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

Derek C. Radisky,[1]* Paraic A. Kenny,[2] and Mina J. Bissell[2]**

[1]Department of Cancer Biology, Mayo Clinic Cancer Center, Jacksonville, Florida
[2]Life Sciences Division, Lawrence Berkeley National Laboratory, Berkeley, California

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.

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]. 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

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*Correspondence to: Derek C. Radisky, Mayo Clinic Cancer Center, 4500 San Pablo Road, Jacksonville, FL 32224.
E-mail: radisky.derek@mayo.edu

**Correspondence to: Mina J. Bissell, Life Sciences Division, Lawrence Berkeley National Laboratory, 1 Cyclotron Road, MS 977-225A, Berkeley, CA 94720.
E-mail: mjbissell@lbl.gov

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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 [Wellings 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

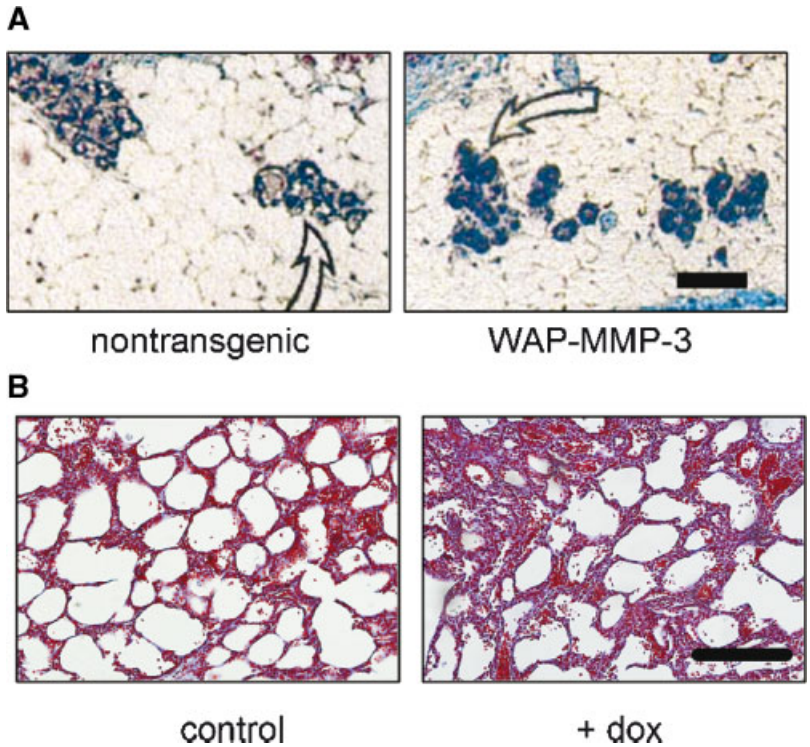

Fig. 1. Induction of fibrosis in breast and lung of MMP-3-transgenic mice. **A**: Accumulation of collagen around ducts of 15-day pregnant MMP-3-transgenic mice, assessed by Gomori's trichrome staining for total collagen (blue) (scale bar = 50 mm). **B**: Alveolar hyperplasia and collagen deposition characteristic of early fibrosis in newborn CCSP-rtTA/tet-MMP-3 transgenic mice induced to express MMP-3 for 7d in utero, assessed by Masson's trichrome stain (scale bar = 200 mm). A: Adapted from Thomasset et al., 1998. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

