

# Tumor-Associated Macrophages Press the Angiogenic Switch in Breast Cancer

Elaine Y. Lin<sup>2</sup> and Jeffrey W. Pollard<sup>1</sup>

<sup>1</sup>Department of Developmental and Molecular Biology, Center of Reproductive Biology and Women's Health and <sup>2</sup>Department of Medicine, Oncology Division, Albert Einstein Cancer Center, Albert Einstein College of Medicine, Bronx, New York

## Abstract

**The development of a supportive vasculature is essential for tumor progression. In a mouse model of breast cancer, we found that tumor-associated macrophages that are recruited to the tumor just before malignant conversion are essential for the angiogenic switch. These findings establish a causal linkage to explain well-documented clinical correlations between macrophages, microvessel density, and poor prognosis in breast tumors.** [Cancer Res 2007;67(11):5064–6]

## Background

The process of developing a high-density vessel network that connects the tumor and host circulation, termed the “angiogenic switch,” is a crucial step for conversion of a tumor from a benign to malignant state (1, 2). This step in malignant conversion assists the tumor by allowing it to gain sufficient nutrients, oxygen, and growth factors for the highly proliferative malignant cells to propagate, as well as facilitating removal of waste products that accumulate due to tumor metabolism. Importantly, this process also facilitates tumor cells to gain access to the host circulation and consequently to “seed” metastases in distant organs.

Multiple steps are involved in the evolution of the tumor to full malignancy. These include the acquisition of growth factor independence, loss of negative proliferative control, apoptosis resistance, limitless replicative potential, and the development of a supportive vasculature (3). Among these, the angiogenic switch has been considered an initial and rate-limiting step in malignant conversion because there is evidence that the accumulation of mutations that define malignancy is necessary but not sufficient for the malignant phenotype to be manifested (4). Indeed, it has been reported that tumor lesions with limited vasculature development are found in high frequency in normal individuals, arguing that these lesions can be maintained in a steady state for many years without further progression to malignancy (4, 5). Why the angiogenic switch is not activated in such dormant lesions is unknown, nor is it fully understood what controls activation of the switch when it happens. In the last few decades, many factors with the potential to promote tumor angiogenesis, such as vascular endothelial growth factor (VEGF), basic fibroblast growth factor, urokinase-type plasminogen activator (uPA), and Ang2, have been identified in many types of malignancy (6). However, how these factors function in driving malignant conversion and how their expression is regulated during this process are still rather unclear.

Naturally occurring tumors do not live as isolated islands but instead are in intimate association with many host cells including fibroblasts, adipocytes, and infiltrating leukocytes. These cells in the tumor microenvironment are known to influence the expression of the malignant phenotype. Of these, macrophages are a major population of infiltrating cells in the tumor stroma, and recent evidence suggests that they, on the whole, have a tumor-supporting role (7). Using an autochthonous mouse model of breast cancer to study their function in breast cancer, we showed that they are required to regulate both the angiogenic switch and tumor progression (8).

## Macrophages Regulate the Angiogenic Switch

In the well-characterized transgenic mouse model of breast cancer, MMTV-PyMT mice, where tumors arise rapidly due to the expression of the polyoma virus middle-T antigen in mammary epithelium driven by the mammary tumor virus promoter (MMTV LTR), one sees a prototypical progression from the benign stages of hyperplasia and adenoma/mammary intraepithelial neoplasia to the malignant stages of early and late carcinoma (9). Notably, a marked increase of macrophage infiltration occurs in primary tumors at the nonmalignant adenoma stage, preceding the transition to malignancy associated with the angiogenic switch. By genetically deleting the colony stimulating factor-1 (CSF-1), a critical macrophage growth factor, we showed that inhibition of macrophage maturation and infiltration into tumors dramatically delayed the angiogenic switch as well as malignant progression in this model (8). Transgenic techniques to induce macrophage infiltration into wild-type tumors that were still at the early stages of premalignancy, characterized by the presence of a sparse vasculature, resulted in a dramatic enhancement of angiogenesis and the formation of a complex vascular structure. Notably, this event accelerated malignant progression, strongly supporting the hypothesis that macrophages regulate tumor progression by establishing conditions that allow tumor angiogenesis to proceed aggressively (8).

