In: Myofibroblasts
Editor: Analise Martinez
ISBN: 978-1-63485-743-7

Chapter 3

MYOFIBROBLASTS IN CANCER AND FIBROSIS: TWO SIDES OF THE SAME COIN?

Bridget Hindman and Qiang Ma*
Receptor Biology Laboratory, Toxicology and Molecular Biology Branch,
Health Effects Laboratory Division,
National Institute for Occupational Safety and Health,
Centers for Disease Control and Prevention, Morgantown, WV, US

ABSTRACT

Increasing evidence reveals that the tumor microenvironment plays a pivotal role in determining how cancer cells proliferate, invade, and metastasize. The role of tumor stromal fibroblasts, known as cancer-associated fibroblasts (CAF), in particular is being investigated in a number of types of cancer. This interest is in part due to the complicated relationship between cancer cells and their extracellular matrix (ECM). Many solid tumors, such as breast cancer, are associated with a significant increase in ECM stiffness. This stiff ECM encourages cancer cell proliferation, invasion, and migration through a process known as mechanoreciprocity. But are cancer cells themselves directly responsible for the stiffening matrix? Or do CAF, which are more canonically associated with matrix changes than epithelial tumor cells, mediate this

* Corresponding Author address: Email: qam1@cdc.gov.

process? CAF are known to have pro-invasive effects on tumor cells, but the molecular basis of these effects are largely unclear. Several studies have shown that certain CAF cells are capable of *de novo* synthesis of α-smooth muscle actin-containing stress fibers that bestow contractility to the cells, and have high capacities of protein synthesis and secretion, both of which are characteristics of myofibroblasts found in wound healing and organ fibrosis. Therefore, at least some CAF appear to behave as activated myofibroblasts. Nonetheless, the role of myofibroblasts in cancer remains a subject of debate, which in part is due to differing terminology used and a lack of a specific marker(s) in defining myofibroblasts. In this review, we summarize the evidence for the presence and role of myofibroblasts in cancer progression and discuss the similarities of myofibroblasts in cancer and fibrosis.

Keywords: myofibroblast, CAF, tumor microenvironment, α-SMA, ECM

INTRODUCTION

Cancer is a collection of devastating diseases that affects millions of people every year and is the focus of billions of dollars in research and healthcare [1]. Cancer, especially "solid tumors," such as breast or liver cancer, mostly derives from epithelial cells, whereas tumors of connective tissues and stromal cells are relatively rare [2]. For this reason, the role of the stroma, especially that of fibroblasts, in cancer development and progression has only begun to be rigorously investigated in the relatively recent past. Nonetheless, this research has yielded a plethora of evidence supporting the idea that the microenvironment, including local fibroblasts, does play a significant role in cancer tumorigenesis and progression [3-5].

Fibroblasts are mesenchymal cells that play an essential role in development and in the maintenance of tissue structure and integrity [6]. These cells are responsible for the production and deposition of the extracellular matrix (ECM) as well as the physical reshaping of the ECM, which impacts other cell types, such as the epithelium, in a myriad of ways [6-8]. Fibroblasts play multiple roles in physiologic wound healing, ranging from recruitment and migration of inflammatory cells into the wound site, to formation of granulation tissue and eventual contracture and scarring of the repaired wound [6]. Fibroblasts and their related cells are also important, sometimes essential, for the development of certain diseases. Cancer is known to bear functional similarities to wound healing, such that it is referred to as "wound that will not

heal" [9, 10]. In this context, it is not surprising that fibroblasts play a part in cancer, which has been a subject of increasing interest in the fields of both cancer research and matrix biology. The role of fibroblasts in organ fibrosis, a pathologic condition that has, perhaps, a more direct resemblance to wound healing gone awry than cancer, has been abundantly clear, as may be gathered from its name [11].

Both cancer and organ fibrosis are associated with substantial changes in the ECM, such as collagen deposition, matrix cross-linking, and contraction, which typically result in a stiffening of the ECM [11, 12]. These functions—i.e., matrix secretion and contractility—are characteristically associated with activated fibroblasts, also known as myofibroblasts [13-15]. Therefore, while epithelial tumor cells have been proven capable of these functions, the more likely culprit of these changes in the cancer environment would be local fibroblasts and their associated cells. From this prospect, as the role of fibroblasts in cancer becomes more evident, the question remains as to whether the stromal fibroblasts in cancer are activated to become myofibroblasts, and if so, whether they express the same markers and have the same functions as the myofibroblasts found in fibrosis. In this review, we discuss the cell markers and functions attributed to cancer-associated fibroblasts (CAF) and myofibroblasts in cancer and fibrosis to determine if these populations of cells are truly distinct or merely two sides of the same coin.

Tumor Microenvironment and Stiffness in Cancer

The tumor microenvironment is the local environment surrounding tumor cells that consists of tissue components, such as blood vessels and the ECM, and cell components, such as immune cells and fibroblasts, all of which directly or indirectly affect tumor cell survival, proliferation, and metastatic behavior in multiple ways [16]. For the purpose of this chapter, we focus our discussion on the role of mesenchymal cells—fibroblasts and their related cells—in cancer development with emphasis on their relations to cancer-promoting physical characteristics—matrix stiffness in particular—of the tumor microenvironment.

The tumor microenvironment is known to have both restrictive and enhancive effects on tumor development and invasion [17-21]. Early in the disease process, the normal tissue surrounding a mutated cell prevents that cell from unchecked proliferation through a variety of chemical and structural cues

[17, 21, 22]. Such restraining effects of a normal microenvironment on cancer development are supported by the observation that many individuals have small, undiagnosed tumors at the time of death that fail to, or have not had the chance to, develop into a full-blown cancer [23-27]. These tumors could simply be slow growing; alternatively, the local microenvironment could have restricted tumor growth. In patients that do develop clinically recognized cancer, the microenvironment may have supported tumorigenesis from early steps. Many tumors have accumulated multiple genetic abnormalities at the time of biopsy and diagnosis [28]. One of these multiple hits could be a change in the microenvironment, wherein an initial genetic mutation or chromosomal abnormality in the tumor cell is encouraged by a tumor-promoting alteration in the surrounding tissue that allows the tumor cell to proliferate [17, 18]. For instance, a dense parenchymal pattern that indicates a stiffness in the breast tissue is a high risk factor for breast cancer [29]. In these patients, the tumor promoting environment may exist even before the tumor-initiating genetic mutation occurs.

