Capstone Paper: *Senescence-associated secretory phenotype (SASP) matrix metalloproteases (MMP) function and mechanism on SASP associated diseases*

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Abstract

Aging is a major risk factor to disease, and its biochemical contribution is still being investigated. One hallmark of aging, senescent cells, are known to contribute to aging through a secretory phenotype known as the senescent-associated secretory phenotype (SASP), which consist of cytokines, chemokines, and proteases. The SASP causes paracrine senescence and increased inflammation. As the effects of the SASP continue to be investigated, one aspect of the SASP that still needs to be better understood in the context of aging are the matrix metalloproteinases (MMPs) that are secreted as part of the SASP. The research demonstrates that these proteases contribute to the progression of pro-inflammatory and pro-fibrotic age-related disease such as atherosclerosis, osteoarthritis, and chronic kidney disease. MMPs secreted by senescent cells degrade the extracellular matrix (ECM) and proteins in the disease microenvironment, trigger phenotypic changes in healthy cells that lead to differentiation into dysfunctional cells and contribute to pro-inflammatory signaling through macrophage recruitment and cellular signaling. This indicates that the MMPs of the SASP may play critical roles in the progression of disease that go beyond degradation of the extracellular matrix. Countering the effects of MMPs through direct inhibition or removal of senescent cells each show potential in altering the course of investigated diseases. MMPs may emerge as a promising therapeutic target to reduce the contribution of senescent cells to the pathogenesis of aging in certain diseases.

Introduction

Aging is a major contributor to the development and poor prognosis of many diseases. Despite being a major risk factor in the development of disease, its precise mechanisms have not been established. Progress toward a greater understanding of aging has focused on an understanding of the hallmarks of aging and their associated processes which can contribute to the development of disease. A major hallmark of aging which is thought to drive its pathology is cellular senescence. Senescent cells are growth arrested cells that no longer progress through the cell cycle even with stimulation. [1] At one time, senescent cells were thought to be a potentially beneficial result of the natural aging process through comparison with the development of cancer. However, further research has shown that they contribute to the development of chronic inflammatory and fibrotic age-related disease like osteoarthritis, atherosclerosis and chronic kidney disease. Senescent cells are not only growth arrested cells. They secrete chemokines, cytokines, proteases, and other molecules which both promote inflammation and change the local microenvironment, driving disease in what is known as the senescence-associated secretory phenotype (SASP). A complete understanding of the contribution of the SASP to diseases of aging is still developing. One component of the SASP that requires further investigation is a group of proteases known as the matrix metalloproteinases (MMP). Historically, they were known for degrading the extracellular matrix, but more effects beyond ECM modification have been discovered. For age-related diseases, the effects of a modified ECM and non-ECM substrate effects from MMP have not been well covered. By connecting the function of SASP MMPs to the pathophysiology of disease, greater clarity into a component of the common role of cellular senescence in age-related diseases may

be found. The goal of this review is to discuss the contribution of SASP MMPs to age-related, SASP-associated diseases and highlight patterns of effects to elucidate MMP function directly related to the senescent cells of these diseases.

Background: Aging

An analysis of the hallmarks of aging has expanded to a list of 12 features that are representative of the aging process.[2] As depicted in Figure 1, there are several hallmarks of aging that can be separated into primary, antagonistic and integrative based on their proposed place in the progression of aging. Primary hallmarks represent an accumulation of dysfunction. Antagonistic hallmarks represent hallmarks that are in response to damage. Integrative hallmarks are the result of damage responses being overwhelmed.[2] The focus of this review is on cellular senescence, which are growth arrested cells that no longer progress through the cell cycle even with stimulation.[1] Senescent cells can be the result of telomere depletion, injury or stress and are considered an antagonistic hallmark. The cell detects dysfunction such as through tumor suppressor proteins including p53, p21, and p16 and exits the cell cycle. As part of the normal wound repair process, senescent cells can be naturally cleared. However, when senescent cells persist, they contribute to disease through pro-inflammatory and pro-fibrotic secretions, leading to further damage and the buildup of unwanted ECM proteins. These effects can cause local or paracrine senescence of other cells. The SASP represents a systemic factor that can drive an aging phenotype in otherwise healthy organisms. This makes the investigation of its components important for potential therapeutic interventions.[2]

Background: Matrix Metalloproteinases and the ECM

Matrix metalloproteinases are zinc-dependent endopeptidases. There are 28 MMPs with 23 of them expressed in humans. They can be classified into families based on their ECM substrate specificity. As shown in Figure 2, the MMP families have broad structural differences that determine their differences in substrate specificity. All of the MMP are secreted in their inactive pro-MMP form until they are activated by cleavage of the pro-peptide sequence. [3]

The extracellular matrix is a non-cellular structural scaffold throughout the body. It goes through dynamic changes during both healthy and diseased states and is composed of collagens and multiple glycoproteins. It is regulated by ECM-associated proteins including MMPs. [4] Cells attach to the extracellular matrix through cell-matrix proteins such as cell-surface integrins and more complex cell-matrix junctions, which consist of several proteins. Cells also attach to each other through cell-cell proteins such as cadherins, or multiprotein cell-cell junctions.[5] MMPs can proteolytically cleave the proteins of the ECM as well as cell-cell and cell-matrix proteins depending on their substrate type.

