DOI:10.1158/0008-5472.CAN-07-1684

Cancer Research

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Cancer Res 2007;67:8429-8432. Published online September 17, 2007.

DOI:10.1158/0008-5472.CAN-07-1684

Tie2-Expressing Monocytes and Tumor Angiogenesis: Regulation by Hypoxia and Angiopoietin-2

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Abstract

Recent findings indicate that tumor-associated macrophages are important drivers of tumor angiogenesis. Here, we review the essential role played by Tie2-expressing monocytes (TEM) in this phenomenon. TEMs are present in human blood and tumors and their elimination in various tumor models suppresses tumor angiogenesis. A ligand for Tie2, angiopoietin-2 (Ang-2), is produced by angiogenic tumor vessels and is a chemoattractant for TEMs. Hypoxia up-regulates Tie2 expression on TEMs and, together with Ang-2, down-regulates their antitumor functions. Learning more about the regulation of TEMs by the tumor microenvironment may yield new strategies to ablate the tumor vasculature. [Cancer Res 2007;67(18):8429–32]

Bone Marrow–Derived Cells Regulate Tumor Angiogenesis

Tumor angiogenesis—the generation of a tumor-associated vascular system from host-derived, preexisting blood vessels—occurs in response to the increasing demand for nutrients and oxygen experienced by proliferating tumor cells. The angiogenic process involves the activation, proliferation, and migration of endothelial cells toward angiogenic stimuli produced by the tumor. Once a primitive endothelial cell network is formed, the recruitment of perivascular cells may enable stabilization of the nascent vessels, functional lumen formation, and blood flow (1, 2). It has recently emerged that a close interplay between cells of the innate immune system and the developing vascular network takes place during tumor angiogenesis (3). Innate immune cells such as macrophages, granulocytes, and dendritic cells are prominent components of both premalignant and malignant tissues and contribute to tumor development by producing several factors that directly or indirectly promote tumor angiogenesis. As early as in 1971, Folkman (4) postulated that inhibition of tumor angiogenesis would be an effective strategy to treat human cancer. The recognition of the critical interactions between immune cells and tumor angiogenesis has led to the suggestion that targeting tumor-infiltrating immune cells may represent a valuable antiangiogenic strategy for cancer treatment (5).

One particular tumor-infiltrating, innate effector, the macrophage, has been shown to play a critical role in tumor development. Monocytes are recruited from the bloodstream into tumors and, as they extravasate across the tumor vasculature, begin to differentiate into macrophages (6). Several studies have now shown that these tumor-associated macrophages (TAM) promote both tumor angiogenesis and metastasis (7). For example, when Lin et al. (8) crossed MMTV-PyMT mice (which spontaneously develop mammary tumors) with *op/op* mice, which lack a functional *CSF-1* gene (a crucial growth factor for macrophages and their precursors), the tumors that grew in these macrophage-depleted mice showed a slower rate of progression to malignancy and formed fewer pulmonary metastases than those in CSF-1 wild-type mice. Moreover, these authors recently showed that TAMs contribute to the "angiogenic switch" that occurs when hyperplastic lesions develop into early-stage carcinomas in MMTV-PyMT mice (9). Most recently, an elegant study by Condeelis' group has used multiphoton imaging to visualize the cooperation between TAMs and tumor cells during the process of tumor cell intravasation and metastasis (10). These data are consistent with clinical findings that high numbers of TAMs often correlate with increased tumor vascularization and/or lymph node involvement in human tumors (11).

TAM involvement in tumor angiogenesis is mediated, at least in part, by their release of a wide array of proangiogenic factors and enzymes, including vascular endothelial growth factor-A (VEGF-A) and matrix metalloproteinase-9 (MMP-9). For example, TAMs are an important source of VEGF-A in mouse mammary (9) and human breast (12) tumors. Moreover, Coussens et al. (13) used a transgenic mouse model of spontaneous skin tumorigenesis (K14-HPV) to show the essential role of MMP-9 production by tumor-associated stromal cells, such as TAMs, in tumor progression. It therefore came as no surprise that ablation of TAMs using a DNA vaccine approach in various murine tumor models resulted in marked reduction in tumor VEGF and MMP-9 levels and the suppression of tumor angiogenesis, growth, and metastasis (14).

