

University of Groningen

Cellular senescence and cardiovascular diseases

Evangelou, Konstantinos; Vasileiou, Panagiotis V.S.; Papaspyropoulos, Angelos; Hazapis, Orsalia; Petty, Russell; Demaria, Marco; Gorgoulis, Vassilis G.

Published in:
Physiological reviews

DOI:
[10.1152/physrev.00007.2022](https://doi.org/10.1152/physrev.00007.2022)

IMPORTANT NOTE: You are advised to consult the publisher's version (publisher's PDF) if you wish to cite from it. Please check the document version below.

Document Version
Publisher's PDF, also known as Version of record

Publication date:
2023

[Link to publication in University of Groningen/UMCG research database](#)

Citation for published version (APA):

Evangelou, K., Vasileiou, P. V. S., Papaspyropoulos, A., Hazapis, O., Petty, R., Demaria, M., & Gorgoulis, V. G. (2023). Cellular senescence and cardiovascular diseases: Moving to the "heart" of the problem. *Physiological reviews*, 103(1), 609-647. Advance online publication. <https://doi.org/10.1152/physrev.00007.2022>

Copyright

Other than for strictly personal use, it is not permitted to download or to forward/distribute the text or part of it without the consent of the author(s) and/or copyright holder(s), unless the work is under an open content license (like Creative Commons).

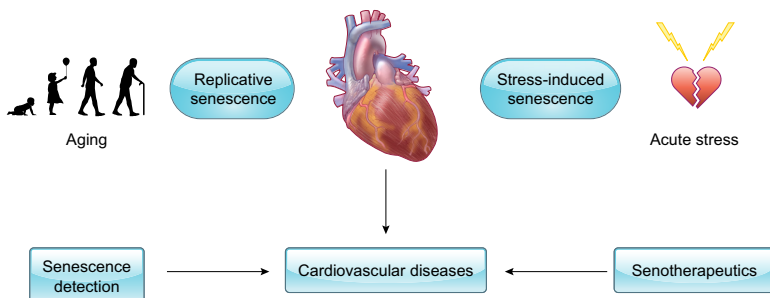
The publication may also be distributed here under the terms of Article 25fa of the Dutch Copyright Act, indicated by the "Taverne" license. More information can be found on the University of Groningen website: <https://www.rug.nl/library/open-access/self-archiving-pure/taverne-amendment>.

Take-down policy

If you believe that this document breaches copyright please contact us providing details, and we will remove access to the work immediately and investigate your claim.

Downloaded from the University of Groningen/UMCG research database (Pure): <http://www.rug.nl/research/portal>. For technical reasons the number of authors shown on this cover page is limited to 10 maximum.

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



AUTHORS

Konstantinos Evangelou,
Panagiotis V. S. Vasileiou,
Angelos Papaspyropoulos, Orsalia Hazapis,
Russell Petty, Marco Demaria,
Vassilis G. Gorgoulis

CORRESPONDENCE

vgorg@med.uoa.gr

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.

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

Konstantinos Evangelou,^{1*} Panagiotis V. S. Vasileiou,^{1,2*} Angelos Papaspyropoulos,^{1*} Orsalia Hazapis,¹ Russell Petty,³ Marco Demaria,⁴ and Vassilis G. Gorgoulis^{1,3,5,6,7,8}

¹Molecular Carcinogenesis Group, Department of Histology and Embryology, Medical School, National and Kapodistrian University of Athens, Athens, Greece; ²Intensive Care Unit, “Sotiria” Thoracic Diseases General Hospital, Athens, Greece; ³Ninewells Hospital and Medical School, University of Dundee, Dundee, United Kingdom; ⁴European Research Institute for the Biology of Ageing (ERIBA), University Medical Center Groningen, Groningen, The Netherlands; ⁵Faculty Institute for Cancer Sciences, Manchester Academic Health Sciences Centre, University of Manchester, Manchester, United Kingdom; ⁶Biomedical Research Foundation, Academy of Athens, Athens, Greece; ⁷Center for New Biotechnologies and Precision Medicine, Medical School, National and Kapodistrian University of Athens, Athens, Greece; and ⁸Faculty of Health and Medical Sciences, University of Surrey, Guildford, United Kingdom

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.

cardiovascular diseases; cellular senescence; senotherapeutics; stress

1. INTRODUCTION	609
2. FUNDAMENTAL ASPECTS OF CELLULAR...	611
3. HALLMARKS OF CARDIOVASCULAR...	615
4. CLINICAL AND EXPERIMENTAL EVIDENCE...	618
5. SENOLYTICS/SENOTHERAPEUTICS	627
6. FUTURE PERSPECTIVES	629

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

*K. Evangelou, P. V. S. Vasileiou, and A. Papaspyropoulos contributed equally to this work.

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.

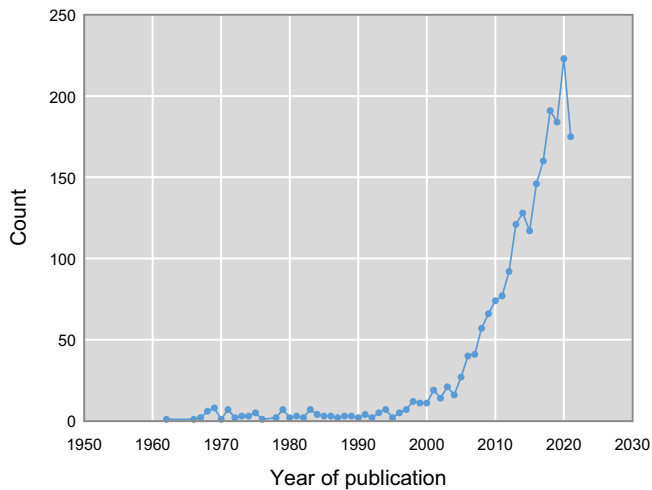


FIGURE 1. Investigation of the involvement of senescence in cardiovascular diseases (CVDs) is a rapidly growing field. Results by year for the search query “cellular senescence AND cardiovascular diseases” in the PubMed platform.

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 multi-marker 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

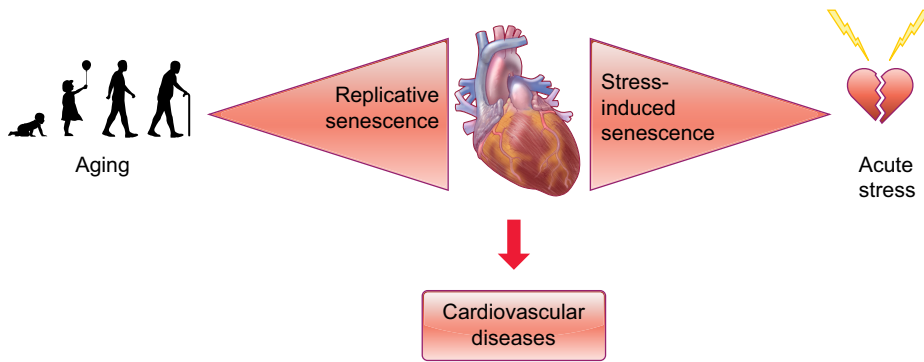


FIGURE 2. Aging vs. senescence. Senescence is triggered acutely by a variety of non-telomere-dependent insults, on a premature basis, earlier than the exhaustion of the cellular replicative potential. In contrast, aging refers to the outcome of the accumulation of changes within the cell due to random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

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, presenescent 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-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- κ B, C/EPB β , 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, dihomoprostaglandins, 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- β 2, 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

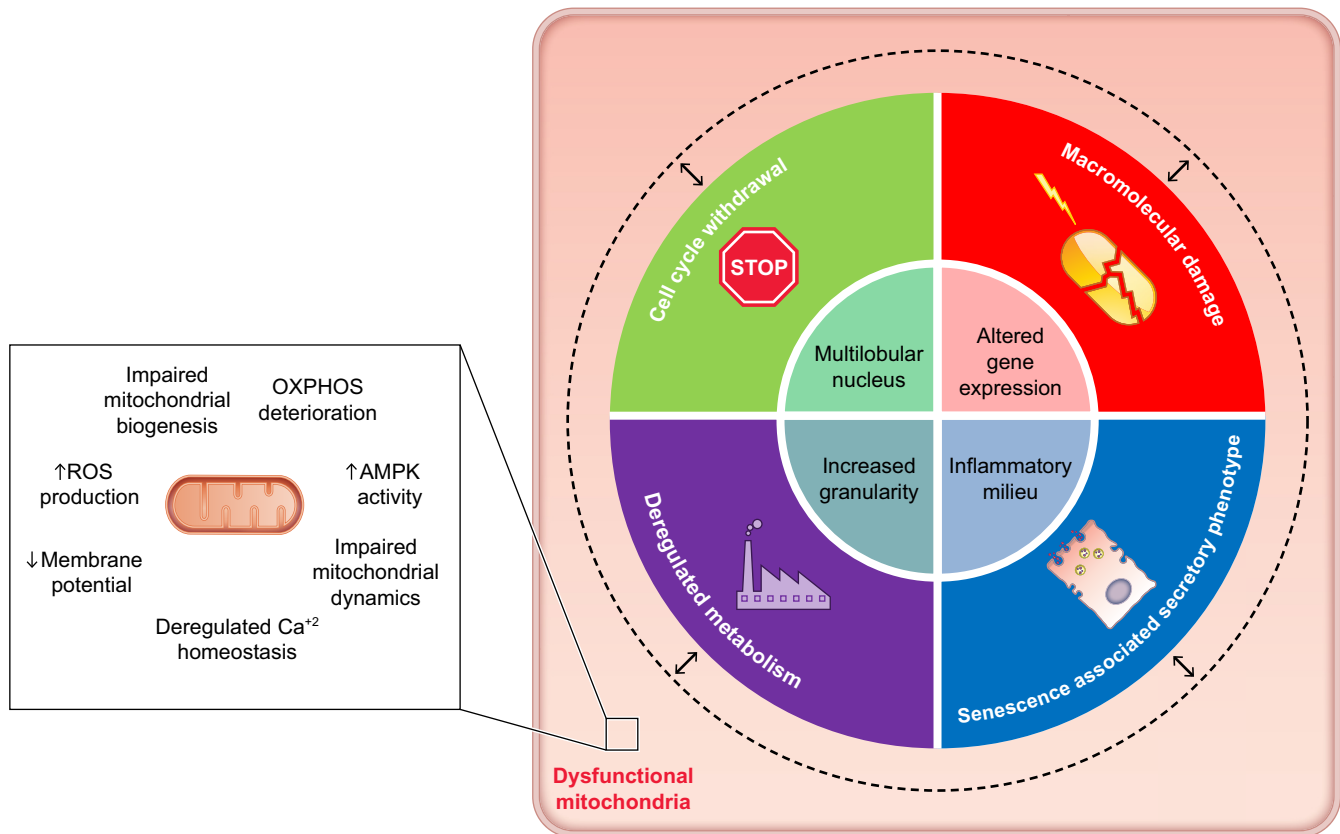


FIGURE 3. Altered mitochondrial activity as a result of deregulated metabolism in senescent cells. Deregulated metabolism is one of the hallmarks of cellular senescence. Disruption of mitochondrial homeostatic mechanisms is a key feature of metabolic deregulation in senescent cells. It is manifested 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 aggregates (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 O₂ (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 p38 α depletion or pharmacological inhibition halts cardiomyogenesis, thereby providing evidence of the important role of p38 α activation in cardiac differentiation (132). A role for p38 α 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 Reg3 γ 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- β 1, a known SASP factor (62, 143). Specifically, TGF- β 1 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- β 1 signaling downregulates FoxO3a expression in cardiac fibroblasts and that TGF- β 1-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- β 1-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- β 1-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 O₂ 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,

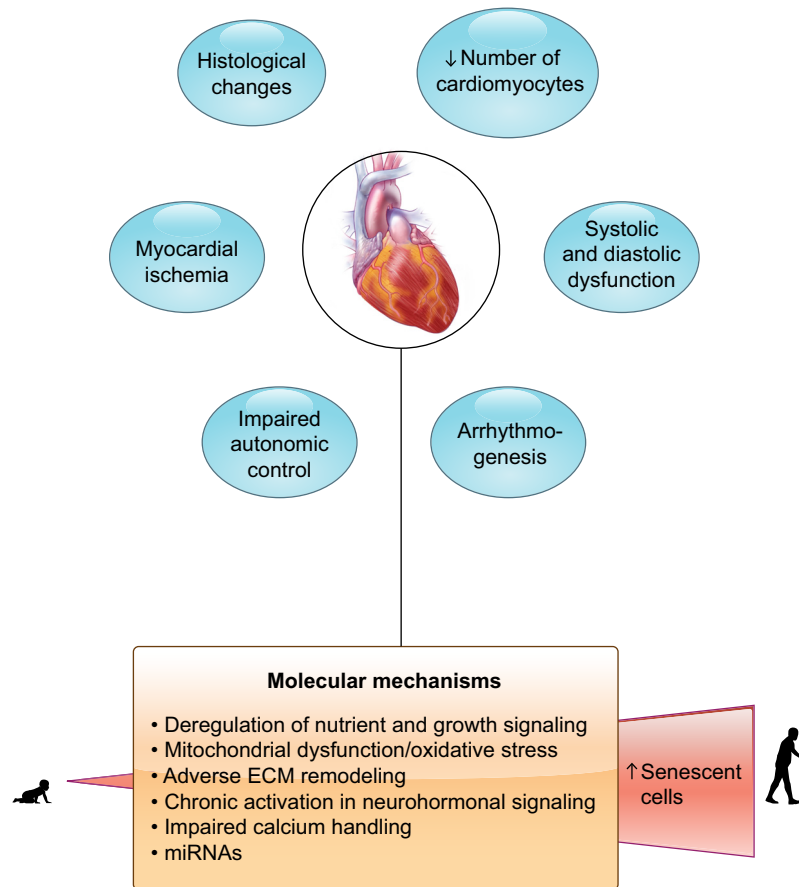


FIGURE 4. Functional and structural changes characterizing the aging heart and molecular pathways implicated. Histological modifications that characterize aged cardiac cells include fibrosis, hypertrophy, calcification, thickening of the tunica media in vessels, amyloid deposits, expansion of the extracellular 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

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**.

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
SERPINE2	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

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- κ B 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 atheroprogession.

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**.

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

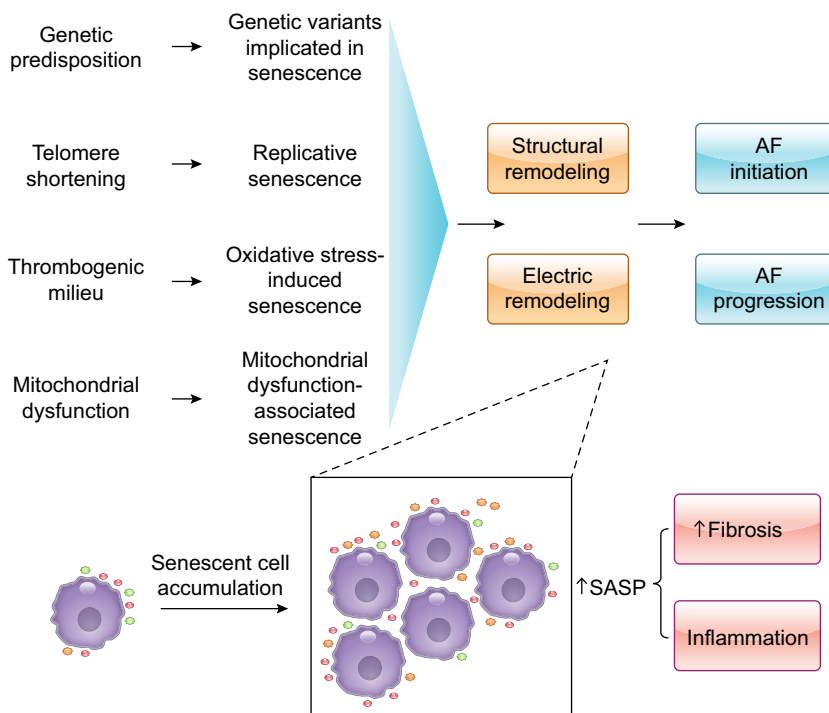


FIGURE 6. Senescence-inducing mechanisms leading to AF initiation and progression. Schematic of main mechanisms leading to cellular senescence, accompanied by structural and electrical remodeling of the heart, which result in AF initiation and progression. Genetic variants predisposing to senescence 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.

