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Evasion of apoptosis by myofibroblasts: a hallmark of fibrotic diseases

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Abstract

Organ fibrosis is a lethal outcome of autoimmune rheumatic diseases such as systemic sclerosis. Myofibroblasts are scar-forming cells that are ultimately responsible for the excessive synthesis, deposition and remodelling of extracellular matrix proteins in fibrosis. Advances have been made in our understanding of the mechanisms that keep myofibroblasts in an activated state and control myofibroblast functions. However, the mechanisms that help myofibroblasts to persist in fibrotic tissues remain poorly understood. Myofibroblasts evade apoptosis by activating molecular mechanisms in response to pro-survival biomechanical and growth factor signals from the fibrotic microenvironment, which can ultimately lead to the acquisition of a senescent phenotype. Growing evidence suggests that myofibroblasts and senescent myofibroblasts, rather than being resistant to apoptosis, are actually primed for apoptosis owing to concomitant activation of cell death signalling pathways; these cells are poised to apoptose when survival pathways are inhibited. This knowledge of apoptotic priming has paved the way for new therapies that trigger apoptosis in myofibroblasts by blocking pro-survival mechanisms, target senescent myofibroblast for apoptosis or promote the reprogramming of myofibroblasts into scar-resolving cells. These novel strategies are not only poised to prevent progressive tissue scarring, but also have the potential to reverse established fibrosis and to regenerate chronically injured tissues.

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Author contributions

The authors contributed equally to all aspects of this article.

Competing interests

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Organ fibrosis is a lethal outcome of autoimmune rheumatic diseases such as systemic sclerosis (SSc, also known as scleroderma) and rheumatoid arthritis (RA)[1–4]. In SSc and RA, autoimmune responses result in chronic inflammation and tissue injury, ultimately leading to the development of fibrosis, mostly in the lungs (in the form of interstitial lung disease (ILD)) and in the skin[1,2,4,5]. ILD is associated with high morbidity and mortality and is the leading cause of death in patients with rheumatic disease-associated fibrotic disorders worldwide[3,6]. ILD is characterized by progressive scarring of the lungs, which severely distorts the normal tissue architecture, resulting in respiratory failure and, ultimately, in death[7]. Nintedanib, an anti-fibrotic drug approved for the treatment of lung fibrosis in patients with idiopathic pulmonary fibrosis (IPF), has now also been approved for the treatment of lung fibrosis in SSc-associated ILD following the positive results of the SENSCIS trial[8]. The efficacy of pirfenidone, another FDA-approved treatment for IPF, in patients with SSc is currently being investigated in clinical trials[9–12]. Although both drugs reduce the rate of progression of IPF, they cannot halt disease progression[8,13]. Despite advances in therapy, destroyed lung architecture and function cannot be regained with currently available treatments and organ transplantation often remains the only option for patients[14]. Thus, an improved understanding of the pathobiological mechanisms promoting the progression of organ fibrosis in autoimmune rheumatic diseases is crucial to provide cellular and molecular targets for novel therapies that not only halt fibrosis, but also restore lost tissue function.

Excessive scarring of skin and lung tissues in rheumatic disease-associated fibrotic disorders is promoted by fibrogenic cells. These so-called myofibroblasts are the ultimate culprits responsible for excessive synthesis, deposition and remodelling of extracellular matrix (ECM) proteins — most notably fibrillar collagens[15,16]. Whereas substantial progress has been made over the past 40 years in understanding the molecular mechanisms that promote myofibroblast formation from different precursor cells[17], considerably less is known about the mechanisms that promote their survival and persistence in fibrotic disorders. Although myofibroblast differentiation was initially regarded as a terminal state for fibroblastic cells, it is now clear that the myofibroblast phenotype can be plastic and reversible. Thus, the term ‘activation’ is used in this Review to describe myofibroblast phenotypic transition.

In this Review, we discuss advances in our knowledge of the mechanisms that keep myofibroblasts in an activated state, control myofibroblast functions and help myofibroblasts evade apoptosis. We focus on pathways and processes that are relevant for the development, progression and persistence of rheumatic disease-associated fibrotic disorders. However, the fundamental processes leading to persistent fibrosis are remarkably universal, so examples from different organ systems are also provided. Furthermore, we discuss novel therapeutic strategies aimed at specifically inducing myofibroblast apoptosis or, alternatively, promoting their deactivation with the aim of re-educating fibrogenic myofibroblasts into scar-resolving cells that reverse the damage inflicted by fibrosis.

Fibroblastic cell types

The generic term fibroblast is often used to describe collagen-producing, heterogeneous cell populations that reside in soft connective tissue, including mesenchymal stromal (or stem)

cells[18], pericytes[19] and bona fide fibroblasts with different phenotypes[20–23]. Because specific molecular markers are lacking and the phenotypic features of these cell types are malleable, one could use the term fibroblast to describe the cells of a tissue that remain after all other cells have been classified according to their unique criteria. The following sections focus on fibroblasts and myofibroblasts, which differ in several ways (TABLE 1). Myofibroblasts are important cells that orchestrate the integration of the complex dynamic biochemical and biophysical cues present within tissue undergoing repair, with the aim of restoring tissue integrity and, ideally, homeostasis. Myofibroblasts are not typically found in healthy connective tissues.

Fibroblasts in tissue homeostasis.

Historically, fibroblasts residing in healthy tissues were considered to be quiescent or dormant, a belief that was partly based on the misperception that the ECM is a permanent scaffold that exists to support cells and only undergoes turnover following tissue injury or during disease. However, normal connective tissue ECM is a highly complex and dynamic structure that is continuously being synthesized, degraded and remodelled by resident fibroblasts[24]. These fibroblast activities enable the adaption of normal organ structures and functions to changing physical demands. For example, in athletes who are training, physiological connective tissue remodelling enables the heart to resist increasing mechanical loads and the lungs to increase in capacity[25,26].

To fulfil their role as homeostats, fibroblasts have developed numerous, complex, positive and negative feedback mechanisms that enable them to sense and respond to changes in the biomechanical state, integrity and biochemical composition of the ECM. For example, weakening of the ECM by matrix metalloproteinase (MMP)-mediated degradation triggers fibroblasts to synthesize and deposit ECM and to secrete ECM crosslinking enzymes such as lysyl oxidases, leading to a mechanically reinforced ECM[27,28]. Moreover, fibroblasts orient ECM fibres during normal tissue turnover, which contributes to the mechanical stability of organs and tissues[29]. These activities are carefully orchestrated and seem to function seamlessly under physiological conditions; however, in acute traumatic conditions, during chronic inflammation or when repeated tissue microdamage occurs, fibroblasts cannot maintain homeostasis and the activity of professional repair cells — myofibroblasts — is required.

Myofibroblasts in tissue repair.

In conjunction with preceding tissue inflammation, changes in ECM biochemistry and/or mechanics that exhaust the homeostatic capacity of fibroblasts cause them to become activated and to phenotypically transition into myofibroblasts[17] (FIG. 1a). The definition of a myofibroblast is equally as broad as that of a fibroblast and is still under debate[30,31]; however, all myofibroblasts share their common spatial association with fibrillar collagen, the formation of contractile actin–myosin bundles and the exertion of contractile forces within the ECM[15,32]. In addition, myofibroblasts are often characterized by the expression of α-smooth muscle actin (α-SMA) in stress fibres, which confers on them their highly contractile phenotype[33]. No known unique cell surface markers exist that distinguish myofibroblasts from other mesenchymal cells, define subsets of myofibroblasts with distinct

physiological repair functions or discriminate ‘good’ wound-healing myofibroblasts from ‘bad’ pro-fibrotic myofibroblasts in fibrosis[31]. Beneath their surface, however, single-cell analysis has revealed that heterogeneous (myo)fibroblast populations have different gene expression and transcription profiles that undergo dynamic changes during tissue repair and fibrosis in various organs[34–40]. Despite the justified excitement around single-cell sequencing and the representation of the resulting complex data in relatively easy-to-grasp t-distributed stochastic neighbour embedding (t-SNE) cluster plots, one should bear in mind that myofibroblast heterogeneity and plasticity are essential for our body to react to unpredictable injury scenarios. Thus, attempts to classify fibroblast and myofibroblast populations might result in the same conclusion as that reached by researchers in the macrophage field[41] — that classifying cells by their functions is preferable to classification by molecular markers.

Myofibroblast heterogeneity is associated with functional diversity, which can either reflect distinct myofibroblast lineages and precursors or different activation states of the same cell type. Notably, myofibroblasts can arise from other cell types, such as epithelial cells, endothelial cells, mesenchymal stem cells, pericytes, pre-adipocytes and adipocytes[17] (FIG. 1a). However, it is still unclear whether the origin of myofibroblasts translates into specific functions during tissue repair or fibrosis. Evidence of different myofibroblast lineages exists in a variety of organs, including the lungs[42–44], heart[25], kidney[45], liver[46], bone marrow[47] and skin[48–50]. In mouse dorsal skin, two dermal fibroblast populations (reticular and papillary) are derived from a common mesenchymal progenitor cell that is present at day 12 during embryonic development[51] (FIG. 1a). Remarkably, these two subsets of fibroblasts not only differ in their anatomical position within the same tissue but also exert distinct functions during tissue repair. Genetic tracing has revealed that reticular fibroblasts of the lower dermis of mouse skin are the first to migrate into the wounded sites, where they activate to become collagen-producing α-SMA$^+$ myofibroblasts. By contrast, papillary fibroblasts seem to be essential for new hair follicle formation later on in the healing process and never express α-SMA[51]. Other lineage markers, such as engrailed 1 and CD26, have been used to reveal how at least two succeeding distinct fibroblast populations contribute to skin development[21], normal wound healing and scarring in mouse models[52]. Whether the same or similar fibroblast lineages contribute differently to the healing of human skin injuries and/or to the development of skin fibrosis in SSc remains elusive. However, one common conclusion of such lineage-tracing studies, despite the use of different genetic markers, is that targeting specific myofibroblast precursor cells has the potential to suppress the development of fibrosis without affecting regenerative fibroblast populations. Notably, distinct fibroblast populations have also been identified by single-cell mRNA analysis in synovium and are thought to have different roles in RA[53]. Thy-1$^+$ fibroblasts in the synovial sublining promoted and sustained inflammation in a mouse model of arthritis without affecting bone and cartilage, whereas a Thy-1$^-$ fibroblast population residing in the synovial lining degraded bone and cartilage ECM without affecting inflammation[53].

An alternative, but not mutually exclusive, hypothesis is that myofibroblasts undergo dynamic transitions or exist in a variety of maturation states that differently affect the outcome of normal and fibrotic tissue repair[15,54–57]. The term ‘proto-myofibroblast’ has been proposed to emphasize that full ‘differentiation’ of fibroblasts into highly contractile α-

SMA$^+$ myofibroblasts is preceded by the transition to a collagen-synthesizing phenotype that has α-SMA$^-$ stress fibres and relatively lower contractile activity[15]. Such phenotypic shifts must be considered when interpreting phenotypic and/or transcriptional myofibroblast profiles obtained at single time-points from cell cultures, healing tissues or fibrotic lesions, which do not take into account the history or future of these cells.

Phenotypic shifts have also been reported for highly activated, α-SMA$^+$ myofibroblasts at later stages of tissue repair and fibrosis — a phenomenon called reversal. For example, subsets of α-SMA$^+$ myofibroblasts undergo phenotypic transition into senescent myofibroblasts that express p16INK4a (a cell cycle regulatory protein) during the resolution phase of cutaneous healing[58,59]. This transition is required to shift the activity of myofibroblasts from ECM production to ECM degradation, and thereby create scar-resolving cells (FIG. 1a). Using single-cell tracing in a mouse model of carbon tetrachloride-induced induced liver fibrosis, myofibroblasts reverted back to a fibroblast-like inactive state in vivo, and fibrosis was resolved once the experimental organ insult was discontinued[60,61]. Irrespective of the underlying mechanism, the termination of myofibroblast ECM secreting and contractile activities has been proposed as an important step in the resolution of organ fibrosis[62,63].

