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INVITED REVIEW

Senescence mechanisms and targets in the heart

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Abstract

Cellular senescence is a state of irreversible cell cycle arrest associated with ageing. Senescence of different cardiac cell types can direct the pathophysiology of cardiovascular diseases (CVDs) such as atherosclerosis, myocardial infarction, and cardiac fibrosis. While age-related telomere shortening represents a major cause of replicative senescence, the senescent state can also be induced by oxidative stress, metabolic dysfunction, and epigenetic regulation, among other stressors. It is critical that we understand the molecular pathways that lead to cellular senescence and the consequences of cellular senescence in order to develop new therapeutic approaches to treat CVD. In this review, we discuss molecular mechanisms of cellular senescence, explore how cellular senescence of different cardiac cell types (including cardiomyocytes, cardiac endothelial cells, cardiac fibroblasts, vascular smooth muscle cells, and valve interstitial cells) can lead to CVD, and highlight potential therapeutic approaches that target molecular mechanisms of cellular senescence to prevent or treat CVD.

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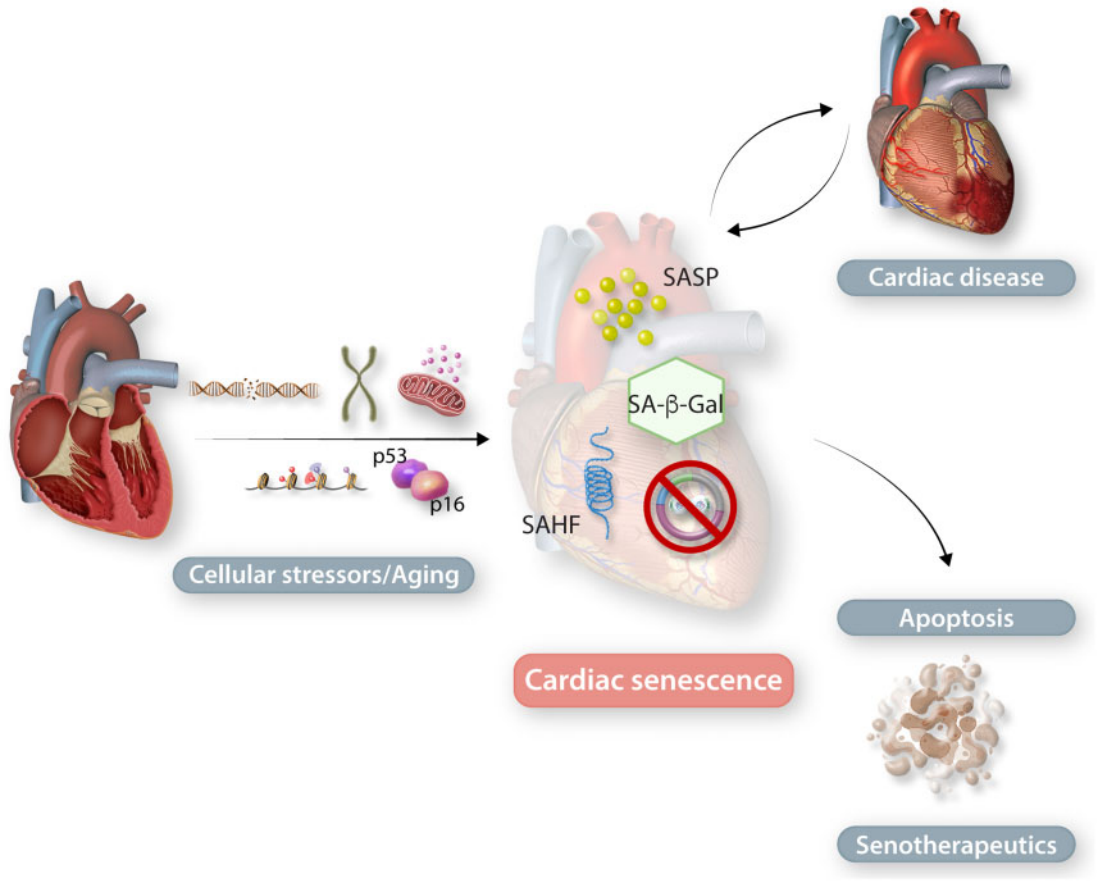
Graphical Abstract



Keywords Senescence • Ageing • Cardiovascular disease • Senotherapy

1. Introduction

Cellular senescence is characterized by irreversible cell cycle arrest and can be initiated by stressors such as ageing, DNA damage, or elevated levels of reactive oxygen species (ROS).[1] Senescence of different cardiac cell types is associated with a wide range of cardiovascular diseases (CVDs) including atherosclerosis,[2] valvular heart disease,[3] cardiomyopathies,[4] and arrhythmias.[5] The initiation and maintenance of cellular senescence in the heart contributes to the severity and progression of cardiac pathologies. Understanding how cellular senescence leads to CVD is important to develop therapies to counter these effects. In this review, we consider mechanisms of cellular senescence in the heart, explore the disease phenotypes associated with senescence of different cardiac cell types, and discuss potential therapeutic approaches.

2. Characteristics of cellular senescence

Cellular senescence is a heterogeneous phenotype that depends on cell type and context, defined as an irreversible loss of proliferative capacity.[6] Senescence can be further classified into sub-types, including replicative senescence, oncogene-induced senescence, and stress-induced premature senescence.[7] Senescence is distinct from quiescence, an adaptive response to changes in nutrient signalling leading to reversible cell cycle arrest that is reviewed elsewhere.[8] The distinguishing characteristic of cellular senescence is its state of irreversible cell cycle arrest in the G1/S or G2 phase, leading to permanent withdrawal from the potential proliferative pool.[6]

Given its heterogeneous phenotype, identification of senescent cells can be challenging. Senescent cells, in the heart and elsewhere, are marked by several key characteristics that indirectly indicate senescence (*Figure 1*). Senescent cells can have up-regulation of p53/p21 or p16/retinoblastoma protein (Rb) pathways and markers associated with activation of the DNA damage response (DDR) [p38 mitogen-activated protein kinase (MAPK), phosphorylated histone 2AX (γH2AX)].[9–11] Some cells display senescence-associated heterochromatin foci, whereupon proliferation-related genes are silenced through distinct heterochromatin alterations.[12] Senescent cells can exhibit a senescence-associated secretory phenotype (SASP), where soluble signalling factors, proteases, and insoluble proteins/extracellular matrix (ECM) components are secreted into the surrounding cellular

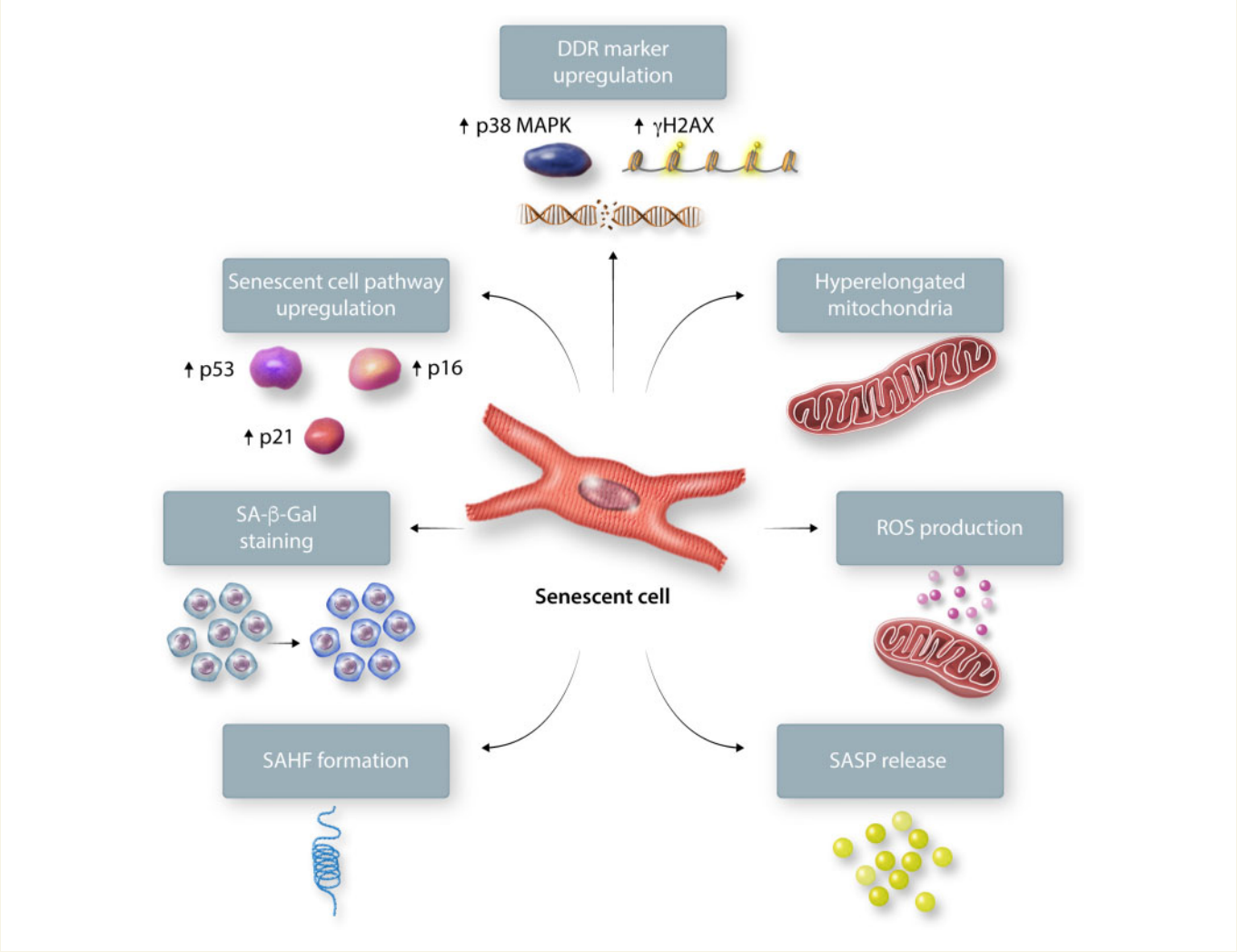


Figure 1 Markers of senescent cells. Senescent cells exhibit irreversible cell cycle arrest, elevation of p53, p21, and p16, elevation of DNA damage response markers p38 MAPK and γH2AX, hyperelongated mitochondria, elevated reactive oxygen species, increased senescence-associated β-galactosidase activity, senescence-associated heterochromatin foci (SAHF), and a senescence-associated secretory phenotype (SASP).

environment.[13] SASP maintains the senescent state while also influencing the surrounding cellular environment through paracrine effects. Senescence-associated β-galactosidase (SA-β-Gal) activity is typically increased in senescence, although SA-β-Gal activity can also be increased in quiescent cells.[14] Future work is needed to identify direct markers of cellular senescence.

It is worth noting that while cellular senescence can exacerbate or accelerate ageing-related diseases, interplay between senescence and apoptotic pathways can also play a key protective role in cancer prevention[15] and is important during development.[16] Doses of stressors likely contribute to pathway selection, as low doses of the chemotherapeutic agent, doxorubicin, induce senescence, while high doses induce apoptosis in neonatal rat cardiomyocytes.[17] Oncogenic stimuli can upregulate p53 or p16 to induce senescence in some cell types to prevent transition to a tumourigenic state.[15] Senescence induction through p53 signalling can also divert the cell death pathway from apoptosis to necrosis in fibroblasts, which may also be beneficial in anticancer therapies.[18] In addition, programmed activation of cellular senescence may be required for normal embryonic development and can work alongside apoptotic pathways to control normal morphogenesis.[16] Furthermore, while SASP is generally associated with negative effects such as inflammation and tumourigenesis,[19] SASP can also have beneficial effects on wound healing, tissue regeneration, and immune responses.[19]

3. Molecular regulators of cellular senescence

In this section, we broadly discuss molecular mechanisms of senescence that can occur across diverse cell types (*Figure 2*). In Section 4, we will discuss how these processes mediate senescence of different cardiac cell types.

3.1 Telomeres and the DNA damage response

Telomeres are tandem repeats that cap chromosomes to protect the chromosome from degradation and end-to-end fusion. The shelterin