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*, *lfitm1*, 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 $\text{Na}_v1.5$, 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 γ H2AX), 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- κ B 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 angiogenic dysfunction by increasing HIF-1 α protein stability and HIF-1 α -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 evoke 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 (SAMMD), 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-1 α , 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

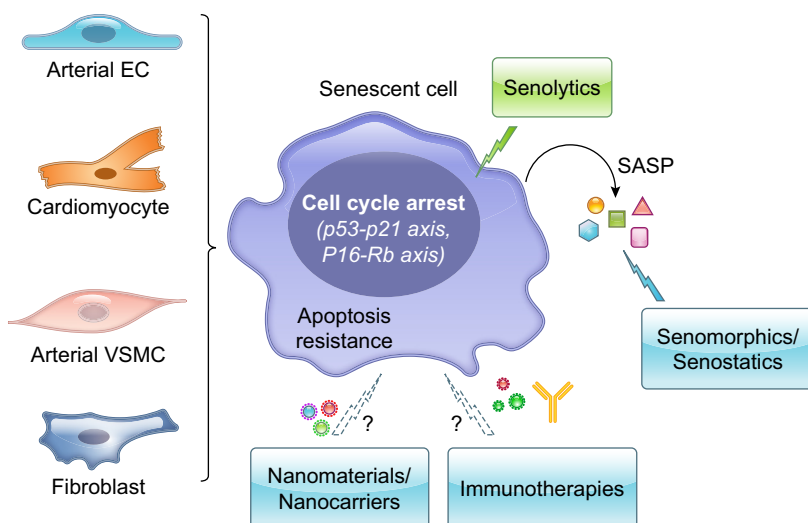


FIGURE 7. Potential therapeutic approaches targeting senescent cells in CVDs. Senescence-associated CVDs are likely to benefit from the development of therapies in the fields of senotherapeutics, nanomedicine, and immunology. Senolytics: dasatinib and quercetin, navitoclax, cardiac glycosides, 17-DMAG;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

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 anti-cancer 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, ouabain, 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 administered 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 irradiation-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 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 glucose 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 through 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 immunosuppressive 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). 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

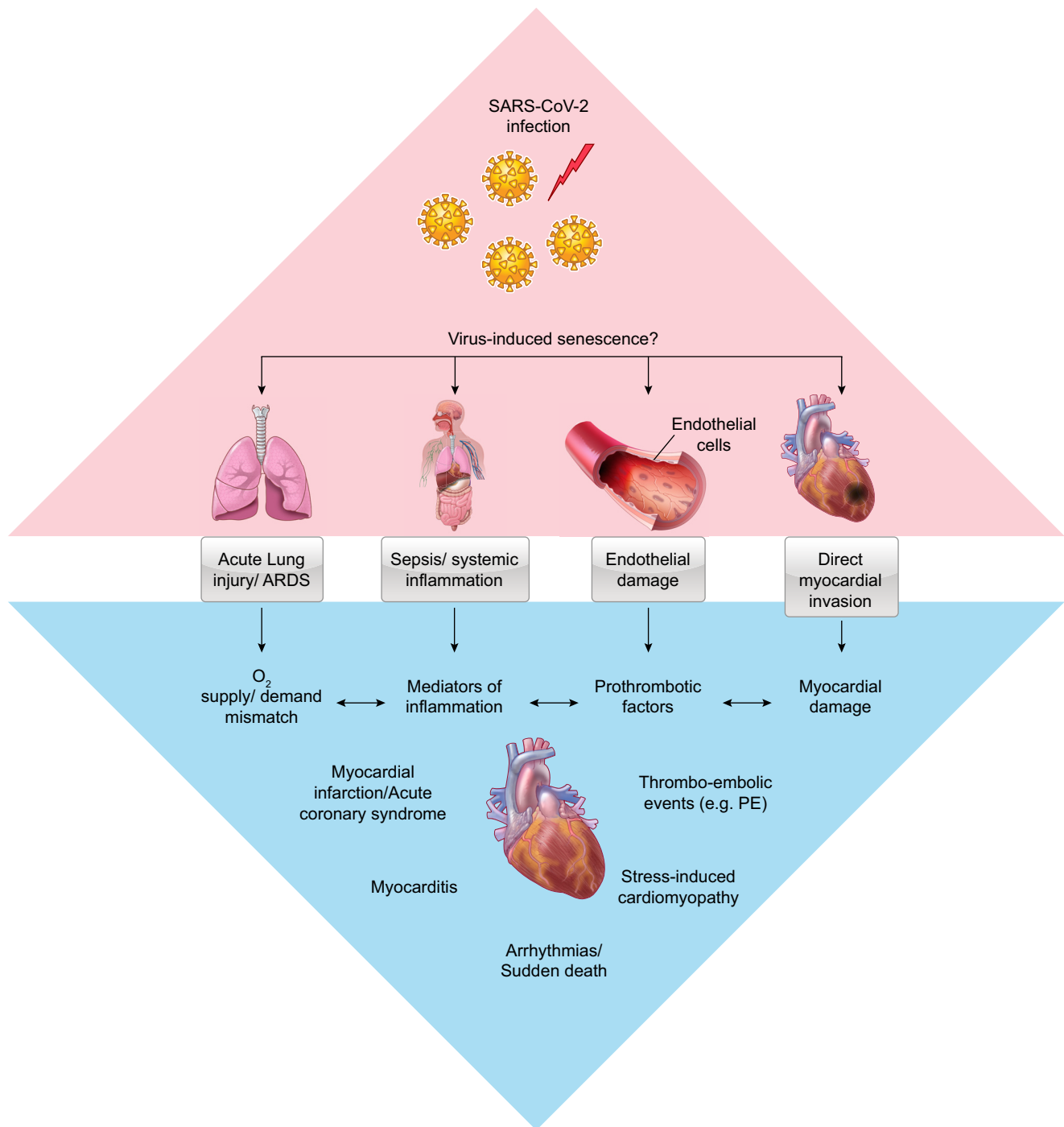


FIGURE 8. SARS-CoV-2 infection induces organ damage through virus-induced senescence. A potential link of SARS-CoV-2-mediated senescence with cardiovascular diseases (CVDs) warrants investigation. ARDS, acute respiratory distress syndrome; PE, pulmonary embolism.

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 antisenesence 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

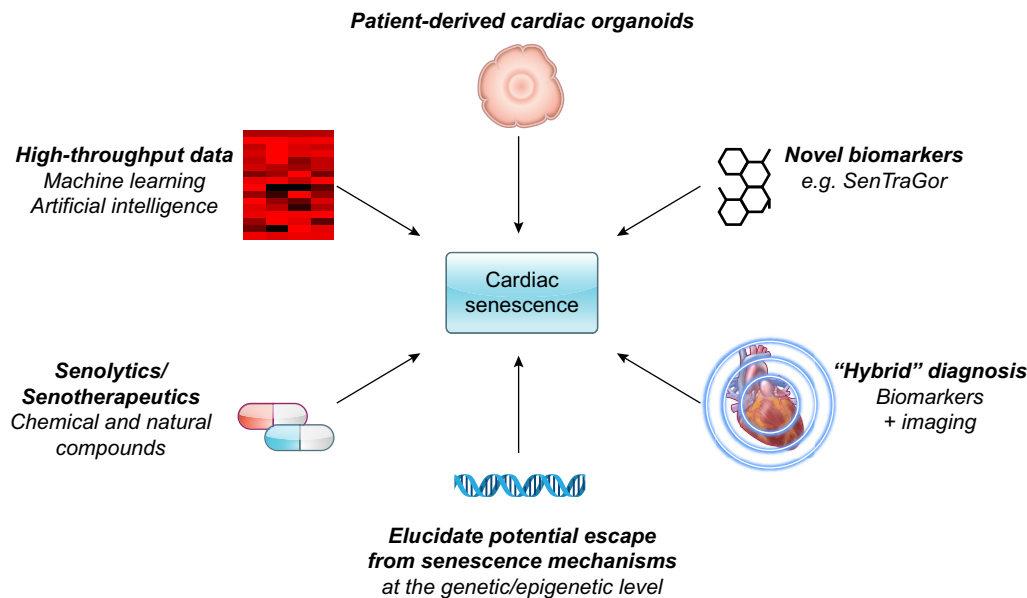


FIGURE 9. Future perspectives on senescence-related cardiovascular diseases (CVDs). The advent of new biomarkers, model systems, technological tools, and therapeutic agents is expected to contribute significantly toward a better understanding and potential treatment of cardiac senescence. Vectors were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

GLOSSARY

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

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

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

CORRESPONDENCE

V. G. Gorgoulis (vgorg@med.uoa.gr).

GRANTS

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

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

AUTHOR CONTRIBUTIONS

K.E., M.D., and V.G.G. conceived the project. O.H., R.P., and V.G.G. analyzed data; P.V.S.V. and A.P. interpreted data; 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.

REFERENCES

- Martin-Puig S, Wang Z, Chien KR. Lives of a heart cell: tracing the origins of cardiac progenitors. *Cell Stem Cell* 2: 320–331, 2008. doi:10.1016/j.stem.2008.03.010.
- Rog-Zielinska EA, Norris RA, Kohl P, Markwald R. The living scar—cardiac fibroblasts and the injured heart. *Trends Mol Med* 22: 99–114, 2016. doi:10.1016/j.molmed.2015.12.006.
- Banerjee I, Fuseler JW, Price RL, Borg TK, Baudino TA. Determination of cell types and numbers during cardiac development in the neonatal and adult rat and mouse. *Am J Physiol Heart Circ Physiol* 293: H1883–H1891, 2007. doi:10.1152/ajpheart.00514.2007.
- Bergmann O, Zdunek S, Felker A, Salehpour M, Alkass K, Bernard S, Sjoström SL, Szewczykowska M, Jackowska T, Dos Remedios C, Malm T, Andrä M, Jashari R, Nyengaard JR, Possnert G, Jovinge S, Druid H, Frisén J. Dynamics of cell generation and turnover in the human heart. *Cell* 161: 1566–1575, 2015. doi:10.1016/j.cell.2015.05.026.
- Pinto AR, Illykh A, Ivey MJ, Kuwabara JT, D'Antoni ML, Debuque R, Chandran A, Wang L, Arora K, Rosenthal NA, Tallquist MD. Revisiting cardiac cellular composition. *Circ Res* 118: 400–409, 2016. doi:10.1161/CIRCRESAHA.115.307778.
- Taegtmeyer H, Lubrano G. Rethinking cardiac metabolism: metabolic cycles to refuel and rebuild the failing heart. *F1000Prime Rep* 6: 90, 2014. doi:10.12703/P6-90.
- Mollova M, Bersell K, Walsh S, Savva J, Das LT, Park SY, Silberstein LE, Dos Remedios CG, Graham D, Colan S, Kühn B. Cardiomyocyte proliferation contributes to heart growth in young humans. *Proc Natl Acad Sci USA* 110: 1446–1451, 2013. doi:10.1073/pnas.1214608110.
- Frangogiannis NG. Matricellular proteins in cardiac adaptation and disease. *Physiol Rev* 92: 635–688, 2012. doi:10.1152/physrev.00008.2011.
- Li AH, Liu PP, Villarreal FJ, Garcia RA. Dynamic changes in myocardial matrix and relevance to disease: translational perspectives. *Circ Res* 114: 916–927, 2014. doi:10.1161/CIRCRESAHA.114.302819.
- Brutsaert DL. Cardiac endothelial-myocardial signaling: its role in cardiac growth, contractile performance, and rhythmicity. *Physiol Rev* 83: 59–115, 2003. doi:10.1152/physrev.00017.2002.
- Hsieh PC, Davis ME, Lisowski LK, Lee RT. Endothelial-cardiomyocyte interactions in cardiac development and repair. *Annu Rev Physiol* 68: 51–66, 2006. doi:10.1146/annurev.physiol.68.040104.124629.
- Bajpai G, Schneider C, Wong N, Bredemeyer A, Hulsmans M, Nahrendorf M, Epelman S, Kreisel D, Liu Y, Itoh A, Shankar TS, Selzman CH, Drakos SG, Lavine KJ. The human heart contains distinct macrophage subsets with divergent origins and functions. *Nat Med* 24: 1234–1245, 2018. doi:10.1038/s41591-018-0059-x.
- Meilhac SM, Buckingham ME. The deployment of cell lineages that form the mammalian heart. *Nat Rev Cardiol* 15: 705–724, 2018. doi:10.1038/s41569-018-0086-9.

14. Gorgoulis VG, Pefani DE, Pateras IS, Trougakos IP. Integrating the DNA damage and protein stress responses during cancer development and treatment. **J Pathol** 246: 12–40, 2018. doi:[10.1002/path.5097](https://doi.org/10.1002/path.5097).
15. Chien KR, Karsenty G. Longevity and lineages: toward the integrative biology of degenerative diseases in heart, muscle, and bone. **Cell** 120: 533–544, 2005. doi:[10.1016/j.cell.2005.02.006](https://doi.org/10.1016/j.cell.2005.02.006).
16. Gorgoulis V, Adams PD, Alimonti A, Bennett DC, Bischof O, Bishop C, Campisi J, Collado M, Evangelou K, Ferbeyre G, Gil J, Hara E, Krizhanovsky V, Jurk D, Maier AB, Narita M, Niedernhofer L, Passos JF, Robbins PD, Schmitt CA, Sedivy J, Vougas K, von Zglinicki T, Zhou D, Serrano M, Demaria M. Cellular senescence: defining a path forward. **Cell** 179: 813–827, 2019. doi:[10.1016/j.cell.2019.10.005](https://doi.org/10.1016/j.cell.2019.10.005).
17. Sapielha P, Mallette FA. Cellular senescence in postmitotic cells: beyond growth arrest. **Trends Cell Biol** 28: 595–607, 2018. doi:[10.1016/j.tcb.2018.03.003](https://doi.org/10.1016/j.tcb.2018.03.003).
18. López-Otín C, Blasco MA, Partridge L, Serrano M, Kroemer G. The hallmarks of aging. **Cell** 153: 1194–1217, 2013. doi:[10.1016/j.cell.2013.05.039](https://doi.org/10.1016/j.cell.2013.05.039).
19. von Zglinicki T, Wan T, Miwa S. Senescence in post-mitotic cells: a driver of aging? **Antioxid Redox Signal** 34: 308–323, 2021. doi:[10.1089/ars.2020.8048](https://doi.org/10.1089/ars.2020.8048).
20. Di Micco R, Krizhanovsky V, Baker D, d’Adda di Fagagna F. Cellular senescence in ageing: from mechanisms to therapeutic opportunities. **Nat Rev Mol Cell Biol** 22: 75–95, 2021. doi:[10.1038/s41580-020-00314-w](https://doi.org/10.1038/s41580-020-00314-w).
21. Hayflick L, Moorhead PS. The serial cultivation of human diploid cell strains. **Exp Cell Res** 25: 585–621, 1961. doi:[10.1016/0014-4827\(61\)90192-6](https://doi.org/10.1016/0014-4827(61)90192-6).
22. Kumari R, Jat P. Mechanisms of cellular senescence: cell cycle arrest and senescence associated secretory phenotype. **Front Cell Dev Biol** 9: 645593, 2021. doi:[10.3389/fcell.2021.645593](https://doi.org/10.3389/fcell.2021.645593).
23. Shay JW, Wright WE. Hayflick, his limit, and cellular ageing. **Nat Rev Mol Cell Biol** 1: 72–76, 2000. doi:[10.1038/35036093](https://doi.org/10.1038/35036093).
24. Hayflick L. The limited in vitro lifetime of human diploid cell strains. **Exp Cell Res** 37: 614–636, 1965. doi:[10.1016/0014-4827\(65\)90211-9](https://doi.org/10.1016/0014-4827(65)90211-9).
25. Chang J, Wang Y, Shao L, Laberge RM, Demaria M, Campisi J, Janakiraman K, Sharpless NE, Ding S, Feng W, Luo Y, Wang X, Aykin-Burns N, Krager K, Ponnappan U, Hauer-Jensen M, Meng A, Zhou D. Clearance of senescent cells by ABT263 rejuvenates aged hematopoietic stem cells in mice. **Nat Med** 22: 78–83, 2016. doi:[10.1038/nm.4010](https://doi.org/10.1038/nm.4010).
26. Yosef R, Pilpel N, Tokarsky-Amiel R, Biran A, Ovadya Y, Cohen S, Vadai E, Dassa L, Shahar E, Condiotti R, Ben-Porath I, Krizhanovsky V. Directed elimination of senescent cells by inhibition of BCL-W and BCL-XL. **Nat Commun** 7: 11190, 2016. doi:[10.1038/ncomms11190](https://doi.org/10.1038/ncomms11190).
27. Childs BG, Baker DJ, Kirkland JL, Campisi J, van Deursen JM. Senescence and apoptosis: dueling or complementary cell fates? **EMBO Rep** 15: 1139–1153, 2014. doi:[10.15252/embr.201439245](https://doi.org/10.15252/embr.201439245).
28. Georgakopoulou E, Evangelou K, Havaki S, Townsend P, Kanavaros P, Gorgoulis VG. Apoptosis or senescence? Which exit route do epithelial cells and fibroblasts preferentially follow? **Mech Ageing Dev** 156: 17–24, 2016. doi:[10.1016/j.mad.2016.03.010](https://doi.org/10.1016/j.mad.2016.03.010).
29. Kohli J, Wang B, Brandenburg SM, Basisty N, Evangelou K, Varela-Irin M, Campisi J, Schilling B, Gorgoulis V, Demaria M. Algorithmic assessment of cellular senescence in experimental and clinical specimens. **Nat Protoc** 16: 2471–2498, 2021. doi:[10.1038/s41596-021-00505-5](https://doi.org/10.1038/s41596-021-00505-5).
30. Galanos P, Vougas K, Walter D, Polyzos A, Maya-Mendoza A, Haagenen EJ, Kokkalis A, Roumelioti FM, Gagos S, Tzetzis M, Canovas B, Igea A, Ahuja AK, Zellweger R, Havaki S, Kanavakis E, Kletsas D, Roninson IB, Garbis SD, Lopes M, Nebreda A, Thanos D, Blow JJ, Townsend P, Sørensen CS, Bartek J, Gorgoulis VG. Chronic p53-independent p21 expression causes genomic instability by deregulating replication licensing. **Nat Cell Biol** 18: 777–789, 2016. doi:[10.1038/ncb3378](https://doi.org/10.1038/ncb3378).
31. Milanovic M, Fan DN, Belenki D, Däbritz JH, Zhao Z, Yu Y, Dörr JR, Dimitrova L, Lenze D, Monteiro Barbosa IA, Mendoza-Parra MA, Kanashova T, Metzner M, Pardon K, Reimann M, Trumpp A, Dörken B, Zuber J, Gronemeyer H, Hummel M, Dittmar G, Lee S, Schmitt CA. Senescence-associated reprogramming promotes cancer stemness. **Nature** 553: 96–100, 2018. doi:[10.1038/nature25167](https://doi.org/10.1038/nature25167).
32. Saleh T, Tyutyunyk-Massey L, Gewirtz DA. Tumor cell escape from therapy-induced senescence as a model of disease recurrence after dormancy. **Cancer Res** 79: 1044–1046, 2019. doi:[10.1158/0008-5472.CAN-18-3437](https://doi.org/10.1158/0008-5472.CAN-18-3437).
33. Komseli ES, Pateras IS, Krejsgaard T, Stawiski K, Rizou SV, Polyzos A, Roumelioti FM, Chiourea M, Mourkioti I, Papatrouna E, Zampetidis CP, Gumeni S, Trougakos IP, Pefani DE, O’Neill E, Gagos S, Eliopoulos AG, Fendler W, Chowdhury D, Bartek J, Gorgoulis VG. A prototypical non-malignant epithelial model to study genome dynamics and concurrently monitor micro-RNAs and proteins in situ during oncogene-induced senescence. **BMC Genomics** 19: 37, 2018. doi:[10.1186/s12864-017-4375-1](https://doi.org/10.1186/s12864-017-4375-1).
34. Halazonetis TD, Gorgoulis VG, Bartek J. An oncogene-induced DNA damage model for cancer development. **Science** 319: 1352–1355, 2008. doi:[10.1126/science.1140735](https://doi.org/10.1126/science.1140735).
35. Myriantopoulos V, Evangelou K, Vasileiou PV, Cooks T, Vassilakopoulos TP, Pangalis GA, Kouloukoussa M, Kittas C, Georgakilas AG, Gorgoulis VG. Senescence and senotherapeutics: a new field in cancer therapy. **Pharmacol Ther** 193: 31–49, 2019. doi:[10.1016/j.pharmthera.2018.08.006](https://doi.org/10.1016/j.pharmthera.2018.08.006).
36. Zampetidis CP, Galanos P, Angelopoulou A, Zhu Y, Polyzou A, Karamitros T, Kotsinas A, Lagopati N, Mourkioti I, Mirzazadeh R, Polyzos A, Garnerone S, Mizi A, Gusmao EG, Sofiadis K, Gál Z, Larsen DH, Pefani DE, Demaria M, Tsirigos A, Crossetto N, Maya-Mendoza A, Pappaspyropoulos A, Evangelou K, Bartek J, Papanonis A, Gorgoulis VG. A recurrent chromosomal inversion suffices for driving escape from oncogene-induced senescence via subTAD reorganization. **Mol Cell** 81: 4907–4923.e8, 2021. doi:[10.1016/j.molcel.2021.10.017](https://doi.org/10.1016/j.molcel.2021.10.017).
37. Zampetidis CP, Papanonis A, Gorgoulis VG. Escape from senescence: revisiting cancer therapeutic strategies. **Mol Cell Oncol** 9: 2030158, 2022. doi:[10.1080/23723556.2022.2030158](https://doi.org/10.1080/23723556.2022.2030158).
38. Wang L, Lankhorst L, Bernards R. Exploiting senescence for the treatment of cancer. **Nat Rev Cancer** 22: 340–355, 2022. doi:[10.1038/s41568-022-00450-9](https://doi.org/10.1038/s41568-022-00450-9).
39. Beauséjour CM, Krtolica A, Galimi F, Narita M, Lowe SW, Yaswen P, Campisi J. Reversal of human cellular senescence: roles of the p53 and p16 pathways. **EMBO J** 22: 4212–4222, 2003. doi:[10.1093/emboj/cdg417](https://doi.org/10.1093/emboj/cdg417).
40. Lee S, Schmitt CA. The dynamic nature of senescence in cancer. **Nat Cell Biol** 21: 94–101, 2019. doi:[10.1038/s41556-018-0249-2](https://doi.org/10.1038/s41556-018-0249-2).

