Cardiac stem cells in the real world

Julia Pouly, MD,^a Patrick Bruneval, MD,^b Chantal Mandet,^b Suzanne Proksch, PhD,^c Séverine Peyrard, MSc,^d Catherine Amrein, MD,^e Véronique Bousseaux, MD,^e Romain Guillemain, MD,^e Alain Deloche, MD,^f Jean-Noel Fabiani, MD, PhD,^g and Philippe Menasché, MD, PhD^g

Objective: Cardiac stem cell transplantation as a potential means of regenerating infarcted myocardium is currently receiving a great deal of interest. However, data on these endogenous cardiac precursors are primarily derived from animal studies, and their clinical relevance still remains elusive.

Methods: We prospectively screened 32 endomyocardial biopsies harvested from heart transplant recipients (off rejection episodes) and 18 right appendage biopsies collected during coronary artery bypass surgery, and processed the tissue specimens for the immunohistochemical detection of markers of stemness (*c-kit*, MDR-1, Isl-1), hematopoietic origin (CD45), mast cells (tryptase), endothelial cells (CD105), and cardiac lineage (Nkx2.5). Confocal microscopy was used for colocalization experiments. Three right appendage biopsies were also cultured for 2 to 3 weeks, at the completion of which *c-kit*-positive cells were sorted by flow cytometry.

Results: In endomyocardial biopsies, a median number of 2.7 (1.8–4) *c-kit*-positive cells/mm² were found, and this number was even significantly smaller in right appendage biopsies (1 [0.5–1.8] *c-kit*-positive cell/mm², *P* = .01). All of these *c-kit*-positive cells co-stained for CD45 and were more specifically identified as mast cells by their positive staining for the specific tryptase marker. However, none of the *c-kit*-positive cells expressed the markers of stemness MDR-1 and Isl-1 or colocalized with CD105. Flow cytometry confirmed the small number of *c-kit*-positive cells in cultured right atrial appendages.

Conclusion: These data raise a cautionary note on the therapeutic exploitation of cardiac stem cells in patients with ischemic cardiomyopathy, who may be the elective candidates for regenerative therapy.

In the field of cardiac development, one of the most controversial concepts developed during the past few years has been the recognition that (in contrast with a long-standing dogma) the heart is not a terminally differentiated organ but that it harbors a population of stem cells ensuring the lifelong renewal of cardiomyocytes and the ability to divide further in response to injury.^{1,2} Aside from the cognitive implications of such a paradigm for a better understanding of cardiac developmental biology, this finding was soon recognized to be important for fostering strategies of regenerative therapies aimed at alleviating the consequences of heart failure. In the ongoing quest for the ideal cell to be used for reconstituting a functional myocardium, these cardiac stem cells (CSCs) might become major players because of their 2 major characteristics: an autologous origin, overcoming the ethical, immunologic, and technical challenges associated with embryonic stem cells, and a cardiogenic differentiation, potentially leading to the generation of new cardiomyocytes expected to express, among others, the gap junction proteins required for electromechanical coupling with those of the host myocardium. As such, these cells should allow the formation of a functional syncytium, which is a prerequisite for the graft to beat in synchrony with the remainder of the heart and thus contribute to augment its pump function.

To the present, studies on CSCs have primarily focused on animal models, and few clinical data are available. The present study was designed to assess whether these cells could be identified in the myocardium in patients (particularly those with

From the Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Department of Cardiovascular Surgery; University Paris-5, Faculty of Medicine,^a Paris, France; Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Department of Pathology; University Paris-5, Faculty of Medicine, INSERM U 872,^b Paris, France; INSERM U 633, Laboratory of Biosurgical Research,^c Paris France; Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Epidemiology and Clinical Research Unit, INSERM CIE4,^d Paris, France; Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Department of Anesthesiology,^e Paris, France; Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Department of Cardiovascular Surgery; University Paris-5, Faculty of Medicine, INSERM U 633,^f Paris, France; and Assistance Publique-Hôpitaux de Paris, Hôpital Européen Georges Pompidou, Department of Cardiovascular Surgery; University Paris-5, Faculty of Medicine,^g Paris, France.