Although the molecular mechanisms that promote the activation of myofibroblasts have been relatively well studied, considerably less is known about the pathways that deactivate myofibroblasts upon successful completion of tissue repair. Once tissue integrity is re-established, myofibroblasts can adopt one of several different functions[17] (FIG. 1a). Myofibroblasts can deactivate and return to the low activity state characteristic of fibroblasts in homeostatic tissues. Alternatively, they can assume roles that were not characteristic of their precursor cells, such as becoming scar-resolving or senescent cells. Myofibroblasts can also initiate self-clearance via apoptosis (also known as programmed cell death), or can continue to remodel tissue beyond what is required for repair, resulting in pathological scarring (FIG. 1b). How these different fates are determined and how the underlying cellular mechanisms are temporally and spatially regulated remain unclear. In the following sections, we discuss factors and pathways that promote myofibroblast persistence beyond the resolution phase of tissue repair, particularly the evasion of apoptosis.

Apoptotic pathways

Apoptosis is a physiological process in which cells are genetically programmed to self-destruct[64–67]. In healthy adult tissue, 50–70 billion cells are cleared every day via apoptosis to balance the rate of cell division in tissues[68]. Although it seems extremely inefficient to continuously destroy so many cells, apoptosis is an important homeostatic mechanism that controls organ size and function. It is also the main cellular mechanism used to eliminate damaged or infected cells, or to control cells that start dividing uncontrollably[69]. The cellular and molecular features of apoptotic cells include cell shrinkage, membrane blebbing, pyknosis and cell disassembly into apoptotic bodies in a process called budding[70]. Apoptotic bodies are subsequently phagocytosed by surrounding cells, including macrophages[71]. Importantly, the engulfment of cells via efferocytosis is associated with decreased production of pro-inflammatory cytokines such as TNF, and increased production

of anti-inflammatory cytokines such as transforming growth factor-β1 (TGFβ1) and IL-10 (REF.[72]).

Apoptosis is a complex process that is tightly controlled by two interconnected molecular pathways: the intrinsic and extrinsic pathways[65] (FIG. 2). The intrinsic pathway of apoptosis is triggered by intracellular death stimuli such as DNA damage, oxidative stress or oncogene activation[65], all of which initiate apoptosis by inducing mitochondrial outer membrane permeabilization (MOMP). Accordingly, the intrinsic pathway is also called mitochondrial apoptosis. MOMP occurs as the membrane potential and pH gradient across the inner mitochondrial membrane is impaired, leading to mitochondrial swelling, rupture of the outer mitochondrial membrane and release of pro-apoptotic proteins such as cytochrome c from the intermembrane space into the cytoplasm. Cytoplasmic cytochrome c binds to and activates apoptotic protease activating factor 1 (APAF1) to form the apoptosome, which mediates activation of caspase-9, ultimately leading to cell death via the activation of caspase-3 and caspase-7. Caspase-9-mediated cleavage and activation of the effectors caspase-3 and caspase-7 can be inhibited by the pro-survival inhibitor of apoptosis (IAP) proteins (such as X-linked IAP (XIAP)), thereby preventing apoptosis[67]. In this intrinsic pathway, the apoptotic threshold of a cell (the likelihood that it will enter apoptosis) is set by dynamic interactions at the mitochondrial outer membrane between distinct members of the BCL-2 family of proteins. These BCL-2 proteins are divided by structure and function into effector, activator, pro-survival and sensitizer proteins[64,65].

Each BCL-2 family member contains one or more of the four conserved BCL-2 homology domains: BH1, BH2, BH3 and BH4 (REF.[65]). BCL-associated X protein (BAX) and BCL-2 homologous antagonist/killer (BAK) are multi-domain effector proteins that initiate apoptosis via MOMP by forming pores in the mitochondrial membrane. The activities of these effectors are tightly regulated by BH3-only activator proteins, such as BCL-2-like protein 11 (BCL2L11; also known as BIM), p53-upregulated modulator of apoptosis (PUMA) and BH3-interacting domain death agonist (BID). Binding of activators to effectors initiates MOMP, whereas MOMP can be prevented by multi-domain pro-survival proteins such as BCL-2, BCL-X_L, BCL-W, induced myeloid leukaemia cell differentiation protein MCL1 (MCL1) and BCL-2-related protein A1 (BCL2A1; also known as BFL1), which can directly bind and inhibit both effectors and activators. MOMP can also be induced in cells even in the presence of pro-survival proteins if another set of BH3-only sensitizer proteins are highly expressed. Sensitizers, such as BCL-2-associated death promoter (BAD), phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1; also known as NOXA), activator of apoptosis harakiri (HRK), PUMA (which is also an activator), BCL-2-interacting killer (BIK) and BCL-2 modifying factor (BMF), indirectly promote apoptosis by blocking pro-survival proteins and thereby freeing activators and effectors to initiate MOMP[73].

The extrinsic, or death-receptor, pathway of apoptosis is activated by the binding of extracellular death ligands such as FAS ligand (FASL), TNF ligand superfamily member 10 (TNFSF10; also known as TRAIL) and TNF (FIG. 2). Typical cell-surface death receptors are FAS, TNF receptors, death receptor 4 (DR4) and DR5 (REF.[66]). In this pathway, the pro-apoptotic signal is transmitted via a multiprotein death-inducing signalling complex, in

which the adaptor proteins FAS-associated death domain (FADD), TNF receptor type 1-associated DEATH domain (TRADD) and regulated IRE1-dependent decay (RIDD) assemble to activate caspase-8 and caspase-10. Both caspases then initiate apoptosis via cleavage and activation of pro-caspase-3 (FIG. 2).

Interestingly, the extrinsic and intrinsic apoptotic pathways can also be cross-activated via BID[74], an activator protein that can be activated via the extrinsic pathway through caspase-8-mediated proteolytic cleavage. Once cleaved, truncated BID triggers the intrinsic pathway of apoptosis by directly binding to and activating the effector proteins BAX and BAK in the mitochondrial membrane. In the following section we elaborate on how the intrinsic and extrinsic apoptosis pathways are regulated in myofibroblasts during tissue repair and fibrosis.

Myofibroblast apoptosis

Clearance of myofibroblasts in tissue repair.

In seminal studies in the 1990s, Gabbiani and colleagues proposed that the majority of myofibroblasts undergo apoptosis upon completion of tissue repair. They supported this concept by demonstrating the accumulation of fragmented DNA in α-SMA$^+$ cells, and of α-SMA$^+$ apoptotic bodies in the phagolysosomes of phagocytic cells, days after the complete closure of skin wounds in rats[75,76]. Subsequent studies suggested that activated myofibroblasts become ‘addicted’ to growth factor receptor-mediated pathways that enable their survival during tissue repair, but promote apoptosis upon ‘withdrawal’. For example, pro-survival growth factors, such as TGFβ1 or platelet-derived growth factor (PDGF), are released by platelets and macrophages at an early stage of the inflammatory phase of wound healing; the depletion of these growth factors during wound healing can potentially induce myofibroblast apoptosis[77,78].

Such growth factor ‘dependence’ is one plausible mechanism limiting myofibroblast survival during wound healing. Alternatively, pro-apoptotic cytokines released during late stages of tissue repair might selectively induce apoptosis of myofibroblasts by directly activating cell death signalling pathways or by inhibiting pro-survival pathways, which regulate the activation of the intrinsic and/or extrinsic pathways of apoptosis in myofibroblasts. For example, fibroblast growth factor 1 (FGF1) induces caspase-3-mediated apoptosis in activated myofibroblasts from skin granulation tissue by inhibiting the phosphorylation of pro-survival protein kinase B (PKB, also known as AKT) and the related focal adhesion kinase (FAK) signalling pathway; by contrast, FGF1 does not induce apoptosis in fibroblasts from healthy skin[79,80]. Similarly, IL-1β and hepatic growth factor (HGF) induce caspase-dependent apoptosis in mouse lung myofibroblasts by inhibiting FAK[77,81]. In the liver, targeted apoptosis of myofibroblasts occurs during liver regeneration, in which hepatic stellate cells upregulate the pro-apoptotic FAS receptor and are rapidly eliminated through the extrinsic pathway of apoptosis[82,83]. Together, the results of these studies[77,81–83] provide evidence that the activation state of myofibroblasts increases their susceptibility to apoptosis during the resolution phase of the wound healing programme.

Evasion of apoptosis by myofibroblasts in fibrosis.

The shift from physiological tissue repair to pathological organ fibrosis in the context of autoimmune disease involves molecular signalling pathways that prevent the termination of myofibroblast activities. By contrast to their counterparts in wound healing, which disappear at the end of physiological repair, the persistence of myofibroblasts in fibrotic disease has led to the suggestion that these cells are apoptosis-resistant[84,85]. Studies from the past 2 years have shown that, rather than resisting apoptosis, myofibroblasts are actually poised to self-destruct in fibrotic conditions. The molecular basis for the pro-apoptotic tendencies of myofibroblasts seems to centre around an increase in mitochondrial apoptotic priming in these cells[86,87] (FIG. 3). Mitochondrial priming refers to the closeness of mitochondria to the apoptosis threshold, and is controlled by the relative expression of effector, activator, pro-survival and sensitizer proteins from the BCL-2 family[88–90]. Importantly, apoptotic priming is not determined by the expression of individual pro-apoptotic or anti-apoptotic proteins, and cell survival is not a binary ‘yes’ or ‘no’ decision. Instead, cell survival is determined by an intricate dynamic molecular rheostat, in which the relative balance between multiple pro-apoptotic and anti-apoptotic BCL-2 family proteins instructs cells as to whether to survive or undergo apoptosis (FIG. 3). For example, pro-apoptotic BH3-only proteins can be induced transcriptionally and/or post-translationally by cytotoxic stress signals, thereby increasing mitochondrial priming[73,89]. When mitochondrial priming is high enough to cross the apoptotic threshold, MOMP and subsequent apoptosis will occur.

However, cells can still survive with a high degree of mitochondrial priming if a pro-survival mechanism is activated. Typically, cells with high mitochondrial priming upregulate anti-apoptotic proteins that sequester pro-apoptotic BH3-only proteins, thus preventing MOMP[91–93]. In myofibroblasts, upregulation of anti-apoptotic BCL-2 proteins such as BCL-X_L in response to the extracellular environment enable survival despite these cells being primed for death[86]. Nevertheless, cells primed for death become dependent on anti-apoptotic proteins for survival, and the inhibition of important pro-survival proteins therapeutically (with, for example, the so-called BH3 mimetic drugs) can rapidly induce apoptosis in such cells[91] (FIG. 3). In the following section, we discuss molecular mechanisms that promote the survival of myofibroblasts that are primed for death in fibrosis.

Myofibroblast survival mechanisms

The list of factors known to activate myofibroblasts in vitro, during normal tissue repair and during organ fibrosis is ever growing and has been extensively reviewed elsewhere[4,17,20,31,94–101]. In this Review, we focus on biomechanical and biochemical factors that not only promote myofibroblast activation, but also have pro-survival functions.

Biomechanical myofibroblast survival factors.

