Molecular Pathways: Connecting Fibrosis and Solid Tumor Metastasis

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Abstract
Pathologic organ fibrosis is a condition that can affect all major tissues and is typically ascribed to the excessive accumulation of extracellular matrix components, predominantly collagens. It typically leads to compromise of organ function and subsequent organ failure, and it is estimated that 45% of deaths in the developed world are linked to fibrotic disease. Fibrosis and cancer are known to be inextricably linked; however, we are only just beginning to understand the common and overlapping molecular pathways between the two. Here, we discuss what is known about the intersection of fibrosis and cancer, with a focus on cancer metastasis, and highlight some of the exciting new potential clinical targets that are emerging from analysis of the molecular pathways associated with these two devastating diseases. Clin Cancer Res; 20(14); 3637–43. ©2014 AACR.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Staff Planners’ Disclosures
The members of the planning committee have no real or apparent conflict of interest to disclose.

Learning Objectives
Upon completion of this activity, the participant should have a better understanding of the intersection between fibrosis and cancer metastasis, which key players are involved, and how these molecules might serve as novel therapeutic targets.

Background

The tissue microenvironment
Homeostasis of the extracellular matrix (ECM) is maintained by the normal stroma through a delicate balance of ECM synthesis, posttranslational modification, remodeling, and degradation. Such tight control ensures the provision of the correct cues to residing cells and mediates many aspects of cellular decision making and behavior (reviewed in ref. 1). However, the notion of the ECM providing potent signals to a cell is not new. Developmental biologists have for decades recognized the power of the cellular environment in regulating cell behavior, long before cancer biologists began to look outside the cell. Nonetheless, over two decades ago, Bissell and colleagues proposed the role for the ECM as a critical regulator of tumor cell fate through growth promoting and suppressive cues (2). Now, the ECM and, more importantly, disruption of ECM homeostasis, are thought to provide one of the biggest extrinsic drivers of tumor progression.

Fibrosis: unbalancing homeostasis
During organ fibrosis, this delicate balance of ECM homeostasis becomes disrupted, resulting in the excess deposition of ECM components and in particular collagens. Organ fibrosis differs from tissue scarring in acute wound repair in the composition and volume of the ECM. In general, fibrosis contains increased concentrations of collagen, a rich blood supply, and the presence of activated myofibroblasts. The underlying cause of tissue fibrosis is typically a chronic inflammatory response, although this has been shown to be dispensable clinically as evidenced in cases of idiopathic pulmonary fibrosis (IPF), where no response to anti-inflammatory therapy is achieved (3). A detailed discussion of the immune component of fibrosis is beyond the scope of this feature and has been covered in depth elsewhere (4, 5). However, where inflammatory
responses are present, these are driven by a plethora of initiating factors, including repeated physical/mechanical and chemical insults, radiation, pathogens, and autoimmune reactions.

At the cellular level, it is generally accepted that the key mediators of tissue fibrosis are activated collagen-secreting myofibroblasts. The origin of the myofibroblast is usually tissue dependent, and myofibroblasts have been shown to originate from tissue-resident mesenchymal, epithelial, and endothelial cells as well as from circulating bone marrow–derived fibrocytes (5). The molecular activators of myofibroblasts and ultimately fibrosis are wide ranging, and include paracrine and autocrine signals from lymphocytes, macrophages, and myofibroblasts, as well as pathogen-released activators such as pathogen-associated molecular patterns. In particular, reactive oxygen species (ROS), TGFβ, lysophosphatidic acid, VEGF, PDGF, MCP-1, IL6 II21, IL13, and MIP-1β have all been implicated in the recruitment and activation of myofibroblasts, many of which are being investigated as potential clinical targets (reviewed in ref. 6). Similarly, biomechanical feedback onto the cells as a result of structural ECM changes is also thought to play a critical role in the development of tissue fibrosis (Fig. 1).