## The Angiogenic Switch Is Required for Malignant Transition

In the MMTV-PyMT model, tumors progress through different, readily identifiable stages, and coupling this process to quantitative measures of vascularization in the whole tumor allowed a new definition of the basis for the angiogenic switch. We observed that, unlike previous reports suggesting that premalignant lesions were “avascular,” tumors before the malignant transition had developed a discernable vasculature. However, at this stage, its density and morphology were largely similar to those found in the surrounding normal tissues. We also observed that when the high-density vessel network developed, it was restricted to the area of the tumor displaying a malignant morphology. This feature was often seen in the center of the lesion. This observation also differed from

**Requests for reprints:** Jeffrey W. Pollard, DMB, 601 Chanin Building, Albert Einstein College of Medicine, 1300 Morris Park Avenue, Bronx, NY 10461. Phone: 718-430-2090; Fax: 718-430-8663; E-mail: pollard@aecom.yu.edu.

©2007 American Association for Cancer Research.  
doi:10.1158/0008-5472.CAN-07-0912

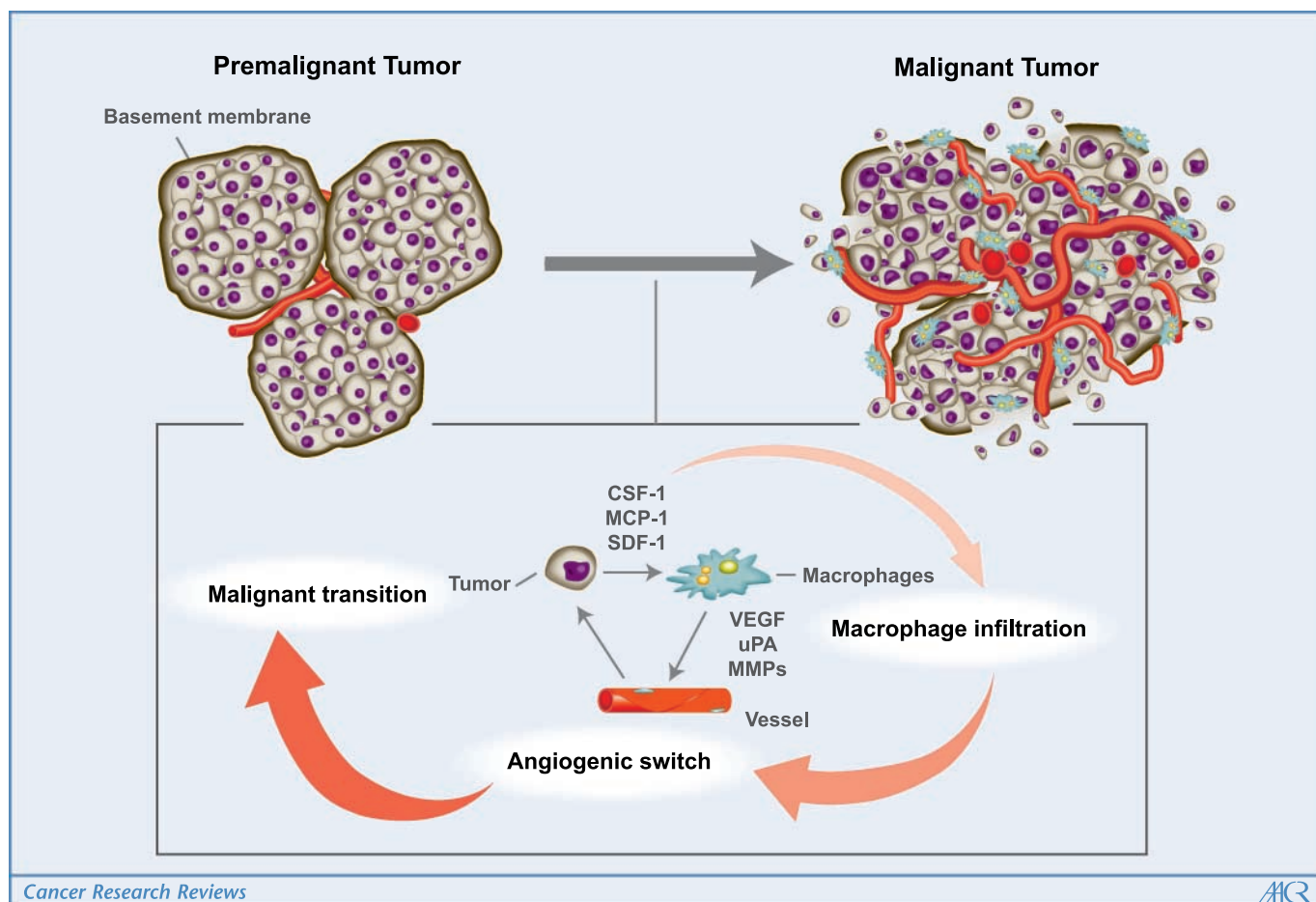
previous studies of transplantable tumors, where the angiogenic switch occurs through the formation of new capillaries that converge toward the tumor (6). These findings highlight an important difference between transplanted tumors and autochthonous transgene-induced tumors: only the latter spontaneously develop and progress within a normal tissue environment. The phenotype observed in these studies would be expected to be closer to that found in human cancers, based on the spontaneous autochthonous nature of the tumors formed in the MMTV-PyMT model.