Collagenases like MMP-1 and MMP-13 are crucial for cleaving collagen types I, II, and III.[3] They are the most effective at degrading collagen and are capable of degrading fibrillar collagen in the triple helical domain, forming a denatured collagen called gelatin.[6] Gelatinases, like MMP-2 and MMP-9, are structurally very similar to collagen, but contain a fibronectin domain that allows for additional cleavage of collagen (denatured collagen is also known as gelatin) in what is known as gelatinolysis. Gelatinases are also well-known to cleave the cell-surface integrins which are important for cell adhesion. Despite having the same broad domain structure as collagenases, stromelysins, like MMP-3 and MMP-10, do not cleave interstitial collagenases.

They are most well-known for stimulating the activity of other collagenases. MMP-3 was initially referred to as a collagenase activator protein.[7] Matrilysins, like MMP-7, lack the hemopexin domain and the hinge region. This makes matrilysins unable to cleave larger and more complex substrates, including certain forms of collagen. However, MMP-7 is able to cleave simpler cell-surface molecules. [3] Other-type like MMP-12 and MMP-20, are among the least well-studied MMPs. MMP-12 is a metalloelastase, known for lysis of elastin in the ECM, reducing flexibility in the interstitial matrix. MMP-20 is known as enamelysin with most of the studies focusing on enamel.[8] The last family are membrane-type MMPs, which are membrane bound and are not secreted by the senescence-associated secretory phenotype.

MMPs are secreted by connective tissues, during fetal development, and during injury by pro-inflammatory tissues. In healthy, resting cells, MMP-levels can be undetectable.[6] During injury, disease, and senescence, the levels of MMPs can contribute to and serve as a biomarker for disease. Despite their name, the non-ECM substrates of MMPs are more abundant than the ECM substrates with only about 40% being ECM substrates. In addition to ECM degradation, MMP substrates include virtually all human chemokines, various cytokines, cell-surface receptors, growth factors, metabolic proteins, and nuclear proteins. [9] This may indicate roles for MMP in influencing cell behavior as well as affecting tissue remodeling, inflammation and innate immunity that go beyond only ECM degradation.[10] For this reason, SASP MMPs in each disease are analyzed for the changes caused to the ECM as well as their influence on non-senescent cells through phenotypic changes and as potential drivers of inflammation.

Background: Age-related Diseases

A basic understanding of the progression of the age-related diseases chosen can help to understand the particular role of SASP MMP. Each disease has been tied to cellular senescence and the production of the SASP.

Osteoarthritis

Osteoarthritis is a disorder of the joint leading to joint pain, articular cartilage degeneration, and joint dysfunction. It is a degenerative disease. It may also involve the development of bone spurs in the joint, known as osteophytes or bone degradation in advanced disease. It is seen most often in weight-bearing joints, but it is no longer considered as only the result of wear and tear. The cells of the joint are known as chondrocytes, which maintain the joint. Senescent chondrocytes have been found to be associated with osteoarthritis[11,12]. Local and systemic inflammation contributes to the breakdown of articular cartilage. Damage associated signals prevent repair while senescent cells contribute to further dysfunction as shown in Figure 3.[13]

Atherosclerosis

Atherosclerosis involves the buildup of lipid in the arterial wall as well as inflammation. This builds up into atherosclerotic plaques which may cause heart attacks or strokes from unstable rupture of the lesions or occlusion of arteries. As shown in Figure 4, the artery consists of a monolayer of vascular endothelial cells (VEC) that contact the blood flow. Beneath that is an acellular intima, followed by vascular smooth muscle cells called the media, and then a fibrous layer. The lipid accumulation happens between the VEC and VSMC in the largely acellular intima. As lipid accumulates, macrophages are recruited to the intima. However, their

phagocytosis of the accumulated lipids turns them into foam cells overwhelmed by the concentration of lipids absorbed. They undergo apoptosis and contribute to necrosis inside the plaque. VSMCs are also affected by the accumulation of lipids, undergoing a transformation from a muscular phenotype to one of several pathological phenotypes. This includes a phenotype that secretes collagen leading to pro-fibrotic activity and migration into the intima, which can form a fibrous cap. For atherosclerosis, the pro-fibrotic activity of certain transformed VSMCs tend to lead to a more stable plaque. This means that it is less likely to rupture causing serious heart attacks or strokes.[14]

Chronic Kidney Disease

Chronic Kidney Disease (CKD) is the progressive loss of the kidneys' filtration ability through the loss of functional nephrons. The number of nephrons decline with aging and with acute kidney injuries (AKI). After an acute injury, there is a further progression of dysfunction that indicates the transition into chronic kidney disease that is increasingly tied to the senescence as the remaining nephrons are forced to deal with increased stress.[15,16] This is associated with reduced filtration ability of the nephrons through hardening of the network of vessels of the glomerulus, known as glomeruloscelrosis, inflammation, tubular atrophy and interstitial fibrosis as nephrons continue to decline in number. These structural changes of late-stage CKD have been tied to the profibrotic effects of the SASP of senescent cells. Podocytes in the glomerulus help to filter proteins and tubular epithelial cells are important for fluid balance. [15]

Osteoarthritis SASP MMP

Chondrocytes are the only cells that make up the articular cartilage of the joint. They help to maintain the extracellular matrix of the joint and their senescence has been tied to the progression of osteoarthritis.[13,17] MMP-1,-3,-12, and -13 have been found to be the MMP components of the SASP secreted by senescent chondrocytes. [12].

ECM Modifications

The collagenases MMP-1 and -13, are highly effective at breaking down collagen. In general, cartilage is high in collagen, collagenases break it down and contribute to the destruction of the membrane. MMP-13 in particular has been found to be highly associated with osteoarthritis, due to its preferential cleavage for type II collagen compared to other MMP, which is abundant in cartilage. [18] MMP-3 as a stromelysin likely enhances the activity of the collagenases, such as MMP-1 for further breakdown.

