Cardiac ageing: extrinsic and intrinsic factors in cellular renewal and senescence

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Abstract | Cardiac ageing manifests as a decline in function leading to heart failure. At the cellular level, ageing entails decreased replicative capacity and dysregulation of cellular processes in myocardial and nonmyocyte cells. Various extrinsic parameters, such as lifestyle and environment, integrate important signalling pathways, such as those involving inflammation and oxidative stress, with intrinsic molecular mechanisms underlying resistance versus progression to cellular senescence. Mitigation of cardiac functional decline in an ageing organism requires the activation of enhanced maintenance and reparative capacity, thereby overcoming inherent endogenous limitations to retaining a youthful phenotype. Deciphering the molecular mechanisms underlying dysregulation of cellular function and renewal reveals potential interventional targets to attenuate degenerative processes at the cellular and systemic levels to improve quality of life for our ageing population. In this Review, we discuss the roles of extrinsic and intrinsic factors in cardiac ageing. Animal models of cardiac ageing are summarized, followed by an overview of the current and possible future treatments to mitigate the deleterious effects of cardiac ageing.

Cardiovascular disease (CVD) is the main cause of death worldwide, accounting for nearly one-third of deaths in the USA annually[1,2]. Projections indicate that, in 12 years, nearly 44% of adults in the USA will have some form of CVD[1,2]. Ageing is a strong risk factor for heart disease; elderly individuals (aged >70 years) comprise approximately two-thirds of those dying from CVD. As the baby-boomer generation transitions into the elderly demographic, demand will increase for anti-ageing strategies and treatments to promote both healthspan and lifespan. Preventing and treating heart disease will be paramount to meeting this demand. Accordingly, understanding the cellular mechanisms of cardiac ageing is critical to the prevention and treatment of CVD in an ageing population[3–5].

In essence, ageing is a process of degeneration at the organismal, cellular, and molecular levels. Events acting at the organismal level, such as the environment or lifestyle, have repercussions on cellular and molecular mechanisms. Conversely, molecular and cellular signalling alterations influence overall cardiac function and health. Although intrinsic factors have previously been viewed as the main drivers of cardiac ageing[6], studies are increasingly examining dynamic interactions between these external and internal influences to understand how they affect each other and thereby find more effective points of intervention for the prevention and management of cardiac ageing.

The nine proposed hallmarks of cellular ageing are subdivided into three stages: causative primary hallmarks, including DNA damage and genomic instability, telomere attrition and dysfunction, and epigenetic changes and decreased protein homeostasis; antagonistic hallmarks comprising deregulated nutrient sensing, mitochondrial dysfunction, and increased cellular senescence; and integrative hallmarks that manifest as ageing, including depletion of the stem-cell pool and changes in intercellular communication[7,8]. Each of these hallmarks reflects dysregulation of essential cellular functions leading to cell death, and they all participate in an interconnected network. Core signals and mechanisms linking these processes include inflammation[9], oxidative stress[10], autophagy[11–13], and metabolic imbalance[14,15]. Recent reviews of the cellular and molecular processes in cardiac ageing are summarized in TABLE 1. Ultimately, these processes converge upon deterioration of chromatin structural organization and function. Epigenetics has a crucial influential role in how DNA is accessed and interpreted.

In this Review, we focus on external factors that have an effect in the nucleus, integrating concepts of how perturbations in DNA and chromatin pertain to cardiac ageing. Aspects of cardiac cellular renewal and senescence

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Key points

- Ageing is a primary risk factor for cardiovascular disease and mortality.
- The capacity of the adult human heart to maintain function and preserve cellular homeostasis declines with age.
- Extrinsic factors of environment, behaviour, and lifestyle can promote or blunt cellular and molecular cardiac ageing.
- Intrinsic processes that promote cellular ageing, such as inflammation and oxidative stress, exacerbate telomere shortening, chromatin remodelling, and epigenetic drift.
- Cardiovascular ageing is inextricably tied to genetic predisposition and the complex interaction of hereditary influences.
- Promising advances to antagonize myocardial ageing connect external factors with intrinsic molecular mechanisms, enabling interventional strategies on both behavioural and cellular levels.

are summarized, and animal models of cardiac ageing are examined for their value in understanding the molecular basis of cardiac ageing. Finally, approaches for exogenous and endogenous modifications intended as anti-ageing interventions are summarized, followed by anticipated areas for future therapeutic discovery.

Cardiac cellular renewal and senescence

Cardiac regeneration research has overturned the long-standing traditional view of the mammalian heart as a postmitotic organ[16–27]. The replicative potential of adult mammalian cardiomyocytes — although limited and inefficient[26,28] — remains an area of investigation for potentiating cardiac repair[29,30]. Likewise, the sometimes contentious field of cardiac progenitor cell biology still offers the potential to develop cardiac cell-based therapies for the treatment of heart disease[31,32]. The integral involvement of telomeres, genetics, and epigenetics influencing replication and reparative processes of cardiomyocytes and noncardiomyocytes provides insight into the mechanisms underlying youthful versus ageing cardiac phenotypes[33].

Cardiomyocyte renewal

Mammalian cardiomyocyte proliferation contributes to heart formation and growth during embryonic development and, to a diminishing extent, shortly after birth, as shown by residual replicative capacity mediating repair in the resected neonatal murine heart[33,34]. Proliferation of mammalian cardiomyocytes later in postnatal development remains an area of active debate[35–38]. Transcriptional and epigenetic profiling of neonatal versus adult cardiomyocytes confirms the postnatal loss of replicative and regenerative capacity[28], possibly owing to downregulation of pro-proliferative growth factors, innervation, and signalling pathways[39]. Cardiomyocyte turnover in the human heart at a rate of ≤1% per year[22] might seem inconsequentially low, but slow cardiomyogenic cellular turnover is highly important for the maintenance of cardiac structure and function in a species with an expected lifespan of several decades[40,41]. Moreover, decreased endogenous cardiomyogenesis with age might be linked to loss of cardiomyocytes and decline of myocardial performance[42–44]. Co-culture of adult cardiomyocytes with neonatal rat ventricular myocytes seems to induce dedifferentiation, proliferation, and redifferentiation of adult cardiomyocytes in vitro[45], although compelling evidence of adult cardiomyocytes undergoing a similar process in vivo after losing intercellular connections following ischaemic cardiac injury remains elusive and remarkably resistant to augmentation[46]. Numerous transgenic animal models overexpressing pro-proliferative proteins in cardiomyocytes show hyperplasia, upregulation of proliferative markers, and resistance to infarction injury, but unequivocal evidence of replication in vivo leading to fully functional cardiomyocytes has not been established[47–51]. Similarly, forced expression of *Tert*, which encodes telomerase reverse transcriptase (TERT), in cardiac myocytes by transgenesis or adenoviral-associated virus results in cardioprotection, cell cycle re-entry, and eventual myocyte hypertrophy but not enhanced cardiomyogenesis in transgenic hearts[52]. Therefore, inherent limitations of adult cardiomyogenesis remain an intractable obstacle for therapeutic interventions intended to antagonize deleterious effects of ageing.

Noncardiomyocyte renewal

The noncardiomyocyte population comprises several cell types, including endothelial, pericyte, smooth muscle, immune, neuronal, and fibroblast cells[53,54]. Cellular and molecular profiles of these nonmyocyte populations provide mechanistic insight into the regenerative potential and roles that these cells have during cardiac development, homeostasis, ageing, and repair[28,54–56].

Vascular cells. Vascular renewal after cardiac injury is essential to recovery and preservation of cardiac function[57,58]. The three main blood vessel cell types — vascular smooth muscle cells, vascular endothelial cells, and pericytes — proliferate in vitro and after injury in vivo, although the role of pericytes in cardiac neovascularization remains unclear[59–61]. Regardless, age-associated build-up of atherosclerotic plaque within coronary arterial blood vessels can lead to wall stiffness, inflammation, myocardial infarction, and consequent vascular cell death, which impair vascular structure and function[62,63].

Interstitial cells. Interstitial cells, such as fibroblasts, pericytes, and mesenchymal cells, constitute a large portion of the cardiac cell population. These cells participate in tissue homeostasis through secretion of paracrine factors and extracellular matrix, and respond to cardiac injury by proliferation and increased production of extracellular matrix to create scar tissue in place of dead myocardium. During cardiac ageing, the extracellular matrix remodels, leading to a stiffer left ventricular wall and compromised cardiac function in elderly individuals[64–66]. At the cellular level, ageing mesenchymal stem cells generate dysfunctional fibroblasts that promote inflammation and collagen deposition[67].

Progenitor cells. The innate reparative capacity of the heart is inadequate to compensate for a wide variety of pathological injuries and conditions, but resident cardiac progenitor cells (CPCs) mount limited myocardial healing responses. CPCs are a subset of the cardiac interstitial cell population, and the specific identities

and biological relevance of these cells remain areas of intense discussion and investigation, with the ongoing quest to find cell types and applications for cell-based therapy in the treatment of heart disease, including age-related deterioration. The motivation for pursuing these strategies rests with the need to augment the insufficient reparative capacity of the adult mammalian myocardium with enhancements to potentiate cardiac repair. Studies revisiting CPC biology demonstrate that myocardial KIT$^+$ cell biology is more complex than previously appreciated[68–70]. Innovative approaches to cardiac cell therapy continue to emerge, including clinical trials using KIT$^+$ cells[71], application of induced pluripotent stem cell (iPSC)-derived myocardial cell sheets[72], combining stem cell types[73], or engineering cells to potentiate reparative capacity[74]. Integrating multiple cardiac cellular mechanisms for tissue homeostasis and repair, including studies of age-related consequences for cardiac interstitial cell populations, such as CPCs, warrants further analyses and continues to be pursued for the development of treatments for heart disease[16].

