ANTIOXIDANTS & REDOX SIGNALING
Volume 8, Numbers 11 & 12, 2006

Forum Review

The Telomere–Telomerase Axis and the Heart

JAN KAJSTURA,[1] MARCELLO ROTA,[1] KONRAD URBANEK,[1]
TORU HOSODA,[1] CLAUDIA BEARZI,[1] PIERO ANVERSA,[1]
ROBERTO BOLLI,[2] and ANNAROSA LERI[1]

ABSTRACT

The preservation of myocyte number and cardiac mass throughout life is dependent on the balance between cell death and cell division. Rapidly emerging evidence indicates that new myocytes can be formed through the activation and differentiation of resident cardiac progenitor cells. The critical issue is the identification of mechanisms that define the aging of cardiac progenitor cells and, ultimately, their inability to replace dying myocytes. The most reliable marker of cellular senescence is the modification of the telomere–telomerase axis, together with the expression of the cell cycle inhibitors $p16^{INK4a}$ and p53. Cellular senescence is characterized by biochemical events that occur within the cell. In this regard, one of the most relevant processes is represented by repeated oxidative stress that may evolve into the activation of the cell death program or result in the development of a senescent phenotype. Thus, the modulation of telomerase activity and the control of telomeric length, together with the attenuation of the formation of reactive oxygen species, may represent important therapeutic tools in regenerative medicine and in prevention of aging and diabetic cardiomyopathies.
Antioxid. Redox Signal. 8, 2125–2141.

INTRODUCTION

THE BALANCE BETWEEN cell death and cell division is crucial for the preservation of cell number and organ mass in prenatal and postnatal life (61). Decreases in the number of cells may be compensated by an increase in the size of the remaining parenchymal cells, but this response can rapidly become maladaptive because of the difficulty of the hypertrophied cells to perform their specialized function efficiently (35). Myocyte death and growth are biologic processes that are present in the adult heart and are enhanced with age and in pathologic states (6). It has been repeatedly shown that myocytes can die by apoptosis and necrosis (48, 67). More recently, the possibility of myocyte autophagy has been introduced (58, 156). Whether this form of intracellular organelle turnover has beneficial or detrimental consequences on cardiac structure and function is not yet understood. However, new myocytes can be formed through the activation and differentiation of resident cardiac progenitor cells (CPCs). Under extreme conditions of myocardial damage, produced by extensive infarcts, and overt cardiac failure, a large number of stem cells have to reenter the cell cycle simultaneously in an attempt to reconstitute the lost myocardium. With age, cell death occurs slowly and over a long period of time, and this may allow CPCs to divide and replace, at least in part, dead myocytes. An efficient regenerative response of the myocardium depends on the size of the CPC pool, which is expected to be intact in the event of acute infarcts but may be severely compromised in chronic heart failure and in the senescent heart (31, 149). Thus, the fraction of functionally competent CPCs conditions the magnitude of myocardial regeneration.

Treatment of cardiac diseases in the elderly has prolonged average life span, but maximum life span has not changed in the last 70 years (131). This suggests that cellular aging plays a role in maximum life span that is more important than generally expected. Several lines of evidence have been obtained in favor of the regeneration potential of the adult and aged myocardium by activation of resident CPCs (13, 60, 90, 95–97, 113, 120, 123, 144). The existence of a mitotic clock that regulates cellular life span independently from organ and organism life span would indicate that aging of CPCs condi-

[1]Cardiovascular Research Institute, Department of Medicine, New York Medical College, Valhalla, New York.
[2]Institute of Molecular Cardiology, University of Louisville, Louisville, Kentucky.

tions myocyte aging and, ultimately, the development of an aging decompensated myopathy.

DO STEM CELLS AGE?

According to a well-established paradigm, stem cells possess unlimited self-renewal capacity with an ability to replace themselves, which exceeds the life span of the organ and organism (41, 49). Stem cells were interpreted as the fountain of youth, were considered to be immortal, and were assumed to be well equipped against any form of aging effect. Initial studies on bone marrow stem cells have contributed to the introduction of this concept. The notion that stem cells do not age was supported by the simple observation that hematopoietic function is maintained in old human beings as well as in aged mice. Additionally, flow cytometric assays have indicated that the number of hematopoietic stem cells (HSCs) increases with age, together with the fraction of cycling HSCs (103). Moreover, transplantation experiments revealed that, when bone marrow from old C57BL/6 mice was transplanted into irradiated young animals, the old bone marrow cells were not at a competitive disadvantage with the survived young cells, but successfully replaced the lymphohematopoietic tissues of the ablated hosts (53, 103). However, it has become clear that the number of old HSCs needed for the repopulation of the bone marrow is higher than that usually required when young HSCs are employed (40). This is most likely due to a reduced capacity of these old cells to home and engraft. Aging effects on HSCs are strain dependent and different results have been observed, creating discrepancy in interpretation. Age negatively influences the growth of HSCs. A measurable and progressive functional deterioration in the self-renewing property of HSCs occurs from early development to adulthood and senescence (50). This does not necessarily imply a reduction in the total number of HSCs (103). Thus, the properties of self-renewal, clonogenicity, and multipotentiality of old HSCs are uniformly compromised in all cells or only a reduced pool of HSCs has maintained functional competence. In both cases, HSCs are subjected to the impact of aging and senescence as all other cells of the organism. Signs of aging are even more evident if other mouse strains are considered: in transplantation studies, old HSCs obtained from DBA/2, BALB/c, and CBA/CaH-T6 mice were consistently at a significant growth disadvantage with respect to their young counterparts (26, 27, 140).

Although the concept of stem cell senescence is more largely accepted than in the past, the mechanisms underlying the morphological, molecular, and functional alterations that characterize old stem cells are still controversial. The debate involves not only stem cells but the wider notion of eukaryotic cellular senescence. The theory of aging based on cellular senescence was originally formulated in 1965 when cell senescence was described as the process that limits the number of doublings that normal human cells can undergo in culture (54). The Hayflick limit takes place after a determined number of cell divisions and results in the arrest of cells in G1; these cells are unable to perform specialized functions. This phenomenon has been defined as replicative senescence. The difficulty in the application of these *in vitro* observations to the *in vivo* condition has led to a heated controversy and questions have been raised concerning the validity of this biological process. The possibility that the loss of proliferative capacity may result from "culture shock" and not from a "mitotic clock" has been suggested and the notion of replicative senescence has been challenged (136). Confusion in the field was potentiated by conflicting results obtained from the study of dermal fibroblasts and epidermal keratinocytes *in vivo* (126). Although a progressive accumulation of senescent cells occurs in the skin of old individuals, cutaneous biopsies collected from the same patients at different ages failed to confirm the original observation (32).

The discovery that loss of telomeric DNA occurs during cell division has given new enthusiasm to the supporters of replicative senescence. In each division, cells duplicate their DNA during the S phase. The semiconservative mechanism of DNA replication has an intrinsic obstacle, consisting of the inability of conventional DNA polymerase to complete the synthesis of the lagging strand of the replication fork (101). The end-replication problem would cause a progressive shortening of the DNA (2). In eukaryotic cells, chromosome integrity is maintained by shielding caps, telomeres, and a specialized DNA polymerase, telomerase (15, 44, 45, 112). Telomeres constitute the physical ends of chromosomes (153) and telomerase is the enzyme capable of preserving telomeric length. This ribonucleoprotein is a reverse transcriptase, which extends the 3′ chromosomal ends utilizing its own RNA as a template (15, 44, 45, 112). The synthesis of telomeric repeats by telomerase protects from loss of nucleic acid and allows complete DNA replication.

To date, the most reliable marker of cellular senescence is the modification of the telomere–telomerase axis, together with the expression of the cell cycle inhibitors $p16^{INK4a}$ and p53 (46). Telomerase activity is present in the normal adult human heart and is increased in premature myocardial aging (Fig. 1) and myocardial hypertrophy (31, 147). This ribonucleoprotein, however, does not necessarily prevent telomere erosion (31, 66); severe telomeric shortening has been detected in both myocytes and CPCs (Fig. 2). These observations are consistent with results obtained in hematopoietic stem cells (HSCs). HSCs express low to moderate levels of telomerase, but telomeres shorten considerably with age (3, 76). Two possibilities have been proposed in an attempt to explain telomeric shortening in the presence of detectable telomerase activity: a suppressor of telomerase function may

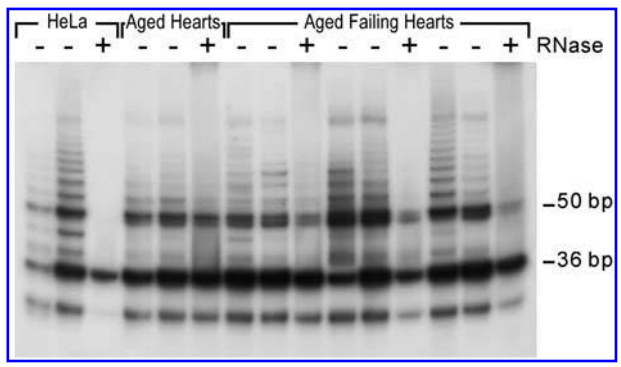

FIG. 1. Telomerase activity measured by the TRAP assay in samples of aged control and aged failing hearts. Products of telomerase activity start at 50 bp and display 6 bp periodicity. Serial dilutions of proteins (1 μg, 0.5 μg) have been used. Samples treated with RNase (+) were used as negative control and HeLa cells as positive control. Note an increase in telomerase activity in aged failing hearts. Modified from Chimenti *et al.* (31).

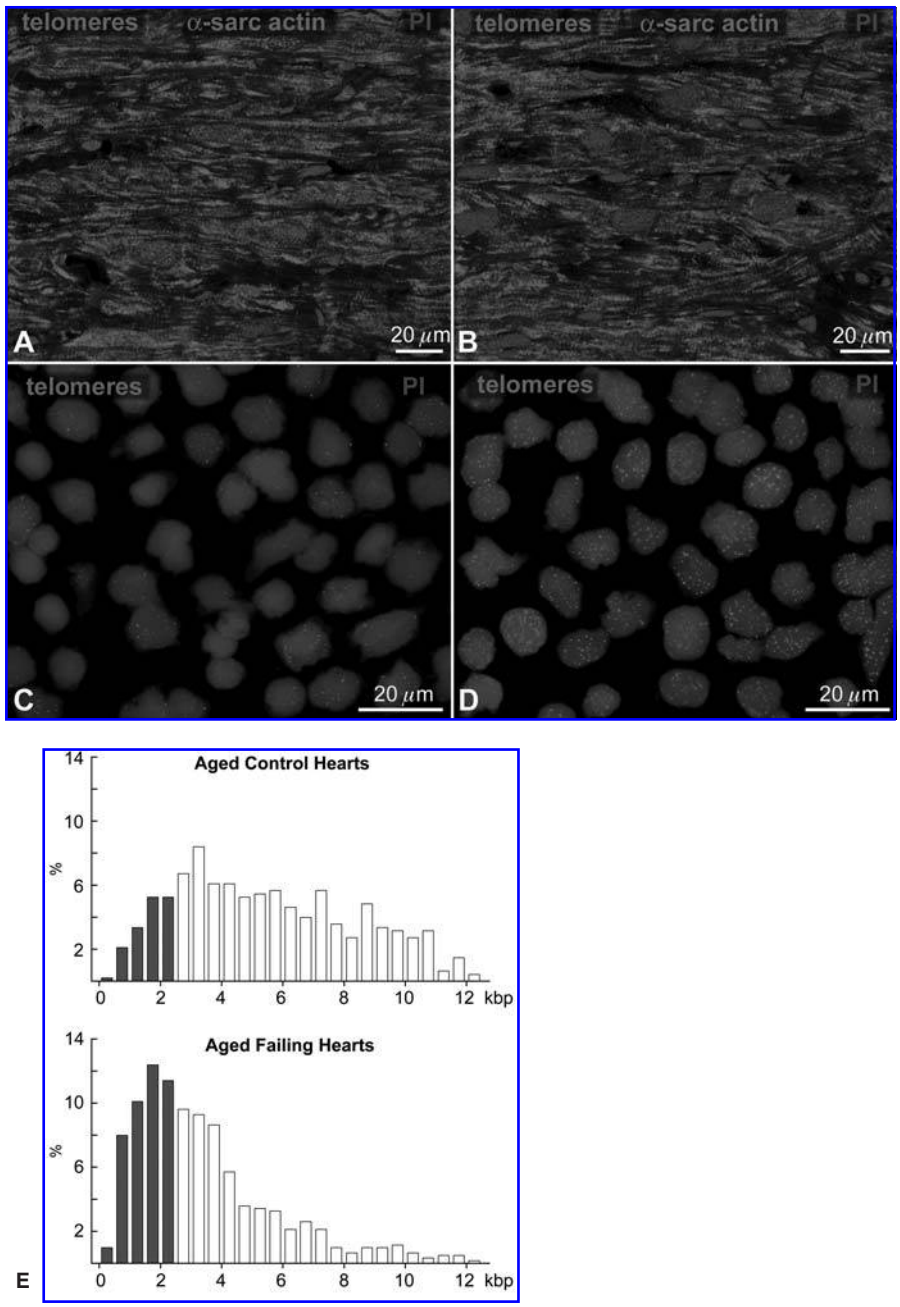

FIG. 2. Telomeric shortening in senescent myocytes demonstrated by quantitative fluorescent in situ hybridization of a PNA probe specific for telomeric sequences. (**A**) corresponds to an aged control heart and (**B**) illustrates an aged failing heart. Dots in nuclei correspond to individual telomeres. The intensity of labeling in myocyte nuclei from the aged failing heart (**B**) is lower than in the aged control heart (**A**). Lymphoma cells with known short (**C**; L5178Y-S cells, 7 kbp) and long (**D**; L5178Y cells, 48 kbp) telomeres are also shown. (**E**) Distribution of telomere lengths in myocyte nuclei of aged control and aged failing hearts. Buckets are 0.5 kbp. *Solid bars* correspond to myocyte nuclei with telomeres <2.5 kbp. Modified from Chimenti *et al.* (31).

be stimulated during the cell cycle or a competition may occur between the reassembly of telomeric chromatin and extension of telomeres by telomerase. In HSCs, telomere shortening occurs at a rate of 50–100 base pairs per population doublings (bp/pd), which is only slightly lower than the 50–150 bp/pd rate characteristic of telomerase-negative cells (76). It is reasonable to assume that the aging effects on CPCs lead to an imbalance between telomerase activity and length of telomeres, resulting in critical telomeric shortening, permanent withdrawal from the cell cycle, and CPC senescence.

