Whether Modern Cell Technologies Can Break Down Biological Limitations of Tissue-Specific Regeneration of the Myocardium

L. M. Nepomnyashchikh, E. L. Lushnikova, and D. V. Goldshtein

Translated from *Kletochnye Tekhnologii v Biologii i Meditsine*, No. 2, pp. 63-74, 2005

The paper reviews modern concepts of physiological and reparative regeneration of the myocardium as a highly specific and highly differentiated tissue system. Special attention was given to evaluation of the proliferative potential of cardiomyocytes, in particular, to the existence of a population of resident cardiac stem cells in the myocardium. Modern approaches to replenishment of massive cardiomyocyte loss via transplantation and transdifferentiation of adult and embryonic stem cells are discussed and the possibilities of using cell technologies for induction of tissue-specific regeneration of the myocardium are analyzed.

Key Words: *myocardial regeneration; cardiomyocyte proliferation; stem cells; transdifferentiation*

Physiological and reparative regeneration of the myocardium and, first of all, cardiomyocytes (CMC) is still a key problem in cardiological studies. The progress in this field will help to answer the questions about the possibilities, pathways, and terms of restoration of heart structure and function after ischemic, toxic, infectious, allergic, and other types of damage [3,11,17,24,34].

These questions are most important in case of massive focal or diffuse CMC death accompanied by the formation of cardiosclerosis (focal or diffuse) and changes in the geometry of the ventricles, interventricular septum, and the heart of the whole, *i.e*. processes determining heart remodeling. Remodeling of the heart is a complex process of adaptive regeneration of CMC, vascular bed, and connective tissue components determined by changes in the hemodynamics and various etiological, epidemiological, neurohormonal, and other factors [104]. Cell death leading to contractile tissue loss and the capacity of CMC and other cell populations to proliferation and hypertrophy are also important biological determinants of heart remodeling [9,16].

Research Institute of Regional Pathology and Pathomorphology, Siberian Division of the Russian Academy of Medical Sciences, Novosibirsk. ***Address for correspondence:*** pathol@soramn.ri. L. M. Nepomnyashchikh

Postinfarction and dilatation types of remodeling are the most prevalent and most severe variants often accompanied by the development of heart failure [22,23].

Heart remodeling characterizes not only pathological state, but also aging. According to epidemiological studies, heart remodeling is the main cause of death in peoples over 65 years old. Changes in heart architectonics and its phenotypic modifications in cardiomyopathy of different genesis, myocardial infarction, and myocarditis differ from structural and functional rearrangements of the heart during physiological aging [15,104]. However, many aspects of heart remodeling induced by pathological processes or accompanying gerontogenesis are still little studied, which makes difficult to evaluate differences between changes caused by pathological processes and biological aging.

PROBLEM OF MYOCARDIAL REGENERATION

Function of all organs and tissue systems depends on their structural homeostasis, which, in turn, is determined by the processes of cell proliferation, death, and tissue specific differentiation. The intensity of cell death should be compensated by the intensity of cell

0007-4888/05/1394-0481

proliferation. Under these conditions, a large portion of cells should be not only highly differentiated, but also terminally differentiated for the maintenance of optimal functional activity of organs and tissues. These general theoretical theses lead to a conclusion: normal functioning of organs and tissues is determined by a critical mass of highly specialized cells ensuring vital needs of the organism at different stages of ontogeny and under varying environmental conditions.

The imbalance between death, proliferation, and differentiation irrespective of its genesis determines the entire spectrum of pathological changes and possible variants of remodeling in organs and tissues. Enhanced cell death against the background of unchanged or reduced cell proliferation (under the effect of cytopathic agents, extreme environmental factors, or functional overstrain) or unchanged intensity of cell death in combination with inhibition of cell proliferation (aging) determine decompensation and functional insufficiency. These shifts underlie various acute and chronic pathological processes. Predominance of proliferative reactions over cell differentiation and death (neoplastic reactions) also leads to functional insufficiency.

Despite the fact that various aspects of myocardial regeneration after damaging influences, especially, after ischemic damage, are extensively studied, no considerable progress in the understanding of the regenerative processes in the heart was attained. We can say, that we know much more about the nature and degree of myocardial damage, forms of CMC death, types of CMC damage, and about molecular, cellular, and genetic mechanisms of heart diseases, than about forms and scales of myocardial regeneration [12, 14], and, more important, about the possibility of inducing CMC proliferation.

Many experimental studies showed that regeneration of the myocardium after focal and diffuse CMC death is realized via hypertrophy of remaining muscle cells and the development of diffuse or focal sclerosis. It was also postulated that the processes of physiological and reparative regeneration are similar by their nature and proceed via intracellular regeneration, which can be intensified under conditions of increased functional load or under the action of some damaging factors and leads to CMC hypertrophy [25].