One subset of monocytes has recently been shown to have a particularly important role in tumor angiogenesis—those expressing Tie2, an angiopoietin receptor thought previously to be restricted mainly to endothelial cells and hematopoietic stem cells (15, 16). The selective elimination of these Tie2-expressing monocytes (TEM) by means of a suicide gene dramatically impaired angiogenesis in mouse tumors and induced substantial tumor regression (15, 16). The presence and phenotype of these TEMs have now been defined in human blood and tumors (15, 17, 18), and their role in tumor angiogenesis investigated using cell transplantation assays. Furthermore, the pronounced effects on TEMs of factors present in the tumor microenvironment, such as hypoxia and angiopoietin-2 (Ang-2), have also now been described (17, 18). These studies are outlined in greater detail below.

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doi:10.1158/0008-5472.CAN-07-1684

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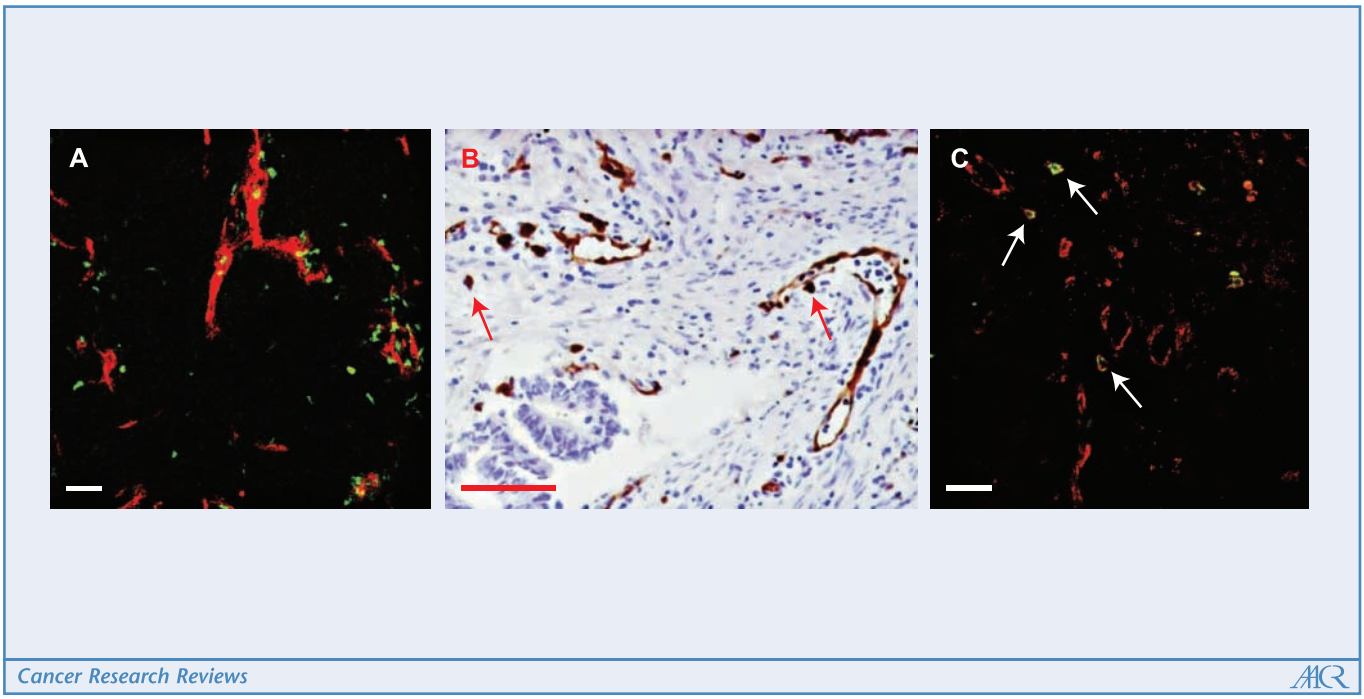

