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Differentiation of circulating endothelial progenitor cells to a cardiomyogenic phenotype depends on E-cadherin

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Abstract Progenitor cells may contribute to cardiac regeneration. Here, we investigated the role of cadherins and integrins for differentiation of human adult circulating endothelial progenitor cells (EPCs) into cardiomyocytes (CM) in a co-culture system. N- and E-cadherin were expressed in EPCs and were localized at the interface between EPCs and CM. Incubation of a blocking antibody against E-cadherin reduced the expression of CM marker protein in EPCs. Blocking antibodies against N- or P-cadherin or the β1- and β2-integrins were not effective. These data suggested that cell-to-cell communication mediated by E-cadherin contributes to the acquirement of a cardiomyogenic phenotype of human endothelial progenitor cells.

Keywords: Differentiation; Endothelial progenitor cells; Cardiomyocytes; Cadherin

1. Introduction

A number of cardiovascular diseases such as myocardial infarction lead to cardiomyocyte loss and consequently deterioration of cardiac function. The replacement of cardiomyocytes by cell therapy would be a promising option to regenerate cardiac tissue. Furthermore, the transplantation of healthy stem cells may compensate for mutated, dysfunctional genes in cardiomyocytes as suggested by first animal studies [1]. Several different types of adult stem and progenitor cells have been shown to differentiate into the cardiomyogenic lineage. Bone marrow-derived hematopoietic stem cells, mesenchymal stem cells and side population (SP)-cells showed expression of cardiac marker proteins after infusion in animal models of myocardial infarction [2–4]. Additionally, tissue resident cardiac progenitor cells have been identified [5,6], which can differentiate into cardiomyocytes. Very recent studies questioned that c-kit$^+$/lin$^-$ bone marrow-derived stem cells can acquire a cardiac phenotype after injection in a mouse model of acute myocardial infarction [7,8]. Moreover, other studies suggested that bone marrow-derived stem cells can fuse with cardiomyocytes [9]. Interestingly, some studies showed that both fusion and differentiation occur in mice after injection of cardiac stem cells in a model of myocardial infarction [6]. The reason for the controversial outcomes between the studies is not yet resolved and likely reflects methodological differences.

In order to determine the molecular mechanism(s) underlying cardiomyogenic differentiation of adult stem or progenitor cells, we used a co-culture system of neonatal rat cardiomyocytes to mimic the cardiac environment [10]. Embryonic endothelial cells [10], human CD34$^+$ cells [11], and human peripheral blood mononuclear cell-derived circulating endothelial progenitor cells (EPCs) [12] were shown to acquire a cardiomyogenic phenotype after co-culture. In this co-culture assay, expression of cardiac genes and Gap-junctional communication between neonatal cardiomyocytes and differentiating stem or progenitor cells was detected [10,12]. Functional activity of the differentiated circulating progenitor cells was documented by showing oscillating calcium transients after pacing [12]. These data suggest that the co-culture system might be useful as an experimental model system to study the mechanism(s) of cardiomyogenic differentiation of adult progenitor cells.

Interestingly, albeit at a lower extent cardiac differentiation was still achieved, when paraformaldehyde-fixed cardiomyocytes were used as matrix for the co-culture assay [12], but was not observed by co-incubation with cardiomyocyte-conditioned medium [10,12] suggesting an important role of cell-to-cell or cell-to matrix interaction for cardiac differentiation. Cellular interactions can be mediated by integrins or cadherins. Particularly, β1-integrin expression was shown to be essential for the differentiation of cardiac muscle cells from embryonic stem cells [13]. Cadherins are also known to regulate signaling processes such as differentiation, proliferation, and migration [14]. Among the classic cadherins, which are calcium dependent and have five repeats of cadherin-specific extracellular modules, N-cadherin and E-cadherin are crucial for cardiogenesis [15,16]. N-cadherin deficiency resulted in cardiovascular defects [15]. Forced N-cadherin or E-cadherin expression using α- or β-MHC promotor rescued the heart development in N-cadherin null mice [16], demonstrating an essential role of cadherins in embryonic cardiogenesis. Moreover, connexin 43-mediated Gap-junctional intercellular communication is regulated by E-cadherin [17]. We had previously reported that adhesion of EPCs to fixed cardiomyocytes

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was calcium dependent [12] suggesting the relevance of calcium-dependent proteins such as integrins or cadherins for cell-to-cell communication between EPC and cardiomyocytes.

Here, we investigated the contribution of integrins and cadherins for the acquirement of a cardiomyogenic phenotype of EPCs after co-culture with neonatal cardiomyocytes. Our data demonstrate an important role of E-cadherin on EPCs differentiation. In particular, E-cadherin blocking antibody significantly inhibited the expression of the cardiac marker α-sarcomeric actinin in the human EPCs after co-culture. In contrast, inhibition of N- or P-cadherin and integrin blocking experiments had no effect. Furthermore, addition of an E-cadherin blocking antibody did not affect cell fusion, suggesting a predominant role of E-cadherin on EPC differentiation.