A crucial factor that enables myofibroblasts primed for death to survive is the specific biomechanical conditions that they generate around themselves in the form of fibrotic ECM. As a result of excessive collagen deposition and incremental myofibroblast contractions, fibrotic ECM becomes stiffer than the ECM of healthy organs. In SSc, the formation of stiff fibrotic scars changes the mechanical properties of the skin, kidney, heart and lungs, which

directly affects organ function[102]. For example, the mechanical obstacle of stiff scar tissue reduces the pumping function of the heart and disturbs electrical transmission. Likewise, stiffening of lung tissue not only causes failure to promote proper gas exchange in the alveolar space, but also reduces distensibility and thus overall lung capacity[102]. In addition to impeding the function of organs that depend on their biomechanical properties, tissue stiffening has a profound influence on the progression of fibrosis by promoting the biomechanical activation of pro-fibrotic TGFβ1 and of myofibroblasts[26,103]. This biomechanical positive feedback loop establishes a vicious cycle that eventually perpetuates fibrosis[26,102,104–106] (FIG. 4). The concept that increased ECM stiffness contributes to the progression of fibrosis, in addition to being its consequence, has intensified mechanobiology research in the context of fibrotic disorders, which has revealed important regulatory elements[102,103,107–110] (discussed below).

Myofibroblast activation culminates in the expression of α-SMA and an associated increase in contractile force (FIG. 4a). Intracellularly, α-SMA protein production is controlled by TGFβ1-induced signalling through TGFβ control elements and mothers against decapentaplegic homolog (SMAD)-binding elements in the *ACTA2* (encoding α-SMA) promoter[111]. Extracellularly, the amount of TGFβ1 activation from latent precursor complexes is dependent on both the biomechanical state of the ECM[112] and the transmission of cellular forces by integrins binding to latent TGFβ1 (REFS[113–115]). In addition to indirect biomechanical stimulation through stress-dependent TGFβ1 activation, the *ACTA2* promoter contains CArG boxes that bind both to myocardin-related transcription factors (MRTFs) and to serum-response factor (SRF), which positively regulate *ACTA2* transcription[116]. MRTF shuttling from the cytosol to the nucleus is controlled by the polymerization state of the actin cytoskeleton (FIG. 4a). In cells under low stress, MRTFs bind to available monomeric G-actin; under high stress, G-actin polymerizes into F-actin stress fibres and releases MRTFs, which travel to the nucleus and upregulate *ACTA2* transcription in conjunction with SRF[117,118]. In addition, the mechanosensitive transcriptional co-activators yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ) positively regulate myofibroblast differentiation from several types of cells[33,119–129] (FIG. 4a).

Whereas ECM stiffening is a pro-myofibroblastic stimulus, the release from extracellular and intracellular stress that occurs during wound healing results in the loss of contractile features and reduced myofibroblast activation[102,103]. In fact, it has been proposed that resolution of stiff scar tissue and the associated tension release serve as important signals for myofibroblasts to terminate their actions and/or to apoptose once normal tissue repair is completed. For example, in vitro, myofibroblast apoptosis can be induced by the relaxing of mechanically restrained fibroblast-populated collagen lattices[130,131]. In vivo, releasing mouse skin wounds from extracellular strain likewise causes myofibroblast apoptosis and, conversely, inhibiting the release of biomechanical stress in healing tissue by splinting the wound inhibits myofibroblast apoptosis and leads to hypertrophic scarring in rodents[132–134].

The molecular pathways that link stress release to myofibroblast apoptosis are still unclear, but potentially involve the inhibition of pro-survival mechanotransduction pathways. Stiffness-activated myofibroblasts undergo rapid apoptosis in vitro upon inhibition of mechanotransduction, and integrin-mediated stress perception and FAK-mediated

mechanosignalling are important components of myofibroblast survival on stiff ECM[86,135] (FIG. 4b). By contrast, blockade of force-dependent mechanosignalling neither interferes with the homeostatic functions of fibroblasts nor causes apoptosis when fibroblasts are plated on soft ECM, further suggesting that the myofibroblast cellular state seems to prime these cells for apoptosis.

Myofibroblasts activated by ECM stiffness are primed for apoptosis by death signals such as the apoptosis activator protein BIM; upregulation of BIM creates a requirement for tonic expression of anti-apoptotic proteins such as BCL-X_L to ensure myofibroblast survival and persistence[86]. BCL-X_L expression in stiffness-activated myofibroblasts seems to be controlled by YAP and TAZ, the translocation of which to the nucleus is induced by integrin-mediated and FAK-mediated mechanotransduction pathways[86] (FIG. 4b). Whereas the evasion of apoptosis by dermal myofibroblasts in skin fibrosis is controlled by the mechanically regulated FAK–YAP–TAZ–BCL-X_L pathway[86], myofibroblast survival in lung fibrosis is mechanically controlled by RHO-associated protein kinase (ROCK)–MRTF–BCL-2 signalling[126]. In addition, TGFβ1–ROCK–MRTF signalling blocks mitochondrial apoptosis by increasing concentrations of XIAP[136–140], which directly inhibits caspases (FIG. 4b). The results of these studies[86,126] support the concept that biomechanical signalling induced by ECM stiffness promotes myofibroblast survival by the upregulation of specific BCL-2 family pro-survival factors in myofibroblasts that are primed for death.

In addition to directly affecting mitochondrial priming, biomechanical signalling can also promote myofibroblast resistance to apoptosis via indirect mechanisms. ECM stiffness induces the expression of the microRNAs miR-21 and miR-29a, which promote the survival of stiffness-activated myofibroblasts by increasing the expression of BCL-2 and reducing the expression of BAX[134,139] (FIG. 4b). MiR-21 is known to be pro-fibrotic in many organs and fibroblastic cells[140–145]. Curiously, miR-21 is also involved in maintaining the contractile phenotype of myofibroblasts that have been mechanically primed on pathological scar-stiff polymer substrates, even weeks after moving and exposing the same cells to soft substrate cultures[134]. This phenomenon has been termed ‘mechanical memory’[95], and it will be interesting to see the results of future investigations into how mechanical priming and priming for death are coordinated.

Extracellular myofibroblast survival factors.

Autocrine production of TGFβ1 and the vasoactive peptide endothelin 1 (ET1) mediates resistance to apoptosis in cultured SSc and IPF fibroblasts via increased activation of the FAK–AKT signalling pathway[146–150]. In promoting myofibroblast survival, the activation of FAK is dependent on β1 integrin[77,135,137,149]. FAK activation requires auto-phosphorylation at tyrosine 397, which creates a binding site for the Src homology 2 (SH2) domains of the p85 regulatory subunit of phosphoinositide 3-kinase (PI3K) that, in turn, leads to activation of the pro-survival PI3K–AKT signalling pathway[150–152]. Alternatively, AKT activation by TGFβ1 in lung myofibroblasts can occur in a FAK-independent, p38 mitogen-activated protein kinase (MAPK)-dependent manner[153]. AKT activation results in phosphorylation and inhibition of the sensitizer protein BAD[77,154,155], thereby inhibiting myofibroblast apoptosis at a crossover point between the extrinsic and intrinsic apoptosis pathways (FIG.

4b). Furthermore, TGFβ1-mediated activation of the transcription factor ABL results in increased amounts of the pro-survival protein BCL-2 and decreased amounts of the effector protein BAX[156]. In addition, PDGF can activate cancer-associated myofibroblasts, while simultaneously increasing their mitochondrial priming and sensitivity to apoptosis[157]. Similarly, the survival of PDGF-activated myofibroblasts in the liver is dependent on BCL-X_L[158], indicating an important role for this pro-survival protein in promoting the survival of fibrogenic myofibroblasts, potentially across different organs. Taken together, the intrinsic mitochondrial pathway is directly inhibited by TGFβ1 and PDGF signalling via transcriptional regulation of pro-survival BCL-2 family proteins, leading to decreased mitochondrial priming in myofibroblasts.

In addition to regulating myofibroblast survival through the intrinsic apoptotic pathway, FAK signalling is also important in blocking the extrinsic apoptotic pathway in response to pro-fibrotic cytokines[86,159]. Both TGFβ1 and ET1 block the extrinsic pathway of apoptosis in SSc and IPF myofibroblasts by inhibiting FAS–FASL signalling[77,154,160–162]. The pro-apoptotic activities of the FAS–FASL pathway in myofibroblasts are mostly mediated by ceramide generation via the activation of acidic sphingomyelinase[163]. In SSc myofibroblasts, autocrine TGFβ1 signalling inhibits sphingomyelinase, thereby protecting these cells from FASL-induced apoptosis despite similar expression levels of FAS receptor compared with healthy fibroblasts[154,160,164] (FIG. 4b). In IPF myofibroblasts, sensitivity to FASL-induced apoptosis is similarly reduced, albeit through different molecular mechanisms, including increased levels of soluble FAS, which is produced as an alternatively spliced variant of FAS and functions as decoy receptor for FASL[162], and upregulation of decoy receptor 3, which binds to FASL and inhibits FASL-induced apoptosis[165].

In addition, reduced sensitivity of fibrotic fibroblasts to FASL-induced apoptosis can be mediated by down-regulation of cyclooxygenase-2 (COX2), which normally promotes apoptosis in fibroblasts by producing prostaglandin E2 (PGE_2)[166], a lipid mediator derived from the metabolism of arachidonic acid. Exogenous administration of PGE_2 restores the sensitivity of fibrotic lung myofibroblasts to FASL-induced apoptosis by decreasing AKT signalling[166,167]. PGE_2 treatment also suppresses the caspase inhibitor XIAP, thereby enhancing FASL-mediated apoptosis in lung myofibroblasts and lending support for a potential mechanistic role for XIAP in myofibroblast evasion of apoptosis[161,167]. Interestingly, ECM stiffening in fibrotic disorders participates in a positive feedback loop with myofibroblasts that leads to suppression of COX2 expression and PGE_2 production, which suggests that the fibrotic biomechanical microenvironment itself might be sufficient to promote myofibroblast survival through this pathway[105]. Thus, diminished PGE_2 production and/or responsiveness contribute to myofibroblast apoptosis resistance in fibrotic disorders.

Intracellular myofibroblast survival factors.

Metabolic reprogramming is emerging as an important mechanism of myofibroblast survival. Myofibroblasts are cells with a high metabolic rate that require energy for the synthesis and contraction of the ECM. Notably, although metabolic reprogramming of myofibroblasts rewires their pro-survival pathways to promote their persistence[168], the

molecular mechanisms promoting myofibroblast survival via metabolic reprogramming remain poorly understood. YAP–TAZ activity is also controlled by metabolic and energy-sensing pathways[169], suggesting that the metabolic status and reprogramming of myofibroblasts might control the activation of mechanosensitive pro-survival pathways.

Myofibroblast bioenergetics are sustained by the induction of glycolysis and increased mitochondrial oxygen consumption, which support their aerobic glycolysis phenotype[169]. In one study[170], this high-energy status of myofibroblasts was associated with fragmented and damaged mitochondria in IPF fibroblasts, which potentially contribute to the increased mitochondrial priming of these cells via poorly understood mechanisms. Loss of activity of the energy sensor AMP-activated protein kinase (AMPK) promoted mitochondrial dysfunction and metabolic reprogramming in myofibroblasts. Accordingly, activation of AMPK by treatment with metformin restored the sensitivity of myofibroblasts to apoptosis, indicating that mitochondrial biogenesis is required to induce apoptosis in primed-for-death myofibroblasts. More importantly, metformin reversed established lung fibrosis in experimental models by promoting myofibroblast deactivation and/or apoptosis[170].

Myofibroblast senescence.

