



Does the Tongue Eloquently Address the Question of Cardiac Regeneration?

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Hearth failure is a major cause of morbidity and mortality worldwide. At present, patients with chronic heart failure have a poor prognosis¹ and a strong likelihood of being readmitted to hospital, despite treatment.^{1,2} The most common cause of heart failure is myocardial infarction-induced remodeling of the left ventricle, which is characterized by dilatation and diminished cardiac performance.³ Therefore, to improve clinical outcomes among patients with myocardial infarction, it will be essential to develop therapies that effectively inhibit the resultant left ventricular remodeling. Cell therapy is a promising strategy for restoring contractile elements to the failing myocardium of the infarcted heart. Autologous transplantation is optimal for this purpose because it obviates tissue rejection, and several types of adult progenitor cells have been proposed as surrogates for cardiomyocytes (Table), some of which are currently undergoing clinical evaluation.⁴⁻⁶

Article p 1219

The first clinically relevant cells to be proposed were skeletal muscle myoblasts, which are undifferentiated proliferation-competent cells that serve as precursors to skeletal muscle.^{7,8} For clinical use, autologous human myoblasts are isolated from thigh muscle biopsies, propagated *ex vivo* for a few days or weeks, and then injected directly into the ventricular wall.⁹ So far, the major safety issue raised by the use of skeletal myoblasts for cardiac repair has been the occurrence of fatal ventricular arrhythmias. Although patients eligible for cell replacement therapy are prone to develop arrhythmias because of their underlying ischemic heart disease, one cannot refute the intrinsic potential arrhythmogenicity of stem cells, which is primarily related to the cells' lack of electromechanical integration into the recipient myocardium.⁹ For skeletal myoblasts, this in part reflects the fact that they downregulate expression of the gap junction protein, connexin 43, as they differentiate into myotubes.¹⁰ Lentivirus-mediated transduction of connexin 43 gene provides gap junction functionality to skeletal myoblasts, thereby reducing their arrhythmicity.¹¹

In this issue of the Journal, Shibuya et al show that stem cell antigen (Sca)-1-positive cells isolated from tongue muscles can present the cardiomyocyte phenotype with beating and *Nkx 2.5* expression.¹² It is noteworthy, moreover, that these cells preserve expression of connexin 43. In their study, the authors characterize for the first time the cardiomyocyte-

like properties of cultured tongue muscle-derived stem cells (TDSCs). In addition to expressing cardiac-specific genes, these cells appear to form gap junctions, as indicated by the transfer of dye and synchronization of calcium transients among adjacent cells. Collectively, these findings strongly suggest TDSCs may be ideal for cell therapy in heart disease (ie, TDSCs appear to differentiate into cardiomyocytes that electrically cooperate with adjacent cardiomyocytes).

Intriguing as the *in vitro* findings of Shibuya et al are, their *in vivo* evaluation of transplanted TDSCs is somewhat superficial.¹² The authors transplanted TDSCs into acutely infarcted mouse hearts and then observed the mice for as long as 12 weeks. Among the TDSC-treated mice, the survival rate

Table. Sources of Reported Stem Cells for Potential Use in Cardiac Repair

Organ/tissue	Cell type
Bone marrow	Hematopoietic stem cells (HSCs) Mesenchymal stem cells (MSCs) Multipotent adult progenitor cells (MAPCs) Bone marrow stem cells (BMSCs) Very small embryonic stem cell-like cells (VSESCs)
Heart	c-Kit ⁺ cells Side population (SP) cells Sca-1 ⁺ cells Islet-1 ⁺ cells
Blood	Endothelial progenitor cells (EPCs) Umbilical cord blood-derived stem cells (UCBSCs) Menstrual blood-derived mesenchymal cells (MMCs)
Other organs/tissues	Skeletal muscle (satellite cells, Sca-1 ⁺ cells, SP cells) Adipose tissue (adipose-derived stem cells (ADSCs)) Placenta (placenta-derived extraembryonic mesodermal cells) Tongue (tongue muscle-derived stem cells)*
Pluripotent stem cells	Embryonic stem cells (ESCs) Inducible pluripotent stem cells (iPSCs) Embryonic germ cells (EGS)

*Introduced by Shibuya et al¹² in this issue of the Journal.

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was higher, and postinfarction cardiac remodeling and dysfunction were significantly attenuated, as compared with the controls. However, the authors' findings do not shed light on the mechanism underlying those beneficial effects. Given the in vitro observations, one would expect that the transplanted TDSCs differentiate into cardiomyocytes and form gap junctions with each other and/or the resident cardiomyocytes, thereby making electrical connections and enabling cooperative contraction. Unfortunately, the authors were unable to confirm this possibility using green fluorescent protein-labeled TDSCs in the treated hearts, as no labeled cells were detected, and the reason for their apparent absence remains unclear. If the transplanted TDSCs had really disappeared, the merit of preserving connexin 43 seems to make no sense. Although the authors also suggest that paracrine effects of the transplanted TDSCs may contribute to their beneficial effects, this is only speculative and is unsupported by evidence. Furthermore, with respect to clinical application, the selection of TDSCs based on Sca-1-positivity is problematic, because Sca-1 is not encoded in the human genome.¹³ Although the study by Shibuya et al does indeed present a novel observation and might significantly contribute to research in the field of cardiac regeneration,¹² further study of TDSCs, especially their behavior in vivo, is clearly needed.

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