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

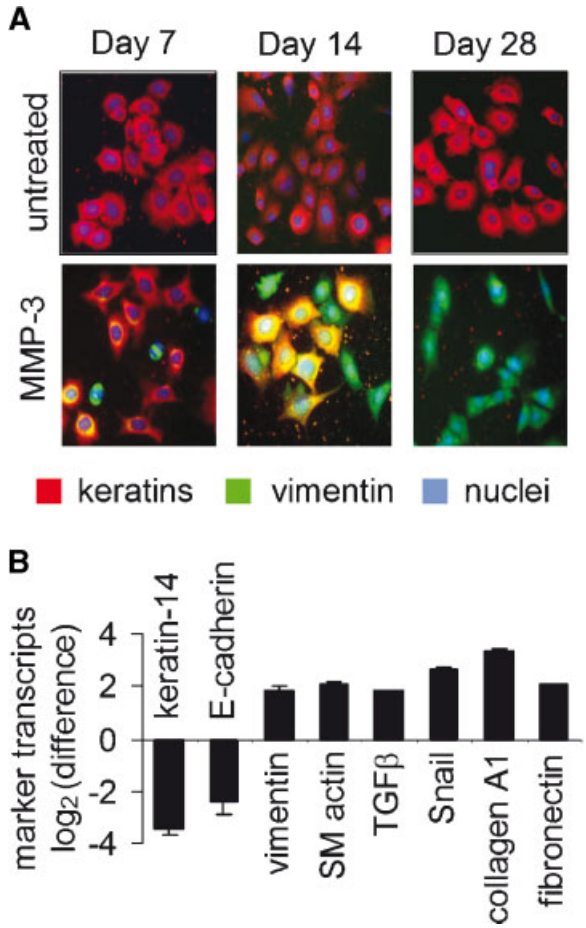

Fig. 2. Properties of MMP-3-induced EMT. **A**: MMP-3-treated SCp2 cells, stained for cytokeratins (red), vimentin (green), and DNA (blue) (scale bar = 50 μm). **B**: Marker transcript levels in cells treated with MMP-3 for 4 days; $P < 0.01$ for all altered expression levels. Adapted from Radisky et al., 2005. [Color figure can be viewed in the online issue, which is available at www.interscience.wiley.com.]

