The view of the heart as a static organ implies that myocyte death and formation play a negligible role in cardiac homeostasis. Although stem cells have been unexpectedly identified in several organs, including the brain, kidney, lung, and skeletal muscle, the search for a cardiac stem cell (CSC) has been perceived as a futile effort, given the acknowledged lack of regenerative potential of the myocardium. Nevertheless, in the past several years, the demonstration of myocyte renewal in the normal and diseased heart has revealed a new, dynamic, and lively picture of this organ. Components of the cell cycle machinery and markers of cell replication have been detected in cardiomyocytes. The demonstration of karyokinesis and cytokinesis involves cells expressing contractile proteins has provided evidence that cardiomyocyte division occurs in the adult heart. More recently, pulse-chase assays with thymidine analogs, lineage tracing protocols, and $^{14}$C birth dating of cells have shown the existence of myocyte turnover, a process that has been found to differ in magnitude according to the methods used for its documentation and quantification.

Over the years, the heart has provided us with the evidence that solves the critical problem of the origin of cardiomyocytes. At this moment in time, paraphrasing Eugenio Montale, we could say that the human heart is "on the verge of betraying [its] final secret offering the opportunity to uncover...the still point of the world, the link that won’t hold, the thread to untangle that will finally lead to the heart of a truth." Newly generated myocytes may derive from division of preexisting parenchymal cells or from activation and differentiation of resident CSCs. Discriminating between these two possibilities is not an easy task. Fate-mapping strategies, which are commonly used to track the origin of cells and their destiny, would represent the ideal retrospective assay for the analysis of cardiomyocyte turnover, inasmuch as the expression of the fluorescent label can be placed under the control of promoter of genes coding for contractile proteins. This protocol provides information at the level of populations of cells that share the reporter gene, but it fails to demonstrate in vivo the self-renewal, clonogenicity, and multipotentiality of single progenitor cells. This inherent limitation makes it impossible to establish the identity of the ancestors of replicating myocytes.

An alternative retrospective protocol is based on the stable integration of proviral integrants in the genome of the infected cells. The insertion site of the viral genome is inherited by the entire population derived from the parental cell. Clonal tracking of individual mouse and human c-kit–positive CSCs has documented that these cells possess the fundamental properties of stem cells in vivo under physiological conditions and after injury. By this approach, the existence of a direct link between human CSCs (hCSCs) and their committed myocyte progeny has been demonstrated. Still, the controversy concerning myocyte regeneration in the adult heart by endogenous progenitor cells has not been resolved, and whether hCSCs can be implemented therapeutically is questioned frequently.

The study by Itzhaki-Alfia and colleagues published in this issue of Circulation deals with these fundamental questions and documents that the human heart contains a pool of cells capable of creating myocytes in vitro and regenerating myocardium in vivo. Successful collection of hCSCs was obtained from all myocardial specimens independently of age, sex, and type and duration of disease, which indicates that a stem cell compartment is present in the decompensated heart. Accordingly, functionally competent hCSCs may be isolated from myocardial samples and, after their expansion, administered back to the patient. Two Phase I clinical trials, testing the safety of c-kit–positive allographic hCSCs and cardiospheres, are in progress.

The recognition that a stem cell compartment exists in the adult human heart answers only in part the question of whether these cells retain the capacity to divide and differentiate throughout life or whether the multiple variables that affect human beings, including age, diseases, and the interrelated effects of genes, environment, and probabilistic changes, interfere with the growth of hCSCs, limiting their therapeutic efficacy. The accumulation of senescent poorly contracting cardiomyocytes in the old heart poses the question of whether aging uniformly affects the hCSC compartment or whether a stem cell subset maintains its youth during the entire lifespan of the organ. Pathological states of ischemic and nonischemic origin, together with the duration and severity of disease, may have profound implications on the availability and function of hCSCs. The geographic distribution of hCSCs may not be uniform within the myocardium, and cells may accumulate preferentially in specific anatomic regions, possibly interfering with cell acquisition. Sex may
represent another important determinant of hCSC growth and lineage commitment. These issues have been addressed carefully in the elegant study by Itzhaki-Alfia and collaborators.8

Surgical specimens and endomyocardial biopsy specimens were obtained from the myocardium of the 4 cardiac chambers. Expansion of the dissociated cells favored the survival and proliferation of c-kit–positive cells, which, at early passages, accounted for 4% to 24% of the unfractionated pool—a value that is much higher than the frequency of lineage-negative c-kit–positive hCSCs found in situ.11 These human cells were negative for hematopoietic markers, excluding their bone marrow origin and mast cell contamination. A fraction of c-kit–positive cells showed various degrees of cardiomyocyte commitment and expressed transcription factors such as GATA4 and sarcomeric proteins. In addition, cardiac cells gave rise to other mesodermal lineages, including adipocytes and osteoblasts. This differentiation potential is consistent with the mesenchymal ancestry of the heart. The nonpermissive environment of the myocardium conceals and suppresses the formation of noncardiac cells in vivo; this phenomenon occurs only under specific culture conditions.12