This study was partly funded by a grant from the Foundation LeDucq (Cardiac Progenitors Transatlantic Alliance network).

Received for publication July 31, 2007; revisions received Sept 26, 2007; accepted for publication Oct 26, 2007.

Address for reprints: Philippe Menasché, MD, PhD, Department of Cardiovascular Surgery, Hôpital Européen Georges Pompidou, 20, rue Leblanc, 75015 Paris, France (E-mail: philippe.menasche@egp.aphp.fr).

J Thorac Cardiovasc Surg 2008;135:673-8
0022-5223/\$34.00

Copyright © 2008 by The American Association for Thoracic Surgery

doi:10.1016/j.jtcvs.2007.10.024

Abbreviations and Acronyms

CABG = coronary artery bypass grafting
 CSC = cardiac stem cell
 EMB = endomyocardial biopsy

ischemic heart disease) in such a way that their subsequent isolation, expansion, and reinjection could become a clinically relevant procedure.

Materials and Methods**Patients**

After informed written consent and board approval were obtained, percutaneous right ventricular endomyocardial biopsies (EMBs) were performed during routine follow-up visits of heart transplant recipients. In another set of experiments, right atrial appendages were removed after right atrial cannulation during elective coronary artery bypass grafting (CABG) in patients with chronic ischemic cardiomyopathy. All samples were fixed in formalin and embedded in paraffin. Tissue sections (5- μ m thick) were subsequently immunostained for the expression of different cell markers.

Immunohistochemistry

Samples were processed as previously described³ using antibodies recognizing markers of stemness (*c-kit*, dilution 1/50, Dako, Trappes, France; MDR-1, 1/250, Proteogenix, Oberhausbergen, France; Isl-1, 1/50, Developmental Studies Hybridoma Bank, Iowa City, Iowa), hematopoietic origin (CD45, 1/100, Dako), mast cells (tryptase, 1/200, Dako), endothelial cells (CD105, 1/100, Abcam, Cambridge, UK), and cardiac lineage (Nkx2.5, 1/50, R&D, Minneapolis, Minn). Both negative and positive controls were used to assess the specificity of the labeling. Negative controls were obtained by replacing primary mouse monoclonal antibodies against MDR-1, Isl-1, CD45, tryptase, Nkx2.5, and CD105 by normal mouse serum and rabbit polyclonal antibody against *c-kit* by normal rabbit serum. Positive controls were as follows: *c-kit*-positive tumor cells in a gastrointestinal stromal tumor; MDR-1-positive tubules in a human kidney obtained from the normal part of a nephrectomy specimen for cancer; Isl-1-positive nuclei in Langerhans islet cells obtained from a pancreas removed for neonatal hypoglycemia; tryptase-positive mast cells in an inflammatory skin lesion; CD105-positive endothelial cells in cardiac tissue; and Nkx2.5-positive nuclei in normal adult cardiac myocytes.

Cell Counts

Whole labeled tissue sections were scanned using an eyepiece grid with a microscope at $\times 20$ magnification. The number of *c-kit*-positive cells was normalized per square millimeter to account for the variable number of examined fields per patient, which in turn reflected the variable size of the tissue samples available for analysis.

Combined Immunofluorescence and Confocal Laser Microscopy

These procedures were performed as previously described.⁴ In brief, the rabbit polyclonal anti-*c-kit* antibody was first incubated and revealed by using a biotinylated anti-rabbit antibody (Dako) and

streptavidin-cyanin-2 (Amersham, Les Ulis, France) or by using an Alexa 555-labeled anti-rabbit antibody (Invitrogen, Cergy-Pontoise, France); monoclonal antibodies against tryptase or CD45 were then incubated and revealed using a cyanin-3-labeled anti-mouse antibody, and Nkx2.5 and CD105 antibodies were revealed using a biotin-labeled antimouse antibody and streptavidin-cyanin-2. Double immunofluorescence was assessed with a confocal laser microscope (Leica, Rueil-Malmaison, France).