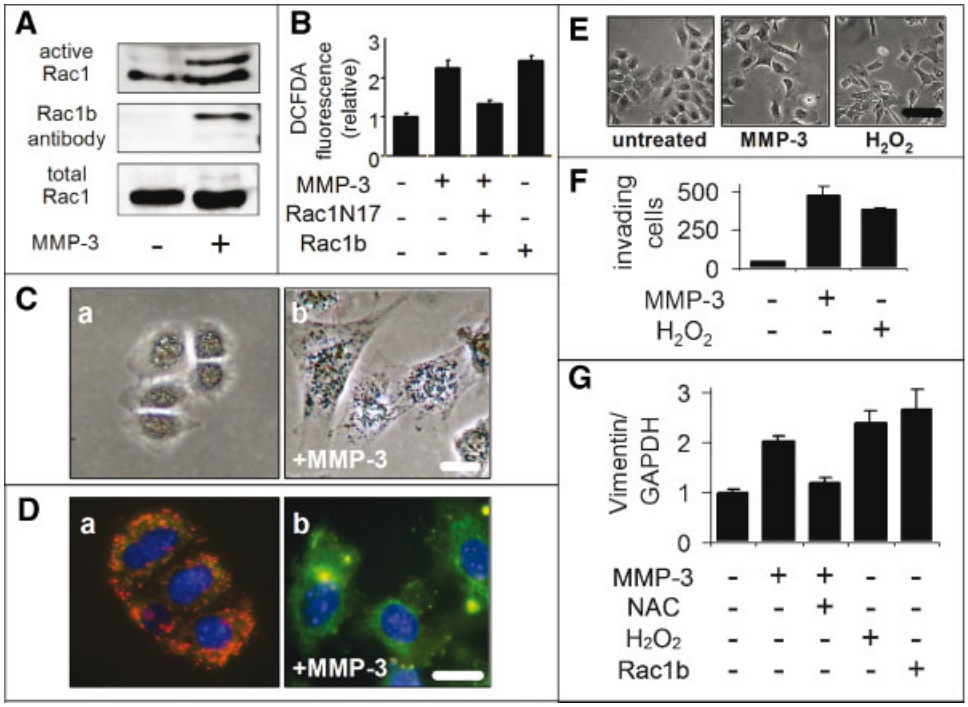

Fig. 3. MMP-3-induced ROS activate EMT. **A**: SCp2 cells treated with MMP-3 stimulate increased production of Rac1b, as assessed by Rac1 activity assay and Western blot with Rac1b-specific antibody. **B**: Exposure to MMP-3 activates redox-sensitive fluorescent dye DCFDA in Rac1/Rac1b-dependent fashion. **C**: MMP-3 treatment induces mitochondrial production of superoxide as shown by precipitation of nitrobluetetrazolium in cells treated with MMP-3 (**b**) as compared to untreated cells (**a**). **D**: MMP-3 treatment induces mitochondrial depolarization as shown by loss of punctuate red staining of the J-aggregate and increased diffuse green staining of the monomeric form in the MMP-3-treated cells (**b**) as compared to untreated (**a**). **E–F**: Exposure to H2O2 induces cell scattering (E) and invasiveness through Matrigel (F), as compared with MMP-3-treated cells. Scale bars for C–D, 10 μm; for E, 50 μm. **G**: MMP-3-induced upregulation of mesenchymal vimentin expression is inhibited by NAC and is reproduced by exposure to H_2O_2 or by expression of Rac1b. 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_2O_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 [Sympson 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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REFERENCES

Alexander CM, Howard EW, Bissell MJ, Werb Z. 1996. Rescue of mammary epithelial cell apoptosis and entactin degradation by a tissue inhibitor of metalloproteinases-1 transgene. J Cell Biol 135:1669–1677.

Artinian V, Kvale PA. 2004. Cancer and interstitial lung disease. Curr Opin Pulm Med 10:425–434.

Bartow SA, Pathak DR, Mettler FA. 1990. Radiographic microcalcification and parenchymal patterns as indicators of histologic "high-risk" benign breast disease. Cancer 66:1721–1725.

Bataller R, Brenner DA. 2005. Liver fibrosis. J Clin Invest 115:209–218.

Bissell DM. 2001. Chronic liver injury, TGF-beta, and cancer. Exp Mol Med 33:179–190.

Boyd NF, Jensen HM, Cooke G, Han HL. 1992. Relationship between mammographic and histological risk factors for breast cancer. J Natl Cancer Inst 84:1170–1179.

Boyd NF, Jensen HM, Cooke G, Han HL, Lockwood GA, Miller AB, Reference Pathologists of the Canadian National Breast Screening Study. 2000. Mammographic densities and the prevalence and incidence of histological types of benign breast disease. Eur J Cancer Prev 9:15–24.

Boyd NF, Dite GS, Stone J, Gunasekara A, English DR, McCredie MR, Giles GG, Tritchler D, Chiarelli A, Yaffe MJ, Hopper JL. 2002. Heritability of mammographic density, a risk factor for breast cancer. N Engl J Med 347:886–894.

Boyd NF, Rommens JM, Vogt K, Lee V, Hopper JL, Yaffe MJ, Paterson AD. 2005. Mammographic breast density as an intermediate phenotype for breast cancer. Lancet Oncol 6:798–808.

Bright RA, Morrison AS, Brisson J, Burstein NA, Sadowsky NS, Kopans DB, Meyer JE. 1988. Relationship between mammographic and histologic features of breast tissue in women with benign biopsies. Cancer 61:266–271.

Brittan M, Hunt T, Jeffery R, Poulsom R, Forbes SJ, Hodivala-Dilke K, Goldman J, Alison MR, Wright NA. 2002. Bone marrow derivation of pericryptal myofibroblasts in the mouse and human small intestine and colon. Gut 50:752–757.

Brittan M, Chance V, Elia G, Poulsom R, Alison MR, MacDonald TT, Wright NA. 2005. A regenerative role for bone marrow following experimental colitis: Contribution to neovasculogenesis and myofibroblasts. Gastroenterology 128:1984–1995.