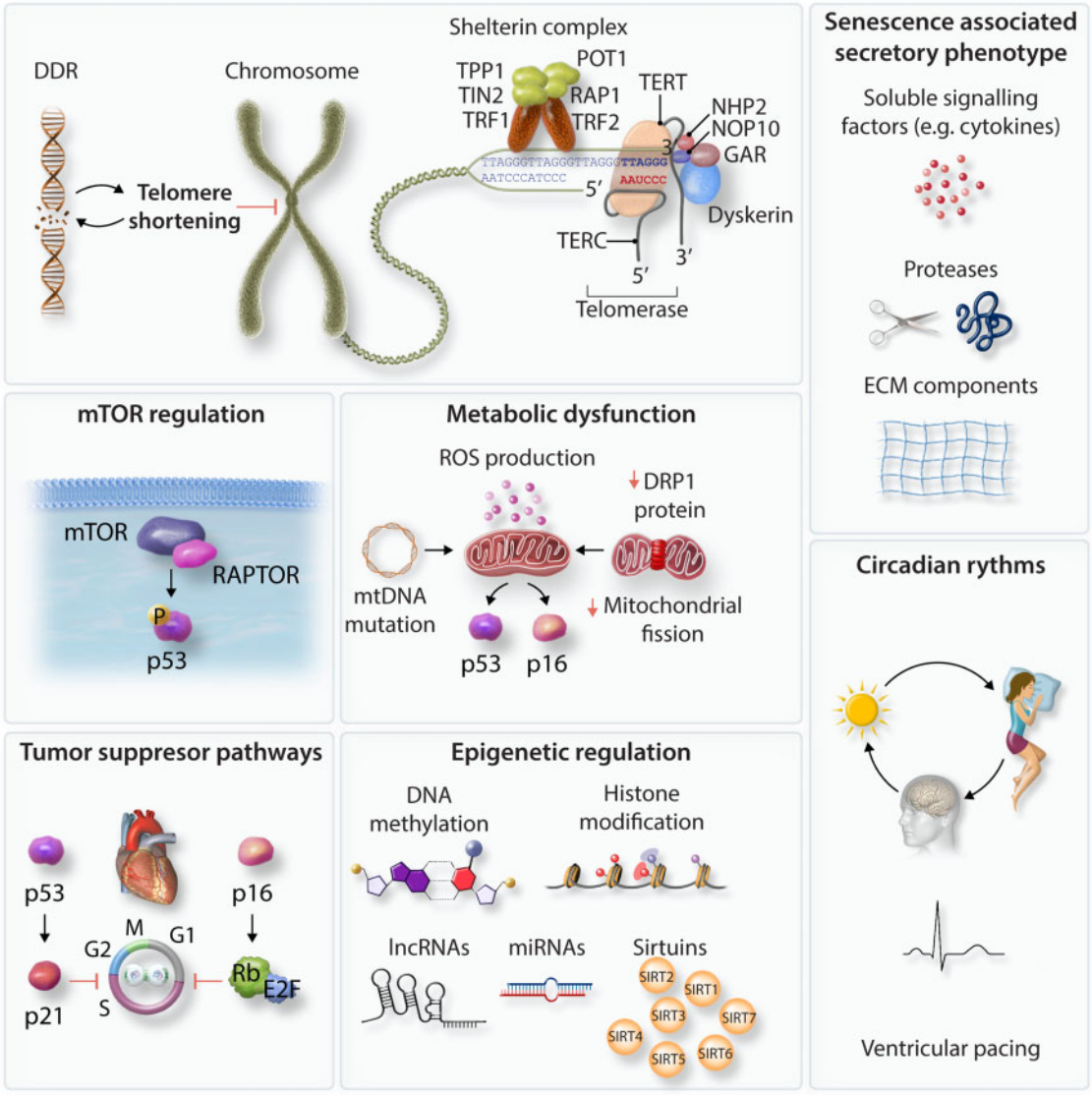


Figure 2 Molecular regulators of cellular senescence. Mechanisms that drive cellular senescence include the DNA damage response/telomere shortening, mTOR activation, metabolic dysfunction, circadian misalignment, SASP, tumour suppressor pathways, and epigenetic changes.

loop complex recognizes telomere structures to prevent DDR mechanisms from recognizing and processing telomeric DNA.[20] Telomeres shorten with each cell cycle repetition, and upon reaching a critical telomeric length, shelterin proteins can no longer be recruited to protect the DNA loop, which activates the DDR system and initiates cell cycle inhibition.[21] Telomere shortening occurs naturally with ageing, leading to replicative senescence, particularly in non-cardiomyocytes.[22]

3.2 Tumour suppressor proteins regulating senescence

Induction of cellular senescence can occur through activation of the p53/p21 and/or p16/Rb pathways, leading to cell cycle arrest (*Figure 3*). p53 is a transcription factor that regulates genes associated with metabolism, autophagy, DDR, cell cycle, and apoptosis.[9] p53 activity is subject to a variety of post-translational modifications, including ubiquitination, phosphorylation, and acetylation.[23] p53 positively regulates p21, a member of the cyclin-dependent kinase (CDK) inhibitors that is required for p53-mediated cell cycle arrest at G1/S or G2/M.[24] By inhibiting cellular apoptosis through binding of caspases, p21 promotes the senescent state.[24] The p16/Rb pathway is regulated by the inhibitory activity of p16, which binds to CDK4/CDK6 to prevent Rb phosphorylation, leading to cell cycle arrest at G1/S.[10] The p16/Rb pathway is also involved in ROS induction through the mitogenic signalling cascade, which activates protein kinase C delta to create a positive feedback loop to maintain senescence.[25]

3.3 Mechanistic target of rapamycin

The mechanistic target of rapamycin (mTOR) is a serine/threonine kinase that responds to a variety of environmental and intracellular cues to regulate growth and metabolism.[26] mTOR can induce

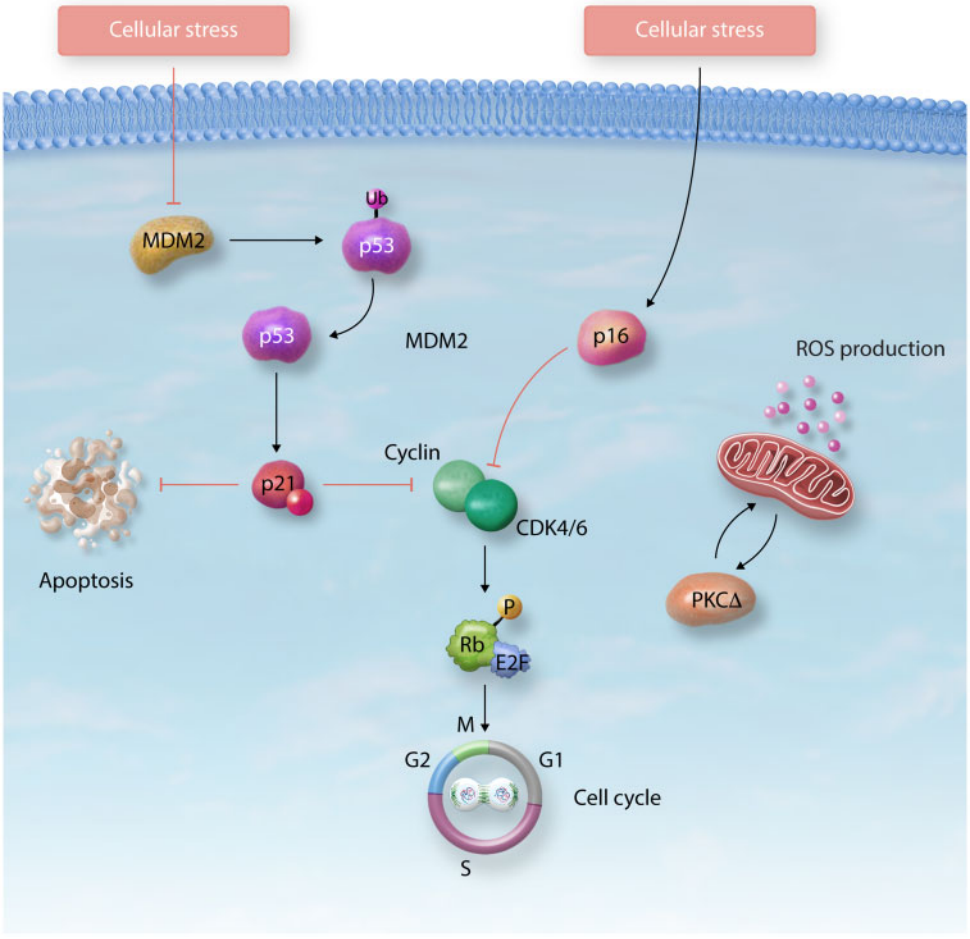


Figure 3 Tumour suppressor regulators of senescence. The p53/p21 and p16/Rb pathways play central roles in senescence via cell cycle arrest, ROS production, and activation of the DNA damage response.

senescence through activation by unchecked proliferation regulators [phosphoinositide 3-kinase (PI3K)/protein kinase B (AKT)] and positive regulation of senescence signalling (p53) during oncogenic activation.[27] MTOR controls the decision between senescence and quiescence: with both p53 and mTOR activation, cells become senescent; however, with p53 activation and mTOR inhibition, cells become quiescent.[28]

3.4 Mitochondrial dynamics and dysfunction

Senescent cells can have hyperelongated mitochondria due to disruption of the balance between mitochondrial fission and fusion proteins.[29,30] A relative decrease in levels of mitochondrial fission proteins FIS1[30] or dynamin-related protein 1 (DRP1)[29] or an increase in mitochondrial fusion proteins, mitofusins 1 and 2 (MFN1/2)[29,31] or OPA1[30] promotes a senescent phenotype with hyperelongated mitochondria. Mitochondrial fusion is necessary to maintain normal mitochondrial and cardiac function,[32] while fission facilitates removal of dysfunctional, depolarized mitochondria via mitophagy.[33] An imbalance of fission and fusion processes towards hyperfusion as seen in senescence leads to retention of dysfunctional mitochondria and accumulation of oxidized proteins[33] which may exacerbate the senescent phenotype. Mitochondrial dysfunction is associated with increased ROS production,[34] leading to increased oxidative damage, which includes sulfhydryl oxidation, lipid peroxidation, and mitochondrial DNA (mtDNA) mutation.[35] Metabolic dysfunction with aberrant mitochondrial dynamics is particularly consequential in directing senescence of cardiomyocytes.

3.5 Epigenetic regulation of cardiac senescence

Epigenetic changes include DNA methylation, histone acetylation, chromatin remodelling, and non-coding RNAs.[36] Compared to proliferating cells, senescent cells retain a very different methylation signature, with DNMT1 regulation leading to hypomethylation of late-replicating gene regions and hypermethylation at certain promoter-proximal regions.[37] These effects are hypothesized to lead to cell cycle repression and subsequent proliferation arrest/cell cycle exit.[38]

Histone modification physically alters chromatin structure while recruiting various adaptor/effector proteins that contain binding domains for further remodelling.[36] Sirtuins (SIRT) are a family of nicotinamide adenine dinucleotide (NAD^+)-dependent histone deacetylases that can protect against a senescent phenotype in multiple cell types. In cardiomyocytes, SIRT1 can suppress transcription of a SASP in cardiomyocytes

through histone deacetylation[39] while in endothelial cells, SIRT1 maintains endothelial cell function through regulation of endothelial nitric oxide synthase (eNOS) to reduce oxidative damage.[40]

Non-coding RNAs [microRNAs (mi-RNA), long non-coding RNAs (lncRNA)] can regulate senescence and CVD progression. For example, miRNA-22, when up-regulated in the ageing heart, induces accelerated cardiac fibroblast senescence and migratory activity.[41] Additionally, miRNA-29 negatively regulates H4K20me3 to maintain the senescent state during cardiac ageing via the transforming growth factor beta (TGF-β)/SMAD signalling pathway.[42] Prior reviews describe the roles of other miRNAs in regulating cardiac senescence during ageing and heart injury.[43] LncRNAs serve important functions in cardiac regeneration and development, working primarily through three main mechanisms: binding to ribonucleoproteins, binding and inhibiting miRNAs (generating the lncRNA-miRNA-target axis), and binding to DNA segments to form nuclear domains.[44] For example, lncRNA H19 promotes cardiomyocyte senescence when overexpressed in ageing hearts by inhibiting miR-19a to stimulate the p53/p21 senescence driver pathway.[45] Furthermore, lncRNA SNHG12 demonstrated mediating activity as a regulator of the DDR via binding with DNA dependent protein kinase (DNA-PK); knockdown of lncRNA SNHG12 caused impaired DNA damage repair and vascular senescence to accelerate atherosclerosis.[46] Thus, lncRNAs may be potential therapeutic targets for senescence-related CVDs.

3.6 Senescence-associated secretory phenotype

SASP contains three main categories of secreted factors: soluble signalling factors, proteases, and insoluble proteins/ECM components. Major cytokines in the soluble signalling family of the SASP include interleukin-6 (IL-6) and IL-1 proteins. IL-6 is thought to be directly regulated by the DDR independently of p53 regulation and can affect neighbouring cells expressing the IL-6R signalling complex on the cell surface.[47] IL-1 proteins are similarly up-regulated during senescence, promoting nuclear factor kappa B (NF-κB)-mediated transcriptional activation of an inflammatory cascade as a major feed-forward mechanism for SASP expression.[48] SASP proteases include matrix metalloproteinases (MMPs) and serine proteases.[13] The SASP mechanism is particularly important in communicating senescence cues from non-cardiomyocytes to cardiomyocytes (*Figure 4*).

3.7 Circadian regulation of senescence

Circadian rhythms may play a role in regulating cellular senescence, as circadian misalignment decreases lifespan and induces immune cell senescence in mice.[49] In the heart, mutation of Per2, a circadian gene, causes vascular senescence via AKT-signalling.[50] Because the circadian clock governs heart rhythmicity through a pacemaker-like function,[51] dysregulation of clock function may have detrimental effects on CVD progression and cardiac senescence. Likewise, senescence of vascular smooth muscle cells (VSMCs) can impair transmission of circadian signals.[52]

4. Senescent cardiac cells in cardiovascular disease

We review phenotypic changes that occur in different cardiac cell types during senescence and how this influences CVD progression. While global disease processes may precede cellular senescence in some scenarios [e.g. acute myocardial infarction (MI)], here we examine the evidence that suggests that in many diseases, cellular senescence initiates a cascade of events leading to the disease phenotype. For each cardiac cell type, we review examples of CVDs associated with cellular senescence.

4.1 Cardiomyocytes

Cardiomyocytes comprise ∼30–40% of the total cellular population in the heart and 80% of the cellular volume.[53] Senescent cardiomyocytes have impaired shortening, increased pacing frequency, contractile dysfunction, metabolic dysfunction,[54] and can secrete SASP factors to induce senescence in neighbouring cell types including fibroblasts.[55] While different mechanisms can induce senescence in cardiomyocytes, metabolic stressors predominate in inducing disease phenotypes in cardiomyocytes.

4.1.1 Molecular mechanisms of senescence in cardiomyocytes

Metabolic dysfunction may have significant consequences for age-related functional decline in the heart, as changes in energy substrates or stressors that affect energy production can lead to cardiomyocyte senescence. Specifically, dysregulation of mitochondrial dynamics or quality control can lead to increased ROS production and increased oxidative damage.[34] Mitochondria are capable of changing morphology via fission or fusion within minutes in response to stimuli.[56] In addition, dysregulation of mitophagy, the process of removing damaged mitochondria to protect the cell from global metabolic dysfunction, can lead to cellular senescence.[57] Mice with cardiomyocyte-specific triple deletion of Drp1/Mfn1/Mfn2 surprisingly survive longer than mice with either Drp1 or Mfn1/Mfn2 removal alone suggesting that an imbalance of fission and fusion processes is more detrimental than global disruption to mitochondrial dynamics.[31]

Prevention of oxidative damage through FOXO3 activation leading to up-regulation of catalase and superoxide dismutase 2 (SOD2) prevents senescence in cardiomyocytes.[58] Catalase overexpression in mitochondria protects the heart from age-associated metabolic dysfunction.[59] Mutations in mtDNA increase with age and lead to increased ROS production.[60] mtDNA that escapes from mitophagy can trigger toll-like receptor 9 (TLR9) signalling and the downstream release of pro-inflammatory cytokines leading to inflammatory responses in cardiomyocytes.[61] These inflammatory responses contribute to SASP maintenance, encouraging CVD progression. This demonstrates the importance of preventing ROS as a therapeutic intervention against ageing-related oxidative stress in the heart, particularly as related to senescent cardiomyocytes.