41. Anderson R, Lagnado A, Maggiorani D, Walaszczyk A, Dookun E, Chapman J, et al. Length-independent telomere damage drives post-mitotic cardiomyocyte senescence. **EMBO J** 38: e100492, 2019. doi:[10.15252/embj.2018100492](https://doi.org/10.15252/embj.2018100492).
42. Benkafadar N, François F, Affortit C, Casas F, Ceccato JC, Menardo J, Venail F, Malfroy-Camine B, Puel JL, Wang J. ROS-induced activation of DNA damage responses drives senescence-like state in postmitotic cochlear cells: implication for hearing preservation. **Mol Neurobiol** 56: 5950–5969, 2019. doi:[10.1007/s12035-019-1493-6](https://doi.org/10.1007/s12035-019-1493-6).
43. Chen YW, Harris RA, Hatahet Z, Chou KM. Ablation of XP-V gene causes adipose tissue senescence and metabolic abnormalities. **Proc Natl Acad Sci USA** 112: E4556–E4564, 2015. doi:[10.1073/pnas.1506954112](https://doi.org/10.1073/pnas.1506954112).
44. Galbiati A, Beauséjour C, d'Adda di Fagagna F. A novel single-cell method provides direct evidence of persistent DNA damage in senescent cells and aged mammalian tissues. **Aging Cell** 16: 422–427, 2017. doi:[10.1111/acer.12573](https://doi.org/10.1111/acer.12573).
45. Rodier F, Campisi J. Four faces of cellular senescence. **J Cell Biol** 192: 547–556, 2011. doi:[10.1083/jcb.201009094](https://doi.org/10.1083/jcb.201009094).
46. Lontos M, Koutsami M, Sideridou M, Evangelou K, Kletsas D, Levy B, Kotsinas A, Nahum O, Zoumpourlis V, Kouloukoussa M, Lygerou Z, Taraviras S, Kittas C, Bartkova J, Papavassiliou AG, Bartek J, Halazonetis TD, Gorgoulis VG. Deregulated overexpression of hCdt1 and hCdc6 promotes malignant behavior. **Cancer Res** 67: 10899–10909, 2007. doi:[10.1158/0008-5472.CAN-07-2837](https://doi.org/10.1158/0008-5472.CAN-07-2837).
47. Dulic V, Beney GE, Frebourg G, Drullinger LF, Stein GH. Uncoupling between phenotypic senescence and cell cycle arrest in aging p21-deficient fibroblasts. **Mol Cell Biol** 20: 6741–6754, 2000. doi:[10.1128/MCB.20.18.6741-6754.2000](https://doi.org/10.1128/MCB.20.18.6741-6754.2000).
48. Mallette FA, Ferbeyre G. The DNA damage signaling pathway connects oncogenic stress to cellular senescence. **Cell Cycle** 6: 1831–1836, 2007. doi:[10.4161/cc.6.15.4516](https://doi.org/10.4161/cc.6.15.4516).
49. Kamijo T, Zindy F, Roussel MF, Quelle DE, Downing JR, Ashmun RA, Grosveld G, Sherr CJ. Tumor suppression at the mouse INK4a locus mediated by the alternative reading frame product p19ARF. **Cell** 91: 649–659, 1997. doi:[10.1016/S0092-8674\(00\)80452-3](https://doi.org/10.1016/S0092-8674(00)80452-3).
50. Lagopati N, Belogiannis K, Angelopoulou A, Papaspyropoulos A, Gorgoulis V. Non-canonical functions of the ARF tumor suppressor in development and tumorigenesis. **Biomolecules** 11: 86, 2021. doi:[10.3390/biom11010086](https://doi.org/10.3390/biom11010086).
51. Bartkova J, Rezaei N, Lontos M, Karakaidos P, Kletsas D, Issaeva N, Vassiliou LV, Kolettas E, Niforou K, Zoumpourlis VC, Takaoka M, Nakagawa H, Tort F, Fugger K, Johansson F, Sehested M, Andersen CL, Dyrskjot L, Ørntoft T, Lukas J, Kittas C, Helleday T, Halazonetis TD, Bartek J, Gorgoulis VG. Oncogene-induced senescence is part of the tumorigenesis barrier imposed by DNA damage checkpoints. **Nature** 444: 633–637, 2006. doi:[10.1038/nature05268](https://doi.org/10.1038/nature05268).
52. Di Micco R, Fumagalli M, Cicalese A, Piccinin S, Gasparini P, Luise C, Schurra C, Garre M, Nuciforo PG, Bensimon A, Maestro R, Pelicci PG, d'Adda di Fagagna F. Oncogene-induced senescence is a DNA damage response triggered by DNA hyper-replication. **Nature** 444: 638–642, 2006. doi:[10.1038/nature05327](https://doi.org/10.1038/nature05327).
53. Kuilman T, Michaloglou C, Mooi WJ, Peeper DS. The essence of senescence. **Genes Dev** 24: 2463–2479, 2010. doi:[10.1101/gad.1971610](https://doi.org/10.1101/gad.1971610).
54. Serrano M, Lin AW, McCurrach ME, Beach D, Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. **Cell** 88: 593–602, 1997. doi:[10.1016/S0092-8674\(00\)81902-9](https://doi.org/10.1016/S0092-8674(00)81902-9).
55. d'Adda di Fagagna F, Reaper PM, Clay-Farrace L, Fiegler H, Carr P, Von Zglinicki T, Saretzki G, Carter NP, Jackson SP. A DNA damage checkpoint response in telomere-initiated senescence. **Nature** 426: 194–198, 2003. doi:[10.1038/nature02118](https://doi.org/10.1038/nature02118).
56. Herbig U, Jobling WA, Chen BP, Chen DJ, Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21^{CIP1}, but not p16^{INK4a}. **Mol Cell** 14: 501–513, 2004. doi:[10.1016/S1097-2765\(04\)00256-4](https://doi.org/10.1016/S1097-2765(04)00256-4).
57. Hewitt G, Jurk D, Marques FD, Correia-Melo C, Hardy T, Gackowska A, Anderson R, Taschuk M, Mann J, Passos JF. Telomeres are favoured targets of a persistent DNA damage response in ageing and stress-induced senescence. **Nat Commun** 3: 708, 2012. doi:[10.1038/ncomms1708](https://doi.org/10.1038/ncomms1708).
58. Sun Y, Coppé JP, Lam EW. Cellular senescence: the sought or the unwanted? **Trends Mol Med** 24: 871–885, 2018. doi:[10.1016/j.molmed.2018.08.002](https://doi.org/10.1016/j.molmed.2018.08.002).
59. Coppé JP, Desprez PY, Krtolica A, Campisi J. The senescence-associated secretory phenotype: the dark side of tumor suppression. **Annu Rev Pathol** 5: 99–118, 2010. doi:[10.1146/annurev-pathol-121808-102144](https://doi.org/10.1146/annurev-pathol-121808-102144).
60. Acosta JC, Banito A, Wuestefeld T, Georgilias A, Janich P, Morton JP, Athineos D, Kang TW, Lasitschka F, Andrulelis M, Pascual G, Morris KJ, Khan S, Jin H, Dharmalingam G, Snijders AP, Carroll T, Capper D, Pritchard C, Inman GJ, Longrich T, Sansom OJ, Benitah SA, Zender L, Gil J. A complex secretory program orchestrated by the inflammasome controls paracrine senescence. **Nat Cell Biol** 15: 978–990, 2013. doi:[10.1038/ncb2784](https://doi.org/10.1038/ncb2784).
61. Kang C, Xu Q, Martin TD, Li MZ, Demaria M, Aron L, Lu T, Yankner BA, Campisi J, Elledge SJ. The DNA damage response induces inflammation and senescence by inhibiting autophagy of GATA4. **Science** 349: aaa5612, 2015. doi:[10.1126/science.aaa5612](https://doi.org/10.1126/science.aaa5612).
62. Kuilman T, Peeper DS. Senescence-messaging secretome: SMS-ing cellular stress. **Nat Rev Cancer** 9: 81–94, 2009. doi:[10.1038/nrc2560](https://doi.org/10.1038/nrc2560).
63. Salama R, Sadaie M, Hoare M, Narita M. Cellular senescence and its effector programs. **Genes Dev** 28: 99–114, 2014. doi:[10.1101/gad.235184.113](https://doi.org/10.1101/gad.235184.113).
64. Freund A, Patil CK, Campisi J. p38MAPK is a novel DNA damage response-independent regulator of the senescence-associated secretory phenotype. **EMBO J** 30: 1536–1548, 2011. doi:[10.1038/emboj.2011.69](https://doi.org/10.1038/emboj.2011.69).
65. Sun L, Wu J, Du F, Chen X, Chen ZJ. Cyclic GMP-AMP synthase is a cytosolic DNA sensor that activates the type I interferon pathway. **Science** 339: 786–791, 2013. doi:[10.1126/science.1232458](https://doi.org/10.1126/science.1232458).
66. Wu J, Sun L, Chen X, Du F, Shi H, Chen C, Chen ZJ. Cyclic GMP-AMP is an endogenous second messenger in innate immune signaling by cytosolic DNA. **Science** 339: 826–830, 2013. doi:[10.1126/science.1229963](https://doi.org/10.1126/science.1229963).
67. Dou Z, Ghosh K, Vizioli MG, Zhu J, Sen P, Wangensteen KJ, Simithy J, Lan Y, Lin Y, Zhou Z, Capell BC, Xu C, Xu M, Kieckhafer JE, Jiang T, Shoshkes-Carmel M, Tanim K, Barber GN, Seykora JT, Millar SE, Kaestner KH, Garcia BA, Adams PD, Berger SL. Cytoplasmic chromatin triggers inflammation in senescence and cancer. **Nature** 550: 402–406, 2017. doi:[10.1038/nature24050](https://doi.org/10.1038/nature24050).
68. Hoare M, Ito Y, Kang TW, Weekes MP, Matheson NJ, Patten DA, Shetty S, Parry AJ, Menon S, Salama R, Antrobus R, Tomimatsu K, Howat W, Lehner PJ, Zender L, Narita M. NOTCH1 mediates a switch between two distinct secretomes during senescence. **Nat Cell Biol** 18: 979–992, 2016. doi:[10.1038/ncb3397](https://doi.org/10.1038/ncb3397).

69. Taegtmeyer H, Lam T, Davogusto G. Cardiac metabolism in perspective. *Compr Physiol* 6: 1675–1699, 2016. doi:[10.1002/cphy.c150056](https://doi.org/10.1002/cphy.c150056).
70. Taegtmeyer H, Wilson CR, Razeghi P, Sharma S. Metabolic energetics and genetics in the heart. *Ann NY Acad Sci* 1047: 208–218, 2005. doi:[10.1196/annals.1341.019](https://doi.org/10.1196/annals.1341.019).
71. Davogusto GE, Salazar RL, Vasquez HG, Karlstaedt A, Dillon WP, Guthrie PH, Martin JR, Vitrac H, De La Guardia G, Vela D, Ribas-Latre A, Baumgartner C, Eckel-Mahan K, Taegtmeyer H. Metabolic remodeling precedes mTORC1-mediated cardiac hypertrophy. *J Mol Cell Cardiol* 158: 115–127, 2021. doi:[10.1016/j.yjmcc.2021.05.016](https://doi.org/10.1016/j.yjmcc.2021.05.016).
72. Kundu BK, Zhong M, Sen S, Davogusto G, Keller SR, Taegtmeyer H. Remodeling of glucose metabolism precedes pressure overload-induced left ventricular hypertrophy: review of a hypothesis. *Cardiology* 130: 211–220, 2015. doi:[10.1159/000369782](https://doi.org/10.1159/000369782).
73. Wende AR, Brahma MK, McGinnis GR, Young ME. Metabolic origins of heart failure. *JACC Basic Transl Sci* 2: 297–310, 2017. doi:[10.1016/j.jacbts.2016.11.009](https://doi.org/10.1016/j.jacbts.2016.11.009).
74. Kodde IF, van der Stok J, Smolenski RT, de Jong JW. Metabolic and genetic regulation of cardiac energy substrate preference. *Comp Biochem Physiol A Mol Integr Physiol* 146: 26–39, 2007. doi:[10.1016/j.cbpa.2006.09.014](https://doi.org/10.1016/j.cbpa.2006.09.014).
75. Emelyanova L, Ashary Z, Cosic M, Negmadjanov U, Ross G, Rizvi F, Olet S, Kress D, Sra J, Tajik AJ, Holmuhamedov EL, Shi Y, Jahangir A. Selective downregulation of mitochondrial electron transport chain activity and increased oxidative stress in human atrial fibrillation. *Am J Physiol Heart Circ Physiol* 311: H54–H63, 2016. doi:[10.1152/ajpheart.00699.2015](https://doi.org/10.1152/ajpheart.00699.2015).
76. Vasileiou PV, Evangelou K, Vlasis K, Fildisis G, Panayiotidis MI, Chronopoulos E, Passias PG, Kouloukoussa M, Gorgoulis VG, Havaki S. Mitochondrial homeostasis and cellular senescence. *Cells* 8: 686, 2019. doi:[10.3390/cells8070686](https://doi.org/10.3390/cells8070686).
77. van de Ven RA, Santos D, Haigis MC. Mitochondrial sirtuins and molecular mechanisms of aging. *Trends Mol Med* 23: 320–331, 2017. doi:[10.1016/j.molmed.2017.02.005](https://doi.org/10.1016/j.molmed.2017.02.005).
78. Wiley CD, Velarde MC, Lecot P, Liu S, Sarnoski EA, Freund A, Shirakawa K, Lim HW, Davis SS, Ramanathan A, Gerencser AA, Verdin E, Campisi J. Mitochondrial dysfunction induces senescence with a distinct secretory phenotype. *Cell Metab* 23: 303–314, 2016. doi:[10.1016/j.cmet.2015.11.011](https://doi.org/10.1016/j.cmet.2015.11.011).
79. Correia-Melo C, Marques FD, Anderson R, Hewitt G, Hewitt R, Cole J, Carroll BM, Miwa S, Birch J, Merz A, Rushton MD, Charles M, Jurk D, Tait SW, Czapiewski R, Greaves L, Nelson G, Bohlooly YM, Rodriguez-Cuenca S, Vidal-Puig A, Mann D, Saretzki G, Quarato G, Green DR, Adams PD, von Zglinicki T, Korolchuk VI, Passos JF. Mitochondria are required for pro-ageing features of the senescent phenotype. *EMBO J* 35: 724–742, 2016. doi:[10.15252/embj.201592862](https://doi.org/10.15252/embj.201592862).
80. Ogrodnik M, Miwa S, Tchkonja T, Tiniakos D, Wilson CL, Lahat A, Day CP, Burt A, Palmer A, Anstee QM, Grellescheid SN, Hoeijmakers JH, Barnhoorn S, Mann DA, Bird TG, Vermeij WP, Kirkland JL, Passos JF, von Zglinicki T, Jurk D. Cellular senescence drives age-dependent hepatic steatosis. *Nat Commun* 8: 15691, 2017. doi:[10.1038/ncomms15691](https://doi.org/10.1038/ncomms15691).
81. Jurk D, Wang C, Miwa S, Maddick M, Korolchuk V, Tsolou A, Gonos ES, Thrasivoulou C, Saffrey MJ, Cameron K, von Zglinicki T. Postmitotic neurons develop a p21-dependent senescence-like phenotype driven by a DNA damage response. *Aging Cell* 11: 996–1004, 2012. doi:[10.1111/j.1474-9726.2012.00870.x](https://doi.org/10.1111/j.1474-9726.2012.00870.x).
82. Ogrodnik M, Zhu Y, Langhi LG, Tchkonja T, Krüger P, Fielder E, Victorelli S, Ruswhandi RA, Giorgadze N, Pirtskhalava T, Podgorni O, Enikolopov G, Johnson KO, Xu M, Inman C, Palmer AK, Schafer M, Weigl M, Ikeno Y, Burns TC, Passos JF, von Zglinicki T, Kirkland JL, Jurk D. Obesity-induced cellular senescence drives anxiety and impairs neurogenesis. *Cell Metab* 29: 1233, 2019. doi:[10.1016/j.cmet.2019.01.013](https://doi.org/10.1016/j.cmet.2019.01.013).
83. Campisi J. Aging, cellular senescence, and cancer. *Annu Rev Physiol* 75: 685–705, 2013. doi:[10.1146/annurev-physiol-030212-183653](https://doi.org/10.1146/annurev-physiol-030212-183653).
84. Wiley CD, Sharma R, Davis SS, Lopez-Dominguez JA, Mitchell KP, Wiley S, Alimrah F, Kim DE, Payne T, Rosko A, Aimontche E, Deshpande SM, Neri F, Kuehnemann C, Demaria M, Ramanathan A, Campisi J. Oxylin biosynthesis reinforces cellular senescence and allows detection of senolysis. *Cell Metab* 33: 1124–1136.e5, 2021. doi:[10.1016/j.cmet.2021.03.008](https://doi.org/10.1016/j.cmet.2021.03.008).
85. Robbins E, Levine EM, Eagle H. Morphologic changes accompanying senescence of cultured human diploid cells. *J Exp Med* 131: 1211–1222, 1970. doi:[10.1084/jem.131.6.1211](https://doi.org/10.1084/jem.131.6.1211).
86. Hernandez-Segura A, de Jong TV, Melov S, Guryev V, Campisi J, Demaria M. Unmasking transcriptional heterogeneity in senescent cells. *Curr Biol* 27: 2652–2660.e4, 2017. doi:[10.1016/j.cub.2017.07.033](https://doi.org/10.1016/j.cub.2017.07.033).
87. Swanson EC, Manning B, Zhang H, Lawrence JB. Higher-order unfolding of satellite heterochromatin is a consistent and early event in cell senescence. *J Cell Biol* 203: 929–942, 2013. doi:[10.1083/jcb.201306073](https://doi.org/10.1083/jcb.201306073).
88. Cui S, Xue L, Yang F, Dai S, Han Z, Liu K, Liu B, Yuan Q, Cui Z, Zhang Y, Xu F, Chen Y. Postinfarction hearts are protected by premature senescent cardiomyocytes via GATA 4-dependent CCN 1 secretion. *J Am Heart Assoc* 7: e009111, 2018. doi:[10.1161/JAHA.118.009111](https://doi.org/10.1161/JAHA.118.009111).
89. Tang X, Li PH, Chen HZ. Cardiomyocyte senescence and cellular communications within myocardial microenvironments. *Front Endocrinol (Lausanne)* 11: 280, 2020. doi:[10.3389/fendo.2020.00280](https://doi.org/10.3389/fendo.2020.00280).
90. Senyo SE, Steinhauser ML, Pizzimenti CL, Yang VK, Cai L, Wang M, Wu TD, Guerin-Kern JL, Lechene CP, Lee RT. Mammalian heart renewal by pre-existing cardiomyocytes. *Nature* 493: 433–436, 2013. doi:[10.1038/nature11682](https://doi.org/10.1038/nature11682).
91. Hu C, Zhang X, Teng T, Ma ZG, Tang QZ. Cellular senescence in cardiovascular diseases: a systematic review. *Aging Dis* 13: 103–128, 2022. doi:[10.14336/AD.2021.0927](https://doi.org/10.14336/AD.2021.0927).
92. Ball AJ, Levine F. Telomere-independent cellular senescence in human fetal cardiomyocytes. *Aging Cell* 4: 21–30, 2005. doi:[10.1111/j.1474-9728.2004.00137.x](https://doi.org/10.1111/j.1474-9728.2004.00137.x).
93. Yang F, Kemp CJ, Henikoff S. Doxorubicin enhances nucleosome turnover around promoters. *Curr Biol* 23: 782–787, 2013. doi:[10.1016/j.cub.2013.03.043](https://doi.org/10.1016/j.cub.2013.03.043).
94. Liu LF. DNA topoisomerase poisons as antitumor drugs. *Annu Rev Biochem* 58: 351–375, 1989. doi:[10.1146/annurev.bi.58.070189.02031](https://doi.org/10.1146/annurev.bi.58.070189.02031).
95. Chai YC, Ashraf SS, Rokutan K, Johnston RB Jr, Thomas JA. S-thiolation of individual human neutrophil proteins including actin by stimulation of the respiratory burst: evidence against a role for glutathione disulfide. *Arch Biochem Biophys* 310: 273–281, 1994. doi:[10.1006/abbi.1994.1167](https://doi.org/10.1006/abbi.1994.1167).

