J_ID: PHYSREV ART NO: PRV-00007-2022 Date: 10-October-22 Page: 41 Total Pages: 41 ini: 6//EP

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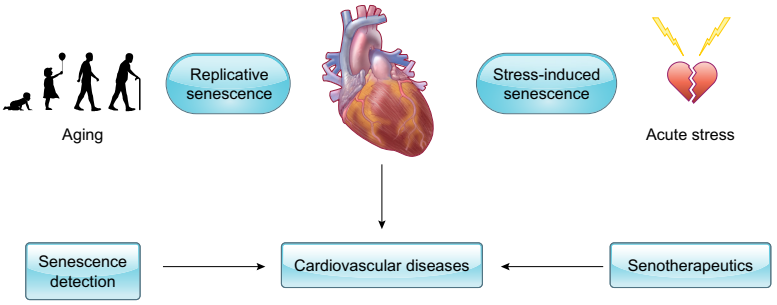
PHYSIOLOGICAL REVIEWS

REVIEW ARTICLE

CELLULAR SENESCENCE AND CARDIOVASCULAR DISEASES: MOVING TO THE "HEART" OF THE PROBLEM



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KEY WORDS

cardiovascular diseases; cellular senescence; senotherapeutics; stress

CLINICAL HIGHLIGHTS

Cellular senescence has been the subject of focused research for over two decades; however, it was not until recently that the role of senescence in cardiovascular disease (CVD) started to be explored. A body of evidence has shown that cellular senescence constitutes a stress-mediated pathophysiological mechanism implicated in a wide range of CVDs at the cellular and molecular levels. In this review, we recapitulate key molecular and clinical findings supporting the involvement of senescence in heart disease and discuss current clinical strategies aimed at eradicating the detrimental effects of senescence on cardiac homeostasis. On the basis of recent evidence, we additionally address how the advent of the senotherapeutics field, in conjunction with the development of novel senescence detection tools in tissues and biological fluids, may now facilitate effectively combating CVDs.

September 1, 2022;
https://doi.org/10.1152/physrev.00007.2022

american physiological society | PHYSIOLOGICAL REVIEWS

Physiol Rev 103: 000–000, 2023
First published September 1, 2022; doi:10.1152/physrev.00007.2022

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CELLULAR SENESCENCE AND CARDIOVASCULAR DISEASES: MOVING TO THE "HEART" OF THE PROBLEM

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Abstract

Cardiovascular diseases (CVDs) constitute the prime cause of global mortality, with an immense impact on patient quality of life and disability. Clinical evidence has revealed a strong connection between cellular senescence and worse cardiac outcomes in the majority of CVDs concerning both ischemic and nonischemic cardiomyopathies. Cellular senescence is characterized by cell cycle arrest accompanied by alterations in several metabolic pathways, resulting in morphological and functional changes. Metabolic rewiring of senescent cells results in marked paracrine activity, through a unique secretome, often exerting deleterious effects on neighboring cells. Here, we recapitulate the hallmarks and key molecular pathways involved in cellular senescence in the cardiac context and summarize the different roles of senescence in the majority of CVDs. In the last few years, the possibility of eliminating senescent cells in various pathological conditions has been increasingly explored, giving rise to the field of senotherapeutics. Therefore, we additionally attempt to clarify the current state of this field with a focus on cardiac senescence and discuss the potential of implementing senolytics as a treatment option in heart disease.

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CLINICAL HIGHLIGHTS

Cellular senescence has been the subject of focused research for over two decades; however, it was not until recently that the role of senescence in cardiovascular disease (CVD) started to be explored. A body of evidence has shown that cellular senescence constitutes a stress-mediated pathophysiological mechanism implicated in a wide range of CVDs at the cellular and molecular levels. In this review, we recapitulate key molecular and clinical findings supporting the involvement of senescence in heart disease and discuss current clinical strategies aimed at eradicating the detrimental effects of senescence on cardiac homeostasis. On the basis of recent evidence, we additionally address how the advent of the senotherapeutics field, in conjunction with the development of novel senescence detection tools in tissues and biological fluids, may now facilitate effectively combating CVDs.

1. INTRODUCTION

To facilitate survival during embryonic development, the heart is the first organ formed in a rapidly growing fetus during the third week of gestation. Hence, the heart exerts a fundamental role for organismal homeostasis and well-being throughout life.

The heart is a mosaic of subsets of differentiated cells, including discrete myocytes (atrial and ventricular myocytes and myocytes of the conduction system), arterial and venous smooth muscle cells, autonomic ganglia, endothelial cells, macrophages, interstitial mesenchymal fibroblast cells, and progenitor/stem cells (1). Notably, it

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is the nonmyocytes that form the majority of cells in the heart (2). Studies in humans (i.e., postmortem hearts from healthy subjects) and animals (rat and mouse models) have shown that cardiomyocytes occupy ~70–85% of the mammalian heart volume but constitute only 30–40% of total heart cells (3–5). Cardiomyocytes comprise a dynamic cell type, capable of being constantly recycled (6). It has been estimated that, despite variability between different proteins and organelles, the entire mammalian heart turns over all of its constituent proteins at least once every 30 days. Thus, through this process of recycling, cardiomyocytes respond and adapt to alterations that jeopardize cardiac homeostasis (e.g., ischemia, infection, and mechanical stress) (6). In addition, the dogma that cardiomyocytes remain in a postmitotic phase has been challenged, since it has been shown that cardiomyocytes still retain a low proliferative capacity (4, 7). For human cardiomyocytes this capacity is highest in early childhood but decreases gradually throughout life to <1% per year in adulthood. Other cell subpopulations exert a more substantial renewal rate, i.e., high turnover rate of endothelial cells throughout life (>15% per year) in comparison with a more limited renewal of mesenchymal cells (<4% per year in adulthood) (4).

Nonmyocytes occupy a relatively small volume fraction, but they are altogether more abundant compared with cardiomyocytes. Furthermore, nonmyocyte cells exert crucial roles in cardiovascular homeostasis by providing the heart with extracellular matrix (ECM) (8, 9). ECM is a scaffold/network of structural and matricellular proteins that confers mechanical support, facilitates intercellular communication and metabolic exchange, and modulates cellular responses such as cell survival, death, proliferation, and differentiation (8, 9). Interestingly, the prevailing view that fibroblasts are the most prevalent subset of noncardiomyocytes in the heart has been challenged based on modern technologies and novel genetic tools (5, 10, 11). Indeed, it has been demonstrated that endothelial cells outnumber all other cardiac cell types, comprising >60% of noncardiomyocyte populations, with an estimated ratio of endothelial cells to cardiomyocytes being 3:1 (5, 10, 11). This suggests a more fundamental role for endothelial cells in cardiac homeostasis and response to injury than previously appreciated (5). The number of fibroblasts seems lower than previously estimated, accounting for <20% of noncardiomyocyte cells. Another important population found in the heart are macrophages (originating by both peripheral monocyte recruitment and local proliferation), which account for 5–10% of noncardiomyocytes (5, 12).

The unique spatiotemporal interplay between different cardiac cell populations is of paramount importance for the proper structure and function of the mammalian heart (13). Over a lifetime, each one of these diverse cells senses and responds to intrinsic and extrinsic stressors that jeopardize tissue homeostasis (14). Ultimately, disease results from the suboptimal ability of diverse cell populations and macromolecules to respond and adapt to stress (15).

In the cardiac context, cellular senescence enters the scene as a stress response mechanism induced by a plethora of stimuli, including telomere attrition, hypoxia, viruses, oxidative stress, mitochondrial dysfunction, perturbed proteostasis, and autophagy impairment (16). Senescent cells display the following interdependent traits: *1*) cell cycle withdrawal, *2*) macromolecular damage, *3*) a unique secretory phenotype (SASP), *4*) deregulated metabolism, *5*) tolerance to apoptosis, and *6*) morphological changes (16). Although cellular senescence was initially described as a process preventing proliferation of stressed/damaged mitotic cells, accumulating evidence suggests a putative role of postmitotic cell senescence (PoMiCS) in health and disease. In organs principally harboring postmitotic cells such as the heart, PoMiCS provides an evolutionary advantage to ensure cellular integrity by restraining stressor-induced tissue degeneration and facilitating tissue repair (17). However, PoMiCS may also promote disease progression, mainly through secretion of SASP factors (17). Importantly, during the last decade emerging evidence has revealed a connection between cellular senescence and cardiovascular disease (CVD), the leading cause of morbidity and mortality worldwide. Of relevance, cellular senescence is also a hallmark of aging, the main nonmodifiable risk factor for CVD (18). Indeed, it has been extensively demonstrated that accumulation of senescent cells with age may be detrimental for tissue homeostasis (17, 19). Even more intriguing is the finding that a class of compounds (i.e., senotherapeutics) that target senescent cells by exploiting molecular pathways implicated in the senescence phenotype can alter the natural history of CVDs.

The involvement of cellular senescence in the pathogenesis of CVD is a relatively novel concept (**FIGURE 1**). Here, we initially discuss the common hallmarks and molecular pathways of cellular senescence and CVDs; because of length restrictions, the implication of cellular senescence in stroke, aortic aneurysm/dissection, peripheral arterial disease, and vascular pathologies such as vasculitis or vascular disorders that occur in the frame of other entities (e.g., chronic obstructive pulmonary disease) are not discussed here. Subsequently, we focus on recent findings dealing with the involvement of senescence in specific cardiovascular scenarios. Finally, we present the potential advantages and limitations of the use of senotherapeutics in CVD.

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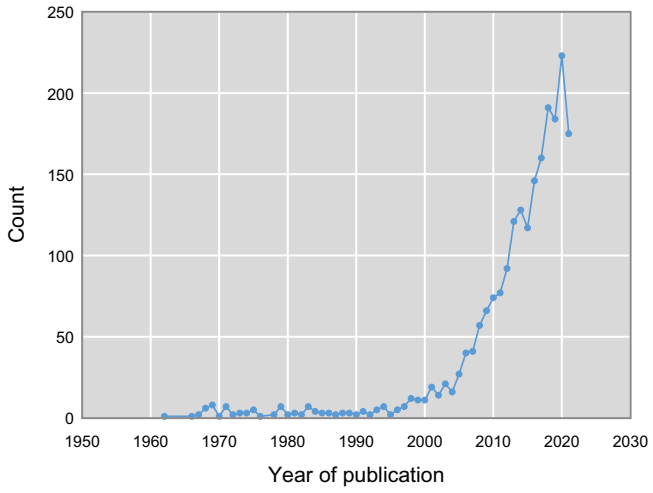

FIGURE 1. Investigation of the involvement of senescence in cardiovascular diseases (CVDs) is a rapidly growing field. Results by year for the search query "cellular senescence AND cardiovascular diseases" in the PubMed platform.

2. FUNDAMENTAL ASPECTS OF CELLULAR SENESCENCE

2.1. Inducers and Global Features of Senescence

Etymologically, senescence stems from the Latin *senex*, meaning "someone of old age." It was originally shown by Hayflick and Moorhead in 1961 that serial passaging of normal human diploid fibroblasts constituted a barrier to their in vitro proliferation after reaching a fixed number of population doublings now known as the Hayflick limit (20–22). Later on, the Hayflick limit was attributed to telomere shortening (23). These observations led to the hypothesis that aging may be the outcome of cells progressively losing their proliferative capacity because of telomere attrition (replicative senescence), thus becoming unable to replace damaged tissues and thereby contributing to organismal dysfunction over time (24).

Since its discovery, cellular senescence has been widely perceived as a cell fate determinant upon a variety of signals. It has been extensively supported that cells undergo senescence in response to various extrinsic and intrinsic insults, such as irradiation, nutrient deprivation, genotoxic and oxidative stress, telomere attrition, telomeric structure modifications, mitogenic signals, oncogene activation, epigenetic modifications, chromatin rearrangements, mitochondrial dysfunction, immune response modulators, infectious agents, and inflammation (16, 22). Senescence is a well-orchestrated process in which cells cease to divide, acquiring a secretory phenotype and distinctive phenotypic alterations linked with changes in morphology, cellular metabolism, epigenetic regulation, and gene expression (16). Moreover, senescent cells display increased resistance to programmed cell death due to the activation of cell survival pathways and modulators, such as the BCL-2 family of antiapoptotic proteins, even after exposure to exogenous stress cues (25, 26). The complete network of molecular events selectively leading to senescence activation over apoptosis remains largely elusive; however, it has been postulated that the intensity and duration of the stimulus, as well as the nature of damage and cell type, are factors dictating cell fate (20, 27, 28).

Over the last decade, extensive research and significant progress in our understanding of the causes and consequences of cellular senescence has occurred, despite the absence of a consensus agreement on what features truly reflect the senescence phenotype and the lack of reliable and specific senescence markers (16). The latter enabled discussions that led to a revised definition of cellular senescence and drove the development of novel markers and approaches for accurate cellular senescence determination (16, 29). Toward this direction, in the last few years a guideline multimarker algorithmic approach for accurate senescent cell assessment has been adopted by the senescence community, rendering detection of senescent cells feasible and precise even in clinical (archival) material (16, 29). The latter is imperative not only to further elucidate the role of senescence in the pathophysiology of various age-associated diseases but also to estimate the effectiveness of therapeutic strategies that target senescent cells.

An important, frequently confusing issue stems from the fact that the term "senescence" was incorrectly used for many years to refer to both cellular and organismal senescence (aging). Currently it is clear that the terms "senescent" and "aged" are not equal and should not be used interchangeably. Senescence can be triggered rather acutely by a variety of non-telomere-dependent insults, on a premature basis, earlier than the exhaustion of the cellular replicative potential (stress-induced senescence) (16). In contrast, aging refers to the outcome of the accumulation of cell-intrinsic changes due to mild but steady damage, which eventually leads to a decline in cellular function (18). Therefore, compared with cellular senescence, the loss of physiological integrity characterizing the aged cell is gradual and progressive (**FIGURE 2**). Moreover, aging is a nonreversible phenomenon, whereas recent findings suggest that cellular senescence represents an occasionally reversible state, given that under certain conditions escape from senescence may occur (18, 30–32). In particular, it has been thoroughly demonstrated in a series of studies that cells undergoing senescence following prolonged oncogenic activation (oncogene-induced senescence), irrespective of cell origin (epithelial or mesenchymal), may "escape" from this cellular state and reenter the cell cycle, thus

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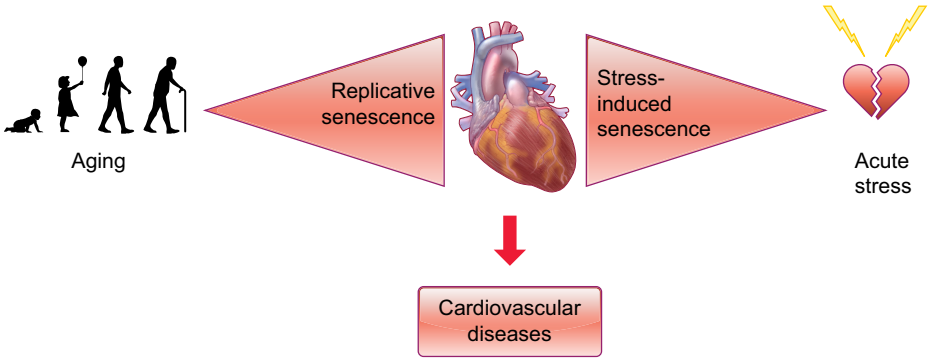

FIGURE 2. Aging vs. senescence. Senescence is triggered acutely by a variety of non-telomere-dependent insults, on a premature basis, earlier than the exhaustion of the cellular replicative potential. In contrast, aging refers to the outcome of the accumulation of changes within the cell due to random damage over time, which leads to a decline in cellular function. Vectors were obtained from www.vecteezy.com.

