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Isolation and Characterization of Cardiac Progenitor Cells from Myocardial Right Atrial Appendage Tissue

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Abstract—Resident cardiac stem cells, known as "cardiogenic progenitor cells" (CPCs), are a heterogeneous population of immature cells residing in the myocardium and capable of self-renewal and differentiation into cardiomyocyte-like and vascular-like cells. CPCs are usually isolated by long enzymatic digestion of heart tissue and selection with stem cell markers. However, long exposure to enzymatic digestion and the small size of a myocardial sample significantly hinder acquiring a large number of viable cells. To avoid these problems, we developed a method based on CPC growth ex vivo and subsequent immunomagnetic selection.

Keywords: cardiac progenitor cells, myocardium regeneration, right atrial appendage

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INTRODUCTION

Cellular cardiomyoplasty employing stem and progenitor cells is a promising approach to treat various heart diseases, such as chronic ischemic heart disease, myocardial infarction, and heart failure. However, numerous studies using postnatal stem somatic cells (progenitor cells) from nonheart tissue (bone marrow, peripheral blood, skeletal myoblasts) show a statistically significant, but very modest, recovery of heart contractile function with a good safety profile (Fisher et al., 2015). The positive effect observed after transplantation of these cells is mediated mostly by their paracrine activity, which inhibits apoptosis of cardiomyocytes and stimulates angiogenesis. Nevertheless, transplanted cells are unable to generate new myocardial cells and replace them with their loss (Kikuchi and Poss, 2012).

The discovery of cardiac residential pool of stem (progenitor) cells involved in myocardial renewal/partial regeneration and with the ability to differentiate into major cardiac cell types opened a new possibility for their use as an optimal material for cellular cardiomyoplasty (Anversa et al., 2013). Expression of the receptor to stem cell factor (c-kit), transcription factor MEF2C, and lack of hematopoietic cell markers (CD45 and CD34) are common properties of these cells. In the myocardium, these cells are localized preferentially in the specific microenvironment of cell niches located in the apex and atria, which are known to be the areas with the least hemodynamic load (Leri et al., 2014). It has been shown that these cells display properties of postnatal stem (progenitor) cells: self-renewal, clonogenity, and multipotency, They are involved in repair of damaged myocardium due to mobilization from niches, working with injured tissue, and differentiation into cardiomyocytes and endothelial and smooth muscle cells (Goichberg et al., 2014).

Cardiac progenitor cells (CPCs) are able to replace lost myocardial cells with new cell elements that significantly improve the systolic function, affect remodeling, and slow down the progression of heart failure. However, the number of autologous residential CPCs obtained from endomyocardial biopsy or heart surgery in volumes safe for patients is very low for clinical application; thus, development of effective isolation methods is required. Most techniques for human CPC isolation rely on enzyme treatment of myocardium samples followed by cell sorting using c-kit or sca-1-like markers (Beltrami et al., 2003; Oh et al., 2003; Smits et al., 2009a). However, the small size of myocardial material and long-term enzymatic treatment limit acquisition of large number of viable cells for clinical application and research.

We have suggested a method of residential CPC isolation that may obtain stem cells from human or

[1] *Abbreviations:* CPC—cardiogenic progenitor stem cells, RA—right atrium, c-kit—receptor to SCF (stem cell factor), PBS—phosphate buffered saline, VEGF—vascular endothelial growth factor, sca-1—stem cell antigen 1.

animal myocardial tissue independent of its size or heart area. This method relies on cell culture in vitro to increase CPC mass followed by immunomagnetic selection.

MATERIALS AND METHODS

CPC isolation and culture. Fifty samples of myocardial appendage from the right atrium (RA) were taken from patients with ischemic heart disease who had undergone examination and routine surgery at the Department of Cardiovascular Surgery, Myasnikov Institute of Clinical Cardiology of the Federal State Institution Russian Cardiology Research and Production Complex (Russian Ministry of Health). The appendage was excised during RA cannulation for heart–lung machine connection during bypass surgery. Excised myocardial pieces were treated with 0.1% collagenase A (Roche diagnostic, United States) for 10 min at 37 °C twice. Culture plates were coated with 20 μg/mL fibronectin (Sigma, United States). Cells were maintained in IMDM medium (Invitrogen, United States) supplemented with 20% fetal calf serum (ATCC, United States), 100 units/mL penicillin and 100 μg/mL streptomycin (Thermo Scientific, United States), 2 mM L-glutamine (Invitrogen, United States), and 3 IU/L erythropoietin (Sigma, United States). Accutase (ICT, United States) was used for the cell detachment. A human Lineage Cell Depletion Kit (Miltenyi Biotec, United States) and human CD117 MicroBead Kit (Miltenyi biotec, United States) were used for magnetic cell sorting. C-kit (+)Lin(–) cells were cultured in Cardiac Cellutions™ Medium (DV Biologics, United States).

Evaluation of immunophenotype of obtained CPCs. CPCs were assayed using flow cytometry as follows. A cell suspension obtained with Accutase™ Enzyme Cell Detachment Medium was stained with antibodies in PBS with 1% bovine serum albumin. The antibody panel included c-kitPeCy7 and sca-1 FITC (Miltenyi Biotec, United States); CD105 FITC, CD90 PeCy5, LinFITC, CD34 FITC, and CD45 FITC (BDBiosciences, United States); VEGFR2/KDRAPC (Biolegend, United States); snf Notch 1 FITC (Abcam, United States). Live (not fixed) cells were incubated with antibodies at +4°C for 30 min, washed with PBS, and assayed with a BD FACSCanto™ II flow cytometer (BD, United States).

