Cytokine Preconditioning Promotes Codifferentiation of Human Fetal Liver CD133+ Stem Cells Into Angiomyogenic Tissue

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Background—CD133 (AC133) is a surface antigen that defines a broad population of stem cells, including myogenic and endothelial progenitors. CD133+ cells are rare in adult tissues, and the factors that support their differentiation into mature angiomyogenic cells are not known. These hurdles have hampered the use of CD133+ cells for therapeutic purposes. Because human fetal liver is a rich source of CD133+ cells, we sought to identify the growth factors that promote codifferentiation of these cells into angiogenic and myogenic cells.

Methods and Results—Human fetal liver CD133+ and CD133− cell subpopulations were cultured with 5′-azacytidine or vascular endothelial growth factor (VEGF165) and/or brain-derived nerve growth factor (BDNF). CD133+ but not CD133− cells from human fetal liver codifferentiated into spindle-shaped cells, as well as flat adherent multinucleated cells capable of spontaneous contractions in culture. The resulting spindle-shaped cells were confirmed to be endothelial cells by immunohistochemistry analysis for von Willebrand factor and by acetylated LDL uptake. Multinucleated cells were characterized as striated muscles by electron microscopy and immunohistochemistry analysis for myosin heavy chain. Presence of VEGF165 and BDNF significantly enhanced angiomyogenesis in vitro. Inoculation of cells derived from CD133+ cells, but not CD133− cells, into the ear pinna of NOD/SCID mice resulted in the formation of cardiomyocytes, as identified by immunostaining with cardiac troponin-T antibody. These cells generated electrical action potentials, detectable by ECG tracing.

Conclusions—CD133 defines a population of human fetal liver cells capable of differentiating into both angiogenic and myogenic cells. Preconditioning of these CD133+ cells with VEGF165 and BDNF enhances the angiomyogenesis. CD133+ fetal liver cells ultimately may be used for therapeutic angiomyogenesis. (Circulation. 2005;111:1175-1183.)

Key Words: angiogenesis ■ endothelium ■ growth substances ■ muscle development

Adult stem cells can contribute to the regeneration of functional vasculature and muscle tissues.1,2 Most clinical trials have focused on transplanting crude populations of adult bone marrow cells directly into ischemic myocardium or atrophic skeletal muscles to induce differentiation of progenitor cells into vascularized muscle tissue.3–8 The preliminary results, however, have not revealed significant long-term clinical benefits from this approach.9 There are several explanations for the incapacity of immature adult bone marrow–derived cells to differentiate into muscle tissue. First, the precise phenotype of the bone marrow–derived muscle precursor cells and the factors responsible for their differentiation into functional striated muscles remain unknown. In addition, it is possible that the number of the adult bone marrow–derived muscle progenitor cells is very low. Thus, in vitro expansion and differentiation of immature progenitors into functional angiomyogenic cells before transplantation to the site of injury might be necessary. The identification of the specific precursor cells, which differentiate into myocytes and myocyte-specific vasculature, as well as the cytokines, which precondition precursor cells to induce their differentiation into angiomyogenic tissue, will lead to more effective stem cell–based therapies for revascularization and cardiac regeneration after myocardial infarction.

Cardiomyogenic progenitors differentiate concurrently with cardiac endothelial cells, and intimate spatial and temporal interactions between endothelial and myogenic cells are essential for myogenesis during embryonic development.10,11 This suggests the existence of a common cardiomyogenic-endothelial precursor; however, the markers that identify this common precursor cell are not known. We previously found that CD133 (AC133, human prominin-1), a surface antigen12...
that is expressed on hematopoietic and neural stem cells, also defines a population of functionally active circulating endothelial progenitor cells. Most recently, it was demonstrated that circulating CD133 cells from normal blood are able to differentiate into myoblasts in vitro and to regenerate skeletal muscles in the murine model of Duchenne muscular dystrophy. In accordance with the above data, we hypothesized that CD133 expression defines a population of human cells that can differentiate into both endothelial and myogenic cells.

