Review

Cardiac stem cell aging and heart failure

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ABSTRACT

A side effect of the medical improvements of the last centuries is the progressive aging of the world population, which is estimated to reach the impressive number of 2 billion people with more than 65 years by 2050. As a consequence, age-related diseases, such as heart failure, will affect more and more patients in the next years. To understand the biological bases of these diseases will be a crucial task in order to find better treatments, and possibly slow age-related morbidity and mortality.

Cardiac stem cells have been at the center of a heated debate and their potential involvement in cardiac homeostasis has been questioned. In this review, we summarize evidence obtained by independent groups, on different animal models and humans, that strongly support the important role played by immature, cardiac resident cells in the cardioprotection against heart failure.

1. Introduction

As a consequence of improvements in reducing perinatal mortality, treating acute diseases, and ameliorating preventive medicine, the world population is rapidly aging, so that it has been estimated that 2 billion people will be over 65 in 2050 [1]. Biological aging is characterized by a progressive decline of the abilities to replace cells lost with normal tissue turnover coupled with an impaired ability to respond to a variety of insults, eventually leading to a condition named frailty. This latter has been described as a syndrome characterized by decreased reserve and resistance to stressors, that causes vulnerability to adverse outcomes. Frailty is not synonymous with comorbidity and disability. However, comorbidities are a risk factor for frailty and disabilities are an outcome of this syndrome [2]. From a pathophys-

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iological standpoint, frailty is the result of alterations of several inter-related physiological systems, that lead to a failure in the function of homeostatic mechanisms [3]. In this regard, the proper regulation of the complex interaction between poorly differentiated stem/progenitor cell populations residing in adult tissues with their specialized microenvironment (i.e. stem cell niches) has been considered to be crucial to generate mature cells adequate in number and function to provide for tissue homeostasis.

2. Stem cell senescence in aging and age-related pathologies

2.1. Senescent stem cells accumulate with aging and in age-related pathologies

Aside from germ cells, that constitute a remarkable exception to cell aging and may hide the secret for cell rejuvenation, the quantity of functional somatic stem cells declines with biological aging [4]. This observation supports the "disposable soma" theory of aging, according to which the finite metabolic resources of each organism should be optimally located between maintenance and repair of either its soma or the germline [5]. Since individuals of species that are protected from those extrinsic causes of mortality, that occur in the wild, live beyond their peak of reproductive fitness, phenotypes of aging and age-related diseases emerge that would not be otherwise apparent. In line, it can be speculated that, for mutations that give selective advantages in older ages, beyond the reproductive age, there is no positive pressure to select enhanced mechanisms of somatic maintenance [6]. Moreover, organismal maturity has been associated with a switch between growth promoting to growth suppressive mechanisms, that leads to a progressive loss of tissue homeostasis. Specifically, aging is coupled with a number of pathophysiological alterations, such as: a progressive loss of muscle mass and function [7], slower wound healing processes [8], defects in immunity [9], reduced neurogenesis [10], reduced reconstruction of hair follicle pigmentary unit [11], alterations in vascular structure and function [12], and a progressive loss of cardiac myocytes [13]. Intriguingly, a parallel accumulation of cells expressing senescent markers has been observed to occur in tissues with aging, while the selective clearance of senescent cells prolongs median lifespan in mice [14]. Cellular senescence is a complex cellular response to a variety of stressors that is believed to trigger tissue remodeling. Senescent cells are resistant to mitogenic stimuli, display a permanent growth arrest coupled with a permanently activated DNA damage response, and release a secretome that is enriched in pro-inflammatory cytokines and matrix remodeling enzymes (reviewed in [15]). Therefore, a transient induction of cellular senescence, followed by the clearance of senescent cells by phagocytic cells, is beneficial, since it can promote tumor regression or tissue remodeling [16,17].

2.2. Why do senescent and dysfunctional stem cells increase their frequency with aging?

An accumulating body of literature is showing that, in many organs, resident stem cells are required for tissue homeostasis and/or repair after injury. The observed decline of stem cell function that ensues with aging has been, therefore, regarded as mechanistic in developing the age related pathophysiological alterations that occur in several tissues.