Clonogenic analysis of c-kit(+)Lin(–) cells. The cell clonogenic potential was assayed in medium composed of 35% IMDM and 65 DMEM/F12 media (Invitrogen, United States) supplemented with 2% B27 (Gibco, United States); 10 ng/mL EGF and 20 ng/mL bFGF (R&D, United States); 40 nM cardiotrophin-1 (Biovision, United States); 40 nM thrombin (Sigma, United States); and 100 U/mL penicillin, 100 μg/mL streptomycin, and 2 mM L-glutamine.

Proliferation assay of c-kit(+)Lin(–) cells. A number of cells of 3×10^4 were seeded in fibronectin-coated in Petri dishes with Cardiac Cellutions™ medium. Every 24 h, the cells were detached with HyQtase (Invitrogen, United States) and counted manually in a hematocytometer under a microscope. The population doubling time was calculated according to the instructions available at http://www.doubling-time.com.

Differentiation potency of c-kit(+)Lin(–) cells. For cardiomyogenic differentiation, 20 000 cells/cm^2 were seeded in Cardiomyocyte Cellutions™ Differentiation Media with 10 μM 5-azacytidine and cultured for 72 h, with the medium being changed every 24 h. The medium was then replaced by Cardiomyocyte Cellutions™ Differentiation Media with 10 ng/mL TGFβ, and the cells were cultured in this medium for 21 days. Endothelial differentiation was induced by c-kit(+)Lin(–) cell plating (20 000 cells/cm^2) in DMEM/F12 medium with 1% fetal calf serum, 1% 100 penicillin/streptomycin, 2 mM L-glutamine, 10-8 dexamethasone, and 10 hg/mL VEGF. The cells were cultured for 21 days changing the medium every 48 h.

Immunofluorescent staining. Cells were fixed and stained according to the protocol recommended by Abcam Co. (www.abcam.com). The primary antibodies were c-kit (Dako and Biolegend, United States), sarcomeric α-actinin and α-actin (Sigma, United States), CD31 and CD34 (BDPharmingen, United States); von Willebrandt factor (Sigma, United States), CD45 and Nkx 2.5 (Abcam and Santa Cruz, United States), and Gata 4 (Santa Cruz, United States). Samples were visualized with an Axiovert 200M fluorescence microscope (Zeiss, Germany). Images were captured with an Axiocam HRC digital video camera (Zeiss, Germany) and analyzed in the Axiovision 3.1 (Zeiss, Germany) and Adobe PhotoShop CS (Adobe Systems, United States) software.

Patient clinical characteristics and CPC number in explant cultures. The amount of c-kit+Lin(–) CPCs in explants was assessed with a BD FACSCanto™ II flow cytometer. The number of CPCs was evaluated in patients according to their sex, age, body mass index, and cardiovascular disease risk factors (diabetes mellitus, smoking, hyperlipidemia, arterial hypertension), as well as echocardiogram exams of the functional heart state. Correlative analysis was performed using the Spearman rank correlation method in the Statistica 10.0 software.

mRNA isolation, reverse transcription, and real-time PCR. mRNA was isolated with the RNeasy Miniprep Kit (Qiagen, Germany). Isolated mRNA was used for cDNA synthesis with the Revert Aid Kit (Fermentas, Latvia). Real-time PCR was performed with reagents from Synthol Co. (Russia). Product accumulation was measured with Applied Biosystems equipment (United States). The primers were *Oct4* gene: forward CTGCAGCAGATCAGCCACATCGCCCAGCAG

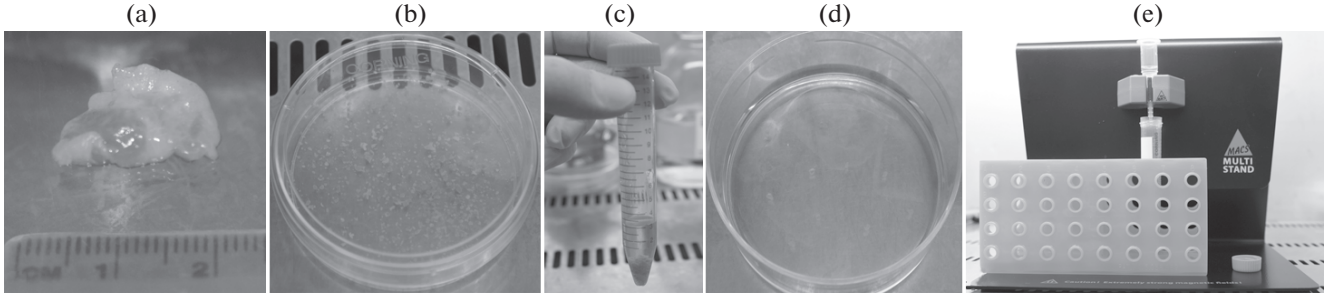

Fig. 1. Stages of human cardiogenic progenitor cells (CPCs) isolation. (a) Right atrial appendage, (b) minced tissue, (c) enzymatic treatment, (d) explant culture, and (e) immunomagnetic selection.

and reverse ACTCGGACCACATCCTTCTCGAGC; *Sox2* gene: forward GGGAAATGGGAGGGGTGCAAAAGAGG, reverse TTGCGTGAGTGTGG; *Klf4* gene: forward ACCCACACAGGTGAGAAACCTTACC, reverse TGATTGTAGTGCTTTCTGGCTGGGCTCC; *c myc* gene: forward CCCGAGCAAGGACGCGACTC, reverse TCGCGGGAGGCTGCTGG; *Nanog* gene: forward CAGCCCTGATTCTTCCACCAGTCCC, reverse TGGAACCAGGTCTTCACCTGTTTG; *β-actin* gene: forward CCTGGCACCCAGCACAAT, reverse GGGCCGGACTCGTCATAC; *GAPDH* gene: forward TGCACCACCAACTG, reverse GGCATGGACTGTGG. Human skin and heart fibroblasts were used as negative controls while lung and kidney cells of human embryos served as positive controls. Fluorescent high-resolution DNA melting curves of PCR products were used to determine the specificity of amplified products in real-time PCR.