In the present study, we have identified a prenatal stem cell population of CD133 cells, isolated from human fetal liver (HFL), that have the capacity to codifferentiate into cardiac muscle and endothelium. We also demonstrated that both vascular endothelial growth factor (VEGF165) and brain-derived nerve growth factor (BDNF) play an important role in the process of myo-endothelial codifferentiation. In addition, we showed the ability of CD133 cells derived from fetal tissue that can differentiate into both endothelial and myogenic cells.

**Methods**

**Fetal Tissue Preparation**

The Investigational Review Board of Weill Medical College of Cornell University approved the use of fetal tissues. Twenty samples of human 12- to 20-week-old fetal livers (Ob/Gyn Department at Weill Medical College of Cornell University, New York, NY; Advanced Bioscience Resources Inc) were mechanically dissociated in X-Vivo 20 medium (BioWhittaker). The cell suspension was filtered with a syringe through a 40-mm nylon mesh (Millipore).

**Isolation of CD133+ Cells From HFL**

Fetal liver CD133 cells were purified from cell suspensions by use of Accu-Prep Lymphocytes (Accurate Chemical & Scientific Corp) and CD133 (AC133) Cell Isolation Kit (Miltenyi Biotec Inc) according to the manufacturers’ instructions. The magnetic separation was performed twice to obtain a 95% pure CD133 population.

**Gene Expression Analysis**

Total RNA was isolated from CD133+ and CD133 cells obtained from 15-week-old HFL by use of High Pure RNA Isolation Kit (Roche Diagnostics GmbH). One microgram of total RNA was used for cDNA synthesis by use of 10 μL of 10 mM dCTP according to the manufacturer’s protocol (Research Genetics/Invitrogen). Unincorporated nucleotides were removed by use of Bio-Spin 6 Chromatography columns (Bio-Rad). Labeled cDNA was hybridized to the gene filter GF21-Human “Named Genes” GeneFilters Microarrays, Release 1 (Research Genetics), containing 4000 known human genes. After hybridization, the gene filter was exposed to a phosphorimaging screen, scanned by PhosphorImager (Amersham Biosciences), and analyzed with Pathways software (Research Genetics). Normalized intensities were calculated by subtracting a constant background value and dividing each point by the average intensity for the GeneFilter.

**Reverse Transcription–Polymerase Chain Reaction Analysis of HFL Cells**

Primers were designed as shown: GATA-4 (forward: 5’-CTCCTCATGCGACACGCA-3’ and reverse: 5’-AACATTGCGATGTATTGC-3’); β-actin (forward: 5’-CATGACACAAATACCCAGCT-3’ and reverse: 5’-ACCTGCGTCTGAAGGACT-3’); CSX/Nkx2.5 (forward: 5’-ACAAGGTGAAGGCCTCCACGCTCTC-3’ and reverse: 5’-CTTGACTACAGCGGCGACT-3’); MyoD1 (forward: 5’-AGACTACAGCGGCGACT-3’ and reverse: 5’-AGGCGGTCCAGCGGACT-3’); Myf-5 (forward: 5’-AAGCGTGGAGAAGACTAT-3’ and reverse: 5’-ATGAGATAAGCCTGGACT-3’); and MEF2C (forward: 5’-CCATCGGATCACTAACAAC-3’ and reverse: 5’-CTGACTGATGCTGACGCCT-3’). Amplification was performed by use of Advantage 2 Polymerase (BD Biosciences Clontech) with cycling conditions as follows: 94°C for 30 seconds; 60°C for 30 seconds; and 72°C for 30 seconds for 35 cycles.

