The ECM path of senescence in aging: components and modifiers
Naama Levi¹, Nurit Papismadov¹, Inna Solomonov², Irit Sagi² and Valery Krizhanovsky¹

1 Department of Molecular Cell Biology, Weizmann Institute of Science, Rehovot, Israel
2 Biological Regulation, Weizmann Institute of Science, Rehovot, Israel

Keywords
aging; cellular senescence; ECM remodeling; extracellular matrix; tissue repair

Introduction
The extracellular matrix (ECM) is a noncellular component in all organs and tissues. In addition to its structural role as a physical scaffold for cellular constituents, the ECM participates in numerous of biological functions. The mammalian ECM is composed of around thousand proteins, including ECM-associated proteins. The ECM is dynamic, both in the normal physiology of tissues, and under pathological circumstances. It is exposed to constant regulation manifested by deposition, modification, and degradation of its components. The main regulatory proteins of the ECM are remodeling enzymes, in particular the matrix metalloproteinases (MMPs) and a disintegrin and metalloproteinase with thrombospondin motifs (ADAMTS) families [1-5]. Cellular senescence is associated with changes in both the ECM components and remodeling enzymes expression and secretion [6].

Cellular senescence is a stable state of cell cycle arrest [7-10]. It was first described by Hayflick and
Moorhead at 1961, who demonstrated that human fibroblasts remain viable for many weeks in culture, even after completion of their finite number of cellular divisions [11]. Various triggers can lead cells to enter into senescence, including DNA replication stress, telomere dysfunction, oncogene activation, oxidative stress, and cell–cell fusion [8,12]. The senescence growth arrest is established and maintained by two tumor suppressor pathways, the p53-p21 and p16-pRB pathways. While both pathways can establish the growth arrest, their relative contribution might differ among cell types and between stimuli used to trigger cellular senescence [12,13]. On one hand, short-term induction of cellular senescence can be beneficial in various settings, for example, in tumor suppression, wound healing and in embryonic development. On the other hand, the long-term persistence of senescent cells can be deleterious and promote tumorigenesis and the development of age-related diseases [8]. Senescent cells have both autocrine and paracrine effects, as they can produce a variety of characteristic secreted factors, such as cytokines, chemokines, growth factors, and proteases. These factors are collectively termed the senescence-associated secretory phenotype (SASP) [14]. While cytokines and growth factors are present in the extracellular space and associated with ECM, they are widely discussed and thus would not constitute part of this review.

At present, little is known about the specific interplay between senescent cells and the ECM. Importantly, changes in ECM structure and content occur in physiological and pathological conditions, in which senescent cells accumulate. Senescent cells in humans and in mice secrete MMPs as part of their SASP [15]. This might imply for duality between the ECM-senescent cells interactions. On the one hand, the ECM can signal and affect senescent cells, and on the other hand, senescent cells affect the ECM composition and structure through their secretome. These secreted pro-inflammatory components can promote age-related fibrosis. Therefore, senescent cells might affect ECM composition in a cell nonautonomous manner. While fibroblasts are main producers of ECM, the changes observed in senescent fibroblasts are not necessarily would be a part of the senescence phenotype in other cell types. Discussion of the current research progress on the boundary between ECM and senescence will facilitate further investigation of this crosstalk that is an important regulator in aging and many pathological processes. Here, we will discuss the effects of different ECM constituents on senescence cells in aging and age-associated diseases.

**Collagens**

Collagens are the most abundant ECM proteins in the organism and major structural proteins of the ECM. They have an essential role in providing structural integrity, tensile strength, and stiffness to the tissues, as well as in processes such as cell adhesion, chemotaxis, and migration [1,4,16,17]. Collagen monomer is a triple helix molecule, which is composed of three α chains, made of domains with a repetitious amino acid sequence Gly-X-Y, where X and Y can be any amino acid [18,19]. In vertebrates, more than 49 genes encode collagen α chains, which allow the formation of at least 28 different collagen types. Collagens assemble into hierarchical structures, forming fibrils (collagen types I, II, III, V, and XI) or networks of different morphologies. Interestingly, collagen expression is decreased in senescent cells in various experimental systems (Fig. 1). In the retina, main ECM producers – retinal pigment epithelial cells (RPE) – can undergo senescence, accompanied by a decreased expression in COL1A1, COL1A2, and COL3A1 mRNA levels. Therefore, the decline in the ECM components

![Fig. 1. Changes in ECM components in senescent cells.](image)

Senescent cells accumulate in different tissues during pathological conditions. The presence of senescent cells affects the levels of collagens, GP, and ECM-associated proteins in the tissues of their presence. The arrows near the names of the ECM components indicate the direction of change of the component as a result of the presence of senescent cells. The changes in the ECM components levels and composition might affect senescent cells as well as indicated by the bidirectional arrows between senescent cells and the ECM components.
expression in senescent RPE cells has a negative effect on the retinal structure, which might cause the development of age-related macular degeneration [20]. In the liver, human senescent hepatic stellate cells (HSCs) have reduced expression in collagen type I mRNA and protein levels [21]. A microarray analysis has also shown a decreased expression of COL3A1, COL4A1, COL4A2, and COL5A1 in senescent HSCs [22]. In the skin, overexpression of the microRNA miR-181 induced senescence in normal human dermal fibroblast cells and downregulated the expression of COL16A1, which is a direct target of the miR-181 [23]. Likewise, collagen levels were decreased when senescent cells are eliminated in a model of liver fibrosis [24]. However, other studies have shown an upregulation of collagen components in senescent cells. For example, the α1(I) procollagen (encoded by the COL1A1 gene) mRNA levels were upregulated in both senescent human diploid fibroblasts and in replicative senescence of Werner syndrome (WS) fibroblasts, indicating that COL1A1 might have a role in the senescence cell cycle arrest [25]. In a different study, the levels of α1(I) procollagen mRNA were increased in WI-38 and IMR-90 HDFs that were exposed to tert-butylhydroperoxide and H2O2 in order to induce senescence [26]. Therefore, the expression of collagens is altered in senescent cells.