RESULTS

RA myocardial appendage tissues were delivered within 1 h after coronary artery bypass surgery (Fig. 1a). Under sterile conditions, tissues were minced with scissors into 1- to 3-mm^3 pieces (Fig. 1b), washed with PBS, treated with 0.1% collagenase A in IMDM medium twice (Fig. 1c), and washed with the culture medium. The cell suspension was seeded in fibronectin-coated plates (Fig. 1d). Every 3 days, the culture medium was partially changed (about half of the total volume). Explants were cultured until transparent round cells appeared on the fibroblast monolayer (21 days) (Fig. 2c). The average number of c-kit(+)Lin(−) cells in these cultures was 3329 ± 823/per 10^5 explanted cells. The CPC c-kit(+)Lin(−) number negatively correlated with end-diastolic dimension ($r = -0.59$, $p = 0.05$) and was significantly less in patients with chronic heart failure (2413 ± 1297 and 3635 ± 304, $p = 0.033$) compared to subjects with preserved left ventricular systolic function. This finding seems to indicate a decline of these cells under insufficient blood supply due to cell death or constant lack of nutrients.

Immunomagnetic selection performed after culture of explanted cell for 21 days enriched the culture with residential c-kit(+)Lin(−) CPC. Bone marrow cells were depleted with a human Lineage Cell Depletion Kit (Miltenyi Biotec, United States). Cell cultures with depleted bone marrow cells were used for residential CPC selection by human CD117 MicroBead Kit. Cultures enriched with c-kit(+)Lin(−) cells were maintained in Cardiac Cellulation Media (Fig. 3). The population doubling time of c-kit(+)Lin(−) cells from the RA appendage was 38.5 ± 4.5 h, and CPCa exhibited major properties of stem (progenitor) cells. For example, CPCs generated cell aggregates in culture with low serum content and 2–5% of c-kit(+)Lin(−) cells were clonogenic, which is comparable with the potential of bone marrow mesenchymal stromal cells.

The cells did not carry hematopoietic markers or markers of mature bone marrow or cardiac mast cells CD34 and CD45 (Fig. 4). Most cells expressed mesenchymal stem cell marker CD105 but not CD90. A small number of cells exhibited KDR and Notch1 markers common for CPCw with vasculogenic potential (Bearzi et al., 2009). c-kit(+)Lin(−) cells did not stain with antibodies to sarcomeric α-actin or α-actinin (cardiomyocyte markers), as well as von Willebrand factor or PEGAM/CD31 (vascular markers). Like embryonic stem cells, c-kit(+) cells expressed pluripotent genes (*Oct4, Sox2, KLF4, c-myc*, and *Nanog*) (Fig. 4) indicating c-kit(+)Lin(−) cells' capability for self-renewal, proliferation, and maintenance of undifferentiated status in vitro.

An important property of residential CPCs is multipotency (Fig. 5). Cardiogenic differentiation of c-kit(+)Lin(−) cells was induced by the cell cultivation in Cardiac Differentiation Media included TGFβ (an embryonic cardial differentiation factor) and synthetic nucleoside 5-aza-cytidine applied as a DNA methylation inhibitor (Smits et al., 2009b). c-kit(+)Lin(−) cell cultivation in this differentiation medium did not induce the appearance of contractile structures but facilitated the expression of Nkx 2.5 transcription factors and sarcomeric α-actin and α-actinin (markers of cardiomyocyte contractile apparatus). C-kit(+)Lin(−) cells cultured in differen-

