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Macrophages: Obligate Partners for Tumor Cell Migration, Invasion, and Metastasis

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Macrophages within the tumor microenvironment facilitate angiogenesis and extracellular-matrix breakdown and remodeling and promote tumor cell motility. Recent studies reveal that direct communication between macrophages and tumor cells leads to invasion and egress of tumor cells into the blood vessels (intravasation). Thus, macrophages are at the center of the invasion microenvironment and are an important drug target for cancer therapy.

In their landmark review, Hanahan and Weinberg (2000) enumerated six traits that cells require for malignant growth. These are self-sufficiency from external growth signals, insensitivity to negative growth signals, resistance to apoptosis, limitless replicative potential, sustained angiogenesis, and acquisition of tissue invasiveness. Although the last two traits depend on heterotypic interactions with nonmalignant cells, the review's emphasis was clearly upon intrinsic genetic changes in tumor cells. Despite considerable success in the molecular definition of these changes, it has become apparent that tumors are complex ecologies of different cell types and that the full manifestation of the malignant potential of transformed epithelial cells requires an appropriate support structure from the stroma. The stroma is complex and changing and depends on the tumor's origin. It can consist of resident fibroblasts, adipocytes, and blood and lymph vessels and may also be infiltrated by a wide range of hematopoietic cells. Recent studies have shown that all of these cell types can influence tumor progression to varying degrees depending on tumor type.

Hematopoietic cells are recruited to most tumors, and one group, the tumor-associated macrophages, can constitute a large portion of the tumor mass (Pollard, 2004). Two strands of evidence derived from clinical and epidemiological studies implicate these macrophages in cancer. First is the association of chronic inflammation, which involves macrophages, with cancer initiation and promotion and the reduction of cancer risk by treatment with anti-inflammatory drugs (Balkwill et al., 2005). Second, a high density of these tumor-associated macrophages correlates with poor prognosis in over 80% of studies published (Bingle et al., 2002).

Macrophages appear to be directly involved in tumor progression and metastasis. Removal of macrophages in mice—through a homozygous null mutation of the gene that encodes the macrophage growth factor, colony-stimulating factor-1 (CSF-1)—reduced the rate of tumor progression and almost completely ablated the metastasis of the tumor in a mouse model of breast cancer (induced by expression of the polyoma middle T oncoprotein in mammary epithelial cells). In contrast, overexpression of CSF-1 accelerated tumor progression and metastasis in this mouse model (Lin et al., 2001). Blocking expression of mouse CSF-1 in a xenograft model (mice engrafted with human tumor cells) reduced the growth and metastatic capacity of the tumor cells, and this was associated with reduced invasion of host-tumor-associated macrophages (Pollard, 2004 and references within). These data suggest that a causal relationship exists between poor prognosis in a variety of reproductive tumors and overexpression of CSF-1, which recruits macrophages. Indeed, in human breast cancers, there is a positive correlation between poor prognosis and the density of tumor-associated macrophages (Lin et al., 2002). Furthermore, recent experiments using specific ablation of a key player in the inflammatory response, NF-κB, in tumor-associated macrophages showed that this signaling pathway is important for tumor initiation and growth (Karin and Greten, 2005). Clinical and experimental evidence therefore links tumor-associated macrophages with tumor progression and metastasis.

Macrophages can provide support for developing tissues through their matrix remodeling capacities, synthesis of growth and angiogenesis factors, and their engulfment of apoptotic cells. Excellent examples of these functions are displayed by the macrophage-derived osteoclasts that remodel bone and for macrophages in mammary development (Figure 1) (Lin et al., 2002). During wound healing, macrophages are sentinel cells that organize immune defenses and coordinate the tissue repair process, which involves epithelial migration, matrix remodeling, and angiogenesis (Coussens and Werb, 2002). We argue here that tumors recruit macrophages and create a microenvironment that causes macrophages to suppress immune functions and instead adopt trophic roles found during development and repair (Figure 1). However, in contrast to normal tissue, tumor cells have lost their "off switches" due to intrinsic mutations

