this point. Macrophages were attractive by colony-stimulating factor 1 (CSF-1) expressed by cancer cells. Graft *et al.* reported that most of the mice implanted with glioma cells expressing cell surface-bound CSF-1 survived; in contrast, all mice that were implanted with glioma cells expressing soluble form of CSF-1 died [34].

TAMs recruited to tumor and educated by microenvironment

Macrophages — TAMs — are recruited to tumors by growth factors and chemokines, which are often produced by the cancer cells and stroma cells in the tumor. Macrophages are an important component of the innate immune system and are derived from myeloid progenitor cells called the colony-forming unit granulocyte-macrophage in the bone marrow. These progenitor cells develop into promonocytes and then differentiate into monocytes. Monocytes then migrate into almost all tissues of the body, where they differentiate into tissue macrophages. Examples of tissue macrophage include Kupfer cells in the liver and alveolar macrophage in the lung. It is thought that TAMs are almost derived from peripheral blood monocytes recruited into the tumor rather than derived from local tissue macrophages.

A number of monocyte chemoattractants derived from tumors have been shown to correlate with increased TAM numbers in many human tumors [35]. Such monocyte chemoattractants include CSF-1, the CC chemokines, CCL2 (formally monocyte chemoattractant protein-1), CCL3, CCL4, CCL5 and CCL8, vascular endothelial growth factor (VEGF), macrophage inflammatory protein-1 alpha (MIP-1α) and macrophage migration inhibition factor (MIF).

Lin *et al.* [16] used transgenic mouse model to study the effect of depleting macrophage in a breast cancer. In this model, mammary tumors are initiated by the mammary epithelial restricted expression of the polyoma virus middle T oncoprotein (PyMT) and the mice are homozygous for a null mutation in the CSF-1. Depletion of CSF-1 markedly decreased the infiltration of macrophages at the tumor site and macrophage depletion resulted in slower progression of preinvasive lesions to malignant lesions and reduced formation of lung metastases. Similarly, using small interfering RNA to inhibit CSF-1 expression in MCF-7 xenografts showed that lower numbers of TAMs were accompanied by a marked reduction in tumor growth and increase of mice survival [36].

Monocytes were recruited into tumor, differentiated into TAMs, and accumulated in hypoxic area of tumor. TAMs respond to hypoxia through up-regulation of transcriptional factors such as hypoxia-inducing factors 1 and 2 which activate many mitogenic, angiogenic, and proinvasive genes.

Interaction of TAMs and cancer cells enhances invasiveness of cancer cells

TAMs promote cancer metastasis through several mechanisms, including (1) promotion of angiogenesis, (2) induction of tumor growth, and (3) enhancement of tumor cell migration and invasion. The mechanisms of TAMs-promoting angiogenesis and tumor growth have been well reviewed by several articles [20, 23,35,37]. It was summarized in Figure 1. Several studies have demonstrated the association between increased tumor vascularity and macrophage infiltration in several human cancers [38-40], suggesting that TAMs enhance the angiogenic potential of tumors. Macrophage infiltration has been shown to correlate with vessel density in endometrial, ovarian, breast and central nervous system malignancies. The potential angiogenesis factors secreted by TAMs have been shown to include chemokines (IL-8, MIF, etc.), VEGF, tumor-necrosis factor-α (TNF-α), and thymidine phosphorylase [41-43]. TAMs also produce a wide variety of growth factors that can stimulate cancer growth. In this review, we focus on the mechanisms of TAMs to enhance cell migration and invasion.

TAMs-derived proteases

In PyMT-induced mammary tumors, macrophages are present in areas of invasion and basement membrane breakdown during the development of early-stage cancer [16]. Up-

