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MMP9 potentiates pulmonary metastasis formation

Tumor cell dissemination to distant organ sites is a complex process involving multiple cell types, soluble growth factors, adhesion receptors, and tissue remodeling. A new study in this issue of *Cancer Cell* shows that MMP9-expressing tumor-associated macrophages play a key role in prepping premetastatic sites for eventual malignant cell growth in a manner dependent upon vascular endothelial growth factor receptor-1 (VEGFR-1).

Metastasis is the primary cause of morbidity and mortality for patients with cancer. While it has been recognized for many years that movement of neoplastic cells is not a random process, the molecular and cellular mechanisms governing their movement, survival through foreign tissue environments, and parameters for choosing and taking up residence at a final destination have remained uncertain. Several contrasting theories have emerged to explain metastatic specificity. The "homing" theory suggests that organs distal to sites of primary malignancy actively attract and/or arrest ("trap") malignant cells via expression of adhesion receptors, e.g., selectins, or by secretion of soluble chemotactic factors, e.g., chemokines. Indeed, there is good experimental evidence implicating chemokine receptors and their ligands for the chemoattraction aspect of the homing theory (Muller et al., 2001; Wilson and Balkwill, 2002). In addition, identification of "molecular addresses" or adhesion receptors on endothelial cells in vascular beds of distal organs that specifically trap circulating malignant cells supports the active "arrest" view of homing (Borsig et al., 2002; Laakkonen et al., 2002). In contrast, the "fertile soil" theory proposes that different organ environments provide optimal growth conditions for specific circulating cell types. Since underlying mechanisms supporting these theories are not mutually exclusive, it seems likely that distinct mechanisms/molecules might govern a malignant cells journey to an ectopic tissue, separate from those regulating its growth and/or survival once its destination has been achieved. An exciting observation by Shibuyu and colleagues (Hiratsuka et al., 2002 [this issue of *Cancer Cell*]) provides some clues into the later aspects of the metastatic cascade implicating an extracellular metalloproteinase, e.g., matrix metalloproteinase-9 (MMP9), made by tumor-associated macrophages (TAMs) and alveolar endothelial cells, in microenvironmental remodeling necessary for metastatic cell survival in the lung.

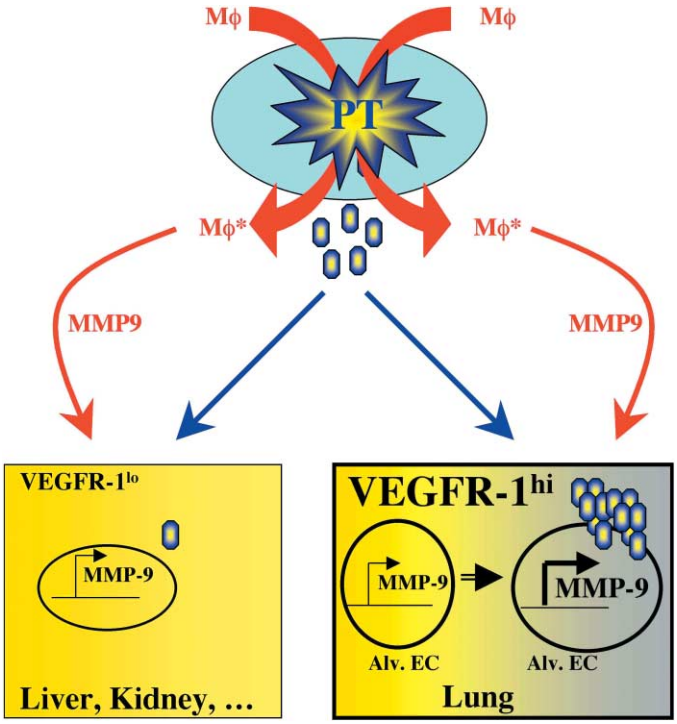

Figure 1. A model for the role of tumor-associated macrophages and MMP9 in priming premetastatic tissue

Macrophages (Mϕ) are recruited to sites of malignant growth. Upon activation by primary tumor (PT) cells, activated macrophages (Mϕ*) expressing MMP9 then reenter the circulation and induce MMP9 expression in (alveolar) endothelial cells (Alv. EC) in a VEGFR-1-dependent manner, thereby prepping premetastatic tissue for malignant cell colonization. Distal organs harboring low-level VEGFR-1 expression are not suitable environments for metastatic growth and fail to induce MMP9 expression in response to the presence of activated macrophages.

