Functional properties of cells obtained from human cord blood CD34+ stem cells and mouse cardiac myocytes in coculture

Alessia Orlandi,1* Francesca Pagani,1,6* Daniele Avitabile,2* Giuseppina Bonanno,3 Giovanni Scambia,3 Elisa Vigna,4 Francesca Grassi,5 Fabrizio Eusebi,5 Sergio Fucile,5 Maurizio Pesce,2 and Maurizio C. Capogrossi1

1Laboratorio di Patologia Vascolare, Istituto Dermopatico dell’ Immacolata, Istituto di Ricovero e Cura a Carattere Scientifico, Rome; 2Laboratorio di Biologia Vascolare e Terapia Genica, Centro Cardiologico Monzino, Istituto di Ricovero e Cura a Carattere Scientifico, Milan; 3Dipartimento di Ginecologia e Ostetricia, Università Cattolica del Sacro Cuore, Rome; 4Istituto per la Ricerca sul Cancro, Istituto di Ricovero e Cura a Carattere Scientifico Candidolo, Candidolo; 5Dipartimento di Fisiologia Umana e Farmacologia, Università di Roma La Sapienza, Rome; and 6Neurophysiology Doctorate Program, Università di Roma La Sapienza, Rome, Italy

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The objective of the present work was to establish whether the CD34+ cell fraction from the human umbilical cord blood (hUCB), which our laboratory (39) and others (25, 30, 45) have shown to contain a myoendothelial stem cell population, is capable of acquiring myocardial functional phenotype in coculture with cardiomyocytes.

MATERIALS AND METHODS

Isolation and culture of CD34+ cells. The collection of human cord blood was performed upon written consent by mothers and in compliance with the Helsinki declaration. hUCB was recovered in EDTA-containing bags immediately after delivery and processed for CD34+ cells magnetic cell sorting. After isolation, CD34+ cells were seeded in 96 wells (BD Biosciences, Falcon, San Jose, CA) and expanded for 2 to 3 days in a serum-free medium (Stem Span; Stem Cell Technologies, Vancouver, Canada) supplemented with (in ng/ml) 100 flt-3 ligand, 100 stem cell factor, 20 IL-3, and 20 IL-6 (all, Stem Cell Technologies, Miltenyi Biotec, Auburn, CA). 100 µM hydrazide was used in dye transfer experiments.

Plasmids, vector production, and CD34+ cells transduction. The third-generation packaging constructs pMD.G/pRRE and pRSVRev pMD.G-VSV.G, the VSVG envelope-encoding plasmid pMD.G, and the self-inactivating transfer vectors pCCL.SIN.cPPT.hPGK.EGFP. WPRE were used as the enhanced green fluorescent protein (EGFP)-labeling system. For further details about lentiviral vector preparation and CD34+ cell infection, see the supplemental material. CD34+/EGFP+ cells were cocultured for 7 days onto cardiomyocytes in MEM containing 10% FCS. After this period, cocultured cells were either analyzed in functional studies or processed for RNA extraction.

Isolation and culture of mouse neonatal cardiac myocytes. The health care of mice used for cardiac myocytes experiments was maintained according to National Institutes of Health guidelines. Neonatal cardiomyocytes were isolated from 1- to 2-day-old Swiss CD-1 as previously described (4). Ca2+ transient recordings. Free intracellular [Ca2+]i ([Ca2+]i) imaging recordings were obtained in cells loaded with the Ca2+ indicator X-rhod-1 AM.

Dye transfer experiments. The red fluorescent tracer Alexa Fluor 594 hydrazide was used in dye transfer experiments.

Real-time RT-PCR. Real-time RT-PCR with human- and mouse-specific primers was used to determine human cardiac genes expression.

For more information on fluorescent-activated cell sorting (FACS) analysis, patch-clamp recordings, Ca2+ transient recordings, dye transfer experiments, and real-time RT-PCR, see the supplementary material provided with the online version of this article.