Originally identified by a haematopoietic lineage-negative and KIT$^+$ phenotype, the adult CPC resides in niches surrounded by myocyte and fibroblast support cells that convey molecular signals about the myocardial environment. Extensive studies of cardiac KIT$^+$ CPCs identified a hierarchy of stemness based on their position within the niche (hypoxic at the core), as well as their expression of cardiac lineage markers. Low levels of CPC self-renewal within quiescent niches maintain CPC number; however, proliferation increases in response to external stimuli such as pathological challenge, wherein CPCs migrate out of the niche towards sites of injury. Relevant to the ageing process, reparative responses are blunted in CPCs with shortened telomeres and elevated levels of cell cycle inhibitors, such as cyclin-dependent kinase inhibitor 2A (CDKN2A; also known as p16^{INK4A}) and cellular tumour antigen p53. CPCs with a youthful phenotype are characterized by long telomeres, high clonogenic and cardiogenic potential, and absence of senescence markers[75–77]. Isolation and expansion of youthful cardiogenic CPCs have led to adoptive transfer studies in multiple animal models and application of autologous CPCs for the treatment of patients with heart failure in phase I clinical trials, demonstrating both feasibility and safety of this cell therapy. Rejuvenation by genetic engineering is a plausible approach to restore functional capacity to CPCs intended for use in cell therapy for patients with heart failure[74,78–81].

Cardiac cellular senescence

Cardiomyocytes. Replicative senescence, originally defined as permanent cell cycle arrest in response to oncogenic stress, is mediated by cell cycle inhibitory tumour-suppressor pathways p53–p21 (also known

Table 1 | **Cellular and molecular processes in cardiac ageing**

Process	Conclusions	Future directions	Refs
Inflammation	• Age-dependent increases in pro-inflammatory signals contribute to cardiac functional decline • Macromolecular degradation decreases with age, induces inflammation, and contributes to cardiac ageing • Inflammageing exacerbates cardiac ageing	• Identify molecular triggers of age-related cardiac inflammation to develop effective interventions • Dissect signalling networks contributing to inflammageing to tailor anti-ageing treatments and lifestyles	96,355,356
Telomere shortening	• Rodent models of telomere biology are useful, but the telomeres are longer than in humans • Telomere shortening is associated with cardiac ageing and disease, but the causal relationship remains undefined • Cellular stress links telomere shortening, senescence, and ageing in nonproliferating cells • Telomere shortening might be a hallmark of heritable cardiomyopathies	• Identify molecular targets specific to the maintenance of cardiac telomere length • Design clinical trials targeting telomere length according to specific cardiac diseases • Characterize various senescent cell types and the role of telomere attrition in vivo • Investigate the telomere–mitochondrial axis and telomere position effect in the context of cardiac ageing	166–168,170
Metabolic imbalance	• All hallmarks of ageing perturb cellular metabolism • Metabolic reprogramming to a catabolic state promotes cellular rejuvenation • Western lifestyle exacerbates ageing • Signalling pathway circuits contribute to metabolic imbalance in cardiac ageing	• Validation of metabolic reprogramming strategies in mammals and humans • Dissect signalling networks contributing to metabolic imbalance to tailor anti-ageing treatments and lifestyles	8,15
Autophagy	• Cardiac autophagy decreases with age • Autophagy-specific markers are difficult to identify • Increased autophagy might be cardioprotective and enhance lifespan	• Design and characterize better in vivo models for autophagy • Identify autophagy-specific targets to develop autophagy-specific activators	13
Mitochondrial dysfunction	• Mitochondrial function declines with age • Dysfunctional mitochondria trigger inflammation and ageing • Dysfunctional mitochondria accumulate with age owing to impaired mitophagy	• Develop mitochondria-centric therapies to maintain or restore mitochondrial function and enhance mitophagy	357,358
Oxidative stress	• A causal relationship between oxidative stress and cardiac ageing has not been definitively established	• Determine how oxidative stress, telomere shortening, inflammation, and cellular senescence contribute to and/or are caused by cardiac ageing to develop effective, targeted anti-ageing treatments	10

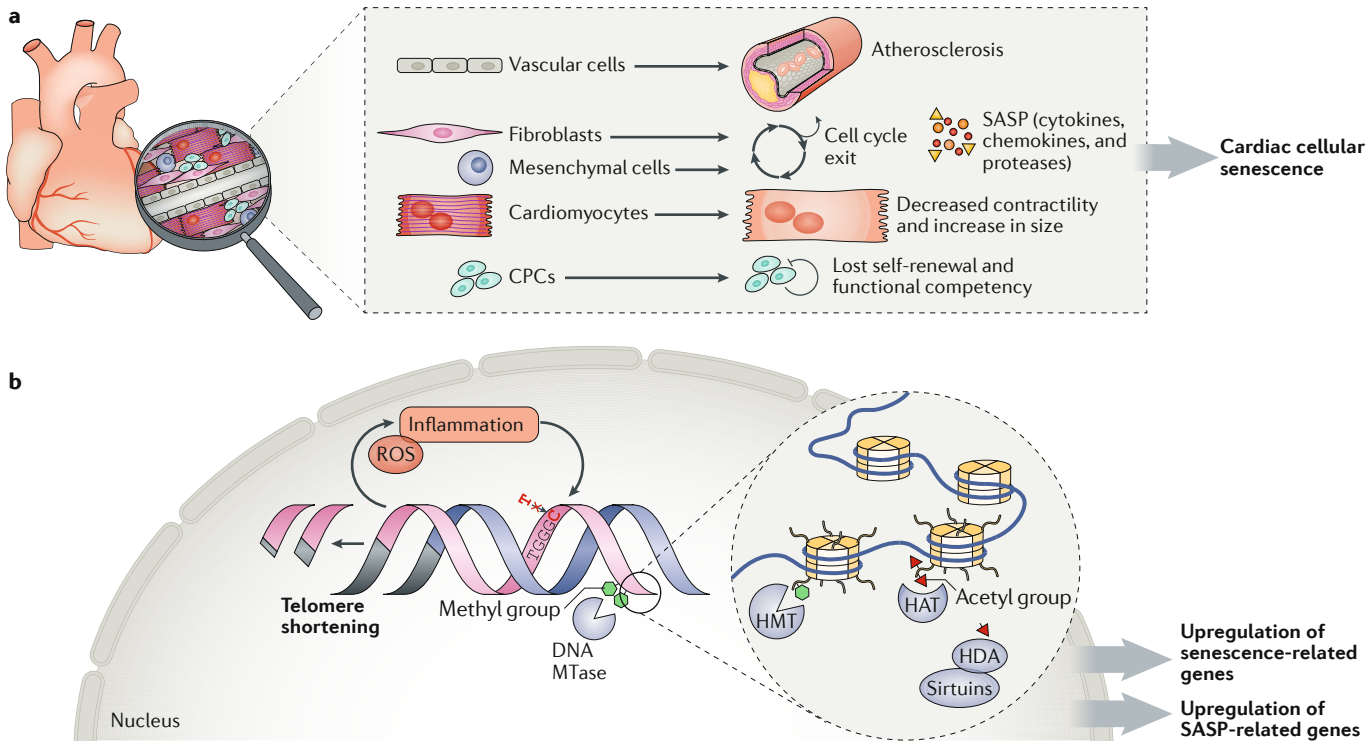

Fig. 1 | **Cellular and molecular signals influencing cardiac ageing.** Cellular senescence and death are mediated by age-dependent signalling that ultimately promotes cardiac failure. **a** | Cardiac cells manifest dysfunction in a cell-specific and tissue-specific fashion: vascular senescence is associated with inflammation and atherosclerosis, interstitial cells exit the cell cycle and secrete senescence-associated secretory phenotype (SASP) factors, myocytes hypertrophy and lose contractile function, and cardiac progenitor cells (CPCs) lose the capacity for self-renewal and differentiation. **b** | At the molecular level, inflammation and reactive oxygen species (ROS) drive DNA damage, telomere shortening, chromatin remodelling, and epigenetic changes, leading to cell cycle exit and expression of SASP and senescence-related genes. Together, these molecular and cellular events compromise cardiac function and accelerate cardiac ageing. DNA MTase, DNA methyltransferase; HAT, histone acetyl transferase; HDA, histone deacetylase; HMT, histone methyltransferase.

as CDKN1A) and $p16^{INK4A}$–retinoblastoma-associated protein. In non-cycling cardiomyocytes, cellular stress induced by inflammation or reactive oxygen species leads to telomere shortening, DNA damage, functional decline, alterations in ploidy, and other molecular changes indicative of senescence[82–84] (FIG. 1). For example, the senescence-associated secretory phenotype (SASP) is a paracrine repertoire comprising pro-inflammatory cytokines, chemokines, growth factors, and proteases[85,86]. Paracrine activity of senescent cells perpetuates tissue senescence or promotes repair after injury, depending on the tissue context[87,88].