**Transwell Cultures of CD133+ HFL Cells**

HFL CD133+ cells were isolated from cell suspensions as described above and grown in growth medium: M199 medium, 20% FCS, 10 ng/mL VEGF165 (Peprotech), 5 ng/mL basic fibroblast growth factor (Peprotech), and 1 μM heparin sulfate at 37°C, 5% CO2 in gelatin-coated 6-well plates. To provide continuous cytokine delivery, we used a coculture system of CD133+ cells and E4 adenovirus–infected human umbilical vein endothelial cells (HUVECs). HUVECs infected with the adenovirus were cocultured with 2×104 HFL cells in a transwell system (Corning Inc). HUVECs were infected with 25 multiplicity of infection (MOI) of VEGF165-containing adenovirus or total 50 MOI of BDNF- and VEGF165-containing adenoviruses, respectively. HUVECs infected with 25 or 50 MOI of adenoviral vector with an empty cassette were used as a negative control. In the 5'-azacytidine induction experiments, CD133 fetal liver cells were cultured in regular growth medium supplemented with 10 μM 5'-azacytidine (Sigma) on gelatin (0.2%)–coated 6-well dishes (Corning). All growth medium was removed after 24 hours, and the nonadherent cells were washed with PBS. The pellet was resuspended in regular growth medium without 5'-azacytidine. Cultures were followed for 2 to 3 weeks until muscle differentiation morphology was detected by phase-contrast microscopy. After the observation of colony formation under phase-contrast microscopy, colonies in each of the different conditions were counted on 10-cm dishes by use of an inverted microscope. Angiomyogenic foci were defined as colonies containing at least 100 differentiated cells.

**Immunohistochemistry of Angiomyogenic Foci**

Methanol-fixed cultures were stained with monoclonal antibodies for human fast myosin heavy chain (F59) hybridoma (1:5 dilution) provided by Don Fishman (Cornell University Medical College); rabbit anti-human von Willebrand factor (1:50) (Dako Corp). Immunocomplexes were visualized by use of biotinylated anti-mouse IgG and the Vectastain Elite ABC kit (Vector Laboratories), followed by the Nova Red substrate (Vector Laboratories), according to the manufacturer’s instructions. Stained wells were examined by light microscopy.

**Transmission Electron Microscopy**

Angiomyogenic foci were fixed in glutaraldehyde, followed by incubation in 1% osmium tetroxide. Samples were dehydrated through graded ethanol solutions and embedded in Spurr’s low viscosity embedding medium. Ultra-thin sections were stained with uranyl acetate and lead citrate and examined with a JEOL 100CX-II electron microscope.

**Functional Analysis of CD133+ HFL-Derived Myocytes in NOD/SCID Mice by In Vivo Biosensing**

The chronotropic function of CD133+ HFL-derived myocytes was tested in a pinnal allograft model that allows the recording of spontaneous electropotentials independent of the endogenous murine heart. HFL CD133+ and CD133+ cells primed with 5'-azacytidine for 24 hours (as described above) were cultured for 3 weeks in the presence of VEGF165 and BDNF. Resulting cultures were trypsinized, and the cell suspension was washed 3 times in sterile 1X PBS. The final cell suspension of 1×106 cells (in 0.5 mL X-Vivo) was injected into the pina of NOD/SCID mice as previously described for embryonic stem cell–derived cardiac myocytes.
TABLE 1. Gene Expression Analysis of CD133+ Cells From Human Fetal Liver

<table>
<thead>
<tr>
<th>Muscle-Specific Genes</th>
<th>Signal Intensity</th>
</tr>
</thead>
<tbody>
<tr>
<td>Caldesmon</td>
<td>247</td>
</tr>
<tr>
<td>drp1</td>
<td>231</td>
</tr>
<tr>
<td>Myosin light chain (smooth muscle isoform)</td>
<td>49</td>
</tr>
<tr>
<td>Tropomyosin, α-chain</td>
<td>44</td>
</tr>
<tr>
<td>Neutral calponin</td>
<td>28</td>
</tr>
<tr>
<td>Skeletal troponin I, slow</td>
<td>15</td>
</tr>
<tr>
<td>Sarcospan-2</td>
<td>13</td>
</tr>
<tr>
<td>Creatine kinase B</td>
<td>12</td>
</tr>
</tbody>
</table>

Hematopoietic genes

- BENE: 196
- CD20: 166
- Class II MHC, DO-β1: 153
- Immunoglobulin-γ3: 153
- Spi-B transcription factor: 146
- Ankyrin 1: 97
- Lymphoid nuclear protein (LAF4): 97
- Monocyte Ig-related receptor (MIR-10): 69
- IL1-β: 45

Pinna were pretreated with 100 ng of platelet-derived growth factor-AB (R&D Systems Inc) in a total volume of 10 μL, 24 hours before cell transplantation by subcutaneous injection, to enhance allograft rheology and chronotropic function.18 Two weeks after cell transplantation, the mouse pinna was analyzed for dynamic independence chronotropic function. Briefly, the electrophysiological activity of the exogenous myocytes was measured after induction of anesthesia with 2.5% tribromoethanol (Avertin). Electrophysiological tracing was acquired for an average of 45 minutes via a 4-channel differential alternating amplifier (model 1700, A-M Systems Inc). The signals were bandpass–filtered between 3.0 and 100.0 Hz, differential alternating amplifier (model 1700, A-M Systems Inc). Notch-filtered at 60.0 Hz, amplified 1000, times and sampled at 500 Hz with the use of a data acquisition board, model AT-MOI-16E10 (National Instruments).