96. Chen K, Wang S, Sun QW, Zhang B, Ullah M, Sun Z. Klotho deficiency causes heart aging via impairing the Nrf2-GR pathway. **Circ Res** 128: 492–507, 2021. doi:10.1161/CIRCRESAHA.120.317348.
97. Gal H, Lysenko M, Stroganov S, Vadai E, Youssef SA, Tzadikевич-Geffen K, Rotkopf R, Biron-Shental T, de Bruin A, Neeman M, Krizhanovsky V. Molecular pathways of senescence regulate placental structure and function. **EMBO J** 38: e100849, 2019. doi:10.15252/embj.2018100849.
98. Muñoz-Espín D, Cañamero M, Maraver A, Gomez-Lopez G, Contreras J, Murillo-Cuesta S, Rodriguez-Baeza A, Varela-Nieto I, Ruberte J, Collado M, Serrano M. Programmed cell senescence during mammalian embryonic development. **Cell** 155: 1104–1118, 2013. doi:10.1016/j.cell.2013.10.019.
99. Storer M, Mas A, Robert-Moreno A, Pecoraro M, Ortells MC, Di Giacomo V, Yosef R, Pilpel N, Krizhanovsky V, Sharpe J, Keyes WM. Senescence is a developmental mechanism that contributes to embryonic growth and patterning. **Cell** 155: 1119–1130, 2013. doi:10.1016/j.cell.2013.10.041.
100. Chuprin A, Gal H, Biron-Shental T, Biran A, Amiel A, Rozenblatt S, Krizhanovsky V. Cell fusion induced by ERVWE1 or measles virus causes cellular senescence. **Genes Dev** 27: 2356–2366, 2013. doi:10.1101/gad.227512.113.
101. Kim KH, Chen CC, Monzon RI, Lau LF. Matricellular protein CCN1 promotes regression of liver fibrosis through induction of cellular senescence in hepatic myofibroblasts. **Mol Cell Biol** 33: 2078–2090, 2013. doi:10.1128/MCB.00049-13.
102. Jun JI, Lau LF. The matricellular protein CCN1 induces fibroblast senescence and restricts fibrosis in cutaneous wound healing. **Nat Cell Biol** 12: 676–685, 2010. doi:10.1038/ncb2070.
103. Fabbri E, An Y, Zoli M, Simonsick EM, Guralnik JM, Bandinelli S, Boyd CM, Ferrucci L. Aging and the burden of multimorbidity: associations with inflammatory and anabolic hormonal biomarkers. **J Gerontol A Biol Sci Med Sci** 70: 63–70, 2015. doi:10.1093/geron/ glu127.
104. Sousa-Victor P, García-Prat L, Serrano AL, Perdiguero E, Muñoz-Canoves P. Muscle stem cell aging: regulation and rejuvenation. **Trends Endocrinol Metab** 26: 287–296, 2015. doi:10.1016/j.tem.2015.03.006.
105. Gnani D, Crippa S, Della Volpe L, Rossella V, Conti A, Lettera E, Rivi S, Omerti M, Frascini G, Bernardo ME, Di Micco R. An early-senescence state in aged mesenchymal stromal cells contributes to hematopoietic stem and progenitor cell clonogenic impairment through the activation of a pro-inflammatory program. **Aging Cell** 18: e12933, 2019. doi:10.1111/ace1.12933.
106. Huang W, Alhenc Gelas F, Osborne-Pellegrin MJ. Protection of the arterial internal elastic lamina by inhibition of the renin-angiotensin system in the rat. **Circ Res** 82: 879–890, 1998. doi:10.1161/01.res.82.8.879.
107. Paneni F, Diaz Cañestro C, Libby P, Lüscher TF, Camici GG. The aging cardiovascular system: understanding it at the cellular and clinical levels. **J Am Coll Cardiol** 69: 1952–1967, 2017. doi:10.1016/j.jacc.2017.01.064.
108. Brouillette SW, Moore JS, McMahon AD, Thompson JR, Ford I, Shepherd J, Packard CJ, Samani NJ; West of Scotland Coronary Prevention Study Group. Telomere length, risk of coronary heart disease, and statin treatment in the West of Scotland Primary Prevention Study: a nested case-control study. **Lancet** 369: 107–114, 2007. doi:10.1016/S0140-6736(07)60071-3.
109. Kurz DJ, Kloeckener-Gruissem B, Akhmedov A, Eberli FR, Bühler I, Berger W, Bertel O, Lüscher TF. Degenerative aortic valve stenosis, but not coronary disease, is associated with shorter telomere length in the elderly. **Arterioscler Thromb Vasc Biol** 26: e114–e117, 2006. doi:10.1161/01.ATV.0000222961.24912.69.
110. Shimizu I, Minamino T. Cellular senescence in cardiac diseases. **J Cardiol** 74: 313–319, 2019. doi:10.1016/j.jjcc.2019.05.002.
111. Camici GG, Savarese G, Akhmedov A, Lüscher TF. Molecular mechanism of endothelial and vascular aging: implications for cardiovascular disease. **Eur Heart J** 36: 3392–3403, 2015. doi:10.1093/eurheartj/ehv587.
112. Kornfeld OS, Hwang S, Disatnik MH, Chen CH, Qvit N, Mochly-Rosen D. Mitochondrial reactive oxygen species at the heart of the matter: new therapeutic approaches for cardiovascular diseases. **Circ Res** 116: 1783–1799, 2015. doi:10.1161/CIRCRESAHA.116.305432.
113. Wattanapitayakul SK, Bauer JA. Oxidative pathways in cardiovascular disease: roles, mechanisms, and therapeutic implications. **Pharmacol Ther** 89: 187–206, 2001. doi:10.1016/s0163-7258(00)00114-5.
114. Zorov DB, Juhaszova M, Sollott SJ. Mitochondrial reactive oxygen species (ROS) and ROS-induced ROS release. **Physiol Rev** 94: 909–950, 2014. doi:10.1152/physrev.00026.2013.
115. Capell BC, Collins FS, Nabel EG. Mechanisms of cardiovascular disease in accelerated aging syndromes. **Circ Res** 101: 13–26, 2007. doi:10.1161/CIRCRESAHA.107.153692.
116. Durik M, Kavousi M, van der Pluijm I, Isaacs A, Cheng C, Verdonk K, et al. Nucleotide excision DNA repair is associated with age-related vascular dysfunction. **Circulation** 126: 468–478, 2012. doi:10.1161/CIRCULATIONAHA.112.104380.
117. Mahmoudi M, Mercer J, Bennett M. DNA damage and repair in atherosclerosis. **Cardiovasc Res** 71: 259–268, 2006. doi:10.1016/j.cardiores.2006.03.002.
118. Botto N, Rizza A, Colombo MG, Mazzone AM, Manfredi S, Masetti S, Clerico A, Biagini A, Andreassi MG. Evidence for DNA damage in patients with coronary artery disease. **Mutat Res** 493: 23–30, 2001. doi:10.1016/s1383-5718(01)00162-0.
119. Zhang W, Song M, Qu J, Liu GH. Epigenetic modifications in cardiovascular aging and diseases. **Circ Res** 123: 773–786, 2018. doi:10.1161/CIRCRESAHA.118.312497.
120. Papat R, Cattaneo P, Kunderfranco P, Greco C, Carullo P, Guffanti A, Viganò V, Stirparo GG, Latronico MV, Hasenfuss G, Chen J, Condorelli G. Genome-wide analysis of histone marks identifying an epigenetic signature of promoters and enhancers underlying cardiac hypertrophy. **Proc Natl Acad Sci USA** 110: 20164–20169, 2013. doi:10.1073/pnas.1315155110.
121. Hofmann C, Katus HA, Doroudgar S. Protein misfolding in cardiac disease. **Circulation** 139: 2085–2088, 2019. doi:10.1161/CIRCULATIONAHA.118.037417.
122. Hetz C. The unfolded protein response: controlling cell fate decisions under ER stress and beyond. **Nat Rev Mol Cell Biol** 13: 89–102, 2012. doi:10.1038/nrm3270.
123. Diteepeng T, Del Monte F, Luciani M. The long and winding road to target protein misfolding in cardiovascular diseases. **Eur J Clin Invest** 51: e13504, 2021. doi:10.1111/eci.13504.
124. Sanbe A, Osinska H, Saffitz JE, Glabe CG, Kayed R, Maloyan A, Robbins J. Desmin-related cardiomyopathy in transgenic mice: a

- cardiac amyloidosis. **Proc Natl Acad Sci USA** 101: 10132–10136, 2004. doi:10.1073/pnas.0401900101.
125. Song H, Conte JV Jr, Foster AH, McLaughlin JS, Wei C. Increased p53 protein expression in human failing myocardium. **J Heart Lung Transplant** 18: 744–749, 1999. doi:10.1016/S1053-2498(98)00039-4.
 126. Birks EJ, Latif N, Enesa K, Folkvang T, Luong LA, Sarathchandra P, Khan M, Ovaa H, Terracciano CM, Barton PJ, Yacoub MH, Evans PC. Elevated p53 expression is associated with dysregulation of the ubiquitin-proteasome system in dilated cardiomyopathy. **Cardiovasc Res** 79: 472–480, 2008. doi:10.1093/cvr/cvn083.
 127. Taglieri DM, Ushio-Fukai M, Monasky MM. P21-activated kinase in inflammatory and cardiovascular disease. **Cell Signal** 26: 2060–2069, 2014. doi:10.1016/j.cellsig.2014.04.020.
 128. Martyn KD, Kim MJ, Quinn MT, Dinauer MC, Knaus UG. p21-activated kinase (Pak) regulates NADPH oxidase activation in human neutrophils. **Blood** 106: 3962–3969, 2005. doi:10.1182/blood-2005-03-0859.
 129. Liu W, Zi M, Naumann R, Ulm S, Jin J, Taglieri DM, Prehar S, Gui J, Tsui H, Xiao RP, Neyses L, Solaro RJ, Ke Y, Cartwright EJ, Lei M, Wang X. Pak1 as a novel therapeutic target for antihypertrophic treatment in the heart. **Circulation** 124: 2702–2715, 2011. doi:10.1161/CIRCULATIONAHA.111.048785.
 130. Monasky MM, Taglieri DM, Patel BG, Chernoff J, Wolska BM, Ke Y, Solaro RJ. p21-activated kinase improves cardiac contractility during ischemia-reperfusion concomitant with changes in troponin-T and myosin light chain 2 phosphorylation. **Am J Physiol Heart Circ Physiol** 302: H224–H230, 2012. doi:10.1152/ajpheart.00612.2011.
 131. Romero-Becerra R, Santamans AM, Folgueira C, Sabio G. p38 MAPK pathway in the heart: new insights in health and disease. **Int J Mol Sci** 21: 7412, 2020. doi:10.3390/ijms21197412.
 132. Aouadi M, Bost F, Caron L, Laurent K, Le Marchand Brustel Y, Binétruy B. p38 mitogen-activated protein kinase activity commits embryonic stem cells to either neurogenesis or cardiomyogenesis. **Stem Cells** 24: 1399–1406, 2006. doi:10.1634/stemcells.2005-0398.
 133. Marais E, Genade S, Huisamen B, Strijdom JG, Moolman JA, Lochner A. Activation of p38 MAPK induced by a multi-cycle ischemic preconditioning protocol is associated with attenuated p38 MAPK activity during sustained ischaemia and reperfusion. **J Mol Cell Cardiol** 33: 769–778, 2001. doi:10.1006/jmcc.2001.1347.
 134. Säkkinen H, Aro J, Kaikkonen L, Ohukainen P, Näpänkangas J, Tokola H, Ruskoaho H, Rysä J. Mitogen-activated protein kinase p38 target regenerating islet-derived 3gamma expression is upregulated in cardiac inflammatory response in the rat heart. **Physiol Rep** 4: e12996, 2016. doi:10.14814/phy2.12996.
 135. Khan R, Sheppard R. Fibrosis in heart disease: understanding the role of transforming growth factor-beta in cardiomyopathy, valvular disease and arrhythmia. **Immunology** 118: 10–24, 2006. doi:10.1111/j.1365-2567.2006.02336.x.
 136. Segura AM, Frazier OH, Buja LM. Fibrosis and heart failure. **Heart Fail Rev** 19: 173–185, 2014. doi:10.1007/s10741-012-9365-4.
 137. Brown EJ, Albers MW, Shin TB, Ichikawa K, Keith CT, Lane WS, Schreiber SL. A mammalian protein targeted by G1-arresting rapamycin-receptor complex. **Nature** 369: 756–758, 1994. doi:10.1038/369756a0.
 138. Sabatini DM, Erdjument-Bromage H, Lui M, Tempst P, Snyder SH. RAFT1: a mammalian protein that binds to FKBP12 in a rapamycin-dependent fashion and is homologous to yeast TORs. **Cell** 78: 35–43, 1994. doi:10.1016/0092-8674(94)90570-3.
 139. Saxton RA, Sabatini DM. mTOR signaling in growth, metabolism, and disease. **Cell** 169: 361–371, 2017. doi:10.1016/j.cell.2017.03.035.
 140. Laberge RM, Sun Y, Orjalo AV, Patil CK, Freund A, Zhou L, Curran SC, Davalos AR, Wilson-Edell KA, Liu S, Limbad C, Demaria M, Li P, Hubbard GB, Ikeno Y, Javors M, Desprez PY, Benz CC, Kapahi P, Nelson PS, Campisi J. MTOR regulates the pro-tumorigenic senescence-associated secretory phenotype by promoting IL1A translation. **Nat Cell Biol** 17: 1049–1061, 2015. doi:10.1038/ncb3195.
 141. Sciarretta S, Forte M, Frati G, Sadoshima J. New insights into the role of mTOR signaling in the cardiovascular system. **Circ Res** 122: 489–505, 2018. doi:10.1161/CIRCRESAHA.117.311147.
 142. Gorski DJ, Petz A, Reichert C, Twarock S, Grandoch M, Fischer JW. Cardiac fibroblast activation and hyaluronan synthesis in response to hyperglycemia and diet-induced insulin resistance. **Sci Rep** 9: 1827, 2019. doi:10.1038/s41598-018-36140-6.
 143. Wang L, Jiang P, He Y, Hu H, Guo Y, Liu X, Qiu H, Ma Q, Ouyang F. A novel mechanism of Smads/miR-675/TGFbetaR1 axis modulating the proliferation and remodeling of mouse cardiac fibroblasts. **J Cell Physiol** 234: 20275–20285, 2019. doi:10.1002/jcp.28628.
 144. Wang L, Wang J, Jin T, Zhou Y, Chen Q. FoxG1 facilitates proliferation and inhibits differentiation by downregulating FoxO/Smad signaling in glioblastoma. **Biochem Biophys Res Commun** 504: 46–53, 2018. doi:10.1016/j.bbrc.2018.08.118.
 145. Vivar R, Humeres C, Anfossi R, Bolivar S, Catalán M, Hill J, Lavandero S, Diaz-Araya G. Role of FoxO3a as a negative regulator of the cardiac myofibroblast conversion induced by TGF-beta1. **Biochim Biophys Acta Mol Cell Res** 1867: 118695, 2020. doi:10.1016/j.bbamcr.2020.118695.
 146. Al-Tamari HM, Dabral S, Schmall A, Sarvari P, Ruppert C, Paik J, DePinho RA, Grimminger F, Eickelberg O, Guenther A, Seeger W, Savai R, Pullamsetti SS. FoxO3 an important player in fibrogenesis and therapeutic target for idiopathic pulmonary fibrosis. **EMBO Mol Med** 10: 276–293, 2018. doi:10.15252/emmm.201606261.
 147. Dai DF, Chen T, Johnson SC, Szeto H, Rabinovitch PS. Cardiac aging: from molecular mechanisms to significance in human health and disease. **Antioxid Redox Signal** 16: 1492–1526, 2012. doi:10.1089/ars.2011.4179.
 148. Lewis-McDougall FC, Ruchaya PJ, Domenjo-Vila E, Shin Teoh T, Prata L, Cottle BJ, Clark JE, Punjabi PP, Awad W, Torella D, Tchkonina T, Kirkland JL, Ellison-Hughes GM. Aged-senescent cells contribute to impaired heart regeneration. **Aging Cell** 18: e12931, 2019. doi:10.1111/accel.12931.
 149. Chiao YA, Rabinovitch PS. The aging heart. **Cold Spring Harb Perspect Med** 5: a025148, 2015. doi:10.1101/cshperspect.a025148.
 150. de Lucia C, Eguchi A, Koch WJ. New insights in cardiac beta-adrenergic signaling during heart failure and aging. **Front Pharmacol** 9: 904, 2018. doi:10.3389/fphar.2018.00904.
 151. Lee WS, Kim J. Insulin-like growth factor-1 signaling in cardiac aging. **Biochim Biophys Acta Mol Basis Dis** 1864: 1931–1938, 2018. doi:10.1016/j.bbdis.2017.08.029.
 152. Miller AJ, Arnold AC. The renin-angiotensin system and cardiovascular autonomic control in aging. **Peptides** 150: 170733, 2022. doi:10.1016/j.peptides.2021.170733.