2. Materials and methods

2.1. Antibodies and peptides for cell culture

Anti-N-cadherin antibody (Ab) (clone NCD-2), anti-E-cadherin Ab (clone HECD-1), anti-P-cadherin Ab (clone NCC-CAD-299) were purchased from Calbiochem (San Diego, CA). Anti-integrin β1 antibody (clone P4C10) was obtained from Chemicon (Temecula, CA). Anti-integrin β2 (CD18) Ab was purchased from Cymbus Biotechnology (UK). RGD peptide (H-Gly-Arg-Gly-Asp-Asn-Pro-OH; GRGDNP) and RGD control peptide (H-Gly-Arg-Gly-Glu-Ser-Pro-OH; GRGESP) were from Bachem. N-cadherin blocking peptide (sequence: RADSFDINGNQV) and control peptide (sequence: RAHAVDINGNQV) were sythesized by Biosyntan (Berlin, Germany). The efficiency of this peptides was confirmed previously [18]. All antibodies and peptides were incubated with EPC before co-cultivating and re-added every 2 days.

2.2. Cell culture experiments

Neonatal ventricular cardiomyocytes were isolated from 0 to 1 day old Wistar rats and cultivated as previously described [12]. Noncardiomyocytes (primarily fibroblasts) were separated from the cardiomyocytes by differential plating onto plastic dishes. EPCs were cultivated from human peripheral blood mononuclear cells from healthy volunteers as described [12]. After 3 days in culture, adherent cells were labeled with Dil-ac-LDL. EPCs have been extensively characterized in our laboratory in previous studies [19]. Expression of endothelial markers, such as vWF, KDR, and eNOS were routinously confirmed [12]. EPCs were pre-incubated with antibodies or isotype IgG for 30 min. Then EPCs (1.5×10^5) and freshly isolated cardiomyocytes were plated onto gelatin coated dishes at a ratio of 1:3 [12]. In some experiments, we fixed cardiomyocytes with 2% paraformaldehye as described [12]. Then, EPCs were added onto fixed cardiomyocytes.

2.3. Immunostaining and flow cytometry analysis

After 6 days of the co-culture, cells were stained with phycoerythrin-conjugated antibodies recognizing human HLA-DR and HLA-class I (both Caltag Laboratories, Burlingame, CA) followed by permeabilization using the Cytofix/Cytoperm kit (BD Pharmingen) and staining with FITC-conjugated (Pierce, Rockford, IL) anti-α-sarcomeric actinin antibody (clone EA-53, Sigma) as described [12]. 20 000 cells were analyzed on a BD FACS Calibur cell sorter (BD Biosciences, San Jose, CA).

For immunostaining, cells were fixed with PLP buffer (2% paraformaldehyde, 0.01 M $NaIO_4$, 0.075 M lysine, 0.037 M phosphate buffer, pH 7.2) for 15 min on ice. After permeabilization with 0.2% saponin (Sigma), cells were incubated with pan-cadherin antibody (Sigma), followed by staining with a FITC-conjugated anti-rabbit IgG (Jackson) and α-sarcomeric actinin antibody (Sigma), followed by staining with Cy5-conjugated anti-mouse IgG (Jackson). Nuclei were counterstained with Sytox Blue (Molecular Probes) according to the manufacture's protocol.

2.4. RNA isolation and RT-PCR analyses

Total RNA from HUVEC, EPCs, and human heart were isolated by using TRIzol (Invitrogen). RNA was subjected to RT-PCR by using M-MLV Reverse Transcriptase (Invitrogen). Primers are as following: E-cadherin (5′-GAACACAGGAGTCATCAGTGTGG-3′/5′-CTGCTACGTGTAGAATGTACTGC-3′), N-cadherin (5′-GCAAGACTGGATTTCCTGAAG-3′/5′-CTGGAGTTTTCTGGCAAGTTG-3′), H-cadherin (5′-TGCGGAAGATATGGCAGAACTCG-3′/5′-GAGTTTTGCCATTGACATCAGTGG-3′), VE-cadherin (5′-AGGTATGAGATCGTGGTGGAAGC-3′/5′-TGGATGTATTCATAATCCAGAGGC-3′), cadherin-19 (5′-ATGCTGACGATCCCTCAAGTGG-3′/5′-TCATCCTCTTCAATGCTGTAATCC-3′), eNOS (5′-CCAGCTAGCCAAAGTCACCAT-3′/5′-GTCTCGGAGCCATACAGGATT-3′), KDR (5′-GGTGCACTGCAGACAGATCTACG-3′/5′-GTCGTCTGATTCTCCAGGTTTCC-3′), GAPDH (5′-TCACCATCTTCCAGGAGCGAGATC-3′/5′-GAGACCACCTGGTGCTCAGTGTAG-3′).

2.5. Detection of cell fusion

Adenoviruses coding for enhanced GFP were generated by using pAdTrack-cytomegalovirus and pShuttle-cytomegalovirus (provided by Vogelstein [20]). One day after isolation cardiomyocytes were infected with adenoviruses (MOI 20). Three days after the infection cardiomyocytes were subsequently used for co-cultivation with Dil-ac-LDL labeled EPCs. GFP^+ cardiomyocytes were counted by immunostaining. After 6 days of cultivation, cells were collected and subjected to FACS analysis. GFP^+/Dil-ac-LDL^+ cells were defined as fused cells. We replated co-cultured cells at day 6 onto dishes with lower concentration to detect the cells on a single cell level. Then we performed immunostaining as described above.