Telomere dysfunction is associated with the upregulation of cell cycle inhibitors, including p53 and $p16^{INK4a}$ (137). Whereas the expression of p53 allows the cell to repair DNA damage and re-enter the cell cycle, the growth arrest induced by $p16^{INK4a}$ is considered irreversible. The role of $p16^{INK4a}$ and its closely related gene $p19^{ARF}$ in organ and organism aging has been documented in a recent study (73). The transcript of these two genes has been examined in multiple tissues of young and old rodents. The mRNA of $p16^{INK4a}$ increased an average 10-fold in the heart while $p19^{ARF}$ increased approximately threefold, and the same pattern of changes was seen for the expression of the corresponding proteins in all tissues except for the lung. Characteristically, the old heart showed a selective and specific increase of the cell cycle inhibitor, $p15^{INK4b}$ that did not change with age in all other organs (73). In cancer cells, epithelial cells, and T-lymphocytes, $p15^{INK4b}$ behaves in a manner very similar to $p16^{INK4a}$: it inhibits cell proliferation, blocks telomerase activity, and promotes replicative senescence (36, 39, 133, 151). However, its role in cardiac aging, if any, remains unknown.

Despite the significant increase in the expression of $p16^{INK4a}$ and $p19^{ARF}$, the quantity of protein and mRNA for these two genes is lower *in vivo* than in early passages of primary cell cultures. This observation points to the existence of powerful inductive stimuli of aging *in vitro* but also to the heterogeneity of the aging process *in vivo* that involves only a small subset of cells at any time in each organ. For example, the expression of $p16^{INK4a}$ is higher in the cortex of the kidney than in the medulla, or in the white than in the red pulpa of the spleen (73). Of relevance, caloric restriction prevents the increase of $p16^{INK4a}$ and $p19^{ARF}$ in cardiac tissue providing further evidence of the link between oxidative stress, on the one hand, and telomeric shortening, cellular senescence and organism aging, on the other.

TELOMERIC LENGTH AND REPLICATIVE SENESCENCE

Cellular senescence is characterized by biochemical events that occur within the cell leading to growth arrest and apoptosis (22). In this regard, repeated oxidative stress may evolve into the activation of the cell death program or result in the development of a senescent phenotype. In the heart, the old cells do not differ from those in other organs. Senescent cells cannot divide, their ability to synthesize proteins or secrete autacoids and hormones is reduced, and the antioxidant defense mechanisms together with the DNA repair system are attenuated. In mature myocytes, in which cell replication is lost, aging promotes a block in the capacity of the cell to hypertrophy (142). Myocyte mechanics and Ca2+ transients are altered, since the systems regulating cell activation, contraction, and relaxation are defective. A preservation of the mechanical behavior of myocytes coupled with a reduced response to norepinephrine has been found in aging Wistar rats (154).

As discussed above, cellular senescence occurs after a cell has undergone a finite number of divisions and has lost the ability to replicate by selective alterations in cell cycle regulatory functions (22). Specifically, replicative senescence corresponds to an irreversible cell cycle block in G0/G1 triggered by integrated actions of the p53/p21/p19 and p16/Rb pathways (72, 133). This phase of viable cell cycle arrest is accompanied by various changes in cell physiology, morphology, and gene expression. The discovery that telomeres shorten with each cell multiplication *in vitro* suggests a mechanism by which a cell can sense its own age. The role of telomeres in the control of the cell replication is exerted at two levels, by counting the number of cell divisions and by acting as cell cycle checkpoint (16, 46). Before fibroblasts reach senescence *in vitro*, their telomeres gradually shorten and foci of DNA damage accumulate specifically within the telomeres (57). Forced expression of fully or partially functional TERT (71) prevents the loss of telomeric DNA and prolongs cellular life span. Conversely, the overexpression of a catalytically inactive form of telomerase reverse transcriptase (TERT) causes premature senescence and apoptosis of human fibroblasts or keratinocytes (71). The senescence of cell culture preparations occurs gradually and necessitates multiple population doublings while at the single-cell level, p16 is upregulated rapidly, exerting its inhibitory function on the target cell. Thus, presenescent cultures represent a combination of old and dividing cells, and the onset of senescence is determined by the frequency by which p16-positive cells accumulate. However, an alternative explanation to the *in vitro* findings and the maximum number of cell divisions set by this system is that the loss of proliferative capacity may result from cumulative trauma imposed by the tissue culture *per se* (136). The nonphysiologic environment, including the disruption of cell-to-cell contacts, the lack of interaction with different cell types, the characteristics of the medium, and the adhesion to a plastic surface, are likely to induce stress responses (136). This combination of factors might dictate the limit of cell divisions.

Whether cellular senescence contributes to organism aging remains controversial (37,127). A recent study in aging baboons has shed light on this complex issue and the occurrence of replicative senescence *in vivo* has been documented (59). Most importantly, the crucial role played by cellular senescence in the aging of organs has been demonstrated. Like humans, baboons have a relatively long life span and show age-dependent telomere shortening (10). Baboon skin fibroblasts undergo replicative senescence upon serial passage in culture, with characteristics identical to those of human fibroblasts. Senescent baboon fibroblasts exhibit DNA strand breaks that are in close association with phosphorylated histone H2AX, activated ataxia–telangiectasia mutated kinase, and p53 binding protein (59, 141). Senescent fibroblasts express p53 mostly in its phosphorylated form at Ser15. Ser15 is the first site in which p53 is phosphorylated upon DNA damage and this posttranslational modification is followed by the addition of phosphate groups in a cluster of serine residues located at the amino-terminal of the transcription factor. The phosphorylation of p53 in Ser15 is accompanied by enhanced expression of $p21^{Cip1}$ to allow the cells to repair, if possible, DNA damage. More than 80% of senescent baboon fibroblasts *in vitro* show signs of telomere dysfunction (59). Three biomarkers of cellular senescence have been employed for the recognition of replicative senescence *in vivo*: telomere dysfunction, activation of the ATM DNA-damage response, and heterochromatinization of the nuclear genome. Cells with these features are senescent and account for >15% of fibroblasts in old animals (59).

Together with telomere dysfunction, chromosome heterochromatinization is considered a fundamental feature of senescent cells (158). Heterochromatinization corresponds to the condensation of eu- and heterochromatin regions (85). It is commonly observed throughout the cell cycle and is associated with changes in gene expression. However, progressive irreversible heterochromatinization occurs with aging being triggered by both replicative and oncogene-induced senescence (110). This process is believed to be irreversible and leads to an increased frequency of foci of transcriptionally silent chromatin called senescence-associated heterochromatin foci (SAHF). The formation of these foci seems to be dictated by the RB pathway to repress the expression of proliferation-promoting E2F target genes, such as cyclin A, DHFR, and Mcm (110). Moreover, the presence of SAHF is coupled with a decreased efficiency of the DNA damage

repair process enhancing the frequency of chromosome aberrations in aging cells (110). The presence of cells with these genetic characteristics has profound physiological consequences on organ function.

Evidence of the *in vivo* association between telomere dysfunction and senescence has been found, but it cannot be excluded *a priori* that telomeric DNA damage may not be exclusively due to replicative exhaustion. In this regard, oxidative stress increases the rate of telomere attrition (17, 159). Aging is typically accompanied by enhanced production of reactive oxygen species in the mitochondrial compartment and reduced cellular defense to oxidative challenge. Thus, telomere dysfunction can be dictated by an increased oxidative challenge and telomeres may serve the dual function *in vivo* of replicative and chronological clocks.

Pathways of telomere-independent senescence have been described. These processes occur in response to a variety of cellular stresses and signaling imbalances. In a manner similar to telomere-dependent aging, they involve the cyclin-dependent kinase inhibitor p16 and the retinoblastoma tumor suppressor RB as the terminal effectors. A well-documented example of this form of telomere-independent aging is cell senescence induced by hyperproliferative signaling elicited by chronic activation of mitogenic signals including Ras or IGF-1 (134). This mechanism is considered to constitute a tumor-defense strategy of the cells and may help in the understanding of the conflicting effects of IGF-1 on aging of lower and higher organisms. Mutation of the homologue of insulin-IGF-1 receptor, *daf-2*, in nematodes or *Inr* in fruitflies delays aging and extends maximum life span in these lower eukaryotes (143). Attenuation of insulin/IGF-1 signaling results in upregulation of DAF-16, which activates a variety of genes implicated in longevity and inhibits selective life-shortening genes (104). Conversely, the overexpression of muscle IGF-1 interferes with Foxo, that is the mammalian homolog of DAF-16, and inactivation of Foxo has a powerful positive therapeutic impact on skeletal muscle growth and cachexia (23).

The molecular mechanism underlying the increased life span in long-lived *daf-2* mutants is largely mediated by the enhanced expression of enzymes that protect and repair oxidative damage (104). Reactive oxygen species (ROS) are formed in several compartments of the cells but approximately 90% of intracellular oxidants are generated within the mitochondria. Strong reductions in IGF-1 signaling in *C. elegans* results in a quiescent state of diapause called dauer, which corresponds to a nonfeeding, stress-resistant larval state (143). Most importantly, nearly 35% of nematodes with modest decreases in *daf-2* become extremely lethargic, dauer-like, and lose spontaneous motility. In both cases, oxidative stress is minimal and longevity may be dictated by the metabolic switch (8). This possibility is consistent with the well-established paradigm of extended life span with severe caloric restriction or reduced fat mass in lower organisms and mammals (19, 69). In yeasts, dietary limitation promotes metabolic changes that invariably increase maximum life span (70). The low metabolic state is regulated by Sir2 that may be part of the IGF-1 pathway (47). The question is whether the IGF-1-IGF-1 receptor system has to be blamed as the cause of premature aging and death in yeasts, nematodes, fruitflies, and mammals or oxidative stress is a more likely determinant of longevity, supporting the notion of the free radical theory of aging.

REPLICATIVE SENESCENCE AND THE AGING HEART

The increase in average life span has resulted in an increased prevalence of cardiovascular diseases. This phenomenon, together with the recognition that telomeric shortening is a major mediator of cellular senescence, has brought the attention of cardiologists and cardiovascular scientists to the potential involvement of changes in telomeric length in the pathologies that typically occur in the elderly. However, the commonly accepted paradigm is that the heart is a postmitotic organ characterized by a predetermined number of myocytes, which is defined shortly after birth and is preserved throughout the life of the organism (30, 108, 109, 116). According to this view, telomeric shortening can occur only during development when myocytes actively replicate. In this regard, early telomere attrition during fetal life has been linked to the small weight of infants and high prevalence of cardiovascular diseases in adults (33). The view of the terminally differentiated state of the heart is in striking contrast to the possibility of replicative senescence. The age of myocytes has to correspond to the age of the organ and organism and the heart has to be composed of a homogenous population of myocytes of identical age. Therefore, myocardial aging has been interpreted as a time-dependent biological process that interacts with ischemic heart disease, hypertension, diabetes, and other pathological conditions, which together define the clinical phenotype (74). Emphasis has been placed on age-associated changes, which increase the chances of cardiovascular events in the elderly.

The problem of cellular aging is complex when the brain and the heart are considered. Because of the presumed postmitotic nature of neurons and cardiomyocytes, the mitotic clock could not be applied to these organs. However, a different view of neuron and cardiomyocyte aging has not been proposed. The recent identification of neural stem cells (21, 121, 138) and CPCs (13, 60, 95–97, 113, 120, 123, 144) points to a new theory of organ aging. Aging of organs with self-renewing properties may be characterized by stem cell damage, forced entry of these cells in a quiescent state, and marked decrease in the number of functionally competent primitive cells (103). Thus, DNA damage and the expression of proteins blocking the cell cycle might increase in stem cells with age, reducing the stem cell pool to a critical value (40, 103, 140). This notion seems to contradict the definition of stem cells as immortal cells. However, this is not the case in the bone marrow (26, 27, 40, 41, 50, 53, 55, 103, 140) and is not the case in the heart (31). Primitive cells positive for $p16^{INK4a}$ have been detected in the heart of patients with cardiac decompensation and signs of precocious organ aging. Apoptosis of c-kit-positive undifferentiated cells was very high in these aged hearts and was always associated with the expression of $p16^{INK4a}$ (31). Moreover, the fraction of cycling CPCs was increased; this phenomenon is consistent with results in bone marrow (103). Similar findings have been col-

lected in the Fischer 344 rat (64). Thus, the activation of old CPCs, which undergo a limited number of doubling reaching cellular senescence and growth arrest, may be viewed as the process underlying cardiac aging.

