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Review Article

Regulation of cellular senescence by extracellular matrix during chronic fibrotic diseases

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The extracellular matrix (ECM) is a complex network of macromolecules surrounding cells providing structural support and stability to tissues. The understanding of the ECM and the diverse roles it plays in development, homoeostasis and injury have greatly advanced in the last three decades. The ECM is crucial for maintaining tissue homoeostasis but also many pathological conditions arise from aberrant matrix remodelling during ageing. Ageing is characterised as functional decline of tissue over time ultimately leading to tissue dysfunction, and is a risk factor in many diseases including cardiovascular disease, diabetes, cancer, dementia, glaucoma, chronic obstructive pulmonary disease (COPD) and fibrosis. ECM changes are recognised as a major driver of aberrant cell responses. Mesenchymal cells in aged tissue show signs of growth arrest and resistance to apoptosis, which are indicative of cellular senescence. It was recently postulated that cellular senescence contributes to the pathogenesis of chronic fibrotic diseases in the heart, kidney, liver and lung. Senescent cells negatively impact tissue regeneration while creating a pro-inflammatory environment as part of the senescence-associated secretory phenotype (SASP) favouring disease progression. In this review, we explore and summarise the current knowledge around how aberrant ECM potentially influences the senescent phenotype in chronic fibrotic diseases. Lastly, we will explore the possibility for interventions in the ECM–senescence regulatory pathways for therapeutic potential in chronic fibrotic diseases.

Introduction

The extracellular matrix (ECM) is a complex network of macromolecules surrounding cells, traditionally recognised for providing structural support and tissue stability [1]. Our understanding of the diverse roles of the ECM has greatly advanced in the last three decades, particularly in the provision of essential biochemical and biomechanical cues required to direct tissue morphogenesis during development, homoeostasis and injury. Mesenchymal cell types, most notably the resident fibroblasts, have the role of maintaining the ECM within tissues throughout an organism's lifespan. Therefore, mesenchymal cells need to respond to localised cues to deposit, maintain and remodel the appropriate ECM to meet the functional needs of the tissue. To facilitate the ECM in its role as a biologically active structure, cells interact with individual matrix proteins through surface receptors such as cell surface proteoglycans (PGs), discoidin domain receptors and specific integrins resulting in outside-in signalling which dictates cell function, fate and phenotype [2,3]. Cells are able to communicate through the ECM by generating mechanical forces or by altering mechanical properties of the ECM such as the stiffness [4].

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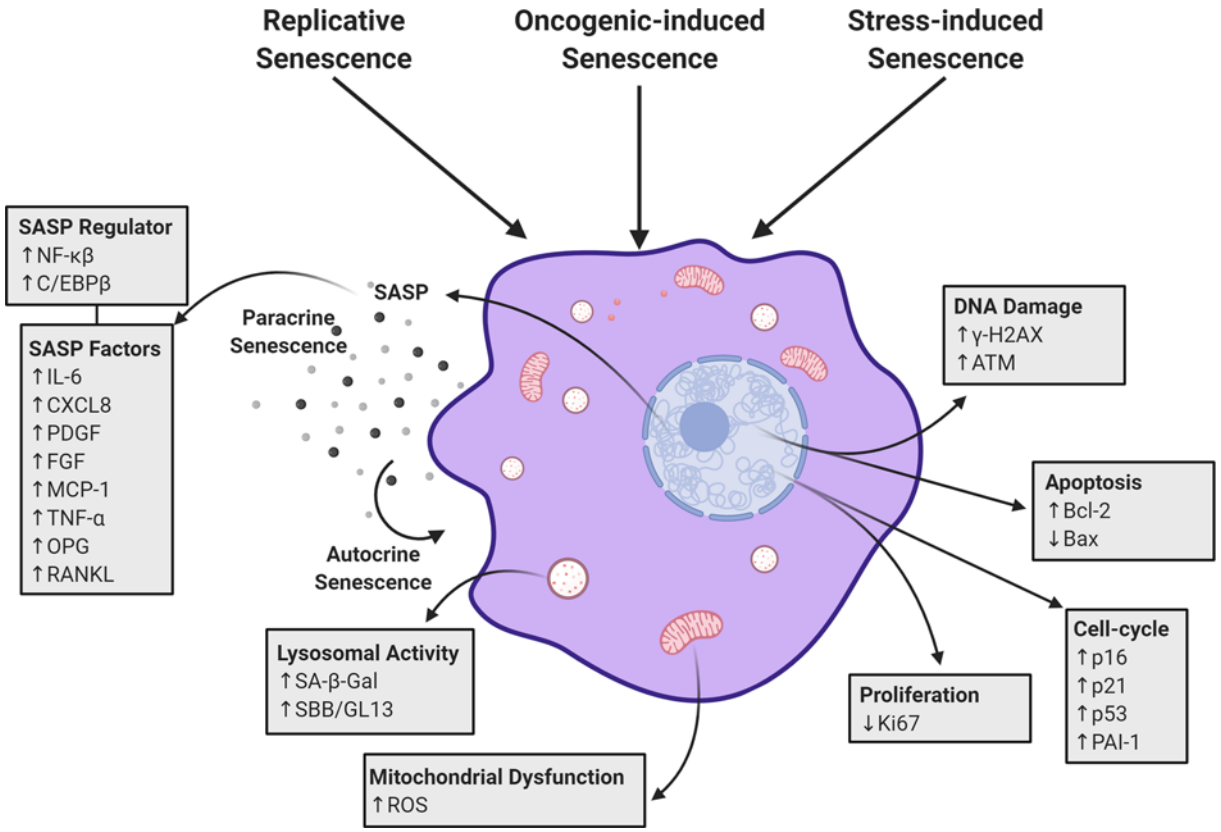

Figure 1. Characteristics of senescent cells

Cells that undergo senescence display several changed features that can be used for identification. An increased secretory phenotype can be detected by measuring growth factors and cytokines. These factors contribute to the spreading of senescence to neighbouring cells or reinforce senescence via an autocrine process. Increased lysosomal activity can be detected using SA-β-Gal and SBB expression. Mitochondrial dysfunction is detectable by measuring ROS levels such as superoxide production. As cells undergo irreversible cell-cycle arrest, markers such as p16, p21 and p53 are increased while proliferation markers like Ki67 decrease. Resistance to apoptosis can be measured by changes in Bcl-2 and Bax expression. In addition, the DDR can be measured by immunofluorescence microscopy for increased formation of phosphorylated ATM and γ-H2AX in the nucleus. Abbreviation: SA-β-Gal, senescence-associated β-galactosidase.

The ECM is crucial for maintaining normal tissue homoeostasis and many pathological conditions arise from dysregulated ECM remodelling as a result of ageing or as an attempt to preserve or restore organ function. Remodelling of the ECM during fibrotic disease, which changes the physical state of the tissue, may contribute to altered cellular responses to mechanical forces such as pressure, stretch and shear force [5,6]. Ageing is characterised by functional decline of tissue over time, leading to progressive deterioration that eventually leads to tissue dysfunction. Many diseases have age as a risk factor such as cardiovascular disease, diabetes, cancer, dementia, glaucoma, chronic obstructive pulmonary disease (COPD) and idiopathic pulmonary fibrosis (IPF) [7–14]. The changes that occur during ageing and/or disease influence the composition, topography and biomechanics of the ECM, thereby contributing to abnormal cellular activation and dysregulated behaviour. For example, aberrant ECM deposition and increased stiffness are observed in fibrotic diseases and cancer, while excessive ECM degradation is linked to osteoarthritis (OA) and COPD [15–17]. The ECM changes in fibrotic lung disease are recognised as a major driver of aberrant cellular responses [18]. In addition, a population of resident fibroblasts in aged tissue show growth arrest and resistance to apoptotic cues, which are indicative of cellular senescence, a hallmark of ageing [19,20]. Cellular senescence is characterised as irreversible cell-cycle arrest in response to various sources of stress such as DNA damage or reactive oxygen species (ROS). During physiological homoeostasis, cellular senescence benefits embryogenesis, tissue repair and defence against tumorigenesis. In contrast, it was recently postulated that senescence contributes to the pathogenesis

of chronic fibrotic diseases in heart, kidney, liver and lung [21–25]. Here, senescent cells negatively impact tissue regeneration while creating a pro-inflammatory environment, as part of a senescence-associated secretory phenotype (SASP) that favours disease progression. Whether the ECM changes in ageing and fibrotic disease are also drivers of the senescent phenotype associated with these conditions is as yet unexplored.

During remodelling and injury, breakdown products of the ECM are released which often act as damage-associated molecular patterns (DAMPs) that activate pattern recognition receptors (PRRs) on cells of the innate immune system. Interestingly, several of these ECM DAMPs have been found to be significantly increased in multiple chronic fibrotic diseases [26–28]. Activation of PRRs by DAMPs induces nuclear factor κB (NF-κB)-mediated pro-inflammatory cytokine release, with a similar cytokine release profile to that associated with the SASP. Moreover, NF-κB is known as a master regulator of the SASP in fibroblasts, which suggests a role of DAMPs in regulating cellular senescence.

In this review, we explore and summarise the current knowledge around how aberrant ECM potentially contributes to the senescent phenotype in chronic fibrotic diseases. A broad overview will be provided about the roles of ECM and senescence during physiological homoeostasis and in chronic fibrotic diseases. An overview of ECM changes in senescence during ageing has previously been described in detail elsewhere [29], and hence will not be extensively covered herein. In this review, the possible mechanisms through which the ECM drives pathological senescence will be outlined, including interactions between cells and the ECM, disruption to mechano-transduction upon ECM remodelling, alterations in integrin–adhesion complexes and the release of ECM DAMPs. Lastly, we will explore the possibility for interventions in the ECM-senescence regulatory pathways for therapeutic potential in chronic fibrotic diseases. While this review will portray several diseases, IPF will be focused upon as an exemplar for chronic fibrotic disease.

Cellular senescence

Cellular senescence is a hallmark of ageing and involves marked changes in cell morphology, phenotype, metabolism and fate [30]. While irreversible cell cycle arrest is the defining feature of senescent cells, they are also resistant to apoptosis and are highly secretory; driving a range of different functions through the SASP [31]. Resistance to apoptosis is mediated by up-regulation of B-cell lymphoma 2 (Bcl-2) and epigenetic suppression of Bcl-2-associated X protein (BAX) [32]. The paracrine activities of senescent cells are crucial for many facets of normal development and tissue function as well as pathologies including fibrosis and tumorigenesis. Senescence is primarily an outcome of genomic damage comprising DNA double-stranded breaks (DSBs). As part of a subsequent DNA damage response (DDR), ataxia-telangiectasia mutated kinase (ATM) phosphorylates p53, leading to the induction of p53-p21 and/or p16-pRB pathways that mediate cell cycle arrest through cyclin-dependent kinase (CDK) inhibition [33]. The transcription factor NF-κB is a pivotal mediator of the SASP and activated by ATM-dependent phosphorylation of NF-κB essential modifier (NEMO) [34].

Types of senescence

Replicative senescence (RS) is a consequence of mitotic ageing and arises from the shortening of telomeres; the repetitive DNA sequences at the end of chromosomes that protect DNA from damage [35]. In a process in somatic cells that was originally described by Hayflick and Moorhead in diploid fibroblasts *in vitro*, telomeres shorten with each successive cell division until DNA damage and senescence eventuate [36,37]. Stress-induced premature senescence (SIPS) is another form of senescence in which insults such as oxidative stress, radiation, mutagens and cytokines elicit directly or indirectly genomic damage and the subsequent DDR [38–41]. While many of these stressors are of external sources, ROS derived from dysfunctional mitochondria can induce and/or reinforce the DDR in SIPS in a cell autonomous manner [42,43]. ROS and SASP mediators released by senescent cells can also evoke 'secondary' senescence in surrounding naïve (non-senescent) cells; a paracrine process termed as 'senescence-induced senescence' [44,45]. Oncogenic-induced senescence (OIS), a form of SIPS comprises a complex senescence program involving the induction of oncogenes such as the *Ras*/*Raf* family [46]. OIS restricts the excessive mitotic signalling of DNA-damaged cells that may otherwise undergo malignant transformation to provide a tumour-suppressing function [47]. Figure 1 summarises the characteristics of cellular senescence. RS is also an anti-tumour mechanism that prevents cell immortalisation. The anti-cancer properties of senescence can be viewed as a 'trade off' with its role in age-related stem cell loss/tissue degeneration [47].

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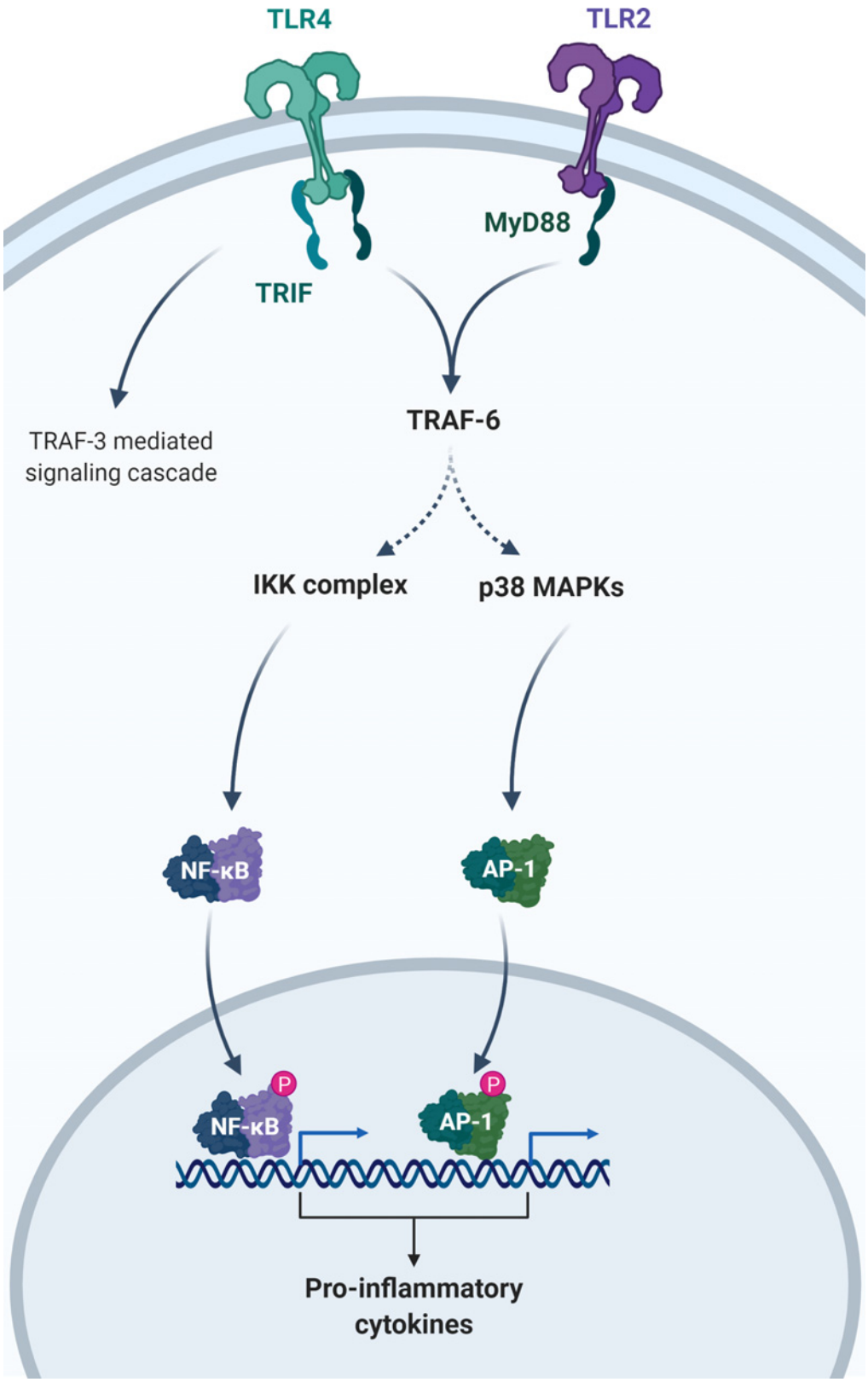

Figure 2. ECM DAMP receptor activation leading to increased pro-inflammatory cytokine release

ECM DAMPs such as soluble decorin, tenascin-c and fibrinogen can activate an inflammatory response by binding to the TLR2 and TLR4 receptors. TLR activation leads to nuclear translocation of NF-κB and subsequent activation of pro-inflammatory cytokines that comprise the SASP such as IL-1β, IL-6, CXCL8, interferon (IFN)-γ and TNF-α.

Cellular senescence in normal homoeostasis

Cellular senescence is an essential, yet transitory feature of normal tissue homoeostasis. In this role, the senescent cell has a relatively short half-life, with its removal by immune cells occurring once its task is completed. This limits overproduction of ECM and contributes to active remodelling to maintain homoeostasis. In embryogenesis, senescence in the apical ectodermal ridge provides an instructive role for tissue development and patterning by the prevention of cell proliferation. This effect is mediated via the release of secreted factors that regulate processes such as neovascularisation and cell migration [48]. Under normal conditions, SASP functionality in tissue homoeostasis changes with time, culminating in the provision of cues that direct senescent cell removal. For example, in skin wounds, senescent cells accumulate and promote wound healing and resolution firstly through secretion of platelet-derived growth factor (PDGF) AA (PDGF-AA) and then subsequently drive their own immune-mediated clearance; suggesting a temporal switch from orchestration of wound repair to inflammatory recruitment of immune cells [49]. Cytokines and other secreted factors from senescent cells are involved in the recruitment of immune cells such as neutrophils, macrophages, natural killer cells and $CD4^+$ T cells that clear senescent cells. The expression of immunogenic stimulatory ligands by senescent cells that control their removal is regulated by autocrine/paracrine activities of the SASP.

