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Triggering the landslide: the tumor-promotional effects of myofibroblasts

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Abstract

Cancers become significantly more dangerous when the tumor progresses from in situ, or contained, to an invasive state, in which the cancer cells acquire the ability to pass through the surrounding basement membrane (BM), a specialized extracellular matrix (ECM) that provides structure and contextual information to the underlying tissue. While the majority of tumors are carcinomas, derived from epithelial cells, it is the stromal cells surrounding the epithelial-derived tumor cells, including fibroblasts and myofibroblasts, vasculature, and immune cells, that are largely responsible for the production and remodeling of the ECM. Here, we will discuss myofibroblasts as key effectors of tumor progression, focusing on recent advances in breast and pancreatic carcinoma, showing how myofibroblasts may function properly in normal tissue remodeling and wound-healing processes, how in the tumor context they can drive cancer invasion and metastasis, and how the pathogenic functions of myofibroblasts may be targeted therapeutically.

Keywords

Myofibroblast; breast cancer; pancreatic cancer; epithelial-mesenchymal transition; extracellular matrix; tissue disruption

Introduction and context

Understanding the key factors in development and progression of a particular cancer type is critical for identifying therapeutic interventions that target cancer at the earliest stages of development. Environmental, nutritional, and hereditary factors that have been identified as associated with increased cancer risk could potentially reveal those processes essential for cancer development. Surprisingly, while cancer risk prediction models that incorporate such risk factors have been found to perform well at the population level, they have not translated as well to individualized cancer risk assessment [1]. Such findings have led to the realization that apart from the cancer cells, the composition and structure of the ECM plays an

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important inhibitory role in cancer development [2]. During a person's lifetime, individual cells may accumulate potentially tumorigenic genetic mutations, but only rarely do these develop into cancer. Cancer development might be viewed as a landslide, where somatic mutations can be viewed as pre-existing stresses, and exposure to carcinogenic toxins as a continuous rain. These mutations may lead to development of abnormalities, and even to carcinoma in situ, but so long as the underlying ECM remains intact, tumor development is slowed. Once myofibroblasts become activated, however the ECM becomes damaged and degraded; the former solid surface becomes loose, leading to system collapses, systemic disease, and death.

The immune system can detect the existence of some oncogenic alterations, in a similar fashion to identification of viruses and bacteria. Initially, the immune system might attempt to “heal” the cancer in the same way it heals wounds, invading the tissue and transforming the phenotypes of resident cells [3]. What works well in wounds, however, can go awry in the innate fight against cancer, where the roles of myofibroblasts and even their origins are not entirely parallel. One major difference between wound myofibroblasts and tumor associated myofibroblasts is the ability of the latter to influence epithelial cells to proliferate and to drive malignant transformation [4]. Another difference is that in wounds, myofibroblasts appear to be differentiated from mesenchymal cells, while in the tumor microenvironment, myofibroblasts can develop directly from the epithelial cells as well as from the mesenchymal cells [5, 6].

In normal wound healing, the initiating damage causes the activation of the coagulation cascade that in turn activates the innate immune response. With the arrival of myeloid-derived immune cells, the signal for cytokine production leads towards activation of the adaptive immune system. Secreted cytokines activate fibroblasts and endothelial cells, which transform into myofibroblasts that stimulate angiogenesis, increase ECM production, and physically contract the wound edges, facilitating the healing process. The initiating factors including key cytokine Transforming Growth Factor- β (TGF- β) are eventually depleted as the wound heals, at which time the myofibroblasts disappear through apoptosis [3, 5]. If myofibroblasts persist, they transform the site into a chronic wound; examples of this phenomenon include skin keloids [7] and fibrosis of organs such as liver, lung or pancreas [8].

In cancer development, myofibroblasts are present in the stroma of most epithelial tumor types [9, 10], where they can act as drivers of malignant transformation. The tumor-associated myofibroblasts express matrix metalloproteinases (MMPs) which can degrade nearly every component of the ECM, including the BM; myofibroblasts also possess the ability to contract the ECM, which disrupts normal epithelial tissue structures and provides pathways for tumor cells to invade [11]. Secreted MMPs interact directly with the carcinoma cells, breaking down cell-cell junctions and adhesions, further facilitating tumor cell movement [12]. MMPs can also directly stimulate tumor progression through promotion of the epithelial mesenchymal transition (EMT) [13], a developmental process that activates a migratory and invasive cellular phenotype in epithelial tumor cells and that can under chronic activation conditions cause epithelial cells to transition entirely into a myofibroblast phenotype [14].