2.6. Statistical analysis

Data are expressed as means ± S.E.M. Unpaired, two-tailed Student's *t*-test was used for the comparison between groups based on the original data.

3. Results

Differentiation of EPCs into cardiomyocytes was induced by co-incubation of EPCs with neonatal rat cardiomyocytes. After 6 days of co-culture, the expression of the cardiac marker protein α-sarcomeric actinin in the human cells was detected by FACS staining against α-sarcomeric actinin and human HLA. The cardiac phenotype of the EPCs was further confirmed by confocal microscopy showing expression of the cardiac markers ANP and MEF-2, RT-PCR directed against ANF and βMHC using human specific primers, and the demonstration of oszillating calcium transients after pacing of the human cells (see our previous publications [12,21]). Moreover, the expression of the cardiac marker protein α-sarcomeric actinin was also detected when fixed cardiomyocytes were co-cultured with human EPCs (Fig. 1A, and [12]).

3.1. β1 and β2 integrins are not required for cardiac differentiation of circulating endothelial progenitor cells

Since β1-integrins are essential for differentiation of embryonic stem cells into cardiomyocytes [13], we determined the contribution of β1-integrin to the co-culture-induced differentiation of human EPCs. Therefore, blocking antibodies (10 μg/ml) or RGD peptides (0.5 mM) were added to the co-culture of EPCs and neonatal cardiomyocytes and expression of the cardiac marker α-sarcomeric actinin was determined after 6 days. However, cardiac differentiation of EPCs was not affected by the β1-blocking antibody or RGD peptide (Fig. 1B). Surprisingly, the survival and adhesion of EPCs in the presence of β1-integrin blocking antibody or RGD peptide was increased (Fig. 1C) suggesting that the blockade of β1-integrins improves adhesion and survival but does not interfere with differentiation. Next, we tested the effects of β2-integrin blocking antibodies. In the presence of β2-integrin antibodies, the total number of human HLA positive cells was signifi-

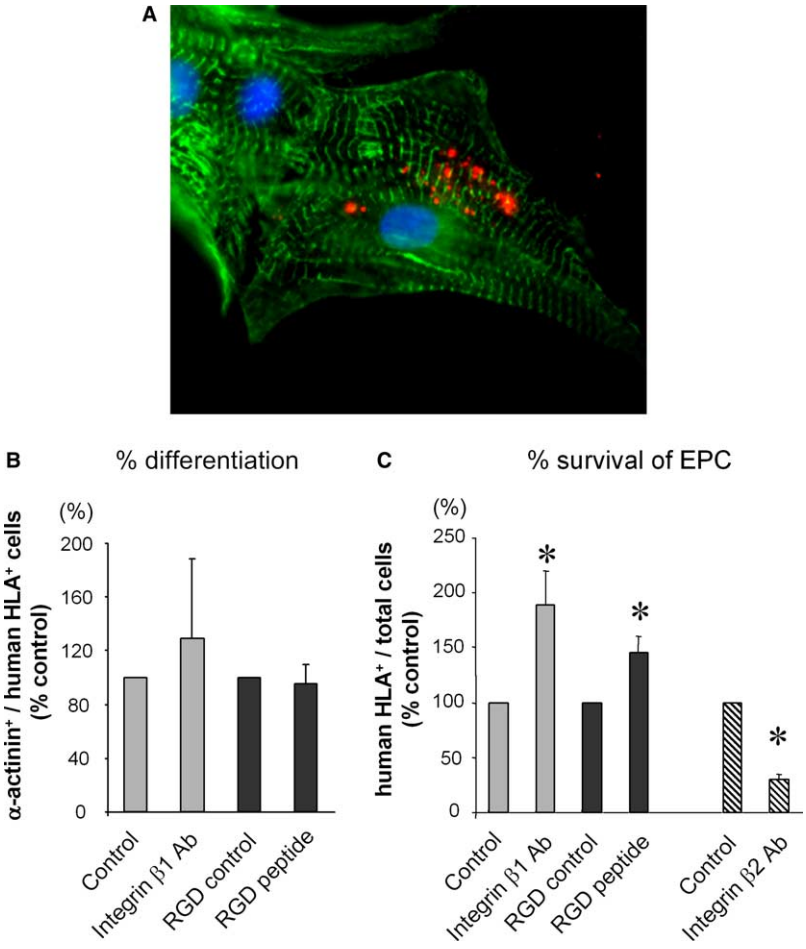

Fig. 1. (A) Representative immunocytochemical picture after 6 days co-culture of human Dil-ac-LDL EPC (red fluorescence) with paraformaldehyde (2%) fixed cardiomyocytes. The cardiac marker α-sarcomeric actinin was detected by using a FITC-conjugated antibody (green fluoresence). Nuclei are stained in blue. (B) Integrin antibodies (β1 integrin; 10 μg/ml, β2 integrin; 1 μg/ml) or RGD peptide (0.5 mM) were incubated with the co-culture for 6 days and EPC differentiation was detected by FACS analysis. Isotype IgG and RGD control peptide were used as control. ($n = 6$). (C) EPCs survival was determined by counting the number of total human HLA positive cells by FACS analysis. $N = 5–6$, $^{*}P < 0.05$ versus control.

cantly decreased (69.7 ± 4.7% reduction, Fig. 1C). Despite the drastic reduction of EPC adhesion and survival, the total number of double positive cells was not changed (64.2 ± 13.7 vs 76.7 ± 23.2; $n = 5$, out of 20000 total cells, respectively). Control experiments confirmed that β1-, and β2-integrin blocking antibodies efficiently and selectively blocked the respective integrins [22].