Clinically, organ fibrosis is a degenerative process leading to an end-stage disease with high morbidity and mortality. Organs typically affected by fibrosis include the lungs (IPF), liver (cirrhosis), kidney (progressive kidney disease), and the heart (cardiovascular fibrosis) as well as connective tissues of the skin, gastrointestinal tract, and other internal organs (systemic sclerosis). Despite extensive efforts, there are still gaps in our understanding of the fundamental molecular pathways involved in fibrotic diseases across multiple organs. Instead, our understanding exists more as separate organ-based disease, rather than a connected pathologic syndrome. Identifying commonalities across organs will aid in discovering pathways related to key disease phenotypes regardless of etiology and tissue specificity.

**Linking fibrosis to cancer and metastasis**

Over a century ago, the “seed and soil” hypothesis was proposed by Paget (7) to explain the seemingly predictable spread of solid tumors. With minor revision (8, 9), this theory still holds and helps to explain the fact that although circulating tumor cells are found in the tumor vasculature of multiple organs, they do not always give rise to metastatic disease. However, other select sites consistently develop metastatic tumor deposits, and as such these sites must be more conducive to overt colonization by tumor cells. Thus, something in the microenvironment of these particular secondary sites must be supporting metastatic colonization. Perhaps a more chilling concept is that changes in secondary microenvironments, as a result of tumor-secreted factors or injury, may drive otherwise inhospitable environments to become supportive of metastatic colonization.

In the context of the primary tumor, the disruption of ECM homeostasis and “tumor–associated fibrosis” is driven by tumor cells themselves, either directly or indirectly through recruitment and activation of nonmalignant host stromal cells such as fibroblasts, macrophages, and leukocytes (10), leading to an expansion of tumor stroma and desmoplasia (11). This desmoplasia is remarkably similar to the increased ECM deposition observed during organ fibrosis, and has been shown to lead to enhanced tumor progression (12). For example, it has been shown that fibrotic breast disease is associated with a predisposition to breast cancer (13), and environmentally induced fibrotic disorders of the lung can increase incidence of lung cancer (14).

In the context of secondary metastatic sites, a rapidly building body of evidence indicates that a similar recruitment and subversion of nonmalignant host cells at future sites of metastasis activate remodeling programs that facilitate subsequent circulating tumor cell colonization. In this Molecular Pathways article, we center our discussion on how changes in the microenvironmental milieu may promote tumor metastasis, not from the primary site, but instead focus on the role of ECM homeostasis and remodeling at secondary sites of future metastasis.

**Priming the metastatic soil**

Recently, it has been shown that tumors can appropriate sites of future metastasis before their arrival in a manner that resembles the development of tissue fibrosis. Kaplan and colleagues (15) originally showed that increased fibronectin expression at sites of future metastasis was associated with VLA-4+/VEGFR1− bone marrow–derived cell (BMDC) recruitment and led to increased angiogenesis at these sites in a C57Bl/6 LLC and C57Bl/6 B16 model. Blocking the adhesion of BMDCs to fibronectin through targeting VLA-4 (integrin α4β1) or by inhibiting VEGFR1 abolished the formation of these BMDC clusters and completely inhibited metastasis, suggesting that fibronectin and BMDC recruitment is a fundamental component in establishing a permissive microenvironment. It was later shown that the collagen cross-linking enzyme lysyl oxidase (LOX) is found elevated (>10-fold) at premetastatic sites and is associated with fibronectin (16). Here, it drives matrix remodeling, resulting in the recruitment of CD11b+BMDCs, and is critical in contributing to the establishment and maintenance of premetastatic niches (reviewed in ref. 17). More recently, this was further strengthened by work from Wong and colleagues (18), who elegantly showed that increased collagen deposition and fiber formation in premetastatic lungs of a human breast cancer model occurred in a LOX family and hypoxia/hypoxia-inducible factor (HIF)-dependent manner and was necessary for BMDC recruitment. Inhibition of HIF1α and HIF2α led to a 50% reduction in collagen cross-linking and subsequent BMDC recruitment. The elevation of fibronectin, collagen secretion, organization, and posttranslational cross-linking and recruitment of immune cells described in premetastatic niche formation are all reminiscent of events occurring during the development of fibrosis and suggest a high degree of overlap in mechanisms between the two processes.

