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The Biology Behind

Tumors and Their Microenvironments: Tilling the Soil

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The Shifting Paradigm
The conventional concept of tumor-host interactions is rooted in the realm of parasitology. A tumor invades an unsuspecting host, and in response to this assault, the host attempts to resist the advances of the tumor by calling on the armamentarium of the immune system. This implies a fundamental opposition between the tumor and host in purpose and intent. However, an accumulation of evidence points to an alternative paradigm, where the tumor microenvironment is not an idle bystander, but actively participates in tumor progression and metastasis (1, 2). In fact, stromal cells and their cytokines coordinate critical pathways that exert important roles in the ability of tumors to invade and metastasize (3).

In this issue, Scott et al. (4) describe a Phase I clinical trial using the radiolabeled antibody sibrotuzumab to target the tumor stromal antigen FAP2 in metastatic lung and colon cancers. Human FAP is unique in its selective expression by tumor stromal fibroblasts in epithelial carcinomas, but not by epithelial carcinoma cells, normal fibroblasts, or other normal tissues. Therefore, FAP is an attractive target for the study of tumor stromal cell biology and provides valuable insights into the roles of the tumor microenvironment. FAP may exemplify pathways by which the microenvironment soil is “tilled” in preparation for tumor invasion and metastasis.

The Tumor Microenvironment Soil
The process of tumor metastasis was described using the “seed and soil” analogy by Paget (5) more than 100 years ago. His landmark paper in 1889 examined 735 patients with breast cancer at autopsy and showed that the distribution of metastases did not occur by chance, but was regulated by the predisposition of congenital soil. For example, the liver had a 14-fold higher incidence of metastases compared with the spleen, although these organs roughly possess the same circulatory volume. Paget (5) proposed that tumor cells, or “seeds,” were randomly scattered by vascular routes, but could only form metastatic deposits if they landed in congenital territory, or “soil.” He hypothesized that tumors have a “semenal influence” on the metastatic microenvironment, and thereby act together with the distant organ to effect tumor metastases. The identity of these seminal influences is a subject of active investigation but remains elusive. However, the importance of tumor-associated fibroblasts, the predominant cell in the microenvironment, has become more evident.

Tumor Stromal Fibroblasts: Contracted Farmers
Both in vivo and in vitro studies have demonstrated that fibroblasts contribute to tumor formation and growth rates (6), and can be thought of as “contracted farmers” used by tumors to prepare the microenvironment. Fibroblasts cocultured with breast or bladder tumor cell lines in nude mice shorten tumor latency and increase tumor growth (7). Fibroblasts cultured from malignant tumors have stimulatory effects on MCF-7 cells, whereas fibroblasts cultured from normal tissue are inhibitory (8). Phenotypic differences among tumor-associated fibroblasts have also been seen. Fibroblasts with smooth muscle differentiation, termed myofibroblasts, are abundant in the stromal cells of malignant breast tissue but are rarely seen in normal breast tissue (9). These findings suggest that tumor-associated fibroblasts are functionally distinct compared with fibroblasts that are not in the tumor microenvironment, and subpopulations of fibroblast may perform specialized functions to coordinate events required for tumor invasion and metastasis. Thus, fibroblasts provide critical frameworks for tumor growth and metastasis.

The microenvironment can also influence tumorogenic transformation. Kinzler and Vogelstein (10) proposed the concept of landscaper defects to explain the increased incidence of colorectal cancer in patients with juvenile polyposis syndrome or ulcerative colitis. In this situation, the proliferating stromal cells predispose the normal epithelial cells to undergo malignant transformation. Thus, stromal inflammatation and proliferation eventually lead to the development of malignant epithelial transformation because of an altered microenvironment terrain. Thus, the gene expression pattern of the fibrotic stroma is distinct from that of the carcinoma cells. Iacobuzio-Donahue et al. (11, 12) used serial analysis of gene expression techniques in breast cancer to show that five regions of invasive tumors can be identified, each with a distinct gene expression profile. These regions consist of the neoplastic epithelium, angioendothelium, inflammatory stroma, panstroma, and juxtatumoral stroma. Furthermore, they identified receptor-ligand pairs between the neoplastic epithelium and the surrounding stroma, implying significant communication between host and tumor compartments. In addition, loss of heterozygosity has been identified in the fibrotic reaction surrounding pancreas cancer that is distinct from the loss of heterozygosity pattern in pancreas adenocarcinoma (13). A number of these genes identified in the