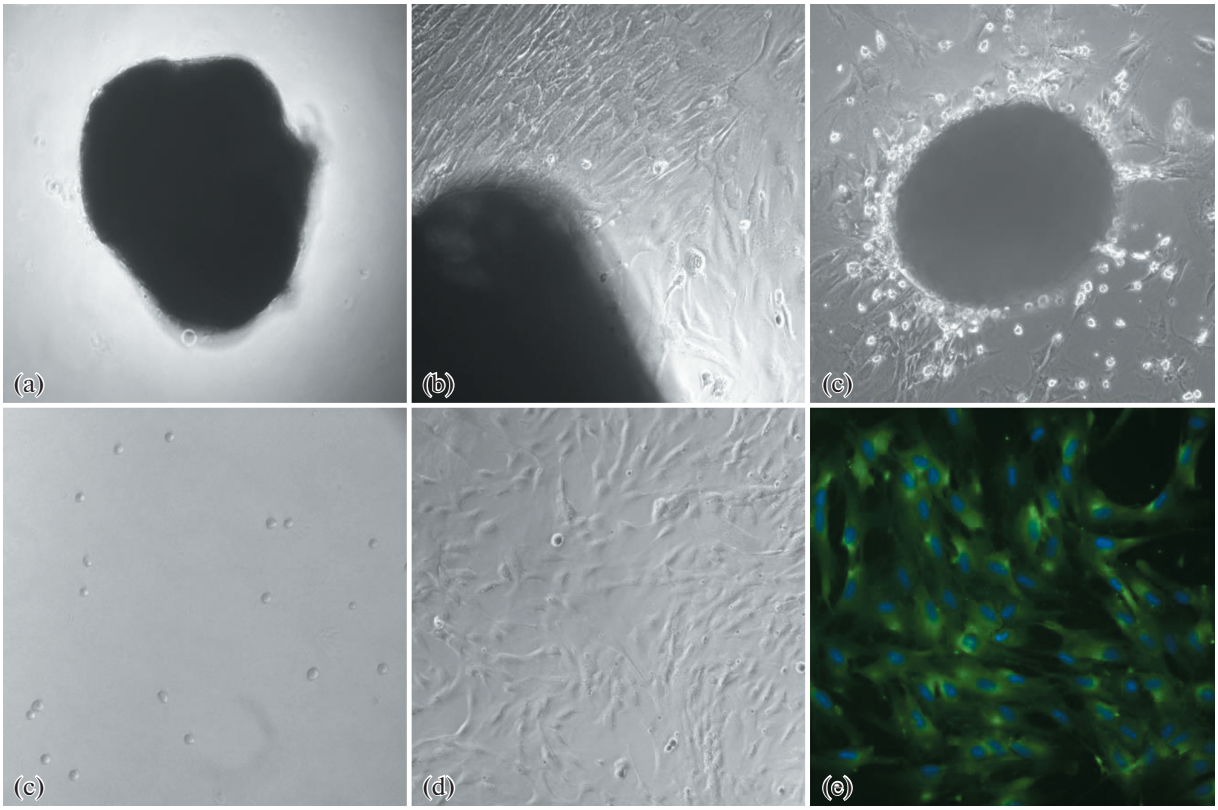

Fig. 2. Explant culture and primary CPC population. (a, b, c) Explant cultures after 48 h and 10 and 19 days of culture, respectively; (d, e) c-kit(+)Lin(−) CPC primary cultures 8 and 10 days after isolation, respectively; and (f) immunofluorescent staining of primary CPC culture with antibodies to c-kit-marker (*green*); nuclei are stained with DAPI (blue).

tiation medium with dexamethasone and VEGF produced capillary-like structures composed of cells expressing endothelial marker CD31 and von Willebrand factor.

DISCUSSION

The discovery of resident CPCs has revolutionized the field of regenerative medicine as drastically changed the idea that the heart is incapable of renewal. It has been shown that new cardiomyocytes are steadily generated from resident stem cells throughout life in states of health and disease (Anversa et al., 2013). However, the rate of renewal is a subject of debate (Bergmann et al., 2009; Kajstura et al., 2010). Nevertheless, endogenous regenerative potential of myocardium is insufficient to maintain cell homeostasis in chronic diseases (coronary heart disease, congestive heart failure, diabetes mellitus) or great myocardial injury (infarction) and aging. Under these conditions, the functional activity of CPCs (proliferation, migration, differentiation) declines and the number of CPCs undergoing apoptosis increases. Altogether, this results in a reduced number of functionally valuable CPCs and ineffective myocardial regeneration, leading to disease progression.

A solution to this problem may be cellular cardiomyoplasty based on autologous residential CPCs obtained from a patient and cultured in vitro to reach applicable volume of cellular mass. CPCs' capacity for cardiomyocyte and vascular differentiation makes them a unique object for restoration of lost myocardial cells. However, CPC isolation can be very challenging, as samples of very small volume (0.1–0.3 mg) may be obtained from a patient's heart, which limits the applicability of CPCs for transplantation.

Typically, intrasurgery myocardial biopsy is used as a source for cell isolation with most methods to isolate progenitor cells relying on enzymatic treatment followed by immunomagnetic selection using beads with antibodies to stem cell markers (Beltrami et al., 2003; Oh et al., 2003; Parfenova et al., 2009) or gradient centrifugation (Oh et al., 2011). However, myocardial biopsy is not a routine patient examination and is applied only under strict medical indications (progressing congestive heart failure of unknown etiology, rhythm and conduction disturbances of unknown origin, myocarditis and cardiomyopathy diagnostics). Therefore, biopsy of myocardial material is not an optimal source for CPC isolation. Moreover, enzymatic treatment of myocardial samples alters the