Cellular senescence in ageing and disease

The numbers of senescent cells in tissue increases with age, contributing to age-related tissue degeneration and disease. Deficiencies in immune surveillance and clearance facilitated by an immune system of deteriorating function and capacity (i.e., a phenomenon termed as 'immuno-senescence') contribute to this accumulation [50]. Senescent cells from aged tissues also express molecules such as human leukocyte antigen E (HLA-E) that inhibit the function of infiltrating immune cells; providing another means by which senescent cells can evade immune-driven apoptosis [51]. Ageing tissues exhibit chronic senescence mediated by SASP that can be increasingly harmful with time, leading to a loss of regenerative potential and subsequent disease [52]. Evidence for a causal role of senescence in ageing and disease comes from murine studies that show that the ablation of senescent cells *in vivo* prolongs lifespan, has a restorative effect on aged tissues and induces protection against insults associated with disease [53–55]. It was shown that glycosaminoglycans (GAGs) content significantly increases with clearance of senescent cells in the knee joint of mice [55]. Accelerated senescence in specific cell types is a feature of certain respiratory and vascular diseases, as well as in OA and in cancer. For example, senescent epithelial cells and fibroblasts accumulate in the lungs of IPF and COPD patients [56,57]. Senescent epithelial cells are proposed to be the crux of the aberrant wound healing response observed in these lung diseases; possibly by hindering re-epithelialisation and/or by the activation of resident fibroblasts [58–62]. A failure to eliminate senescent fibroblasts by apoptosis or immune cell clearance may also impede wound resolution and contribute to disease progression in fibrotic diseases [58,63,64]. In OA, senescent chondrocytes with a pro-inflammatory SASP accumulate and contribute to the damage of articular cartilage tissue and joints [55]. Senescent endothelial cells at atherosclerotic lesions are a prominent feature of vascular disease, potentially contributing to aberrations in barrier function, platelet aggregation and fibrinolysis. While senescence limits cancer initiation in a cell autonomous manner, it can also promote cancer in ageing tissues non-autonomously via the SASP by altering the microenvironment to allow for cancer progression [7,65]. The remodelling of ECM plays a critical role in facilitating tumour cell migration and spread via metastasis to other tissues. A causal role of senescent cells in cancer is supported by studies that show senescent fibroblasts stimulate human tumour cell proliferation when co-injected into immunocompromised mice [65,66]. The deleterious contribution of senescence in age-related pathology is an example of antagonistic pleiotropy; a process that has evolved to be beneficial in youth and increases reproductive fitness (i.e., by the prevention of malignancies); but is detrimental in older, post-reproductive age [52].

The ECM in tissue morphogenesis and homoeostasis

The composition of ECM comprises well over 300 proteins including fibrous proteins (collagens and elastins), PGs, GAGs and glycoproteins such as fibronectin [67]. Based on the location and function, ECM can be separated into two main types: interstitial connective tissue matrix and basement membrane. Interstitial ECM is mainly composed of collagen I, elastin and fibronectin to give support for surrounding cells, and to provide structural scaffolding for tissue. Interstitial ECM is deposited, maintained and remodelled by mesenchymal cells. The basement membrane is a specialised form of compact ECM that separates the epithelium or endothelium from the surrounding stroma. The basement membrane consists mainly of collagen IV, laminins, heparin sulphate PGs (HSPs) and proteins such as entactin and nidogen that are synthesised by epithelial cells and endothelial cells [1].

In addition to giving support to cells, the ECM provides important mechanical and biomechanical cues that direct tissue morphogenesis during development, homoeostasis and injury. More evidence is accumulating that ECM plays

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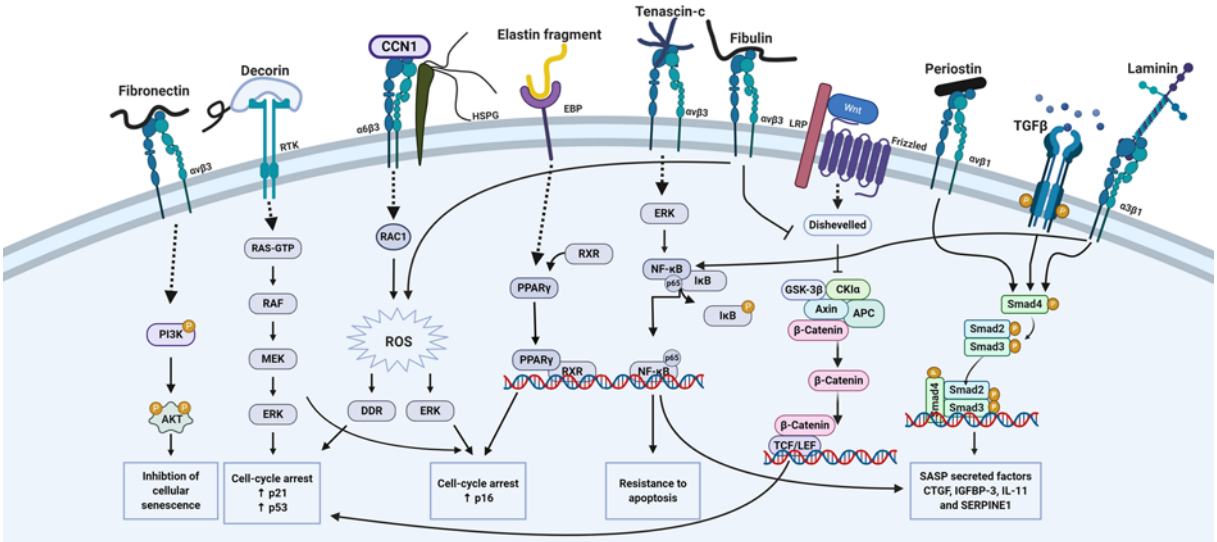

Figure 3. ECM–cell interactions and their downstream targets potentially contribute to cellular senescence

The diagram highlights the different pathways that ECM proteins activate and the downstream targets that potentially contribute to cellular senescence in fibrosis. Fibronectin binds to integrin $\alpha v\beta 3$ and activates the PI3k/Akt pathway, resulting in the inhibition of cellular senescence. Decorin binds to receptor tyrosine kinase and activates the RAS/RAF pathway, which eventually leads to the up-regulation of the cell-cycle inhibitors p16, p21 and phospo-p53. CCN1 binds to the $\alpha 6\beta 3$ integrin which colocalises with HSPG to activate the downstream RAC1 pathway, resulting in increases in ROS production. Subsequently, increased levels of ROS leads to a DNA damage repair response that induces cell-cycle arrest via increases in p16, p21 and phospo-p53. Elastin fragments binds to EBP to interact with PPARγ, which lead to cell-cycle arrest via changes in the expression of p16. Tenascin-c binds to $\alpha v\beta 3$ and induce the activation and nuclear translocation of NF-κB which leads to increases in apoptosis resistance as well as the up-regulation of growth factor and cytokine expression. Fibulin-1 may increase ROS production leading to cell-cycle arrest while limiting the Wnt/β-catenin pathway which protects against senescence. Periostin can directly activate the TGF-β pathway which leads to increase production of profibrotic factors. Laminin can directly activate the TGF-β pathway leading to profibrotic factor production and NF-κB activation which reinforce growth factor production and resistance to apoptosis.

an active role in pathological conditions such as cancer, OA and fibrosis across multiple organs which emphasises the importance of understanding the developmental origins of ECM during tissue morphogenesis, homoeostasis and remodelling [17,68–70]. During development, many organs such as lung, kidney, nervous system and several internal glands undergo several changes such as lengthening, narrowing, bending, folding and branching which is guided by the ECM by a process called branching morphogenesis [71]. Formation of epithelial clefts and buds involves invasion of surrounding embryonic ECM, which is subjected to continuous change and distribution over time. Many different factors have been identified that are involved in branching, such as bone morphogenetic factors (BMPs), sonic hedgehog (Shh), fibroblast growth factors (FGFs), wingless and int-1 (Wnt) and retinoic acid [72]. Not only are these factors involved in development, they also play an important role during injury, remodelling and disease in adult tissue.

Mechanical forces play an important role in tissue morphogenesis and an essential role in generating cytoskeletal prestress contributing to cell, tissue and organ shape stability [73]. Cells are able to interact and respond to changes in the local microenvironment by contracting and pulling on the ECM or neighbouring cells. The contractile forces generate signals that propagate rapidly within the cell showing an advantage in activating signalling pathways compared with chemical stimuli [74]. Contractile forces within a cell are generated by the interaction of activated non-muscle myosin II and actin creating the actomyosin machinery regulating cellular shape and thus cellular function. Cellular contractility is controlled by the Rho-associated coiled-coil containing kinase (ROCK). ROCK consists of two subtypes, ROCK 1 and 2, which both have a critical function in regulating the actin cytoskeleton assembly [75]. ROCK may directly phosphorylate myosin light chain 2 (MLC2) or indirectly regulates MLC by phosphorylating the myosin-binding subunit of myosin phosphate (MYPT1) which is necessary for its interaction with actin [76]. In the embryonic lung of mice, inhibition of ROCK reduced branching and resulted in loss of basement membrane thickness and cell proliferation. Interestingly, ROCK inhibition in ureteric bud formation reported promotion of branching

[77]. Another study reported that the lack of both ROCK1 and 2 protein using conditional knockout mice lead to a flattened and bigger morphology and loss of proliferation suggesting the potential influence on senescence [76]. It was shown that ROCK inhibition *in vitro* induced cellular proliferation and alleviation of senescence-associated cell-cycle arrest [78]. These studies suggest the cell-type specific role of ROCK in the cellular response to the environment, especially during pathological conditions in fibrosis.

ECM homoeostasis is a complicated balance of synthesis, modification and degradation. The importance of this intricate balance has been demonstrated in various diseases that originate from alterations in ECM [79]. Specific proteins which constitute the ECM are well conserved across species but show dramatic changes between individual tissue and organs in the same species. This suggests that the function of cells, tissues and organs is directly related to the mechanical and biomechanical cues of the surrounding ECM [80]. Several studies evaluating cardiac, renal and lung fibrotic disease have demonstrated that ageing, compositional and structural changes to the ECM can greatly influence cell phenotype, and function contributing to disease progression [25,68,81]. The intricate balance is maintained by cells residing in the ECM that produce various key enzymes such as metalloproteinases (MMPs) and its inhibitor endogenous tissue inhibitor of metalloproteinases (TIMPs), a disintegrin and metalloproteinase (ADAM), and ADAMs with thrombospondin motifs (ADAMTS) [67,82]. While the basement membrane is maintained by epithelial or endothelial cells, fibroblasts can contribute to basal lamina by producing nidogen while the interstitial matrix in the lung, kidney or heart is maintained by resident fibroblasts and hepatic stellate cells in the liver [83,84]. Activity of proteolytic enzymes is generally low during homoeostasis but increases during specific developmental events, injury and disease. The expression and activity of proteolytic enzymes is tightly regulated and is cell type- and tissue-specific [82]. During development, when ECM remodelling is necessary for tissue growth and alignment, there is a greater proteolytic profile compared with adult tissue which shows a higher anti-proteolytic profile. In diseases such as cancer, fibrosis and cardiovascular disease the intricate balance of homoeostasis is lost leading to an altered ECM and studies have shown that this is associated somewhat paradoxically with increased MMP activity. In cardiac remodelling, high levels of MMP1 have been linked to decreased collagen expression which contributes to loss of contractility and ultimately cardiomyopathy [85,86]. In IPF, increased MMP expression and activity is an important feature of the disease which has been identified as part of the SASP in fibrosis [25].

The ECM–cell interactions that potentially drive cellular senescence

Fibrotic diseases are characterised by deposition of excessive ECM after injury resulting in organ dysfunction. While little is known about the exact mechanisms that result in excessive ECM deposition and accumulation in fibrotic disease, there is increasing evidence that cellular senescence is implicated in fibrosis [22,23,69]. One major under-recognised element in fibrosis is the contribution of the ECM itself. It is known that the altered composition and increased cross-linking leads to an altered topography and stiffness. These changes alter cellular behaviour and might potentially drive cells to become senescent contributing to an environment favouring disease progression. Increased ECM stiffness is a feature of fibrosis and is thought to result from the quantity of ECM deposition and the degree of its cross-linking. The mechanical properties of areas of wounded tissue significantly change in rat liver fibrosis. It was suggested that this was the result of increased cross-linking of the ECM fibres, rather than increased ECM deposition after the initial insult [87]. These results suggest that cross-linking of ECM has a greater impact on stiffness and thus altered mechanical properties than the quantity of deposited ECM alone. In normal homoeostasis, cross-linking of collagen I and III provides stability which leads to the generation of strong fibrils providing tensile strength and elastic properties. Cross-linking of ECM can happen spontaneously by glycation which is a slow process or by further processing via post-translational modifications by enzymes such as lysyl oxidases (LOs) and transglutaminases (TGs) which will be described in more detail in the next section and its potential regulation of cellular senescence.

Cross-linking of ECM

The LO family of enzymes consist of lysyl oxidase (LOX) and four additional LOX-like proteins (LOXL1–4) which are copper-dependent amine oxidases responsible for covalent cross-linking of collagen and elastin. The process of ECM cross-linking is tightly regulated in homoeostasis with each oxidase being differently expressed across organs. LOX, LOXL-1 and LOXL-2 are expressed in nearly all tissues while LOXL-3 is not expressed in cardiac and skin tissue. LOXL-4 is only expressed in lung, kidney, pancreas and adipose tissue [88]. LOX family proteins are involved in several biological processes including tumour suppression, cell motility, cell transduction and cell adhesion. Dysregulation of LOX activity has been linked to pathological conditions such as cancer and fibrosis in lungs and liver [89–93]. TG

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is an enzyme responsible for cross-linking proteins such as collagen and fibronectin between glutamine and lysine residues or a primary amino group in a calcium-dependent manner. There are eight isoforms of TG, factor XIIIa and TG isoform 1–7 which are expressed throughout the whole body. The post-translational modification mediated by TG is essential for several processes including signal transduction, cell adhesion, skin barrier formation and the ECM assembly. It has been demonstrated that TGs are overexpressed in lungs of IPF patients and inhibition of TGs results in reduced inflammation and pulmonary fibrosis in bleomycin-treated mice [94]. In a recent study it was shown that TG isoform 1 was increased in both epithelium and interstitial space while TG isoform 2 was only increased in the extracellular space in the fibrotic kidney [95]. Inhibition of TGs reduced and preserved function in chronic kidney disease demonstrating the importance of cross-linking in pathological conditions [96].

Increased ECM stiffness

In fibrotic disease, the increased cross-linking has not only been associated with increased stiffness but also in higher resistance to proteolytic degradation as the conformational changes lead to less accessible epitopes for MMPs [97,98]. The stiffness of the ECM directly influences the behaviour and function of cells including increased fibroblast proliferation, migration and contraction [17]. Furthermore, a stiffer ECM leads to an increase in latent transforming growth factor-β (TGF-β) activation which reinforces fibrosis and it has been linked to cellular senescence [99–101]. TGF-β consists of three different isoforms (TGF-β1–3) which are produced in a precursor form bound to the latency associated peptide which are attached to the latent TGF-β binding protein (LTBP). This keeps TGF-β in an inactive form until it is activated/released from the matrix by proteases such as MMP2, MMP9 and thrombospondin. Non-protease release of the active form is facilitated by fibroblasts pulling on the matrix in response to increased ECM stiffness. Upon activation, TGF-β binds the TGF-β receptor 2 (TGFR2) and subsequently recruits TGFR1 to active downstream signalling via activated mother against decapentaplegic proteins (Smads). A role for TGF-β in senescence has already been suggested by Frippiat et al. in 2001 while Acosta et al. demonstrated the importance of TGF-β in paracrine induced-senescence of fibroblasts [44,102]. More recently it was described that TGF-β contributes to the induction of senescence or the acceleration of transformation into senescence in various cell types [103,104]. The senescence promoting effect of TGF-β could be explained by down-regulation of human telomerase reverse transcriptase (hTERT) and proliferation factor c-Myc. Additionally, there is up-regulation of CDKs p15, p21, p27 and p53 and ROS production which are characterised as part of the senescent phenotype [101]. In a recent study, it was shown that signal transducer and activation of transcription 3 (STAT3) was a downstream target of TGF-β in a SMAD-independent pathway suggesting an important role of STAT3 in liver fibrosis [105]. Indeed, not only has STAT3 been linked to fibrosis in the liver but it is also an important regulator in a model of oxidative stress-induced senescence in primary human lung fibroblasts [41]. Recently, it was suggested that STAT3 potentially is involved in epithelial cell senescence [106]. In addition to regulation by TGF-β, it was concluded that matrix stiffness has a substantial impact on the intrinsic and extrinsic activation of the STAT3 pathway in hepatocellular carcinoma cells [107]. Moreover, activation of TGF-β by a stiffer matrix leads to up-regulation of LOX gene expression and enzyme activity [108]. Several studies have linked the increase in LOX expression by TGF-β to decreased expression of the microRNA (miR) 29 family [109,110]. The miR-29 family has been described to have strong antifibrotic effects in organs such as heart, kidney and lung. It is thought that miR-29 can regulate and modulate apoptosis, cell proliferation, differentiation, migration and the immune response [109,111,112]. Decreased levels of miR-29 have been shown to play a critical, common role in myocardial infarction, systemic sclerosis and patients with advanced fibrosis [112]. Furthermore, miR-29 can increase growth factor and inflammatory cytokine expression including PDGF, tumour necrosis factor α (TNF-α), interleukin (IL) 4 and master regulator of the SASP transcription factor NF-κB. The interplay between these factors and miR-29 maximises up-regulation of collagen and ECM related genes and induction of senescence.