Functional decline of senescent cardiomyocytes, such as decreased contractility, increased size, mitochondrial dysfunction, and telomere shortening, negatively affects myocardial performance. As these poorly functioning senescent cells accumulate with age, they interfere with intercellular communication, contribute to compromised tissue function, and promote chronic inflammation, leading to cell death and cardiomyocyte loss[88,89]. Interestingly, inflammation — not telomere shortening — was implicated as the main ageing culprit in a study to identify predictors of longevity in Japanese centenarian individuals[90]. At the cardiac level, antagonizing the progression of cardiomyocyte senescence is a central theme in approaches to blunt the deleterious consequences of ageing on myocardial structure and function[91–93].

Nonmyocytes. Senescence in vascular and interstitial cardiac cell populations manifests as exit from the cell cycle, increased inflammation, expression of $p16^{INK4A}$, telomere shortening, and acquisition of SASP. In the vasculature, accumulation of senescent cells and calcification are characteristic of atherosclerotic plaques[62,63,94,95]. Ageing-associated increases in inflammation and collagen deposition lead to fibrosis, myocardial stiffness, altered calcium handling, arrhythmias, and poor cardiac function[96,97]. Impaired function of senescent cardiac fibroblasts also contributes to adverse remodelling in the ageing heart[98]. CPCs lose the capacity to self-renew with age as a consequence of telomere shortening, dysregulated cell division, and upregulation of senescence markers[76], thereby contributing to overall cardiac ageing[99,100]. Replicative lifespan was decreased in telomerase-deficient haematopoietic stem cells during serial transplantation[101]. Similarly, bone-marrow-derived mononuclear cells from patients with ischaemic heart disease had several indicators of cellular senescence, including shortened telomeres, blunted myeloid differentiation capacity, and increased

p21 and p16^{INK4A} expression[102]. Implications for autologous stem or progenitor cell therapy are clear: patients with CVD often have cells with inherently poor regenerative capacity. An extreme example of restoring youthful characteristics to aged mouse and human somatic cells involves reprogramming into iPSCs[103,104] and, although iPSC therapy has been challenging to implement as a treatment for heart disease, iPSCs derived from ageing systems can provide valuable molecular insights into the ageing process[105]. Collectively, all indications point towards restoration of functional capacity for underperforming aged stem cells as a critical bottleneck for the development of optimized and efficacious personalized regenerative medicine.

Extrinsic factors in cardiac ageing

External factors, such as air pollution, exercise, diet, and psychological stress, all contribute to the modulation of cardiac ageing at the cellular level (BOX 1; FIG. 2). Pathways linking the effect of extrinsic influences with molecular mechanisms often involve canonical regulatory circuits of ageing, as described in this section.

Environment

Pollution. The effect of air pollution on cardiovascular health is an increasing concern in rapidly developing countries with large populations, such as Brazil, China, and India. Long-term exposure to air pollution, specifically fine particulate matter, increases the risk of CVD and death[106]. Likewise, high levels of air pollution counteract the benefits of walking exercise in individuals aged >60 years, regardless of cardiopulmonary disease status[107]. Conversely, improving air quality by air conditioner filtration can improve cardiovascular health in polluted urban settings[108,109]. Exposure to traffic-related pollution has also been linked to peripheral artery disease and hypertension in men and women[110], and is associated with shorter leukocyte telomere length (LTL), which is a biomarker of systemic ageing[111]. Likewise, LTL is negatively correlated with exposure to indoor air pollution in individuals using solid fuel for cooking[112]. Inflammation and oxidative stress have been implicated as molecular mechanisms linking air pollution to telomere shortening[113]. Continued research efforts to identify specific cellular and molecular signals that connect environmental pollution to predisposition to disease will be essential for designing interventions to mitigate the acceleration of cardiovascular ageing.

Lifestyle

Nutrition. Diet has a central role in cardiovascular health, integrating metabolism with cellular function and longevity. Intervention at the nutritional level is a major focus of public health research. For example, the Mediterranean diet, which is rich in fruits, vegetables, nuts, legumes, fish, and unsaturated fats (especially virgin olive oil), has long been associated with anti-ageing and heart-healthy benefits. Molecular mechanisms underlying the beneficial effects of the Mediterranean diet include telomere preservation, anti-inflammatory effects, antioxidant properties, beneficial autophagy, and improved metabolic and lipid profiles[114]. The PREDIMED-NAVARRA trial[115] showed that adherence to the Mediterranean diet for 5 years was correlated with longer LTL in women aged 55–80 years at high risk of CVD. Nutritional genomic approaches applied to the Mediterranean diet identified potentially responsive single nucleotide polymorphisms (SNPs), but prescribing individualized dietary patterns requires further study[114,116]. Interestingly, the Mediterranean diet might also confer cardioprotective changes at the epigenetic level by modulating DNA methylation in leukocytes[117]. The omics analysis of cardiac ageing and disease might reveal the molecular underpinnings of the benefits of the Mediterranean diet and the necessary steps for these observations to be applied to promote cardiovascular functional longevity.

Calorie restriction is a dietary strategy thought to improve quality of life and prolong lifespan. Intermittent fasting imparts cardiovascular benefits to fruitflies[118,119], rodents[120–125], and humans[126–129]. Purported mechanisms yielding beneficial effects in *Drosophila* were associated with regulation of circadian clock genes, a chaperonin complex, and the electron transport chain[118,119,130]. Cardioprotective effects of fasting were observed both before and after myocardial infarction in rats, including higher survival with preservation of cardiac structure and function as well as decreased cell death and increased

Box 1 | Extrinsic influences for a youthful heart

Environment

Chemical exposure
- Clean air
  - Telomere preservation

Lifestyle

Nutrition
- Mediterranean diet
  - Inflammation reduction
  - Epigenetic influence
- Calorie restriction
  - Increased efficiency and capacity of electron transport chain
  - Preserved cardiac structure and function
  - DNA methylation drift delay
  - Oxidative stress protection
  - Insulin level and resistance decreased
- Dietary supplements
  - TA-65 (telomerase activator)
  - Sirtuin activators

Exercise
- Telomere length preserved
- Telomerase activity improved
- Senescence-associated secretory phenotype decrease
- DNA repair improved
- Stress reduction

Mental health
- Relaxation response
  - Inflammation reduction
  - Reduced DNA methylation patterns
  - Telomere preservation
  - Reduced oxidative stress
- Proper sleep
- Social integration

levels of the pro-survival cytokine adiponectin[120–122]. Likewise, intermittent fasting for 6 weeks was protective against ischaemia–reperfusion injury mediated by protective preconditioning to induce autophagy–lysosome machinery[124] as well as tissue-specific redox regulation to protect the heart from oxidative stress[123].

Calorie restriction also delays age-related DNA methylation drift, defined as changes in DNA methylation patterns over time, in mice and rhesus monkeys[125]. The negative correlation between DNA methylation drift and lifespan in mice, monkeys, and humans suggests a molecular mechanism for the beneficial effects of calorie restriction on lifespan and cardiovascular health. However, the potential value of all this experimental research for humans remains debatable, as a systematic literature review concluded that more research is required before recommending fasting as a health intervention[126]. Nonetheless, the AHA published a scientific statement concluding that intermittent fasting might be useful for weight loss and for lowering triglyceride levels, blood pressure, fasting insulin levels, and insulin resistance[128]. Consistent with this view, a combined regimen of intermittent fasting and a high-protein, low-calorie diet reduced body weight, improved the blood lipid profile, and reduced arterial stiffness, and this regimen maintained these cardiovascular benefits after 1 year better than the standard heart-healthy diet (<35% of calorie intake as fat, 50–60% of calorie intake as carbohydrates, <200 mg/dl of dietary cholesterol, and 23–30 g per day of fibre, consistent with dietary guidelines of the National Cholesterol Education Program Therapeutic Lifestyles Changes diet) in individuals with obesity[127].

Translating these lessons to molecular interventions, calorie restriction mimetics are being developed to simulate the beneficial effects of calorie restriction without requiring reduced food intake[131]. Calorie restriction and calorie restriction mimetics produce tissue-specific transcriptional effects, with L-carnitine being the only calorie restriction mimetic shown to produce transcriptional changes in the heart similar to those of calorie restriction[131]. In summary, intermittent fasting seems to have cardioprotective effects in animal models and might improve cardiovascular health in humans, but more rigorous, randomized, controlled clinical trials are necessary before intermittent fasting can be recommended as a medical intervention in the treatment of heart disease[129].