The adult heart is largely composed of terminally differentiated myocytes. Damaged and old units of this highly specialized compartment of contracting cells are constantly replaced by new younger elements. The heart is, in fact, a dynamic organ where myocyte death and growth are tightly regulated to maintain physiologic homeostasis. Mitosis and cytokinesis have been recognized in mature myocytes and in poorly differentiated cells with a thin subsarcolemmal halo of myofibrils, in combination with telomerase activity (4, 14, 31, 65, 147). These *in vivo* findings, together with observations *in vitro* (13), have indicated that replicating myocytes are transient amplifying cells derived from lineage commitment of CPCs. When a stem cell divides, two daughter cells are formed; they may maintain stem cell properties or become amplifying cells (159). Amplifying cells divide rapidly and simultaneously undergo differentiation. They derive from stem cells but have a limited number of population doublings. Such a restriction in cell division is conditioned by the progressive downregulation of telomerase with the progression of differentiation. Thus, the heart is a self-renewing organ in which the ability to replenish cells is maintained by the existence of a stem cell compartment. Telomerase activity has been documented in the decompensated aged rat heart (84) or in the presence of cardiac failure in the dog (78). In humans, myocardial hypertrophy with modest ventricular dysfunction (147) and the prematurely aged heart with severe functional impairment (31) are characterized by an increase in telomerase activity. This enzyme, however, does not necessarily prevent telomere erosion.

Results obtained in HSCs support these observations. HSCs express low to moderate levels of telomerase, but telomeres shorten considerably with age (3, 20, 76, 103). It is unknown why, despite a detectable telomerase activity, HSCs undergo telomere shortening. Two hypotheses have been suggested: a suppressor of telomerase function could be stimulated during the cell cycle or a competition may occur between the reassembly of telomeric chromatin and extension of telomeres by telomerase (20). In HSCs, telomere shortening occurs at a rate of 50–100 base pairs per population doublings (bp/pd), which is only slightly lower than the 50–150 bp/pd rate characteristic of telomerase-negative cells. Undoubtedly, telomerase has the crucial role to extend the life span of HSCs. Forced expression of telomerase in HSCs prevents replicative aging and maintain telomere length in HSCs (3). Thus, it is reasonable to assume that aging effects on CPCs lead to an imbalance between telomerase activity and length of telomeres, resulting in critical telomeric shortening, permanent withdrawal from the cell cycle, and CPC senescence.

The concept that aging is a process that hits all myocytes in a simultaneous and uniform fashion is not consistent with the progressive increase in heterogeneity of the myocyte compartment observed in the senescent heart. The continuous turnover of myocytes with age results in a heterogeneous cell population consisting of young, adult, old, and senescent myocytes. Data in humans suggest that this subdivision corresponds to cells of different sizes, raising the possibility that the life span of a cell is associated with a progressive increase in volume of the cell. Young amplifying dividing myocytes are <180 μm^2 in cross-sectional area, adult nondividing cells are 200–250 μm^2 in cross-sectional area, old nondividing $p16^{INK4a}$ negative cells are 300–500 μm^2 in cross-sectional area, and senescent nondividing $p16^{INK4a}$ positive cells are 600–900 μm^2 in cross-sectional area (147). Myocyte length, however, remains relatively constant in all these cell categories, varying, at most, from 90 to 120 μm. Importantly, young myocytes do not express inhibitors of the cell cycle such as p53 and $p16^{INK4a}$ (31, 145, 147). In contrast, a large fraction of these cells, 10–15%, is cycling as demonstrated by BrdU, Ki67, MCM5, and Cdc6 labeling (147). Adult myocytes are unable to reenter the cell cycle since they are terminally differentiated. They express $p21^{Cip1}$ but not p53 and $p16^{INK4a}$. Old myocytes express p53 and p21 in the absence of $p16^{INK4a}$ and senescent myocytes express p53 and $p16^{INK4a}$ (31, 81, 145, 147). $p16^{INK4a}$ and p53 are markers of cellular aging. Myocyte aging and the concomitant increase in myocyte volume typically result in a severe depression in cell function and calcium metabolism (145).

An upper limit to myocyte growth exists. Ventricular myocytes of 4-month-old rats were separated according to their dimension by gravity sedimentation in isotonic Percoll solution and found to vary in size from 4,000 to 110,000 μm^3 (107). In almost all cases, myocytes 90,000 μm^3 in volume and larger were positive for $p16^{INK4a}$, a protein that inhibits irreversibly the reentry into the cell cycle and is a marker of cellular aging. These large cells expressing $p16^{INK4a}$ are more prone to apoptotic death while myocytes 15,000 μm^3 in volume and smaller rarely express $p16^{INK4a}$ and rarely undergo apoptosis. Thus, cell death is correlated with the size of the cell and with the distribution and level of this marker of cellular senescence. The inability of old myocytes to hypertrophy and/or replicate becomes apparent when myocardial infarction is induced in young adult animals. At 7 days after infarction, DNA replication, detected by BrdU incorporation, is restricted to small cells and decreases progressively with increasing cell size (107). $p16^{INK4a}$ positive cells do not reenter the cell cycle, and cycling cells do not express $p16^{INK4a}$. Neither hypertrophy nor replication occurs in very large myocytes. Myocytes 35,000 μm^3 in volume and larger do not reenter the cell cycle and their growth response is limited to cellular hypertrophy. The fraction of myocytes with extreme dimensions and impaired growth increases with age, progressively affecting the response of the old heart to pathological stimuli (29, 142). The age-dependent increase in myocyte death, coupled with reduction in the coronary vasculature, further deteriorates the functional adaptation of the senescent heart (7). These phenomena may explain why coronary heart disease and its complications are major risk factors in the elderly, and myocardial infarction is associated with increased morbidity and mortality in this population. Thus, cells with cross-sectional areas 300–500 μm^2 do not replicate but can hypertrophy, whereas myocytes 600–900 μm^2 do not incorporate BrdU, do not hypertrophy, and express the cell cycle inhibitors and senescence-associated proteins $p16^{INK4a}$ and/or p53.

The observation that cycling myocytes are typically of smaller size than fully mature adult cells has raised the hy-

pothesis that newly formed myocytes do not derive from preexisting myocytes but from the activation, replication, and differentiation of CPCs. And the negative impact of aging on myocytes and organ function may involve defects at the level of the regulating cell (*i.e.*, the CPC). Alterations in the stem cell compartment can lead to inadequate generation of committed progeny and inefficient replacement of senescent mechanically depressed myocytes. If the physiological turnover of CPCs is attenuated, myocytes age and cellular aging is paralleled by an increase in myocyte volume together with the expression of inhibitors of the cell cycle and markers of cellular senescence. An increase in old and senescent myocytes has detrimental effects on cardiac performance and may cause the development of an aging myopathy.

TELOMERASE, TELOMERES, AND ADULT MYOCYTES

The classic view of the heart as a postmitotic organ implies that the myocardium is composed exclusively of terminally differentiated myocytes that, at birth, permanently withdraw from the cell cycle. According to this notion, telomerase should not be expressed in myocytes and its enzymatic activity should be present only in the embryonic and fetal heart, but it is expected to disappear rapidly postnatally. Surprisingly, telomerase activity was found in left ventricular myocytes from fetal, neonatal, young adult, fully mature adult, and senescent male and female rats (84). Female and male myocytes have comparable telomerase activity at birth, but a sharp decline occurs in both genders with postnatal maturation. From adulthood to senescence, active telomerase complexes were consistently higher in female than male myocytes. These findings imposed a reinterpretation of cardiac biology. Telomerase activity is detected only in cycling cells (102, 155, 157), which belong to three categories: stem cells, progenitor cells, and transient amplifying cells. However, because of the low number of cell cycles that stem cells experience, this cell pool contributes only in minimal part to the level of enzymatic activity detected in a tissue. Progenitor cells derive from stem cells but have lost the multipotential property and are, therefore, less primitive cells that undergo a significant number of population doublings. Together with transient amplifying cells that divide rapidly and differentiate at the same time, progenitor cells are responsible for the catalytic activity of telomerase in organs.

The enzymatic activity of telomerase is usually detected via the incorporation of radiolabeled nucleotides onto a telomeric single-stranded DNA substrate. Using this biochemical assay, telomerase activity has now been detected in numerous progenitor cell types in humans and rodents (102, 155, 157). TRAP assay is a procedure that involves polymerase chain reaction (PCR), and contamination from nonmyocytes could affect the validity of the results. However, pure preparations of enzymatically dissociated myocytes were employed, indicating that the parenchymal compartment of the adult heart contains telomerase-competent transient amplifying muscle cells in combination with telomerase-negative terminally differentiated parenchymal cells. Although telomerase is a feature of cycling cells, it is not a measurement of the number of cells that have reentered the cell cycle. Enzyme activity may vary among cycling cells and may be influenced by different factors, such as age and sex. The marked decrease in telomerase activity shortly after birth appears to depend on the rapid expansion in cardiac mass mediated by the generation of a large number of amplifying cells with reduction in stem cell pool size in both genders. Additionally, the number of telomerase competent myocytes and telomerase activity per cell are influenced by age, modifying the growth reserve of the old male and female heart.

As in the hematopoietic system, the presence of telomerase does not prevent telomeric shortening in aging rat myocytes. Nearly 16% of myocytes of senescent Fischer 344 rats exhibit telomeric shortening, raising the possibility that this fraction of cells experiences multiple divisions throughout life (66). Oxidative stress can also induce erosion of telomeres (17, 159). However, data have been accumulated concerning the ability of adult ventricular myocytes to reenter the cell cycle and undergo karyokinesis and cytokinesis (4, 14, 31, 65, 89, 135, 147), supporting the possibility that telomeric shortening occurs, at least in part, because of cell multiplication.

Different conclusions were reached in a study performed in the mouse heart, in which the postnatal downregulation of telomerase activity and TERT expression was interpreted as a further proof of the terminally differentiated state of the adult myocardium (114). The absence of the enzyme in the 8-week-old heart was considered responsible for the massive telomeric shortening and the onset of replicative senescence that occur simultaneously in all cardiomyocytes shortly after birth. The forced overexpression of TERT in cardiomyocytes maintained telomerase activity in the adult heart, delaying the exit of ventricular myocytes from the cell cycle in the first month of life. However, this genetic manipulation did not induce cell cycle reentry later in life but, unexpectedly, promoted increase in myocyte size. Hypertrophy was also elicited by TERT in cultured cardiac myocytes after viral transduction. This *in vivo* and *in vitro* response was seen as a novel function of the reverse transcriptase. It remains unclear whether normal levels of TERT promote hypertrophy in the heart. The important issue of the physiological role of TERT in myocytes cannot be answered by the transgenic mouse model employed in this study. The TERT transgene was placed under the control of the α-myosin heavy chain promoter in cells that, by definition, had reached complete or almost complete maturation. Mitogenic stimulation of terminally differentiated myocytes has been attempted by various means (1, 117, 139), resulting, in the vast majority of cases, in the reentry of cell into the S phase and DNA synthesis. However, the cells do not traverse the G2/M checkpoint and undergo apoptosis or abortive mitosis. This outcome is the inevitable consequence of terminally differentiated myocytes forced to replicate. A similar phenomenon is present in the mammalian heart in the absence of genetic manipulation. In heart failure, when the demand for new myocyte generation is high, terminally differentiated, hypertrophied senescent myocytes attempt to divide but cannot complete successfully cell replication. A typical feature of senescent failing myocytes is the formation of chromosomal bridges during anaphase (unpublished observations). Senescent cells possess all the fea-

tures that favor the illegitimate rejoining of DNA broken ends: critical shortening of telomeres, uncapping of telomeres for reduced expression of Pot1 or other telomere-related factors, or foci of phosphorylated histone H2AX (150). This chromosomal instability leads to apoptotic cell death.

In rodents, telomerase protects chromosomes from telomere erosion, maintains cell replication, and opposes cell death. Telomerase-competent myocytes are cycling, and telomerase identifies young actively growing myocytes. Conversely, alterations in telomeres result in inhibition of cell replication, myocyte senescence, and death. The effects of changes in telomerase activity and telomere length have been evaluated also in a large animal model of ventricular failure (78). In the failing dog heart, ventricular dysfunction is coupled with activation of myocyte proliferation. The marked increase in telomerase level and activity that accompanies the rapid onset of failure is sufficient to preserve telomere length. Thus, telomerase activity plays an important role in repairing dying myocardium. A subpopulation of canine myocytes expresses telomerase, suggesting that they had the potential of reentering the cell cycle and undergoing multiple mitotic divisions. This contention is supported by the large fraction of Ki67-positive cycling myocytes, which were also telomerase competent. The identification of telomerase in more than one-fifth of myocytes of the canine myocardium, combined with their ability to multiply and express enzyme activity, suggests that proliferation of a subgroup of myocytes contributes significantly to cardiac growth. The preservation of telomeric length found in young dogs with rapidly developing cardiac failure differs from myocardial aging. In fact, regeneration of myocytes with age in rodents leads to progressive telomeric shortening (66).

IGF-1 AND MYOCYTE AGING

IGF-1 belongs to the insulin family of peptides and acts as a growth factor in many tissues and tumors. A locally acting isoform of IGF-1 targeted to skeletal muscle enhances muscle growth and differentiation, prevents age-related muscle atrophy, and potentiates regeneration following injury (105, 106). Similarly, cardiac-restricted expression of IGF-1 increases the formation of ventricular myocytes, attenuates myocyte death, and delays the development of an aging myopathy (86, 88, 122, 145). In the heart, the IGF-1–IGF-1 receptor system induces division of cardiac stem cells (CPCs), upregulates telomerase activity, hinders replicative senescence, and preserves the pool of functionally competent CPCs (12, 145, 148). Following muscle injury, IGF-1 promotes the activation, mobilization, and differentiation of satellite cells that, together with the recruitment of bone marrow progenitor cells, contribute to skeletal muscle regeneration in old animals (105, 106). Spontaneous repair of damaged muscle typically occurs in young animals and is severely impaired with age. Importantly, heart failure leads to a catabolic state characterized by a progressive loss in skeletal muscle mass and the appearance of cardiac cachexia in the late stages of the disease.