3.2. Expression and functional contribution of cadherins

Since integrin blocking antibodies had no effect on EPC differentiation, we determined the expression of cadherins in the co-culture system by confocal microscopy. Cadherins were specifically expressed at the border between cardiomyocytes and neighbouring EPCs (Fig. 2A: yellow arrow). Interestingly, the non-differentiated human cells, which do not express the cardiac marker protein α-sarcomeric actinin, did lack the specific staining of cadherins at the border to the cardiomyocytes (Fig. 2A: white arrow). Next, we assessed the specific expression of cadherins in cardiomyocytes and EPCs by using RT-PCR. As previously described, cardiomyocytes express N-cadherin (Fig. 2B). Additionally expression of E-cadherin was documented in human heart (Fig. 2B). EPC expressed various different cadherin genes including E-cadherin, N-cadherin, and VE-cadherin. In contrast, H-cadherin and Cad-19 were only expressed in human heart but were low to absent in EPC (Fig. 2C). Interestingly, E-cadherin expression was significantly higher in EPC (424 ± 69.8%, $P = 0.004$) compared to human umbilical venous endothelial cells, which lack the progenitor cell characteristics (Fig. 2B).

In order to assess whether cadherins are essential for EPC differentiation, we used cadherin blocking antibodies. E-cadherin blocking antibodies alone significantly inhibited the EPC differentiation, whereas N- and P-cadherin antibodies had no effect on EPC differentiation (Fig. 3A). The mixture of N-, P-, E-cadherin blocking antibodies inhibited EPC differentiation but did not further significantly reduce expression of the cardiac marker protein α-sarcomeric actinin as compared to E-cadherin antibodies (Fig. 3A). Since N-cadherin plays an essential role in embryonic heart development, we confirmed the negative results obtained with the N-cadherin blocking peptides by using a previously described peptide with a sequence specific for the extracellular domain of N-cadherin [18]. However, N-cadherin blocking peptides did not exert any effect (Fig. 3C). Control experiments showed that the survival of EPC was similar in the different antibody treatment regimens (data not shown).

