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The mighty fibroblast and its utility in scleroderma research

Sara M. Garrett, DeAnna Baker Frost, and Carol Feghali-Bostwick

Department of Medicine, Division of Rheumatology and Immunology, Medical University of South Carolina, Charleston, SC - USA

Abstract

Fibroblasts are the effector cells of fibrosis characteristic of systemic sclerosis (SSc, scleroderma) and other fibrosing conditions. The excess production of extracellular matrix (ECM) proteins is the hallmark of fibrosis in different organs, such as skin and lung. Experiments designed to assess the pro-fibrotic capacity of factors, their signaling pathways, and potential inhibitors of their effects that are conducted in fibroblasts have paved the way for planning clinical trials in SSc. As such, fibroblasts have proven to be valuable tools in the search for effective anti-fibrotic therapies for fibrosis. Herein we highlight the characteristics of fibroblasts, their role in the etiology of fibrosis, utility in experimental assays, and contribution to drug development and clinical trials in SSc.

Keywords

Extracellular matrix (ECM); Fibroblasts; Fibrosis; Scleroderma; Systemic sclerosis (SSc)

Fibroblasts

Fibroblasts are derived from primitive mesenchyme, are ubiquitous throughout the body, and are the primary effector cells in a variety of fibrotic conditions (1-3). They exhibit a spindle-shaped morphology and are essential constituents in connective tissue, one of the four main types of tissue in the body (4). A primary function of the fibroblast is to maintain tissue homeostasis through regulation of the extracellular matrix (ECM) via production and maintenance of its components (5). Additionally, fibroblasts provide a supportive function and have varying roles in angiogenesis, inflammation, wound healing, and restoration of tissue integrity (5-7). Fibroblasts are generally identified by a combination of marker inclusion and exclusion, as there is no one singular marker utilized conclusively to identify fibroblasts. Largely, fibroblasts express vimentin, fibroblast-specific protein-1 (FSP1/S100A4), heat shock protein 47 (HSP47, positive in dermal fibroblasts), nestin, Thy1/CD90, and prolyl-4-hydroxylase β (positive in activated fibroblasts), while some also exhibit markers of mesenchymal cells (CD9, CD39, CD44, CD105, etc.) (1, 3, 8-12). The following markers are generally used for exclusionary purposes: alpha smooth muscle actin (α SMA), desmin, and certain lineage-specific cell surface markers (i.e., CD11b, CD31, CD34, CD45,

Corresponding author: Carol Feghali-Bostwick, Department of Medicine, Division of Rheumatology & Immunology, Medical University of South Carolina, 96 Jonathan Lucas St, MSC 637, Charleston, 29425 SC, USA, feghalib@musc.edu.

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CD326, used variably to characterize endothelial cells, hematopoietic cells, stem cells, etc.) (1, 8, 9, 13-15).

Fibroblast heterogeneity in normal physiology

Distinct subpopulations of fibroblasts exist within healthy adult skin, each exhibiting varying characteristics in the niche of their anatomical location within the dermis, whether superficial (papillary), deep (reticular), or hair follicle-adjacent (5, 16, 17). For instance, reticular fibroblasts in the deep dermis exhibit diminished proliferative capacity, have greater collagen lattice contractility, and are contact-inhibited compared to genomically and morphologically identical papillary fibroblasts from site-matched superficial dermis (17). Secretion, composition, and organization of ECM components thus vary between these subpopulations of fibroblasts: type III collagen predominates in organized bundles within the reticular dermis, whereas thin, loosely organized type I and III collagens compose the papillary dermis (5, 18). Additionally, there are differences in response to and production of growth factors and physiologically active compounds, such as prostaglandin (PGE₂), as well as differential expression of intermediate filaments within these subpopulations (3, 18-20).