Altered core ECM composition in fibrosis

Pathological increase in ECM deposition and remodelling contributes to an increase in ECM stiffness causing a mechanical gradient between fibrotic and normal tissue. It is known that these changes in stiffness favour proliferation and migration in different cell types. However, currently little is known how the pathological change in composition specifically contributes to the senescent phenotype. Several core and matricellular ECM proteins have been reported to be differentially expressed and these will now be highlighted and discussed as to how they potentially could contribute to cellular senescence.

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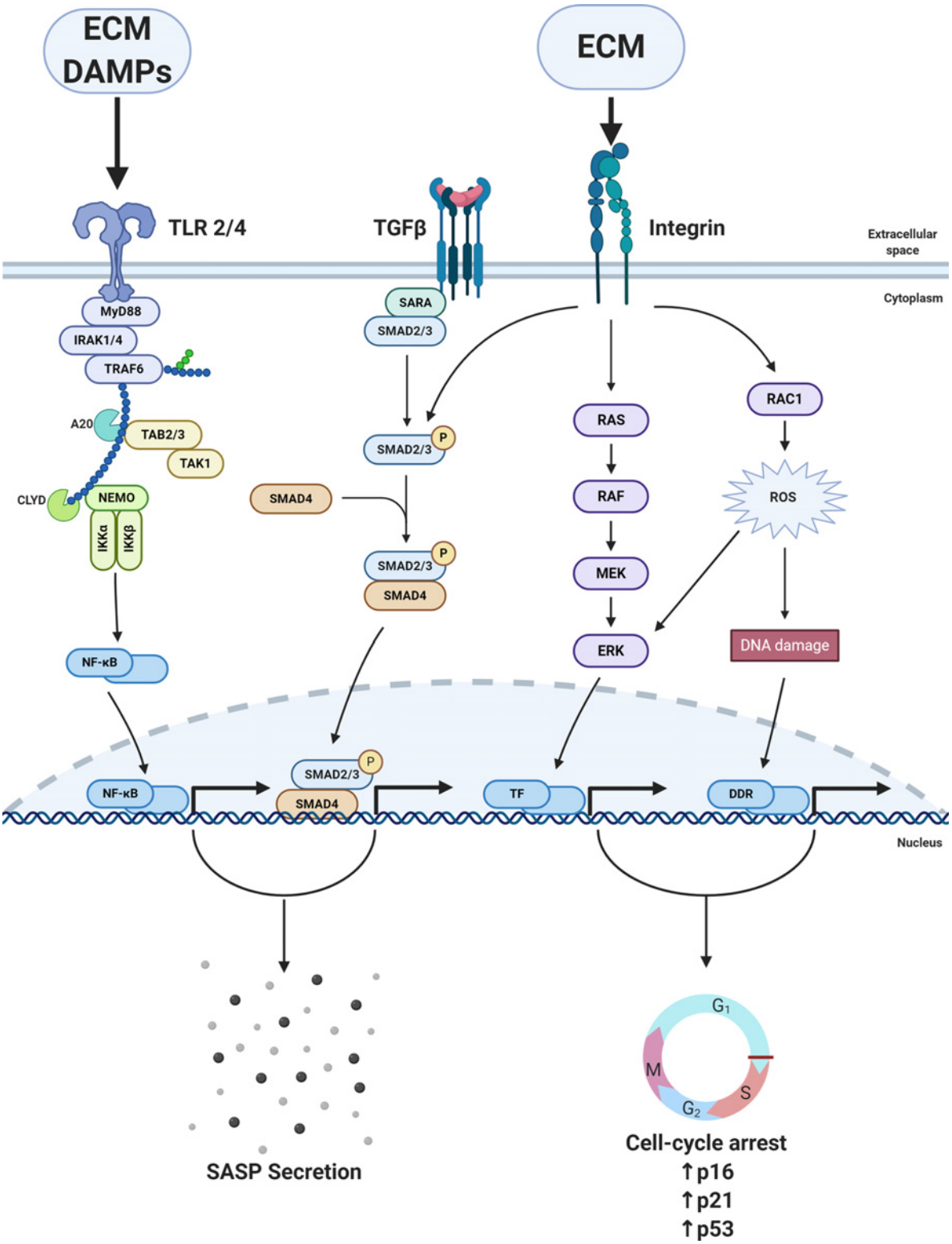

Figure 4. Summary of ECM–cell interaction that contribute to cellular senescence

The binding of ECM DAMPs to TLR2/4 activates the production of pro-inflammatory cytokines that comprise the SASP of senescent cells in fibrosis. Binding of ECM proteins to their respective receptors (i.e. integrins or RTK) leads to the induction of cell-cycle arrest and an increase in apoptosis resistance. Additionally, up-regulation of profibrotic/pro-inflammatory cytokines reinforces the senescent phenotype in an autocrine dependent manner while also spreading senescence to neighbouring cells as part of a feedback loop.

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Collagen

Collagens are the most common ECM protein and are composed of three procollagen chains configured in a triple helix. In connective tissue collagen I and III are the most abundant types of collagens providing structure and tensile strength. Increased collagen III deposition is associated with wound healing, in renal fibrosis both type I and type III are increased while in IPF collagen type I is found in end-stage fibrosis [113,114]. Several studies have reported that collagen expression is differentially expressed in senescent cells. Schafer et al. reported that human IPF lungs express more collagen type I α-1 (COL1A1) while collagen expression in senescent HSCs was reduced [25,115]. Other studies have shown an up-regulation of collagen gene expression in fibroblasts in RS and Werner Syndrome fibroblasts implying a possible role for COL1A1 in cell-cycle arrest [116]. These studies suggest bi-directional cross-talk between senescent cells and collagen type I. Murine vascular smooth muscle cells expressing a collagenase-resistant collagen type I were more sensitive to stress-induced senescence compared with wildtype (WT) cells suggesting a role for increased collagen deposition in premature senescence. In a study investigating glycated collagen, it was shown that early-passage human umbilical vein endothelial cells (HUVECs) grown on glycated collagen-coated dishes expressed hallmarks of premature cell senescence including p14, p53 and increased senescence-associated β-galactosidase (SA-β-Gal) staining compared with non-coated dishes [117]. In cardiac fibroblasts, glycated collagen is associated with integrin α11 expression which modulates activation of TGF-β2 signalling pathway through Smad2/3 which is important for myofibroblast activation and fibrosis [118]. There is accumulating evidence that collagen in different cell types might contribute to premature senescence, however, the exact mechanisms remain unclear. Taken together, it appears that increased deposition of collagen and a stiffer ECM might contribute to the senescent phenotype in fibrosis.

Elastin

Elastin is the main component of elastic fibres which provides compliance and elasticity to a range of tissues. In addition to providing mechanical integrity it has critical regulatory functions of cell behaviour. It is produced by a wide variety of cells including fibroblasts, epithelial cells and smooth muscle cells. Elastin is secreted as tropoelastin monomers and subsequently aligns with collagen fibres before cross-linking by LOX to become part of the fibril assembly [16,119]. As part of the elastic fibre, elastin has several functions; providing elasticity and resilience to connective tissue, regulating the activity of TGF-β as well as having roles in cell migration, survival and differentiation [120,121]. During ageing the expression of elastin decreases. For example in older tendons, there is less elastin while the arterial wall stiffens due to degradation and fragmentation of elastic fibres [122,123]. Pathological increase in deposition and degradation of elastin fragments have been associated with fibrotic disease and poor survival in IPF [124–126]. A more recent study in mice demonstrated that elastin was increasingly expressed in active areas of lung fibrosis induced by bleomycin. In addition, the increased expression was associated with fibroblast activation and differentiation into myofibroblasts that potentially creates a feedback loop to reinforce fibrosis [127]. Elastin does not directly interact with cell surface receptors such as integrins, instead it uses a receptor complex named elastin-binding protein (EBP). During hydrolysis, elastin is cleaved into small peptides such as Val-Gly-Val-Ala-Pro-Gly (VGVAPG) and Val-Val-Gly-Pro-Gly-Ala (VVGPGA). It has been shown that these peptides induce several biological effects such as cell proliferation, migration and differentiation [128]. Furthermore, these peptides lead to chemotaxis of neutrophils and monocytes contributing to a self-propagating cycle of inflammation and ECM proteolysis [108,129]. A recent study reported that VVGPGA can induce expression of IL-α, IL-1β and IL-6, which have been identified as part of the SASP, in ligamentum flavum cells [130–132]. In addition, VGVAPG increased the caspase-1 activity in astrocytes while decreasing IL-1β release *in vitro* [132]. Caspase-1 has been identified as part of the SASP and is strongly associated with fibrosis [133,134]. The most promising receptor activated by VGVPAPG in relation to fibrosis and cellular senescence is peroxisome proliferation-activated receptor-γ (PPARγ) [135]. PPARγ activation leads to the induction of senescence, characterised by increased levels of G_1 cell-cycle arrest, p16 protein expression and an increased SA-β-Gal expression all of which are characteristics of senescent cells [136]. During fibrosis there is increased production and degradation of elastin which might pathologically increase released peptides and contribute to the senescent phenotype.

Fibronectin

Fibronectin is a glycoprotein that is expressed by multiple cell types and plays a significant role in cell adhesion and migration. Fibronectin as well as collagens are needed for normal embryonic development. During wound healing the first active secreted matrix is fibronectin which replaces the fibrin clot formed after the initial insult. To restore homoeostasis, fibroblasts produce fibronectin and collagen as a provisional matrix which is eventually replaced by

collagen abundant ECM. Fibronectin is necessary for collagen fibril formation and *in vitro* added collagen was organised into matrix fibres that co-localised with pre-existing fibronectin fibrils indicating the important function of both ECM proteins [137]. In addition, fibronectin can bind to itself or other ECM proteins such as fibrin, heparin and tenascin. Fibronectin can act as a reservoir for growth factors by binding vascular endothelial growth factor (VEGF), hepatocyte growth factor (HGF), FGF-2, PDGF and BMP [138]. Dysregulated fibronectin has been implicated in diseases such as cancer and fibrosis [139,140]. A recent study showed that fibronectin is able to influence cell-cycle progression and could inhibit cellular senescence and apoptosis in glioma cells via the PI3K/Akt signalling pathway [141]. Fibronectin acts through integrin αvβ3 and it was recently demonstrated that the β3 subunit is involved in cellular senescence via its role in TGF-β activation. During OIS there is a dynamic increase in subunit β3 while down-regulation of β3 reduced OIS and therapy-induced senescence. An αvβ3 antagonist was shown to attenuate the SASP without affecting proliferation in glioma cells. While the exact mechanism remains unclear, there is strong evidence showing that fibronectin is involved in senescence and its increased abundance in fibrosis could potentially contribute to the senescent phenotype.

Laminin

Laminins are proteins that are secreted by epithelial cells into the basement membrane. They contain an α-chain, a β-chain and a γ-chain and there are several splice variants found. Laminins are an integral part of the ECM structure and they mediate cell adhesion, migration, differentiation and proliferation [142]. During wound repair they are critical players in re-epithelialisation and angiogenesis, reviewed in detail by Lorio et al. [143]. The expression of laminins is altered in patients with IPF, and in a small number of patients the distribution of laminin α3 subunit was lost [144]. Interestingly, increased markers of senescence were found in fibroblastic foci and localised clusters of basal cells expressing high levels of laminin 5 γ2 [145], suggesting a potential association between cellular senescence and laminin deposition. Moreover, fibroblastic foci show co-localised expression between laminin and markers of senescence. Increased expression of laminin was also reported in other diseases such as cancer and Sjörgren's syndrome [146,147]. Laminin is able to activate various signal pathways; for example in murine melanoma cells, laminin-1 results in activation of phospholipase D and subsequent induction of phosphatidic acid leading to MMP-2 production favouring tumour invasion [148]. Laminin signalling is mediated via integrins and the 67-kDa laminin receptor [149]. Laminin subunit α1 (Lama1), part of the laminin 111 complex was originally thought to be only expressed during morphogenesis. However, Lama1 was recently shown to be a genetic modifier of TGF-β activity in the context of pulmonary fibrosis [150]. In kidney development, the absence of Lama1 increased TGF-β induced Smad2 phosphorylation in knockout mesangial cells in addition to increased proliferation [151]. These studies demonstrate a strong association between Lama1 and TGF-β. In a study using Schwann cells, Armstrong et al. reported that NF-κB is induced by laminin which had a positive impact on neurite outgrowth [152]. When looking at ageing in endothelial cells of murine origin, a shift was found in expression of laminin β1 and laminin β2. Culture of endothelial cells on laminin produced a difference in response to β1 and β2-containing laminin showing reduced endothelial cell proliferation and migration [153]. While the crucial function of laminin has been described extensively in morphogenesis and wound repair, its involvement in senescence remains poorly understood. There is some evidence suggesting that laminin is involved in senescence and this might be in a TGF-β-dependent way or via activation of NF-κB, but this needs to be elucidated.

Matricellular proteins

Matricellular proteins are dynamically expressed and secreted into the extracellular environment but they do not contribute directly to the major fibrillar structure of the ECM. These proteins modulate cell function by binding to a large variety of cell-surface receptors, cytokines and ECM proteins. They are well-recognised proteins such as CCN family member 1 (CCN1), fibulin, periostin, thrombospondin, tenascin-C and decorin.

CCN1 and CCN2

The best characterised matricellular protein in relation to senescence is CCN1. The CCN family consists of six different types of which CCN1 and CCN2 are the most studied in relation to senescence, fibrosis and cancer [154,155]. During each stage of wound repair CCN1 plays a role in inducing senescence in fibroblasts and endothelial cells. During the proliferation phase fibroblasts undergo senescence to promote formation of granulation tissue. During the maturation phase myofibroblasts undergo senescence to initiate matrix remodelling and limiting fibrosis through up-regulation of MMPs and down-regulation of ECM proteins [156]. Downstream targets ras-related C3 botulinum toxin substrate 1 (RAC1) and NADPH oxidase 1 (NOX1) of CCN1 are activated through the interaction with α6β1 integrin resulting in ROS accumulation leading to senescence via the p53 and p16 pathways [157,158]. Recently,

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Leask et al. reported that loss of CCN1 expression in fibroblasts caused resistance to bleomycin-induced fibrosis but did not impair myofibroblast induction [159]. Interestingly, endogenous CCN1 leads to matrix organisation and stability while CCN2 induces myofibroblast differentiation [160]. In contrast, Grazioli et al. reported that CCN1 was found to be increased in COPD, IPF and animal models of lung fibrosis exacerbating lung injury while inhibition was found to be protective of fibrosis in the kidney [161,162]. In a study using IPF fibroblasts the essential role of CCN1 in mediating profibrotic gene expression via the TGF-β/SMAD3 pathway was demonstrated [154]. CCN2 has been reported to be a pro-fibrotic mediator in several organs and acts downstream of TGF-β [163]. The role of CCN1 and CCN2 has been demonstrated to be tissue- and cell type-specific and how they contribute to fibrotic disease remains unclear. It is plausible that activation of CCN1 and CCN2 contributes to fibrosis by inducing senescence followed by failure of immune clearance during repair leading to pathological senescence.

Periostin

Periostin is a matricellular protein which has been associated with various disease and pathological conditions including cancer and congestive heart failure and is highly expressed in fibrotic disease [164–168]. In IPF, periostin is highly expressed in fibroblastic foci and *in vitro* upon stimulation with IL-4 or IL-13, increased secretion in the supernatant of fibroblasts but not epithelial cells was found [169]. In a murine model, neutralising antibodies prevented bleomycin-induced fibrosis indicating the importance in early onset fibrosis. Periostin binds to ECM including collagen type 1 and fibronectin and transfers signals from the ECM to the cell via specific integrins (i.e., αvβ1, αvβ3, αvβ5 and α6β4) to regulate cell adhesion, proliferation and migration [164]. In cancer, high expression of periostin is associated with increased proliferation, cell invasion and metastasis through activation of the integrin-PI3K/Akt pathway [170,171]. Periostin up-regulates TGF-β and in turn TGF-β may up-regulate periostin providing a complex positive feedback [172]. In a recent study using human fibroblasts, Nanri et al. found that the cross-talk between periostin and TGF-β via SMAD3 resulted in up-regulation of CTGF, IGFBP-3, IL-11 and SERPINE1 in pulmonary fibrosis [173]. Furthermore, it was shown by Li et al. using rat cells that age-related periostin expression from cardiac fibroblasts promotes senescence in cardiomyocytes via the angiotensin II-TGF-β/mitogen-activated protein kinase (MAPK)/ERK pathway [174]. More recently Kim et al. demonstrated that angiotensin II mediated CCN1 induced senescence via the angiotensin II type 1 receptor (AT1R)-p53 dependent pathway in human coronary artery smooth muscle cells. Angiotensin II induces senescence via activation of Erk1/2 and p38 MAPK leading to p16 and p53 expression while CCN1 mediates between Erk1/2 and p53 [175]. While there is clear evidence about the crucial role of periostin in fibrosis and its regulatory effect on TGF-β, there is little knowledge about the influence on cellular senescence.