Statistical analysis. Cell culture experiments and FACS analyses were performed in triplicate in at least three different experiments. Patch-clamp data were sampled and analyzed using pCLAMP 9.0 (Axon Instruments). Significance was calculated by Student’s t-test using Microsoft Excel software. A P of <0.05 was taken to indicate statistical significance. All results are given as means ± SE.

RESULTS

Choice of viral vector. In previous reports using coculture systems, stem cells have been labeled using dyes such as 1,1'-dioctadecyl-3,3',3',-tetramethylindocarbocyanine perchlorate (4, 26). The use of lipophilic dyes may cause, although infrequently, the loading of cells cocultured with stem cells, thereby raising doubts about the origin of the labeled differentiated cells in coculture. To avoid this problem, we genetically labeled hUCB CD34+ using a lentivirus carrying the EGFP expression cassette; the transduction efficiency was 70–80% as previously reported (1). To have the definitive proof that no cross infection occurred by lentivirus still present in the medium used for the final washing of the stem cells, we incubated cardiac myocytes with this medium, in the absence of CD34+ cells, and no EGFP+ cardiomyocytes were found after 1 wk (not shown). It is noteworthy that when this experiment was performed with a replication-deficient adenovirus vector carrying the EGFP expression cassette and CD34+ cells were infected with 500 multiplicity of infection, it was not possible to completely avoid viral vector contamination of the conditioned medium up to 2 days after infection and after three washings, as shown by the ability of the conditioned medium of these cells to infect other cells (data not shown). This artifact would have led to the presence of EGFP+ cardiomyocytes in coculture and to the erroneous conclusion that CD34+ transdifferentiation into functional myocardial cells had occurred. Since this problem can be an important cause of artifact, in the present work a lentiviral vector was used to ensure that all EGFP+ cells represented hUCB-derived CD34+ cells.

Morphology and passive electrophysiological properties of CD34+/EGFP+ cells cocultured onto mouse neonatal cardiac myocytes. During the first days in coculture, EGFP+ cells remained essentially round, but thereafter some of them firmly adhered to cardiac myocytes and acquired an elongated morphology, as previously described for mouse c-kit+ cells (27). At 1 wk of coculture, some EGFP+ cells exhibited contractions in synchrony with surrounding EGFP− cells. Contractions were never observed in EGFP− cells that did not come in firm contact with surrounding cardiac myocytes.

To assess the functional differentiation of EGFP+ cells, we first analyzed their passive electrical properties, specifically the membrane capacitance (Cm), which provides a measure of the cell surface, and the resting potential (Vr). In coculture, the Cm of the EGFP+ cells increased (Table 1). Based on the fitting of the capacitative transient with the sum of exponential functions (Fig. 1), we distinguished between EGFP+ cells that had a monoclonal capacitative transient (44/64) and that can be therefore considered electrically isolated cells (13) and EGFP+ cells that displayed a biexponential capacitative transient (20/64). These cells are possibly electrically coupled to the surrounding myocytes. As shown in Table 1, there was no statistical difference between the Cm of EGFP+ cells and cardiomyocytes.

Table 1. Passive membrane properties in CD34+ cells and cardiomyocytes

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Cm (1 Exponential), pF</th>
<th>Cm (2 Exponentials), pF</th>
<th>Vr, mV</th>
</tr>
</thead>
<tbody>
<tr>
<td>CD34+</td>
<td>22.7 ± 2.4 (12)*</td>
<td>-11 ± 2.5 (10)*</td>
<td></td>
</tr>
<tr>
<td>EGFP+ cocultured</td>
<td>40.2 ± 3.4 (44)*</td>
<td>170 ± 16 (20)†</td>
<td>-24.4 ± 2.3 (48)*</td>
</tr>
<tr>
<td>EGFP+ excitable</td>
<td>40.9 ± 8.8 (5)†</td>
<td>154 ± 17 (6)‡</td>
<td>-36.3 ± 6.1 (11)‡</td>
</tr>
<tr>
<td>Cardiomyocytes</td>
<td>75 ± 24 (17)*</td>
<td>136 ± 34 (12)‡</td>
<td>-44.5 ± 3.4 (32)‡</td>
</tr>
</tbody>
</table>