In addition to avoiding apoptosis, myofibroblasts can persist by becoming senescent (FIG. 1). Growing evidence suggests that progressive fibrosis might result from accumulation of pro-fibrotic senescent myofibroblasts, which are prevalent in age-related fibrotic disorders such as IPF[171–173]. Cellular senescence was initially defined as cell-cycle arrest and first described in human lung fibroblasts by Hayflick in 1965 (REF.[174]). The main function of cellular senescence is to prevent the division of damaged cells as a protective mechanism against tumorigenesis. Senescence locks cells in a permanent cell cycle arrest by increasing expression of cell cycle and tumour suppressor inhibitors in response to excessive stressors, such as telomeric dysfunction (replicative senescence), oncogene activation and genotoxic or oxidative stresses[175]. These processes are promoted by senescence-activating signalling pathways that include the cyclin-dependent kinase inhibitors p53, p16 or p21 (REFS[176,177]).

Because senescent cells are also destined for elimination, cellular senescence is conceptually similar to apoptosis, albeit operating via different clearance mechanisms. Senescent cells are characterized by the acquisition of a senescence-associated secretory phenotype[178]. This phenotype is molecularly controlled by transcription factor NF-κB signalling and characterized by the secretion of pro-inflammatory cytokines and chemokines such as IL-6, CC-chemokine ligand 2 (CCL2) and TGFβ1, which collectively trigger a pro-inflammatory response that leads to the elimination of senescent cells by immune cells[179]. The senescence-associated secretome also comprises pro-inflammatory mediators and growth factors involved in tissue fibrogenesis (such as IL-1α, IL-1β, IL-8, various CCLs, TGFβ1, PDGF and IL-6), proteins involved in ECM turnover (such as fibronectin, collagens and MMPs) and ECM proteins[178,180]. Both senescence-associated ECM and cytokines can ‘spread’ senescence between pro-inflammatory immune cells and stromal cells in mouse models of repair and fibrosis[177,181]. Myofibroblast senescence has been implicated in physiological wound healing, tissue regeneration and fibrosis. During liver regeneration, the transition of hepatic stellate cells that have activated to become myofibroblasts into a

senescent phenotype facilitates their elimination by natural killer (NK) cells and limits the progression of liver fibrosis[182]. Similarly, induction of myofibroblast senescence by the matricellular protein CCN1 limits skin and liver scarring by inducing secretion of MMPs that degrade the fibrotic scar[183,184].

Senescent myofibroblasts have also been implicated in the development of lung fibrosis[172,185–189]. In senescent lung myofibroblasts isolated from patients with IPF, p16 and p21 are upregulated in a TGFβ1-independent manner and seem to promote fibrosis by fostering a pro-inflammatory and pro-fibrotic microenvironment[190]. Indeed, cellular senescence in IPF myofibroblasts might be induced in response to telomere dysfunction as part of the DNA damage response[172]. In human fibroblasts, telomere shortening triggers p53-mediated p21 upregulation, whereas p16 expression is upregulated in a telomere-independent and DNA damage-independent manner[191]. In addition, suppression of telomerase expression is sufficient to promote α-SMA expression, suggesting that replicative senescence is associated with the acquisition of the myofibroblast phenotype[192–194]. By contrast, increased telomerase activity promotes the proliferation and survival of α-SMA$^-$ fibroblasts[193]. Accordingly, mice deficient in telomerase reverse transcriptase have decreased proliferation of α-SMA$^-$ fibroblasts and are protected from bleomycin-induced lung fibrosis[192,195]. Notably, myofibroblast senescence can occur in the absence of shortened telomeres[196], suggesting that mechanisms other than replicative senescence might be behind this pathological phenotype during tissue fibrogenesis. For example, the pro-senescence factor CCN1 is induced by TGFβ1 in lung fibroblasts, suggesting that the acquisition of a senescent myofibroblast phenotype can be caused by telomere-independent mechanisms[196]. Importantly, genetic approaches have been used to ablate p16$^+$ senescent cells in mouse lungs and kidneys, thereby mitigating fibrosis[197]. These results[197] support the idea of using drugs that selectively induce the death of senescent cells (senolytic drugs) for the treatment of organ fibrosis[186,198,199]. However, care must be taken as senescent (myo)fibroblasts also seem to have beneficial functions in promoting normal tissue repair and in impeding fibrosis[180]. For example, inducing myofibroblast senescence by miR-34a inhibited lung fibrosis in mouse models of disease[173]. Timing — as always — seems to be critical for success when therapeutically interfering with senescence.

Resolution of fibrosis

Treatments that halt fibrosis are not necessarily effective in restoring organ function because fibrotic ECM contains biomechanical and biochemical factors that promote myofibroblast activation. To regain function, such mis-instructive and ‘diseased’ ECM needs to be removed and/or replaced with ‘healthy’ ECM in a controlled manner[63,200]. Although originally considered to be irreversible[201], established fibrosis can regress in both mouse models of disease and human fibrotic diseases – particularly in the liver[202]. Although, human livers at late stages of cirrhosis remain untreatable, clear evidence exists that treatment of hepatitis C infection at early-to-mid stages of cirrhosis results in spontaneous reversion of liver fibrosis in humans[203,204]; ECM degradation is an important event in this process[205,206]. Likewise, liver fibrosis reverses spontaneously in mouse models of liver injury induced by alcohol intoxication, carbon tetrachloride or bile duct ligation[60,207,208]. Fibrosis can also regress in

organs with lower regenerative capacity than the liver, such as the lungs[63,209]. In humans, lung fibrosis resolves substantially in patients with acute respiratory syndrome[210,211], mimicking the biology of mouse models of acute lung injury induced by injection of bacterial wall saccharides[212]. Although lung fibrosis resolves spontaneously in mouse models of fibrogenic injury such as intratracheal administration of bleomycin, asbestos fibres or fluorescein isothiocyanate[209], the progressive lung fibrosis seen in patients with IPF or SSc-associated ILD seems to be generally irreversible without treatment[213]. However, the results of randomized controlled trials in SSc-associated ILD consistently show subsets of patients with improved pulmonary function[214,215], suggesting that tissue regeneration or regression of pre-existing fibrosis occurs. Clinical studies have also shown that skin fibrosis can regress in patients with SSc both spontaneously and after treatment[216,217]. Likewise, mouse skin fibrosis caused by subcutaneous injection of bleomycin resolves shortly after the fibrogenic stimulus is removed[218]. Overall, the capacity for fibrosis resolution might be organ-specific and differ depending on the nature of the fibrogenic stimulus, as well as host-specific factors including genetic susceptibility, age, sex and immunocompetence[96].

Fibrosis resolution is characterized by ECM degradation and restoration of the biomechanical and biochemical properties of the ECM. Although it is known that targeted apoptosis of myofibroblasts results in fibrosis resolution in mouse models, the cellular and molecular mechanisms regulating ECM degradation and fibrosis resolution following myofibroblast apoptosis remain largely unknown. Macrophages, fibroblasts, NK cells and neutrophils have been previously implicated in ECM degradation following injury through the secretion of MMPs[219]. Degradation of fibrotic scar tissue, mostly composed of crosslinked type I collagen fibres, is initiated by collagenases (MMP-1, MMP-2, MMP-8, MMP-13, MMP-14, MMP-15 and MMP-16) and is followed by further cleavage by gelatinases, MMP-2 or MMP-9, as well as by other proteases such as cathepsin K[220–222]. Following degradation, collagen fragments are taken up by various cell types including fibroblasts and macrophages[223]. In fibroblasts, collagen fragments are recognized by C-type mannose receptor 2 (also known as ENDO180)[224–227], which promotes collagen internalization and lysosomal degradation in a non-phagocytic manner. Alternatively, fibroblasts can also phagocytose large collagen particles via α1β1, α2β1 and α3β1 integrins prior to lysosomal degradation[228–232].

Turning activated myofibroblasts into scar-resolving cells.

Whereas several mechanisms are known to deactivate myofibroblasts[63], less is known about potential mechanisms that promote the transition of myofibroblasts into scar-resolving cells in vitro and in vivo. In fact, the reprogramming of scar-forming myofibroblasts into scar-resolving cells is an important cellular mechanism for scar resolution during physiological tissue repair (FIG. 1a). Fibroblasts acquire an ECM-degrading phenotype upon YAP–TAZ inhibition, which is associated with an increased expression of ECM-degrading enzymes such as plasmin, MMP-14 and cathepsin K[122,231,232]. Support for an important role for fibroblasts in degrading fibrillar collagen in vivo comes from studies that found impaired resolution of SSc-like fibrosis in mice with fibroblast-selective deletion of *MMP14* (REF. [231]). In addition, mice deficient in cathepsin K develop increased lung fibrosis compared with wild-type mice, a phenotype that has been explained by decreased collagenolytic

activity of fibroblasts[232]. Conversely, excessive cathepsin K activity of synovial fibroblasts mediates pathological collagen degradation in patients with RA[233], indicating that temporal and spatial regulation of fibroblast ECM-degrading enzymes is essential to achieve tissue homeostasis. Concomitantly, expression of the transcription factor PU.1 rapidly causes pro-inflammatory ECM-degrading fibroblasts to become scar-forming pro-fibrotic fibroblasts[234]. Inhibition of PU.1 reprogrammes pro-fibrotic fibroblasts and promotes fibrosis regression in a mouse model of SSc[234], suggesting an attractive novel anti-fibrotic strategy based on stimulating scar-resolving activities in fibroblasts. However, further research is needed to evaluate the plasticity of human pro-fibrotic fibroblasts across the spectrum of inflammatory, homeostatic and fibrotic phenotypes.

Macrophages in myofibroblast survival and fibrosis resolution.

Although fibroblastic cells can phagocytose collagen and other ECM components, they are not considered to be professional phagocytes. Instead, macrophages have been studied as professional phagocytes in the context of scar tissue resolution. In macrophages, the recognition and internalization of degraded collagen products is regulated by the extracellular bridging glycoprotein lactadherin (also known as MFGE8), which mediates collagen uptake and endocytosis via its first discoidin domain[235]. Accordingly, MFGE8-deficient mice develop severe pulmonary fibrosis after bleomycin-induced lung injury as a result of severe defects in collagen clearance and uptake by macrophages[235]. Similarly, the collagen receptor mannose receptor C type 1 is involved in collagen uptake by repair-associated macrophages during homeostasis in mice[236]; however, its role in fibrosis is poorly understood.

Collagen uptake and degradation trigger a rheostat mechanism in which collagen opsonization and endocytosis lead to increased production of collagen-degrading enzymes, supporting a positive feedback loop of ECM degradation[237]. However, whether these anti-fibrotic ECM degradation mechanisms are reactivated during fibrosis resolution is unclear. In support of this possibility, previous studies have shown that the ECM-degrading activity of macrophages can be induced by apoptotic bodies, thereby promoting the resolution of experimental models of pulmonary or biliary fibrosis in vivo[238,239]. Furthermore, although pulmonary delivery of TNF to mice does not directly target myofibroblasts for apoptosis, it still triggers lung fibrosis resolution by reprogramming pro-fibrotic $CD11c^{+}CD11b^{var}F4/80^{+}$ macrophages to a pro-resolving phenotype[240]. Thus, modulating macrophage phenotypes might serve as a novel strategy for promoting fibrosis resolution. Other studies suggest that $CD11b^{hi}Ly6C^{lo}F4/80^{int}$ macrophages are of particular therapeutic interest owing to their capacity to induce myofibroblast apoptosis and to secrete MMPs in the context of liver fibrosis[241,242]. In addition, NK cells have an important role in the resolution of liver fibrosis by promoting myofibroblast apoptosis; NK cells expressing cell death ligands such as TRAIL and FASL trigger the extrinsic pathway of apoptosis in hepatic stellate cells, ultimately promoting fibrosis resolution[243,244]. Overall, fibrosis resolution involves the activation of pro-resolving macrophages and NK cells, which promote ECM degradation, collagen uptake and the immunological clearance of myofibroblasts. Targeted therapies aimed at inducing myofibroblast apoptosis or reprogramming the immune system could reactivate these mechanisms in vivo. It will be interesting to see whether complex

biomechanical and biochemical macrophage–myofibroblast interactions are involved in promoting myofibroblast and macrophage survival and the resolution of fibrosis[31,245–247].

