Tumor progression: **Defining the soil round the tumor seed**
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The tumor microenvironment, or stroma, is known to contribute to tumor progression. Two recent studies have shown that the stromal protein matrix metalloproteinase MMP-9 has a role in the early stages of tumor growth and angiogenesis.

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Enormous advances have been made over the past several decades in identifying the molecular determinants of carcinogenesis. Alterations in specific oncogenes and tumor suppressor genes have been identified and shown to have causal roles in the initiation, maintenance and progression of tumors, in systems such as chemically induced mouse skin tumors and human colorectal cancer [1,2]. This genome-centric view of tumor progression, however, has largely ignored the substantial contribution of the tumor microenvironment to the malignant phenotype [3–5]. Although the 'seed and soil' hypothesis of Paget [6] dates to 1889, the molecular determinants of the 'seed' are much better delineated than those of the 'soil' for either primary or metastatic lesions. But two groups [7,8] have now identified an enzyme produced by the normal cellular component of the tumor that is a critical player in skin and pancreatic islet carcinogenesis.

Matrix metalloproteinases are a family of over 20 enzymes that are characterized by their ability to degrade the extracellular matrix (ECM) and their dependence upon Zn^{2+} binding for proteolytic activity [9]. These proteinases have been implicated in a variety of normal and pathological cellular processes, including organ involution, wound healing and tumor cell invasion. Because of their matrix-degrading activity, and the correlation between high levels of their enzymatic activity and elevated tumor metastasis, matrix metalloproteinases were initially thought to act by destroying the basement membrane and other components of the ECM, and thereby facilitating tumor cell invasion and metastasis. Recent work, however, has shown that matrix metalloproteinases make other contributions to tumor progression, for example by effects on cell proliferation, survival and angiogenesis [10].

At the same time as we have begun to understand the multiple roles played by matrix metalloproteinases in tumor progression, a broadening picture has developed of the functions of both the ECM and the non-matrix substrates of matrix metalloproteinases. The latter substrates include other matrix metalloproteinases, which are activated by proteolysis, as well as cell-surface and matrix-bound growth factors, which are released by proteolysis [11]. Thus, today matrix metalloproteinases are known to function at multiple steps of tumor progression through mechanisms that include the proteoylsis of both matrix and non-matrix targets.

Matrix metalloproteinases have been considered promising targets for anti-cancer drugs, and a number of synthetic matrix metalloproteinase inhibitors have been developed and tested [12,13]. Because of the unexpected side effects of such inhibitors — joint pain and immobility — it has become of critical importance to identify the specific matrix metalloproteinases involved in tumor progression. Progress towards this end has recently been reported by two groups [7,8] who have effectively combined use of well-characterized mouse models of tumorigenesis with targeted ablation of individual matrix metalloproteinases. The two groups both used mice with targeted mutations of the *MMP-9* gene, but they used distinct tumor models: skin tumorigenesis in one case [7], and pancreatic islet cancer in the other [8].

MMP-9, otherwise known as gelatinase B or 92 kDa type IV collagenase, is characterized by its ability to cleave basement membrane and denatured collagens (gelatin) [9]. MMP-9 is produced by a variety of cell types, including epithelial cells, fibroblasts, endothelial cells and inflammatory cells. Targeted ablation of *MMP-9* is not lethal in mice, but it does cause a mild skeletal defect. The skeletal growth plate of the mutant mice showed a delayed development, characterized by a delay in apoptosis, vascularization and ossification in the developing hypertrophic chondrocytes [14]. The existence of viable, healthy *MMP-9* null mice allowed investigation into the specific contribution of MMP-9 to tumor progression.

Coussens *et al.* [7] examined the effects of MMP-9 ablation in a transgenic skin model of tumorigenesis, in which the early gene regions of the human papilloma type 16 (HPV16) genome are expressed under the control of the keratin 14 promoter. These mice develop hyperplasias in the squamous epithelium on their trunk and ears, which progress into squamous cell carcinomas with a 50% incidence by one year of age. In earlier work, the group [15] demonstrated that tumor progression in this mouse model is accompanied by angiogenesis coupled with an influx of mast cells and enhanced levels of active MMP-9. Now

Coussens *et al.* [7] have gone on to determine the contribution of MMP-9 to skin tumorigenesis directly, by crossing the *MMP-9* null mice with the *K14-HPV16* mice.