Deep sequencing experiments have shown that somatic mutations are frequently observed in a large fraction of circulating cells [18–20]. Consistently, it has been estimated that hematopoietic stem cells (HSC) accumulate 10 mutations per year [18]. Although most of these mutations has a neutral effect, part of them is deleterious and leads to HSC death or senescence, while others confers a selective proliferative advantage to the affected HSC. This finding has been associated with a progressive collapse of polyclonal hematopoiesis to a quasi-monoclonal one with aging. In fact, it has been suggested that every individual with >90 years of age has a single dominant Hematopoietic Stem Cell (HSC) clone generating the majority of blood cells [6]. Intriguingly, individuals with clonal hematopoiesis are also characterized by an increased incidence of type 2 diabetes, and of both coronary heart disease and ischemic stroke [20]. Long term effects may arise even as a result of a correctly repaired DNA damage, since DNA repair may alter DNA methylation patterns, and promote changes in histone variants, histone post-translational modifications and histone density, leaving an epigenetic "scar" on chromatin [21]. Consistently, HSC obtained from aged mice display typical modifications of epigenetic marks and, as a consequence, of gene expression patterns, which are associated with sustained self-renewal and loss of differentiation [22]. Intriguingly, features of tissue aging coupled with modifications in gene expression (partially recapitulating those occurring with aging) have been observed following the controlled induction of DNA double-strand breaks *in vivo* [23]. To be mentioned, genes modulated following DNA damage are enriched in ontology terms related with inflammation, immune response, leukocyte proliferation, and chemotaxis [23]. This finding is reminiscent of the biologic activity promoted by the modified secretome released by senescent cells (i.e. senescence activated secretory phenotype or SASP). As anticipated and excellently reviewed in [17], the final scope of SASP release is the elimination of senescent cells by infiltrating leukocytes. Recent findings, however, indicate that, in the context of a *Pten* null background, an immune suppressive SASP is secreted by senescent cells, which prevents the immune-mediated ablation of senescent cells [24]. Additionally, aging exerts a profound effect on the immune system, that includes: a skewing of the hematopoiesis towards the myeloid lineages, and a decrease of NK, and macrophage cell functions [25]. Therefore, the accumulation of senescent cells that occurs with aging may be a consequence of either an immune suppressive SASP or of the reduced function of key immune cell types that are involved in the removal of senescent cells. This latter event, that may be experimentally inhibited by means of a pharmacologic or genetic inhibition of immune-mediated senescent cell disposal, is associated with deleterious effects, such as: impaired tumor regression [26] or persistent fibrosis [27].

2.3. How does an altered environment impacts on stem cell function and aging?

Age-related changes in stem cell microenvironment (also known as niche) could limit the reparative performance of this regenerative pool of cells. Humoral factors and immune cells play a double role in aging, since they may spread inflammation and promote cell senescence in a paracrine fashion, but are required to eliminate senescent cells too.

A clear example of the impact exerted by the environment on stem cell function and tissue aging arises from parabiosis experiments, where the flanks of two animals are sutured in order to create anastomoses between the two circulatory systems [28]. Early observations demonstrated that fluids and humoral factors could be exchanged between the parabionts; although a difference in the transmission of soluble factors was also described (e.g. steroidal gonadal hormones do not cross the "parabiotic barrier", whereas hypophyseal hormones do) [28]. Hematopoietic cells too are exchanged between the animals, although chimerism requires seven to ten days of parabiosis in order to reach its maximum [29]. By suturing animals of different ages (i.e. heterochronic parabiosis), investigators were thus able to demonstrate that blood-borne fac-

tors could exert both positive and negative effects on age-related alterations. Specifically, it was shown that exposure to an aged systemic milieu reduces neurogenesis, hepatocyte proliferation, and muscle repair (promoting fibrosis). This experimental approach additionally showed that a converse "rejuvenation" of old tissues led to improved tissue turnover and healing in the brain, liver and skeletal muscle [30–32], while it was additionally able to reduce age-related cardiac hypertrophy [33]. Additionally, heterochronic cell transplantation experiments performed both in the skeletal muscle and hematopoietic system have led to the conclusion that the age of the host, rather than the age of the cell donor, dictates the efficacy of the treatment [34].