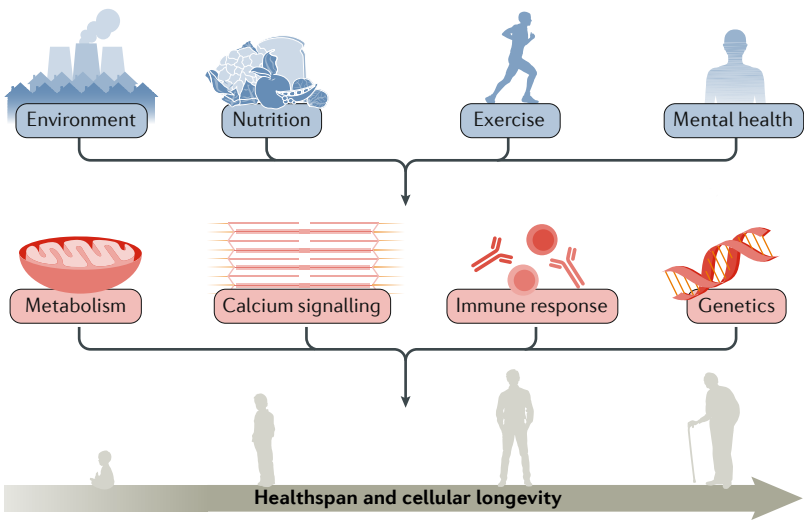

Fig. 2 | **Extrinsic and intrinsic influences contributing to cellular longevity.** Healthspan is enhanced by maintaining a youthful cellular phenotype. Extrinsic factors, including environment, nutrition, exercise, and psychology (shown in blue), influence the functional capacity of cells through changes in metabolism, calcium signalling, immune response, and gene function (shown in red). Optimizing lifestyle choices by living in a non-polluted environment, eating healthy food, exercising regularly, and minimizing psychological stress promotes youthful intracellular and molecular signalling to mitigate the molecular effects of cardiac cellular ageing.

Exercise. Physical inactivity is a known risk factor for CVD and premature death[132]. Exercise confers multiple anti-ageing benefits, particularly in the cardiovascular system, at the systemic and cellular levels[133,134]. Of note, increased LTL clearly associates with physical activity[135–143]. Even moderate-to-vigorous physical activity is positively associated with LTL and cardiorespiratory fitness[144]. Molecular mechanisms accounting for the cardioprotective effects of physical exercise might involve increased levels of TERT, telomeric repeat-binding factor 2, and telomerase; activated pro-survival insulin-like growth factor I signalling; and decreased expression of p53 and $p16^{INK4A}$ observed in mice[135]. Likewise, vasculoprotective effects of voluntary running increase telomerase activity, decrease telomere erosion, and blunt expression of senescence markers in mice[135] as well as in human endurance runners[136]. Even acute exercise influences shelterin complex genes, DNA damage and repair genes, and p38 mitogen-activated protein kinase (MAPK) signalling, indicating an early adaptive response for telomere protection[145]. Consistent with these findings, high levels of physical activity can confer cellular youthfulness with a 9-year ageing advantage relative to inactivity based on an analysis of National Health and Nutrition Examination Survey (NHANES) data[142]. Collectively, the benefits of exercise undoubtedly include preserving a youthful phenotype at the cellular and molecular levels, and this preservation can help to slow the degenerative effects of ageing at multiple levels. Clearly, one size does not fit all in terms of exercise regimens for anti-ageing effects. Tailoring fitness recommendations to individuals requires consideration of age, sex, and cultural disparities. Devising research models that address heterogeneity of cardiac ageing at the cellular and molecular levels will be important to understand how best to apply physical activity to diverse patient populations to improve healthspan.

Mental state. Psychological stress, such as chronic stressors from the workplace environment or social context, increases the risk of coronary heart disease[146–148], possibly through increased inflammation[149–151]. Indeed, the brain–heart interaction is an emerging area of interest in cardiovascular medicine[152]. One particularly compelling example of the brain–heart pathogenesis link is mental-stress-induced myocardial ischaemia or Takotsubo cardiomyopathy[153–155]. Exercises that confer psychological benefit via stress reduction, such as meditation, mitigate ageing and cardiac disease. In support

of meditative therapy, the number of years of regular meditation is significantly inversely correlated with an accelerated epigenetic clock as defined by DNA methylation patterns[156]. Meditation, yoga, and repetitive prayer empower personal ability to calm the body, termed the relaxation response, which relieves stress[157,158]. Molecular mechanisms of the relaxation response include upregulation of telomere maintenance and metabolism, whereas inflammation-associated and stress-associated gene expression is downregulated[159]. Similarly, practitioners of yoga are observed to have longer LTL, higher antioxidant status, and lower levels of the oxidative stress indicators homocysteine and malondialdehyde[160]. The effect of stress is also observed in murine models, in which psychological stress induced by physical restraint exacerbated vascular senescence and reduced neovascularization in a hindlimb model of ischaemia[161]. This finding suggested a potential molecular intervention through the dipeptidyl peptidase 4–glucagon-like peptide 1–adiponectin signalling axis[161]. Sleep deprivation is another aspect of stress affecting the brain–heart axis contributing to cardiac ageing, as investigated in a mouse model of circadian rhythm disruption. Mice expressing mutant *Clock*, which encodes an important protein for the mechanism of circadian rhythm, developed age-dependent cardiomyopathy[162]. Finally, the physiological effect of social integration throughout life is emerging as a powerful influence on cardiovascular health and longevity[163]. Collectively, these studies point to the importance of everyday behaviour and mindset for overall health at the organismal and cellular levels. As public health, clinical, and psychological studies continue to uncover the connections between mental state and cardiovascular health, in vivo and in vitro models deciphering the underlying cellular and molecular mechanisms will be critical for identifying molecular targets to counteract the effects of stress on cardiac ageing.

Box 2 | **Intrinsic influences for a youthful heart**

Metabolism
Mitochondria and sirtuins
- Oxidative stress regulation
- Metabolic process regulation

Mechanistic target of rapamycin (mTOR) inhibition
- Amelioration of laminopathy
- Autophagy upregulation

Calcium signalling
Contractility
Cellular remodelling

Immune response
mTOR inhibition
- Improved immune function

Senescence-associated secretory phenotype regulation
- Reduction of senescent cells
- Inflammatory cytokine signalling decreased

Genetics
Telomere preservation
- Stabilization of shelterin complex
- Preservation of telomerase activity

DNA maintenance
- Repair of DNA strand breaks
- Genome stability
- Lamin-A/C stability
- Longevity loci activity
- Single nucleotide polymorphisms promoting disease resistance

Epigenetics
Limited chromatin remodelling
Limited DNA methylation drift
Limited CpG methylation at promoters
Limited transcriptional drift
Histone preservation

Intrinsic factors in cardiac ageing

Cardiac cellular ageing manifests as functional decline and lost replicative capacity as a consequence of molecular responses to cellular stressors, such as oxidative stress and inflammation (BOX 2; FIG. 2). Telomere shortening, genetic predisposition, and epigenetic modifications comprise fundamental intrinsic processes driving cellular senescence.

Telomeres

Telomeres, first described by Hermann Muller in 1938, are the protective, stabilizing ends of eukaryotic chromosomes and are composed of short DNA repeats, telomerase RNA component (TERC), and TERT. Several associated proteins collectively referred to as the shelterin complex contribute to telomere maintenance and stability. Telomeres become shorter with each round of cell division or in response to inflammation and oxidative stress, eventually reaching a critical minimum length, the consequences of which include cell cycle arrest, possible chromosome damage, differentiation, or cellular senescence. Telomere length is perhaps the best-known cellular marker of ageing, with an emerging role in human ageing and disease[164]. Entire fields of study and industries are based on the premise that telomere length indicates cellular replicative capacity and, by extension, tissue and organismal age. TERT expression, telomerase activity, and telomere length decrease dramatically in postnatal mammalian cardiomyocytes[36]. Consequently, telomere shortening is primarily driven by inflammation and oxidative stress in the postnatal heart[165]. The role of telomeres in cardiac disease and ageing, linking inflammation and oxidative stress with telomere attrition, cellular senescence, and death, has been reviewed previously[166–170] (FIG. 1).

Telomere length in noncardiac tissue is increasingly being used as a clinical diagnostic tool to assess cardiac age and disease[171,172]. For example, circulating blood provides an abundant, accessible tissue source for assaying patient telomere length, such that LTL has become a common indicator of systemic ageing in humans and is thought to be a predictive measure of age-associated disease[172,173]. The first systematic review and meta-analysis of the association between LTL and CVD included 24 studies published up to 2014 and confirmed an inverse relationship between LTL and risk of coronary heart disease[174]. An analysis of NHANES data revealed

that suboptimal cardiovascular health is associated with shorter LTL[175] and confirmed that LTL is an indicator of cardiovascular ageing in association with biomarkers of CVD, such as adiposity, insulin resistance, and blood pressure[176]. Additionally, shortened LTL is a molecular marker of vascular ageing associated with arterial stiffness[177] and adverse cardiac outcomes in patients with coronary artery disease[172]. LTL is a useful but indirect diagnostic measure of cardiac cellular ageing, providing preliminary evidence for how environmental factors affect myocardial function at the cellular and molecular levels. As cellular senescence continues to emerge as a complex and heterogeneous cellular state, closer evaluation of the connection between LTL and myocardial cellular ageing phenotypes will be necessary to establish the clinical relevance and predictive value of LTL as a biomarker for cardiac ageing and risk of CVD.

