If I Can Stop One Heart From Breaking

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-Emily Dickinson

In the Ode to Broken Things, Pablo Neruda tenders a powerful metaphor of existence. It is not difficult to recognize the unwinding of human life in the broken clock that was once “the secret thread of our weeks” and now, with its “blue guts” exposed and its “wide heart unsprung,” is the symbol of agony and death. The beginning and ending of life are embraced in a poem about simple and ordinary things that accompany human beings throughout life and then are lost, together with the feelings and significance that were assigned to them. All things break, even the heart. What to do for broken things and broken hearts? The poet suggests collecting our treasures and sinking them in the ocean with the hope that the “long labor of its tides” may give back wholeness to the fragments. While we wait for the sea to reveal its strength and miraculous effects, the discovery that stem cells repair broken organs projects a more hopeful view of medicine and the way it is practiced.

Soon after the first experimental evidence that bone marrow cells (BMCs) induce cardiac repair in the postinfarcted heart, unfractionated mononuclear BMCs and CD34-positive cells were given to patients affected by acute myocardial infarction or chronic ischemic heart failure. Results accumulated so far have documented the feasibility of this therapeutic approach with indications of potential beneficial effects on cardiac function and critical clinical end points. Although the mechanisms by which BMCs improve ventricular performance in humans are currently unknown, experimental evidence favors cardiac regeneration and the production of paracrine factors by these cells. New myocytes and coronary vessels are formed by transdifferentiation of the delivered cells, a process that reflects the conversion of adult progenitor cells into functionally competent cells that do not belong to the organ of origin of the administered cells. The recognition that BMCs regenerate infarcted myocardium strongly suggests that the heart is a permissive organ for the multilineage differentiation of exogenous primitive cells. This discovery has prompted the search for a cardiac-specific progenitor cell that resides in the heart, controls the physiological turnover of cardiac cells, and to a limited extent, restores the integrity of areas of myocardial injury. Several laboratories have identified cardiac primitive cells, but the controversy concerning myocyte regeneration in the adult heart by exogenous or endogenous progenitor cells has not been resolved. Most important, whether resident progenitor cells can be implemented therapeutically is open to question.

The study by Smith and colleagues published in this issue of Circulation deals with these fundamental problems and demonstrates that the human heart contains a pool of cells capable of creating myocytes and coronary vascular cells in vitro and in vivo. This finding is highly relevant biologically and clinically. The perennial conviction of the heart as a postmitotic organ is defeated once more by these observations that project a more realistic view of cardiac biology and pathophysiology. Additionally, the present study raises the possibility that activation of the endogenous cardiac stem cell pool locally within the myocardium or the in vivo delivery of a previously expanded cell population in vitro may be introduced in the management of the human disease. Myocytes can be formed together with coronary vessels, and necrotic and scarred tissue can be replaced by new, better-functioning myocardium.

Different cell types have been proposed for the reconstitution of the damaged heart: skeletal myoblasts, fibroblasts, smooth muscle cells, fetal myocytes, embryonic stem cells, and BMCs. These cell classes except BMCs have failed to integrate, acquire the cardiomyocyte phenotype, or generate concurrently cardiomyocytes and coronary vasculature. Remarkably, the totipotent embryonic stem cells appear to engraft into the myocardium but die rapidly because of the lack of vessel formation. These negative results emphasize the importance of the study by Smith et al. For a long time, this group has been at the forefront in the search for the most effective cell for cardiac repair. The most logical and potentially powerful cell to be used to treat the failing heart is a resident primitive cell. Myocardial regeneration would be accomplished by enhancing the normal turnover of cardiac cells, and the need for the slow, time-consuming process of transdifferentiation of exogenous stem cells would be overcome. However, difficulties exist in the acquisition of myocardial samples in humans and in the isolation and expansion of cardiac progenitor cells in quantities that can be used therapeutically. These 2 significant issues have been addressed in the work by Smith and colleagues, and elegant solutions are presented: Progenitor cells were obtained from small endomyocardial biopsies and were expanded to a clinically relevant number in a timely fashion with a suspension culture method. The endomyocardial biopsy was introduced as diagnostic procedure >40 years ago and is currently used for evaluating...
myocarditis and dilated, hypertrophic, or restrictive cardiomyopathy, as well as monitoring rejection in the transplanted heart. In adult patients, this procedure is safe, with a very low incidence of complications. As reported in the present study, the successful isolation and expansion of cardiac cells in 69 of 70 cases raises the possibility that a therapeutically relevant quantity of cells can be generated with a protocol that avoids invasive surgery. This is an important aspect of cell therapy for the heart.

The isolation of cells from self-renewing organs and their culture in serum-free media on nonadhesive substrates lead to the formation of spherical clusters of cells known as floating spheres. This suspension culture method is used for largescale amplification of stem/progenitor cells as an alternative technique to single-cell deposition and clonal expansion. The suspension protocol, however, does not reflect the 3-dimensional structures. The sphere-forming assay has an intrinsic limitation: These spheres correspond to aggregates of nonhomogeneous populations of cells in which undifferentiated progenitors are present within the aggregates (Figure 1). This peculiar form of anchorage-independent growth typically occurs with neural stem cells and corneal endothelial cell precursors. A central core of proliferating cells is commonly surrounded by quiescent cells with restricted developmental options. Characteristically, neurospheres contain a small number of undifferentiated multipotent stem cells that can initiate new colonies and a large number of neural progenitors that can differentiate into neural and glial cells.