Values are means ± SE (number of cells). Cell capacitance (Cm) and membrane resting potential (Vr) were measured in CD34+ cells cultured in isolation (CD34+) and in 7-day-cocultured enhanced green fluorescent protein (EGFP+) cells or cardiomyocytes. Cells are grouped based on the fit of their capacitative transient with 1 or 2 exponential components. All CD34+ cells had a monoclonal transient. In coculture, both groups were present. The Cm and Vr values of the subset of EGFP+ cells showing inward voltage-gated currents (EGFP+ excitable) are given separately. These values are also included in the overall mean (EGFP+ cocultured). All 1 exponential Cm values were statistically different from 2 exponentials Cm values. *Values statistically different from all the other values (P < 0.03); †Cm values different from CD34+ and cardiomyocytes (P < 0.03); ‡values not statistically different (P > 0.2).
myocytes with a biexponential decay of the capacitative transient, suggesting an electric coupling between cardiomyocytes and differentiated CD34+/H11001-derived cells.

The analysis of $V_r$ showed a hyperpolarization of cocultured stem cells (CD34+/H11001 coculture) compared with CD34+ cells cultured in isolation for 4 days (CD34+; Table 1). In particular, the subsets of CD34+ cells that displayed transient inward currents and/or spontaneous action potentials (APs; CD34+ excitable) had a mean $V_r$ not statistically different from that of cardiomyocytes (Table 1).

In CD34+ cells, the coculture condition was essential to induce a voltage-gated current profile recapitulating that was observed during cardiac differentiation. Freshly isolated CD34+ cells did not display any voltage-evoked current (data not shown), whereas some cocultured EGFP+ cells acquired voltage-dependent activity. In these cells, depolarizing steps evoked either no response (42/62; data not shown), only outward currents (13/62; 21%), or inward and outward currents (7/62; 11%). The outward current had a mean amplitude of 0.45 ± 0.16 nA ($n = 20$; Fig. 2A, top traces) at +50 mV.
similar to the value measured in EGFP− cells (0.62 ± 0.12 nA, n = 12; Fig. 2A, bottom traces). The small amplitude of delayed rectifier K+ currents in neonatal cardiomyocytes has already been reported (35). The inward currents had the typical kinetics and voltage dependence of Na+ currents (Fig. 2, A and B). When present, these currents were so large that we applied the stimulation protocol from a holding potential of −60 mV, to improve the voltage- and space clamp, as described (35). The maximal inward current had a mean peak amplitude of −1.19 ± 0.22 nA (n = 7; Fig. 2A, top traces), comparable with that measured in EGFP− cells (−0.74 ± 0.12 nA, n = 8; Fig. 2A, bottom traces). The current-voltage relationship peaked in both cell types at −10 or 0 mV (Fig. 2B). Interestingly, two of the EGFP+ cells (2/62; 3%) exhibiting inward and outward currents had a monoexponential capacitative transient, suggesting that in these two cells there was a specific expression of cardiac channels. It is noteworthy that although these two cells were electrically isolated from surrounding cells, their physical connection, for instance through nanotubes, cannot be excluded.

Some differentiated EGFP+ cells contracted spontaneously. From four of these cells, we were able to record APs. When compared with cardiomyocytes, the APs recorded in EGFP+ cells had a lower frequency, a smaller amplitude, a slower rise time, and a longer duration, suggesting that EGFP+ -contracting cells are more immature than cardiomyocytes (Table 2). In contrast, the firing threshold was similar in EGFP+ cells and cardiomyocytes, in good agreement with the observed similarity of the voltage dependence of inward Na+ currents. When cocultures were superfused with Ca2+-free extracellular solution, APs reversibly disappeared (Fig. 2C).