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adopting aggressive features (16, 30, 33). During carcinogenesis senescence in preneoplastic cells restrains the propagation of incipient cancer cells, whereas in "full-blown" cancers senescent cells represent a pool of dormant cancer cells that need to be eliminated to prohibit further tumor progression and relapse (34–37). Therefore, novel anticancer strategies aimed at senescent cell elimination, complementary to classical therapeutic interventions, have been recently proposed (37). It should be noted that escape from senescence represents a phenomenon entirely different from that described as senescence "bypass." Senescence bypass pertains to the circumvention of senescence entry occurring during tumorigenic transformation (38).

Although senescence is a dynamic process in which, under certain conditions, senescence-associated cell cycle arrest may be reversed, the term "senescence reversal" mainly refers to a state of "light" senescence accompanied by low $p16^{INK4A}$ levels in which "reversed" cells more or less recapitulate their original, presenescence features (39). In contrast, "escape" refers to cell cycle reentry following a prolonged period of "deep" senescence leading to cells with a significantly altered genetic and epigenetic landscape compared with their presenescent state, also reflected in their biological behavior (16, 36, 39, 40). In this respect, although the various types of cell state conversions are not yet fully elucidated, escape from senescence appears highly distinct from senescence reversal.

Cells of any age can undergo senescence. In fact, this probably occurs throughout our life; in developing and young organisms cellular senescence prevents the propagation of damaged cells and contributes to tissue formation and homeostasis, whereas in old organisms senescent cells start to accumulate because either the rate of their formation is increased or a deregulated immune system fails to remove them (16). It is noteworthy that, contrary to the dogma that senescence is restricted to proliferating cells, a number of senescence markers have also been identified in postmitotic cells, such as cardiomyocytes, neurons, and adipocytes, a phenomenon termed postmitotic senescence (41–43). Conclusively, even though cellular senescence is a hallmark of aging and senescent cells become more abundant in aged tissues, the extent to which senescence drives the aging process remains unknown. It is important to underline that since cellular senescence can occur both independently of age (i.e., stress-induced premature senescence) or correlated with age (i.e., replicative senescence), not all senescent cells are aged cells.

2.2. Hallmarks of Cellular Senescence

Cell cycle arrest is a cardinal trait of the senescence phenotype, driven by the sequential activation of the $p53/p21^{WAF1/Cip1}$ and the $Rb\text{-}p16^{INK4A}$ axes (16). DNA damage is a common but not universal trigger of cell cycle withdrawal given that it is absent in certain types of senescence (e.g., developmental senescence) (16). In senescent cells, DNA damage is evident in the form of persistent (irreparable) DNA damage and double-strand breaks, leading to continuous activation of the DNA damage response (DDR) pathway, reflected in persistent DDR foci formation (16, 20, 44). Colocalization of DDR foci with promyelocytic leukemia (PML) nuclear bodies has been suggested to be a senescence marker (45, 46). DDR blocks cell cycle progression by exerting checkpoint functions, to ensure that only intact genomic information is inherited by daughter cells (20). It has been found that senescence entry occurs via the DDR component p53, which activates its downstream target $p21^{WAF1/Cip1}$ upon ATM/ATR stimulation, thereby eliciting cell cycle arrest, whereas $p16^{INK4A}$ may further maintain senescence by functioning as a CDK4/6 inhibitor (39, 47). Of note, inhibition of the DDR signaling kinases ATM, ATR, and CHK1/2 in senescent cells leads to cell cycle reentry (20, 48). Moreover, the tumor suppressor ARF acts as a p53 stabilizer, leading to senescence induction (49, 50). Other types of DNA damage evident in senescent cells include cytoplasmic chromatin fragments (CCFs) and mitochondrial DNA (mtDNA) damage (16). In the case of oncogenes, their aberrant activation results in a hyperproliferative cellular state that is

inherently linked with deregulated DNA replication (replication stress) leading to DNA damage, DDR pathway stimulation, and manifestation of senescence, a process collectively known as oncogene-induced senescence (OIS) (34, 51, 52). Oncogene-mediated cell cycle arrest also occurs through the tumor suppressors $p16^{INK4A}$ and ARF, which are both encoded by the *CDKN2A* genomic locus (53, 54). Moreover, the DDR pathway and ARF have been found to synergize during OIS, with ARF requiring a higher oncogenic load than the DDR (14).

Telomere shortening and dysfunctional telomeres constitute a key feature of cellular senescence linked with persistent DNA damage termed telomere dysfunction-induced foci (TIFs) (16, 20, 22). The DNA replication machinery lacks the capacity to fully replicate telomeric DNA; thus in the absence of mechanisms ensuring accurate telomere maintenance such as telomerase expression or recombination among telomeric DNA, each round of cell division results in telomere attrition (55, 56). As critically shortened telomeres are deprived of their protective structures and telomere capping factors, they are recognized as one-ended DSBs (persistent DNA damage) by components of the DDR pathway, thereby activating DDR in a similar fashion as DSBs (55, 56). Importantly, persistent telomeric DDR activation may result in senescence and can be found not only at the gradually shortened telomeres of proliferating cells but also at the telomeres of certain types of noncycling cells, regardless of telomere length (57). In the latter case, a persistent DDR is often observed at the telomeres of aging cells or nondividing cells that have been exposed to genotoxic insults, as repair is considerably less efficient when DSBs are found within telomeres; when those telomeric DSBs persist, cells enter senescence (57).

Upon entering senescence, cells exhibit an enlarged and flattened morphology and rewire their metabolic activity despite being in a cell growth arrest state (16). Not only is senescence accompanied by intracellular effects, but secreted signals from senescent cells also have the capacity to affect their surrounding microenvironment, including neighboring nonsenescent cells that respond to a variety of secreted factors (22, 58). The secretome of senescent cells undergoes significant changes, and as a result senescent cells display a distinct secretory phenotype named the senescence-associated secretory phenotype (SASP), which constitutes a key senescence hallmark (16, 59). SASP consists of a large variety of soluble signaling factors such as chemokines, proinflammatory cytokines, angiogenic factors and growth modulators, extracellular matrix components, matrix metalloproteinases (MMPs), and bioactive lipids (59, 60). Among the main SASP drivers are transcription factors such as NF-κB, C/EPBβ, and GATA4 (16, 61–63), signaling pathways including the mammalian target of rapamycin (mTOR) and p38 mitogen-activated protein kinase (p38MAPK) pathways (62, 64), and DNA sensors such as the cGMP-AMP (cGAMP) synthase (cGAS), which activates the adaptor protein STING, leading to production of type I interferons and inflammatory cytokines (65, 66). Upstream signals activating SASP vary depending on the senescence inducer; however, they include cytoplasmic chromatin fragments (CCFs) that mediate inflammation (67). Several cell surface markers have been identified in regulating the SASP, such as Notch1 in OIS and dipeptidyl-peptidase 4 (DPP4) in replicative senescence and OIS (68). It has been demonstrated that senescent cells communicate with their microenvironment via NOTCH signaling, as well as through the production of reactive oxygen species (ROS), formation of cytoplasmic bridges, and secretion of extracellular vesicles such as exosomes (16). Thus, characterizing the senescent secretome in several biological settings is a promising strategy in deriving senescence-related molecular signatures.

The metabolic demands of the heart are the largest compared with all organs; even though the heart accounts for only ~0.5% of body weight, it is responsible for roughly 8% of the overall energy consumption (69). Cellular metabolism is deregulated during senescence, which is at least partly associated with mitochondrial dysfunction, aberrant proteostasis, altered autophagic properties, and the presence of dysfunctional lysosomes, thus leading to the accumulation of macromolecular damage (14, 16). In the postnatal heart, substrate switching and metabolic flexibility are features of normal function (70). Of great interest, metabolic reprogramming induces functional and structural remodeling in the heart, upon stress (71, 72). Indeed, an early trait of the maladapted heart is loss of metabolic flexibility (70). The healthy myocardium uses mainly fatty acids as its major energy source, with little contribution of glucose. However, lactate, ketone bodies, amino acids, or even acetate can be oxidized in heart cells under certain circumstances. For this reason, the heart is considered to be a metabolic "omnivore" (73). In addition, metabolic signals regulate transcriptional, translational, and posttranslational signaling in the heart (70). Developmental and/or pathophysiological stimuli regulate the expression of genes implicated in cardiac metabolism via specific nuclear receptor transcription factors and coactivators, including peroxisome proliferator-activated receptors (PPARs) and their nuclear receptor coactivator, estrogen-related receptors, and hypoxia-inducible transcription factor 1 (HIF-1) (74). Importantly, mitochondria constitute

30% of the myocardial mass and represent the main sites of ATP production. Thus, mitochondria retain a central role in cardiac metabolism and heart muscle function (75). In keeping with this, mitochondrial dysfunction frequently accompanies the senescence phenotype (76). Mitochondrial sirtuins are evolutionarily conserved proteins that function as histone deacetylases (HDACs) and regulate several aspects of aging across different species (77). It has been shown that inactivation of sirtuins as well as selective perturbation of mitochondrial functions drives adoption of the senescence phenotype (20, 78). Additionally, a body of evidence supports the presence of a reciprocal relationship between mitochondrial dysfunction and DNA damage (79). Interestingly, a distinct senescence phenotype named MiDAS (mitochondrial dysfunction-associated senescence), which is regulated by the NAD-AMPK-p53 pathway, has been found to display a cell-autonomous program likely resulting in deregulated adipocyte differentiation encountered in aged animals (78).

Mitochondrial dysfunction also leads to ROS-mediated lipid damage, increase of lipid deposits, and accumulation of lipofuscin (14, 79, 80). Besides oxidation, lipid-derived aldehyde modifications have been identified in senescent cells (81), whereas depletion of senescent cells in either obese or aged mouse models restricted hepatic and brain lipid accumulation (80, 82). Although a strong association between cellular senescence and lipid accumulation is evident, little is known about lipid metabolism in senescent environments (83). Recently, it was demonstrated that senescent cells activate the biosynthesis of several oxylipins, dihomo-prostaglandins, and leukotrienes, promoting SASP and reinforcing cell cycle arrest (84). However, given the high variability of senescence-related lipid profiles, the implementation of lipid modification detection methods may be of limited use. Similarly to mitochondria, lysosomes are severely affected by senescence, as indicated by their increased number and size within senescent cells (85). The increased lysosomal content leads to an increased organelle mass, which is associated with senescence-dependent β-galactosidase (SA-β-gal) activity (86). SA-β-gal has been widely used as a senescence biomarker; however, its abundant expression in senescent cells provides no indication of its requirement in the manifestation of the senescence phenotype (86).

Most senescent cells exhibit profound epigenetic and chromatin organization changes, which are associated with cell-autonomous and paracrine features of senescence-mediated cell cycle arrest (20). A type of chromatin foci called senescence-associated heterochromatin foci (SAHFs) is one of the senescence hallmarks that predominantly appear as nuclear structures in repressive chromatin marks and proteins, such as trimethylated histone H3 Lys9 (H3K9me3), high-mobility group protein A (HMGA) players, heterochromatin protein 1 (HP1), histone variant macroH2A, and histone cochaperones (20). Moreover, striking decondensation of peri/centromeric satellite heterochromatin termed senescence-associated distension of satellites (SADS) is commonly observed in various species upon different means of senescence induction (87). Senescence-related chromatin structure modifications are not usually accompanied by changes in histone marks but rather by structural modifications occurring in nuclear proteins, such as nuclear lamina degradation (87).

2.3. *The Role of Cellular Senescence in Cardiomyocytes*

Cellular senescence is commonly associated with both hypertrophic growth and fibrosis in cardiomyocytes (88, 89). Given that the majority of cardiomyocytes become postmitotic soon after birth, investigating how they enter cellular senescence adopting PoMiCS is particularly interesting. As cardiomyocyte cell divisions and turnover rates in both mice and humans are extremely low (90), telomere shortening-mediated replicative senescence is unlikely to occur. However, both human and mouse cardiomyocytes display a senescent phenotype where DNA damage is observed at telomere regions (41). It was found that this length-independent telomere damage is responsible for activating the senescence-promoting $p21^{WAF1/Cip1}$ and $p16^{INK4A}$ pathways in cardiomyocytes, thereby leading to an atypical profibrotic and prohypertrophic SASP (41). Interestingly, it was also shown that elimination of senescent cells in mice may rescue both fibrotic and hypertrophic cardiac phenotypes (41).

By conducting a SASP factor analysis in purified senescent cardiomyocytes, Anderson et al. (41) identified considerable differences between cardiomyocytes and whole heart homogenates. Specifically, in contrast to canonical SASP, the inflammatory factors Il-6 and Cxcl1 were not found to be elevated, whereas noncanonical SASP factors such as Edn3 and TGF-β2, known to promote cardiac hypertrophy, were found to be upregulated (41). This suggested that SASP complexes in cardiomyocytes may include not only proinflammatory cytokines but also cardiac remodeling molecules (91).

Cellular senescence induces distinct morphological alterations in cardiomyocytes. Fetal senescent cardiomyocytes display enlargement and vacuolization of their cell bodies (92). It was found that hypoxia treatment was able to confer such morphological changes in cardiomyocytes, accompanying senescence entry (91). Moreover, the anticancer agent doxorubicin was shown to promote cardiomyocyte senescence, with cells displaying increased cell

volume, flattened morphology, and the presence of vacuoles (91). The anthracycline DNA intercalator doxorubicin is a known DSB inducer and topoisomerase II inhibitor, while it has also been shown to enhance nucleosome turnover (93, 94). Cardiomyocyte senescence following doxorubicin treatment was invariably accompanied by cardiotoxicity (91).

Along those lines, senescent cardiomyocytes also exhibit contractile dysfunction, linked with accumulated endoplasmic reticulum (ER) stress (89). ER stress activates the unfolded protein response (UPR) and, furthermore, contributes to apoptosis and cardiomyocyte hypertrophic growth (89). Interestingly, ER stress attenuation protects against cardiomyocyte senescence, by concomitantly improving cardiomyocyte contractility (89).

A means of preventing cardiomyocyte senescence was based on inhibiting oxidative stress, a known inducer of cellular senescence (16, 91). Glutathione reductase (GR) is a homodimeric flavoprotein disulfide oxidoreductase that plays a critical role in maintaining the antioxidant capacity of cells by modulating the reduced glutathione (GSH)-to-oxidized glutathione (GSSG) ratio (95). It was recently shown that GR repression was implicated in cardiomyocyte senescence in mice following inactivation of the aging suppressor gene *Klotho* (96). Inversely, GR overexpression reduced oxidative stress and rescued the senescent phenotype as indicated by decreased $p16^{INK4A}$ levels in Klotho-deficient mice (96).

2.4. Physiological and Pathophysiological Implications of Cellular Senescence

Senescence is considered to be a stress response mechanism, implicated in a variety of important biological functions. One of the most beneficial roles of senescence is reflected in embryogenesis, where senescence pathways are transiently activated to regulate growth and patterning in the mammalian embryo (mesonephros and the endolymphatic sac of the inner ear) and placenta, thus highlighting the significant contribution of senescence to morphogenesis (97–100). Besides its important role in normal development, later in life acute or transient senescence becomes fundamental in tissue repair: homeostasis and tumor suppression (20). Damaged or stressed (senescent) cells are removed by cells of the immune system via SASP, thus maintaining the structural integrity and function of tissues after injury. For instance, in liver fibrosis, which is associated with tissue scarring and functional retardation of hepatic cells, senescence restricts the growth of ECM-producing stellate cells, thus limiting damage (101). It was also found that senescence limits fibrosis in skin wound healing (102).