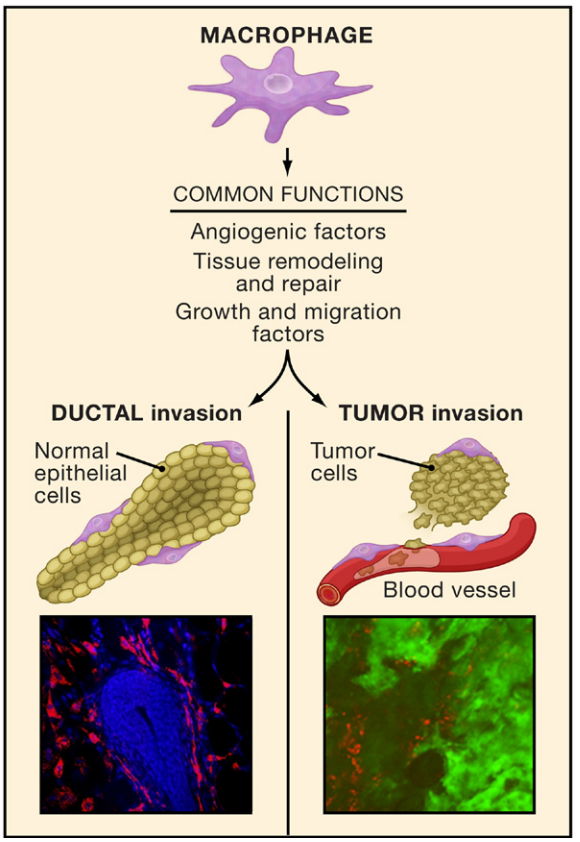

Figure 1. Tumor Cells Co-opt Macrophage Functions
Macrophages aid in the invasion of epithelial cells during morphogenesis (left) and are co-opted by tumor cells during metastasis (right). Multiphoton image at the bottom left: epithelial cells (blue) of a terminal end bud in a mouse mammary gland during normal glandular development. The bud is surrounded by macrophages (red) that precede the invasion of the fat pad by the epithelial cells. Multiphoton image at the bottom right: tumor-associated macrophages (red) and tumor cells (green) at an invasive edge in a mouse mammary tumor. Adapted from Wyckoff et al. (2004) and Lin et al. (2002).

and no longer respond to positional information. Therefore, they continue to grow, invade the surrounding tissue, and escape to distant sites. Here, we propose six extrinsic traits conferred by macrophages that enhance tumor incidence, progression, and metastasis. These are chronic inflammation, matrix remodeling, tumor cell invasion, intravasation, angiogenesis, and seeding at distant sites (Figure 2).

Inflammation

Infections that cause chronic inflammation are responsible for >15% of cancers worldwide (Coussens and Werb, 2002). Among the best documented of these are the causal relationships between the bacterium *Helicobacter pylori* and stomach cancer and between the helminth worm *Schistosoma hematobium* and bladder cancer. Furthermore, compounds such as asbestos and cigarette smoke cause a chronic inflammatory state that promotes tumorigenesis. Additionally, there are strong associations between increased cancer risk and genetic conditions that cause