Histological Examination and Immunohistochemistry of Ear Pinna of NOD/SCID Mice

Paraffin sections were stained with Accustain trichrome, Masson (Sigma), for histological examination. Immunostaining of serial sections was performed by use of connexin-43 polyclonal antibody (Sigma) or polyclonal antibodies for troponin T (Santa Cruz Biotechnology Inc), which are specific for cardiac muscle but do not cross-react with fast or slow skeletal troponin T.

Results

CD133+ Subpopulation of HFL Cells Expresses Muscle-Specific Genes

We performed gene expression analysis on the CD133+ cells obtained from 15-week-old HFLs by gene filter microarray. Remarkably, CD133+ HFL cells, in addition to expressing hematopoietic genes, also express muscle-specific genes (Table 1). On the basis of these data, we hypothesized that in addition to their role in hematopoiesis and angiogenesis, CD133+ cells could also contribute to myogenesis.

HFL Cells Express Cardiac and Skeletal Muscle Markers

HFL cells were further characterized by reverse transcription–polymerase chain reaction for the presence of cardiac and skeletal muscle–specific markers (Table 2). This analysis revealed that HFL cells are positive for muscle marker MEF-2C and cardiac markers TBX-5 and GATA-4, as well as skeletal muscle marker Myo-D in undifferentiated cells. We were not able to detect cardiac marker CSX (Nkx2.5) or skeletal marker Myf-5 at this stage; however, the expression of Nkx2.5 was upregulated after differentiation of CD133+ fetal liver cells into striated muscles (data not shown). These data suggest that subsets of HFL CD133+ cells have the capacity to differentiate into striated muscles with skeletal and cardiac features.

CD133+ Subpopulation of Fetal Liver Cells Differentiates Into Angiomyogenic Cells

To verify the capability of CD133+ cells to differentiate into endothelial and myogenic cells, 5′-azacytidine–primed or nonprimed HFL CD133+ and CD133- cell subpopulations were cultured with VEGF165 and/or BDNF. After 2 weeks, proliferating CD133+ cells formed dense cellular colonies (Figure 1A), which evolved into an endothelium-like monolayer with round, nodular, nonadherent cells (Figure 1B). By 2 weeks, the nonadherent cells codifferentiated into large numbers of flat adherent multinucleated cells, which exhibited spontaneous contractions in culture (Figure 1, C and D; Data Supplement Movie). These cells developed further into several long, multinucleated cells, reminiscent of myotubes (Figure 1E). The presence of multinucleated myotubes was confirmed by staining of differentiated cells with F59 antibody specific for myosin heavy chain (Figure 1, F–I). F59 antibody recognizes both skeletal and cardiac muscles, but not smooth muscles.29 We also confirmed the presence of endothelial cells by staining with monoclonal antibody to von Willebrand factor (Figure 1J) and by demonstrating metabolic acetylated LDL uptake (Figure 1, K and L). On the basis of immunohistochemistry, we determined that the ratio of muscle to endothelial cells was 4:1.

Electron microscopy analysis of angiomyogenic foci showed the presence of multinucleated myotubes with sarcomeres, which are characteristic of striated muscle cells (Figure 1, M and N). Granules of glycogen and a high frequency of mitochondria were also observed, further confirming that the CD133+ cells had differentiated into myocyte-like cells (Figure 1, O and P).