The crosstalk between senescent cells and collagen levels is not limited to the effect of senescence on collagen expression. In fact, collagen levels might affect the entrance of cells to senescence. The vascular smooth muscle cells (SMCs) in the aortic wall of Col1A1+/− mice, which express collagenase-resistant form of collagen type I, were more sensitive to stress-induced senescence than SMCs in wild-type (WT) mice. Additionally, culturing vascular SMCs from patients with mouse embryonic fibroblasts (MEFs) that were produced from Col1A1+/− promote senescence of the SMCs [27]. This study indicates that upregulation of collagen might play a role in induction of senescence in vivo. All the aforementioned findings imply for a bidirectional interaction of collagen and senescent cells. On the one hand, senescent cells show a decreased expression in different collagens and on the other hand, the senescence state can be induced by elevation in collagen. Further research will provide better understanding of the interactions between senescent cells and collagen.

**Glycoproteins (GP)**

Glycoproteins (GP) is a subcategory of ECM components. They participate in ECM structural formation, assist in ECM–cell interactions, and retain different growth factors that can be activated by remodeling enzymes (Fig. 1).

**Fibronectin**

Fibronectin (FN) is a large highly abundant GP, which plays one of the major roles in proper ECM assembly. It can bind a variety of ECM molecules such as collagen I and III, gelatin, thrombospondin, heparin, decorin, and latent transforming growth factor-β protein-1 [29-32]. FN is secreted as a dimer, but assembles in interwoven fibers which are a functional structure of the protein [28]. FN has an important role in multiple cell functions including cell adhesion, migration, growth, and differentiation [1,33]. FN mediates cellular activities through interaction with integrin receptors or syndecans located on the cell surface. While FN is encoded by a single gene, the protein was found in many different forms since its mRNA can undergo alternative splicing [34]. Changes in FN levels have been found in senescent cells. Interestingly, the trend of these changes is highly similar to the trend that has been reported regarding collagen. In the liver fibrosis studies mentioned above reduction in FN levels expression and secretion was found in human HSCs using a variety of experimental methods [21,22]. On the other hand, several experimental systems showed upregulation in FN levels during senescence. Both senescent HDFs and replicative senescent WS fibroblasts exhibited upregulation of FN mRNA levels [25,26]. An increase in FN mRNA levels was also observed in HDFs exposed to repeated stresses with Ultraviolet B (UVB) [35]. These results, which present the duality in FN expression in senescent cells, similarly to collagen expression, might be explained by the difference between cell populations. In the case of human HSCs, for example, the reduction in FN levels can reflect strong inflammatory phenotype of the HSCs, instead of a fibrogenic phenotype [21,22]. Therefore, FN and collagens might be coregulated in senescent cells.

**Laminin**

Laminins are large heterotrimeric GP. They are major component of the basal lamina, one of the layers of the basement membrane, and are essential to its proper structure and function. Laminins help to mediate processes such as cell adhesion, migration, differentiation, and proliferation, using several specific surface receptors. Each isoform of laminin consists of
three chains, α, β, and γ, and is named by its chain composition. At least 16 different such isoforms are known in vertebrates [36,37]. Several studies have reported an increase in laminin levels in correlation with cellular senescence. In senescent bovine aortic endothelial cells (BAEC), the laminin-γ mRNA was highly expressed compared to early passage BAEC [38]. In the age-related pathology of idiopathic pulmonary fibrosis (IPF), high expression of cellular senescence markers, such as p16, p21, and SA-β-gal activity, was found in sites of fibroblast foci and localized to discrete clusters of bronchiolar basal cells, expressing very high levels of laminin-5-γ2-chain (LAM5γ2) [39,40]. Additionally, in the skin, p16 and LAM5γ2 have been reported to play a role in keratinocytes cells hypermotility and growth arrest, both activated during the process wound healing [41]. Altogether, laminins are upregulated in senescent cells. This upregulation might contribute to the progression of age-related pathologies.

Elastin

Elastin is a self-assembling protein, synthesized as a monomer called tropoelastin that is converted into an elastin polymeric fibers through irreversible crosslinking reaction by the enzyme lysyl oxidase. It has a central role in providing tensile strength, firmness, and suppleness of tissues, which are essential for proper organ functions [42]. Elastin is the most abundant ECM protein in the lung and is crucial for the formation of the alveoli in mice [4]. As a structural component of the alveoli, it is strongly dysregulated during chronic obstructive pulmonary disease (COPD). COPD is an age-related lung pathology, which cause a progressive airflow limitation in the lungs. COPD pathology is characterized by the accumulation of senescent cells and by a reduction in the proliferative capacity of the mesenchymal precursors in the alveolar parenchyma. Combined, these two factors limit ECM production in the lungs, including elastin. One major pathological component of COPD is emphysema, a progressive dilation of the alveolar spaces and parenchymal remodeling. It has been suggested that emphysema is linked to the reduction in both ECM components production and regulation through remodeling. Reduction in ECM remodeling eventually interrupts the repair and maintenance of the connective and epithelial tissues and leads to a connective tissue insufficiency. Cellular senescence might be the cause for the reduction in ECM remodeling, mainly by effecting mesenchymal cells function. That leads to a progressive reduction in the production of ECM proteins [39]. These findings reflect the importance of elastin in maintaining normal tissue function and provide an insight into the destructive implications of its senescence-associated downregulation.