153. Hamilton S, Terentyev D. Altered intracellular calcium homeostasis and arrhythmogenesis in the aged heart. *Int J Mol Sci* 20: 2386, 2019. doi:10.3390/ijms20102386.
154. Sharov VS, Dremina ES, Galeva NA, Williams TD, Schöneich C. Quantitative mapping of oxidation-sensitive cysteine residues in SERCA in vivo and in vitro by HPLC-electrospray-tandem MS: selective protein oxidation during biological aging. *Biochem J* 394: 605–615, 2006. doi:10.1042/BJ20051214.
155. Quiat D, Olson EN. MicroRNAs in cardiovascular disease: from pathogenesis to prevention and treatment. *J Clin Invest* 123: 11–18, 2013. doi:10.1172/JCI62876.
156. Berenji Ardestani S, Eftedal I, Pedersen M, Jeppesen PB, Nørregaard R, Matchkov VV. Endothelial dysfunction in small arteries and early signs of atherosclerosis in ApoE knockout rats. *Sci Rep* 10: 15296, 2020. doi:10.1038/s41598-020-72338-3.
157. Wolf D, Ley K. Immunity and inflammation in atherosclerosis. *Circ Res* 124: 315–327, 2019. doi:10.1161/CIRCRESAHA.118.313591.
158. Pantsulaia I, Ciszewski WM, Niewiarowska J. Senescent endothelial cells: potential modulators of immunosenescence and ageing. *Ageing Res Rev* 29: 13–25, 2016. doi:10.1016/j.arr.2016.05.011.
159. Stojanović SD, Fiedler J, Bauersachs J, Thum T, Sedding DG. Senescence-induced inflammation: an important player and key therapeutic target in atherosclerosis. *Eur Heart J* 41: 2983–2996, 2020. doi:10.1093/eurheartj/ehz919.
160. Denver JW, Reed SF, Porges SW. Methodological issues in the quantification of respiratory sinus arrhythmia. *Biol Psychol* 74: 286–294, 2007. doi:10.1016/j.biopsycho.2005.09.005.
161. Donato AJ, Morgan RG, Walker AE, Lesniewski LA. Cellular and molecular biology of aging endothelial cells. *J Mol Cell Cardiol* 89: 122–135, 2015. doi:10.1016/j.yjmcc.2015.01.021.
162. Rossman MJ, Kaplon RE, Hill SD, McNamara MN, Santos-Parker JR, Pierce GL, Seals DR, Donato AJ. Endothelial cell senescence with aging in healthy humans: prevention by habitual exercise and relation to vascular endothelial function. *Am J Physiol Heart Circ Physiol* 313: H890–H895, 2017. doi:10.1152/ajpheart.00416.2017.
163. Bhayadia R, Schmidt BM, Melk A, Hömme M. Senescence-induced oxidative stress causes endothelial dysfunction. *J Gerontol A Biol Sci Med Sci* 71: 161–169, 2016. doi:10.1093/gerona/glv008.
164. Weber C, Noels H. Atherosclerosis: current pathogenesis and therapeutic options. *Nat Med* 17: 1410–1422, 2011. doi:10.1038/nm.2538.
165. Childs BG, Baker DJ, Wijshake T, Conover CA, Campisi J, van Deursen JM. Senescent intimal foam cells are deleterious at all stages of atherosclerosis. *Science* 354: 472–477, 2016. doi:10.1126/science.aaf6659.
166. Jakubzick CV, Randolph GJ, Henson PM. Monocyte differentiation and antigen-presenting functions. *Nat Rev Immunol* 17: 349–362, 2017. doi:10.1038/nri.2017.28.
167. Zhao Y, Zou W, Du J, Zhao Y. The origins and homeostasis of monocytes and tissue-resident macrophages in physiological situation. *J Cell Physiol* 233: 6425–6439, 2018. doi:10.1002/jcp.26461.
168. Feil S, Fehrenbacher B, Lukowski R, Essmann F, Schulze-Osthoff K, Schaller M, Feil R. Transdifferentiation of vascular smooth muscle cells to macrophage-like cells during atherogenesis. *Circ Res* 115: 662–667, 2014. doi:10.1161/CIRCRESAHA.115.304634.
169. Shankman LS, Gomez D, Cherepanova OA, Salmon M, Alencar GF, Haskins RM, Swiatlowska P, Newman AA, Greene ES, Straub AC, Isakson B, Randolph GJ, Owens GK. KLF4-dependent phenotypic modulation of smooth muscle cells has a key role in atherosclerotic plaque pathogenesis. *Nat Med* 21: 628–637, 2015. doi:10.1038/nm.3866.
170. Wang J, Uryga AK, Reinhold J, Figg N, Baker L, Finigan A, Gray K, Kumar S, Clarke M, Bennett M. Vascular smooth muscle cell senescence promotes atherosclerosis and features of plaque vulnerability. *Circulation* 132: 1909–1919, 2015. doi:10.1161/CIRCULATIONAHA.115.016457.
171. Sorokin V, Vickneson K, Kofidis T, Woo CC, Lin XY, Foo R, Shanahan CM. Role of vascular smooth muscle cell plasticity and interactions in vessel wall inflammation. *Front Immunol* 11: 599415, 2020. doi:10.3389/fimmu.2020.599415.
172. Stefanadis C, Antoniou CK, Tsiachris D, Pietri P. Coronary atherosclerotic vulnerable plaque: current perspectives. *J Am Heart Assoc* 6: e005543, 2017. doi:10.1161/JAHA.117.005543.
173. Park SY, Kim IS. Engulfment signals and the phagocytic machinery for apoptotic cell clearance. *Exp Mol Med* 49: e331, 2017. doi:10.1038/emm.2017.52.
174. Lin D, Kang X, Shen L, Tu S, Lenahan C, Chen Y, Wang X, Shao A. Efferocytosis and its associated cytokines: a light on non-tumor and tumor diseases? *Mol Ther Oncolytics* 17: 394–407, 2020. doi:10.1016/j.omto.2020.04.010.
175. Thorp E, Vaisar T, Subramanian M, Mautner L, Blobel C, Tabas I. Shedding of the Mer tyrosine kinase receptor is mediated by ADAM17 protein through a pathway involving reactive oxygen species, protein kinase Cdelta, and p38 mitogen-activated protein kinase (MAPK). *J Biol Chem* 286: 33335–33344, 2011. doi:10.1074/jbc.M111.263020.
176. Zhang Y, Wang Y, Zhou D, Zhang LS, Deng FX, Shu S, Wang LJ, Wu Y, Guo N, Zhou J, Yuan ZY. Angiotensin II deteriorates advanced atherosclerosis by promoting MerTK cleavage and impairing efferocytosis through the AT1R/ROS/p38 MAPK/ADAM17 pathway. *Am J Physiol Cell Physiol* 317: C776–C787, 2019. doi:10.1152/ajpcell.00145.2019.
177. Rymut N, Heinz J, Sadhu S, Hosseini Z, Riley CO, Marinello M, Maloney J, MacNamara KC, Spite M, Fredman G. Resolvin D1 promotes efferocytosis in aging by limiting senescent cell-induced MerTK cleavage. *FASEB J* 34: 597–609, 2020. doi:10.1096/fj.201902126R.
178. Schmid M, Gemperle C, Rimann N, Hersberger M. Resolvin D1 polarizes primary human macrophages toward a proresolution phenotype through GPR32. *J Immunol* 196: 3429–3437, 2016. doi:10.4049/jimmunol.1501701.
179. Kojima Y, Volkmer JP, McKenna K, Civelek M, Lusic AJ, Miller CL, Direnzo D, Nanda V, Ye J, Connolly AJ, Schadt EE, Quertermous T, Betancur P, Maegdefessel L, Matic LP, Hedin U, Weissman IL, Leeper NJ. CD47-blocking antibodies restore phagocytosis and prevent atherosclerosis. *Nature* 536: 86–90, 2016. doi:10.1038/nature18935.
180. Sartore S, Chiavegato A, Faggini E, Franch R, Puato M, Ausoni S, Paoletto P. Contribution of adventitial fibroblasts to neointima formation and vascular remodeling: from innocent bystander to active participant. *Circ Res* 89: 1111–1121, 2001. doi:10.1161/hh2401.100844.
181. Wilcox JN, Scott NA. Potential role of the adventitia in arteritis and atherosclerosis. *Int J Cardiol* 54, Suppl: S21–S35, 1996. doi:10.1016/s0167-5273(96)02811-2.

182. Muñoz-Espín D, Serrano M. Cellular senescence: from physiology to pathology. *Nat Rev Mol Cell Biol* 15: 482–496, 2014. doi:10.1038/nrm3823.
183. Zalewski A, Shi Y. Vascular myofibroblasts. Lessons from coronary repair and remodeling. *Arterioscler Thromb Vasc Biol* 17: 417–422, 1997. doi:10.1161/01.atv.17.3.417.
184. Shan H, Li T, Zhang L, Yang R, Li Y, Zhang M, Dong Y, Zhou Y, Xu C, Yang B, Liang H, Gao X, Shan H. Heme oxygenase-1 prevents heart against myocardial infarction by attenuating ischemic injury-induced cardiomyocytes senescence. *EBioMedicine* 39: 59–68, 2019. doi:10.1016/j.ebiom.2018.11.056.
185. Dookun E, Walaszczyk A, Redgrave R, Tual-Chalot S, Yausep O, Spyridopoulos I, Owens A, Arthur H, Passos J, Richardson G. Accumulation of cardiomyocyte senescence following ischaemia-reperfusion injury (IRI); a potential therapeutic target (Abstract)? *Heart* 104: A103, 2018.
186. Feng T, Meng J, Kou S, Jiang Z, Huang X, Lu Z, Zhao H, Lau LF, Zhou B, Zhang H. CCN1-induced cellular senescence promotes heart regeneration. *Circulation* 139: 2495–2498, 2019. doi:10.1161/CIRCULATIONAHA.119.039530.
187. Paspaspyropoulos A, Angelopoulou A, Mourkioti I, Polyzou A, Pankova D, Toskas K, Lanfredini S, Pantazaki AA, Lagopati N, Kotsinas A, Evangelou K, Chronopoulos E, O'Neill E, Gorgoulis V. RASSF1A disrupts the NOTCH signaling axis via SNURF/RNF4-mediated ubiquitination of HES1. *EMBO Rep* 23: e51287, 2021. doi:10.15252/embr.202051287.
188. Paspaspyropoulos A, Bradley L, Thapa A, Leung CY, Toskas K, Koennig D, Pefani DE, Raso C, Grou C, Hamilton G, Vlahov N, Grawenda A, Haider S, Chauhan J, Buti L, Kanapin A, Lu X, Buffa F, Dianov G, von Kriegsheim A, Matallanas D, Samsonova A, Zernicka-Goetz M, O'Neill E. RASSF1A uncouples Wnt from Hippo signalling and promotes YAP mediated differentiation via p73. *Nat Commun* 9: 424, 2018. doi:10.1038/s41467-017-02786-5.
189. van der Weyden L, Paspaspyropoulos A, Poulgiannis G, Rust AG, Rashid M, Adams DJ, Arends MJ, O'Neill E. Loss of RASSF1A synergizes with deregulated RUNX2 signaling in tumorigenesis. *Cancer Res* 72: 3817–3827, 2012. doi:10.1158/0008-5472.CAN-11-3343.
190. Jia L, Zhang W, Ma Y, Chen B, Liu Y, Piao C, Wang Y, Yang M, Liu T, Zhang J, Li T, Nie S, Du J. Haploinsufficiency of Ataxia Telangiectasia Mutated accelerates heart failure after myocardial infarction. *J Am Heart Assoc* 6: e006349, 2017. doi:10.1161/JAHA.117.006349.
191. Sawaki D, Czibik G, Pini M, Ternacle J, Suffee N, Mercedes R, Marcelin G, Surenaud M, Marcos E, Gual P, Clément K, Hue S, Adnot S, Hatem SN, Tsuchimochi I, Yoshimitsu T, Hénégat C, Derumeaux G. Visceral adipose tissue drives cardiac aging through modulation of fibroblast senescence by osteopontin production. *Circulation* 138: 809–822, 2018. doi:10.1161/CIRCULATIONAHA.117.031358.
192. Litvin J, Blagg A, Mu A, Matiwala S, Montgomery M, Berretta R, Houser S, Margulies K. Periostin and periostin-like factor in the human heart: possible therapeutic targets. *Cardiovasc Pathol* 15: 24–32, 2006. doi:10.1016/j.carpath.2005.09.001.
193. Segers VF, Lee RT. Protein therapeutics for cardiac regeneration after myocardial infarction. *J Cardiovasc Transl Res* 3: 469–477, 2010. doi:10.1007/s12265-010-9207-5.
194. Li Q, Liu X, Wei J. Ageing related periostin expression increase from cardiac fibroblasts promotes cardiomyocytes senescent. *Biochem Biophys Res Commun* 452: 497–502, 2014. doi:10.1016/j.bbrc.2014.08.109.
195. Childs BG, Li H, van Deursen JM. Senescent cells: a therapeutic target for cardiovascular disease. *J Clin Invest* 128: 1217–1228, 2018. doi:10.1172/JCI95146.
196. Abbas M, Jesel L, Auger C, Amoura L, Messas N, Manin G, Rumig C, León-González AJ, Ribeiro TP, Silva GC, Abou-Merhi R, Hamade E, Hecker M, Georg Y, Chakfe N, Ohlmann P, Schini-Kerth VB, Toti F, Morel O. Endothelial microparticles from acute coronary syndrome patients induce premature coronary artery endothelial cell aging and thrombogenicity: role of the Ang II/AT1 receptor/NADPH oxidase-mediated activation of MAPKs and PI3-kinase pathways. *Circulation* 135: 280–296, 2017. doi:10.1161/CIRCULATIONAHA.116.017513.
197. Regina C, Panatta E, Candi E, Melino G, Amelio I, Balistreri CR, Annicchiarico-Petruzzelli M, Di Daniele N, Ruvolo G. Vascular ageing and endothelial cell senescence: molecular mechanisms of physiology and diseases. *Mech Ageing Dev* 159: 14–21, 2016. doi:10.1016/j.mad.2016.05.003.
198. Alam P, Haile B, Arif M, Pandey R, Rokvic M, Nieman M, Maliken BD, Paul A, Wang YG, Sadayappan S, Ahmed RP, Kanisicak O. Inhibition of senescence-associated genes Rb1 and Meis2 in adult cardiomyocytes results in cell cycle reentry and cardiac repair post-myocardial infarction. *J Am Heart Assoc* 8: e012089, 2019. doi:10.1161/JAHA.119.012089.
199. Levi N, Papismadov N, Solomonov I, Sagi I, Krizhanovsky V. The ECM path of senescence in aging: components and modifiers. *FEBS J* 287: 2636–2646, 2020. doi:10.1111/febs.15282.
200. Heusch G. Myocardial ischaemia-reperfusion injury and cardioprotection in perspective. *Nat Rev Cardiol* 17: 773–789, 2020. doi:10.1038/s41569-020-0403-y.
201. Dookun E, Walaszczyk A, Redgrave R, Palmowski P, Tual-Chalot S, Suwana A, Chapman J, Jirkovsky E, Donastorg Sosa L, Gill E, Yausep OE, Santin Y, Mialet-Perez J, Andrew Owens W, Grieve D, Spyridopoulos I, Taggart M, Arthur HM, Passos JF, Richardson GD. Clearance of senescent cells during cardiac ischemia-reperfusion injury improves recovery. *Ageing Cell* 19: e13249, 2020. doi:10.1111/ace1.13249.
202. Borden A, Kurian J, Nickoloff E, Yang Y, Troupes CD, Ibeti J, Lucchese AM, Gao E, Mohsin S, Koch WJ, Houser SR, Kishore R, Khan M. Transient introduction of miR-294 in the heart promotes cardiomyocyte cell cycle reentry after injury. *Circ Res* 125: 14–25, 2019. doi:10.1161/CIRCRESAHA.118.314223.
203. Saheera S, Potnuri AG, Nair RR. Modulation of cardiac stem cell characteristics by metoprolol in hypertensive heart disease. *Hypertens Res* 41: 253–262, 2018. doi:10.1038/s41440-018-0015-7.
204. Staerk L, Sherer JA, Ko D, Benjamin EJ, Helm RH. Atrial fibrillation: epidemiology, pathophysiology, and clinical outcomes. *Circ Res* 120: 1501–1517, 2017. doi:10.1161/CIRCRESAHA.117.309732.
205. Virani SS, Alonso A, Aparicio HJ, Benjamin EJ, Bittencourt MS, Callaway CW, et al. Heart Disease and Stroke Statistics—2021 Update: a report from the American Heart Association. *Circulation* 143: e254–e743, 2021. doi:10.1161/CIR.0000000000000950.
206. Feghaly J, Zakka P, London B, MacRae CA, Refaat MM. Genetics of atrial fibrillation. *J Am Heart Assoc* 7: e009884, 2018. doi:10.1161/JAHA.118.009884.
207. Oyen N, Ranthe MF, Carstensen L, Boyd HA, Olesen MS, Olesen SP, Wohlfahrt J, Melbye M. Familial aggregation of lone atrial fibrillation in young persons. *J Am Coll Cardiol* 60: 917–921, 2012. doi:10.1016/j.jacc.2012.03.046.