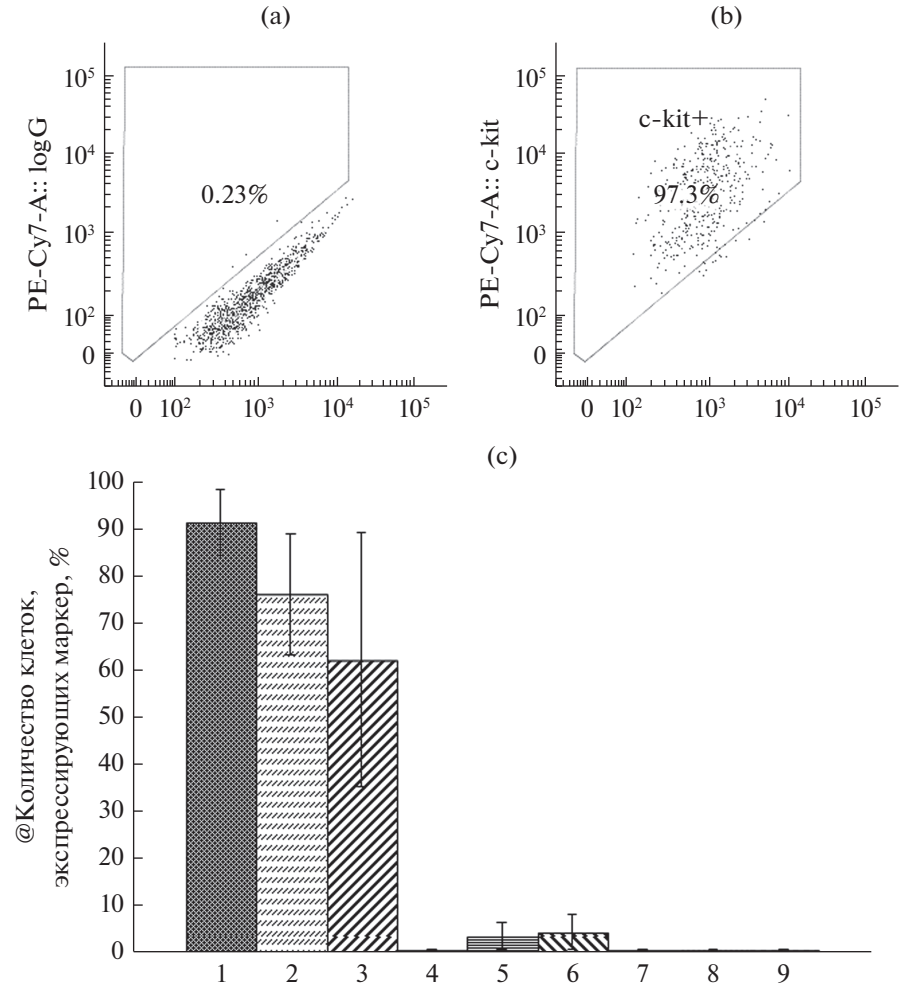

Fig. 3. Immunophenotype of human cardiac progenitor cells. CPC c-kit(+) distribution, flow cytometry. (a) Control antibodies, (b) antibodies to c-kit, and (c) quantification of various markers in CPC population: (1) c-kit, (2) sca-1, (3) D105, (4) CD90, (5) KDR, (6) Notch1, (7) CD34, (8) CD25, and (9) Lin.

expression of cell surface molecules, modifying CPC viability and proliferative potential.

Another approach to acquiring CPCs is cardiosphere production, which provides CPCs in numbers sufficient for autotransplantation (Messina et al., 2004). However, it also has drawbacks, as cardiospheres are cell aggregates composed of cells with different phenotypes and at various differentiation stages. These cells express markers of myocardial progenitor cells, as well as mesenchymal and endothelial cells and fibroblasts. It has been suggested that interaction between these cells facilitates myocardial progenitor cell differentiation into cardiomyocytes, endothelial and smooth muscle cells. Cardiomyocytes obtained from mouse and human cells have been seen to exhibit spontaneous contraction (Messina et al., 2004). To the contrary, some authors have failed to obtain functionally mature cardiomyocytes differentiated from cardiospheres (Shenje et al., 2008). In these studies, cardiospheres induced to differentiation did not exhibit expression of stem cell markers (c-kit, sca-1, SSEA-1) or the proliferation and differentiation potency common for stem cells. Another group (Andersen et al., 2009) also stated that cardiospheres do not possess cardiomyogenic potential and cardiosphere cell properties are explained by culture contamination with fragments of initial myocardial material or bone marrow stem cells rather than CPC proliferation and differentiation.

The CPC isolation method that we have described can be used to obtain progenitor cells from myocardium pieces from any human heart segments independent their size. This allows one to increase ex vivo the yield of residential CPCs by explant culture followed by selection of specific stem cell population using immunomagnetic sorting. We used the method of explants relying on the suggestion that microenvironment paracrine factors regulate the viability and proliferation of stem cells both in vitro and in vivo (Urbanek et al., 2006). Thereby, explant culture may

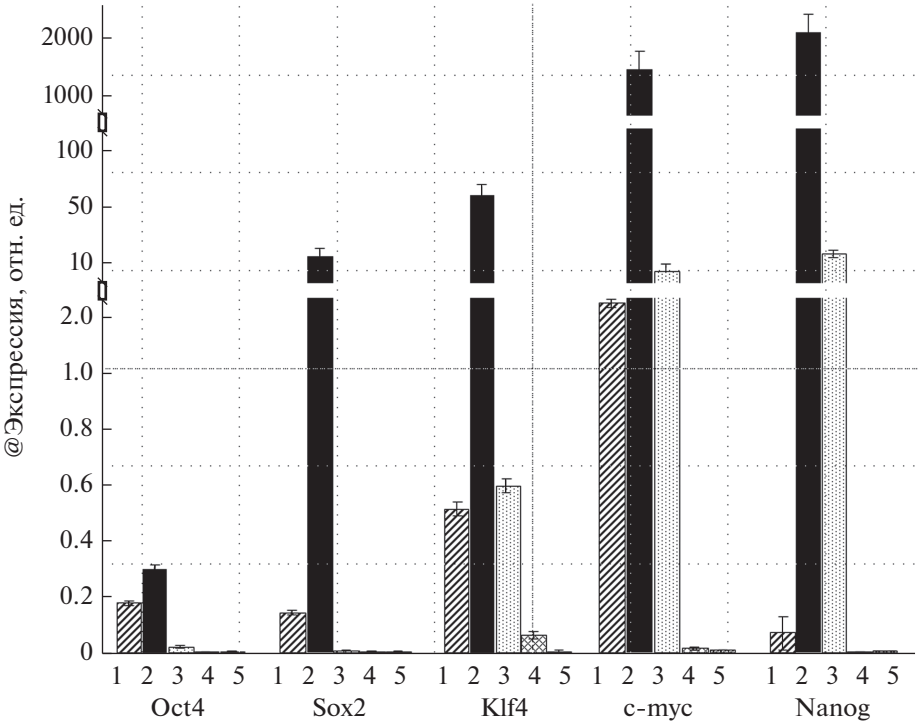

Fig. 4. Expression of pluripotent genes in (1) human CPCs, (2) embryonic lung cells, (3) embryonic kidney cells, (4) skin fibroblasts, and (5) heart fibroblasts.