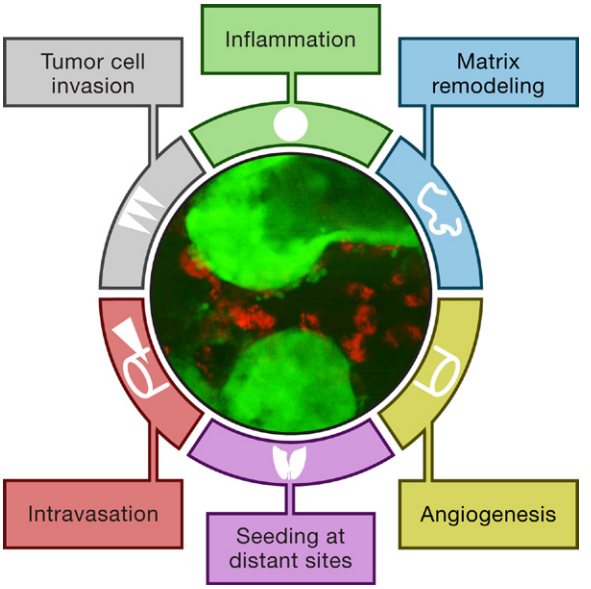

Figure 2. Six Traits for Malignancy Promoted by Macrophages
Tumors direct macrophages to adopt a trophic role that facilitates six traits that are extrinsic to the intrinsic genetic changes of tumor cells. The wheel (in deference to Hanahan and Weinberg, 2000) can turn in either direction, allowing macrophages to contribute to invasion, intravasation, angiogenesis, and extravasation equally. The image in the center is a multiphoton micrograph of a mammary tumor (green) and associated macrophages (red) in a living mouse.

continuous inflammatory disorders such as Crohn's disease; conversely, there is a reduction in cancer risk with the use of nonsteroidal anti-inflammatory drugs. In cases of chronic inflammation, the persistent recruitment of immune cells is thought to establish a microenvironment that is mutagenic through the production of reactive oxygen and nitrogen species that can initiate malignant changes in nearby epithelial cells. Furthermore, this microenvironment is rich in growth factors and cytokines that can stimulate proliferation and survival of the mutated premalignant cells, enabling them to accumulate further genetic changes on the path to becoming frank malignancies. The role of inflammation in cancer initiation and promotion has been recently reviewed (Balkwill et al., 2005; Coussens and Werb, 2002; Pollard, 2004).

Matrix Remodeling, Tumor Cell Invasion, and Intravasation

In the early stages of tumorigenesis, macrophages are found at points of basement-membrane breakdown during the transition to malignancy and at the invasive front of more advanced tumors. This suggests that tumors exploit the normal matrix remodeling capacities (Figure 1) of macrophages, enabling them to egress into and migrate through the surrounding stroma (Lin et al., 2002, Pollard, 2004, Wyckoff et al., 2004). Multiphoton imaging has revealed remarkable interactions between tumor cells, macrophages, and blood vessels, supporting the notion that macrophages enhance tumor cell migration and invasion (Condeelis and Segall, 2003). Additionally, macrophages are found throughout mammary tumors and in association with blood vessels. In

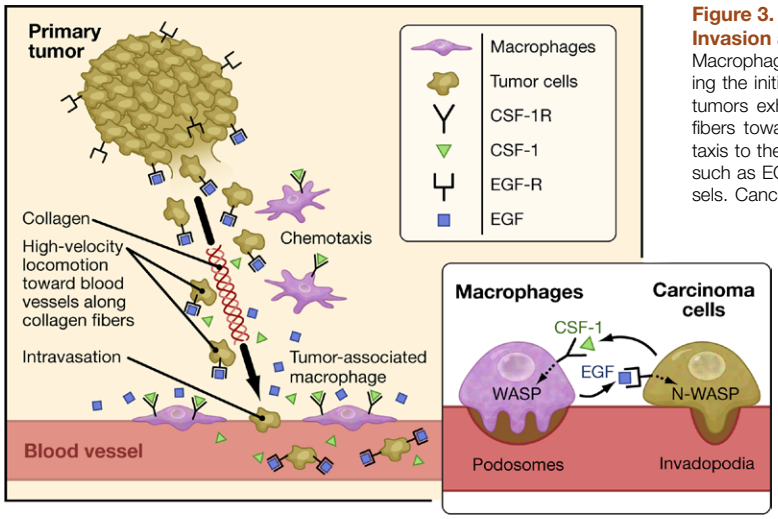

Figure 3. Macrophages Play a Direct Role in the Invasion and Intravasation of Mammary Tumor Cells