This situation can be explained by some objective and subjective factors: the absence of reliable methods for detection of low differentiated cells, insufficient resolution of usual light microscopes determining underestimation of mitotically active CMC, the absence of universal methodical basis for the studies of structural and functional changes in the heart, and insufficient use of quantitative methods for the studies of structural mechanisms of heart remodeling.

PROLIFERATIVE POTENTIAL OF CARDIOMYOCYTES

It was accepted for a long time that mammalian CMC are highly differentiated postmitotic cells, which normally do not enter mitosis in adults. This conclusion was based on the data obtained in studies of postnatal cardiomyogenesis and reparative processes in the myocardium. Proliferative activity of CMC in adult mammals was routinely evaluated by incorporation of ^{3}H-thymidine and 5-bromodeoxyuridine (BrdU) and calculation of the mitotic index.

Some Russian and foreign scientists showed that DNA synthesis in CMC nuclei recorded by ^{3}H-thymidine incorporation rapidly decreases within 2-3 weeks of postnatal ontogeny and drops below 1% [24]. Other researchers showed that DNA synthesis in mouse and rat CMC stops by the first or even second month after birth [92]. The mitotic index of CMC in the myocardium of small mammals at this term decreases from 1.5-2% to 0.3% or even to 0.05%.

Due to considerable hyperplasia of CMC during the first 3 weeks of postnatal ontogeny, the total number of CMC in rat heart increases 2-fold [81]. It should be noted that this parameter considerably varies and its values depend on the method of cell counting. Evaluation of the total number of CMC in rat heart by the density of cell nuclei and heart weight showed that this parameter increases 4-fold: from 5×10^{6} after birth to 2×10^{7} in adult animals [98]. There are data that the left ventricle of young rats consists of 13×10^{6} CMC [36]. Heart weight increases proportionally to CMC number [89]. Further increase in the heart weight and size and regenerative processes are associated with CMC hypertrophy.

However, quantitative analysis of CMC population carried out by us in the dynamics of anthracycline cardiomyopathy in mature Wistar rats (single injection of 10 mg/kg doxorubicin) showed that the total number of CMC in the heart not only decreases (by 20-25%) under the effect of cytotoxic agents, but can return to the control level at certain terms of the experiment [7]. This compensatory increase in CMC number after their massive doxorubicin-induced death can be associated only with CMC proliferation. In young animals at the early stages of postnatal ontogeny this proliferative response of CMC to the damaging action of doxorubicin (single injection in a dose of 5 mg/kg) is more pronounced: after transient decrease the number of CMC after 7-8 weeks increases by 23-34% compared to the control level.

Replenishment of CMC population after their apoptotic or necrotic death is evidently, a continuous process and an integral element of physiological regeneration of the myocardium. There are data that in

the heart of young rats 94,200 CMC die every day via necrosis and apoptosis, *i.e.* 2.83×10^6 cells per month [36]. In the absence of CMC proliferation their population should disappear within 5-7 months. But it is not the case. According to our data, the total number of CMC in rat heart does not considerably change throughout the life: in 24-33-month-old rats with signs of cardiac decompensation the total number of CMC does not differ from that in 4-month-old animals [8].

The concept of CMC as a terminally differentiated cell population is revised in recent years after the appearance of new technologies, *e.g.* confocal microscopy and wide set of monoclonal antibodies enabling detection of markers of CMC proliferation, differentiation, and death. The use of laser scans of sections prepared for light microscopy made it possible to increase the volume of analyzed tissue and to correctly evaluate mitotic activity of CMC. Now, CMC proliferation and DNA replication can be evaluated by using mono- and polyclonal antibodies to proliferation markers Ki-67, MCM5, Cdc6, cyclin B1. These markers are nuclear protein expressed at different stages of the cell cycle.

It was demonstrated that the mitotic index of CMC in the heart of adult humans is 0.0011-0.0015% [40, 57,109]. Enhanced CMC proliferation is observed at the terminal stages of heart failure (dilated cardiomyopathy, postinfarction remodeling) and during decompensation of hypertrophied myocardium; mitotic index increases 3-4 and even 10-fold (to 0.015%)

Expression of some genes regulating phases of the cell cycle was detected in CMC after myocardial infarction, *e.g.* the level of cyclins A, I, and E and activity of the corresponding cyclin-dependent kinases increase [35].

The concept on proliferative activity of CMC under conditions of physiological and reparative regeneration is confirmed by the data on changes in telomerase activity in cardiac muscle cells. In some experimental models of heart remodeling increased telomerase activity was found in 20-30% CMC. This enzyme is expressed during cell proliferation [66], it preserves telomere length and prevents chromosome disorganization and premature cell aging [72]. Since in rapidly renewing cells and precursors telomerase activity is always high [68], increased telomerase activity in CMC under conditions of myocardial remodeling can be considered as an indirect marker of their proliferative activity. Decreased telomerase activity and telomere shortening in CMC were observed in aged animals during decompensation of hypertrophied heart [58].

