The traditional concept of the heart as a terminally differentiated postmitotic organ has been largely based on an influential study published in 1925 that reported the paucity or near-absence of cell division and mitosis in the human heart, which was in striking contrast with the regenerative capacity of, e.g., liver tissue. Additional arguments thought to support this concept are the absence of significant tissue regeneration after damage such as myocardial infarction and the response to increased demand of the heart by hypertrophy of cells, rather than by hyperplasia. This concept was, however, challenged a number of years ago. Using improved imaging methods for apoptosis, mitosis, and cellular senescence, Anversa and colleagues provided new quantitative data well beyond what had been reported before; cardiac cells were shown to be capable of reentering the replicative phase, and the incidence of mitotic figures was increased several-fold in the failing heart. Observations, together with the demonstration of host male cells in gender-mismatched human cardiac transplant recipients, formed the basis for developing a new concept of the heart as a dynamic, potentially self-renewing organ. Generation of new myocytes was reported to contribute to remodeling processes.

Several studies in support of this new paradigm provided evidence for cell division using microscopic confocal imaging. Interestingly, these replicating cells showed a much smaller volume than fully differentiated cardiac myocytes, and it seemed unlikely that the latter would reenter the cell cycle. Subsequently, confocal analysis as well as improved cell isolation and separation techniques identified the existence of cardiac stem cells (CSC) distributed in small clusters between cardiomyocytes. When isolated and cultured under specific conditions, these cells can differentiate into myocytes, smooth muscle, and endothelial cells. CSC with cardiogenic potential and capable of cardiac repair were recently also isolated from small endomyocardial biopsies of human hearts. Such cells could represent the reservoir for continuous self-renewal, and could potentially be harnessed to provide for the much-needed tissue repair after myocardial infarction. However, cardiac stem cells lack migratory and homing capacity to translocate to sites of injury but were recently reported to respond to essential growth factors that facilitate migration (hepatocyte growth factor [HGF]) and survival in an ischemic microenvironment (insulin-like growth factor-1 [IGF-1]). Local injection of these growth factors in the myocardium of mice 5 hours after myocardial infarction and 2 days after retroviral inoculation with enhanced green fluorescence protein (EGFP, to tag cycling cardiomyocytes) induced formation of new EGFP-positive vessels and new EGFP-positive myocytes in a time-dependent manner. This study was the first to propose that the newly formed myocytes from translocated cardiac stem cells possessed specific functional properties (such as higher shortening velocity) and contributed to improved cardiac performance and survival after MI. A study published earlier this year from the laboratory of Dr Houser in collaboration with Dr Anversa’s laboratory, reported that the properties of small myocytes present in the adolescent feline ventricle were distinct from the population of larger cells and also represent newly-formed myocytes.

In the current issue Rota et al expand on the concept of the heart as an organ in continuous self-renewal. To do so, the authors have looked at markers for ‘age’ of myocytes of different sizes and have performed extensive functional characterization of myocytes of different sizes, this time in the heart of the mouse, at 3 months of age. Myocytes were subdivided according to volume. Small myocytes tended to be mononucleated, whereas larger myocytes had 2 or more nuclei. The majority, 91%, of the myocytes were binucleated. There was an inverse correlation between cell volume and telomere length suggesting loss of telomerase function in larger, old myocytes. On average, after classifying cells by volume, these larger myocytes also had a higher expression of p16INKa, a marker of senescence.

Are These Indeed ‘Young’ Versus ‘Old’ Cells?

The evidence for assigning the label ‘young’ to small cells, versus ‘senescent’ or ‘old’ to large myocytes, is based on a statistical predominance of certain age-associated characteristics according to cell volume. This is a compelling and statistically sound approach, relying on a large amount of data. Yet not all small cells have long telomeres, or signs of mitosis (the latter incidence is not given but is not 100%); the presence of shorter telomeres in larger cells is more consistent, but again, not all large cells are p16INKa-positive. The presence of more than 2 nuclei is another sign of senescence, and here as well, the correlation with cell size shows that there is quite a bit of overlap between categories. Because all functional data are correlated with size, ‘large/senescent’ cells will include both bi- and multinucleated cells, and ‘small/young’ cells will be a mix of mono- and bi-nucleated cells. The decision to relate the functional data to cell size is
understandably based on a pragmatic approach, given that these are living cells. To establish the relation between function and ‘age’ more directly, the authors fixed myocytes after functional measurements and stained for telomeres and p16INK4a. This is an extraordinary accomplishment and the data support the larger statistical analysis. Nevertheless this remains a small sample and hence a note of caution is justified.

Perhaps impossible to finally resolve with currently available techniques, the major question remains as to the rate and magnitude of turnover of individual myocardial cells during normal cardiac homeostasis. There has been an ongoing controversy regarding the continuous renewal of myocytes in the heart and the existence and fundamental biological role of early committed cells. Much of this debate relates to the early evidence which was mostly based on histologic data using stem cell-related surface markers and variable isolation protocols with often discrepant results. The present study is consistent with the authors’ previously published data, but does not provide additional direct proof of the transition from stem cell to young and eventually senescent myocytes. This will have to await novel techniques.