Fibulin

Fibulin is a secreted glycoprotein important for the stabilisation and binding of other ECM proteins such as fibronectin, PGs and laminins during collagen deposition [172]. The human fibulin family consist of seven members (fibulin-1–7) of which fibulin-1 expression is associated with fibrosis [176,177]. Fibulin-1 is predominantly expressed in connective tissue that highly expresses elastic fibres such as vessels, skin and lung [178]. The soluble form of fibulin-1 can be found in plasma and interacts with fibrinogen. Soluble fibulin-1 is associated with disease severity in IPF and has been identified as a potential marker of kidney disease and diabetes [176,179]. Four different isoforms of fibulin-1 (FBLN1A, B, C and D) are known, each with a different function [178]. In a recent study it was shown that the activation of fibroblasts to myofibroblasts by TGF-β was substantially reduced in the absence of FBLN1C [177]. Furthermore, FBLN1C can increase MMP activity leading to increased production of ECM fragments which could act as DAMPs [177]. Histological staining in healthy and control lung has shown that fibulin-1c is highly expressed in fibrotic areas of the lung while non-fibrotic areas show little expression. Given that fibulins are responsible for the stabilisation of ECM proteins, it is possible that fibulin-1c creates or contributes to an environment that favours induction of senescence in fibroblastic foci. Whether this is via TGF-β activation or by direct binding of specific integrins is unknown. While the evidence suggests a strong role of fibulin-1 in fibrosis, other members within the family are of interest as regulators of cell function. Fibulin-5 can block angiogenesis by direct binding to the fibronectin receptors α5β1 and α4β1, and in endothelial cells it can increase the integrin-induced production of ROS [180]. In addition, fibulin-5 can interact with LOXL-1, -2 and -4 to tether LOXL on to elastic fibres [181]. A role for fibulin-3 is suggested in the pathogenesis of OA where it has been shown that reduced levels are associated with age and high levels with bone marrow mesenchymal stromal cells (BM-MSCs) [182]. Moreover, Chen et al. reported that fibulin-3 functions as a suppressor of cancer invasion via inhibitory effects on ERK and Wnt/β-catenin and that both fibulin-3 and 5 are down-regulated in invasive lung cancer and metastasis [183]. Over the years several lines of evidence have suggested that Wnt/β-catenin signalling plays crucial roles in ageing and cellular senescence [184–187]. Modulation

of Wnt/β-catenin signalling in systemic lupus erythematosus BM-MSCs leads to a decrease in SA-β-Gal expression and the p53/p21 pathway, and increased proliferation [188]. More recently, chronic Wnt/β-catenin signalling was linked to senescence induction in lung epithelial cells [189]. Evidence is accumulating that the fibulin family might have a potential role in regulation of cellular senescence by altering expression of the Wnt/β-catenin signalling and increasing ROS production both of which are linked to senescence and age-related pathologies.

Aggrecan

Aggrecan is a major glycoprotein predominantly found in articular cartilage of the joint providing a hydrated gel structure that allows the joint to withstand tremendous pressure. Aggrecan consists of three disulphide bonded globular regions with keratin-sulphate and chondroitin-sulphate linked to its core. Aggrecan does not exist in free form but is found in PG aggregates, which is composed of hyaluronic acid (HA) with multiple aggrecan molecules attached to it. While aggrecan is best understood for its function in cartilage and pathogenesis of OA, there is evidence suggesting a role in arterial remodelling [190]. In a recent study by Yasmin et al. it was suggested that loss of aggrecan functionality could impact viscoelastic properties, ECM disorganisation and collagen fibril redistribution to modulate vessel stiffness [191]. Additionally, it was unexpectedly shown that aggrecan was expressed in normal skin and to be significantly increased during ageing [192]. While it has been reported that PG deposition is altered in disease, the exact role of aggrecan in fibrosis is poorly understood [193,194]. It is possible that aggrecan in serum could play a role in fibrosis. CD44 is able to bind aggrecan and is necessary for an invasive fibroblast phenotype in fibrosis [195]. Furthermore, Tsuneki and colleagues described that CD44 influences fibroblast behaviour via modulation of the Survivin and Hippo pathways [196]. The contribution of aggrecan remains poorly understood with little to no evidence present that could suggest a role in fibrosis let alone cellular senescence.

Tenascin

Tenascins are a family of giant proteins found in the ECM of embryonic tissues, wounds, tumours and in fibrosis. There are four different proteins named tenascin-C, -R, -W and -X, which somewhat overlap in adult tissue [197]. Tenascins bind to different ECM proteins including fibronectin, PGs and cell-surface receptors. Their function is cell type specific and tenascin can promote or inhibit cell adhesion. Tenascin-R is predominantly found in the central nervous system during development while the others are more widespread and can be found in connective and soft tissue [198]. Tenascin-X and -Y are mainly found in skeletal muscle connective tissue during development while tenascin-C and -W are expressed in a variety of developing tissues [199]. The focus of tenascin research has predominantly been in cell migration and tissue remodelling, tumour development or fibrosis. This review will focus on tenascin-C because of its association in pathological conditions such as vascular hypertension, myocardial infarction and fibrosis [200–204]. There is little or no tenascin-C expression in healthy adult tissue but there is *de novo* expression after tissue injury. Tenascin-C can interact with several cell-surface receptors such as integrins α9β1, αvβ3 and αvβ6, and toll-like receptor 4 (TLR4) to control cell adhesion, motility and proliferation. Cell adhesion can be modulated by tenascin-C by direct interaction with fibronectin and integrin α5β1 to diminish focal adhesions. Cell proliferation can be modulated through direct binding and activation of EGF-receptor and tenascin-C can act as a DAMP by activating TLR-4 [205,206]. It has been reported that TLR4 is able to up-regulate TGF-β expression in fibrosis across multiple organs [207,208]. In a recent study persistent tenascin-C deposition was shown to lead to activation of TLR4-dependent profibrotic responses in skin and lung fibrosis [208]. This included enhanced expression of collagen, myofibroblast activation, fibroblast migration and contraction, and secreted factors such as IL-6 and TGF-β. Activation of TLR4 using lipopolysaccharide (LPS) and TGF-β has shown a different profibrotic response between control and IPF lung fibroblasts, perhaps as a consequence of reduced TGF-β receptor type 1 expression on IPF fibroblasts [209]. In pancreatic cancer Shi and colleagues described that tenascin-C can induce resistance to apoptosis through activation of the Erk1/2/NF-κβ/p65 pathway [210]. Moreover, it was reported that tenascin-C is able to bind several soluble factors including PDGF, VEGF, FGF and TGF-β [211]. The affinity for growth factors could be due to the large role it plays during tissue repair and disease. Furthermore, growth factors bound by tenascin-C have distinct roles in fibrosis, have been described to be part of the SASP and more importantly they are targeted by the antifibrotic drug nintedanib [212]. Taken together it may suggested that tenascin-C plays an important role during fibrosis by increasing collagen production, fibroblast activation and creating a profibrotic environment potentially contributing to cellular senescence.

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Decorin

Decorin is a small leucine-rich PG that is widely expressed in different tissues by fibroblasts and its function is required for normal fibrillogenesis of collagen. Decorin is involved in many different biological and physiological processes including proliferation, differentiation, muscular development, regulation of inflammation and autophagy, and fibrosis of kidney and liver [213,214]. Decorin interacts with other matrix proteins and a variety of cell surface receptors such as EGF-receptor, insulin-growth like receptor, HGF-receptor, VEGF-receptor and PDGF-receptor. Reszegi and colleagues recently reported that decorin has a protective role in liver cancer and could theoretically be used as a physical tyrosine kinase receptor inhibitor [215]. The antifibrotic properties of decorin have been well characterised in the last two decades. Decorin binds and forms a complex with TGF-β, which prevents activation of the TGF-β receptor type 1 and subsequent signalling via SMAD2/3-Erk1/2 protein [216]. The antifibrotic properties further extend by down regulating production of fibronectin, thrompospondin-1, inhibition of collagen maturation and increased production of MMP-1 [217]. Using a colon carcinoma cell-line it was shown that decorin can induce growth suppression by up-regulating p21 and CDK in a p53 and TGF-β independent pathway [218]. Overexpression of decorin in the adenocarcinoma cell-line A549 resulted in an increase in p21 and p53 expression and decreased phosphorylation of EGF-receptor leading to inhibition of metastasis [219,220]. In a study comparing COPD to control fibroblasts it was suggested that cellular senescence is associated with deregulation of ECM expression. A negative correlation was shown between decorin and p16 expression [221]. The reported antifibrotic effect of decorin has been documented in the literature but the effect on cell-cycle progression has been given much less attention. Interestingly, decorin also has the ability to induce premature apoptosis in dermal fibroblasts contributing to hypertrophic scarring [222]. It is possible that decorin induces cell-cycle inhibition in pre-senescent cells but fails to induce apoptosis in this specific phenotype. In addition it was reported that cleaved fragments from decorin in serum correlate with disease severity in both cancer and fibrosis [223]. In the next section of the review the role of decorin as a DAMP will be discussed in more detail.

ECM-derived DAMPs inducing Pro-inflammatory responses in IPF

It has become increasingly evident that the ECM has important immunomodulatory properties. The ECM can act as a reservoir for cytokines and matrikines which can be liberated from the ECM. Matrikines are small peptides proteolytically cleaved from the ECM macromolecules in order to regulate cellular functions, including cell migration, proliferation, and death [224]. Another important immunomodulatory function of the ECM is to signal damage and danger, and subsequently alert and activate the immune system. The signalling of damage and danger is done by the release of danger signals or DAMPs from the ECM [225]. DAMPs were first proposed by Polly Matzinger in 1994 when she described the danger theory [226]. This theory states that not only molecular products derived from 'strangers', or pathogen-associated molecular patterns (PAMPs) can activate the immune system, but similar molecular patterns derived from damaged or death host-cells can also activate the immune system using the same receptor and signalling pathways. The list of currently known DAMPs is extensive and still growing [225,227,228]. However, all DAMPs have in common that they are actively or passively released upon cellular or structural damage and that they elicit pro-inflammatory functions by activation of PRRs upon release which are distinct from their physiological functions [225]. Currently, there are five main classes of PRRs: (1) TLRs, transmembrane proteins located at the cell surface or intracellularly on endosomes; (2) NOD-like receptors (NLRs), located in the cytoplasm; (3) C-type lectin receptors (CLRs), which are transmembrane receptors located at the cell surface; (4) RIG-I-like receptors (RLRs), which are also located intracellularly and (5) the Receptor for Advanced Glycation End products (RAGE) a transmembrane protein located at the cell surface [229]. Upon activation of PRRs by DAMPs, downstream signalling pathways are activated, including NF-κB, MAPK and type I interferon pathways, leading to the release of pro-inflammatory cytokines and chemokines (e.g. IL-6, chemokine ligand (CXCL) 8 and TNF-α), ultimately resulting in activation of the immune system and attraction of immune cells to the site of damage [230]. Next to DAMPs derived from intracellular compartments upon release during cell death, it has been shown that the proteolytic cleavage of ECM macromolecules leads to the release of pro-inflammatory peptides, coined ECM DAMPs [231,232]. Examples of ECM DAMPs are peptides from (1) GAGs such as, low-molecular weight hyaluronan and heparan sulphate, (2) PGs such as, decorin, biglycan, aggrecan and versican and (3) glycoproteins such as, fibronectin, fibrinogen and tenascin-C. All these peptides can be cleaved by proteases, such as MMPs, ADAMs or more specific proteases, including hyaluronidase and heparanase and that they can induce a pro-inflammatory response by activation of PRRs such as TLRs, RAGE and NLRP3. Which proteases cleave the different ECM DAMPs and the exact receptors and downstream signalling pathways are reviewed in detail elsewhere [231,232].

Little is known about the role of extracellular DAMPs in the pathophysiology of IPF. Given the fact that IPF is characterised by the exaggerated deposition of ECM within lung tissue it is enticing to speculate about the role of ECM DAMPs in IPF. Although many studies have been performed investigating individual ECM DAMPs in the context of IPF, no studies have been performed investigating the role of ECM DAMPs in a more structured and controlled fashion. Most studies have investigated the levels of specific ECM DAMPs by either measuring the intracellular expression levels by western blot or mRNA expression or the protein concentration within the ECM by immunohistochemistry. While an increased intracellular expression or increased ECM deposition of ECM DAMPs may be indicative for increased cleavage of pro-inflammatory ECM DAMP fragments it is not directly correlated. In order to study ECM DAMPs they should be assessed in the context of fragments released into the extracellular space, for instance by measuring ECM DAMPs in serum, plasma, sputum, bronchoalveolar lavage fluid (BALF) or epithelial lining fluid.

Several studies have been performed investigating the extracellular fluid levels of ECM proteins such as fibronectin. Fibronectin has several isoforms, which all originate from the same gene. One of these isoforms is referred to as fibronectin-EDA [233]. Fibronectin-EDA is enriched at sites where tissue remodelling due to inflammation is present and has the ability to activate the immune system by binding TLR4, thereby acting as a DAMP [234,235]. Although the levels of fibronectin-EDA have not been measured in extracellular fluids of IPF patients to date, using a targeted mass-spectrometry approach it was shown that the plasma levels of fibronectin were significantly lower in plasma of IPF patients compared with matched healthy controls [236]. In contrast, the levels of fibronectin in BALF were shown to be two- to three-fold increased in IPF patients compared with healthy controls [237]. This was supported by a second study showing a two-fold increase in fibronectin in BALF of IPF patients, however the study lacked the statistical power to reach significance [238]. Together, these studies indicate that fibronectin is locally released in lung tissue in IPF patients, but the leakage of extracellular fibronectin into the circulation is limited. This may lead to a local inflammatory response attracting innate immune cells to the site of release.

Another ECM glycoprotein with the ability to activate TLR4 upon release from the ECM is fibrinogen. Bargagli and colleagues described that the levels of fibrinogen were more than three times as high in patients with IPF compared with healthy controls. Interestingly, the levels were not different between IPF patients in stable disease and IPF patients which experienced an acute exacerbation [239]. Tenascin-C is another glycoprotein with DAMP properties, that can activate TLR4 when released from the ECM [231]. One study investigating the levels of tenascin-C in serum and BALF in patients with idiopathic interstitial pneumonias found no differences in both serum and BALF levels of tenascin-C between healthy controls and IPF patients [240]. However, the serum tenascin-C levels were significantly increased in patients with cryptogenic organising pneumonia (COP) and the BALF tenascin-C levels were increased in both COP and non-specific interstitial pneumonia patients [240].

Several studies have been performed investigating decorin in the context of IPF. Decorin has been shown to be immunostimulatory when it is proteolytically cleaved by MMP3, MMP13 or cathepsin-S and released from the ECM into the extracellular space [241,242]. Cleaved decorin has a small protein core which is up to 42 kDa and contains a leucine-rich-repeat that is also present on TLRs and NLRs, enabling it to ligate and activate these PRRs [243]. In soluble form, decorin can activate TLR2 and TLR4, increasing activity of the p38 MAPK and ERK pathways and resulting in the synthesis of pro-inflammatory cytokines like IL-1β and TNF-α [244] (Figure 2). Interestingly, Kehlet and colleagues reported in two independent cohorts that decorin fragments specifically degraded by cathepsin-S are significantly increased in serum of IPF patients compared with healthy controls [223]. This was shown by an elegantly designed ELISA assay that specifically detects decorin fragments that are cleaved by cathepsin-S [223]. Another study found that serum decorin levels significantly correlated with oxygenation, and that IPF patients with low serum decorin levels showed a significantly higher survival rate compared with IPF patients with high serum decorin levels [223]. These data indicate that in IPF there is increased breakdown of decorin which produces increased circulating levels of pro-inflammatory decorin fragments, which may contribute to the development of IPF.

Although the data listed above indicate that IPF patients display increased ECM breakdown, that increases the concentration of pro-inflammatory ECM DAMPs both systemically as well as locally, much is unknown about the role of ECM DAMPs in the pathophysiology of IPF. Furthermore, although the link between increased senescence and increased ECM DAMP production seems logical, as both are associated with release of the same pro-inflammatory mediators, no studies have been performed to date to investigate the connection between ECM DAMP release and senescence in IPF. A systematic study investigating the levels of multiple ECM DAMPs both locally and systemically in the same cohort is needed to assess the importance of ECM DAMPs in IPF.

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Targeting senescence as a potential therapeutic approach for the treatment of fibrotic disease

Current therapeutic approaches for modulating senescence aim to specifically kill aberrant cells that have entered the senescent state and are having a detrimental effect on the tissue microenvironment. Such agents are being newly developed for this purpose, but also include several drugs (for example quercetin and dasatinib) that were originally developed for targeting tumours. However, as some of the markers that are present in malignant cells are also specifically expressed in senescent cells these agents have now been repurposed as senolytic agents [245]. These drugs have been shown to effectively reduce the number of senescent cells, and in some cases decrease fibrosis, at least in preclinical models, potentially through modulation of the pro-inflammatory SASP released by the senescent cells [61,246]. In a first human, open label pilot study, the potential application of dasatinib and quercetin was tested in 14 patients with IPF [247]. One patient had a serious adverse event during the three-week trial and mild-moderate adverse events included respiratory symptoms, skin irritation/bruising and gastrointestinal complaints. There was a significant improvement in the physical condition of the patients, but the pulmonary function of the patients did not change within this short trial period. Circulating SASP factors were measured, and while no significant change was reported there was a suggestion of reduced levels of selected proteins important for fibrotic remodelling, including IL-6, MMP and TIMP2 in at least 8 of the patients. Intriguingly, changes in pulmonary function and physical condition of the patients correlated with changes in the circulating levels of matrix remodelling proteins. While this is a very early study that needs to be validated in a much larger population in a randomised controlled trial, these observations further suggest that the role of the ECM should not be overlooked when striving to understand the regulation of senescence in fibrosis.

