Fibrosis and Cancer: Do Myofibroblasts Come Also From Epithelial Cells Via EMT?

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Abstract
Myofibroblasts produce and modify the extracellular matrix (ECM), secrete angiogenic and pro-inflammatory factors, and stimulate epithelial cell proliferation and invasion. Myofibroblasts are normally induced transiently during wound healing, but inappropriate induction of myofibroblasts causes organ fibrosis, which greatly enhances the risk of subsequent cancer development. As myofibroblasts are also found in the reactive tumor stroma, the processes involved in their development and activation are an area of active investigation. Emerging evidence suggests that a major source of fibrosis- and tumor-associated myofibroblasts is through transdifferentiation from non-malignant epithelial or epithelial-derived carcinoma cells through epithelial-mesenchymal transition (EMT). This review will focus on the role of EMT in fibrosis, considered in the context of recent studies showing that exposure of epithelial cells to matrix metalloproteinases (MMPs) can lead to increased levels of cellular reactive oxygen species (ROS) that stimulate transdifferentiation to myofibroblast-like cells. As deregulated MMP expression and increased cellular ROS are characteristic of both fibrosis and malignancy, these studies suggest that increased MMP expression may stimulate fibrosis, tumorigenesis, and tumor progression by inducing a specialized EMT in which epithelial cells transdifferentiate into activated myofibroblasts. This connection provides a new perspective on the development of the fibrosis and tumor microenvironments. J. Cell. Biochem. 101: 830–839, 2007. © 2007 Wiley-Liss, Inc.

Key words: epithelial-mesenchymal transition; matrix metalloproteinases; extracellular matrix; tumor microenvironment

Following identification of tumors near scar tissue [Friedrich, 1939], fibrosis has been investigated for its role in tumor formation and development. Investigations of the mechanisms governing fibrosis development and the relationship between fibrosis and tumor development are focusing on myofibroblasts, as these cells are most responsible for the development of fibrosis and are also abundant in the reactive tumor stroma [Ronnov-Jessen et al., 1995; Faouzi et al., 1999; Powell et al., 1999; Phan, 2002; Desmouliere et al., 2003; Petersen et al., 2003]. Originally characterized for their role in wound healing [Gabbiani et al., 1971], myofibroblasts have been found to play critical roles in an array of pathological processes [Powell et al., 1999; Desmouliere et al., 2005]. Recently characterized for their role in wound healing [Gabbiani et al., 1971], myofibroblasts have been found to play critical roles in a number of pathological processes [Powell et al., 1999; Desmouliere et al., 2005]. Recent findings that myofibroblasts can be derived from epithelial cells [Lee and Joo, 1999; Oldfield et al., 2001; Petersen et al., 2003; Li et al., 2004; Nightingale et al., 2004; Willis et al., 2005; Kim et al., 2006; Selman and Pardo, 2006] have provided a new impetus for investigating the processes involved in myofibroblast formation in the fibrotic and malignant context. These discoveries have paralleled an increasing awareness of the role of EMT in the control of tissue function in many organ systems [Gudjonsson et al., 2005].

Matrix metalloproteinases (MMPs) are so named because of their ability to cleave almost
all elements of the extracellular matrix (ECM), but MMPs can also break down molecules that mediate cell–cell and cell–ECM interactions, and can cleave and activate growth factors and growth factor receptors [Lochter et al., 1997a; Lochter et al., 1998; Overall and Kleifeld, 2006]. Many of the MMPs were initially cloned as cancer-specific genes [McCawley and Matrisian, 2000], and a causal relationship between upregulation of MMPs and progression to malignancy has long been suspected [Coussens and Werb, 2002]. A number of studies have suggested a causal relationship between MMP expression and lung fibrosis [reviewed in Pardo and Selman, 2006], and transgenic mouse experiments have shown that MMPs stimulate mammary fibrosis as well [Thomasset et al., 1998; Sternlicht et al., 1999; Ha et al., 2001]. In this review, we focus on the role of MMP-induced fibrosis and malignancy in the lung and breast, although it is clear that MMPs and fibrosis are involved in the development of malignancy in other organs as well [Bissell, 2001].