In a similar manner, Smith and colleagues have shown that progenitors resident in the human myocardium respond to the mitogenic stimulation of fibroblast growth factor and epidermal growth factor, divide, and create cardiospheres. Spheroids are obtained in poly-L-lysine-coated dishes, suggesting that cardiac primitive cells have to adhere to the substrate before detaching and proliferating in suspension. This dynamic phenotypic transition from a “mesenchymal” monolayer state to an “epithelial” floating state is commonly seen in culture of bone marrow mesenchymal stem cells. When cells are not plated at limiting dilution, cardiospheres develop on aggregation of a small number of cardiac cells. But whether cardiospheres are clonal or oligoclonal in nature, the cardiosphere-derived cells represent the progeny of the most primitive cells within the aggregates. In fact, a fraction of cells express the stem cell antigen c-kit. However, the c-kit-positive cells within the aggregates do not correspond to a uniform class of progenitors because of the heterogeneity dictated by the uncommitted or early committed state of the cells, their quiescent or cycling condition, or migratory properties.

The c-kit receptor tyrosine kinase originally was detected in a class of murine hematopoietic stem cells with long-term reconstituting ability in irradiated recipients. More recently, c-kit has been found in several populations of stem cells in the adult liver, brain, and pancreas. In the heart, this stem cell antigen identifies a pool of resident cardiac stem cells that are self-renewing, clonogenic, and multipotent in vitro and in vivo. These cardiac stem cells replace infarcts, with functionally competent myocardium restoring ventricular performance experimentally. Whether an identical cell with these biological and functional characteristics exists in the human heart remains to be determined. As nicely documented by Smith et al, the outer layer of the cardiospheres is composed of cells positive for CD105, a membrane glycoprotein commonly expressed in vascular cells at sites of active vessel growth. CD105 binds several members of the transforming growth factor-β superfamily, a cytokine that regulates multiple cell responses, including proliferation, differentiation, and migration. CD105 has recently been included as one of the typical markers of mesenchymal stem cells. The coexpression of CD105 and c-kit identifies a population of bone marrow mesenchymal stem cells in the mouse.

As shown for the neurospheres, the cardiospheres contain a fraction of differentiating cells located at the periphery of the 3-dimensional structures. Cardiosphere-derived cells undergo spontaneous maturation toward the myocyte lineage, and the process of commitment can be coaxed by coculture with neonatal ventricular myocytes. Connexin 43 is expressed between highly dividing cells within the cardiospheres and in the expanded differentiating cardiosphere-derived cells, suggesting that junctional communications between cells may play a dual role. The expression of connexin 43 in undifferentiated progenitors favors their proliferation, whereas connexin 43 in cells committed to the myocyte phenotype
promotes electrical coupling with the surroundings and the acquisition of functional competence. The presence of gap junctions between uncommitted and differentiated cells within the cardiospheres raises the possibility that the differentiated cells may function as supporting cells. If this were the case, the cardiospheres would reconstitute in vitro the complex structure of the cardiac niches identified in vivo.

The work of Smith et al raises an important question concerning the recognition of the most appropriate cell for the treatment of the failing heart of ischemic origin. It is currently unknown whether the use of a pure population of cardiac stem cell is preferable to the use of cells already committed to the myocyte, endothelial cell, and smooth muscle cell lineages. A pool of progenitors and partially differentiated cells, mimicked by the cardiospheres, may provide a greater and faster regenerative response. Figure 2 illustrates these 2 possibilities: a clonogenic single cell–derived cluster is shown on the left; a heterogeneous population of undifferentiated and partially differentiated cells is documented on the right. A mixture of cells with clearly defined properties but with the potential for proliferation may be more effective in reconstituting infarcted myocardium or replacing poorly contracting myocytes of the chronically failing heart with younger, better-functioning cells and vascular supply. Therefore, new myocytes, arterioles, and capillaries may be rapidly developed to improve tissue oxygenation and cardiac pump function.

Amplifying cells at early stage of commitment express nuclear and/or cytoplasmic markers of myocytes, endothelial cells, and smooth muscle cells and are highly proliferating. Experimental studies show that clonogenic cardiac stem cells (derived from a single founder cell) and a mixture of cardiac stem cells and rapidly growing amplifying cells (derived from several founder cells) both result in a robust reconstitution of damaged myocardium. A careful comparison of the 2 protocols has never been performed, however, and it is not known whether 1 of the 2 cell populations gives rise to more mature myocytes and coronary vessels, resulting in a faster recovery of the infarcted heart. Clonogenic cells have a large growth reserve but need more time to acquire a differentiated state. Committed cells have a reduced proliferative capacity but may acquire the adult phenotype more rapidly. Cell preparations have to be well characterized, and accurate studies have to be performed to identify the reparative potential of distinct progenitor cell types.

The work by Smith et al proves unequivocally that a mixed cell pool has regenerative capacity but, because of the nature and composition of the cardiospheres, does not resolve the dilemma of the most effective cell(s) for cardiac repair. The same uncertainty persists about the therapeutic efficacy of BMCs clinically, although recent experimental studies point to the CD34-positive cell fraction as the preferential subset for transdifferentiation and cardiac regeneration. Cardiospheres are a unique tool for the analysis of these crucial issues in vitro and in vivo. The answer to these questions may dramatically change the prognosis of acute and chronic ischemic cardiomyopathy and heart failure. And the ineluctable fate of things that are inevitably destined to break because they are pushed by “an invisible, deliberate smash-er” may apply to the broken heart no longer.

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