Fibroblasts within the skin exist not only as subpopulations within a single anatomical location (intrasite heterogeneity), but differ across skin sites throughout the body (intersite heterogeneity), exhibiting distinct genome-wide gene expression patterns for ECM synthesis, metabolism, proliferation, and migration (6). Features of this 'positional memory' translate to cultured outgrowth of fibroblasts from skin cultures (5). Likewise, fibroblast heterogeneity extends to other organs, including the lungs, exhibiting unique anatomical and physiological aspects within and across pulmonary regions, essentially forming functional subsets (3, 16). Like skin fibroblasts, pulmonary fibroblasts are heterogeneous for morphology, proliferative capacity, cytokine production, plasma membrane marker expression, role in immune function, and production of collagen (16, 19). Also, there exist large differences between fetal and adult fibroblasts; most notably, fetal fibroblasts produce and express very low levels of certain cytokines and growth factors, such as transforming growth factor β 1 (TGF β 1) (5).

Pathology in tissue fibrosis: differentiation into the myofibroblast

The intricate nature of the fibroblast can be further complicated by its plasticity. Fibroblasts are not terminally differentiated, retaining the ability to adopt alternative phenotypes. For instance, papillary fibroblasts in long-term in vitro culture adopt a more reticular fibroblastic phenotype, suggesting that reticular fibroblasts are more differentiated than their more plastic papillary fibroblast counterparts (18). Upon induction of an epithelial tissue injury (by a physical, environmental, chemical, inflammatory, genetic, or other trauma-inducing agent), autocrine and paracrine TGF β is transiently upregulated in order to differentiate resident-tissue fibroblasts into myofibroblasts to facilitate wound healing, in part through abundant secretion of ECM proteins, including collagen type I and III, glycosaminoglycans, and fibronectin as part of the normal immune response (1, 20-25). This conversion has been recapitulated with TGF β treatment of in vitro cultures of fibroblasts (2, 24-26). Myofibroblasts usually undergo deactivation and apoptosis, dedifferentiation, or senescence

in the latter stages of the wound healing process, the time when matrix reabsorption and TGF β signaling terminate and redox homeostasis is restored (5, 7, 21, 23, 24, 27-31). The danger in fibroblast plasticity arises when the precipitating injury leads to excessive fibrogenic milieu activation wherein myofibroblast differentiation or persistence occurs, leading to wound strengthening through disproportionate collagen deposition, fiber alignment, and excessive force contraction, in part because myofibroblasts contribute to wound repair, not tissue regeneration (3, 7, 28). Chronic fibroblast or myofibroblast activation or enhanced survival can cause progressive fibrosis, scarring, organ dysfunction (due to blockage of normal blood supply and disruption of normal architecture), and promote tumor progression in the skin, lungs, and other tissues, leading to significant morbidity and/or mortality (3, 24, 27, 28, 30, 32-34).

Myofibroblast characteristics

Differentiation of fibroblasts into proto-myofibroblasts can be marked by progressive accumulation of cytoplasmic microfilament bundles (stress fibers), expression of α SMA, exhibition of cell-to-cell and cell-to-matrix adhesion complexes, presence of collagen secretion granules, dense rough endoplasmic reticulum, increased cell motility, and resistance to apoptotic signaling (3, 4, 27, 28, 30). Full differentiation into myofibroblasts generally requires exogenous stimuli such as TGF β 1 or mechanical stress (ECM stiffness, often as a result of scar tissue) (1-3, 7, 26, 30). These two signals are ultimately responsible for amplified ECM production/deposition and α SMA expression, which enhance contractile force to attract open wound edges together to facilitate wound closure (7, 27).

In large part, myofibroblasts are positive for caldesmon, c-Met receptor expression, mesenchymal markers like cadherins-2 and -11, collagen (types 1 and III), and vimentin; desmin in some tissues/pathologies; endothelin (ET)-1; ezrin; extra domain-A fibronectin splice variant; FSP-1; glial fibrillary acidic protein; moesin; paladin; paxillin; platelet-derived growth factor (PDGF); PDGF α - and β -receptors; tenascin-C; and vimentin; though they generally lack expression of smooth muscle markers h(eavy)-caldesmon, myosin heavy chain, and smoothelin (2-4, 22, 24, 27, 28, 30, 32, 35). Myofibroblasts are generally identified by a combination of markers and not by the presence or absence of any singular marker.