The tumor microenvironment, in particular matrix stiffness, has an effect on cancer progression. Stiffness can be altered by three types of changes in the matrix: increased deposition of matrix components such as collagen, increased cross-linking by enzymes such as lysyl oxidase (LOX), and architectural changes of matrix components such as collagen bundling [16, 30, 31]. In a normal tissue, there is a delicate balance maintained between tissue stiffness and cellular stiffness, a phenomenon known as tensional homeostasis or mechanoreciprocity [7, 32]. When this tensional homeostasis is lost, an increase in stiffness of the surrounding matrix causes an increase in cellular tension as the cells attempt to regain that balance, which is evidenced in many cancers [33, 34]. Increased cellular tension enhances cell proliferation via a FAK-Rho-ERK signaling axis. Changes in matrix stiffness can also have direct effects on cell signaling, which makes separating the mechanical and chemical effects a difficult task. As an example, increased collagen deposition leads to a stiffened matrix and, at the same time, activates integrin signaling that boosts cell survival and proliferation [35, 36].

Altered microenvironments and stiffness may also directly affect cancer cells in a variety of other ways. For instance, putting normal epithelial cells into stiff matrices was sufficient to induce malignant phenotypes, such as the appearance of invasive behavior and loss of polarization, in a 3D culture [35]. Culturing lung cancer cells on fibroblast-derived matrices has effects on cell growth, and perhaps more notably, expression of markers of epithelial-mesenchymal transition, an important phenomenon in cancer cell biology that

is generally thought to yield cells with high metastatic potential [37]. Therefore, cancer microenvironment and stiffness can contribute to the initial stages of tumorigenesis where normal epithelial cells transform into malignant cancer cells, as well as later stages where cancer invasion and metastasis take place.

The role of tumor microenvironment and stiffness in cancer invasion and metastasis has been studied in a somewhat more detailed fashion. Increased collagen deposition provides an ideal scaffold for cell migration, along which tumor cells can migrate to invade the surrounding tissues [9, 12, 13]. Fibers aligned in a perpendicular fashion to the edge of a tumor increase the invasive potential of cells, likely because they present a convenient path for migration with less matrix manipulation on the part of the tumor cells [38]. In a process known as durotaxis, cells tend to migrate from softer substrates towards stiffer substrates [39, 40]. In order for cancer cells to efficiently migrate through a matrix, they must form mature focal adhesions, which create the physical connections between the cell and its matrix in a force-dependent manner [39, 41, 42]. A stiffer substrate, such as the altered ECM surrounding tumors, allows cells to exert the force needed for focal adhesions to mature. There are also physical constraints a dense matrix places on cells to make cancer cells migrate at a faster rate and with more persistence [40]. Thus, stiffened matrix and increased physical constraints would increase cancer cell invasion, migration, and metastasis, which are poor prognostic factors for cancer patients clinically [43, 44].

Apart from the apparent importance of tumor microenvironment and stiffness in cancer, insights into how they are altered can be difficult to interpret. There is some evidence that cancer cells themselves are capable of interacting with and altering the surrounding matrix. For instance, increased expression of the cross-linking enzyme LOX by tumor cells has been shown in several types of cancer [45-47]. Cancer cells are also capable of secreting ECM components, such as hyaluronan, fibronectin, and collagen, as well as matrix metalloproteins that degrade the matrix [16, 48, 49]. Finally, cancer cells can directly manipulate the surrounding matrix fibers, rearranging them to generate a stiffer collagen matrix [38, 50, 51]. Nevertheless, many of these functions—matrix protein deposition, secretion of matrix modulating enzymes, and physical manipulation of the ECM through cell contractility—are characteristically and developmentally associated with fibroblasts, especially activated myofibroblasts [6, 15, 52, 53]. This raises the question of how much of the matrix changes surrounding a tumor is due to the cancer cells themselves, and how much to tumor stromal fibroblasts.

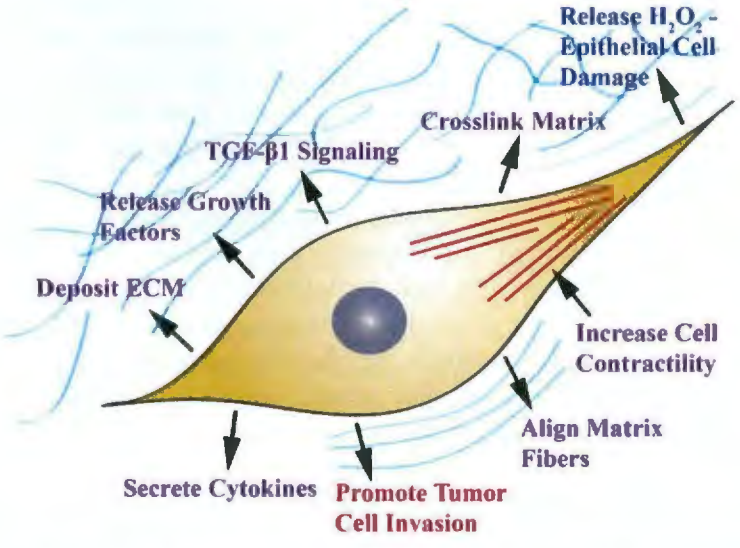

Figure 1. Functions of Myofibroblasts in Cancer and Fibrosis. Cancer-specific functions are listed in red, whereas fibrosis-specific functions are listed in blue and functions shared between the two diseases in purple.

FIBROBLASTS IN CANCER

The contribution of stromal fibroblasts to the progression of cancer has been of increasing interest in recent years [5, 54, 55]. Many investigators have referred to these cells as CAF [3, 5, 54], whereas others have used peritumoral fibroblasts or cancer-associated myofibroblasts for the cells. In fact, all three terms have been used somewhat interchangeably [52]. This differing terminology is perhaps in part due to the relatively new and sparse literature directly studying the cells that are not the tumor cells themselves. For the purpose of this discussion, we refer to these cells as CAF.

CAF are believed to contribute to disease progression in a number of ways (see Figure 1 for a graphic summary). Activated fibroblasts are highly secretory cells that deposit ECM proteins and enzymes, including collagens, fibronectin, and LOX [3, 56, 57]. In cancer, active CAF are capable of generating a dense and rigid ECM surrounding the tumor called desmoplasia [30, 58] and the response is termed the desmoplastic response [3]. This reaction and the ECM generated are very similar to what occurs during normal wound healing. In fact, the fibrillary networks generated are nearly identical between cancer and wound healing [59]. The role of CAF in altering tumor

ECM also extends beyond depositing matrix proteins. Activated fibroblasts are highly contractile cells. This contractility allows CAF to physically rearrange the ECM, such as the bundling and re-aligning of the collagen fibers, which promotes tumor growth and invasion [56, 60].