Although these biological effects are largely undisputed, the mechanisms linked to tissue rejuvenation remain uncompletely understood and controversial. Among these, it has been shown, in the skeletal muscle, that the proinflammatory cytokine TNFα (that is upregulated with aging, in a proinflammatory status known as inflammaging) reduces the activation of the Delta1/Notch axis, which normally occurs in response to injury and is dampened with aging [35,36]. Moreover, the excess of TGFβ1, that is found in the serum of aged mice and humans, induces cyclin dependent kinase inhibition in old muscle stem cells [36]. Wnt signaling, instead, deserves a special mention. In the skeletal muscle, its activity is increased both with physiological aging and in the Klotho-deficient mouse models of accelerated aging [30,37]. Consistently, serum from aged animals can activate the canonical Wnt pathway, likely binding to the Frizzled receptors [30], while Wnt16B, that is secreted by senescent cells (i.e. is a SASP component), can activate the canonical Wnt/β-catenin pathway *in vitro* [38]. As a result of the increased Wnt signaling, a myogenic to fibrogenic conversion of skeletal muscle stem cells has been described [30]. More complex is the impact exerted by Wnt signaling on the cardiovascular system (we refer the reader to specific reviews [39,40]), which is consistent with the dual and stage-specific role that is played by this signal transduction pathway during the cardiogenic process. In fact, although the canonical Wnt signaling promotes the expansion of immature cardiovascular progenitors, it subsequently exerts an inhibitory effect on cardiomyocyte maturation [41]. To add a layer of complexity, the age related changes of the cardiac-specific Wnt signaling are still controversial, since both an enhanced [42] and a reduced activation of the pathway have been reported to occur [43]. Nonetheless, independent studies have demonstrated the importance of Wnt signaling in promoting repair and fibrosis following myocardial injury, possibly acting on cardiac progenitors [44,45]. Intriguingly, Wnt is inhibited by the Hippo/YAP pathway [46], an integrator of biochemical and biophysical stimuli that may be altered by aging and pathologic conditions [47].

With regard to neural stem cells, it has been shown that the proinflammatory chemokine CCL11 increases with aging and impairs neurogenesis in mice [31].

A more heated debate and controversy surrounds the role of Growth Differentiation Factor (GDF) 11 in aging. This latter is a member of TGFβ family, which shares a 90% sequence identity with the mature form of GDF8 (also known as myostatin), that has been shown, in recent reports, to decrease with age [33,48,49]. A series of seminal studies has shown that supplementing old mice with GDF11 may reverse age-related cardiac hypertrophy, could restore the functional impairment of skeletal muscle stem cells, improve cerebral vasculature, and enhance neurogenesis [33,48,50]. However, these data were recently challenged by other reports that showed that, as opposed to what previously reported, GDF11 levels increase with age [51,52]. Furthermore, data from different laboratories indicate that GDF11 does not stimulate or could even inhibit skeletal muscle regeneration [51,53]. These conflicting reports, however could only denote the complexity of GDF11 effects in the body. In line, the claimed beneficial effects of GDF11 on the brain have not been confuted yet [54].

An important player, that could be responsible for the beneficial effects of parabiosis, is the immune system. Indeed, following acute muscle injury, both pro-inflammatory (M1) and anti-inflammatory (M2) macrophages infiltrate the damaged area, and exert distinct, but integrated roles in promoting skeletal muscle repair [55]. Although, it should be acknowledged that the description of macrophage activation is still confusing and a matter of debate [56], regulatory T cells (Treg), that are attracted to the injured area by IL-33 [57], promote tissue repair both by regulating M1–M2 switch, and releasing growth-promoting factors that stimulate satellite cells [58]. During aging, the reduced capacity for muscle repair is coupled with a reduced capacity of Treg to accumulate in sites of tissue damage. This condition may be reversed by IL33 supplementation [57]. We consider these findings of particular relevance, since it has been recently shown that mesenchymal stem cell therapy increases the frequency of reparative M2 macrophages in the infarcted myocardium [59]. Moreover, macrophages were shown to be essential both for: healing and repair of acute myocardial infarction in the adult mammalian heart [59], and for the regeneration of the neonatal mouse heart [60]. Intriguingly, the plasma concentrations of soluble ST2 (a decoy receptor for IL33, that is induced when a mechanical strain is imposed to cardiomyocytes and fibroblasts) are associated with increased risk for cardiovascular mortality, in patients with acute and chronic heart failure, and even in the low-risk, population-based cohort of the Dallas Heart Study [61].