After detection of proliferative activity in CMC a question arised: which subpopulation of muscle cells in the heart can divide? In the heart of adult mammals CMC are terminally differentiated cells (low mitotic index), therefore proliferative activity can be exhibited by remaining non-terminally differentiated CMC and/or resident stem cells (SC) or precursor cells committed towards differentiation into CMC. In the first case, cell forms of CMC regeneration are restricted due to limited number of division of differentiated cells, which enter the cell cycle, most likely, in the acute phase of damage. In the second case, primitive cells carrying SC markers should rapidly divide after stimulation and ensure the appearance of CMC at different stages of differentiation in the myocardium [109].

POPULATION OF RESIDENT CARDIAC STEM CELLS

The existence of a population of resident cardiac SC in the myocardium is a principal question for evaluation of the regenerative potential of the myocardium as a highly specialized tissue system. Earlier, similar studies were performed on hepatocytes. Similarly to CMC, hepatocytes belong to highly specialized postmitotic cells. However, unlike CMC hepatocytes can proliferate more intensively and ensure organ-specific regeneration of the liver. It was found that replicative reserve of the liver consists of unipotent committed precursor cells (differentiated hepatocytes and biliary epithelium) and bipotent nonparenchymal precursors (oval nondifferentiated cells), which are considered to be hepatic SC [27,28,77]. It was shown that hepatic SC are multipotent and can transdifferentiate [70]. Facultative stem compartment plays little role in normal regeneration of the liver parenchyma. It was assumed that transient expression of necessary growth factors occurs in SC, which can induce hepatocyte replication.

About 15-20% CMC in the myocardium can be assigned (by telomerase activity) to non-terminally differentiated (young) CMC retaining proliferation potential and capable of hypertrophic growth [58,66]. Moreover, immunohistochemical analysis revealed nondifferentiated cells expressing SC markers: c-kit (receptor of SC factor), MDR1 (P-glycoprotein extruding dyes, toxic substances, and drugs), Sca-1 (protein involved into signal transduction and cell adhesion) [36]. These nondifferentiated cells with a diameter of 6±2 μ do not carry markers of bone marrow cells ($CD45^+$) and hemopoietic SC ($CD34^+$) [45, 109]. Detection of nondifferentiated cells with SC antigens and cells expressing cardiac transcription factors (GATA4 and MEF2) and cardiac myosin in the myocardium of patients with aortal stenosis allowed us to hypothesize the existence of consecutive linear committing of primitive cells into cardiac precursor cells, CMC progenitor cells, and CMC precursors in the heart [109]. SC antigens are not detected on amplifying CMC,

which are larger than CMC precursors, but smaller than mature CMC.

Proliferation of cardiac SC and CMC precursors yields clusters consisting of 6-12 cells characterized by high telomerase activity and asynchronous differentiation. Clonogenic expansion of nondifferentiated cells attests to the existence of a stem reserve. It was shown that the number of proliferating nondifferentiated cells (with markers c-kit, MDR1, and Sca-1) in the myocardium of patients with aortic stenosis increases 70-140-fold, while the number of CMC precursors increases 65-240-fold [109]. On the whole, in the hypertrophied myocardium of patients with aortic stenosis CMC derived from cardiac SC and CMC precursors constitute 15% cells. Similar estimates (12-18% of the total number of cells) of the effect of SC on myocardial regeneration were obtained when studying chimerism of transplanted hearts from female donors to male recipients [95].

Theoretically and practically, it is important to know, whether newly formed CMC are descendants of cardiac SC accumulated in the heart during the early ontogeny, or they originate from hemopoietic SC migrating into the myocardium from the circulation at later terms. Migration of cells carrying c-kit$^+$ receptor and formation of colonies of these cells in different organs, including the heart, during embryo development confirm the first assumption [62,106]. The second assumption is confirmed by the fact that hemopoietic SC due to SC factor-mediated chemotaxis migrate into certain zones and are preserved there as *cardia primordia* remnants [110]. These primitive cells can then undergo both symmetrical and asymmetrical division [79], thus increasing the pool of nondifferentiated cells in adult heart.

The existence of cardiac SC implies that so-called terminally differentiated tissue systems have a stem reserve maintaining proliferation activity of CMC under physiological conditions and undergoing considerable activation under conditions of massive CMC death. The use of stem reserve for myocardial regeneration is more effective, compared to other SC, because these cells are predetermined for differentiation into CMC, whereas hemopoietic SC should be reprogrammed for transdifferentiation under the effect of microenvironment.