Genetics

A causal link between genetics in human longevity and progression of cardiac ageing has not been conclusively established. Genetic factors are estimated to underlie one-quarter of the variance in human lifespan, the rest of which is determined by dynamic interactions between genes, environment, lifestyle, and epigenetics[178]. Animal studies identifying metabolic pathways affecting longevity have provided indications of human genes that influence lifespan and ageing[179–182]. Genome-wide association studies (GWAS) have been used to identify novel genetic loci and pathways associated with late-onset CVD, creating new directions of research into mechanisms and potential therapeutic targets for the prevention and treatment of cardiac ageing and disease[183–185]. Whereas so-called good genes are vaguely presumed to predispose individuals to longevity, genetic mutations leading to progeria or Werner syndrome undoubtedly predispose patients to premature ageing[186]. Indeed, diseases of accelerated ageing often include cardiomyopathy in the phenotype, which provides insights into various cellular and molecular mechanisms underlying shortened lifespan. Mutations in the *LMNA* gene, which encodes the precursor to lamin-A/C (LMNA), result in an unstable, irregular nuclear envelope, DNA damage, and subsequent premature cell death in childhood progeria (Hutchinson–Gilford progeria syndrome; HGPS). Individuals with this condition have classic ageing phenotypes during childhood and are predisposed to CVD. LMNA cardiomyopathies involve dysregulated gene expression as well as mechanical or conduction defects[187]. Werner syndrome, another disease of premature ageing, is caused by mutations in the *WRN* gene, encoding a helicase protein for repairing double-stranded DNA breaks and maintaining genome stability[188], and is frequently associated with atherosclerosis[189]. Animal models of these genetic diseases have been very useful in dissecting molecular mechanisms contributing to cardiac ageing and are discussed in more detail below.

The converse condition to premature ageing is longevity, and longevity-associated candidate genes, such as *APOE* and *FOXO3*, continue to be identified in increasingly rigorous genomic analyses[190,191]. For example, four genetic variants associated with longevity, including candidate loci *ABO*, *APOE–TOMM40*, *CDKN2B–ANRIL*, and *SH2B3–ATXN2*, were identified using informed GWAS, a novel statistical analysis of human disease genomic data[192]. The gene encoding the cell-cycle inhibitor cyclin-dependent kinase 4 inhibitor B (CDKN2B; also known as $p15^{INK4B}$) is located in the same genomic region as the gene encoding the senescence marker $p16^{INK4A}$, and the *ABO* blood group locus is associated with cardiovascular disorders[193]. Other meta-analyses of DNA from elderly individuals of European descent identified 5q33.3 as a novel locus associated with low blood pressure in middle age and a decreased risk of cardiovascular-related death[194], whereas a GWAS of Han Chinese centenarian individuals revealed 11 independent, longevity-associated loci, including a novel locus with *IL6* as the nearest gene. A comparison of Chinese, European, and US longevity associations identified eight overlapping SNPs and confirmed *APOE* and 5q33.3 as longevity-associated loci, and this analysis also identified four pathways (carbohydrate metabolism, immune response, MAPK signalling, and calcium signalling) associated with longevity in Han Chinese individuals, suggesting that favourable genotypes mediating defensive mechanisms against toxins, pathogens, or inflammation contribute to longevity in this population[195]. Although candidate longevity-associated loci are shared by all humans, these studies also highlight the contributions of cultural and environmental factors in the phenotypic manifestation of cardiac health and lifespan.

Not surprisingly, genetic overlap exists between traits of longevity and age-related traits, such as coronary artery disease. Whether the DNA of long-lived individuals lacks disease-associated SNPs, carries beneficial SNPs that promote disease resistance, or contains some combination of both remains unknown. Evidence is accumulating to show that lifespan is influenced by both genetics and modifiable risk factors and that no single genetic profile guarantees longevity. A genome-wide association meta-analysis using parental survival and lifespan data identified two genomic regions associated with longevity as well as specific behaviours and socioeconomic traits affecting life expectancy[196], specifically the *LPA* locus encoding lipoprotein(a), which is known to influence cardiovascular health, and the human leukocyte antigen (HLA) region within the major histocompatibility complex. This meta-analysis showed genetic correlations between complex traits and longevity. Specifically, disease-based measures, such as smoking, diabetes mellitus, and coronary artery disease, showed a negative genetic correlation with lifespan, whereas particular socioeconomic traits, such as education and openness to experience, showed a positive genetic correlation with longevity.

GWAS and exome array analyses have revealed novel locus–protein associations, including one between circulating levels of apolipoprotein E and transcription factor protein phosphatase 1 G (PPM1G), which was confirmed experimentally. This approach of combining proteomic and genomic profiling from two population-based studies associated with the Framingham Risk Score for developing CVD[197] is publicly available in

the National Center for Biotechnology Information Database of Genotypes and Phenotypes[198]. Identification of the genetic underpinnings of cardiovascular ageing continues to evolve with increasingly complex analyses of large databases of human data. However, genetics alone cannot overcome dynamic interactions with environment and lifestyle choices that collectively determine individual lifespan. Genome-wide analyses provide an overview of population and individual genetics but no integration of tissue or cellular heterogeneity, which reflects the more interactive dynamic between external factors and organismal ageing. Further integrative analyses linking genomic, epigenetic, transcriptional, and proteomic data sets will help to reveal how cardiac ageing manifests in diverse populations in response to environmental and behavioural cues. Applying these integrative analyses to animal models of ageing will be useful for deciphering molecular signals that orchestrate cardiac ageing and understanding how therapeutic strategies can mitigate the decline in healthspan with age.

Epigenetics

Epigenetics refers to changes in gene function that occur not as a consequence of changes in DNA sequence, and includes changes resulting from DNA methylation, heterochromatin or chromatin remodelling, histone modifications, and non-coding RNAs. Epigenetic changes can be stable and heritable or can occur more rapidly and reversibly in response to cellular or environmental conditions[199]. During ageing, these changes influence chromatin availability, gene expression, and genome stability. The epigenetics of ageing is an emerging area of research that relates epigenome responses to environmental cues over time to effects on the ageing process[200–202]. Understanding epigenetic mechanisms associated with ageing might reveal potential biomarkers for ageing as well as therapeutic targets for reversing or ameliorating ageing pathologies, including CVD[201,203].

DNA methylation. Chromatin modifications alter chromatin architecture and gene availability. DNA methylation has been extensively studied in the context of gene regulation and with respect to ageing and cardiac disease. DNA methyltransferases attach methyl groups to cytosine rings in CpG dinucleotides of the DNA backbone. Methylated CpG islands — groups of CpG sites often located near to gene transcription start sites — typically suppress gene expression by blocking transcription factor access to promoters or by facilitating repressive chromatin modifications. DNA methylation tends to increase (hypermethylation) in localized sites, such as promoter CpG islands, and decrease (hypomethylation) globally during replicative senescence[201,204].

In cardiac disease, inhibition of important genes by DNA methylation contributes to atherosclerosis[205]. Likewise, elevated levels of homocysteine, a precursor to methionine, are implicated in coronary artery disease such that plasma homocysteine level correlates with global DNA hypermethylation in patients with coronary artery disease[206], and hyperhomocysteinaemia is an independent risk factor for CVD[207,208]. Global differences in DNA methylation profiles of patients with HGPS or Werner syndrome independent of *LMNA* or *WRN* genetic mutations, respectively, indicate a nongenetic role for DNA methylation in these cardiomyopathy-prone ageing syndromes[209]. The role of DNA methylation in ageing is further highlighted by the finding that DNA methylation drift increases with age in cells and tissues[210]. The rate of DNA methylation drift negatively correlates with lifespan in mice, monkeys, and humans, producing a biomarker of lifespan. Interestingly, calorie restriction delays DNA methylation drift and promotes a younger epigenetic age in mice and monkeys, illustrating the dynamic interaction between external factors (nutrition), internal factors (methylation drift), and phenotypic outcome (lifespan)[125].

CpG methylation and histone modifications have been identified as epigenetic regulators of gene expression in human cardiomyocyte nuclei throughout development, but only histone changes distinguish nonfailing from failing adult cardiomyocyte epigenetic signatures[211]. Further molecular information delineating methylomics of normal aged versus pathologically challenged cardiomyocytes will provide valuable insight that might discriminate between healthy adulthood and disease states. Experimental models that recreate tissue-specific and cell-specific changes in methylation patterns with age will help to reveal the precise roles of DNA methylation and DNA methylation drift in cardiac ageing.

Histones. Histones are core proteins around which eukaryotic DNA is packaged and organized into units called nucelosomes. Replication-dependent canonical histones are incorporated into DNA during the S phase of cell division, whereas replication-independent histone variants can be integrated into chromatin throughout the cell cycle and regulate transcription in postmitotic cells[212]. Histone depletion is an established feature of cellular ageing, and reduced histone expression, maturation, or deposition during replication can all contribute to age-associated canonical histone depletion. Global histone depletion is triggered by DNA damage during replicative senescence caused by telomere attrition in human primary fibroblasts, mechanistically linking these cellular ageing phenomena[213]. Global histone loss and attendant decreased nucleosome occupancy increase with age across species, contributing to age-associated chromatin disorganization and genomic instability. Age-associated changes in chromatin content and configuration involve histone variants in a wide variety of experimental models, including nematodes[214], human cell culture[215,216], mice[217], and zebrafish[218]. In some cases, these changes have been implicated in myocardial regenerative processes, such as replacement of canonical histone 3 (H3) with histone variant H3.3, which is associated with activated chromatin in proliferating cardiomyocytes during cardiac regeneration, although replicative capacities might come at the cost of faulty DNA repair and induction of the SASP[219]. Another histone variant associated with ageing, histone H3-like centromeric protein A (CENPA), replaces H3 in

centromere nucleosomes and is critical for kinetochore formation and function during cell division[220]. CENPA levels decline dramatically with age in human pancreatic islet cells[221] and in mouse CPCs[222]. Knockdown of *Cenpa* in mouse CPCs induces premature cellular senescence and exacerbates cell death in committed CPCs. Whereas simple interventions, such as overexpressing histones, can increase lifespan in yeast[223], a more nuanced approach involving selected histone variants that alter chromatin accessibility, such as H3.3 or CENPA, might provide an opportunity for intervention in myocardial ageing processes.