Thrombospondin 1

Thrombospondins (TSPs) are multidomain GP. The TSPs family consists of five members, TSP-1-5. They can be divided into two general groups based on their structure. Group A consists TSP-1 and TSP-2, which form homotrimers, and group B, which include TSP-3-5, form homopentamers. TSPs have multiple functions and can bind a variety of ECM molecules. The first member of this family is TSP-1, which we will discuss in this review. TSP-1 takes a part in processes such as inflammatory response, platelet aggregation, and angiogenesis. It is expressed in senescent cells and can affect the senescence state [43,44]. Senescent human peritoneal mesothelial cells (HPCMs) show increased TSP-1 mRNA and protein levels as well as increased release of the protein into these cells’ culture media [45]. Another study has shown the importance of TSP-1 in a mouse model of Kras-induced senescence [46]. KrasG12D;Tsp-1+/− and KrasG12D;Tsp-1−/− mice were administered with adenovirus-Cre recombinase (Ad-Cre) through intranasal inhalation in order to initiate tumor formation in the lungs. Lung lesions of KrasG12D;Tsp-1−/− presented lack of Bromodeoxyuridine (BrdU) incorporation and positive SA-β-gal staining compared to KrasG12D;Tsp-1+/−, which exhibited the opposite trend. Additionally, lung fibroblasts that were produced from KrasG12D;Tsp-1+/+ mice and exposed to the Ad-Cre, showed increased SA-β-gal activity and typical senescent morphology compared to the KrasG12D;Tsp-1−/− cells, indicating that Kras-induced senescence is abolished with the loss of TSP-1 [46]. TSP-1 is a potent inhibitor of angiogenesis and its activity is mediated by its receptor CD47. A recent study has examined the role of TSP1-CD47 signaling pathway in the senescence state. Experiments on primary endothelial cells (ECs) that were produced from WT or CD47−/− mice brains, revealed that in addition to angiogenesis inhibition, the TSP1-CD47 pathway also accelerates replicative senescence in WT ECs. This finding shines a light on EC involvement in age-associated diseases and suggests a novel strategy of altering the TSP1-CD47 signaling pathway, as an approach to alleviate these diseases [47]. Altogether, TSP-1 plays an important role in mediating cell senescence, in particular in promoting their cell cycle arrest.
**Osteonectin**

Osteonectin, also known as secreted protein acidic and rich in cysteine or BM-40, is expressed in many types of cells and among its functions are cell cycle inhibition and growth factor activity regulation. It is a counter-adhesive protein, meaning, that under certain conditions, it can alter cell-substrate interactions. In adults, osteonectin expression is largely limited to tissues that undergo repair or remodeling [48,49]. Osteonectin is encoded by a single gene, which is highly conserved between species. It composed of three distinct domains: N-terminal acidic domain, Cys-rich domain, follistatin-like domain, and an extracellular calcium binding domain [49]. According to several studies, osteonectin levels are upregulated in senescence cells. Similarly to collagen and FN, osteonectin mRNA levels were found to be increased in senescent HDFs and in replicative senescent WS fibroblasts [25,50], as well as in senescent WI-38 and IMR-90 HDFs compared to nonsenescent cells [26]. In addition, similar to FN, the mRNA levels of osteonectin were also upregulated in senescent HDFs that were induced by UVB [35]. Interestingly, osteonectin has been reported to be involved in the senescence mechanism of colorectal cancer cells. In response to low concentration of the chemotropic drug, Irinotecan (CPT-11), cancer cells underwent cell cycle arrest and entered cellular senescence, in an osteonectin-dependent manner [51]. The activity of osteonectin has been found to be mediated by p53. Furthermore, mice with xenografts tumor treated with CPT-11 and osteonectin appeared to have an increase in the number senescent cells, accompanied by tumor regression [51]. The role of osteonectin as a cell cycle inhibitor and the fact that it is induced by stress are consistent with its upregulation in senescent cells. The identification of its anticancer activity by senescence induction following the combination treatment might lead to further research, in order to understand whether such mechanism can be exploited for cancer treatment.

**ECM-associated proteins**

ECM-associated proteins provide multiple inputs into cells to control survival, proliferation, differentiation, shape, polarity, and motility of cells. They include ECM-modifying enzymes, such as proteases, or cross-linking enzymes, growth factors and different secreted factors (Fig. 1).

**CCN family**

The CCN proteins are the family of secreted ECM-associated proteins that are involved in a wide range of biological processes such as proliferation, adhesion, migration, apoptosis, ECM production, angiogenesis, chondrogenesis, and osteogenesis [52,53]. The CCN proteins are also involved in tissue pathology-related processes and fibrotic disorders. The family consists of six members, CCN1-6, which are named by their order of discovery. In general, each protein is assembled from the following modules: an insulin-like growth factor binding protein, a Von Willebrand factor domain, a thrombospondin-homology domain and a cysteine knot, heparin-binding domain, modules 1-4, respectively [52-54]. Research from the recent years has found that CCN1 and CCN2 can induce senescence and mediate their action, at least partly, through interaction with integrin receptors [55]. CCN1 has also been reported to take part in collagen homeostasis in replicative senescent human skin dermal fibroblasts. Senescent dermal fibroblasts have been shown to elevate CCN1 and MMP1 mRNA and protein levels, and to reduce procollagen type 1 mRNA and protein levels, compared to presenescent cells. Nevertheless, performing a knockdown of CCN1 in replicative senescent dermal fibroblasts decreased the elevation of MMP1 protein level and increased the procollagen protein expression. These findings indicate the regulatory role of CCN1 both in production of collagen and the ability to its degradation through regulation of protease expression [56]. Examination of Ccn1^dm/dm (CCN1 mutant, unable to bind integrin α6β1) mice and WT mice granulation tissues during cutaneous wound healing revealed the requirement of CCN1 for the accumulation of senescent fibroblast cells. In addition, granulation tissues of Ccn1^dm/dm mice have been more fibrogenic than the WT mice tissues, while topical application of CCN1 induced senescence and reversed the profibrotic phenotype. Additionally, CCN1 induced senescence in human BJ fibroblasts through integrin-mediated signaling [57]. CCN1 also limited liver fibrosis through induction of myofibroblasts senescence. Ccn1^A Hep mice, which express hepatocyte-specific Ccn1 deletion, exhibited more fibrogenic tissues with less amount of senescence cells following induction of liver fibrosis by either CCL4 or bile duct ligation. In both cases, Ccn1^A Hep mice developed stronger fibrosis, indicating that CCN1 induces cellular senescence in order to limit liver fibrosis [58]. Similarly to CCN1, CCN2 can induce senescence in human BJ fibroblasts. Moreover, CCN2 treatment of cutaneous wounds of Ccn1^dm/dm mice led to an increase in the senescent cells amount and a decrease in the fibrogenic phenotype [59]. Therefore, CCN1 and CCN2 might limit fibrosis through senescence induction. These findings may constitute a platform for new treatments for...
fibrotic diseases based on those proteins. Furthermore, given that collagen may be downregulated in aging, CCN1 could be a potential therapeutic approach for age-related skin pathologies.