Some properties of resident cardiac SC were studied using subcultures of cells from biopsy specimens of postnatal cardiac atria and ventricles and from mouse hearts [75]. Authors reported that nondifferentiated cells grew as self-attaching clusters (cardiospheres), formed clones, expressed antigens/markers of SC and endothelial precursor cells, and probably exhibited properties of adult cardiac SC. These cells were capable of self-renewing over a long time and differentiated *in vitro* and after ectopic (dorsal subcutaneous connective tissue) and orthotopic (myocardial infarction zone) transplantation into specialized cardiac and blood vessel cells (CMC, endotheliocytes, and smooth muscle cells).

Other researchers isolated, sorted, and cultured Sca-1-positive cells from adult heart [74]. Sca-1-positive cells proliferated slowly, but after addition of oxytocin to the culture medium expression of cardiac transcription factors and contractile proteins, formation of sarcomeres, and spontaneous contractions were observed in these cells. Isoproterenol increased contractile activity of these cells, which was accompanied by enhanced intracellular Ca^{2+} currents. Expression of oxytocin receptor mRNA in Sca-1-positive cells increased after oxytocin treatment. It is known that the oxytocin system plays and important role in cardiomyogenesis [56].

Adult cardiac SC, similarly to other SC, are multipotent cells and after transplantation into ischemic heart can differentiate not only into CMC, but also into endothelial and smooth muscle cells [39].

Aging of cardiac SC (termination of cell division and apoptotic death) is an important biological characteristic of these cells. Analysis of subpopulation of c-kit-positive cells in endomyocardial biopsy specimens from the left ventricles of elderly patients without symptoms of heart failure and patients with age-associated dilated cardiomyopathy (which differs from idiopathic dilated cardiomyopathy) showed that $p16^{INK4a}$ protein, a marker of aging, is present in 14 and 59% these cells, respectively [45]. $p16^{INK4a}$ protein is a cyclin-dependent kinase inhibitor, a blocker of Cdk4 and Cdk6 maintaining retinoblastoma protein in the hyperphosphorylated state, which results in cell cycle arrest [54]. On the other hand, it was shown that in the myocardium of elderly patients without symptoms of heart failure and patients with age-associated dilated cardiomyopathy $p16^{INK4a}$ protein is expressed in 16 and 48% CMC, which also attests to aging of population of differentiated CMC. Telomeres in the nuclei of these cells were shortened by 39%. Only $p16^{INK4a}$-positive SC and CMC underwent apoptotic and necrotic death.

Analysis of recent studies allowed us to make a conclusion on phenotypic heterogeneity of CMC population in the heart of adult mammals. Three subpopulation of CMC are present in mammalian myocardium at all stages of postnatal ontogeny: highly specialized terminally differentiated (postmitotic) CMC, highly specialized non-terminally differentiated CMC, cardiac SC and CMC precursors. Replicative reserve of the myocardium is presented by non-terminally differentiated CMC, which can enter the cell cycle, cardiac SC, and CMC precursors. Replicating CMC, SC, and precursor cells can undergo premature aging, which manifests in termination of cell division.

Despite the absence of precise quantitative studies of the pool of replicating CMC and cardiac SC, it can be assumed that replicative potential of the myocardium constitutes 30-35% of the total number of CMC. This potential can be exhausted with age and under the action of adverse (cardiotoxic factors). These circumstances probably determine insufficient (incomplete) regeneration of the myocardium in massive focal or diffuse damage, which manifests in the development of large sclerotic foci or diffuse cardiosclerosis. An important factor disturbing tissue-specific regeneration in myocardial infarction is the absence of blood supply to the infarction zone for a long time caused by occlusion of coronary arteries, which considerably inhibits migration of SC, their replication and differentiation in the damaged area.

STEM CELLS — FACULTATIVE OR OBLIGATE PROLIFERATIVE RESERVE OF THE MYOCARDIUM?

The absence of tissue-specific regeneration of the myocardium in the majority of myocardial infarction cases due to insufficient proliferation of CMC and cardiac SC prompted the search for new approaches to induction of cellular forms of CMC regeneration, in particular, stimulated the development of cell technologies based on the use of embryonic and neonatal CMC and SC characterized by unique ability to differentiate into specialized cell types [4,6,10,18-21,31,48,82].

SC are self-renewing cells with clonogenic activity, which can differentiate into various cell lines [48,113]. Many modern studies are devoted to evaluation of the morphogenetic capacities of both embryonic and adult SC differing by their biological characteristics.

Embryonic SC in mammals are formed from totipotent cells at the early stages of embryonic development. These cells can be isolated from mammalian embryos at the stage of blastocyst (up to day 5 of embryonic development). These cells can give rise to any cell line of each of the three major germ layers, including CMC [5,71,80, 115], The term "embryonic SC" was introduced in order to distinguish these cells from pluripotent embryonic carcinoma cells (teratocarcinoma derivatives) [107]. Human embryonic cells express some universal cell markers typical of primitive embryonic cell and embryonic carcinoma cells of other mammalian species, *e.g*. SSEA-3 (studies-specific embryonic antigen), SSEA-4, TRA-1, TRA-1-81, and alkaline phosphatase. It should be noted that human embryonic cells are characterized by high telomerase activity.