Buchanan JB, Weisberg BF, Sandoz JP, Gray LA, Sr., Bland KI. 1981. Selected prognostic variables for mammographic parenchymal patterns. Cancer 47:2135–2137.

Campana F, Zervoudis S, Perdereau B, Gez E, Fourquet A, Badiu C, Tsakiris G, Koulaloglou S. 2004. Topical superoxide dismutase reduces post-irradiation breast cancer fibrosis. J Cell Mol Med 8:109–116.

Choi YW, Munden RF, Erasmus JJ, Park KJ, Chung WK, Jeon SC, Park CK. 2004. Effects of radiation therapy on the lung: Radiologic appearances and differential diagnosis. Radiographics 24:985–997; discussion 998.

Corbel M, Caulet-Maugendre S, Germain N, Molet S, Lagente V, Boichot E. 2001. Inhibition of bleomycin-induced pulmonary fibrosis in mice by the matrix metalloproteinase inhibitor batimastat. J Pathol 193: 538–545.

Coussens LM, Werb Z. 2002. Inflammation and cancer. Nature 420:860–867.

Daniels CE, Jett JR. 2005. Does interstitial lung disease predispose to lung cancer? Curr Opin Pulm Med 11:431–437.

Delanian S, Porcher R, Rudant J, Lefaix JL. 2005. Kinetics of response to long-term treatment combining pentoxifylline and tocopherol in patients with superficial radiation-induced fibrosis. J Clin Oncol 23:8570–8579.

Demedts M, Behr J, Buhl R, Costabel U, Dekhuijzen R, Jansen HM, MacNee W, Thomeer M, Wallaert B, Laurent F, Nicholson AG, Verbeken EK, Verschakelen J, Flower CD, Capron F, Petruzzelli S, De Vuyst P, van den Bosch JM, Rodriguez-Becerra E, Corvasce G, Lankhorst I, Sardina M, Montanari M. 2005. High-dose acetylcysteine in idiopathic pulmonary fibrosis. N Engl J Med 353:2229–2242.

Desmouliere A, Darby IA, Gabbiani G. 2003. Normal and pathologic soft tissue remodeling: Role of the myofibroblast, with special emphasis on liver and kidney fibrosis. Lab Invest 83:1689–1707.

Desmouliere A, Guyot C, Gabbiani G. 2004. The stroma reaction myofibroblast: A key player in the control of tumor cell behavior. Int J Dev Biol 48:509–517.

Desmouliere A, Chaponnier C, Gabbiani G. 2005. Tissue repair, contraction, and the myofibroblast. Wound Repair Regen 13:7–12.

Desprez P-Y, Roskelley C, Campisi J, Bissell MJ. 1993. Isolation of functional cell lines from a mouse mammary epithelial cell strain: The importance of basement membrane and cell-cell interactions. Mol Cell Differ 1: 99–110.

Direkze NC, Hodivala-Dilke K, Jeffery R, Hunt T, Poulsom R, Oukrif D, Alison MR, Wright NA. 2004. Bone marrow contribution to tumor-associated myofibroblasts and fibroblasts. Cancer Res 64:8492–8495.

Elkington PT, Friedland JS. 2006. Matrix metalloproteinases in destructive pulmonary pathology. Thorax 61:259–266.

Faouzi S, Le Bail B, Neaud V, Boussarie L, Saric J, Bioulac-Sage P, Balabaud C, Rosenbaum J. 1999. Myofibroblasts are responsible for collagen synthesis in the stroma of human hepatocellular carcinoma: An in vivo and in vitro study. J Hepatol 30:275–284.

Fata JE, Werb Z, Bissell MJ. 2004. Regulation of mammary gland branching morphogenesis by the extracellular matrix and its remodeling enzymes. Breast Cancer Res 6:1–11.

Fiegen D, Haeusler LC, Blumenstein L, Herbrand U, Dvorsky R, Vetter IR, Ahmadian MR. 2004. Alternative splicing of Rac1 generates Rac1b, a self-activating GTPase. J Biol Chem 279:4743–4749.