**MMPs**

Matrix metalloproteinases are a family of zinc-dependent endoproteinases, which are found across all kingdoms of life. The prototype of MMPs, collagenase, was first discovered at 1962 in amphibian tissue. To date, 23 MMPs have been identified in humans, including, among others, the subgroups of collagenases, gelatines, stromelysins, matrilysins, and membrane-type MMPs [60,61]. In general, most of these enzymes consist of three well-conserved domains: amino-terminal propeptide domain, catalytic domain, and hemopexin-like domain [61,62]. MMPs have the ability irreversibly degrade the ECM components and shed ectodomains of cell surface receptors. In addition, MMPs process and activate cytokines, chemokines, and other ECM-associated proteins. In healthy tissues, MMP expression and activity are tightly regulated at different levels, starting from gene expression all the way to zymogen activation and endogenous inhibition [63]. In pathological conditions such as wounds, inflammation, vascular diseases, and cancer, MMPs dysregulation is observed at all the levels [62,64-66]. Numerous studies have linked cellular senescence with increased expression of MMP family members. Late passage senescent and WS fibroblasts upregulated collagenase MMP1 and the stromelysin MMP3 on mRNA and protein levels in comparison to early passage fibroblasts [67]. Senescent HSCs upregulate the mRNA levels of MMP1, MMP2, and MMP10 compared to HSCs that are immortalized by ectopic expression of telomerase. Furthermore, MMP8 and MMP12 mRNA levels and MMP2 protein levels were higher in senescent HSCs compared to early-activated HSCs [21]. Microarray analysis has shown upregulation of MMP1, MMP3, MMP10, and MMP12 in senescent activated HSCs compared to proliferating cells [22]. Examination of the conditioned media (CM) of human replicated and ionizing irradiated senescent cells and mice ionizing irradiated senescent cells revealed an increase in the mRNA expression levels of MMP1, MMP3, MMP10, and MMP12, in comparison with human and mice presenescent cells. The protein expression level of MMP3 was also upregulated in human and mice ionizing irradiated senescent cells compared to presenescent cells [15]. Apparently, senescent cells upregulate multiple MMP proteins. Other studies have reported that downregulation of MMPs can promote cellular senescence. RNAi-mediated MMP9 knockdown in DAOY medulloblastoma cancer cell line resulted in inhibition of cell proliferation and invasion, as well as in promotion of cellular senescence, mediated by the p16 and ERK pathway [68]. A study that examined Mmp14 deficient mice identified cellular senescence in the kidney, adipose tissue, and fibroblasts which were produced from these mice. Of note, these Mmp14−/− mice display, among others, a failure in growth, cardiac defects, and severe metabolic changes. Treatment of Mmp14−/− fibroblasts with retinoic acid succeeded to delay the senescent phenotype in those cells [69]. Additional study has shown that mice that developed cigarette smoke-induced COPD-like condition presented increase mRNA levels of the senescence marker p16, as well as MMP12 [70]. Several studies connected MMPs expression and senescence in human pathologies. MMPs were upregulated in senescent cells isolated from patients suffering from intervertebral disc (IVD) degeneration, which might serve as a major cause to low back pain. Degenerated IVD tissues presented accelerated senescence, which was manifested, among others, by elevation in SA-β-gal and p16 staining compared to age-matched nondegenerated IVDs. A significant positive correlation has been found between p16 and MMP13, showing the correlation between the senescence phenotype to matrix degrading enzyme expression [71]. Examination of IVD cells has shown that a long-term exposure of these cells to H2O2 induced premature senescence and resulted in increased expression levels of MMP1, MMP2, and MMP9 and, in contrast to the former study, a decrease in the expression levels of MMP13 [72]. Examination of human oral submucous fibrosis (OSMF) biopsies, an oral precancerous condition, showed elevated production of MMP1 and MMP2 in the senescent OSMF fibroblasts population [73]. Moreover, an active form of MMP2 was detected in the CM of senescent cancer-associated fibroblasts (CAFs) derived from genetically unstable oral squamous cell carcinomas, and not in the CM of nonsenescent fibroblasts derived from normal oral mucosa or genetically stable carcinomas. Inhibition of MMP2 in senescent CAFs CM in vitro was found to reduce the capacity of these cells to induce keratinocytes dissociation as well as to induce invasion. These results show the role of MMP2 in mediating the more aggressive cancer phenotype induced by senescent CAFs [74]. Altogether, expression of MMPs is highly regulated in senescent cells under different circumstances, including various pathological conditions such as WS, IVD degeneration, fibrosis, and cancer. However, there the studies about MMPs activity in these conditions are
limited. The multifunctionality of these enzymes reflects the importance of further studies of their mechanism of action in cellular senescence.

**ADAMs and ADAMTSs**

A disintegrin and metalloproteinases transmembrane (ADAMs) and secreted ADAMs thrombospondin motifs (ADAMTSs) are families of enzymes that share the metalloproteinase domain with MMPs. ADAMs are well known for their ectodomain shedding activity but also for mediating nonproteolytic ligand binding. These functions are important in many biological processes such as cell fate determination, migration, proliferation, angiogenesis, wound healing, interaction between sperm and egg, heart development, and more. Forty ADAMs have been recognized in the mammalian genome [75,76]. One of the family members involved in a variety of physiological and pathophysiological processes, ADAM17, activates the release of two prominent SASP components, tumor necrosis factor receptor I and the epidermal growth factor receptor ligand, amphiregulin. This role of ADAM17 is independent of the cell type and of stimuli used for senescence induction [77]. Further research will help to uncover the molecular mechanisms, governed by ADAMs, that are activated in senescent cells, and how do they affect senescent cells, and their microenvironment in particular.