TABLE 2. Expression Analysis of Cardiac and Skeletal Specific Genes in Human Fetal Liver by RT PCR

<table>
<thead>
<tr>
<th>Muscle-Specific Genes</th>
<th>Heart</th>
<th>Skeletal Muscles</th>
<th>Fetal Liver</th>
</tr>
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<tbody>
<tr>
<td>GATA-4</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>TBX5</td>
<td>+</td>
<td>-</td>
<td>+</td>
</tr>
<tr>
<td>CSX (Nkx2.5)</td>
<td>+</td>
<td>-</td>
<td>-</td>
</tr>
<tr>
<td>MyoD</td>
<td>-</td>
<td>+</td>
<td>+</td>
</tr>
<tr>
<td>Myf-5</td>
<td>-</td>
<td>+</td>
<td>-</td>
</tr>
<tr>
<td>MEF-2C</td>
<td>+</td>
<td>+</td>
<td>+</td>
</tr>
</tbody>
</table>
**VEGF$_{165}$ and BDNF Enhance Angiomyogenesis**

Incubation of CD133$^+$ cells in culture with VEGF$_{165}$ or a combination of VEGF$_{165}$ and BDNF profoundly enhanced the formation of angiomyogenic colonies. We quantified this process by counting the number of angiomyogenic foci in a transwell cytokine interphase system (Figure 2A). The combination of VEGF$_{165}$ and BDNF was found to be more effective (1800 foci) than VEGF$_{165}$ alone (1200 foci). CD133$^+$ cells grown in serum-free, cytokine-free X-Vivo medium formed considerably fewer angiomyogenic foci (600 foci). In contrast, CD133$^+$ HFL cells formed almost no angiomyogenic foci, with or without cytokines (Figure 2B). These data indicate that preconditioning of a CD133$^+$ immature population of progenitors with VEGF$_{165}$/BDNF supports...
their codifferentiation into endothelial cells and striated muscles. The enhanced angiomyogenic potential of VEGF165/BDNF-primed CD133/H11001 cells provides a powerful tool for generating a large number of transplantable cells to induce rapid regeneration of vascularized cardiac tissue.

Myocytes Derived From CD133+ Cells Display Myocytic Activity In Vivo
Functional analysis of CD133+ HFL-derived myocytes in NOD/SCID mice was performed by in vivo biosensing (Figure 2C). Pinna of NOD/SCID mice were primed with platelet-derived growth factor-AB injection to enhance vascularity as described in our previous study, followed by injection of differentiated CD133/H11001 HFL cells. Histological examination of the ear pinna revealed the formation of vasculature and striated muscles at the site of the injection (Figure 3). Immunostaining of mouse ear pinna sections with connexin-43 and cardiac-specific troponin T showed that muscle cells derived from CD133+ HFL have characteristic features of myocardial cells.

ECG tracing of the endogenous recipient mouse heart showed QRS complexes compatible with cardiac contractions, whereas simultaneous ECG tracing of exogenous myocytic tissue in the left ear of the mouse showed the presence of action potentials, compatible with myocardial contractions. CD133+ cells injected into the right ear failed to generate electrical action potentials. These data indicate that CD133+ cells could differentiate into striated muscle cells with structural and functional features of cardiomyocytes.

Discussion
Although recent preclinical and clinical trials have suggested that stem cell transplantation through therapeutic cellular cardiomyoplasty can restore short-term cardiac function, the development of strategies for induction of angiomyogenesis has been confronted with several hurdles. First, the precise phenotype of the human angiomyogenic stem cell that can readily differentiate and contribute to regeneration of vascularized muscle tissue has not been identified. Second, the scarcity of adult stem cells has forced the use of large numbers of whole bone marrow mononuclear cells, the majority of which are composed primarily of nonangiomyogenic cells. In this report, we demonstrate that fetal liver CD133+ cells constitute a population of cells that can differentiate into large numbers of angiomyogenic cells. Preconditioning of fetal liver CD133+ cells with BDNF and VEGF for 14 days promotes codifferentiation of these cells into functional angiomyogenic foci. Therefore, our findings lay the foundation for development of strategies to improve clinical applications of cellular cardiomyoplasty.

Recent findings suggest that hematopoietic, endothelial, and muscle precursor cells derive from the aorta-gonadomesonephros region and subsequently migrate to the fetal liver, and later, to bone marrow. Fukada et al showed...
that fetal liver contains significantly higher numbers of myogenic stem cells than does bone marrow. Therefore, at certain stages of embryogenesis, fetal liver could be a rich source for muscle stem cells.