Friedrich G. 1939. Periphere Lungenkrebse auf dem Bodem pleuranaher. Virchows Arch Path Anat 230–246.

Gabbiani G, Ryan GB, Majne G. 1971. Presence of modified fibroblasts in granulation tissue and their possible role in wound contraction. Experientia 27:549–550.

Gudjonsson T, Adriance MC, Sternlicht MD, Petersen OW, Bissell MJ. 2005. Myoepithelial cells: Their origin and function in breast morphogenesis and neoplasia. J Mammary Gland Biol Neoplasia 10:261–272.

Ha HY, Moon HB, Nam MS, Lee JW, Ryoo ZY, Lee TH, Lee KK, So BJ, Sato H, Seiki M, Yu DY. 2001. Overexpression of membrane-type matrix metalloproteinase-1 gene induces mammary gland abnormalities and adenocarcinoma in transgenic mice. Cancer Res 61:984–990.

Hashimoto N, Jin H, Liu T, Chensue SW, Phan SH. 2004. Bone marrow-derived progenitor cells in pulmonary fibrosis. J Clin Invest 113:243–252.

Heldin CH, Rubin K, Pietras K, Ostman A. 2004. High interstitial fluid pressure—an obstacle in cancer therapy. Nat Rev Cancer 4:806–813.

Hinz B. 2006. Masters and servants of the force: The role of matrix adhesions in myofibroblast force perception and transmission. Eur J Cell Biol 85:175–181.

Hinz B, Gabbiani G. 2003. Mechanisms of force generation and transmission by myofibroblasts. Curr Opin Biotechnol 14:538–546.

Jordan P, Brazao R, Boavida MG, Gespach C, Chastre E. 1999. Cloning of a novel human Rac1b splice variant with increased expression in colorectal tumors. Oncogene 18:6835–6839.

Kim KH, Burkhart K, Chen P, Frevert CW, Randolph-Habecker J, Hackman RC, Soloway PD, Madtes DK. 2005. Tissue inhibitor of metalloproteinase-1 deficiency amplifies acute lung injury in bleomycin-exposed mice. Am J Respir Cell Mol Biol 33:271–279.

Kim KK, Kugler MC, Wolters PJ, Robillard L, Galvez MG, Brumwell AN, Sheppard D, Chapman HA. 2006. Alveolar epithelial cell mesenchymal transition develops in vivo during pulmonary fibrosis and is regulated by the extracellular matrix. Proc Natl Acad Sci USA 103(35):13180–13185.

Lagace R, Grimaud JA, Schurch W, Seemayer TA. 1985. Myofibroblastic stromal reaction in carcinoma of the breast: Variations of collagenous matrix and structural glycoproteins. Virchows Arch A Pathol Anat Histopathol 408:49–59.

Lee EH, Joo CK. 1999. Role of transforming growth factor-beta in transdifferentiation and fibrosis of lens epithelial cells. Invest Ophthalmol Vis Sci 40:2025–2032.

Li JH, Wang W, Huang XR, Oldfield M, Schmidt AM, Cooper ME, Lan HY. 2004. Advanced glycation end products induce tubular epithelial-myofibroblast transition through the RAGE-ERK1/2 MAP kinase signaling pathway. Am J Pathol 164:1389–1397.

Lim DH, Cho JY, Miller M, McElwain K, McElwain S, Broide DH. 2006. Reduced peribronchial fibrosis in allergen-challenged MMP-9-deficient mice. Am J Physiol Lung Cell Mol Physiol 291:L265–L271.

Lochter A, Galosy S, Muschler J, Freedman N, Werb Z, Bissell MJ. 1997a. Matrix metalloproteinase stromelysin-1 triggers a cascade of molecular alterations that leads to stable epithelial-to-mesenchymal conversion and a premalignant phenotype in mammary epithelial cells. J Cell Biol 139:1861–1872.