[Ca2+]-dependent mechanism in beating EGFP+ cells. To further assess the functional differentiation of EGFP+ cells, we performed [Ca2+] imaging experiments by labeling cells with X-rhod-1 AM, a cell permanent Ca2+-sensitive dye, the red fluorescence intensity of which increases upon binding Ca2+. We observed X-rhod-1 fluorescence intensity oscillations in EGFP+ cells (Fig. 3 and supplemental video 1). It is important to underline that we only analyzed mononucleated EGFP+ cells, identified a priori by Hoechst 33342 nuclear staining, to exclude cytoplasmic cell fusion (Fig. 3A). When EGFP+ and EGFP− cells were in close proximity, changes in [Ca2+]i concentration were coincident in both cell types, suggesting a coupling between EGFP+ cells and cardiomyocytes. Supplemental video 1 shows that Ca2+ release was accompanied by the contraction of both EGFP+ and EGFP− cells, suggesting the presence of a mature contractile apparatus. To investigate the contribution of intracellular Ca2+ stores, we used ryanodine (1 μM), a drug that binds the ryanodine receptors expressed on the sarcoplasmic reticulum of cardiac myocytes and induces Ca2+ release from this store (14). Under these conditions, [Ca2+]i transients were impaired or abolished both in EGFP+ and in EGFP− cells (Fig. 4A). After 120 s of exposure, ryanodine was washed out, and its effect on [Ca2+]i oscillations was slowly reversed (Fig. 4A). Extracellular Ca2+ also contributed to [Ca2+]i transients, which were abolished when EGFP+ or EGFP− cells were superfused with Ca2+-free extracellular solution. In this case, the oscillations were fully restored as soon as the extracellular Ca2+ was again added to the medium (Fig. 4, B and C).

The synchronicity of Ca2+ transients in EGFP+ cells and the surrounding EGFP− cells suggested the existence of cell-to-cell communications, probably mediated by gap junctions. To demonstrate the presence of such junctions, we diazylized through the patch-pipette EGFP+ cells with the fluorescent tracer Alexa Fluor 594, a red fluorescent dye that diffuses through gap junctions (n = 4). As shown in Fig. 5A, dye injection into a morphologically differentiated EGFP+ cell resulted in a dye diffusion to a neighboring EGFP− cell (starting from frame at time 4 min). In contrast, no dye spread was observed following the injection into a round EGFP+ cell (n = 3; Fig. 5B). These results suggest that EGFP+ cells developed gap junctions, whereas rounded EGFP− cells retained a more undifferentiated phenotype.

Human cardiac gene expression in cocultured CD34+ cells. Several reports have called caution about whether the acquisition of cardiac markers or even physiological features typical of cardiac myocytes in adult stem cells is the consequence of transdifferentiation rather than fusion events. To investigate this issue, we analyzed the expression of the early cardiac genes hNkx2.5 and hGATA-4, before and after coculture onto neonatal mouse cardiomyocytes by real-time RT-PCR (17), using primers designed to span two exons and unable to amplify unspecific genomic signals (28) (supplementary Fig. 1). PGK1 constitutively expressed gene was used as control for the presence of mRNA in the analyzed sample. The specificity of these assays was confirmed by cross-species amplification (mouse primers tested on human RNA and vice versa). As an additional control for the expression of cardiac genes, the same CD34+ cells used in coculture experiments were maintained alone in expansion medium or in the same medium used in the coculture experiments.

Although the expression of human and mouse genes was correctly detected but not cross detected in human and mouse tissues, we did not notice a de novo induction of human cardiac-specific genes (n = 4). In fact, as shown in Table 3, in one out of four and two out of four experiments, we detected the expression of hNkX2.5 and hGATA-4, respectively, in freshly isolated CD34+ cells. In addition, the expression of human cardiac genes was not enhanced when coculturing CD34+ cells onto cardiac myocytes, but at least in the case of hGATA-4, it was extinguished over time.