These findings showed that malignant conversion and the angiogenic switch are closely associated events initiated within the tumor. In experiments wherein we prematurely induced the angiogenic switch in premalignant tumors, the acceleration in malignant conversion that occurred argued that the angiogenic switch is essential and causal for malignant conversion. Indeed, in the relative absence of macrophages, tumors can grow very large; however, if macrophages are not recruited, the angiogenic switch does not occur and these tumors do not progress to malignancy.

## Implications

Over a hundred years ago, English surgeon Stephen Paget proposed a “seed and soil” hypothesis to explain the nonrandom

pattern of metastasis. This model pointed out the importance of the interaction between tumor cells and the organ microenvironment during tumor metastasis. Recent studies have shown that such interactions between the tumor and its microenvironment are also very important for the survival, growth, and progression for primary tumors. Our research has provided insight into such cross-talk and identified a key role of macrophages in the initiation of the malignant transition, as illustrated by the model (Fig. 1). We propose that premalignant lesions are not avascular as they are sometimes referred to but that the vasculature developed in these lesions is largely similar to that observed in the surrounding normal tissue (suggesting that no additional blood supply is required to maintain these lesions). Nevertheless, at a certain stage of tumor progression, probably as a result of oncogenic mutations, tumor cells start to produce factors that act as macrophage chemoattractants such as CSF-1 or CC chemokine ligand-2, which induce macrophage infiltration and activation in the tumor. What triggers such changes in the tumor is still unclear. However, recent studies have shown that hypoxia may induce the expression of certain macrophage chemoattractants (10). In this manner, the newly recruited macrophages are then positioned to initiate the angiogenic switch by promoting the formation of new capillary network in the adjacent area surrounding the malignant cells that



**Figure 1.** Macrophages promote tumor progression through stimulation of angiogenic switch. The development of a high-density vasculature is required for tumor progression to malignancy (top). The initiation of this process, termed the angiogenic switch, results from the interaction between the tumor and its microenvironment. Tumor-derived chemoattractants such as CSF-1 and monocyte chemoattractant protein 1 (MCP-1 or CCL2) induce the infiltration of macrophages. These macrophages then promote the development of a high-density vascular network through the secretion of multiple angiogenic growth factors and proteinases. The mature tumor vasculature provides support for tumor progression and metastasis.

produce these chemoattractants. Through this mechanism, tumor-associated macrophages can facilitate a rapid expansion of a more malignant population of cells, allowing them to progress further toward a metastatic phenotype.