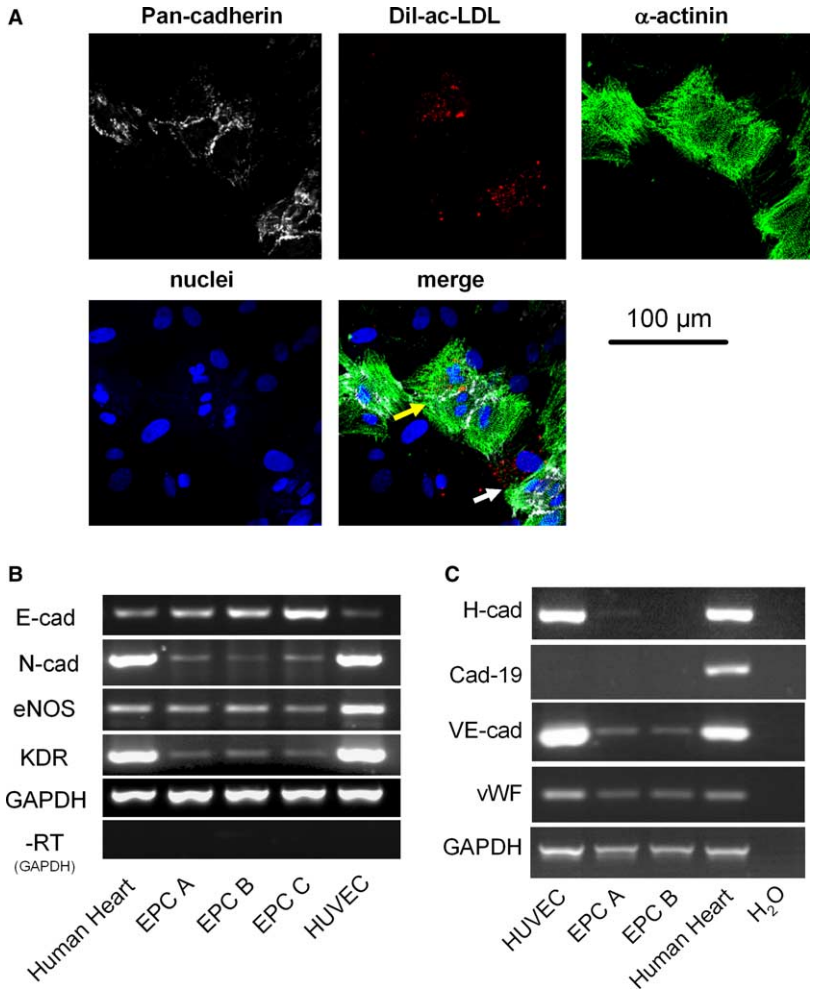

Fig. 2. Expression of Cadherins. (A) Representative immunocytochemical picture after 6 days of the co-culture. Human EPCs were labeled by Dil-ac-LDL (red) and cardiomyocytes were detected by α-sarcomeric actinin (green). Pan-cadherin expression is shown in white. Nuclei are stained in blue. Representative confocal images from $n = 5$ experiments are shown. Pan-cadherin was expressed at the border between cardiomyocytes and neighbouring EPCs (yellow arrow). Non-differentiated human cells (white arrow), which do not express α-sarcomeric actinin, lack the specific staining of cadherins at the border to the cardiomyocytes. Bar indicates 100 μm. (B/C) Expression of cadherins was detected in human umbilical venous endothelial cells (HUVEC), three different donor-derived EPC preparations (EPC A, EPC B, and EPC C), human heart. The endothelial NO-synthase (eNOS), the VEGF-receptor-2 (KDR) and von Willebrand factor (vWF) were used as endothelial markers. Gene expressions were controlled by GAPDH. "-RT" indicates samples without addition of reverse transcriptase. A representative figure out of $N = 3$–6 individual experiments is shown.

3.3. Effects of cadherins on fusion

Because some investigators suggested the involvement of fusion in the process of transformation of stem/progenitor cells into cardiomyocytes, we tested whether cadherin blocking antibodies could inhibit cellular fusion. For detecting fusion, cardiomyocytes were transduced with an adenovirus coding for GFP for 1 week and were then co-cultured for 6 days with Dil-ac-LDL labeled EPCs. Importantly 98.8 ± 1.24% of the cardiomyocytes did express GFP 1 week after transduction. After 6 days of co-culture, cells positive for α-sarcomeric actinin, GFP and Dil-ac-LDL were considered as fused cells, whereas cells only positive for α-actinin and Dil-ac-LDL but negative for GFP represent differentiated EPCs. Since GFP and Dil-ac-LDL are not transported via Gap junctions, GFP^+/Dil-ac-LDL^+ cells may only derive from cellular fusion or transmembrane fusion allowing the exchange of organelles and/or proteins. Using this criterion, fusion was observed in 2.9 ± 6% of the human cells, whereas 6.0 ± 0.7% of the cells were positive for human HLA and α-sarcomeric actinin at day 6 [21]. Thus, fusion represents roughly 50% of the cells with cardiomyogenic phenotype. However, cadherin blocking antibodies in the same concentration as used in the previous experiments did not affect the number of GFP^+/Dil-ac-LDL^+ fused cells, suggesting that E-cadherin specifically contributed to EPCs differentiation but not to cell fusion (Fig. 3D).

4. Discussion

The data of this study disclose an important role of E-cadherin for EPC differentiation into cardiomyocytes. Cadherin expression was confirmed by immunocytochemistry and RT-PCR. Blocking experiments using an E-cadherin antibody significantly inhibited EPC differentiation, but not fusion of EPC with cardiac myocytes. Although integrin β1 was essential for the differentiation of cardiac muscle cells from embryonic stem