Circulating CD133+ cells from peripheral blood have been shown to differentiate into myotubes in vitro and in vivo. These cells were able to participate in muscle regeneration in scid/mdx mice, a murine model of muscular dystrophy. These results

Figure 2. Functional properties of angiomyogenic colonies. A, Schematic illustration of transwell coculture system used for adenoviral cytokine delivery. HUVECs on bottom wells were infected with VEGF165 and/or BDNF containing adenoviral constructs to ensure constant production of those cytokines. Adenoviral vector with an empty cassette was used as a negative control. CD133- and CD133+ HFL cells were incubated in transwells hanging above cytokine-secreting HUVECs. B, Absolute numbers of angiomyogenic foci; cytokine delivery was achieved by use of VEGF165- and BDNF-containing adenoviruses; adenoviral vector with an empty cassette is indicated as “Vehicle”; data shown from 3 independent experiments. C, Functional analysis of myocytes derived from CD133+ HFL cells in NOD/SCID mice by in vivo biosensing. Two electrodes were positioned on tips of ear, with grounding on tail. ECG represents voltage vs time tracing for transplants of CD133- and CD133+ cells in left and right ears of recipient NOD/SCID mice. Arrows point to QRS complexes generated in left ear. No QRS complexes were detected in right ear. ECG from endogenous heart is shown as a reference.

Figure 3. Histological examination of ear pinna of NOD-SCID mice in CD133+ cell transplantation experiments. A and B, Ear pinna sections were stained with trichrome stain (Masson). ×1000 magnification reveals clearly visible striations typical of striated muscles. C, Stainings with cardiac-specific troponin-T antibody, and D, connexin-43 antibody demonstrate cardiac nature of muscles derived from CD133+ HFL cells.
strongly support our data that CD133+ stem cells can differentiate into striated muscle and indicate that depending on the preconditioning regimen, CD133+ cells from different sources may still share similar differentiation pathways.

Using gene microarray and reverse transcription–polymerase chain reaction analysis, we showed that CD133+ HFL cells express muscle-specific genes. Consequently, we hypothesized that these cells could participate in myogenesis. In agreement with our hypothesis, we showed that CD133+ cells, but not CD133- cells, form angiomyogenic colonies in vitro and in vivo. This finding indicates that CD133 defines the population of fetal liver cells capable of angiomyogenic differentiation.

Although it was shown that bone marrow stromal cells can differentiate into muscles on treatment with 5'-azacytidine, until now, little was known about the role of cytokines in support of angiomyogenesis. We demonstrated that fetal myocardium and endothelial cells express VEGF-A receptors and TrkB. This observation led us to explore the significance of those receptors and their ligands in angiomyogenesis. VEGF165, the ligand for VEGFR-2, is one of several cytokines long known to be important in the regulation of angiogenesis and angiogenic sprouting. BDNF, the ligand for TrkB, is best known for its survival and differentiative effect on neurons. We showed previously, however, that BDNF is an endothelial cell survival factor, required for intramyocardial vessel stabilization. BDNF deficiency in embryonic vasculature reduces endothelial cell-cell contact, leading to intraventricular wall hemorrhage and depressed cardiac contractility. On the basis of these data, we hypothesized that VEGF165 and BDNF may play a role in promoting angiomyogenesis. Indeed, we showed that the number of angiomyogenic colonies increases dramatically after constant stimulation of CD133+ cells with VEGF165 and BDNF, confirming our hypothesis that these cytokines are permissive mediators of angiomyogenesis. Thus, although priming of the CD133+ cells with 5'-azacytidine significantly enhances the formation of angiomyogenic foci, the stimulation of the nonprimed CD133+ cells with BDNF and VEGF165 is sufficient by itself to promote angiomyogenesis. It is conceivable that although VEGF165 and BDNF may be permissive for generation of large numbers of angiomyogenic cells, other, as yet unrecognized, factors may determine the initial commitment of immature CD133+ cells into mature angiomyogenic cells. Nonetheless, VEGF165- and BDNF-mediated codifferentiation of CD133+ cells into a large number of functional angiomyogenic colonies might provide an effective means to generate angiomyogenic cells for delivery into the avascular infarcted myocardium.