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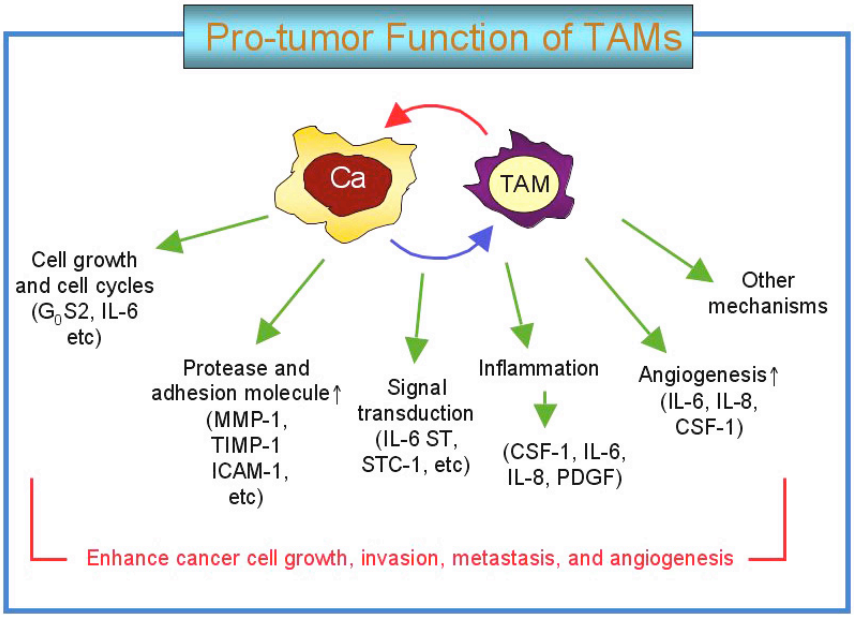

Figure 1: Potential pro-tumor effects of TAMs on cancer cells. The interaction between TAMs and cancer cells may enhance cancer cell growth, invasion, metastasis and angiogenesis by stimulating cancer cells or TAMs to express multiple gene products that are involved in the regulation of tumor-associated angiogenesis, cell cycle, inflammation, signal transduction, invasion, and activities of protease and adhesion molecules. G_0S2, G_0/G_1 switch gene 2; TIMP-1, matrix metalloproteinase tissue inhibitor-1; ICAM-1, intercellular adhesion molecule-1; IL-6 ST, interleukin-6 signal transducer; STC-1, stanniocalcin-1; PDGF, platelet-derived growth factor [55].

regulation of proteolytic enzymes in macrophages present in these locations indicates that TAMs could be involved in the invasion of tumor cells into surrounding normal tissue. It has been generally assumed that tumor cell-derived matrix metalloproteinases (MMPs) are important to allow cancer cells to penetrate the basement membrane and invade the ECM, and metastasize. MMPs are a family of matrix-degrading enzymes including collagenase (MMP-1), gelatinase A (MMP-2), stromelysin (MMP-3), matrilysin (MMP-7), gelatinase B (MMP-9), and other MMPs. The MMP expression has been implicated in tumor progression through enhancing angiogenesis, tumor invasion and metastasis [44,45]. TAMs have been reported to correlate with the metastatic potential of a variety of human cancers, and they have also been shown to be a major source of MMP-9. In addition, urokinase-type plasminogen activator is a serine protease synthesized by TAMs in various human tumor types [46]. The levels of urokinase-type plasminogen activator have been shown to correlate with reduced relapse-free and overall survival in cancer [47]. TAMs can also secrete cysteine-type lysosomal proteases. Traditionally, lysosomal cysteine proteases are considered to execute nonspecific bulk proteolysis within the lysosomes. However, there is growing evidence that lysosomal proteases are secreted extracellularly in cancer. Vasiljeva *et al.* demonstrated that macrophages increased cathepsin B (one of cysteine-type lysosomal protease) expression on being recruited to the tumor and thus promoted tumor growth and lung metastasis of PyMT-induced breast cancer [48].

Paracrine signaling networks between TAMs and cancer cells

Hagemann *et al.* [49] have reported that coculture of macrophage with breast cancer cells stimulated the macrophage and up-regulated production of TNF-α and MMP-2, -3, -7, and -9 in the macrophages; subsequently enhanced cancer cells invasion. It was shown that coculture of macrophages with breast cancer cells led to TNF-α-dependent activation of c-Jun-NH2-kinase and nuclear factor-κB signaling pathways in cancer cells [50]. Downstream targets in tumor cells included MIF and extracellular MMP inducer (EMMPRIN). These proteins then act in turn to enhance the local release of MMPs by macrophages [50,51]. Pukrop *et al.* further reported that Wnt 5a is up-regulated in macrophages upon coculture with breast cancer cells and Wnt 5a is necessary for production of MMP-7 and TNF-α by macrophages [52]. Wnt 5a is essential for macrophage-induced invasiveness, because it regulates tumor cell migration as well as proteolytic activity of the macrophages.