Strategies to reverse fibrosis

Advances in myofibroblast and fibrosis research have led to several exciting new therapeutic approaches and have revealed new potential targets to treat fibrotic disorders, including those associated with SSc[1,30,94,248]. Targeting myofibroblast apoptosis is emerging as one of these novel therapeutic strategies to reverse established fibrosis[86,126,185]. In this section, we discuss the molecular mechanisms behind novel therapeutic strategies aimed at inducing myofibroblast apoptosis (TABLE 2). These strategies might not only prevent the progression of organ fibrosis, but also have the potential to reverse established fibrosis.

Drugs targeting biomechanical signalling.

ECM remodelling by activated fibroblastic cells contributes to progressive tissue stiffening which, in turn, provides biomechanical feedback control over myofibroblast function and phenotype[103,249]. Simply put, myofibroblasts adapt their contractile force to the resistance they experience using their mechano-sensing and mechano-transducing cytoskeletal machinery (FIG. 4a). Several molecular elements of this machinery have been considered and tested as therapeutic targets to interrupt the fibrotic cycle by returning myofibroblasts to a relaxed state or inducing their apoptosis[120,122–126,129,250–255]. For example, direct functional inhibition of α-SMA stress fibres with specific acetylated tetrapeptides (Ac-EEED) reduced myofibroblast contraction and subsequent *COL1A1* transcription in vitro[256]. Therapeutic blockade of myofibroblast integrins using small-molecule inhibitors or biological drugs can potentially have dual effects by reducing αv integrin-mediated TGFβ1 activation and by disturbing myofibroblast mechanosensing[114,257–260]. Inhibition or deletion of the transcription factor MRTF-A suppresses fibrogenesis in cell culture and the development of fibrosis in the skin, lungs, colon and heart in vivo[253,261–263], and therapeutic targeting of TAZ and YAP ameliorates skin fibrosis in mice[264]. Similarly, inhibition of FAK and ROCK mechanotransduction pathways using small molecules induces myofibroblast apoptosis and has been used to treat fibrosis in various experimental models[126,135,147,152,265–271] (TABLE 2).

Drugs targeting the intrinsic apoptosis pathway.

The discovery that apoptotic mitochondrial priming is heightened in pro-fibrotic myofibroblasts has paved the way for these cells to be targeted by pro-apoptotic approaches in fibrotic disorders[86,87]. Studies over the past few years have demonstrated that inducing apoptosis in myofibroblasts can revert tissue fibrosis in mouse models of disease[86,126,160,185], and this approach is now emerging as a novel therapeutic strategy to reverse established fibrosis in human fibrotic diseases. BH3 mimetic drugs can selectively promote the intrinsic apoptotic pathway in myofibroblasts by targeting specific pro-survival BCL-2 family proteins. Importantly, only cells that are primed for death and have increased amounts of pro-apoptotic proteins in their mitochondria are sensitive to these drugs.

BH3 mimetic drugs are small molecules that inhibit interactions between pro-survival proteins and activators or effectors[64,65] by directly binding to a shallow, hydrophobic groove in pro-survival BCL-2 family proteins. Differences in the structure of this hydrophobic groove in individual pro-survival BCL-2 family proteins have guided the design and development of selective BH3 mimetic drugs that target specific anti-apoptotic proteins[65]. Multiple BH3 mimetic drugs have been investigated in preclinical studies and several are currently being tested in clinical trials for various types of cancer. ABT-737 and its orally available analogue ABT-263 (navitoclax) bind to and block BCL-2, BCL-X_L and BCL-W with subnanomolar affinity[272,273]. Treatment with ABT-263 resulted in selective induction of myofibroblast apoptosis in areas of dermal fibrosis and reversion of fibrosis in a mouse model of SSc dermal fibrosis[86]. ABT-263 also promoted apoptosis in PDGF-activated hepatic stellate cells[157,158] and senescent lung fibroblasts[274], and in mice, ABT-263 reversed radiation-induced lung fibrosis[275]. Consistently, the BCL-X_L-specific BH3 mimetic drug A-1331852 abrogated biliary liver fibrosis in *Mdr2*$^{-/-}$ mice by promoting apoptosis of liver myofibroblasts[276]. As BCL-X_L is important in promoting myofibroblast resistance to apoptosis, inhibition of BCL-X_L might be a particularly potent therapeutic strategy for SSc and other fibrotic disorders.

However, the potential adverse effects of BH3 mimetic drugs must be considered. For example, human embryonic stem cells have increased mitochondrial priming and low survival ability in vitro compared with fully differentiated somatic cells[277], a mechanism that prevents the propagation of genetic mutations by inducing apoptosis in injured stem cells. Although these in vitro findings suggest that BH3 mimetic therapy could potentially disrupt the balance of stem cell homeostasis owing to the high predisposition of these cells for apoptosis, in vivo studies have shown that BH3 mimetic therapy paradoxically rejuvenates prematurely aged mice by inducing apoptosis of senescent haematopoietic stem cells (HSCs)[278]. Thus, mitochondrial priming seems to be directly regulated by ageing in stem cells; in fact, mitochondrial priming is increased in HSCs from young mice compared with HSCs from aged mice[279]. However, further research is needed to understand how mitochondrial priming is regulated in tissue-specific stem cells during homeostasis and age-related fibrotic diseases, and what the potential effects of BH3 mimetic therapy might be on stem cell biology in the context of tissue repair and fibrotic disease.

The fact that BH3 mimetic drug ABT-263 causes reversible dose-limiting thrombocytopenia[273] has also limited its use in the treatment of haematological malignancies, which require high doses for therapeutic efficacy. The ABT-263 target protein in chronic lymphocytic leukaemia is BCL-2, rather than BCL-X_L, a fact that led to the development of BCL-2-specific BH3 mimetic drugs such as ABT-199. ABT-199 does not harm platelets and has been approved by the FDA for the treatment of chronic lymphocytic leukaemia in patients with a specific chromosomal abnormality[280,281]. Importantly, solid tumours, and particularly cancer-associated fibroblasts, rely on BCL-X_L rather than BCL-2 to survive, thus providing enduring interest in using BCL-X_L-specific BH3 mimetic drugs (such as ABT-263) to target BCL-X_L in desmoplastic tumours[282]. The risk of thrombocytopenia seems to be manageable in this indication given its reversibility and the high doses needed (above 100 mg/kg) to cause platelet depletion.

Collectively, targeting apoptotic pathways in activated myofibroblasts with BH3 mimetic drugs reverses established fibrosis in preclinical models of skin, lung and liver fibrosis, suggesting that these drugs could be a selective, safe and potent anti-fibrotic tool to reverse organ fibrosis (TABLE 2). However, further clinical studies are needed to determine the efficacy of BH3 mimetic drugs in human fibrotic diseases.

Drugs targeting the extrinsic apoptosis pathway.

Unlike the growing body of evidence in support of targeting the intrinsic apoptosis pathway in myofibroblasts, less is known about the potential to target the control of myofibroblast apoptosis via the extrinsic pathway. Previous studies have found reduced expression of TNF receptors I and II and reduced sensitivity to FASL-induced and TNF-induced apoptosis in pro-fibrotic fibroblasts[160–162,283,284]. Although the results of these studies suggest that the extrinsic pathway of apoptosis is generally suppressed in myofibroblasts and cannot be exploited for therapeutic intervention, extrinsic apoptosis can still be triggered via activation of the TRAIL–death receptor pathway. In this mechanism, TGFβ1-mediated myofibroblast activation causes increased expression of DR4 and DR5, which are receptors for TRAIL. Accordingly, selective agonism of DR4 and DR5 with the recombinant TRAIL ligand TLY012 induces apoptosis in myofibroblasts and reverses established liver and skin fibrosis in mouse models of fibrotic disease[285,286], highlighting the therapeutic potential of targeting myofibroblasts for apoptosis via activation of the extrinsic apoptosis pathway (TABLE 2).

Drugs targeting senescence.

Senolytic drugs are defined as therapeutic agents capable of inducing apoptosis in senescent cells by targeting pro-survival pathways in these cells[175,176,287,288]. The first class of senolytic drugs includes dasatinib and quercetin[289], which increase lung function and partially reduce lung fibrosis in mouse models of fibrotic disease when administered together (D+Q therapy)[186,199,290]. In addition, the decreased sensitivity to FASL-induced apoptosis of IPF fibroblasts can be restored by treatment with quercetin[291], suggesting that senolytic drugs might be a viable therapeutic option for IPF and other age-related diseases that progress with the accumulation of senescent myofibroblasts. Notably, the mechanism of action behind the senolytic activity of D+Q therapy remains poorly understood. Dasatinib is an ATP-competitive inhibitor that targets several kinases, including BCR-ABL, c-KIT, ephrin receptors, SRC, LYN, FYN and LCK[292–294], whereas quercetin is a natural flavonoid that is found in a number of foods and has potent antioxidant effects that modulate the NF-κB and PI3K–AKT pathways[295,296]. Several studies have also shown that D+Q therapy only removes 30% of senescent cells[289], suggesting that it targets an as-yet-unidentified subset of senescent cells.

Advances in understanding the molecular basis for the survival and accumulation of senescent cells have led to the development of a new generation of senolytic agents that promote apoptosis in senescent cells by targeting pro-survival BCL-2 family proteins. Senescent primary human fibroblasts become dependent on BCL-X_L and BCL-W expression for survival regardless of what triggered the senescent phenotype (DNA-damage-induced senescence, replicative senescence or oncogene-induced senescence)[274]. Accordingly, blockade of BCL-X_L and BCL-W with the BH3 mimetic ABT-737 induces

apoptosis in senescent cells formed following DNA damage in the lungs of mice[274]. The BCL-X_L-specific mimetic A1331852 reduced liver fibrosis in vivo by targeting senescent cholangiocytes for apoptosis[158]. Furthermore, ABT-263 showed potent senolytic activity in a mouse model of premature ageing[278,297]. Together, the potent senolytic and anti-fibrotic effects of ABT-263 suggest that the ability of this BH3 mimetic drug to reverse organ fibrosis might be caused by specific 'fibro-senolytic' effects on senescent myofibroblasts. Interestingly, the superoxide-generating enzyme NADPH oxidase 4 (NOX4) is induced in senescent lung fibroblasts, and pharmacological inhibition of NOX4 can target senescent fibroblast for apoptosis and reverse established lung fibrosis in aged mice[185]. Overall, these exciting preclinical data demonstrate the potential efficacy of targeting senescent myofibroblasts for apoptosis with fibro-senolytic agents to reverse established fibrosis. Accordingly, the results of the first-in-human clinical trial evaluating the effects of senolytic drugs on IPF were published in 2019 (TABLE 2). In this pilot study, D+Q therapy for 3 weeks improved physical function (evaluated by the 6-minute walk test and walking speed) in a small cohort of 14 patients with IPF[298]. Although these data are promising, results should be interpreted with caution and require further investigation.