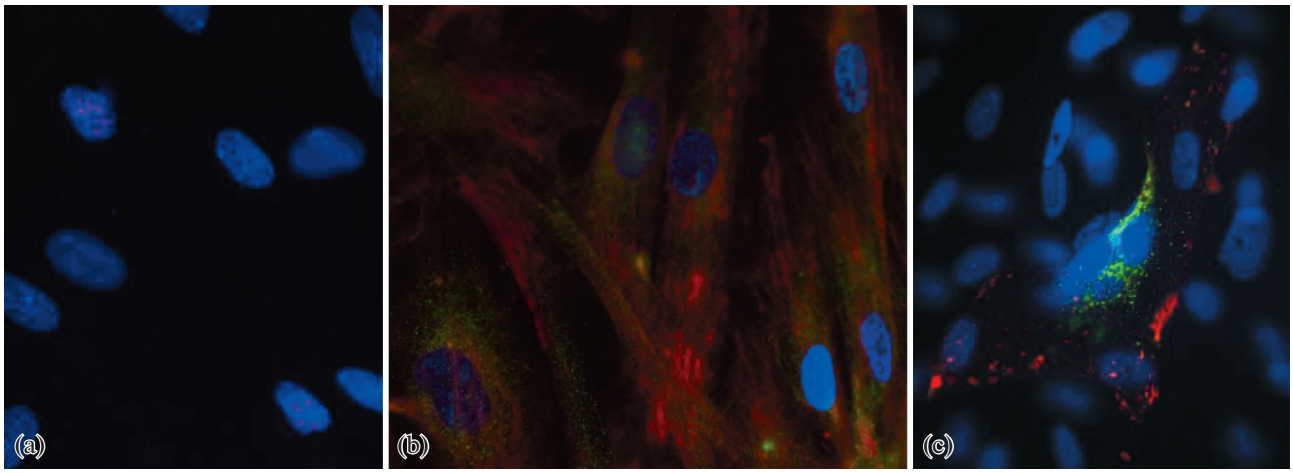

Fig. 5. Expression of cardiomyocyte and endothelial markers in CPCs cultured in differentiation media. Immunofluorescent staining with antibodies to (a) transcription factor Nkx2.5 (red); (b) sarcomeric actin (red) and actinin (green), cardiomyocyte markers; and (c) CD31 (red), von Willebrand factor (green), endothelial markers PECAM(green). Nuclei are stained with DAPI (blue).

mimic the cellular niche ex vivo maintaining proliferation of resident CPCs by autocrine/paracrine factors of the microenvironment.

The second stage of the protocol is hematopoietic cell depletion and selection of residential CPCs from explanted cells. A C-kit—a receptor of stem cell factor (SCF)—was used for identification and isolation of resident CPCs from rats, mice, dogs, and human beings (Beltrami et al., 2003; Oh et al., 2003; Linke et al., 2005; Urbanek et al., 2005). The properties of myocardial c-kit(+) cells (clonogenicity, migratory and differentiation potential) are significantly better than those of other CPC types (sca-1(+) and MDR1(+) cells) (Linke et al., 2005).

CPCs isolated from explant culture express c-kit receptor on their surface and are capable of renewal in vitro. Clones of kit(+)Lin(−) cells from the RA appendage express pluripotent genes common to embryonic stem cells (*Oct4, Sox2, Klf4, c–myc, Nanog*) required for self-renewal of stem cells (Nichols et al., 1998; Avilion et al., 2003; Miyagi et al., 2008). Expression of these genes regulates the undifferentiated state of embryonic stem cells, mediates their unlimited proliferation in vitro, and indicates low differentiation of CPCs.

It should be noted that the number of CPCs in explant culture was reduced in samples obtained from patients with systolic heart failure. This may be because CPCs undergo cell death by necrosis or apoptosis in patients with heart failure due to insufficient blood supply. Release of active compounds and inflammation products during cell death in the injured area inhibits CPC proliferation and differentiation, resulting in even further decline of their number (Liu et al., 2011). Another major issue may be increased angiotensin 2 production by hypertrophic cardiomyocytes (Traktuev et al., 2009; Casellas, 2011), as its binding to AT1 receptor accelerates CPC aging and may reduce their survival potency (Renny et al., 2014; Chalothorn et al., 2010).

In our study, myocadial c-kit(+)Lin(−) cells obtained from patients with ischemic heart disease were clonogenic and prone to differentiation into vascular and cardiomyocyte lineages, indicating their multipotency. CPCs cultured in medium with 5-azacytidine and TGF-β expressed mature cardiomyocyte proteins (Nkx 2.5, sarcomeric actin, and α-actinin). However, we observed no cells with cross-striated sarcomeres capable of spontaneous contraction; i.e., the differentiation process was incomplete and no mature cardiomycytes were produced. Differentiation agents triggered endothelial differentiation in c-kit(+)Lin(−) cells to cells expressing mature endothelial markers—CD31 and vWF. C-kit and Sca-1 are considered markers of endothelial cell progenitors (Takamiya et al., 2006; Kaminski et al., 2008), and it has been suggested that c-kit(+)Lin(−) cells from the RA appendage are endothelial progenitor cells with limited capacity to differentiate into cardiomyocytes (Sandstedt et al., 2010). The authors found that c-kit(+) cells carried CD31, CD34, CXCR4, and Flk-1 surface markers and had an expression pattern resembling mature endothelium. Our results show that c-kit(+)Lin(−) cells from the AR appendage do not express endothelial markers ex vivo and endothelial differentiation may be triggered only by specific differentiation agents, which is typical for multipotent cells.