On the other hand, persistent or chronic senescence is related to a plethora of detrimental phenomena at the tissue level including mild chronic inflammation, impaired renewal capacity due to stem cell niche exhaustion, extracellular matrix degeneration, paracrine senescence, immunosenescence, and tissue fibrosis (16). Particularly, although SASP facilitates tissue homeostasis, its prolonged activity sets the groundwork for the establishment of a deregulated tissue microenvironment, ultimately leading to age-related disorders (16, 22). Indeed, elevated levels of IL-6, IL-1 receptor antagonist (IL-1RA), as well as tumor necrosis factor (TNF) receptor, which are all SASP mediators, have emerged as biomarkers of chronic disease (103). Senescence might also impact on tissue repair and regeneration by posing a barrier to the proliferative capacity of stem cell progenitors. For example, muscle progenitor cells, which display elevated $p16^{INK4A}$ expression upon DNA damage and subsequently enter senescence, exhibit limited potential in contributing to tissue regeneration following injury (104). In accordance with these observations, it was found that hematopoietic stem cell (HSC) clonogenic properties were perturbed after exposure to SASP factors originating from senescent stromal cells (105), also implying the presence of a paracrine regulation of tissue regeneration driven by cellular senescence.

3. HALLMARKS OF CARDIOVASCULAR DISEASES AND KEY SIGNALING MOLECULES/MOLECULAR PATHWAYS IMPLICATED

3.1. Hallmarks of CVDs

One of the major hallmarks of CVDs relates to telomere shortening leading to senescence. Senescent cell accumulation in the heart and vascular wall leads to CV system deterioration with age (106, 107). It has been found that reduced leukocyte telomere length (LTL) correlates with vascular cell senescence, aortic valve stenosis, and increased atherothrombotic risk regardless of race, age, and sex (108, 109). Short LTL was additionally correlated with increased incidence of ischemic and hemorrhagic stroke compared to individuals with longer LTLs, which was further supported by meta-analyses confirming a higher risk for coronary and cerebrovascular disease as a result of telomere attrition (107).

Mitochondrial dysfunction and ROS constitute another hallmark of CVDs (**FIGURE 3**). When the antioxidant capacity of the human system becomes unable to neutralize the effects of free radicals, the resulting oxidative F3

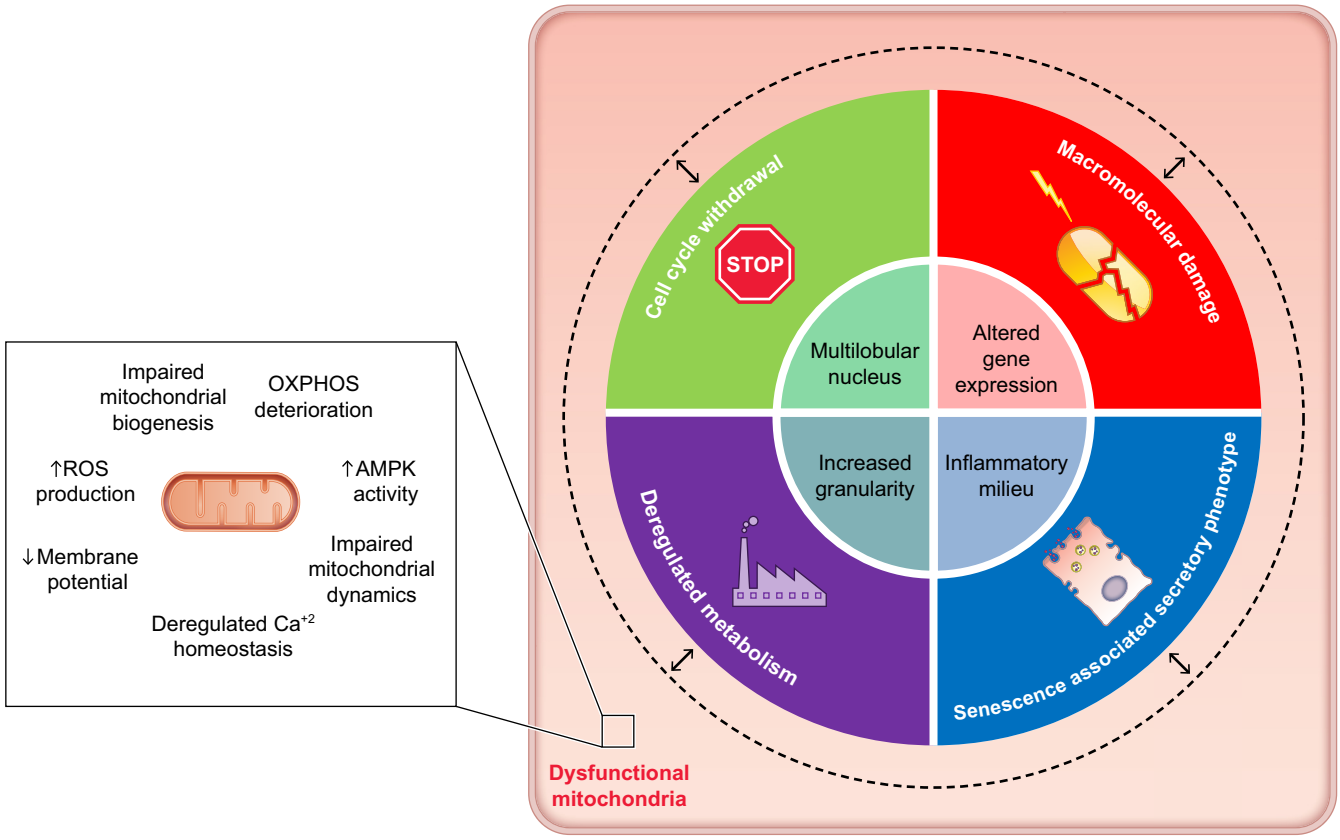

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FIGURE 3. Altered mitochondrial activity as a result of deregulated metabolism in senescent cells. Deregulated metabolism is one of the hallmarks of cellular senescence. Disruption of mitochondrial homeostatic mechanisms is a key feature of metabolic deregulation in senescent cells. It is manifested as impaired mitochondrial dynamics and biogenesis, as well as increased ROS production, leakage of mitochondrial enzymes, deterioration of oxidative phosphorylation, and deregulation of membrane potential. Mitochondrial dysfunction per se has been recognized as a driver of the senescent phenotype. For example, increased ROS production due to dysfunctional mitochondria accelerates telomere shortening (replicative senescence) and/or enhances DNA damage and DDR signaling pathways (premature senescence). See GLOSSARY for abbreviations. Vectors were obtained from www.vecteezy.com.

stress causes cardiac tissue injury leading to the onset of several CVDs such as endothelial dysfunction, atherosclerosis, and ischemia (110). Those deleterious effects are mediated through the formation of the highly reactive products O_2^- and H_2O_2, which, apart from inducing DNA damage, are also implicated in inflammation and cell death pathways (111, 112). It has been reported that in ischemia or hypoxia the mitochondrial electron transport (MET) becomes imbalanced, resulting in increased ROS production (113). Compared with other cell types, cardiac myocytes have a higher number of mitochondria and upon pathological conditions may become a source of oxidative stress themselves, as the increased amount of ROS they release may target the surrounding tissue microenvironment (114).

Genomic instability and epigenetic alterations are a frequent underlying cause of CVDs. With regard to genomic instability, Hutchinson–Gilford progeria patients display enormous levels of nuclear DNA damage, which has been related to premature atherosclerosis resulting in myocardial infarction or stroke before the age of 13 yr (115). Along the same lines, defective expression of the nucleotide excision repair genes *ERCC1* and *XPD* yields genomic instability in mice, which is associated with cellular senescence, hypertension, and vascular stiffness at an early age (116). In this context, cardiovascular aging was observed as a consequence of endothelial nitric oxide synthase (eNOS) and sirtuin deregulation, while nicotinamide adenine dinucleotide phosphate (NADPH) oxidase was increased (116). Apart from genetic diseases, numerous studies have observed DNA damage not only in the plaques of atherosclerotic patients but also in circulating cells (117), whereas peripheral blood mononuclear cells (PBMCs) from coronary heart disease patients display nuclear and mitochondrial DNA damage linked to disease severity (118). These observations indicate that sporadic genomic mutations that are accumulated throughout life may have a considerable impact on CVDs on account of genomic instability (107).

With the implementation of novel multiomics approaches, global histone modification signatures were derived from human cardiomyocytes from failing hearts (119). Analysis of

those signatures revealed that, under these pathological conditions, gene expression was regulated by both active (H3K9ac, H3K27ac, H3K4me3, and H3K36me3) and repressive (H3K27me3) histone mark modifications (120). For instance, downregulation of genes involved in transcription regulation and oxidative stress pathways was likely attributed to increased H3K9me3/H3K27me3 and decreased H3K9ac (120). Moreover, a recent body of evidence has demonstrated that the chromatin remodeling SWI/SNF complex is closely associated with cardiovascular pathophysiology, which renders the SWI/SNF complex a putative clinical target against cardiac hypertrophy and heart failure (119). In line with this, a significant decrease in the expression levels of the SWI/SNF subunit BRG1 has been identified in the myocardium of congenital heart disease patients (119).

Protein misfolding is considered to be another hallmark of CVDs. Proteostasis is ensured by a number of protective mechanisms that are differentially activated in various subcellular compartments; however, those mechanisms are found partially or fully impaired in diseased heart tissue (121). Under normal conditions, protein synthesis is a stringent process that takes place either at the endoplasmic reticulum or on free ribosomes and culminates in the production of properly folded, fully functional proteins (121). Nevertheless, cellular stress or tissue injury frequently yields misfolded, dysfunctional proteins, which are accumulated in aggresomes (121). During the UPR, misfolded proteins are normally resolved or degraded via an ER-dependent mechanism with the contribution of mitochondrial UPR, heat shock response (HSR) proteins, the ubiquitin-proteasome system, and autophagy (122). Amyloid cardiomyopathy, where the light chain of immunoglobulin or transthyretin is deposited in cardiac tissue, comprises one of the hallmark disorders related to intracardiac aggregation of misfolded proteins leading to heart failure (121). With regard to the vasculature, proteostasis defects in the arterial wall have been found to be responsible for the onset and progression of atherosclerosis, whereas hypertrophic/nonischemic or idiopathic dilated cardiomyopathy patients carry misfolded protein oligomers in their cardiac myocytes (121, 123).

Misfolded protein-mediated cardiotoxicity has been investigated in several experimental settings, including in animal models (123). With transgenic mice carrying a mutation in the heat shock gene *CryAB* (*CryAB*^{R120G}), it was demonstrated that impaired *CryAB* function led to the formation of preamyloid oligomeric intermediates (PAOs) that were sufficient to induce cardiomyocyte apoptosis and lead to heart failure (124). However, in most of those animal studies it remains unclear which forms of misfolded proteins (e.g., soluble or insoluble) may be cardiotoxic (121). Additionally, protein misfolding may act as the trigger or the outcome of disease, depending on the case; for example, ATP depletion may trigger protein misfolding in ischemia, whereas the increased demand for protein folding in hypertrophic cardiomyopathy may result in misfolded aggregates (121). Besides protein misfolding within the cell, protein aggregates of extracardiac origin may additionally contribute to disease through directly deteriorating cardiac function (121).

A number of recent studies have shown that postmitotic human cardiomyocytes display senescence-like phenotypes after the age of the mid-40s, accompanied by persistent telomere DNA damage, p21$^{WAF1/Cip1}$/p16^{INK4A}-mediated cell cycle arrest, reduced expression of mitochondrial genes related to the electron transport chain such as *SOD2*, autophagy/mitophagy dysfunction leading to increased SA-β-gal activity, epigenetic modifications, as well as establishment of a nontypical SASP (19, 41).

3.2. Key Signaling Molecules and Cascades in Cardiac Dysfunction

A growing body of evidence has indicated the common pathways between cellular senescence and CVDs. The levels of the tumor suppressor and "guardian of the genome" p53 (110) are markedly increased in end-stage heart failure patients and implicated in promoting apoptosis in the myocardium (125). Along the same lines, cardiac cells of hypertrophic/dilated cardiomyopathy patients display higher p53 levels compared with normal counterparts (126), implying p53 involvement in cardiac homeostasis.

p21$^{WAF1/Cip1}$-activated kinases (PAKs) are serine-threonine kinases that are implicated in inflammatory and cardiovascular disease (127). PAK1 and PAK2 have been found to regulate the NADPH oxidase (NOX) in neutrophils (128). NOX is an enzyme complex involved in ROS generation, via catalyzing electron transfer from NADPH to O_2 (127). Although the NOX complex has been involved in a plethora of biological processes, such as cellular development, migration, and the immune response (127), NOX overexpression leads to manifestation of CVDs such as hypertension, atherosclerosis, myocardial infarction, and cardiac hypertrophy, indicating that NOXs may comprise potential targets for pharmacological inhibition, at least in that context (127). Interestingly, PAK1 has been also found to be implicated in cardiac ischemia-reperfusion injury as well as hypertrophy (129, 130).

Another established cellular senescence marker is elevated expression of p38 mitogen-activated protein kinase (p38MAPK) (131). Although all p38 family members have been found to contribute to various aspects

of cardiomyocyte differentiation and growth, it is now known that they may have either protective or deleterious effects on the myocardium, which is largely dependent on the identity of the p38 family member involved in each case (131). To date, most studies investigating the effect of p38 on CVDs have implemented generic inhibitors, and there is little knowledge regarding how different p38 family members may regulate cardiac physiology (131). Nevertheless, it has been demonstrated that p38α depletion or pharmacological inhibition halts cardiomyogenesis, thereby providing evidence of the important role of p38α activation in cardiac differentiation (132). A role for p38α in cardiac hypertrophy has also been proposed, as the p38 pathway is triggered in response to ischemia-reperfusion (133). In addition, p38 promotes upregulation of the Reg3γ protein, a cardiac inflammatory response player (134). Importantly, the p38 pathway is activated in cardiac remodeling culminating in cardiac arrhythmia observed in the failing heart, which is in line with the observed role of p38 in regulating cardiac contractility (131). However, a negative correlation between p38 activation and extracellular matrix remodeling or cardiac fibrosis, which both lead to heart failure, has been suggested by previous studies (135, 136).

The mammalian target of rapamycin (mTOR) is implicated in the regulation of several biological processes, such as protein synthesis, proliferation, cellular metabolism, and SASP (137–140). mTOR has been found to interact with adaptor proteins to form two complexes named mTOR complex 1 (mTORC1) and 2 (mTORC2) (139). The mTOR pathway is required for cardiovascular development during embryogenesis and is a master regulator of cardiac homeostasis in postnatal life (141). It has been shown that mTORC1 activation is required for adaptive cardiac hypertrophy, whereas mTORC2 is indispensable for normal cardiac function ensuring the survival of cardiomyocytes upon pressure overload (141). In accordance with this, mTORC1 inactivation rescues pressure overload-related heart failure and chronic myocardial infarction, while it also ameliorates metabolic disorder-mediated cardiac cell dysfunction resulting in extended life span in mice (141). It is hence suggested that pharmacological inhibition of mTOR signaling constitutes a potential therapeutic approach to cardioprotection; however, this remains to be thoroughly validated at the clinical level (141).

