With the publication of *De Humani Corporis Fabrica* in 1543, Andreas Vesalius bestowed some of the greatest advancements of anatomic understanding since the time of Galen, correcting major misconceptions, eg, the notion that the great vessels originated in the liver. It took nearly 1500 years for this evolution in anatomic thinking to transpire.

In 1973, Radovan Zak of the University of Chicago stated unequivocally, “The adult heart enlarges only by enlargement of its component muscle cells. Division of ventricular muscle cells in mammals is not activated after cardiac injury.” This conclusion was largely consistent with work by Linzbach in 1960; however, in this earlier comprehensive histological analysis, there was a suggestion that under certain circumstances the number of cardiac muscle fibers might increase.

The Anversa laboratory began suggesting the possibility of cardiomyocyte proliferation in 1980, and by 1992, the group began to document clear evidence for replication of cardiac muscle cells in response to physiological stress and myocardial injury in both experimental models and humans. These early works included such bold statements as “the recognition of factors controlling myocyte proliferation may provide a new approach for regeneration of damaged and lost myocardium, a phenomenon considered impossible for several decades.” Although some recognized the power and implications of these observations, the Pfetters’ call for open-mindedness often fell on deaf ears. Nevertheless, Anversa et al had opened the door to the era of cardiovascular regenerative medicine.

Apparent unsatisfied with the disruption to the cardiovascular orthodoxy it had already created, the Anversa laboratory confronted us yet again with evidence for the existence of cardiac stem cells in animals and humans. The claim now was that the mature myocyte pool was not the source of cells for senescent cell replacement; rather, a resident bone fide stem cell population was responsible for cardiomyocyte homeostasis.

Although some embraced this emerging understanding of the heart as an organ capable of repair, many remained skeptical. In 2009, however, Bergmann et al, using carbon-14 dating, documented what many felt to be unequivocal evidence of cardiomyocyte proliferation. The renewal rate of adult human cardiomyocytes was quantified using measurement of carbon-14 integration into nDNA and mathematical modeling to estimate chronologic age of DNA synthesis in explanted human myocardial cells. This approach relies on the rise and fall of radioactive atmospheric carbon-14 concentrations surrounding 1963 and the proportional incorporation of these radiolabeled molecules into actively dividing cells during postnatal DNA synthesis. Using this method, Bergmann et al reported that the rate of adult human cardiomyocyte turnover ranged from 1% annually until 25 years of age to 0.45% annually by 75 years of age. This resulted in an estimate of up to 50% cardiomyocyte turnover over a normal lifespan.

In the article in this issue, Anversa and colleagues investigate this calculation in collaboration with one of the original Bergmann investigators. They hypothesize that given the requisite balance of cell death, regeneration, and hypertrophy required to maintain the observed preservation of cardiac mass over time, true cardiomyocyte renewal rates must be much higher than initially calculated. They point out that by current estimations, the human heart reaches its adult composition of $\approx 8 \times 10^9$ cardiomyocytes by 20 years of age with up to a 4.5-fold cellular hypertrophy observed in the study cohort by this age. This implies that cardiac mass could theoretically be preserved over time even after 80% cardiomyocyte loss via maximal observed hypertrophy. However, $0.006\%$ of myocytes undergo apoptosis per day, resulting in a natural loss of at least $2.2\%$ myocytes per year, or much greater than $80\%$ cell loss after 20 years of over an average lifespan of 75 years. This results in a net negative cellular balance over an average lifespan when previously reported cardiomyocyte turnover rates are used.

The present study was therefore designed to independently quantify cardiomyocyte proliferation rates in healthy aging and in failing human hearts. A total of 19 healthy and 17 failing human hearts were obtained within 24 hours of death, and cells from well-perfused regions of the left ventricle were digested, purified, and sorted. Cell ploidy was documented with fluorescence-activated cell sorter analysis and confirmed by confocal microscopy. Decay-corrected carbon-14 concentrations were measured with accelerator mass spectroscopy. Myocyte, endothelial cell, and fibroblast fractions were de-
terminated with immunocytochemistry and spectral analysis. The main analyses included patterns of polyploidy, average carbon-14–determined cell ages, and the number of cycling/mitotic myocytes by patient age in both the healthy aging and failing heart cohorts.