In some organs, such as the lungs, the origin of myofibroblasts appears to be resident fibroblasts (21). However, myofibroblast etiology isn't restricted to fibroblasts, let alone one lineage or precursor cell type. Other cell types populate the myofibroblast progenitor pool, with the cell source likely depending on the organ affected, the activation occurring within the tissue, and juxtaposition to surrounding tissue type. Stromal progenitor cell differentiation, smooth muscle cell loss of a contractile phenotypic, heterogeneous mesenchymal stromal precursor differentiation, pericyte conversion, differentiation of circulating bone marrow-derived fibrocytes, and epithelial-to-mesenchymal or endothelial-to-mesenchymal transition are a few of the mechanisms involved in conversion into a myofibroblastic phenotype (2, 3, 7, 20, 21, 27, 28, 30, 36).

As there exist subsets of fibroblasts, it has been postulated that there are subsets of phenotypically diverse myofibroblasts, in varying levels of activation with regard to contractility, ECM production, cytokine secretion, migration, and proliferation (3, 7, 20, 28). Regardless of their origins or method of activation, myofibroblasts are key end-effector cells of fibrogenesis in a multitude of fibrotic diseases and their accumulation is directly proportional to the extent of fibrosis, which largely inversely correlates with clinical prognosis.

Tissue fibrotic response

Profibrotic processes within tissues are generally driven by a delicate interplay of autocrine and paracrine signals secreted by a variety of cell types. The likely source of the principle fibrotic element, TGF β , is predominantly macrophage or epithelial cell in origin (4, 21). In addition to TGF β , a variety of other signals have been implicated in fibroblast and myofibroblast activation and/or differentiation, including but not limited to: angiotensin (Ang) II, cytokines (i.e., granulocyte macrophage colony stimulating factor), chemokines (CC and CXC, including CCL-11, -21, -24, and -26), endoplasmic reticulum stress, endostatin, ET-1, epigenetic factors (DNA methylation, histone modification, microRNA regulation), ezrin, growth factors (connective tissue growth factor, fibroblast growth factor [FGF]-2, hepatocyte growth factor, insulin-like growth factor [IGF]-II, and PDGF), several interleukins (IL-1, -4, -6, -13, -33), IGF binding proteins (IGFBP-3 and -5), lysophosphatidic acid, moesin, paxillin, signaling pathway activation (Fizz1, Hedgehog, mitogen-activated protein kinases, Notch, canonical SMAD, Wnt/ β -catenin), reactive oxygen species (ROS) via nicotinamide adenine dinucleotide phosphate (NADPH) oxidase 4, serotonin, shear forces, toll-like receptor 4 activation, and thrombin (2-5, 7, 20, 25-28, 30, 32, 35-40). Due to the dynamic nature of fibroblast and myofibroblast activation, there are numerous unique approaches targeting these cell types to assess the effect of potential therapeutic interventions for fibrotic conditions with fibroblast pathology.

Culture of fibroblasts and representative model systems

Isolation of fibroblasts

Fibroblasts can be cultured from different tissues relevant for scleroderma research, such as from skin and lung (41). There are two main methods used for obtaining fibroblasts from tissues: the outgrowth method and the digestion/enzymatic method. In the outgrowth method, tissues from skin (42) or lung (43) are minced and pieces are allowed to adhere to cell culture dishes for approximately 30 minutes prior to the addition of culture medium containing 10% fetal bovine serum (FBS) or fetal calf serum and antibiotic/antimycotic. Over time, fibroblasts will migrate out of the tissue and populate the culture dish. The digestion/enzymatic method can also be utilized to obtain fibroblasts from skin (44) or lung (45). This method for the release of fibroblasts requires the use of enzymes, such as collagenase and deoxyribonuclease. Tissues are incubated with the enzymes at 37°C, the mixture is plated, and adherent cells are cultured. Regardless of the method employed, passaging of cells at near-confluence involves trypsinization with 0.25% trypsin (or use of non-trypsin based cell lifting reagents) and expanding or cryopreserving the fibroblasts. Use

of Dulbecco's modified Eagle's medium (DMEM) with high glucose and FBS allows fibroblasts to outgrow keratinocytes from skin or epithelial cells from lung samples, respectively. For primary fibroblasts, the expansion/split ratio should not exceed 1:4 to avoid premature senescence. Primary fibroblasts maintained in culture in this manner can readily reach passage 15 or greater, although most experiments should be done in fibroblasts under passage 10 to avoid the use of senescent cells. Both the outgrowth and the digestion methods have advantages and disadvantages. The outgrowth method likely selects for the growth of fibroblasts that are more active and migratory, while the digestion method may allow the growth of all adherent cells, not just fibroblasts. Furthermore, the digestion method requires a longer processing time and can result in over-digestion, reducing cell viability.