In a follow-up article (19), Cox and colleagues went on to explore the concept of tissue preappropriation before tumor

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cell arrival from a different angle. In these studies, they showed that underlying tissue fibrosis, driven as a result of acute injury (chemical and radiologic) in both pulmonary and hepatic tissue, led to extensive remodeling of the ECM and created a favorable microenvironment in these tissues that is capable of enhancing circulating tumor cell colonization and outgrowth. The authors used two models of lung fibrosis in Balb/c mice; bleomycin, the antineoplastic antibiotic, which drives increased expression of proinflammatory mediators and ECM components through a TGFβ mechanism (20), and thoracic irradiation, which leads to free radical-mediated DNA damage and induction of TGFβ (21). In both models, there was elevated deposition of fibronectin and type I collagen, and increased secretion and activity of LOX by resident fibroblast and fibroblast-like cells in a TGFβ-dependent manner. This led to the deposition of excess fibrillar collagen and development of fibrosis. The development of fibrosis by either mechanism led to a doubling in metastatic colonization of the tissue by circulating 4T1 tumor cells. In a second model of dimethyl-nitrosamine-induced liver fibrosis, Cox and colleagues also show that the development of hepatic fibrosis is LOX dependent and leads to an approximately 50% increase (P < 0.01) in the development of liver micrometastases in the 4T1 orthotopic model of breast cancer (19). This work is supported by Olaso and colleagues, who showed that in the liver, myofibroblast activation is associated with the arrival of B16 melanoma cells into the liver and that this activation occurs through a paracrine mechanism, which leads to enhanced colonization of the organ (22). Taken together, both studies strongly support the notion of targeting liver fibrosis mechanisms in preventing the establishment and development of liver metastases.

All of the above studies show that the underlying mechanisms of fibrosis are capable of creating a permissive "soil" for metastasising tumor cells to colonize. The parallels between unrelated organ fibrosis at secondary metastatic sites and desmoplasia at primary tumors in terms of the ECM are striking, in that both harbor a perpetual activation of myofibroblasts, the recruitment of host immune cells, and the significant alteration in ECM architecture and dynamics, which collectively support the growth of tumor cells.

**Fibrosis and primary tumor progression**

At the primary tumor, it is becoming clear that activated fibroblasts and fibroblast-mediated ECM remodeling have a prominent role in defining the rate and extent of cancer progression. This was clearly shown by Levental and colleagues (23), who established that the structural organization of the ECM at the tumor-stroma interface is significantly altered. The deposition and linearization of collagens at this interface, driven by the activation of resident fibroblasts and subsequent secretion of LOX, are required for malignant progression in the MMTV-Neu mammary cancer model. Building on these findings, Pickup and colleagues (24) confirmed in the PyMT model of mammary carcinoma that the source of LOX and collagen secretion is activated fibroblasts in the tumor stroma, is TGFβ dependent, increases ECM stiffness, and acts to enhance metastasis (by ~4-fold) in this model through increasing primary tumor escape. Thus, it is without doubt that the same cells are responsible for driving tissue fibrosis and creating fibrotic milieus at both primary tumor and secondary metastatic sites that enhance tumor progression and metastasis.

**Fibrosis and cancer dormancy**

Some excellent studies have been carried out in the context of tumor cell dormancy. Barkan and colleagues provide evidence to support the role for reactivation of dormant D2.0R mouse mammary cancer cells seeded to the lung of nude mice, upon the initiation of pulmonary fibrosis driven by adenoviral TGFβ expression (25). Their findings suggest that disseminated tumor cells may lie dormant for many years and even decades until normal tissue repair mechanisms are activated and act to reawaken dormant tumor cells kick-starting aggressive growth programs. Their observations suggest that establishment of a fibrotic-like microenvironment via TGFβ-driven collagen I production may generate a fertile soil around dormant tumor cells that then drives the transition from dormancy to metastatic growth through a β1-integrin signaling mechanism. The clinical implications of this are profound in terms of giving patients an "all-clear" following successful treatment of a primary tumor. These events are also eerily reminiscent of so-called "sleeper agents" used by intelligence agencies, whereby spies infiltrate their targets and "go to sleep," sometimes for many years before being "activated." Just as it is almost impossible to detect these sleeper agents and know which may pose a risk in a population, detecting and monitoring the dissemination and activation state of single dormant tumor cells across multiple tissues pose an enormous clinical problem.