Received 3/25/03; accepted 3/28/03.
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2 The abbreviations used are: FAP, fibroblast activation protein.
stroma had not been associated previously with pancreatic adenocarcinoma. These studies imply that tumor stroma may contribute independently to tumor development through mutation-inducing phenotypes. Such considerations challenge preexisting notions of a passive stroma that is permissive of local tumor growth.

FAP: A Tractor

The current study by Scott et al. (4) demonstrates the ability to safely administer the humanized anti-FAP antibody sibrotuzumab to patients with FAP-positive malignancies with tumor-specific uptake as seen by biodistribution images. This clinical trial is an extension of the groundbreaking work by Garin-Chesa et al. in discovering the unique distribution of FAP on tumor stromal fibroblasts (14), and has been confirmed by Welt et al. (15) as a clinical target. FAP, also known as seprase (16), is a M, 95,000 cell surface glycoprotein expressed transiently in healing wounds, and abundantly on reactive stromal fibroblasts in >90% of human epithelial carcinomas (breast, lung, colorectal, and ovary; Ref. 14). Neural and lymphoid cells, as well as surrounding normal tissue are FAP-negative. Bone and soft tissue sarcomas occasionally express FAP (17), consistent with the mesenchymal origin of FAP. FAP expression in normal tissue has only been seen in a subset of pancreatic endocrine cells and transiently in healing wounds. The specificity of FAP for tumor fibroblasts but not tumor cells has been confirmed by immunohistochemical staining in colorectal cancer patients (15, 18), breast cancer patients (19), reverse transcription-PCR of pancreas, lung, and renal cell xenografts (20), mixed hemadsorption rosetting assays from breast cancer-derived cultured fibroblasts (21), and renal cancer-derived fibroblasts (22). FAP exhibits both dipeptidyl peptidase and collagenase proteolytic activity (18). Thus, FAP can cleave NH₂-terminal dipeptides from polypeptides with L-proline or collagenase proteolytic activity (18). The clinical antibody sibrotuzumab to patients with FAP-positive malignancies with tumor-specific uptake as seen by biodistribution images. This clinical trial is an extension of the groundbreaking work by Garin-Chesa et al. in discovering the unique distribution of FAP on tumor stromal fibroblasts (14), and has been confirmed by Welt et al. (15) as a clinical target. FAP, also known as seprase (16), is a M, 95,000 cell surface glycoprotein expressed transiently in healing wounds, and abundantly on reactive stromal fibroblasts in >90% of human epithelial carcinomas (breast, lung, colorectal, and ovary; Ref. 14). Neural and lymphoid cells, as well as surrounding normal tissue are FAP-negative. Bone and soft tissue sarcomas occasionally express FAP (17), consistent with the mesenchymal origin of FAP. FAP expression in normal tissue has only been seen in a subset of pancreatic endocrine cells and transiently in healing wounds. The specificity of FAP for tumor fibroblasts but not tumor cells has been confirmed by immunohistochemical staining in colorectal cancer patients (15, 18), breast cancer patients (19), reverse transcription-PCR of pancreas, lung, and renal cell xenografts (20), mixed hemadsorption rosetting assays from breast cancer-derived cultured fibroblasts (21), and renal cancer-derived fibroblasts (22). FAP exhibits both dipeptidyl peptidase and collagenase proteolytic activity (18). Thus, FAP can cleave NH₂-terminal dipeptides from polypeptides with L-proline or L-alanine in the penultimate position (23), as well as degrade gelatin and native type I collagen (24). Mutation of the catalytic serine residue abolishes FAP dipeptidyl peptidase and collagenase activity (18). The clinical antibody sibrotuzumab does not appear to affect FAP enzymatic function, one of the most intriguing aspects of this target.

