Niche-Dependent Translineage Commitment of Endothelial Progenitor Cells, Not Cell Fusion in General, Into Myocardial Lineage Cells

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Objective—Previous studies from our laboratory have shown therapeutic potential of ex vivo expanded endothelial progenitor cells (EPCs) for myocardial ischemia. Our purpose was to investigate the mechanisms regulating EPC contribution to myocardial regeneration.

Methods and Results—To evaluate niche-dependent expression profiles of EPCs in vitro, we performed coculture using cultured EPCs derived from human peripheral blood and rat cardiac myoblast cell line (H9C2). Reverse-transcription polymerase chain reaction (PCR) disclosed the expression of human-specific cardiac markers as well as human-specific smooth muscle markers. Cytoimmunochemistry presented several cocultured cells stained with human specific cardiac antibody. To prove this translineage differentiation in vivo, human cultured EPCs were injected into nude rat myocardial infarction model. Reverse-transcription PCR as well as immunohistochemistry of rat myocardial samples demonstrated the expression of human specific cardiac, vascular smooth muscle, and endothelial markers. We observed the distribution of colors (Qtracker; Quantum Dot Corp) in coculture to detect the fused cells, and the frequency of cell fusion was $<1\%$.

Conclusions—EPCs can contribute to not only vasculogenesis but also myogenesis in the ischemic myocardium in vivo. Transdifferentiation, not cell fusion, is dominant for EPCs commitment to myocardial lineage cells. Ex vivo expanded EPCs transplantation might have enhanced therapeutic potential for myocardial regeneration. **(*Arterioscler Thromb Vasc Biol*. 2005;25:1388-1394.)**

Key Words: cardiovascular diseases ■ endothelial ■ myocardium ■ regeneration ■ stem cells ■ vasculogenesis

Somatic stem and progenitor cells have recently demonstrated the flexibility in lineage commitment for tissue regeneration. Although bone marrow cells presented multiple lineage potential, hematopoietic stem cell demonstrated translineage commitment into other lineage cells, such as vascular cell,[1,2] neural cell,[3,4] hepatic cell,[5,6], and mesenchymal cell lineages.[2] Neural stem cell has also shown the adaptability for another lineages.[7,8] These were followed by reports that differentiated endothelial cells, either freshly isolated from mouse dorsal aorta at embryonic day 9 or established as homogenous cells in culture, differentiate into cardiomyocytes, and express cardiac markers when cocultured with neonatal rat cardiomyocytes or when injected into postischemic adult mouse heart. They also demonstrated that human umbilical vein endothelial cells also differentiate into cardiomyocytes.[9]

Bone marrow-derived endothelial progenitor cells (EPCs)[10,11] have shown the regenerative potential in myocardial ischemic animal model[1,12] via ex vivo expansion and incorporation into foci of neovascularization. The study from our laboratory[1] has demonstrated ex vivo expanded EPCs transplantation into ischemic hearts resulted in enhanced myocardial neovasculalization, as well as improved cardiac function (such as reduction in left ventricular dilatation). Histological findings supported that there occurred not only vascular regeneration but also myocardial regeneration, contributing to favorable effects of EPCs on cardiac function. Given these results, we believe EPC, which is considered the cell source for vascular regeneration, might reveal favorable potential in heart tissue regeneration, such as cardiomyocyte and vascular smooth muscle cell lineages.

Recently, Badorff et al have reported transdifferentiation of EPC into cardiomyocytes.[13] The results encourage the possibility of EPC translineage commitment into cardiomyocyte for the treatment of myocardial ischemic disease. However, they lack the in vivo evidence of this translineage commitment for organogenesis by EPC transplantation to ischemic disease patients. Furthermore, we consider the necessity of

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pursuing not only myocardial but smooth muscle lineage commitment, which is required for the stabilization of newly formed vasculatures by EPCs themselves. Very recently, Yeh et al have reported transdifferentiation of $CD34^+$-enriched cell into cardiomyocyte and smooth muscle cell in vivo.[14] Their results have shown transdifferentiation of human peripheral blood $CD34^+$ cell into cardiomyocyte was enhanced in the injured heart compared with in the heart without injury, although they did not indicate any functional significance of transdifferentiation.