In vitro culture conditions

Lung and skin fibroblasts can be isolated from tissue to establish in vitro cultures. The typical media in which fibroblasts are grown is DMEM supplemented with 10% FBS and 1% antibiotic/antimycotic (46). However, FBS and other sera contain proteins and growth factors that can change the response of fibroblasts through activation of intracellular signal transduction pathways, thus altering cellular excitation, proliferation, differentiation and apoptosis (47). To avoid these confounding effects that can occur with FBS supplementation, most researchers initially grow cells in the presence of FBS, serum-starve the cells for a period of time, then conduct experiments in serum-free conditions. However, for experiments that require long-term fibroblast culture (>7 days), low serum or serum-free conditions may not be optimal; therefore, alternative culture conditions for fibroblasts have been explored (48). Use of media designed to stimulate fibroblast proliferation, containing 4% FBS, antibiotic/antimycotic, ascorbic acid derivative, epidermal growth factor (EGF), hydrocortisone, and insulin led researchers to note differences in dermal fibroblast morphology and proliferation compared to fibroblasts cultured in standard DMEM/10% FBS primary human fibroblast maintenance media (46). These differences may be due, in part, to modulation of collagen levels by insulin, warranting discrete use of this media formulation (49). A different culture media system, which utilizes KnockOut DMEM, KnockOut serum replacement (containing insulin), FGF, EGF, hydrocortisone, and fibronectin-coated plates resulted in typical spindle morphology of growing dermal fibroblasts, compared to the large, flat morphology typically seen in senescing fibroblasts maintained in standard culture media (48). Therefore, depending on outcome measures, discretion should be used when analyzing data from fibroblasts cultured under these alternative conditions.

2D and 3D in vitro model systems

In addition to differences in culture media conditions, different culture model systems have been utilized to best mimic the in vivo milieu of fibroblasts. Most studies involve using a 2D in vitro technique of plating fibroblasts on plastic surfaces to evaluate the response of fibroblasts without the influence of other cell types, in contrast to using feeder cultures. However, using plastic cell culture dishes in a 2D capacity has its limitations. Fibroblasts behave differently when attaching to a stiff surface (i.e., plastic) versus a pliable surface (i.e., collagen) (50). Also, the ECM in vivo provides mechanical forces that are not completely recapitulated in 2D cell culture-based systems (51, 52). Finally, cells often have differing gene expression in a 2D system compared to the in vivo milieu (51).

Gel-based matrices have been used to mimic the in vivo environment, thus serving as a unique 3D culture system. Due to the asymmetric characteristics of gel matrices, these 3D systems more closely mimic the in vivo ECM environment and provide a more effective study method compared to 2D systems (51). Often, 3D matrices are used to enhance or validate findings from 2D systems. For instance, fibroblasts seeded in gel matrix exhibited cell motility and a more spindled-shaped morphology compared to those cultured on plastic dishes, enhancing the value of the experimental results (53). Additionally, innate collagen synthesis and regulation in human lung fibroblasts from systemic sclerosis (SSc) patients were found to be increased in a 3D matrix gel compared to fibroblasts from control patients, validating the 2D monolayer experimental results obtained with these same fibroblasts (54). Two types of 3D gels have been described: attached gels and floating gels; generally, attached gels cover the growth surface of a plastic culture dish, while floating gels remain suspended in the culture medium (53). In either method, the cells can be seeded onto the surface of the matrix or embedded within the matrix, thus allowing for a 2D or a 3D culture. The first gel systems incorporated several types of porous substances, including polyacrylamide and ECM proteins, such as collagen, which serve as ligands for cell adhesion (53). Collagen gel matrices (collagen hydrogels), first described in 1972 and prepared using 0.1% collagen, have several positive features. Use of collagen hydrogels allows fibroblasts to rearrange and contract the collagen into a denser region excluding water, exhibiting characteristics seen in wound healing (55). Study of cellular responses to the mechanical properties of the gel matrix (i.e., stiffness) has been confounded by the fact that the collagen composing these matrices can interact with the cell surface receptors of fibroblasts; the alternative use of polyacrylamide to form gel matrices has facilitated in this endeavor (52). Flexibility in gel matrices can be achieved through variation in the monomer:cross-linker ratio of polyacrylamide gels, or matrix stiffness can be altered by mixing defined amounts of gel matrix found in commercially available basement membrane extract (though batch-to-batch variability can affect experimental reproducibility) (51, 52, 56, 57). Due to the variability of composition and mechanical properties of naturally occurring ECM molecules, synthetic gels (i.e., recombinant fibrinogen, elastin, polymer fibers, etc.) are being utilized for a more reproducible environment (52). Use of synthetic fibers allows for the formation of nanofibers and nanopores that closely mimic the ECM structure (51). Exogenous addition of biologically active molecules (i.e., growth factors, proteases, etc.) to synthetic gel preparations can closely imitate physiological conditions, allowing for matrix viscoelasticity, growth factor binding, and matrix degradation (52). Comparison of fibroblast morphology and confluence when grown on a plastic surface, collagen-coated plastic surface, and in a 3D matrix is shown in Figure 1.