In addition to histone levels and composition, histone post-translational modifications affect chromatin structure and ageing[224]. Histone post-translational modifications include (but are not limited to) enzymatic acetylation or methylation of residues in histone amino-terminal tail regions. Typically, acetylation acts as an activating modification that changes with age[200,225], whereas methylation (depending on the residue) leads to chromatin inactivation. Histone lysine acetylation or methylation influences longevity[213,226]. Age-related changes in histone acetylation patterns contribute to transcriptional dysregulation and ageing-associated diseases. Histone acetyltransferases add an acetyl group to histone lysines, loosening the nucleosome and rendering DNA more available to transcription factors, thereby upregulating gene transcription. Conversely, removal of acetyl groups by histone deacetylases tightens histone–DNA interactions and increases nucleosome compaction. Histone deacetylases are grouped into four classes, the enzymatic activity of which relies on either zinc (class I, II, or IV) or NAD^+ (class III or sirtuins) as a cofactor. Sirtuins are discussed in more detail below, whereas the role of zinc-dependent histone deacetylases in cardiac ageing has been reviewed in detail previously[227]. Histone methylation affects the lifespan and ageing via transcriptional regulation and influences cellular processes such as autophagy, cellular senescence, DNA damage, and environmental stress responses associated with ageing[228]. Enzymes that directly orchestrate histone methylation and acetylation, such as histone methyltransferases, demethylases, and deacetylases, are candidates for mitigating ageing processes in the cardiac context.

Sirtuins. NAD-dependent protein deacetylase sirtuins have been extensively studied in association with calorie restriction and longevity, and have been previously reviewed in the context of cardiac disease and ageing[229–231]. Sirtuins comprise seven family members (SIRT1–SIRT7), of which SIRT1–SIRT3, SIRT6, and SIRT7 exert protective effects in the heart[229,232]. SIRT1, SIRT6, and SIRT7 localize primarily to the nucleus, SIRT2 is cytosolic, and SIRT3–SIRT5 reside in the mitochondria. SIRT1, the most thoroughly characterized sirtuin because of its pro-longevity and cardioprotective activity[229,233–238], deacetylates histone 3 lysine 9 (H3K9), H3K56, H4K16, and H1K26, as well as many non-histone proteins. Cardiomyocyte-specific knockout of *Sirt1* recapitulates an ageing cardiac metabolic phenotype in response to ischaemia–reperfusion[239]. SIRT2, a cytoplasmic sirtuin, has a role in metabolic processes[240] and attenuates cardiac hypertrophy in ageing and angiotensin II-treated mice via deacetylation of serine/threonine-protein kinase STK11 and activation of 5′-AMP-activated protein kinase (AMPK) signalling. Likewise, SIRT2 mediates beneficial effects of metformin, commonly used to treat type 2 diabetes, through downstream AMPK signalling[232]. Mitochondrial SIRT3–SIRT5, which have been previously reviewed in the context of cardiac disease[241–243], regulate oxidative stress, mitochondrial metabolic processes, and mitochondrial dynamics, which all mediate CVD and ageing[154]. Anti-ageing effects of SIRT6 include telomere preservation, DNA repair, enhanced genomic stability, resistance to oxidative stress and inflammation, inhibition of endothelial cell senescence and vascular atherogenesis, and improved glucose metabolism[237,244]. Severe premature ageing phenotypes, including cardiomyopathy, appear in mice that are deficient in SIRT6 (REFS[244,245]) or SIRT7 (REFS[246–248]). Interestingly, SIRT7 is localized primarily to the nucleolus along with nucleostemin (also known as guanine nucleotide-binding protein-like 3) and nucleophosmin, which participate in the cardiac nucleolar stress response and maintenance of youthful phenotypes in CPCs[249–252]. SIRT6 and SIRT7 deacetylate nucleophosmin, which becomes increasingly acetylated with cellular senescence as levels of SIRT6 and SIRT7 decline[253]. Finally, *SIRT5* and *SIRT7* have been identified as candidate longevity genes in SNP analysis of genes responsive to calorie restriction[254].

In summary, the sirtuin family participates in a complex web of intracellular signalling pathways and has multiple roles in cardiac homeostasis, ageing, disease, and metabolism, comprising an array of molecular targets throughout the cell for anti-ageing intervention. Dietary supplements claiming to modulate sirtuin activity are popular products advertised to reverse cellular ageing and improve metabolism. Sirtuins have gained the attention of healthy lifestyle advocates who promote eating foods rich in purported sirtuin activators — the Sirtfood diet — to improve metabolism and overall health. Whether through lifestyle choices or targeted pharmaceuticals, optimizing the activity of specific sirtuins offers the potential to develop therapies that restore a youthful cellular phenotype and facilitate myocardial survival and rejuvenation and to combat ageing and disease.

Animal models of cardiac ageing

Clinically relevant animal models that reflect human ageing and CVD are critical tools for identifying potential therapeutic targets and developing effective health-care strategies for improving human lifespan and healthspan[4,255–257] (TABLE 2).

Telomere models

The discovery that telomere shortening underlies replicative senescence[258–261] led to the development of mouse models of ageing in which components of telomerase were genetically deleted[262–269]. Telomerase-deficient mice display ageing pathologies over subsequent generations as the capacity to maintain telomere length

fails. *Terc*$^{-/-}$ mice, which lack the RNA component of telomerase, had decreased cardiac function by the fifth generation relative to second-generation *Terc*$^{-/-}$ mice and wild-type controls. Interestingly, the CAST mouse strain, which naturally has telomere lengths similar to those of humans, has a premature cardiac ageing phenotype. Cardiac cells from the *Terc*$^{-/-}$ and CAST mouse models have significantly shorter telomeres, increased expression of p53, decreased proliferative signalling, and increased cell death in vitro and in vivo compared with nonstransgenic controls or standard inbred mouse strains, consistent with an ageing, diseased cardiac profile[77,270]. Additionally, cardiac arterial blood pressure increases in both first-generation and third-generation *Terc*$^{-/-}$ mice (the only generations assessed in these studies) compared with wild-type controls, together with increased circulating levels of endothelin 1 owing to increased expression of endothelin-converting enzyme[271]. However, some animal genetic models of ageing and disease do not adequately recapitulate the human condition, such as the mdx mouse model of Duchenne muscular dystrophy (DMD)[272,273]. Although mdx mice harbour genetic dystrophin deficiency, longer telomeres mitigate stem cell replicative exhaustion and cardiac defects observed in humans with DMD. However, crossing mdx mice with *Terc*$^{-/-}$ mice to shorten telomere length results in severe muscular dystrophy with ageing[274] and the cardiac defects associated with DMD[275], including dilated cardiomyopathy, by age 32 weeks. Oxidative stress contributes to cardiomyopathy in the mdx/*Terc*$^{-/-}$ model given that antioxidant treatment ameliorates cardiac dysfunction, consistent with postnatal cardiomyocyte telomere length decline accompanied by DNA damage response, activation of the p53–p21 pathway, and mitochondrial dysfunction[276]. Telomere length also overrides the manifestation of human disease symptoms in mouse models of aortic valve disease driven by neurogenic locus Notch homologue protein 1 (NOTCH1) deficiency (*Notch1*$^{+/-}$ mice)[277], probably involving a telomere-dependent mechanism with dysregulation of several genes encoding telomere-contacting promoters. These mouse models collectively support a role for telomere maintenance in cardiac ageing and disease but also highlight limitations of mouse models for human disease. Combining telomerase-deficient backgrounds with genetic mouse models of human ageing offers a promising approach to simulate more accurately in mice how the genetically equivalent disease progresses in humans.

Laminopathies

Lamin-A/C, an intermediate filament protein, contributes structural integrity to the nuclear lamina, thereby participating in intracellular communication between the nucleus, cytoplasm, and cell surface. Laminopathies caused by *LMNA* mutations frequently manifest as premature ageing diseases, such as HGPS. Associated cardiomyopathies are common, although the underlying mechanisms have not been definitively established. Current thinking hypothesizes that disruption of mechanical function and/or electrical conduction is the cause of lamin-related cardiomyopathies. From a mechanical standpoint, cardiomyocytes with compromised nuclear lamina cannot withstand the physical stress of muscle contraction, resulting in myocardial cellular and tissue failure. Likewise, disorganized connexin channels impair conduction of electrical signals among myocytes, further interfering with contractility and myocardial function. Lamin-A/C also has a role in adult stem cell maintenance such that age-related deterioration or truncation of lamin-A/C disrupts adult stem cell function and reparative capacity[278,279].