**MMPs, FIBROSIS, AND CANCER**

Epithelial injuries can be repaired through formation of granulation tissue, a provisional structure generated by ECM deposition, fibroblast proliferation, angiogenesis, and immune cell influx [reviewed in Thannickal et al., 2004]. Myofibroblasts are the key cells involved in the creation of granulation tissue, producing MMPs and other ECM-degrading enzymes that degrade the damaged tissue, as well as synthesizing collagen I, fibronectin, hyaluronic acid, and other components of the wound provisional ECM. An additional function of myofibroblasts is ECM contraction: through the formation of stress fibers containing smooth muscle actin, myofibroblasts can exert substantial remodeling force on the granulation tissue [Tomasek et al., 2002; Hinz and Gabbiani, 2003; Hinz, 2006]. Normally, completion of injury repair is followed by degradation of the provisional ECM and apoptosis of the myofibroblasts; however, sustained myofibroblast activation stimulates dysfunctional repair mechanisms, leading to accumulation of fibrotic ECM that is rich in collagens that coalesce into fibrous bundles resistant to degradation [Thannickal et al., 2004]. The fibrotic ECM disrupts cell polarity and stimulates cell proliferation, creating a context for cancer formation and development—the myofibroblast-induced inflammation and angiogenesis facilitating tumor growth and progression [Sieweke et al., 1990; Bissell, 2001; Desmouliere et al., 2004]. It is unsurprising, then, that the presence of fibrotic lesions significantly increases the risk of cancer in many tissues, including lung [Artinian and Kvale, 2004; Daniels and Jett, 2005], liver [Bissell, 2001; Bataller and Brenner, 2005], and breast [Boyd et al., 2002; Boyd et al., 2005].

In addition to contributing to the earlier stages of transformation, myofibroblast-induced fibrosis and contraction of the interstitial space are also believed to be key contributors to the higher interstitial fluid pressure frequently found in solid tumors [Heldin et al., 2004]. Unlike normal tissues, in which there is a slightly negative transcapillary pressure gradient facilitating the outward flow of solutes from the blood stream, the high interstitial pressure in tumors forms a substantial impediment to this flow. This has implications for the delivery of anti-cancer drugs to the tumor site, and suggests that approaches which target myofibroblasts or reduce fibrosis might enhance the accessibility of the tumor to the drugs.

Lung fibrosis has been extensively studied and classified [Thannickal et al., 2004]. Some lung fibrosis syndromes are caused by exposure to toxic or infectious agents, or by traumatic injuries, and these can generally be resolved by removal of the inducing agent and/or anti-inflammatory treatments. However, persistent idiopathic pulmonary fibrosis (IPF) can occur in the absence of any obvious external agent, and in these cases, clinical treatments have proven less successful. IPF is also distinct from other lung fibrotic syndromes in that it is typified by extensive collagen deposition, loss of basement membrane, and epithelial and fibroblast proliferation, but very little inflammation [Thannickal et al., 2004]. Incidence of IPF in the US alone has been estimated as high as 50,000 affected individuals, with median survival time from diagnosis of less than 3 years; nearly half of those affected by IPF may develop lung cancer [Daniels and Jett, 2005]. Fibroblastic foci containing myofibroblasts are often localized at the leading edge of lung fibrosis, and incidence of fibroblastic foci is an indicator of poor prognosis and decreased survival. The most common animal models of lung fibrosis use treatment with bleomycin,
paraquat/hyperoxia, asbestos, or silica; however, these inflammation-driven models may differ significantly from clinical IPF, which appears to be primarily an epithelial/mesenchymal disorder [Pardo and Selman, 2006].

While breast fibrosis is a common occurrence following anti-cancer radiation therapy [Choi et al., 2004], breast fibrosis as a spontaneously occurring clinical phenomenon is much less characterized than lung fibrosis. However, a number of studies have suggested a link between the common phenomenon of high density regions on mammograms and breast cancer risk [Boyd et al., 2005]; histological analyses of radiographically opaque areas of the breast have revealed increased fibrous tissue, collagen deposition, and fibroblast accumulation [Wellsing and Wolfe, 1978; Buchanan et al., 1981; Bright et al., 1988; Urbanski et al., 1988; Bartow et al., 1990; Boyd et al., 1992; Boyd et al., 2000], characteristic markers of fibrosis. Myofibroblasts are also associated with non-invasive breast hyperplasias and in the stroma surrounding breast carcinoma [Lagace et al., 1985; Sappino et al., 1988].