In this regard, to evaluate niche-dependent expression profiles of EPCs in vitro, we performed coculture of EPCs derived from human peripheral blood and rat cardiac myoblast cell line (H9C2). We also evaluated the frequency of cell fusion phenomenon in the coculture system. Furthermore, to prove equivalent translineage commitment in vivo, human cultured EPCs were transplanted into nude rat myocardial infarction model to sample for transcriptional and expressional evidences.

Methods

Coculture With EPC and H9C2 Cell Line

Total peripheral blood mononuclear cells were isolated from human volunteers by density gradient centrifugation. All procedures were in accordance with the institutional committee. After 4 days in culture, nonadherent cells were removed by washing with phosphate-buffered saline (PBS), new media was applied, and the culture was maintained through day 7 or later. In the culture of EPC after day 7, reseeding was performed once per week.[15] Rat cardiac myoblast cell line (H9C2) was cultivated in DMEM with 10% fetal bovine serum and 5% horse serum. EPC was detached at day 7 and re-seeded onto semi-confluent H9C2 monolayer. Coculture was maintained in the feeding medium of H9C2 for 7 days with 1-time application of new media and sampled for reverse-transcription polymerase chain reaction (RT-PCR) or cytoimmunochemistry. All cells were incubated under normoxia (pO_2, 152 mm Hg) condition.

Sorting of Cultured EPCs and Coculture Subpopulation of EPCs and H9C2

We sorted cultured EPCs to determine which subpopulation of EPCs mainly contributed to cardiac lineage commitment. Briefly, we sorted day 7 cultured EPCs by CD31antibody (BD Pharmingen, San Jose, Calif) using BD FACSAria Cell-Sorting System (BD Biosciences, San Jose, Calif), or CD34 (BD Pharmingen) antibody using autoMACS (Miltenyi Biotec, Bergisch Gladbach, Germany) and obtained positive and negative fractions, respectively. Then we performed coculture with each fraction and H9C2 on 4-chamber glass wells. Cardiac lineage commitment was evaluated by cytoimmunochemistry 7 days after coculture.

RT-PCR

Cocultured cells were lysed in RNA lysis buffer (Ambion, Austin, Tex). However, EPC or PBS-injected myocardial samples were homogenized in RNA lysis buffer. RNA was extracted using RNA extraction kit (Ambion). DNAase digestion was performed after RNA extraction. The RT-PCR was performed by a system according to the manufacture (Clontech, Palo Alto, Calif). Briefly, each primer was amplified for 35 cycles. In every case, each cycle consisted of 95°C for 30 seconds, followed by 65°C for 3 minutes. The primers for RT-PCR were designed as shown in the Table. These primers other than GAPDH and mGAPDH were designed to identify human specific expression of each target.

Cytoimmunochemistry

Day 7 coculture (human EPCs plus H9C2) on a 4-chamber slide was fixed with ice-cold 100% methanol for 7 minutes and washed with PBS 3 times. Cytoimmunochemistry was performed using cardiac antibodies, α/β-ventricular myosin heavy chain (Chemicon, Temecula, Calif), brain natriuretic protein (kindly provided from Dr Itoh, Kyoto University, Japan), cTn-I (Chemicon), smooth muscle lineage antibody, α-SMA (clone 1A4) (Sigma, Saint Louis, Mo), endothelial lineage antibody, CD31 (DAKO, Carpinteria, Calif), and human leukocyte antigen (HLA)-ABC (BD Biosciences Pharmingen) for detecting human cells. Antibodies except for cTn-I and α-SMA are reactive only for humans. We used human-specific α/β-ventricular MHC antibody to evaluate cardiac lineage commitment in coculture with sorted subpopulation of EPCs and H9C2. Proportion of cardiac lineage commitment was evaluated by counting α/β-ventricular MHC-positive cells per total seeded sorting cells in each chamber slide.

Rat Myocardial Infarction Model

Athymic nude rats (Harlan, Indianapolis, Ind) aged 7 weeks and weighing 135 to 140 grams were anesthetized with ketamine and xylazine intraperitoneally. After operatively induced myocardial ischemia,[1] the arrhythmic nude rats each received systemic (1×10^6) or intramuscular injection of 2.5×10^5 culture-expanded human EPCs in 2 sites of myocardial ischemic lesions; 2.5×10^5 EPCs were suspended in 25 μL of PBS, and only 25 μL of PBS was injected in control group. We performed EPC transplantation in 10 rats (5 for systemic injection, 5 for intramuscular injection), and injected PBS in 5 rats (control). Three weeks after operation and injection, these rats were euthanized and myocardial samples were put into OCT compound (Sakura, Torrance, Calif) for frozen tissue section (immunohistochemistry) or directly frozen in liquid nitrogen for RNA extraction (RT-PCR).