Macrophages are involved in a paracrine loop with tumor cells during the initial stages of metastasis. Carcinoma cells of metastatic tumors exhibit high-velocity polarized movement along collagen fibers toward blood vessels as a result of chemotaxis. Chemotaxis to the blood vessel occurs in response to chemoattractants such as EGF secreted by macrophages associated with the vessels. Cancer cells express the EGF receptor (EGFR) and secrete CSF-1, which attracts macrophages and induces them to express EGF, thereby completing the paracrine loop. Collagen-containing fibers support tumor-associated vessels like a spider web with the vessel at the center. The convergence of these fibers on vessels has the effect of directing macrophage-induced tumor cell movement to the vessels. (Adapted from Lin et al., 2002; Condeelis and Segall, 2003.) Inset: signaling between the macrophage and the tumor cell affects the activity of actin regulators such as WASP and N-WASP, resulting in the formation of podosomes in macrophages and invadopodia in tumor cells to promote tumor cell intravasation.

mammary carcinomas derived from implanted tumor cells and in tumors induced by expression of polyoma middle T oncogene in mouse mammary epithelium, tumor cells migrate toward blood vessels, suggesting a chemotactic stimulus originating from blood vessels. Invasive tumor cells and macrophages within primary mammary tumors of rats and mice have been found to migrate together, but only in response to EGF and CSF-1 (Wang et al., 2005). Inhibition of either CSF-1 or EGF receptor signaling blocks migration of both cell types in vivo. The expression patterns of these receptors and their ligands in tumor cells and macrophages (Condeelis et al., 2005) and experiments demonstrating that tumor cells and macrophages migrate toward each other and together penetrate a dense collagen matrix suggest the existence of an EGF/CSF-1 paracrine signaling loop. A key observation is that the stimulation of tumor cells by EGF and macrophages by CSF-1 induces invadopod and podosome formation, respectively. Thus, each cell type is endowed with exaggerated extracellular-matrix remodeling activity and invasive properties at the same time that their migratory activity increases (Yamaguchi et al., 2006) (Figure 3).

Macrophages also enhance the ability of tumor cells to enter blood vessels (intravasation). Importantly, intravasation efficiency is directly correlated with the migration of tumor cells toward blood vessels and with macrophage density in the primary tumor. Furthermore intravasation can be blocked by pharmacologic inhibition of EGFR signaling. This suggests that the paracrine loop, via CSF-1 and EGF receptor signaling in macrophages and tumor cells, respectively, is operating during intravasation (Wyckoff et al., 2004).

These data show that macrophages control tumor intravasation, and this is one explanation for the inhibition of metastasis when macrophages are ablated from experimental tumor models (Lin et al., 2002). These data are also consistent with correlative clinical data in breast cancer showing that overexpression of CSF-1 in tumor cells and EGF in macrophages are both independent predictors of poor prognosis (Lin et al., 2002; Leek and Harris, 2002). These results directly implicate macrophages in the induction of tumor cell migration, invasion, and intravasation in the primary tumor (Figure 3).

Gene-expression profiling of mammary tumor cells that migrate with macrophages indicates that macrophages may help to elicit a particular "invasion signature" of gene expression in these tumor cells. This identifies the tumor cells as neither proliferating nor apoptotic, but they have heightened chemotaxis to EGF. The genes in the invasion signature fall into coordinately regulated pathways that suggest that these tumor cells can communicate with and follow macrophages during invasion (Condeelis et al., 2005). Based on this signature, a tumor microenvironment invasion model has been proposed in which tumorigenesis leads to the development of microenvironments within the tumor, which presumably result from the stable gene-expression patterns seen by whole-tumor profiling. These stable expression patterns might lead, for example, to increased inflammation and macrophage involvement in tumor progression and to the invasion microenvironment, which in turn would elicit transient gene-expression patterns in tumor cells that support invasion (similar to the way in which transient patterns of gene expression support the invasion of the fat pad by mammary ductal epithelial cells during normal development) (Figure 1). An interesting prediction of the tumor microenvironment model is that the early and uniform expression of certain genes could lead to the random appearance, in time and location, of an invasion microenvironment. This would cause repeated episodes of invasion and micrometastasis that increase in frequency as the tumor progresses but that are not necessarily limited to late stage carcinomas (Wang et al., 2005 and references within).