The restriction in developmental options of hCSCs was confirmed here in vivo after myocardial infarction.8 Nearly 7% of c-kit–negative cells displayed the Isl1 transcription factor in vitro. This finding is at variance with previous reports that indicated that Isl1–positive cells are present exclusively in the human fetal heart, sharply decreasing after midgestation and becoming undetectable at birth.13 The expression of Isl1 in cultured cardiac cells corresponds to the onset of myocyte commitment, inasmuch as Isl1, together with GATA-4, is a transcriptional activator of the myocyte transcription factor MEF2C.14 It is important to note that the upregulation of Isl1 in vitro does not imply that similar cells are present in situ in the human myocardium. Isl1–positive cells are myocyte precursors and lack the properties of stem cells. Their absence in the normal and failing human heart excludes any potential therapeutic use of this forming cardiomyocyte subset (Figure 1).

An important aspect of the study by Itzhaki-Alfia et al8 involves the recognition that the distribution of hCSCs in different anatomic regions of the heart conditions their number and functional behavior. The right atrium is the richest source of c-kit–positive cells, and these hCSCs are endowed with superior growth and differentiation potential. This finding has great clinical implications and is in harmony with the nonuniform localization of cardiac progenitors in the hearts of animals and humans.2,9,11 Clusters of hCSCs were collected from all myocardial samples, and they respond to these needs by increasing the number of dividing hCSCs. Although prolonged pathological states alter the niche microenvironment and/or progenitor cells, a class of hCSCs was collected from all myocardial samples, which points to a previously unappreciated growth reserve of the human heart. It defeats common thinking and projects a novel perspective for the management of the decompensated human heart.

As reported by Itzhaki-Alfia and colleagues,8 myocardial samples obtained from female donors produced a larger number of hCSCs. These observations support speculation that the lower incidence of heart failure in women may depend on a greater or more efficient pool of hCSCs that may be present at birth and persist throughout life. This difference between the sexes may enhance cardiac cell turnover in adulthood and senescence, delaying the onset of the aging myopathy. Similarly, the adult female heart is more effective at sustaining pathological loads than is the male heart; the cardiomyocyte compartment in females is composed of younger, better-functioning cells because hCSCs may more efficiently replace old dying myocytes with new differentiated progeny.15,16 Although only 4 cases of end-stage heart failure were studied, the left ventricle was still the richest anatomic source of hCSCs.8 This is a critical and novel finding. It is difficult to envision how the cause of heart
failure and the unpredictable path of the disease influence the hCSC compartment and its distribution. Ischemic heart disease and hypertension are the major causes of congestive heart failure; in both cases, prolonged overload on the myocardium may result in activation and proliferation of hCSCs located at the border zone of the necrotic or scarred infarct or scattered throughout the myocardium of the hypertensive heart. This possibility has been shown to be valid in animals17 and humans,1,18 which suggests that progenitor cells initiate an intense, albeit inadequate, regenerative response to counteract cell death and restore cardiac muscle mass.

The study by Itzhaki-Alfia and collaborators8 emphasizes once more the critical role of hCSCs in the modulation of myocardial homeostasis and regeneration after injury. Different classes of stem and progenitor cells have been characterized in the embryonic, fetal, postnatal, and adult hearts, but whether they represent distinct categories of undifferentiated cells with diverse functional behavior is currently unknown. Various surface antigens and transcription factors have been used to identify these cells and their distribution in several anatomic regions of the heart (Figure 2). Although these findings have begun to untangle “the thread [. . .] that will finally lead to the heart of a truth,” a heated controversy over myocyte regeneration persists.19 This scenario does not differ from the debate that in the early 1970s questioned the existence of hematopoietic stem cells, which were considered from the debate that in the early 1970s questioned the existence of hematopoietic stem cells, which were considered elusive and enigmatic. As discussed in Lancet,20 over the years, however, “the fierce controversies about the details of blood cell development . . . have quite died down. The textbooks no longer begin with descriptions and illustrations of rival theories; instead there seems general agreement on the existence of a stem cell which can give rise to all varieties of hematopoietic cells . . . One of the reasons for its neglect is that most of the work has been done on rodents or birds . . . Perhaps, experimental hematologists should now devote more of their resources to the direct study of human disease.” We should probably follow this suggestion.


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Human Cardiac Stem Cells: The Heart of a Truth
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