Coevolution of cancer and stromal cellular responses

It is now becoming apparent that multiple types of stromal cells, including macrophages, mast cells, adipocytes, and fibroblasts make pivotal contributions to carcinogenesis. In the May 6 issue of *Cell*, Orimo and colleagues (Orimo et al., 2005) show that carcinoma-associated fibroblasts can promote epithelial tumorigenesis by secreting the chemokine SDF-1α (CXCL12). SDF-1α stimulates carcinoma cell proliferation and recruitment of endothelial precursor cells.

A remarkable evolution in thinking about epithelial-derived cancer has taken place over the past several years. From an oncogene- and tumor suppressor gene-centric view of cancer initiation and progression has come the realization that the tumor microenvironment is a coconspirator in the carcinogenic process. The idea that the "host" stroma (mesenchyme) talks to and coevolves with the mutated epithelium (ectoderm) during progression comes as no surprise to developmental biologists, who have been dealing with instructive and permissive interactions between the two compartments for a century. However, except for a couple of lonely prescient scientists, it is only recently that tumor biologists have latched on to this idea. So which cell types of the microenvironment are involved, do they have a specific "activated" state, and what molecules do they use to communicate?

It is becoming accepted that the stromal microenvironment contributes to tumorigenesis in the cancers that are epithelial in origin. Although the initiating mutation usually occurs in the epithelium, the event that promotes tumor progression involves the stroma. In fact, in some cases, the trigger for neoplastic progression may come from signals within the stromal microenvironment (reviewed in Bhowmick et al., 2004b). There are several known mechanisms for generating aberrant tumor-promoting stroma in vivo. The classic way is to expose stroma to tumor promoters such as phorbol esters, which trigger the inflammatory response. Another way is to irradiate the stroma. Genetic evidence for stromal contribution comes from studies on fibroblast secreted protein-1 (FSP1, also called S100A4 or mts1), which is upregulated in carcinoma-associated fibroblasts (CAFs) during tumor progression. Metastatic carcinoma cells injected into *Fsp1*$^{-/-}$ mice are less likely to form tumors and do not metastasize. Interestingly, coinjection of *Fsp1*$^{+/+}$ fibroblasts with the tumor cells restores tumor development and metastasis (Grum-Schwensen et al., 2005). This suggests that Fsp1, which is secreted by the fibroblasts, alters the stromal microenvironment, making it more favorable for tumor progression. In contrast, mice with a cell-specific ablation of the TGF-β receptor II in a subset of stromal fibroblasts, which renders them unresponsive to TGF-β signaling, develop neoplasias and carcinomas in the absence of any additional induced mutations in the epithelium (Bhowmick et al., 2004a). This suggests that TGF-β not only prevents proliferation of both the fibroblasts and epithelial cells, but also somehow protects the epithelium from developing hyperplasias or even carcinomas. However, the action of TGF-β is cell type specific; ablation of the TGF-β receptor II in epithelial cells inhibits tumor progression (Forrester et al., 2005).

Irradiation of the mammary gland stroma also induces nonreversible changes in the stroma that contribute to neoplasia: nontransformed mammary epithelial cells injected into irradiated mammary stromal fat pads have greatly increased tumor growth compared to those injected into the contralateral, nonirradiated mammary fat pads (Barcellos-Hoff and Ravani, 2000).

The above-mentioned studies show that there are both tumor-promoting and tumor-inhibiting activities mediated by CAFs. Now, Orimo and coworkers (Orimo et al., 2005) have identified another mechanism whereby stromal CAFs can promote tumorigenesis, through the secretion of stromal cell-derived factor 1α (SDF-1α, also known as CXCL12). SDF-1α acts directly on the mammary carcinoma cells stimulating proliferation through

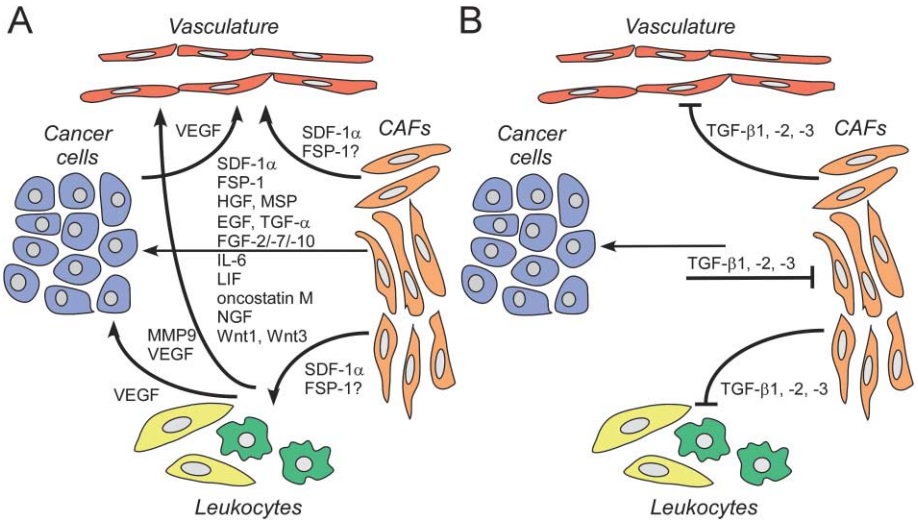

Figure 1. Molecular mediators of stromal-epithelial interactions in tumorigenesis

The cells in the tumor tissue communicate during tumor progression through the secretion of growth factors, chemokines, and cytokines.