Immunohistochemistry

Frozen slides were prepared by Criostat (Microm, HM505E; Walldorf, Germany) and stained with cardiac antibodies (α/β-ventricular MHC) (Biocytex, Marseille, France), BNP (kindly provided by Dr Itoh, Kyoto University, Japan), cTn-I (Biomeda, Foster City, Calif), or smooth muscle lineage antibody, Calponin (DAKO), or endothelial lineage antibody, CD31(DAKO). Both α/β-ventricular MHC and cTn-I are different antibodies used in cytoimmunochemistry. Connexin43 (BD Biosciences Pharmingen) was used for experiment of gap junction and double-stained with HLA-ABC (BD Biosciences Pharmingen) for detecting human cells. Antibodies except for connexin 43 were active only for humans. We used DAB system (brown) for single antibody staining for visualizing the signals. Double staining was performed using DAB system for HLA antibody and VIP system (purple) for connexin 43 antibody.

Evaluation of Frequency of Cell Fusion in Coculture System

We performed coculture using Qtracker (Quantum Dot Corp, Hayward, Calif), which is a nanocrystal labeling marker incorporated in the vesicles of the cytoplasm. EPCs were labeled with the Qtracker 565 and H9C2 cells were labeled with Qtracker 655. We observed the distribution of colors to detect the fused cells by fluorescent microscopy (Olympus IX71; Tokyo, Japan). When the cells fuse, the fused cell has both colors. Because the nanocrystals are larger than organic dyes, they are not transferred between cells, so each cell type would maintain the single color until they fuse. EPC fusion ratio was detected by counting the number of fused cells that indicated yellow in cytoplasmic area out of the number of total labeled EPCs in 10 different high-power fields (×200). The data were shown as the mean±SD.

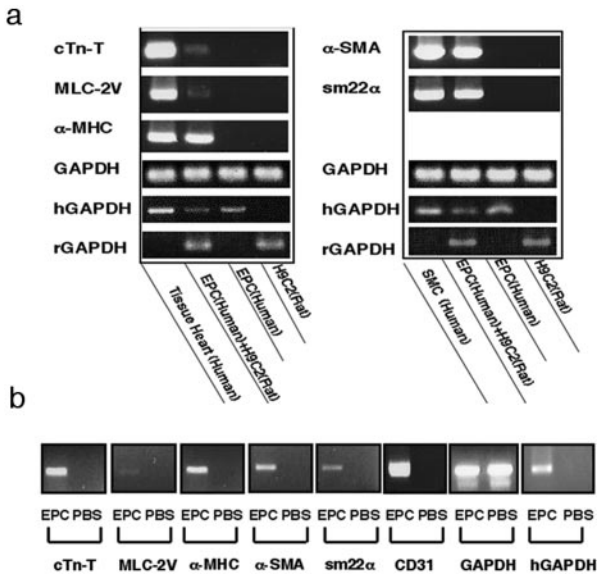

Figure 1. Expression of human-specific cardiac and smooth muscle markers in coculture system and in EPC-injected rat heart by RT-PCR. RNA samples from various culture conditions were analyzed by RT-PCR. cTn-T, MLC-2V, and α-MHC are specific markers for cardiomyocyte, and α-SMA and sm22α are specific markers for smooth muscle cells. Left lane of each column shows positive control, and right lane of each column shows negative control (only rat-derived H9C2). Human EPC did not express both cardiac and smooth muscle cell markers. After coculture with human EPC and rat H9C2, both cardiac and smooth muscle markers were observed (a). RNA samples were obtained from ischemic nude rat heart injected with human EPC or PBS. RT-PCR was performed using the same condition as used in coculture samples. Left lane of each column shows the data from EPC injected heart sample. Right lane of each column shows the data from PBS-injected heart sample (b). GAPDH served as internal standard. GAPDH recognizes both human and rat, and hGAPDH only recognized human.