In other coculture experiments, macrophages and cancer cells co-migrated and invaded into a collagen matrix. Invasion of cancer cells was increased by macrophages that synthesized epidermal growth factor (EGF). Cancer cells expressed the EGF receptor and secreted CSF-1, which attracted macrophages and promoted the expression of EGF by macrophages [53,54]. In addition, EGF promoted the expression of CSF-1 by cancer cells, thereby generating a positive feedback loop. The CSF-1/EGF paracrine loop is required for breast cancer cells invasion [53,54].

Anti-tumor function of TAMs

On the other hand, some investigations showed that the TAMs might play an important role in anti-tumor activity in human malignancy [14]. Although most studies have shown that the TAMs have the pro-tumorigenesis activity, may promote the invasion activity, and are negatively associated with patients' survival in a variety of human cancers, there are some investigations showing that TAMs was associated with good patients' prognosis as mentioned above. The possible anti-tumor function of TAMs may be through enhancing MIF nuclear translocation, GM-CSF, and IL-12 expression [14,17,35] and is summaries in Figure 2. Differentiation of TAMs to cytotoxic macrophge subpopulation (i.e. macrophages in tumor islet) may also contribute to the anti-tumor function of TAMs.

Future potentials of TAMs

Our understanding of the cellular and molecular events in the interaction of cancer cells and stromal cells is improving significantly. Although many studies have shown TAMs have a variety of functions, further study is needed to clarify the interaction network of cancer cells and TAMs.

Genome-wide screening of genes regulated by interaction of cancer cells and macrophages

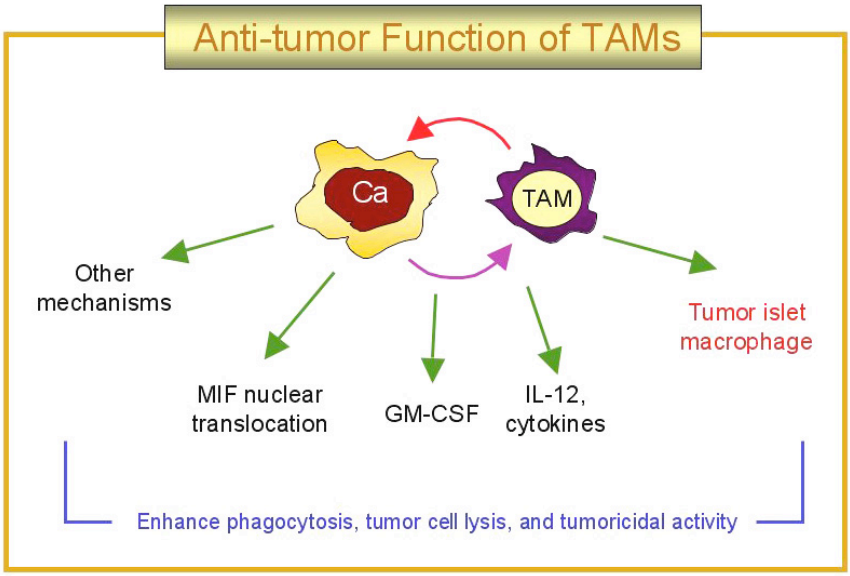

Figure 2: Potential anti-tumor effects of TAMs on cancer cells. Several investigations have demonstrated that TAMs may play an important role in inducing tumor cell lysis. The interaction between TAMs and cancer cells may enhance the tumor cell phagocytosis, tumor cell lysis and tumoricidal activity of TAMs by inducing expression or translocation of GM-CSF, MIF and other cytokines, or other unknown mechanisms. The macrophages distributed in tumor islet may stand for cytotoxic macrophage subpopulation of TAMs.

To explore the other possible mechanisms by which TAMs increase tumor progression in NSCLC, we use cDNA microarray to investigate the tumor invasiveness and gene expression profiles of several NSCLC cancer cell lines after coculture with macrophages [55]. We identified about 50 genes that were up-regulated more than 2-fold in cancer cells after interaction with macrophages. These up-regulated genes included genes involved in angiogenesis and lymphangiogenesis, cytokine and inflammation, adhesion and protease, signal transduction, cell growth and cell cycle regulation, metabolism and unknown functions. The examples of these up-regulated genes were IL-6, IL-7R, IL-8, NF-κB, ICAM-1, MMP-1, MMP-9, VEGF-A, VEGF-C, etc.