Figure 1. TEM recruitment to tumors. *A,* a human glioblastoma orthotopically injected in the mouse brain shows several TEMs (labeled by green fluorescent protein, *green*) associated with the tumor blood vessels (*red*, stained by an anti-CD31 monoclonal antibody). *B,* a human gastric adenocarcinoma stained with an anti-Tie2 monoclonal antibody. Note the $Tie2^+$ blood vessels and the presence of scattered $Tie2^+$ mononuclear cells (TEMs, *arrows*) in the stroma. Section was counterstained by H&E. *C,* a human colon carcinoma stained with a fluorescent anti-Tie2 monoclonal antibody (*red*) shows $Tie2^+$ blood vessels and scattered $Tie2^+CD14^+$ TEMs (*arrows*). *Green,* $CD14^+$ monocytes were stained with a FITC-conjugated anti-CD14 monoclonal antibody. Double-positive cells (i.e., TEMs) appear *yellow*. Bar, 50 μm.

Identification of TEMs

Studies by De Palma et al. (15, 16) have identified TEMs in several mouse tumor models, including s.c. tumors, human glioblastoma grown in the mouse brain, and spontaneous pancreatic tumors developing in RIP1-Tag2 transgenic mice. In these, TEMs were found to constitute a small subpopulation of the total tumor-infiltrating, $CD11b^+$ myeloid cells that could be distinguished from the majority of TAMs by their surface marker profile ($Tie2^+Sca\text{-}1^+CD11b^+$), their preferential localization to areas of angiogenesis, and their marked proangiogenic activity (15). These studies have now been extended to show the existence of TEMs in a variety of human tumors where they represented a small subset of tumor-infiltrating leukocytes found largely in perivascular and avascular, viable areas of human tumors. These cells were largely missing in nonneoplastic tissues adjacent to tumors (17).

In addition to tumors, TEMs can be detected at low frequency in the peripheral blood of both mice and humans (15, 17, 18). Whereas only 1% to 2% of total leukocytes are $Tie2^+$, a substantial fraction (~20%) of circulating monocytes express Tie2 (17, 18). In mice, the circulating $Tie2^+CD45^+$ hematopoietic cells are mostly $CD11b^+Gr\text{-}1^{low/neg}$ (15), whereas in humans they express CD14, CD16, and CD11c (17, 18). The surface marker profile of mouse and human TEMs is similar to that of the so-called "resident monocytes," a monocyte population distinct from the classic inflammatory monocytes and thought to comprise precursors of tissue macrophages (19). Previous studies have reported that a subset of monocytes has the ability to differentiate into endothelial cells on an appropriate stimulus and expresses some endothelial cell markers, including Tie2 (20). However, we found that the vast majority of the circulating $Tie2^+$ cells lacked the expression of the endothelial cell/endothelial cell precursor markers VEGFR-2, AC133, CD146 (17), and CD34 (18), whereas they expressed pure hematopoietic markers, such as CD45. Although a certain degree of phenotypical and functional heterogeneity may exist among circulating $Tie2^+$ cells, these data indicate that the prominent fraction of peripheral blood $Tie2^+$ cells are bona fide monocytes.