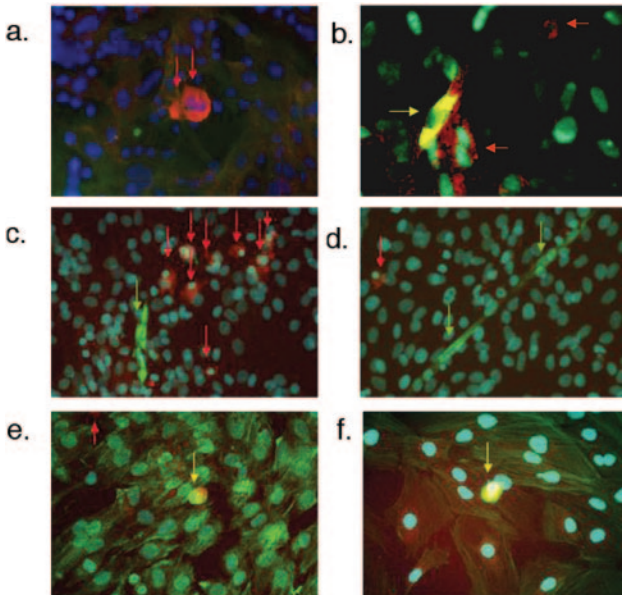

Figure 2. Expression of cardiac smooth muscle and endothelial lineage markers in culture system by cytoimmunochemistry. After fixation of coculture cells (human EPC and rat H9C2), these cells were stained with human-specific cardiac antibody (α/β-ventricular MHC) and α-SMA antibody. a, Positive cells for cardiac marker (red; arrow). b, Positive cells for both cardiac and smooth muscle markers (yellow; arrow) and the only positive cell for cardiac marker (red; arrow). c and d, Staining with human-specific cardiac antibody (α/β-ventricular MHC) (green; arrow) and human-specific endothelial lineage antibody (CD31) (red: arrow). Coculture cells were stained with both cardiac antibody (cTn-I) (green) and human cell antibody (HLA-ABC) (red; arrow), or smooth muscle lineage marker (green) and human cell antibody (HLA-ABC). Double-stained cell shows cardiac marker-positive cell derived from human cell (yellow; arrow) (e) and smooth muscle lineage marker-positive cell derived from human cell (yellow; arrow) (f). Blue shows DAPI.

Results

Coculture of Human EPCs and Rat Cardiac Myoblasts (H9C2) Expressed Human-Specific Cardiac and Smooth Muscle Markers

We performed RT-PCR to evaluate the human specificity of the primers. Cardiac-specific markers such as cTn-T, MLC-2V, and α-MHC were expressed in human heart RNA (Clontech) but not expressed in RNA from H9C2 and human EPCs. Smooth muscle markers such as sm22α and α-SMA were expressed in RNA from human smooth muscle cells, but not expressed in RNA from rat smooth muscle cells and human EPCs. Endothelial marker such as CD31 was expressed in RNA from human umbilical vein endothelial cells and human EPCs, but not expressed in RNA from rat endothelial cells (data not shown). Also, these primers except for CD31 were not expressed in RNA from human EPCs alone. RT-PCR was performed using these primers 7 days after initiating coculture of human EPCs and H9C2. RT-PCR from coculture samples disclosed the expression of cardiac markers (cTn-T, MLC-2V, α-MHC) and smooth muscle markers (sm22α, α-SMA) (Figure 1a). These data suggested that coculture condition induced human EPCs to express cardiac and smooth muscle lineage-specific genes. We designed 3 types of GAPDH primers, human-specific, mouse-/rat-specific, and both human and rat cross-reactive, to standardize the amount of DNA in each lane.

Cocultured Cells Stained With Cardiac and Smooth Muscle Antibody

Cytoimmunochemistry was performed after fixation of cocultured cells. Human-specific cardiac antibody (α/β-ventricular MHC) stained human EPCs 7 days after initiating coculture with H9C2. The morphology of α/β-ventricular MHC-positive cells was round or spindle, and the frequency was $\approx$0.1% (Figure 2a). Several cocultured EPCs double-stained with both human-specific cardiac antibody (α/β-ventricular MHC) and α-SMA antibodies were observed (Figure 2b). The morphology of these double-stained cells was spindle and the frequency was $<$0.08%. We observed positive cells for human cardiac antibody besides human endothelial lineage-positive cells (Figure 2c and 2d). Based on the identification of human-derived cells by HLA antibody, Tn-I and HLA double-stained cardiac lineage-positive cells derived from human cells (Figure 2e), and α-SMA and HLA double-stained human-derived smooth muscle lineage cell (Figure 2f). These in vitro data suggested that coculture condition induced human EPCs to express both cardiac and smooth muscle lineage-specific proteins. Furthermore, we