Angiogenesis

Clinical evidence shows a correlation between local macrophage density and areas of intense angiogenesis defined by the presence of microvessels, suggesting a role for macrophages in this process (Leek and Harris, 2002). In the polyoma middle T oncoprotein model for mammary cancer, depletion of macrophages inhibits the

angiogenic switch that occurs during the malignant transition and the subsequent remodeling of the vasculature as the tumors become late carcinomas (E.Y. Lin and J.W.P., unpublished data). Tumor-derived signals appear to recruit a subset of monocytes that express a marker normally restricted to endothelial cells, Tie2, and that are responsible for neoangiogenesis. The ablation of this specialized cell type remarkably reduced angiogenesis in the tumor and caused its regression (De Palma et al., 2005). Signals for this macrophage recruitment include hypoxia caused by the tumor's outgrowing the vascular supply (Murdoch et al., 2004). Hypoxia induces the HIF transcription factors in these cells whose targets include genes for many angiogenic factors, such as VEGF, whose action enhances angiogenesis in these avascular areas. Indeed, in a mouse model for cervical cancer, inhibition of the matrix metalloproteinase MMP9 in macrophages blocked the release of VEGF and thereby inhibited angiogenesis and tumor growth (Giraudo et al., 2004). These data strongly suggest that macrophages play a role in both initiation of angiogenesis in avascular areas and in the remodeling of the vasculature once formed to give coherent vascular flow. Indeed, similar functions of macrophages appear to be used in wound healing and during the remodeling of the vasculature in the eye during postnatal development (Lobov et al., 2005), coupling pathological angiogenesis in tumors with the normal physiological role of macrophages.

Thus, macrophages not only increase vascularization to provide sustenance to the tumor but also promote metastasis by enhancing tumor cell movement toward and intravasation into an increased number of vessels. This places macrophages at the center of an invasion microenvironment.

Seeding at Distant Sites

The events that occur at distant sites are similar to those at the primary tumor. Circulating tumor cells, in either the lymphatic or blood system, are believed to exit vessels (extravasate) to establish a proliferative niche where angiogenesis is necessary for sustained growth. There is evidence that suggests that macrophages play an important role during these processes, including clinical observations that the number of macrophages associated with metastases in the lymph nodes correlates well with poor survival (Oberg et al., 2002). Primary tumors induce the expression of MMP9 in macrophages at sites of lung metastasis, thereby causing the release of bound VEGF, which promotes angiogenesis (Pollard, 2004 and references within). Furthermore, depletion of macrophages in the peritoneum reduced the ability of carcinoma cells introduced into the portal vein to seed and grow in the lung (Oosterling et al., 2005). Although these data showing that macrophages promote metastatic lesions are still scant, they are consistent with the poor capacity for metastasis of mammary tumors in macrophage-depleted mice (Lin et al., 2001).

Conclusion

The trophic activities of macrophages may play a causative role in defining the invasion microenvironment of mammary and other types of tumors. Macrophages do not harbor malignant mutations and therefore have a stable genome; they thus are much less likely to develop drug resistance. This makes them a good target for cytostatic treatment of tumor progression to malignancy using small molecule inhibitors of selected macrophage functions. Studying the signaling pathways that allow macrophages to contribute to tumor progression will lead to new insights into the evolution of the microenvironments supporting invasion and metastasis, thereby providing targets for anticancer therapies.

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