The prevailing dogma in cardiac biology for almost a century has been that the adult mammalian heart is a terminally differentiated organ. According to this paradigm, a 100-year-old human heart is comprised of a population of cardiomyocytes that are as old as the individual. Over the last decade, work from the laboratory of Piero Anversa and others has challenged this dogma.1 Cardiac biologists are now confronted with an alternative conceptual framework proposing the heart as a dynamic, self-renewing organ, capable of cardiomyocyte turnover throughout life.

Heart disease is the leading cause of death in the developed world. A major reason for this is that the adult mammalian heart has limited regenerative capacity following injury. The default healing response to myocyte loss in the adult mammalian heart (eg, following myocardial infarction) involves replacement of myocytes with fibrous, noncontractile scar tissue. The consequent loss of contractile units compromises cardiac function and ultimately leads to heart failure. Absence of regeneration in the adult mammalian heart contrasts with the impressive regenerative potential of lower vertebrates, such as urodele amphibians and teleost fish, which are capable of regenerating significant portions of ventricular myocardium following injury.2,3 Although studies in adult rodents and humans suggest that some myocyte repopulation occurs after myocardial infarction,4,5 this response is clearly limited and incapable of circumventing large-scale myocyte loss, fibrosis, and ultimately organ failure.

Cardiomyocyte loss accompanies aging. The heart undergoes a series of structural and morphological changes during normal aging that are characterized by cardiomyocyte loss (through apoptosis, necrosis, and autophagy) and hypertrophy of remaining viable myocytes, as well as interstitial and perivascular fibrosis.6 Aging also renders the heart more susceptible to major cardiovascular events. Therefore, studies investigating the physiological turnover and aging of myocytes are important. Recent experiments using radiocarbon dating of DNA from humans subjected to nuclear fallout showed that aging is associated with increased rates of myocyte apoptosis from 19 to 104 years of age.8,9 Intriguingly, myocyte loss exceeds myocyte formation in men, whereas a perfect balance between myocyte renewal and death appears to exist in females up to 90 years of age.8 The present study by Kajstura et al confirms earlier work by the same group and demonstrates that aging is associated with increased rates of myocyte apoptosis (measured by TdT staining) and replicative senescence (based on p16INK4a expression) in males and females. The incidence of apoptosis was higher in men than women at all time points tested, although the rate of increase in myocyte apoptosis from 19 to 104 years of age did not differ with gender. The rate of increase in p16INK4a-positive myocytes was 31% higher in men, potentially reflecting earlier replicative senescence of myocytes in the male heart.

On the basis of these observations, Anversa and colleagues reach the stunning conclusion that the entire cardiomyocyte compartment is replaced 15 times in women and 11 times in men from 20 to 100 years of age! Earlier studies of the aging process in human hearts showed that aging is associated with increased rates of apoptosis in male and female hearts.8 Intriguingly, myocyte loss exceeds myocyte formation in men, whereas a perfect balance between myocyte renewal and death appears to exist in females up to 90 years of age.8 The present study by Kajstura et al confirms earlier work by the same group and demonstrates that aging is associated with increased rates of myocyte apoptosis (measured by TdT staining) and replicative senescence (based on p16INK4a expression) in males and females. The incidence of apoptosis was higher in men than women at all time points tested, although the rate of increase in myocyte apoptosis from 19 to 104 years of age did not differ with gender. The rate of increase in p16INK4a-positive myocytes was 31% higher in men, potentially reflecting earlier replicative senescence of myocytes in the male heart. On the basis of these rates of apoptosis and senescence, coupled with the time for myocytes to complete apoptosis in vitro (~4 hours), Kajstura et al calculated that only 5% of cardiomyocytes would persist in the human heart at 63 and 48 years of age, whereas the majority of cardiomyocytes are as old as the organ and organism. Although these intriguing findings supported the concept of myocyte regeneration, several questions remain unresolved. For example, the magnitude of cardiomyocyte turnover contrasts with some previous estimates of myocyte death and reports of high rates of myocyte replacement in the human heart.9 Moreover, the source of newly formed cardiomyocytes remains unclear.

In this issue of Circulation Research, Kajstura et al offer some fascinating insights into the turnover of cardiac stem cells and cardiomyocytes with aging.10 Through comprehensive immunohistochemical analysis of postmortem hearts from patients who died from causes other than cardiovascular disease, the authors shed new light on the effects of age and gender on cardiac cell turnover. In their article, the authors determine the fraction of cardiomyocytes and cardiac stem cells (Lin−c-kit+) undergoing cellular senescence, apoptosis, and proliferation from heart samples collected from patients 19 to 104 years of age. The results from these analyses indicate remarkably high myocyte turnover rates of 7%, 12%, and 32% per year at 20, 60, and 100 years of age, respectively, in males, and even higher rates of turnover in females. On the basis of these observations, Anversa and colleagues reach the stunning conclusion that the entire myocyte compartment is replaced 15 times in women and 11 times in men from 20 to 100 years of age!
years of age in women and men, respectively, implying that myocyte regeneration must play a major role in the preservation of tissue mass and function with aging.