Human *LMNA* mutations and equivalent mouse models are summarized in a review of laminopathies and related cardiac phenotypes[187]. Briefly, seven *Lmna* mouse models are available: four knock-in point mutations, two cardiomyocyte-specific transgenic point mutations, and one deletion mutant. Six models die prematurely, two develop progeria, and all show cardiac remodelling, failure, or arrhythmia[280–287]. Global knockout of *Lmna* in mice leads to early death from heart failure[288]. Interestingly, rapamycin treatment improves cardiac function and extends lifespan in *Lmna*$^{-/-}$ mice by enhancing autophagy and clearance of excess desmin[289]. Disruption of prelamin-A/C processing, required for correct incorporation of lamin-A/C into the nuclear lamina, results in lamin toxicity. For example, truncated prelamin-A/C, or progerin, underlies common HGPS phenotypes and is thought to contribute to vascular senescence, heterochromatin instability, disrupted nuclear morphology, and compromised DNA repair. Overall, these *Lmna* mouse models and humans with *LMNA* mutations provide valuable insights for deciphering the cellular and molecular mechanisms underlying cardiac disease and ageing in a clinically relevant context.

Senescent cell ablation

Accumulation of senescent cells exacerbates tissue ageing by interfering with regeneration and spreading the senescence phenotype through SASP signalling. Consequently, deletion of senescent cells is a potential anti-ageing strategy[290]. For example, feeding ABT263 (a senolytic drug) to aged mice depleted senescent haematopoietic and muscle stem cells, and rejuvenated stem cell populations in both normal and aged mice[291]. Cardiovascular function modestly improved in aged mice after a single dose of the senolytic drugs dasatinib and quercitin, both of which are approved for use in humans[292]. Peptide disruption of the forkhead box protein O4 (FOXO4)–p53 interaction targeted senescent cells for apoptosis in vitro and in vivo, counteracting ageing features in progeroid and naturally aged mice[293]. Genetic ablation of senescent cells further confirms this approach. The cell cycle inhibitor p16^{INK4A} is a classic marker of senescence[44]. Transgenic mouse models designed to target senescent cells for destruction utilize the *Cdkn2a* promoter to drive peptidyl-prolyl cis-trans isomerase FKBP–caspase 8-inducible activation of cell death pathways[294,295]. Delayed onset of ageing phenotypes but not extended lifespan was observed following transgenic clearance of senescent cells in a progeroid model (*Bub1b* hypomorphs) with shortened lifespan from cardiac failure[294]. However, treatment

Table 2 | **Cardiac phenotypes in animal models of ageing**

Category	Model	Cardiac phenotype	Associated human disease	Refs
Premature ageing mouse strains	CAST	Diastolic dysfunction, cardiac hypertrophy, fibrosis, and cellular senescence	Premature ageing	77
	SAMP8	Diastolic dysfunction, inflammation, and oxidative stress	Premature ageing	359
Gene deletions	*Terc*$^{-/-}$	Cardiac dysfunction and remodelling	Premature ageing	270
	Sirt6$^{-/-}$	Cardiac hypertrophy, ageing, and failure	SIRT6 level is lower in failing hearts than in healthy hearts	245
	Sirt7$^{-/-}$	Cardiac hypertrophy and inflammatory cardiomyopathy	NA	246
	Pim1$^{-/-}$, *Pim2*$^{-/-}$, and *Pim3*$^{-/-}$	Premature ageing and cardiomyocyte senescence	Cardiac senescence owing to metabolic dysfunction	360
	Lmna$^{-/-}$	Cardiac arrhythmia and premature death	EDMD	288
Laminopathies and other progeroid models	*Lmna*$^{GT-/-}$	Cardiomyopathy and premature death	*LMNA* nonsense mutation fatal in neonates	361
	Lmna^{H222P}	DCM	*LMNA*^{H222P} AD-EDMD	281
	Lmna^{N195K}	Cardiac dystrophy, DCM, and cardiac arrhythmia	*LMNA*^{N195K} and DCM	280
	Lmna^{L530P}	Myocardial degeneration	HGPS	280
	Lmna^{C609G}	Premature death and cardiovascular abnormalities	HGPS	285
	Myh6–Lmna^{M371K}	Fragmentation of cardiac myofibrils	Mimics accelerated AD-EDMD	283
	Zmpste24$^{-/-}$ progerin	DCM	HGPS	362
	Wrn$^{-/-}$ with *Terc* deletion	Diabetes mellitus; CVD not assessed but probable	Werner syndrome	363
	Bub1b$^{+/GTTA}$ missense mutation mouse	Premature ageing and cardiac arrhythmia	MVA syndrome	364
	Bub1b$^{H/H}$ hypomorphic mouse	Premature ageing, cardiac arrhythmia, and vascular ageing	BUB1β level declines with age; vascular ageing	294,365
Engineered gene mutations	Mdx with *Terc* deletion	DCM	DMD cardiomyopathy	274
	Notch1 haploinsufficiency with *Terc* deletion	Premature calcification of the aortic valve	Calcific aortic valve disease	277
	Wrn$^{-/-}$ with *Terc* deletion	Diabetes mellitus; CVD not assessed but probable	Werner syndrome	363
	Sirt1 deletion in cardiomyocytes	Impaired AMPK activation after ischaemia, similar to ageing	NA	239
	Ndufs4 deletion in cardiomyocytes	Disruption of complex I and severe hypertrophic cardiomyopathy	NA	366
Mitochondrial mutations	*Polg* knock-in mutator mouse	Premature ageing and cardiomyopathy	Multiple *POLG*-related syndromes and premature ageing	367,368
	Taz deletion or inducible knockdown mouse	DCM and poor cardiac function	Barth syndrome and early death	369,370
	Slc25a4$^{-/-}$ mouse	Cardiac hypertrophy and mitochondrial cardiomyopathy	*SLC25A4* frameshift leading to *SLC25A4* deletion	371,372
Rodent models of diabetes and obesity	SHR	Hypertension with age, cardiac hypertrophy, and fibrosis	Hypertension and age-associated cardiac phenotypes	373,374
	SHR and STZ	Hypertension, diabetes, and cardiomyopathy	Diabetes, hypertension, and diabetic cardiomyopathy	375
	Zucker fa/fa obese rat	Obesity, diabetes, and premature cardiac metabolic dysfunction	Obesity-related premature cardiac ageing	376,377
	Ob/ob diabetic obese mouse	Diabetes, obesity, cardiac diastolic dysfunction, premature cardiomyocyte apoptosis, and premature death	Obesity-related premature cardiac ageing	378,379
	Db/db diabetic mouse	Age-dependent cardiac and metabolic dysfunction, premature cardiomyocyte apoptosis, and premature death	Age-dependent diabetic cardiomyopathy	379,380

Table 2 (cont.) | **Cardiac phenotypes in animal models of ageing**

Category	Model	Cardiac phenotype	Associated human disease	Refs
Large animal models of CAD, diabetes, and obesity	Canine MMVD	Mitral valve degeneration and CVD	MMVD	381
	Porcine experimental aortic valve insufficiency	Lower cardiac output and valve regurgitation	Age-related aortic valve insufficiency	382
	Porcine STZ/HFD model of diabetes and high cholesterol levels	Atherosclerosis and diabetes	Accelerated atherosclerosis and diabetes	383,384
Chemically induced ageing	D-Galactose-induced ageing in rat and mouse	Increased cardiac cellular senescence, inflammation, and oxidative stress, resulting in cardiac dysfunction	Ageing cardiac disease phenotype	385
Inactivity models	Experimental rodent models of physical inactivity	Increased abdominal fat in young rats and cardiac remodelling	Obesity with inactivity and ageing, and CVD	132,386
	Simulated microgravity in mice	Cardiac remodelling	Cardiac and ageing effects of space flight and age-dependent effects in cardiac progenitor cells	387,388
	Human weightlessness in space flight, microgravity, and head-down bed rest	NA	Ageing effects, cardiac remodelling, and arrhythmia	389–392
Mental stress models	Social isolation	Age-dependent hypertension and CVD	Decreased healthspan owing to social isolation	163,393
	Physical-restraint stress in mice	Increased vascular senescence and impaired angiogenesis	Impaired vascular healing owing to psychological stress	161

AD, autosomal dominant; AMPK, 5′-AMP-activated protein kinase; BUB1β, mitotic checkpoint serine/threonine-protein kinase BUB1β; CAD, coronary artery disease; CVD, cardiovascular disease; DCM, dilated cardiomyopathy; DMD, Duchenne muscular dystrophy; EDMD, Emery–Dreifuss muscular dystrophy; HFD, high-fat diet; HGPS, Hutchinson–Gilford progeria syndrome; MMVD, myxomatous mitral valve degeneration; MVA, mosaic variegated aneuploidy; NA, not applicable; SIRT6, NAD-dependent protein deacetylase sirtuin 6; SHR, spontaneously hypertensive rat; STZ, streptozotocin.

of non-progeroid mice aged 1 year to clear senescent cells did extend lifespan[295]. These pharmacological and genetic models provide proof of concept for senescent cell ablation as an anti-ageing mechanism but will require further refinement for translational application as the field progresses towards human clinical studies[290,296–299]. Establishing the safety and efficacy of pharmacological senolytics in preclinical and large-animal models and defining cardiac SASP cell heterogeneity will be necessary for senescent cell ablation to become a clinically relevant therapy in the treatment of cardiac ageing and disease.