Cardiac fibroblasts, which are required for extracellular matrix (ECM) homeostasis, are normally quiescent and secrete ECM components. However, upon cardiac tissue damage cardiac fibroblasts differentiate toward cardiac myofibroblasts (CMFs), which represent a more metabolically active cell type (142). CMF differentiation, which is a common feature of cardiac fibrosis encountered in heart failure and diabetic cardiomyopathy, is elicited through involvement of TGF-β1, a known SASP factor (62, 143). Specifically, TGF-β1 signaling activates the SMAD nuclear factors, which in conjunction with Forkhead box type O (FoxO) proteins facilitate CMF conversion (143, 144). It was recently shown that TGF-β1 signaling downregulates FoxO3a expression in cardiac fibroblasts and that TGF-β1-mediated regulation of FoxO3a relies on active SMAD3, as well as the ERK1/2 and Akt serine/threonine kinases (145). This was not an unexpected finding, as FoxO3a is a known member of the FoxO family implicated in several fibrotic processes, such as in pulmonary tissue (146). Interestingly, FoxO1 was found to be essential in TGF-β1-induced FoxO3a upregulation and that CMF conversion was perturbed by FoxO3a expression, implying that a FoxO1-FoxO3a regulation may have a negative effect on TGF-β1-mediated CMF differentiation (145).

4. CLINICAL AND EXPERIMENTAL EVIDENCE REGARDING THE ROLE OF SENESCENCE IN CARDIOVASCULAR DISEASES

A growing body of clinical and experimental studies highlights the involvement of cellular senescence in the pathogenesis of a plethora of cardiac diseases. The rationale relies heavily upon the view that cellular senescence represents an adaptation to stress. In addition, there are several functional and structural senescence-mediated changes characterizing the aging heart, involving a number of molecular pathways (**FIGURE 4**). The progressive deterioration that characterizes the aging heart is attributed to modifications at the cellular, subcellular, and macroscopic levels. For example, aged cardiac cells exert specific microscopic traits such as fibrosis, hypertrophy, amyloid deposits, and lipofuscin accumulation (147). The decreased number of cardiomyocytes along with the low self-renewal capacity of cardiac progenitor cells justifies a diminished regenerative capacity of the aged heart upon stress or damage (148). At the macroscopic level, the aged heart exerts systolic and diastolic dysfunction, impaired autonomic control, and a propensity to arrhythmias. Systolic dysfunction is associated with an O_2 supply-demand mismatch that can be attributed to pressure overload, remodeling of the myocardial microvasculature, and decreased coronary perfusion, whereas the diastolic dysfunction is due to impaired active relaxation (149). Chronic activation of nutrient and growth signaling pathways, including the renin-angiotensin-aldosterone system, TGF-β, mTOR,

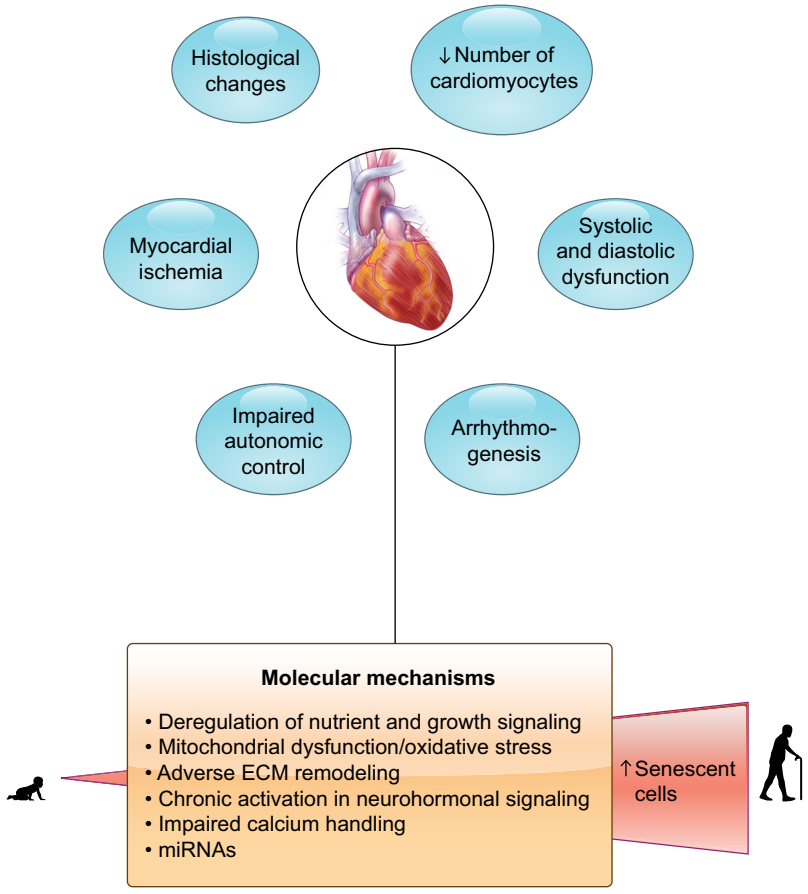

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FIGURE 4. Functional and structural changes characterizing the aging heart and molecular pathways implicated. Histological modifications that characterize aged cardiac cells include fibrosis, hypertrophy, calcification, thickening of the tunica media in vessels, amyloid deposits, expansion of the extracellular matrix/collagen, and lipofuscin accumulation. Overall, the number of cardiomyocytes in the aged heart is diminished because of increased necrosis and apoptosis, impaired regenerative capacity of heart cell populations, and decreased self-renewal capacity of cardiac progenitor cells. Pressure overload, remodeling of the myocardial microvasculature, and decline in coronary perfusion result in O_2 supply-demand mismatch and, consequently, myocardial ischemia. In addition, age-related left ventricle hypertrophy, left ventricle systolic reserve capacity fall-off, and impaired early diastolic filling further aggravate systolic and diastolic dysfunction of the aging heart. The aging process is also accompanied by a number of changes in the autonomic control of the heart, favoring enhanced cardiac sympathetic tone along with parasympathetic withdrawal and dampened cardio-vagal baroreflex sensitivity, due to decreased plasma clearance of norepinephrine and β-adrenergic desensitization. Finally, the aged heart is more prone to arrhythmogenic events associated with atrioventricular and intra- and interventricular conduction abnormalities. Several critical molecular mechanisms have been involved in cardiac aging, such as chronic activation in neurohormonal signaling (RAAS, ANG II), nutrient and growth signaling deregulation (mTOR, IGF-1), mitochondrial dysfunction/oxidative stress, impaired calcium handling, adverse extracellular matrix remodeling (TGF-β, MMPs) and a number of miRNAs. Of great importance, the accumulation of senescent cells is a hallmark of the aging process and drives these changes. See GLOSSARY for abbreviations. Vectors were obtained from www.vecteezy.com.

and insulin-like growth factor-1 (IGF-1) signaling, is detrimental for the heart and drives both hypertrophy and fibrosis as well as relaxation impairment (150–152). Another molecular mechanism underlying age-dependent heart dysfunction is impaired active relaxation due to Ca^{2+} handling deregulation, which is associated with reduced sarco(endo)plasmic reticulum Ca^{2+}-ATPase 2 (SERCA2) expression and posttranslational SERCA2 modifications (153, 154). In addition, a growing body of evidence suggests that microRNAs are important regulators of cardiac aging (155).

However, it has not yet been fully elucidated in which scenarios cellular senescence is the causative factor or an epiphenomenon of pathological phenotypes or, even more intriguingly, in which context cellular senescence may exert a beneficial role.

To present findings in a comprehensive way, in each of the following subsections we initially provide a brief introduction regarding the pathophysiological basis of the clinical entities presented therein and then appose evidence linking cellular senescence with key molecular players in the cardiac cell

AQ: 8 subtypes involved in each case. A schematic summary of the key molecular players involved in senescence-associated CVDs in individual cardiac cell types is provided in **FIGURE 5**.

F5

4.1. *Ischemia: from Atherosclerosis to Myocardial Infarction*

Atherosclerosis refers to a process of arterial vessel wall remodeling secondary to atheromatous plaque formation mainly driven by chronic inflammation (156, 157). Overall, senescent cells are main drivers of this pathological condition, acting mainly as inflammatory hotspots throughout the natural progress of the disease. Different cell populations acquire a senescent program as disease progresses to more advanced stages. These are endothelial cells, macrophage foam cells, immune cells (monocytes/macrophages, neutrophils, T cells), vascular smooth muscle cells (VSMCs), and adventitial fibroblasts (158). Senescence in this context can be induced either prematurely by stress (e.g., oxidative

Cell membrane	Cell type	ER	Cell type	Nuclear factors	Cell type
PECAM-1	endothelial cells	CALR	endothelial cells	**Nucleus**	
ICAM	endothelial cells			CDKN2A	adult stem cells
VCAM	endothelial cells	**Mitochondria**	**Cell type**	P21	cardiomyocytes
SELP	endothelial cells	ALDH2	cardiomyocytes	TAF	cardiomyocytes
CD31	apoptotic cells	SIRT2	cardiomyocytes	RBM20	cardiomyocytes
TM1	macrophages	SIRT3	cardiomyocytes	P16	cardiomyocytes
SIPR1	macrophages	FXN	endothelial cells	RB1	cardiomyocytes
TAM	macrophages			MEIS2	cardiomyocytes
MERTK	macrophages	**Cytoplasm**	**Cell type**	GATA4	cardiomyocytes
VEGF	macrophages	TGFB1-induced MAPK	adult stem cells	NKX2-5	cardiomyocytes
IL6	myofibroblasts	TFB1M		TBX5	cardiomyocytes
IL13	myofibroblasts	**Cytoskeleton**		PITX2	cardiomyocytes
THBS1	myofibroblasts	CFL2	cardiomyocytes	P53	cardiomyocytes
TGFB	myofibroblasts	**Membrane**		P53	cardiomyocytes
IGFBP5	muscle satellite cells	PKP2	cardiomyocytes	HIF1A	cardiomyocytes
PAI1	vascular and blood cells	**Myofibril**		CFL2	cardiomyocytes
F2	vascular and blood cells	MYH6	cardiomyocytes	TMEM43	cardiomyocytes, fibroblasts
HTRA4	endothelial cells	MYH7	cardiomyocytes	LEMD2	cardiomyocytes, fibroblasts
IGFBP3	endothelial cells			NFKB	endothelial cells
SERPINE1	endothelial cells			PCNA	endothelial cells
SERPINEB2	endothelial cells			CHEK2	endothelial cells
CC15	muscle satellite cells			NOX4	endothelial cells
PARP1	myofibroblasts			CDKN2C	endothelial cells
CCN1	fibroblasts			P53	fibroblasts
POSTN	myofibroblasts			HIFs	macrophages
				Nuclear membrane	
				LAMIN A/C	cardiomyocytes
				EMD	cardiomyocytes

Color coding correlating genes with diseases

atherosclerosis
myocardial infarction
atrial fibrillation
valvular heart disease
dilated cardiomyopathy
hypertrophic cardiomyopathy
peripartum cardiomyopathy
diabetic cardiomyopathy

COLOR

FIGURE 5. Summary of key molecular players involved in senescence-associated cardiovascular diseases (CVDs) in the various cardiac cell types. Key molecular players are categorized on the basis of their subcellular function. Color coding is used to distinguish the different types of CVDs affected by the function of each protein in the respective cardiac cell type. ER, endoplasmic reticulum.

stress, mitochondrial dysfunction, infection) or naturally by replicative exhaustion (159).

There are two main pillars responsible for atherosclerotic lesion formation: *1*) endothelial dysfunction and *2*) lipid retention and oxidative modification within the arterial vessel wall. Endothelial dysfunction, which has been identified as an early event in atherosclerosis (160, 161), is associated with accumulation of endothelial cell senescence (162, 163). Increased ROS production within endothelial cells leads to NF-κB activation, a master regulator of inflammatory responses (161). Elevated oxidative and inflammatory stress seem to be the pathways through which many traditional CVD risk factors (increased LDL, elevated blood pressure, blood glucose and sodium intake, old age, obesity) induce endothelial senescence (161).

Additionally, endothelial senescent cells upregulate expression of homing molecules-receptors (ICAM, VCAM, P-selectin) and cytokines/chemokines through their SASP, thus allowing recruitment of immune cells including neutrophils (important for disease initiation) and monocytes (important for disease progression through their differentiation in dendritic and inflammatory macrophages) (164). Overall, the senescent endothelium acquires a proinflammatory, proatherosclerotic, prothrombotic, and prooxidant phenotype that facilitates oxidation of the built-up lipids, leading to xanthoma formation.

Oxidative modification of lipids creates neoepitopes that trigger activation of the immune response. Initially, neoepitopes of ox-LDL, ox-phospholipids, and ox-triglycerides (164), accumulated in the subendothelium, are recognized as foreign bodies by resident dendritic cells and macrophages. Macrophages, after lipid uptake, are transformed into foam cells that become senescent and display deleterious properties throughout all disease stages (165). Chemokine release by the abovementioned immune cells and endothelial senescent cells through SASP results in neutrophil chemotactic recruitment. Neutrophils, in turn, recruit classical monocytes [i.e., inflammatory monocytes that differentiate into inflammatory macrophages (166)], which migrate into the inflamed vessel wall (164, 167) to regulate the inflammatory process by clearing apoptotic cells, including apoptotic neutrophils.

Persistent inflammation activates VSMCs to migrate toward the lesion site, where a portion of VSMCs transdifferentiate to alternative cell lineages of macrophage-like and mesenchymal stem cell-like phenotype and another portion become senescent (168, 169), acquiring a secretory phenotype (SASP) that involves production of metalloproteinases (MMPs) and ECM remodeling, responsible for pathological intimal thickening and fibrous plaque formation.

Depending on the number of VSMCs and on the degree of deposited fibrin and ECM consistency, plaque stability is determined based on the thickness of the fibrous cap. Of great importance, the process of fibrous cap thinning is causally linked to senescence via increased MMP production by senescent endothelial-like, macrophage-like, and VSMC-like foam cells (165, 170). In line with this, thinning of the fibrous cap is paralleled by a progressive decrease in the VSMC-to-macrophage ratio due to VSMC senescence and increased phagocyte recruitment (171).