It is interesting to note that fetal liver CD133+ cells may also, under specific permissive conditions, differentiate into nonangiomyogenic tissues. Hao et al. used human astrocyte culture–conditioned medium as well as a double-chamber coculture with human astrocytes separated from nonadherent hematopoietic stem cells by a semipermeable membrane to differentiate HFL CD133+ ‘CD34+’CD3- cells into neural cells and astrocytes. However, the majority of the cells generated in our experiments from CD133+ fetal liver cells by use of VEGF165, BDNF, and 5-azacytidine expressed either endothelium-specific or muscle-specific markers. Although our data suggest that VEGF165 and BDNF induce formation of muscle and endothelial cells, it is likely that incubation of subsets of CD133+ cells with specific cytokines could permit their differentiation into neuronal or hematopoietic cells.

To study the mechanism by which CD133 expression is regulated in different stem cells, we cloned the human CD133 promoter. Remarkably, we found that CD133 expression is regulated by several alternative promoters, which are selectively activated in a tissue-specific manner. It is likely that cytokines may trigger the activation of different alternative CD133 promoters in a tissue-specific manner, leading to the differentiation of CD133+ progenitor cells in different directions.

Myocardial infarction results in the loss of irreplaceable cardiomyocytes and the regression of regional vascular supply. Therefore, exogenous delivery of both cardiomyocyte and endothelial progenitors is necessary to rebuild and revascularize a large zone of avascular damaged tissue, which could be inaccessible to the circulatory cells. The importance of endothelial progenitor cells to the generation of vasculature in ischemic cardiac tissues is being investigated. One recent clinical study showed that transplantation of bone marrow–derived CD133+ cells improves the function of infarcted myocardium. The authors proposed that angiogenesis was the key factor in this process, although the precise mechanisms were not investigated. Our data suggest that the essential role of CD133+ endothelial progenitor transplantation on rejuvenation of infarcted myocardium could be a result of not only angiogenesis but also regeneration of cardiac muscles as a result of local delivery of a population of stem cells capable of angiomyogenic differentiation. Indeed, using cardiac troponin-T antibody and functional in vivo tests, we demonstrated that CD133+ cells differentiate into striated muscle with cardiac features.
Recent studies have shown that introduction of a purified population of immature hematopoietic stem cells into the infarcted myocardium may not replace damaged cardiac muscle,\(^3\) as previously demonstrated.\(^5\) Although transplantation of bone marrow does appear to improve the function of injured myocardium, experiments with genetic tags showed that transplanted bone marrow cells may not differentiate into cardiac myocytes.\(^6,7\) The cells used for transplantation by different groups, although phenotypically similar, may have profound differences in their maturation and viability potential. We believe that transplanting the pure population of immature progenitors to the site of injury may be insufficient to induce their differentiation into mature myocytes. Ischemic or necrotic myocardium may lose tissue-specific molecular cues, which could be critical for the progenitor cells to initiate angiomyogenesis. In contrast, cytokine-preconditioned stem cells, committed to differentiate into angiogenicogenic tissue, may recognize the damaged myocardium more readily. Therefore, preconditioning of angiogenicogenic cells might be necessary to rebuild functional vascularized, electromechanically-coupled cardiac tissue. We also expect that preconditioning of bone marrow– or peripheral blood–derived progenitors with VEGF\(_{65}\) and BDNF may provide a clinically feasible platform to enhance the success of therapeutic cellular cardiomyoplasty interventions.

In conclusion, our data indicate that the CD133 biomarker defines a population of myo-endothelial progenitors that can be instructed to codifferentiate into functional angiogenicogenic colonies in vitro and in vivo. We have also identified cytokines that facilitate differentiation of fetal tissue–derived progenitors into angiogenicogenic cells. This finding extends the concept of the differentiation potential of CD133\(^+\) cells (Figure 4) by discovery of myoangioblasts in addition to previously reported hemangioblasts. Further in vivo work will clarify the regenerative potential of CD133\(^+\) cells and their use as therapeutic agents for muscle repair.

Acknowledgments

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