Initially, cultured embryonic cells form embryonic bodies consisting of derivatives of the three major germ layers. Outgrowths of these bodies exhibiting spontaneous contractile activity contain CMC [53]. These CMC can be isolated from these bodies and their morphological and electrical characteristics can be evaluated.

Human embryonic cells cultured for 40-95 days differentiate into embryonic CMC, which can be divided into three classes by their action potential: nodal, atrial, and ventricular [53]. Mouse embryonic cells considerably differ from human embryonic cells by the stage-specific antigens (express SSEA-1) and the ability of leukemia-inhibiting factor to maintain them in a nondifferentiated state [107]. These cells differentiated into embryonic CMC (nodal, atrial, ventricular, and Purkinje cells).

Analysis of CMC differentiation from embryonic SC showed that this process is triggered by a limited set of tissue-specific transcription factors (e.g. Nkx-2.5 and GATA-4 [41,52]) determining the complex picture of the expression of tissue-specific genes [111].

Adult SC are a part of population of tissue-specific cells in the postnatal body committed to a certain type of differentiation. Despite high plasticity of embryonic SC far surpassing that of adult SC, the latter are more often used in clinical and experimental studies for the induction of regenerative processes and replacement of irreversibly damaged terminally differentiated cell populations. This can be explained by the existence of ethical and technical difficulties for the use of embryonic SC and the possibility of using autologous adult SC, which improves the take of cell transplants in the target organs.

Several types of adult SC were phenotypically characterized, in particular, hemopoietic SC (differentiate into bone marrow cells and all lymphohemopoietic blood cells), mesenchymal SC (differentiate into bones, cartilage, ligaments, adipose tissue, muscles, bone marrow stroma, neural cells), neural SC (neurons, astrocytes, oligodendrocytes), hepatic SC (oval cells differentiating into hepatocytes and ductal epitheliocytes), pancreatic SC (β-cell), skeletal and muscle SC or satellite cells (skeletal muscle fibers), skin SC (epidermal cells and hair follicles), epithelial SC of the lungs (goblet cells, ciliary cells, types I and II alveolocytes), intestinal epithelial cells (Paneth cells, enterocytes, secretory cells, and villous enteroendocrine cells) [59].

Hemopoietic SC. Biological effects of hemopoietic SC (carry $CD34^+$ marker) are most comprehensively studied. According to the classical paradigm, there is a strict hierarchical organization of hemopoietic cells: multipotent self-renewing SC give rise to committed precursor cells (result of asymmetrical division), which, in turn, give rise to precursor cells with limited linear differentiation, and these latter to terminally differentiated cells [29,30]. However, recent studies cast doubt on the classical paradigm that dif-

ferentiation of hemopoietic SC is restricted to some organ-specific cell lineages: the possibility of their differentiation into cell lineages not belonging to this tissue type and germ layer was demonstrated. This property of hemopoietic SC was called as developmental plasticity [59].

Due to their high migration capacity, hemopoietic SC easily migrate from the bloodstream into different organs and tissues and can under certain conditions repopulate them. Molecular mechanisms determining "switching" of the differentiation from one type to another are still little studied.

The use of hemopoietic SC for myocardial and vascular reparation is based on some biological properties of these cells, in particular, their intensive migration into damaged focus (inflammation) and transdifferentiation under the effect of microenvironment. Considerably increased number of circulating $CD34^+$ mononuclear cells and increased content of endothelial growth factor were detected in patients with acute myocardial infarction [102].

For the evaluation of the plasticity of hemopoietic SC different authors used different methods of isolation and preparation of these cells (isolation of nonselected cells from the bone marrow, sorting of hemopoietic SC, *etc*.), which determined controversy of their data. This can be explained by the fact that hemopoietic tissues (bone marrow and peripheral blood) contain a heterogeneous SC population consisting hemopoietic SC, mesenchymal SC, multipotent adult precursor cells, and endothelial precursor cells.

The first *in vivo* studies of the plasticity of hemopoietic SC were carried out on labeled nonselected cells from the bone marrow. After transplantation of these cells to animals and accompanying conditioning therapy, these cells gave rise to muscle fibers [47], hepatocytes [64,91,100], microglia and astroglia [46], and neuronal tissue [43,76].

At present, a large body of evidence is accumulated on transplantation of hemopoietic SC into the heart, where they differentiate into CMC, endotheliocytes, and smooth muscle cells [55,63,83,84,99]. Most often, massive damage to CMC are reproduced on the model of myocardial infarction.