Last, a related mechanism that could influence the immune system, is the bioavailability of trace elements, such as copper (Cu) and zinc (Zn). Indeed, the Cu to Zn ratio (CZr) is incremented in acute inflammation, increases as a function of age, and is associated with the risk of cardiovascular disease death [62]. As recently suggested, inflammaging and the SASP released by senescent cells may be involved in raising CZr levels [62]. Importantly, Zn deficiency is associated with reduced polymorphonuclear chemotaxis and decreased phagocytosis. Additionally, Zn promotes monocyte adhesion and is important for the production of pro-inflammatory cytokines, increases the differentiation of $CD34^+$ cells toward NK cells and increases NK cytotoxicity. Altogether, these functions suggest that Zn deficiency may be associated with a defective ability of the immune system to clear senescent cells [63].

3. (Stem) cell senescence in heart failure

3.1. Role of stem cells in the turnover of post-natal cardiomyocytes

Although we are still far away from an unambiguous quantification of the cardiac turnover, the evidence that cardiac myocytes can be generated postnatally has been nowadays accepted and is no longer viewed as heresy [64,65]. Moving from a large body of literature showing that mature cardiomyocytes are withdrawn from the cell cycle soon after birth [66,67], several independent investigators have hypothesized that, in analogy with what has been described for other tissues, including the skeletal muscle [68], resident, undifferentiated stem/progenitor cells could be responsible for the observed adult myocyte turnover. In support to this hypothesis, elegant genetic labeling experiments, that took advantage of the αMHC-MerCreMer x Z/EG mouse model to permanently label cardiomyocytes before injury [69], have repeatedly shown the contribution of immature cells to myocyte replacement [69–71]. These findings were consistent with earlier ones, which described cardiomyocyte chimerism in recipients of sex-mismatched cardiac transplants [72,73]. However, to inflame the debate, more

recent findings from Lee's laboratory, using the same animal model they employed before [65,69,71], have subsequently claimed that a subset of adult cardiomyocytes would be responsible for cardiomyocyte renewal [65]. Nonetheless, in almost 15 years of work, several laboratories worldwide have independently shown that cells with stem/progenitor properties reside in the myocardium of mammalians, including humans, and may generate cardiomyocytes, smooth muscle cells, endothelial cells, and fibroblasts both *in vitro* and *in vivo* (reviewed in [74–76]). Cardiac primitive cells were thus isolated and cultured on the basis of different antigenic profiles and protocols (e.g. c-Kit, Sca1, PDGFRα, cardiospheres) [74–76]. Of these, cKit expressing cells have been at the center of an intense debate. In fact, while Ellison and coll. have shown that: $cKit^+$ cells promote the spontaneous wave of myocardial regeneration that is observed following isoproterenol infusion, in a mouse model of Takotsubo cardiomyopathy, while ablation of $cKit^+$ cells impairs myocardial repair [70], other reports, that employed lineage tracking experiments, have been considered as a definitive proof against the stem cell nature of cKit expressing cells [77]. We will not address technical issues and difference in data interpretation that may have led investigators to draw this conclusion and refer the reader to two commentaries [78,79]. However, we should point out that, as suggested by Hatzistergos and Hare, 4 independent genetic studies have demonstrated the presence of $cKit^+$ cardiomyogenic progenitors in the embryonic and postnatal mouse hearts [78]. However, according to Hatzistergos and coll., bone morphogenetic protein signaling may restrict the cardiomyogenic potential of $cKit^+$ cells, suggesting that external influences may limit the cardiomyocyte differentiation of cells that have the potency to do so (in line with the principles of potentiality and actuality, already introduced by Aristotle around 300 B.C.) [80]. Accordingly, several reports have demonstrated that the beneficial effect of cardiac cell therapy may result from the ability of implanted cells to unlock the endogenous potential to repair [81]. Published results from a collaboration between us and Madeddu's lab have shown that the stimulation of cardiomyocyte proliferation from endogenous sources, together with the protection of ischemic cardiomyocytes from apoptotic cell death, are a potent mechanism of action of human cardiac stem cells [82,83]. Intriguingly, the exact mechanism of paracrine action of cardiac progenitors has not been fully identified yet, but may involve actors (i.e. miRNAs and exosomes) able to target with one hit multiple molecular pathways in target cells [84,85].