Other features pointing toward rupture are intraplaque neoangiogenesis, hemorrhage, and increased necrotic core size, all associated with cellular senescence. Neoangiogenesis and hemorrhage, late features in the disease course, are mainly the result of HIFs secreted by hypoxic macrophages located deep within the necrotic core (172), coupled with vascular endothelial growth factor (VEGF) and other SASP-related vessel-trophic growth factor effects (59). Necrotic core size is highly dependent on the functional state of efferocytosis, a phagocytic process eliminating apoptotic cells. During the initial disease stages, efferocytosis protects against atherosclerosis via indirect and direct mechanisms; indirect mechanisms refer to decreases in ROS and proinflammatory cytokines, whereas direct mechanisms are related to enhancement of anti-inflammatory cytokines and antioxidant actions. Of note, the efficiency of efferocytosis decreases over time, especially in advanced lesions. In these lesions, “eat-me” molecules [i.e., engulfment signals displayed by cells marked out for destruction, which recruit the phagocytic machinery (173)] produced by apoptotic cells, including foam cells, and bridging molecules (produced by recruited effector macrophages) are decreased (174). Interestingly, analysis of genes linked to senescence with the SeneQuest database suggests that many of these signals are differentially expressed in senescent cells (16), suggesting that efferocytosis deficiency is causally linked to cellular senescence. Specifically, eat-me signaling such as CD31, the macrophage receptors Tim1 and Sphingosine 1 receptor 1, and TAM, a bridging molecule expressed by macrophages that facilitates efferocytosis, appears to be downregulated during cellular senescence (174). In addition, MERTK, a macrophage receptor that recognizes eat-me signals, is thought to be proteolytically cleaved by ADAM17, an enzyme commonly upregulated in senescent cells (175, 176). It has also been shown that MERTK is downregulated in macrophages treated with senescent cell-conditioned medium, showing decreased efferocytosis and a dysbalanced RvD1 (Resolvin D1, a pro-resolving lipid mediator produced by ω-3 docosahexaenoic acid)-to-LTB4 (leukotriene B4, a proinflammatory lipid mediator) ratio that favors inflammation persistence over resolution (177, 178). Moreover, the most well-known “don’t-eat-me” signaling pathway mediated by CD47 is

upregulated in cellular senescence, and CD47-blocking antibodies were able to restore efferocytosis and abrogate atherosclerotic lesions in multiple mouse models (179). Additionally, protracted TNF-α production, a main SASP component, induces CD47 expression, rendering vascular cells resistant to efferocytosis. All the above are indicative of how cellular senescence may potentially regulate efferocytosis directly via modulation of effector molecules involved in the process, unveiling another mechanism by which cellular senescence supports atherogenesis and atheroprogression.

The role of activated myofibroblasts residing in the adventitia of blood vessels in atherosclerosis has been lately acknowledged (180, 181). Intriguingly, these cells are largely regarded as senescent cells since they harbor profibrotic and prosecretory properties involved in wound healing and repair (182, 183). Adventitial fibroblasts are therefore an additional cell population whose senescence is likely involved in the process of atherosclerosis, although the disease stage at which this occurs still remains elusive.

Myocardial infarction (MI) typically results in large-scale cardiomyocyte loss. Ischemic injury triggers DNA damage, oxidative stress, and mitochondrial dysfunction, which predispose to cardiomyocyte senescence (184). Since cardiomyocytes are terminally differentiated cells, cell cycle arrest is not a hallmark of cardiomyocyte senescence. In fact, senescent cardiomyocytes express other senescence-associated features including DNA damage and repair response, ROS accumulation, metabolic maladaptation, endoplasmic reticulum stress, mitochondrial dysfunction, impaired contractile function, hypertrophic growth, and SASP (89). Topologically, cellular senescence has been identified within the surviving myocardium, proximal to the infarcted region and in border areas, early after the ischemic event, as demonstrated by an increase in senescence markers SA-β-Gal, $p21^{WAF1/Cip1}$, and $p16^{INK4A}$ and TAF accumulation (88, 185).

During the weeks following an acute ischemic insult, lost cardiomyocytes are replaced by noncontractile scar tissue. Scar formation induces a remodeling process that progressively alters ventricular architecture, impairs systolic function, and causes heart failure with reduced ejection fraction. Besides cardiomyocyte senescence, fibroblast senescence also occurs in the adult heart after myocardial infarction. The matricellular protein CCN1 has been reported to induce fibroblast senescence via p53 and $p16^{INK4A}$ activation (102). It has been suggested that CCN1-induced senescence may have a beneficial role in acute ischemia by inhibiting myofibroblast proliferation, thus preventing adverse cardiac remodeling and improving cardiac function. In a model of infarcted adult mice, CCN1-treated hearts exhibited more senescent cells and fewer proliferating fibroblasts in the ischemic regions. Moreover, infarct size was attenuated and cardiac function improved (186). In line with these findings, senescence mediated by the GATA-binding factor 4/CCN1 axis averts postinfarct myocardial fibrosis and preserves heart function (88). In agreement, aldehyde dehydrogenase 2 (ALDH2) knockout impaired the beneficial effects of myocardial senescence by blocking the GATA-binding factor 4/CCN1 pathway (88). As a regulatory interplay has been identified between GATA factors and Hippo pathway components (187–189), it would be interesting to explore the potential impact of the Hippo pathway on senescence in the MI context.

Interestingly, as mentioned above, pathological involvement of cellular senescence is context dependent, and this notion justifies, at least in part, some contradictory findings (110). For example, although senescent endothelial cells are consistently reported as detrimental in the aging cardiovascular system, this is not always observed for fibroblasts. Inhibition of cellular senescence in fibroblasts exerts detrimental effects in cardiac tissues by promoting fibrosis in noninfarcted areas and, subsequently, remodeling (190, 191). Activated but nonsenescent fibroblasts (myofibroblasts) highly express periostin, an ECM protein that appears to play a critical role in myocardial fibrosis and inflammation. Of note, increased levels of periostin and associated myocardial fibrosis were observed in individuals with myocardial infarction and hypertrophy (192, 193). Intriguingly, increased periostin expression from activated cardiac fibroblasts promotes senescence in cardiomyocytes and is associated with increased expression of IL-6 and IL-13 (194).

Cellular senescence of endothelial cells has also been implicated in myocardial ischemia. Senescent endothelial cells *1*) induce endothelial dysfunction that predisposes to angina pectoris and ischemic heart injury (195), *2*) release endothelium-derived microparticles increasing the expression and activity of tissue factors and augmenting the aggregation of platelets, eventually leading to thrombogenicity (196), and *3*) affect cellular proliferation and angiogenesis, thus impairing repair after MI (197). Of great interest, recent evidence suggests that silencing of senescence-associated genes Rb1 and Meis2 in adult cardiomyocytes results in cell cycle reentry and cardiac repair in the context of ischemic injury. The improvement of cardiac function after infarction was attributed to both the reduced infarct size and enhanced peri-infarct angiogenesis (198).

Of great importance, cellular senescence of host heart cells also affects the local microenvironment by releasing proinflammatory factors. Senescent cellular populations and the local microenvironment interact through a vicious cycle in which the inflammatory milieu produced by senescent cells triggers and enhances a proportionate

response by the microenvironment, thus promoting myocardial tissue dysfunction (89). After an ischemic injury, the response of myocardial ECM, a key component of the local microenvironment, is divided into three phases: *1*) early injury response, *2*) proliferation, and *3*) late maturation. During the first phase, endogenous ligands released by damaged cells trigger host innate immunity, which in turn leads to the release of cytokines and chemokines, promoting the trafficking of inflammatory cells to the site of injury. Degradation products of matrix proteins can also modulate inflammation and repair. During the proliferation phase, TGF-β and thrombospondin-1 stimulate proliferation of myofibroblasts. During the maturation phase, continuous collagen deposition results in scar formation and healing (9). Interestingly, cellular senescence has also been associated with changes in both ECM component expression and ECM remodeling enzyme secretion under various pathological conditions (199). In line with this view, thrombospondin, TGF-β, and MMPs represent pleiotropic signaling peptides whose involvement in cellular senescence is well established.

The current standard of care for MI is early reperfusion of the occluded vessel with angioplasty or thrombolysis to reverse ischemia and increase the number of surviving myocytes. However, cardiac ischemia-reperfusion injury generates ROS products that have detrimental effects on viable myocardium (200). In this regard, ischemia-reperfusion events induce cellular senescence in both cardiomyocytes and interstitial cell populations, which contributes to impaired heart function and adverse remodeling because of SASP (201). In respect to heart recovery, it was recently reported that ectopic transient expression of miR-294 promotes cell cycle reentry, leading to augmented cardiac function in mice after myocardial infarction (202). Besides early reperfusion therapy, angiotensin-converting enzyme (ACE) inhibitors and beta-blockers are used to prevent remodeling after MI and progression to heart failure. Notably, treatment with metoprolol attenuates oxidative stress and senescence of cardiac stem cells, thus delaying progressive cardiac remodeling (203).

4.2. Atrial Fibrillation

Atrial fibrillation (AF) is a supraventricular arrhythmia characterized by irregular heart rhythm driven by the predominance of a high-frequency atrial activity over the normal pacemaker of the heart (i.e., the sinus node) (204). Despite major milestones in the management of AF, this arrhythmia remains one of the major causes of stroke, heart failure, premature death, and cardiovascular morbidity worldwide. Increasing population age, together with several conditions predisposing to AF (such as hypertension, heart failure, coronary artery disease, valvular heart disease, obesity, diabetes mellitus, or chronic kidney disease), seems to drive the epidemiological explosion of AF (205). A schematic summarizing key pathways and mechanisms linking AF with senescence is provided in **FIGURE 6**. F6

Occasionally, genetic predispositions and a strong heritable component are documented (206, 207). Many

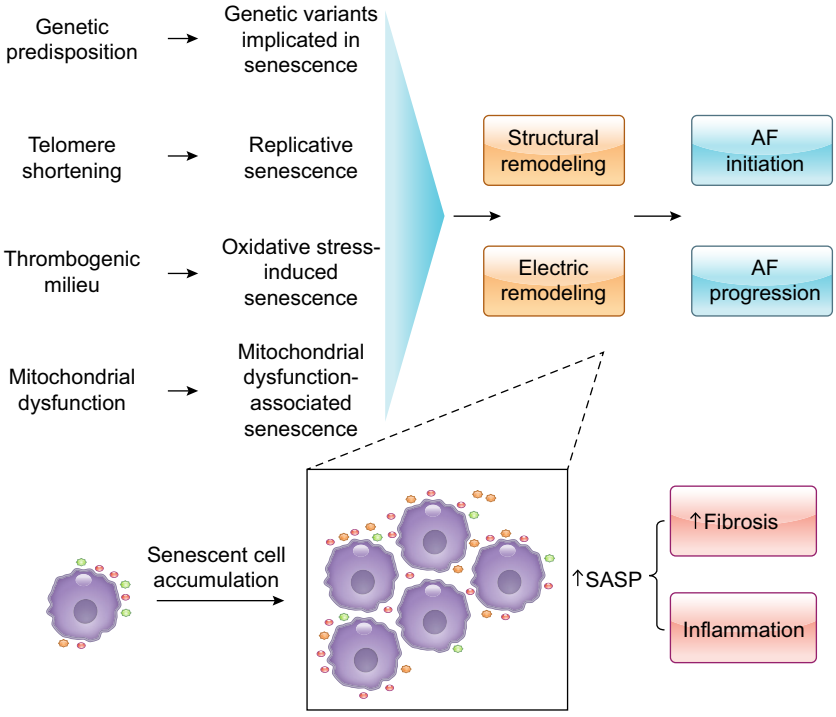

FIGURE 6. Senescence-inducing mechanisms leading to AF initiation and progression. Schematic of main mechanisms leading to cellular senescence, accompanied by structural and electrical remodeling of the heart, which result in AF initiation and progression. Genetic variants predisposing to senescence lead to reduced repair capacity, conduction defects, and decreased antioxidant scavengers; replicative senescence results in early vascular stiffness, increased ventricular filling pressures, and autonomic nervous system dysfunction; oxidative stress-induced senescence drives upregulation of the local angiotensin system and inflammatory marker increase; mitochondrial dysfunction-associated senescence leads to energetic failure, calcium overload, and oxidative injury. See GLOSSARY for abbreviations.

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common genetic variants have been identified as AF associated, many of which are proximal to deleterious mutations causing serious heart defects (i.e., *GATA4*, *MYH6*, *NKX2-5*, *PITX2, TBX5*) or near genes important for striated muscle function and integrity (i.e., *CFL2*, *MYH7*, *PKP2*, *RBM20*, *SGCG*, *SSPN*) (208, 209). The most important genetic variants predisposing to AF (up to 7-fold increased risk for AF) are located close to the paired-like homeodomain transcription factor 2 (*Pitx2*) gene on chromosome 4q25 (210, 211). Reduced Pitx2 expression results in proarrhythmic cardiac electrical modifications such as atrial action potential shortening and a depolarized atrial resting membrane, two established causes of arrhythmia. Furthermore, Pitx2 deficiency leads to structural remodeling of the atria and compromises the repair capacity of the heart, both of which may influence AF through divergent mechanisms (212). Of note, progressive loss of Pitx2c, the predominant isoform of Pitx2 expressed in the heart, occurs with age (213, 214). In cardiomyocytes, Pitx2 plays a critical role in the regulation of antioxidant scavenger genes (215). Of relevance, mutation of Pitx2/3 in adult muscle satellite cells leads to failure of muscle regeneration due to deregulation of their redox state and triggers senescence as measured by upregulation of senescence-associated genes (Igfbp5, Ifitm1, or Ccl5), increased number of cells positive for the Hp1-gamma marker of heterochromatin foci, and activation of SA-β-Gal activity (216).

In addition, changes in the activity of splicing factors and the production of key splice variants have been implicated in cellular senescence as well as in the aging process (217). In particular, functional association between p53, IGF-1, SIRT1, and ING-1 splice variants and both senescence and aging has been observed (217). Defective splicing leads to CVDs such as aortic aneurysm and arrhythmias (218). Missplicing of *SCN5A*, the gene that encodes the sodium channel $Na_v1.5$, causes conduction defects and arrhythmias (219). Thus, defective splicing may represent another possible nonmutational mechanism by which cellular senescence and AF are intertwined.

Given that the prevalence of AF is age dependent and that telomere shortening is a surrogate marker of biological aging, the implication of replicative senescence in the pathogenesis of AF seems to be an attractive hypothesis to be tested. Shorter telomere lengths contribute to early vascular stiffness and elevated ventricular filling pressures, two pathophysiological findings that characterize AF-induced cardiomyopathy (220, 221). Shorter telomere lengths have also been linked with decreased sympathetic tone and accelerated autonomic nervous system dysfunction, well-established mechanisms that drive the onset of paroxysmal AF (222). Recently, subjects with a history of AF were shown to have shorter telomeres compared with individuals in normal sinus rhythm. Indeed, the association remained after adjustment for age and cardiovascular risk factors. Surprisingly, the mean telomere-to-single gene ratio (t/s) for paroxysmal AF was significantly shorter than for persistent or permanent/long-standing AF, implying that shortened telomeres are involved in AF onset but not in AF progression (223). In addition, telomere length was longer and mRNA levels of the senescence-related proteins sirtuin-2 and -3 were higher in lone AF (LAF) than in AF patients with structural heart disease, suggesting a distinct arrhythmogenic substrate between these two subgroups of AF patients (224).

The functional and structural alterations taking place in the atria of AF patients along with the stasis of blood due to the unorganized and ineffective atrial contraction (especially in the left atrial appendage) generate and conserve a thrombogenic milieu (225). This generalized prothrombotic state is manifested by high circulating levels of fibrinolytic degradation products, increased plasminogen activator inhibitor-1 (PAI-1) expression, and enhanced thrombin-antithrombin complex and procoagulant microparticles (MPs) released to the bloodstream (226, 227). Indeed, endothelial cell-derived MPs facilitate cellular senescence through NOX and mitochondrion-derived reactive oxygen species (228). In addition, circulating MPs exert direct effects on vascular and blood cells, thereby triggering thrombin generation (229). Recent findings indicate that thrombin, at concentrations achieved during vascular injury associated with thrombus formation, induces oxidative stress and premature atrial endothelial senescence (230). Atrial endothelial senescence induced by thrombin is characterized by the acquisition of a prothrombotic, proadhesive, profibrotic, and proremodeling phenotype (230). Therefore, beyond its role in coagulation, thrombin also emerges as a key molecule for the development of the arrhythmogenic substrate that creates and maintains AF.