**Clinical implications of cancer fibrosis cross-talk**

Cross-talk between tumor cells and the ECM is fundamental to the advancement of cancer, and represents one of the most robust avenues for therapeutic intervention. The ability to prevent disseminating tumor cells from colonizing secondary sites and preventing reactivation of already resident dormant tumor cells represents a very real and achievable target. Therapies that modulate the biosynthesis of ECM components such as fibronectin and collagen, ECM physical organization and remodeling, and posttranslational modification, such as cross-linking, and degradation, offer a platform upon which to establish nonpermissive microenvironments as well as to block and potentially revert the generation of promotastatic environments. Indeed, it may also be possible to block tumor cells from capitalizing on these prometastatic environments, through blocking cell surface receptors such as integrins. Because metastatic colonization of secondary tissues represents the dominant rate-limiting step of the metastatic cascade, with an estimated <0.02% of disseminating tumor cells successfully colonizing tissues (26), targeting this stage of the cascade should increase our success in treating metastatic
disease. Such approaches will act to render tumor cells, in effect, homeless, and exposed, allowing the body’s own defense the greatest chance of eradicating them.

However, from the viewpoint of metastasis, understanding prometastatic ECM changes in future sites of metastasis requires that we are able to reliably find them first. Alterations in the expression of ECM-related genes and proteins can easily be identified in primary tumors and linked to subsequent prognosis and metastasis (27); however, very little has been done to look at secondary sites. Although we can predict the organ of future metastasis, identifying at the microscopic level the exact sites of future metastasis really is the needle in the haystack. By its very nature, a “site of future metastasis” exists arbitrarily as a likelihood of an event occurring (metastatic colonization), in a manner similar to that of knowing the exact position of an electron orbiting a nucleus. In essence, metastatic sites only come into reality once tumor cells actually colonize, and thus they are no longer “prometastatic.” Although we can detect early changes that predict a likely hotspot for metastasis, we do not fully understand how many “future sites” may exist at any single time point, their time course of evolution and devolution, whether they are truly necessary, required, or merely sufficient, and whether all, or only some are fully capable of supporting metastatic colonization and to what extent. Thus, through understanding the parallels between whole organ ECM changes, such as in the case of tissue fibrosis, and the microscopic changes at prometastatic sites, we can increase our understanding of the very early changes in tissue ECM homeostasis that may be responsible for allowing circulating tumor cells to gain a selective advantage for colonizing often physiologically distinct and hostile secondary environments.

Fibrosis is accompanied by matrix stiffening, through increased collagen I deposition and cross-linking, and this abnormal ECM stiffness clearly plays an important role in cancer progression. Looking at studies from the prometastatic niche, it is apparent that both increased collagen deposition and LOX-mediated collagen fiber formation, as well as increased local stiffness in the tissue, are important (Cox and colleagues; unpublished data). These events are all strangely reminiscent of the development of fibrosis, and we speculate that prometastatic niche formation, at the microscopic level, mimics local fibrotic foci. This hypothesis is strongly supported by findings from us and others whereby organ fibrosis and prometastatic niche formation share many commonalities (16, 19). A key factor that links fibrosis to cancer initiation and progression is the potent and pleotropic growth factor TGFβ. Many of the overlaps between the two diseases center around TGFβ and TGFβ signaling. Both Col1A1/Col1A2 and LOX expression are induced by TGFβ mechanisms, and both are critical to organ fibrosis and tumor-associated desmoplasia. Furthermore, ROS, one of the byproducts of LOX enzymatic activity, have been shown to drive myofibroblast activation (28–31), typically in conjunction with and by modulating TGFβ pathways. Thus, hypoxia-induced tumor secretion of LOX may initiate fibrotic cascades in surrounding tissues and, combined with increases in ECM biomechanics, create a self-reinforcing feedback loop driving the development of fibrosis. However, we have only just begun to decipher how different cell types respond to changes in ECM biomechanics and which receptors are critically involved. Determining whether ECM biomechanics can be restored to that of normal tissue within the cancer context and how this may effect treatment prognosis has yet to be uncovered.