Table 2. Action potential characteristics in EGFP+ cells and cardiomyocytes

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>Frequency, Hz</th>
<th>Threshold, mV</th>
<th>Spike Amplitude, mV</th>
<th>Rise Slope, mV/ms</th>
<th>APD50, ms</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cardiomyocytes</td>
<td>16±0.4</td>
<td>19.7±0.4</td>
<td>102.8±0.5</td>
<td>38.6±1.0</td>
<td>57.1±1.3</td>
</tr>
<tr>
<td>EGFP+ cells</td>
<td>0.6±0.2</td>
<td>18.7±0.5</td>
<td>87.2±2.2</td>
<td>12.1±1.2</td>
<td>110.3±3.8</td>
</tr>
</tbody>
</table>

Values are means ± SE; n = 4 cells for each cell type. For the action potentials, we measured the frequency of occurrence, the mean depolarization to threshold, the total spike amplitude, the maximal slope of the rising phase, and the time the spike exceeded half-amplitude action potential duration (APD50). All the values were statistically different between cardiomyocytes and EGFP+ cells, except for the threshold values (P > 0.1).
Taken together, these data suggest that early cardiac markers were occasionally expressed in CD34<sup>+</sup>/H11001 cells before and after growth alone in the medium used for coculture and that cell contact with cardiac myocytes did not promote the expression of human cardiac genes. Alternatively, it is possible that CD34<sup>+</sup>/H11001 cell transdifferentiation into myocardial cells may have affected just a few cells and result below the detection ability of the RT-PCR analysis.

**DISCUSSION**

This work reports that some CD34<sup>+</sup> stem cells from the hUCB, upon coculture with neonatal mouse cardiac myocytes, form gap junctions with the cardiac cells and exhibit electrophysiological properties of myocardial cells as well as spontaneous [Ca<sup>2+</sup>]<sub>i</sub> oscillations from the sarcoplasmic reticulum. It is noteworthy that the issue of HSC differentiation into myo-
cardiac cells in vitro is still highly controversial. The vast majority of the in vitro studies have been performed on HSCs cocultured with cardiac cells, i.e., under experimental conditions that make cell fusion possible; however, these studies either failed to exclude cell fusion (16, 26) or did not address this issue (15, 42). Moreover, most studies examined only whether HSCs acquire myocardial cell markers as evidenced by the expression of myocardium-restricted transcription factor mRNA or proteins (7, 15, 40, 49). To our knowledge, only two studies examined the functional properties as well as gene and/or myocardial protein expression of HSCs that supposedly had transdifferentiated into myocardial cells; both studies were carried out with HSCs that had been cocultured with cardiomyocytes (4, 42), and neither study excluded cell fusion. In contrast, a prior work from our laboratory showed that BM-derived murine c-kit+ cells cocultured with mouse neonatal cardiac myocytes did not develop gap junctions and exhibited neither [Ca2+]i nor APs (27). Another recent study showed that human peripheral blood CD34+ cells and HL-1 mouse cardiomyocytes form hybrid cells through c-kit+β1 integrin and vascular cell adhesion molecule-1 interaction (50). Thus, except for embryonic stem cells (24), murine spermatogonial (18) and cardiac-derived (33) stem cells, which have been found to differentiate in a cell-autonomous way into myocardial lineages, i.e., in the absence of coculture with cardiomyocytes and drugs administration, all in vitro studies have failed to convincingly exclude fusion or other potential artifacts as mechanisms for their results and have not demonstrated that BM-derived stem cells, i.e., HSCs and EPCs, can both express cardiac antigens and also acquire the functional properties of cardiac cells. In the present study, it was examined whether human CD34+ cells from hUCB, which our laboratory (39) and others (25, 45) have found to contain a stem cell fraction and others (25, 45) have found to contain a stem cell fraction, were able to give rise to skeletal muscle and endothelial lineages. cocultured onto cardiac myocytes do acquire electrophysiological features of myocardial cells. Under our experimental conditions, cocultured CD34+ cells were able to integrate in the cardiomyocyte layer and appeared to start a cardiac differentiation program, as suggested by resting potential hyperpolarization, the presence of spontaneous APs, and [Ca2+]i, intracellular transients. However, comparing CD34+/EGFP+ cells and cardiomyocytes parameters, as AP frequency, we also found several differences suggesting a functional immaturity of the EGFP+ cells.