A: Examples of stimulators of tumorigenesis secreted by one cell type and acting on another.

B: TGF-β is unique in its ability to both promote and inhibit tumorigenesis, depending on the cell type it is acting on. CAFs, carcinoma-associated fibroblasts; VEGF, vascular endothelial growth factor; SDF-1α, stromal derived factor 1α; FSP-1, fibroblast-specific protein-1; HGF, hepatocyte growth factor; MSP, macrophage-stimulating protein; EGF, epidermal growth factor; TGF-α, transforming growth factor-α; TGF-β, transforming growth factor-β; FGF, fibroblast growth factor; IL-6, interleukin 6; LIF, leukemia inhibitory factor; NGF, nerve growth factor (Bhowmick et al., 2004b, and references therein).

the SDF-1α receptor CXCR4 found on the cancer cells, but SDF-1α secretion also leads to recruitment of endothelial cell precursors (EPCs) to the growing tumor, thereby promoting angiogenesis. SDF-1α's ability to recruit the EPCs and promote angiogenesis might be the result of the activation of matrix metalloproteinase-9 (MMP-9). SDF-1α treatment activates MMP-9 in bone marrow cells, and *MMP-9*$^{-/-}$ mice are unresponsive to SDF-1α-induced recruitment of the hematopoetic and endothelial precursor cells (Heissig et al., 2002).

TGF-β, FSP-1, SDF-1α, VEGF, MMP-9, and a number of other stromal factors have been implicated in epithelial tumor progression (Figure 1), but we do not know if these factors are coconspirators with each other, if they are additive, synergistic, or are independent pathways of varying importance in different tumors. Interestingly, there are clear links between TGF-β1 and SDF-1, which complicates the interpretation of the role of CAFs: TGF-β1 upregulates the SDF-1/CXCL12 receptor CXCR4 on macrophages, eosinophils, NK cells, T cells, and hematopoietic progenitor cells, thereby enhancing SDF-1α's effects (e.g., Chen et al., 2005). Thus, TGF-β and SDF-1α likely act synergistically, at least when it comes to their effects on inflammatory cells.

We still know very little about what these tumor-promoting CAFs are and what distinguishes them from their counterpart fibroblasts found from the same tissue only a few centimeters away. Like true fibroblasts, CAFs express vimentin. However, they also express α-smooth muscle actin and can contract collagen gels in vitro, thus resembling myofibroblasts. Thus, the origin of these cells is unclear. They could be derived from fibroblasts, fibroblast precursors, myofibroblasts, or a different cell type, such as preadipocytes or smooth muscle cells. Interestingly, the CAFs isolated by Orimo and coworkers maintain their ability to stimulate tumor progression through several cell passages, but show no evidence of genetic alterations and senesce normally in culture. Thus, the CAFs could not have evolved from the cancer cells, and the signals in the early tumor that result in their expansion are not required to preserve the characteristics of the cells. It is tempting to speculate that the CAFs are an expanded population of an early developmental precursor initially present in the normal precancerous mammary gland, a population that expands in response to signals from the cancer cells.

While we have focused on the contributions of the fibroblast-like cells, the CAFs, to tumor evolution, both the fibroblasts and the carcinoma cells exist in the context of a tumor "tissue" with multiple cell types, including leukocytes, endothelial cells, and adipocytes, all of which have been shown to adapt to, coevolve with, and shape tumor progression. In animal experiments, it has profound effects on tumor progression when one of these stromal cell types is removed or their behavior changed by genetic alterations. Thus, we can deduce that signaling takes place between virtually all of the cell types in the tumor tissue, although only a limited number of signaling molecules involved in the communications between cell types within the tumor tissue has been identified (Figure 1). Orimo and colleagues used a xenograft model employing immune-compromised mice for their studies and did not address signaling links between CAFs and leukocytes mediated by SDF-1α. The regulation of tumor progression by leukocytes has been shown in numerous studies, and SDF-1α is a well-established chemoattractant for leukocytes. Thus, it is very likely that SDF-1α would have additional effects acting through leukocytes if studied in the context of a full cellular immune response.

Significantly, genes expressed by the stromal cells in the tumors are prognostic predictors in human breast cancer (West et al., 2005). Thus, the stromal contribution to cancer is not only of academic interest to a cancer biologist working with animal models; it has huge relevance for cancer patients and offers great potential for treatment of cancer. Whereas a normal stroma may protect the epithelium from tumorigenesis, an aberrant stroma can initiate tumorigenesis. Stunningly, restoration of abnormal microenvironmental signaling can reverse the malignant phenotype even though the tumor cells retain all their mutations (Bissell and Radisky, 2001). We are still far from developing strategies to interrupt signaling between CAFs and the carcinoma cells. However, drugs that target endothelial cells and thereby inhibit angiogenesis are showing significant effects on cancer patient survival, even if the first generation of drugs is no magical bullet to cure cancer. The complex signaling network within the cancer cells has long been studied and is the target of drug development. The complex signaling between cells in the tumor tissue could be next.

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