Interestingly, thrombin-induced senescence has been shown to promote the upregulation of the local angiotensin system in atrial endothelial cells (230). Respectively, both angiotensin II type 1 receptor (AT_1R) blockade and ACE inhibition significantly dampen thrombin-dependent induction of oxidative stress and cellular senescence (230). In addition, thrombin exerts pleiotropic cellular effects in hemostasis, inflammation, cellular growth, and proliferation, by activating protease-associated receptor-1 (PAR-1) (231). Of great importance, thrombin inhibitors and PAR-1 antagonists prevent atrial remodeling and reduce AF susceptibility (231). In line with these findings, cardiac glycosides (CGs), which were commonly used in AF treatment, were recently found to exert senolytic properties, thus

suggesting a causal involvement of senescence in the pathophysiology of AF (232).

Another possible link between cellular senescence and AF appears to be mitochondrial dysfunction. Deregulation of mitochondrial homeostatic mechanisms is considered to be a hallmark of both cellular senescence and aging (see sects. 2.1, 2.2, and 3.1) (76). At the same time, a selective reduction in the activity of the mitochondrial electron transport chain drives oxidative stress and facilitates AF development (75). Moreover, mitochondrial function decline in the elderly predisposes to AF due to enhanced sensitivity of the myocardium to energetic failure, calcium overload, and oxidative injury during stress (233, 234). In accordance with this, loss of mtDNA in cardiac muscle is pronounced in AF patients, further implying that mitochondrial dysfunction is involved in the pathogenesis of AF (235).

4.3. Nonischemic Cardiomyopathies

Nonischemic cardiomyopathies comprise a diverse group of inherited cardiac disorders that frequently lead to death or heart failure requiring cardiac transplantation. Further elucidation of the fundamental mechanisms involved in the onset and progression of nonischemic cardiomyopathies is urgently needed to optimize targeted therapy (236, 237). Surprisingly, emerging evidence supports the involvement of cellular senescence in several of these clinical entities. In this section we only refer to those nonischemic cardiomyopathies exhibiting the strongest links with senescence to date.

4.3.1. Hypertrophic cardiomyopathy.

Hypertrophic cardiomyopathy (HCM) is a complex heart disease that is most commonly caused by a single mutation in genes encoding sarcomeric proteins (238). HCM is characterized by left ventricular hypertrophy, adverse cardiac remodeling, fibrosis, and atrial fibrillation and may ultimately culminate in heart failure (239). Accumulation of damaged and misfolded proteins due to impairment of the ubiquitin-proteasome system are key processes in HCM pathogenesis. In addition, patients with HCM show higher levels of p53 expression compared with control subjects (240) and have shorter cardiomyocyte telomeres (241). Cardiac telomere attrition is cell type specific (identified only in cardiomyocytes within diseased human hearts) and is associated with increased cardiomyocyte DNA damage (241). Of interest, telomere length was found to be associated with disease severity in the obstructive HCM subtype (242).

HCM with heart failure is the most common cause of early death in Friedreich ataxia, a progressive cardio- and neurodegenerative disease typically diagnosed in midchildhood. Mutations in the Frataxin gene (*FXN*) are considered to be the main cause of Friedreich ataxia (243). FXN deficiency correlates with attenuated ventricular contractility (244). Interestingly, mitochondrial dysfunction induced by FXN deficiency has been associated with cellular senescence and abnormal calcium metabolism (245). Additionally, FXN deficiency promotes endothelial replication stress, sustained DDR (including ATR, CHK1, CHK2, p53, and γH2AX), and S-phase arrest, thus triggering endothelial senescence in pulmonary hypertension (246).

4.3.2. Arrhythmogenic cardiomyopathy.

Arrhythmogenic cardiomyopathy (ACM) encompasses a genetically heterogeneous group of myocardial diseases that are morphologically characterized by apparent patches of apoptotic tissue in the right, and to a lesser extent, left ventricles along with fibro-adipogenic infiltration. Mutations in the TMEM43 transmembrane protein are known to cause ACM (247). Of great interest, cardiomyocyte-restricted heterozygous deletion of the *TMEM43* gene in mice leads to activation of the DDR/TP53 pathways and expression of SASP, which is associated with a late-onset senescence-associated cardiomyopathy characterized by cardiac dilation, systolic dysfunction, myocardial fibrosis, and apoptosis (248). Recently, a new homozygous missense mutation in LEM Domain Nuclear Envelope Protein 2 (LEMD2) leading to juvenile cataract and a severe form of arrhythmic cardiomyopathy with variable onset was reported in people of the Hutterite population (249). Cardiac tissue and fibroblasts from affected patients exhibited abnormally shaped and elongated nuclei as well as disorganized, condensed heterochromatin. Mutant fibroblasts displayed reduced proliferative capacity and cell senescence but no increased apoptosis, suggesting an involvement of mutant LEMD2 in chromatin remodeling processes and premature aging (249).

4.3.3. Diabetic cardiomyopathy.

Diabetes mellitus results in the development of cardiac myopathy, a distinct clinical entity characterized by a decrease in muscle mass, chamber dilation, and impaired ventricular function, in the absence of coronary artery disease (CAD). Pathophysiological factors that contribute to the development and progression of diabetic cardiomyopathy include, among others, impaired cardiac insulin metabolic signaling, mitochondrial dysfunction, increased oxidative stress, inflammation,

endoplasmic reticulum stress, and microvascular dysfunction (250). These pathophysiological abnormalities promote cardiac stiffness, hypertrophy, and fibrosis, resulting in subclinical pathological cardiac remodeling that initially manifests itself as isolated diastolic dysfunction but with time progresses to systolic dysfunction eventually leading to overt heart failure (250). Multiple proinflammatory pathways regulate the development of diabetic cardiomyopathy such as activation of protein kinase C (PKC), MAPK, and NF-κB signaling pathways, microRNA (miRNA) dysregulation, and exosome secretion (251). Upregulation or downregulation of specific miRNAs has been involved in insulin sensitivity, systemic glucose metabolism, cardiac diastolic dysfunction, as well as cardiomyocyte hypertrophy and interstitial fibrosis, in clinical and preclinical diabetic models (251). In accordance, in type 2 diabetic hearts, the exosomal transfer of miR-320 into coronary endothelial cells caused reduced NO production and inhibition of angiogenesis (252). Systemic and cardiac cell inflammation promotes oxidative stress and mitochondrial dysfunction leading, subsequently, to cardiomyocyte contractile dysfunction, metabolic imbalance, hypertrophy, and death (251).

Within this context, the diabetic heart is characterized by premature senescence of cardiac progenitor cells, as documented by telomeric shortening and expression of the senescence-associated proteins p53 and $p16^{INK4A}$. This, in turn, is responsible for the increase of the number of senescent myocytes and therefore premature myocardial aging and heart failure (253). Diabetes mellitus inhibits the regenerative potential of multipotent cardiac stem/progenitor cells (CSCs) through the induction of cellular senescence and SASP independently of aging (254). Interestingly, ablation of senescent CSCs suspends SASP and restores a fully proliferative and differentiation-competent human CSC pool in type 2 diabetes mellitus (T2DM), thus improving cardiac function (254). Epigenetic regulation of cardiac progenitor cell senescence is another mechanism involved in the pathogenesis of diabetic cardiomyopathy. Those epigenetic mechanisms include DNA and histone modifications as well as noncoding RNA-mediated effects (through microRNAs and long-noncoding RNAs) (255, 256). DNA and histone modifications pertain to hypermethylation of CpG islands as well as increased trimethylation of histones H3K9, H3K27, and H4K20 and deacetylation of H3K9 and K27, which convert active chromatin to its inactive form, thus inhibiting the transcription of genes involved in cell growth and proliferation and finally triggering senescence in cardiac progenitor cells (CPCs) in diabetes (255).

The role of p53-dependent pathways in the pathogenesis of diabetic cardiomyopathy is of great interest. In a type 1 diabetes (T1DM) mouse model in which diabetes is induced by streptozotocin (STZ), inhibition of p53 prevented cardiac apoptosis during early-stage diabetes, attenuated diabetes-induced cell senescence, and prevented glycolytic and angiogenetic dysfunction by increasing HIF-1α protein stability and HIF-1α-mediated genomic transcription (257).

Furthermore, in type 2 diabetes, both myocardial and immune cells undergo metabolic remodeling characterized by low metabolic flexibility and impaired adaptive capacity to nutrient and oxygen availability. In this respect, metabolic stress-induced immunosenescence, which is a trait of the prolonged low-grade systemic inflammation characterizing type 2 diabetes, has been recognized as a key contributing factor to cardiac dysfunction in diabetic cardiomyopathy (258, 259).

4.4. *Implication of Cellular Senescence in Cancer Therapy-Induced Cardiotoxicity*

Classical/conventional chemotherapy and radiotherapy along with novel antitumor treatment modalities such as immunotherapy pose a considerable risk of cardiotoxicity (260). The term “cardiotoxicity” here refers to cardiovascular complications that are manifested after the implementation of an anticancer treatment and include a wide spectrum of cardiac pathologies, from asymptomatic systolic dysfunction to clinically overt heart failure, as well as myocarditis, arrhythmias, valvulopathies, pericardial effusions, and arterial or pulmonary hypertension. The molecular mechanisms by which cardiotoxicity is mediated are constantly investigated and involve, among others, mitochondrial dysfunction, oxidative stress, autophagy deregulation, and telomere dysfunction, all of which are also well-established triggers of cellular senescence (261–263). Consequently, cellular senescence has been recently acknowledged as a key player in the development of cardiotoxicity due to cancer treatment. Not only cardiomyocytes but also cardiovascular endothelial cells and fibroblasts are susceptible to the senescence-inducing effect of anticancer agents (262, 264, 265). It has been recently reported that accelerated cardiomyocyte senescence due to mitochondrial DNA damage contributes to doxorubicin-induced impairment of heart function (262). Of note, anticancer therapy-induced senescent cells exert common features with cells triggered to senesce by other stimuli, including persistent hypoproliferation, upregulation of $p16^{INK4A}$, persistent DNA damage, and transcriptional activation of genes encoding many SASP factors, thus contributing to local and systemic inflammation (264). Of clinical relevance, elimination of senescent cells can alleviate cardiac systolic

dysfunction (264–266). From another point of view, molecular signatures/patterns of senescent cardiovascular cells and detection of specific molecules (such as SASP components or lipofuscin, a by-product of the metabolism of senescent cells) in blood samples and other biological fluids hold the potential to serve as novel biomarkers in the field of cardio-oncology (267).

5. SENOLYTICS/SENOTHERAPEUTICS

Transient cellular senescence is beneficial throughout embryonic and normal life, whereas the accumulation of chronic senescent cells in the organism exerts deleterious effects compromising tissue and organ homeostasis and eventually leading to age-related diseases and aging (16). Genetic elimination of senescent cells resulted in delayed aging phenotypes and attenuation of age-associated pathologies (268, 269), prompting the development of reagents that selectively kill senescent cells (senolytics) or neutralize their adverse effects in tissues and organs (senomorphics). In particular, the development of mouse models such as INK-ATTAC or p16-3MR, which enable pharmacogenetic ablation of senescent $p16^{INK4A}$-positive cells, was crucial to determining the impact of senescence in heart integrity and how $p16^{INK4A}$ loss ameliorates those effects in preclinical models (270, 271). Based on those model systems, it was shown that senescence contributes to cardiac aging and cardiomyocyte hypertrophy in naturally aged mice (268) and cardiac dysfunction after therapy (264). Regarding the cardiovascular system, accumulating evidence has highlighted the importance of senescence in the onset, development, and deterioration of CVDs (272, 273). Therefore, senotherapies are emerging as a promising strategy to prevent or cure CVDs (**FIGURE 7**) (274).

Senotherapeutics are chemical compounds primarily derived from other research and clinical purposes such as cancer, idiopathic pulmonary fibrosis, and chronic kidney disease (drug repurposing) or well-characterized natural bioactive compounds and derivatives (35, 275). Although the majority of these compounds/drugs have been successfully evaluated in cellular systems and animal models, administration in humans is now under investigation in clinical trials, with some of these molecules possibly exerting unfavorable outcomes (272). Apart from cytotoxicity reflected by neutropenia and thrombocytopenia, senotherapeutic treatments raise additional concerns given that neutralization of senescent cells may evocate putative oncogenic mutations (276). Therefore, their translation to clinical trial testing should be limited to a controlled context, while the discovery of a suitable drug for the treatment of a specific clinical entity/setting still remains a challenging task. Senotherapeutics are classified as follows (35, 275): *1*) Senolytics are agents that promote cell death by inhibiting cardinal senescence prosurvival pathways. Senescent cells exhibit resistance to apoptosis, a property that extends their life span compared with normal cells (27, 277). Processes such as DNA damage and DDR activation, altered metabolic traits, senescence-associated mitochondrial dysfunction (SAMD), and SASP, commonly evident in senescent cells, are associated with the activation of a variety of prosurvival pathways, termed senescent cell antiapoptotic pathways (SCAPs). These include signaling cascades mediated by $p53/p21^{WAF1/Cip1}$/serpins, BCL-2/Bcl-XL, PI3K/AKT/ceramide, HIF-1α, and the heat shock protein HSP90, rendering senescent cells resistant to their own proinflammatory secretome (278, 279). SCAPs are considered to be the "Achilles' heel" of senescent cells, rendering senescent cells vulnerable to cell death when targeted with senolytics (280). Inactivation of more than one SCAP may lead to elimination of senescent cells

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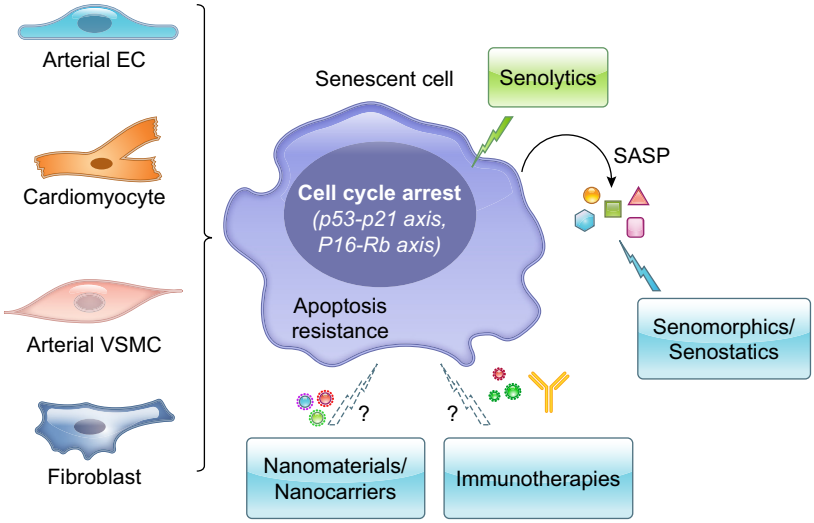

FIGURE 7. Potential therapeutic approaches targeting senescent cells in CVDs. Senescence-associated CVDs are likely to benefit from the development of therapies in the fields of senotherapeutics, nanomedicine, and immunology. Senolytics: dasatinib and quercetin, navitoclax, cardiac glycosides, 17-DMAG; senomorphics/senostatics: rapamycin, metformin, statins, polyphenols, SRT1720. See GLOSSARY for abbreviations.