In the mouse heart overexpressing IGF-1 (*Igf*+/+), telomeric length is better preserved than in WT littermates. Telomeric shortening affects a progressively larger percentage of mouse myocytes with age in both genders. However, at 20 months of age, telomeres in female *Igf*+/+ are the least affected by shortening, while telomeres in male WT reach the shortest value. Telomere length appears to be a crucial modulator of myocyte division and survival. By plotting telomere length in myocyte nuclei, cell cross section, and p16INK4a and p53 expression as a function of age, an inverse relationship between myocyte size and telomere length can be found in mice (145). In old animals, larger cells with shorter telomeres were p16INK4a and p53 positive. IGF-1 interfered with the age-dependent increases in myocyte size, telomeric shortening, and p16INK4a and p53 proteins. In comparison with young cells, telomere length decreased more in old WT than in TG myocytes.

The changes in telomere length may dictate the differences in myocyte necrosis and apoptosis observed in the mouse heart as a function of age, sex, and IGF-1 expression (145). Overexpression of IGF-1 is coupled with higher levels of the telomerase catalytic subunit. Similarly, telomerase activity, which decreases in WT myocytes with age, is increased in the presence of IGF-1. IGF-1 overexpression preserves high enzyme levels in male and female myocytes at 20 months (145). Complex is the understanding of the heterogeneous adaptation of telomerase in myocytes with sex, age, IGF-1 quantities, and species. Several factors have to be considered: (a) Telomerase activity is present only in a fraction of myocardial cells (12, 51); (b) telomerase-competent cells do not reflect the number of cycling cells and/or their individual enzymatic activity (51, 52); (c) IGF-1 expression and/or state of IGF-1 receptor phosphorylation may not be similar in males and females and may influence the number of telomerase-positive cells, percentage of cycling stem/progenitor/amplifying cells, and degree of catalytic activity (68, 146); and (d) differences in telomerase function between rats and mice (84, 145) may be due to longer telomeres in mouse cells (128, 152).

The myocyte-restricted overexpression of IGF-1 offers the opportunity to study the PI3K-Akt pathway, which may be a fundamental signaling mechanism for the phosphorylation and activation of telomerase in myocytes (145). A consensus site for Akt phosphorylation is present in the mouse telomerase, suggesting that Akt may potentiate telomerase function (145). Four observations were made by performing an Akt-kinase assay employing WT and TG myocytes as well as myocytes infected with an adenovirus carrying a nuclear (Akt-nuc) or membrane (Akt-myr) targeted Akt or a dominant–negative form of Akt (Akt-DN): (a) Noninfected TG myocytes had higher levels of phosphorylated telomerase sequence than WT myocytes; (b) the nuclear-targeted form of Akt is involved in the phosphorylation of telomerase sequence of WT myocytes; (c) Akt-DN decreases substrate phosphorylation in TG myocytes; and (d) peptide phosphorylation was proportional to Akt-nuc–infected WT myocyte proteins. *In vivo*, phosphorylated telomerase decreases only in WT myocytes with age but increases as a function of age in TG myocytes. A similar pattern of changes was observed for phospho-Akt levels. These results are consistent with the attenuation and increase in telomerase activity observed in aging WT and TG myocytes, respectively. To confirm phospho-Akt function in the regulation of telomerase, a TRAP assay was performed in old WT myocytes infected with Akt-nuc or Akt-myr. Only Akt-nuc expression resulted

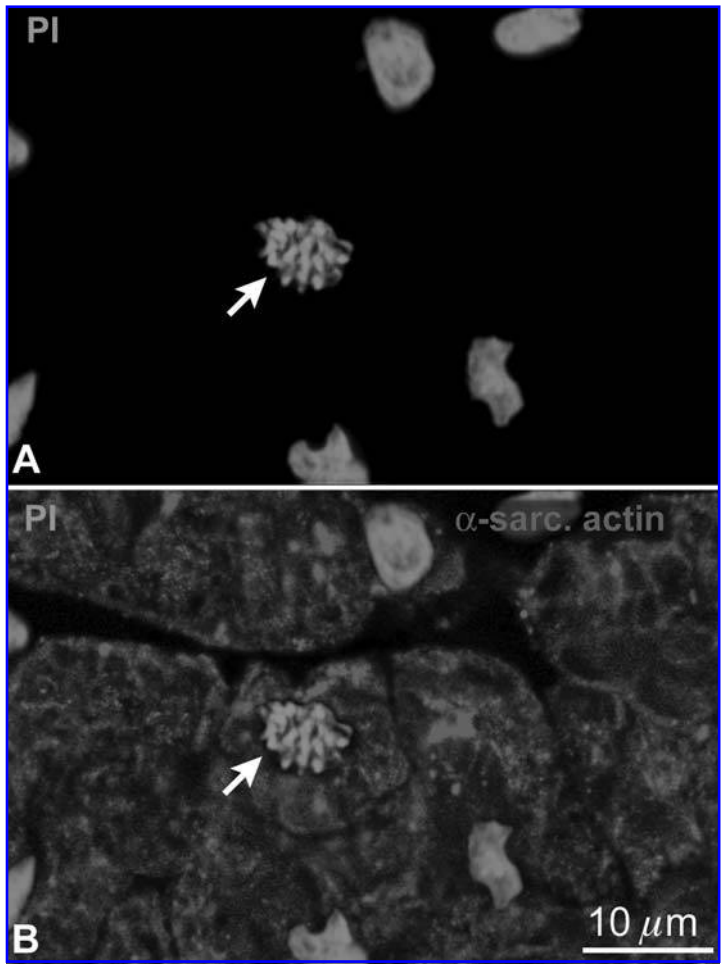

FIG. 3. Myocyte division in an aged failing heart. Metaphase chromosomes are stained with propidium iodide (PI, *arrows*; **A** and **B**) and the myocytes are recognized by the cytoplasmic localization of α-sarcomeric actin staining (**B**). Modified from Chimenti *et al.* (31).

in a marked elevation in telomerase activity. Increasing degrees of Akt-nuc infection of WT myocytes were accompanied by increases in telomerase activity. Akt-myr infection did not modify baseline telomerase in WT myocytes. Telomerase activity in old TG myocytes was severely depressed after infection with the Akt-DN adenovirus (145).

Telomere function is modulated by specific telomere proteins that participate in the prevention of end-to-end fusion of chromosomes (43, 75). The two telomeric related factors, TRF1 and TRF2, bind preferentially to the DNA sequence located at the junction between the telomeric repeat sequence and the G-strand overhang. Alterations in the binding of TRF1 and TRF2 to DNA occur during cellular senescence (18) and deletion of TRF proteins induces premature senescence in murine and human cells by activating the p53 and $p16^{INK4a}$ pathways, which lead to growth arrest and cell death. Accumulation of G-rich single-stranded DNA fragments, together with attenuation in the expression of TRF1 and TRF2, leads to erosion of telomeres and chromosomal instability (130), possibly promoting the formation of intercellular anaphase bridges. In addition to TRF1 and TRF2, telomeres are protected by uncleaved poly(ADP-ribose) polymerase (PARP), and DNA-dependent protein kinase (DNA-PK). Telomere-binding proteins have been recently identified in myocytes and CPCs. Decreased expression of several telomere-associated proteins has been found in the human decompensated heart (31, 147, 149). In the mouse heart, aging was associated with a marked reduction in the quantity of TRF2, and telomere dysfunction was aggravated by downregulation of the components of DNA-PK with age, the heterodimeric complex Ku86/Ku70 and the DNA-PK catalytic subunit (145). Consistently, cleaved PARP increased with age, exceeding the uncleaved protective form. TRF1 was not influenced by aging. The impact of aging on telomeric proteins was attenuated by IGF-1 overexpression. It is unknown whether IGF-1 has a direct or indirect effect on the transcription of these proteins, or promotes post-translational modifications that oppose their degradation.

CARDIAC PROGENITOR CELL AGING

Cardiac aging reflects the attenuation of the growth reserve of the myocardium dictated by senescent CPCs. This negatively affects myocyte turnover favoring cell loss and the accumulation of old, poorly contracting cells. Cellular senescence is characterized by biochemical events that occur within the cell leading to growth arrest and loss of specialized functions (3, 22, 46, 54). In addition to the loss in myocyte replication, aging leads to a block in cellular hypertrophy (142). Senescent myocytes express p53, $p16^{INK4a}$ and hypophosphorylated Rb, together with short telomeres. Low

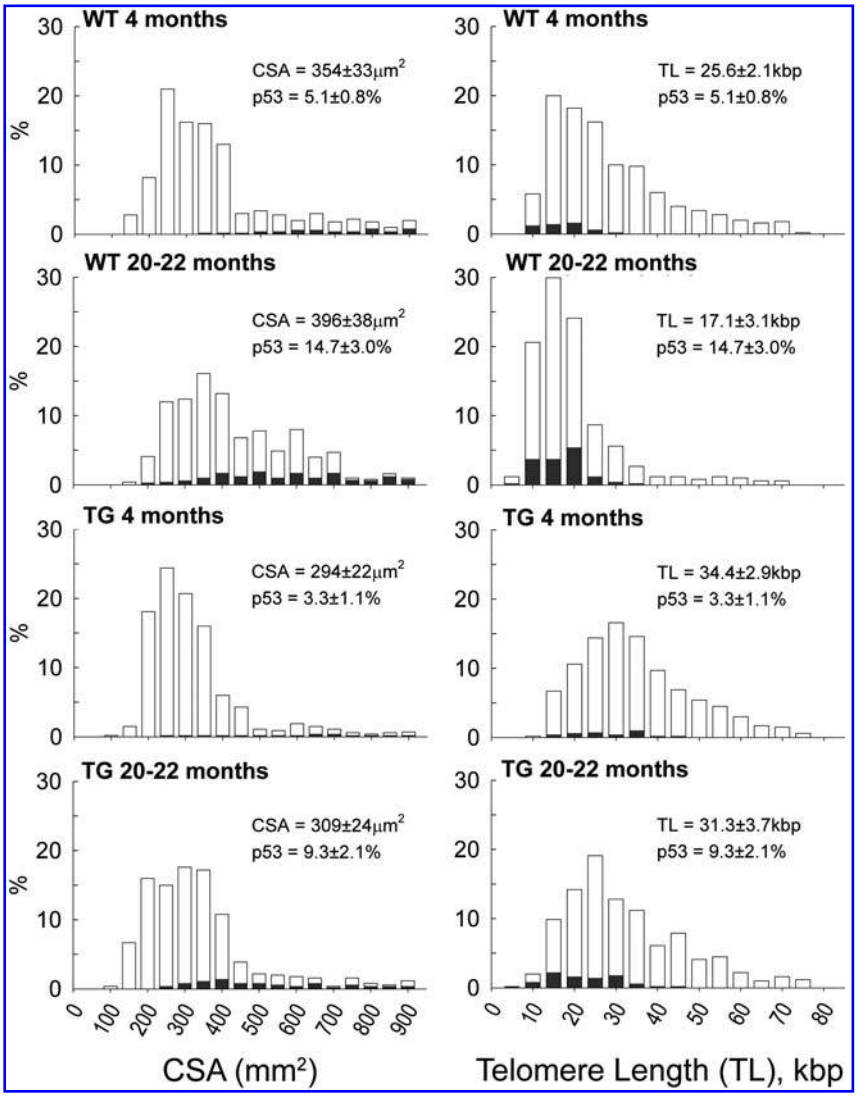

FIG. 4. IGF-1 overexpression and distribution of myocyte cross-sectional areas (CSA) and telomere lengths. Aging in wild-type (WT) mice results in a shift in the distribution of CSA to the *right* and telomere length to the *left*. The solid portions of the bars correspond to p53 positive myocytes. Note that p53 is preferentially expressed in cells with larger CSA and with shorter telomeres. In transgenic mice with cardiac expression of IGF-1 (TG), the age-dependent changes in CSA and telomeric length are markedly attenuated. The numerical values in each graph correspond to average ± standard deviation. Modified from Torella *et al.* (145).

telomerase activity is coupled with a diminished nuclear expression of phospho-Akt, the positive modulator of telomerase. There are three possibilities regarding the deficiency in myocardial regeneration with age: (a) CPC growth and differentiation are attenuated with age; this notion assumes that CPCs are subjected to aging effects, as are the other cells of the myocardium. (b) The ability of CPCs to migrate in the old heart is diminished; this implies that aged CPCs do not respond, as do young CPCs, to growth factors promoting their activation and translocation. (c) The number of functionally competent CPCs decreases with age by growth arrest, apoptotic, and/or necrotic death of old CPCs; thus an attenuation of survival factors occurs in aged CPCs.

DNA damage and the expression of proteins blocking the cell cycle increase in stem cells with age, reducing the pool of functionally competent cells. This notion seems to contradict the definition of stem cells. According to traditional views, stem cells possess an unlimited self-renewal capacity, which exceeds the life span of the organ and organism. However, this is not the case in bone marrow (103) and in the heart (31). CPC aging mediated by telomeric shortening triggers the expression of several gene products, which interfere with the cell cycle but mostly with the G1–S transition. The typical marker of cellular senescence $p16^{INK4a}$ is identified in old CPCs. DNA damage produced by telomere erosion upregulates p53, which together with $p16^{INK4a}$ represents the molecular phenotype of aged CPCs. Primitive cells positive for $p16^{INK4a}$ have been detected in the heart of patients with cardiac decompensation and signs of precocious organ aging. Apoptosis of c-kit-positive undifferentiated cells is very high in these aged hearts and is consistently associated with the expression of $p16^{INK4a}$ (31). As in bone marrow (137), the fraction of cycling putative CPCs is increased, suggesting that activated primitive cells undergo a limited number of doubling, and then reach cellular senescence and growth arrest.