Ex vivo organ culture

The response of fibroblasts can also be assessed in skin maintained in organ culture, which more closely mimics the in vivo environment than 2D or 3D culture systems (34, 58, 59). When maintained in primary human fibroblast culture media, human skin can respond to fibrotic triggers, such as TGF β 1 and IGFBP-5, as measured via increase in dermal thickness and collagen content (34, 58). This model not only facilitates the identification of pro-fibrotic factors, but also the testing of potential therapies that can reduce fibrosis, as was successfully reported for the endostatin-derived peptide E4 (34). Testing anti-fibrotic

therapies in ex vivo skin models renders findings directly relevant to human tissue and extends results from in vitro and in vivo testing. This model is gaining traction in its application, at least in academic centers where human skin might be readily available. Implementation of the organ culture model decreases risks inherent to human clinical trials that are based on in vitro or in vivo studies in rodents by more accurately predicting the likelihood of a response in humans.

Direct translation of fibroblast research to clinical trials

Results of experimental manipulation of fibroblasts have facilitated translation of research in SSc from the bench to the clinic. Multiple recent and ongoing clinical trials were initiated based on findings from in vitro studies using fibroblasts. A comprehensive description of all recently completed and ongoing clinical trials is beyond the scope of this review; however, a few examples are described below.

Neoplastics and immunomodulators

several known anti-cancer and immunosuppressive drugs have been considered for SSc patients based on primary research conducted with fibroblasts. Dasatinib is a dual Bcr-Abl/Src family tyrosine kinase inhibitor. Utilizing fibrotic human lung fibroblasts from IPF patients, dasatinib attenuated differentiation of myofibroblasts and blocked both α SMA and fibronectin production in vitro through modulation of the Src kinase/serum response factor pathway (60). In dermal fibroblasts derived from SSc patients, dasatinib significantly reduced ECM component mRNA and proteins; in a bleomycin mouse model, dasatinib significantly reduced dermal thickness, myofibroblast formation, and skin collagen content (61). Following these studies, dasatinib was evaluated in clinical trials in scleroderma patients with pulmonary fibrosis, but the results of the study are not yet available (NCT00764309).

The immunomodulatory drug thalidomide significantly abrogated the TGF β -induced fibrotic effects of increased hydroxyproline, collagen, and α SMA in human fetal lung fibroblasts and hydroxyproline and α SMA in a bleomycin mouse model (62). A phase 1 clinical trial was established to test collagen reduction by thalidomide in SSc, and a phase 2 trial with a thalidomide derivative, pomalidomide, is under way in SSc-associated interstitial lung disease (ILD; NCT00418132, NCT01559129) (63). Results from thalidomide treatment of idiopathic pulmonary fibrosis (IPF) led to statistically significant reduction in cough and improved respiratory-related quality of life (64).