CAF can also impact cancer cell behavior through other means. CAF secrete several growth factors that promote proliferation, including HGF, EGF, FGF, and TGF-β1 [4, 61, 62]. The hormone leptin has been implicated in CAF's influence on tumor proliferation, where CAF-derived leptin drove cancer cell proliferation in a co-culture of CAF and breast cancer cells [63]. Conditioned media from CAF can stimulate estradiol processing by breast cancer cells that overexpress the estrogen receptor, a common subtype of breast cancer. In this scenario, increased estradiol processing would boost breast cancer cell proliferation [22]. Cytokines are another class of molecules secreted by CAF that influence tumor cell behavior in multiple ways. Certain cytokines and chemokines, such as CXCL13, are pro-inflammatory and recruit leukocytes such as macrophages that are immunosuppressive and prevent the cytotoxic T cells from attacking the tumor [54, 64, 65]. Increased secretion of growth factors and chemokines also promote tumor invasion and metastasis by stimulating the epithelial to mesenchymal transition and by acting as a chemoattractant to lure cells towards the blood vessels, both of which foster cancer cells to ultimately metastasize to distant organs, which accounts for a majority of cancer mortality [4, 54, 62].

In addition, CAF can take a more direct role in tumor invasion. Fibroblasts are uniquely capable of migrating through a dense matrix as part of their normal physiological function [61, 66, 67]. The fibroblasts surrounding tumors are recruited to forge the path, as it were, for cancer cells [3, 54, 62, 68]. In aggregate, CAF play an important role in cancer cell proliferation, invasion, and metastasis through multiple means.

CAF AS MYOFIBROBLASTS

Myofibroblasts are the activated fibroblasts that have high capacities of both ECM-secretion and contraction. Although considerable progress has been made in understanding these cells over the past several decades, there are many unanswered questions regarding myofibroblasts. For instance, there is a lack of a truly specific maker(s) for the cells. Rather, the identification of the cells relies on the use of a combination of markers and functions, making it difficult to isolate this population of cells and their functions from the milieu

of a tumor and its microenvironment in the case of cancer. Even in fibrosis, where the function of myofibroblasts has been well established, there is a debate on the origin of these cells. Are resident fibroblasts activated to the active state? Do other types of cells transdifferentiate? There is evidence for both possibilities, and these questions are even murkier for cancer.

Recent in depth studies of tumor stromal fibroblasts on their function, origin, genetics, and expression of marker proteins reveal that there are different subpopulations of these cells, some of which are in the active, myofibroblast state [4, 61, 69]. As these cells undergo closer investigation, the similarity of the more active CAF to myofibroblasts becomes more evident. In fact, many of the markers used to identify CAF are also used for identification of active myofibroblasts in other diseases (see Table 1 for a brief summary).

Activated fibroblasts that contribute to cancer progression have the capacity of *de novo* synthesis of α-smooth muscle actin (α-SMA) that incorporates into the cytoskeletal stress fiber of the cell. α-SMA in stress fibers is considered a major characteristic of myofibroblasts, although other types of cells, such as smooth muscle cells of small blood vessels and some myoepithelial cells, also express this protein. Increased expression of α-SMA-containing stress fibers in CAF can be seen morphologically in breast cancer and many other types of cancers (Figure 2) [58, 62, 67, 83]. Moreover, the expression of this contractile cytoskeletal protein is often associated with more advanced cancer stages and poorer clinical outcome in patients and animal models [55, 83], which is illustrated in Figure 2 for breast cancer.

Table 1. Commonly used markers of CAF and myofibroblasts

Marker	Cell Type	References
α-SMA	CAF, Myofibroblast	[70, 71]
FSP-1	CAF, Myofibroblast	[72, 73]
PDGFR	CAF, Myofibroblast	[70, 74, 75]
Vimentin	CAF, Myofibroblast	[70, 76]
Col1A1	CAF, Myofibroblast	[69-71]
Podoplanin	CAF	[62, 77]
Desmin	CAF	[67, 78]
Cad11	CAF	[67, 79]
FAP	CAF	[80, 81]
Tenascin-C	CAF	[62, 82]

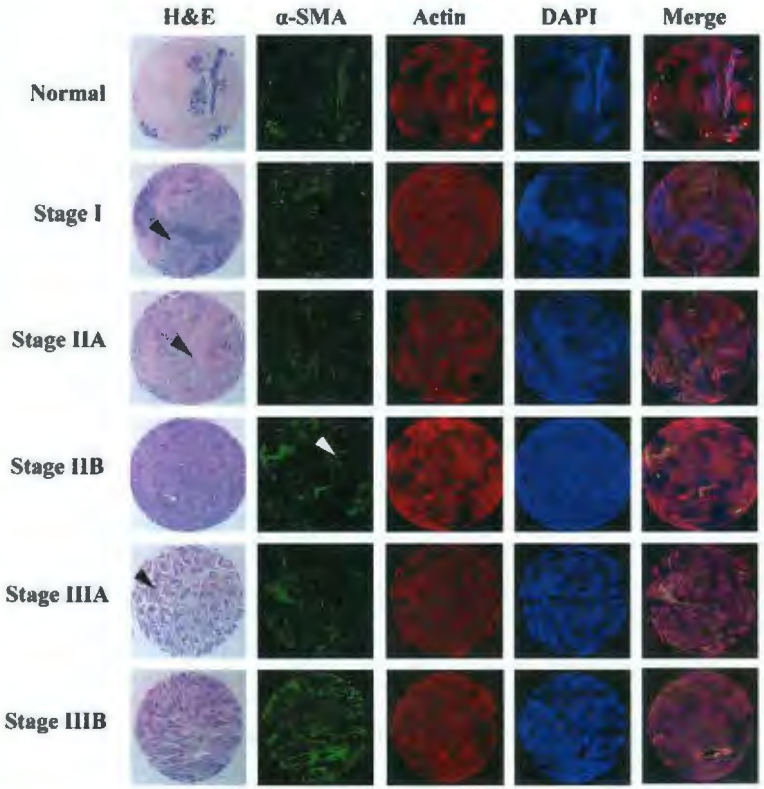

Figure 2. Formation of myofibroblast in breast cancer. An array of breast samples were stained with hematoxylin and eosin to distinguish architecture features of normal and increasing stages of cancer tissues of human breast. Arrays of adjacent sections were then immunostained for α-SMA to investigate the presence of activated fibroblasts, actin to delineate cell borders and tissue components, and DAPI for counterstaining of the nucleus. In normal breast sections and in the initial stages of breast cancer, α-SMA staining was confined to the smooth muscle cells surrounding blood vessels and the myoepithelial cells of the duct. Beginning at stage IIB, there were α-SMA-positive fibroblasts that are not associated with either of these tissue components, indicating formation of cancer-associated myofibroblasts.