Lochter A, Srebrow A, Sympson CJ, Terracio N, Werb Z, Bissell MJ. 1997b. Misregulation of stromelysin-1 expression in mouse mammary tumor cells accompanies acquisition of stromelysin-1-dependent invasive properties. J Biol Chem 272:5007–5015.

Lochter A, Sternlicht MD, Werb Z, Bissell MJ. 1998. The significance of matrix metalloproteinases during early stages of tumor progression. Ann N Y Acad Sci 857:180–193.

Lund LR, Romer J, Thomasset N, Solberg H, Pyke C, Bissell MJ, Dano K, Werb Z. 1996. Two distinct phases of apoptosis in mammary gland involution: Proteinase-

independent and -dependent pathways. Development 122:181–193.
Matos P, Collard JG, Jordan P. 2003. Tumor-related alternatively spliced Rac1b is not regulated by Rho-GDP dissociation inhibitors and exhibits selective downstream signaling. J Biol Chem 278:50442–50448.
McCawley LJ, Matrisian LM. 2000. Matrix metalloproteinases: Multifunctional contributors to tumor progression. Mol Med Today 6:149–156.
Moinfar F, Man YG, Arnould L, Bratthauer GL, Ratschek M, Tavassoli FA. 2000. Concurrent and independent genetic alterations in the stromal and epithelial cells of mammary carcinoma: Implications for tumorigenesis. Cancer Res 60:2562–2566.
Moody SE, Perez D, Pan TC, Sarkisian CJ, Portocarrero CP, Sterner CJ, Notorfrancesco KL, Cardiff RD, Chodosh LA. 2005. The transcriptional repressor snail promotes mammary tumor recurrence. Cancer Cell 8:197–209.
Mori K, Shibanuma M, Nose K. 2004. Invasive potential induced under long-term oxidative stress in mammary epithelial cells. Cancer Res 64:7464–7472.
Mori L, Bellini A, Stacey MA, Schmidt M, Mattoli S. 2005. Fibrocytes contribute to the myofibroblast population in wounded skin and originate from the bone marrow. Exp Cell Res 304:81–90.
Nieto MA. 2001. The early steps of neural crest development. Mech Dev 105:27–35.
Nightingale J, Patel S, Suzuki N, Buxton R, Takagi KI, Suzuki J, Sumi Y, Imaizumi A, Mason RM, Zhang Z. 2004. Oncostatin M, a cytokine released by activated mononuclear cells, induces epithelial cell-myofibroblast transdifferentiation via Jak/Stat pathway activation. J Am Soc Nephrol 15:21–32.
Oldfield MD, Bach LA, Forbes JM, Nikolic-Paterson D, McRobert A, Thallas V, Atkins RC, Osicka T, Jerums G, Cooper ME. 2001. Advanced glycation end products cause epithelial-myofibroblast transdifferentiation via the receptor for advanced glycation end products (RAGE). J Clin Invest 108:1853–1863.
Overall CM, Kleifeld O. 2006. Tumour microenvironment—opinion: Validating matrix metalloproteinases as drug targets and anti-targets for cancer therapy. Nat Rev Cancer 6:227–239.
Pardo A, Selman M. 2006. Matrix metalloproteases in aberrant fibrotic tissue remodeling. Proc Am Thorac Soc 3:383–388.
Parks WC, Shapiro SD. 2001. Matrix metalloproteinases in lung biology. Respir Res 2:10–19.
Petersen OW, Nielsen HL, Gudjonsson T, Villadsen R, Rank F, Niebuhr E, Bissell MJ, Ronnov-Jessen L. 2003. Epithelial to mesenchymal transition in human breast cancer can provide a nonmalignant stroma. Am J Pathol 162:391–402.
Phan SH. 2002. The myofibroblast in pulmonary fibrosis. Chest 122:286S–289S.
Powell DW, Mifflin RC, Valentich JD, Crowe SE, Saada JI, West AB. 1999. Myofibroblasts. I. Paracrine cells important in health and disease. Am J Physiol 277:C1–C9.
Radisky DC. 2005. Epithelial-mesenchymal transition. J Cell Sci 118:4325–4326.