In addition, cell cycle regulators can promote mitochondrial dysfunction, further supporting senescence. Cytosolic p53 can promote mitochondrial dysfunction through inhibition of Parkin-mediated mitophagy.[62] p53 activation during senescence disrupts glycolysis and glucose transport through GLUT1/4 in cardiomyocytes, leading to impaired glucose metabolism.[63] P53 inhibition in diabetic mice prevents senescence and cardiomyopathy.[64] Cell cycle inhibitors Rb1 and Meis homeobox 2 (Meis2) can drive senescence, and inhibition of Rb1 and Meis2 following MI can enhance proliferation of adult rat cardiomyocytes and reduce infarct size.[65]

In addition to mitochondrial dysfunction, telomeric shortening is associated with cardiomyocytes and DNA damage during heart failure.[66] Also, epigenetic factors can affect cardiomyocyte senescence. For

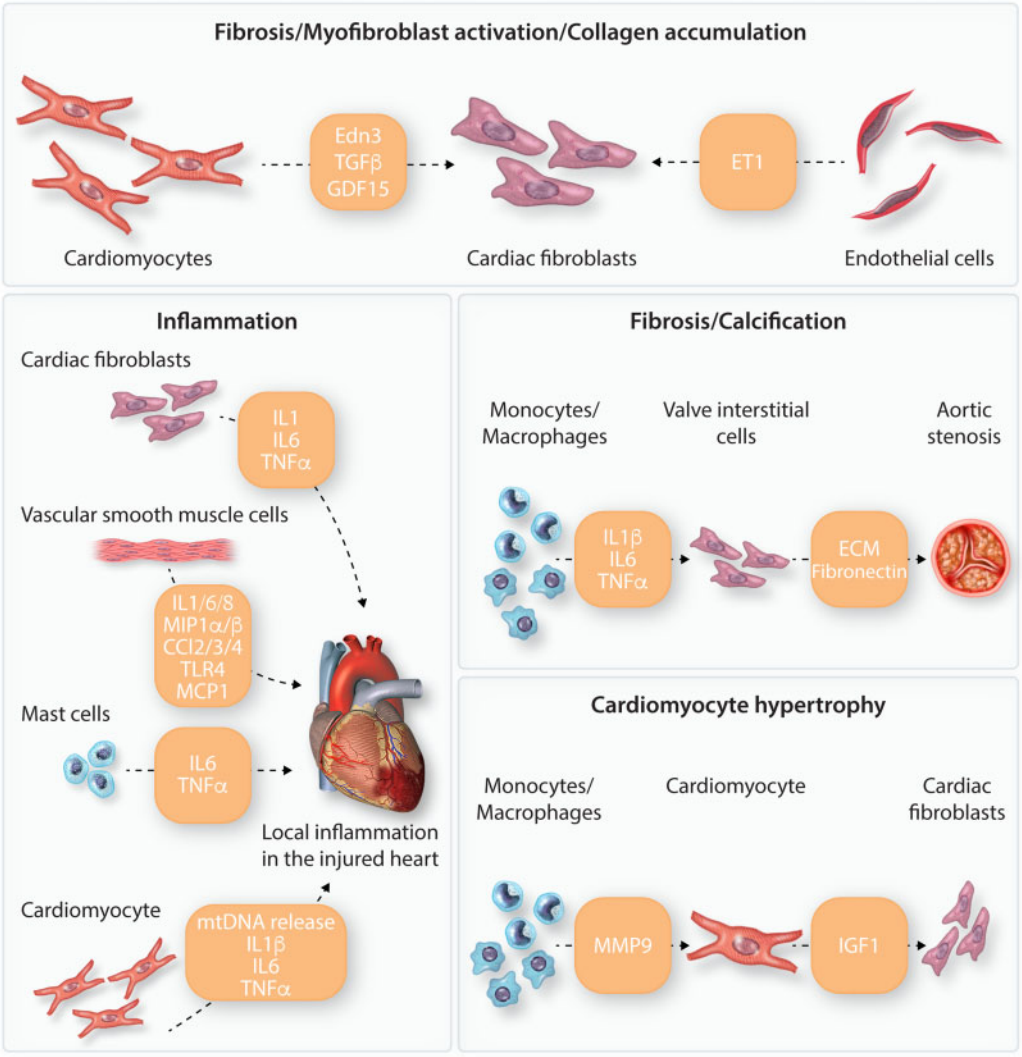


Figure 4 Secretome of different cell types in the heart and examples of associated phenotypic changes. Senescent cardiac cells secrete different senescence-associated secretory phenotypes leading to paracrine signalling and exacerbation of the senescent phenotype. Several consequences of these secreted factors include fibrosis, myofibroblast activation, collagen synthesis, calcification, hypertrophy, and inflammation.

example, loss of H3K9me3 in adult cardiomyocytes with overexpression of histone demethylase, KDM4D, results in increased proliferation and cell cycle gene expression, reversing cell cycle exit.[67] The miRNA-34 family is up-regulated in ageing hearts and contributes to reduced cardiomyocyte contractility.[68] The miRNA-17-92 family has a cardiomyocyte-specific decrease in expression during age-associated heart failure leading to fibrotic remodelling and functional decline.[69] Moderate SIRT1 overexpression protects against cardiac hypertrophy, apoptosis, dysfunction, and senescence.[70] SIRT7 also interacts with p53 to deacetylate p53, leading to protection against apoptosis and potential anti-ageing effects in cardiomyocytes.[71]

The secretome of senescent cardiomyocytes includes the inflammatory cytokines IL-1 and IL-6, tumour necrosis factor α (TNF-α), endothelin 3 (Edn3), growth and differentiation factor 15 (GDF15), and TGF-β.[55,72] While the inflammatory markers (IL-1, IL-6, and TNF-α) induce local inflammation in the surrounding cardiac microenvironment,[72] Edn3, GDF15, and TGF-β were shown to induce fibrosis and myofibroblast activation in cardiac fibroblasts.[55] Thus, SASP associated with cardiomyocytes has extensive paracrine effects on the surrounding cardiac cell populations.

4.1.2 Clinical consequences of cardiomyocyte senescence

Senescent cardiomyocytes have impaired contractility and abnormal conduction patterns, leading to cardiomyopathies or arrhythmias.[73] For example, in a model of Duchenne muscular dystrophy (DMD), cardiomyocytes from mice lacking dystrophin demonstrate a senescent phenotype.[74] Furthermore, mutations in lamin A (LMNA) are associated with dilated cardiomyopathy, likely related to genomic instability from disruption of the nuclear membrane with increased regions of open chromatin.[4] Also, anthracyclines are a class of chemotherapeutic agents used to treat cancer that can lead to a dilated cardiomyopathy phenotype associated with cardiomyocyte senescence of cardiomyocytes.[75]

Cardiomyocytes from rats administered doxorubicin demonstrate increased mtDNA damage and increased senescent markers 6 months after drug exposure.[75]

Cardiomyocyte senescence may also increase risk of ventricular arrhythmias.[5] Senescent rat ventricular cardiomyocytes have increased ROS that impair their ability to couple with electrical pacing, suggesting increased arrhythmia risk.[76] In addition, following acute MI, patients who develop ventricular arrhythmias exhibit cardiomyocytes with increased telomere dysfunction suggestive of cellular senescence, compared to post-MI patients without ventricular arrhythmias.[77] Mutations in TMEM43 (transmembrane protein 43) are associated with arrhythmogenic cardiomyopathy and cardiomyocyte senescence.[78] Thus, cardiomyocyte senescence may not only lead to intrinsic cardiomyocyte dysfunction but also induce senescent phenotypes of other cardiac cell types via paracrine signalling.

4.2 Endothelial cells

Endothelial cells comprise ~60% of the non-cardiomyocytes in the heart.[53] Endothelial cells regulate vasodilation and vascular tone through secretion of vasoactive compounds and growth factors.[79] Senescent endothelial cells are observed in atherosclerotic plaques, failing hearts (particularly those with diastolic dysfunction), and in hearts with atrial fibrillation.

4.2.1 Molecular mechanisms of senescence in cardiac endothelial cells

Endothelial cell senescence can be triggered by oxidative stress or vascular inflammation.[80] Metabolic factors such as hyperuricaemia observed in high fat/high carbohydrate Western diets or dysregulation of the renin–angiotensin system can also promote endothelial cell senescence.[80] Epigenetic regulation via miRNAs or histone acetylation also contributes to endothelial cell senescence. miR-217 down-regulates eNOS leading to endothelial cell dysfunction and exacerbating atherosclerosis in *apoE* null mice.[81] miR-217 stimulates a senescent phenotype with SIRT1 down-regulation.[82] In endothelial cells, SIRT1 deacetylates p53 to deactivate the replicative senescence pathway.[83]

Senescent endothelial cells show increased endothelin-1 (ET-1) production and decreased nitric oxide production leading to significant crosstalk between senescent endothelial cells and surrounding cardiac cell populations.[79] This leads to vascular inflammation and impaired vasodilation in a positive feedback loop whereupon accumulation of senescent endothelial cells leads to vascular dysfunction, and vice versa.[80] ET-1 secretion causes increased collagen synthesis in senescent cardiac fibroblasts, leading to collagen and fibronectin accumulation characteristic of age-related cardiac hypertrophy.[84] Therapeutically, targeting senescent endothelial cell populations remains an attractive approach to avoiding age-related vascular dysfunction. When treated with neuregulin-1 (NRG1), endothelial senescence was attenuated via ErbB tyrosine kinase receptors.[85] Similarly, expression of telomerase rescued senescence-related endothelial dysfunction.[22] By mediating endothelial cell senescence, this may avoid downstream effects of induced senescence in other cardiac cell populations.

4.2.2 Clinical consequences of endothelial cell senescence in the heart

Endothelial cell senescence leads to impaired vasodilation and vascular dysfunction, leading to disorders such as atherosclerosis, heart failure with preserved ejection fraction (HFpEF) or pulmonary hypertension. SIRT6 deficiency or miR-217 overexpression can promote endothelial cell senescence leading to atherosclerosis.[81,86] HFpEF risk increases with age[87] and is associated with endothelial cell senescence[88] and myocardial fibrosis.[89] Senescence-accelerated prone mice (SAMP8/TaHsd mice with premature ageing formed naturally through selective inbreeding of AKR/J mice[90]) have diastolic dysfunction, left ventricular hypertrophy and interstitial fibrosis by 24 weeks of age, with endothelial cells exhibiting p53 acetylation and up-regulation of SA-β-gal activity.[88]

Congenital heart defects involving aortopulmonary shunts lead to elevated pulmonary arterial pressures that can lead to chronic pulmonary arterial hypertension if left untreated.[91] Pulmonary arterial hypertension can be reversed through surgical correction of aortopulmonary shunts if performed when the child is young[91]; however, late correction leads to irreversible damage to the pulmonary vasculature and chronic pulmonary hypertension develops (i.e. Eisenmenger's syndrome). Loss of the ability of the pulmonary vasculature to remodel is associated with a senescent phenotype in pulmonary endothelial cells.[91]

Atrial fibrillation risk increases with age and can lead to morbidity and mortality associated with thromboembolic events or heart failure.[92] Atrial fibrillation is associated with both endothelial cell and fibroblast senescence.[92,93] eNOS is down-regulated with atrial fibrillation, which is associated with endothelial cell dysfunction.[92] Circulating factors such as thrombin can induce cellular senescence in porcine atrial endothelial cells via an angiotensin II-mediated pathway, initiating a pro-inflammatory, pro-fibrotic, and pro-thrombotic response.[94] Interestingly, expression levels of senescence markers p53 and p16 were positively correlated with severity of atrial fibrillation.[92]

4.3 Cardiac fibroblasts

Cardiac fibroblasts contribute ~20% of the non-cardiomyocyte population in the heart.[53] Cardiac fibroblasts regulate ECM remodelling and reorganization, as well as paracrine communication in the cardiac microenvironment.[95] Senescence induction of cardiac fibroblasts can be beneficial in the setting of chronic wound healing following MI, or detrimental in the setting of myocardial fibrosis during ageing.