Conclusions

Organ fibrosis is a lethal outcome of SSc, an autoimmune rheumatic disease characterized by persistent activation of scar-forming myofibroblasts. Intensive research into the mechanisms that promote and maintain myofibroblast activation has revealed novel therapeutic targets and prompted the development of promising first-in-class anti-fibrotic therapies for the treatment of fibrosis — a condition previously thought to be progressive and irreversible. As a result, a growing pipeline of small molecules and biologic drugs are now being tested in multiple phase II clinical trials on the basis of their ability to slow the progression of fibrosis in preclinical models. However, therapies that halt fibrosis are not necessarily effective at restoring organ function, and researchers have been investigating mechanisms of fibrosis resolution and how to reactivate the regenerative capacity of chronically damaged organs in an effort to restore function to fibrotic organs.

In established fibrosis, myofibroblast persistence, rather than activation, seems to be the disease mechanism that prevents tissue regeneration. Myofibroblasts escape death by activating pro-survival mechanisms in response to biomechanical and growth factor signals from the fibrotic microenvironment. Chronic activation of myofibroblasts can ultimately lead to the acquisition of a senescent phenotype. In addition, (senescent) myofibroblasts, rather than being apoptosis-resistant, are primed for apoptosis owing to concomitant activation of cell death signalling pathways. This knowledge of apoptotic priming has paved the way to specifically trigger apoptosis in myofibroblasts by blocking specific pro-survival BCL-2 family proteins. Mechanotherapeutic strategies that reduce the expression of pro-survival proteins by targeting biomechanical signalling and the direct inhibition of BCL-2 family proteins with BH3 mimetic drugs and senolytic agents trigger myofibroblast apoptosis and reverse established fibrosis in mouse models of disease. This growing body of research has set the stage for the development of a second generation of anti-fibrotic therapies that have the potential to reverse established fibrosis and to regenerate chronically injured tissues.

The initial enthusiasm around such drugs has been justified by studies in humans showing that established fibrosis can regress in fibrotic diseases such as liver cirrhosis and dermal SSc. Because ECM degradation is a crucial event in the process of reversing fibrosis, further efforts are needed to understand the cellular and molecular mechanisms that control ECM degradation during fibrosis resolution. Professional phagocytic cells such as macrophages would be expected to have an important role in ECM degradation upon the therapeutic removal of (senescent) myofibroblasts. Alternatively, scar-forming myofibroblasts could potentially be reprogrammed into scar-resolving cells, which would promote ECM degradation by harnessing the natural capacity of fibroblasts to synthesize and degrade ECM. Future research into the mechanisms that promote fibrosis resolution will increase our molecular and cellular understanding of this process and bring potential new therapies to reverse organ fibrosis.

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Key points

- Organ fibrosis is a lethal outcome of autoimmune rheumatic diseases such as systemic sclerosis (SSc).
- Myofibroblasts are scar-forming cells that are responsible for the excessive synthesis, deposition and remodelling of extracellular matrix proteins in SSc.
- Persistent myofibroblast activity leads to progressive tissue fibrosis and distortion of the normal tissue architecture, resulting in organ failure and, ultimately, in death.
- The termination of myofibroblast activity is suppressed in fibrotic disease by biomechanical and biochemical cues, which assist myofibroblast escape from apoptosis, thereby halting their elimination.
- Evasion of apoptosis results in persistent myofibroblast activation and/or the differentiation of myofibroblasts into a pro-fibrotic or pro-inflammatory senescent phenotype, thereby preventing fibrosis resolution.
- Targeting myofibroblast apoptosis and reprogramming these cells to become scar-resolving cells are emerging as novel therapeutic strategies to reverse established fibrosis.

Stress fibres

Contractile bundles composed of actomyosin filaments.

t-Distributed stochastic neighbour embedding

A technique for visualizing high-dimensional datasets, often displayed in the form of clusters.

Membrane blebbing

Protrusions of the cell membrane that occur during apoptosis.

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Pyknosis

Condensation of the nuclear chromatin during apoptosis.

Apoptosome

A multi-protein complex that mediates the initiation of apoptosis.

Granulation tissue

New connective tissue that forms during wound healing.

Molecular rheostat

A system that maintains and controls critical biological processes such as cell death and survival.

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CArG boxes

Repeating [$CC(A/T)_6GG$] DNA sequences present within gene promoters.

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Secretome

The collection of molecules secreted by cells into the extracellular space.

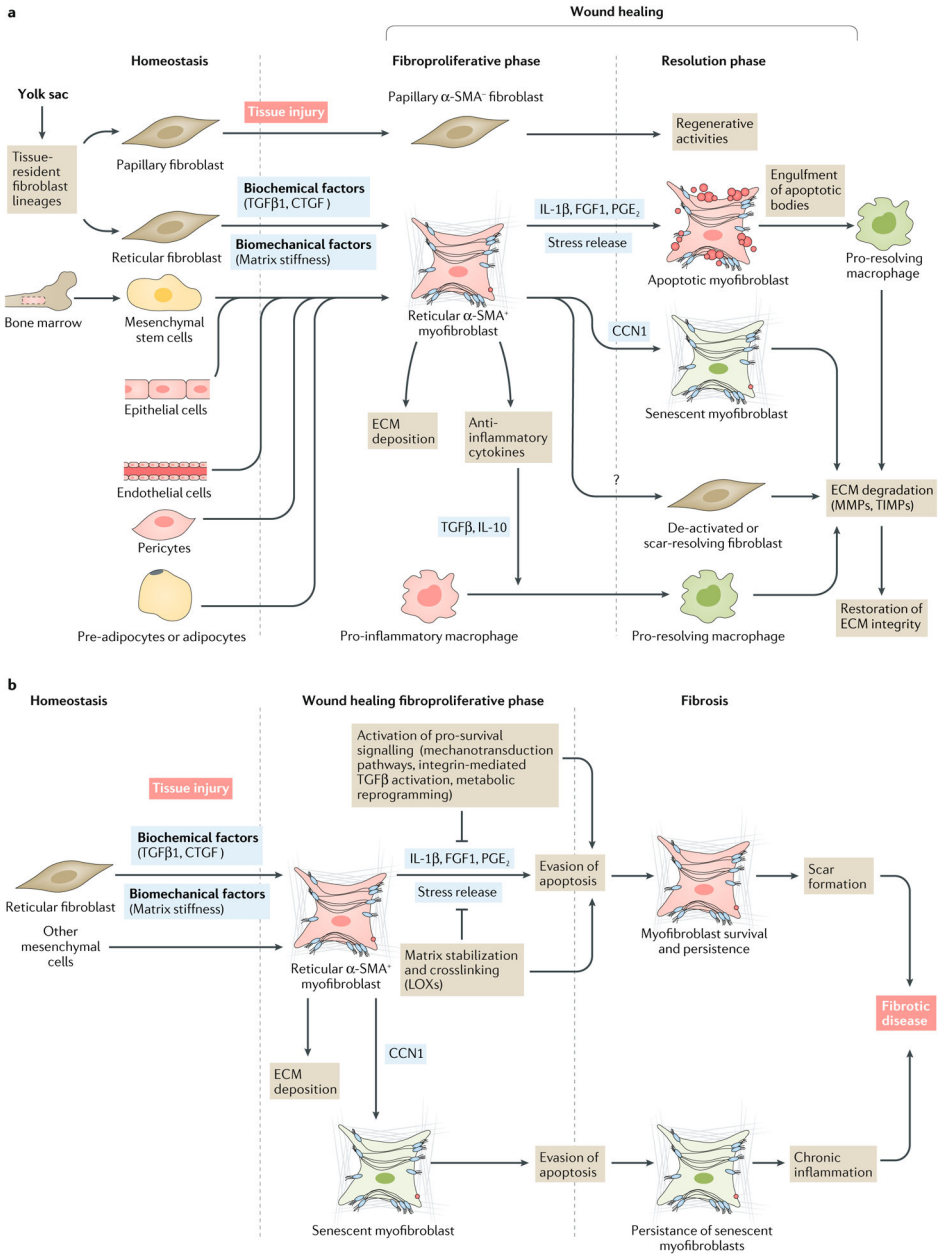

Fig. 1 |. Origin, functions and fate of myofibroblasts during tissue repair and fibrosis.

a | Activated myofibroblasts are important orchestrators of wound healing. During tissue injury, fibroblasts can differentiate into myofibroblasts. In the skin, two dermal fibroblast populations (reticular and papillary), which are derived from a common embryonic origin, have distinct functions during tissue repair. Whereas papillary fibroblasts do not express α-smooth muscle actin (α-SMA) and are involved in the regeneration of skin structures such as hair follicles, reticular fibroblasts differentiate into collagen-producing α-SMA$^+$ myofibroblasts by the action of biomechanical (extracellular matrix (ECM) stiffness) and biochemical factors such as transforming growth factor-β1 (TGFβ1) and connective tissue growth factor (CTGF). In specific conditions and experimental models, myofibroblasts can also arise from epithelial and endothelial cells via epithelial-to-mesenchymal transition or

endothelial-to-mesenchymal transition, respectively, and from mesenchymal stem cells, pericytes and pre-adipocytes or adipocytes. Myofibroblasts are characterized by increased synthesis of ECM proteins and by the expression of α-SMA, which confers a contractile phenotype that promotes wound closure. Activated myofibroblasts also produce anti-inflammatory cytokines such as TGFβ1 and IL-10, thereby shifting macrophages towards an ECM-degrading phenotype. During the resolution of wound healing, myofibroblasts can follow several different cell fates. Myofibroblasts can die via apoptosis, a process induced by ECM softening (stress release) or soluble pro-apoptotic factors such as IL-1β, fibroblast growth factor 1 (FGF1) and prostaglandin E2 (PGE_2). Alternatively, they can deactivate, become scar-resolving fibroblasts or become senescent via the action of CCN family member 1 (CCN1); these cell types all participate in ECM degradation in partnership with pro-resolving macrophages. **b** | Myofibroblasts and senescent myofibroblasts can also escape apoptosis and continue to remodel tissue beyond repair, which results in pathological scarring and the development of fibrotic disease. LOXs, lysyl oxidases; MMPs, matrix metalloproteinases; TIMPs, tissue inhibitors of metalloproteinases.