Radisky DC, Levy DD, Littlepage LE, Liu H, Nelson CM, Fata JE, Leake D, Godden EL, Albertson DG, Nieto MA, Werb Z, Bissell MJ. 2005. Rac1b and reactive oxygen species mediate MMP-3-induced EMT and genomic instability. Nature 436:123–127.
Rahman I, Skwarska E, Henry M, Davis M, O'Connor CM, FitzGerald MX, Greening A, MacNee W. 1999. Systemic and pulmonary oxidative stress in idiopathic pulmonary fibrosis. Free Radic Biol Med 27:60–68.
Rhyu DY, Yang Y, Ha H, Lee GT, Song JS, Uh ST, Lee HB. 2005. Role of reactive oxygen species in TGF-beta1-induced mitogen-activated protein kinase activation and epithelial-mesenchymal transition in renal tubular epithelial cells. J Am Soc Nephrol 16:667–675.
Ronnov-Jessen L, Petersen OW, Koteliansky VE, Bissell MJ. 1995. The origin of the myofibroblasts in breast cancer. Recapitulation of tumor environment in culture unravels diversity and implicates converted fibroblasts and recruited smooth muscle cells. J Clin Invest 95:859–873.
Russo FP, Alison MR, Bigger BW, Amofah E, Florou A, Amin F, Bou-Gharios G, Jeffery R, Iredale JP, Forbes SJ. 2006. The bone marrow functionally contributes to liver fibrosis. Gastroenterology 130:1807–1821.
Sappino AP, Skalli O, Jackson B, Schurch W, Gabbiani G. 1988. Smooth-muscle differentiation in stromal cells of malignant and non-malignant breast tissues. Int J Cancer 41:707–712.
Schnelzer A, Prechtel D, Knaus U, Dehne K, Gerhard M, Graeff H, Harbeck N, Schmitt M, Lengyel E. 2000. Rac1 in human breast cancer: Overexpression, mutation analysis, and characterization of a new isoform, Rac1b. Oncogene 19:3013–3020.
Selman M, Pardo A. 2006. Role of epithelial cells in idiopathic pulmonary fibrosis: From innocent targets to serial killers. Proc Am Thorac Soc 3:364–372.
Shook D, Keller R. 2003. Mechanisms, mechanics and function of epithelial-mesenchymal transitions in early development. Mech Dev 120:1351–1383.
Sieweke MH, Thompson NL, Sporn MB, Bissell MJ. 1990. Mediation of wound-related Rous sarcoma virus tumorigenesis by TGF-beta. Science 248:1656–1660.
Singh A, Karnoub AE, Palmby TR, Lengyel E, Sondek J, Der CJ. 2004. Rac1b, a tumor associated, constitutively active Rac1 splice variant, promotes cellular transformation. Oncogene 23:9369–9380.
Sternlicht MD, Lochter A, Sympson CJ, Huey B, Rougier JP, Gray JW, Pinkel D, Bissell MJ, Werb Z. 1999. The stromal proteinase MMP3/stromelysin-1 promotes mammary carcinogenesis. Cell 98:137–146.
Sternlicht MD, Bissell MJ, Werb Z. 2000. The matrix metalloproteinase stromelysin-1 acts as a natural mammary tumor promoter. Oncogene 19:1102–1113.
Sympson CJ, Talhouk RS, Alexander CM, Chin JR, Clift SM, Bissell MJ, Werb Z. 1994. Targeted expression of stromelysin-1 in mammary gland provides evidence for a role of proteinases in branching morphogenesis and the requirement for an intact basement membrane for tissue-specific gene expression. J Cell Biol 125:681–693.
Talhouk RS, Bissell MJ, Werb Z. 1992. Coordinated expression of extracellular matrix-degrading proteinases and their inhibitors regulates mammary epithelial function during involution. J Cell Biol 118:1271–1282.
Tan RJ, Fattman CL, Niehouse LM, Tobolewski JM, Hanford LE, Li Q, Monzon FA, Parks WC, Oury TD. 2006. Matrix metalloproteinases promote inflammation

and fibrosis in asbestos-induced lung injury in mice. Am J Respir Cell Mol Biol 35:289–297.