Interestingly, circulating human TEMs do not express CCR2 (17), the receptor for CCL2 [also known as monocyte chemoattractant protein-1 (MCP-1)], a chemokine that regulates the recruitment of monocytes to inflamed tissues and tumors. Although the recruitment of TEMs to inflamed tissues has yet to be investigated, it is tempting to speculate on the non-MCP-1 circuits that may govern the recruitment of TEMs to tumors. TEMs may be attracted to tumors in a CCR2-independent manner by signals produced by the tumor cells or stromal components of the tumor, such as the blood vessels themselves. For example, as discussed below, Ang-2, a Tie2 ligand up-regulated by tumors, could play a role in this (17, 18).

Role of TEMs in Tumor Angiogenesis: Insights from Mouse Tumor Models

TEMs have been shown to promote tumor angiogenesis in various murine tumor models (15, 16). For example, murine TEMs isolated by cell sorting from the peripheral blood and injected with Matrigel s.c. in mice were more angiogenic than their Tie2-negative counterparts. Moreover, when TEMs were isolated from murine mammary tumors and injected with tumor cells s.c. in mice, the early-stage tumors that developed from the injected cells were significantly more vascularized than the tumors originating from

DOI:10.1158/0008-5472.CAN-07-1684

tumor cells alone or tumor cells coinjected with $CD11b^{+}Tie2^{neg}$ cells. Together, these data indicate that both circulating and tumor-infiltrating TEMs have marked proangiogenic activity and suggest that circulating TEMs might represent the precursors of the tumor TEM population. Similar to murine cells, human TEMs also have such proangiogenic activity (17). Indeed, $Tie2^{+}CD14^{+}$ cells isolated from human peripheral blood by cell sorting, and coinjected with human glioma cells s.c. in nude mice, markedly promoted tumor vascularization, whereas $CD14^{+}Tie2^{neg}$ monocytes failed to show such activity (17).

To investigate the importance of TEMs in tumor angiogenesis, transgenic mice were generated that express the conditionally toxic gene *thymidine kinase* (*tk*) under the control of the murine Tie2 promoter/enhancer (Tie2-tk transgenic mice; ref. 15). In this mouse model, proliferating Tie2-expressing cells can be selectively killed by administration of the prodrug ganciclovir (GCV). Hematopoietic progenitors cells from these Tie2-tk transgenic mice were transplanted into wild-type mice and, a few weeks later, inoculated with either s.c. mammary tumors or orthotopic human gliomas. When GCV was given to eliminate TEMs during the early stages of tumor growth, tumors in GCV-treated mice were significantly smaller and less vascularized than those of untreated mice, indicating that TEM depletion was sufficient to inhibit tumor angiogenesis and growth (15). One interesting finding was that TEM elimination did not affect the overall number of TAMs, making it unlikely that TEMs are simply precursors for TAMs or tissue macrophages in general. Rather, it seems from these studies that TEMs represent a distinct monocyte subset with inherent proangiogenic activity. Interestingly, when GCV treatment was halted, s.c. mammary tumors remained dormant for more than 2 weeks before resuming their growth, suggesting that reconstitution of the TEM pool was required before the tumors could switch angiogenesis back on and resume their growth (15).

In addition to TEMs, other studies reported proangiogenic activity of selected myeloid cell subsets in mouse tumors, including $VEGFR\text{-}1^{+}CD11b^{+}$ hematopoietic progenitors, $Gr\text{-}1^{+}CD11b^{+}$ myeloid suppressor cells, and $CD11c^{+}MHC\text{-}II^{+}$ dendritic cell precursors (3). Because many cell surface markers are broadly expressed among myeloid cells, it is difficult to establish whether these proangiogenic cells represent distinct hematopoietic populations, rather than overlapping myeloid cell subsets, possibly comprising the TEMs.

Because some TEMs preferentially localize to the vicinity of tumor blood vessels in viable tumor areas (17), it can be envisaged that they may provide paracrine support to nascent blood vessels in these areas during the angiogenic process. In human glioma xenografts, for instance, perivascular TEMs produce high levels of the proangiogenic factor basic fibroblast growth factor (15). As mentioned previously, other studies have suggested that the proangiogenic activity of myeloid cells in tumors involves the production of growth factors and matrix-remodeling proteins that stimulate the angiogenic process in a paracrine manner (3). In addition to these functions, TEMs could function as pathfinders for activated endothelial cells or form provisional endothelium-like structures for blood vessel formation.