We have demonstrated that FAP overexpression in an animal model results in a greater propensity for tumor development, a shortened latency period, and an enhanced growth rate compared with mock-transfected controls (24). Because FAP is not expressed by epithelial carcinomas, but only by tumor fibroblasts, this provides the proof-of-principle that this stroma-specific protein can alter tumor growth properties. If tumor fibroblasts can be thought of as contracted farmers hired by tumors to prepare the microenvironment soil, then FAP would be analogous to the tractors the fibroblasts use to till the soil so tumors can flourish.

Fallow Ground of Wound Healing and Desmoplasia

FAP is also expressed in healing wounds and in desmoplasia. The congenial territory that Paget (5) described may be demonstrated clinically in the fallow ground of wound healing and desmoplasia (Fig. 1B). An activated environment in the absence of malignant transformation, such as that seen in healing wounds, can provide fertile soil for malignant cell “homing.” Experimental animal models demonstrate that tumor metastases arise preferentially in healing wounds (25, 26). The incidence of wound recurrences is proportional to the extent of wound trauma, as larger wounds and wounds that are not surgically repaired exhibit greater frequencies of metastasis. This has been confirmed clinically in the occurrences of surgical wound and port site metastases (27, 28). Given the ability of the wound healing environment to promote tumor cell proliferation (29), tumors may subvert normal wound healing machinery to activate factors necessary for tumor metastases. Therefore, tumor cells may initiate a permissive microenvironment by inducing expression of permeability factors, procoagulants, or chemotactic and mitogenic factors.

Similarly, the fibrotic reaction seen in desmoplasia tends to confer a worse prognosis and may represent a histopathologic marker of fertilized soil. Invasive ductal carcinoma of the breast with a fibrotic focus has more aggressive clinical characteristics than carcinoma without fibrosis (30, 31). The identification of fibrotic foci predicts worse overall survival, a higher incidence of lymph node and distant organ metastases, greater angiogenic activity, and a higher tumor cell proliferation rate. Similar observations have correlated desmoplasia of colorectal carcinomas with liver metastases (32) and worse prognosis (33). In addition, focal fibrosis correlates with the proliferative activity of tumor-associated fibroblasts (34). These studies suggest that proliferating fibroblasts in the tumor microenvironment generate fibrotic foci and increase the invasive characteristics of carcinomas. The mechanisms by which this occurs are unknown, although the many similarities of tumor stroma desmoplasia with wound healing pathways have been noted (29).

Because fibroblasts are responsible for the desmoplastic reaction (35), as well as the production of the majority of proteinases in the tumor microenvironment (36, 37), it is reasonable to hypothesize that the desmoplastic reaction is a histopathologic marker of proteinase activity by the tumor fibroblasts. In such a scenario, tumor cells induce surrounding fibroblasts to express a number of proteases to facilitate tumor invasion by remodeling extracellular matrix, inducing angiogenesis, and promoting motility. This protease milieu initiates a fibrotic reaction in the tumor microenvironment, with subsequent scarring that is manifested histologically as the fibrosis of desmoplasia. Thus, the tumor stroma provides critical factors, distinct from those produced by the neoplastic cells, that are required for tumor invasion and metastases.

FAP and Frogs

FAP has been identified not only in wound healing and desmoplasia, but also in amphibian metamorphosis. Amphibian tail resorption in tadpoles induces a set of complex developmental changes similar to those seen with tumor invasion and metastasis. These changes include epithelial proliferation, apoptosis, and tissue remodeling with redistribution. The invasion of the collagen basement lamella by mesenchymal cells during tail resorption is strikingly similar to tumor invasion. Proteolytic enzymes that are commonly seen in the tumor stroma have been identified as part of the tail resorption program. These include collagenase 3, stromelysin 3, and FAP (38). In fact, FAP ex-
Fig. 1 FAP: a model for tumor-stromal epithelial interactions.
pression is highest within the subepidermal fibroblast layer of the resorbing tail (39). This is consistent with the tumor fibroblast localization of FAP in the tumor microenvironment. Thus, there is a striking convergence of FAP expression in the tumor microenvironment, in healing wounds, and in physiological organ remodeling.