Phenotypic Changes

While chondrocytes are the only cell type in the joint, the phenotype of chondrocytes can vary. There are proliferative chondrocytes, pre-hypertrophic chondrocytes and hypertrophic chondrocytes.[19] Hypertrophic chondrocytes are the key contributor to endochondral ossification, cartilage, and bone development. This contributes to osteophyte development or bone spurs in osteoarthritis. The differentiation of chondrocytes for matrix mineralization may rely on MMP-13.[20] However, MMP-13 likely promotes chondrocyte hypertrophy but is not required for its development. [17,21] Senescent chondrocytes produce MMP-13, allowing for hypertrophic chondrocyte differentiation. Hypertrophic differentiation is a terminal differentiation that overlaps with senescence, producing cytokines and proteases, and

eventually leads to chondrocyte senescence. [22] This may indicate a cyclical pattern that promotes increased hypertrophy and paracrine senescence.

Inflammation and Immune Recruitment

In osteoarthritis, the presence of MMP-12, which cleaves osteopontin[23] may attract innate immune cells including neutrophils, monocytes and macrophages. Once cleaved, it serves as a chemoattractant for these cells, promotes survival, and promotes differentiation, such as monocyte differentiation into macrophages. This may also contribute to the low-level inflammation in osteoarthritis.[24]

Atherosclerosis SASP MMP

Atherosclerosis involves many cell types, each of which can become senescent. Some of these cells include vascular endothelial cells, vascular smooth muscle cells, and macrophages/foam cells. The MMP expressed by studies on senescent vascular endothelial cells are MMP-1,-3 from oxidative stress[25] as well as mmp-9,-13 from mouse model of atherosclerosis with nitric oxide (NO) knockout. Senescent foam cells secrete MMP-2, -3, -12, -13. [26] Senescent VSMC are associated with MMP-1,-3,-9. [27]

ECM Modifications

Vascular endothelial cells form a barrier between the blood and the vessel wall. Endothelial senescence impairs the barrier function of the endothelial cells, allowing for the infiltration of LDL and immune cells. The SASP, including SASP MMP, disrupts the tight junctions between the cells. [28] Tight junctions are cell-cell junctions which form a highly selective barrier through reducing the space between cells until it is almost nonexistent. This forms a barrier on the top

layer of cells, such as the endothelial layer in blood vessels.[29] MMP-9 has been shown to degrade the tight junctions, [30] which can contribute to endothelial cells being detached and loosened and facilitate infiltration.

In general, MMPs themselves degrade the ECM. The fibrous cap can be destabilized from MMP secretions, such as from senescent VSMCs and macrophages that have migrated to the fibrous cap.[27] MMP-1, -3, -9 are each associated with destabilization of the fibrous cap of plaques, making them more unstable, overlapping particularly with the VSMCs.[31] Senescent VSMCs are one cell phenotype that can migrate to the fibrous cap.

Phenotype Changes

Vascular smooth muscle cells maintain plasticity that can be facilitated by separation from the basement membrane. They transition into several different cell types that play roles in the progression of atherosclerosis as shown in Figure 5. The initial step is the transition from the healthy contractile VSMC phenotype to the dedifferentiated, synthetic VSMC phenotype. This synthetic phenotype then differentiates into other pathological phenotypes. MMP-2 and MMP-9 are known to degrade the basement membrane, freeing VSMCs. Senescent cells expressing these MMP can contribute to their ability to migrate into the intima and undergo phenotypic transition [32] [33]. This can lead to pro- and anti-fibrotic effects in the formation of a fibrous cap depending on further differentiation. VSMCs can differentiate into foam-like cells or macrophage-like cells and accumulate lipids. They can differentiate in osteoblast-like cells causing calcification of the plaque. They can differentiate into myofibroblast like cells, leading to increased fibrosis and a thicker, more stable fibrous cap. They can also become senescent,

leading to increases MMP production, which can proteolytically cleave the ECM proteins in the cap leading to a more unstable plaque.[34]

Inflammation and Immune Recruitment

MMP-3,-9, and -12 have been shown to be able to cleave osteopontin.[23,35,36] The cleavage of osteopontin may contribute to the recruitment of macrophages to the atherosclerotic plaque by SASP MMPs through chemoattraction of innate immune cells.[37] MMP-9 has been shown to release growth factors such as vascular endothelial growth factor (VEGF) and fibroblast growth factor (FGF).[30] VEGF is associated with increased cardiovascular risk and may contribute to the formation of leaky blood vessels within atherosclerotic plaques which increase plaque growth and instability.[26,28]

Chronic Kidney Disease

Because of the extensive similarities mechanisms of CKD and renal aging, CKD can be seen as a form of premature aging. The MMP implicated in the CASP were MMP-2,-7,-9,-20.[38] Tubular epithelial cells found to have increased expression of MMP-2, MMP-9 [16] and MMP-7, [39] which is also used as a key biomarker of disease severity. [40]

ECM Modifications

As shown in Figure 6,[41] MMP-pathways in the kidneys have diverse effects that can lead to the hallmarks of disease. For kidney fibrosis, MMP activity, such as MMP-2 and MMP-9 have fibrinolytic activity. This can reduce fibrosis, but similarly to atherosclerosis, it also contributes to increased migration for a phenotypic transition.