Interestingly, ECM stiffening also occurs as part of the normal aging process in some organs, where tissues exhibit increased deposition and abrogated posttranslational modification [typically nonenzymatic covalent cross-linking, so-called advanced glycation end-products (AGE)] of ECM proteins, leading to increases in mechanical compliance of the tissue. This process, albeit to a lesser extent, has many of the classic hallmarks of fibrosis. Postulating on this, one might assume that the development of cancer in an aged individual is more likely to be accompanied by metastasis than if the cancer was to develop at a younger stage of life. Such a hypothesis would revolve around a central theme, that a loss of active remodeling, replacement with passive cross-linking, and posttranslational modification of ECM components lead to the enhancement of metastatic potential of the tissue. Supporting this hypothesis is a recently published article by Maller and colleagues (32). These authors address a critical clinical question about the reduction in breast cancer risk in postpregnancy women. Despite the observation of an approximately 50% increase ($P < 0.05$) in abundance of collagen I in postparous rat mammary tissue, and in clinical samples, there is a decreased level of collagen I linearization. The authors conclude that collagen I organization, and subsequently stiffness, play a role in parity-induced protection. These findings would complement a hypothesis that the active remodeling brought about by pregnancy-induced remodeling of the mammary gland acts to reset the accumulation of passive age-linked cross-linking.

The development of these age-associated AGES in the ECM can be accelerated by exogenous dietary-related sources such as in the case of hyperglycemia seen in diabetes mellitus patients. Indeed, in a recent article by De Bruijn and colleagues (33), the authors undertake a global meta-analysis of diabetes mellitus and cancer incidence and mortality in more than 1.9 million patients with breast and colorectal carcinoma. Their findings reveal that diabetes mellitus is a risk factor for breast and colorectal cancer–specific mortality [HR 1.38 (1.20–1.58) for breast cancer and 1.30 (1.15–1.47) for colorectal cancer]. The mechanism remains to be investigated, but in the context of the above, one might consider the role of diabetes-driven AGE formation in the ECM, in which a fibrosis-mimicking event may act as a driver of tumor initiation and progression.

The development of a primary solid tumor from normal to premalignant to malignant takes time—decades in some cases—and incidence of diagnosis is typically associated with age. The development of fibrosis can rapidly enhance
this process, yet a rather frightening concept would be that during this extended latency, the "normal" aging of secondary tissues creates organs that are incrementally becoming more susceptible to metastasis. Thus, normal ECM aging may lead to increased support for metastatic colonization. Developmental programs that actively remodel the ECM, such as that described above, may act to reset this process, delaying tumor initiation and progression. In this vein, it would be of great interest to study whether aging tissues are inherently more likely to develop primary tumors, irrespective of genetic status, and subsequently whether aged secondary tissues are more likely to succumb to metastases than are younger tissues, independent of primary tumor formation.

At present, very few clinical and epidemiologic studies have looked into whether conditions of underlying fibrosis may enhance seeding of metastatic tumor cells. In light of what we have discussed, fibrotic environments may provide a foothold for tumor cells to infiltrate, generating either a protective environment within which they can escape immune surveillance until reactivated, or provide cues to switch them to a dormant state. Determining causality in such situations where metastases may arise decades later will, however, prove difficult.

Clinical–Translational Advances

Given the significant overlap in pathways associated with fibrosis and cancer, summarized in Fig. 1, we should simultaneously evaluate agents that target the tumor microenvironment and fibrosis for potential roles in both settings. Indeed, there are several commercial interests in testing antifibrotic agents that may have direct translational benefit in a cancer context, and these agents represent an important new direction for cancer therapy. However, although numerous treatments are available to tackle cancer, there are currently no clinically approved and deployed treatments that directly target the mechanisms of fibrosis despite the apparent overlap in potential mechanisms regulating ECM homeostasis. By targeting both deposition and post-translational modification, such as collagen secretion and cross-linking, or degradation, such as through targeting the metzincin superfamily, TGFβ, CTGF, uPAR, cathepsins, or the LOX family, regaining control of ECM homeostasis offers a way of controlling mechanisms central to both fibrotic diseases and cancer.