It is generally accepted that upregulation of MMPs likely contributes to lung fibrosis in humans [Pardo and Selman, 2006], but dissecting the specific mechanisms by which MMPs contribute to development and progression of fibrosis has been challenging. A number of experimental models have been used to evaluate the role of MMPs in the sequence of events following treatment with fibrosis-inducing agents [Elkington and Friedland, 2006]. Transcriptional profiling analysis has identified matrilysin (MMP-7) as significantly upregulated in bleomycin-induced lung fibrosis [Zuo et al., 2002], and a suite of MMPs are upregulated in asbestos-induced lung fibrosis [Tan et al., 2006]. Induction of fibrosis is enhanced in mice lacking expression of tissue inhibitors of metalloproteinases (TIMPs) [Kim et al., 2005], and reduced in mice treated with pharmacologic inhibitors of MMPs [Corbel et al., 2001; Lim et al., 2006; Tan et al., 2006] or in mice genetically deficient for MMPs [Zuo et al., 2002; Lim et al., 2006].

We have found that expression of MMP-3 in the mammary glands and lungs of transgenic mice is sufficient to induce fibrosis even in the absence of any additional challenge (Fig. 1 and Thomasset et al., [1998]), and similar results have been observed in mice expressing MT1-MMP [Ha et al., 2001]. While these results suggest MMPs as therapeutic targets for treatment of fibrosis, non-specific inhibition of MMPs is likely to be problematic: MMPs are involved in many critical physiological processes, activity of MMPs is likely to be necessary to remove the excess collagen depositions present in fibrosis and MMPs are necessary for lung alveolarization and mammary branching [Parks and Shapiro, 2001; Fata et al., 2004], so inhibition of MMPs may actually prevent tissue repair and block recovery. Accordingly, identifying and targeting the processes stimulated by MMPs may be required in combination.

**EMT AS A SOURCE OF MYOFIBROBLASTS IN FIBROSIS AND CANCER**

The origin of myofibroblasts has been an active topic of debate almost since their identification. Their morphological similarities to tissue fibroblasts have led to the assumption that myofibroblasts are derived from these cells; indeed, in culture, fibroblasts can be induced to express myofibroblast markers and to adopt morphological properties of myofibroblasts following treatment with specific cytokines, of which transforming growth factor-β (TGFβ) has been the most studied [Ronnov-Jessen et al., 1995; Tomasek et al., 2002; Willis et al., 2006]. However, more recent results are suggesting that while myofibroblasts may sometimes develop from existing interstitial fibroblasts, this viewpoint may be too narrow. Animal studies using transplanted bone marrow precursors have shown that cells with myofibroblast characteristics can be derived from myeloid precursor cells [Brittan et al., 2002; Direkze et al., 2004; Hashimoto et al., 2004; Brittan et al., 2005; Mori et al., 2005; Yamaguchi et al., 2005; Russo et al., 2006], suggesting a potential role for bone marrow-derived stem/progenitor cells in tissue fibrosis.

Emerging evidence, however, suggests that epithelial cells are also an important source of myofibroblasts in fibrosis and cancer [Selman and Pardo, 2006]. Epithelial-myofibroblast transition can be induced in cultured epithelial cells from a number of organ systems [Lee and Joo, 1999; Oldfield et al., 2001; Petersen et al., 2003; Li et al., 2004; Nightingale et al., 2004; Willis et al., 2005; Kim et al., 2006]. Analysis of tissue samples from patients with IPF revealed many cells with shared epithelial and...
myofibroblast markers [Willis et al., 2005], and genetic tests showed that mesenchymal cells with myofibroblast characteristics isolated from human breast cancer biopsies were found to be derived from the epithelial tumor cells [Petersen et al., 2003]. These findings are further supported by the demonstration that stromal cells of breast tumors can share genetic lesions in common with the tumor epithelium [Moinfar et al., 2000]. Recently, transgenic mice were created in which lung epithelial cells were permanently tagged for expression of β-galactosidase; induction of pulmonary fibrosis in these mice by TGFβ revealed that the increases in myofibroblasts were largely due to transdifferentiation from epithelial cells [Kim et al., 2006]. The accumulating evidence of epithelial-myofibroblast transdifferentiation has profound implications for our understanding of the processes involved in fibrosis and cancer development. An important goal now is to define the extracellular mediators that induce epithelial-myofibroblast transdifferentiation, and the signaling pathways involved in this process.