Fibrosis and myofibroblasts

Myofibroblasts are ubiquitously found in the human body, in the skin during wound healing [8, 15], the heart [16, 17] and the intestines [4]. They are detectable by week 21 during embryogenesis [4] while in adults, stellate cells in liver, pancreas, and lung possess myofibroblast characteristics. During activation, myofibroblasts develop actin stress fibers and express α -smooth muscle actin (α SMA) [3, 15, 18, 19]. In 1972 Gabbiani et al. described fibroblasts in their ability to become contractile cells by undergoing essential changes of their inner architecture as well as their membrane structure [20]. In the years after the first description this cell type was found in all types of connective and soft tissue disorders such as fibrosarcoma [21], arterial dysplasia [22] and also in organ specific parenchymal disorders such as alcoholic cirrhosis [23].

Fibrosis is defined as the disproportionate increase of fibrous connective tissue, collagens, and fibronectin [19]. Usually occurring first around inflamed or injured tissue, fibrosis can be found in benign skin conditions such as Dupuytren's contracture [24], and can be a life-threatening disease when it affects the internal organs such as lung, liver, kidneys, and pancreas [8]. Myofibroblasts are the key players in the development of fibrosis through their ability to produce large amounts of ECM and to modulate contraction, leading to a very dense tissue appearance as seen for example in fibrosis of the liver, lung, or breast [5, 25–27]. Expression of TGF- β is central to fibrosis generation and maintenance. The three isoforms of TGF- β (β 1, β 2, β 3) bind to TGF- β receptor II (TGF- β RII) which will then generate a complex with TGF β RI; this heteromeric receptor complex then phosphorylates Smad transcription factors which in turn translocate into the nucleus to control transcription [28–30]. TGF- β 1 has been the best studied of the isoforms for its role in activation and signaling pathways of myofibroblasts [31]

TGF- β 1 can induce myofibroblast differentiation from fibroblasts; inhibiting TGF- β 1 signaling in fibrosis leads to reduced expression of myofibroblast markers and decreased tissue contraction [15, 16, 32]. The activating TGF- β 1 can derive from invading immune cells, epithelial cells, or through fibroblast autocrine pathways [33]. Other cytokines such as TNF- α have been shown to facilitate TGF- β induced myofibroblast activation and fibrosis promotion [34]. In addition reactive oxygen species (ROS) have also been implicated in TGF- β -induced myofibroblast formation, and the antioxidant N-acetylcysteine has been assessed as a potential antifibrosis therapeutic [35]. The mechanical and oxidative stress that leads to the production of ROS can alter the gene expression in fibroblasts as well [36, 37].

Cancer and myofibroblasts

Cancer-associated myofibroblasts possess many of the features found in nonmalignant tissue. However, there is an important difference: in normal wound healing, elevated levels of TGF- β 1 in the wound microenvironment has a powerful cytostatic effect on epithelial cells, but once the epithelial cells have acquired specific mutations, the cytostatic effect of TGF- β is lost [38, 39]. Additionally, the metaplastic characteristic of tumors allows for a wider range of cellular differentiation processes, and many instances of epithelial-derived, cancer-associated myofibroblasts have been found. In many cases, these may differentiate

from the cancer cells themselves through EMT, which is facilitated both by the cytokines produced by cancer cells, the invading immune cells [40, 41], and by the elevated levels of ROS found in tumors [42]. ROS can stimulate the expression of Snail, which can activate the EMT program, including downregulation of E-cadherin, increased invasiveness, and expression of mesenchymal markers [43–45]. TGF- β 1 can stimulate ROS production in endothelial cells as well, triggering conversion to myofibroblasts through autocrine and paracrine effects [46]. ROS also directly activate fibroblasts, producing myofibroblasts with mitochondrial dysfunction and alterations in aerobic glycolysis that increase production of l-lactate, ketone bodies, and glutamine; these nutrients function as energy sources for the cancer cells' anabolic growth and oxidative mitochondrial metabolism [47–50].

Breast cancer

Myofibroblasts are found in all subtypes of breast cancer [12, 51, 52], and are a major component of the breast cancer mass. Myofibroblast abundance correlates with lymph node metastasis, higher clinical grade, and poor overall survival prognosis [53]. Most breast cancer cell lines can be induced to undergo EMT, but ongoing activation of EMT is particularly evident in metaplastic breast carcinomas, a breast cancer subtype with particularly poor prognosis [54]. Direct experimental evidence for generation of myofibroblasts from tumor cells came from experiments in which fibroblast cell lines were derived from a metaplastic breast tumor [6]. These cell lines were found to have common patterns of X-chromosome inactivation with the parental tumor, indicating a common cellular origin, but were found to express mesenchymal markers. Furthermore, the lines behaved *in vitro* and *in vivo* as normal fibroblasts, but when combined with tumor cells, expressed α SMA and acquired the phenotypic appearance of myofibroblasts. Strikingly, the tumor-derived fibroblasts were nonmalignant when injected into immunocompromised mice, but substantially enhanced tumor growth when coinjected with MCF7 breast cancer cells [6]. MMPs, abundant in breast cancer, can also directly activate myofibroblast characteristics in nonmalignant mammary epithelial cells through a pathway involving increased expression of the splice isoform Rac1b and increased production of ROS [43].