ADAMs thrombospondin motifs enzymes participate in collagen processing, matrix proteoglycans cleavage, angiogenesis inhibition, and blood coagulation homoeostasis. They are important in tissue development and maintenance, while mutations in the enzymes have been found to be associated with diseases. In mammals, the ADAMTS family consists of nineteen members [78,79]. There is only limited evidence for coordination between senescence and ADAMTS. Human IVD degenerated NP cells presented an increased expression in p16 and ADAMTS5 genes, and exhibited a significant positive correlation in the expression patterns, while no expression has been observed in nondegenerated cells [71]. Additionally, human NP IVD H2O2-induced premature senescent cells showed an increase in ADAMTS5 mRNA expression levels compared to control untreated cells [72]. The elevation in the degrading enzyme ADAMTS5, in correlation with induction of senescence in NP cells, might imply the role of cellular senescence in IVD degeneration pathology. Future investigation and understanding of the mechanism of how senescent cells promote degeneration in human IVD might lead to the development of therapeutic treatments.

**TIMPs**

Tissue inhibitors of metalloproteinases (TIMPs) are a family of endogenous inhibitors of MMPs [80]. As suggested by their name, they are inhibitors of metalloproteinases and several of the disintegrin-metalloproteinases, ADAMs and ADAMTSs, and therefore play an important role in the regulation of ECM turnover. Nonetheless, they also show activities that are not related to protease inhibition. TIMPs participate in modulation of cell growth, proliferation and migration and inhibition of cellular invasion, tumorigenesis, metastasis, and angiogenesis. The human TIMPs family consists of four members, which are also present in other mammals, named TIMP1-4. TIMPs are broad-spectrum inhibitors, since they are able to interact with a number of MMPs and ADAMs.

The computational and experimental studies of the TIMP1 and TIMP2 binding interface to MMPs showed its nonoptimal free energies of binding to eight MMP targets, suggesting that this is a property of all proteins evolved for binding to multiple targets [61,80-83]. Inverse expression pattern to MMPs, TIMPs might be downregulated in senescent cells. Lower expression levels of TIMPs were observed in replicative senescent human fibroblasts and WS fibroblasts [67,84]. Additionally, H2O2-induced premature senescent IVD cells have shown a reduction in the mRNA expression levels of TIMP1, TIMP2, and TIMP3 [72]. However, a study that found upregulation of MMP2 protein in human senescent HSCs has also found an increase of TIMP1 in these cells [21].

TIMPs are known to be highly inducible in response to many cytokines and hormones, suggesting that the autocrine activity of SASP might contribute to upregulation of their expression. A coordinated expression between MMPs and TIMPs in senescent cells might be explained by the importance of TIMPs in regulation of MMPs activity. The balance between MMPs and TIMPs affects tissue remodeling in physiological processes, as well as in pathological conditions that senescent cells are involved in.

**Conclusions**

In this review, we discussed evidence for multiple ECM–senescence relations. This crosstalk was observed in a wide range of human and mouse tissues and cells, and it is important in various pathophysiological conditions.

In senescent cells, there are diverse changes in the expression of different structural proteins. Collagen and FN have shown differential expression patterns in
Senescent cells depending on the cell type and the pathological condition cells where they are expressed. Remarkably, ECM-associated proteins, containing CCN family and proteases, were found to be upregulated in senescence in most conditions that were studied. However, downregulated expression of these components was also occasionally observed. Increased levels of the different MMPs, ADAM17, and ADAMTS5 were detected in different models of senescence. Further studies will identify the activity of these enzymes in these models. In contrast, their inhibitors, TIMPs, were mostly downregulated in senescence. Altogether, these observations reflect the complex interplay between different ECM components and ECM remodeling enzymes, and the presence of senescent cells. Both ECM and senescence play a role in aging, and in particular, in age-related pathologies such as OSMF, IPF, COPD, liver fibrosis, wound healing, IVD, and cancer.

The ECM-senescent cell crosstalk might contribute to the regulation of the presence of senescent cells in aging and age-related diseases. On one hand, ECM can influence cells entrance to senescence and therefore regulate the amount of senescent cells in the tissue. On the other hand, senescent cells secrete metalloproteinases as part of SASP, which may cause changes in the ECM and exacerbate pathology. Therefore, MMP inhibitors might provide a strategy to reduce ECM changes resulted from the presence of senescent cells and, together with senescent cells elimination [85], improve multiple pathological conditions.

Acknowledgements
VK was supported by grants from the Israel Science Foundation (634-15) and Sagol Institute for Longevity Research. VK is an Incumbent of The Georg F. Dukowitz Professorial Chair. ISa. is an Incumbent of the Maurizio Pontecorvo Professorial Chair and wants to acknowledge European Research Council (ERC) under the European Union’s Horizon 2020 research and innovation program (grant agreement No 695437), Israel Science Foundation (1800/19), the USA-Israel Binational Science Foundation (712506-01), Minerva and The Rising Tide Foundation.

Conflict of interest
The authors declare no conflict of interest.

Author contributions
NL, ISa, and VK came up with the idea of reviewing this topic. NL wrote the manuscript. NP and ISa contributed to the writing of the manuscript. VK and ISa contributed to writing the manuscript and supervised the project.