The authors next tested the possibility that a pool of c-kit–positive cardiac stem cells contributes to myocyte turnover during normal human aging. By defining functional competence and lack of senescence by the absence of p16INK4a staining and combining the number of proliferating stem cells with the duration of the cell cycle (determined in vitro), the authors noted an increase in the pool of “functionally competent” c-kit–positive stem cells in the old and senescent myocardium. When the authors extended their analysis to myocyte progenitors and precursors (defined by the expression of cardiac-restricted transcription factors and contractile proteins), a similar age-dependent increase in this cell compartment was also apparent. Interestingly, the rate of increase in myocyte progenitor/precursor formation with age was significantly higher in the female myocardium. Furthermore, proliferating cardiomyocytes (detected by Ki67, phospho–histone H3, and aurora B kinase staining) were identified at all ages tested. Once again, there was a time-dependent increase in the number of mitotic myocytes, which was higher in females than males. Collectively, these findings suggest that myocardial aging is characterized by a significant degree of myocyte regeneration. These observations also raised an interesting conundrum: there was an apparent contradiction between the increase in the number of senescent myocytes and the increase in myocyte renewal with age.

In an attempt to resolve this apparent paradox between the rates of myocyte senescence and renewal with aging, Kajstura et al measured the length of telomeres in cardiac stem cells and myocytes from young and old hearts. Telomeres are specialized DNA structures located at the ends of chromosomes and serve as protective caps preventing DNA damage, which can eventually lead to cellular senescence or apoptosis. Telomeres lose 30 to 150 base pairs of DNA during each cell division, so telomere length effectively marks the “life history” of a cell and has been used as a marker of cellular aging.11 Kajstura et al demonstrate that telomeric shortening occurs in both cardiac stem cells and myocytes with aging. The magnitude of telomere shortening with aging was greater in men than women. These findings raise the possibility that, although the senescent heart contains a larger pool of proliferating cardiac stem cells, older stem cells may give rise to older myocyte progeny, which rapidly acquire a senescent phenotype and die.

The latest findings by Kajstura et al will surely provoke much interest and prompt reconsideration of the biological processes that contribute to cardiac senescence. There is an emerging consensus that myocyte renewal occurs at measurable levels throughout life, disputing the old dogma that the adult mammalian heart is terminally differentiated. However, estimates of myocyte turnover rates vary markedly from 0.4% to 1% per year7 to 7% to 40% per year (present study).10

There are many potential explanations for these discrepancies, most of which reflect the inherent difficulties associated with measuring slow cell turnover rates in a species such as our own, which can live for more than 100 years. Such longitudinal studies of myocyte turnover are theoretically much easier in experimental model organisms such as the mouse, but intrinsic species differences may limit extrapolation of results obtained in mice (which live for only 2 years) to humans. Nevertheless, thymidine-labeling studies in the mouse showed that the rate of DNA synthesis in the adult ventricle of this species is 100000%12, yielding an annual renewal rate of approximately 1.1% of cardiomyocytes, which is similar to estimates of myocyte renewal from carbon-dating experiments in humans.7,13 Although Kajstura et al highlight some of the limitations of carbon birth-dating experiments and provide a comprehensive immunohistochemical analysis of the aging heart, this approach also has limitations. For instance, histological analyses were restricted to sections of 4 μm in thickness, which may have excluded the majority of multinucleated cardiomyocytes from the analysis. A recent study by Bergmann and colleagues suggests that the majority of adult human cardiomyocytes are multinucleated cells with a transverse diameter of >4 μm.13 In contrast, Kajstura et al claim that <20% of mature cardiomyocytes in the adult myocardium are binucleated. Therefore, large myocytes with multiple nuclei and higher ploidy levels may have been excluded from the present analyses, which could affect estimates of myocyte turnover. Moreover, estimates of myocyte turnover rates were determined by mathematical modeling, which required significant assumptions about the inherent nature of c-kit–positive stem cells, the duration of the cardiac cell cycle, and myocyte ploidy levels. As the authors note, the length of the cardiac cell cycle was determined in vitro and may differ in vivo. Furthermore, the precise lineage origin of the newly formed cardiomyocytes is difficult to definitively assess in humans. Although Kajstura et al provide data implicating c-kit–positive stem cells in the aging phenotype, the precise lineage relationship of these stem cells to the newly formed myocytes is less clear. It is possible that the proliferating c-kit–positive cells, myocyte precursors, and mature cardiomyocytes represent distinct cell populations that do not share a direct lineage relationship and are not hierarchically connected. Genetic lineage–tracing techniques in mice may help resolve these outstanding questions, but current models suggest that stem cells do not contribute to myocyte turnover during normal aging in mice.4

Aging is an inevitable fact of life, yet we know little about how getting old affects the very cells that keep us alive. Although the present study by Kajstura et al is unlikely to completely resolve ongoing debates regarding the degree of myocyte repopulation in the human heart, this study provides some fascinating clues that point toward a previously unrecognized role for cardiac stem cells in the aging process. Intriguingly, the female heart appears to harbor a superior ability to sustain stem cell and myocyte function with age. Understanding these sex differences and age-dependent changes may ultimately open cardionic stem cell populations to therapeutic exploitation to enhance cardiac regenerative capacity during aging and disease.

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None.

References

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