Future treatments for cardiac ageing

CVD and ageing are major growing health-care challenges for industrialized nations and are intimately intertwined, given that ageing populations have a higher incidence of heart disease, leading to premature death[300]. Optimizing healthspan in an ageing population requires a balance between promoting cellular youthfulness and maintaining appropriate control of cellular proliferation[301,302]. Too much of the former and the risk of cancerous transformation increases, not enough and cellular senescence prevails, exacerbating tissue and organismal ageing. Treatments to mitigate deleterious consequences of ageing and CVD integrate extrinsic (BOX 1) and intrinsic (BOX 2) therapeutic targets, from environmental protection and lifestyle changes to pharmacological interventions and cell-based therapies[303]. Ongoing research directions and therapies under development to mitigate cardiovascular ageing include a range of environmental, dietary, and molecular interventional strategies.

Dietary supplements

Given the importance of nutrition in ageing, dietary supplements offer a popular and convenient method for maintaining or restoring youthfulness to an ageing population[304]. For example, spermidine increased lifespan and improved cardiac function in aged mice and rats, correlating with a reduced incidence of CVD in humans[305,306]. Telomerase activator TA-65 can be purchased as a dietary supplement[307–309], and metformin, which promoted healthy ageing in mice[310], will be tested in the TAME trial as an anti-ageing therapy for humans[311]. Although metformin is routinely prescribed for the treatment of type 2 diabetes, a known risk factor for cardiac ageing, the effectiveness of telomerase activators to counteract ageing in non-proliferative tissues, such as myocardium, remains unclear.

Increasing sirtuin activity is a strategy for mitigating the effects of cardiac ageing. Supplementation of NAD^+ to boost sirtuin activity is being investigated as a potential treatment for cardiac disease[312]. Similarly, dietary supplementation with sirtuin activators was shown to increase longevity and improve metabolism in mice fed a high-fat diet[313]. Additional sirtuin activators, such as resveratrol, curcumin, and other phytochemicals, have cardioprotective and anti-ageing properties[314–316]. New nanoformulations of resveratrol seem to increase *SIRT1* and *SIRT6* expression in blood cells and improve serum lipid and insulin profiles in humans[317]. Treatment with rikkunshito, a ghrelin signalling activator, increased SIRT1 protein levels in the heart and improved cardiac symptoms of ageing in mouse models of ageing and senescence[318]. Collectively, these nutraceuticals to combat cellular ageing are gaining popularity, and dietary

manipulations will undoubtedly factor into explorations for anti-ageing targets and therapies. Additionally, further mechanistic studies are needed to establish specific cellular and molecular modes of action, safety profiles, and efficacy in preserving a youthful cardiac phenotype.

Metabolic signalling

Related directly to the influence of diet, metabolic pathways are important regulatory mechanisms controlling cardiac homeostasis. Mechanistic target of rapamycin (mTOR) is a central mediator of cardiac disease and ageing[319,320]. Pharmacological inhibitors of mTOR (known as rapalogues), such as rapamycin, have therapeutic potential in mitigating imbalances in cardiac mTOR signalling[321,322]. Manipulation of proline-rich AKT1 substrate 1 (PRAS40; also known as AKT1S1), an endogenous inhibitor of mTOR complex 1 (mTORC1) signalling, also confers cardioprotection after ischaemic damage and prevents diabetic cardiomyopathy in obese mice[323]. Similarly, inhibition of mTOR signalling ameliorates laminopathy-based cardiac symptoms through upregulation of autophagy. Unfortunately, the adverse effects of pharmacological mTOR inhibitors preclude their development as anti-ageing treatments, although low doses of one rapalogue safely improved immune function in elderly patients[324]. Further studies searching for less-toxic mTOR inhibitors and dosing regimens might reveal ways to promote healthy cardiac phenotypes while minimizing adverse effects.

Epigenetic modifiers

Epigenetics link chromatin to the outside world, mediating how our DNA responds to environmental and cellular influences. As such, targeting molecular ageing at the epigenetic level offers multiple avenues for therapeutic intervention. For example, treatment of cells from patients with HPGS using a histone demethylase inhibitor increases the heterochromatin markers H3K9me2, H3K9me3, and H3K27me3, and ameliorates nuclear deformation in HPGS cells[325]. RVX-208, a small molecule bromodomain and extra-terminal motif (BET) inhibitor in clinical trials for the treatment of CVD, epigenetically derepresses *APOA1* expression and targets several CVD-related pathways, such as inflammation, atherosclerosis, and thrombosis[326]. BET inhibitors are one facet of pharmacoepigenomics — an emerging area of epigenetics-based drug development with potential for applications in personalized medicine[327]. The ageing epigenome is an important area of investigation for developing preventive and regenerative medicine for an ageing population[328].

Gene editing

New technologies are advancing the molecular understanding and therapeutic approaches to treat cardiac disease and ageing. The advent of iPSCs and gene editing methods (such as CRISPR–Cas9) makes it possible to correct deleterious mutations in cells from human patient samples and test for functional improvement. Correction of *DMD* mutations by exon skipping, or myoediting, restores dystrophin expression and myocyte function in 3D heart muscle preparations engineered from iPSCs derived from patients with DMD[329]. Although currently limited to proof of concept, these cutting-edge investigations hint at the potential for targeted gene therapy to treat genetically based defects and potentially also have applications in ageing[330]. However, off-target effects and associated unintended consequences preclude the clinical application of gene editing in its current form.

Circulating factors

Repair of damaged myocardium has long been achieved using cardioprotective and angiogenic cytokines, forming the basis for several therapeutic strategies[331]. Heterochronic parabiosis uses systemic-level intervention to promote youthful restoration via humoral factors that influence tissue repair and stem cell regenerative capacity[332–334]. A parabiotic lifestyle of elderly people coupled to young donors is clearly not an ethically or clinically viable option, but that reality has not hindered searches for rejuvenating factors from young blood, notably the controversial growth differentiation factor 11 (REFS[335–339]). Ultimately, any humoral-based rejuvenation is likely to depend on multiple factors and physiological determinants beyond our current level of mastery for practical interventional strategies to combat ageing.

Cell-based therapy

Cardiac stem cells are synonymous with cardiac research and regenerative medicine[340]. Phase I clinical trials for autologous KIT$^+$ CPC therapy in patients with heart failure have been completed[341,342], and a multicentre, phase II, clinical trial is in preparation. To date, the measurable clinical benefits of CPC therapy have been modest, in part because stem cells from diseased and aged patients have diminished reparative capacity[343]. Rejuvenation of these aged CPCs through genetic engineering[78,79,81,344,345], preconditioning with hypoxia[346,347], or using them in combination with other stem cells[73,348] is a future direction for improving the effectiveness of autologous stem cells as a treatment for age-related cardiac disease. Stimulating endogenous CPCs with drugs, growth factors, exosomes, or exogenously applied stem cells that secrete paracrine factors is another stem cell-based strategy in the treatment of cardiac ageing[349]. Exosomes produced by iPSC-derived cardiomyocytes could be a future direction for the use of autologous iPSCs in the treatment of the ageing myocardium[350,351]; however, further preclinical studies are required to demonstrate the feasibility of this approach.

Literature mining and bioinformatics

Summarizing the scientific literature increasingly requires computer algorithms and integrated bioinformatics analyses to reveal novel research directions and clinically relevant targets. Ageing databases created to achieve this aim include the JenAge Ageing Factor Database (AgeFactDB)[352], Atlas of Gene Expression in Mouse Aging Project (AGEMAP)[353], Digital Ageing Atlas (DAA), and Human Ageing Genomic Resources (HAGR). Development of novel computer programmes combining literature mining, bioinformatics, and experimental science will continue to reveal previously unappreciated associations between candidate ageing

genes, molecular pathways, and CVDs that might point towards novel therapeutic targets and research directions for the treatment of cardiac ageing[354].

Conclusions

Ageing and heart disease are the main health-care burdens of industrialized nations in the 21st century. Reductionist research focusing on any single facet of these complex conditions might identify specific components of the whole; however, integrated strategies incorporating epidemiological, environmental, social, and biological studies are increasingly essential to understand the bidirectional extrinsic–intrinsic interactions. From air pollution to nutrition down to molecular signalling and chromatin remodelling, each aspect influences the others. Epidemiologists, public health researchers, clinicians, and basic research scientists need to collaborate to understand and treat heart disease and ageing in a more holistic way to achieve meaningful change. At the cellular and molecular levels, epigenetics performs this function inasmuch as it represents how our chromatin responds to changes in our behaviour and external environment. In this Review, we have examined components of external and internal macro and micro influences on cardiac ageing in the context of how each contributes to cellular and organismal ageing and how they influence each other. Our ability to mitigate cardiac ageing will depend on a far deeper comprehension of the world in which we live, the molecular processes occurring within us, and how these external and internal influences are integrated.

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N.A.G. and M.A.S. researched data for the article, discussed its content, wrote the manuscript, and reviewed and edited it before submission. K.M.B. and F.F. contributed to creation of the display items before submission.

Competing interests
K.M.B. has a significant interest in CardioCreate, and M.A.S. is a founding member of CardioCreate. N.A.G. and F.F. declare no competing interests.

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