In Figure 2, an array of human breast cancer tissues were immunostained for α-SMA, in comparison with histopathological sections of the same core, to determine if α-SMA expressing cells were associated with the tissue and matrix changes of progressive cancer stages. In the normal breast tissue section (top panels) where ducts are clear of cells, α-SMA-positive cells are confined to the myoepithelial cells surrounding the mammary ducts and the smooth muscle cells associated with blood vessels. In stage I breast cancer

sections, the mammary duct is enlarged and filled with tumor cells (indicated by black arrow head) with α-SMA expression primarily associated with smooth muscle and myoepithelial cells. At stage IIA, the tumor cells have clearly begun to invade through the ductal wall into the surrounding tissue (black arrow head), but α-SMA expression is still confined to the myoepithelial cells surrounding the ducts. At stage IIB, stromal infiltration is evident and there is some faint α-SMA immunostaining that is not associated with either the myoepithelial cells of the ducts or the smooth muscle cells of the blood vessels (white arrowhead in α-SMA panel). At stage IIIA, in which lymph node involvement becomes significant, there is significant α-SMA staining among tumor-associated stromal cells together with evident collagen deposition (black arrowheads in histology panel). This pattern—increased α-SMA expression in stromal cells and increased collagen deposition surrounding tumor cells—is continued in the stage IIIB samples. This gradual increase in stromal infiltration, collagen deposition surrounding tumor cells, and α-SMA expression as the severity of the disease progresses agrees with the notion that myofibroblast activation is associated with more advanced stages of breast cancer.

Increased expression of α-SMA can also be demonstrated in cultured fibroblasts isolated from lung tumors compared to fibroblasts from normal lungs [84]. Cancer fibroblasts also express other proteins associated with active myofibroblasts, including FSP-1, podoplanin, and PDGFR [61, 62, 67, 69]. It is worth noting that not all these markers are expressed in CAF and not all CAF express these markers. These findings indicate that there exist different subpopulations of CAF, some of which are more active in promoting tumor progression, and it is these more active CAF that express higher levels of α-SMA fibers and other myofibroblast markers [85].

In addition to expressing myofibroblastic markers, CAF share a similar functionality to myofibroblasts in two aspects. First, it is known that myofibroblasts are highly contractile through their prominent α-SMA-containing stress fibers that are capable of generating a large amount of force; and evidence reveals that at least some fibroblasts isolated from lung tumors exhibit similar ultrastructure and demonstrate contractile potential in *in vitro* gel contraction assays [13, 85, 86]. Second, both myofibroblasts and CAF secrete ECM proteins including collagen and fibronectin to construct the ECM in normal or cancer and other disease states [4, 13, 14, 68, 84, 87]. These similarities indicate that CAF have a lot in common with the myofibroblasts associated with normal processes such as wound healing, and that the more

active CAF that express such markers as α-SMA and contractile functionality are indeed similar enough in function to be qualified as myofibroblasts.

Comparison of Myofibroblasts in Fibrosis and Cancer

Myofibroblasts are also highly involved in the disease progression of fibrosis, which bears some similarities to cancer except that it does not possess uncontrolled epithelial cell growth (see Figure 1 for a graphic illustration of the functions of myofibroblasts in both disease processes). In fibrosis, an initial inflammatory response to a trigger activates myofibroblasts in the tissue. Such activators include: fibrogenic chemicals, such as the cancer chemotherapeutic agent bleomycin and the herbicide paraquat, and inhaled particles and fibers, such as silica and asbestos, both of which induce lung fibrosis [88-91]; pre-existing medical conditions, such as non-alcoholic fatty liver disease that results in liver cirrhosis [92]; auto-immune abnormalities, such as systemic sclerosis that includes organ fibrosis as a severe outcome and cause of lethality [93]; and idiopathy, such as idiopathic pulmonary fibrosis or IPF [94].

Like the desmoplastic response in cancer, the initial stages of fibrosis are similar to normal wound healing. Fibroblasts are activated and begin to deposit ECM components while recruiting immune cells. As with cancer, this process fails to reach an end point. Rather than the fibroblasts being "turned off" through mechanisms like apoptosis when the job is done, as seen in physiologic wound healing, the cells are persistently present and remain activated to continue to deposit ECM until organ function is inhibited from pathologic scarring and deformation [11, 12, 36, 86]. Cell markers of activated myofibroblasts are also shared between cancer and fibrosis, with the most often used being α-SMA-containing stress fibers (see Table 1 for comparison) [67].

There are also similarities in the activation of myofibroblasts in both diseases, the most prominent being TGF-β1. TGF-β1 has complex and paradoxical effects on carcinogenesis. Early in the disease process, TGF-β1 has a cytostatic effect on tumor cells, thus acting as a tumor suppressor. However, when dysregulated it is a well-known driver of carcinogenesis by way of inducing EMT and promoting invasive and metastatic behaviors through its microenvironment effects as well as its direct effects on the cancer

cells [95, 96]. TGF-β is an extremely potent pro-fibrogenic molecule and is upregulated in nearly all forms of fibrosis, and therefore is arguably the most relevant endogenous inducer of fibrosis [66, 91, 97, 98]. It also has a unique place in the transforming potential of the altered ECM in both cancer and fibrosis, being the center of a feed-forward loop in both diseases. Secreted TGF-β1 is sequestered in the matrix, bound to ECM components like fibronectin by its latency associated peptide [36, 53, 99]. When activated myofibroblasts contract the surrounding matrix, TGF-β1 is released from the matrix and is free to bind to its receptor on both fibroblasts and cancer cells, driving myofibroblast differentiation and cancer cell proliferation, as well as epithelial-to-mesenchymal transition implicated in both fibrosis and cancer. Myofibroblasts and cancer cells that have undergone EMT are able to secrete more TGF-β1, which would amplify this cycle of latency, contraction, and signaling [13, 53, 97, 99]. In addition to the above discussed similarities, chronic fibrosis may directly lead to cancerous transformation, which suggests that, not only is the fibrogenic matrix conducive to cancer development, but also the pathways in these two diseases are similar enough to be indistinguishable in certain conditions [11].

Despite these considerable overlaps in the function and mode of action of myofibroblasts in cancer and fibrosis, there are certain notable differences between the two populations of cells. For instance, in idiopathic pulmonary fibrosis, myofibroblasts have been shown to produce and release hydrogen peroxide, which leads to apoptosis of local epithelial cells [100]. On the other hand, in cancer, it is the cancer cells themselves that release this reactive oxygen species from their altered metabolism. In this latter scenario, the hydrogen peroxide produced aids cancer cells in maintaining their altered state, and may actually increase cancer cell proliferation [78]. The origin of myofibroblasts is especially complex and uncertain in many cancers [62, 66, 86, 87, 101], though it is also under some debate in fibrosis. Genetic studies in cancer indicate that these cells may derive from the cancer cells, or surrounding stromal cells [69]. In fibrosis, myofibroblasts are thought to originate from normal resident fibroblasts or possibly transdifferentiation of myoepithelial cells or hematopoietic cells [84, 87, 102]. Therefore, there are clear differences regarding the source of myofibroblasts in cancer and fibrosis, but a clearer picture of the origin and fate of the myofibroblasts in the two disease states awaits further investigations, which would be facilitated by the use of new and more sensitive and specific markers and molecular tools.