Using this approach, Anversa and colleagues report an ≈8-fold turnover of cardiomyocytes and fibroblasts and a 6-fold turnover of endothelial cells over an average lifespan. Briefly, their methods revealed the following 3 major findings. First, the average myocyte age in healthy human hearts increased postnatally from a cell age of 8 months during childhood to a cell age of 7.9 years between the ages of 33 and 46 years. Average myocyte age remained relatively constant during adulthood but subsequently decreased progressively to a cell age of 2.6 years by 68 to 78 years of age. This translates into calculated cardiomyocyte turnover rates ranging from 7% to 23% of cells per year (peak turnover before 20 years of age, nadir during adulthood, and subsequent increased cardiomyocyte turnover with aging). Second, the average myocyte age in failing human hearts follows a similar pattern over time as in healthy hearts, but absolute cell ages are significantly lower in failing than in healthy hearts, indicating up to a 2-fold greater cardiomyocyte turnover rate in decompensated heart failure. This elevated cardiomyocyte regeneration rate is measured in the setting of an overall decrease in number of cardiomyocytes per unit volume in heart failure resulting from fibrosis and cell hypertrophy. Third, an average frequency of 23% multinucleated adult myocytes was demonstrated, the majority of which were binucleated and developed by 25 years of age. This finding was independent of heart health. These values indicate a degree of adult cardiomyocyte turnover that is more than an order of magnitude greater than previously reported. Such a high degree of endogenous regenerative capacity would represent an attractive biological pathway target for therapeutic modulation for cardiac regeneration. Some will argue that the “true” degree of cardiomyocyte turnover may lie somewhere between the previously reported and current estimations; however, the current analysis by Anversa and colleagues more appropriately satisfies homeostatic equations as described earlier, and the importance of defining biologically plausible rates of endogenous cardiomyocyte repair, as the present study has done, cannot be understated.

There are notable methodological differences between the study in this issue of Circulation and previous work that may account for differences in findings. First, Anversa et al had access to fresh cardiac tissue, which meant that all data needed for carbon-14 analysis could be acquired. In the Bergmann et al study, mathematical modeling was required with the use of correction factors owing to the lack of availability of all data on the preserved specimens. Differences in calculated cell turnover rates between the 2 studies are additionally influenced by measure of cell polyploidy rates. Multinucleated or polyploid cells by definition carry greater carbon-14 DNA mass and may be interpreted as replicating cells, thereby falsely elevating estimations of cell turnover. Estimates of cell turnover are therefore corrected (decreased) in proportion to the frequency of cellular polyploidy measured. In the previously published study, the polyploid fraction was estimated to be 100% in patients >10 years of age rather than the 12% polyploid rate in those >25 years of age directly measured by the gold standard of fluorescence-activated cell sorter analysis by Anversa et al. Finally, choices in mathematical modeling undoubtedly influence calculated regeneration rates, and the previous study analysis was notable for using an exponential decay equation to model cell turnover. Such a function was not applied to average cardiomyocyte age measurements by Anversa et al.

The staggering burden of cardiovascular morbidity and mortality today reflects a threshold of ischemic and hemodynamic injury above which the adult heart exceeds its capacity for endogenous repair. Yet these natural repair mechanisms are quite elegant and robust, relying not only on adequate diffusion of oxygen and nutrients to at-risk tissue but also on dynamic compensatory genetic/molecular signaling and a degree of cellular plasticity maintained throughout adulthood. At what point are these mechanisms overwhelmed? What calculi can we use to predict whether an injury is “too great” to allow favorable remodeling and cellular recovery? Many variables in this equation remain unknown. A clearer understanding of the natural plasticity of myocardium is needed to allow better quantification of injury and anticipated repair and to guide the strategic development and application of our cellular, paracrine, and biomaterials armamentarium. Knowledge of this “natural capacity” for endogenous regeneration also serves as a benchmark against which the scale and success of our exogenous regenerative therapies can be measured and as a starting point for therapies that seek to leverage or expand this capacity. In this issue of Circulation, Anversa et al have redefined the scope of natural repair in the aging and decompensated heart, helping us to better define the calculus of myocardial regeneration. This new understanding of the natural history of myocardial tissue homeostasis will serve us well on the road from scientific understanding to clinical application.

Disclosures

None.

References


Key Words: Editorials ■ aging ■ myocardium ■ stem cells ■ regenerative medicine
On the Fabric of the Human Body
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Circulation. 2012;126:1812-1814; originally published online September 6, 2012;
doi: 10.1161/CIRCULATIONAHA.112.136127
Circulation is published by the American Heart Association, 7272 Greenville Avenue, Dallas, TX 75231
Copyright © 2012 American Heart Association, Inc. All rights reserved.
Print ISSN: 0009-7322. Online ISSN: 1524-4539

The online version of this article, along with updated information and services, is located on the
World Wide Web at:
http://circ.ahajournals.org/content/126/15/1812

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