The second approach for modulating senescence *in vivo* strives to interfere with the impact of the released elements of the SASP using drugs named senomorphics. This approach aims to block the components of the SASP from signalling to surrounding cells and in this way reduce the damaging effects of the senescent cells. As ECM remodelling components are recognised elements of the SASP, an innovative approach would be to begin exploring how blocking the activity of these proteins and enzymes impacts the fibrotic disease phenotype. In line with this, there is a need to investigate the therapeutic options of inhibiting either the release or activity of ECM DAMPs in fibrosis. DAMPs, have been well characterised in terms of their role in inflammaging, particularly RAGE ligands, which have a well characterised role in innate immune driven pathologies such as COPD, OA and asthma [248–251]. However, the contribution of DAMPs and the effect of targeting this therapeutically in fibrosis is less well explored. Majewski and colleagues examined the levels of IL-25, IL-33 and thymic stromal lymphopoietin (TSLP) in patients with IPF and followed them after one year of treatment with the antifibrotic drugs nintedanib or pirfenidone [252]. The baseline levels of TSLP were elevated in IPF patients compared with controls, while IL-25 and IL-33 levels did not vary. Strikingly, patients showed reduced levels of both TSLP and IL-25 in exhaled breath condensate after twelve months of treatment with pirfenidone, but not nintedanib. The investigators did not measure the levels of ECM DAMPs in this study leaving an open question as to the likely impact of these antifibrotic therapeutics on ECM DAMPs.

An unexplored realm for modulating senescence in fibrotic diseases, is through modulating the ECM changes directly. It is recognised that the SASP released by senescent cells can induce ECM structural changes and stiffening of the local tissue microenvironment [253] (reviewed in [254]). These changes are well characterised in fibrotic tissues and are known to drive aberrant cell functions including, but not limited to, enhanced proliferation, migration, cytokine/growth factor production and ECM deposition and crosslinking [17,255,256]. Given the role of senescence in regulating tissue repair, it would seem logical that the altered ECM would also give rise to a greater number of senescent cells as part of the natural defence response. Cell interactions with the ECM, via integrin $\alpha 6\beta 1$ and the matricellular protein CCN1 [157,257,258], or integrin $\alpha v\beta 3$ [259] exemplify interactions that induce senescence. Therefore, exploring therapeutic options for targeting these cell-matrix interactions orchestrated via these, and possibly other, integrins may hold promise for regulating senescence.

The influence of the currently approved antifibrotic therapeutics nintedanib and pirfenidone on the induction of senescence has not been directly examined. Both of these drugs are known to modulate the production of major ECM proteins including collagen I and fibronectin in *in vitro* or *ex vivo* models [260,261]. *In vivo*, administration of both antifibrotic therapies did not modulate the senescence markers p16 or p21 nor the deposition of dense collagen deposits in lung tissues collected at the time of lung transplantation. The lack of effectiveness of these therapies in this instance may reflect the end stage disease tissues examined in this study [262]. Encouragingly, both nintedanib and pirfenidone inhibited collagen fibril formation *in vitro*, an important step in the early stages of the deposition of collagen fibres driving the development of fibrosis, and returned the microscopic appearance of the fibres deposited by fibroblasts derived from donors with IPF to an appearance more like those deposited by fibroblasts from a healthy

donor [261]. The structural organisation and crosslinking of the ECM, as directed by LOX family enzymes, deposited by IPF fibroblasts dictated the subsequent response, in terms of attachments and proliferation, of other fibroblasts that were then exposed to this environment [263]. While this study did not examine the senescence characteristics of cells exposed to the IPF-derived ECM it would be an important follow up for understanding the potential for modulating the ECM microenvironment as a tool for controlling senescence in the fibrotic environment.

This review has highlighted the potential feedback loop resulting in enhanced senescence driven by cellular responses to their altered ECM microenvironment. As our understanding of the mechanisms underlying the effectiveness of the approved antifibrotic therapies for lung fibrosis, nintedanib and pirfenidone, and further exploration of novel antifibrotic therapies advances, new possibilities for modulating levels of senescence with increasing finesse should emerge.

Concluding remarks

In this review, we discussed the current knowledge about the ECM as a modulator of cell function and how this could potentially contribute to the senescent phenotype observed in chronic fibrotic diseases. The potential of ECM as a regulator of cellular senescence is described in several ECM components across a variety of tissues while some ECM components may prevent cellular senescence (Figure 3). Moreover, the ability to regulate cell function was not always directly attributed to the ECM component itself i.e. binding to cell-surface receptors, but rather by the ability to bind SASP factors and serve as a reservoir for growth factors/cytokines. Furthermore, some ECM components are described as having a strong regulatory effect once cleaved and released as danger molecules in the environment (Figure 3).

While our knowledge has tremendously increased over the last three decades, much remains unclear as to how the specific ECM components play a role in regulating or contributing to pathological disease states such as fibrosis. Much of what we know about the function of ECM has been discovered in relation to cancer research, which shares similar mechanisms with fibrosis. It is interesting that the majority of matricellular proteins described in the literature show a stronger regulatory effect towards cellular senescence than the core proteins collagen, elastin and laminin. Although these proteins have been extensively investigated, their association with senescence remains poorly understood considering the important role they play for providing structure and stability. The matricellular proteins CCN1, decorin, periostin and tenascin-C all have in common that they might contribute to senescence by regulating cell-cycle, apoptosis and production of secreted factors (Figure 4). In contrast fibronectin and fibulin can act both as inhibitor and promotor of cellular senescence. Lastly most DAMPs released from matricellular proteins lead to a pro-inflammatory response via TLR receptors contributing to up-regulation of secreted factors which are shared with the SASP from senescent cells.

Cellular senescence and the regulation or induction by aberrant ECM contributes to a negative feedback loop in which both reinforce each other. It is difficult to know if senescence is a result of dysregulated ECM deposition, or dysregulated ECM is a consequence of senescent cells. There remain many mysteries as to how the different ECM components can regulate cellular senescence in chronic fibrotic disease. New therapies that combine antifibrotics and senolytics might be able to interrupt the negative feedback loop and interfere with both the aberrant production of ECM and aid in clearance of senescent cells to ultimately halt or reverse disease progression.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Author Contribution

K.E.C.B. prepared the figures. K.E.C.B., S.D.P., M.S. and J.K.B. drafted the manuscript. K.E.C.B., S.D.P., M.S., D.A.K. and J.K.B. edited and revised the manuscript. K.E.C.B., S.D.P., M.S., D.A.K. and J.K.B. approved the final version of manuscript.

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Abbreviations

ADAM, a disintegrin and metalloproteinase; ATM, ataxia-telangiectasia mutated kinase; BALF, bronchoalveolar lavage fluid; Bcl-2, B-cell lymphoma 2; BM-MSC, bone marrow mesenchymal stromal cell; BMP, bone morphogenetic factor; CCN1, CCN family member 1; CDK, cyclin-dependent kinase; COL1A1, collagen type I α-1; COP, cryptogenic organising pneumonia; COPD, chronic obstructive pulmonary disease; DAMP, damage-associated molecular pattern; DDR, DNA damage response; ECM, extracellular matrix; FGF, fibroblast growth factor; GAG, glycosaminoglycan; HGF, hepatocyte growth factor; IL, interleukin; IPF, idiopathic pulmonary fibrosis; LO/LOX, lysyl oxidase; LOXL, LOX-like protein; MAPK, mitogen-activated protein kinase; miR, microRNA; MMP, metalloproteinase; NF-κB, nuclear factor κB; NLR, NOD-like receptor; OA, osteoarthritis; OIS, oncogenic-induced senescence; PDGF, platelet-derived growth factor; PG, proteoglycan; PPARγ, peroxisome proliferation-activated receptor-γ; PRR, pattern recognition receptor; RAGE, receptor for advanced glycation end product; ROCK, Rho-associated coiled-coil containing kinase; ROS, reactive oxygen species; RS, replicative senescence; SASP, senescence-associated secretory phenotype; SA-β-Gal, senescence-associated β-galactosidase; SIPS, stress-induced premature senescence; STAT3, signal transducer and activation of transcription 3; TG, transglutaminase; TGF-β, transforming growth factor-β; TIMP, thrombospondin motif; TLR4, toll-like receptor 4; TNF-α, tumour necrosis factor α; TSLP, thymic stromal lymphopoietin; VEGF, vascular endothelial growth factor; Wnt, wingless and int-1.

References

1 Bonnans, C., Chou, J. and Werb, Z. (2014) Remodelling the extracellular matrix in development and disease. *Nat. Rev. Mol. Cell Biol.* **15**, 786–801, https://doi.org/10.1038/nrm3904
2 Jansen, K.A., Atherton, P. and Ballestrem, C. (2017) Mechanotransduction at the cell-matrix interface. *Semin. Cell Dev. Biol.* **71**, 75–83, https://doi.org/10.1016/j.semcdb.2017.07.027
3 Leiphart, R.J. et al. (2019) Mechanosensing at cellular interfaces. *Langmuir* **35**, 7509–7519, https://doi.org/10.1021/acs.langmuir.8b02841
4 Sapir, L. and Tzlil, S. (2017) Talking over the extracellular matrix: how do cells communicate mechanically? *Semin. Cell Dev. Biol.* **71**, 99–105, https://doi.org/10.1016/j.semcdb.2017.06.010
5 Tschumperlin, D.J. et al. (2018) Mechanosensing and fibrosis. *J. Clin. Invest.* **128**, 74–84, https://doi.org/10.1172/JCI93561
6 Alam, S.G. et al. (2016) The mammalian LINC complex regulates genome transcriptional responses to substrate rigidity. *Sci. Rep.* **6**, 38063, https://doi.org/10.1038/srep38063
7 Campisi, J. (2013) Aging, cellular senescence, and cancer. *Annu. Rev. Physiol.* **75**, 685–705, https://doi.org/10.1146/annurev-physiol-030212-183653
8 McHugh, D. and Gil, J. (2018) Senescence and aging: causes, consequences, and therapeutic avenues. *J. Cell Biol.* **217**, 65–77, https://doi.org/10.1083/jcb.201708092
9 Tuomilehto, J. (2004) Impact of age on cardiovascular risk: implications for cardiovascular disease management. *Atheroscler. Suppl.* **5**, 9–17, https://doi.org/10.1016/j.atherosclerosissup.2004.03.006
10 Alva, M.L. et al. (2017) Identifying risk for type 2 diabetes in different age cohorts: does one size fit all? *BMJ Open Diabetes Res. Care* **5**, e000447, https://doi.org/10.1136/bmjdrc-2017-000447
11 Corrada, M.M. et al. (2010) Dementia incidence continues to increase with age in the oldest old: The 90+ study. *Ann. Neurol.* **67**, 114–121, https://doi.org/10.1002/ana.21915
12 McMonnies, C.W. (2017) Glaucoma history and risk factors. *J. Optometry* **10**, 71–78, https://doi.org/10.1016/j.optom.2016.02.003
13 Brandsma, C.-A. et al. (2017) Lung ageing and COPD: is there a role for ageing in abnormal tissue repair? *Eur. Respir. Rev.* **26**, 170073, https://doi.org/10.1183/16000617.0073-2017
14 Zaman, T. and Lee, J.S. (2018) Risk factors for the development of idiopathic pulmonary fibrosis: a review. *Curr. Pulmonol. Rep.* **7**, 118–125, https://doi.org/10.1007/s13665-018-0210-7
15 Zhen, G. and Cao, X. (2014) Targeting TGFbeta signaling in subchondral bone and articular cartilage homeostasis. *Trends Pharmacol. Sci.* **35**, 227–236, https://doi.org/10.1016/j.tips.2014.03.005
16 Frantz, C., Stewart, K.M. and Weaver, V.M. (2010) The extracellular matrix at a glance. *J. Cell Sci.* **123**, 4195–4200, https://doi.org/10.1242/jcs.023820
17 Burgess, J.K. et al. (2016) The extracellular matrix - the under-recognized element in lung disease? *J. Pathol.* **240**, 397–409, https://doi.org/10.1002/path.4808
18 Parker, M.W. et al. (2014) Fibrotic extracellular matrix activates a profibrotic positive feedback loop. *J. Clin. Invest.* **124**, 1622–1635, https://doi.org/10.1172/JCI71386
19 Im, J. et al. (2016) Idiopathic pulmonary fibrosis fibroblasts become resistant to Fas ligand-dependent apoptosis via the alteration of decoy receptor 3. *J. Pathol.* **240**, 25–37, https://doi.org/10.1002/path.4749
20 Hernandez-Segura, A., Nehme, J. and Demaria, M. (2018) Hallmarks of cellular senescence. *Trends Cell Biol.* **28**, 436–453, https://doi.org/10.1016/j.tcb.2018.02.001
21 Schafer, M.J. et al. (2018) Targeting senescent cells in fibrosis: pathology, paradox, and practical considerations. *Curr. Rheumatol. Rep.* **20**, 3, https://doi.org/10.1007/s11926-018-0712-x
22 Meyer, K. et al. (2016) Essential role for premature senescence of myofibroblasts in myocardial fibrosis. *J. Am. Coll. Cardiol.* **67**, 2018–2028, https://doi.org/10.1016/j.jacc.2016.02.047

23 Luo, C. et al. (2018) Wnt9a promotes renal fibrosis by accelerating cellular senescence in tubular epithelial cells. *J. Am. Soc. Nephrol.* **29**, 1238–1256, https://doi.org/10.1681/ASN.2017050574
24 Ezhilarasan, D., Sokal, E. and Najimi, M. (2018) Hepatic fibrosis: it is time to go with hepatic stellate cell-specific therapeutic targets. *Hepatobiliary Pancreat. Dis. Int.* **17**, 192–197, https://doi.org/10.1016/j.hbpd.2018.04.003
25 Schafer, M.J. et al. (2017) Cellular senescence mediates fibrotic pulmonary disease. *Nat. Commun.* **8**, 14532, https://doi.org/10.1038/ncomms14532
26 Anders, H.J. and Schaefer, L. (2014) Beyond tissue injury-damage-associated molecular patterns, toll-like receptors, and inflammasomes also drive regeneration and fibrosis. *J. Am. Soc. Nephrol.* **25**, 1387–1400, https://doi.org/10.1681/ASN.2014010117
27 Turner, N.A. (2016) Inflammatory and fibrotic responses of cardiac fibroblasts to myocardial damage associated molecular patterns (DAMPs). *J. Mol. Cell Cardiol.* **94**, 189–200, https://doi.org/10.1016/j.yjmcc.2015.11.002
28 Ellson, C.D. et al. (2014) Danger-associated molecular patterns and danger signals in idiopathic pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* **51**, 163–168
29 Levi, N. et al. (2020) The ECM path of senescence in aging: components and modifiers. *FEBS J.*, https://doi.org/10.1111/febs.15282
30 Herranz, N. and Gil, J. (2018) Mechanisms and functions of cellular senescence. *J. Clin. Invest.* **128**, 1238–1246, https://doi.org/10.1172/JCI95148
31 Lopes-Paciencia, S. et al. (2019) The senescence-associated secretory phenotype and its regulation. *Cytokine* **117**, 15–22, https://doi.org/10.1016/j.cyto.2019.01.013
32 Westphal, D. et al. (2011) Molecular biology of Bax and Bak activation and action. *Biochim. Biophys. Acta Mol. Cell Res.* **1813**, 521–531, https://doi.org/10.1016/j.bbamcr.2010.12.019
33 Mirzayans, R. et al. (2010) Single-cell analysis of p16(INK4a) and p21(WAF1) expression suggests distinct mechanisms of senescence in normal human and Li-Fraumeni Syndrome fibroblasts. *J. Cell. Physiol.* **223**, 57–67
34 Salminen, A., Kauppinen, A. and Kaarniranta, K. (2012) Emerging role of NF-kappaB signaling in the induction of senescence-associated secretory phenotype (SASP). *Cell. Signal.* **24**, 835–845, https://doi.org/10.1016/j.cellsig.2011.12.006
35 Herbig, U. et al. (2004) Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21CIP1, but not p16INK4a. *Mol. Cell* **14**, 501–513, https://doi.org/10.1016/S1097-2765(04)00256-4
36 Hayflick, L. and Moorhead, P.S. (1961) The serial cultivation of human diploid cell strains. *Exp. Cell Res.* **25**, 585–621, https://doi.org/10.1016/0014-4827(61)90192-6
37 Bernadotte, A., Mikhelson, V.M. and Spivak, I.M. (2016) Markers of cellular senescence. Telomere shortening as a marker of cellular senescence. *Aging* **8**, 3–11, https://doi.org/10.18632/aging.100871
38 Kojima, H. et al. (2013) IL-6-STAT3 signaling and premature senescence. *JAKSTAT* **2**, e25763
39 Wang, Y., Boerma, M. and Zhou, D. (2016) Ionizing radiation-induced endothelial cell senescence and cardiovascular diseases. *Radiat. Res.* **186**, 153–161, https://doi.org/10.1667/RR14445.1
40 Gluck, S. et al. (2017) Innate immune sensing of cytosolic chromatin fragments through cGAS promotes senescence. *Nat. Cell Biol.* **19**, 1061–1070, https://doi.org/10.1038/ncb3586
41 Waters, D.W. et al. (2019) STAT3 regulates the onset of oxidant-induced senescence in lung fibroblasts. *Am. J. Respir. Cell Mol. Biol.* **61**, 61–73, https://doi.org/10.1165/rcmb.2018-0328OC
42 Correia-Melo, C. et al. (2016) Mitochondria are required for pro-ageing features of the senescent phenotype. *EMBO J.* **35**, 724–742, https://doi.org/10.15252/embj.201592862
43 Schuliga, M. et al. (2018) Mitochondrial dysfunction contributes to the senescent phenotype of IPF lung fibroblasts. *J. Cell. Mol. Med.* **22**, 5847–5861, https://doi.org/10.1111/jcmm.13855
44 Acosta, J.C. et al. (2013) A complex secretory program orchestrated by the inflammasome controls paracrine senescence. *Nat. Cell Biol.* **15**, 978–990, https://doi.org/10.1038/ncb2784
45 Nelson, G. et al. (2018) The senescent bystander effect is caused by ROS-activated NF-kappaB signalling. *Mech. Ageing Dev.* **170**, 30–36, https://doi.org/10.1016/j.mad.2017.08.005
46 Gorgoulis, V.G. and Halazonetis, T.D. (2010) Oncogene-induced senescence: the bright and dark side of the response. *Curr. Opin. Cell Biol.* **22**, 816–827, https://doi.org/10.1016/j.ceb.2010.07.013
47 Chan, A.S.L. and Narita, M. (2019) Short-term gain, long-term pain: the senescence life cycle and cancer. *Genes Dev.* **33**, 127–143, https://doi.org/10.1101/gad.320937.118
48 Storer, M. et al. (2013) Senescence is a developmental mechanism that contributes to embryonic growth and patterning. *Cell* **155**, 1119–1130, https://doi.org/10.1016/j.cell.2013.10.041
49 Demaria, M. et al. (2014) An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. *Dev. Cell* **31**, 722–733, https://doi.org/10.1016/j.devcel.2014.11.012
50 Ventura, M.T. et al. (2017) Immunosenescence in aging: between immune cells depletion and cytokines up-regulation. *Clin. Mol. Allergy* **15**, 21, https://doi.org/10.1186/s12948-017-0077-0
51 Pereira, B.I. et al. (2019) Senescent cells evade immune clearance via HLA-E-mediated NK and CD8(+) T cell inhibition. *Nat. Commun.* **10**, 2387, https://doi.org/10.1038/s41467-019-10335-5
52 Childs, B.G. et al. (2015) Cellular senescence in aging and age-related disease: from mechanisms to therapy. *Nat. Med.* **21**, 1424–1435, https://doi.org/10.1038/nm.4000
53 Baker, D.J. et al. (2016) Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. *Nature* **530**, 184–189, https://doi.org/10.1038/nature16932
54 Hashimoto, M. et al. (2016) Elimination of p19(ARF)-expressing cells enhances pulmonary function in mice. *JCI Insight* **1**, e87732, https://doi.org/10.1172/jci.insight.87732