TAMs may promote epithelial-mesenchymal transition of cancer cells

Epithelial–mesenchymal transition (EMT) is a process that allows epithelial cells to separate from their neighbors and migrate to distal regions during embryonic development [56,57]. The EMT confers migratory and invasive properties to epithelial cells and has also been suggested to play a fundamental role during invasion and metastasis of carcinoma cells. Loss of E-cadherin, a major phenomenon of EMT, decreases adhesiveness and releases cancer cells from the primary locus into distant sites. Recently, Lin *et al.* reported that the conditioned medium of activated macrophage altered the morphology of HepG2 cells. These cells showed loss of epithelial morphology, became dissociated from the epithelial clusters, and acquired a mesenchymal phenotype [58]. Furthermore, both the Src family kinase inhibitor and the EGFR inhibitor abrogated the activated macrophage conditioned medium-induced down-regulation of E-cadherin and β-catenin at the adherens junction, providing a mechanistic explanation for the TAMs-induced cancer cell EMT and invasiveness. This result is in line with our observation that upon coculture of macrophage and lung adenocarcinoma cell line, the expression of E-cadherin decreased and the expression of Slug increased in cancer cells (Yuan *et al.*, unpublished data).

TAMs may be the cause of chemoresistance and anti-hormone resistance of cancer cells

Recently, Paulus *et al.* provided evidence that TAMs may be associated with chemoresistance of cancer [59]. Mice bearing human chemoresistant MCF-7 breast cancer xenograft were treated with chemotherapy and recombinant anti-CSF-1 antigen-binding fragment (Fab). Anti-CSF-1 Fab reversed chemoresistance of MCF-7 xenograft and down-regulated expression of chemoresistance genes like breast cancer-related protein, multiple resistance gene and glucosylceramide synthase, and prolonged mouse survival. Furthermore, Zhu *et al.* disclosed that the interaction between macrophage and prostate cancer cells mediated anti-androgen resistance [60] and provided another possible function of TAMs in regulation of cancer cells.

Closing remarks

Cancer metastasis is a complex process. In addition to the cancer cell intrinsic factors, the cancer microenvironments, including many tumor-associated stoma cells and the ECM, influence the behavior of cancer cells. Substantial evidence suggests that TAMs can interact with cancer cells, modify the ECM, and promote cancer cell invasion and metastasis. McDaniel *et al.* demonstrated that the ECM from involuting mammary glands could enhance invasiveness and metastasis of breast cancer cells [61]. Multiple cellular components of tumor interact to each other within the ECM, whereas growth factors, cytokines and chemokines provide the information required for the formation of complex tumor tissues. Therefore, we should treat the tumor as a whole rather than just try to eliminate the cancer cells. Further investigations are needed to elucidate the mechanism for regulation of ECM and stromal cell function in human malignancies, and this may help for the design of novel adjunctive cancer therapy in the future.

References

1. Steeg, PS. Metastasis suppressors alter the signal transduction of cancer cells. *Nat Rev Cancer* 3: 55-63, 2003.
2. Yoshida BA, Sokoloff MM, Welch DR, Rinker-Schaefer CW. Metastasis-suppressor genes: a review and perspective. *J Natl Cancer Inst* 92: 1717–1730, 2000.
3. Woodhouse EC, Chuaqui RF, Liotta LA. General mechanisms of metastasis. *Cancer* 80: 1529–1537, 1997.
4. Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. *Nat Med* 10: 789-799, 2004.
5. Cahill DP, Kinzler KW, Vogelstein B, Lengauer C. Genetic instability and darwinian selection in tumours. *Trends Cell Biol* 9: M57-60, 1999.
6. Shih JY, Yang SC, Hong TM, Yuan A, Chen JJ, Yu CJ, Chang YL, Lee YC, Peck K, Wu CW, Yang PC. Collapsin response mediator