It is generally accepted that TGFβ is the central mediator of, and essential for the pathogenesis of fibrosis. Thus, it has naturally emerged as an attractive therapeutic target. Several strategies are being taken for TGFβ inhibition, including neutralizing antibodies, soluble TGFβ receptors, small-molecule TGFβR kinase inhibitors, and inhibitors of downstream Smad signaling (reviewed in ref. 6). However, the pleiotropic and multifunctional effects of TGFβ and its critical role in normal tissue homeostasis, immunity, and cellular decision-making have raised serious concerns about potential side effects that may be caused by systemic TGFβ blockade.

We believe that the next generation of therapeutic agents will focus more closely on normalizing tissue environments at secondary metastatic sites as well as primary tumor sites to impede and even prevent tumor progression and metastasis. Indeed, some clinical studies have indicated that chemotherapy for hepatocarcinoma would be more effective if therapies to target underlying liver fibrosis were also used (34). Taking this a step further, we postulate that in patients with advanced metastatic disease, adjuvant therapies that target both tumor and fibrotic pathways, or indeed antifibrotic agents alone, will prove useful in the medical arsenal for treating solid tumor metastases. To that end, the use of LOX/LOXL-targeting therapies has been shown to be efficacious in treating primary tumor growth and metastasis, and now fibrosis and fibrosis-enhanced metastasis (19, 35). The LOX family member LOX-like 2 (LOXL2) has also been shown to be important in the development of the pathogenic tumor and fibrotic microenvironment through the activation of fibroblasts through a TGFβ mechanism Barry-Hamilton and colleagues show that blocking LOXL-2 immunologically acts to decrease tumor-driven and chemically induced fibrosis in the C57Bl/6, bleomycin lung fibrosis and Balb/c, carbon tetrachloride liver fibrosis models (35). LOXL-2 inhibition improves both survival and hepatic function, respectively. This LOXL2-targeting antibody is in phase II clinical trials for patients with fibrotic diseases and is now entering clinical trials for patients with cancer. Several other LOX/LOXL2 drug development programs are currently under way, and we expect them to enter clinical trials in patients with both disease types in the not-too-distant future.

Although decades of basic and clinical research have aimed at curbing tumor growth, metastasis remains the foremost reason why patients with cancer succumb to their disease. Despite our drive to uncover more of solid tumor metastasis and the rapidly expanding body of knowledge, one often feels that the more we uncover, the less we truly understand, except that metastasis is more complex than it appears. Lessons from the clinic have taught us that targeting a single molecule in a disease network will result in "network compensation" and subsequent failure of the therapeutics (36). In the case of cancer and fibrosis, the 3-dimensional microenvironment contains many overlapping mechanisms that help to maintain its functional disorder. Thus, there is a need to better understand the position of key individual and collective players within these disease networks, how these nodes respond to the dynamics of and perturbations to the network, and, more importantly, how microenvironmental and cellular inputs come together to modulate disease progression (37). In terms of cancer metastasis, by improving our understanding of the fundamental requirements of tumor cells to undergo metastatic colonization of seemingly distinct and inhospitable environments, we will be a step closer to eradicating the clinical complications presented by metastases, diminishing patient suffering and prolonging life.
Figure 1. Under normal conditions, insult to healthy tissue activates transient mechanisms that repair the damaged tissue. Chronic or repetitive insult can lead to the onset of tissue fibrosis through persistent activation of myofibroblasts. These activated myofibroblasts remodel the surrounding ECM, generating a high linearized collagen-containing microenvironment with increased biomechanical stiffness as a result of increased ECM deposition and posttranslational modification. Concomitantly, the release of several growth factors and cytokines drives changes in angiogenesis and cell growth within the affected tissue. These changes lead to the generation of prometastatic microenvironments that support the colonization of circulating tumor cells, and also activation of already resident dormant tumor cells. At the primary tumor, similar mechanisms of stromal cell recruitment and activation lead to the development of tumor-associated desmoplasia facilitating tumor initiation and progression to metastasis. Finally, the active remodeling at secondary premetastatic sites mimics tissue fibrosis mechanisms, albeit at a microscopic scale, and acts to enhance tumor cell colonization and growth.

Authors' Contributions
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