208. Nielsen JB, Thorolfsdottir RB, Fritsche LG, Zhou W, Skov MW, Graham SE, et al. Biobank-driven genomic discovery yields new insight into atrial fibrillation biology. **Nat Genet** 50: 1234–1239, 2018. doi:10.1038/s41588-018-0171-3.
209. Roselli C, Chaffin MD, Weng LC, Aeschbacher S, Ahlberg G, Albert CM, et al. Multi-ethnic genome-wide association study for atrial fibrillation. **Nat Genet** 50: 1225–1233, 2018. doi:10.1038/s41588-018-0133-9.
210. Gudbjartsson DF, Arnar DO, Helgadóttir A, Gretarsdóttir S, Holm H, Sigurdsson A, et al. Variants conferring risk of atrial fibrillation on chromosome 4q25. **Nature** 448: 353–357, 2007. doi:10.1038/nature06007.
211. Lubitz SA, Lunetta KL, Lin H, Arking DE, Trompet S, Li G, et al. Novel genetic markers associate with atrial fibrillation risk in Europeans and Japanese. **J Am Coll Cardiol** 63: 1200–1210, 2014. doi:10.1016/j.jacc.2013.12.015.
212. Syeda F, Kirchhof P, Fabritz L. PITX2-dependent gene regulation in atrial fibrillation and rhythm control. **J Physiol** 595: 4019–4026, 2017. doi:10.1113/JP273123.
213. Kirchhof P, Kahr PC, Kaese S, Piccini I, Vokshi I, Scheld HH, Rotering H, Fortmueller L, Laakmann S, Verheule S, Schotten U, Fabritz L, Brown NA. PITX2c is expressed in the adult left atrium, and reducing Pitx2c expression promotes atrial fibrillation inducibility and complex changes in gene expression. **Circ Cardiovasc Genet** 4: 123–133, 2011. doi:10.1161/CIRCGENETICS.110.958058.
214. Wang J, Klysis E, Sood S, Johnson RL, Wehrens XH, Martin JF. Pitx2 prevents susceptibility to atrial arrhythmias by inhibiting left-sided pacemaker specification. **Proc Natl Acad Sci USA** 107: 9753–9758, 2010. doi:10.1073/pnas.0912585107.
215. Tao G, Kahr PC, Morikawa Y, Zhang M, Rahmani M, Heallen TR, Li L, Sun Z, Olson EN, Amendt BA, Martin JF. Pitx2 promotes heart repair by activating the antioxidant response after cardiac injury. **Nature** 534: 119–123, 2016. doi:10.1038/nature17959.
216. L'honoré A, Commère PH, Negroni E, Pallafacchina G, Friguet B, Drouin J, Buckingham M, Montarras D. The role of Pitx2 and Pitx3 in muscle stem cells gives new insights into P38alpha MAP kinase and redox regulation of muscle regeneration. **Elife** 7: e32991, 2018. doi:10.7554/eLife.32991.
217. Deschênes M, Chabot B. The emerging role of alternative splicing in senescence and aging. **Aging Cell** 16: 918–933, 2017. doi:10.1111/ace1.12646.
218. Dlamini Z, Tshidino SC, Hull R. Abnormalities in alternative splicing of apoptotic genes and cardiovascular diseases. **Int J Mol Sci** 16: 27171–27190, 2015. doi:10.3390/ijms161126017.
219. Pang PD, Alsina KM, Cao S, Koushik AB, Wehrens XH, Cooper TA. CRISPR-mediated expression of the fetal Scn5a isoform in adult mice causes conduction defects and arrhythmias. **J Am Heart Assoc** 7: e010393, 2018. doi:10.1161/JAHA.118.010393.
220. Denil SL, Rietzschel ER, De Buyzere ML, Van Daele CM, Segers P, De Bacquer D, Van Criekinge W, Bekaert S, Gillebert TC, De Meyer T; Asklepios Investigators. On cross-sectional associations of leukocyte telomere length with cardiac systolic, diastolic and vascular function: the Asklepios study. **PLoS One** 9: e115071, 2014. doi:10.1371/journal.pone.0115071.
221. Xie J, Chen Y, Hu C, Pan Q, Wang B, Li X, Geng J, Xu B. Premature senescence of cardiac fibroblasts and atrial fibrosis in patients with atrial fibrillation. **Oncotarget** 8: 57981–57990, 2017. doi:10.18632/oncotarget.19853.
222. Révész D, Verhoeven JE, Milaneschi Y, de Geus EJ, Wolkowitz OM, Penninx BW. Dysregulated physiological stress systems and accelerated cellular aging. **Neurobiol Aging** 35: 1422–1430, 2014. doi:10.1016/j.neurobiolaging.2013.12.027.
223. Carlquist JF, Knight S, Cawthon RM, Le VT, Jared Bunch T, Horne BD, Rollo JS, Huntinghouse JA, Brent Muhlestein J, Anderson JL. Shortened telomere length is associated with paroxysmal atrial fibrillation among cardiovascular patients enrolled in the Intermountain Heart Collaborative Study. **Heart Rhythm** 13: 21–27, 2016. doi:10.1016/j.hrthm.2015.07.032.
224. Batlle M, Calvo N, Castellà M, Sandoval E, Ramos P, Pérez-Villa F, Ramírez J, Guasch E, Brugada J, Mont L. Structural and senescence changes in the atria of lone atrial fibrillation patients (Abstract). **Circulation** 132: A17987, 2015. doi:10.1161/circ.132.suppl_3.17987.
225. Lim HS, Willoughby SR, Schultz C, Gan C, Alasady M, Lau DH, Leong DP, Brooks AG, Young GD, Kistler PM, Kalman JM, Worthley MI, Sanders P. Effect of atrial fibrillation on atrial thrombogenesis in humans: impact of rate and rhythm. **J Am Coll Cardiol** 61: 852–860, 2013. doi:10.1016/j.jacc.2012.11.046.
226. Jesel L, Abbas M, Toti F, Cohen A, Arentz T, Morel O. Microparticles in atrial fibrillation: a link between cell activation or apoptosis, tissue remodelling and thrombogenicity. **Int J Cardiol** 168: 660–669, 2013. doi:10.1016/j.ijcard.2013.03.031.
227. Watson T, Shantsila E, Lip GY. Mechanisms of thrombogenesis in atrial fibrillation: Virchow's triad revisited. **Lancet** 373: 155–166, 2009. doi:10.1016/S0140-6736(09)60040-4.
228. Burger D, Kwart DG, Montezano AC, Read NC, Kennedy CR, Thompson CS, Touyz RM. Microparticles induce cell cycle arrest through redox-sensitive processes in endothelial cells: implications in vascular senescence. **J Am Heart Assoc** 1: e001842, 2012. doi:10.1161/JAHA.112.001842.
229. Morel O, Toti F, Hugel B, Bakouboula B, Camoin-Jau L, Dignat-George F, Freyssinet JM. Procoagulant microparticles: disrupting the vascular homeostasis equation? **Arterioscler Thromb Vasc Biol** 26: 2594–2604, 2006. doi:10.1161/01.ATV.0000246775.14471.26.
230. Hasan H, Park SH, Auger C, Belcastro E, Matsushita K, Marchandot B, Lee HH, Qureshi AW, Kauffenstein G, Ohlmann P, Schini-Kerth VB, Jesel L, Morel O. Thrombin induces angiotensin II-mediated senescence in atrial endothelial cells: impact on pro-remodeling patterns. **J Clin Med** 8: 1570, 2019. doi:10.3390/jcm8101570.
231. Jumeau C, Rupin A, Chieng-Yane P, Mougnot N, Zahr N, David-Duflho M, Hatem SN. Direct thrombin inhibitors prevent left atrial remodeling associated with heart failure in rats. **JACC Basic Transl Sci** 1: 328–339, 2016. doi:10.1016/j.jacpts.2016.05.002.
232. Triana-Martínez F, Picallos-Rabina P, Da Silva-Álvarez S, Pietrocola F, Llanos S, Rodilla V, Soprano E, Pedrosa P, Ferreirós A, Barradas M, Hernandez-Gonzalez F, Lalinde M, Prats N, Bernado C, Gonzalez P, Gomez M, Ikonopoulou MP, Fernandez-Marcos PJ, Garcia-Caballero T, Del Pino P, Arribas J, Vidal A, Gonzalez-Barcia M, Serrano M, Loza MI, Dominguez E, Collado M. Identification and characterization of cardiac glycosides as senolytic compounds. **Nat Commun** 10: 4731, 2019. doi:10.1038/s41467-019-12888-x.
233. Lai LP, Tsai CC, Su MJ, Lin JL, Chen YS, Tseng YZ, Huang SK. Atrial fibrillation is associated with accumulation of aging-related common type mitochondrial DNA deletion mutation in human atrial tissue. **Chest** 123: 539–544, 2003. doi:10.1378/chest.123.2.539.
234. Montaigne D, Marechal X, Lefebvre P, Modine T, Fayad G, Dehondt H, Hurt C, Coisne A, Koussa M, Remy-Jouet I, Zerimech F, Boulanger E, Lacroix D, Staels B, Neviere R. Mitochondrial

- dysfunction as an arrhythmogenic substrate: a translational proof-of-concept study in patients with metabolic syndrome in whom post-operative atrial fibrillation develops. **J Am Coll Cardiol** 62: 1466–1473, 2013. doi:10.1016/j.jacc.2013.03.061.
235. Lin PH, Lee SH, Su CP, Wei YH. Oxidative damage to mitochondrial DNA in atrial muscle of patients with atrial fibrillation. **Free Radic Biol Med** 35: 1310–1318, 2003. doi:10.1016/j.freeradbiomed.2003.07.002.
236. Braunwald E. Cardiomyopathies: an overview. **Circ Res** 121: 711–721, 2017. doi:10.1161/CIRCRESAHA.117.311812.
237. McKenna WJ, Maron BJ, Thiene G. Classification, epidemiology, and global burden of cardiomyopathies. **Circ Res** 121: 722–730, 2017. doi:10.1161/CIRCRESAHA.117.309711.
238. Marian AJ, Braunwald E. Hypertrophic cardiomyopathy: genetics, pathogenesis, clinical manifestations, diagnosis, and therapy. **Circ Res** 121: 749–770, 2017. doi:10.1161/CIRCRESAHA.117.311059.
239. Authors/Task Force members, Elliott PM, Anastakis A, Borggreve M, Borggrefe M, Cecchi F, Charron P, Hagege AA, Lafont A, Limongelli G, Mahrholdt H, McKenna WJ, Mogensen J, Nihoyannopoulos P, Nistri S, Pieper PG, Pieske B, Rapezzi C, Rutten FH, Tillmanns C, Watkins H. 2014 ESC Guidelines on diagnosis and management of hypertrophic cardiomyopathy: the Task Force for the Diagnosis and Management of Hypertrophic Cardiomyopathy of the European Society of Cardiology (ESC). **Eur Heart J** 35: 2733–2779, 2014. doi:10.1093/eurheartj/ehu284.
240. Predmore JM, Wang P, Davis F, Bartolone S, Westfall MV, Dyke DB, Pagani F, Powell SR, Day SM. Ubiquitin proteasome dysfunction in human hypertrophic and dilated cardiomyopathies. **Circulation** 121: 997–1004, 2010. doi:10.1161/CIRCULATIONAHA.109.904557.
241. Sharifi-Sanjani M, Oyster NM, Tichy ED, Bedi KC Jr, Harel O, Margulies KB, Mourkioti F. Cardiomyocyte-specific telomere shortening is a distinct signature of heart failure in humans. **J Am Heart Assoc** 6: e005086, 2017. doi:10.1161/JAHA.116.005086.
242. Chatterjee S, de Gonzalo-Calvo D, Derda AA, Schimmel K, Sonnenschein K, Bavendiek U, Bauersachs J, Bär C, Thum T. Leukocyte telomere length correlates with hypertrophic cardiomyopathy severity. **Sci Rep** 8: 11227, 2018. doi:10.1038/s41598-018-29072-8.
243. Payne RM, Wagner GR. Cardiomyopathy in Friedreich ataxia: clinical findings and research. **J Child Neurol** 27: 1179–1186, 2012. doi:10.1177/0883073812448535.
244. Wong AO, Wong G, Shen M, Chow MZ, Tse WW, Gurung B, Mak SY, Lieu DK, Costa KD, Chan CW, Martelli A, Nabhan JF, Li RA. Correlation between frataxin expression and contractility revealed by in vitro Friedreich's ataxia cardiac tissue models engineered from human pluripotent stem cells. **Stem Cell Res Ther** 10: 203, 2019. doi:10.1186/s13287-019-1305-y.
245. Bolinches-Amorós A, Mollá B, Pla-Martín D, Palau F, Gonzalez-Cabo P. Mitochondrial dysfunction induced by frataxin deficiency is associated with cellular senescence and abnormal calcium metabolism. **Front Cell Neurosci** 8: 124, 2014. doi:10.3389/fncel.2014.00124.
246. Culley MK, Zhao J, Tai YY, Tang Y, Perk D, Negi V, Yu Q, Woodcock CC, Handen A, Speyer G, Kim S, Lai YC, Satoh T, Watson AM, Aaraj YA, Sembrat J, Rojas M, Goncharov D, Goncharova EA, Khan OF, Anderson DG, Dahlman JE, Gurkar AU, Lafyatis R, Fayyaz AU, Redfield MM, Gladwin MT, Rabinovitch M, Gu M, Bertero T, Chan SY. Frataxin deficiency promotes endothelial senescence in pulmonary hypertension. **J Clin Invest** 131: e136459, 2021. doi:10.1172/JCI136459.
247. Merner ND, Hodgkinson KA, Haywood AF, Connors S, French VM, Drenckhahn JD, Kupprion C, Ramadanova K, Thierfelder L, McKenna W, Gallagher B, Morris-Larkin L, Bassett AS, Parfrey PS, Young TL. Arrhythmogenic right ventricular cardiomyopathy type 5 is a fully penetrant, lethal arrhythmic disorder caused by a missense mutation in the TMEM43 gene. **Am J Hum Genet** 82: 809–821, 2008. doi:10.1016/j.ajhg.2008.01.010.
248. Rouhi L, Cheedipudi SM, Chen SN, Fan S, Lombardi R, Chen X, Coarfa C, Robertson MJ, Gurha P, Marian AJ. Haploinsufficiency of Tmem43 in cardiac myocytes activates the DNA damage response pathway leading to a late-onset senescence-associated pro-fibrotic cardiomyopathy. **Cardiovasc Res** 117: 2377–2394, 2021. doi:10.1093/cvr/cvaa300.
249. Abdelfatah N, Chen R, Duff HJ, Seifer CM, Buffo I, Huculak C, Clarke S, Clegg R, Jassal DS, Gordon PM, Ober C, Frosk P, Gerull B; Care4Rare Canada Consortium. Characterization of a unique form of arrhythmic cardiomyopathy caused by recessive mutation in LEMD2. **JACC Basic Transl Sci** 4: 204–221, 2019. doi:10.1016/j.jacbts.2018.12.001.
250. Tan Y, Zhang Z, Zheng C, Wintergerst KA, Keller BB, Cai L. Mechanisms of diabetic cardiomyopathy and potential therapeutic strategies: preclinical and clinical evidence. **Nat Rev Cardiol** 17: 585–607, 2020. doi:10.1038/s41569-020-0339-2.
251. Jia G, Hill MA, Sowers JR. Diabetic cardiomyopathy: an update of mechanisms contributing to this clinical entity. **Circ Res** 122: 624–638, 2018. doi:10.1161/CIRCRESAHA.117.311586.
252. Wang X, Huang W, Liu G, Cai W, Millard RW, Wang Y, Chang J, Peng T, Fan GC. Cardiomyocytes mediate anti-angiogenesis in type 2 diabetic rats through the exosomal transfer of miR-320 into endothelial cells. **J Mol Cell Cardiol** 74: 139–150, 2014. doi:10.1016/j.yjmcc.2014.05.001.
253. Rota M, LeCapitaine N, Hosoda T, Boni A, De Angelis A, Padin-Iruegas ME, Esposito G, Vitale S, Urbanek K, Casarsa C, Giorgio M, Lüscher TF, Pellicci PG, Anversa P, Leri A, Kajstura J. Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by deletion of the p66shc gene. **Circ Res** 99: 42–52, 2006. doi:10.1161/01.RES.0000231289.63468.08.
254. Marino F, Scalise M, Salerno N, Salerno L, Molinaro C, Cappetta D, Torella M, Greco M, Foti D, Sasso FC, Mastroberto P, De Angelis A, Ellison-Hughes GM, Sampaolesi M, Rota M, Rossi F, Urbanek K, Nadal-Ginard B, Torella D, Cianflone E. Diabetes-induced cellular senescence and senescence-associated secretory phenotype impair cardiac regeneration and function independently of age. **Diabetes** 71: 1081–1098, 2022. doi:10.2337/db21-0536.
255. Satthenapalli VR, Lamberts RR, Katare RG. Concise review: challenges in regenerating the diabetic heart: a comprehensive review. **Stem Cells** 35: 2009–2026, 2017. doi:10.1002/stem.2661.
256. Pappaspyropoulos A, Lagopati N, Mourkioti I, Angelopoulou A, Kyriazis S, Lontos M, Gorgoulis V, Kotsinas A. Regulatory and functional involvement of long non-coding RNAs in DNA double-strand break repair mechanisms. **Cells** 10: 1506, 2021. doi:10.3390/cells10061506.
257. Gu J, Wang S, Guo H, Tan Y, Liang Y, Feng A, Liu Q, Damodaran C, Zhang Z, Keller BB, Zhang C, Cai L. Inhibition of p53 prevents diabetic cardiomyopathy by preventing early-stage apoptosis and cell senescence, reduced glycolysis, and impaired angiogenesis. **Cell Death Dis** 9: 82, 2018. doi:10.1038/s41419-017-0093-5.
258. Henson SM, Aksentijevic D. Senescence and type 2 diabetic cardiomyopathy: how young can you die of old age? **Front Pharmacol** 12: 716517, 2021. doi:10.3389/fphar.2021.716517.