Print ISSN 1816-0735; Online ISSN 1817-4256

protein-1 and the invasion and metastasis of cancer cells. *J Natl Cancer Inst* 93: 1392-1400, 2001.
7. Chang CC, Shih JY, Jeng YM, Su JL, Lin BZ, Chen ST, Chau YP, Yang PC, Kuo ML. Connective tissue growth factor and its role in lung adenocarcinoma invasion and metastasis. *J Natl Cancer Inst* 96: 364-375, 2004.
8. Shih JY, Tsai MF, Chang TH, Chang YL, Yuan A, Yu CJ, Lin SB, Liou GY, Lee ML, Chen JJ, Hong TM, Yang SC, Su JL, Lee YC, Yang PC. Transcription repressor slug promotes carcinoma invasion and predicts outcome of patients with lung adenocarcinoma. *Clin Cancer Res* 11: 8070-8078, 2005.
9. Su JL, Yang PC, Shih JY, Yang CY, Wei LH, Hsieh CY, Chou CH, Jeng YM, Wang MY, Chang KJ, Hung MC, Kuo ML. The VEGF-C/Flt-4 axis promotes invasion and metastasis of cancer cells. *Cancer Cell* 9: 209-223, 2006.
10. Su JL, Yang CY, Shih JY, Wei LH, Hsieh CY, Jeng YM, Wang MY, Yang PC, Kuo ML. Knockdown of contactin-1 expression suppresses invasion and metastasis of lung adenocarcinoma. *Cancer Res* 66: 2553-2561, 2006.
11. Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet* 357: 539–545, 2001.
12. Coussens LM, Werb Z. Inflammation and cancer. *Nature* 420: 860–867, 2002.
13. Murdoch C, Lewis CE. Macrophage migration and gene expression in response to tumor hypoxia. *Int J Cancer* 117: 701–8, 2005.
14. Bingle L, Brown NJ, Lewis CE. The role of tumour-associated macrophages in tumour progression: Implications for new anticancer therapies. *J Pathol* 196: 254-265, 2002.
15. Murdoch C, Giannoudis A, Lewis CE. Mechanisms regulating the recruitment of macrophages into hypoxic areas of tumors and other ischemic tissues. *Blood* 104: 2224–2234, 2004.
16. Lin EY, Nguyen AV, Russell RG, Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 193: 727–740, 2001.
17. Leek RD, Harris AL. Tumour associated macrophages in breast cancer. *J Mamm Gland Biol Neoplasia* 7: 177–189, 2002.
18. Pollard JW. Tumour-educated macrophages promote tumour progression and metastasis. *Nat Rev Cancer* 4: 71–78, 2004.
19. Balkwill F. Cancer and the chemokine network. *Nat Rev Cancer* 4: 540–550, 2004.
20. Lewis CE, Murdoch C. Macrophage responses to hypoxia: implications for tumor progression and anti-cancer therapies. *Am J Pathol* 167: 627–635, 2005.
21. van Kempen LC, de Visser KE, Coussens LM. Inflammation, proteases and cancer. *Eur J Cancer* 42: 728-734, 2006.
22. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 66: 605-612, 2006.
23. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 124: 263-266, 2006.
24. Koide N, Nishio A, Sato T, Sugiyama A, Miyagawa S. Significance of macrophage chemoattractant protein-1 expression and macrophage infiltration in squamous cell carcinoma of the esophagus. *Am J Gastroenterol* 99: 1667–1674, 2004.
25. Hanada T, Nakagawa M, Emoto A, Nomura T, Nasu N, Nomura Y. Prognostic value of tumor-associated macrophage count in human bladder cancer. *Int J Urol* 7: 263–269, 2000.
26. Lissbrant IF, Stattin P, Wikstrom P, Damber JE, Egevad L, Bergh A. Tumor associated macrophages in human prostate cancer: relation to clinicopathological variables and survival. *Int J Oncol* 17: 445–451, 2000.
27. Ohno S, Ohno Y, Suzuki N, Kamei T, Koike K, Inagawa H, Kohchi C, Soma G, Inoue M. Correlation of histological localization of tumor-associated macrophages with clinicopathological features in endometrial cancer. *Anticancer Res* 24: 3335–3342, 2004.
28. Leek RD, Landers RJ, Harris AL, Lewis CE. Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. *Br J Cancer* 79: 991–995, 1999.
29. Chen JJ, Yao PL, Yuan A, Hong TM, Shun CT, Kuo ML, Lee YC, Yang PC. Upregulation of tumor interleukin-8 expression by infiltrating macrophages: Its correlation with tumor angiogenesis and patient survival in non-small cell lung cancer. *Clin Cancer Res* 9: 729-737, 2003.
30. Toomey D, Smyth G, Condron C, Kelly J, Byrne AM, Kay E, Conroy RM, Broe P, Bouchier-Hayes D. Infiltrating immune cells, but not tumour cells, express FasL in non-small cell lung cancer: No association with prognosis identified in 3-year follow-up. *Int J Cancer* 103: 408-412, 2003.
31. Funada Y, Noguchi T, Kikuchi R, Takeno S, Uchida Y, Gabbert HE. Prognostic significance of CD8+ T cell and macrophage peritumoral infiltration in colorectal cancer. *Oncol Rep* 10: 309-313, 2003.
32. Ohno S, Inagawa H, Dhar DK, Fujii T, Ueda S, Tachibana M, Suzuki N, Inoue M, Soma G, Nagasue N. The degree of macrophage infiltration into the cancer cell nest is a significant predictor of survival in gastric cancer patients. *Anticancer Res* 23: 5015-5022, 2003.