Tumor cells produce various cytokines and chemokines that attract leukocytes (macrophages, neutrophils, dendritic cells, eosinophils, and mast cells) that are variably loaded with molecules affecting primary neoplastic growth, e.g., cytokines, cytotoxic mediators, serine-, cysteine-, and metallo-proteases, membrane-perforating agents, and soluble mediators of cell killing, such as tumor necrosis factor-α (TNF-α), interleukins (IL), and interferons (IFNs) (Wilson and Balkwill, 2002). TAMs play a dual role in neoplasms. Whereas TAMs

may kill neoplastic cells following activation by IL-2, IFN, or IL-12, they produce a number of potent angiogenic and lymphangiogenic growth factors, cytokines, and proteases that potentiate neoplastic development. The functional significance of TAM recruitment to developing neoplasms was established by intercrossing transgenic mice susceptible to mammary cancer (PyMT mice) with mice containing a recessive null mutation in the colony stimulating factor-1 (CSF-1) gene (*Csf1^{op}*) (Lin et al., 2001). Whereas absence of CSF-1 during early neoplastic growth was without consequence, development of late-stage invasive carcinoma and their metastatic pulmonary derivatives were significantly attenuated. The key difference between PyMT mice and PyMT/*Csf1^{op}*/*Csf1^{op}* mice was in the failure to recruit mature macrophages into neoplastic tissue in the absence of CSF-1, implying that circulating macrophages and molecule(s) they secrete are necessary for productive metastatic growth. Studies by Hiratsuka et al. (2002) now provide evidence that macrophages are potentiators of lung metastases through MMP9 and vascular endothelial cell receptor-1 (VEGFR-1)-dependent mechanisms (Figure 1).

Using a mouse model of experimental metastasis formation, Hiratsuka et al. (2002) have reported that following recruitment to sites of primary tumor growth, TAMs circulate to distal organs. Distal organs exhibiting low-level expression of VEGFR-1 fail to induce MMP9 in response to TAM presence and are therefore not suitable environments for subsequent metastatic cell growth. On the other hand, distal organs that are VEGFR-1-positive and contain a population of endothelial cells capable of inducing expression of MMP9 above that supplied by circulating TAMs are fertile sites for productive metastatic growth. While induced expression of the VEGFR-1 ligand VEGF-A does not appear to be involved, presence of an active VEGFR-1 tyrosine kinase domain is necessary; thus, it seems reasonable based on previous reports (Bergers et al., 2000) that activated MMP9 releases matrix-sequestered VEGF-A, rendering it bioavailable for interaction with its receptors and thereby stimulating efficient vascular remodeling and angiogenesis necessary for metastatic cell growth and survival. Administration of a synthetic metalloproteinase inhibitor (BB-94) attenuates metastatic growth in the lung in this model, suggesting that the enzymatic activity of MMP9 is also a necessary component of the pathway.

While VEGF-A is known to be a matrix-sequestered molecule, it is likely that other growth factors, cytokines, and perhaps chemokines may similarly have limited bioavailability characteristics based on their affinities toward diverse matrix molecules. Chemokines are certainly involved in regulating chemotaxis of leukocytes (Wilson and Balkwill, 2002) and are now also known to impart organ-specific metastatic potential on chemokine receptor-positive neoplastic cells (Muller et al., 2001). Thus, one outstanding question is whether MMP9 also regulates the bioavailability characteristics of lung-specific chemokines involved in recruiting malignant cells, or alternatively, whether it regulates the activities of an adhesion receptor of the selectin family necessary for "trapping" malignant cells as they circulate.

The current report by Hiratsuka et al. (2002) offers a new perspective on the multifaceted activities of MMP9 and the role of TAMs in prepping a distal organ for arrival and eventual metastatic colonization. Historical literature reporting on MMP9 function(s) in vitro has provided a compelling story implicating MMP9 as a rate-limiting extracellular protease involved in cell migration across basement membranes. Likewise, the work by Hiratsuka et al. (2002) also reveals a role for MMP9 in facilitating tumor cell invasion; however, it is not clear if this is a mechanistic role or if MMP9 activity creates an environment amenable for migration. Studies of cellular invasion in tumor-prone mouse models harboring homozygous null deletions in the MMP9 gene or in animals (or humans) treated with broad spectrum MMP inhibitors do not suffer from impaired migratory or invasive behavior of malignant cells (Bergers et al., 2000; Coussens et al., 2000, 2002). Instead, cells growing in MMP9-deficient environments have altered proliferative or differentiation capacity, largely due to altered growth factor or survival factor bioavailability (Bergers et al., 2000; Coussens et al., 2000; Liu et al., 1998; Vu et al., 1998). In sum, the combined implication of this data suggests that TAM-induced MMP9 expression, in alveolar endothelial cells as well as in TAMs, fertilizes the soil necessary for secondary malignant cell growth, dependent upon the presence and activation of the VEGF-VEGFR signaling cascade.

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