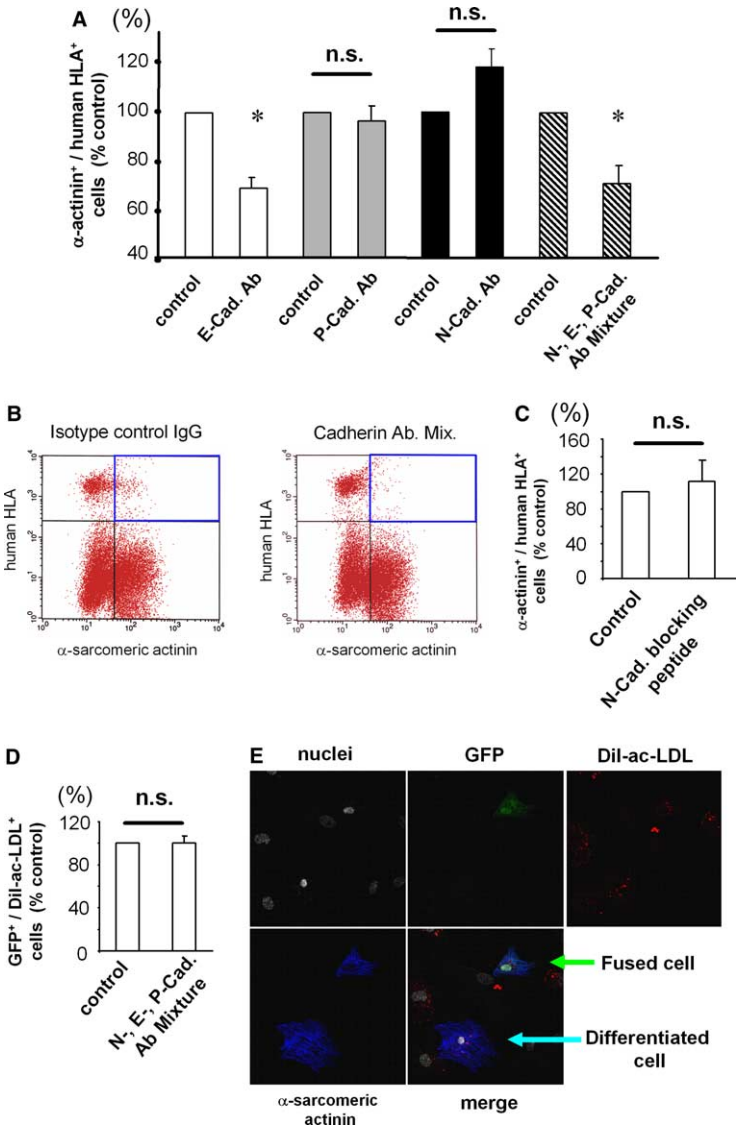

Fig. 3. (A/B) Cadherin blocking antibodies (10 μg/ml each) were incubated with the co-culture of human EPC and neonatal rat cardiac myocytes. After 6 days of co-culture, FACS analysis was performed to detect the expression of the cardiac marker gene α-sarcomeric actinin in the human HLA-positive cells. Isotype control IgG was used as control ($n = 5$). $^{*}P < 0.05$ versus control IgG. Panel B shows a representative FACS analysis after incubation of the co-culture with anti N-, E-, P-cadherin antibodies (10 μg/ml each). Upper right (blue box), indicates α-sarcomeric actinin and human HLA double positive cells. (C) The effect of N-cadherin blocking peptide (sequence: RADSFDINGNQV) on α-sarcomeric actinin expression after 6 days of co-culture as assessed by FACS analysis. A control peptide (sequence: RAHAVDINGNQV) was used as a negative control. $n = 8$ experiments. D: Effect of mixture of N-, E-, P-cadherin antibodies (10 μg/ml each) on cell fusion. Cardiac myocytes were transduced with an adenovirus encoding GFP. After 1 week, cardiac myocytes were extensively washed and were co-incubated with Dil-ac-LDL labeled human EPC. Double positive cells expressing GFP and show Dil-ac-LDL represent fused cells. $n = 3$ experiments. (E) Representative immunostaining of fused cell and non-fused differentiated cell. Co-cultured cells of Dil-ac-LDL (red) labeled EPCs and GFP-transduced CM (green) were replated in a low density to detect cells on a single cell level. Double positive cells for Dil-ac-LDL (red) and α-sarcomeric actinin (blue) were detected in GFP positive (green arrow) and GFP negative cells (blue arrow). Nuclei are stained in white. Representative confocal images from $n = 3$ experiments are shown.

cells [13], integrin β1 and RGD peptide had no effect on EPC differentiation in the co-culture system.

Classic cadherins promote calcium-dependent cell-to-cell adhesion [23]. Furthermore, cadherins are also known to regulate diverse signaling processes such as differentiation, proliferation, and migration [14]. Since adhesion of EPCs to fixed cardiomyocytes was calcium dependent [12], we hypothesized that classic calcium-dependent cadherins were involved in cell-to-cell communication between EPCs and cardiomyocytes. Indeed, cadherins were expressed at the border between differentiated EPCs and cardiomyocytes (Fig. 2A). As classic cadherins such as N-cadherin and E-cadherin are crucial for cardiogenesis [15–17], we examined the effect of N-, E-, and P-cadherin blocking antibodies on EPC differentiation. Surprisingly, only the blockade of E-cadherin significantly inhibited the expression of cardiomyocyte markers in human EPCs. Cardiomyocytes predominantly express N-cadherin. And it is not entirely clear, whether cardiomyocytes express substantial amounts of E-cadherin. Thus, E-cadherin may be potentially activated by heterophilic interactions [24].

Intracellular signaling of classic cadherins is mainly mediated via β-catenin. β-catenin is a transcription cofactor of T

cell factor/ lymphoid enhancer factor (TCF/LEF). Phosphorylation of β-catenin by glycogen synthase kinase 3β (GSK3β) leads to ubiquitination of β-catenin and its subsequent degradation [23]. Activation of cadherins blocks the degradation of β-catenin by inhibiting GSK3β activity [23]. However, we have recently observed that inhibition of GSK3β by LiCl did not affect EPC differentiation [21]. These data suggested that the signaling of EPC differentiation is unlikely to be mediated via the GSK3β/β-catenin pathway. If so, how does cadherin contribute to GSK3β/β-catenin-independent signal transduction? Recently, it had been reported that presenilin-1 cleaved the N-cadherin intracellular domain resulting in degradation of the transcriptional coactivator CREB binding protein [25]. E-cadherin could also be cleaved by cellular stress [26] or presenilin-1 [27]. One may speculate that a C-terminal cleavage fragment of E-cadherin might directly influence the intracellular signaling independent on β-catenin. However, we could not detect a E-cadherin cleavage fragment by Western blot analysis in the co-cultured cells under the condition tested (data not shown). Thus, additional mechanisms appear to be mediated by E-cadherin.

Recently, some investigators questioned that bone marrow-derived hematopoietic stem cells can acquire a cardiac phenotype in vivo [7,8]. Moreover, other studies suggested that bone marrow-derived stem cells can fuse with cardiomyocytes [9]. However, at least in vitro, stem/progenitor cells are capable to differentiate into other lineages after co-culturing with fixed cells as shown in previous studies [12,28], thus indicating that differentiation certainly exists. Furthermore, both fusion and differentiation were shown to occur in mice after injection of cardiac stem cells in a model of myocardial infarction [6]. We also confirmed that fusion processes (either cellular or transmembrane fusion) existed in our co-culture assay representing roughly 50% of α-sarcomeric actinin-positive EPCs. However, E-cadherin blocking antibodies did not affect fusion. Thus, E-cadherin appears to rather selectively contribute to EPC differentiation but not fusion.