In experiments with ligation of the left coronary artery (infarction area attained 70% of free left ventricular wall), injection of bone marrow SC (Lin^- $c\text{-}kit^+$) carrying a gene encoding green fluorescent protein 5 h after intervention led to the appearance of strips of regenerating myocardium in the necrotic zone after 9 days [83]. Developing CMC expressed cardiospecific transcription factors GATA-4, Csx/Nkx2.5, and MEF-2, cardiac myosin, α-actin of sarcomeres, and connexin-43 (a component of intercalated disks). These morphogenetic events promoted the formation of structural and functional syncytium typical of mature myocardium. Myocardial regeneration was also observed after modeling of myocardial infarction in mice, in whom hemopoietic SC were labeled with β-galactosidase gene [55]. These cells migrated into the necrotic zones and differentiated into CMC and endotheliocytes. On the whole, β-galactosidase-positive cells constituted no more than 0.02% of the total number of myocardial cells, which attests to low efficiency of spontaneous transdifferentiation of hemopoietic SC.

The low level of spontaneous transdifferentiation of SC migrating into the myocardial necrotic zone correlated with low proliferative activity of resident CMC and cardiac SC. This phenomenon can be explained by the facts that only low number of hemopoietic SC administered via different routes (into coronary vessels, into left ventricle, and intravenously) survive, proliferate, and differentiate into CMC in the myocardium. Analysis of distribution of ^{111}In-labeled hemopoietic SC in the body showed that only 1% labeled cells were detected in the heart [44]. High percent of radiolabeled cells was detected in the lungs (17% after 1 h postinjection) and in other organs.

Mesenchymal SC. Mesenchymal SC are spindle-shaped cells with irregular processes demonstrating positive reaction for $CD13^+$, $CD29^+$, $CD44^+$, and $CD71^+$ markers and negative reaction for $CD3^-$, $CD14^-$, $CD15^-$, $CD33^-$, $CD34^-$, $CD38^-$, $CD45^-$, and HLA-DR markers [26,117]. These cells differ from hemopoietic SC by $CD34^-$ marker. Human mesenchymal SC can be isolated from the bone marrow or peripheral blood; their advantage is low immunogenicity, allowing wider use of allogenic cell transplants. Under certain conditions mesenchymal SC differentiate into CMC, endotheliocytes, and smooth muscle cells [65,69].

After intravenous administration mesenchymal SC primarily migrate into the lungs; after injection into the cavity of infarcted left ventricle SC colonize only the infarction zone and boundary area and their number 4 h after administration did not surpass 1% of the total number of injected cells [37].

Implantation of autologous or allogenic porcine mesenchymal SC after myocardial infarction and their differentiation into CMC inhibit ventricular remodeling, prevented thinning of the ventricular wall, and improved cardiac function [101]. Injection of mesenchymal SC into the infarction zone also prevents heart remodeling and promotes healing [93]. Transplantation of mesenchymal SC with untreated bone marrow into the infarcted myocardium increases expression of cardiac tenascin and sympathetic hyperinnervation [86]. At the same time, differentiation of mesenchymal SC in the connective tissue scar was never studied.

Evaluation of the transplantation capacity of β-galactosidase-labeled human mesenchymal SC and

their ability to differentiate into CMC in the heart of immunodeficient mice showed that 4 days after injection of 5-10×10^5 cells into the left ventricular cavity the majority of them were detected in the spleen, liver, and lungs [108]. In the heart mesenchymal cells were distributed diffusely, after 4 days only 0.44% cells took, at later terms the percent of β-galactosidase-positive cells in the myocardium was even lower. The morphology of β-galactosidase-positive cells changes, their size increased from 20-30 μ in diameter to 50-70 μ in length. After 14-60 days immunohistochemical analysis revealed expression of desmin, cardiac troponin, and α-actinin (a component of Z disks), and phospholamban in these cells.

The use of autologous mononuclear bone marrow cells in addition to standard therapy of myocardial infarction or after aortocoronary bypass surgery and other coronary interventions showed safety and efficiency of this method [2,90,97,103]. The authors explain the improvement of the general state and normalization of some hemodynamic parameters by neovascularization (revascularization) and regeneration of the myocardium stimulated by bone marrow SC. However, the formation of calcifications in the myocardium is an unfavorable morphogenetic side effect of transplanted nonselected bone marrow SC [118].

Hepatic SC. Model experiments with adult hepatic SC (WB-F344, a cloned SC strain obtained from the liver of adult mice and labeled with β-galactosidase) transplanted into the myocardium demonstrated *in vivo* transdifferentiation of these cells into mature CMC under the effects of microenvironment [70]. The length of β-galactosidase-positive CMC varied from 20 to 110 μ, these cells expressed troponin T and contained well organized myofibrils. CMC originating from SC formed intercalated disks and connected to preexisting cells, *i.e.* can form functional syncytium.