Macrophages may promote angiogenesis by producing angiogenic growth factors and proteinases including VEGF, matrix metalloproteinase-9, and uPA (7). Alternatively, they may recruit and interact with other cells in the tumor microenvironment (e.g., neutrophils that have also been shown to regulate the angiogenic switch in the RIP-Tag model of pancreatic cancer; ref. 11). Macrophages also have other functions that promote tumor progression (12). These include a stimulation of tumor cell migration, invasion, and, ultimately, intravasation into the circulation (13). Together, these observations suggest that tumor-associated macrophages confer not only the ability to develop a vasculature with its increase in vessel density but also a means of escape for tumor cells into the circulation. The ability of

macrophages to enhance metastasis in this manner may explain why macrophage depletion in this mouse model resulted in a significant inhibition of metastatic capacity (14).

In summary, these studies show that macrophages are key regulators of the angiogenic switch in tumors, suggesting why the density of tumor-associated macrophages is correlated with microvascular density and poor prognosis in human tumors (15–17). Based on these findings, the development of antimacrophage therapeutics that target specific pathways associated with angiogenesis might contribute valuable additions to the armamentarium of agents for treating cancer or preventing the conversion of benign lesions to malignant tumors.

## Acknowledgments

Received 3/7/2007; accepted 3/16/2007.

Supported by NIH grants CA94173 and CA100324, and Albert Einstein Cancer Center Core grant P30 CA13330.

## References

1. Folkman J, Watson K, Ingber D, Hanahan D. Induction of angiogenesis during the transition from hyperplasia to neoplasia. *Nature* 1989;339:58–61.
2. Hanahan D, Folkman J. Patterns and emerging mechanisms of the angiogenic switch during tumorigenesis. *Cell* 1996;86:353–64.
3. Hanahan D, Weinberg RA. The hallmarks of cancer. *Cell* 2000;100:57–70.
4. Naumov GN, Akslen LA, Folkman J. Role of angiogenesis in human tumor dormancy: animal models of the angiogenic switch. *Cell Cycle* 2006;5:1779–87.
5. Black WC, Welch HG. Advances in diagnostic imaging and overestimations of disease prevalence and the benefits of therapy. *N Engl J Med* 1993;328:1237–43.
6. Ribatti D, Nico B, Crivellato E, Roccaro AM, Vacca A. The history of the angiogenic switch concept. *Leukemia* 2007;21:44–52.
7. Pollard JW. Tumor educated macrophages promote tumor progression and metastasis. *Nat Rev Cancer* 2004; 4:71–8.
8. Lin EY, Li JF, Gnatovskiy L, et al. Macrophages regulate the angiogenic switch in a mouse model of breast cancer. *Cancer Res* 2006;66:11238–46.
9. Lin EY, Jones JG, Li P, et al. Progression to malignancy in the polyoma middle T oncoprotein mouse breast cancer model provides a reliable model for human diseases. *Am J Pathol* 2003;163:2113–26.
10. Crowther M, Brown NJ, Bishop ET, Lewis CE. Microenvironmental influence on macrophage regulation of angiogenesis in wounds and malignant tumors. *J Leukoc Biol* 2001;70:478–90.
11. Nozawa H, Chiu C, Hanahan D. Infiltrating neutrophils mediate the initial angiogenic switch in a mouse model of multistage carcinogenesis. *Proc Natl Acad Sci U S A* 2006;103:12493–8.
12. Condeelis J, Pollard JW. Macrophages: obligate partners for tumor cell migration, invasion, and metastasis. *Cell* 2006;124:263–6.
13. Wyckoff J, Wang W, Lin EY, et al. A paracrine loop between tumor cells and macrophages is required for tumor cell migration in mammary tumors. *Cancer Res* 2004;64:7022–9.
14. Lin EY, Nguyen AV, Russell RG, Pollard JW. Colony-stimulating factor 1 promotes progression of mammary tumors to malignancy. *J Exp Med* 2001;193:727–40.
15. Lewis CE, Pollard JW. Distinct role of macrophages in different tumor microenvironments. *Cancer Res* 2006;66: 605–12.
16. Leek RD, Harris AL. Tumor-associated macrophages in breast cancer. *J Mammary Gland Biol Neoplasia* 2002; 7:177–89.
17. Leek RD, Lewis CE, Whitehouse R, Greenall M, Clarke J, Harris AL. Association of macrophage infiltration with angiogenesis and prognosis in invasive breast carcinoma. *Cancer Res* 1996;56:4625–9.