Nephrin forms a barrier between podocytes in the kidney. Nephrin is not technically a part of the extracellular matrix as a cell-to-cell junction, but it acts as a filter for proteins in podocyte function. However, its cleavage by MMP-7 leads to a breakdown of the slit diaphragm, preventing effective filtration of protein in the glomerulus. [41]

Phenotype Changes

Tubular epithelial cells contribute to kidney fibrosis through an epithelial to mesenchymal transition that can be mediated through MMP-7 which involves the degradation of epithelial proteins like E-cadherin, integrins, laminin, and collagen.[42] E-cadherin is cell-cell protein that forms cellular adhesions between epithelial cells. Cell-surface integrins are cell-matrix proteins that help to adhere the cell to the ECM.[5] Laminin is a major component of the basement membrane, which is involved in cell-matrix adhesion. Collagen is a major component of the ECM.[4] Disruption to cell-cell and cell-matrix connections help to facilitate the phenotypic transition of epithelial cells, as shown in Figure 7, through Epithelial-to-Mesenchymal transition (EMT).[43] There is evidence that MMP-9 can also trigger EMT through similar disruption of cell-surface proteins.[16] in addition, MMP-7 cleaves the Fas ligand, preventing apoptotic signals through Fas/FasL interaction, which may contribute to increased dysfunction and senescence.

Inflammation and Immune Recruitment

MMP-7 and MMP-9 cleaves osteopontin, leading to innate immune cell recruitment to the kidney, including macrophages.[35,41] Osteopontin serves as a chemoattractant for these cells, indicating a contribution from these SASP MMPs to further inflammation and fibrosis. [37] MMP-9 can activate cytokines and growth factors, including transforming growth factor-β (TGF-β), which mediates a key pathway of fibrosis as part of kidney fibrosis.[16,44]

Conclusion

MMPs represent a direct-acting component of the SASP that can help in the understanding of the pathology of disease. Their contribution to inflammatory diseases goes beyond serving as a biomarker for disease or only extracellular degradation. They contribute to pro-fibrotic phenotypic switching, immune cell recruitment, inflammation and other pro and anti-fibrotic effects. They may serve as an important therapeutic target in the progression of disease. In the past, broad spectrum MMP inhibitors were used for the prevention of cancer with extreme side effects.[45] Future investigations into MMP inhibition will need to be very selective and chosen based on an understanding of the course of the disease so as to avoid inhibiting beneficial MMP. However, the overlapping effect of aging and senescence may offer another avenue into therapeutics. SASP MMPs have been shown to have a balance between pro- and anti- fibrotic effects, but also to mostly contribute to inflammation, fibrosis, and the development of disease. Senolytic therapies which remove the chronic negative effects of the cells producing MMPs may be a promising method for attenuating the progression of these age-related diseases. Progress along that front with senolytics like quercetin, fisetin have shown potential promise including a reduction in harmful MMP generation. [46–48] The pathological overlap found between the effects of SASP on disease can help to elucidate the effects of aging. This understanding may help for future interventions into disease and for reducing the negative effects of this severe risk factor.

Figures:

Figure 1. Hallmarks of Aging, expanded

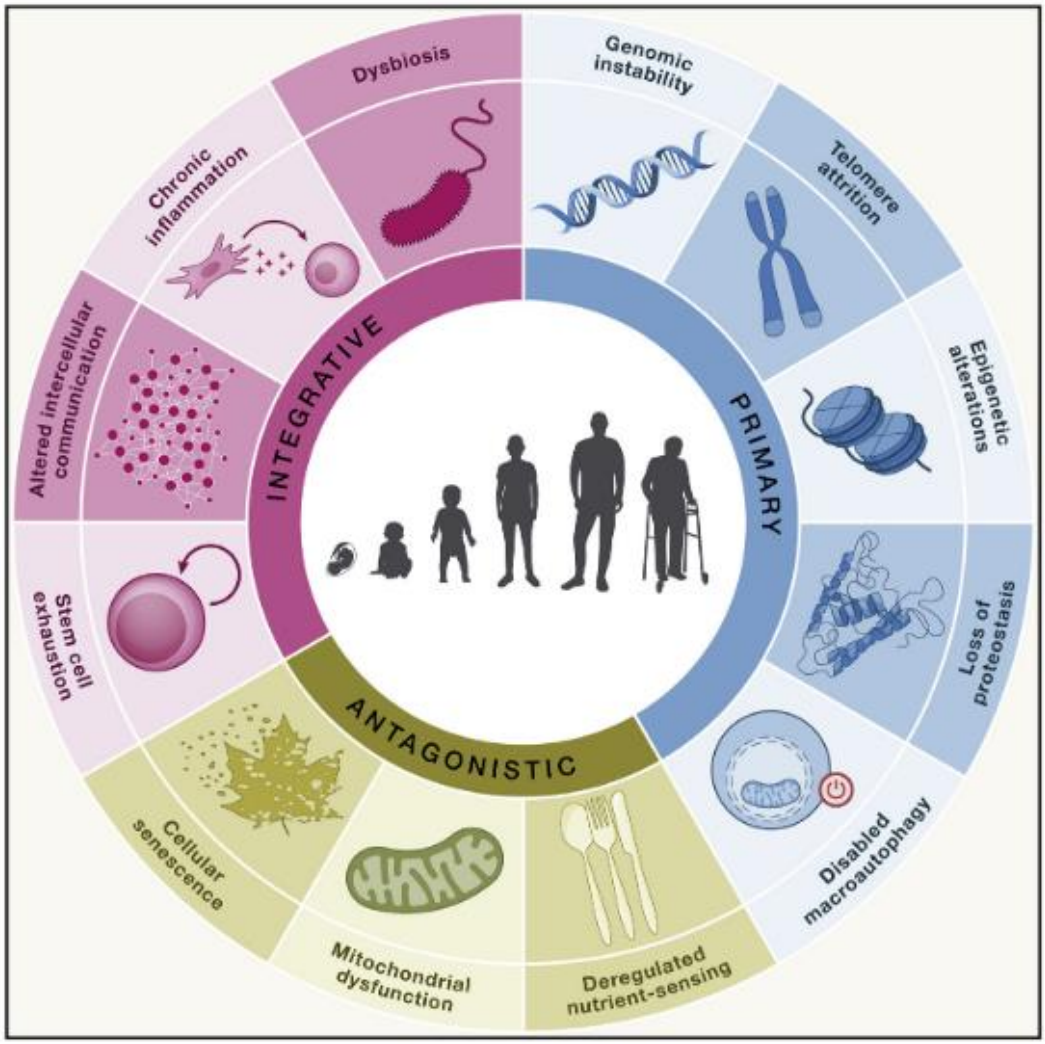

Fig 1. There are 5 primary hallmarks: genomic instability, telomere attrition, epigenetic alteration, loss of proteostasis, and disabled macroautophagy. There are 3 antagonistic hallmarks: cellular senescence, mitochondrial dysfunction, and deregulated nutrient sensing. There are also 4 integrative hallmarks: stem cell exhaustion, altered intracellular communication, chronic inflammation, and dysbiosis. Cellular senescence is an antagonistic hallmark, representing a response to damage. While it may be beneficial in acute responses, the chronic presence of cellular senescence contributes to aging. While it is an important component of aging, it is one of a number of hallmarks that are still being understood.s Adapted from reference 2.

Figure 2. MMP Family Structures

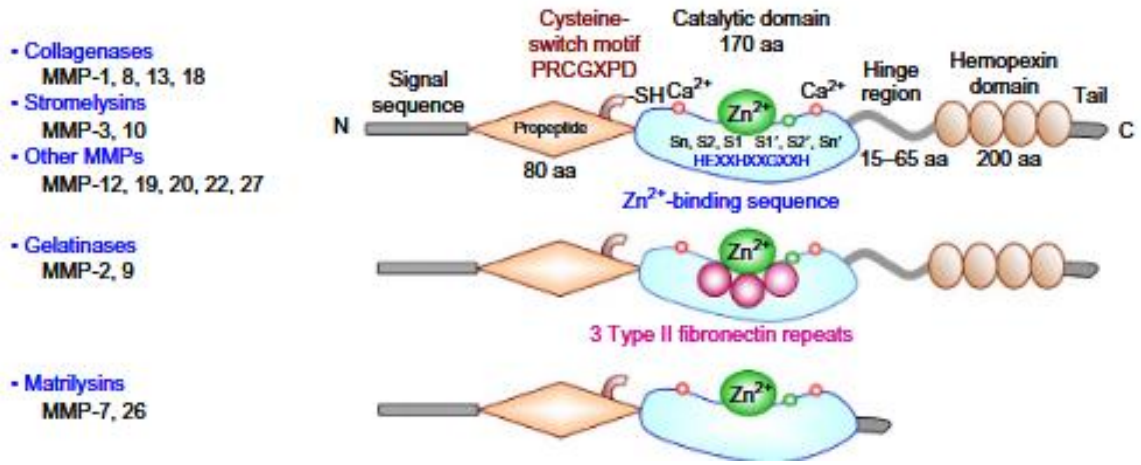

Fig 2. MMP families highlighted in this review, including collagenases, stromelysins, other, gelatinases, and matrilysins. Each MMP exists in the inactive zymogen form until the propeptide is cleaved. Each MMP is a zinc-dependent endopeptidase. The Zn^{2+} characteristic binding sequence is HEXXHXXGXXH. Gelatinases contain fibronectin repeats that assist in gelatinolysis. Matrilysins lack the hinge region and hemopexin domain, preventing the cleavage of more complex subtrates. The structure of collagenases, stromelysins and other-type MMPs are highly similar. MMP-1, 2 ,3, 7, 9, 12, 13, and 20 are covered in this review. Adapted from reference 3.