4.3.1 Molecular mechanisms of senescence in cardiac fibroblasts

By expressing integrins and MMPs, cardiac fibroblasts maintain ECM structure and integrity for adhesion.[96] Up-regulation of the p53/p21 pathways after acute MI induces senescence of cardiac fibroblasts.[97] The SASP protein, cellular communication network factor 1 (CCN1), suppresses myocardial fibrosis likely through induction of senescence in fibroblasts following MI, suggesting that senescence of fibroblasts may actually have a beneficial effect in some situations.[72,98] In contrast, NEIL3 is a DNA glycosylase that minimizes oxidative damage to DNA through removal of oxidative bases, and *Neil3*$^{-/-}$ mice have increased expression of MMP2, which promotes ECM degradation leading to cardiac rupture.[99]

Cardiac fibroblasts also communicate through paracrine signalling to regulate proliferation, hypertrophic growth, and cardiomyocyte senescence.[95] During heart injury, cardiac fibroblasts up-regulate secretion of the inflammatory cytokines (IL-1, IL-6), and TNF-α, as well as pro-fibrotic TGF-β. Insulin-like growth factor-1 (IGF-1) is also produced by cardiac fibroblasts, which promotes collagen synthesis and cardiomyocyte hypertrophy.[100]

4.3.2 Clinical consequences of cardiac fibroblast senescence

Following acute MI, the phenotype of activated cardiac fibroblasts is dynamic, transitioning from a pro-inflammatory state to an anti-inflammatory state, ultimately supporting scar formation.[101] Premature senescence of cardiac fibroblasts limits collagen expression, which may limit reparative fibrosis during early wound healing but also may prevent excessive fibrosis during chronic wound healing.[97,102] Genetic inactivation of the fibroblast senescence program leads to cardiac dysfunction and increased fibrosis; conversely, induction of senescence in cardiac fibroblasts through CCN1 overexpression leads to decreased fibrosis and improved heart function.[102] However, beneficial effects from fibroblast senescence need to be balanced by potentially detrimental effects of senescence, as clearance of senescent cells with the systemic treatment with the senolytic agent, navitoclax, during ischaemia/reperfusion injury improves outcomes.[103] Interestingly, the risk of cardiac rupture is increased despite hyperproliferation of cardiac fibroblasts in mice with ablation of *Neil3*.[104] Thus while fibroblasts may remain active in the cell cycle in this setting, oxidative DNA damage from *Neil3* deletion may trigger a senescent phenotype in the cardiac microenvironment through SASP-mediated paracrine signalling that promotes ECM degradation and cardiac rupture.[104] While senescence of cardiac fibroblasts may have beneficial effects on chronic wound healing through fibrosis reduction, secretion of SASP factors such as MMP2 from non-senescent fibroblasts may adversely affect ECM remodelling to increase risk of cardiac rupture.[104]

Although premature senescence of fibroblasts in acute injury may reduce fibrosis, a senescent phenotype in chronic conditions may exacerbate cardiac fibrosis. Myocardial fibrosis increases with age and is associated with HFpEF.[105] miR-1468-3p increases senescence of cardiac fibroblasts with increased p53 and p16 expression, increased SA-β-gal activity and increased collagen deposition and cardiac fibrosis via signalling through TGF-β1.[106] SIRT6 deficiency also promotes conversion of fibroblasts to myofibroblasts via TGF-β signalling to increase myocardial fibrosis.[107]

4.4 Vascular smooth muscle cells

VSMCs coordinate vascular function with endothelial cells to control blood pressure, vascular tone, and blood flow.[108] Senescent VSMCs play an important role in development of atherosclerosis and may also promote development of pulmonary hypertension.

4.4.1 Molecular mechanisms of senescence in vascular smooth muscle cells

Senescence in VSMCs can be induced by telomeric shortening, DNA damage, oxidative stress, and autophagic dysfunction.[109,110] Senescent VSMCs have increased SA-β-Gal activity and p16/p21/Rb protein transcriptome regulation.[111] Abnormal processing of prelamin A to lamin A leads to improper formation of the nuclear lamina, making cells more susceptible to DNA damage.[112] Prelamin A up-regulation resulting from mutations in LMNA (Lamin A/C) or an adverse effect from HIV protease inhibitors leads to VSMC senescence via decreased expression of ZMPSTE24, a metalloprotease that processes prelamin A.[113] Continuous DNA damage signalling via prelamin A accumulation encourages secretion of pro-osteogenic cytokines VSMCs leading to VSMC mineralization and subsequent vascular calcification.[114] SIRT6 deficiency can also lead to VSMC senescence due to hyperacetylation of histone 3 at K9 and K27 (H3K9 and H3K27) leading to telomere DNA damage.[115]

Senescent VSMCs have increased expression of inflammatory cytokines. Both IL-6 and C-C motif chemokine ligand 2 (CCL2) are up-regulated in aged VSMCs, as is TLR4-mediated signalling.[116] The SASP of VSMCs additionally contains increased levels of monocyte chemoattractant protein 1 (MCP1), macrophage inflammatory protein -1α/β (MIP1α/β), CCL3/4, and IL-1/IL-6/IL-8, with down-regulation of anti-inflammatory cytokines.[117] IL-1α expression causes neighbouring cells to activate SASP and increase IL-6 secretion, indicating a paracrine effect of senescent VSMCs on inducing local inflammation in the cardiac microenvironment.[117]

4.4.2 Clinical consequences of vascular smooth muscle cell senescence

Like endothelial cells, VSMC senescence leads to diseases of blood vessels, including atherosclerosis or pulmonary hypertension.[2] Plaque VSMCs have shortened telomeres, increased expression of p16 and p21, increased SA-β-gal activity, and increased oxidative DNA damage compared to normal medial VSMCs.[111] Secretion of MCP1, MIP1α/β, and CCL3/4 promotes recruitment of monocytes, macrophages, and lymphocytes, thus accelerating plaque growth and risk of rupture.[118,119] Plaque VSMCs also have reduced SIRT6 expression, while SIRT6 overexpression prevents senescence of VSMCs.[115] Accumulation of prelamin A in VSMCs leads to the clinical phenotype seen in Hutchinson-Gilford progeria syndrome, where patients have severe atherosclerosis leading to accelerated ageing and premature death.[120] Furthermore, cellular senescence of VSMCs may also contribute to the pathophysiology of pulmonary hypertension via SASP.[121]

4.5 Valve interstitial cells

Valve interstitial cells (VICs) make up the majority of the cells in heart valves,[122] and senescence of VICs can lead to valve dysfunction.[123] The prevalence of aortic stenosis due to calcification of the aortic valve leaflets increases with age.[124] Calcified aortic valves have increased expression of p16 and p53 suggestive of a senescence mechanism regulating disease phenotype.[125] Secretion of TNF-α, IL-1β, and IL-6 from pro-inflammatory macrophages induces a transient osteogenic phenotype in VICs leading to valve fibrosis and calcification.[126] Likewise, MAPK activation via TGF-β1 induces a senescent phenotype in porcine aortic VICs that increases ROS and formation of a calcifying nodule *in vitro*.[3] VICs from calcified aortic valves also exhibit hyperacetylation of histones 3 and 4[127] and down-regulation of miR-214 leads to osteogenic differentiation of VICs.[128] Mitral valve VICs in myxomatous mitral valve disease exhibit down-regulation of miR-17, miR-20a, miR-30d, and let-7c, with increased expression of p21 and SA-β-gal activity, reduced viability, and myofibroblastic differentiation.[123]

4.6 Immune cell interactions with senescent resident cardiac cells

The roles of immune cells in senescence regulation in the heart is complex and is reviewed in detail elsewhere.[54,129,130] Here, we address the role of immune cells in regulating senescence of resident cardiac cells rather than senescence of the immune cells themselves. Immune cells have seemingly dual roles—at times, they facilitate elimination of senescent cardiac cells,[131] but at other times, they promote senescence through inflammatory processes. For example, neutrophils mediate polarization of macrophages into an anti-inflammatory phenotype following

myocardial injury[132]; however, the acute inflammatory neutrophil response can also have detrimental effects on myocardial injury.[133] Cardiac macrophages remove senescent or damaged cardiac cells to maintain cardiac homeostasis.[130] However, during ageing, resident cardiac macrophages also have increased secretion of factors such as MMP9, which in turn promotes cardiomyocyte hypertrophy and dysregulated ECM homeostasis with excessive collagen deposition suggestive of fibroblast senescence.[134] Following acute MI, regulatory CD4+ T-cells secrete cytokines that promote wound healing and cardiomyocyte proliferation.[135] However, T cells may also contribute to myocardial dysfunction with ageing, as elderly wild-type mice have worse systolic function compared to elderly mice deficient in CD4+ T cells, possibly due to higher levels of inflammatory cytokines such as TNF.[136] Mast cells release tryptase post-MI which regulates protein kinase A activity to preserve cardiac function by preventing hyperphosphorylation of troponin I and myosin-binding protein.[137] However, in diabetic cardiomyopathy, mast cells have an adverse effect by secreting TNF-α and IL-6 to promote cardiomyocyte apoptosis and collagen synthesis via cardiac fibroblast TGF-β signalling.[138] Further understanding of how to harness the beneficial effects of immune cells may offer therapeutic strategies to decelerate cardiac ageing.

5. Animal models of cardiac senescence

Mouse models with accelerated senescence can be used to study cardiac senescence and age-related CVDs and have been reviewed previously.[139] *Lmna*$^{-/-}$ mice have mitochondrial dysfunction and cardiomyopathy and die by 6–8 weeks of age,[140] while mice with deletion of *Zmpste24*, a metallopeptidase that facilitates processing of lamin A, have conduction abnormalities (bradycardia, QRS complex prolongation) but normal cardiac function.[141] Mice with deletion of Cu/Zn-superoxide dismutase (*Sod1*) in mice are more susceptible to ischaemia–reperfusion injury due to increased ROS.[142] BubR1 is a mitotic checkpoint protein that is required for embryonic development[143]; mice with hypomorphic *BubR1* alleles have premature death with muscle wasting and cardiac arrhythmias.[144] Also, senescence-accelerated prone mice (SAMP), developed at Kyoto University formed by selective inbreeding of AKR/J mice, have premature senescence and ageing.[90] The study of these murine models of cardiac senescence can further elucidate the physiological changes of senescent cardiac cell populations and their role in aggravating age-related CVDs.

6. Current challenges, targeted therapies, and future directions

6.1 Prevention of senescence following chemotherapy

Anthracyclines, including doxorubicin, can induce senescence in multiple cell types in the heart leading to late heart failure after successful treatment of cancers.[145] Doxorubicin induces damage to mtDNA in rat neonatal cardiomyocytes, and increased p16 expression and SA-β-gal activity are seen in the left ventricle of doxorubicin-treated rats.[75] Doxorubicin-induced senescence of VSMCs reduces SIRT1 expression and decreases AMP-activated kinase (AMPK) activation, leading to a pro-inflammatory state.[146] Prednisolone can prevent VSMC senescence following doxorubicin exposure.[146] LncRNA-MALAT1 prevents miRNA-92a-3p from inhibiting autophagy-regulating protease 4a (ATG4a); ATG4a suppresses senescence and improves mitochondrial function in doxorubicin-exposed cardiomyocytes.[147] Strategies aimed at preventing cellular senescence during chemotherapy may prevent late cardiac effects from anthracycline toxicity.

6.2 Senotherapies

Senescent cells have increased glycolysis, increased DDRs and exhibit SASP,[148] providing potential targets for drugs to remove senescent cells (senolytics) or suppress the senescent phenotype (senostatics). Potential drug candidates have been reviewed in detail previously.[149–151] Here we highlight strategies that have been tested to minimize the effects of senescent cells in models of CVD (*Figure 5*).

6.2.1 Dasatinib and quercetin

Two of the first senolytics identified were dasatinib and quercetin, identified while screening drugs known to facilitate apoptosis.[148] Dasatinib is a tyrosine kinase inhibitor that induces apoptosis in senescent cells by preventing interaction of ephrin ligands with ephrin receptors.[148] Quercetin is a flavonol that inhibits PI3K, increases expression of SIRT1[152] and inhibits mTOR signalling.[153] Quercetin decreases lipid deposition and increases SIRT1 expression in aortas of ApoE$^{-/-}$ mice.[154] Combined quercetin and dasatinib administration reduces p16-expressing senescent cells *in vivo* in mice after exposure to ionizing radiation[148] and improves left ventricular systolic function in aged mice.[148] Similarly, quercetin and dasatinib treatment reduced senescent markers in mice with hypercholesterolaemia and also improved vasomotor function and reduced aortic calcification.[155]

6.2.2 Navitoclax (ABT-263)

Navitoclax inhibits activity of the anti-apoptotic BCL-2 family of proteins and induces apoptosis of senescent lung fibroblasts but not preadipocytes.[156] Navitoclax promotes clearance of senescent cardiomyocytes following MI and improves survival and reduces fibrosis in mice.[157] Navitoclax also improves left ventricular systolic function, reduces cardiac fibrosis and hypertrophy, and improves conduction velocity in mice with Ang II-induced heart failure.[158]

6.2.3 Cardiac glycosides

The Na+/K+ ATPase pump utilizes ATP to move sodium out of the cell and potassium into the cell against each of their concentration gradients.[159] Cardiac glycosides (including digoxin, ouabain, and proscillaridin A) are a class of compounds derived from the foxglove plant that inhibit activity of the Na+/K+ ATPase pump leading to depolarization and disruption of the electrochemical gradient.[160] Senescent cells have a more depolarized plasma membrane than non-senescent cells, thus are more prone to the effects of cardiac glycosides.[160] Digoxin can eliminate senescent pulmonary fibroblasts in a mouse model of pulmonary fibrosis.[160]

6.2.4 Metabolic regulators

The mTOR pathway controls conversion from a quiescent to a senescent state,[161] and when inhibited, can prolong lifespan in mice.[162] Rapamycin can delay senescence and promote clearance of progerin via enhanced autophagy in fibroblasts from patients with Hutchinson-Gilford progeria syndrome.[163] Celastrol was shown to inhibit mTOR signalling and increase autophagy in VSMCs, leading to reduced intracellular

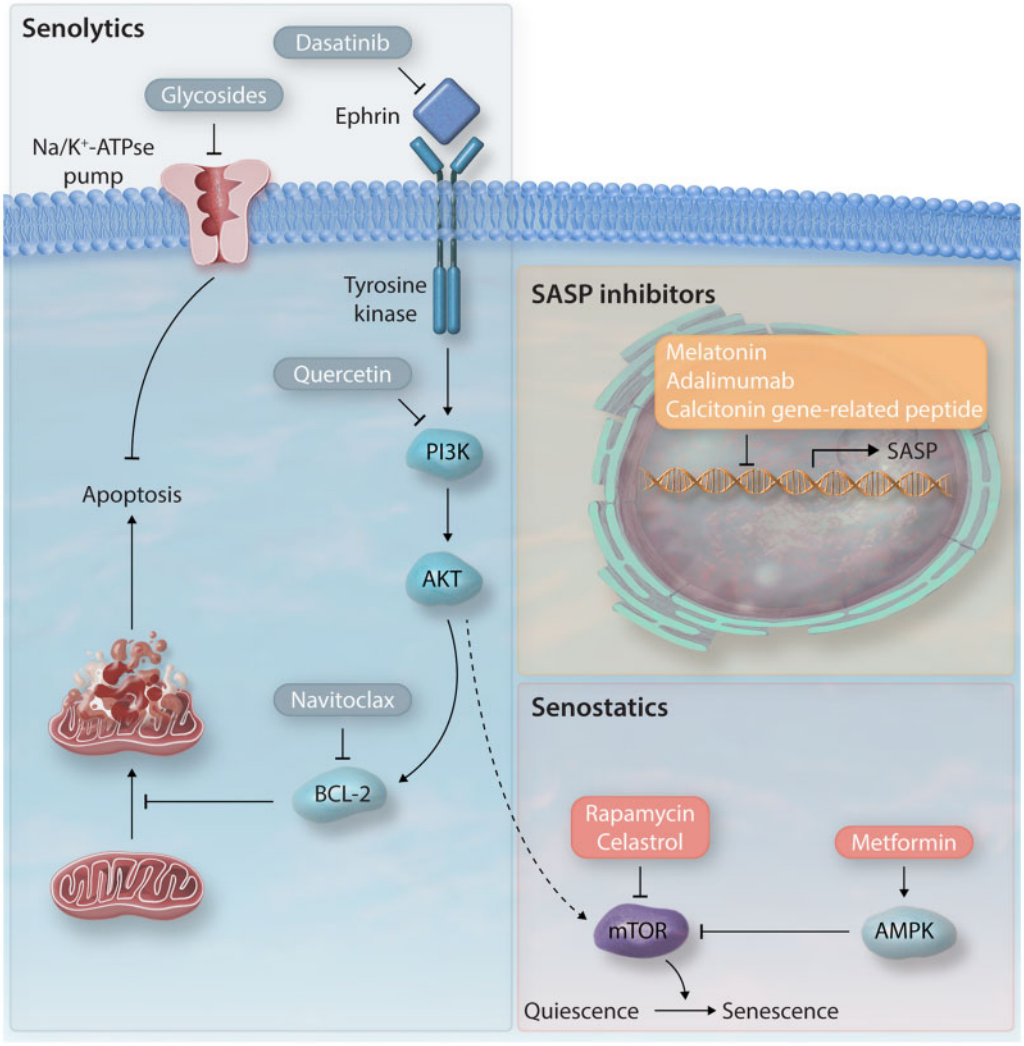


Figure 5 Senotherapeutic drugs. Senotherapies aim to eliminate senescent cells (senolytics), prevent acquisition of a senescent state (senostatics), or delay progression of senescence (SASP inhibitors).