Transdifferentiation of myofibroblasts from epithelial cells is a specialized version of epithelial-mesenchymal transition (EMT), a process in which epithelial cells can take on the characteristics of mesenchymal cells [Radisky, 2005]. EMT was originally identified in development: in gastrulation, EMT enables the embryonic epithelium to give rise to the mesoderm; in delamination of the neural crest, EMT produces a population of highly mobile cells that migrate to and are incorporated into many different tissues [Nieto, 2001; Shook and Keller, 2003]. More recently, EMT regulators identified in developmental studies are being found to be involved in key steps of tumor development in vivo and in culture [Yang et al., 2004; Moody et al., 2005; Radisky et al., 2005]. It should be noted that the role of EMT in tumor invasion and metastasis is currently a topic of active debate [Tarin et al., 2005; Thompson et al., 2005]. Although this may be because investigations have primarily focused on tumor EMT as a mechanism for stimulating epithelial cell invasion or metastasis through a
transient mesenchymal state, consideration that a significant function of tumor EMT is to create stromal myofibroblasts that may not be intrinsically malignant but which significantly stimulate progression of the remaining epithelial tumor cells could redefine these controversies. The identification of a mechanism by which MMPs, long associated with fibrosis induction and tumor progression, also stimulate EMT suggests a connection between these processes.

**MATRIX METALLOPROTEINASE INDUCTION OF EPITHELIAL-MESENCHYMAL TRANSITION**

The non-tumorigenic mouse mammary epithelial cell line SCp2 has been used to study processes involved in the induction of EMT [Desprez et al., 1993; Lochter et al., 1997a,b]. SCp2 cells contain mutated p53, but are phenotypically normal: they undergo alveolus-like development and induce milk protein synthesis when cultured in laminin-rich three-dimensional gels supplemented with lactogenic hormones [Desprez et al., 1993; Lochter et al., 1997b]. Exposure of these cells to MMP-3 results in loss of intact E-cadherin, increased motility and invasiveness, downmodulation of epithelial markers, and upregulation of mesenchymal markers [Lochter et al., 1997a,b; Radisky et al., 2005]. SCp2 cells exposed to MMP-3 also develop anchorage independence, as demonstrated by growth in soft agarose [Lochter et al., 1998], and grow into tumors when injected into the cleared mammary fat pads of immunocompromised mice [Sternlicht et al., 1999].

Treatment of mouse mammary epithelial cells with MMP-3 causes a range of phenotypic alterations associated with EMT, including increased motility and invasiveness [Lochter et al., 1997a,b], alterations of cytoskeletal composition, including downmodulation of epithelial cytokeratins and induction of mesenchymal vimentin (Fig. 2A), and altered expression of a number of EMT-related genes, including, significantly, smooth muscle actin, collagen A1, and fibronectin (Fig. 2B), characteristic markers of myofibroblasts. Elucidation of the cellular pathways connecting MMP-3 treatment with induction of EMT provided further insight into the role of this process in MMP-induced fibrosis and tumorigenesis. We found that the stimulation of EMT by MMP-3 was due to elevated cellular reactive oxygen species (ROS) caused by increased levels of Rac1b [Radisky et al., 2005]. Cells treated with MMP-3 showed increased Rac1b (Fig. 3A), an activated splice variant of Rac1 that has been found in breast and colorectal tumors [Jordan et al., 1999; Schnelzer et al., 2000; Matos et al., 2003; Fiegen et al., 2004; Singh et al., 2004]. MMP-3-induced Rac1b stimulated elevated production of ROS, as assessed with the redox-sensitive dye dichlorodihydrofluoresceine diacetate (DCFDA) (Fig. 3B). The elevated cellular ROS in cells treated with MMP-3 were caused by mitochondrial activation [Radisky et al., 2005], as assessed by precipitation of nitrobluetetrazolium (Fig. 3C) and cytoplasmic

