Editorial

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Regenerative Medicine

Controversy in myocardial regeneration

"Chronological age does not coincide with biological aging and the myocyte pool is highly heterogeneous comprising cells with a different birth date and variable telomere length."

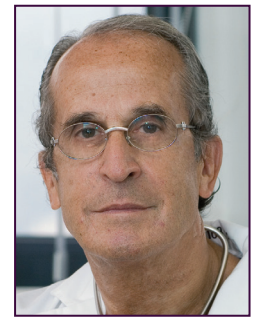
Keywords: cardiac hypertrophy • cardiomyocytes • cell death • cell dedifferentiation • cell proliferation • c-Kit • lineage tracing • stem cells • telomere length

Men and women 90–100 years of age and older are increasing consistently in our society and the turnover of sarcomeric and nonsarcomeric proteins has been considered the exclusive mechanism involved in the preservation of the structural and mechanical behavior of cardiomyocytes. Cardiomyocytes persist for the entire lifespan of the organ and organism and can only be lost as a result of a disease process that, in turn, triggers a hypertrophic response of the remaining viable cells. Myocyte enlargement is the restricted mechanism available to increase the cardiac muscle mass. Because of this notion, over the past several decades, a tremendous effort has been made to dissect the molecular pathways of myocyte hypertrophy, neglecting the possibility that myocyte formation occurs in the adult heart.

Based on this premise, the myocardium is composed throughout life of cardiomyocytes of identical age which are present at birth and persist largely until death of the organ and organism, overlooking the principle of cellular senescence and cardiomyocyte loss. The biological event of cellular senescence occurs after a cell has undergone a finite number of divisions and is controlled by an intrinsic 'replicometer' [1], strictly connected to telomere shortening. Physiologically, the growth arrest of aged cells is irreversible, although molecular manipulations involving the activation of the cell cycle machinery induce division of postmitotic cardiomyocytes [2].

Despite the presence of telomerase, loss of telomeric DNA at the end of chromosomes occurs in proliferating cells at a rate of 100–130 bp for each round of division. Growth arrest and replicative senescence, a phenomenon originally defined as the Hayflick limit, is coupled with telomere dysfunction, which may be dictated by extreme shortening and/or uncapping of telomeric DNA repeat sequences. The disruption of the D-loop–T-loop architecture activates the DNA damage repair response, cellular aging and, ultimately, apoptosis. The accumulation of unrepaired DNA lesions results in prolonged DNA damage repair signaling, which involves the recruitment of γ-H2A.X at the sites of DNA strand breaks and the sustained upregulation of p53 and its target genes [3]. The length of telomeres represents a biomarker of the replicative or postmitotic phenotype of any given cell. The identification of short telomeres in cardiomyocytes is in contrast with the possibility that cardiomyocytes derive from a pool of amplifying cells that have the ability to divide and simultaneously differentiate. Cardiomyocytes should be a highly homogenous compartment containing cells of the same age carrying relatively long telomeres. But chronological age does not coincide with biological aging and the myocyte pool is highly heterogeneous comprising cells with a different birth date and variable telomere length [4].

There is a general consensus in the scientific community that cardiomyocyte apoptosis and necrosis are commonly found in the myocardium at all stages of life, although they increase significantly with aging and cardiac pathology [5]. The recognition that cardiomyocyte death comprises a significant part of this cell population implies that car-



Tiziano Moccetti
Fondazione Cardiocentro Ticino, University of Zurich, Lugano 6900, Switzerland

Annarosa Leri
Departments of Anesthesia & Medicine, & Division of Cardiovascular Medicine, 75 Francis Street, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115, USA

Piero Anversa
Author for correspondence:
Departments of Anesthesia & Medicine, & Division of Cardiovascular Medicine, 75 Francis Street, Brigham & Women's Hospital, Harvard Medical School, Boston, MA 02115, USA
panversa@partners.org

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diomyocyte formation has to be present to preserve cardiac mass and function. Physiologically, the degree of myocyte regeneration has to be commensurated to the extent of cell death and, following sudden increases in work-load dictated by ischemic events and large myocardial losses, the process, to be effective, has to be fast and capable of creating a significant number of myocytes and coronary vessels. In the absence of this growth response, ventricular dysfunction and heart failure supervene.

We will consider now the evidence in favor of myocyte death and the potential origins of cardiomyocytes in an attempt to provide a realistic view of the biology of the adult human heart. In spite of the identification by numerous laboratories of cardiac progenitor cells (CPCs) and their ability to differentiate into the various cardiac cell lineages [6], a major effort has recently been made to reestablish the old concept professing the lack or irrelevant magnitude of myocyte renewal in the adult mammalian heart [7]. The desire to promote old beliefs contrasts with the clinical implementations of CPCs and, based on encouraging results obtained in a Phase I trial [8], the NIH is sponsoring through the CCTRN consortium a new Phase II trial in patients with chronic ischemic cardiomyopathy and reduced ejection fraction (ClinicalTrials.gov Identifier: NCT02501811).

> "The recognition that cardiomyocyte death occurs throughout life implies that cardiomyocyte formation has to be present to preserve cardiac mass and function."