Rapamycin is an anti-neoplastic immunosuppressive macrolide, which has previously been shown to significantly block ECM production in lung fibroblasts (65). In another study, rapamycin had significant effects in two mouse models of SSc, decreasing dermal thickness, blocking T-cell infiltration, inhibiting skin and lung fibrosis, and decreasing mRNA expression of interleukins and TGF β (66). These same gene expression changes were recapitulated in fibroblasts from two murine SSc models treated with rapamycin, which also significantly reduced fibroblast proliferation (66). Success of rapamycin treatment in fibroblasts and in vivo models spurred translation to the clinical setting. Rapamycin was studied in diffuse SSc (67) and is in an ongoing trial for IPF (NCT01462006). Overall global

assessment (via Health Assessment Questionnaire Disability Index), modified Rodnan Skin Score (mRSS), and forced vital capacity (FVC) significantly improved with rapamycin treatment in patients with diffuse SSc (67).

The antineoplastic imatinib mesylate inhibits specific tyrosine kinases, as well as the TGF β and PDGF pathways (68, 69). Dermal fibroblasts from SSc patients have abnormal signaling through PDGF, likely contributing to fibrosis (22). Imatinib mesylate and structurally related nilotinib significantly decreased collagen and ECM component mRNA and protein levels in normal and SSc dermal fibroblasts, as well as dermal thickness, myofibroblast number, and ECM proteins in a bleomycin mouse model of dermal fibrosis (61, 68). Safety and efficacy of imatinib mesylate and nilotinib have been evaluated in clinical trials for SSc (NCT00479934, NCT00613171, NCT00506831). In SSc patients with ILD, imatinib improved the mRSS and showed a trend toward improvement of FVC that was not statistically significant (70). Studies using fibroblasts additionally resulted in a phase IIa, open-label clinical trial, which showed significant improvement in mRSS and stabilized FVC in SSc patients with ILD (71).

Nintedanib is an inhibitor of the receptors for PDGF, FGF, and vascular endothelial growth factor. In fibroblasts from healthy and SSc patients, nintedanib reduced TGF β - and PDGF-induced cellular proliferation, migration, myofibroblast formation, and collagen release, and significantly ameliorated fibrosis in several mouse models (72). A clinical trial in IPF has been completed and current recruitment for a clinical trial for patients with SSc-related lung fibrosis is underway (NCT02597933). FDA-approved for IPF, nintedanib resulted in significantly improved FVC compared to placebo, consistent with a reduction in disease progression in IPF patients (73).

Dermal fibroblasts from SSc patients constitutively express high levels of IL-6, a pro-fibrotic cytokine (42). In conjunction with in vivo findings showing that blockade of IL-6 significantly reduced bleomycin-induced murine fibrosis (74, 75), these results led to the development of targeted anti-IL-6 therapy, more recently using tocilizumab (76). In patients with early diffuse SSc, tocilizumab showed non-significant improvement in mRSS, the primary endpoint of the trial, but in an exploratory endpoint, tocilizumab significantly reduced decline in lung function in patients compared to placebo (77).

Due to the overabundance of fibrosis in scleroderma, targeting of the proteasomal system was originally tested in fibroblasts. Proteasome inhibitors led to significant upregulation of matrix metalloproteinase-1 (MMP1) and significant downregulation of both collagen and tissue inhibitor of metal-loproteinase-1 (TIMP1) in normal and SSc dermal fibroblasts (78). Bortezomib, a proteasome inhibitor, significantly blocked SMAD-mediated transcription and increased PPAR γ levels and activity in normal, ILD lung, and SSc skin fibroblasts; furthermore, bortezomib significantly blocked bleomycin-induced skin and lung fibrosis in mice (79). Recruitment for a phase 2 clinical trial for bortezomib in scleroderma is underway (NCT02370693).

Receptor modulation

wide study of the endothelin receptors has led to a myriad of clinical trials based upon basic research conducted with fibroblasts. Levels of the vasoactive peptide ET-1 and procontractile proteins are significantly increased in SSc fibroblasts, which enhance their ability to contract collagen matrix compared to normal fibroblasts (26, 31, 32). Treatment of normal and SSc human dermal fibroblasts with ET-1 promoted significant ECM production and matrix contraction through the endothelin A (ETA) receptor (32, 80). Severity of the pulmonary fibrotic phenotype has been linked to increased levels of circulating ET-1 (33). Lung fibroblasts from normal and SSc patients treated with the ETA/ETB receptor dual antagonist bosentan showed alterations in gene expression and reduced fibroblast adhesion to collagen matrix, contraction of collagen matrix, and expression of pro-adhesive and pro-contractile proteins (32, 33). In a prospective open-label, non-comparative trial, bosentan significantly decreased skin fibrosis, seen as a reduction in mRSS in patients with SSc (81).