Functional Characteristics of ‘Young’ and ‘Old’ Cells in the Young Heart

Contractile properties of small/young versus large/old cells are shown in a correlation analysis, in relation to cell size. This analysis points to larger and faster contractions in small cells, with larger Ca$^{2+}$ transients. Extent of shortening is shown in micrometer, in absolute numbers, unlike in most studies where data are normalized to cell length. Such normalization would have steepened the relationship, though surprisingly, the sarcomere shortening, which is independent of cell length, was not steeper. Analysis of the Ca$^{2+}$ transients shows a similar trend, but the caffeine-induced Ca$^{2+}$ transient, as a measure for SR Ca$^{2+}$ content, is similar for all cells. In voltage clamp analysis the L-type Ca$^{2+}$ current is not different according to cell size. However, action potential profiles are different with smaller cells having longer action potentials with reduced early repolarization, which allows for a larger Ca$^{2+}$ influx during the course of the action potential. This is proposed to be responsible for the larger Ca$^{2+}$ transients and contractions of the smaller cells.

While these data clearly identify differences in functional properties among the cell population, a few questions remain regarding the mechanisms underlying the specific Ca handling of small/young cells. A first point is the comparison of the present data obtained in mouse versus the earlier data obtained in cat. In this latter study, small mononucleated myocytes had smaller slower Ca transient that were, as in the present study, attributed to reduced K channels and reduced early repolarization. This makes more sense than the present concept, because reduced early repolarization indeed makes for reduced Ca$^{2+}$ release. Whereas Rota et al rightfully state that longer APs have larger Ca$^{2+}$ influx, a positive effect on contraction results from an increase in SR Ca$^{2+}$ content, not from an effect on the trigger for this release. Such increase in SR Ca$^{2+}$ content was, however, not observed and the larger Ca$^{2+}$ transients remain therefore incompletely explained.

A second remaining question concerns the distribution of Ca$^{2+}$ channels and RyR in small/young versus large/old cells. Studies of neonatal hearts have demonstrated that immature cells lack T-tubules. A lack of T-tubules is associated with dyssynchronous and slower Ca$^{2+}$ release, and such reduced synchrony was noted earlier by Chen et al. These aspects were not investigated in the present study, but the small cells had a slower time to 50% of the Ca$^{2+}$ transients. However, this was measured in the field stimulation experiments, not in the voltage clamp studies, which would be more revealing as they eliminate the AP differences. What is somewhat unexpected is the preserved surface/volume (S/V) relation throughout the cell population, because low amounts of T-tubules reduce the S/V. A future specific analysis of T-tubule density and S/V stratified to number of nuclei might provide a better understanding.

Do ‘Old’ Cells Resemble Myocytes From Aged Hearts?

The authors predominantly report data obtained in mice at 3 months of age but also include data from mice at 22 months of age. For a mouse this is close to end of lifespan, yet also in these hearts small cells were present. A distribution analysis of cell size shows an increase in the fraction of larger cells, but not a simple shift of the curves with chronological age. How can we compare data on old/large cells to previous studies on ageing? Earlier studies do not report on a range of sizes of cells studied, but we can assume that the random sampling reflects properties of the majority of cells. Two comparisons can be made. The first one compares the data on old/large cells of the current study to earlier data on samples of ageing hearts. Most data for chronological ageing were obtained in rodents, and were summarized a few years ago by one of the major contributors in the field. Typically a reduction of contraction is observed, with prolongation of the action potential and reduction of the transient outward K+ current. This is seen as compensation to maintain SR Ca$^{2+}$ load and contraction, in the presence of reduced SERCA. So these observations are at odds with what is seen in old/large cells of the young adult animal.

The second comparison is the effect of chronological ageing as reported in the present study versus earlier reports on ageing. The main emphasis of Rota et al is on the presence of a range of cells with different properties in the hearts of these old and young animals alike. The comparison of functional properties of myocytes from old and young animals is made as well, but harder to interpret given a different classification (3 classes in young animals, but only 2 in old animals). Because the majority of cells in a young heart, as can be seen from the distribution curves, are in the small-to-middle range of size, and in the old hearts in the middle-to-large range of sizes, a comparison of properties of these respective groups should more or less correspond to the more randomly sampled earlier studies. The data however don’t correspond. In the current study, APD$_{90}$ of cells $< 20 000 \mu m^3$ of young animals is around 20 ms, whereas APD$_{90}$ of cells $> 20 000 \mu m^3$ in old animals is on average 10 ms, suggesting chronological ageing leads to a shortening of the AP, or no change, at odds with previous studies. Only when comparing
within 1 class, namely the cells smaller than 20,000 μm², there is a trend toward prolongation of the AP in the older animals. An important lesson to be learnt from the present study is that in comparing animals of different age, we may need to study larger cell populations. This probably applies also for disease conditions. For basic properties such as cell contraction this is feasible, but for the more detailed analysis of excitation-contraction coupling this presents a major challenge.

In conclusion, the study by Rota et al is a major contribution in its detailed analysis of populations of constituent myocardial cells. It identifies different classes of myocytes with distinctive functional and molecular properties, consistent with the earlier study of Chen et al. The data are strongly supportive of the concept of cells in transition, though definite proof awaits novel techniques that may eventually identify and follow directly the lifespan of individual myocytes. The electrophysiological properties of small/young versus large/old myocytes do not correspond to previous reports on chronological ageing, suggesting that different processes superimpose in the ageing animal or that the differences in the specified classes of myocytes are not a simple matter of ‘age’.

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Disclosures
None.

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How Old Is Your Heart?
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