The average telomeric length provides information on the replicative history of the cell. However, this value cannot be interpreted in terms of the growth reserve of the cell and its ability to divide further in the future. The shortest telomere, and not the average telomeric length, is critical for cell viability and chromosome integrity (87). Similarly, the fate of a CPC may be linked to the shortest telomere present in the cell. Loss of DNA sequences and telomeric shortening do

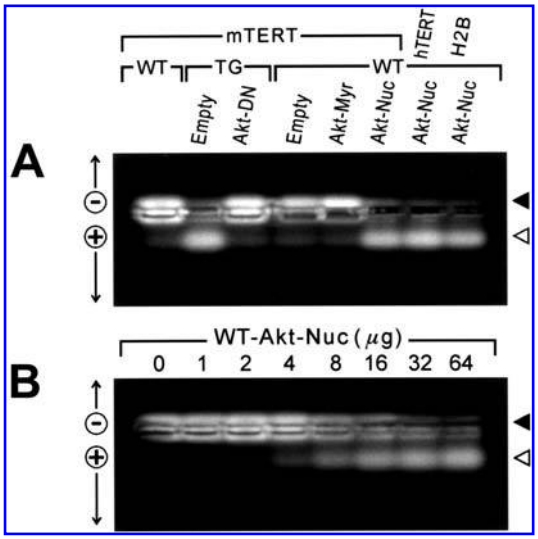

FIG. 5. Activation of telomerase by Akt. (**A**) illustrates the Akt kinase assay of wild-type (WT) and IGF-1 transgenic (TG) myocyte nuclear lysates, and nuclear lysates of WT and TG myocytes infected with empty, Akt-myr, Akt-nuc, or Akt-DN vectors. The substrate utilized in the reaction corresponds to the sequence 585–594 of the mouse TERT. Human TERT (hTERT) and histone 2B (H2B) were used as positive controls. Note that the noninfected myocytes from IGF-1 transgenic mice had higher levels of phosphorylated telomerase (*open arrow*). (**B**) Increasing amounts of proteins were employed to confirm the specificity of the reaction. Modified from Torella *et al.* (145).

not affect all telomeres homogeneously. Shortening preferentially occurs in a fraction of telomeres (56, 77, 87, 94, 160). CPCs may have comparable mean telomeric lengths but exhibit significant differences in the length of individual telomeres.

In conclusion, the process of CPC senescence follows a pattern similar to that identified in myocytes of rodents and humans. This process is characterized by the expression of $p16^{INK4a}$ and telomeric shortening. Because of this CDK inhibitor, a large number of c-kit-positive cells cannot divide and maintain the CPC pool. The increase in c-kit-positive cells undergoing senescence and death interferes with the needs for myocyte replacement in the old heart. This deficiency promotes differentiation of the remaining $p16^{INK4a}$-negative c-kit-positive cells into amplifying myocytes. The higher fraction of CPCs undergoing myocyte commitment is consistent with the increase in BrdU labeling of myocytes with age. CPC senescence, on the one hand, and differentiation, on the other, leads to a progressive depletion of the growth reserve of the heart. The overexpression of the secreted form of IGF-1 had an impact not only on myocyte aging but also on CPC function. In fact, IGF-1 accumulates at high concentration in myocytes where it is then secreted in the interstitium (122). Released IGF-1 binds to IGF-1 receptors present on CPCs (12, 148). The IGF-1/IGF-1 receptor system induces CPC division, enhances telomerase activity, delays senescence, and preserves the reservoir of functionally competent CPCs (145). Importantly, IGF-1 expression increases in the surviving myocytes after infarction and this response prevents the activation of myocyte apoptosis in the overloaded spared myocardium positively interfering with side-side slippage, ventricular dilation, and wall thinning of the postinfarcted heart. Thus, CPCs and IGF-1 protect myocardial regeneration and delay organ aging and heart dysfunction.

CELL DEATH, AGING, AND CARDIAC DISEASE

A relevant question concerns the pattern of cell death and its consequences on the heart. Apoptosis is a rapid phenomenon, which allows sudden anatomical adaptation in response to abrupt changes in loading. The increase in diastolic stress is coupled with the immediate activation of apoptosis, side-to-side slippage of cells, mural thinning, and chamber dilation (7). Apoptosis is very effective in removing undesired cells during development, and the same may be true in the adult heart. Conversely, necrosis develops over a longer period and it is not implicated in the precipitous restructuring of the wall required for the preservation of stroke volume and cardiac output after infarction and other pathologic states (6). Stretching of myocytes *in vitro* does not induce necrosis but triggers apoptosis, promoting an architectural rearrangement of the myocardium (28, 80, 82, 83, 129). Apoptosis does not alter the architecture and composition of the myocardium, whereas cell necrosis results in an inflammatory reaction, vascular proliferation, and collagen deposition leading to myocardial scarring. Although apoptosis and necrosis are two different biological processes, similar stimuli can initiate both forms of cell death. Ischemic insults induce myocyte apoptosis and necrosis, but the timing is different. The intensity of the death stimulus is responsible for the choice of the cell to die by either mechanism. This is the case for oxidative stress. Different levels of ROS trigger apoptosis or necrosis. An increased formation of ROS can cause DNA damage together with covalent modifications of cellular proteins inducing double DNA strand breaks with blunt ends, typical of cell necrosis (34). Oxidative stress characterizes cardiac aging as well as several cardiomyopathies of ischemic and nonischemic origin.

Alterations in Ca^{2+} and mitochondrial respiration occur with heart failure, potentiating the production of superoxide anion, $O_2\bullet-$, and hydrogen peroxide, H_2O_2 (62, 93). Additionally, NO can interact with $O_2\bullet-$, forming peroxynitrite, or ONOO− (11). ONOO- reacts with cellular proteins generating nitrotyrosine, an end product of oxidative damage. Antioxidant enzymes decrease in the decompensated heart (92, 132), depressing its defense mechanisms against these sources of oxidative challenge. Importantly, reactive O_2 may trigger apoptotic cell death (25). The effects of oxygen free radicals are regulated at least in part by the expression of a member of the Shc family of proteins, $p66^{shc}$, which modulates the oxidative stress response (98). Moreover, reactive O_2 causes DNA strand breaks and the injured DNA is recognized by p53 (42, 111). This link stimulates p53 via autoproteolytic activity and cleavage of this protein (100). p53 fragments lacking the C terminus, p50 (ΔC), may bind to single DNA strand breaks, promoting $p21^{Cip1}$ expression and DNA repair

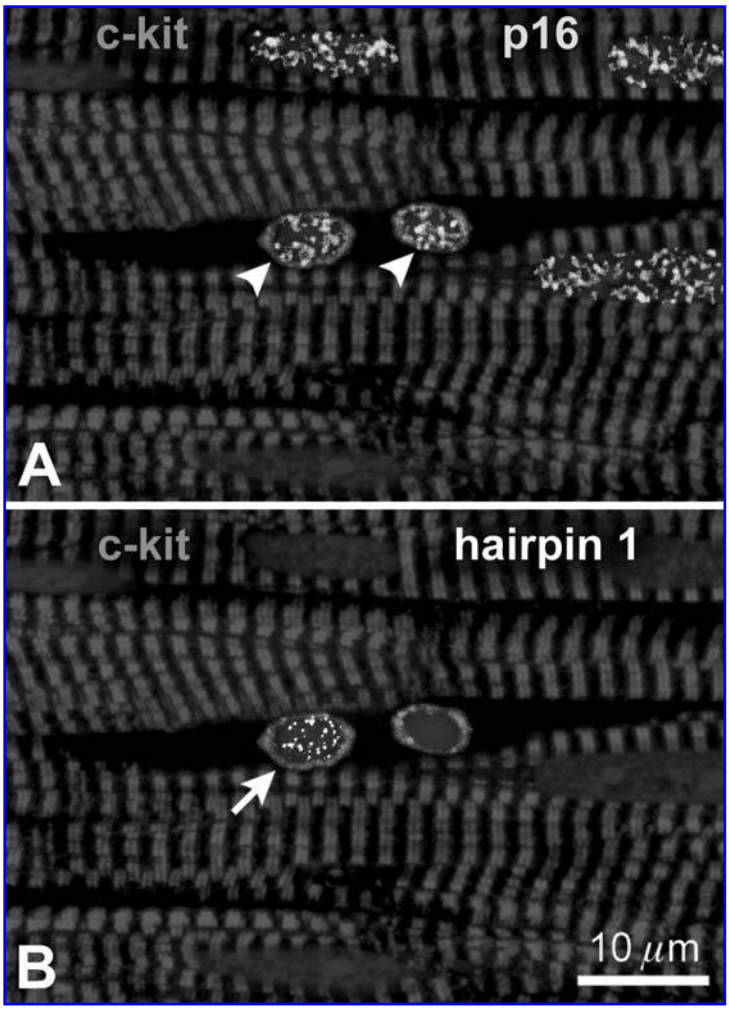

FIG. 6. Senescence and death of cardiac stem cells. (**A**) and (**B**) illustrate the same field in an aging mouse heart. Two cells that have c-kit at the level of the plasma membrane also express $p16^{INK4a}$ (nuclear dots, *arrowheads*). One c-kit-$p16^{INK4a}$ positive cell is labeled by hairpin 1 (nuclear dots, *arrow*). Modified from Torella *et al.* (145).

(115). In contrast, p53 fragments with N-terminal cleavage, p50 (ΔN), may interact with double DNA strand breaks, upregulating Bax and leading to apoptosis (115). Alternatively, reactive O_2 may facilitate the mitochondrial release of cytochrome c, activating the caspase cascade (9). This mechanism of oxidative stress-induced myocyte death may be operative in heart failure and has been documented in a large model of tachycardia-induced ventricular decompensation.

In the canine failing heart, enhanced oxidative stress led to DNA damage, formation of p53 fragments, cytochrome c release, caspase activation, and ultimately cell death. Apoptotic cardiac cell death became apparent at 1 week of pacing, increasing progressively with time (24). The extent of cell death was higher in myocytes than in endothelial cells and fibroblasts. Endothelial cell death may reflect disappearance of capillaries and regional reduction in blood flow and decreased tissue oxygenation (7). Groups of myocytes may also die by ischemia leading to collagen accumulation. In this regard, the serum of patients with heart failure has the capacity to enhance endothelial cell death *in vitro* (124). Moreover, the death of fibroblasts may influence the interconnection between myocytes, facilitating wall restructuring, mural thinning, and chamber dilation (7). Thus, the chronic deterioration in function with pacing appears to be the result of interrelated phenomena initiated by oxidative stress and culminating in cardiac cell death. Cardiac cell death is expected to precede ventricular dysfunction and subsequently contribute to its evolution to terminal failure.

The progressive telomeric shortening documented in pacing myocytes may depend not only on the rapid replication of amplifying cells but also on the significant level of oxidative stress. This correlation has been documented in CPCs during diabetes, a condition characterized by elevated production of ROS. Hyperglycemia leads to enzymatic O-glycosylation and activation of the transcription factor p53, which upregulates the local renin–angiotensin system and the synthesis of Ang II (38). Binding to AT_1 receptors and p53 phosphorylation by p38 MAP-kinase stimulate chronically the tumor suppressor and the formation of Ang II. Hormone secretion and receptor binding increase cytosolic Ca^{2+}, promote the generation of ROS and initiate cell death (38).

Oxygen toxicity and DNA damage alter telomeres, resulting in telomere shortening, cellular senescence, and death (5). In this regard, hyperglycemia led to a significant decrease of telomeric length in myocytes and CPCs after 28 days of diabetes. This adaptation was prevented in knockout mice in which the p66 gene was deleted (125). Consistently, the expression of p53 and p16 increased only in CPCs and myocytes from WT

mice but did not vary in p66 null mice. $p66^{shc}$ is a member of the Shc family of proteins, which include three splicing isoforms encoded by the same genetic locus (99). $p46^{shc}$ and $p52^{shc}$ activate the Ras oncoprotein (91), MAP kinases, and cell division (99, 118). Conversely, $p66^{shc}$ does not upregulate MAP kinases, inhibits c-fos promoter activity (98, 99), and contrasts cell replication (119). Thus, the Shc proteins exert opposite functions: $p46^{shc}$ and $p52^{shc}$ promote cell proliferation while $p66^{shc}$ negatively affects cell growth and enhances ROS-mediated cell injury. Because of $p66^{shc}$ function, the metabolic dysregulation of diabetes was corrected at least in part by ablation of this gene, which prevented oxidative stress.

Ablation of $p66^{shc}$ in CPCs and myocytes may have dramatic beneficial consequences on the diabetic heart; $p66^{shc}$ deletion may decrease the extent of cell death and change cell death, from necrosis to apoptosis. The CPC pool may be protected and, thereby, myocyte regeneration and vessel formation can occur. If apoptosis rather than cell necrosis is the predominant outcome of diabetes in $p66^{shc-/-}$ mice, interstitial fibroblasts may not be activated, and collagen accumulation, myocardial scarring, and cardiac remodeling may be largely avoided. In this regard, cardiac performance is moderately altered 1 week after the onset of diabetes and severe ventricular dysfunction is apparent at 4 weeks in wild-type mice. Conversely, cardiac performance is protected up to 4 weeks in diabetic $p66^{shc-/-}$ mice. Additionally, ventricular dilation is prevented and the myocyte number is preserved (125). These findings suggest that the impact of ROS on the heart is attenuated in $p66^{shc-/-}$ mice, delaying the onset of a diabetic myopathy.

A similar phenomenon was observed in transgenic mice overexpressing IGF-1. With diabetes, IGF-1 protected CPCs and myocytes from both apoptotic and necrotic cell death by interfering with the upregulation of the cardiac RAS, limiting the production of ROS and, ultimately, attenuating cell death. For these reasons, IGF-1 opposes or delays the development of diabetic cardiomyopathy. In the presence of IGF-1, ROS formation is only moderately increased and modest levels of myocyte apoptosis have been detected. Because of this negative influence of IGF-1 on p53 function, local RAS, Ang II production, oxidative stress, and myocyte death, no appreciable alterations in ventricular hemodynamics are found in IGF-1 transgenic mice at 7 and 30 days after streptozotocin administration (63). IGF-1 does not interfere with p53 glycosylation but enhances the expression of the p53-inducible gene mdm2 by upregulating Akt and Akt-dependent phosphorylation of the N-terminus of p53. The formation of p53-Mdm2 inactive complexes led to a decreased synthesis of Ang II, reduced oxidative stress and cell death (63). This mechanism of action of IGF-1 is operative chronically with diabetes, providing long-term beneficial effects on the diabetic heart.