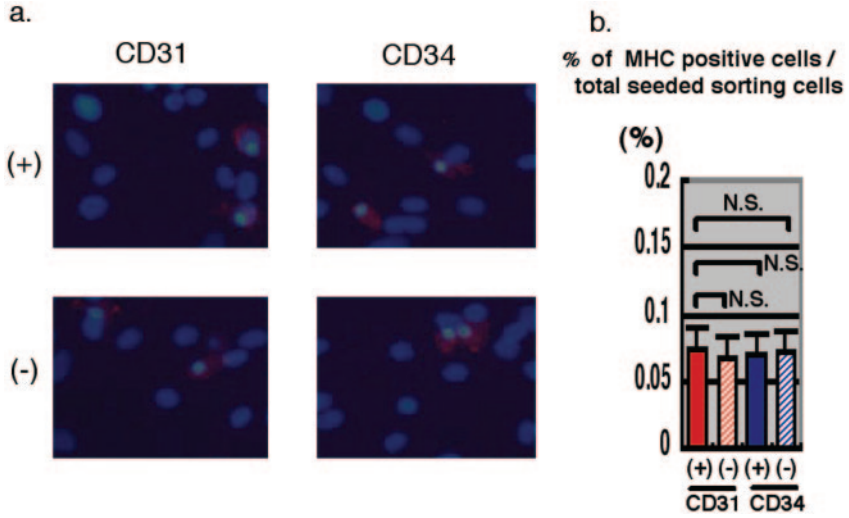

Figure 3. Evaluation of cardiac lineage differentiation frequency in coculture with H9C2 and CD31 or CD34 sorted cells from ex vivo expanded endothelial progenitor cells. After fixation of coculture cells (rat H9C2 with CD31-positive cells, CD31-negative cells, CD34-positive cells, and CD34-negative cells, respectively) (a), these cells were stained with human-specific cardiac antibody (α/β-ventricular MHC). The positive cells (red) were counted and the proportion was evaluated as percent MHC-positive cells/total seeded sorting cells (b). Blue shows DAPI.

designed coculture with subpopulation of cultured EPCs and H9C2 to define the endothelial marker subpopulation that will mainly contribute to translineage commitment. We sorted CD31 positive fraction and negative fraction, or CD34 positive fraction and negative fraction, and then cocultured with H9C2 in each fraction (Figure 3a). Cytoimmunochemistry disclosed the frequency of cardiac lineage commitment in each sorting fraction, and no difference was observed among the coculture for cardiac lineage commitment in coculture with both CD31 and CD34 fractioning (Figure 3b).

RT-PCR of EPC-Injected Myocardial Samples Demonstrated the Expression of Human-Specific Cardiac and Vascular Smooth Muscle Markers

RT-PCR using human EPC-injected rat myocardial samples disclosed the expression of cardiac (cTn-T, MLC-2V, α-MHC) and smooth muscle-specific (sm22α, α-SMA) genes. However, RT-PCR using PBS-injected rat myocardial samples did not express any cardiac and smooth muscle genes (Figure 1b). The data confirmed RT-PCR using coculture samples in vitro. We designed human-specific GAPDH to standardize the DNA amount derived from transplanted human cells.

Immunohistochemistry of EPC-Injected Myocardial Samples Demonstrated the Expression of Cardiac and Vascular Smooth Muscle Markers

Frozen sections of EPC-injected myocardial samples were stained with human-specific cardiac antibodies (α/β-ventricular MHC, cTn-I, BNP) (Figure 4a-1, 4b-1, 4c-1, respectively), human-specific smooth muscle cell antibody (Calponin) (Figure 4d-1), and human-specific endothelial marker (CD31) (Figure 4e-1). For gap junction experiment, connexin 43 and HLA antibodies were used for double staining because connexin 43 antibody had cross-reactivity with human and rat. In PBS-injected rat myocardium, connexin 43 stained gap