Table 3. Human cardiac gene expression in cocultured CD34+ cells

<table>
<thead>
<tr>
<th>Sample</th>
<th>m-Pgk-1</th>
<th>m-Nkx2.5</th>
<th>m-GATA4</th>
<th>h-Pgk-1</th>
<th>h-Nkx2.5</th>
<th>h-GATA4</th>
</tr>
</thead>
<tbody>
<tr>
<td>Mouse cardiomyocytes</td>
<td>+ (2/2)</td>
<td>+ (2/2)</td>
<td>+ (3/3)</td>
<td>- (2/2)</td>
<td>- (2/2)</td>
<td>- (2/2)</td>
</tr>
<tr>
<td>Human heart</td>
<td>- (2/2)</td>
<td>- (2/2)</td>
<td>- (2/2)</td>
<td>+ (2/2)</td>
<td>- (2/2)</td>
<td>- (2/2)</td>
</tr>
<tr>
<td>T0</td>
<td>+ (4/4)</td>
<td>- (3/4)</td>
<td>+ (1/4)</td>
<td>- (2/4)</td>
<td>+ (2/4)</td>
<td>- (3/4)</td>
</tr>
<tr>
<td>T5</td>
<td>+ (4/4)</td>
<td>- (3/4)</td>
<td>+ (1/4)</td>
<td>- (3/4)</td>
<td>+ (1/4)</td>
<td>- (4/4)</td>
</tr>
<tr>
<td>T12</td>
<td>+ (4/4)</td>
<td>- (3/4)</td>
<td>+ (1/4)</td>
<td>- (3/4)</td>
<td>+ (1/4)</td>
<td>- (4/4)</td>
</tr>
<tr>
<td>T12 MEM</td>
<td>+ (4/4)</td>
<td>- (3/4)</td>
<td>+ (1/4)</td>
<td>- (3/4)</td>
<td>+ (1/4)</td>
<td>- (4/4)</td>
</tr>
</tbody>
</table>

Expression analysis of the early cardiac genes Nkx2.5 and GATA-4 was determined on human CD34+ cells before and after their coculture onto neonatal mouse cardiomyocytes by real-time RT-PCR (more details in Materials and Methods). T0, freshly isolated CD34+ cells; T5, CD34+ cultured for 5 days in expansion medium; T12, CD34+ cells cultured for 12 days in expansion medium; T12 MEM, CD34+ cells cultured for 12 days in DMEM. The first 2 lines display the absence of cross-reaction between mouse primers tested on human RNA and vice versa. In 2 out of 4 independent experiments, GATA-4 was detected (+) in T0. Cells lost the expression (−) of this early cardiac marker during the in vitro expansion. DMEM containing 10% FBS, the same medium used in coculture experiments, caused a slow proliferation of CD34+ cells (data not shown) and favored GATA-4 expression retention, as observed in 1 out of 4 cases at T12 MEM. In contrast, the expression of Nkx2.5 was already detected at T0 only in 1 out of 4 cases and maintained over time as demonstrated by the presence of a detectable signal at T5, T12, and T12 MEM. Although mouse genes and human PGK1 were always present, human cardiac markers were never detectable in cocultured cells (4 out of 4 experiments), suggesting that contact between CD34+ and cardiomyocytes was not sufficient to enhance the expression of human cardiac genes.
Our findings suggest that cell adhesion is necessary for a full differentiation of EGFP+ cells, since only cells firmly associated to cardiac myocytes formed gap junctions and became contractile (Fig. 5). This hypothesis is in line with previous observations that even in the presence of molecules inducing cardiac differentiation, such as Wnt-11, physical contact with cardiac myocytes is required to trigger EPC cardiac differentiation (26). Actually, we observed inward currents in two electrically isolated cells. However, these cells were morphologically differentiated, and we cannot exclude that they were connected to surrounding cardiomyocytes by tiny cytoplasmic contacts or nanotubes.