Conclusion

Cancer and organ fibrosis bear several similarities to each other. Relevant to this discussion, both diseases are associated with wound healing gone out of control [8, 10]. At the cellular level, activated myofibroblasts wreak havoc in both diseases, resulting in the desmoplastic response and palpable solid tumors in cancer and fibrosing deformation and loss of function of organs in fibrosis [10, 68, 87, 89]. Studies of both diseases use similar markers for fibroblast activation, especially α-SMA and increased cellular contractility [9, 14, 84]. There is sufficient evidence to support the notion that at least some populations of activated CAF behave as myofibroblasts. Moreover, myofibroblasts in cancer and fibrosis share considerable common features and functionality. It is evident from current studies that these cells play a major role in both diseases. However, while the role of myofibroblasts in fibrosis is well accepted, it is much less well defined and less understood in cancer. Thus, there is still much to learn about these cell populations. Given that both cancer and organ fibrosis are highly refractory to most therapeutic agents currently available and both have exceedingly high rates of mortality and disability, the function of these cells and their possible targeting by therapeutic agents are important avenues of future research.

Disclaimer

The findings and conclusions in this report are those of the authors and do not necessarily represent the views of the National Institute for Occupational Safety and Health.

References

[1] American Cancer Society. Cancer Facts and Figures 2016. Atlanta: American Cancer Society; 2016.

[2] Siegel RL, Miller KD, Jemal A, (2015). Cancer statistics, 2015. *CA Cancer J Clin*. 65. 5-29.

[3] Karagiannis GS, Poutahidis T, Erdman SE, Kirsch R, Riddell RH, Diamandis EP, (2012). Cancer-associated fibroblasts drive the

progression of metastasis through both paracrine and mechanical pressure on cancer tissue. *Mol Cancer Res.* 10. 1403-18.

[4] Polanska UM, Orimo A, (2013). Carcinoma-associated fibroblasts: non-neoplastic tumour-promoting mesenchymal cells. *J Cell Physiol.* 228. 1651-7.

[5] Shiga K, Hara M, Nagasaki T, Sato T, Takahashi H, Takeyama H, (2015). Cancer-Associated Fibroblasts: Their Characteristics and Their Roles in Tumor Growth. *Cancers (Basel).* 7. 2443-58.

[6] McAnulty RJ, (2007). Fibroblasts and myofibroblasts: their source, function and role in disease. *Int J Biochem Cell Biol.* 39. 666-71.

[7] Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, et al., (2005). Tensional homeostasis and the malignant phenotype. *Cancer Cell.* 8. 241-54.

[8] Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, et al., (2008). Collagen density promotes mammary tumor initiation and progression. *BMC Med.* 6. 11.

[9] Schafer M, Werner S, (2008). Cancer as an overhealing wound: an old hypothesis revisited. *Nat Rev Mol Cell Biol.* 9. 628-38.

[10] Dvorak HF, (1986). Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med.* 315. 1650-9.

[11] Rybinski B, Franco-Barraza J, Cukierman E, (2014). The wound healing, chronic fibrosis, and cancer progression triad. *Physiol Genomics.* 46. 223-44.

[12] Radisky DC, Kenny PA, Bissell MJ, (2007). Fibrosis and cancer: do myofibroblasts come also from epithelial cells via EMT? *J Cell Biochem.* 101. 830-9.

[13] Van De Water L, Varney S, Tomasek JJ, (2013). Mechanoregulation of the Myofibroblast in Wound Contraction, Scarring, and Fibrosis: Opportunities for New Therapeutic Intervention. *Adv Wound Care (New Rochelle).* 2. 122-41.

[14] Grinnell F, (1994). Fibroblasts, myofibroblasts, and wound contraction. *J Cell Biol.* 124. 401-4.

[15] Baum J, Duffy HS, (2011). Fibroblasts and myofibroblasts: what are we talking about? *J Cardiovasc Pharmacol.* 57. 376-9.

[16] Allen M, Louise Jones J, (2011). Jekyll and Hyde: the role of the microenvironment on the progression of cancer. *J Pathol.* 223. 162-76.

[17] Petersen OW, Ronnov-Jessen L, Howlett AR, Bissell MJ, (1992). Interaction with basement membrane serves to rapidly distinguish

growth and differentiation pattern of normal and malignant human breast epithelial cells. *Proc Natl Acad Sci U S A*. 89. 9064-8.

[18] Bissell MJ, Hines WC, (2011). Why don't we get more cancer? A proposed role of the microenvironment in restraining cancer progression. *Nat Med*. 17. 320-9.

[19] Berenblum I, Shubik P, (1949). An experimental study of the initiating state of carcinogenesis, and a re-examination of the somatic cell mutation theory of cancer. *Br J Cancer*. 3. 109-18.

[20] Folkman J, Kalluri R, (2004). Cancer without disease. *Nature*. 427. 787.

[21] Weaver VM, Petersen OW, Wang F, Larabell CA, Briand P, Damsky C, et al., (1997). Reversion of the malignant phenotype of human breast cells in three-dimensional culture and in vivo by integrin blocking antibodies. *J Cell Biol*. 137. 231-45.

[22] Adams EF, Newton CJ, Braunsberg H, Shaikh N, Ghilchik M, James VH, (1988). Effects of human breast fibroblasts on growth and 17 beta-estradiol dehydrogenase activity of MCF-7 cells in culture. *Breast Cancer Res Treat*. 11. 165-72.

[23] Rich AR, (1979). Classics in oncology. On the frequency of occurrence of occult carcinoma of the prostate: Arnold Rice Rich, M. D., *Journal of Urology* 33:3, 1935. *CA Cancer J Clin*. 29. 115-9.

[24] Sakr WA, Haas GP, Cassin BF, Pontes JE, Crissman JD, (1993). The frequency of carcinoma and intraepithelial neoplasia of the prostate in young male patients. *J Urol*. 150. 379-85.

[25] Nielsen M, Thomsen JL, Primdahl S, Dyreborg U, Andersen JA, (1987). Breast cancer and atypia among young and middle-aged women: a study of 110 medicolegal autopsies. *Br J Cancer*. 56. 814-9.