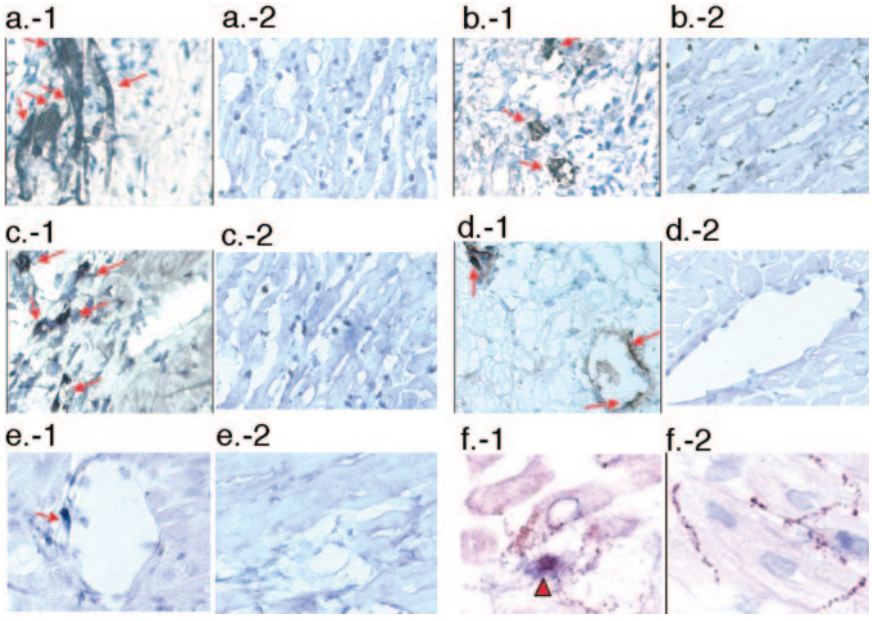

Figure 4. Expression of human-specific cardiac and smooth muscle markers in EPC-injected rat heart by immunohistochemistry. After fixation of ischemic nude rat heart injected with human EPCs or PBS, samples were stained with human-specific cardiac antibodies (a, α/β-ventricular MHC; b, cTn-I; c, BNP), human-specific smooth muscle antibody (d, calponin), and human-specific endothelial marker (e, CD31). For the experiment of gap junction, tissue sample was stained with connexin 43 in rat myocardium (f-2: connexin 43) or double-stained with connexin 43 and HLA antibodies in EPC-transplanted rat myocardium (f-1, connexin 43 and HLA, arrowhead). Left photo of each group shows rat heart with human EPCs, and right shows rat heart with PBS. The right photo of each group shows negative control for each human-specific antibody (Figure 4a-1 through 4e-1). DAB staining system was used for HLA and VIP staining system was used for connexin 43. All other immunohistochemical stainings (each staining by single antibody) were performed using chemical (DAB) method. Nuclei were stained by hematoxylin staining. Photos from (a to e) $\times$400 magnification. Photos (f-1 and f-2) $\times$1000 magnification.

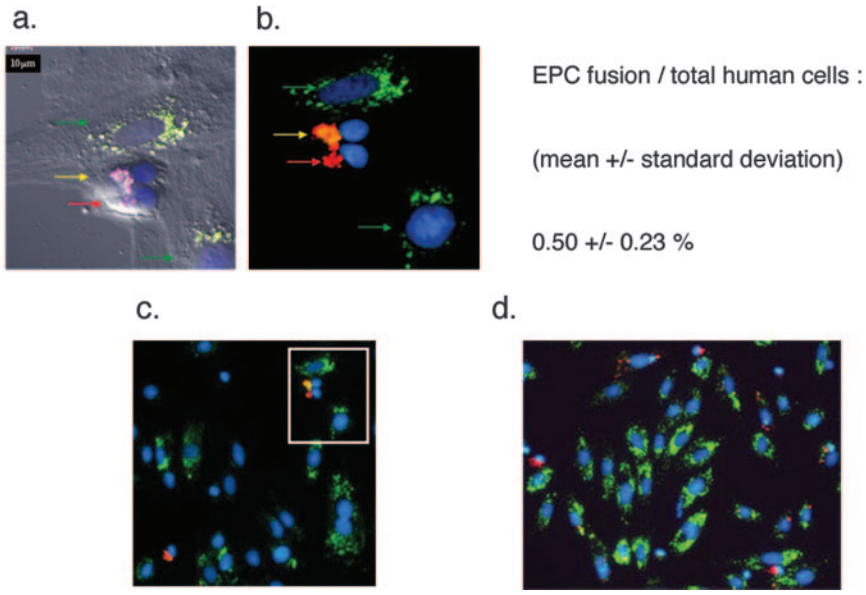

Figure 5. Evaluation of frequency of cell fusion in coculture system. We performed coculture using Qtracker (Quantum Dot Corp), which is a nanocrystal labeling marker incorporated in the vesicles of the cytoplasm. EPCs were labeled with the Qtracker 565(red arrow) and H9C2 cells were labeled with Qtracker 655 (green arrows) (a and b). Cell fusion was observed in the EPCs attached to H9C2 (yellow arrow) (a and b). Low-magnification photo was indicated (c), and the white square (c) was equivalent to (b). EPC fusion ratio was evaluated by counting fusion cells out of total human-derived cells and indicated the frequency as mean±SD (d).