259. Shakeri H, Lemmens K, Gevaert AB, De Meyer GR, Segers VF. Cellular senescence links aging and diabetes in cardiovascular disease. **Am J Physiol Heart Circ Physiol** 315: H448–H462, 2018. doi:10.1152/ajpheart.00287.2018.
260. Abdelgawad IY, Sadak KT, Lone DW, Dabour MS, Niedernhofer LJ, Zordoky BN. Molecular mechanisms and cardiovascular implications of cancer therapy-induced senescence. **Pharmacol Ther** 221: 107751, 2021. doi:10.1016/j.pharmthera.2020.107751.
261. Chatterjee S, Hofer T, Costa A, Lu D, Batkai S, Gupta SK, Bolesani E, Zweigerdt R, Megias D, Streckfuss-Bömeke K, Brandenberger C, Thum T, Bär C. Telomerase therapy attenuates cardiotoxic effects of doxorubicin. **Mol Ther** 29: 1395–1410, 2021. doi:10.1016/j.ymthe.2020.12.035.
262. Mitry MA, Laurent D, Keith BL, Sira E, Eisenberg CA, Eisenberg LM, Joshi S, Gupte S, Edwards JG. Accelerated cardiomyocyte senescence contributes to late-onset doxorubicin-induced cardiotoxicity. **Am J Physiol Cell Physiol** 318: C380–C391, 2020. doi:10.1152/ajpcell.00073.2019.
263. Russo M, Bono E, Ghigo A. The interplay between autophagy and senescence in anthracycline cardiotoxicity. **Curr Heart Fail Rep** 18: 180–190, 2021. doi:10.1007/s11897-021-00519-w.
264. Demaria M, O’Leary MN, Chang J, Shao L, Liu S, Alimirah F, Koenig K, Le C, Mitin N, Deal AM, Alston S, Academia EC, Kilmarx S, Valdovinos A, Wang B, de Bruin A, Kennedy BK, Melov S, Zhou D, Sharpless NE, Muss H, Campisi J. Cellular senescence promotes adverse effects of chemotherapy and cancer relapse. **Cancer Discov** 7: 165–176, 2017. doi:10.1158/2159-8290.CD-16-0241.
265. Ghosh AK, Rai R, Park KE, Eren M, Miyata T, Wilsbacher LD, Vaughan DE. A small molecule inhibitor of PAI-1 protects against doxorubicin-induced cellular senescence. **Oncotarget** 7: 72443–72457, 2016. doi:10.18632/oncotarget.12494.
266. Zhang S, Wu P, Liu J, Du Y, Yang Z. Roflumilast attenuates doxorubicin-induced cardiotoxicity by targeting inflammation and cellular senescence in cardiomyocytes mediated by SIRT1. **Drug Des Devel Ther** 15: 87–97, 2021. doi:10.2147/DDDT.S269029.
267. Vasileiou PVS, Siasos G, Gorgoulis VG. Molecular biomarkers in cardio-oncology: where we stand and where we are heading. **Bioessays** 44: e2100234, 2022. doi:10.1002/bies.202100234.
268. Baker DJ, Childs BG, Durik M, Wijers ME, Sieben CJ, Zhong J, Saltness RA, Jeganathan KB, Verzosa GC, Pezeshki A, Khazaie K, Miller JD, van Deursen JM. Naturally occurring p16(Ink4a)-positive cells shorten healthy lifespan. **Nature** 530: 184–189, 2016. doi:10.1038/nature16932.
269. He S, Sharpless NE. Senescence in health and disease. **Cell** 169: 1000–1011, 2017. doi:10.1016/j.cell.2017.05.015.
270. Baker DJ, Wijshake T, Tchkonja T, LeBrasseur NK, Childs BG, van de Sluis B, Kirkland JL, van Deursen JM. Clearance of p16Ink4a-positive senescent cells delays ageing-associated disorders. **Nature** 479: 232–236, 2011. doi:10.1038/nature10600.
271. Demaria M, Ohtani N, Youssef SA, Rodier F, Toussaint W, Mitchell JR, Laberge RM, Vijg J, Van Steeg H, Dollé ME, Hoeijmakers JH, de Bruin A, Hara E, Campisi J. An essential role for senescent cells in optimal wound healing through secretion of PDGF-AA. **Dev Cell** 31: 722–733, 2014. doi:10.1016/j.devcel.2014.11.012.
272. Owens WA, Walaszczyk A, Spyridopoulos I, Dookun E, Richardson GD. Senescence and senolytics in cardiovascular disease: promise and potential pitfalls. **Mech Ageing Dev** 198: 111540, 2021. doi:10.1016/j.mad.2021.111540.
273. Song S, Lam EW, Tchkonja T, Kirkland JL, Sun Y. Senescent cells: emerging targets for human aging and age-related diseases. **Trends Biochem Sci** 45: 578–592, 2020. doi:10.1016/j.tibs.2020.03.008.
274. Childs BG, Gluscevic M, Baker DJ, Laberge RM, Marquess D, Dananberg J, van Deursen JM. Senescent cells: an emerging target for diseases of ageing. **Nat Rev Drug Discov** 16: 718–735, 2017. doi:10.1038/nrd.2017.116.
275. Evangelou K, Gorgoulis VG. Senescence. In: **Encyclopedia of Molecular Pharmacology**, edited by Offermanns S, Rosenthal W. Cham, Switzerland: Springer International Publishing, 2020, p. 1–12.
276. Xu M, Pirtskhalava T, Farr JN, Weigand BM, Palmer AK, Weivoda MM, et al. Senolytics improve physical function and increase lifespan in old age. **Nat Med** 24: 1246–1256, 2018. doi:10.1038/s41591-018-0092-9.
277. Soto-Gamez A, Quax WJ, Demaria M. Regulation of survival networks in senescent cells: from mechanisms to interventions. **J Mol Biol** 431: 2629–2643, 2019. doi:10.1016/j.jmb.2019.05.036.
278. Lozano-Torres B, Estepa-Fernández A, Rovira M, Orzáez M, Serrano M, Martínez-Mañez R, Sancón F. The chemistry of senescence. **Nat Rev Chem** 3: 426–441, 2019. doi:10.1038/s41570-019-0108-0.
279. Robbins PD, Jurk D, Khosla S, Kirkland JL, LeBrasseur NK, Miller JD, Passos JF, Pignolo RJ, Tchkonja T, Niedernhofer LJ. Senolytic drugs: reducing senescent cell viability to extend health span. **Annu Rev Pharmacol Toxicol** 61: 779–803, 2021. doi:10.1146/annurev-pharmtox-050120-105018.
280. Zhu Y, Tchkonja T, Pirtskhalava T, Gower AC, Ding H, Giorgadze N, et al. The Achilles’ heel of senescent cells: from transcriptome to senolytic drugs. **Ageing Cell** 14: 644–658, 2015. doi:10.1111/accel.12344.
281. Kirkland JL, Tchkonja T. Senolytic drugs: from discovery to translation. **J Intern Med** 288: 518–536, 2020. doi:10.1111/joim.13141.
282. Paez-Ribes M, González-Gualda E, Doherty GJ, Muñoz-Espín D. Targeting senescent cells in translational medicine. **EMBO Mol Med** 11: e10234, 2019. doi:10.15252/emmm.201810234.
283. Kirkland JL, Tchkonja T. Cellular senescence: a translational perspective. **EBioMedicine** 21: 21–28, 2017. doi:10.1016/j.ebiom.2017.04.013.
284. Kirkland JL, Tchkonja T, Zhu Y, Niedernhofer LJ, Robbins PD. The clinical potential of senolytic drugs. **J Am Geriatr Soc** 65: 2297–2301, 2017. doi:10.1111/jgs.14969.
285. Roos CM, Zhang B, Palmer AK, Ogronnik MB, Pirtskhalava T, Thalji NM, Hagler M, Jurk D, Smith LA, Casaclang-Verzosa G, Zhu Y, Schafer MJ, Tchkonja T, Kirkland JL, Miller JD. Chronic senolytic treatment alleviates established vasomotor dysfunction in aged or atherosclerotic mice. **Ageing Cell** 15: 973–977, 2016. doi:10.1111/accel.12458.
286. Singh R, Letai A, Sarosiek K. Regulation of apoptosis in health and disease: the balancing act of BCL-2 family proteins. **Nat Rev Mol Cell Biol** 20: 175–193, 2019. doi:10.1038/s41580-018-0089-8.
287. Zhu Y, Zhang YJ, Liu WW, Shi AW, Gu N. Salidroside suppresses HUVECs cell injury induced by oxidative stress through activating the Nrf2 signaling pathway. **Molecules** 21: 1033, 2016. doi:10.3390/molecules21081033.
288. Walaszczyk A, Dookun E, Redgrave R, Tual-Chalot S, Victorelli S, Spyridopoulos I, Owens A, Arthur HM, Passos JF, Richardson GD.

- Pharmacological clearance of senescent cells improves survival and recovery in aged mice following acute myocardial infarction. **Aging Cell** 18: e12945, 2019. doi:10.1111/ace1.12945.
289. Guerrero A, Herranz N, Sun B, Wagner V, Gallage S, Guiho R, Wolter K, Pombo J, Irvine EE, Innes AJ, Birch J, Glegola J, Manshaei S, Heide D, Dharmalingam G, Harbig J, Olona A, Behmoaras J, Dauch D, Uren AG, Zender L, Vernia S, Martínez-Barbera JP, Heikenwalder M, Withers DJ, Gil J. Cardiac glycosides are broad-spectrum senolytics. **Nat Metab** 1: 1074–1088, 2019. doi:10.1038/s42255-019-0122-z.
 290. Martin N, Soriani O, Bernard D. Cardiac glycosides as senolytic compounds. **Trends Mol Med** 26: 243–245, 2020. doi:10.1016/j.molmed.2020.01.001.
 291. Fuhrmann-Stroissnigg H, Ling YY, Zhao J, McGowan SJ, Zhu Y, Brooks RW, Grassi D, Gregg SQ, Stripay JL, Dorronsoro A, Corbo L, Tang P, Bukata C, Ring N, Giacca M, Li X, Tchkonja T, Kirkland JL, Niedernhofer LJ, Robbins PD. Identification of HSP90 inhibitors as a novel class of senolytics. **Nat Commun** 8: 422, 2017. doi:10.1038/s41467-017-00314-z.
 292. Lazaro I, Oguiza A, Recio C, Mallavia B, Madrigal-Matute J, Blanco J, Egido J, Martin-Ventura JL, Gomez-Guerrero C. Targeting HSP90 ameliorates nephropathy and atherosclerosis through suppression of NF-kappaB and STAT signaling pathways in diabetic mice. **Diabetes** 64: 3600–3613, 2015. doi:10.2337/db14-1926.
 293. Gardner SE, Humphry M, Bennett MR, Clarke MC. Senescent vascular smooth muscle cells drive inflammation through an interleukin-1alpha-dependent senescence-associated secretory phenotype. **Arterioscler Thromb Vasc Biol** 35: 1963–1974, 2015. doi:10.1161/ATVBAHA.115.305896.
 294. Chakraborty S, Galla S, Cheng X, Yeo JY, Mell B, Singh V, Yeoh B, Saha P, Mathew AV, Vijay-Kumar M, Joe B. Salt-responsive metabolite, beta-hydroxybutyrate, attenuates hypertension. **Cell Rep** 25: 677–689.e4, 2018. doi:10.1016/j.celrep.2018.09.058.
 295. Han YM, Bedarida T, Ding Y, Somba BK, Lu Q, Wang Q, Song P, Zou MH. beta-Hydroxybutyrate prevents vascular senescence through hnRNP A1-mediated upregulation of Oct4. **Mol Cell** 71: 1064–1078.e5, 2018. doi:10.1016/j.molcel.2018.07.036.
 296. Barzilai N, Crandall JP, Kritchevsky SB, Espeland MA. Metformin as a tool to target aging. **Cell Metab** 23: 1060–1065, 2016. doi:10.1016/j.cmet.2016.05.011.
 297. Flynn JM, O’Leary MN, Zambataro CA, Academia EC, Presley MP, Garrett BJ, Zykovich A, Mooney SD, Strong R, Rosen CJ, Kapahi P, Nelson MD, Kennedy BK, Melov S. Late-life rapamycin treatment reverses age-related heart dysfunction. **Aging Cell** 12: 851–862, 2013. doi:10.1111/ace1.12109.
 298. Singh M, Jensen MD, Lerman A, Kushwaha S, Rihal CS, Gersh BJ, Behfar A, Tchkonja T, Thomas RJ, Lennon RJ, Keenan LR, Moore AG, Kirkland JL. Effect of low-dose rapamycin on senescence markers and physical functioning in older adults with coronary artery disease: results of a pilot study. **J Frailty Aging** 5: 204–207, 2016. doi:10.14283/jfa.2016.112.
 299. Yin M, van der Horst IC, van Melle JP, Qian C, van Gilst WH, Silljé HH, de Boer RA. Metformin improves cardiac function in a nondiabetic rat model of post-MI heart failure. **Am J Physiol Heart Circ Physiol** 301: H459–H468, 2011. doi:10.1152/ajpheart.00054.2011.
 300. Evangelisti C, Cenni V, Lattanzi G. Potential therapeutic effects of the MTOR inhibitors for preventing ageing and progeria-related disorders. **Br J Clin Pharmacol** 82: 1229–1244, 2016. doi:10.1111/bcp.12928.
 301. Walters HE, Deneka-Hannemann S, Cox LS. Reversal of phenotypes of cellular senescence by pan-mTOR inhibition. **Aging (Albany NY)** 8: 231–244, 2016. doi:10.18632/aging.100872.
 302. Nafisa A, Gray SG, Cao Y, Wang T, Xu S, Wattoo FH, Barras M, Cohen N, Kamato D, Little PJ. Endothelial function and dysfunction: impact of metformin. **Pharmacol Ther** 192: 150–162, 2018. doi:10.1016/j.pharmthera.2018.07.007.
 303. Nesti L, Natali A. Metformin effects on the heart and the cardiovascular system: a review of experimental and clinical data. **Nutr Metab Cardiovasc Dis** 27: 657–669, 2017. doi:10.1016/j.numecd.2017.04.009.
 304. Torimoto K, Okada Y, Tanaka Y. [Type 2 diabetes and vascular endothelial dysfunction]. **J UOEH** 40: 65–75, 2018. doi:10.7888/juoeh.40.65.
 305. Gager GM, von Lewinski D, Sourij H, Jilma B, Eyileten C, Filipiak K, Hülsmann M, Kubica J, Postula M, Siller-Matula JM. Effects of SGLT2 inhibitors on ion homeostasis and oxidative stress associated mechanisms in heart failure. **Biomed Pharmacother** 143: 112169, 2021. doi:10.1016/j.biopha.2021.112169.
 306. Bennaceur K, Atwill M, Al Zhrany N, Hoffmann J, Keavney B, Breault D, Richardson G, von Zglinicki T, Saretzki G, Spyridopoulos I. Atorvastatin induces T cell proliferation by a telomerase reverse transcriptase (TERT) mediated mechanism. **Atherosclerosis** 236: 312–320, 2014. doi:10.1016/j.atherosclerosis.2014.07.020.
 307. Gurău F, Baldoni S, Prattichizzo F, Espinosa E, Amenta F, Procopio AD, Albertini MC, Bonafè M, Olivieri F. Anti-senescence compounds: a potential nutraceutical approach to healthy aging. **Ageing Res Rev** 46: 14–31, 2018. doi:10.1016/j.arr.2018.05.001.
 308. Mattison JA, Wang M, Bernier M, Zhang J, Park SS, Maudsley S, An SS, Santhanam L, Martin B, Faulkner S, Morrell C, Baur JA, Peshkin L, Sosnowska D, Csiszar A, Herbert RL, Tilmont EM, Ungvari Z, Pearson KJ, Lakatta EG, de Cabo R. Resveratrol prevents high fat/sucrose diet-induced central arterial wall inflammation and stiffening in nonhuman primates. **Cell Metab** 20: 183–190, 2014. doi:10.1016/j.cmet.2014.04.018.
 309. Chen HZ, Wang F, Gao P, Pei JF, Liu Y, Xu TT, Tang X, Fu WY, Lu J, Yan YF, Wang XM, Han L, Zhang ZQ, Zhang R, Zou MH, Liu DP. Age-associated Sirtuin 1 reduction in vascular smooth muscle links vascular senescence and inflammation to abdominal aortic aneurysm. **Circ Res** 119: 1076–1088, 2016. doi:10.1161/CIRCRESAHA.116.308895.
 310. Imai S, Guarente L. NAD⁺ and sirtuins in aging and disease. **Trends Cell Biol** 24: 464–471, 2014. doi:10.1016/j.tcb.2014.04.002.
 311. van der Veer E, Ho C, O’Neil C, Barbosa N, Scott R, Cregan SP, Pickering JG. Extension of human cell lifespan by nicotinamide phosphoribosyltransferase. **J Biol Chem** 282: 10841–10845, 2007. doi:10.1074/jbc.C700018200.
 312. Gao D, Zuo Z, Tian J, Ali Q, Lin Y, Lei H, Sun Z. Activation of SIRT1 attenuates Klotho deficiency-induced arterial stiffness and hypertension by enhancing AMP-activated protein kinase activity. **Hypertension** 68: 1191–1199, 2016. doi:10.1161/HYPERTENSIONAHA.116.07709.
 313. Sanada F, Muratsu J, Otsu R, Shimizu H, Koibuchi N, Uchida K, Taniyama Y, Yoshimura S, Rakugi H, Morishita R. Local production of activated factor X in atherosclerotic plaque induced vascular smooth muscle cell senescence. **Sci Rep** 7: 17172, 2017. doi:10.1038/s41598-017-17508-6.