CONCLUSIONS

The documentation that short telomeres contribute to different pathologies points to the possibility that effective therapeutic strategies of multiple human diseases are based on the temporary reactivation of telomerase. Target cells would be those cell types that normally divide to maintain organ homeostasis, such as stem cells, which, although telomerase competent, are unable to maintain telomere length over time. Since telomeres constitute mitotic and chronological clocks of cellular senescence, telomerase has also been proposed as an anti-aging therapy. In senescent cells, the elongation of critically short telomeres would decrease chromosomal instability, increase cell viability, and prevent cell death. In contrast, chronic upregulation of telomerase has been demonstrated in the vast majority of human tumors, in which the maintenance of telomere length allows neoplastic cells to divide indefinitely. In these cases, telomerase inhibition could be an effective way to abolish tumor growth. Thus, the modulation of telomerase activity and the control of telomeric length may represent an important therapeutic tool in regenerative medicine, anti-aging strategies, and anti-cancer treatments.

ACKNOWLEDGMENTS

Supported by National Institute of Health Grants HL-38132, HL-65577, HL65573, HL-75480, AG-17042, AG-26107, HL-81737, HL-78825, HL-55757, HL68088, HL-70897, and AG-23071.

ABBREVIATIONS

ATM, ataxia-telangiectasia mutated; BrdU, bromodeoxyuridine; CPC, cardiac progenitor cell; DAF-16, Decay Accelerating Factor; DHFR, dihydrofolate reductase; DNA-PK, DNA-dependent protein kinase; HSC, hematopoietic stem cell; IGF-1, insulin-like growth factor-1; MAP, mitogen-activated protein; PARP, poly(ADP-ribose) polymerase; ROS, reactive oxygen species; SAHF, senescence-associated heterochromatin foci; TERT, telomerase reverse transcriptase; TRAP, telomeric repeat analysis protocol; TRF, telomeric related factor.

REFERENCES

1. Agah R, Kirshenbaum LA, Abdellatif M, Truong LD, Chakraborty S, and Schneider MD. Adenoviral delivery of E2F-1 directs cell cycle reentry and p53-independent apoptosis in post-mitotic adult myocytes *in vivo*. *J Clin Invest* 100: 2722–2728, 1997.
2. Allsopp RC, Chang E, Kashefi–Aazam M, Rogaev EI, Piatyszek MA, Shay JW, and Harley CB. Telomere shortening is associated with cell division *in vitro* and *in vivo*. *Exp Cell Res* 220: 194–200, 1995.
3. Allsopp RC and Weissman IL. Replicative senescence of hematopoietic stem cells during serial transplantation: does telomere shortening play a role? *Oncogene* 21: 3270–3273, 2002.
4. Anversa P and Kajstura J. Ventricular myocytes are not terminally differentiated in the adult mammalian heart. *Circ Res* 83: 1–14, 1998.
5. Anversa P, Kajstura J, Leri A, and Bolli R. Life and death of cardiac stem cells: a paradigm shift in cardiac biology. *Circulation* 113: 1451–1463, 2006.
6. Anversa P, Leri A, and Kajstura J. Cardiac regeneration. *J Am Coll Cardiol* 47: 1769–1776, 2006.
7. Anversa P and Olivetti G. Cellular basis of physiological and pathological myocardial growth. In: *Handbook of Physiology, Section 2*: *The Cardiovascular System: The Heart, Volume 1*,

edited by Page E, Fozzard HA, Solaro RJ. Oxford University Press. 2002, pp. 75–144.
8. Arantes–Oliveira N, Berman JR, and Kenyon C. Healthy animals with extreme longevity. *Science* 302: 611, 2003.
9. Atlante A, Calissano P, Bobba A, Azzariti A, Marra E, and Passarella S. Cytochrome *c* is released from mitochondria in a ROS-dependent fashion and can operate as a ROS scavenger and as a respiratory substrate in cerebellar neurons undergoing excitotoxic death. *J Biol Chem* 275: 37159–37166, 2000.
10. Baerlocher GM, Mak J, Roth A, Rice KS, and Lansdorp PM. Telomere shortening in leukocyte subpopulations from baboons. *J Leukoc Biol* 73: 289–296, 2003.
11. Beckman JS and Koppenol WH. Nitric oxide, superoxide, and peroxynitrite: the good, the bad, and ugly. *Am J Physiol* 271: C1424–C1437, 1996.
12. Belair GD, Yeager TR, Lopez PM, and Reznikoff CA. Telomerase activity: a biomarker of cell proliferation, not malignant transformation. *Proc Natl Acad Sci USA* 94: 13677–13682, 1997.
13. Beltrami AP, Barlucchi L, Torella D, Baker M, Limana F, Chimenti S, Kasahara H, Rota M, Musso E, Urbanek K, Leri A, Kajstura J, Nadal–Ginard B, and Anversa P. Adult cardiac stem cells are multipotent and support myocardial regeneration. *Cell* 114: 763–766, 2003.
14. Beltrami AP, Urbanek K, Kajstura J, Yan, SM, Finato N, Bussani R, Nadal–Ginard B, Silvestri F, Leri A, Beltrami CA, and Anversa P. Evidence that human cardiac myocytes divide after myocardial infarction. *New Engl J Med* 334: 1750–1757, 2001.
15. Blackburn EH: Telomerases. *Annu Rev Biochem* 61: 113–129, 1992.
16. Blackburn EH. Telomere states and cell fates. *Nature* 408: 53–56, 2000.
17. Blackburn EH. Telomeres and telomerase: their mechanisms of action and the effects of altering their functions. *FEBS Lett* 579: 859–862, 2005.
18. Blasco MA. Telomeres and human disease: ageing, cancer and beyond. *Nat Rev Genet* 6: 611–622, 2005.
19. Bluher M, Kahn BB, and Kahn CR. Extended longevity in mice lacking the insulin receptor in adipose tissue. *Science* 299: 572–574, 2003.
20. Brummendorf TH, Rufer N, Holyoake TL, Maciejewski J, Barnett MJ, Eaves CJ, Eaves AC, Young N, and Lansdorp PM. Telomere length dynamics in normal individuals and in patients with hematopoietic stem cell-associated disorders. *Ann NY Acad Sci* 938: 293–303, 2001.
21. Caldwell MA, He X, Wilkie N, Pollack S, Marshall G, Wafford KA, and Svendsen CN. Growth factors regulate the survival and fate of cells derived from human neurospheres. *Nat Biotechnol* 19: 475–479, 2001.
22. Campisi J. The biology of replicative senescence. *Eur J Cancer* 33: 703–709, 1997.
23. Carter CS, Ramsey MM, and Sonntag WE. A critical analysis of the role of growth hormone and IGF-1 in aging and lifespan. *Trends Genet* 18: 295–301, 2002.
24. Cesselli D, Jakoniuk I, Barlucchi L, Beltrami AP, Hintze TH, Nadal–Ginard B, Kajstura J, Leri A, and Anversa A. Oxidative stress-mediated cardiac cell death is a major determinant of ventricular dysfunction and failure in dog dilated cardiomyopathy. *Circ Res* 89: 279–286, 2001.
25. Chandra J, Samali A, and Orrenius S. Triggering and modulation of apoptosis by oxidative stress. *Free Radic Biol Med* 29: 323–333, 2000.
26. Chen J, Astle CM, and Harrison DE. Development and aging of primitive hematopoietic stem cells in BALB/cBy mice. *Exp Hematol* 27: 928–935, 1999.
27. Chen J, Astle CM, and Harrison DE. Genetic regulation of primitive hematopoietic stem cell senescence. *Exp Hematol* 28: 442–450, 2000.
28. Cheng W, Li B, Kajstura J, Li P, Wolin MS, Sonnenblick EH, Hintze TH, Olivetti G, and Anversa P. Stretch-induced programmed myocyte cell death. *J Clin Invest* 96: 2247–2259, 1995.
29. Cheng W, Reiss K, Li P, Chun MJ, Kajstura J, Olivetti G, and Anversa P. Aging does not affect the activation of the myocyte insulin-like growth factor-1 autocrine system after infarction and ventricular failure in Fischer 344 rats. *Circ Res* 78: 536–546, 1996.
30. Chien KR. Stem cells: lost in translation. *Nature* 428: 607–608, 2004.
31. Chimenti C, Kajstura J, Torella D, Urbanek K, Heleniak H, Colussi C, Di Meglio F, Nadal–Ginard B, Frustaci A, Leri A, Maseri A, and Anversa P. Senescence and death of primitive cells and myocytes lead to premature cardiac aging and heart failure. *Circ Res* 93: 604–613, 2003.
32. Cristofalo VJ, Allan RG, Pignolo RJ, Martin BG, and Beck JC. Relationship between donor age and replicative lifespan in culture, a reevaluation. *Proc Natl Acad Sci USA* 95: 10614–10619, 1998.
33. Demerath EW, Cameron N, Gillman MW, Towne B, and Siervogel RM. Telomeres and telomerase in the fetal origins of cardiovascular disease: a review. *Hum Biol* 76: 127–146, 2004.
34. Didenko VV and Hornsby PJ. Presence of double-strand breaks with single-base 3′ overhangs in cells undergoing apoptosis but not necrosis. *J Cell Biol* 135: 1369–1376, 2006.
35. Dolznig H, Grebien F, Sauer T, Beug H, and Mullner EW. Evidence for a size-sensing mechanism in animal cells. *Nat Cell Biol* 6: 899–903, 2004.
36. Erickson S, Sangfelt O, Heyman M, Castro J, Einhorn S, and Grander D. Involvement of the Ink4 proteins p16 and p15 in T-lymphocyte senescence. *Oncogene* 17: 595–602, 1998.
37. Faragher RGA and Kipling D. How might replicative senescence contribute to human ageing? *BioEssays* 20: 985–991, 1998.
38. Fiordaliso F, Leri A, Cesselli D, Limana F, Safai B, Nadal–Ginard B, Anversa P, and Kajstura J. Hyperglycemia activates p53 and p53-regulated genes leading to myocyte cell death. *Diabetes* 50: 2363–2375, 2001.
39. Fuxe J, Akusjarvi G, Goike HM, Roos G, Collins VP, and Petterson RF. Adenovirus-mediated expression of p115INK4B inhibits human glioma cell growth, induces replicative senescence, and inhibits telomerase activity similarly to p16INK4a. *Cell Growth Differ* 11: 373–384, 2000.
40. Geiger H and Van Zant G. The aging of lympho-hematopoietic stem cells. *Nat Immunol* 3: 329–333, 2002.
41. Globerson A. Hematopoietic stem cells and aging. *Exp Gerontol* 34: 137–146, 1999.
42. Götz C and Montenarh M. p53: DNA damage, DNA repair, and apoptosis. *Rev Physiol Biochem Pharmacol* 127: 65–95, 1995.
43. Greenwood MJ and Lansdorp PM. Telomeres, telomerase, and hematopoietic stem cell biology. *Arch Med Res* 34: 489–495, 2003.
44. Greider CW and Blackburn EH. Identification of a specific telomere terminal transferase activity in *Tetrahymena* extracts. *Cell* 43: 405–413, 1985.
45. Greider CW. Telomerase is processive. *Mol Cell Biol* 11: 4572–4580, 1991.
46. Greider CW. Cellular responses to telomere shortening: cellular senescence as a tumor suppressor mechanism. *Harvey Lect* 96: 33–50, 2000–2001.
47. Guarente L and Picard F. Calorie restriction–the SIR2 connection. *Cell* 120: 473–482, 2005.
48. Guerra S, Leri A, Wang X, Finato N, Di Loreto C, Beltrami CA, Kajstura J, and Anversa P. Myocyte death in the failing human heart is gender dependent. *Circ Res* 85: 856–866, 1999.
49. Harrison DE. Normal function of transplanted mouse erythrocyte precursors for 21 months beyond donor life spans. *Nature New Biol* 237: 220–222, 1972.
50. Harrison DE. Long-term erythropoietic repopulating ability of old, young, and fetal stem cells. *J Exp Med* 157: 1496–1504, 1983.
51. Harrison DE, Astle CM, and Delaittre JA. Loss of proliferative capacity in immunohaemopoietic stem cells caused by serial transplantation rather than ageing. *J Exp Med* 147: 1526–1531, 1978.
52. Harrison DE, Astle CM, and Lerner C. Number and continuous proliferative pattern of transplanted primitive immuno-haemopoietic stem cells. *Proc Natl Acad Sci USA* 85: 822–826, 1988.
53. Harrison DE, Zhong RK, Jordan CT, Lemischka IR, and Astle CM. Relative to adult marrow, fetal liver repopulates nearly five