[26] Harach HR, Franssila KO, Wasenius VM, (1985). Occult papillary carcinoma of the thyroid. A "normal" finding in Finland. A systematic autopsy study. *Cancer*. 56. 531-8.

[27] Manser RL, Dodd M, Byrnes G, Irving LB, Campbell DA, (2005). Incidental lung cancers identified at coronial autopsy: implications for overdiagnosis of lung cancer by screening. *Respir Med*. 99. 501-7.

[28] Knudson AG, (2001). Two genetic hits (more or less) to cancer. *Nat Rev Cancer*. 1. 157-62.

[29] Wolfe JN, (1976). Risk for breast cancer development determined by mammographic parenchymal pattern. *Cancer*. 37. 2486-92.

[30] Egeblad M, Rasch MG, Weaver VM, (2010). Dynamic interplay between the collagen scaffold and tumor evolution. *Curr Opin Cell Biol*. 22. 697-706.

[31] Frantz C, Stewart KM, Weaver VM, (2010). The extracellular matrix at a glance. *J Cell Sci*. 123. 4195-200.

[32] Butcher DT, Alliston T, Weaver VM, (2009). A tense situation: forcing tumour progression. *Nat Rev Cancer*. 9. 108-22.

[33] Chaudhuri O, Koshy ST, Branco da Cunha C, Shin JW, Verbeke CS, Allison KH, et al., (2014). Extracellular matrix stiffness and composition jointly regulate the induction of malignant phenotypes in mammary epithelium. *Nat Mater*. 13. 970-8.

[34] Baker EL, Bonnecaze RT, Zaman MH, (2009). Extracellular matrix stiffness and architecture govern intracellular rheology in cancer. *Biophys J*. 97. 1013-21.

[35] Schwartz MA, (2010). Integrins and extracellular matrix in mechanotransduction. *Cold Spring Harb Perspect Biol*. 2. a005066.

[36] Ma Q, Dong J, Hindman B. Integrins: form and role in myofibroblast differentiation and function. Myofibroblasts: origin, function and role in disease. New York: Nova Science Publishers, Inc.; 2016.

[37] Scherzer MT, Waigel S, Donninger H, Arumugam V, Zacharias W, Clark G, et al., (2015). Fibroblast-Derived Extracellular Matrices: An Alternative Cell Culture System That Increases Metastatic Cellular Properties. *PLoS One*. 10. e0138065.

[38] Provenzano PP, Eliceiri KW, Inman DR, Keely PJ, (2010). Engineering three-dimensional collagen matrices to provide contact guidance during 3D cell migration. *Curr Protoc Cell Biol*. Chapter 10. Unit 10 7.

[39] Pelham RJ, Jr., Wang Y, (1997). Cell locomotion and focal adhesions are regulated by substrate flexibility. *Proc Natl Acad Sci U S A*. 94. 13661-5.

[40] Irimia D, Toner M, (2009). Spontaneous migration of cancer cells under conditions of mechanical confinement. *Integr Biol (Camb)*. 1. 506-12.

[41] Berrier AL, Yamada KM, (2007). Cell-matrix adhesion. *J Cell Physiol*. 213. 565-73.

[42] Hernandez-Varas P, Berge U, Lock JG, Stromblad S, (2015). A plastic relationship between vinculin-mediated tension and adhesion complex area defines adhesion size and lifetime. *Nat Commun*. 6. 7524.

[43] Miron-Mendoza M, Seemann J, Grinnell F, (2010). The differential regulation of cell motile activity through matrix stiffness and porosity in three dimensional collagen matrices. *Biomaterials*. 31. 6425-35.

[44] Zaman MH, Trapani LM, Sieminski AL, Mackellar D, Gong H, Kamm RD, et al., (2006). Migration of tumor cells in 3D matrices is governed

by matrix stiffness along with cell-matrix adhesion and proteolysis. *Proc Natl Acad Sci U S A*. 103. 10889-94.

[45] Wuest M, Kuchar M, Sharma SK, Richter S, Hamann I, Wang M, et al., (2015). Targeting lysyl oxidase for molecular imaging in breast cancer. *Breast Cancer Res*. 17. 107.

[46] Kasashima H, Yashiro M, Kinoshita H, Fukuoka T, Morisaki T, Masuda G, et al., (2016). Lysyl oxidase is associated with the epithelial-mesenchymal transition of gastric cancer cells in hypoxia. *Gastric Cancer*. 19. 431-42.

[47] Miller BW, Morton JP, Pinese M, Saturno G, Jamieson NB, McGhee E, et al., (2015). Targeting the LOX/hypoxia axis reverses many of the features that make pancreatic cancer deadly: inhibition of LOX abrogates metastasis and enhances drug efficacy. *EMBO Mol Med*. 7. 1063-76.

[48] Stivarou T, Patsavoudi E, (2015). Extracellular molecules involved in cancer cell invasion. *Cancers (Basel)*. 7. 238-65.

[49] Naba A, Clauser KR, Lamar JM, Carr SA, Hynes RO, (2014). Extracellular matrix signatures of human mammary carcinoma identify novel metastasis promoters. *Elife*. 3. e01308.

[50] Provenzano PP, Inman DR, Eliceiri KW, Trier SM, Keely PJ, (2008). Contact guidance mediated three-dimensional cell migration is regulated by Rho/ROCK-dependent matrix reorganization. *Biophys J*. 95. 5374-84.

[51] Hindman B, Goeckeler Z, Sierros K, Wysolmerski R, (2015). Non-Muscle Myosin II Isoforms Have Different Functions in Matrix Rearrangement by MDA-MB-231 Cells. *PLoS One*. 10. e0131920.

[52] 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. 509-17.

[53] Wipff PJ, Rifkin DB, Meister JJ, Hinz B, (2007). Myofibroblast contraction activates latent TGF-beta1 from the extracellular matrix. *J Cell Biol*. 179. 1311-23.

[54] Zhou L, Yang K, Andl T, Wickett RR, Zhang Y, (2015). Perspective of Targeting Cancer-Associated Fibroblasts in Melanoma. *J Cancer*. 6. 717-26.

[55] Roswall P, Pietras K, (2012). Of mice and men: a comparative study of cancer-associated fibroblasts in mammary carcinoma. *Ups J Med Sci*. 117. 196-201.

[56] Dumont N, Liu B, Defilippis RA, Chang H, Rabban JT, Karnezis AN, et al., (2013). Breast fibroblasts modulate early dissemination, tumorigenesis, and metastasis through alteration of extracellular matrix characteristics. *Neoplasia*. 15. 249-62.

[57] Jodele S, Blavier L, Yoon JM, DeClerck YA, (2006). Modifying the soil to affect the seed: role of stromal-derived matrix metalloproteinases in cancer progression. *Cancer Metastasis Rev*. 25. 35-43.