Clinical Science (2020) **134** 2681–2706
https://doi.org/10.1042/CS20190893

55 Jeon, O.H. et al. (2017) Local clearance of senescent cells attenuates the development of post-traumatic osteoarthritis and creates a pro-regenerative environment. *Nat. Med.* **23**, 775–781, https://doi.org/10.1038/nm.4324
56 Tsuji, T., Aoshiba, K. and Nagai, A. (2006) Alveolar cell senescence in patients with pulmonary emphysema. *Am. J. Respir. Crit. Care Med.* **174**, 886–893, https://doi.org/10.1164/rccm.200509-1374OC
57 Armanios, M.Y. et al. (2007) Telomerase mutations in families with idiopathic pulmonary fibrosis. *N. Engl. J. Med.* **356**, 1317–1326, https://doi.org/10.1056/NEJMoa066157
58 Chilosi, M. et al. (2013) Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. *Transl. Res.* **162**, 156–173, https://doi.org/10.1016/j.trsl.2013.06.004
59 Naikawadi, R.P. et al. (2016) Telomere dysfunction in alveolar epithelial cells causes lung remodeling and fibrosis. *JCI Insight* **1**, e86704, https://doi.org/10.1172/jci.insight.86704
60 Disayabutr, S. et al. (2016) miR-34 miRNAs regulate cellular senescence in type II alveolar epithelial cells of patients with idiopathic pulmonary fibrosis. *PLoS ONE* **11**, e0158367, https://doi.org/10.1371/journal.pone.0158367
61 Lehmann, M. et al. (2017) Senolytic drugs target alveolar epithelial cell function and attenuate experimental lung fibrosis ex vivo. *Eur. Respir. J.* **50**, 1602367–1602367, https://doi.org/10.1183/13993003.02367-2016
62 Murtha, L.A. et al. (2019) The role of pathological aging in cardiac and pulmonary fibrosis. *Aging Dis.* **10**, 419–428, https://doi.org/10.14336/AD.2018.0601
63 Hecker, L. et al. (2014) Reversal of persistent fibrosis in aging by targeting Nox4-Nrf2 redox imbalance. *Sci. Transl. Med.* **6**, 231ra47, https://doi.org/10.1126/scitranslmed.3008182
64 Yanai, H. et al. (2015) Cellular senescence-like features of lung fibroblasts derived from idiopathic pulmonary fibrosis patients. *Aging (Albany N.Y.)* **7**, 664–672, https://doi.org/10.18632/aging.100807
65 Liu, D. and Hornsby, P.J. (2007) Senescent human fibroblasts increase the early growth of xenograft tumors via matrix metalloproteinase secretion. *Cancer Res.* **67**, 3117–3126, https://doi.org/10.1158/0008-5472.CAN-06-3452
66 Krtolica, A. et al. (2001) Senescent fibroblasts promote epithelial cell growth and tumorigenesis: a link between cancer and aging. *Proc. Natl. Acad. Sci. U.S.A.* **98**, 12072–12077, https://doi.org/10.1073/pnas.211053698
67 Zhou, Y. et al. (2018) Extracellular matrix in lung development, homeostasis and disease. *Matrix Biol.* **73**, 77–104, https://doi.org/10.1016/j.matbio.2018.03.005
68 Lu, L. et al. (2017) Cardiac fibrosis in the ageing heart: contributors and mechanisms. *Clin. Exp. Pharmacol. Physiol.* **44**, 55–63, https://doi.org/10.1111/1440-1681.12753
69 Hoare, M., Das, T. and Alexander, G. (2010) Ageing, telomeres, senescence, and liver injury. *J. Hepatol.* **53**, 950–961, https://doi.org/10.1016/j.jhep.2010.06.009
70 Farris, A.B. and Colvin, R.B. (2012) Renal interstitial fibrosis: mechanisms and evaluation. *Curr. Opin. Nephrol. Hypertens.* **21**, 289–300, https://doi.org/10.1097/MNH.0b013e3283521cfa
71 Munjal, A. and Lecuit, T. (2014) Actomyosin networks and tissue morphogenesis. *Development* **141**, 1789–1793, https://doi.org/10.1242/dev.091645
72 Kim, H.Y. and Nelson, C.M. (2012) Extracellular matrix and cytoskeletal dynamics during branching morphogenesis. *Organogenesis* **8**, 56–64, https://doi.org/10.4161/org.19813
73 Mammoto, T. and Ingber, D.E. (2010) Mechanical control of tissue and organ development. *Development* **137**, 1407–1420, https://doi.org/10.1242/dev.024166
74 Kasza, K.E. and Zallen, J.A. (2011) Dynamics and regulation of contractile actin-myosin networks in morphogenesis. *Curr. Opin. Cell Biol.* **23**, 30–38, https://doi.org/10.1016/j.ceb.2010.10.014
75 Joo, H.J. et al. (2012) ROCK suppression promotes differentiation and expansion of endothelial cells from embryonic stem cell-derived Flk1(+) mesodermal precursor cells. *Blood* **120**, 2733–2744, https://doi.org/10.1182/blood-2012-04-421610
76 Kumper, S. et al. (2016) Rho-associated kinase (ROCK) function is essential for cell cycle progression, senescence and tumorigenesis. *eLife* **5**, e12994, https://doi.org/10.7554/eLife.12203
77 Moore, K.A. et al. (2005) Control of basement membrane remodeling and epithelial branching morphogenesis in embryonic lung by Rho and cytoskeletal tension. *Dev. Dyn.* **232**, 268–281, https://doi.org/10.1002/dvdy.20237
78 Park, J.T. et al. (2018) A crucial role of ROCK for alleviation of senescence-associated phenotype. *Exp. Gerontol.* **106**, 8–15, https://doi.org/10.1016/j.exger.2018.02.012
79 Sonbol, H.S. (2018) Extracellular matrix remodeling in human disease. *J. Microsc. Ultrastruct.* **6**, 123–128, https://doi.org/10.4103/JMAU.JMAU˙4˙18
80 Daley, M.C., Fenn, S.L. and Black, L.D. (2018) Applications of cardiac extracellular matrix in tissue engineering and regenerative medicine. In *Cardiac Extracellular Matrix: Fundamental Science to Clinical Applications* (Schmuck, E.G., Hematti, P. and Raval, A.N., eds), pp. 59–83, Springer International Publishing, Cham
81 Sturmlechner, I. et al. (2017) Cellular senescence in renal ageing and disease. *Nat. Rev. Nephrol.* **13**, 77–89, https://doi.org/10.1038/nrneph.2016.183
82 Lampi, M.C. and Reinhart-King, C.A. (2018) Targeting extracellular matrix stiffness to attenuate disease: from molecular mechanisms to clinical trials. *Sci. Transl. Med.* **10**, eaao0475, https://doi.org/10.1126/scitranslmed.aao0475
83 Zhang, C.-Y. et al. (2016) Liver fibrosis and hepatic stellate cells: etiology, pathological hallmarks and therapeutic targets. *World J. Gastroenterol.* **22**, 10512, https://doi.org/10.3748/wjg.v22.i48.10512
84 Pollard, T.D. (2017) Extracellular matrix molecules. *Cell Biology*, pp. 505–524, Elsevier

85 Turner, N.A. and Porter, K.E. (2012) Regulation of myocardial matrix metalloproteinase expression and activity by cardiac fibroblasts. *IUBMB Life* **64**, 143–150
86 Foronjy, R.F. et al. (2008) Transgenic expression of matrix metalloproteinase-1 inhibits myocardial fibrosis and prevents the transition to heart failure in a pressure overload mouse model. *Hypertens Res.* **31**, 725–735
87 Georges, P.C. et al. (2007) Increased stiffness of the rat liver precedes matrix deposition: implications for fibrosis. *Am. J. Physiol. Gastrointest. Liver Physiol.* **293**, G1147–G1154, https://doi.org/10.1152/ajpgi.00032.2007
88 Molnar, J. et al. (2003) Structural and functional diversity of lysyl oxidase and the LOX-like proteins. *Biochim. Biophys. Acta* **1647**, 220–224, https://doi.org/10.1016/S1570-9639(03)00053-0
89 Wang, C. et al. (2019) Lysyl oxidase-like protein 2 promotes tumor lymphangiogenesis and lymph node metastasis in breast cancer. *Neoplasia* **21**, 413–427, https://doi.org/10.1016/j.neo.2019.03.003
90 Tjin, G. et al. (2017) Lysyl oxidases regulate fibrillar collagen remodelling in idiopathic pulmonary fibrosis. *Dis. Models Mech.* **10**, 1301–1312
91 Espindola, M.S. et al. (2019) Targeting lysyl oxidase-like 2 in idiopathic pulmonary fibrosis. *Cold Spring Harb. Lab.*, https://doi.org/10.1101/813907
92 Ikenaga, N. et al. (2017) Selective targeting of lysyl oxidase-like 2 (LOXL2) suppresses hepatic fibrosis progression and accelerates its reversal. *Gut* **66**, 1697–1708, https://doi.org/10.1136/gutjnl-2016-312473
93 Wang, T.-H., Hsia, S.-M. and Shieh, T.-M. (2016) Lysyl oxidase and the tumor microenvironment. *Int. J. Mol. Sci.* **18**, 62, https://doi.org/10.3390/ijms18010062
94 Gilhodes, J.-C. et al. (2017) Quantification of pulmonary fibrosis in a bleomycin mouse model using automated histological image analysis. *PLoS ONE* **12**, e0170561, https://doi.org/10.1371/journal.pone.0170561
95 Tatsukawa, H. et al. (2018) Isozyme-specific comprehensive characterization of transglutaminase-crosslinked substrates in kidney fibrosis. *Sci. Rep.* **8**, 7306, https://doi.org/10.1038/s41598-018-25674-4
96 Johnson, T.S. et al. (2007) Transglutaminase inhibition reduces fibrosis and preserves function in experimental chronic kidney disease. *J. Am. Soc. Nephrol.* **18**, 3078–3088, https://doi.org/10.1681/ASN.2006070690
97 Trackman, P.C. (2016) Lysyl oxidase isoforms and potential therapeutic opportunities for fibrosis and cancer. *Expert Opin. Ther. Targets* **20**, 935–945, https://doi.org/10.1517/14728222.2016.1151003
98 Schmelzer, C.E.H. et al. (2019) Lysyl oxidase-like 2 (LOXL2)-mediated cross-linking of tropoelastin. *FASEB J.* **33**, 5468–5481, https://doi.org/10.1096/fj.201801860RR
99 Hassona, Y. et al. (2013) Progression of genotype-specific oral cancer leads to senescence of cancer-associated fibroblasts and is mediated by oxidative stress and TGF-beta. *Carcinogenesis* **34**, 1286–1295, https://doi.org/10.1093/carcin/bgt035
100 Zhang, Y., Alexander, P.B. and Wang, X.-F. (2017) TGF-β family signaling in the control of cell proliferation and survival. *Cold Spring Harb. Perspect. Biol.* **9**, a022145, https://doi.org/10.1101/cshperspect.a022145
101 Tominaga, K. and Suzuki, H.I. (2019) TGF-β signaling in cellular senescence and aging-related pathology. *Int. J. Mol. Sci.* **20**, 5002, https://doi.org/10.3390/ijms20205002
102 Frippiat, C. et al. (2001) Subcytotoxic H_2O_2 stress triggers a release of transforming growth factor-beta 1, which induces biomarkers of cellular senescence of human diploid fibroblasts. *J. Biol. Chem.* **276**, 2531–2537, https://doi.org/10.1074/jbc.M006809200
103 Senturk, S. et al. (2010) Transforming growth factor-beta induces senescence in hepatocellular carcinoma cells and inhibits tumor growth. *Hepatology* **52**, 966–974, https://doi.org/10.1002/hep.23769
104 Minagawa, S. et al. (2011) Accelerated epithelial cell senescence in IPF and the inhibitory role of SIRT6 in TGF-beta-induced senescence of human bronchial epithelial cells. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **300**, L391–L401, https://doi.org/10.1152/ajplung.00097.2010
105 Tang, L.-Y. et al. (2017) Transforming growth factor-β (TGF-β) directly activates the JAK1-STAT3 axis to induce hepatic fibrosis in coordination with the SMAD pathway. *J. Biol. Chem.* **292**, 4302–4312, https://doi.org/10.1074/jbc.M116.773085
106 Dutta, P. et al. (2020) Unphosphorylated STAT3 in heterochromatin formation and tumor suppression in lung cancer. *BMC Cancer* **20**, 145, https://doi.org/10.1186/s12885-020-6649-2
107 Schrader, J. et al. (2011) Matrix stiffness modulates proliferation, chemotherapeutic response, and dormancy in hepatocellular carcinoma cells. *Hepatology* **53**, 1192–1205, https://doi.org/10.1002/hep.24108
108 Wells, R.G. (2013) Tissue mechanics and fibrosis. *Biochim. Biophys. Acta* **1832**, 884–890, https://doi.org/10.1016/j.bbadis.2013.02.007
109 Zhang, Y. et al. (2014) MiR-29b inhibits collagen maturation in hepatic stellate cells through down-regulating the expression of HSP47 and lysyl oxidase. *Biochem Biophys Res Commun* **446**, 940–944
110 Fang, Y. et al. (2016) Transforming growth factor-beta1 increases lysyl oxidase expression by downregulating MIR29A in human granulosa lutein cells. *Reproduction* **152**, 205–213, https://doi.org/10.1530/REP-16-0144
111 Kriegel, A.J. et al. (2012) The miR-29 family: genomics, cell biology, and relevance to renal and cardiovascular injury. *Physiol. Genomics* **44**, 237–244, https://doi.org/10.1152/physiolgenomics.00141.2011
112 Cushing, L., Kuang, P. and Lu, J. (2015) The role of miR-29 in pulmonary fibrosis. *Biochem. Cell. Biol.* **93**, 109–118, https://doi.org/10.1139/bcb-2014-0095
113 Rasmussen, D.G.K. et al. (2019) Collagen turnover profiles in chronic kidney disease. *Sci. Rep.* **9**, 16062, https://doi.org/10.1038/s41598-019-51905-3
114 Merl-Pham, J. et al. (2019) Quantitative proteomic profiling of extracellular matrix and site-specific collagen post-translational modifications in an in vitro model of lung fibrosis. *Matrix Biol. Plus* **1**, 100005, https://doi.org/10.1016/j.mbplus.2019.04.002
115 Krizhanovsky, V. et al. (2008) Senescence of activated stellate cells limits liver fibrosis. *Cell* **134**, 657–667, https://doi.org/10.1016/j.cell.2008.06.049