Cell Cultures

Three intraoperatively harvested right appendages were cultured according to previously described techniques developed to grow CSCs^{5,13,14} and assessed for the expression of *c-kit* by flow cytometry. Data were collected with CellQuest software and analyzed with ModFitLT2.0 software (BD Biosciences, Le-Pont-de-Claix, France).

Statistics

The results are expressed as means $\pm$ 1 standard deviation or median values (interquartile range). Comparison of *c-kit*-positive cell numbers between EMB and right appendage samples was performed by the Wilcoxon test, and the level of statistical significance was set at the .05 level.

Results

EMBs were obtained in 32 heart transplant recipients. The mean ages of these patients and their donors were 45.8 ± 11 years and 40.1 ± 10 years, respectively. The time since transplantation was 73.5 ± 77 months, and the percentages of grade 0 and 1A pathologic rejection were 62.5% and 37.5%, respectively. Right appendage tissue was collected in 18 patients (mean age: 65.3 ± 8.1 years; male/female ratio: 16/2) undergoing elective isolated CABG.

The median number of microscopic fields assessed per patient was 14 (range: 10.5–17.5) for EMBs and 30 (17–45) for right appendage biopsies. In EMBs, a median number of 2.7 [1.8;4] *c-kit*-positive cells/mm² were found, regardless of the age of the donor and the time interval between transplantation and biopsy (Figure 1, A). This number was significantly smaller in right appendage biopsies (1 [0.5;1.8] *c-kit*-positive cell/mm², $P = .01$ vs EMB, Figure 1, B). Colocalization experiments demonstrated that all these *c-kit*-positive cells were of hematopoietic origin, as evidenced by their positive staining for CD45 (Figure 1, C). Their perivascular location suggested that the *c-kit*-positive cells could be mast cells, and this phenotype was confirmed by a consistently positive staining for the specific tryptase marker (Figure 1, D). However, none of the *c-kit*-positive cells identified in these tissue samples expressed the markers of stemness (MDR-1, Isl-1), whereas the antibodies used strongly stained control tissues (Figure 2, A and B). Furthermore, the *c-kit*-positive cells did not colocalize with the cardiac lineage marker Nkx2.5 (Figure 2, C). Likewise, the CD105 marker stained endothelial cells in both EMBs and right atrial biopsies, but there was no overlay between this staining and the expression of the stem cell marker *c-kit* (Figure 2, D). The culture data tended

Figure 1. A, Detection of the *c-kit* marker by immunohistochemistry. Two mast cells (arrows) are identified in the interstitium of the myocardium in an EMB ($\times 250$). B, Detection of the *c-kit* marker by immunohistochemistry. Mast cell (arrow) is identified in a right atrial appendage specimen ($\times 200$). C, Mast cell (arrow) co-expresses *c-kit* and the hematopoietic lineage marker CD45 (combined immunofluorescence with rabbit polyclonal anti-*c-kit* antibody revealed with cyanin-2 green fluorescence and mouse monoclonal anti-CD45 antibody revealed with cyanin-3 red fluorescence) ($\times 150$). D, Mast cell co-expresses *c-kit* and the mast cell lineage marker tryptase (combined immunofluorescence with rabbit polyclonal anti-*c-kit* antibody revealed with cyanin-2 green fluorescence and mouse monoclonal anti-tryptase antibody revealed with cyanin-3 red fluorescence) ($\times 2,000$).

to support the scarcity of *c-kit*-positive cells in right appendage tissue because they represented only $0.58\% \pm 0.67\%$ of cells identified by flow cytometry after a 3- to 4-week period of cultures.