33. Welsh TJ, Green RH, Richardson D, Waller DA, O'Byrne KJ, Bradding P. Macrophage and mast-cell invasion of tumor cell islets confers a marked survival advantage in non-small-cell lung cancer. *J Clin Oncol* 23: 8959-8967, 2005.
34. Graf MR, Jadus MR, Hiserodt JC, Wepsic HT, Granger GA. Development of systemic immunity to glioblastoma multiforme using tumor cells genetically engineered to express the membrane-associated isoform of macrophage colony-stimulating factor. *J Immunol* 163: 5544-5551, 1999.
35. van der Bij GJ, Oosterling SJ, Meijer S, Beelen RH, van Egmond M. The role of macrophages in tumor development. *Cell Oncol* 27: 203-213, 2005.
36. Aharinejad S, Paulus P, Sioud M, Hofmann M, Zins K, Schafer R, Stanley ER, Abraham D. 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.
37. Crowther M, Brown NJ, Bishop ET, Lewis CE. Microenvironmental influence on macrophage regulation of angiogenesis in wounds and malignant tumors. *J Leukoc Biol* 70: 478-490, 2001.
38. Orre M, Rogers PA. Macrophages and microvessel density in tumors of the ovary. *Gynecol Oncol* 73: 47–50, 1999.
39. Leek RD, Landers RJ, Harris AL, Lewis CE. Necrosis correlates with high vascular density and focal macrophage infiltration in invasive carcinoma of the breast. *Br J Cancer* 79: 991–995, 1999.
40. Hashimoto I, Kodama J, Seki N, Hongo A, Miyagi Y, Yoshinouch M and Kudo T. Macrophage infiltration and angiogenesis in endometrial cancer. *Anticancer Res* 20: 4853–4856, 2000.
41. White ES, Strom SRB, Wys NL, Arenberg DA. Non-small cell lung cancer cells induce monocytes to increase expression of angiogenic activity. *J Immunol* 166: 7549–7555, 2001.
42. Ueno T, Toi M, Saji H, Muta M, Bando H, Kuroi K, Koike M, Inadera H, Matsushima K. Significance of macrophage chemoattractant protein-1 in macrophage recruitment, angiogenesis, and survival in human breast cancer. *Clin Cancer Res* 6: 3282–3289, 2000.
43. Tanaka Y, Kobayashi H, Suzuki M, Kanayama N, Suzuki M, Terao T. Thymidine phosphorylase expression in tumor-infiltrating macrophages may be correlated with poor prognosis in uterine endometrial cancer. *Hum Pathol* 33: 1105–1113, 2002.
44. Naylor MS, Stamp GW, Davies BD, Balkwill FR. Expression and activity of MMPS and their regulators in ovarian cancer. *Int J Cancer* 58: 50–56, 1994.
45. Wang FQ, So J, Reierstad S, Fishman DA. Matrilysin (MMP-7) promotes invasion of ovarian cancer cells by activation of progelatinase. *Int J Cancer* 114: 19–31, 2005.
46. Hildenbrand R, Wolf G, Bohme B, Bleyl U, Steinborn A. Urokinase plasminogen activator receptor (CD87) expression of tumor-associated macrophages in ductal carcinoma in situ, breast cancer, and resident macrophages of normal breast tissue. *J Leukoc Biol* 66: 40–49, 1999.
47. Hildenbrand R, Dilger I, Horlin A, Stutte HJ. Urokinase and macrophages in tumour angiogenesis. *Br J Cancer* 72: 818–823, 1995.
48. Vasiljeva O, Papazoglou A, Kruger A, Brodoefel H, Korovin M, Deussing J, Augustin N, Nielsen BS, Almholt K, Bogyo M, Peters C, Reinheckel T. Tumor cell-derived and macrophage-derived cathepsin B promotes progression and lung metastasis of mammary cancer. *Cancer Res* 66: 5242-5250, 2006.
49. Hagemann T, Robinson SC, Schulz M, Trumper L, Balkwill FR, Binder C. Enhanced invasiveness of breast cancer cell lines upon cocultivation with macrophages is due to TNF-α dependent up-regulation of matrix metalloproteases. *Carcinogenesis* 25: 1543–1549, 2004.
50. Hagemann T, Wilson J, Kulbe H, Li NF, Leinster DA, Charles K, Klemm F, Pukrop T, Binder C, Balkwill FR. Macrophages induce invasiveness of epithelial cancer cells via NF-κB and JNK. *J Immunol* 175: 1197–1205, 2005.
51. Tang Y, Kesavan P, Nakada MT, Yan L. Tumor stroma interaction: positive feedback regulation of extracellular matrix metalloproteinase inducer (EMMPRIN) expression and matrix metalloproteinase dependent generation of soluble EMMPRIN. *Mol Cancer Res* 2: 73–80, 2004.
52. Pukrop T, Klemm F, Hagemann T, Gradl D, Schulz M, Siemes S, Trumper L, Binder C. Wnt 5a signaling is critical for macrophage-induced invasion of breast cancer cell lines. *Proc Natl Acad Sci USA* 103: 5454-5459, 2006.
53. Wyckoff J, Wang W, Lin EY, Wang Y, Pixley F, Stanley ER, Graf T, Pollard JW, Segall J, Condeelis J. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* 64: 7022–7029, 2004.
54. Goswami S, Sahai E, Wyckoff JB, Cammer M, Cox D, Pixley FJ, Stanley ER, Segall JE, Condeelis JS. Macrophages promote the invasion of breast carcinoma cells via a colony-stimulating factor-1/epidermal growth factor paracrine loop. *Cancer Res* 65: 5278–5283, 2005.
55. Chen JJ, Lin YC, Yao PL, Yuan A, Chen HY, Shun CT, Tsai MF, Chen CH, Yang PC. Tumor-associated macrophages: the dou-