Analysis of experimental and clinical studies of transplantation of embryonic and adult SC into the myocardium for induction of its regeneration in massive focal and diffuse damage showed that the use these technologies can partially replenish CMC loss (repopulation) and promote revascularization of the infarction zone and newly formed myocardium. Complete restoration of the geometry of infarcted ventricles and tissue architectonics after macrofocal infarction is hardly possible, because CMC loss is accompanied by the loss of vascular net and connective tissue framework characterized by a peculiar 3D organization and largely determining the direction of morphogenetic processes. Evaluation of CMC loss and transplantation of an equivalent number of SC, as well as creation of special conditions of successful colonization of new tissue niches are important for myocardial repopulation.

POSSIBILITIES OF INDUCTION OF CARDIOMYOGENESIS AND TISSUE-SPECIFIC REGENERATION IN MASSIVE MYOCARDIAL DAMAGE

Mobilization of hemopoietic and mesenchymal SC and their migration into the damaged area (including infarcted myocardium) are natural processes for these cells. However, the number of migrated cells is usually insufficient for complete or considerable regeneration of CMC and replenishment of the lost myocardial cells. Therefore, special technological approaches increasing the number of SC and special conditioning therapy (modeling paracrine regulation) determining the direction of cell differentiation (in particular, into CMC) are required. The development of technologies of mobilization of autologous SC from tissue niches, in particular, bone marrow SC, is of crucial importance for wider use of cell therapy, because this approach excludes the use of donor cells, which is associated with the risk of transmission of infectious agents and activation of immune reactions.

Some cytokines, *e.g.* granulocyte colony-stimulating factor (G-CSF) and granulocyte-macrophage colony-stimulating factor (GM-CSF) are now widely used for mobilization of hemopoietic SC and bone marrow precursor cells. The mechanisms underlying the mobilizing effects of G-CSF and GM-CSF on hemopoietic SC and bone marrow precursor cells are unknown, because these cells, except bone marrow stromal cells do not express receptors for these cytokines [61].

Transplantation of G-CSF to mice after experimental myocardial infarction increased the number of SC in the infarction area and had no effect on the intensity of migration of inflammatory cells [33]. Combined administration of G-CSF and SC factor 250-fold increased the number of circulating Lin$^-$ c-kit$^+$ cells [42]. The use of this protocol in mice with experimental infarction reduced animal mortality by 68%, decreased the infarction zone by 40%, determined less pronounced dilation of the left ventricle, and lowers the diastolic strain in the left-ventricular wall [85]. These changes resulted from reparation of the infarcted myocardium due to proliferation and differentiation of hemopoietic SC and, probably, other SC, which led to the formation of 15×10^6 new CMC and their integration into the functional syncytium. These processes were accompanied by the formation of arterioles and capillaries connecting to the vascular system of the heart.

However, when patients with severe chronic heart failure were treated with Neupogen (recombinant human G-CSF), in none patients the appearance of regeneration zones was noted [1]. The observed positive chan-

ges in the function of the left ventricle in some patients were probably determined by neoangiogenesis.

Other cytokines, *e.g.* IL-3, IL-8, IL-11, SC factor (SCF), fit-3-ligand, or macrophagic inflammatory protein-1α can also be used for mobilization of hemopoietic SC [61]. However, these agents are inferior to G-CSF and GM-CSF by their mobilization capacity. It was shown that CSF (c-kit ligand) can activate migration of hemopoietic SC carrying tyrosine kinase receptor c-kit. This factor is expressed in the heart during embryonic and neonatal periods [73] and in adult heart (in mast cells) and macrophages under conditions of ischemia-reperfusion [49,88]. However, expression of CSF is probably insufficient for mobilization and migration of hemopoietic SC into the myocardial infarction zone.

Mobilization and migration of bone marrow $CD34^+$ cells is modulated by various chemokines and adhesion molecules. Adhesive interactions between hemopoietic SC and components of the bone marrow microenvironment play a central role in migration, circulation, and proliferation of SC [94,112]. Among adhesion molecules, β1- and β2-integrins, selectin, and superimmunoglobulin families are involved into these processes. The corresponding ligands are expressed on endothelial cells and stromal bone marrow cells or form zones in the extracellular matrix of the bone marrow microenvironment. Platelet endothelial cell adhesion molecule-1 and CD44 are also involved into adhesion processes. Ligands of CD44, hyaluronic acid, and fibronectin are secreted by stromal cells.

The use of monoclonal antibodies to adhesion molecules increases mobilization of SC from the bone marrow. For instance, a 200-fold increase in the level of circulating hemopoietic precursor cells was observed in primates 24 h after injection of antibodies to α4-integrin [87]. Expression of adhesion molecules can be suppressed or blocked with antisence oligonucleotides hybridizing with complimentary sequence of target mRNA [51]. It should be noted that antiadhesion technologies can be used only at the initial stages of cell therapy for more potent mobilization of SC from the bone marrow. At later stages, colonization of the target organs and tissues with SC and their differentiation can be stimulated via directed activation of chemokine and adhesion factors in the damaged zone.