Surprisingly, only E-cadherin did affect EPC differentiation. Of note, the E-cadherin expression pattern is different from other cadherins, when EPCs were compared with mature endothelial cells (HUVEC) (Fig. 2B). E-cadherin expression was significantly higher in EPCs compared to HUVEC (0.85 ± 0.07 vs 0.21 ± 0.04, $P < 0.05$, $n = 3$), whereas other cadherins such as N-cadherin and VE-cadherin were preferentially expressed in mature endothelial cells. In contrast to a previous study showing a transdifferentiation of freshly isolated HUVEC to cardiomyocyte [10], cultivated HUVEC (>3 passages) used in the present study failed to acquire a cardiomyogenic phenotype (data not shown). Thus, it is possible that the selective expression of E-cadherin in progenitor cells compared to HUVEC might contribute to the capacity of the progenitor cells to acquire a cardiomyogenic phenotype.

Taken together, the present study describes that E-cadherin, but not P-, N-cadherin as well as β1 or β2 integrins contributes to the expression of cardiac marker proteins within human EPCs after co-culture with neonatal rat cardiomyocytes. E-cadherin blocking antibodies significantly inhibited EPC differentiation but not fusion, suggesting that cell-to-cell communication via E-cadherin is involved in differentiation processes rather than cell fusion.

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References

[1] Gussoni, E., Soneoka, Y., Strickland, C.D., Buzney, E.A., Khan, M.K., Flint, A.F., Kunkel, L.M. and Mulligan, R.C. (1999) Dystrophin expression in the mdx mouse restored by stem cell transplantation. Nature 401, 390–394.
[2] Jackson, K.A., Majka, S.M., Wang, H., Pocius, J., Hartley, C.J., Majesky, M.W., Entman, M.L., Michael, L.H., Hirschi, K.K. and Goodell, M.A. (2001) Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells. J. Clin. Invest. 107, 1395–1402.
[3] Makino, S., Fukuda, K., Miyoshi, S., Konishi, F., Kodama, H., Pan, J., Sano, M., Takahashi, T., Hori, S., Abe, H., Hata, J., Umezawa, A. and Ogawa, S. (1999) Cardiomyocytes can be generated from marrow stromal cells in vitro. J. Clin. Invest. 103, 697–705.
[4] Orlic, D., Kajstura, J., Chimenti, S., Jakoniuk, I., Anderson, S.M., Li, B., Pickel, J., McKay, R., Nadal-Ginard, B., Bodine, D.M., Leri, A. and Anversa, P. (2001) Bone marrow cells regenerate infarcted myocardium. Nature 410, 701–705.
[5] Beltrami, A.P., Barlucchi, L., Torella, D., Baker, M., Limana, F., Chimenti, S., Kasahara, H., Rota, M., Musso, E., Urbanek, K., Leri, A., Kajstura, J., Nadal-Ginard, B. and Anversa, P. (2003) Adult cardiac stem cells are multipotent and support myocardial regeneration. Cell 114, 763–776.
[6] Oh, H., Bradfute, S.B., Gallardo, T.D., Nakamura, T., Gaussin, V., Mishina, Y., Pocius, J., Michael, L.H., Behringer, R.R., Garry, D.J., Entman, M.L. and Schneider, M.D. (2003) Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. Proc. Natl. Acad. Sci. USA 100, 12313–12318.
[7] Balsam, L.B., Wagers, A.J., Christensen, J.L., Kofidis, T., Weissman, I.L. and Robbins, R.C. (2004) Haematopoietic stem cells adopt mature haematopoietic fates in ischaemic myocardium. Nature 428, 668–673.
[8] Murry, C.E., Soonpaa, M.H., Reinecke, H., Nakajima, H., Nakajima, H.O., Rubart, M., Pasumarthi, K.B., Virag, J.I., Bartelmez, S.H., Poppa, V., Bradford, G., Dowell, J.D., Williams, D.A. and Field, L.J. (2004) Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. Nature 428, 664–668.
[9] Nygren, J.M., Jovinge, S., Breitbach, M., Sawen, P., Roll, W., Hescheler, J., Taneera, J., Fleischmann, B.K. and Jacobsen, S.E. (2004) Bone marrow-derived hematopoietic cells generate cardiomyocytes at a low frequency through cell fusion, but not transdifferentiation. Nat. Med. 10, 494–501.
[10] Condorelli, G., Borello, U., De Angelis, L., Latronico, M., Sirabella, D., Coletta, M., Galli, R., Balconi, G., Follenzi, A., Frati, G., Cusella De Angelis, M.G., Gioglio, L., Amuchastegui, S., Adorini, L., Naldini, L., Vescovi, A., Dejana, E. and Cossu, G. (2001) Cardiomyocytes induce endothelial cells to trans-differentiate into cardiac muscle: implications for myocardium regeneration. Proc. Natl. Acad. Sci. USA 98, 10733–10738.
[11] Yeh, E.T., Zhang, S., Wu, H.D., Korbling, M., Willerson, J.T. and Estrov, Z. (2003) Transdifferentiation of human peripheral blood $CD34^+$-enriched cell population into cardiomyocytes, endothelial cells, and smooth muscle cells in vivo. Circulation 108, 2070–2073.
[12] Badorff, C., Brandes, R.P., Popp, R., Rupp, S., Urbich, C., Aicher, A., Fleming, I., Busse, R., Zeiher, A.M. and Dimmeler, S. (2003) Transdifferentiation of blood-derived human adult endothelial progenitor cells into functionally active cardiomyocytes. Circulation 107, 1024–1032.
[13] Fassler, R., Rohwedel, J., Maltsev, V., Bloch, W., Lentini, S., Guan, K., Gullberg, D., Hescheler, J., Addicks, K. and Wobus, A.M. (1996) Differentiation and integrity of cardiac muscle cells are impaired in the absence of beta 1 integrin. J. Cell Sci. 109 (Pt 13), 2989–2999.