3.2. (Stem) cell senescence is associated with aging and age-related pathologies

The progressive decline of the ability to adequately maintain tissue homeostasis or to repair tissues after injury is associated with the occurrence of age-related diseases (such as heart failure, HF) and, eventually, with frailty [15]. As summarized in Fig. 1, we and other authors have tested the hypothesis that the progressive accumulation of dysfunctional and senescent stem cells (and their differentiated progeny) plays a crucial role in the pathogenesis of cardiac aging and HF. Several lines of evidence seem to support this view. Since it has been postulated [76] and experimentally shown that different cardiac resident stem cell populations show a large degree of gene expression overlap [86], we will discuss evidence of resident stem cell senescence, not distinguishing between the different (sub)populations of primitive cells.

Senescent primitive cells and cardiomyocytes accumulate in the myocardium of aged patients with severe systolic dysfunction, in the absence of a history suggestive of ischemia, diabetes, hypertension, valvulopathy or myocarditis [87]. Additionally, histological and morphometric data suggested that patients affected by HF secondary to chronic ischemia were characterized by the accumulation of senescent stem cells in the left ventricle [88]. This finding prompted us to isolate, propagate in culture and analyze cardiac primitive cells both from recipients of cardiac transplantation, affected by end stage HF, and from normal, donated hearts. Histological and *in vitro* studies showed that both aging and heart failure deplete human atria of primitive cells. Furthermore, age and pathology increased the rate of cardiac stem cell senescence and dysfunction, reducing thier proliferative, migratory and differentiation abilities [82,89,90]. In line, Cossu and coll. demonstrated the reduced clonogenicity and differentiation capacity of primitive cells isolated from the heart of patients affected by mitral and aortic regurgitation with ensuing cardiac hypertrophy [91]. With regard to comorbidities, diabetes too, a relevant age-related disease, impairs the *in vitro* proliferative and differentiation capability of resident stem cells, further accelerating their senescence even when compared with ischemic, non diabetic patients [92]. Last, anthracyclin based chemotherapy, a condition that has been associated with accelerated aging [93] and HF, is characterized by cardiac progenitor cell senescence [94].

3.3. Is stem cell senescence good for the heart?

Although the association between stem cell senescence and HF has been repeatedly proven, recent data and a provocative review article of Muñoz-Espín and Serrano [16] have suggested that the accumulation of senescent fibroblasts that is observed after myocardial infarction may indeed be beneficial, since it would limit cardiac fibrosis [95]. Nonetheless, while an acute induction of cell senescence may be required to stimulate regeneration (as suggested in the same review [16]), an increasing level of evidence indicates that the persistence of senescent stem cells limits cardiac repair and may lead to HF. In line, the selective clearance of senescent, $p16^{InkA}$ positive, cells that accumulate in mouse tissues (including the heart) with aging is associated with a reduction of cardiac hypertrophy, and an increased resistance of the heart to β-adrenergic stress [14].

With regard to the specific involvement of cardiac resident stem cells in tissue repair, Ellison and coll. have demonstrated that the selective ablation of $cKit^+$ cells reduces the ability of acutely injured hearts to recover [79]. In line with these data, focusing on anthracyclin cardiomyopathy, De Angelis and coll. have recently observed that, while the direct injection of healthy cardiac stem cells improves the cardiac function and anatomy of Doxorubicin (DOXO) treated rats, senescent cardiac stem cells, that were exposed to DOXO before *in vivo* administration, did not promote myocardial repair in the same animal model [94].

