Editorials

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Primitive Cells and Tissue Regeneration

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In the last few years, several experimental studies have used stem cells of different sources to reconstitute damaged tissues. The brain and the heart have been the most investigated organs because of the long-standing view of the lack of regenerating potential of neurons and myocytes. Bone marrow stem cells (BMSCs) have been reported capable of transdifferentiating in various cell lineages distinct from the site of origin and, because of this property, they may constitute a new form of cellular therapy. Neuronal and myocardial growth mediated by bone marrow cells (BMCs) has been demonstrated, but these results have been challenged and the issue of BMSC transdifferentiation has become highly controversial. Heated debates at scientific meetings, letters in high-profile journals, and reports with contradicting observations have raised questions on the plasticity of BMSCs. If negative results would be more cautiously interpreted instead of being blown out of context, it is likely that the actual role that adult stem cells play in the repair of tissues and organs would be better understood and appreciated. This is particularly relevant when negative data are dropped as “valid” statements from the podium and are quoted before they undergo peer review and publication.

A good example of the opposite approach is found in the study of Chen and colleagues in this issue of Circulation Research. The authors have utilized, among other sophisticated techniques, confocal microscopy to identify and characterize an important new function of human pluripotent adult mesenchymal BMSCs. In this report, an unequivocal demonstration was obtained on the ability of these cells to deliver vascular endothelial growth factor (VEGF) to an ischemic region of the brain. VEGF accumulation coupled with endogenous activation of endothelial cells and VEGF synthesis promoted vessel formation after stroke. BMSCs via VEGF secretion acted as a chemoattractant for circulating and resident endothelial progenitor cells. Homing of these early committed cells led to the reconstitution of a vascular network within the damaged portion of the brain. Similarly, BMSCs stimulated the growth of the coronary vasculature and microvasculature, and the regeneration of cardiomyocytes improving the performance of the postinfarcted heart. It is noteworthy that administration of VEGF by different modalities in various aspects of ischemic heart disease enhances coronary blood flow and cardiac hemodynamics, mimicking the effects of human mesenchymal BMSCs on the infarcted brain. The broad developmental potential of BMCs has recently been confirmed and extremely well documented in the brain, skeletal muscle, retina, and intestinal epithelium. However, the identity of the cells involved has not been clearly elucidated. The quandary of transdifferentiation of adult primitive cells into cell lineages different from those of the tissue of origin remains to be completely resolved, although accumulating evidence favors this possibility.

Growth and Differentiation of Adult Stem Cells

A relevant area of debate concerns the mechanisms by which exogenous adult stem cells grow and differentiate in order to repair injured organs. The early studies in which BMCs have been used to induce regeneration of brain, heart, skeletal muscle, and liver have assumed that primitive cells of bone marrow origin had the ability to reprogram themselves and subsequently give rise to the cellular components of the host microenvironment. This logic and simple biological process has been questioned by in vitro studies suggesting that BMCs might fuse with resident embryonic stem cells or with parenchymal cells of the target organ before cell proliferation. If this were the case, tissue reconstitution would have to be interpreted not as a direct consequence of BMC implantation or homing through the systemic circulation, but as a secondary event initiated by transacting factors from the differentiated cell acting on and activating the differentiation pathways of the BMC nuclei. Although this issue has recently been discussed in some detail, a few comments can be made. Whether embryonic stem cells are scattered throughout the brain or the heart cannot be excluded. However, the presence of these highly dividing and multipotent cells would suggest that limited areas of damage in the brain or the heart should be constantly repaired. Unfortunately, there is no demonstration of spontaneous regeneration of injured cerebral and cardiac tissue. Moreover, neuron and myocyte loss occurs early in life and increases with age pointing to a minimal role, if any, of the vestigia of embryonic development in the brain and the heart. Fusion of a BMC with a cerebral or cardiac parenchymal cell would inevitably increase the number of nuclei per cell or the DNA content per nucleus and/or ploidy formation. Increased ploidy does not facilitate cell division, suggesting that this might be a rather unlikely consequence of tissue repair by BMCs. The phenomenon of cell fusion is
important and needs to be specifically addressed in the future. Of relevance, regenerating cells in the heart are small (Figure 1) with normal DNA content (unpublished data) suggesting that cell fusion may contribute minimally, if at all, to myocardial replacement after injury.\textsuperscript{10,18} Similarly, in the study by Chen and collaborators, \textsuperscript{1%} of endothelial cells in newly formed vessels in the injured brain were derived from human mesenchymal BMSCs. Rat nuclear antigens were not identified in these vascular cells.\textsuperscript{8} Cell fusion has also been excluded in transdifferentiation of human adult endothelial progenitors in functionally competent myocytes.\textsuperscript{19}