In conclusion, this method based on explant culture and immunomagnetic separation is applicable for isolation of c-kit(+) cells from a human RA appendage or ventricular tissue. These cells in vitro show clonogenicity and capability for self-renewal and express pluripotency genes common to embryonic stem cells, indicating the presence of poorly differentiated cells in the human CPC population. Certain clones of these cells may be induced to differentiate into cardiomyocyte and endothelial lineages, yet cardiomyocyte-like cells were immature in terms of their contractile function. Isolation of human c-kit(+) CPC using the method that has been elaborated will facilitate evaluation of their reparative properties in animal models so as to advance the application of stem cells towards in patients.

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REFERENCES

Andersen, D.C., Andersen, P., Schneider, M., Jensen, H.B., and Sheikh, S.P., Murine "cardiospheres" are not a source of stem cells with cardiomyogenic potential, *Stem Cells*, 2009, vol. 27, pp. 1571–1581.

Anversa, P., Kajstura, J., Rota, M., and Leri, A., Regenerating new heart with stem cells, *J. Clin. Invest.*, 2013, vol. 123, pp. 62–70.

Avilion, A.A., Nicolis, S.K., Pevny, L.H., Perez, L., Vivian, N., and Lovell-Badge, R., Multipotent cell lineages in early mouse development depend on SOX2 Function, *Gen. Dev.*, 2003, vol. 17, pp. 126–140.

Bearzi, C., Rota, M., Hosoda, T., Tillmanns, J., Nascimbene, A., De Angelis, A., Yasuzawa-Amano, S., Trofimova, I., Siggins, R.W., Lecapitaine, N., Cascapera, S., Beltrami, A.P., D'Alessandro, D.A., Zias, E., Quaini, F., Urbanek, K., Michler, R.E., Bolli, R., Kajstura, J., Leri, A., and Anversa, P., Human cardiac stem cells, *Proc. Natl. Acad. Sci. U. S. A.*, 2007, vol. 104, pp. 14068–14073.

Bearzi, C., Leri, A., Lo Monaco, F., Rota, M., Gonzalez, A., Hosoda, T., Pepe, M., Qanud, K., Ojaimi, C., Bardelli, S., D'Amario, D., D'Alessandro, D.A., Michler, R.E., Dimmeler, S., Zeiher, A.M., Urbanek, K., Hintze, T.H., Kajstura, J., and Anversa, P., Identification of a coronary vascular progenitor cell in the human heart, *Proc. Natl. Acad. Sci. U. S. A.*, 2009, vol. 106, pp. 15885–15890.

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., Adult cardiac stem cells are multipotent and support myocardial regeneration, *Cell*, 2003, vol. 114, pp. 763–768.

Bergmann, O., Bhardwaj, R.D., Bernard, S., Zdunek, S., Barnabé-Heider, F., Walsh, S., Zupicich, J., Alkass, K, Buchholz, B.A., Druid, H., Jovinge, S., and Frisén, J., Evidence for cardiomyocyte renewal in humans, *Science*, 2009, vol. 324, pp. 98–102.

Casellas, J., Inbred mouse strains and genetic stability: a review, *Animal*, 2011, vol. 5, pp. 1–7.

Chalothorn, J.E. and Faber, J.E., Strain-dependent variation in collateral circulatory function in mouse hindlimb, *Physiol. Genomics*, 2010, vol. 42, pp. 469–479.

Fisher, S.A., Zhang, H., Doree, C., Mathur, A., and Martin-Rendon, E., Stem cell treatment for acute myocardial infarction, *Cochrane Database Syst. Rev.*, 2015, vol. 9, pp. 6536–6551.

Goichberg, P., Chang, J., Liao, R., and Leri, A., Cardiac stem cells: biology and clinical applications, *Antiox. Redox. Signal.*, 2014, vol. 21, pp. 2002–2017.

Kajstura, J., Urbanek, K., Perl, S., Hosoda, T., Zheng, H., Ogórek, B., Ferreira-Martins, J., Goichberg, P., Rondon-Clavo, C., Sanada, F., D'Amario, D., Rota, M., Del, Monte, F., Orlic, D., Tisdale, J., Leri, A., and Anversa, P., Cardiomyogenesis in the adult human heart, *Circ. Res.*, 2010, vol. 107, pp. 305–315.

Kaminski, A., Ma, N., Donndorf, P., Lindenblatt, N., Feldmeier, G., Ong, L.L., Furlani, D., Skrabal, C.A., Liebold, A., Vollmar, B., and Steinhoff, G., Endothelial NOS is required for SDF-1alpha/CXCR4-mediated peripheral endothelial adhesion of C-kit+ bone marrow stem cells, *Lab. Invest.*, 2008, vol. 88, pp. 58–69.

Kikuchi, K. and Poss, K.D., Cardiac regenerative capacity and mechanisms, *Ann. Rev. Cell Dev. Biol.*, 2012, vol. 28, pp. 719–741.

Leri, A., Rota, M., Hosoda, T., Goichberg, P., and Anversa, P., Cardiac stem cell niches, *Stem Cell Res.*, 2014, vol. 13, pp. 631–646.

Linke, A., Müller, P., Nurzynska, D., Casarsa, C., Torella, D., Nascimbene, A., Castaldo, C., Cascapera, S., Böhm, M., Quaini, F., Urbanek, K., Leri, A., Hintze, T.H., Kajstura, J., and Anversa, P., Stem cells in the dog heart are self-renewing, clonogenic, and multipotent and regenerate infarcted myocardium, improving cardiac function, *Proc. Natl. Acad. Sci. U. S. A.*, 2005, vol. 102, pp. 8966–8971.