Tarin D, Thompson EW, Newgreen DF. 2005. The fallacy of epithelial mesenchymal transition in neoplasia. Cancer Res 65:5996–6000; discussion 6000-1.

Thannickal VJ, Toews GB, White ES, Lynch JP, 3rd, Martinez FJ. 2004. Mechanisms of pulmonary fibrosis. Annu Rev Med 55:395–417.

Thomasset N, Lochter A, Sympson CJ, Lund LR, Williams DR, Behrendtsen O, Werb Z, Bissell MJ. 1998. Expression of autoactivated stromelysin-1 in mammary glands of transgenic mice leads to a reactive stroma during early development. Am J Pathol 153:457–467.

Thompson EW, Newgreen DF, Tarin D. 2005. Carcinoma invasion and metastasis: A role for epithelial-mesenchymal transition? Cancer Res 65:5991–5995; discussion 5995.

Tomasek JJ, Gabbiani G, Hinz B, Chaponnier C, Brown RA. 2002. Myofibroblasts and mechano-regulation of connective tissue remodelling. Nat Rev Mol Cell Biol 3: 349–363.

Urbanski S, Jensen HM, Cooke G, McFarlane D, Shannon P, Kruikov V, Boyd NF. 1988. The association of histological and radiological indicators of breast cancer risk. Br J Cancer 58:474–479.

Walter N, Collard HR, King TE Jr. 2006. Current perspectives on the treatment of idiopathic pulmonary fibrosis. Proc Am Thorac Soc 3:330–338.

Wellings SR, Wolfe JN. 1978. Correlative studies of the histological and radiographic appearance of the breast parenchyma. Radiology 129:299–306.

Willis BC, Liebler JM, Luby-Phelps K, Nicholson AG, Crandall ED, du Bois RM, Borok Z. 2005. Induction of epithelial-mesenchymal transition in alveolar epithelial cells by transforming growth factor-beta1: Potential role in idiopathic pulmonary fibrosis. Am J Pathol 166:1321–1332.

Willis BC, duBois RM, Borok Z. 2006. Epithelial origin of myofibroblasts during fibrosis in the lung. Proc Am Thorac Soc 3:377–382.

Witty JP, Wright JH, Matrisian LM. 1995. Matrix metalloproteinases are expressed during ductal and alveolar mammary morphogenesis, and misregulation of stromelysin-1 in transgenic mice induces unscheduled alveolar development. Mol Biol Cell 6:1287–1303.

Yamaguchi Y, Kubo T, Murakami T, Takahashi M, Hakamata Y, Kobayashi E, Yoshida S, Hosokawa K, Yoshikawa K, Itami S. 2005. Bone marrow cells differentiate into wound myofibroblasts and accelerate the healing of wounds with exposed bones when combined with an occlusive dressing. Br J Dermatol 152:616–622.

Yang J, Mani SA, Donaher JL, Ramaswamy S, Itzykson RA, Come C, Savagner P, Gitelman I, Richardson A, Weinberg RA. 2004. Twist, a master regulator of morphogenesis, plays an essential role in tumor metastasis. Cell 117:927–939.

Zuo F, Kaminski N, Eugui E, Allard J, Yakhini Z, Ben-Dor A, Lollini L, Morris D, Kim Y, DeLustro B, Sheppard D, Pardo A, Selman M, Heller RA. 2002. Gene expression analysis reveals matrilysin as a key regulator of pulmonary fibrosis in mice and humans. Proc Natl Acad Sci USA 99:6292–6297.