The progeny of this cross that are both null for *MMP-9* and carry the *K14-HPV16* transgene were found, at one year of age, to have a reduced incidence of squamous cell carcinoma and reduced cell proliferation, compared to wild-type, *K14-HPV16* transgenic counterparts. Inflammatory cells — particularly mast cells, neutrophils, and macrophages — were the primary sites of MMP-9 production. Using a strategy previously shown to reverse the effects of *MMP-9* ablation on skeleton [14], bone marrow cells from wild-type mice were introduced into the *MMP-9* null, *K14-HPV16* transgenic animals [7]. The response was striking. All of the wild-type phenotypes were restored, including the proliferative response and the incidence of squamous cell carcinoma at one year of age [7]. This suggests that *MMP-9*-expressing bone marrow cells are involved in regulating cell proliferation and tumor progression in the *K14-HPV16* model of skin tumorigenesis.

Bergers *et al.* [8] took a similar approach to investigating the role of MMP-9 in pancreatic islet carcinoma. They used a transgenic model of pancreatic cancer, *RIP1-Tag2* mice whose insulin-producing beta cells express SV40 T antigen. The islets of these transgenic mice undergo a well-defined progression from hyperplastic, precancerous nodules to large invasive carcinomas over a 14 week time period. An angiogenic response is a critical step in the progression of these tumors. An earlier study [16] investigated the effects of the synthetic, broad-spectrum matrix metalloproteinase inhibitor BB-94 on tumor progression in this model system. BB-94 was found to reduce angiogenesis in precancerous islets and also growth of tumors when given at an early stage in progression.

In their more recent study, Bergers *et al.* [8] addressed the roles of MMP-9 and MMP-2, another gelatinase, in pancreatic islet carcinoma by crossing MMP-9 or MMP-2-deficient animals with *RIP1-Tag2* mice [8]. The MMP-9-deficient, *RIP1-Tag2* transgenic progeny showed reduced angiogenesis and tumor growth; but while the MMP-2-deficient, *RIP1-Tag2* transgenic progeny also showed reduced tumor growth, they developed similar angiogenic lesions in the hyperproliferative islet cells to wild-type controls. The distinction between the angiogenic response of pancreatic islets in the presence of MMP-9 or MMP-2 was also apparent *in vitro*: treatment of normal islets with MMP-9, but not MMP-2, could generate a morphogenic response in endothelial cells. Interestingly, *MMP-9* expression was localized, not to the transgene-expressing epithelial cells, but to a small number of cells in close apposition to the vasculature characteristic of infiltrating inflammatory cells. These results indicate that stromal MMP-9 plays an important role in establishing the tumor vasculature.

The substrate responsible for MMP-9's activity in angiogeneis has not been clearly established, but has been suggested to be vascular endothelial growth factor (VEGF) which is released from vascular basement membrane and the ECM in the *RIP1-Tag2* model. Bergers *et al.* [8] showed that VEGF is critical for the angiogenic switch, but that the growth factor and its receptors are expressed constitutively in *RIP1-Tag2* mice. They found that *MMP-9* null mice show reduced coupling of VEGF with one of its receptors in the angiogenic pre-cancerous islets, and *in vitro* studies indicated that the release of VEGF into the supernatant is influenced by MMP-9 [8]. The role of MMP-9 in this system thus appears to be to facilitate the accessibility of angiogenic molecules to endothelial cells, an activity that represents 'seed and soil' interactions.

One insight from these studies [7,8] that is of particular interest is the early stage of tumor progression at which matrix metalloproteinase ablation was effective. In contrast to the notion that matrix metalloproteinases facilitate tumor invasion and metastasis, the new studies [7,8] support the view that MMP-9 plays a role in the control of cell proliferation and the angiogenic switch, and is a determinant of the overall tumor burden. In fact, in the *K14-HPV16* skin tumorigenesis model, although fewer tumors developed in the absence of MMP-9, a higher proportion of those that did develop had a phenotype characteristic of more highly malignant cancers [7]. Furthermore, administration of the matrix metalloproteinase inhibitor BB-94 at both early and intermediate stages of tumor progression in the *RIP1-Tag2* model reduced the tumor burden, but had no effect on large, established tumors [16]. Thus, the absence of MMP-9 retarded tumor development in early stages of establishment and growth, but did not impede the progression of well established tumors to a more advanced stage.