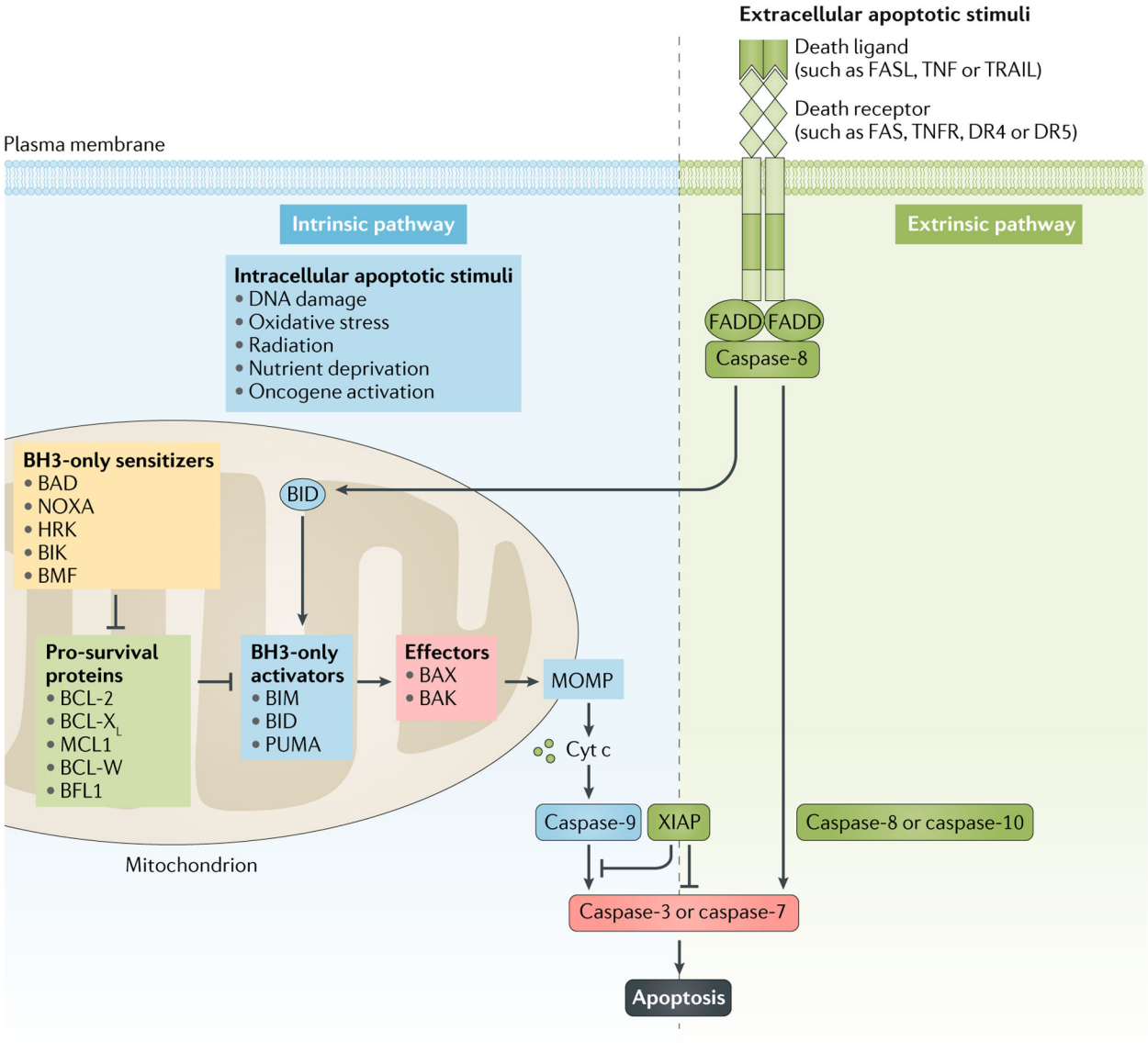

Fig. 2 |. Intrinsic and extrinsic apoptosis pathways.

Apoptosis is controlled by two distinct yet connected pathways. The intrinsic pathway is triggered by intracellular death stimuli, such as DNA damage, radiation, nutrient deprivation, oxidative stress or oncogene activation, which induce apoptosis by promoting mitochondrial outer membrane permeabilization (MOMP) and cytochrome c (cyt c)-dependent activation of caspase-9, which then activates caspase-3 and caspase-7. This pathway of apoptosis is tightly controlled by the BCL-2 family of proteins, which are classified on the basis of their structural homology and function into sensitizers, pro-survival proteins, activators and effectors. MOMP is controlled by homo-oligomerization of the effector proteins BCL-associated X protein (BAX) and BCL-2 homologous antagonist/killer (BAK), which are activated by the activator proteins BCL-2-like protein 11 (BCL2L11; also known as BIM), p53-upregulated modulator of apoptosis (PUMA) and BH3-interacting domain death agonist (BID). Pro-survival proteins such as BCL-2, BCL-X_L, BCL-W,

induced myeloid leukaemia cell differentiation protein MCL1 (MCL1) and BCL-2-related protein A1 (BCL2A1; also known as BFL1) can bind and sequester both activators and effectors, thereby preventing their interaction and the induction of MOMP. Sensitizer proteins (such as BCL-2-associated death promoter (BAD), BCL-2 interaction killer (BIK), BCL-2 modifying factor (BMF), activator of apoptosis harakiri (HRK) and phorbol-12-myristate-13-acetate-induced protein 1 (PMAIP1; also known as NOXA) are a distinct group of proteins that promote apoptosis by binding and blocking pro-survival proteins, thereby releasing formerly bound activator and effector proteins. The extrinsic pathway is initiated by extracellular ligands such as FAS ligand (FASL), TNF and TNF ligand superfamily member 10 (TNFSF10; also known as TRAIL), which bind to the cell-surface receptors FAS, TNF receptors (TNFRs) and death receptor 4 (DR4) and DR5, respectively. The signal is transmitted via FAS-associated death domain (FADD), which triggers the activation of caspase-8 and caspase-10, ultimately initiating apoptosis through cleavage and activation of pro-caspase-3 and pro-caspase-7. BH3, BCL-2 homology domain 3; XIAP, X-linked inhibitor of apoptosis protein.

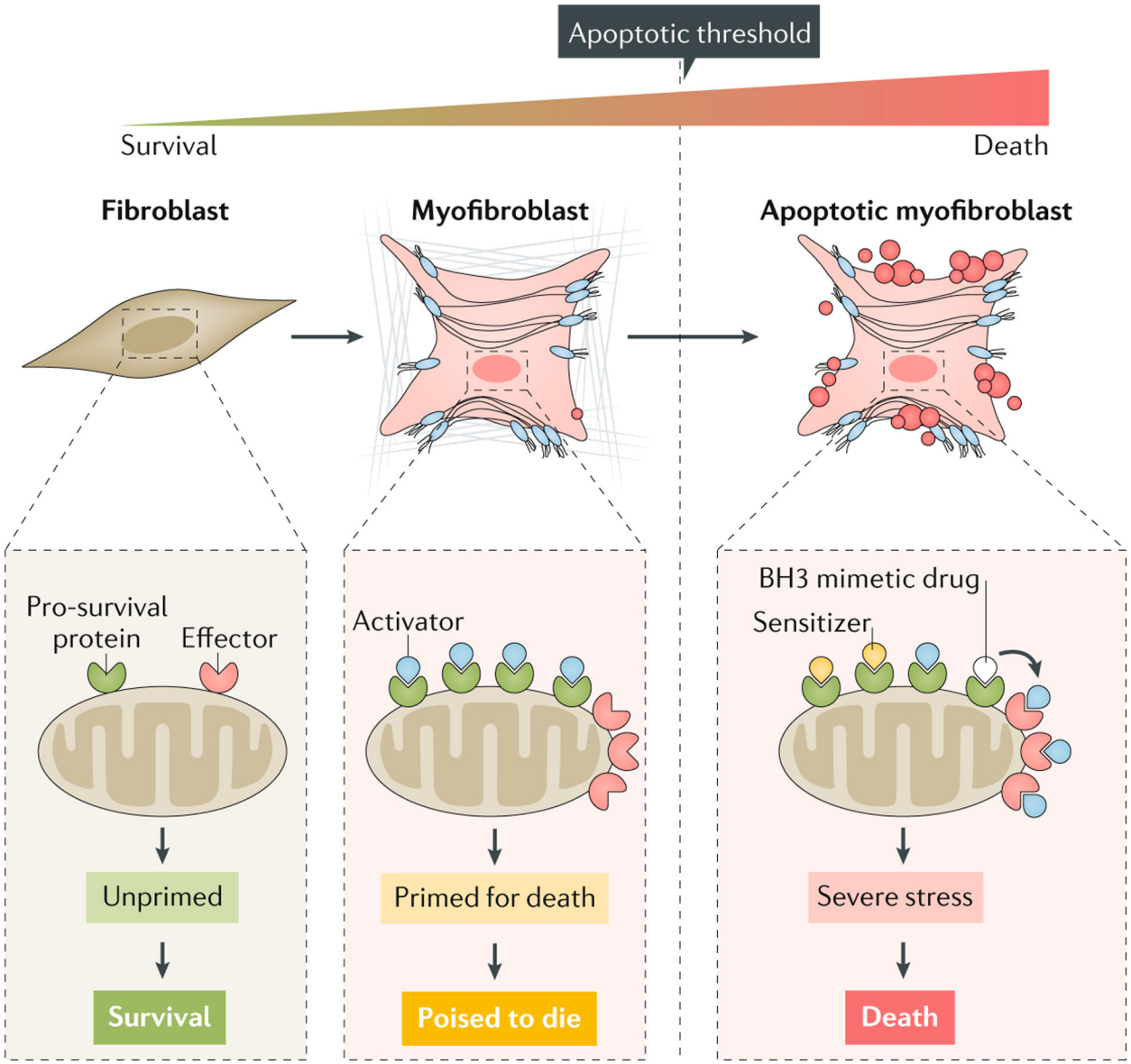

Fig. 3 |. Mitochondrial priming of myofibroblasts.

Mitochondrial priming refers to the proximity of mitochondria to the apoptosis threshold and is determined by the relative expression of pro-apoptotic (effectors, activators and sensitizers) and pro-survival members of the BCL-2 family of proteins. 'Unprimed' fibroblasts express low amounts of activators and/or effectors, leading to an apoptosis-resistant phenotype. Conversely, high expression of sensitizers, activators and/or effectors results in high mitochondrial priming, ultimately triggering cytochrome-c-mediated mitochondrial outer membrane permeabilization and apoptosis. However, myofibroblasts can survive with high mitochondrial priming if a pro-survival mechanism is activated. In this cellular state, known as 'primed for death', cells are poised to die and become dependent on one or more pro-survival proteins to sequester pro-apoptotic proteins and ensure survival. BCL-2 homology domain 3 (BH3) mimetic drugs can trigger the intrinsic pathway of

apoptosis in primed-for-death myofibroblasts by binding to pro-survival proteins, resulting in the release of activator proteins, which can then bind to and activate effector proteins.

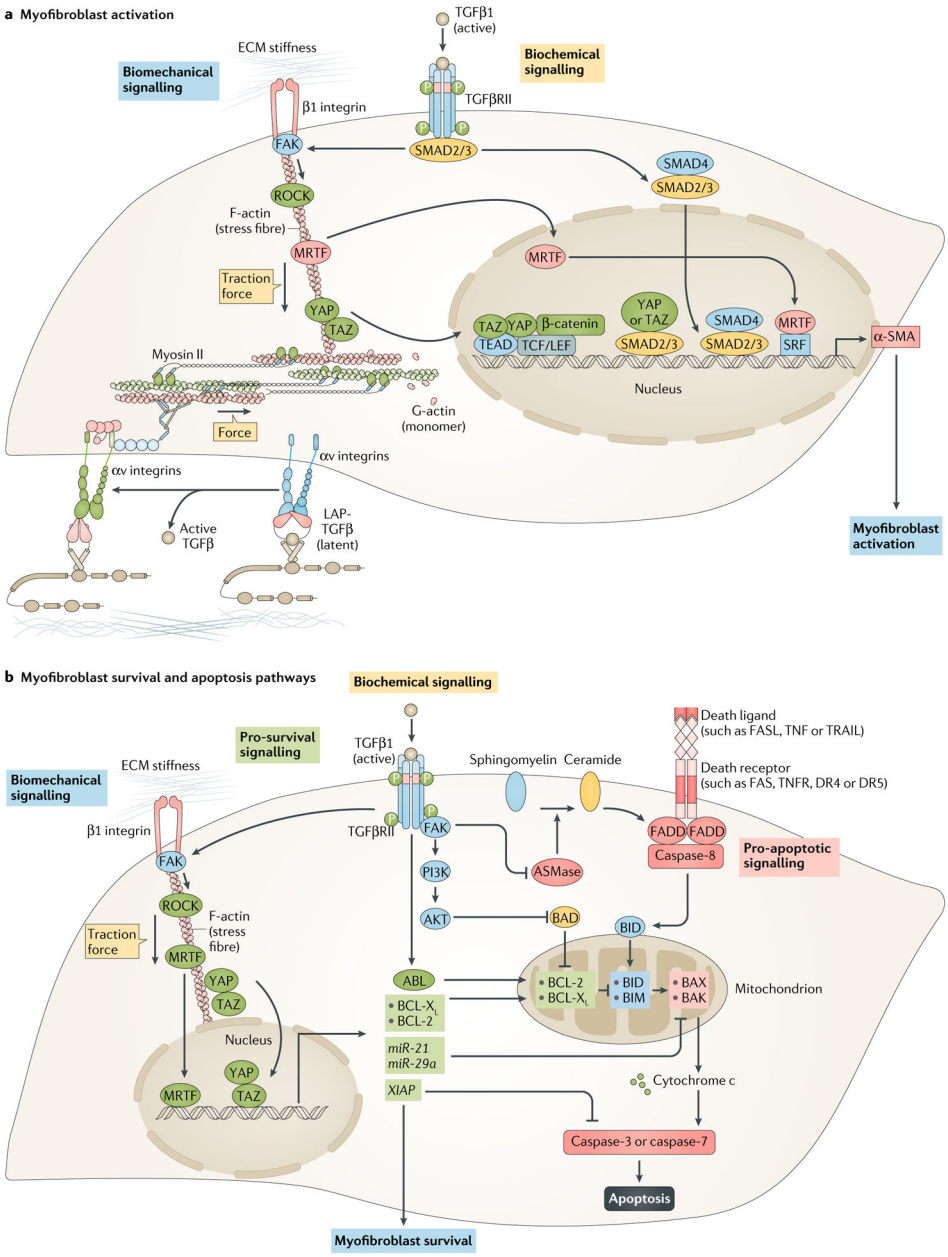

Fig. 4 |. Molecular control of myofibroblast activation and survival.