Liu, L. and Rando, T.A., Manifestations and mechanisms of stem cell aging, *J. Cell Biol.*, 2011, vol. 193, pp. 257–266.

Messina, E., De, Angelis, L., Frati, G., Morrone, S., Chimenti, S., Fiordaliso, F., Salio, M., Battaglia, M., Latronico, M.V., Coletta, M., Vivarelli, E., Frati, L., Cossu, G., and Giacomello, A., Isolation and expansion of adult cardiac stem cells from human and murine heart, *Circ. Res.*, 2004, vol. 95, pp. 911–921.

Miyagi, S., Masui, S., Niwa, H., Saito, T., Shimazaki, T., Okano, H., Nishimoto, M., Muramatsu, M., Iwama, A., and Okuda, A., Consequence of the loss of Sox2 in the developing brain of the mouse, *FEBS Lett.*, 2008, vol. 582, pp. 2811–2815.

Nichols, J., Zevnik, B., Anastassiadis, K., Niwa, H., Klewe-Nebenius, D., Chambers, I., Schöler, H., and Smith, A., Formation of pluripotent stem cells in the mammalian embryo depends on the POU transcription factor Oct4, *Cell*, 1998, vol. 95, pp. 379–391.

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., Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction, *Proc. Natl. Acad. Sci. U. S. A.*, 2003, vol. 100, pp. 12313–1238.

Oh, H., Tateishi, K., and Matsubara, H., Pluripotent stem cell derived from cardiac tissue, Patent EP 2295541 A1 (Kyoto University), 2011.

Parfyonova, E.V., Tkachuk, V.A., Akchurin, R.S., Rubina, K.A., Kalinina, N.I., Melihova, V.S., Rahmat-Zade, T.M., Tsokolaeva, Z.I., and Sysoeva, V.U., A method of isolation and directed differentiation of multipotent cell culture for cell therapy and / or tissue engineering in the area of myocardial ischemia, RF Patent no. 2366706, 2010.

Renny, M., Julio, N.B., Bernardi, S.F., Gardenal, C.N., and Oyarzabal, M.I., Estimation of genetic variability level in inbred CF1 mouse lines selected for body weight, *J. Genet.*, 2014, vol. 93, pp. 483–487.

Sandstedt, J., Jonsson, M., Lindahl, A., Jeppsson, A., and Asp, J., C-kit+ CD45-cells found in the adult human heart represent a population of endothelial progenitor cells, *Basic Res. Cardiol.*, 2010, vol. 105, pp. 545–556.

Shenje, L.T, Field, L.J, Pritchard, C.A, Guerin, C.J, Rubart, M., Soonpaa, M.H., Ang, K.L., and Galiñanes, M., Lineage tracing of cardiac explant derived cells, *PLoS One*, 2008, vol. 3, p. 1929.

Smits, A.M., van Laake, L.W., den Ouden, K., Schreurs, C., Szuhai, K., van Echteld, C.J., Mummery, C.L., Doevendans, P.A., and Goumans, M.J., Human cardiomyocyte progenitor cell transplantation preserves long-term function of the infarcted mouse myocardium, *Cardiovasc. Res.*, 2009a, vol. 83, pp. 527–535.

Smits, A.M., van Vliet, P., Metz, C.H., Korfage, T., Sluijter, J.P., Doevendans, P.A., and Goumans, M.J., Human cardiomyocyte progenitor cells differentiate into functional mature cardiomyocytes: an in vitro model for studying human cardiac physiology and pathophysiology. *Nat. Protoc.*, 2009b, vol. 4, pp. 232–243.

Takamiya, M., Okigaki, M., Jin, D., Takai, S., Nozawa, Y., Adachi, Y., Urao, N., Tateishi, K., Nomura, T., Zen, K, Ashihara, E., Miyazaki, M., Tatsumi, T., Takahashi, T., and Matsubara, H., Granulocyte colony-stimulating factor-mobilized circulating C-Kit+/Flk-1+ progenitor cells regenerate endothelium and inhibit neointimal hyperplasia after vascular injury, *Arterioscler. Thromb. Vasc. Biol.*, 2006, vol. 26, pp. 751–757.

Traktuev, D.O, Prater, D.N., Merfeld-Clauss, S., Sanjeevaiah, A.R., Saadatzadeh, M.R., Murphy, M., Johnstone, B.H., Ingram, D.A., and March, K.L., Robust functional vascular network formation in vivo by cooperation of adipose progenitor and endothelial cells, *Circ. Res.*, 2009, vol. 104, pp. 1410–1420.

Urbanek, K., Rota, M., Cascapera, S., Bearzi, C., Nascimbene, A., De Angelis, A., Hosoda, T., Chimenti, S., Baker, M., Limana, F., Nurzynska, D., Torella, D., Rotatori, F., Rastaldo, R., Musso, E., Quaini, F., Leri, A., Kajstura, J., and Anversa, P., Cardiac stem cells possess growth factor–receptor systems that after activation regenerate the infarcted myocardium, improving ventricular function and long–term survival, *Circ. Res.*, 2005, vol. 97, pp. 663–673.

Urbanek, K., Cesselli, D., Rota, M., Nascimbene, A., De, Angelis, A., Hosoda, T., Bearzi, C., Boni, A., Bolli, R, Kajstura, J., Anversa, P., and Leri, A., Stem cell niches in the adult mouse heart, *Proc. Natl. Acad. Sci. U. S. A.*, 2006, vol. 103, pp. 9226–9231.

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