These results have important implications for the use of matrix metalloproteinase inhibitors in a clinical setting. Several matrix metalloproteinase inhibitors have reached the stage of clinical trials as anti-angiogenic or anti-metastatic agents, and have been tested for their efficacy in the treatment of advanced pancreatic, gastric, prostate and lung cancer [12]. The results of the phase III trials with matrix metalloproteinase inhibitors have been disappointing, with no beneficial effects of matrix metalloproteinase inhibition; indeed, in one study, patients treated with a matrix metalloproteinase inhibitor performed worse than placebo-treated controls [13,17]. An exception to this gloomy scenario is gastric cancer, where treatment with the matrix metalloproteinase inhibitor marimastat was found to have a beneficial effect on the survival of patients with no evidence of metastatic disease [18].

The clinical data are therefore consistent with the results reported by Coussens *et al.* [7] and Bergers *et al.* [8,16]:

Figure 1
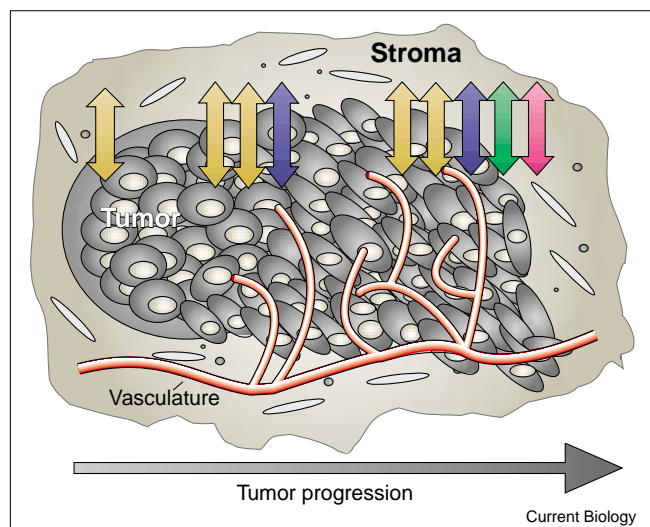
Tumor–stroma communication during tumor progression. The diagram illustrates tumor progression from a hyperplastic, premalignant stage through the 'angiogenic switch' to an invasive, metastatic cancer. The colored arrows indicate lines of communication between tumor cells and surrounding stromal components of the tumor, including fibroblasts, endothelial cells, inflammatory cells and structural matrix components. The gold arrows may be representative of the contribution of matrix metalloproteinases, which represent a greater proportion of the communication signals in early, as compared to later stages of tumor progression.

matrix metalloproteinase inhibition has little impact on advanced-stage tumors, but it can be effective if the inhibitor is administered during earlier stages of tumor progression. Matrix metalloproteinase inhibition has also been shown to be effective in the early stages of intestinal tumor formation. In a mouse model of intestinal tumor progression, animals that were either deficient in MMP-7 (also known as matrilysin) or treated with a synthetic matrix metalloproteinase inhibitor were found to develop a reduced number of benign tumors [19,20]. Matrix metalloproteinase inhibition could thus be considered as a strategy for preventing the progression of tumors from a premalignant to a malignant state.

Why does MMP-9 activity appear to be more critical in early stages of tumor progression? This may mean that matrix metalloproteinase activity is essential early on, when the tumor is being established and harnessing critical functions of the surrounding stroma, but does not play an indispensable role in maintenance of the tumor. A number of genetic and epigenetic changes occur throughout tumorigenesis [1–5]. As the tumor progresses, there are more signals serving redundant functions and any one of these becomes less critical for maintaining overall tumor survival (Figure 1). The recent studies [7,8] suggest that MMP-9 has a critical role in early stages of pancreatic

islet and skin tumorigenesis; loss of just one matrix metalloproteinase, however, does not eliminate tumor formation altogether. The results support the view that matrix metalloproteinase inhibitors would be more efficacious in cancer treatment if given at an earlier stage, and in combination with other therapies.

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