a | Myofibroblast activation is jointly promoted by biomechanical and biochemical cues. Biomechanical signalling induced by extracellular matrix (ECM) stiffness activates mechanotransduction pathways that directly control α-smooth muscle actin (α-SMA) transcription. These pathways involve β1 integrin, focal adhesion kinase (FAK) and RHO-associated protein kinase (ROCK), together with increasing traction forces in fibroblasts. Increased actomyosin activity causes the nuclear translocation of myocardin-related transcription factor (MRTF), as well as the transcriptional co-activators yes-associated protein (YAP) and transcriptional co-activator with PDZ-binding motif (TAZ), which regulate α-SMA expression by binding and activating other transcription factors, such as serum response factor (SRF), TEA domain family member (TEAD), T cell factor/lymphoid

enhancer-binding factor (TCF/LEF) and β-catenin. ECM stiffness and mechanical forces also regulate force-dependent activation of latent transforming growth factor-β1 (TGFβ1) by increasing resistance to traction forces generated by fibroblasts. In this mechanism, extracellular latent TGFβ1 (TGFβ1 with its latency-associated peptide (L AP)) is released from latent TGFβ1 binding protein stores when αv integrins respond to mechanical pulling forces. Once activated, TGFβ1 binds to TGFβ receptors and promotes canonical mothers against decapentaplegic homolog 3 (SMAD3) activation. Activated SMAD3 binds to SMAD4 and translocates to the nucleus, where it binds to SMAD-binding elements in the promoters of fibrogenic genes, such as *ACTA2* (encoding α-SMA). Together, myofibroblast activation is controlled by both the TGFβ–SMAD pathway, as well as biomechanical pathways such as integrin–FAK–ROCK–MRTF–YAP–TAZ signalling. **b** | Mitochondria in activated myofibroblasts contain large amounts of pro-apoptotic factors, which force these cells to activate pro-survival mechanisms to ensure survival. The intrinsic pathway of apoptosis is directly inhibited by TGFβ1 via activation of ABL signalling, which increases the amount of the pro-survival proteins BCL-2 and BCL-X_L. TGFβ1 also blocks the intrinsic pathway by inhibiting the pro-apoptotic protein BCL-2-associated death promoter (BAD) via the FAK–PI3K–AKT signalling pathway, and TGFβ1-mediated FAK signalling blocks the extrinsic pathway of apoptosis by inhibiting sphingomyelinase (ASMase), an enzyme that controls FAS-mediated apoptosis by generating ceramide from sphingomyelin. Biomechanical signalling similarly inhibits the intrinsic pathway via the biomechanically regulated TGFβ–FAK–YAP1–TAZ–BCL-X_L and TGFβ–ROCK–MRTF–BCL-2 pathways. Moreover, ECM stiffness induces expression of the microRNAs miR-21 and miR-29a, which promote the survival of myofibroblasts by increasing the expression of pro-survival BCL-2 proteins. Both ECM stiffness and TGFβ1 can also block apoptosis by increasing amounts of X-linked inhibitor of apoptosis protein (XIAP), a direct caspase inhibitor. AKT, pro-survival protein kinase B; BAK, BCL-2 homologous antagonist/killer; BAX, BCL-associated X protein; BID, BH3-interacting domain death agonist; BIM, BCL-2-interacting mediator of cell death (also known as BCL2L11); PI3K, phosphoinositide 3-kinase; TGFβRII, transforming growth factor-β receptor II.

Table 1 |

Characteristics of fibroblasts and myofibroblasts

Characteristic	Fibroblasts	Myofibroblasts	Refs
Distribution	Ubiquitous in connective tissues	Damaged tissues, fibrosis and cancer	299
Morphology	Large, flat and elongated (spindleshaped) cells that contain cortical actin microfilaments	Smooth muscle-like cells that contain contractile actin-myosin bundles and have specialized adhesion structures	15
Origin	Mesoderm	Tissue-resident fibroblasts, epithelial cells, endothelial cells, pericytes and mesenchymal stem cells	17
Functions	Maintain ECM homeostasis by secreting ECM proteins and matrix-degrading enzymes; proliferate and migrate into the wound bed to deposit granulation tissue during tissue repair	Produce large amounts of type I collagen during tissue repair; contract wound margins to facilitate re-epithelialization and restoration of tissue integrity; their persistence contributes to the pathogenesis of fibrosis and cancer	1,30,300
Molecular markers	Vimentin^{+}α-SMA^{-} cells that secrete ECM proteins and produce MMPs and TIMPs	Fibronectin ED-A^{+}α-SMA^{+} cells that synthesize type I collagen and produce matrix crosslinking enzymes	98
Proliferative capacity	High (during wound healing)	Low	301
Metabolic activity	Low	High (aerobic glycolysis)	168
Biomechanical activity	Low	High (hypercontractile phenotype)	302
Apoptosis sensitivity	Low	High (primed for death)	86

ECM, extracellular matrix; fibronectin ED-A, fibronectin extra domain A; MMP, matrix metalloproteinase; TIMP, tissue inhibitor of metalloproteinase; α-SMA, α-smooth muscle actin.

Table 2 |

Therapeutic strategies to target myofibroblasts

Therapy	Target	Effect on myofibroblasts	Mouse models used to show anti-fibrotic effect	Development stage	Refs
Drugs targeting biomechanical signalling					
CWHM 12	αV integrin	Inhibits TGFβ activation	CCL4-induced liver fibrosis; bleomycin-induced lung fibrosis	Preclinical	115
Abituzumab	αV integrin	Inhibits TGFβ activation	Not tested	Phase II	303
C8	αVβ1 integrin	Inhibits TGFβ activation	Bleomycin-induced lung fibrosis; CCL4-induced liver fibrosis	Preclinical	304
Cilengitide	αVβ3 and αVβ5 integrins	Inhibits mechanotransduction	Bile duct ligation-induced liver fibrosis	Preclinical	305
6.3G9 (STX-100)	αVβ6 integrin	Inhibits TGFβ activation	Bleomycin-induced lung fibrosis; irradiation-induced lung fibrosis; bile duct ligation-induced liver fibrosis; DDC-induced liver fibrosis; Alport mouse model *Col4A3*$^{-/-}$	Phase II	306–311
PF-562,271 (VS-6062)	FAK	Inhibits mechanotransduction	Bleomycin-induced lung fibrosis; CCL4-induced liver fibrosis; acute myocardial infarction	Preclinical	135,312
PF-573,228	FAK	Inhibits mechanotransduction	Bleomycin-induced lung fibrosis; hypertrophic skin scarring; acute myocardial infarction	Preclinical	267,269,313
TAE-226	FAK	Inhibits mechanotransduction	Bleomycin-induced lung fibrosis	Preclinical	265
VS-4718	FAK	Inhibits mechanotransduction	Pancreatic ductal adenocarcinoma	Phase II	266,314
Fasudil (ROCK1 and ROCK2)	ROCK	Inhibits mechanotransduction	Hypoxia-induced lung fibrosis; bleomycin-induced lung fibrosis; HClO-induced skin fibrosis; UUO-induced kidney fibrosis	Preclinical	315–319
Relaxin	ROCK	Inhibits mechanotransduction	Bleomycin-induced lung fibrosis	Phase III	271,320
KD025 (ROCK2)	ROCK	Inhibits mechanotransduction	Not tested	Phase II	321
CCG-1423	MRTF-Aand MRTF-B	Inhibits mechanotransduction	Peritoneal fibrosis model	Preclinical	322
CCG-203971	MRTF-Aand MRTF-B	Inhibits mechanotransduction	Bleomycin-induced lung fibrosis; bleomycin-induced skin fibrosis	Preclinical	262,323
Verteporfin	YAP and TAZ	Inhibits mechanotransduction	UUO-induced kidney fibrosis	Preclinical	324
Dimethyl fumarate	YAP and TAZ	Inhibits mechanotransduction	Bleomycin-induced skin fibrosis	Phase I	264,325
Ac-EEED peptide	α-SMA	Inhibits mechanotransduction	Full-thickness splinted skin wound healing model	Preclinical	256
Drugs targeting the intrinsic apoptosis pathway					
ABT-263 (navitoclax)	BCL-X_L, BCL-2 and BCL-W/	Activates intrinsic apoptosis pathway	Bleomycin-induced skin fibrosis; radiation-induced lung fibrosis	Preclinical	86,275
A-1331852	BCL-X_l	Activates intrinsic apoptosis pathway	*Mdr2*$^{-/-}$ rodent model of primary sclerosing cholangitis	Preclinical	158
Drugs targeting the extrinsic apoptosis pathway					

Therapy	Target	Effect on myofibroblasts	Mouse models used to show anti-fibrotic effect	Development stage	Refs
AT-406	XIAP	Activates extrinsic apoptosis pathway	Bleomycin-induced lung fibrosis	Preclinical	138
TLY012	TRAIL	Activates extrinsic apoptosis pathway	Bleomycin-induced skin fibrosis; CCL4-induced liver fibrosis	Preclinical	285,286
Drugs targeting senescence					
Dasatinib and quercetin dual therapy	Multiple kinases	Activates apoptotic pathways	Bleomycin-induced lung fibrosis	Phasel	186,326
ABT-263 and A-1331852	BCL-X_l	Activates intrinsic apoptosis pathway	Radiation-induced lung fibrosis; *Mdr2*$^{-/-}$ rodent model of primary sclerosing cholangitis	Preclinical	158,275
GKT137831	NOX4	Activates apoptotic pathways	Bleomycin-induced lung fibrosis	Phase II	185,327

α-SMA, α-smooth muscle actin; CCL4, CC-chemokine ligand 4; DDC, 3,5-diethoxycarbonyl-1,4-dihydrocollidine; FAK, focal adhesion kinase; HClO, hypochlorous acid; MRTF, myocardin-related transcription factor; NOX4, NADPH oxidase 4; ROCK, RHO-associated protein kinase; TAZ, transcriptional co-activator with PDZ-binding motif; TGFβ, transforming growth factor-β; TRAIL, TNF-related apoptosis-inducing ligand (also known as TNFSF10); UUO, unilateral ureteral obstruction; XIAP, X-linked IAP; YAP, yes-associated protein.