Regulation of TEMs by the Tumor Microenvironment

In two recent studies (17, 18), Ang-2 has been shown to stimulate migration of $Tie2^{+}$ monocytes *in vitro* and to elicit this effect, at least in part, via activation of Tie2 (17, 18). As Ang-2 is up-regulated by tumor blood vessels (21), it may recruit TEMs into tumors by attracting them toward the tumor vasculature. This scenario might help to explain why TEMs are seen in clusters around angiogenic vessels in some tumor models (15).

Lewis and her colleagues have shown that hypoxia up-regulates Tie2 expression on human TEMs (18). Moreover, Naldini's group has shown that TEMs isolated from human tumors express Tie2 to a higher extent than circulating TEMs (17). Together, these findings suggest that hypoxia in the tumor microenvironment may up-regulate Tie2 expression by TEMs, making them more responsive to Ang-2 in hypoxic (i.e., highly angiogenic) tumor areas. Lewis's group showed that Ang-2 also modulates cytokine secretion by TEMs. For example, it inhibits their release of tumor necrosis factor-α (TNF-α), an interesting finding given that elevated levels of TNF-α promote apoptosis of both tumor and endothelial cells (22). The down-regulation of TNF-α by TEMs near $Ang\text{-}2^{+}$ tumor blood vessels may thus enhance tumor and endothelial cell survival, thereby promoting metastasis and angiogenesis. Furthermore, in the presence of hypoxia, Ang-2 inhibits the expression of the antiangiogenic cytokine interleukin-12 by TEMs (18), thereby highlighting an important mechanism by which the tumor microenvironment may down-regulate the antiangiogenic activities of tumor-infiltrating macrophages. It remains to be investigated whether hypoxia, Ang-2, and/or other Tie2 ligands also play a role in regulating the activity of TEMs in tumors.

Concluding Remarks

The studies outlined above have unearthed the essential role played by a small subset of tumor-infiltrating monocytes, those that express Tie2, in regulating tumor angiogenesis. They may lead to several new therapeutic approaches in anticancer treatment. From the data discussed here, it follows that conventional antiangiogenic therapy may be improved by drugs that concomitantly target this subset of proangiogenic monocytes. Assessing the specific contribution of TEMs to human neoplastic disease, together with the identification of selective targets for these cells, may herald the design of new anticancer therapies. Monocytes/macrophages are genetically stable cells that are less likely to develop drug resistance than cancer cells, so drugs that inhibit selected macrophage functions should hold promise for effective anticancer therapies. The challenge now, however, is to understand which targets could safely and effectively distinguish TEMs from other effector cells in the body that regulate immune functions and tissue homeostasis. Furthermore, it will be important to determine whether TEM inhibition would affect physiologic processes. Given their tumor-homing ability and selectivity, TEMs may be used as surrogate markers to monitor angiogenesis *in vivo* or as gene delivery vehicles to restrict antiangiogenic or antitumor gene expression to the tumor site (16).

Acknowledgments

Received 5/8/2007; revised 6/19/2007; accepted 6/19/2007.

Grant support: Associazione Italiana per la Ricerca sul Cancro (52-2005), European Union (Tumor-Host Genomics, LSHC-CT-2005-518198), and Telethon Institute for Gene Therapy (L. Naldini) and Associazione Italiana per la Ricerca sul Cancro (M. De Palma).

We thank M.A. Venneri for helpful discussions on the manuscript. C.E. Lewis thanks the Yorkshire Cancer Research and the Biotechnology and Biological Sciences Research Council, United Kingdom, for their generous grant support of her group's work in this and related areas.

DOI:10.1158/0008-5472.CAN-07-1684

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