[58] Kang N, Shah VH, Urrutia R, (2015). Membrane-to-Nucleus Signals and Epigenetic Mechanisms for Myofibroblastic Activation and Desmoplastic Stroma: Potential Therapeutic Targets for Liver Metastasis? *Mol Cancer Res*. 13. 604-12.

[59] Schurch W, Seemayer TA, Gabbiani G, (1998). The myofibroblast: a quarter century after its discovery. *Am J Surg Pathol*. 22. 141-7.

[60] Provenzano PP, Eliceiri KW, Campbell JM, Inman DR, White JG, Keely PJ, (2006). Collagen reorganization at the tumor-stromal interface facilitates local invasion. *BMC Med*. 4. 38.

[61] Marsh T, Pietras K, McAllister SS, (2013). Fibroblasts as architects of cancer pathogenesis. *Biochim Biophys Acta*. 1832. 1070-8.

[62] Buchsbaum RJ, Oh SY, (2016). Breast Cancer-Associated Fibroblasts: Where We Are and Where We Need to Go. *Cancers (Basel)*. 8. DOI 10.3390/cancers8020019.

[63] Barone I, Catalano S, Gelsomino L, Marsico S, Giordano C, Panza S, et al., (2012). Leptin mediates tumor-stromal interactions that promote the invasive growth of breast cancer cells. *Cancer Res*. 72. 1416-27.

[64] Mishra P, Banerjee D, Ben-Baruch A, (2011). Chemokines at the crossroads of tumor-fibroblast interactions that promote malignancy. *J Leukoc Biol*. 89. 31-9.

[65] Ammirante M, Shalapour S, Kang Y, Jamieson CA, Karin M, (2014). Tissue injury and hypoxia promote malignant progression of prostate cancer by inducing CXCL13 expression in tumor myofibroblasts. *Proc Natl Acad Sci U S A*. 111. 14776-81.

[66] Okabe H, Hayashi H, Nakagawa S, Imai K, Nitta H, Arima K, et al., (2016). Inducible factors for cancer-associated fibroblasts in liver cancer versus myofibroblasts in inflammatory liver disease. *Histol Histopathol*. 31. 141-8.

[67] De Wever O, Demetter P, Mareel M, Bracke M, (2008). Stromal myofibroblasts are drivers of invasive cancer growth. *Int J Cancer*. 123. 2229-38.

[68] Otranto M, Sarrazy V, Bonte F, Hinz B, Gabbiani G, Desmouliere A, (2012). The role of the myofibroblast in tumor stroma remodeling. *Cell Adh Migr*. 6. 203-19.

[69] Augsten M, (2014). Cancer-associated fibroblasts as another polarized cell type of the tumor microenvironment. *Front Oncol*. 4. 62.

[70] Sugimoto H, Mundel TM, Kieran MW, Kalluri R, (2006). Identification of fibroblast heterogeneity in the tumor microenvironment. *Cancer Biol Ther*. 5. 1640-6.

[71] Zhang K, Rekhter MD, Gordon D, Phan SH, (1994). Myofibroblasts and their role in lung collagen gene expression during pulmonary fibrosis. A combined immunohistochemical and in situ hybridization study. *Am J Pathol*. 145. 114-25.

[72] Spaeth EL, Dembinski JL, Sasser AK, Watson K, Klopp A, Hall B, et al., (2009). Mesenchymal stem cell transition to tumor-associated fibroblasts contributes to fibrovascular network expansion and tumor progression. *PLoS One*. 4. e4992.

[73] Strutz F, Okada H, Lo CW, Danoff T, Carone RL, Tomaszewski JE, et al., (1995). Identification and characterization of a fibroblast marker: FSP1. *J Cell Biol*. 130. 393-405.

[74] Ostman A, Heldin CH, (2007). PDGF receptors as targets in tumor treatment. *Adv Cancer Res*. 97. 247-74.

[75] Bonner JC, (2004). Regulation of PDGF and its receptors in fibrotic diseases. *Cytokine Growth Factor Rev*. 15. 255-73.

[76] Schurch W, Seemayer TA, Lagace R, Gabbiani G, (1984). The intermediate filament cytoskeleton of myofibroblasts: an immunofluorescence and ultrastructural study. *Virchows Arch A Pathol Anat Histopathol*. 403. 323-36.

[77] Pula B, Jethon A, Piotrowska A, Gomulkiewicz A, Owczarek T, Calik J, et al., (2011). Podoplanin expression by cancer-associated fibroblasts predicts poor outcome in invasive ductal breast carcinoma. *Histopathology*. 59. 1249-60.

[78] Corvigno S, Wisman GB, Mezheyeuski A, van der Zee AG, Nijman HW, Avall-Lundqvist E, et al., (2016). Markers of fibroblast-rich tumor stroma and perivascular cells in serous ovarian cancer: inter- and intra-patient heterogeneity and impact on survival. *Oncotarget*. 7. 18573-84.

[79] McAndrews KM, Yi J, McGrail DJ, Dawson MR, (2015). Enhanced Adhesion of Stromal Cells to Invasive Cancer Cells Regulated by Cadherin 11. *ACS Chem Biol*. 10. 1932-8.

[80] Lai D, Ma L, Wang F, (2012). Fibroblast activation protein regulates tumor-associated fibroblasts and epithelial ovarian cancer cells. *Int J Oncol*. 41. 541-50.

[81] Wolf BB, Quan C, Tran T, Wiesmann C, Sutherlin D, (2008). On the edge of validation--cancer protease fibroblast activation protein. *Mini Rev Med Chem*. 8. 719-27.

[82] Ishihara A, Yoshida T, Tamaki H, Sakakura T, (1995). Tenascin expression in cancer cells and stroma of human breast cancer and its prognostic significance. *Clin Cancer Res*. 1. 1035-41.

[83] Yamashita M, Ogawa T, Zhang X, Hanamura N, Kashikura Y, Takamura M, et al., (2012). Role of stromal myofibroblasts in invasive breast cancer: stromal expression of alpha-smooth muscle actin correlates with worse clinical outcome. *Breast Cancer*. 19. 170-6.

[84] Hinz B, Phan SH, Thannickal VJ, Prunotto M, Desmouliere A, Varga J, et al., (2012). Recent developments in myofibroblast biology: paradigms for connective tissue remodeling. *Am J Pathol*. 180. 1340-55.

[85] Karvonen HM, Lehtonen ST, Sormunen RT, Lappi-Blanco E, Skold CM, Kaarteenaho RL, (2014). Lung cancer-associated myofibroblasts reveal distinctive ultrastructure and function. *J Thorac Oncol*. 9. 664-74.

[86] Kendall RT, Feghali-Bostwick CA, (2014). Fibroblasts in fibrosis: novel roles and mediators. *Front Pharmacol*. 5. 123.