Figure 3. Drivers of senescent chondrocytes in osteoarthritis

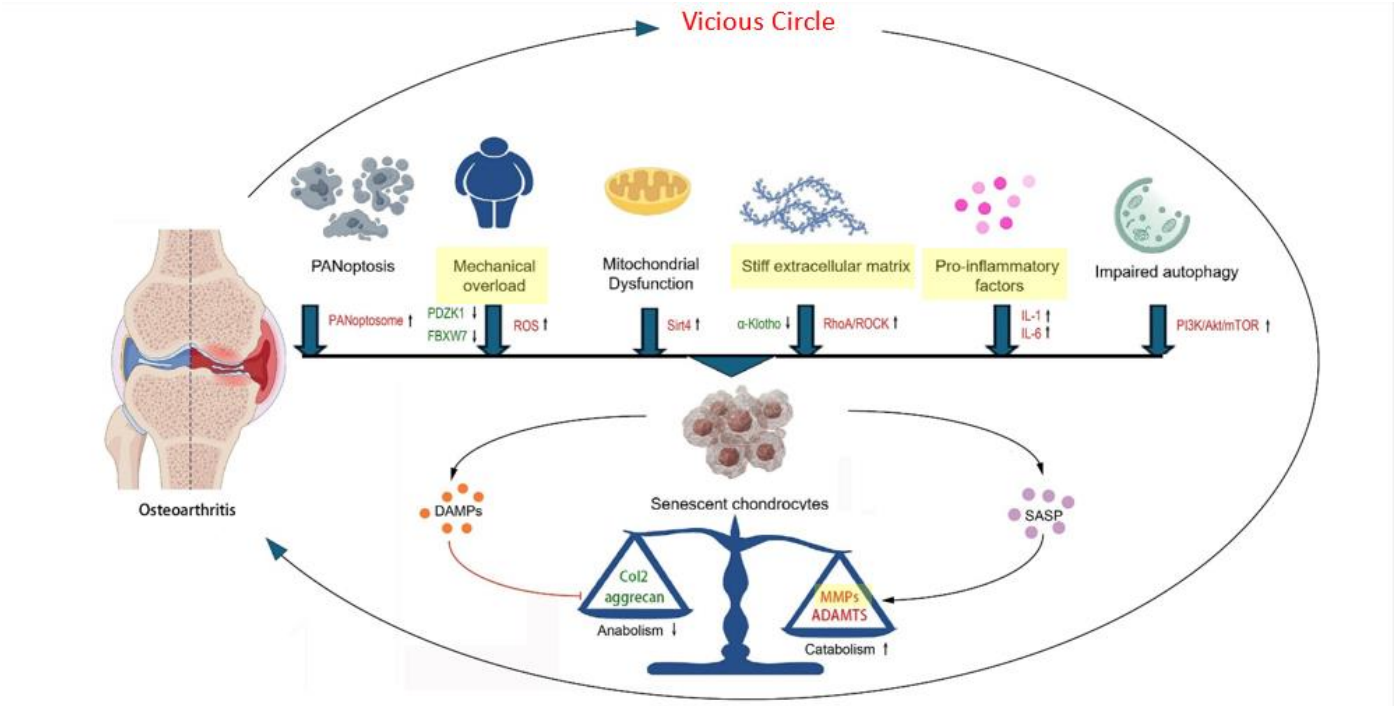

Fig 3. Drivers of senescent chondrocytes in osteoarthritis. Increased catabolism from SASP MMPs contributes to disease and further senescence. As highlighted, MMP degrade the extracellular matrix of the joint, leading to a weaker joint, a stiffer ECM and the recruitment of pro-inflammatory factors. These factors also lead further senescence of chondrocytes, leading to a cyclical pathological process. Adapted from reference 13.

Figure 4. Atherosclerosis Overview

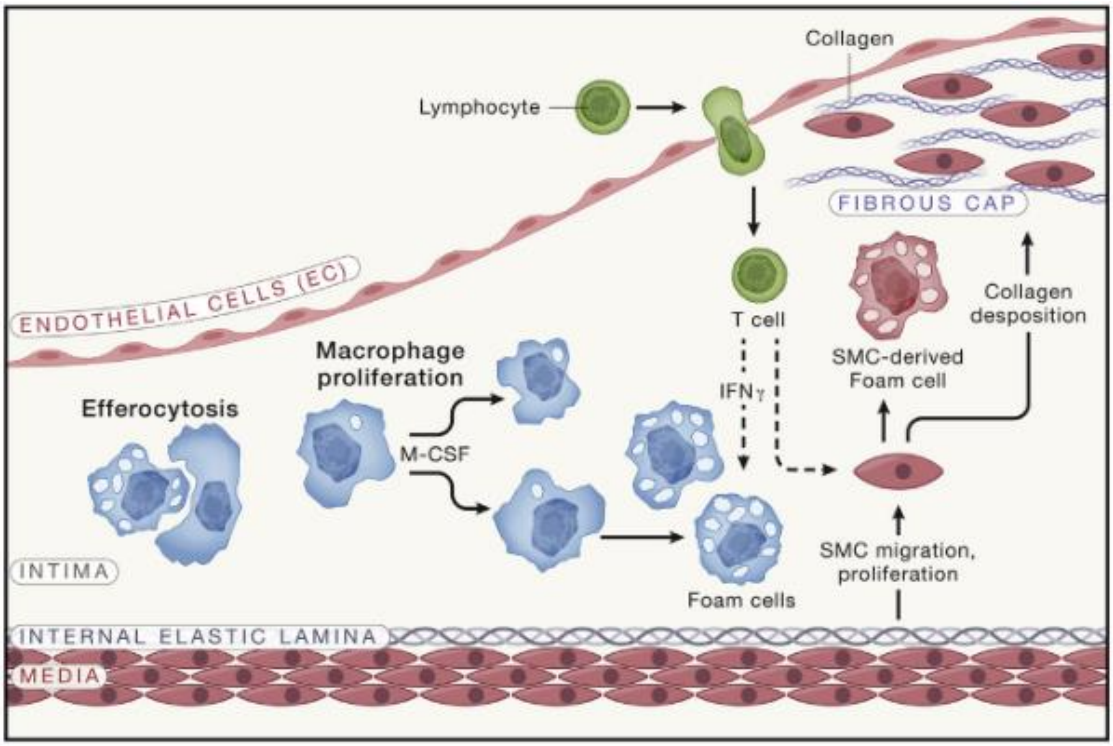

Fig 4. A qualitative depiction of the vascular wall environment in the development of atherosclerosis. After disruption of endothelial cells, there is a buildup of lipid in the intima located between the endothelial cells and the vascular smooth muscle cells of the media. Macrophages progress to pathological foam cells. VSMC migrate from the media and differentiate. These contribute to plaque formation and the formation of the fibrous cap. Adapted from reference 14.