Discussion

The major finding of the present study is that cells expressing the classic markers of stemness are rare in the right append-

age and septal walls of adult patients, particularly those with ischemic heart disease, and that their phenotype is consistent with that of mast cells and not of precursors of the cardiomyocytic lineage.

Methodologic Issues

One major issue raised by CSCs is the multiplicity of markers that have been described to characterize them. At least 4

Figure 2. A, Anti-MDR-1 immunohistochemistry. In the normal kidney used as positive control tissue, the MDR-1 antibody detects a strong signal in the tubules ($\times 350$). B, Anti-islet-1 immunohistochemistry. In the neonatal pancreas used as positive control tissue, islet-1 is expressed in the nuclei of the Langerhans islets ($\times 500$). C, *c-kit*-positive mast cells (arrows) do not express the cardiac myocyte lineage marker Nkx2.5 (arrowheads) (combined immunofluorescence with rabbit polyclonal anti-*c-kit* antibody revealed with cyanin-3 red fluorescence and mouse monoclonal anti-Nkx2.5 antibody revealed with cyanin-2 green fluorescence) ($\times 200$). D, *c-kit*-positive mast cells (arrows) do not express the endothelial cell lineage marker CD105 (arrowheads) (combined immunofluorescence with rabbit polyclonal anti-*c-kit* antibody revealed with Alexa 555-red fluorescence and mouse monoclonal anti-CD105 antibody revealed with cyanin-2 green fluorescence) ($\times 350$).

different markers, which are at times mutually exclusive, have been described: *c-kit*,^{5,6} Sca-1,⁷ side population,^{8,9} and Isl-1.¹⁰ It is difficult to reconcile the multiplicity of these phenotypic patterns with the limited (if any) self-regenerative capacity of the heart. Because the CSC population is not characterized in a more consistent way, we selected to identify these cells on the basis of *c-kit*, the receptor of stem cell factor, because experimentally clonogenic *c-kit*-positive cells have been reported to feature a greater ability to generate myocardial cells.⁵

Our results show that there are few *c-kit*-positive cells in the right septal wall of presumably normal adult hearts (those used for transplantation) and that this number is still lower in patients undergoing CABG for ischemic heart disease, who are actually better representatives of the target population

for regenerative therapy. The perivascular location of these *c-kit*-positive cells was consistent with a mast cell phenotype, which was confirmed by their expression of the panhematopoietic marker CD45, the mast cell-specific marker tryptase (and an additional positive staining for toluidine blue; data not shown). By itself, this finding is not unexpected because the right appendage has been shown to harbor 0.5% to 1.5% of mast cells,¹¹ and both cyclosporine and FK506 have been reported to up-regulate the expression of *c-kit* on a mast cell line,¹² which could account for the greater number of the *c-kit*-positive cells in our EMB-derived tissue samples. The important observation is that none of these *c-kit*-positive cells expressed any of the other tested markers of stemness (MDR-1 and Isl-1) or of the more specific cardiac differentiation pathway (Nkx 2.5), thereby raising serious doubts

about the presence (or the persistence) of a reservoir of CSCs in the adult myocardium. These findings are consistent with those of Yamabi and colleagues,¹³ who reported that *c-kit*-positive cells isolated from human fetal hearts were negative for cardiac markers. Their results differ from the experimental observation that cardiac *c-kit*-positive cells are lineage-negative but overall fit the paradigm that these progenitors are rare, on the order of 1 per 1×10^4 myocytes.¹⁴ Whether the hematopoietic *c-kit*-positive cells that we have identified normally reside in cardiac niches or dynamically migrate from the bone marrow cannot be determined from the present study. Likewise, our findings do not exclude that an initially present pool of CSCs was subsequently exhausted because of senescence or after its mobilization for repairing ischemic injuries because cardiac failure in humans has been reported to cause a 10-fold increase in the number of dividing cardiomyocytes compared with healthy hearts.¹⁵ This compensatory phenomenon may have emptied the reservoir of CSCs. The hypothesis that cardiac progenitors may disappear over time is also supported by the finding made in tissue samples retrieved during pediatric heart operations that Isl-1-positive cells have only been detected up to the age of 8 weeks.¹⁰