Focusing on the molecular mechanisms associated with cardiac stem cell dysfunction, independent groups have additionally shown that biological aging and diabetes are coupled with a reduced expression of the serine/threonine kinase Pim1 [96,97]. Importantly, the forced expression of this protein reversed cardiac dysfunction and diminished the frequency of senescent cardiac primitive cells [96,97]. Pim1 regulates the expression of nucleostemin (a stem cell-related nucleolar protein required for cell cycle progression and proliferation, that is downregulated following differentiation) via cMyc. Intriguingly, aging and replicative senescence are associated with a progressive loss of nucleostemin expression, while mice that are haploinsufficient for nucleostemin are characterized by an accelerated cardiac remodeling leading to HF, coupled with cell senescence [98]. To further advance our understanding of the mechanisms coupled with stem cell senescence and dysfunction in HF, we took advantage of gene expression profiling and biostatistical analysis of genes differentially expressed between cardiac resident stem cells cultured from normal organs vs. failing hearts [82,89,90]. With this approach, we have shown that the hyperactivation of the TORC1 com-

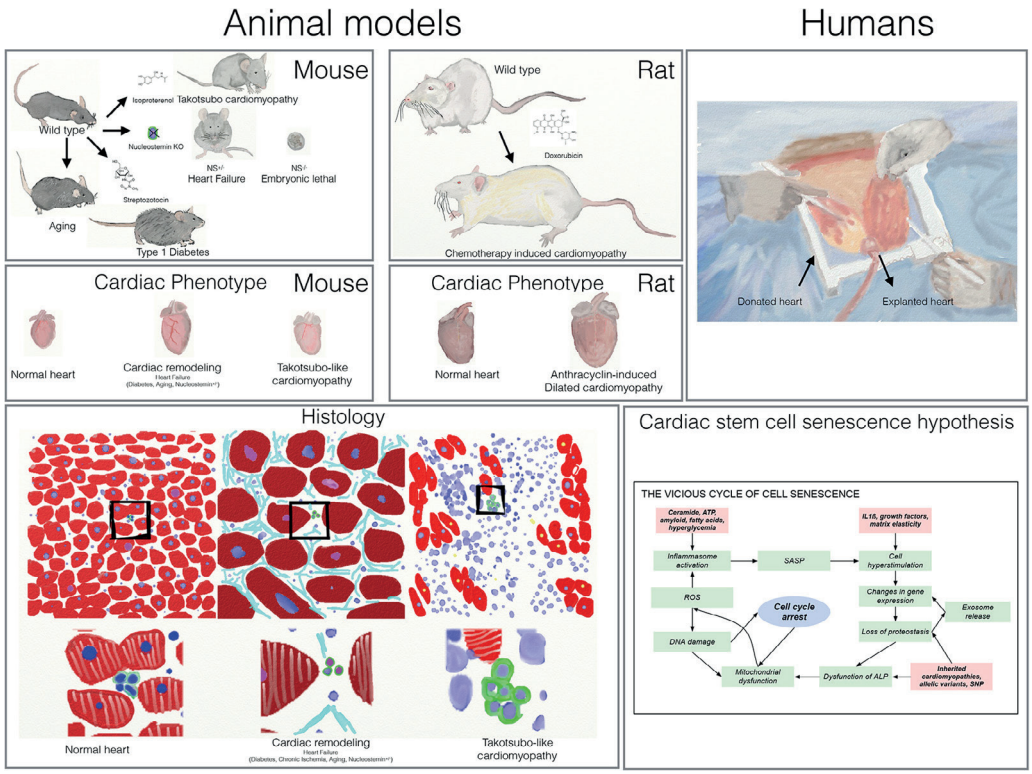

Fig. 1. Involvement of stem cell senescence in aging and heart failure. Cartoon summarizing the experimental evidence, collected both on animal models and humans, indicating the involvement of cardiac stem cells in cardiac pathology. The function, activation, and senescence of cardiac resident stem cells was evaluated in mice, rats, and humans. Specifically, mouse and rat models of drug induced injury (i.e. isoproterenol, doxorubicin or streptozotocin administration to mimic Takotsubo cardiomyopathy, chemotherapy induced cardiomyopathy or type 1 diabetes, respectively), aged mice, and mice heterozygous for the expression of nucleostemin were studied. Samples of donated and explanted human hearts collected after cardiac transplantation were analyzed as well. Histologically, cardiac remodeling is characterized by hypertrophy of cardiomyocytes (depicted in red), fibrosis (shown in cyan), and by the accumulation of cardiac resident stem cells (labeled in green) that express senescent markers (labeled in pink). Takotsubo-like cardiomyopathy, induced by the acute administration of isoproterenol, is characterized by acute cell injury and death (yellow labeling of nuclei), inflammatory cell infiltration, and by cardiac stem cell activation.