References
1 Frantz C, Stewart KM & Weaver VM (2010) The extracellular matrix at a glance. J Cell Sci 123 (Pt 24), 4195–4200.
2 Lu P, Takai K, Weaver V m & Werb Z (2011) Extracellular matrix degradation and remodeling in development and disease. Cold Spring Harb Perspect Biol 3, a005058.
3 Bosman FT & Stamenkovic I (2003) Functional structure and composition of the extracellular matrix. J Pathol 200, 423–428.
4 Bonnans C, Chou J & Werb Z (2014) Remodelling the extracellular matrix in development and disease. Nat Rev Mol Cell Biol 15, 786–801.
5 Hynes RO (2009) The extracellular matrix: not just pretty fibrils. Science 326, 1216–1219.
6 Campisi J (1998) The role of cellular senescence in skin aging. J Invest Dermatol Symp Proc 3, 1–5.
7 Campisi J (2013) Aging, cellular senescence, and cancer. Annu Rev Physiol 75, 685–705.
8 Burton DG & Krizhanovsky V (2014) Physiological and pathological consequences of cellular senescence. Cell Mol Life Sci 71, 4373–4386.
9 Munoz-Espin D & Serrano M (2014) Cellular senescence from physiology to pathology. Nat Rev Mol Cell Biol 15, 482–496.
10 Naylor RM, Baker DJ & van Deursen JM (2013) Senescent cells: a novel therapeutic target for aging and age-related diseases. Clin Pharmacol Ther 93, 105–116.
11 Hayflick L & Moorhead PS (1961) The serial cultivation of human diploid cell strains. Exp Cell Res 25, 585–621.
12 Campisi J & d’Adda di Fagagna F (2007) Cellular senescence: when bad things happen to good cells. Nat Rev Mol Cell Biol 8, 729–740.
13 Rodier F & Campisi J (2011) Four faces of cellular senescence. J Cell Biol 192, 547–556.
14 Coppe JP, Patil CK, Rodier F, Sun Y, Muñoz DP, Goldstein J, Nelson PS, Desprez P-Y & Campisi J (2008) Senescence-associated secretory phenotypes reveal cell-nonautonomous functions of oncogenic RAS and the p53 tumor suppressor. PLoS Biol 6, 2853–2868.
15 Coppe JP, Patil CK, Rodier F, Krutolica A, Beauséjour CM, Parrinello S, Hodgson JG, Chin K, Desprez P-Y & Campisi J (2010) A human-like senescence-associated secretory phenotype is conserved in mouse cells dependent on physiological oxygen. PLoS ONE 5, e9188.
16 Rozario T & DeSimone DW (2010) The extracellular matrix in development and morphogenesis: a dynamic view. Dev Biol 341, 126–140.
Senescent cells and ECM during aging

17 Gelse K, Poschl E & Aigner T (2003) Collagens—structure, function, and biosynthesis. *Adv Drug Deliv Rev* **55**, 1531–1546.
18 Heino J (2007) The collagen family members as cell adhesion proteins. *BioEssays* **29**, 1001–1010.
19 Gordon MK & Hahn RA (2010) Collagens. *Cell Tissue Res* **339**, 247–257.
20 Shelton DN, Chang E, Whittier PS, Choi D & Funk WD (1999) Microarray analysis of replicative senescence. *Curr Biol* **9**, 939–945.
21 Schnabl B, Purbeck CA, Choi YH, Hagedorn CH & Brenner D (2003) Replicative senescence of activated human hepatic stellate cells is accompanied by a pronounced inflammatory but less fibrogenic phenotype. *Hepatology* **37**, 653–664.
22 Krizhanovsky V, Yon M, Dickins RA, Hearn S, Simon J, Miething C, Yee H, Zender L & Lowe SW (2008) Senescence of activated stellate cells limits liver fibrosis. *Cell* **134**, 657–667.
23 Mancini M, Saintigny G, Mahé C, Annicchiarioco-Petruselli M, Melino G & Candi E (2012) MicroRNA-152 and -181a participate in human dermal fibroblasts senescence acting on cell adhesion and remodeling of the extra-cellular matrix. *Aging* **4**, 843–853.
24 Yosef R, Pipel N, Papismadov N, Gal H, Ovadya Y, Vadai E, Miller S, Porat Z, Ben-Dor S & Krizhanovsky V (2017) p21 maintains senescent cell viability under persistent DNA damage response by restraining JNK and caspase signaling. *EMBO J* **36**, 2280–2295.
25 Murano S, Thweatt R, Shmookler Reis RJ, Jones RA, Moerman EJ & Goldstein S (1991) Diverse gene sequences are overexpressed in werner syndrome fibroblasts undergoing premature replicative senescence. *Mol Cell Biol* **11**, 3905–3914.
26 Dumont P, Burton M, Chen QM, Gonos ES, Frippiat C, Mazarati J-B, Eliuares F, Remacle J & Toussaint O (2000) Induction of replicative senescence biomarkers by sublethal oxidative stresses in normal human fibroblast. *Free Radic Biol Med* **28**, 361–373.
27 Vafaie F, Yin H, O’Neil C, Nong Z, Watson A, Arpino J-M, Chu MWA, Wayne Holdsworth D, Gros R & Pickering JG (2014) Collagenase-resistant collagen promotes mouse aging and vascular cell senescence. *Aging Cell* **13**, 121–130.
28 Zollinger AJ & Smith ML (2017) Fibronectin, the extracellular glue. *Matrix Biol* **60–61**, 27–37.
29 Schmidt G, Hauser H & Kresse H (1991) Interaction of the small proteoglycan decorin with fibronectin. Involvement of the sequence NKISK of the core protein. *Biochem J* **280** (Pt 2), 411–414.
30 Velling T, Risteli J, Wennerberg K, Mosher DF & Johansson S (2002) Polymerization of type I and III collagens is dependent on fibronectin and enhanced by integrins α1β1 and α2β1. *J Biol Chem* **277**, 37377–37381.
31 Dallas SL, Sivakumar P, Jones CJ, Chen Q, Peters DM, Mosher DF, Humphries MJ & Kiely CM (2005) Fibronectin regulates latent transforming growth factor-beta (TGF beta) by controlling matrix assembly of latent TGF beta-binding protein-1. *J Biol Chem* **280**, 18871–18870.
32 Pankov R & Yamada KM (2002) Fibronectin at a glance. *J Cell Sci* **115** (Pt 20), 3861–3863.
33 Takahashi S, Leiss M, Moser M, Ohashi T, Kitao T, Heckmann D, Pfeifer A, Kessler H, Takagi J, Erickson HP et al. (2007) The RGD motif in fibronectin is essential for development but dispensable for fibril assembly. *J Cell Biol* **178**, 167–178.
34 Ffrench-Constant C (1995) Alternative splicing of fibronectin—many different proteins but few different functions. *Exp Cell Res* **221**, 261–271.
35 Chainiaux F, Magalhaes J-P, Eliaers F, Remacle J & Toussaint O (2002) UVB-induced premature senescence of human diploid skin fibroblasts. *Int J Biochem Cell Biol* **34**, 1331–1339.
36 Kim HJ, Ji B-R, Kim J-S, Lee H-N, Ha D-H & Kim C-W (2012) Proteomic analysis of proteins associated with cellular senescence by calorie restriction in mesenchymal stem cells. *In Vitro Cell Dev Biol Anim* **48**, 186–195.
37 Domogatskaya A, Rodin S & Tryggvason K (2012) Functional diversity of laminins. *Annu Rev Cell Dev Biol* **28**, 523–553.
38 Augustin-Voss HG, Voss AK & Pauli BU (1993) Senescence of aortic endothelial cells in culture: effects of basic fibroblast growth factor expression on cell phenotype, migration, and proliferation. *J Cell Physiol* **157**, 279–288.
39 Chilosi M, Carloni A, Rossi A & Poletti V (2013) Premature lung aging and cellular senescence in the pathogenesis of idiopathic pulmonary fibrosis and COPD/emphysema. *Transl Res* **162**, 156–173.
40 Chilosi M, Zamò A, Doglioni C, Reghellin D, Lestani M, Montagna L, Pedron S, Ennas MG, Cancellieri A, Murer B et al. (2006) Migratory marker expression in fibroblast foci of idiopathic pulmonary fibrosis. *Respir Res* **7**, 95.
41 Natarajan E, Omobono JD, Guo Z, Hopkinson S, Lazar AJF, Brenn T, Jones JC & Rheinwald JG (2006) A keratinocyte hypermotility/growth-arrest response involving laminin 5 and p16INK4A activated in wound healing and senescence. *Am J Pathol* **168**, 1821–1837.
42 Bellingham C & Keeley F (2004) Self-ordered polymerization of elastin-based biomaterials. *Cur Opin Solid State Mater Sci* **8**, 135–139.
43 Bornstein P (1992) Thrombospondins: structure and regulation of expression. *FASEB J* **6**, 3290–3299.
44 Adams JC & Lawler J (2004) The thrombospondins. *Int J Biochem Cell Biol* **36**, 961–968.
Senescent cells and ECM during aging