Comparison With Previous Studies

Studies performed in rat,⁶ dog,⁵ and pig¹⁶ have demonstrated that CSCs differentiated into cardiomyocytes after engraftment into infarcted myocardium, reduced infarct size, attenuated remodeling, and improved left ventricular function. In contrast, human data are limited to 3 clinical studies. In the study by Urbanek and colleagues,¹⁷ samples were retrieved from the hypertrophied left ventricular wall of patients undergoing aortic valve surgery, which makes the comparison difficult with our data. Conversely, the study by Messina and colleagues¹⁸ is more similar to ours in that it entailed derivation of human tissue from atrial or ventricular biopsy specimens taken during cardiac operations and subsequent culture that resulted in self-adherent clusters called *cardiospheres*. These cardiospheres expressed endothelial and stem cell markers (including *c-kit*), and cells derived from them displayed morphologic and phenotypical patterns characteristic of cardiomyocytes. A similar approach was subsequently described by Smith and colleagues,¹⁶ who used human right ventricular biopsy specimens to grow cardiospheres made of a mix population of cardiac progenitors, fibroblasts, and mesenchymal cells. These cardiospheres were primarily defined by a consistent expression of *c-kit* and CD105, which contrasts with our finding that these 2 markers were not coexpressed by the same cells and suggests that CD105 expression in cardiospheres may have reflected a contamination by endothelial cells. Although the putative CSCs did not spontaneously beat unless they were cocultured with rat cardiomyocytes in these 2 studies, their injection of the border zones of acute myocardial infarction in immunodeficient mice resulted in improved function and histologic

patterns of cardiac regeneration. Altogether, these data have raised the appealing hypothesis that CSCs could be retrieved from an EMB¹⁶ or pharmacologically mobilized by growth factors⁵ and, after in vitro expansion, reinjected to effect myocardial regeneration.

However, the results of these studies should be interpreted cautiously because of the possibility of phenotypic changes induced by repeat passages, which could explain why the antigen expression pattern of these cultured cells was different from that of the unprocessed cells examined in the present study and possibly closer to the biological reality. Furthermore, the fact that CSCs only acquired their cardiomyocyte phenotype after co-cultures with rat cardiomyocytes makes it difficult to exclude the possibility that the cardiomyocytic differentiation of the engrafted cardiosphere-derived CSCs was not a mere consequence of fusion events.⁷ It is noteworthy that the patient demographics were not clearly detailed in any of the above mentioned studies (in one study, it was simply reported that the patients' ages ranged from 1 month to 80 years¹⁸).

Study Limitations

This study was primarily based on immunostaining, which may always be fraught with technical artifacts, particularly when colocalization experiments are performed. Efforts were made to minimize these shortcomings with the use of confocal microscopy. Furthermore, the unequivocal expression of the cardiac markers Nkx2.5, MDR-1, and Isl-1 in our positive controls makes it unlikely that these markers would have been missed if they had been expressed by the *c-kit*-positive cells. We also acknowledge that deriving quantitative data from immunohistochemical observations may not be accurate, but it is unlikely that it may have led to a gross underestimation of the number of CSCs, given our method of counting. Second, one could argue that we did not test the whole set of stemness or cardiac differentiation markers. We did not assess the expression of Sca-1 because the cardiac differentiation of Sca-1-positive cells requires exposure to 5-azacytidine,⁷ which is clinically irrelevant. Furthermore, the ability of cells expressing this marker to give rise to cardiomyocytes is 6 times less than that of *c-kit*-positive cells.⁵ We did not screen the tissue samples for GATA-4 or MEF-2 because of the lack of specific monoclonal antibodies that were required for the co-staining experiments with our anti-*c-kit* polyclonal antibody. A third limitation of the results obtained in the surgical group of patients is that sampling was performed in the right atrium. This was intentionally done to mimic a clinical scenario whereby CSCs would be harvested by an EMB and does not allow the exclusion of the presence of resident cardiac progenitors in other areas of the heart, for which the accessibility would then be more technically challenging. Finally, one could argue that we only provided a snapshot of the *c-kit*-positive cell content in biopsies without subsequent sorting and single cell clonal