times more effectively long-term than short-term. *Exp Hematol* 25: 293–297, 1997.
54. Hayflick L. The limited *in vitro* lifetime of human diploid cell strains. *Exp Cell Res* 37: 614–636, 1965.
55. Hellman S, Botnick LE, Hannon EC, and Vigneulle RM. Proliferative capacity of murine hematopoietic stem cells. *Proc Natl Acad Sci USA* 75: 490–494, 1978.
56. Hemann MT, Strong MA, Hao L-Y, and Greider CW. The shortest telomere, not average telomere length, is critical for cell viability and chromosome stability. *Cell* 107: 67–77, 2001.
57. Herbig U, Jobling WA, Chen BP, Chen DJ, and Sedivy JM. Telomere shortening triggers senescence of human cells through a pathway involving ATM, p53, and p21(CIP1), but not p16(INK4a). *Mol Cell* 14: 501–513, 2004.
58. Hein S, Arnon E, Kostin S, Schonburgh M, Elsasser A, Ployakova V, Bauer EP, Klovekorn WP, and Schaper J. Progression from compensated hypertrophy to failure in the pressure-overloaded human heart: structural deterioration and compensatory mechanisms. *Circulation* 107: 984–991, 2003.
59. Herbig U, Ferreira M, Condel L, Carey D, and Sedivy JM. Cellular senescence in aging primates. *Science* 311: 1257, 2006.
60. Hierlihy AM, Seale P, Lobe CG, Rudnicki MA, and Megeney LA. The postnatal heart contains a myocardial stem cell population. *FEBS Lett* 530: 239–243, 2002.
61. Hipfner DR and Cohen SM. Connecting proliferation and apoptosis in development and disease. *Nat Rev Mol Cell Biol* 5: 805–815, 2004.
62. Ide T, Tsutsui H, Kinugawa S, Suematsu N, Hayashidani S, Ichikawa K, Utsumi H, Machida Y, Egashira K, and Takeshita A. Direct evidence for increased hydroxyl radicals originating from superoxide in the failing myocardium. *Circ Res* 86: 152–157, 2000.
63. Kajstura J, Fiordaliso F, Andreoli AM, Li B, Chimenti S, Medow MS, Limana F, Nadal–Ginard B, Leri A, and Anversa P. IGF-1 overexpression inhibits the development of diabetic cardiomyopathy and angiotensin II-mediated oxidative stress. *Diabetes* 50: 1414–1424, 2001.
64. Kajstura J, Leri A, Castaldo C, Quaini F, Mancarella S, Nadal–Ginard B, and Anversa P. Cardiac stem cells mediate myocyte replication in the young and senescent rat heart. *Circulation* 104: II–220, 2001.
65. Kajstura J. Leri A, Finato N, Di Loreto C, Beltrami CA, and Anversa P. Myocyte proliferation in end-stage cardiac failure in humans. *Proc Natl Acad Sci USA* 95: 8801–8805, 1998.
66. Kajstura J, Pertoldi B, Leri A, Beltrami C-A, Deptala A, Darzynkiewicz Z, and Anversa P. Telomere shortening is an *in vivo* marker of myocyte replication and aging. *Am J Pathol* 156: 813–819, 2000.
67. Kang PM and Izumo S. Apoptosis and heart failure: A critical review of the literature. *Circ Res* 8: 1107–1113, 2000.
68. Kang SS, Kwon T, Kwon DY, and Do SI. Akt protein kinase enhances human telomerase activity through phosphorylation of telomerase reverse transcriptase subunit. *J Biol Chem* 274: 13085–13090, 1999.
69. Kenyon C. A conserved regulatory system for aging. *Cell* 105: 165–168, 2001.
70. Kenyon C. The plasticity of aging: insights from long-lived mutants. *Cell* 120: 449–460, 2005.
71. Kim M, Xu L, and Blackburn EH. Catalytically active human telomerase mutants with allele-specific biological properties. *Exp Cell Res* 288: 277–287, 2003.
72. Kim H, You S, Farris J, Kong BW, Christman SA, Foster LK, and Foster DN. Expression profiles of p53-, p16(INK4a)-, and telomere-regulating genes in replicative senescent primary human, mouse, and chicken fibroblast cells. *Exp Cell Res* 272: 199–208, 2002.
73. Krishnamurthy J, Torrice C, Ramsey MR, Kovalev GI, Al–Regaiey K, Su L, and Sharpless NE. Ink4a/Arf expression is a biomarker of aging. *J Clin Invest* 114: 1299–1307, 2004.
74. Lakatta EG. Cardiovascular regulatory mechanisms in advanced age. *Physiol Rev* 73: 413–467, 1993.
75. de Lange T. T-loops and the origin of telomeres. *Nat Rev Mol Cell Biol* 5: 323–329, 2004.
76. Lansdorp PM. Telomere length and proliferation potential of hematopoietic stem cells. *J Cell Sci* 108: 1–6, 1995.
77. Lansdorp PM, Verwoerd NP, van de Rijke FM, Dragowska V, Little MT, Dirks RW, Raap AK, and Tanke HJ. Heterogeneity in telomere length of human chromosomes. *Hum Mol Genet* 5: 685–691, 1996.
78. Leri A, Barlucchi L, Limana F, Deptala A, Darzynkiewicz Z, Hintze TH, Kajstura J, Nadal–Ginard B, and Anversa P. Telomerase expression and activity are coupled with myocyte proliferation and preservation of telomeric length in the failing heart. *Proc Natl Acad Sci USA* 98: 8626–8631, 2001.
79. Leri A, Bearzi C, Cascapera S, Torella D, Cesselli D, Rastaldo R, Quaini F, Urbanek K, and Anversa P. Symmetric and asymmetric division of cardiac stem cells in the mouse heart. *Circulation* 108: IV-33, 2003.
80. Leri A, Fiordaliso F, Setoguchi M, Limana F, Bishopric NH, Kajstura J, Webster K, and Anversa P. Inhibition of p53 function prevents renin-angiotensin system activation and stretch-mediated apoptosis. *Am J Pathol* 157: 843–857, 2000.
81. Leri A, Franco S, Zacheo A, Barlucchi L, Chimenti S, Limana F, Nadal–Ginard B, Kajstura J, Anversa P, and Blasco MA. Ablation of telomerase and telomere loss leads to cardiac dilatation and heart failure associated with p53 upregulation. *EMBO J* 22: 131–139, 2003.
82. Leri A, Liu Y, Claudio PP, Kajstura J, Wang X, Wang S, Kang P, Malhotra A, and Anversa P. Insulin-like growth factor-1 induces Mdm2 and down-regulates p53, attenuating the myocyte renin-angiotensin system and stretch-mediated apoptosis. *Am J Pathol* 154: 567–580, 1999.
83. Leri A, Liu Y, Wang X, Kajstura J, Meggs LG, and Anversa P. Overexpression of insulin-like growth factor-1 attenuates the myocyte renin-angiotensin system in transgenic mice. *Circ Res* 84: 752–762, 1999.
84. Leri A, Malhotra A, Liew C-C, Kajstura J, and Anversa P. Telomerase activity in rat cardiac myocytes is age and gender dependent. *J Mol Cell Cardiol* 32: 385–390, 2000.
85. Lezhava TA. Heterochromatinization as a key factor in aging. *Mech Ageing Dev* 28: 279–287, 1984.
86. Li B, Setoguchi M, Wang X, Andreoli AM, Leri A, Malhotra A, Kajstura J, and Anversa P. Insulin-like growth factor-1 attenuates the detrimental impact of nonocclusive coronary artery constriction on the heart. *Circ Res* 84: 1007–1019, 1999.
87. Li H and Liu JP. Signaling on telomerase: a master switch in cell aging and immortalization. *Biogerontology* 3: 107–116, 2002.
88. Li Q, Li B, Wang X, Leri A, Jana KP, Liu Y, Kajstura J, Baserga R, and Anversa P. Overexpression of insulin-like growth factor-1 in mice protects from myocyte death after infarction, attenuating ventricular dilation, wall stress and cardiac hypertrophy. *J Clin Invest* 100: 1991–1999, 1997.
89. Limana F, Urbanek K, Chimenti S, Quaini F, Leri A, Kajstura J, Nadal–Ginard B, Izumo S, and Anversa P. bcl-2 overexpression promotes myocyte proliferation. *Proc Natl Acad Sci USA* 99: 6257–6262, 2002.
90. Linke A, Muller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, Castaldo C, Cascapera S, Bohm M, Quaini F, Urbanek K, Leri A, Hintze TH, Kajstura J, and Anversa P. Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infracted myocardium, improving cardiac function. *Proc Natl Acad Sci USA* 102: 8966–8971, 2005.
91. Lotti LV, Lanfrancone L, Migliaccio E, Zompetta C, Pelicci G, Salcini AE, Falini B, Pelicci PG, and Torrisi MR. Shc proteins are localized on endoplasmic reticulum membranes and are redistributed after tyrosine kinase receptor activation. *Mol Cell Biol* 16: 1946–1954, 1996.
92. Mailer K, MacLeod I, and Morris W. Age related changes in anti-oxidative enzymes in cardiomyopathic hamster hearts. *Mech Ageing Dev* 59: 37–45, 2000.
93. Marks AR. Cardiac intracellular calcium release channels role in heart failure. *Circ Res* 87: 8–11, 2000.
94. Martens UM, Zijlmans JM, Poon SS, Dragowska W, Yui J, Chavez EA, Ward RK, and Lansdorp PM. Short telomeres on human chromosome 17p. *Nat Genet* 18: 76–80, 1998.

95. Martin CM, Meeson AP, Robertson SM, Hawke TJ, Richardson JA, Bates S. Goetsch SC, Gallardo TD, and Garry DJ. Persistent expression of the ATP-binding cassette transporter, Abcg2, identifies cardiac SP cells in the developing and adult heart. *Dev Biol* 265: 262–275, 2004.
96. Matsuura K, Nagai T, Nishigaki N, Oyama T, Nishi J, Wada H, Sano M, Toko H, Akazawa H, Sato T, Nakaya H, Kasanuki H, and Komuro I. Adult cardiac Sca-1-positive cells differentiate into beating cardiomycytes. *J Biol Chem* 279: 11384–11391, 2004.
97. Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, Salio M, Battaglia M, Latronico MVG, Coletta M, Vivarelli E, Frati L, Cossu G, and Giacomello A. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res* 95: 911–921, 2004.
98. Migliaccio E, Giorgio M, Mele S, Pelicci G, Reboldi P, Pandolfi PP, Lanfrancone L, and Pelicci PG. The p66^{shc} adaptor protein controls oxidative stress response and life span in mammals. *Nature* 402: 309–313, 1999.
99. Migliaccio E, Mele S, Salcini AE, Pelicci G, Lai KM, Superti–Furga G, Pawson T, DiFiore PP, Lanfrancone L, and Pelicci PG. Opposite effects of the p52^{shc}/p46^{shc} splicing isoforms on the EGF receptor-MAP kinase-fos signaling pathway. *EMBO J* 16: 706–716, 1997.
100. Molinari M, Okorokov AL, and Milner J. Interaction with damaged DNA induces selective proteolytic cleavage of p53 to yield 40 kDa and 35 kDa fragments competent for sequence-specific DNA binding. *Oncogene* 13: 2077–2086, 1996.
101. Morin GB. The implications of telomerase biochemistry for human disease. *Eur J Cancer* 33: 750–760, 1997.
102. Morrison SJ, Prowse KR, Ho P, and Weissmann IL. Telomerase activity in hematopoietic cells is associated with self-renewal potential. *Immunity* 5: 207–216, 1996.
103. Morrison SJ, Wandycz AM, Akashi K, Globerson A, and Weissman IL. The aging of hematopoietic stem cells. *Nat Med* 2: 1011–1016, 1996.
104. Murphy CT, McCarroll SA, Bargmann CI, Fraser A, Kamath RS, Ahringer J, Li H, and Kenyon C. Genes that act downstream of DAF-16 to influence the lifespan of *Caenorhabditis elegans*. *Nature* 424: 277–283, 2003.
105. Musaro A, Giacinti C, Borsellino G, Dobrowolny G, Pelosi L, Cairns L, Ottolenghi G, Cossu G, Bernardi G, Battistini L, Molinaro M, and Rosenthal N. Stem cell-mediated muscle regeneration is enhanced by local isoform of insulin-like growth factor 1. *Proc Natl Acad Sci USA* 101: 1206–1210, 2004.
106. Musaro A, McCullagh K, Paul A, Houghton L, Dobrowolny G, Molinaro M, Barton ER, Sweeney HL, and Rosenthal N. Localized Igf-1 transgene expression sustains hypertrophy and regeneration in senescent skeletal muscle. *Nat Genet* 27: 195–200, 2001.
107. Nadal–Ginard B, Kajstura J, Leri A, and Anversa P. Myocyte death, growth, and regeneration in cardiac hypertrophy and failure. *Circ Res* 92: 139–150, 2003.
108. Nadal–Ginard B and Mahdavi V. Molecular basis of cardiac performance. *J Clin Invest* 84: 1693–1700, 1989.
109. Nakamura T and Schneider MD. The way to a human's heart is through the stomach. Visceral endoderm-like cells drive human embryonic stem cells to a cardiac fate. *Circulation* 107: 2638–-2639, 2003.
110. Narita M. Nunez S, Heard E, Narita M, Lin AW, Hearn SA, Spector DL, Hannon GJ, and Lowe SW. Rb-mediated heterochromatin formation and silencing of E2F target genes during cellular senescence. *Cell* 13: 703–716, 2003.
111. Newcomb TG and Loeb LA. Oxidative DNA damage and mutagenesis. In: *DNA damage and repair,* edited by Nickoloff J and Hoekstra M. Totowa, NJ, Humana Press, 1996, pp. 65–84.
112. Nugent CI and Lundblad V. The telomerase reverse transcriptase: components and regulation. *Genes Dev* 12: 1073–1085, 1998.
113. Oh, H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, Pocius J, Michael LH, Behringer RR, Garry DJ, Entman NL, and Schneider MD. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci USA* 100: 12313–12318, 2003.
114. Oh H, Taffet GE, Youker KA, Entman ML, Overbeek PA, Michael LH, and Schneider MD. Telomerase reverse transcriptase promotes cardiac muscle cell proliferation, hypertrophy, and survival. *Proc Natl Acad Sci USA* 98: 10308–10313, 2001.
115. Okorokov AL, Ponchel F, and Milner J. Induced N- and C-terminal cleavage of p53: a core fragment of p53, generated by interaction with damaged DNA, promotes cleavage of the N-terminus of full-length p53, whereas ssDNA induces C-terminal cleavage of p53. *EMBO J* 16: 6008–6017, 1997.
116. Olson EN and Schneider MD. Sizing up the heart: development redux in disease. *Genes Dev* 17: 1937–1956, 2003.
117. Pasumarthi KB, Nakajima H, Nakajima HO, Soonpaa MH, and Field LJ. Targeted expression of cyclin D2 results in cardiomyocyte DNA synthesis and infarct regression in transgenic mice. *Circ Res* 96: 110–119, 2005.
118. Pelicci G, Dente L, De Giuseppe A, Verducci–Galletti B, Giuli S, Mele S, Vetriani C, Giorgio M, Pandolfi PP, Cesareni G, Pelicci PG. A family of Shc related proteins with conserved PTB, CH1 and SH2 regions. *Oncogene* 13: 633–641, 1996.
119. Pellegrini M, Pacini S, and Baldari CT. p66SHC: the apoptotic side of Shc proteins. *Apoptosis* 10: 13–18, 2005.
120. Pfister O, Mouquet F, Jain M, Summer R, Helmes M, Fine A, Colucci WS, and Liao R. CD31- but not CD31+ cardiac side population cells exhibit functional cardiomyogenic differentiation. *Circ Res* 97: 52–61, 2005.
121. van Praag H, Schinder AF, Christie BR, Toni N, Palmer TD, and Gage FH. Functional neurogenesis in the adult hippocampus. *Nature* 415: 1030–1034, 2002.
122. Reiss K, Cheng W, Ferber A, Kajstura J, Li P, Li B, Olivetti G, Homcy CJ, Baserga R, and Anversa P. Overexpression of insulin-like growth factor-1 in the heart is coupled with myocyte proliferation in transgenic mice. *Proc Natl Acad Sci USA* 93: 8630–8635, 1996.
123. Rosenblatt–Velin N, Lepore MG, Cartoni C, Beermann F, and Pedrazzini T. FGF-2 controls the differentiation of resident cardiac precursors into functional cardiomyocytes. *J Clin Invest* 115: 1724–1733, 2005.
124. Rossig L, Haendeler J, Mallat Z, Hugel B, Freyssinet JM, Tedgui A, Dimmeler S, and Zeiher AM. Congestive heart failure induces endothelial cell apoptosis: protective role of carvedilol. *J Am Coll Cardiol* 36: 2081–2089, 2000.
125. Rota M, LeCapitaine N, Hosoda T, Boni A, De Angelis A, Padin–Iruegas E, Esposito G, Vitale S, Urbanek K, Casarsa C, Giorgio M, Luscher TF, Pelicci PG, Anversa P, Leri A, and Kajstura J. Diabetes promotes cardiac stem cell aging and heart failure, which are prevented by the deletion of the p66^{shc} gene. *Circ Res* 99: 42–52, 2006.
126. Rubin H. Cell aging *in vivo* and *in vitro. Mech Ageing Dev* 98: 1–35, 1997.
127. Rubin H. The disparity between human cell senescence *in vitro* and lifelong replication *in vivo. Nat Biotechnol* 20: 675–681, 2002.
128. Rudolph KL, Chang S, Lee HW, Blasco M, Gottlieb GJ, Greider C, and DePinho RA. Longevity, stress response, and cancer in aging telomerase-deficient mice. *Cell* 96: 701–712, 1999.
129. Sadoshima J, Xu Y, Slayter HS, and Izumo S. Autocrine release of angiotensin II mediates stretch-induced hypertrophy of cardiac myocytes *in vitro*. *Cell* 75: 977–984, 1993.
130. Samper E, Fernandez P, Eguia R, Martin–Rivera L, Bernad A, Blasco MA, and Aracil M. Long-term repopulating ability of telomerase-deficient murine hematopoietic stem cells. *Blood* 99: 2767–2775, 2002.
131. Sanderson WC and Scherbov S. Average remaining lifetimes can increase as human populations age. *Nature* 435: 811–813, 2005.
132. Schimke I, Schikora M, Meyer R, Dubel HP, Modersohn D, Kleber FX, and Baumann G. Oxidative stress in the human heart is associated with changes in the antioxidative defense as shown after heart transplantation. *Mol Cell Biochem* 204: 89–96, 2000.
133. Schwarze SR. Shi Y, Fu VX, Watson PA, and Jarrard DF. Role of cyclin-dependent kinase inhibitors in the growth arrest at senescence in human prostate epithelial and uroepithelial cells. *Oncogene* 20: 8184–8192, 2001.
134. Serrano M, Lin AW, McCurrach ME, Beach D, and Lowe SW. Oncogenic ras provokes premature cell senescence associated with accumulation of p53 and p16INK4a. *Cell* 88: 593–602, 1997.