314. Borissoff JI, Spronk HM, ten Cate H. The hemostatic system as a modulator of atherosclerosis. **N Engl J Med** 364: 1746–1760, 2011. doi:[10.1056/NEJMra1011670](https://doi.org/10.1056/NEJMra1011670).
315. Connolly SJ, Eikelboom JW, Bosch J, Dagenais G, Dyal L, Lanan F, et al. Rivaroxaban with or without aspirin in patients with stable coronary artery disease: an international, randomised, double-blind, placebo-controlled trial. **Lancet** 391: 205–218, 2018. doi:[10.1016/S0140-6736\(17\)32458-3](https://doi.org/10.1016/S0140-6736(17)32458-3).
316. Lutgens E, Atzler D, Döring Y, Duchene J, Steffens S, Weber C. Immunotherapy for cardiovascular disease. **Eur Heart J** 40: 3937–3946, 2019. doi:[10.1093/eurheartj/ehz283](https://doi.org/10.1093/eurheartj/ehz283).
317. Li T, Liang W, Xiao X, Qian Y. Nanotechnology, an alternative with promising prospects and advantages for the treatment of cardiovascular diseases. **Int J Nanomedicine** 13: 7349–7362, 2018. doi:[10.2147/IJN.S179678](https://doi.org/10.2147/IJN.S179678).
318. Muñoz-Espín D, Rovira M, Galiana I, Gimenez C, Lozano-Torres B, Paez-Ribes M, Llanos S, Chaib S, Munoz-Martin M, Uceró AC, Garaulet G, Mulero F, Dann SG, VanArsdale T, Shields DJ, Bernardos A, Murguía JR, Martínez-Manez R, Serrano M. A versatile drug delivery system targeting senescent cells. **EMBO Mol Med** 10: 9355, 2018. doi:[10.15252/emmm.201809355](https://doi.org/10.15252/emmm.201809355).
319. González-Gualda E, Pàez-Ribes M, Lozano-Torres B, Macías D, Wilson JR 3rd, Gonzalez-Lopez C, Ou HL, Miron-Barroso S, Zhang Z, Lerida-Viso A, Blandez JF, Bernardos A, Sancenon F, Rovira M, Fruk L, Martins CP, Serrano M, Doherty GJ, Martínez-Manez R, Muñoz-Espín D. Galacto-conjugation of Navitoclax as an efficient strategy to increase senolytic specificity and reduce platelet toxicity. **Aging Cell** 19: e13142, 2020. doi:[10.1111/acer.13142](https://doi.org/10.1111/acer.13142).
320. Guerrero A, Guiho R, Herranz N, Uren A, Withers DJ, Martínez-Barbera JP, Tietze LF, Gil J. Galactose-modified duocarmycin prodrugs as senolytics. **Aging Cell** 19: e13133, 2020. doi:[10.1111/acer.13133](https://doi.org/10.1111/acer.13133).
321. Mehdizadeh M, Aguilar M, Thorin E, Ferbeyre G, Nattel S. The role of cellular senescence in cardiac disease: basic biology and clinical relevance. **Nat Rev Cardiol** 19: 250–264, 2022. doi:[10.1038/s41569-021-00624-2](https://doi.org/10.1038/s41569-021-00624-2).
322. Dimri GP, Lee X, Basile G, Acosta M, Scott G, Roskelley C, Medrano EE, Linskens M, Rubelj I, Pereira-Smith O. A biomarker that identifies senescent human cells in culture and in aging skin in vivo. **Proc Natl Acad Sci USA** 92: 9363–9367, 1995. doi:[10.1073/pnas.92.20.9363](https://doi.org/10.1073/pnas.92.20.9363).
323. Georgakopoulou EA, Tsimaratou K, Evangelou K, Fernandez Marcos PJ, Zoumpourlis V, Trougakov IP, Kletsas D, Bartek J, Serrano M, Gorgoulis VG. Specific lipofuscin staining as a novel biomarker to detect replicative and stress-induced senescence. A method applicable in cryo-preserved and archival tissues. **Aging (Albany NY)** 5: 37–50, 2013. doi:[10.18632/aging.100527](https://doi.org/10.18632/aging.100527).
324. Evangelou K, Lougiakis N, Rizou SV, Kotsinas A, Kletsas D, Muñoz-Espín D, Kastrinakis NG, Pouli N, Marakos P, Townsend P, Serrano M, Bartek J, Gorgoulis VG. Robust, universal biomarker assay to detect senescent cells in biological specimens. **Aging Cell** 16: 192–197, 2017. doi:[10.1111/acer.12545](https://doi.org/10.1111/acer.12545).
325. Evangelou K, Veroutis D, Paschalaki K, Foukas PG, Lagopati N, Dimitriou M, Papaspyropoulos A, Konda B, Hazapis O, Polyzou A, Havaki S, Kotsinas A, Kittas C, Tzioufas AG, de Leval L, Vassilakos D, Tsiodras S, Stripp BR, Papantonis A, Blandino G, Karakasioti I, Barnes PJ, Gorgoulis VG. Pulmonary infection by SARS-CoV-2 induces senescence accompanied by an inflammatory phenotype in severe COVID-19: possible implications for viral mutagenesis. **Eur Respir J** 60: 2102951, 2022. doi:[10.1183/13993003.02951-2021](https://doi.org/10.1183/13993003.02951-2021).
326. Galanos P, Pappas G, Polyzos A, Kotsinas A, Svolaki I, Giakoumakis NN, Glytsou C, Pateras IS, Swain U, Souliotis VL, Georgakilas AG, Geacintov N, Scorrano L, Lukas C, Lukas J, Livneh Z, Lygerou Z, Chowdhury D, Sørensen CS, Bartek J, Gorgoulis VG. Mutational signatures reveal the role of RAD52 in p53-independent p21-driven genomic instability. **Genome Biol** 19: 37, 2018. doi:[10.1186/s13059-018-1401-9](https://doi.org/10.1186/s13059-018-1401-9).
327. Patel PL, Suram A, Mirani N, Bischof O, Herbig U. Derepression of hTERT gene expression promotes escape from oncogene-induced cellular senescence. **Proc Natl Acad Sci USA** 113: E5024–E5033, 2016. doi:[10.1073/pnas.1602379113](https://doi.org/10.1073/pnas.1602379113).
328. Yu Y, Schleich K, Yue B, Ji S, Lohneis P, Kemper K, Silvis MR, Qutob N, van Rooijen E, Werner-Klein M, Li L, Dhawan D, Meierjohann S, Reimann M, Elkahoulou A, Treitschke S, Dörken B, Speck C, Mallette FA, Zon LI, Holmen SL, Peeper DS, Samuels Y, Schmitt CA, Lee S. Targeting the senescence-overriding cooperative activity of structurally unrelated H3K9 demethylases in melanoma. **Cancer Cell** 33: 785, 2018. doi:[10.1016/j.ccell.2018.03.009](https://doi.org/10.1016/j.ccell.2018.03.009).
329. Angelopoulou A, Papaspyropoulos A, Papantonis A, Gorgoulis VG. CRISPR-Cas9-mediated induction of large chromosomal inversions in human bronchial epithelial cells. **STAR Protoc** 3: 101257, 2022. doi:[10.1016/j.xpro.2022.101257](https://doi.org/10.1016/j.xpro.2022.101257).
330. Seoane R, Vidal S, Bouzaher YH, El Motiam A, Rivas C. The interaction of viruses with the cellular senescence response. **Biology (Basel)** 9: 455, 2020. doi:[10.3390/biology9120455](https://doi.org/10.3390/biology9120455).
331. Baz-Martínez M, Da Silva-Álvarez S, Rodríguez E, Guerra J, El Motiam A, Vidal A, Garcia-Caballero T, Gonzalez-Barcia M, Sanchez L, Muñoz-Fontela C, Collado M, Rivas C. Cell senescence is an antiviral defense mechanism. **Sci Rep** 6: 37007, 2016. doi:[10.1038/srep37007](https://doi.org/10.1038/srep37007).
332. Kelley WJ, Zemans RL, Goldstein DR. Cellular senescence: friend or foe to respiratory viral infections? **Eur Respir J** 56: 2002708, 2020. doi:[10.1183/13993003.02708-2020](https://doi.org/10.1183/13993003.02708-2020).
333. Kohli J, Veenstra I, Demaria M. The struggle of a good friend getting old: cellular senescence in viral responses and therapy. **EMBO Rep** 22: e52243, 2021. doi:[10.15252/embr.202052243](https://doi.org/10.15252/embr.202052243).
334. Lagopati N, Tsioli P, Mourkioti I, Polyzou A, Papaspyropoulos A, Zafiroopoulos A, Evangelou K, Sourvinos G, Gorgoulis VG. Sample pooling strategies for SARS-CoV-2 detection. **J Virol Methods** 289: 114044, 2021. doi:[10.1016/j.jviromet.2020.114044](https://doi.org/10.1016/j.jviromet.2020.114044).
335. Lee S, Yu Y, Trimpert J, Benthani F, Mairhofer M, Richter-Pechanska P, et al. Virus-induced senescence is a driver and therapeutic target in COVID-19. **Nature** 599: 283–289, 2021. doi:[10.1038/s41586-021-03995-1](https://doi.org/10.1038/s41586-021-03995-1).
336. Karakasioti I, Lagopati N, Evangelou K, Gorgoulis VG. Cellular senescence as a source of SARS-CoV-2 quasispecies. **FEBS J**. In press. doi:[10.1111/febs.16230](https://doi.org/10.1111/febs.16230).
337. Tzekaki EE, Geromichalos G, Lavrentiadou SN, Tsantariotiou MP, Pantazaki AA, Papaspyropoulos A. Oleuropein is a natural inhibitor of PAI-1-mediated proliferation in human ER-/PR- breast cancer cells. **Breast Cancer Res Treat** 186: 305–316, 2021. doi:[10.1007/s10549-020-06054-x](https://doi.org/10.1007/s10549-020-06054-x).
338. Tzekaki EE, Papaspyropoulos A, Tsolaki M, Lazarou E, Kozori M, Pantazaki AA. Restoration of BMI1 levels after the administration of early harvest extra virgin olive oil as a therapeutic strategy against Alzheimer's disease. **Exp Gerontol** 144: 111178, 2021. doi:[10.1016/j.exger.2020.111178](https://doi.org/10.1016/j.exger.2020.111178).

339. Zhu F, Li Y, Zhang J, Piao C, Liu T, Li H-H, Du J. Senescent cardiac fibroblast is critical for cardiac fibrosis after myocardial infarction. **PLoS One** 8: e74535, 2013. doi:10.1371/journal.pone.0074535.
340. Grosse L, Wagner N, Emelyanov A, Molina C, Lacas-Gervais S, Wagner KD, Bulavin DV. Defined p16(High) senescent cell types are indispensable for mouse healthspan. **Cell Metab** 32: 87–99.e6, 2020. doi:10.1016/j.cmet.2020.05.002.
341. Drakhlis L, Biswanath S, Farr CM, Lupanow V, Teske J, Ritzenhoff K, Franke A, Manstein F, Bolesani E, Kempf H, Liebscher S, Schenke-Layland K, Hegemann J, Nolte L, Meyer H, de la Roche J, Thiemann S, Wahl-Schott C, Martin U, Zweigerdt R. Human heart-forming organoids recapitulate early heart and foregut development. **Nat Biotechnol** 39: 737–746, 2021. doi:10.1038/s41587-021-00815-9.
342. Giacomelli E, Meraviglia V, Campostrini G, Cochrane A, Cao X, van Helden RW, Krotenberg Garcia A, Mircea M, Kostidis S, Davis RP, van Meer BJ, Jost CR, Koster AJ, Mei H, Míguez DG, Mulder AA, Ledesma-Terrón M, Pompilio G, Sala L, Salvatori DC, Sliker RC, Sommariva E, de Vries AA, Giera M, Semrau S, Tertoolen LG, Orlova VV, Bellin M, Mummery CL. Human-iPSC-derived cardiac stromal cells enhance maturation in 3D cardiac microtissues and reveal non-cardiomyocyte contributions to heart disease. **Cell Stem Cell** 26: 862–879.e11, 2020. doi:10.1016/j.stem.2020.05.004.
343. Thavandiran N, Dubois N, Mikryukov A, Massé S, Beca B, Simmons CA, Deshpande VS, McGarry JP, Chen CS, Nanthakumar K, Keller GM, Radisic M, Zandstra PW. Design and formulation of functional pluripotent stem cell-derived cardiac microtissues. **Proc Natl Acad Sci USA** 110: E4698–E4707, 2013. doi:10.1073/pnas.1311120110.
344. Paspaspyropoulos A, Tsolaki M, Foroglou N, Pantazaki AA. Modeling and targeting Alzheimer's disease with organoids. **Front Pharmacol** 11: 396, 2020. doi:10.3389/fphar.2020.00396.
345. Sachs N, Paspaspyropoulos A, Zomer-van Ommen DD, Heo I, Böttinger L, Klay D, et al. Long-term expanding human airway organoids for disease modeling. **EMBO J** 38: e100300, 2019. doi:10.15252/emj.2018100300.
346. Sato T, Stange DE, Ferrante M, Vries RG, Van Es JH, Van den Brink S, Van Houdt WJ, Pronk A, Van Gorp J, Siersema PD, Clevers H. Long-term expansion of epithelial organoids from human colon, adenoma, adenocarcinoma, and Barrett's epithelium. **Gastroenterology** 141: 1762–1772, 2011. doi:10.1053/j.gastro.2011.07.050.
347. Sato T, Vries RG, Snippert HJ, van de Wetering M, Barker N, Stange DE, van Es JH, Abo A, Kujala P, Peters PJ, Clevers H. Single Lgr5 stem cells build crypt-villus structures in vitro without a mesenchymal niche. **Nature** 459: 262–265, 2009. doi:10.1038/nature07935.
348. Wiener DJ, Basak O, Asra P, Boonekamp KE, Kretzschmar K, Paspaspyropoulos A, Clevers H. Establishment and characterization of a canine keratinocyte organoid culture system. **Vet Dermatol** 29: 375–e126, 2018. doi:10.1111/vde.12541.
349. Jacob F, Salinas RD, Zhang DY, Nguyen PT, Schnoll JG, Wong SZ, Thokala R, Sheikh S, Saxena D, Prokop S, Liu DA, Qian X, Petrov D, Lucas T, Chen HI, Dorsey JF, Christian KM, Binder ZA, Nasrallah M, Brem S, O'Rourke DM, Ming GL, Song H. A patient-derived glioblastoma organoid model and biobank recapitulates inter- and intra-tumoral heterogeneity. **Cell** 180: 188–204.e22, 2020. doi:10.1016/j.cell.2019.11.036.
350. Sakellaropoulos T, Vougas K, Narang S, Koinis F, Kotsinas A, Polyzos A, Moss TJ, Piha-Paul S, Zhou H, Kardala E, Damianidou E, Alexopoulos LG, Aifantis I, Townsend PA, Panayiotidis MI, Sfrikakis P, Bartek J, Fitzgerald RC, Thanos D, Mills Shaw KR, Petty R, Tsigirigou A, Gorgoulis VG. A deep learning framework for predicting response to therapy in cancer. **Cell Rep** 29: 3367–3373.e4, 2019. doi:10.1016/j.celrep.2019.11.017.
351. Vougas K, Sakellaropoulos T, Kotsinas A, Foukas GP, Ntargaras A, Koinis F, Polyzos A, Myrianthopoulos V, Zhou H, Narang S, Georgoulis V, Alexopoulos L, Aifantis I, Townsend PA, Sfrikakis P, Fitzgerald R, Thanos D, Bartek J, Petty R, Tsigirigou A, Gorgoulis VG. Machine learning and data mining frameworks for predicting drug response in cancer: An overview and a novel in silico screening process based on association rule mining. **Pharmacol Ther** 203: 107395, 2019. doi:10.1016/j.pharmthera.2019.107395.