ROS and prevention of Ang II-induced senescence.[164] Metformin, a drug commonly used for treatment of diabetes, also demonstrates effects on longevity via AMPK activation.[165] When administered to *ApoE*$^{-/-}$ mice, metformin inhibits endothelial cell senescence and inhibited formation of atherosclerotic plaques.[166]

6.2.5 SASP inhibition

SASP factors are diverse and context-dependent, but inhibition of SASP can prevent downstream inflammatory responses that further accelerate cellular ageing.[151] Melatonin can suppress SASP in human primary foetal lung fibroblast and human embryonic fibroblast cells via preventing acetylation of H2BK120 mediated by poly(ADP-ribose) polymerase-1 (PARP-1) and CREB-binding protein, thereby preventing up-regulation of SASP genes.[167] Calcitonin gene-related peptide can prevent senescence and SASP in cardiac fibroblasts via up-regulation of klotho, an anti-ageing protein.[168] Inhibiting SASP without eliciting adverse effects is challenging because many pathways that can broadly activate SASP (such as NF-κB or mTOR) are also important in regulating other physiological processes such as tumour surveillance or the immune system.[149]

6.3 Personalized therapies

Precision medicine aims to provide personalized therapies for patients based on the disease phenotype and molecular mechanisms of disease.[169] Molecular mechanisms that promote senescence of individual cardiac cell types can potentially be targeted to prevent or treat disease. For example, lack of dystroglycan in DMD promotes senescence, likely due to dysregulation of the nuclear architecture with subsequent genomic instability.[170] Precision medicines have recently been developed for DMD patients who have a frame shift mutation in the dystrophin gene leading to a premature stop codon by delivering antisense oligonucleotides to skip or bypass the mutant region of DNA.[171] Our understanding of epigenetic, proteomic, metabolomic, and microbiomic mechanisms of disease is expanding rapidly and further discussion of how this knowledge can be applied to specific CVD phenotypes has been reviewed previously.[172]

6.4 Cell replacement

Cell replacement strategies aim to deliver differentiated human cells derived from stem cells to improve function of the dysfunctional cell type. In the heart, because the majority of cardiomyocytes do not actively proliferate, there is much interest in delivering induced pluripotent stem cell-derived cardiomyocytes to improve cardiac function in heart failure.[173] In another approach, bone marrow stem cell antigen 1 (Sca1)+ cells from young mice transplanted into aged mice suppresses senescence and promotes rejuvenation of cardiac endothelial cells in aged mice.[174] Interplay of immune cells with resident cardiac cell types should be considered when designing cell-based strategies to promote cardiac rejuvenation.

7. Conclusions

Cellular senescence is becoming increasingly recognized as a possible cause of disease pathophysiology throughout the cardiovascular system. Understanding how senescence of individual cardiac cell types contributes to specific disease phenotypes will allow us to develop improved, targeted therapies to delay or prevent ageing-associated diseases in the heart. Current therapeutic approaches are being studied that target global senescence suppression and/or removal of senescent cells. Further advances in genetic, epigenetic or metabolomic mechanisms of cellular senescence in the heart may provide further personalization to treat patients with an array of CVDs.

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References

1. Davalli P, Mitic T, Caporali A, Lauriola A, D'Arca D. ROS, cell senescence, and novel molecular mechanisms in aging and age-related diseases. *Oxid Med Cell Longev* 2016;**2016**:3565127.
2. Wang JC, Bennett M. Aging and atherosclerosis: mechanisms, functional consequences, and potential therapeutics for cellular senescence. *Circ Res* 2012;**111**:245–259.
3. Das D, Holmes A, Murphy GA, Mishra K, Rosenkranz AC, Horowitz JD, Kennedy JA. TGF-beta1-Induced MAPK activation promotes collagen synthesis, nodule formation, redox stress and cellular senescence in porcine aortic valve interstitial cells. *J Heart Valve Dis* 2013;**22**:621–630.
4. Lee J, Termglinchan V, Diecke S, Itzhaki I, Lam CK, Garg P, Lau E, Greenhaw M, Seeger T, Wu H, Zhang JZ, Chen X, Gil IP, Ameen M, Sallam K, Rhee JW, Churko JM, Chaudhary R, Chour T, Wang PJ, Snyder MP, Chang HY, Karakikes I, Wu JC. Activation of PDGF pathway links LMNA mutation to dilated cardiomyopathy. *Nature* 2019;**572**:335–340.
5. Chadda KR, Ajijola OA, Vaseghi M, Shivkumar K, Huang CL, Jeevaratnam K. Ageing, the autonomic nervous system and arrhythmia: from brain to heart. *Ageing Res Rev* 2018;**48**:40–50.
6. Mao Z, Ke Z, Gorbunova V, Seluanov A. Replicatively senescent cells are arrested in G1 and G2 phases. *Aging (Albany NY)* 2012;**4**:431–435.
7. Paluvai H, Di Giorgio E, Brancolini C. The histone code of senescence. *Cells* 2020;**9**:466.
8. Marescal O, Cheeseman IM. Cellular mechanisms and regulation of quiescence. *Dev Cell* 2020;**55**:259–271.
9. Rufini A, Tucci P, Celardo I, Melino G. Senescence and aging: the critical roles of p53. *Oncogene* 2013;**32**:5129–5143.
10. Rayess H, Wang MB, Srivatsan ES. Cellular senescence and tumor suppressor gene p16. *Int J Cancer* 2012;**130**:1715–1725.
11. Freund A, Patil CK, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. *EMBO J* 2011;**30**:1536–1548.
12. Narita M, Nunez S, Heard E, Narita M, Lin AW, Hearn SA, Spector DL, Hannon GJ, Lowe SW. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* 2003;**113**:703–716.
13. Coppé J-P, Desprez P-Y, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. *Annu Rev Pathol* 2010;**5**:99–118.
14. Yang NC, Hu ML. The limitations and validities of senescence associated-beta-galactosidase activity as an aging marker for human foreskin fibroblast Hs68 cells. *Exp Gerontol* 2005;**40**:813–819.
15. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 1997;**88**:593–602.
16. Storer M, Mas A, Robert-Moreno A, Pecoraro M, Ortells MC, Di Giacomo V, Yosef R, Pilpel N, Krizhanovsky V, Sharpe J, Keyes WM. Senescence is a developmental mechanism that contributes to embryonic growth and patterning. *Cell* 2013;**155**:1119–1130.
17. Spallarossa P, Altieri P, Aloi C, Garibaldi S, Barisione C, Ghigliotti G, Fugazza G, Barsotti A, Brunelli C. Doxorubicin induces senescence or apoptosis in rat neonatal cardiomyocytes by regulating the expression levels of the telomere binding factors 1 and 2. *Am J Physiol Heart Circ Physiol* 2009;**297**:H2169–H2181.
18. Seluanov A, Gorbunova V, Falcovitz A, Sigal A, Milyavsky M, Zurer I, Shohat G, Goldfinger N, Rotter V. Change of the death pathway in senescent human fibroblasts in response to DNA damage is caused by an inability to stabilize p53. *Mol Cell Biol* 2001;**21**:1552–1564.
19. Watanabe S, Kawamoto S, Ohtani N, Hara E. Impact of senescence-associated secretory phenotype and its potential as a therapeutic target for senescence-associated diseases. *Cancer Sci* 2017;**108**:563–569.
20. Lange T. Shelterin: the protein complex that shapes and safeguards human telomeres. *Genes Dev* 2005;**19**:2100–2110.
21. Fyhrquist F, Saijonmaa O, Strandberg T. The roles of senescence and telomere shortening in cardiovascular disease. *Nat Rev Cardiol* 2013;**10**:274–283.
22. Minamino T, Miyauchi H, Yoshida T, Ishida Y, Yoshida H, Komuro I. Endothelial cell senescence in human atherosclerosis. *Circulation* 2002;**105**:1541–1544.
23. Bode AM, Dong Z. Post-translational modification of p53 in tumorigenesis. *Nat Rev Cancer* 2004;**4**:793–805.
24. Yosef R, Pilpel N, Papismadov N, Gal H, Ovadya Y, Vadai E, Miller S, Porat Z, Ben-Dor S, Krizhanovsky V. p21 maintains senescent cell viability under persistent DNA damage response by restraining JNK and caspase signaling. *EMBO J* 2017;**36**:2280–2295.
25. Takahashi A, Ohtani N, Yamakoshi K, Iida S-I, Tahara H, Nakayama K, Nakayama KI, Ide T, Saya H, Hara E. Mitogenic signalling and the p16INK4a-Rb pathway cooperate to enforce irreversible cellular senescence. *Nat Cell Biol* 2006;**8**:1291–1297.
26. Sciarretta S, Volpe M, Sadoshima J. Mammalian target of rapamycin signaling in cardiac physiology and disease. *Circ Res* 2014;**114**:549–564.
27. Jung SH, Hwang HJ, Kang D, Park HA, Lee HC, Jeong D, Lee K, Park HJ, Ko Y-G, Lee J-S. mTOR kinase leads to PTEN-loss-induced cellular senescence by phosphorylating p53. *Oncogene* 2019;**38**:1639–1650.
28. Korotchkina LG, Leontieva OV, Bukreeva EI, Demidenko ZN, Gudkov AV, Blagosklonny MV. The choice between p53-induced senescence and quiescence is determined in part by the mTOR pathway. *Aging (Albany NY)* 2010;**2**:344–352.
29. Park YY, Lee S, Karbowski M, Neutzner A, Youle RJ, Cho H. Loss of MARCH5 mitochondrial E3 ubiquitin ligase induces cellular senescence through dynamin-related protein 1 and mitofusin 1. *J Cell Sci* 2010;**123**:619–626.
30. Lee S, Jeong SY, Lim WC, Kim S, Park YY, Sun X, Youle RJ, Cho H. Mitochondrial fission and fusion mediators, hFis1 and OPA1, modulate cellular senescence. *J Biol Chem* 2007;**282**:22977–22983.
31. Song M, Franco A, Fleischer JA, Zhang L, Dorn GW 2nd. Abrogating mitochondrial dynamics in mouse hearts accelerates mitochondrial senescence. *Cell Metab* 2017;**26**:872–883.e5.
32. Chen Y, Liu Y, Dorn GW 2nd. Mitochondrial fusion is essential for organelle function and cardiac homeostasis. *Circ Res* 2011;**109**:1327–1331.
33. Twig G, Elorza A, Molina AJ, Mohamed H, Wikstrom JD, Walzer G, Stiles L, Haigh SE, Katz S, Las G, Alroy J, Wu M, Py BF, Yuan J, Deeney JT, Corkey BE, Shirihai OS. Fission and selective fusion govern mitochondrial segregation and elimination by autophagy. *EMBO J* 2008;**27**:433–446.
34. Lesnefsky EJ, Chen Q, Hoppel CL. Mitochondrial metabolism in aging heart. *Circ Res* 2016;**118**:1593–1611.