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depending on the cell type and the senescence phenotype, due to the degree of redundancy that these exert (281). Over the last decades, nanomaterials have emerged as a rapidly growing field that, among others, has greatly influenced the area of biomedicine. Nanoparticles/nanocarriers can efficiently carry and accurately deliver therapeutic agents or biological factors to targeted sites such as a specific cell type, tissue, or organ. In this context, nanoparticles carrying senolytics and specifically targeting senescent cells by promoting SCAP inactivation have recently been developed, providing innovative therapeutic perspectives in translational medicine (282). *2*) Senomorphics or senostatics are compounds or specific antibodies that act as SASP suppressors (on SASP factors or SASP signaling cascades) or modifiers of the senescent phenotype. *3*) Modifiers/enhancers of the immune system are immunotherapeutic and immunomodulatory interventions aiming to increase the immunogenicity of senescent cells or reactivate exhausted immune cell populations.

A body of evidence from cell culture and in vivo studies suggests that senotherapeutics can be a promising and innovative strategy to prevent the onset of CVDs or delay their progression, while clinical trials implementing senotherapeutics are soon expected to contribute to a deeper understanding of the field (283, 284). Pharmacological elimination of senescent cells has been linked with reversal of phenotypic changes associated with aging, via improvement of cardiac dysfunction, stimulation of cardiomyocyte regenerative capacity, and inhibition of heart fibrosis (41, 148).

Among the senotherapeutics, the tyrosine kinase inhibitor dasatinib and the flavonoid quercetin (polyphenol, PI3K inhibitor) were the first drugs tested for CVD treatments (276, 285). Both exert senolytic properties, and their combination has been proven to be accompanied by maximum senolytic effects while they do not affect the viability of proliferating or quiescent cells. Quercetin alone is more effective in eliminating senescent human umbilical vein endothelial cells (HUVECs) than dasatinib (280). Treatment with dasatinib + quercetin (D+Q) has been demonstrated to drastically decrease TAF-positive senescent VSMCs in the aorta media in aged and hypercholesterolemic mice and improve cardiac function (left ventricular ejection fraction and fractional shortening) in aged mice (280). At the same time, D+Q treatment has been found to improve vasomotor function and diminish aortic calcification (280, 285). Moreover, significantly improved vascular endothelial function and vascular smooth muscle sensitivity to nitroprusside were observed after D+Q administration, without any effect on smooth muscle contractile function (280). On the other hand, D+Q treatment did not influence the senescent cell burden in established intimal atherosclerotic plaques or their size in atherosclerotic *ApoE*$^{-/-}$ mice receiving a western diet (285).

Another class of compounds with proven senolytic effects in the context of CVDs includes the BH3 mimetics, small molecules that act as inhibitors of the B cell lymphoma 2 (BCL-2) family proteins (BCL-2, BCL-W, and BCL-XL) (26, 286). ABT-263 (navitoclax) selectively increases apoptosis of senescent but not of proliferating HUVECs (287). Navitoclax has been demonstrated to dramatically prevent the onset of atherogenesis in the aorta of *Ldlr*$^{-/-}$ mice receiving a high-fat diet (165). In addition, ABT-263 treatment of aged mice eliminates senescent cardiomyocytes and diminishes fibrosis and cardiomyocyte hypertrophy (41). Notably, senescent cell removal by ABT-263 restores myocardial remodeling and improves diastolic function and survival in a myocardial infarction mouse model (288).

Cardiac glycosides (CGs) have also been described as senolytic compounds with potentially beneficial effects in atherosclerosis, pulmonary fibrosis, and anticancer treatments (232, 289). They act as inhibitors of the Na^+-K^+-ATPase, a well-known plasma membrane (PM) pump that mediates cellular exit of Na^+ and K^+ influx into cells against concentration gradients, thereby promoting cell membrane depolarization and cytosol acidification (232). Senescent cells are sensitive to CG-induced apoptosis, as they demonstrate a depolarized plasma membrane and a lower pH than nonsenescent cells (232). GCs additionally seem to regulate a variety of Na^+-K^+-ATPase-dependent signaling pathways including SRC, ITPR, and AKT (290). Among the most widely used GCs are digoxin, quabain, and digitoxin (290). Digoxin, through its senolytic activity, has been demonstrated to reduce cancer cell senescence and lung fibrosis (290). Ouabain and digoxin administration has been linked with increased expression of proapoptotic BCL2 family members such as NOXA through activation of the JNK, GSK-3β, and P38 pathways (290). Treatment of mice with ouabain reduced senescent cell burden and improved parameters related to inflammation, metabolism, and physical fitness normally compromised during aging (290). Digitoxin has been found to exert senolytic properties at doses similar to those administrated to patients for the treatment of heart failure and atrial fibrillation (290).The HSP90 inhibitors ganetespib and 17-DMAG have been shown to have senolytic properties in IR-induced senescent HUVECs and improve atherosclerosis in mice (291, 292). Moreover, the glucose analog 2-deoxy-D-glucose (2DG) exhibits senolytic properties, by inhibiting ATP synthesis and thus determining cell cycle arrest and cell death in senescent VSMCs. 2DG seems to additionally impact the progression of atherosclerosis

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by influencing the metabolic activity of senescent cells (293). Notably, β-hydroxybutyrate restrains vascular cell senescence occurring during fasting and caloric restriction, thus rendering this compound putatively beneficial for CVD prevention (294, 295).

Among compounds with senomorphic activity, rapamycin (mTOR inhibitor) and metformin (an antidiabetic drug that functions directly or indirectly on the liver to decrease glycose production) act by preventing CVD development or improving disease progress (296–299). Metformin and rapamycin (sirolimus) interfere with the SASP and alleviate the proinflammatory environment (283). Moreover, in mice rapamycin reduces cell senescence burden and accompanying adverse effects including atherosclerosis, hence extending life span (300, 301). Vascular function is ameliorated in diabetic patients treated with metformin and pioglitazone (302–304). In addition, TA-1887, a SGLT2 inhibitor, can have cardioprotective effects in diabetic patients (305). Statins have also been demonstrated to prevent SASP (306). Polyphenols have antioxidant and anti-inflammatory effects, neutralizing prooxidant and proinflammatory signaling (307). In particular, resveratrol has been proposed not only as a cell senescence suppressor but also as an inhibitor of cardiovascular complications (308). Resveratrol is known to exert its effect though Sirtuin1 (SIRT1) signaling cascades. Interestingly, resveratrol-mediated SIRT1 activation results in inhibition of both arterial wall inflammation and stiffening in primates (308). SIRT1 downregulation is evident in vascular smooth cells from patients affected by abdominal aortic aneurysm, whereas SIRT1 activation is accompanied by low levels of VSMC senescence and vascular inflammation (309–311). Other SIRT1 inducers, such as SRT1720, reduce hypertension and arterial stiffness in mice (312). Similarly, rivaroxaban, an anticoagulant agent, can attenuate VSMC senescence (313) and has been shown to prevent CVDs (314, 315).

Other unconventional treatments that are under investigation include a variety of anti-inflammatory and immune-modulatory approaches (immunotherapy, antibodies, and vaccines) that can inhibit CVD-related inflammatory pathways while displaying limited immunesuppressive side effects (316). Those treatments are mainly focused on atherosclerotic cardiovascular diseases (316). Whether cellular senescence and SASP could be targeted or neutralized by such interventions to prevent the development of or treat CVDs remains to be elucidated in clinical settings. Recent technological advances have enabled the development of a variety of nanomaterials for the treatment of cardiovascular disorders such as ischemic heart failure and atherosclerosis (317). Interestingly, encapsulation of doxorubicin in senotherapeutic nanoparticles reduced the cardiotoxicity of the free drug in mice (318). Similar results were achieved by the incorporation of activatable senolytic prodrugs in nanocarriers (319, 320). Although for the time being clinical studies with nanoparticles/carriers specifically targeting senescence in cells of the cardiovascular system are lacking, the adoption of such approaches for the treatment of CVDs seems not so far away.

6. FUTURE PERSPECTIVES

A robust body of evidence has demonstrated the impact of cellular senescence on heart disease progression and patient outcome, leading to the design of numerous potentially druggable approaches aimed at eradicating senescent cell pools (321). However, several aspects of CVD-related senescence still remain elusive, such as whether senescence may be implicated in stent thrombosis and restenosis following balloon angioplasty. Additionally, although the detrimental effects of cardiovascular senescence have been largely established (272, 321), it remains unclear how individual cell types may lead to different clinical outcomes upon induction of senescence, as well as whether ablation of senescent cells can be pursued in all CVD cases. Along those lines, the clinical and experimental evidence connecting senescence with important clinical entities such as valvular heart disease or some of the nonischemic cardiomyopathies, such as dilated cardiomyopathy, inflammatory cardiomyopathies, and peripartum cardiomyopathy, is still not strong enough or inconclusive. Thus, additional research is required to shed light on the role of cellular senescence in the various clinical settings of CVDs, including those of a congenital nature.

Precise detection and quantification of senescent cells in clinical material is of paramount importance to develop diagnostic as well as therapeutic strategies specifically targeting senescent cell pools. However, for many years the most widely used senescence markers were either nonspecific or characterized by lack of accuracy and significant limitations (182, 322, 323). To that end, the development of novel biomarkers capable of tracking cellular senescence in sensitive and easy-to-perform assays emerged as an imperative and challenging task. In this context, the GL13 (SenTraGor) reagent, which is a lipophilic, biotin-linked Sudan Black B (SBB) analog, was synthesized a few years ago. The GL13-based method allows for specific detection of senescent cells by interacting with lipofuscin, a hallmark of senescent cells, not only in fresh tissues but in any biological sample including clinical material (324). Although it only recently became available, the value of GL13 has been extensively proven, especially for ex vivo senescence

detection, given that it allows for concurrent staining of tissues with other markers involved in senescence phenotypes (29, 324). Based on these facts, lipofuscin identification via GL13 staining has recently been adopted in the guideline multimarker algorithm for accurate detection of senescent cells proposed by the senescence community (16, 29). Our laboratory is currently optimizing the use of a hydrophilic analog of GL13 in biochemical applications such as flow cytometry and cell sorting through FACS, for which GL13 has been suboptimal because of its lipophilic nature. Replacing GL13/SA-β-Gal with a hydrophilic analog in our senescence detection algorithm is of paramount importance in the field, as it will unlock the possibility of sorting senescent cells or even performing senescent cell tracking in vivo. Implementation of the use of such approaches in basic research and clinical investigations is expected to add significantly to our understanding of the impact of senescence in several disease settings, including CVDs. Indeed, precisely following the algorithm, we recently identified that SARS-CoV-2-infected alveolar cells undergo senescence in COVID-19 patients (325) (see also below). Along those lines, it will be possible to establish modern hybrid techniques for senescence evaluation in CVDs, by combining classical imaging methods with the high level of sensitivity conferred by novel senescence biomarkers. Thus, the potential of adopting a robust, senescence-oriented approach in CVD diagnosis and treatment is increasingly becoming visible.

The available body of evidence on the impact of senescence on heart tissue has suggested diverse outcomes with regard to disease, which is mainly attributed to the different cell types affected in the various CVD types (see sect. 4). As the effects of senescence on cardiac homeostasis seem to be cell type specific, it is currently challenging to determine whether depletion of senescent cells in CVDs may yield beneficial or detrimental phenotypes. To that end, machine learning approaches would be useful in identifying a potentially differing expression pattern of predicted senescence-related regulatory genes among the various cardiac cell populations, in conditions leading to heart failure versus normal heart tissue. The SeneQuest database, which constitutes an information hub for gene-to-senescence associations derived from the literature (16), already contains machine learning predictions for CVDs (https://github.com/VGlabUOA/prediction_senescence_CVD). The rapid accumulation of "-omics" data in the field of CVDs and senescence is expected to further increase the accuracy of predictions carried out via artificial intelligence-based approaches, thus culminating in the development of better-targeted therapeutic strategies.

Previous research in the cancer field has shown that, under certain conditions, OIS cells may exit senescence and reenter the cell cycle in a process collectively referred to as escape from OIS (30, 31, 326–328). It was recently demonstrated that as a result of genomic instability and chromatin refolding changes in OIS cells, activation of the circadian gene *BHLHE40* was sufficient to drive the escape from OIS, leading to the manifestation of aggressive oncogenic behavior (36, 329). Given that genomic instability and epigenetic alterations are a frequent underlying cause of CVDs (107, 119, 120), it would be important to investigate whether escape from senescence is a possibility in cardiac disease and to delineate the molecular mechanisms through which it may occur. As different senescence pathways are activated in the various CVD settings and cardiac cell types, the outcome of such research efforts would be extremely valuable in dissecting heart disease at the genetic and epigenetic levels.

Although a correlation between viral infections and CVDs has already been demonstrated (330), evidence regarding virus-induced senescence (VIS) is still sparse in human diseases (100, 331–333). This matter is predicted to be of huge interest and importance in the next decades given that the emergence of new infectious agents and the outbreak of new pandemics in the forthcoming years seems currently a very realistic scenario. Urged by the SARS-CoV-2 pandemic (334), we demonstrated in vivo, following the abovementioned algorithm, that in severe cases of COVID-19 infection of lung epithelial cells with SARS-CoV-2 induces senescence, a finding also confirmed by others (325, 335). Importantly, our observations support that SARS-CoV-2-induced senescence is accompanied by secretion of proinflammatory cytokines/inflammation and viral mutagenesis (325, 335). Apart from the intriguing findings, this work highlights the significance of applying the algorithmic workflow in clinical samples to accurately identify which cells are truly senescent and how they are actually involved in the pathophysiology of human diseases. As the potentially harmful immediate and long-term effects of SARS-CoV-2 infections or infections mediated by other viral strains on cardiac function are continuously being investigated, elucidating the exact mechanisms through which viral infections elicit senescence would be insightful (**FIGURE 8**). F8
Additionally, since the establishment of cellular senescence may be proportionally linked to the severity of SARS-CoV-2-mediated infection (325, 336), the development of therapeutic protocols aimed at eliminating senescent cells may fulfill a topical medical need.