Figure 5. Synthetic VSMC Plasticity

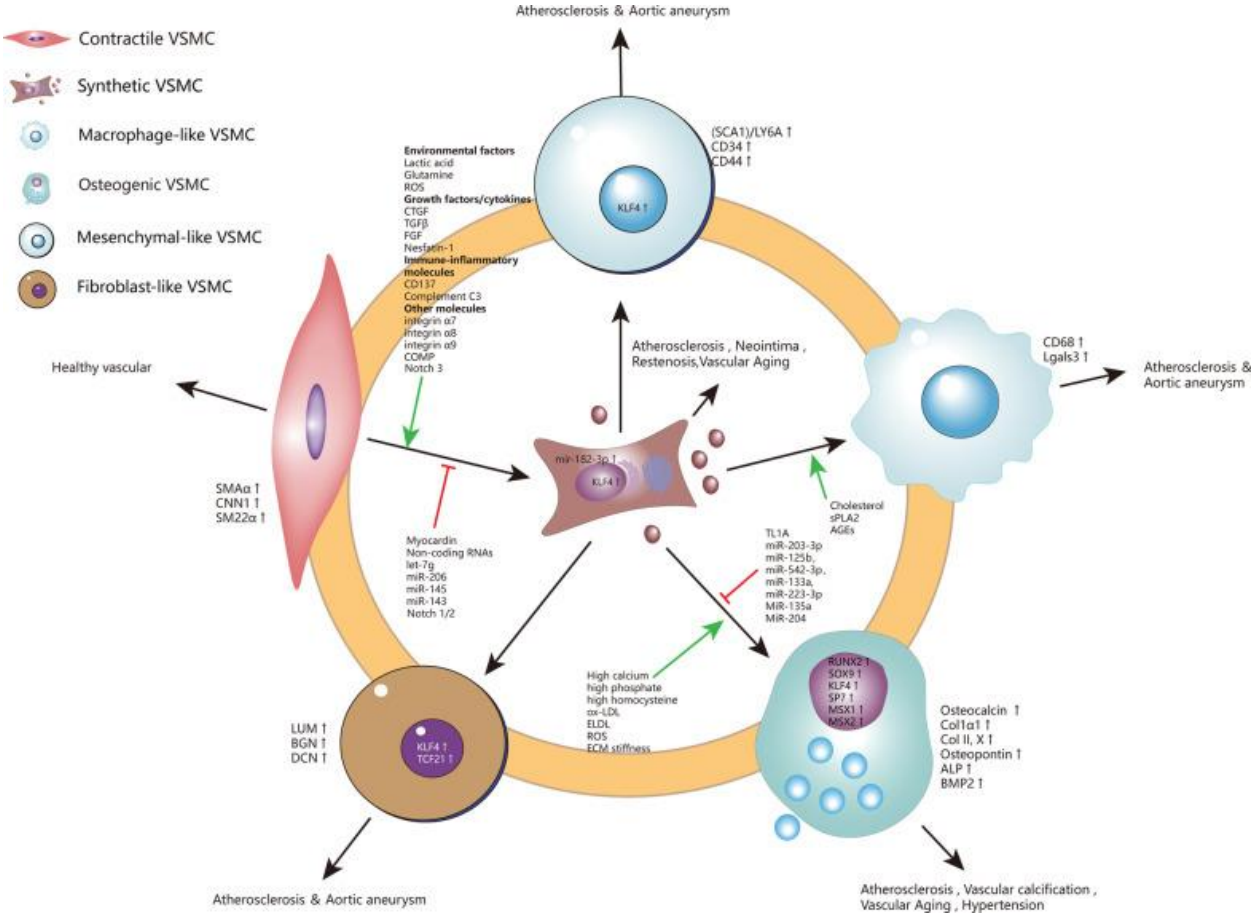

Fig 5. VSMC plasticity leads to a highly diverse contribution of differentiated VSMC to the pathogenesis of atherosclerosis. The central synthetic phenotype is surrounded by the contractile phenotype, the macrophage-like phenotype, the osteogenic phenotype, the mesenchymal-like phenotype, and the fibroblast-like phenotype. Research which indicates that MMPs contribute to the formation of the synthetic VSMC indicates a role for MMP in the facilitation of VSMC differentiation into various pathological phenotypes. MMP disruption of the basement membrane promotes a VSMC synthetic state and helps to mediate further differentiation into various phenotypes. Adapted from reference 33.