The brain\textsuperscript{20} and the heart\textsuperscript{21} contain primitive cells with stem cell characteristics (Figure 2). Under proper stimulation, these undifferentiated cells undergo lineage commitment and differentiate into mature neurons and cardiomyocytes. VEGF can exert a paracrine function on progenitor endothelial cells resident in the brain\textsuperscript{8} and in the heart.\textsuperscript{11} However, the identification of growth factors, which could have the ability to activate stem cells and mobilize them to reach the injured regions of the brain or heart would have a significant clinical impact. Through this mechanism, cerebral and cardiac tissues composed of parenchymal cells and vascular structures could be generated, ultimately

\begin{figure}[h]
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\includegraphics[width=\textwidth]{image1.png}
\caption{Cross section of human myocardium. Large hypertrophied myocytes surround a cluster of small myocytes (arrows and arrowheads). Bright fluorescence in myocyte nuclei (arrowheads) corresponds to the expression of Ki67, a protein indicative of cell division. Nuclei are stained by the blue fluorescence of propidium iodide, and the myocyte cytoplasm is recognized by the red fluorescence of $\alpha$-sarcomeric actin. Scale bar=10 $\mu$m.}
\end{figure}

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{image2.png}
\caption{Cross section of human myocardium. Large hypertrophied myocytes surround a group of 5 undifferentiated small cells, expressing the stem cell antigen c-kit on the surface membrane (green, arrowheads). Nuclei are stained by the blue fluorescence of propidium iodide, and the myocyte cytoplasm is recognized by the red fluorescence of $\alpha$-sarcomeric actin. Three small developing myocytes are located in proximity of the c-kit-positive cells. Scale bar=10 $\mu$m.}
\end{figure}
leading to a true form of organ repair. Examples of this approach exist in both the brain and the heart. After destruction of the substantia nigra and the induction of a Parkinson-like disease in rats, the local infusion of transforming growth factor-α (TGF-α) mobilized endogenous primitive cells from the forebrain, which translocated and homed to the site of injury.22 The subsequent reconstitution of the substantia nigra by these cells was accompanied by the disappearance of the neurological manifestations. Additionally, intraventricular infusion of fibroblast growth factor-2 (FGF-2) and epidermal growth factor (EGF) in the rat brain triggers extensive regeneration of hippocampal neurons after ischemia.23 In a similar manner, the systemic administration of cytokines in mice affected by myocardial infarction induced the mobilization of stem cells and progenitor cells and their colonization within the dead tissue.10 By this process, the infarct was replaced by contracting myocardium composed of new myocytes and coronary vessels functionally connected with the viable portion of the ventricular wall. Mortality was also decreased in this animal model. Thus, the long-term objective of tissue regeneration is reconstitution of the main components of the organ and not partial recovery of a particular brain or heart cell type, which is limited in rebuilding the complex and integrated structural organization of these two organs.

The understanding of the partial recovery of function after ischemic injury in the brain or in the heart is difficult. In both organs, this improvement could be due to the reappearance of activity in areas of the brain or the heart that were not irreversibly damaged. Alternatively, a growth response may occur in the viable tissue adjacent to the cerebral and cardiac infarct regenerating new brain and new myocardium. A combination of these two adaptations is also feasible. New myocyte formation has been shown in the postinfarcted human heart acutely and chronically.24 Similarly, spontaneous neuronal regeneration in the human brain has been demonstrated.25 The study of Chen and collaborators8 and their previous work emphasize the high plasticity of the brain and in particular the formation of new vessels in the infarcted zone. The recovery from stroke is enhanced in patients who constitutively possess an increased numerical density of vessels in the brain. Whether cerebral vessel formation is coupled with neuronal regeneration has not yet been determined.

Conclusions

There is accumulating evidence in the brain, skeletal muscle, liver, and heart that BMCs have a high level of plasticity. The microenvironment of the host organ might influence their commitment and differentiation in cell lineages separate from the site of origin of these primitive cells. The study of Chen and colleagues in the present issue of Circulation Research emphasizes the paracrine role that human mesenchymal BMSCs have in promoting vessel formation after stroke. Angiogenesis has the ability to interfere in a positive manner on the outcome of ischemic brain injury. In this editorial, the brain has been compared with the heart because both organs have been considered for almost a century postmitotic with essentially no potential for additional growth after birth. Despite the obvious importance of BMSCs in the repair process of various tissues, a goal of cellular therapy has to be directed at the identification of resident stem cells and progenitor cells in target organs. It is conceptually more powerful from a therapeutic viewpoint to establish whether pluripotent cells nested in an organ can be activated in order for them to translocate to regions of damage, home within the destroyed tissue, and reconstitute a healthy organ. In brief, cell turnover of organs such as the brain and the heart might be regulated by stem cell growth and differentiation. The identification of growth factors that selectively trigger stem cell locomotion and proliferation may lead to the recognition of novel therapies, which we could have not been predicted only a few years ago.

References


Key Words: stem cell plasticity ■ transdifferentiation ■ growth factors ■ brain ■ heart
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