Some of the same processes that regulate tumor cell malignancy can also affect how myofibroblasts function. Examination of p16^{INK4A} levels in multiple samples of tumor associated breast fibroblasts revealed that a significant proportion showed downregulation of protein expression [55]. p16^{INK4A} is a cyclin dependent kinase inhibitor that regulates cell cycle progression and is expressed in cells undergoing senescence, an intrinsic antiproliferative mechanism. These experiments showed further, that reduced p16^{INK4a} was associated with reduced expression of α SMA. Conditioned media from fibroblasts with downregulated p16^{INK4a} stimulated proliferation of epithelial cells *in vitro* and when coinjected with MDAMB231 cells in orthotopic xenografts lead to faster tumor progression [55]. Myofibroblasts and breast cancer cells can interact with each other in complex ways. During tumor invasion, TGF- β 1 signaling causes “single strand migration” of myofibroblasts and tumor cells, while inhibition of TGF- β results in compact movement of the invasion front of the tumor [56]. As another example, breast cancer cells can induce laminin 332 and integrin β 4 expression in myofibroblasts, enhancing anoikis resistance for single detaching cells, thus stabilizing the surrounding ECM for the tumor[57].

Pancreatic cancer

In the pancreas, myofibroblasts are derived principally from pancreatic stellate cells (PSC). These cells are also important in the formation of chronic pancreatitis, a common comorbidity of pancreatic cancer and known predisposing factor in cancer development. PSCs are needed under normal circumstances to organize the ECM and to maintain the structural integrity of the organ. With tissue injury (caused by excessive alcohol, toxins, intake of fatty acids, or hypoglycemia), PSCs become activated to myofibroblasts, with characteristic development of microfilaments, expression of α SMA, increased production of ECM and MMPs, and increased contractility [58]. Similarly, in adenocarcinoma α SMA is found in the typical fibrillar pattern in the myofibroblasts, and in regions with high amounts of desmoplasia; procollagen I is also detected in activated myofibroblasts [59] (Figure 1).

Specific mechanisms for activating pancreatic cancer-associated myofibroblasts have recently been identified: expression of paladin by pancreatic cancer cells stimulates myofibroblast activation [11], and expression in pancreatic myofibroblasts of the adhesion molecule L1CAM causes increased migratory ability and chemoresistance in pancreatic cancer cells [60, 61]. Pancreatic cancer-associated myofibroblasts may also act to drive metastasis by directly seeding the metastatic bed, as revealed by identification of Y-chromosomal positive pancreatic tumor-derived myofibroblasts in female mice in distant metastases [62].

Current challenges, and future directions

The near ubiquitous co-occurrence of fibrosis with cancer in the pancreas [63], liver [19, 64], and kidney [45], and the fact that chronic fibrosis is a predisposing factor for cancer development in these and other organ systems [5, 8, 45, 65] points to a central role of myofibroblasts in tumor development. The recent identification of additional direct interactions between tumor cells and myofibroblasts that potentiate tumor malignancy further highlights the importance of these cells. Therapeutic approaches that blocked myofibroblast development or function could have substantial value for treatment of fibrosis or cancer. However, while myofibroblasts are easy to identify in the tumor context, they are very difficult to study in culture, as their phenotype is very fluid, and it is unclear how much tissue-specificity is retained when separated from the *in vivo* context. Markers of myofibroblast tissue specificity are needed, as are more sophisticated culture methods for differentiating essential functions of myofibroblasts in the normal context from those that occur in chronic fibrosis and tumors. Even existing anticancer therapies may be more effective if we can block the destructive degradative action of the pathologic myofibroblasts, halting the landslide.

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Abbreviations

BM	Basement membrane
ECM	Extracellular matrix
MMPs	Matrix metalloproteinases
TGF-β	Transforming growth factor- β
ROS	Reactive oxygen species

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Figure 1. Myofibroblast localization identified by smooth muscle actin staining (α SMA) in pancreatic ductal carcinoma

(A,B) Normal pancreatic tissue, hematoxylin and eosin (H&E) staining (A) shows normal acinar cell and duct formation; in healthy tissue α SMA staining appears mainly in endothelial cells with rare presence of α SMA staining in connective tissue (B). (C,D) Ductal pancreatic carcinoma, H&E staining (C) shows disrupted gland formation with malignant cells, and large amounts of tumor surrounding stroma; strong positive staining of α SMA (D) reveals abundant myofibroblasts.