Intracellular modulators

fibroblast research has elucidated several intracellular signaling pathways important in the development of scleroderma and other fibrotic conditions, which has ultimately led to preclinical and clinical use of modulators of these pathways, including cell-permeable peptides, pathway inhibitors, hormones, and protein activators. A cell-permeable peptide corresponding to the carboxyl terminus of endostatin, called E4 was found to antagonize TGF β -induced fibrosis in human skin explants, primary human fibroblasts, and mouse models of SSc (34). Use of recombinant endostatin or E4 significantly abrogated TGF β -induced upregulation of ECM proteins and reduced the levels of the crosslinking enzyme lysyl oxidase in normal human fibroblasts, and decreased dermal thickness in human skin (34, 82). Recently, an observational clinical study in patients with SSc was completed with measurement of endostatin levels as the primary outcome; results have yet to be published (NCT00668473).

Relaxin, a protein hormone and cytokine, decreased collagen expression in fibroblasts derived from scleroderma patients (83). In relaxin-deficient mice, dermal fibrosis and collagen content were increased with age, and dermal fibroblasts derived from these mice also exhibited amplified collagen content (84). Early intervention with relaxin in these mice significantly reversed dermal fibrosis. Phase 2 and 3 clinical trials with recombinant human relaxin in SSc have been completed (NCT00704665, NCT00004380). Recombinant human relaxin reduced dermal thickening and improved FVC in scleroderma patients in one trial, yet resulted in no significant improvement in a follow-up trial (85, 86).

Mycophenolate mofetil (MMF) is another drug whose initial effects were tested using fibroblasts. In vitro experiments utilizing human lung fibroblasts treated with MMF showed a reduction of type I collagen and α SMA and diminished fibroblast motility and contractility (87). As a result, MMF has been used in observational studies that showed a statistically significant improvement in mRSS in patients with diffuse SSc (88, 89). A recent larger clinical trial comparing MMF to cyclophosphamide in scleroderma-related ILD patients showed a comparable improvement of mRSS in both groups, yet MMF was tolerated better with less toxicity to patients (90).

In addition to the utility of fibroblast studies in guiding clinical trials, some studies are leading the way for the identification of biomarkers. For example, fibroblasts cultured from the dermal scars or affected areas of the skin of SSc patients exhibit significantly increased expression of the class 4 membrane-bound glycoprotein semaphorin (Sema4A), procontractile proteins (α SMA, ezrin, moesin, paxillin), and demonstrate high contractility on collagen gel (29). Treatment of normal human lung fibroblasts with Sema4A caused upregulated expression of procontractile proteins, enhanced collagen gel matrix contraction, and apoptotic resistance. These findings formed the basis of an ongoing clinical trial assessing the utility of semaphorins as biomarkers in SSc (NCT01943968).

Closing remarks

In summary, fibroblasts represent a complex group of cells capable of driving fibrosis across different organs, including skin and lung. Recent evidence suggests that the reversal of the myofibroblast phenotype is possible and represents a plausible therapeutic intervention for organ fibrosis (20, 30). Fibroblasts continue to provide an important tool for identifying pro-fibrotic mediators, assessing their effects on these effector cells, and identifying therapeutic modalities that can ultimately be implemented in the clinic. By utilizing and targeting fibroblasts and myofibroblasts, we can increase the chances of success in clinical outcome in current and future trials for SSc and related fibrosing diseases.

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Fig. 1. Morphology of primary human lung fibroblasts grown on tissue culture-treated plastic, collagen-coated plastic, and in a 3D collagen matrix (PureCol). Cells were plated at equal densities and images were taken 24-h and 72-h post-seeding. Note that cells in PureCol have migrated within the 3D matrix and not all are visible within one focal plane.