expansion and that (although initially small) the number of these cells could be expanded in vitro. This is conceivable, although not supported by our flow cytometry data that demonstrated only a small proportion of *c-kit*-positive cells after culture. Given the minute amount of starting materials, sorting of the candidate cells and their expansion (without loss of the cardiac differentiation potential) under Good Manufacturing Practice are likely to be technically challenging and time-consuming procedures, notwithstanding the delay in onset of cell therapy, which may be a concern in these often critically ill patients. The individual variability in cell functionality and the cost of customized quality controls may further complicate the clinical practicality of this patient-specific approach.

Conclusions

Cell-based interventions can be oriented toward paracrine or structural objectives. The paracrine objective implies that the transplant has to primarily supply a missing mediator, such as insulin or dopamine in the cases of diabetes and Parkinson disease, respectively. In these settings, the replacement cells must not necessarily adopt a phenotype that fully matches that of the diseased cells as long as the missing substance is appropriately secreted. Conversely, the structural objective implies that the grafted cells ensure the true regeneration of a dead tissue. In this case, an optimal outcome can only be expected if the grafted cells adopt the phenotype of the cells they are intended to replace. This is especially true in the context of heart failure in which improvement of left ventricular function requires that large areas of dysfunctional myocardium be repopulated by new cells featuring a contractile activity and able to electromechanically couple with the host cardiomyocytes. The limited plasticity of adult cells^{19,20} and the results of fetal cardiomyocyte transplantation suggest that cells that recapitulate the developmental cardiomyogenic pathway are the best candidates for generating new myocardium.^{21,22} Although the use of CSCs seems appealing in this setting, the present results raise a cautionary word about the possibility of therapeutically exploiting these cells in patients who might need them most and call for additional investigation of adult or embryonic stem cells capable of featuring a cardiomyogenic differentiation potential after intramyocardial engraftment.

We acknowledge the expert secretarial assistance of Emilie Floc'h.