Cardiomyocyte apoptosis at a rate of 0.001% and higher has consistently been found in the nondiseased human myocardium [9]. Importantly, this mechanism of cell death is completed in less than 5 h [10] so that in 30 years, apoptosis alone would reduce the number of cardiomyocytes by approximately 50%, a value that is inconsistent with quantitative measurements of myocyte number in the human heart from 17 to 90 years of age [5]. Moreover, values of myocyte apoptosis ranging from 0.12 to 0.70% have been measured in New York Heart Association classes III–IV heart failure (HF) [11] indicating that, in the absence of significant cardiomyocyte formation, the entire heart would disappear in a few years.

The blood levels of cardiac troponin are routinely employed to assess the degree of myocyte necrosis in patients with acute or recurrent myocardial ischemia, and minimal elevations in the serum concentration of troponin predict adverse clinical events [12]. Recently, a high sensitive troponin assay has been introduced and, by this sophisticated methodology, it has been possible to establish that detectable levels of troponin correlate with the incidence of HF and cardiovascular death in patients with stable coronary artery disease [13]. In older adults without cardiovascular diseases, increases in the concentration of cardiac troponin are associated with HF and cardiovascular death [12]. Moreover, in older adults, in the absence of ventricular dysfunction, a low concentration of cardiac troponin predicts defects in systolic performance, HF and cardiovascular death [13].

It is impossible to reconcile the ongoing magnitude of myocyte death by apoptosis and necrosis with the contention that myocyte regeneration is a negligible component of cardiac homeostasis and myocardial recovery following tissue damage. Apparently sophisticated methodologies, requiring the implementation of complex model systems and inevitable assumptions [14,15], should not be interpreted and accepted at face value, neglecting the incontrovertible reality of cardiomyocyte loss. As an example, an organ in which a relative balance between cell death and cell regeneration has been found is the small intestine [16]. More importantly, quantitative studies have shown repeatedly that the hypertrophied decompensated human heart contains a significantly larger number of ventricular cardiomyocytes [17]. Despite ongoing apoptotic and necrotic myocyte death, the population of cardiomyocytes doubles in pathological conditions characterized by a prolonged and sustained pressure/volume overload on the myocardium. These critical observations are in conflict with the principle that the heart is a static organ with little or none regenerative reserve. What has changed is that there is somehow a general consensus that myocyte turnover does occur in the human heart, although profound disagreement persists on the magnitude of the process.

In our view, the evidence that myocyte renewal is a relevant component of cardiac homeostasis is no longer debatable and this position raises the challenging question regarding the mechanism of myocyte formation. The disagreement on the degree of myocyte regeneration is equally intense when the source of cardiomyocytes is considered. And the groups involved in the debate are the same. The laboratories sustaining the stem cell origin of cardiomyocytes are those in favor of the high rate of myocyte turnover [5], and the laboratories supporting myocyte self-duplication as the mechanism of myocyte formation are those claiming that cardiomyocyte renewal is modest physiologically and pathologically [7].

The recognition that the adult heart in animals and humans contains a compartment of primitive c-Kit-positive cells, which are self-renewing, clonogenic and multipotent *in vitro* and are able to form cardiomyocytes and coronary vessels *in vivo* has provided the fundamental information that has changed the under-

standing of myocardial biology [18,19]. The fate of the heart appeared to be controlled by a pool of stem cells which regulate the turnover of cardiac cells and determine the magnitude and limits of tissue regeneration following injury. A cellular hierarchy was identified in which stem cells give rise to myocyte, endothelial cell and smooth muscle progenitor cells which then become amplifying cells and ultimately reach terminal differentiation and growth arrest. This novel and relatively simple organization of the myocardium has been challenged and the existence and role of resident CPCs has been questioned. Cell regeneration has been argued to derive exclusively by self-duplication of postmitotic cells [14]. A lineage tracing study in the mouse has supported this view since only a very small number of cardiomyocytes have been found to derive from c-Kit-positive cells [20]. However, the knock-in strategy employed with downregulation of c-Kit expression in CPCs, together with the appearance of a white belly spot and severe atrophy of the testes (unpublished observation), questions the validity of this transgenic mouse model, which mimics the aspects commonly found in mice with spontaneous mutations of the c-Kit receptor [21]. In this genetic system, the cardiac repair process after infarction is severely compromised [22].

Interestingly, the repeatedly documented CPC function was rejected and a new theory was advanced to support the concept of self-duplication of cardiomyocytes. Terminally differentiated postmitotic cardiomyocytes were alleged to be capable of dedifferentiating and dramatically altering the composition of the cytoplasm so that a proliferative cell phenotype could be reacquired [14,23]. These dedifferentiated cardiomyocytes were considered the exclusive source of new parenchymal cells. An important caveat is that the proponents of this theory have failed to document the presence of dedifferentiated replicating cardiomyocytes within the myocardium *in vivo*. Additionally, amplifying cardiomyocytes derived from activation and lineage specification of CPCs are immature dividing cells, which progressively reached the adult phenotype. Currently, there is no protocol able to identify dedifferentiated cardiomyocytes *in vivo* [5]. Notably, there is no precedent in which self-renewing, clonogenic and multipotent stem cells are nested within an organ and have no implications in tissue homeostasis and repair.

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