Table 1
Experimental interventions of "rejuvenation" that have been reported in the literature.

Type of intervention	Intervention modality	Mechanism of action	Reference
Systemic intervention	Genetic ablation of $p16^{INK4A}$ positive cells (i.e. senolytic approach).	Activation of a drug inducible suicide transgene (i.e. INK-ATTAC) that becomes activated in senescent cells.	[14,101]
	Pharmacological removal of senescent cells (i.e. use of senolytic drugs).	Inhibition of prosurvival, anti-apoptotic pathways in senescent cells (e.g. by dasatinib, quercetin, navitoclax, and TW-37)	[99,102]
	Restoration of a "youthful" microenvironment.	Administration of systemic factors (e.g. GDF11, oxytocin or BPIFB4) or inhibition of pathways altered with aging (e.g. TGFβ or Wnt). However, the impact of these interventions on cardiovascular system rejuvenation is still speculative or unclear.	[33,103–106]
Cell therapy	Administration of young, healthy stem cells to a diseased heart.	Protection from cardiomyocyte senescence and improved cardiac repair.	[82,83,107]
Ex vivo rejuvenation of reparative cells	*In vitro* pharmacologic pre-treatment of stem cells isolated from old, diseased animals followed by their *in vivo* delivery.	Inhibition or activation of pathways that are modified by the aging process (e.g. inhibition of mTOR signaling, Sirt1 activation). Use of epigenetic drugs able to revert, at least partially, the alterations that are associated with systemic diseases (e.g. diabetes).	[82,92,94]
	Genetic modification of stem cells to enhance their function.	Overexpression of either the prosurvival kinase Pim-1 or nucelostemin to reverse cellular senescence. Overexpression of CAMKIIδB to enhance stress resistance.	[97,98,108]

plex, coupled with altered proteostasis, and a dysfunction of the autophagy/lysosome pathway are central players leading to cardiac stem cell dysfunction. Importantly, these alterations can be reversed by means of a pharmacological treatment, that reduces the frequency of senescent stem cells and restores the *in vivo* reparative properties of cardiac stem cells [82]. In line with our data, Vecellio and coll. have recently demonstrated that the altered function of cardiac resident stem cells isolated from diabetic patients is associated with a modified epigenetic landscape. Importantly, epigenetic interventions could reverse these alterations *in vitro* [94]. Last, activation of the class III histone deacetylase Sirt1 was able to prevent the senescence of cardiac stem cells exposed to doxorubicin, partially restoring their *in vivo* reparative properties [94].

4. Cardiac rejuvenation

Given these premises, several investigators have tried to restore cardiac structure and function with different strategies that have been collectively named cardiac "rejuvenation".

As summarized in Table 1, the experimental approaches could be broadly classified into four distinct groups: organism rejuvenation by means of systemic interventions, cardiac rejuvenation by means of cell therapy, and *ex vivo* rejuvenation of reparative cells.

Among these interventions, the identification of senolytic drugs, able to clear senescent cells inducing their apoptosis is certainly one of the most exciting areas of investigation [99]. It is tempting to speculate that this approach will be translated to the clinic, especially if it will combine *in vitro* screening assays on relevant cell types, such as cardiac progenitors cultured from human cardiac biopsies, with appropriate animal models [100].

5. Conclusion

Aging and heart failure are associated with a progressive accumulation of senescent tissue resident cardiac stem cells. The mechanisms that lead to this outcome are part cell intrinsic, part due to systemic changes associated with organism aging. A heated debate has questioned the role of resident stem cells in tissue homeostasis and the causative role of the persistence of senescent cells in cardiac dysfunction. In this review, we have briefly reported data from a large number of studies, obtained from different laboratories, on independent animal models (including humans), that support the notions that: resident stem cells sustain cardiac repair after damage; aging and HF are coupled with the accumulation of epigenetic, gene expression, and biological changes of cardiac resident stem cells that accelerate their senescence rate, impairing their protective function. Finally, and most importantly, we have understood that the alterations imposed on resident cells by pathology are not irreversible, but could be reversed by pharmacological therapy, opening the possibility of employing patient-based cellular models to better tailor cardioprotective drugs, able to stimulate the primitive cell compartment.

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