135. Setoguchi M, Leri A, Wang S, Liu Y, De Luca A, Giordano A, Hintze TH, Kajstura J, and Anversa P. Activation of cyclins and cyclin-dependent kinases, DNA synthesis and myocyte mitotic division in pacing-induced heart failure in dogs. *Lab Invest* 79: 1545–1558, 1999.
136. Sherr CJ and De Pinho RA. Cellular senescence: mitotic clock or culture shock. *Cell* 102: 407–410, 2000.
137. Smogorzewska A and de Lange T. Different telomere damage signaling pathways in human and mouse cells. *EMBO J* 21: 4338–4348, 2002.
138. Song HJ, Stevens CF, and Gage FH. Neural stem cells from adult hippocampus develop essential properties of functional CNS neurons. *Nat Neurosci* 5: 438–445, 2002.
139. Soonpaa MH, Koh GY, Pajak L, Jing S, Wang H, Franklin MT, Kim KK, and Field LJ. Cyclin D1 overexpression promotes cardiomyocyte DNA synthesis and multinucleation in transgenic mice. *J Clin Invest* 99: 2644–2654, 1997.
140. Sudo K, Ema H, Morita Y, and Nakauchi H. Age-associated characteristics of murine hematopoietic stem cells. *J Exp Med* 192: 1273–1280, 2000.
141. Suzuki M, Suzuki K, Kodama S, and Watanabe M. Phosphorylated histone H2AX foci persist on rejoined mitotic chromosomes in normal human diploid cells exposed to ionizing radiation. *Radiat Res* 165: 269–276, 2006.
142. Takahashi T, Schunkert H, Isoyama S, Wei JY, Nadal–Ginard B, Grossman W, and Izumo S. Age related differences in the expression of protooncogene and contractile protein genes in response to pressure overload in the rat myocardium. *J Clin Invest* 89: 939–946, 1992.
143. Tatar M, Bartke A, and Antebi A. The endocrine regulation of aging by insulin-like signals. *Science* 299: 1346–1351, 2003.
144. Tomita Y, Matsumura K, Wakamtsu Y, Matsuzaki Y, Shibuya I, Kawaguchi H, Ieda M, Kanakubo S, Shinmazaki T, Ogawa S, Osumi N, Okano H, and Fukuda K. Cardiac neural crest cells contribute to the dormant multipotent stem cell in the mammalian heart. *J Cell Biol* 170: 1135–1146, 2005.
145. Torella D, Rota M, Nurzynska D, Musso E, Monsen A, Shiraishi I, Zias E, Walsh K, Rosenzweig A, Sussman MA, Urbanek K, Nadal–Ginard B, Kajstura J, Anversa P, and Leri A. Cardiac stem cell and myocyte aging, heart failure and IGF-1 overexpression. *Circ Res* 94: 514–524, 2004.
146. Tu W, Zhang DK, Cheung PT, Tsao SW, and Lau YL. Effect of insulin-like growth factor 1 on PHA-stimulated cord blood mononuclear cell telomerase activity. *Br J Haemat* 104: 785–794, 1999.
147. Urbanek K, Quaini F, Tasca G, Torella D, Castaldo C, Nadal–Ginard B, Leri A, Kajstura J, Quaini E, and Anversa P. Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *Proc Natl Acad Sci USA* 100: 10440–10445, 2003.
148. Urbanek K, Rota M, Cascapera S, Bearzi C, Nascimbene A, De Angelis A, Hosoda T, Chimenti S, Baker M, Limana F, Nurzynska D, Torella D, Rotatori F, Rastaldo R, Musso E, Quaini F, Leri A, Kajstura J, and Anversa P. Cardiac stem cells possess growth factor-receptor systems that following activation regenerate the infarcted myocardium improving ventricular function and long-term survival. *Circ Res* 97: 663–673, 2005.
149. Urbanek K, Torella D, Sheikh F, De Angelis A, Nurzynska D, Silvestri F, Beltrami CA, Bussani R, Beltrami AP, Quaini F, Bolli R, Leri A, Kajstura J, and Anversa P. Myocardial regeneration by activation of multipotent cardiac stem cells in ischemic heart failure. *Proc Natl Acad Sci USA* 102: 8692–8697, 2005.
150. Veldman T, Etheridge KT, and Counter CM. Loss of hPot1 function leads to telomere instability and a cut-like phenotype. *Curr Biol* 14: 2264–2270, 2004.
151. Vijayachandra K, Lee J, and Glick AB. Smad3 regulates senescence and malignant conversion in a mouse multistage skin carcinogenesis model. *Cancer Res* 63: 3447–3452, 2003.
152. Weinrich SL, Pruzan R, Ma L, Ouellette M, Tesmer VM, Holt SE, Bodnar AG, Lichtesteiner S, Kim NW, Trager JB, Taylor RD, Carlos R, Andrews WH, Wright WE, Shay JW, Harley CB, and Morin GB. Reconstitution of human telomerase with the template RNA component hTR and the catalytic protein subunit hTRT. *Nat Genet* 17: 498–502, 1997.
153. Wellinger RJ and Sen D. The DNA structures at the ends of eukaryotic chromosomes. *Eur J Cancer* 33: 735–749, 1997.
154. Xiao RP, Spurgeon HA, O'Connor F, and Lakatta EG. Age-associated changes in beta-adrenergic modulation on rat cardiac excitation-contraction coupling. *J Clin Invest* 94: 2051–2054, 1994.
155. Yamada O, Takanashi M, Ujihara M, and Mizoguchi H. Down-regulation of telomerase activity is an early event of cellular differentiation without apparent telomeric DNA change. *Leukemia Res* 22: 711–717, 1998.
156. Yan L, Vatner DE, Kim SJ, Ge H, Masurekar M, Massover WH, Yang G, Matsui Y, Sadoshima J, and Vatner SF. Autophagy in chronically ischemic myocardium. *Proc Natl Acad Sci USA* 102: 13807–13812, 2005.
157. Yui J, Chiu CP, and Lansdorp PM. Telomerase activity in candidate stem cells from fetal liver and adult bone marrow. *Blood* 91: 3255–3262, 1998.
158. Zhang R, Poustovoitov, M, Ye X, Santos H, Chen W, Daganzo S, Erzberger JP, Serebriiskii I, Canutescu A, Dunbrack R., Pehrson JR, Berger JM, Kaufman PD, and Adams PD. Formation of MacroH2A-containing senescence-associated heterochromatin foci and senescence driven by ASF1a and HIRA. *Dev Cell* 8: 19–30, 2005.
159. von Zglinicki. Oxidative stress shortens telomeres. *Trends Biochem Sci* 27: 339–344, 2002.
160. Zulmans JMJ, Martens UM, Poon SSS, Raap AK, Tanke HJ, Ward RK, and Lansdorp PM. Telomeres in the mouse have large inter-chromosomal variations in the number of T_2AG_3 repeats. *Proc Natl Acad Sci USA* 94: 7423–7428, 1997.

Address for reprint requests:
Jan Kajstura, Ph.D.
Cardiovascular Research Institute
Department of Medicine
Vosburgh Pavilion, Room 302
New York Medical College
Valhalla, New York 10595

E-mail: jan_kajstura@nymc.edu

Date of first submission to ARS Central, July 5, 2006; date of acceptance, July 8, 2006.

This article has been cited by:

1. Virginija Jazbutyte, Jan Fiedler, Susanne Kneitz, Paolo Galuppo, Annette Just, Angelika Holzmann, Johann Bauersachs, Thomas Thum. 2012. MicroRNA-22 increases senescence and activates cardiac fibroblasts in the aging heart. *AGE* . [CrossRef]
2. Yin SHI, Yun-hua CUI, Huan-gan WU, Wei ZHANG, Chen ZHAO, Hui-rong LIU, Lan-qin GUO, Bei-ling WU, Ai-zhen YU, Ying-ying ZHANG. 2012. Effects of mild-warming moxibustion on Bcl-2 and PKC expressions of peripheral blood in elderly people. *Journal of Traditional Chinese Medicine* **32**:1, 45-51. [CrossRef]
3. Y. Shi, M. Moon, S. Dawood, B. McManus, P.P. Liu. 2011. Mechanisms and management of doxorubicin cardiotoxicity. *Herz* **36**:4, 296-305. [CrossRef]
4. Piero Anversa, Jan Kajstura, Annarosa LeriCellular Basis for Myocardial Repair and Regeneration 48-72. [CrossRef]
5. Gabriela M. Kuster , Stéphanie P. Häuselmann , Berit I. Rosc-Schlüter , Vera Lorenz , Otmar Pfister . 2010. Reactive Oxygen/Nitrogen Species and the Myocardial Cell Homeostasis: An Ambiguous Relationship. *Antioxidants & Redox Signaling* **13**:12, 1899-1910. [Abstract] [Full Text HTML] [Full Text PDF] [Full Text PDF with Links]
6. Yi-Wei Zhang, Jianjian Shi, Yuan-Jian Li, Lei Wei. 2009. Cardiomyocyte death in doxorubicin-induced cardiotoxicity. *Archivum Immunologiae et Therapiae Experimentalis* **57**:6, 435-445. [CrossRef]
7. Jan Kajstura, Konrad Urbanek, Marcello Rota, Claudia Bearzi, Toru Hosoda, Roberto Bolli, Piero Anversa, Annarosa Leri. 2008. Cardiac stem cells and myocardial disease. *Journal of Molecular and Cellular Cardiology* **45**:4, 505-513. [CrossRef]