ble-edged sword in cancer progression. ***J Clin Oncol*** **23: 953-964, 2005.**

56. Savanger P. Leaving the neighborhood: molecular mechanisms involved during epithelial-mesenchymal transition. *BioEssays* 23: 912-923, 2001.

57. Thiery JP. Epithelial-mesenchymal transitions in tumor progression. *Nat Rev Cancer* 2: 442-454, 2002.

58. Lin CY, Lin CJ, Chen KH, Wu JC, Huang SH, Wang SM. Macrophage activation increases the invasive properties of hepatoma cells by destabilization of the adherens junction. *FEBS Lett* 580: 3042-3050, 2006.

59. Paulus P, Stanley ER, Schafer R, Abraham D, Aharinejad S. Colony-stimulating factor-1 antibody reverses chemoresistance in human MCF-7 breast cancer xenografts. *Cancer Res* 66: 4349-4356, 2006.

60. Zhu P, Baek SH, Bourk EM, Ohgi KA, Garcia-Bassets I, Sanjo H, Akira S, Kotol PF, Glass CK, Rosenfeld MG, Rose DW. Macrophage/cancer cell interactions mediate hormone resistance by a nuclear receptor derepression pathway. *Cell* 124: 615-629, 2006.

61. McDaniel SM, Rumer KK, Biroc SL, Metz RP, Singh M, Porter W, Schedin P. Remodeling of the mammary microenvironment after lactation promotes breast tumor cell metastasis. *Am J Pathol* 168: 608-620, 2006.

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