35. Floyd RA, West M, Hensley K. Oxidative biochemical markers; clues to understanding aging in long-lived species. *Exp Gerontol* 2001;**36**:619–640.
36. Zhang W, Song M, Qu J, Liu G-H. Epigenetic modifications in cardiovascular aging and diseases. *Circ Res* 2018;**123**:773–786.
37. Cruickshanks HA, McBryan T, Nelson DM, VanderKraats ND, Shah PP, van Tuyn J, Rai TS, Brock C, Donahue G, Dunican DS, Drotar ME, Meehan RR, Edwards JR, Berger SL, Adams PD. Senescent cells harbour features of the cancer epigenome. *Nat Cell Biol* 2013;**15**:1495–1506.
38. Irizarry RA, Ladd-Acosta C, Wen B, Wu Z, Montano C, Onyango P, Cui H, Gabo K, Rongione M, Webster M, Ji H, Potash J, Sabunciyan S, Feinberg AP. The human colon cancer methylome shows similar hypo- and hypermethylation at conserved tissue-specific CpG island shores. *Nat Genet* 2009;**41**:178–186.
39. Hayakawa T, Iwai M, Aoki S, Takimoto K, Maruyama M, Maruyama W, Motoyama N. SIRT1 suppresses the senescence-associated secretory phenotype through epigenetic gene regulation. *PLoS One* 2015;**10**:e0116480.
40. Man AWC, Li H, Xia N. The role of Sirtuin1 in regulating endothelial function, arterial remodeling and vascular aging. *Front Physiol* 2019;**10**:1173.
41. Jazbutyte V, Fiedler J, Kneitz S, Galuppo P, Just A, Holzmann A, Bauersachs J, Thum T. MicroRNA-22 increases senescence and activates cardiac fibroblasts in the aging heart. *AGE* 2013;**35**:747–762.
42. Lyu G, Guan Y, Zhang C, Zong L, Sun L, Huang X, Huang L, Zhang L, Tian X-L, Zhou Z, Tao W. TGF-β signaling alters H4K20me3 status via miR-29 and contributes to cellular senescence and cardiac aging. *Nat Commun* 2018;**9**:2560.
43. Florio MC, Magenta A, Beji S, Lakatta EG, Capogrossi MC. Aging, microRNAs, and heart failure. *Curr Probl Cardiol* 2020;**45**:100406.
44. Lozano-Vidal N, Bink DI, Boon RA. Long noncoding RNA in cardiac aging and disease. *J Mol Cell Biol* 2019;**11**:860–867.
45. Zhuang Y, Li T, Xiao H, Wu J, Su S, Dong X, Hu X, Hua Q, Liu J, Shang W, Ju J, Sun F, Pan Z, Lu Y, Zhang M. LncRNA-H19 drives cardiomyocyte senescence by targeting miR-19a/socs1/p53 axis. *Front Pharmacol* 2021;**12**:631835.
46. Haemmig S, Yang D, Sun X, Das D, Ghaffari S, Molinaro R, Chen L, Deng Y, Freeman D, Moullan N, Tesmenitsky Y, Wara AKM, Simion V, Shvartz E, Lee JF, Yang T, Sukova G, Marto JA, Stone PH, Lee WL, Auwerx J, Libby P, Feinberg MW. Long noncoding RNA SNHG12 integrates a DNA-PK-mediated DNA damage response and vascular senescence. *Sci Transl Med* 2020;**12**:eaaw1868.
47. Rodier F, Coppé J-P, Patil CK, Hoeijmakers WAM, Muñoz DP, Raza SR, Freund A, Campeau E, Davalos AR, Campisi J. Persistent DNA damage signalling triggers senescence-associated inflammatory cytokine secretion. *Nat Cell Biol* 2009;**11**:973–979.
48. Lau L, Porciuncula A, Yu A, Iwakura Y, David G. Uncoupling the senescence-associated secretory phenotype from cell cycle exit via interleukin-1 inactivation unveils its protumorigenic role. *Mol Cell Biol* 2019;**39**:e00586-18.
49. Inokawa H, Umemura Y, Shimba A, Kawakami E, Koike N, Tsuchiya Y, Ohashi M, Minami Y, Cui G, Asahi T, Ono R, Sasawaki Y, Konishi E, Yoo SH, Chen Z, Teramukai S, Ikuta K, Yagita K. Chronic circadian misalignment accelerates immune senescence and abbreviates lifespan in mice. *Sci Rep* 2020;**10**:2569.
50. Wang CY, Wen MS, Wang HW, Hsieh IC, Li Y, Liu PY, Lin FC, Liao JK. Increased vascular senescence and impaired endothelial progenitor cell function mediated by mutation of circadian gene Per2. *Circulation* 2008;**118**:2166–2173.
51. Monfredi O, Lakatta EG. Complexities in cardiovascular rhythmicity: perspectives on circadian normality, ageing and disease. *Cardiovasc Res* 2019;**115**:1576–1595.
52. Kunieda T, Minamino T, Katsuno T, Tateno K, Nishi J, Miyauchi H, Orimo M, Okada S, Komuro I. Cellular senescence impairs circadian expression of clock genes *in vitro* and *in vivo*. *Circ Res* 2006;**98**:532–539.
53. Pinto AR, Ilinykh A, Ivey MJ, Kuwabara JT, D'Antoni ML, Debuque R, Chandran A, Wang L, Arora K, Rosenthal NA, Tallquist MD. Revisiting cardiac cellular composition. *Circ Res* 2016;**118**:400–409.
54. Tang X, Li P-H, Chen H-Z. Cardiomyocyte senescence and cellular communications within myocardial microenvironments. *Front Endocrinol (Lausanne)* 2020;**11**:280.
55. Anderson R, Lagnado A, Maggiorani D, Walaszczyk A, Dookun E, Chapman J, Birch J, Salmonowicz H, Ogrodnik M, Jurk D, Proctor C, Correia-Melo C, Victorelli S, Fielder E, Berlinguer-Palmini R, Owens A, Greaves LC, Kolsky KL, Parini A, Douin-Echinard V, LeBrasseur NK, Arthur HM, Tual-Chalot S, Schafer MJ, Roos CM, Miller JD, Robertson N, Mann J, Adams PD, Tchkonia T, Kirkland JL, Mialet-Perez J, Richardson GD, Passos JF. Length-independent telomere damage drives post-mitotic cardiomyocyte senescence. *EMBO J* 2019;**38**:e100492.
56. Miyazono Y, Hirashima S, Ishihara N, Kusukawa J, Nakamura KI, Ohta K. Uncoupled mitochondria quickly shorten along their long axis to form indented spheroids, instead of rings, in a fission-independent manner. *Sci Rep* 2018;**8**:350.
57. Vasileiou PVS, Evangelou K, Vlasis K, Fildisis G, Panayiotidis MI, Chronopoulos E, Passias PG, Kouloukoussa M, Gorgoulis VG, Havaki S. Mitochondrial homeostasis and cellular senescence. *Cells* 2019;**8**:686.
58. Chang ZS, Xia JB, Wu HY, Peng WT, Jiang FQ, Li J, Liang CQ, Zhao H, Park KS, Song GH, Kim SK, Huang R, Zheng L, Cai DQ, Qi XF. Forkhead box O3 protects the heart against paraquat-induced aging-associated phenotypes by upregulating the expression of antioxidant enzymes. *Aging Cell* 2019;**18**:e12990.
59. Dai D-F, Santana LF, Vermulst M, Tomazela DM, Emond MJ, MacCoss MJ, Gollahon K, Martin GM, Loeb LA, Ladiges WC, Rabinovitch PS. Overexpression of catalase targeted to mitochondria attenuates murine cardiac aging. *Circulation* 2009;**119**:2789–2797.
60. Hahn A, Zuryn S. Mitochondrial genome (mtDNA) mutations that generate reactive oxygen species. *Antioxidants (Basel)* 2019;**8**:392.
61. Oka T, Hikoso S, Yamaguchi O, Taneike M, Takeda T, Tamai T, Oyabu J, Murakawa T, Nakayama H, Nishida K, Akira S, Yamamoto A, Komuro I, Otsu K. Mitochondrial DNA that escapes from autophagy causes inflammation and heart failure. *Nature* 2012;**485**:251–255.
62. Hoshino A, Mita Y, Okawa Y, Ariyoshi M, Iwai-Kanai E, Ueyama T, Ikeda K, Ogata T, Matoba S. Cytosolic p53 inhibits Parkin-mediated mitophagy and promotes mitochondrial dysfunction in the mouse heart. *Nat Commun* 2013;**4**:2308.
63. Gu J, Wang S, Guo H, Tan Y, Liang Y, Feng A, Liu Q, Damodaran C, Zhang Z, Keller BB, Zhang C, Cai L. Inhibition of p53 prevents diabetic cardiomyopathy by preventing early-stage apoptosis and cell senescence, reduced glycolysis, and impaired angiogenesis. *Cell Death Dis* 2018;**9**:82.
64. Mak TW, Hauck L, Grothe D, Billia F. p53 regulates the cardiac transcriptome. *Proc Natl Acad Sci USA* 2017;**114**:2331–2336.
65. Alam P, Haile B, Arif M, Pandey R, Rokvic M, Nieman M, Maliken BD, Paul A, Wang YG, Sadayappan S, Ahmed RPH, Kanisicak O. Inhibition of senescence-associated genes Rb1 and Meis2 in adult cardiomyocytes results in cell cycle reentry and cardiac repair post-myocardial infarction. *J Am Heart Assoc* 2019;**8**:e012089.
66. Sharifi-Sanjani M, Oyster NM, Tichy ED, Bedi KC, Harel O, Margulies KB, Mourkioti F. Cardiomyocyte-specific telomere shortening is a distinct signature of heart failure in humans. *JAHA* 2017;**6**:e005086.
67. El-Nachef D, Oyama K, Wu Y-Y, Freeman M, Zhang Y, MacLellan WR. Repressive histone methylation regulates cardiac myocyte cell cycle exit. *J Mol Cell Cardiol* 2018;**121**:1–12.
68. Boon RA, Iekushi K, Lechner S, Seeger T, Fischer A, Heydt S, Kaluza D, Tréguer K, Carmona G, Bonauer A, Horrevoets AJG, Didier N, Girmatsion Z, Biliczki P, Ehrlich JR, Katus HA, Müller OJ, Potente M, Zeiher AM, Hermeking H, Dimmeler S. MicroRNA-34a regulates cardiac ageing and function. *Nature* 2013;**495**:107–110.
69. Almen GCv, Verhesen W, Leeuwen REWv, Vrie M, Eurlings C, Schellings MWM, Swinnen M, Cleutjens JPM, Zandvoort M, Heymans S, Schroen B. MicroRNA-18 and microRNA-19 regulate CTGF and TSP-1 expression in age-related heart failure. *Aging Cell* 2011;**10**:769–779.
70. Alcendor RR, Gao S, Zhai P, Zablocki D, Holle E, Yu X, Tian B, Wagner T, Vatner SF, Sadoshima J. Sirt1 regulates aging and resistance to oxidative stress in the heart. *Circ Res* 2007;**100**:1512–1521.
71. Vakhrusheva O, Smolka C, Gajawada P, Kostin S, Boettger T, Kubin T, Braun T, Bober E. Sirt7 increases stress resistance of cardiomyocytes and prevents apoptosis and inflammatory cardiomyopathy in mice. *Circ Res* 2008;**102**:703–710.
72. Cui S, Xue L, Yang F, Dai S, Han Z, Liu K, Liu B, Yuan Q, Cui Z, Zhang Y, Xu F, Chen Y. Postinfarction hearts are protected by premature senescent cardiomyocytes via GATA4-dependent CCN1 secretion. *JAHA* 2018;**7**:e009111.
73. Gude NA, Broughton KM, Firouzi F, Sussman MA. Cardiac ageing: extrinsic and intrinsic factors in cellular renewal and senescence. *Nat Rev Cardiol* 2018;**15**:523–542.
74. Chang AC, Ong SG, LaGory EL, Kraft PE, Giaccia AJ, Wu JC, Blau HM. Telomere shortening and metabolic compromise underlie dystrophic cardiomyopathy. *Proc Natl Acad Sci USA* 2016;**113**:13120–13125.
75. Mitry MA, Laurent D, Keith BL, Sira E, Eisenberg CA, Eisenberg LM, Joshi S, Gupte S, Edwards JG. Accelerated cardiomyocyte senescence contributes to late-onset doxorubicin-induced cardiotoxicity. *Am J Physiol Cell Physiol* 2020;**318**:C380–C391.
76. Masoud S, McDonald F, Bister D, Kotecki C, Bootman MD, Rietdorf K. Examining cardiomyocyte dysfunction using acute chemical induction of an ageing phenotype. *Int J Mol Sci* 2019;**21**:197.
77. Sawhney V, Campbell NG, Brouilette SW, Coppen SR, Harbo M, Baker V, Ikebe C, Shintani Y, Hunter RJ, Dhinoja M, Johnston A, Earley MJ, Sporton S, Bendix L, Suzuki K, Schilling RJ. Telomere shortening and telomerase activity in ischaemic cardiomyopathy patients—potential markers of ventricular arrhythmia. *Int J Cardiol* 2016;**207**:157–163.
78. Rouhi L, Cheedipudi SM, Chen SN, Fan S, Lombardi R, Chen X, Coarfa C, Robertson MJ, Gurha P, Marian AJ. Haplo-insufficiency of Tmem43 in cardiac myocytes activates the DNA damage response pathway leading to a Late-Onset Senescence-Associated pro-fibrotic cardiomyopathy. *Cardiovasc Res* 2020;cvaa300. (online ahead of print)
79. Colliva A, Braga L, Giacca M, Zacchigna S. Endothelial cell–cardiomyocyte crosstalk in heart development and disease. *J Physiol* 2020;**598**:2923–2939.
80. Jia G, Aroor AR, Jia C, Sowers JR. Endothelial cell senescence in aging-related vascular dysfunction. *Biochim Biophys Acta Mol Basis Dis* 2019;**1865**:1802–1809.
81. de Yebenes VG, Briones AM, Martos-Folgado I, Mur SM, Oller J, Bilal F, Gonzalez-Amor M, Mendez-Barbero N, Silla-Castro JC, Were F, Jimenez-Borreguero LJ, Sanchez-Cabo F, Bueno H, Salaices M, Redondo JM, Ramiro AR. Aging-associated miR-217 aggravates atherosclerosis and promotes cardiovascular dysfunction. *Arterioscler Thromb Vasc Biol* 2020;**40**:2408–2424.
82. Menghini R, Casagrande V, Cardellini M, Martelli E, Terrinoni A, Amati F, Vasa-Nicotera M, Ippoliti A, Novelli G, Melino G, Lauro R, Federici M. MicroRNA 217