Clinical Science (2020) **134** 2681–2706
https://doi.org/10.1042/CS20190893

116 Murano, S. et al. (1991) Diverse gene sequences are overexpressed in werner syndrome fibroblasts undergoing premature replicative senescence. *Mol Cell Bio* **11**, 3905–3914
117 Chen, J. et al. (2002) Glycated collagen I induces premature senescence-like phenotypic changes in endothelial cells. *Circ. Res.* **90**, 1290–1298, https://doi.org/10.1161/01.RES.0000022161.42655.98
118 Talior-Volodarsky, I. et al. (2015) Glycated collagen induces α11 integrin expression through TGF-β2 and Smad3. *J. Cell. Physiol.* **230**, 327–336, https://doi.org/10.1002/jcp.24708
119 Halper, J., Kjaer, M. and Halper, J. (2014) Basic components of connective tissues and extracellular matrix: elastin, fibrillin, fibulins, fibrinogen, fibronectin, laminin, tenascins and thrombospondins. *Progress in Heritable Soft Connective Tissue Diseases*, pp. 31–47, Springer, Netherlands: Dordrecht
120 Kielty, C.M. (2006) Elastic fibres in health and disease. *Expert Rev. Mol. Med.* **8**, 1–23, https://doi.org/10.1017/S146239940600007X
121 Muiznieks, L.D., Weiss, A.S. and Keeley, F.W. (2010) Structural disorder and dynamics of elastin. *Biochem. Cell. Biol.* **88**, 239–250, https://doi.org/10.1139/009-161
122 Sun, Z. (2015) Aging, arterial stiffness, and hypertension. *Hypertension* **65**, 252–256, https://doi.org/10.1161/HYPERTENSIONAHA.114.03617
123 Sherratt, M.J. (2009) Tissue elasticity and the ageing elastic fibre. *AGE* **31**, 305–325, https://doi.org/10.1007/s11357-009-9103-6
124 Enomoto, N. et al. (2013) Amount of elastic fibers predicts prognosis of idiopathic pulmonary fibrosis. *Respir. Med.* **107**, 1608–1616, https://doi.org/10.1016/j.rmed.2013.08.008
125 Kanta, J. (2016) Elastin in the liver. *Front. Physiol.* **7**, 491, https://doi.org/10.3389/fphys.2016.00491
126 Sun, Q. et al. (2019) Elastin imaging enables noninvasive staging and treatment monitoring of kidney fibrosis. *Sci. Transl. Med.* **11**, eaat4865, https://doi.org/10.1126/scitranslmed.aat4865
127 Blaauboer, M.E. et al. (2014) Extracellular matrix proteins: a positive feedback loop in lung fibrosis? *Matrix Biol.* **34**, 170–178
128 Le Page, A. et al. (2019) The role of elastin-derived peptides in human physiology and diseases. *Matrix Biol.* **84**, 81–96, https://doi.org/10.1016/j.matbio.2019.07.004
129 Senior, R.M. (1984) Val-Gly-Val-Ala-Pro-Gly, a repeating peptide in elastin, is chemotactic for fibroblasts and monocytes. **99**, 870–874
130 Debret, R. et al. (2006) Elastin fragments induce IL-1β upregulation via NF-κB pathway in melanoma cells. *J. Invest. Dermatol.* **126**, 1860–1868, https://doi.org/10.1038/sj.jid.5700337
131 Kobayashi, K. et al. (2017) Elastinderived peptides are involved in the processes of human temporomandibular disorder by inducing inflammatory responses in synovial cells. *Mol. Med. Rep.* **16**, 3147–3154, https://doi.org/10.3892/mmr.2017.7012
132 Szychowski, K.A. and Gmiński, J. (2019) The elastin-derived peptide VGVAPG does not activate the inflammatory process in mouse cortical astrocytes in vitro. *Neurotox. Res.* **37**, 136–145, https://doi.org/10.1007/s12640-019-00114-x
133 Wiggins, K.A. et al. (2019) IL-1α; cleavage by inflammatory caspases of the noncanonical inflammasome controls the senescence-associated secretory phenotype. *Aging Cell.* **18**, e12946, https://doi.org/10.1111/acel.12946
134 Artlett, C.M. et al. (2011) The inflammasome activating caspase 1 mediates fibrosis and myofibroblast differentiation in systemic sclerosis. *Arthritis Rheum* **63**, 3563–3574
135 Szychowski, K.A. and Gminski, J. (2019) Impact of elastin-derived VGVAPG peptide on bidirectional interaction between peroxisome proliferator-activated receptor gamma (Ppargamma) and beta-galactosidase (beta-Gal) expression in mouse cortical astrocytes in vitro. *Naunyn Schmiedebergs Arch. Pharmacol.* **392**, 405–413, https://doi.org/10.1007/s00210-018-1591-4
136 Gan, Q. et al. (2008) PPAR accelerates cellular senescence by inducing p16INK4 expression in human diploid fibroblasts. *J. Cell Sci.* **121**, 2235–2245, https://doi.org/10.1242/jcs.026633
137 Kubow, K.E. et al. (2015) Mechanical forces regulate the interactions of fibronectin and collagen I in extracellular matrix. *Nat. Commun.* **6**, 8026, https://doi.org/10.1038/ncomms9026
138 Zhu, J. and Clark, R.A.F. (2014) Fibronectin at select sites binds multiple growth factors and enhances their activity: expansion of the collaborative ECM-GF paradigm. *J. Invest. Dermatol.* **134**, 895–901, https://doi.org/10.1038/jid.2013.484
139 Yousif, N.G. (2014) Fibronectin promotes migration and invasion of ovarian cancer cells through up-regulation of FAK-PI3K/Akt pathway. *Cell Biol. Int.* **38**, 85–91, https://doi.org/10.1002/cbin.10184
140 Valiente-Alandi, I. et al. (2018) Inhibiting fibronectin attenuates fibrosis and improves cardiac function in a model of heart failure. *Circulation* **138**, 1236–1252, https://doi.org/10.1161/CIRCULATIONAHA.118.034609
141 piekLiao, Y.X. et al. (2018) Effects of fibronectin 1 on cell proliferation, senescence and apoptosis of human glioma cells through the PI3K/AKT signaling pathway. *Cell. Physiol. Biochem.* **48**, 1382–1396
142 Givant-Horwitz, V., Davidson, B. and Reich, R. (2005) Laminin-induced signaling in tumor cells. *Cancer Lett.* **223**, 1–10, https://doi.org/10.1016/j.canlet.2004.08.030
143 Iorio, V., Troughton, L.D. and Hamill, K.J. (2015) Laminins: roles and utility in wound repair. *Adv. Wound Care* **4**, 250–263, https://doi.org/10.1089/wound.2014.0533
144 Morales-Nebreda, L.I. et al. (2015) Lung-specific loss of α3 laminin worsens bleomycin-induced pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* **52**, 503–512, https://doi.org/10.1165/rcmb.2014-0057OC
145 Chilosi, M. et al. (2006) Migratory marker expression in fibroblast foci of idiopathic pulmonary fibrosis. *Respir. Res.* **7**, 95, https://doi.org/10.1186/1465-9921-7-95
146 Qiu, X. et al. (2018) Laminin is over expressed in breast cancer and facilitate cancer cell metastasis. *J. Cancer Res. Ther.* **14**, 1170–1172
147 Daniels, P.J. et al. (1999) Cytokine-mediated stimulation of laminin expression and cell-growth arrest in a human submandibular gland duct-cell line (HSG). *Arch. Oral Biol.* **44**, 603–615, https://doi.org/10.1016/S0003-9969(99)00024-2

148 Reich, R., Blumenthal, M. and Liscovitch, M. (1995) Role of phospholipase D in laminin-induced production of gelatinase A (MMP-2) in metastatic cells. *Clin. Exp. Metastasis* **13**, 134–140, https://doi.org/10.1007/BF00133618
149 Digiacomo, V. and Meruelo, D. (2016) Looking into laminin receptor: critical discussion regarding the non-integrin 37/67-kDa laminin receptor/RPSA protein. *Biol. Rev.* **91**, 288–310, https://doi.org/10.1111/brv.12170
150 Lee, C.-M. et al. (2018) Laminin α1 is a genetic modifier of TGF-β1–stimulated pulmonary fibrosis. *JCI Insight* **3**, e99574, https://doi.org/10.1172/jci.insight.99574
151 Ning, L. et al. (2014) Laminin α1 regulates age-related mesangial cell proliferation and mesangial matrix accumulation through the TGF-β pathway. *Am J Pathol* **184**, 1683–1694
152 Armstrong, S.J. et al. (2008) Laminin activates NF-kappaB in Schwann cells to enhance neurite outgrowth. *Neurosci. Lett.* **439**, 42–46, https://doi.org/10.1016/j.neulet.2008.04.091
153 Wagner, J.U.G. et al. (2018) Switch in laminin β2 to laminin β1 isoforms during aging controls endothelial cell functions—brief report. *Arteriosclerosis Thrombosis Vasc. Biol.* **38**, 1170–1177, https://doi.org/10.1161/ATVBAHA.117.310685
154 Kurundkar, A.R. et al. (2016) The matricellular protein CCN1 enhances TGF-beta1/SMAD3-dependent profibrotic signaling in fibroblasts and contributes to fibrogenic responses to lung injury. *FASEB J.* **30**, 2135–2150, https://doi.org/10.1096/fj.201500173
155 Kim, H., Son, S. and Shin, I. (2018) Role of the CCN protein family in cancer. *BMB Rep.* **51**, 486–492, https://doi.org/10.5483/BMBRep.2018.51.10.192
156 Kim, K.H. et al. (2018) The matricellular protein CCN1 in tissue injury repair. *J. Cell Commun. Signal.* **12**, 273–279, https://doi.org/10.1007/s12079-018-0450-x
157 Jun, J.I. and Lau, L.F. (2010) The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. *Nat. Cell Biol.* **12**, 676–685, https://doi.org/10.1038/ncb2070
158 Jun, J.I. and Lau, L.F. (2017) CCN2 induces cellular senescence in fibroblasts. *J. Cell Commun. Signal.* **11**, 15–23, https://doi.org/10.1007/s12079-016-0359-1
159 Quesnel, K. et al. (2019) CCN1 expression by fibroblasts is required for bleomycin-induced skin fibrosis. *Matrix Biol. Plus* **3**, 100009, https://doi.org/10.1016/j.mbplus.2019.100009
160 Leask, A. (2013) CCN2: a novel, specific and valid target for anti-fibrotic drug intervention. *Expert Opin. Ther. Targets* **17**, 1067–1071, https://doi.org/10.1517/14728222.2013.812074
161 Grazioli, S. et al. (2015) CYR61 (CCN1) overexpression induces lung injury in mice. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **308**, L759–L765, https://doi.org/10.1152/ajplung.00190.2014
162 Lai, C.F. et al. (2014) Blockade of cysteine-rich protein 61 attenuates renal inflammation and fibrosis after ischemic kidney injury. *Am. J. Physiol. Renal Physiol.* **307**, F581–F92, https://doi.org/10.1152/ajprenal.00670.2013
163 Riser, B.L., Barnes, J.L. and Varani, J. (2015) Balanced regulation of the CCN family of matricellular proteins: a novel approach to the prevention and treatment of fibrosis and cancer. *J. Cell Commun. Signal.* **9**, 327–339, https://doi.org/10.1007/s12079-015-0309-3
164 Ashley, S.L. et al. (2017) Periostin regulates fibrocyte function to promote myofibroblast differentiation and lung fibrosis. *Mucosal Immunol.* **10**, 341–351, https://doi.org/10.1038/mi.2016.61
165 An, J.N. et al. (2019) Periostin induces kidney fibrosis after acute kidney injury via the p38 MAPK pathway. *Am. J. Physiol. Renal Physiol.* **316**, F426–F437, https://doi.org/10.1152/ajprenal.00203.2018
166 Zhao, S. et al. (2014) Periostin expression is upregulated and associated with myocardial fibrosis in human failing hearts. *J. Cardiol.* **63**, 373–378, https://doi.org/10.1016/j.jjcc.2013.09.013
167 O'Dwyer, D.N. and Moore, B.B. (2017) The role of periostin in lung fibrosis and airway remodeling. *Cell. Mol. Life Sci.* **74**, 4305–4314, https://doi.org/10.1007/s00018-017-2649-z
168 González-González, L. and Alonso, J. (2018) Periostin: a matricellular protein with multiple functions in cancer development and progression. *Front. Oncol.* **8**, 225, https://doi.org/10.3389/fonc.2018.00225
169 Okamoto, M. et al. (2011) Periostin, a matrix protein, is a novel biomarker for idiopathic interstitial pneumonias. *Eur. Respir. J.* **37**, 1119–1127, https://doi.org/10.1183/09031936.00059810
170 Utispan, K. et al. (2012) Periostin activates integrin alpha5beta1 through a PI3K/AKTdependent pathway in invasion of cholangiocarcinoma. *Int. J. Oncol.* **41**, 1110–1118, https://doi.org/10.3892/ijo.2012.1530
171 Hu, Q. et al. (2015) Periostin mediates TGF-β-induced epithelial mesenchymal transition in prostate cancer cells. *Cell. Physiol. Biochem.* **36**, 799–809, https://doi.org/10.1159/000430139
172 Tsuda, T. (2018) Extracellular interactions between fibulins and transforming growth factor (TGF)-β in physiological and pathological conditions. *Int. J. Mol. Sci.* **19**, 2787, https://doi.org/10.3390/ijms19092787
173 Nanri, Y. et al. (2020) Cross-talk between transforming growth factor-beta and periostin can be targeted for pulmonary fibrosis. *Am. J. Respir. Cell Mol. Biol.* **62**, 204–216, https://doi.org/10.1165/rcmb.2019-0245OC
174 Li, Q., Liu, X. and Wei, J. (2014) Ageing related periostin expression increase from cardiac fibroblasts promotes cardiomyocytes senescent. *Biochem. Biophys. Res. Commun.* **452**, 497–502, https://doi.org/10.1016/j.bbrc.2014.08.109
175 Kim, I., Park, C.S. and Lee, H.-Y. (2019) Angiotensin II type 1 receptor blocker, fimasartan, reduces vascular smooth muscle cell senescence by inhibiting the CYR61 signaling pathway. *Korean Circ. J.* **49**, 615, https://doi.org/10.4070/kcj.2018.0379
176 Jaffar, J. et al. (2014) Fibulin-1 predicts disease progression in patients with idiopathic pulmonary fibrosis. *Chest* **146**, 1055–1063, https://doi.org/10.1378/chest.13-2688
177 Liu, G. et al. (2019) Fibulin-1c regulates transforming growth factor-beta activation in pulmonary tissue fibrosis. *JCI Insight* **5**, e124529, https://doi.org/10.1172/jci.insight.124529