Data reported in Table 3 show that using mutually exclusive human and mouse primers in real-time RT-PCR experiments, we never found a substantial upregulation of human genes in coculture. This suggests that although EGFP+ cells do acquire the ability to contract simultaneously with surrounding neonatal myocytes, which are connected by functional gap junctions to them and respond to similar bathing [Ca2+] changes and pharmacological stimulations, the acquisition of these features may be the result of cell fusion. However, since it was not possible to examine both cardiac gene expression and electrophysiological functional properties in the same cells, we cannot rule out the possibility that CD34+ transdifferentiation associated with cardiac gene expression may be an infrequent event below the detection limit of our RT-PCR analysis. Some EGFP+ cells exhibited spontaneous [Ca2+]i oscillations, due to Ca2+ release from the sarcoplasmic reticulum. In mature cardiomyocytes, the increase in cytosolic Ca2+ causes a depolarization of the membrane potential due to the activation of the electrogenic Na+/Ca2+ exchange, which can reach a threshold for the opening of fast Na+ channels and trigger an AP (8). Indeed, spontaneous sarcoplasmic reticulum Ca2+ oscillations have been shown to be a key mechanism both for the normal pacemaker activity of the cardiac conduction tissue (6, 29) and for the development of different types of cardiac arrhythmias (9, 19). Therefore, their presence in HSCs cocultured with the myocardial cell may both offer an opportunity for the development of a biological pacemaker and pose a threat for the potential risk associated with spontaneously depolarizing tissue in the heart. However, neither the numerous animal nor the clinical studies in which HSCs have been injected in the heart have evidenced an increase in rhythm disturbances, possibly because of the integration of HSCs with the surrounding myocytes through gap junctions. This is in contrast to the marked increase in ventricular arrhythmias observed in patients treated with the intramyocardial injection of autologous skeletal muscle cells (32, 43), which in fact lack gap junctional coupling to the host heart cells.

In conclusion, it is important to discuss some technical limitations of the present work. All functional studies were performed with cells that were both EGFP+ and mononucleated; this would suggest that CD34+ cell transdifferentiation into cardiomyocytes had occurred. In keeping with this conclusion, the functional properties of some of these cells differed both from those of hUCB CD34+ cells, which were not cocultured with heart cells and were similar albeit not identical, to those of cultured cardiomyocytes. However, it cannot be excluded that EGFP transfer via nanotubes (26) or nuclear fusion (50) had led to the formation of EGFP+ contractile cells. Furthermore, RT-PCR analysis showed no expression of human-specific cardiac genes upon coculture. This result argues against CD34+ cell transdifferentiation into cardiomyocytes, but it is possible that transdifferentiation may have been limited to only a limited number of cells, below the RT-PCR sensitivity of human cardiac gene expression. Conclusive proof of transdifferentiation would have required fluorescence in situ hybridization as well as other analyses of the same cell, which exhibited myocardial functional properties; this is a very complex technical task that could not be successfully accomplished in this work. Because of these limitations, it cannot be conclusively established whether cell fusion or transdifferentiation had occurred. Nevertheless, the results suggest that hUCB CD34+ cells cocultured with neonatal mouse cardiomyocytes may acquire functional properties similar to those of myocardial cells. Studies using double-color labeling of cells by lentiviral gene transfer and high-throughput FACS are an option to further address the issue of fusion.

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