There is rapidly growing evidence on novel putative therapies against CVDs, which holds great promise for clinical trials involving senotherapeutics (321). Provided that their safety and efficacy are ensured and the

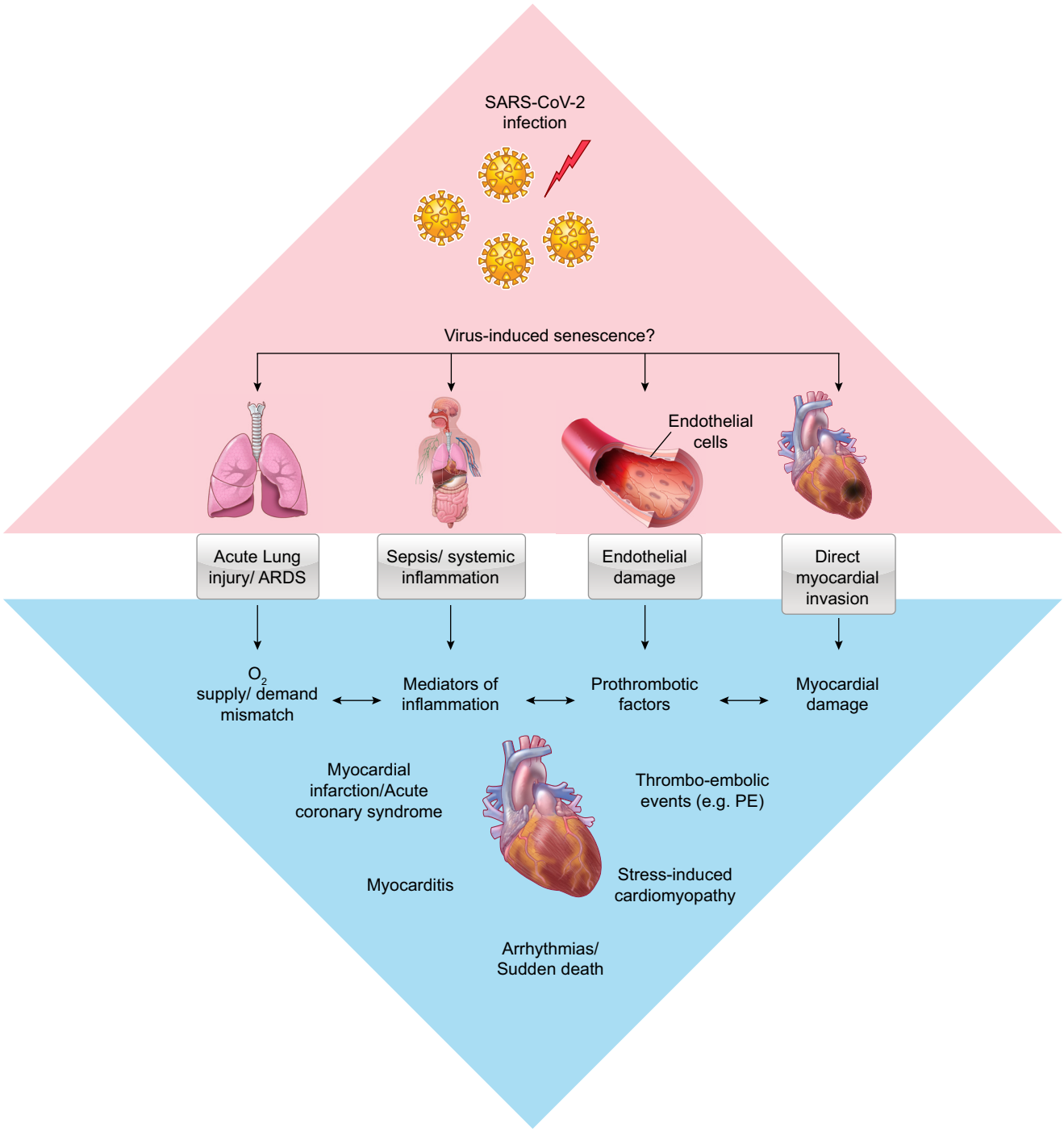

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FIGURE 8. SARS-CoV-2 infection induces organ damage through virus-induced senescence. A potential link of SARS-CoV-2-mediated senescence with cardiovascular diseases (CVDs) warrants investigation. ARDS, acute respiratory distress syndrome; PE, pulmonary embolism.

appropriate administration patterns are determined for human use, senotherapeutics may prove to become valuable therapeutic interventions, capable of completely eliminating senescence and senescence-associated CVD pathologies and complications at their onset. Apart from chemical compounds that are currently being investigated as potential senotherapeutic agents, the inclusion of nontoxic herb- or plant-based natural compounds in the toolbox against senescence-associated CVDs may additionally be of considerable value. On several occasions, natural compounds were found to exert significant neuroprotective or anticancer effects in clinical trials and in vitro studies (337, 338); therefore potential antisenescence effects in CVDs deserve to be thoroughly explored. It should be stressed, however, that senescence has also been shown to produce beneficial effects, for instance by limiting cardiac fibrosis after infarction (339). Use of senolytic

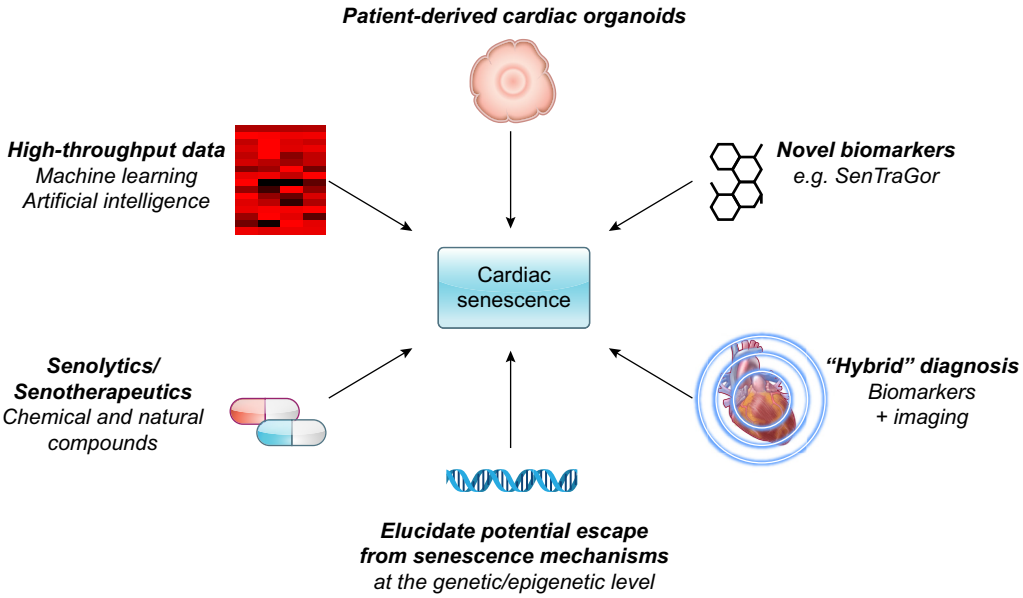

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FIGURE 9. Future perspectives on senescence-related cardiovascular diseases (CVDs). The advent of new biomarkers, model systems, technological tools, and therapeutic agents is expected to contribute significantly toward a better understanding and potential treatment of cardiac senescence. Vectors were obtained from www.vecteezy.com.

agents in such cases might result in unwanted effects, and, additionally, it is tempting to speculate that the elimination of cardiac or vascular cells in the context of aging or disease could potentially compromise the integrity of the tissue. The latter has been recently shown in senescent liver sinusoidal cells (340).

One of the challenges of current research in the CVD field is the lack of appropriate ex vivo systems capable of faithfully recapitulating the in vivo tissue organization and complex interactions between the different cardiac cell types. The most successful attempts to mimic the in vivo situation in the heart have relied on induced pluripotent stem cell (iPSC) technology, where somatic cells are forced to dedifferentiate into an embryonic-like state before being guided to differentiate again into a desirable cell type (341–343). However, this methodology is not free of disadvantages such as genomic instability and maintenance of epigenetic memory, raising several biosafety concerns (344). In the past decade, a three-dimensional (3-D) in vitro culture system for several epithelial tissues such as intestine, lung, and skin (345–348) has been established by utilizing the self-renewal properties of adult stem cells residing in those tissues. Subjecting patient stem cells to defined culture conditions is sufficient to generate 3-D minitissues called organoids. Primary tissue-derived organoids are genetically stable, and their generation does not rely on iPSCs, thereby rendering this system unique in resembling the in vivo situation better than any other cell culture to date (344). Given that, to our knowledge, patient-derived cardiac organoids have yet to be established, the study of CVDs would benefit highly from expanding the existing organoid technology to additionally encompass heart tissue, as was recently achieved for other types of nonepithelial tissue (349). Since organoid systems are being successfully used as drug treatment platforms and can be genetically manipulated to dissect mechanisms of disease (344), the establishment of patient-derived cardiac organoids would vastly contribute to the development of personalized treatment approaches in the battle against senescence-driven CVDs. The advent of new tools for the analysis of high-throughput data based on artificial intelligence and machine learning approaches is expected to strengthen personalized medicine efforts (350, 351).

In conclusion, there is still much unanswered with regard to the impact of senescence on cardiac physiology and disease, and a lot more clinical and basic research is required to determine the clinical environment in which senotherapeutics may provide effective solutions (**FIGURE 9**). The implementation of novel biomarkers enabling detection of cellular senescence with increased sensitivity and precision, as well as the development of comprehensive ex vivo systems to model cardiac disease in depth, will undoubtedly contribute to further elucidation of the molecular mechanisms of senescence in the CVD context and design of targeted treatment approaches at the patient level.

F9

GLOSSARY

2DG	2-Deoxy-D-glucose
ACE	Angiotensin-converting enzyme

ACM	Arrhythmogenic cardiomyopathy
ADAM	A disintegrin and metalloproteinase
AF	Atrial fibrillation
AKT	AKT serine/threonine kinase
ALDH2	Aldehyde dehydrogenase 2
ANG II	Angiotensin II
ApoE	Apolipoprotein E
ARF	Alternative reading frame
AT_1R	Angiotensin II type 1 receptor
ATM	Ataxia-Telangiectasia mutated
ATP	Adenosine triphosphate
ATR	Ataxia Telangiectasia and Rad3-related protein
BCL-2	B cell lymphoma 2
BRG1	Brahma-related gene 1
C/EPBβ	CCAAT/enhancer-binding protein β
CAD	Coronary artery disease
CCF	Cytoplasmic chromatin fragment
CCL5	C-C Motif Chemokine Ligand 5
CCN1	Cellular Communication Network Factor 1
CDK	Cyclin-dependent kinase
CG	Cardiac glycoside
cGAS	cGMP-AMP synthase
CMF	Cardiac myofibroblast
CPC	Cardiac progenitor cell
CryABR120G	Heat shock mutant gene CryABR120G
CSC	Cardiac stem cell
CVD	Cardiovascular disease
CXCL1	C-X-C Motif Chemokine Ligand 1
DDR	DNA damage response
DPP4	Dipeptidyl-peptidase 4
DSB	Double-strand break
ECM	Extracellular matrix
EDN3	Endothelin 3
eNOS	Endothelial nitric oxide synthase
ERCC1	Excision repair cross complementation group 1
ERK	Extracellular signal-regulated kinase
FACS	Fluorescence-activated cell sorting
FoxO	Forkhead box type O
FXN	Frataxin
GATA4	GATA Binding Protein 4
GL13	SenTraGor
GR	Glutathione reductase
GSK-3β	Glycogen synthase kinase 3β
GSSG	Glutathione disulfide
H3K9me3	Trimethylated histone H3 Lys9
HCM	Hypertrophic cardiomyopathy
HDAC	Histone deacetylase
HGPS	Hutchinson–Gilford progeria syndrome
HIF-1	Hypoxia-inducible transcription factor 1
HIF-1α	Hypoxia-inducible factor 1α
HMGA	High-mobility group protein A
HP1	Heterochromatin protein 1
HSC	Hematopoietic stem cell
HSR	Heat shock response
HUVEC	Human umbilical vein endothelial cell
ICAM	Intercellular adhesion molecule
IFITM1	Interferon Induced Transmembrane Protein 1
IGFBP5	Insulin-Like Growth Factor Binding Protein 5
IGF-1	Insulin-like growth factor-1
IL-1RA	IL-1 receptor antagonist
IL-6	Interleukin 6
ING-1	Inhibitor Of Growth Family Member 1
iPSC	Induced pluripotent stem cell
ITPR	Inositol 1,4,5-trisphosphate receptor type 1
JNK	c-Jun NH_2-terminal kinase
LEMD2	Lamina-Associated Polypeptide-Emerin-MAN1 Domain Nuclear Envelope Protein 2
LTB4	Leukotriene B4
LTL	Leukocyte telomere length
MERTK	MER Proto-Oncogene, Tyrosine Kinase
MET	Mitochondrial electron transport
MGI	Mouse Genome Informatics
MiDAS	Mitochondrial dysfunction-associated senescence
miRNA	MicroRNA
MMP	Matrix metalloproteinase
mtDNA	Mitochondrial DNA
mTOR	Mechanistic target of rapamycin
mTORC1	mTOR complex 1
mTORC2	mTOR complex 2
NADPH	Nicotinamide adenine dinucleotide phosphate
NF-κB	Nuclear factor-κB
NO	Nitrogen monoxide
NOX	NADPH oxidase
OIS	Oncogene-induced senescence
p38MAPK	p38 mitogen-activated protein kinase
PAI-1	Plasminogen activator inhibitor-1
PAK	p21-activated kinase
PAO	Preamyloid oligomer
PAR-1	Protease-associated receptor-1
PBMC	Peripheral blood mononuclear cell
PI3K	Phosphatidylinositol-3-OH kinase
PITX2	Paired-like homeodomain transcription factor 2
PKC	Protein kinase C
PLM	Promyelocytic leukemia
PM	Plasma membrane
PoMiCS	Postmitotic cell senescence
RAAS	Renin-angiotensin-aldosterone system
ROS	Reactive oxygen species
RvD1	Resolvin D1
SA-β-gal	Senescence-dependent β-galactosidase
SADS	Senescence-associated distension of satellites
SAMD	Senescence-associated mitochondrial dysfunction
SASP	Senescence-associated secretory phenotype
SBB	Sudan Black B
SCAP	Senescent cell antiapoptotic pathway
scATAC-seq	Single-cell ATAC-seq
scRNA-seq	Single-cell RNA-seq
SERCA2	Sarco(endo)plasmic reticulum Ca^{2+}-ATPase 2
SGLT2	Sodium glucose cotransporter 2
SIRT1	Sirtuin1

SMAD	Mothers against decapentaplegic homolog proteins
SOD2	Superoxide dismutase 2
SRC	SRC Proto-Oncogene, Non-Receptor Tyrosine Kinase
STING	Stimulator of interferon genes
STZ	Streptozotocin
SWI/SNF	SWItch/Sucrose Non-Fermentable
T1DM	Type 1 diabetes
TAF	Telomere-associated DNA damage focus
TAM	Tyro3, Axl, and MerTk receptor
TGF	Transforming growth factor
TIF	Telomere dysfunction-induced focus
TNF	Tumor necrosis factor
UMAP	Uniform manifold approximation and projection
UPR	Unfolded protein response
VCAM	Vascular cell adhesion molecule
VEGF	Vascular endothelial growth factor
VIS	Virus-induced senescence
VSMC	Vascular smooth muscle cell
XPD	Xeroderma pigmentosum group D

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GRANTS

AQ: 12 We acknowledge support by the National Public Investment Program of the Ministry of Development and Investment/ General Secretariat for Research and Technology, in the framework of the Flagship Initiative to address SARS-CoV-2 (2020ΣΕ01300001); the European Regional Development Fund of the European Union and Greek national funds through the Operational Program Competitiveness, Entrepreneurship and Innovation, under the call RESEARCH–CREATE–INNOVATE (project code: T2EDK-02939); H. Pappas donation; Hellenic Foundation for Research and Innovation (HFRI) Grant Nos. 775 and 3782; Welfare Foundation for Social & Cultural Sciences, Athens, Greece (KIKPE); and NKUA SARG Grant 70/3/8916. P.V.S.V. has been awarded a Hellenic Cardiological Society Scholarship for Research in the field of cellular senescence. A.P. is cofunded by the Foundation for Education and European Culture (IPEP).

DISCLOSURES

AQ: 13 No conflicts of interest, financial or otherwise, are declared by the authors.

AUTHOR CONTRIBUTIONS

AQ: 14 K.E., M.D., and V.G.G. conceived and designed research; O.H. performed experiments; O.H., R.P., and V.G.G. analyzed data; P.V.S.V. and A.P. interpreted results of experiments; K.E., A.P., and O.H. prepared figures; K.E., P.V.S.V., A.P., R.P., and M.D. drafted manuscript; A.P., R.P., M.D., and V.G.G. edited and revised manuscript; K.E., A.P., O.H., R.P., M.D., and V.G.G. approved final version of manuscript.

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