Clinical Science (2020) **134** 2681–2706
https://doi.org/10.1042/CS20190893

178 Timpl, R. et al. (2003) Fibulins: a versatile family of extracellular matrix proteins. *Nat. Rev. Mol. Cell Biol.* **4**, 479–489, https://doi.org/10.1038/nrm1130
179 Scholze, A. et al. (2013) Plasma concentrations of extracellular matrix protein fibulin-1 are related to cardiovascular risk markers in chronic kidney disease and diabetes. *Cardiovasc. Diabetol.* **12**, 6, https://doi.org/10.1186/1475-2840-12-6
180 Yanagisawa, H., Schluterman, M.K. and Brekken, R.A. (2009) Fibulin-5, an integrin-binding matricellular protein: its function in development and disease. *J. Cell Commun. Signal.* **3**, 337–347, https://doi.org/10.1007/s12079-009-0065-3
181 Liu, X. et al. (2004) Elastic fiber homeostasis requires lysyl oxidase-like 1 protein. *Nat. Genet.* **36**, 178–182, https://doi.org/10.1038/ng1297
182 Hasegawa, A. et al. (2017) Role of Fibulin 3 in aging-related joint changes and osteoarthritis pathogenesis in human and mouse knee cartilage. *Arthritis Rheumatol.* **69**, 576–585, https://doi.org/10.1002/art.39963
183 Chen, X. et al. (2014) Fibulin-3 suppresses Wnt/beta-catenin signaling and lung cancer invasion. *Carcinogenesis* **35**, 1707–1716, https://doi.org/10.1093/carcin/bgu023
184 Liu, H. et al. (2007) Augmented Wnt signaling in a mammalian model of accelerated aging. *Science* **317**, 803–806, https://doi.org/10.1126/science.1143578
185 Zhang, D.-Y., Wang, H.-J. and Tan, Y.-Z. (2011) Wnt/β-catenin signaling induces the aging of mesenchymal stem cells through the DNA damage response and the p53/p21 pathway. *PLoS ONE* **6**, e21397, https://doi.org/10.1371/journal.pone.0021397
186 Zhang, D.Y. et al. (2013) Wnt/beta-catenin signaling induces the aging of mesenchymal stem cells through promoting the ROS production. *Mol. Cell. Biochem.* **374**, 13–20, https://doi.org/10.1007/s11010-012-1498-1
187 Miao, J. et al. (2019) Wnt/beta-catenin/RAS signaling mediates age-related renal fibrosis and is associated with mitochondrial dysfunction. *Aging Cell* **18**, e13004, https://doi.org/10.1111/acel.13004
188 Gu, Z. et al. (2014) Wnt/beta-catenin signaling mediates the senescence of bone marrow-mesenchymal stem cells from systemic lupus erythematosus patients through the p53/p21 pathway. *Mol. Cell. Biochem.* **387**, 27–37, https://doi.org/10.1007/s11010-013-1866-5
189 Lehmann, M. et al. (2020) Chronic WNT/beta-catenin signaling induces cellular senescence in lung epithelial cells. *Cell. Signal.* **70**, 109588, https://doi.org/10.1016/j.cellsig.2020.109588
190 Suna, G. et al. (2018) Extracellular matrix proteomics reveals interplay of aggrecan and aggrecanases in vascular remodeling of stented coronary arteries. *Circulation* **137**, 166–183, https://doi.org/10.1161/CIRCULATIONAHA.116.023381
191 Yasmin, X. et al. (2018) The matrix proteins aggrecan and fibulin-1 play a key role in determining aortic stiffness. *Sci. Rep.* **8**, 8550, https://doi.org/10.1038/s41598-018-25851-5
192 Haydont, V. et al. (2019) Genome-wide profiling of adult human papillary and reticular fibroblasts identifies ACAN, Col XI α1, and PSG1 as general biomarkers of dermis ageing, and KANK4 as an exemplary effector of papillary fibroblast ageing, related to contractility. *Mech. Ageing Dev.* **177**, 157–181, https://doi.org/10.1016/j.mad.2018.06.003
193 Bensadoun, E.S. et al. (1996) Proteoglycan deposition in pulmonary fibrosis. *Am. J. Respir. Crit. Care Med.* **154**, 1819–1828, https://doi.org/10.1164/ajrccm.154.6.8970376
194 El-Arman, M.M. et al. (2010) Aggrecan and cartilage oligomeric matrix protein in serum and synovial fluid of patients with knee osteoarthritis. *HSS J.* **6**, 171–176, https://doi.org/10.1007/s11420-010-9157-0
195 Li, Y. et al. (2011) Severe lung fibrosis requires an invasive fibroblast phenotype regulated by hyaluronan and CD44. *J. Exp. Med.* **208**, 1459–1471, https://doi.org/10.1084/jem.20102510
196 Tsuneki, M. and Madri, J.A. (2016) CD44 influences fibroblast behaviors via modulation of cell-cell and cell-matrix interactions, affecting Survivin and Hippo pathways. *J. Cell. Physiol.* **231**, 731–743, https://doi.org/10.1002/jcp.25123
197 Tucker, R.P. et al. (2006) Phylogenetic analysis of the tenascin gene family: evidence of origin early in the chordate lineage. *BMC Evol. Biol.* **6**, 60, https://doi.org/10.1186/1471-2148-6-60
198 Tucker, R.P. and Chiquet-Ehrismann, R. (2009) The regulation of tenascin expression by tissue microenvironments. **1793**, 888–892
199 Schellings, M. (2004) Matricellular proteins in the heart: possible role during stress and remodeling. *Cardiovasc. Res.* **64**, 24–31, https://doi.org/10.1016/j.cardiores.2004.06.006
200 Podesser, B.K. et al. (2018) Tenascin-C promotes chronic pressure overload-induced cardiac dysfunction, hypertrophy and myocardial fibrosis. *J. Hypertens.* **36**, 847–856, https://doi.org/10.1097/HJH.0000000000001628
201 Shimojo, N. et al. (2015) Tenascin-C may accelerate cardiac fibrosis by activating macrophages via the integrin αVβ3/nuclear factor–κB/interleukin-6 axis. *Hypertension* **66**, 757–766, https://doi.org/10.1161/HYPERTENSIONAHA.115.06004
202 Kasprzycka, M., Hammarström, C. and Haraldsen, G. (2015) Tenascins in fibrotic disorders—from bench to bedside. *Cell Adh Migr* **9**, 83–89
203 Fu, H. et al. (2017) Tenascin-C is a major component of the fibrogenic niche in kidney fibrosis. *J. Am. Soc. Nephrol.* **28**, 785–801, https://doi.org/10.1681/ASN.2016020165
204 Midwood, K.S. et al. (2011) Advances in tenascin-C biology. *Cell. Mol. Life Sci.* **68**, 3175–3199, https://doi.org/10.1007/s00018-011-0783-6
205 Zuliani-Alvarez, L. et al. (2017) Mapping tenascin-C interaction with toll-like receptor 4 reveals a new subset of endogenous inflammatory triggers. *Nat. Commun.* **8**, 1595, https://doi.org/10.1038/s41467-017-01718-7
206 Swindle, C.S. et al. (2001) Epidermal growth factor (EGF)-like repeats of human tenascin-C as ligands for EGF receptor. *J. Cell Biol.* **154**, 459–468, https://doi.org/10.1083/jcb.200103103
207 Bhattacharyya, S. et al. (2013) Toll-like receptor 4 signaling augments transforming growth factor-β responses. *Am J Pathol.* **182**, 192–205
208 Bhattacharyya, S. et al. (2016) Tenascin-C drives persistence of organ fibrosis. *Nat. Commun.* **7**, 11703, https://doi.org/10.1038/ncomms11703
209 Ebener, S. et al. (2017) Toll-like receptor 4 activation attenuates profibrotic response in control lung fibroblasts but not in fibroblasts from patients with IPF. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **312**, L42–L55, https://doi.org/10.1152/ajplung.00119.2016

210 Shi, M. et al. (2015) Tenascin-C induces resistance to apoptosis in pancreatic cancer cell through activation of ERK/NF-kappaB pathway. *Apoptosis* **20**, 843–857, https://doi.org/10.1007/s10495-015-1106-4
211 De Laporte, L. et al. (2013) Tenascin C promiscuously binds growth factors via its fifth fibronectin type III-like domain. *PLoS ONE* **8**, e62076, https://doi.org/10.1371/journal.pone.0062076
212 Wollin, L. et al. (2015) Mode of action of nintedanib in the treatment of idiopathic pulmonary fibrosis. *Eur. Respir. J.* **45**, 1434–1445, https://doi.org/10.1183/09031936.00174914
213 Yamaguchi, Y., Mann, D.M. and Ruoslahti, E. (1990) Negative regulation of transforming growth factor-beta by the proteoglycan decorin. *Nature* **346**, 281–284, https://doi.org/10.1038/346281a0
214 Ruoslahti, E. and Yamaguchi, Y. (1991) Proteoglycans as modulators of growth factor activities. *Cell* **64**, 867–869, https://doi.org/10.1016/0092-8674(91)90308-L
215 Reszegi, A. et al. (2020) Protective role of decorin in primary hepatocellular carcinoma. *Front. Oncol.* **10**, 645, https://doi.org/10.3389/fonc.2020.00645
216 Baghy, K., Iozzo, R.V. and Kovalszky, I. (2012) Decorin-TGF B axis in hepatic fibrosis and cirrhosis. *J Histochem Cytochem.* **60**, 262–268, https://doi.org/10.1369/0022155412438104
217 Huttenlocher, A. et al. (1996) Decorin regulates collagenase gene expression in fibroblasts adhering to vitronectin. *Matrix Biol.* **15**, 239–250, https://doi.org/10.1016/S0945-053X(96)90115-8
218 De Luca, A. et al. (1996) Decorin-induced growth suppression is associated with up-regulation of p21, an inhibitor of cyclin-dependent kinases. *J. Biol. Chem.* **271**, 18961–18965, https://doi.org/10.1074/jbc.271.31.18961
219 Liang, S. et al. (2013) Human decorin regulates proliferation and migration of human lung cancer A549 cells. *Chin. Med. J.* **126**, 4736–4741
220 Shi, X. et al. (2015) Decorin is responsible for progression of non-small-cell lung cancer by promoting cell proliferation and metastasis. *Tumour Biol.* **36**, 3345–3354, https://doi.org/10.1007/s13277-014-2968-8
221 Woldhuis, R.R. et al. (2020) Link between increased cellular senescence and extracellular matrix changes in COPD. *Am. J. Physiol. Lung Cell. Mol. Physiol.* **319**, 48–60, https://doi.org/10.1152/ajplung.00028.2020
222 Honardoust, D. et al. (2012) Deep dermal fibroblasts refractory to migration and decorin-induced apoptosis contribute to hypertrophic scarring. *J. Burn Care Res.* **33**, 668–677, https://doi.org/10.1097/BCR.0b013e31824088e3
223 Kehlet, S.N. et al. (2017) Cathepsin-S degraded decorin are elevated in fibrotic lung disorders – development and biological validation of a new serum biomarker. *BMC Pulm. Med.* **17**, 110, https://doi.org/10.1186/s12890-017-0455-x
224 Maquart, F.X. et al. (2004) An introduction to matrikines: extracellular matrix-derived peptides which regulate cell activity. Implication in tumor invasion. *Crit. Rev. Oncol. Hematol.* **49**, 199–202, https://doi.org/10.1016/j.critrevonc.2003.06.007
225 Pouwels, S.D. et al. (2014) DAMPs activating innate and adaptive immune responses in COPD. *Mucosal Immunol.* **7**, 215–226, https://doi.org/10.1038/mi.2013.77
226 Matzinger, P. (1994) Tolerance, danger, and the extended family. *Annu. Rev. Immunol.* **12**, 991–1045, https://doi.org/10.1146/annurev.iy.12.040194.005015
227 Patel, S. (2018) Danger-associated molecular patterns (DAMPs): the derivatives and triggers of inflammation. *Curr. Allergy Asthma Rep.* **18**, 63, https://doi.org/10.1007/s11882-018-0817-3
228 Venereau, E., Ceriotti, C. and Bianchi, M.E. (2015) DAMPs from cell death to new life. *Front. Immunol.* **6**, 422, https://doi.org/10.3389/fimmu.2015.00422
229 Gong, T. et al. (2020) DAMP-sensing receptors in sterile inflammation and inflammatory diseases. *Nat. Rev. Immunol.* **20**, 95–112, https://doi.org/10.1038/s41577-019-0215-7
230 Chen, G.Y. and Nunez, G. (2010) Sterile inflammation: sensing and reacting to damage. *Nat. Rev. Immunol.* **10**, 826–837, https://doi.org/10.1038/nri2873
231 Frevert, C.W. et al. (2018) Danger-associated molecular patterns derived from the extracellular matrix provide temporal control of innate immunity. *J. Histochem. Cytochem.* **66**, 213–227, https://doi.org/10.1369/0022155417740880
232 De Lorenzo, G. et al. (2018) Extracellular DAMPs in plants and mammals: immunity, tissue damage and repair. *Trends Immunol.* **39**, 937–950, https://doi.org/10.1016/j.it.2018.09.006
233 Pankov, R. and Yamada, K.M. (2002) Fibronectin at a glance. *J. Cell Sci.* **115**, 3861–3863, https://doi.org/10.1242/jcs.00059
234 Okamura, Y. et al. (2001) The extra domain A of fibronectin activates Toll-like receptor 4. *J. Biol. Chem.* **276**, 10229–10233, https://doi.org/10.1074/jbc.M100099200
235 Bennett, V.D., Pallante, K.M. and Adams, S.L. (1991) The splicing pattern of fibronectin mRNA changes during chondrogenesis resulting in an unusual form of the mRNA in cartilage. *J. Biol. Chem.* **266**, 5918–5924
236 Moodley, Y.P. et al. (2019) Analysis by proteomics reveals unique circulatory proteins in idiopathic pulmonary fibrosis. *Respirology* **24**, 1111–1114, https://doi.org/10.1111/resp.13668
237 Nakstad, B., Boye, N.P. and Lyberg, T. (1990) Distribution of bronchoalveolar cells and fibronectin levels in bronchoalveolar lavage fluids from patients with lung disorders. *Scand. J. Clin. Lab. Invest.* **50**, 587–593, https://doi.org/10.3109/00365519009089175
238 Willems, S. et al. (2013) Multiplex protein profiling of bronchoalveolar lavage in idiopathic pulmonary fibrosis and hypersensitivity pneumonitis. *Ann. Thorac. Med.* **8**, 38–45
239 Bargagli, E. et al. (2014) Serum analysis of coagulation factors in IPF and NSIP. *Inflammation* **37**, 10–16, https://doi.org/10.1007/s10753-013-9706-z
240 Hisatomi, K. et al. (2009) Elevated levels of tenascin-C in patients with cryptogenic organizing pneumonia. *Intern. Med.* **48**, 1501–1507, https://doi.org/10.2169/internalmedicine.48.2233

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https://doi.org/10.1042/CS20190893

241 Boukpessi, T. et al. (2008) The effect of stromelysin-1 (MMP-3) on non-collagenous extracellular matrix proteins of demineralized dentin and the adhesive properties of restorative resins. *Biomaterials* **29**, 4367–4373, https://doi.org/10.1016/j.biomaterials.2008.07.035
242 Monfort, J. et al. (2006) Degradation of small leucine-rich repeat proteoglycans by matrix metalloprotease-13: identification of a new biglycan cleavage site. *Arthritis Res. Ther.* **8**, R26, https://doi.org/10.1186/ar1873
243 Inohara, N. et al. (2005) NOD-LRR proteins: role in host-microbial interactions and inflammatory disease. *Annu. Rev. Biochem.* **74**, 355–383, https://doi.org/10.1146/annurev.biochem.74.082803.133347
244 Moreth, K., Iozzo, R.V. and Schaefer, L. (2012) Small leucine-rich proteoglycans orchestrate receptor crosstalk during inflammation. *Cell Cycle* **11**, 2084–2091, https://doi.org/10.4161/cc.20316
245 Cazzola, M. et al. (2018) Senolytic drugs in respiratory medicine: is it an appropriate therapeutic approach? *Expert Opin. Investig. Drugs* **27**, 573–581, https://doi.org/10.1080/13543784.2018.1492548
246 Knoppert, S.N. et al. (2019) Cellular senescence and the kidney: potential therapeutic targets and tools. *Front. Pharmacol.* **10**, 770, https://doi.org/10.3389/fphar.2019.00770
247 Justice, J.N. et al. (2019) Senolytics in idiopathic pulmonary fibrosis: results from a first-in-human, open-label, pilot study. *EBioMedicine* **40**, 554–563, https://doi.org/10.1016/j.ebiom.2018.12.052
248 Hoonhorst, S.J.M. et al. (2016) Advanced glycation endproducts and their receptor in different body compartments in COPD. *Respir Res.* **17**, 46, https://doi.org/10.1186/s12931-016-0363-2
249 Faiz, A. et al. (2019) AGER expression and alternative splicing in bronchial biopsies of smokers and never smokers. *Respir. Res.* **20**, https://doi.org/10.1186/s12931-019-1038-6
250 Millerand, M., Berenbaum, F. and Jacques, C. (2019) Danger signals and inflammaging in osteoarthritis. *Clin. Exp. Rheumatol.* **37**, 48–56
251 Wang, Z.N. et al. (2020) Potential role of cellular senescence in asthma. *Front. Cell Dev. Biol.* **8**, 59, https://doi.org/10.3389/fcell.2020.00059
252 Majewski, S. et al. (2019) Epithelial alarmins in serum and exhaled breath in patients with idiopathic pulmonary fibrosis: a prospective one-year follow-up cohort study. *J. Clin. Med.* **8**, 1590, https://doi.org/10.3390/jcm8101590
253 Calhoun, C. et al. (2016) Senescent cells contribute to the physiological remodeling of aged lungs. *J. Gerontol. A Biol. Sci. Med. Sci.* **71**, 153–160, https://doi.org/10.1093/gerona/glu241
254 Fane, M. and Weeraratna, A.T. (2020) How the ageing microenvironment influences tumour progression. *Nat. Rev. Cancer* **20**, 89–106, https://doi.org/10.1038/s41568-019-0222-9
255 Burgstaller, G. et al. (2017) The instructive extracellular matrix of the lung: basic composition and alterations in chronic lung disease. *Eur. Respir. J.* **50**, https://doi.org/10.1183/13993003.01805-2016
256 Sivakumar, P., Kitson, C. and Jarai, G. (2019) Modeling and measuring extracellular matrix alterations in fibrosis: challenges and perspectives for antifibrotic drug discovery. *Connect. Tissue Res.* **60**, 62–70, https://doi.org/10.1080/03008207.2018.1500557
257 Feng, T. et al. (2019) CCN1-induced cellular senescence promotes heart regeneration. *Circulation* **139**, 2495–2498, https://doi.org/10.1161/CIRCULATIONAHA.119.039530
258 Kim, K.H. et al. (2013) Matricellular protein CCN1 promotes regression of liver fibrosis through induction of cellular senescence in hepatic myofibroblasts. *Mol. Cell. Biol.* **33**, 2078–2090, https://doi.org/10.1128/MCB.00049-13
259 Rapisarda, V. et al. (2017) Integrin Beta 3 regulates cellular senescence by activating the TGF-beta pathway. *Cell Rep.* **18**, 2480–2493, https://doi.org/10.1016/j.celrep.2017.02.012
260 Lehmann, M. et al. (2018) Differential effects of Nintedanib and Pirfenidone on lung alveolar epithelial cell function in ex vivo murine and human lung tissue cultures of pulmonary fibrosis. *Respir. Res.* **19**, 175, https://doi.org/10.1186/s12931-018-0876-y
261 Knuppel, L. et al. (2017) A novel antifibrotic mechanism of nintedanib and pirfenidone. Inhibition of collagen fibril assembly. *Am. J. Respir. Cell Mol. Biol.* **57**, 77–90, https://doi.org/10.1165/rcmb.2016-0217OC
262 Zhang, Y. et al. (2019) Histopathological and molecular analysis of idiopathic pulmonary fibrosis lungs from patients treated with pirfenidone or nintedanib. *Histopathology* **74**, 341–349, https://doi.org/10.1111/his.13745
263 Philp, C.J. et al. (2018) Extracellular matrix cross-linking enhances fibroblast growth and protects against matrix proteolysis in lung fibrosis. *Am. J. Respir. Cell Mol. Biol.* **58**, 594–603, https://doi.org/10.1165/rcmb.2016-0379OC