[87] Hinz B, Phan SH, Thannickal VJ, Galli A, Bochaton-Piallat ML, Gabbiani G, (2007). The myofibroblast: one function, multiple origins. *Am J Pathol*. 170. 1807-16.

[88] Dong J, Yu X, Porter DW, Battelli LA, Kashon ML, Ma Q, (2016). Common and distinct mechanisms of induced pulmonary fibrosis by particulate and soluble chemical fibrogenic agents. *Arch Toxicol*. 90. 385-402.

[89] Dong J, Porter DW, Batteli LA, Wolfarth MG, Richardson DL, Ma Q, (2015). Pathologic and molecular profiling of rapid-onset fibrosis and inflammation induced by multi-walled carbon nanotubes. *Arch Toxicol*. 89. 621-33.

[90] Dong J, Ma Q, (2015). Advances in mechanisms and signaling pathways of carbon nanotube toxicity. *Nanotoxicology*. 9. 658-76.

[91] He X, Wang L, Szklarz G, Bi Y, Ma Q, (2012). Resveratrol inhibits paraquat-induced oxidative stress and fibrogenic response by activating the nuclear factor erythroid 2-related factor 2 pathway. *J Pharmacol Exp Ther*. 342. 81-90.

[92] Goh GB, McCullough AJ, (2016). Natural History of Nonalcoholic Fatty Liver Disease. *Dig Dis Sci.* 61. 1226-33.

[93] Murdaca G, Contatore M, Gulli R, Mandich P, Puppo F, (2016). Genetic factors and systemic sclerosis. *Autoimmun Rev.* 15. 427-32.

[94] Raghu G, Collard HR, Egan JJ, Martinez FJ, Behr J, Brown KK, et al., (2011). An official ATS/ERS/JRS/ALAT statement: idiopathic pulmonary fibrosis: evidence-based guidelines for diagnosis and management. *Am J Respir Crit Care Med.* 183. 788-824.

[95] Lebrum J-J, (2012). The Dual Role of TGFb in Human Cancer: From Tumor Suppression to Cancer Metastasis. *ISRN Mol Biol.* 381428.

[96] Nagaraj NS, Datta PK, (2010). Targeting the transforming growth factor-beta signaling pathway in human cancer. *Expert Opin Investig Drugs.* 19. 77-91.

[97] Liu RM, Desai LP, (2015). Reciprocal regulation of TGF-beta and reactive oxygen species: A perverse cycle for fibrosis. *Redox Biol.* 6. 565-77.

[98] O'Connor JW, Gomez EW, (2014). Biomechanics of TGFbeta-induced epithelial-mesenchymal transition: implications for fibrosis and cancer. *Clin Transl Med.* 3. 23.

[99] Klingberg F, Chow ML, Koehler A, Boo S, Buscemi L, Quinn TM, et al., (2014). Prestress in the extracellular matrix sensitizes latent TGF-beta1 for activation. *J Cell Biol.* 207. 283-97.

[100] Waghray M, Cui Z, Horowitz JC, Subramanian IM, Martinez FJ, Toews GB, et al., (2005). Hydrogen peroxide is a diffusible paracrine signal for the induction of epithelial cell death by activated myofibroblasts. *FASEB J.* 19. 854-6.

[101] Gonda TA, Varro A, Wang TC, Tycko B, (2010). Molecular biology of cancer-associated fibroblasts: can these cells be targeted in anti-cancer therapy? *Semin Cell Dev Biol.* 21. 2-10.

[102] Mack M, Yanagita M, (2015). Origin of myofibroblasts and cellular events triggering fibrosis. *Kidney Int.* 87. 297-307.

BIOGRAPHICAL SKETCH

Name: Bridget Hindman

Affiliation: Receptor Biology Laboratory, Toxicology and Molecular Biology Branch, Health Effects Laboratory Division, National Institute

for Occupational Safety and Health, Centers for Disease Control and Prevention

Education: University of Texas at Dallas, Molecular Biology and Criminology, B.S. 2009

West Virginia University, Cancer Cell Biology, Ph.D., 2015

Research and Professional Experience:

Oct 2015 – present Regular Fellow – Research involves *in vivo* studies of pulmonary fibrosis post-exposure to nanoparticles to determine how different particle charactersistics influence their fibrogenicity.

Aug 2009 – Jul 2015 Graduate Assistant – Research on the role of non-muscle myosin II isoforms in breast cancer cell interactions with the collagen matrix, migration, and metastatic potential.

June 2005 – Aug 2005 Clark Summer Scholar – Research on the physical properties, especially tensile strength, of fibers formed from carbon nanotubes

Professional Appointments:

Since October 2015 Regular Fellow, NIOSH

August 2009-July 2015 Graduate Research Assistant, West Virginia University

Honors:

Feb 2016	Keystone Symposia Scholarship Awardee
2014-2015	NanoSAFE Graduate Fellow
2011-2013	NHLBI T32 Grant, Cardiovascular and Pulmonary Diseases Graduate Trainee
Oct 2012	WVU Health Magazine Scientific Images Contest Winner
2009-2010	NanoBridge Award Recipient

Publications Last 3 Years:

Hindman B, Goeckeler Z, Sierros K, Wysolmerski R (2015). Non-muscle myosin II isoforms have different functions in matrix rearrangement by MDA-MB-231 cells. *PLoS One.* 10(7).

Analise Martinez
Editor

Myofibroblasts

Origin, Function and Role in Disease

Contributors

Qiang Ma
Jie Dong
Bridget Hindman
Véronique J. Moulin
Viktor Shtilbans

ISBN 978-1-63485-743-7

www.novapublishers.com

CELL BIOLOGY RESEARCH PROGRESS

MYOFIBROBLASTS

ORIGIN, FUNCTION AND ROLE IN DISEASE

ANALISE MARTINEZ
EDITOR

New York

NOTICE TO THE READER

Library of Congress Cataloging-in-Publication Data

Names: Martinez, Analise, editor.
Title: Myofibroblasts : origin, function and role in disease / editor, Analise Martinez.
Description: Hauppauge, New York : Nova Science Publisher's, Inc., [2016] | Includes index.
Identifiers: LCCN 2016029795 (print) | LCCN 2016032513 (ebook) | ISBN 9781634857437 (softcover) | ISBN 9781634857666 (ebook) | ISBN 9781634857666 ()
Subjects: LCSH: Wound healing. | Myofibroblasts.
Classification: LCC RD94 .M96 2016 (print) | LCC RD94 (ebook) | DDC 617.1/4--dc23
LC record available at https://lccn.loc.gov/2016029795

Published by Nova Science Publishers, Inc. † New York