Page 5

45 Mikula-Pietrasik J, Sosińska P, Janus J, Rubiś B, Brewińska-Olchowiak M, Piołoca K & Kiszałek K (2013) Bystander senescence in human peritoneal mesothelium and fibroblasts is related to thrombospondin-1-dependent activation of transforming growth factor-beta1. Int J Biochem Cell Biol 45, 2087–2096.

46 Baek KH, Bhang D, Zaslavsky A, Wang L-C, Vachani A, Kim CF, Albelda SM, Evan GI & Ryeom S (2013) Thrombospondin-1 mediates oncogenic Ras-induced senescence in premalignant lung tumors. J Clin Invest 123, 4375–4389.

47 Gao Q, Chen K, Gao L, Zheng Y & Yang Y-G (2016) SPARC, a matricellular glycoprotein with important biological functions. J Histochem Cytochem 47, 1495–1506.

48 Brecken RA & Sage EH (2000) SPARC, a matricellular protein: at the crossroads of cell-matrix. Matrix Biol 19, 569–580.

49 Yan Q & Sage EH (1999) SPARC, a matricellular glycoprotein with important biological functions. J Histochem Cytochem 47, 1495–1506.

50 Lecka-Czerni B, Moerman EJ, Jones RA & Goldstein S (1996) Identification of gene sequences overexpressed in senescent and Werner syndrome human fibroblasts. Exp Gerontol 31, 159–174.

51 Chan JM, Ho SH & Tai IT (2010) Secreted protein acidic and rich in cysteine-induced cellular senescence in colorectal cancers in response to irinotecan is mediated by P53. Carcinogenesis 31, 812–819.

52 Brigstock DR (1999) The connective tissue growth factor/cysteine-rich 61/nephroblastoma overexpressed (CCN) family. Endocr Rev 20, 189–206.

53 Eskinazi C, Abraham DJ (2006) All in the CCN family: essential matricellular signaling modulators emerge from the bunker. J Cell Sci 119 (Pt 23), 4803–4810.

54 Perbal B (2004) CCN proteins: multifunctional signalling regulators. Lancet 363, 62–64.

55 Lau LF & Lam SC (1999) The CCN family of angiogenic regulators: the integrin connection. Exp Cell Res 248, 44–57.

56 Quan T, Qin Z, Voorhees JJ & Fisher GJ (2012) Cysteine-rich protein 61 (CCN1) mediates replicative senescence-associated aberrant collagen homeostasis in human skin fibroblasts. J Cell Biolchem 113, 3011–3018.

57 Jun Ji & Lau LF (2010) The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. Nat Cell Biol 12, 676–685.

58 Kim KH, Chen C-C, Monzon RI & Lau LF (2013) Matricellular protein CCN1 promotes regression of liver fibrosis through induction of cellular senescence in hepatic myofibroblasts. Mol Cell Biol 33, 2078–2090.

59 Jun Ji & Lau LF (2017) CCN2 induces cellular senescence in fibroblasts. J Cell Commun Signal 11, 15–23.

60 Page-McCaw A, Ewald AJ & Werb Z (2007) Matrix metalloproteinases and the regulation of tissue remodelling. Nat Rev Mol Cell Biol 8, 221–233.