junctions in cardiomyocytes (Figure 4f-2). However, double-stained cells disclosed the connection between rat cardiomyocyte and human-derived cell (Figure 4f-1, arrowhead). To test the human specificity of the antibodies, immunohistochemistry was performed using PBS-injected rat myocardium as the negative control. Each antibody did not react with rat cardiomyocytes (Figure 4a-2, 4b-2, 4c-2), rat smooth muscle cells (Figure 4d-2), and rat endothelial cells (Figure 4e-2). These in vivo data suggested that human EPCs transplantation caused multi-lineage differentiation into cardiac, smooth muscle, and endothelial lineages in the ischemic myocardium. Immunostaining by using connexin 43 and HLA revealed that transdifferentiated EPCs connected to other surviving rat derived cardiomyocytes (Figure 4f-1).

Evaluation of Frequency of Cell Fusion in Coculture System

We performed coculture using Qtracker (Quantum Dot Corp), which is a nanocrystal labeling marker incorporated in the vesicles of the cytoplasm. EPCs were labeled with the Qtracker 565 (red) and H9C2 cells were labeled with Qtracker 655 (green) (Figure 5c). Only EPCs attached to H9C2 incorporated both green and red dye markers (white square in Figure 5c and yellow arrow in Figure 5a and 5b). However, the frequency of cell fusion was very low from these data. We evaluated EPC fusion ratio by counting fusion cells out of total human-derived cells and indicated the frequency as mean±SD (0.50±0.23%) (Figure 5d). These data were equivalent to the result demonstrated by Badorff et al.[13]

Discussion

Previous studies from our and another laboratories showed therapeutic potential of ex vivo expanded EPCs for myocardial ischemia.[1,12] We hypothesized that EPCs can contribute to not only vasculogenesis but also myogenesis in the ischemic myocardium. Although differentiated endothelial cells were the candidate for therapeutic application of ischemic disease, EPCs proved themselves as much more effective by animal model experiment[16].

Considering the flexibility of somatic stem and progenitor cells for lineage commitments,[1,8,13] we investigated whether the translineage commitment of EPCs contribute to cardiomyogenesis and vasculogenesis for functional improvement after EPC transplantation. To elucidate the mechanism of translineage commitment, we developed the detection system to differentiate target cell transcription and expression. Established coculture system detected human myocardial and smooth muscle lineage profiles from the cell population derived from human EPCs and rat cardiomyocytes without cross-reactivity between species. Rat cardiac myoblast cell line (H9C2) was cocultured with human EPCs for RT-PCR to distinguish species-specific markers. The RT-PCR system detected only human-specific cardiac and smooth muscle markers but not rat cardiac and smooth muscle markers. Using this system, translineage commitment from EPC to cardiac and smooth muscle lineages was detected precisely.

Using human-specific cardiac antibody (α/β-ventricular MHC), the percentage of positively stained EPCs was $\approx$0.1% among incubated EPCs by immunohistochemical determination. In addition, using both human-specific cardiac and smooth muscle antibodies, the percentage of double-positively stained EPCs was $<$0.08%. This indicates the phenomenon of EPC translineage commitment is not a common differentiation cascade during in vitro condition cocultured with myocardial lineage cells. Despite that we have already found the therapeutic potential of cultured EPCs in ischemic animal models, it still remains the issue which subpopulation of EPCs mainly contributes to cardiac lineage commitment. To address this point, we performed the sorting of cultured EPCs using CD34 or CD31 surface marker as one of the candidate markers for EPC and also established markers for endothelial cells. It should be noted that no specific markers are available for purifying EPCs yet, although a lot of challenges have been reported from various laboratories around the world. However, it could be possible

to compare positive and negative fractions and evaluate the tendency regarding cardiac lineage commitment. Our findings suggested that coculture in positive or negative fractions with cardiac lineage cells (H9C2) revealed no difference in cardiac lineage commitment in the case of both CD31 and CD34 fractioning as shown in Figure 3b. In this experiment, we conclude that at least both CD31 and CD34 are not key markers to determine the contribution of cardiac lineage commitment, and that the chance of contamination of mesenchymal stem cells is excluded because negative fraction that is supposed to include mesenchymal stem cells is incompetent in cardiac lineage commitment compared with positive fraction of CD31 or CD34. We will make effort to identify the precise marker for purifying EPCs in our next research endeavors.