[14] Steinberg, M.S. and McNutt, P.M. (1999) Cadherins and their connections: adhesion junctions have broader functions. Curr. Opin. Cell Biol. 11, 554–560.
[15] Radice, G.L., Rayburn, H., Matsunami, H., Knudsen, K.A., Takeichi, M. and Hynes, R.O. (1997) Developmental defects in mouse embryos lacking N-cadherin. Dev. Biol. 181, 64–78.
[16] Luo, Y., Ferreira-Cornwell, M., Baldwin, H., Kostetskii, I., Lenox, J., Lieberman, M. and Radice, G. (2001) Rescuing the N-cadherin knockout by cardiac-specific expression of N- or E-cadherin. Development 128, 459–469.
[17] Jongen, W.M., Fitzgerald, D.J., Asamoto, M., Piccoli, C., Slaga, T.J., Gros, D., Takeichi, M. and Yamasaki, H. (1991) Regulation of connexin 43-mediated gap junctional intercellular communication by Ca^{2+} in mouse epidermal cells is controlled by E-cadherin. J. Cell Biol. 114, 545–555.
[18] Shapiro, L., Fannon, A.M., Kwong, P.D., Thompson, A., Lehmann, M.S., Grubel, G., Legrand, J.F., Als-Nielsen, J., Colman, D.R. and Hendrickson, W.A. (1995) Structural basis of cell–cell adhesion by cadherins. Nature 374, 327–337.
[19] Dimmeler, S., Aicher, A., Vasa, M., Mildner-Rihm, C., Adler, K., Tiemann, M., Rutten, H., Fichtlscherer, S., Martin, H. and Zeiher, A.M. (2001) HMG-CoA reductase inhibitors (statins) increase endothelial progenitor cells via the PI 3-kinase/Akt pathway. J. Clin. Invest. 108, 391–397.
[20] He, T.C., Zhou, S., da Costa, L.T., Yu, J., Kinzler, K.W. and Vogelstein, B. (1998) A simplified system for generating recombinant adenoviruses. Proc. Natl. Acad. Sci. USA 95, 2509–2514.
[21] Koyanagi, M., Haendeler, J., Badorff, C., Brandes, R.P., Hoffmann, J., Pandur, P., Zeiher, A.M., Kuhl, M. and Dimmeler, S. (2005) Non-canonical Wnt signaling enhances differentiation of human circulating progenitor cells to cardiomyogenic cells. J. Biol. Chem. 280, 16838–16842.
[22] Chavakis, E., Aicher, A., Heeschen, C., Sasaki, K., Kaiser, R., El Makhfi, N., Urbich, C., Peters, T., Scharffetter-Kochanek, K., Zeiher, A.M., Chavakis, T. and Dimmeler, S. (2005) Role of beta2-integrins for homing and neovascularization capacity of endothelial progenitor cells. J. Exp. Med. 201, 63–72.
[23] Rowlands, T.M., Symonds, J.M., Farookhi, R. and Blaschuk, O.W. (2000) Cadherins: crucial regulators of structure and function in reproductive tissues. Rev. Reprod. 5, 53–61.
[24] Zeschnigk, M., Kozian, D., Kuch, C., Schmoll, M. and Starzinski-Powitz, A. (1995) Involvement of M-cadherin in terminal differentiation of skeletal muscle cells. J. Cell. Sci. 108 (Pt 9), 2973–2981.
[25] Marambaud, P., Wen, P.H., Dutt, A., Shioi, J., Takashima, A., Siman, R. and Robakis, N.K. (2003) A CBP binding transcriptional repressor produced by the PS1/epsilon-cleavage of N-cadherin is inhibited by PS1 FAD mutations. Cell 114, 635–645.
[26] Keller, S.H. and Nigam, S.K. (2003) Biochemical processing of E-cadherin under cellular stress. Biochem. Biophys. Res. Commun. 307, 215–223.
[27] Marambaud, P., Shioi, J., Serban, G., Georgakopoulos, A., Sarner, S., Nagy, V., Baki, L., Wen, P., Efthimiopoulos, S., Shao, Z., Wisniewski, T. and Robakis, N.K. (2002) A presenilin-1/gamma-secretase cleavage releases the E-cadherin intracellular domain and regulates disassembly of adherens junctions. EMBO J. 21, 1948–1956.
[28] Wurmser, A.E., Nakashima, K., Summers, R.G., Toni, N., D'Amour, K.A., Lie, D.C. and Gage, F.H. (2004) Cell fusion-independent differentiation of neural stem cells to the endothelial lineage. Nature 430, 350–356.