61 Visse R & Nagase H (2003) Matrix metalloproteinases and tissue inhibitors of metalloproteinases: structure, function, and biochemistry. Circ Res 92, 827–839.

62 Wang X & Khalil RA (2018) Matrix metalloproteinases, vascular remodeling, and vascular disease. Adv Pharmacol 81, 241–330.

63 Gaffney J, Solomonov I, Zehorai E & Sagi I (2015) Multilevel regulation of matrix metalloproteinases in tissue homeostasis indicates their molecular specificity in vivo. Matrix Biol 44–46, 191–199.

64 Krishnaswamy VR, Mintz D & Sagi I. (2017) Matrix metalloproteinases: the sculptors of chronic cutaneous wounds. Biochim Biophys Acta Mol Cell Res 1864(11 Pt B), 2220–2227.

65 Manicone AM & McGuire JK (2008) Matrix metalloproteinases as modulators of inflammation. Semin Cell Dev Biol 19, 34–41.

66 Gialeli C, Theocharis AD & Karamanos NK (2011) Roles of matrix metalloproteinases in cancer progression and their pharmacological targeting. FEBS J 278, 16–27.

67 Millis AJ, McCue HM, Kumar S & Baglioni C (1992) Metalloproteinase and TIMP-1 gene expression during replicative senescence. Exp Gerontol 27, 425–428.

68 Rao JS, Bhooopathi P, Chetty C, Gujarati M & Lakka SS (2007) MMP-9 short interfering RNA induced senescence resulting in inhibition of medulloblastoma growth via p16(INK4a) and mitogen-activated protein kinase pathway. Cancer Res 67, 4956–4964.

69 Gutierrez-Fernandez A, Soria-Valles C, Osorio FG, Gutiérrez-Abril J, Garabaya C, Aguirre A, Fuetu A, Fernández-García MS, Puente X & López-Otín C (2015) Loss of MT1-MMP causes cell senescence and nuclear defects which can be reversed by retinoic acid. EMBO J 34, 1875–1888.

70 Rashid K, Sundar IK, Gerloff J, Li D & Rahman I (2018) Lung cellular senescence is independent of aging in a mouse model of COPD/pleymysema. Sci Rep 8, 9023.

71 Le Maître CL, Freemont AJ & Hoyland JA (2007) Accelerated cellular senescence in degenerate intervertebral discs: a possible role in the pathogenesis of intervertebral disc degeneration. Arthritis Res Ther 9, R45.

72 Dimozi A, Mavrogmatou E, Sklirou A & Kletsas D (2015) Oxidative stress inhibits the proliferation, induces premature senescence and promotes a catabolic phenotype in human nucleus pulposus intervertebral disc cells. Eur Cell Mater 30, 89–102; discussion 103.

73 Pitiyage GN, Slijepcevic P, Gabrani A, Chianea YG, Lim KP, Prime SS, Tilakaratne WM, Fortune F & Parkinson EK (2011) Senescent mesenchymal cells

The FEBS Journal 287 (2020) 2636–2646 © 2020 The Authors. The FEBS Journal published by John Wiley & Sons Ltd on behalf of Federation of European Biochemical Societies
accumulate in human fibrosis by a telomere-independent mechanism and ameliorate fibrosis through matrix metalloproteinases. J Pathol 223, 604–617.

74 Hassona Y, Cirillo N, Heesom K, Parkinson E k & Prime SS (2014) Senescent cancer-associated fibroblasts secrete active MMP-2 that promotes keratinocyte disconnection and invasion. Br J Cancer 111, 1230–1237.

75 Reiss K & Saftig P (2009) The "a disintegrin and metalloprotease" (ADAM) family of sheddases: physiological and cellular functions. Semin Cell Dev Biol 20, 126–137.

76 Edwards DR, Handsley MM & Pennington CJ (2008) The ADAM metalloproteinases. Mol Aspects Med 29, 258–289.

77 Effenberger T, Heyde J, Bartsch K, Garbers C, Schulze-Osthoff K, Chalaris A, Murphy G, Rose-John S & Rabe B (2014) Senescence-associated release of transmembrane proteins involves proteolytic processing by ADAM17 and microvesicle shedding. FASEB J 28, 4847–4856.

78 Porter S, Clark IM, Kevorkian L & Edwards DR (2005) The ADAMTS metalloproteinases. Biochem J 386 (Pt 1), 15–27.

79 Kelwick R, Desanlis I, Wheeler GN & Edwards DR (2015) The ADAMTS (A Disintegrin and Metalloproteinase with Thrombospondin motifs) family. Genome Biol 16, 113.

80 Brew K & Nagase H (2010) The tissue inhibitors of metalloproteinases (TIMPs): an ancient family with structural and functional diversity. Biochim Biophys Acta 1803, 55–71.

81 Brew K, Dinakarpandian D & Nagase H (2000) Tissue inhibitors of metalloproteinases: evolution, structure and function. Biochim Biophys Acta 1477, 267–283.

82 Grossman M, Tworowski D, Dym O, Lee M-H, Levy Y, Murphy G & Sagi I (2010) The intrinsic protein flexibility of endogenous protease inhibitor TIMP-1 controls its binding interface and affects its function. Biochemistry 49, 6184–6192.

83 Sharabi O, Shirian J, Grossman M, Lebendiker M, Sagi I & Shifman J (2014) Affinity- and specificity-enhancing mutations are frequent in multispecific interactions between TIMP2 and MMPs. PLoS ONE 9, e93712.

84 West MD, Pereira-Smith OM & Smith JR (1989) Replicative senescence of human skin fibroblasts correlates with a loss of regulation and overexpression of collagenase activity. Exp Cell Res 184, 138–147.

85 Ovadya Y & Krizhanovsky V (2018) Strategies targeting cellular senescence. J Clin Invest 128, 1247–1254.