modulates endothelial cell senescence via silent information regulator 1. *Circulation* 2009;**120**:1524–1532.
83. Ota H, Akishita M, Eto M, Iijima K, Kaneki M, Ouchi Y. Sirt1 modulates premature senescence-like phenotype in human endothelial cells. *J Mol Cell Cardiol* 2007;**43**: 571–579.
84. Wang X, Guo Z, Ding Z, Khaidakov M, Lin J, Xu Z, Sharma SG, Jiwani S, Mehta JL. Endothelin-1 upregulation mediates aging-related cardiac fibrosis. *J Mol Cell Cardiol* 2015;**80**:101–109.
85. Shakeri H, Gevaert AB, Schrijvers DM, De Meyer GRY, De Keulenaer GW, Guns P-J, Lemmens K, Segers VF. Neuregulin-1 attenuates stress-induced vascular senescence. *Cardiovasc Res* 2018;**114**:1041–1051.
86. Lee OH, Woo YM, Moon S, Lee J, Park H, Jang H, Park YY, Bae SK, Park KH, Heo JH, Choi Y. Sirtuin 6 deficiency induces endothelial cell senescence via downregulation of forkhead box M1 expression. *Aging (Albany NY)* 2020;**12**: 20946–20967.
87. Oktay AA, Rich JD, Shah SJ. The emerging epidemic of heart failure with preserved ejection fraction. *Curr Heart Fail Rep* 2013;**10**:401–410.
88. Gevaert AB, Shakeri H, Leloup AJ, Van Hove CE, De Meyer GRY, Vrints CJ, Lemmens K, Van Craenenbroeck EM. Endothelial senescence contributes to heart failure with preserved ejection fraction in an aging mouse model. *Circ Heart Fail* 2017;**10**:e003806.
89. Ovchinnikov AG, Arefieva TI, Potekhina AV, Filatova AY, Ageev FT, Boytsov SA. The molecular and cellular mechanisms associated with a microvascular inflammation in the pathogenesis of heart failure with preserved ejection fraction. *Acta Naturae* 2020;**12**:40–51.
90. Takeda T, Hosokawa M, Higuchi K. Senescence-accelerated mouse (SAM): a novel murine model of senescence. *Exp Gerontol* 1997;**32**:105–109.
91. van der Feen DE, Bossers GPL, Hagdorn QAJ, Moonen JR, Kurakula K, Szulcek R, Chappell J, Vallania F, Donato M, Kok K, Kohli JS, Petersen AH, van Leusden T, Demaria M, Goumans MTH, De Boer RA, Khatri P, Rabinovitch M, Berger RMF, Bartelds B. Cellular senescence impairs the reversibility of pulmonary arterial hypertension. *Sci Transl Med* 2020;**12**:eaaw4974.
92. Jesel L, Abbas M, Park SH, Matsushita K, Kindo M, Hasan H, Auger C, Sato C, Ohlmann P, Mazzucotelli JP, Toti F, Kauffenstein G, Schini-Kerth V, Morel O. Atrial fibrillation progression is associated with cell senescence burden as determined by p53 and p16 expression. *J Clin Med* 2019;**9**:36.
93. Xie J, Chen Y, Hu C, Pan Q, Wang B, Li X, Geng J, Xu B. Premature senescence of cardiac fibroblasts and atrial fibrosis in patients with atrial fibrillation. *Oncotarget* 2017;**8**:57981–57990.
94. Hasan H, Park SH, Auger C, Belcastro E, Matsushita K, Marchandot B, Lee HH, Qureshi AW, Kauffenstein G, Ohlmann P, Schini-Kerth VB, Jesel L, Morel O. Thrombin induces angiotensin II-mediated senescence in atrial endothelial cells: impact on pro-remodeling patterns. *J Clin Med* 2019;**8**:1570.
95. Saucerman JJ, Tan PM, Buchholz KS, McCulloch AD, Omens JH. Mechanical regulation of gene expression in cardiac myocytes and fibroblasts. *Nat Rev Cardiol* 2019; **16**:361–378.
96. Civitarese RA, Kapus A, McCulloch CA, Connelly KA. Role of integrins in mediating cardiac fibroblast–cardiomyocyte cross talk: a dynamic relationship in cardiac biology and pathophysiology. *Basic Res Cardiol* 2016;**112**:6.
97. Zhu F, Li Y, Zhang J, Piao C, Liu T, Li H-H, Du J. Senescent cardiac fibroblast is critical for cardiac fibrosis after myocardial infarction. *PLoS One* 2013;**8**:e74535.
98. Shibamoto M, Higo T, Naito AT, Nakagawa A, Sumida T, Okada K, Sakai T, Kuramoto Y, Yamaguchi T, Ito M, Masumura Y, Higo S, Lee JK, Hikoso S, Komuro I, Sakata Y. Activation of DNA damage response and cellular senescence in cardiac fibroblasts limit cardiac fibrosis after myocardial infarction. *Int Heart J* 2019;**60**: 944–957.
99. Ozcan S, Alessio N, Acar MB, Mert E, Omerli F, Peluso G, Galderisi U. Unbiased analysis of senescence associated secretory phenotype (SASP) to identify common components following different genotoxic stresses. *Aging (Albany NY)* 2016;**8**: 1316–1329.
100. Horio T, Maki T, Kishimoto I, Tokudome T, Okumura H, Yoshihara F, Suga S-I, Takeo S, Kawano Y, Kangawa K. Production and autocrine/paracrine effects of endogenous insulin-like growth factor-1 in rat cardiac fibroblasts. *Regul Pept* 2005;**124**: 65–72.
101. Daseke MJ 2nd, Tenkorang MAA, Chalise U, Konfrst SR, Lindsey ML. Cardiac fibroblast activation during myocardial infarction wound healing: fibroblast polarization after MI. *Matrix Biol* 2020;**91–92**:109–116.
102. Meyer K, Hodwin B, Ramanujam D, Engelhardt S, Sarikas A. Essential role for premature senescence of myofibroblasts in myocardial fibrosis. *J Am Coll Cardiol* 2016; **67**:2018–2028.
103. Dookun E, Walaszczyk A, Redgrave R, Palmowski P, Tual-Chalot S, Suwana A, Chapman J, Jirkovsky E, Donastorg Sosa L, Gill E, Yausep OE, Santin Y, Mialet-Perez J, Andrew Owens W, Grieve D, Spyridopoulos I, Taggart M, Arthur HM, Passos JF, Richardson GD. Clearance of senescent cells during cardiac ischemia-reperfusion injury improves recovery. *Aging Cell* 2020;**19**:e13249.
104. Olsen MB, Hildrestrand GA, Scheffler K, Vinge LE, Alfsnes K, Palibrk V, Wang J, Neurauter CG, Luna L, Johansen J, Ogaard JDS, Ohm IK, Slupphaug G, Kusnierczyk A, Fiane AE, Brorson SH, Zhang L, Gullestad L, Louch WE, Iversen PO, Ostlie I, Klungland A, Christensen G, Sjaastad I, Saetrom P, Yndestad A, Aukrust P, Bjoras M, Finsen AV. NEIL3-dependent regulation of cardiac fibroblast proliferation prevents myocardial rupture. *Cell Rep* 2017;**18**:82–92.
105. Schelbert EB, Fridman Y, Wong TC, Abu Daya H, Piehler KM, Kadakkal A, Miller CA, Ugander M, Maanja M, Kellman P, Shah DJ, Abebe KZ, Simon MA, Quarta G, Senni M, Butler J, Diez J, Redfield MM, Gheorghiade M. Temporal relation between myocardial fibrosis and heart failure with preserved ejection fraction: association with baseline disease severity and subsequent outcome. *JAMA Cardiol* 2017;**2**: 995–1006.
106. Lin R, Rahtu-Korpela L, Magga J, Ulvila J, Swan J, Kemppi A, Pakanen L, Porvari K, Huikuri H, Junttila J, Kerkela R. miR-1468-3p promotes aging-related cardiac fibrosis. *Mol Ther Nucleic Acids* 2020;**20**:589–605.
107. Maity S, Muhamed J, Sarikhani M, Kumar S, Ahamed F, Spurthi KM, Ravi V, Jain A, Khan D, Arathi BP, Desingu PA, Sundaresan NR. Sirtuin 6 deficiency transcriptionally up-regulates TGF-beta signaling and induces fibrosis in mice. *J Biol Chem* 2020; **295**:415–434.
108. Brozovich FV, Nicholson CJ, Degen CV, Gao YZ, Aggarwal M, Morgan KG. Mechanisms of vascular smooth muscle contraction and the basis for pharmacologic treatment of smooth muscle disorders. *Pharmacol Rev* 2016;**68**:476–532.
109. Grootaert MOJ, da Costa Martins PA, Bitsch N, Pintelon I, De Meyer GRY, Martinet W, Schrijvers DM. Defective autophagy in vascular smooth muscle cells accelerates senescence and promotes neointima formation and atherogenesis. *Autophagy* 2015;**11**:2014–2032.
110. Basatemur GL, Jørgensen HF, Clarke MCH, Bennett MR, Mallat Z. Vascular smooth muscle cells in atherosclerosis. *Nat Rev Cardiol* 2019;**16**:727–744.
111. Matthews C, Gorenne I, Scott S, Figg N, Kirkpatrick P, Ritchie A, Goddard M, Bennett M. Vascular smooth muscle cells undergo telomere-based senescence in human atherosclerosis. *Circ Res* 2006;**99**:156–164.
112. Ragnauth CD, Warren DT, Liu Y, McNair R, Tajsic T, Figg N, Shroff R, Skepper J, Shanahan CM. Prelamin A acts to accelerate smooth muscle cell senescence and is a novel biomarker of human vascular aging. *Circulation* 2010;**121**:2200–2210.
113. Afonso P, Auclair M, Boccara F, Vantyghem MC, Katlama C, Capeau J, Vigouroux C, Caron-Debarle M. LMNA mutations resulting in lipodystrophy and HIV protease inhibitors trigger vascular smooth muscle cell senescence and calcification: role of ZMPSTE24 downregulation. *Atherosclerosis* 2016;**245**:200–211.
114. Liu Y, Drozdov I, Shroff R, Beltran Leilani E, Shanahan Catherine M. Prelamin A accelerates vascular calcification via activation of the DNA damage response and senescence-associated secretory phenotype in vascular smooth muscle cells. *Circ Res* 2013;**112**:e99–e109.
115. Grootaert MOJ, Finigan A, Figg NL, Uryga AK, Bennett MR. SIRT6 protects smooth muscle cells from senescence and reduces atherosclerosis. *Circ Res* 2021;**128**: 474–491.
116. Song Y, Shen H, Schenten D, Shan P, Lee PJ, Goldstein DR. Aging enhances the basal production of IL-6 and CCL2 in vascular smooth muscle cells. *Arterioscler Thromb Vasc Biol* 2012;**32**:103–109.
117. Gardner SE, Humphry M, Bennett MR, Clarke MC. Senescent vascular smooth muscle cells drive inflammation through an interleukin-1α-dependent senescence-associated secretory phenotype. *Arterioscler Thromb Vasc Biol* 2015;**35**:1963–1974.
118. Gosling J, Slaymaker S, Gu L, Tseng S, Zlot CH, Young SG, Rollins BJ, Charo IF. MCP-1 deficiency reduces susceptibility to atherosclerosis in mice that overexpress human apolipoprotein B. *J Clin Invest* 1999;**103**:773–778.
119. Potteaux S, Combadière C, Esposito B, Lecureuil C, Ait-Oufella H, Merval R, Ardouin P, Tedgui A, Mallat Z. Role of bone marrow-derived CC-chemokine receptor 5 in the development of atherosclerosis of low-density lipoprotein receptor knockout mice. *Arterioscler Thromb Vasc Biol* 2006;**26**:1858–1863.
120. Hamczyk MR, Andres V. Vascular smooth muscle cell loss underpins the accelerated atherosclerosis in Hutchinson-Gilford progeria syndrome. *Nucleus* 2019;**10**:48– 34.
121. Saker M, Lipskaia L, Marcos E, Abid S, Parpaleix A, Houssaini A, Validire P, Girard P, Noureddine H, Boyer L, Vienney N, Amsellem V, Marguerit L, Maitre B, Derumeaux G, Dubois-Rande JL, Jourdan-Lesaux C, Delcroix M, Quarck R, Adnot S. Osteopontin, a key mediator expressed by senescent pulmonary vascular cells in pulmonary hypertension. *Arterioscler Thromb Vasc Biol* 2016;**36**:1879–1890.
122. Liu AC, Joag VR, Gotlieb AI. The emerging role of valve interstitial cell phenotypes in regulating heart valve pathobiology. *Am J Pathol* 2007;**171**:1407–1418.
123. Yang VK, Tai AK, Huh TP, Meola DM, Juhr CM, Robinson NA, Hoffman AM. Dysregulation of valvular interstitial cell let-7c, miR-17, miR-20a, and miR-30d in naturally occurring canine myxomatous mitral valve disease. *PLoS One* 2018;**13**: e0188617.
124. Eveborn GW, Schirmer H, Heggelund G, Lunde P, Rasmussen K. The evolving epidemiology of valvular aortic stenosis. the Tromso study. *Heart* 2013;**99**: 396–400.
125. Oh KS, Febres-Aldana CA, Kuritzky N, Ujueta F, Arenas IA, Sriganeshan V, Medina AM, Poppiti R. Cellular senescence evaluated by P16INK4a immunohistochemistry is a prevalent phenomenon in advanced calcific aortic valve disease. *Cardiovasc Pathol* 2021;**52**:107318.
126. Grim JC, Aguado BA, Vogt BJ, Batan D, Andrichik CL, Schroeder ME, Gonzalez-Rodriguez A, Yavitt FM, Weiss RM, Anseth KS. Secreted factors from proinflammatory macrophages promote an osteoblast-like phenotype in valvular interstitial cells. *Arterioscler Thromb Vasc Biol* 2020;**40**:e296–e308.