**Not All Seeds Germinate**

Multiple tumor-stromal interactions and extensive cross-talk are required to coordinate the multiple events of the metastatic process. As shown in Fig. 1, epithelial cells in the normal quiescent state are not transformed, and the stromal compartment is in homeostasis. Through the accumulation of multiple mutations, malignant epithelial transformation occurs. However, cancer cells require assistance to accomplish the complex series of events required for invasion and subsequent metastasis. Malignant cells usurp their microenvironments to synthesize necessary growth factors, cytokines, angiogenesis molecules, adhesion molecules, and proteases to break down the extracellular matrix. If malignant cells are unable to activate these determinants of metastases, clinical metastases do not occur, and these cells remain in a dormant condition. This setting is akin to metastases before initiation of the “angiogenic switch” (40, 41) and can be observed clinically in autopsy findings of malignant cells lodged in the pulmonary vasculature, but without frank metastasis (42).

Experience with peritoneovenous shunts has provided clinically relevant confirmation that metastases are actually rare events. Animal studies have demonstrated that large numbers of tumor cells are shed from primary tumors into the vascular system (43, 44). However, only a fraction of these circulating tumor cells form metastatic tumor deposits. Peritoneovenous shunts alleviate abdominal distension from malignant ascites by returning fluid from the peritoneal cavity into the venous circulation through one-way valves, infusing large numbers of malignant tumor cells directly into the venous circulation. In a study of 29 patients with locally advanced abdominal tumors requiring peritoneovenous shunts for malignant ascites (42), >100 million malignant cells were infused into the circulating blood per week. However, half of these patients did not show histological evidence of hematogenous metastases at autopsy. The observation that these patients were not routinely overwhelmed by massive widespread metastases supports the notion that the appropriate tissue microenvironment is crucial for metastasis development. These results imply that relatively few circulating tumor cells develop into clinically overt metastases because they experience difficulty in finding an appropriate microenvironmental soil. Thus, induction of the stroma is crucial to provide an environment conducive for malignant growth and for tumor metastases to form.

**Targeting the Stroma: Can Treatment Be Effective after the Horse Has Left the Barn?**

Once the stroma has been activated, therapeutic maneuvers aimed at the microenvironment, such as angiogenesis, matrix metalloproteinase inhibition, or FAP inhibitors, may have limited effectiveness given the overwhelming force of diverse growth potentiating factors. Therapeutic interventions directed against the stroma are more likely to have clinical benefit by altering dormant metastasis progression into metastatic storm, rather than by arresting metastases once the microenvironment has been activated. Indeed, we have shown that only moderate tumor growth attenuation can be achieved in an animal model using FAP inhibitory antibodies (24). Many clinical trials that have targeted the tumor microenvironment, including large randomized Phase III clinical trials testing both antiangiogenic agents (45) and matrix metalloproteinase inhibitors (46), have resulted in limited clinical success. This may be partially explained by the redundancy of tumor: microenvironment interactions, as the inhibition of any single protease family or pathway is diluted by the ability of tumors to use other mechanisms to accomplish similar ends.

One approach to overcoming this obstacle is to inhibit multiple stations along any given pathway or to target multiple discrete pathways. This strategy has been used in a wide assortment of clinical treatments, such as antibiotics (e.g., trimethoprim-sulfamethoxazole), HIV disease (e.g., protease inhibitor mixtures), and some combination chemotherapy programs for prostate cancer (e.g., estramustine/paclitaxel). These approaches use noncross-resistant treatments targeting the same mechanistic pathway, such as folic acid synthesis for trimethoprim-sulfamethoxazole or microtubule formation for prostate cancer treatment. Clinical efficacy can be demonstrated if the pathway targeted is of sufficient centrality to the disease process. Therefore, identification of the crucial stations for targeting tumor-stromal interactions is critically important.

In conclusion, tumor invasion and metastasis is a complex process requiring the coordination of multiple events. Given the complexity of events required for clinical metastases to develop, cancers cells cannot metastasize without assistance from host elements. Therefore, cancers induce stromal elements, in particular tumor fibroblasts, as active participants in the process of invasion and metastasis by expressing stromal proteins such as FAP. The process of tumor invasion and metastasis is thereafter dependent on the microenvironment and its communication with neoplastic cells. Such tumor-stromal interactions may provide a rich new source of important targets for cancer therapy.

**References**

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