References

1. Leri A, Kajstura J, Anversa P. Cardiac stem cells and mechanisms of myocardial regeneration. *Physiol Rev*. 2005;85:1373-416.
2. Beltrami AP, Urbanek K, Kajstura J, Yan SM, Finato N, Bussani R, et al. Evidence that human cardiac myocytes divide after myocardial infarction. *N Engl J Med*. 2001;344:1750-7.
3. Bariety J, Mandet C, Hill GS, Bruneval P. Parietal podocytes in normal human glomeruli. *J Am Soc Nephrol*. 2006;17:2770-80.
4. Lucien N, Bruneval P, Lasbennes F, Belair MF, Mandet C, Cartron JP, et al. UT-B1 urea transporter is expressed along the urinary and gastrointestinal tracts of the mouse. *Am J Physiol Regul Integr Comp Physiol*. 2005;288:R1046-56.
5. Linke A, Muller P, Nurzynska D, Casarsa C, Torella D, Nascimbene A, et al. 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;102:8966-71.
6. Dawn B, Stein AB, Urbanek K, Rota M, Whang B, Rastaldo R. Cardiac stem cells delivered intravascularly traverse the vessel barrier, regenerate infarcted myocardium, and improve cardiac function. *Proc Natl Acad Sci U S A*. 2005;102:3766-71.
7. Oh H, Bradfute SB, Gallardo TD, Nakamura T, Gaussin V, Mishina Y, et al. Cardiac progenitor cells from adult myocardium: homing, differentiation, and fusion after infarction. *Proc Natl Acad Sci U S A*. 2003;100:12313-8.
8. Martin CM, Meeson AP, Robertson SM, Hawke TJ, Richardson JA, Bates S, et al. Persistent expression of the ATP-binding cassette transporter, *Abcg2*, identifies cardiac SP cells in the developing and adult heart. *Dev Biol*. 2004;265:62-75.
9. Hierlihy AM, Seale P, Lobe CG, Rudnicki MA, Megeney LA. The postnatal heart contains a myocardial stem cell population. *FEBS Lett*. 2002;530:239-43.
10. Laugwitz KL, Moretti A, Lam J, Gruber P, Chen Y, Woodard S, et al. Postnatal Isl1+ cardioblasts enter fully differentiated cardiomyocyte lineages. *Nature*. 2005;433:647-53. Erratum in: *Nature*. 2007;446:934.
11. Sperr WR, Bankl HC, Mundigler G, Klappacher G, Grossschmidt K, Agis H, et al. The human cardiac mast cell: localization, isolation, phenotype, and functional characterization. *Blood*. 1994;84:3876-84.
12. Ito F, Toyota N, Sakai H, Takahashi H, Iizuka H. FK506 and cyclosporin A inhibit stem cell factor-dependent cell proliferation/survival while inducing upregulation of c-kit expression in cells of the mast cell line MC/9. *Arch Dermatol Res*. 1999;291:275-83.
13. Yamabi H, Lu H, Dai X, Lu Y, Hannigan G, Coles JG. Overexpression of integrin-linked kinase induces cardiac stem cell expansion. *J Thorac Cardiovasc Surg*. 2006;132:1272-9.
14. Parmacek MS, Epstein JA. Pursuing cardiac progenitors: regeneration redux. *Cell*. 2005;120:295-8.
15. Kajstura J, Leri A, Finato N, Di Loreto C, Beltrami CA, Anversa P. Myocyte proliferation in end-stage cardiac failure in humans. *Proc Natl Acad Sci U S A*. 1998;95:8801-5.
16. Smith RR, Barile L, Cho HC, Leppo MK, Hare JM, Messina E, et al. Regenerative potential of cardiosphere-derived cells expanded from percutaneous endomyocardial biopsy specimens. *Circulation*. 2007;115:896-908.
17. Urbanek K, Quaini F, Tasca G, Torella D, Castaldo C, Nadal-Ginard B, et al. Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy. *Proc Natl Acad Sci U S A*. 2003;100:10440-5.
18. Messina E, De Angelis L, Frati G, Morrone S, Chimenti S, Fiordaliso F, et al. Isolation and expansion of adult cardiac stem cells from human and murine heart. *Circ Res*. 2004;95:911-21.
19. Reinecke H, Poppa V, Murry CE. Skeletal muscle stem cells do not transdifferentiate into cardiomyocytes after cardiac grafting. *J Mol Cell Cardiol*. 2002;34:241-9.
20. Murry CE, Soonpaa MH, Reinecke H. Haematopoietic stem cells do not transdifferentiate into cardiac myocytes in myocardial infarcts. *Nature*. 2004;428:664-8.
21. Leor J, Patterson M, Quinones MJ, Kedes LH, Kloner RA. Transplantation of fetal myocardial tissue into the infarcted myocardium of rat. A potential method for repair of infarcted myocardium? *Circulation*. 1996;94(9 Suppl):II332-6.
22. Scorsin M, Hagege AA, Marotte F, Mirochnik N, Copin H, Barnoux M, et al. Does transplantation of cardiomyocytes improve function of infarcted myocardium? *Circulation*. 1997;96(9 Suppl):II-188-93.