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**Fig. 2.** Properties of MMP-3-induced EMT. A: MMP-3-treated SCp2 cells, stained for cytokeratins (red), vimentin (green), and DNA (blue) (scale bar = 50 μm). B: Marker transcript levels in cells treated with MMP-3 for 4 days; P<0.01 for all altered expression levels. Adapted from Radisky et al., 2005. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]
localization of JC-1 (Fig. 3D). Elevation of ROS by treatment with H$_2$O$_2$ was also sufficient to induce EMT-related cell scattering and invasiveness (Fig. 3E,F), and MMP-3/Rac1b-induced ROS were found to be specifically required for induction of the mesenchymal vimentin (Fig. 3G) as well as other myofibroblast genes [Radisky et al., 2005].

The role of MMP-3 as an inducer of fibrosis is seen clearly in the WAP-MMP-3 transgenic mouse [Thomasset et al., 1998]. Normally, MMP-3 is produced primarily by mammary stromal cells, with highest levels during the post-lactational developmental stage, a time of maximal ECM remodeling and regression of glandular structure [Talhouk et al., 1992; Witty et al., 1995; Lund et al., 1996]. Expression of the MMP-3 transgene in mammary epithelial cells stimulated increased expression of endogenous MMP-3 in the tumor stroma and development of fibrosis, associated with collagen deposition, increased angiogenesis, and inflammatory influx; these stromal alterations were coupled with increased proliferation and branching morphogenesis of ductal epithelial cells and apoptosis of secretory alveolar cells [Symson et al., 1994; Witty et al., 1995; Alexander et al., 1996; Thomasset et al., 1998; Sternlicht et al., 1999; Sternlicht et al., 2000]. Similar effects were also seen in the MMTV-MT1-MMP transgenic mouse [Ha et al., 2001]. Analysis by comparative genomic hybridization of the tumors that developed in MMP-3 transgenic mice revealed significant chromosomal amplifications and deletions [Sternlicht et al., 1999], demonstrating that exposure to MMP-3 and the MMP-3-induced fibrotic microenvironment also caused genomic instability in epithelial cells. Thus, long-term induction of MMP-3 can lead to self-sustaining alterations in the microenvironment that are sufficient to cause development of tumors and progression to full malignancy.
EPITHELIAL-MYOFIBROBLAST TRANSDIFFERENTIATION AS A THERAPEUTIC TARGET

Recognition of epithelial transdifferentiation as a possible major source of myofibroblasts in fibrosis provides an essential new target for therapeutic strategies. Even with aggressive anti-inflammatory treatment regimens, IPF remains an irreversible and progressive disease [Walter et al., 2006], and many patients with IPF develop lung cancer. Breast fibrosis, though not intrinsically fatal, presents a substantial risk factor for subsequent tumor development. Experiments using cultured cells and transgenic animals showed that the MMP-3-induced EMT and stimulation of tumor growth was initially reversible upon MMP withdrawal, but eventually became permanent [Lochter et al., 1997a; Sternlicht et al., 1999]; these results suggest that elevations in myofibroblast numbers, a transient phenomenon under normal physiological conditions, also may be reversible at early stages of fibrosis and tumor genesis. We found that cellular ROS were essential for MMP-induced EMT [Radisky et al., 2005], and ROS have been implicated as mediators of EMT in other models as well [Mori et al., 2004; Rhyu et al., 2005]. Human lung fibrosis is associated with oxidative stress, with evidence of oxidant/antioxidant imbalance in alveolar air spaces [Rahman et al., 1999], and inhibitors of ROS have shown promise in clinical trials targeting fibrosis [Campana et al., 2004; Delanian et al., 2005; Demedts et al., 2005]. It may be that combining specific inhibitors of MMPs and agents that inhibit ROS may be even more efficacious. Further investigation of the processes governing epithelial-myofibroblast transdifferentiation will likely suggest additional approaches.

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