C

urrently, 3 types of adult progenitor cells are used in clinical trials to treat pa-
tients who have sustained acute myocardial infarction or who have chronic
ischemic heart disease. They consist of unpurified bone marrow cells, pe-
ripheral blood cells that are highly enriched for expression of the CD34 marker, and
mesenchymal stem cells. Many of these studies have demonstrated clinical benefit—
Improved ejection fraction, improved perfusion,1-6 or both. So far, there has been only
a single study of what actually happens inside the heart after adult progenitor cells
have been injected. This study,7 however, showed only that angiogenesis had occurred
at the site of injection. It was difficult to determine whether the adult progenitor cells
had become cardiomyocytes, because it was not possible to verify the transplantation
of autologous cells. Before we can advance to clinical studies, it is important to dem-
onstrate in preclinical studies the long-term fate of the adult progenitor cells, the mech-
anism whereby the progenitor cells transform into different cardiac lineages, and the
critical importance of angiogenesis, myogenesis, or paracrine effects—or any combi-
nation of these—for cardiac repair.

We injected human peripheral blood CD34+ cells into the peri-infarct regions of se-
vere combined immune deficient (SCID) mice after the mice had undergone experi-
mental myocardial infarction (MI). Using the human leukocyte antigen (HLA) as
a convenient marker of the human cells, we could easily distinguish the transplanted
cells from the endogenous murine cells. We showed that the CD34+ cells had the po-
tential to turn into cardiomyocytes, endothelial cells, and smooth muscle cells.8

To further understand the mechanism of the transformation of the CD34+ cells, we
digested SCID mouse hearts that had been obtained at various times after experi-
mental MI and cell injection. These single-cell preparations enabled us to separate
the cells using unique cell-surface or intracellular markers to distinguish human-derived
cells (HLA-positive) from endogenous mouse cells (HLA-negative); and they enabled
us, further, to distinguish cardiomyocytes (troponin T-positive) from endothelial cells
(VE-cadherin-positive). We showed, on the basis of the surface expression of HLA,
that about 1% of the total cardiac cells were derived from human progenitor cells.9
Of these, 70% were troponin T-positive (human-derived cardiomyocytes) and 20%
were VE-cadherin-positive (human-derived endothelial cells). Using probes that label
human or mouse X-chromosome, we showed that human-derived endothelial cells
directly differentiate from human progenitor cells, whereas only 30% of human-
derived cardiomyocytes are direct descendents of the injected progenitors. Remark-
ably, 70% of the human-derived cardiomyocytes result from fusion between the
human progenitor cells and resident mouse cardiomyocytes.

Next, we developed an in vitro model of cell fusion and demonstrated that this pro-
cess is driven by an adhesion molecule pair (VCAM-1 and α4β1). Indeed, cell fusion
can be blocked by an antibody specific for VCAM-1 or α4β1, but not by an antibody
specific for vascular endothelial growth factor (VEGF), either in vitro or in vivo.10
In contrast, only the directly human-derived endothelial cells are blocked by anti-VEGF
in vivo. Thus, we can distinguish the process of myogenesis from that of angiogenesis,
paracrine effect, or both by selective antibody blocking, at least in CD34+ cells (Fig. 1).
This insight enabled us to design studies to determine the relative contributions of
angiogenesis, myogenesis, and paracrine effect by comparing the ejection fractions
(by magnetic resonance imaging) and the final cellular compositions of the inject-
ed animals (by fluorescent activated cell sortor). Preliminary studies suggested that
angiogenesis or paracrine effect (or both) are more important than myogenesis in contributing to improvement in ejection fraction.

We have also developed a molecular imaging technique: we transfect the progenitor cells with genes that encode for luciferase and thymidine kinase; then we track the long-term fate of the injected cells with bioluminescence (luciferase) or positron emission tomography (thymidine kinase). In our preliminary studies, we showed that human CD34⁺ cells can persist in the hearts of SCID mice for up to 1 year after experimental MI and cellular injection. Furthermore, there is a long-term improvement (up to 26 weeks) in ejection fraction following the injection of CD34⁺ cells.

The experimental model and methods that we developed for human peripheral blood CD34⁺ cells can be used to study other adult progenitor cells, such as mesenchymal stem cells and induced pluripotent stem cells (iPS cells). We believe that thorough studies of mechanisms and side-by-side comparisons in a well-established small-animal model are important steps toward large-animal preclinical studies and successful human clinical trials.

References