Although we are interested in the emergence of double-lineage marker expressing (α/β-ventricular MHC and α-SMA) cell in vitro as the process of translineage commitment, in early heart development multiple smooth muscle lineage genes are reported to be expressed as regulators of muscle differentiation. α-SMA as well as sm22-α, a calponin-related protein, is expressed in cell lines derived from embryonic and adult hearts.[17] These protein detections might reflect early phase of myocardial lineage differentiation in this coculture system.

As discussed for years, we are still clueless regarding the mechanism of translineage differentiation. Along with formerly discussed transdifferentiation and de-differentiation, several groups have recently reported spontaneous cell fusion occurring in coculture between embryonic stem cells and bone marrow cells,[18] or between embryonic stem cells and brain-derived cells.[19] Cell fusion has long been known to achieve effective reprogramming of cells. Terada et al have reported that the frequency of spontaneous cell fusion was very low. Nevertheless, Lagasse et al have reported robust (30% to 50%) levels of transdifferentiation.[6] To define the frequency of cell fusion in this coculture condition, we used Qtracker system to determine the population of cell fusion. The frequency of cell fusion was rarely seen ($0.50\pm0.23\%$) though Qtracker system clearly disclosed the phenomenon of cell fusion. Transdifferentiation, but not cell fusion, is the main mechanism in our coculture system. Our finding regarding cell fusion is compatible with the data reported by Badorff et al.[13] They have concluded that cell-to-cell contact, but not cellular fusion, mediated EPC transdifferentiation. Although our data indicated lower proportion of cardiac lineage commitment, it could be the difference in methods, for example, EPC culture method, evaluation method, and antibodies used for the evaluation. Yeh et al have not investigated the cell fusion issue in their article; however, they have also suggested that phenotypic conversion of the injected $CD34^+$ cells may occur predominantly through transdifferentiation.[14]

We have expanded in vitro experiments to deduce whether this is a pathophysiological phenomenon observed in vivo. After transplantation of human EPCs to rat ischemic heart models, myocardial samples disclosed both human cardiac and smooth muscle, as well as endothelial lineage gene expressions detected by RT-PCR and immunohistochemistry. We also performed the experiment to confirm the cross-talk between ischemic rat cardiomyocyte and transplanted human-derived EPC by immunohistological staining with connexin 43, one of the gap junctional molecules. The functional connection was observed between rat cardiomyocyte and human EPC in ischemic region.

The evidence that translineage commitment of EPCs into cardiomyocyte and smooth muscle cell lineages in vivo encourages therapeutic application of EPCs for myocardial ischemic diseases. The results indicate the occurrence of niche-dependent translineage differentiation of EPCs for vasculogenesis and cardiomyogenesis for heart regeneration. Because the severely damaged myocardium requires significant heart organogenesis, the potency of EPCs to supplement myocardial and smooth muscle lineage cells is very reasonable to regenerate heart tissues. The emergence of newly formed cardiomyocyte may reconstitute destroyed myocardium and provide cross-talk signaling toward vasculogenesis. Furthermore, the occurrence of smooth muscle lineage supports the maturation and maintenance of newly formed blood vessels by original endothelial lineage cells derived from EPCs. Recent publication suggested CD34 transdifferentiation into cardiomyocytes, smooth muscle cells, and endothelial cells in ischemic rat heart.[14] These generated systemic biological cross-talk between lineages are proceleusmatic for the ischemic heart disease treatment. These data suggest that EPC transplantation therapy has beneficial effects via both blood flow improvement and myogenesis in myocardial regeneration.

However, the frequency of myogenesis observed in this study is not enough to encourage functional improvement by translineage differentiation of EPCs themselves. Further mechanistic investigation is necessary to improve the transdifferentiation ratio and apply for clinical trial.

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