Figure 6. Metalloproteinase Pathways in Kidney Disease

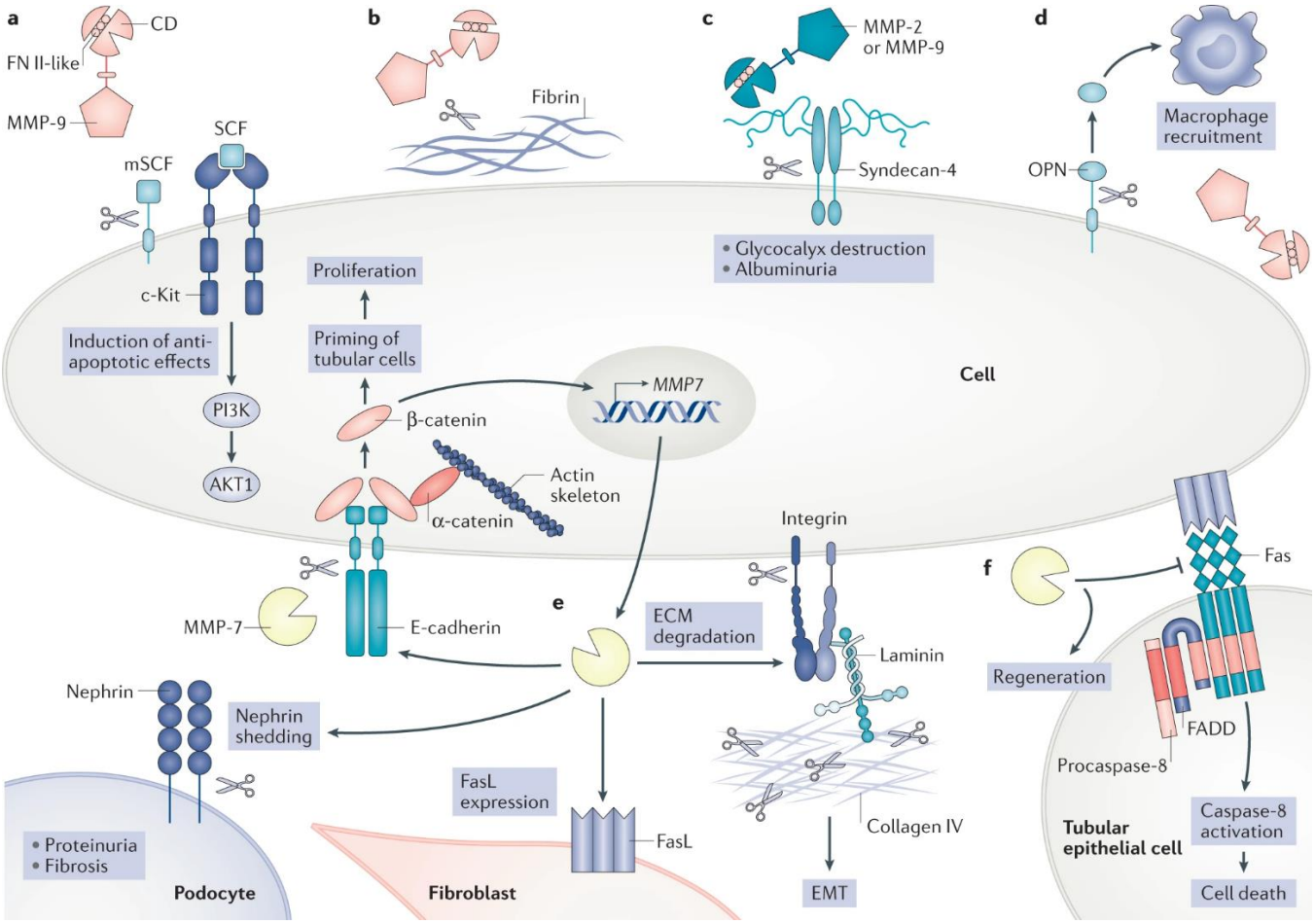

Fig 6. MMP have many effects related to the pathology of Chronic Kidney Disease including fibrinolytic activity from MMP-9. Osteopontin cleavage by MMP leads to macrophage recruitment. Nephrin shedding in podocytes from MMP-7 leads to disruption of the slit diaphragm and proteinuria. E-cadherin shedding, integrin shedding as well as laminin and collagen IV degradation leads to disruption of cell-cell and cell-matrix connections that facilitate EMT. MMP-7 sheds the Fas ligand, preventing apoptosis of damaged or senescent cells. Adapted from reference 41.

Figure 7. Epithelial-to-Mesenchymal Transition

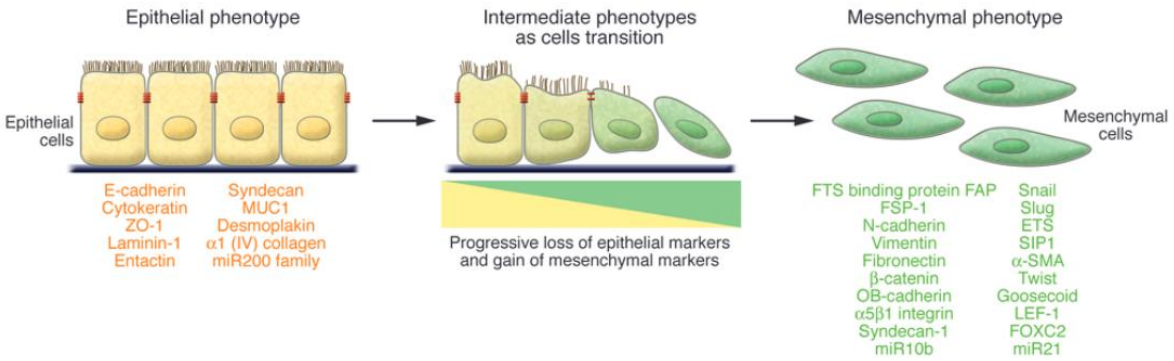

Fig. 7 A qualitative depiction of epithelial-to-mesenchymal transition. As the cell-cell and cell-matrix proteins are disrupted, epithelial cells progressively lose genes that serve as epithelial markers (such as E-cadherin and Laminin-1) and transition to those that serve as mesenchymal markers (such as fibronectin and α-SMA). This is associated with increased fibrosis as the cells detach from the ECM. Adapted from reference 43.

Academic Integrity and AI Use:

AI tools were not used to generate scientific content. All scientific content, analysis, and conclusions were reviewed and revised by the author.

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