127. Gu J, Lu Y, Deng M, Qiu M, Tian Y, Ji Y, Zong P, Shao Y, Zheng R, Zhou B, Sun W, Kong X. Inhibition of acetylation of histones 3 and 4 attenuates aortic valve calcification. *Exp Mol Med* 2019;**51**:1–14.
128. Li N, Bai Y, Zhou G, Ma Y, Tan M, Qiao F, Li X, Shen M, Song X, Zhao X, Liu X, Xu Z. miR-214 attenuates aortic valve calcification by regulating osteogenic differentiation of valvular interstitial cells. *Mol Ther Nucleic Acids* 2020;**22**:971–980.
129. Swirski FK, Nahrendorf M. Cardioimmunology: the immune system in cardiac homeostasis and disease. *Nat Rev Immunol* 2018;**18**:733–744.
130. Li Y, Li Q, Fan GC. Macrophage efferocytosis in cardiac pathophysiology and repair. *Shock* 2021;**55**:177–188.
131. Song P, An J, Zou MH. Immune clearance of senescent cells to combat ageing and chronic diseases. *Cells* 2020;**9**:671.
132. Horckmans M, Ring L, Duchene J, Santovito D, Schloss MJ, Drechsler M, Weber C, Soehnlein O, Steffens S. Neutrophils orchestrate post-myocardial infarction healing by polarizing macrophages towards a reparative phenotype. *Eur Heart J* 2017;**38**:187–197.
133. Liao Y-H, Xia N, Zhou S-F, Tang T-T, Yan X-X, Lv B-J, Nie S-F, Wang J, Iwakura Y, Xiao H, Yuan J, Jevallee H, Wei F, Shi G-P, Cheng Xl. 17A contributes to myocardial ischemia/reperfusion injury by regulating cardiomyocyte apoptosis and neutrophil infiltration. *J Am Coll Cardiol* 2012;**59**:420–429.
134. Ma Y, Chiao YA, Clark R, Flynn ER, Yabluchanskiy A, Ghasemi O, Zouein F, Lindsey ML, Jin Y-F. Deriving a cardiac ageing signature to reveal MMP-9-dependent inflammatory signalling in senescence. *Cardiovasc Res* 2015;**106**:421–431.
135. Weirather J, Hofmann UD, Beyersdorf N, Ramos GC, Vogel B, Frey A, Ertl G, Kerkau T, Frantz S. Foxp3+ CD4+ T cells improve healing after myocardial infarction by modulating monocyte/macrophage differentiation. *Circ Res* 2014;**115**:55–67.
136. Ramos GC, van den Berg A, Nunes-Silva V, Weirather J, Peters L, Burkard M, Friedrich M, Pinnecker J, Abesser M, Heinze KG, Schuh K, Beyersdorf N, Kerkau T, Demengeot J, Frantz S, Hofmann U. Myocardial aging as a T-cell-mediated phenomenon. *Proc Natl Acad Sci USA* 2017;**114**:E2420–E2429.
137. Ngkelo A, Richart A, Kirk JA, Bonnin P, Vilar J, Lemitre M, Marck P, Branchereau M, Le Gall S, Renault N, Guerin C, Ranek MJ, Kervadec A, Danelli L, Gautier G, Blank U, Launay P, Camerer E, Bruneval P, Menasche P, Heymes C, Luche E, Casteilla L, Cousin B, Rodewald HR, Kass DA, Silvestre JS. Mast cells regulate myofilament calcium sensitization and heart function after myocardial infarction. *J Exp Med* 2016;**213**:1353–1374.
138. He A, Fang W, Zhao K, Wang Y, Li J, Yang C, Benadjaoud F, Shi G-P. Mast cell-deficiency protects mice from streptozotocin-induced diabetic cardiomyopathy. *Transl Res* 2019;**208**:1–14.
139. Yousefzadeh MJ, Melos KI, Angelini L, Burd CE, Robbins PD, Niedernhofer LJ. Mouse models of accelerated cellular senescence. *Methods Mol Biol* 2019;**1896**:203–230.
140. Sullivan T, Escalante-Alcalde D, Bhatt H, Anver M, Bhat N, Nagashima K, Stewart CL, Burke B. Loss of A-type lamin expression compromises nuclear envelope integrity leading to muscular dystrophy. *J Cell Biol* 1999;**147**:913–920.
141. Rivera-Torres J, Calvo CJ, Llach A, Guzman-Martinez G, Caballero R, Gonzalez-Gomez C, Jimenez-Borreguero LJ, Guadix JA, Osorio FG, Lopez-Otin C, Herraiz-Martinez A, Cabello N, Vallmitjana A, Benitez R, Gordon LB, Jalife J, Perez-Pomares JM, Tamargo J, Delpon E, Hove-Madsen L, Filgueiras-Rama D, Andres V. Cardiac electrical defects in progeroid mice and Hutchinson-Gilford progeria syndrome patients with nuclear lamina alterations. *Proc Natl Acad Sci USA* 2016;**113**:E7250–E7259.
142. Yoshida T, Maulik N, Engelman RM, Ho YS, Das DK. Targeted disruption of the mouse Sod I gene makes the hearts vulnerable to ischemic reperfusion injury. *Circ Res* 2000;**86**:264–269.
143. Wang Q, Liu T, Fang Y, Xie S, Huang X, Mahmood R, Ramaswamy G, Sakamoto KM, Darzynkiewicz Z, Xu M, Dai W. BUBR1 deficiency results in abnormal megakaryopoiesis. *Blood* 2004;**103**:1278–1285.
144. Wijshake T, Malureanu LA, Baker DJ, Jeganathan KB, van de Sluis B, van Deursen JM. Reduced life- and healthspan in mice carrying a mono-allelic BubR1 MVA mutation. *PLoS Genet* 2012;**8**:e1003138.
145. De Angelis A, Urbanek K, Cappetta D, Piegari E, Ciuffreda LP, Rivellino A, Russo R, Esposito G, Rossi F, Berrino L. Doxorubicin cardiotoxicity and target cells: a broader perspective. *Cardiooncology* 2016;**2**:2.
146. Sung JY, Kim SG, Kim JR, Choi HC. Prednisolone suppresses adriamycin-induced vascular smooth muscle cell senescence and inflammatory response via the SIRT1-AMPK signaling pathway. *PLoS One* 2020;**15**:e0239976.
147. Xia W, Chen H, Xie C, Hou M. Long-noncoding RNA MALAT1 sponges microRNA-92a-3p to inhibit doxorubicin-induced cardiac senescence by targeting ATG4a. *Aging (Albany NY)* 2020;**12**:8241–8260.
148. Zhu Y, Tchkonia T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, Palmer AK, Ikeno Y, Hubbard GB, Lenburg M, O'Hara SP, LaRusso NF, Miller JD, Roos CM, Verzosa GC, LeBrasseur NK, Wren JD, Farr JN, Khosla S, Stout MB, McGowan SJ, Fuhrmann-Stroissnigg H, Gurkar AU, Zhao J, Colangelo D, Dorronsoro A, Ling YY, Barghouthy AS, Navarro DC, Sano T, Robbins PD, Niedernhofer LJ, Kirkland JL. The Achilles' heel of senescent cells: from transcriptome to senolytic drugs. *Aging Cell* 2015;**14**:644–658.
149. Childs BG, Gluscevic M, Baker DJ, Laberge R-M, Marquess D, Dananberg J, van Deursen JM. Senescent cells: an emerging target for diseases of ageing. *Nat Rev Drug Discov* 2017;**16**:718–735.
150. Kim E-C, Kim J-R. Senotherapeutics: emerging strategy for healthy aging and age-related disease. *BMB Rep* 2019;**52**:47–55.
151. Zhu M, Meng P, Ling X, Zhou L. Advancements in therapeutic drugs targeting of senescence. *Ther Adv Chronic Dis* 2020;**11**:204062232096412.
152. Tang J, Lu L, Liu Y, Ma J, Yang L, Li L, Guo H, Yu S, Ren J, Bai H, Yang J. Quercetin improve ischemia/reperfusion-induced cardiomyocyte apoptosis *in vitro* and *in vivo* study via SIRT1/PGC-1α signaling. *J Cell Biochem* 2019;**120**:9747–9757.
153. Olson ER, Melton T, Dickinson SE, Dong Z, Alberts DS, Bowden GT. Quercetin potentiates UVB-induced c-Fos expression: implications for its use as a chemopreventive agent. *Cancer Prev Res* 2010;**3**:876–884.
154. Jiang Y-H, Jiang L-Y, Wang Y-C, Ma D-F, Li X. Quercetin attenuates atherosclerosis via modulating oxidized LDL-induced endothelial cellular senescence. *Front Pharmacol* 2020;**11**:512.
155. Roos CM, Zhang B, Palmer AK, Ogrodnik MB, Pirtskhalava T, Thalji NM, Hagler M, Jurk D, Smith LA, Casaclang-Verzosa G, Zhu Y, Schafer MJ, Tchkonia T, Kirkland JL, Miller JD. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. *Aging Cell* 2016;**15**:973–977.
156. Zhu Y, Tchkonia T, Fuhrmann-Stroissnigg H, Dai HM, Ling YY, Stout MB, Pirtskhalava T, Giorgadze N, Johnson KO, Giles CB, Wren JD, Niedernhofer LJ, Robbins PD, Kirkland JL. Identification of a novel senolytic agent, navitoclax, targeting the BCL-2 family of anti-apoptotic factors. *Aging Cell* 2016;**15**:428–435.
157. Walaszczyk A, Dookun E, Redgrave R, Tual-Chalot S, Victorelli S, Spyridopoulos I, Owens A, Arthur HM, Passos JF, Richardson GD. Pharmacological clearance of senescent cells improves survival and recovery in aged mice following acute myocardial infarction. *Aging Cell* 2019;**18**:e12945.
158. Jia K, Dai Y, Liu A, Li X, Wu L, Lu L, Bao Y, Jin Q. Senolytic agent navitoclax inhibits angiotensin II-induced heart failure in mice. *J Cardiovasc Pharmacol* 2020;**76**:452–460.
159. Fozzard HA, Sheets MF. Cellular mechanism of action of cardiac glycosides. *J Am Coll Cardiol* 1985;**5**:10A–15A.
160. Triana-Martínez F, Picallos-Rabina P, Da Silva-Álvarez S, Pietrocola F, Llanos S, Rodilla V, Soprano E, Pedrosa P, Ferreirós A, Barradas M, Hernández-González F, Lalinde M, Prats N, Bernadó C, González P, Gómez M, Ikonomopoulou MP, Fernández-Marcos PJ, García-Caballero T, del Pino P, Arribas J, Vidal A, González-Barcia M, Serrano M, Loza MI, Domínguez E, Collado M. Identification and characterization of Cardiac Glycosides as senolytic compounds. *Nat Commun* 2019;**10**:4731.
161. Castilho RM, Squarize CH, Chodosh LA, Williams BO, Gutkind JS. mTOR mediates Wnt-induced epidermal stem cell exhaustion and aging. *Cell Stem Cell* 2009;**5**:279–289.
162. Harrison DE, Strong R, Sharp ZD, Nelson JF, Astle CM, Flurkey K, Nadon NL, Wilkinson JE, Frenkel K, Carter CS, Pahor M, Javors MA, Fernandez E, Miller RA. Rapamycin fed late in life extends lifespan in genetically heterogeneous mice. *Nature* 2009;**460**:392–395.
163. Cao K, Graziotto JJ, Blair CD, Mazzulli JR, Erdos MR, Krainc D, Collins FS. Rapamycin reverses cellular phenotypes and enhances mutant protein clearance in Hutchinson-Gilford Progeria Syndrome Cells. *Sci Transl Med* 2011;**3**:89ra58.
164. Xu X-J, Zhao W-B, Feng S-B, Sun C, Chen Q, Ni B, Hu H-Y. Celastrol alleviates angiotensin II-mediated vascular smooth muscle cell senescence via induction of autophagy. *Mol Med Rep* 2017;**16**:7657–7664.
165. Piskovatska V, Storey KB, Vaiserman AM, Lushchak O. The use of metformin to increase the human healthspan. In: PC Guest, ed. *Reviews on New Drug Targets in Age-Related Disorders*. Cham: Springer International Publishing, 2020:319–332.
166. Karnewar S, Neeli PK, Panuganti D, Kotagiri S, Mallappa S, Jain N, Jerald MK, Kotamraju S. Metformin regulates mitochondrial biogenesis and senescence through AMPK mediated H3K79 methylation: relevance in age-associated vascular dysfunction. *Biochim Biophys Acta Mol Basis Dis* 2018;**1864**:1115–1128.
167. Yu S, Wang X, Geng P, Tang X, Xiang L, Lu X, Li J, Ruan Z, Chen J, Xie G, Wang Z, Ou J, Peng Y, Luo X, Zhang X, Dong Y, Pang X, Miao H, Chen H, Liang H. Melatonin regulates PARP1 to control the senescence-associated secretory phenotype (SASP) in human fetal lung fibroblast cells. *J Pineal Res* 2017;**63**:e12405.
168. Li W-Q, Tan S-L, Li X-H, Sun T-L, Li D, Du J, Wei S-S, Li Y-J, Zhang B-K. Calcitonin gene-related peptide inhibits the cardiac fibroblasts senescence in cardiac fibrosis via up-regulating klotho expression. *Eur J Pharmacol* 2019;**843**:96–103.
169. Leopold JA, Loscalzo J. Emerging role of precision medicine in cardiovascular disease. *Circ Res* 2018;**122**:1302–1315.
170. Jimenez-Gutierrez GE, Mondragon-Gonzalez R, Soto-Ponce LA, Gómez-Monsiváis WL, García-Aguirre I, Pacheco-Rivera RA, Suárez-Sánchez R, Brancaccio A, Magaña JJ, C.R. Perlingeiro R, Cisneros B. Loss of dystroglycan drives cellular senescence via defective mitosis-mediated genomic instability. *Int J Mol Sci* 2020;**21**:4961.
171. Fortunato F, Rossi R, Falzarano MS, Ferlini A. Innovative therapeutic approaches for duchenne muscular dystrophy. *J Clin Med* 2021;**10**:820.
172. Cresci S, Pereira NL, Ahmad F, Byku M, de Las Fuentes L, Lanfear DE, Reilly CM, Owens AT, Wolf MJ. Heart failure in the era of precision medicine: a scientific statement from the American Heart Association. *Circ Genom Precis Med* 2019;**12**:458–485.
173. Selvakumar D, Clayton ZE, Chong JJH. Robust cardiac regeneration: fulfilling the promise of cardiac cell therapy. *Clin Ther* 2020;**42**:1857–1879.
174. Li J, Li SH, Dong J, Alibhai FJ, Zhang C, Shao ZB, Song HF, He S, Yin WJ, Wu J, Weisel RD, Liu SM, Li RK. Long-term repopulation of aged bone marrow stem cells using young Sca-1 cells promotes aged heart rejuvenation. *Aging Cell* 2019;**18**:e13026.