An important role in the migration of hemopoietic SC is played by α-stromal-derived factor (SDF-1), a common SC chemokine acting as a ligand for chemokine receptor CXCR4 [32,67]. SDF-1 plays an important role in vasculogenesis and hemopoiesis. Expression of CXCR4 in hemopoietic SC depends on the differentiation stage (expression in higher in less mature cells). Analysis of gene expression in the heart after myocardial infarction revealed enhanced expression of some genes, in addition to SDF-1 (vascular endothelial growth factor, metalloproteinase-9, intercellular adhesion molecule-1, and vascular cellular molecule 1), which also participate in recruitment of bone marrow SC into the damaged zone [32].

Survival of transplanted and mobilized SC and their differentiation into CMC are important events determining regeneration of the damaged myocardium. Survival after transplantation, differentiation, and functional maturation of SC in the myocardium is promoted by a number of growth factors. Injection of a suspension of mouse embryonic SC together with insulin-like growth factor (IGF-1) to mice with experimental myocardial infarction improves cardiac functions and prevents thinning of the left-ventricular wall [60]. Histological analysis of differentiating CMC revealed expression of sarcomere α-actin.

The efficiency of cell cardiomyoplastics in rats with experimental myocardial infarction increased after combined administration of neonatal CMC and HVJ-lysosome-plasmid complex containing human hepatocyte growth factor (hHGF) [78]. Thickening of the left-ventricular wall, appearance of newly formed myocardial tissue, and stimulation of angiogenesis were noted in these animals.

Differentiation of embryonic SC into CMC increases after activation of the TGF-b/BMP-signal pathway [38] and after stimulation of opioid κ-receptors [111]. Accelerated expression of cardiospecific genes in embryonic SC was observed after treatment with retinoid or ascorbic acid [105,114].

An important moment in the differentiation of bone marrow SC into CMC is their contact with microenvironment, including other CMC [50,96,116]. Contact growth of embryonic nondifferentiated or low differentiated cells is always accompanied by enhanced differentiation and maturation of cell population.

CONCLUSION

New technologies of regenerative cell therapy of cardiovascular pathology and, first of all, myocardial infarction are based on the results of fundamental studies of the processes of physiological and reparative regeneration in organs and tissues. An important result of these studies is deciphering of the universal hierarchical organization of proliferative processes in differentiated tissue systems: the existence of SC, committed precursor cells, and non-terminally differentiated specialized cells. Despite well-known difficulties of studies of human heart, first of all, vital morphological studies and evaluation of the proliferative reserve of the myocardium, further development of cell technologies and their introduction into clinical

practice is necessary and well substantiated, because these approaches more closely simulate biological regularities of regenerative reactions.

REFERENCES

1. Yu. N. Belenkov, F. T. Ageev, B. Yu. Mareev, and V. G. Savchenko, *Kardiologiya*, No. 3, 7-12 (2003).
2. L. A. Bokeriya, Yu. I. Buziashvili, S. T. Matskeplishvili, and D. Kh. Kamardinov, *Ibid*., No. 9, 16-22 (2004).
3. V. Ya. Brodskii, *Byull. Eksp. Biol Med*., **119**, No. 5, 454-459 (1995).
4. A. E. Vermel', *Klin. Med.*, No. 1, 5-11 (2004).
5. I. A. Grivennikov and A. A. Shkumatov, *Probl. Reproduktsii,* No. 3, 16-25 (2002).
6. V. V. Egorov, A. A. Ivanov, and M. A. Pal'tsev, *Molek. Med*., No. 2, 3-13 (2003).
7. E. L. Lushnikova, M. G. Klinnikova, O. P. Molodykh, and L. M. Nepomnyashchikh, *Byull. Eksp. Biol. Med*., **138**, No. 12, 684-689 (2004).
8. E. L. Lushnikova, L. M. Nepomnyashchikh, and M. G. Klinnikova, *Ibid*., **132**, No. 12, 685-691 (2001).
9. E. L. Lushnikova, L. M. Nepomnyashchikh, and V. D. Rozenberg, *Morphological, Molecular, and Genetic Bases of Dilated Cardiomyopathy* [in Russian], Moscow (2004).
10. L. N. Maslov, V. V. Ryabov, and S. I. Sazonova, *Vestn. Transplantol. Iskusstv. Organov,* No. 4, 78-86 (2003).
11. L. N. Maslov, V. V. Ryabov, and S. I. Sazonova, *Uspechi Fiziol. Nauk.*, No. 3. 50-60 (2004).
12. N. V. Naryzhnaya, E. V. Krivoshchekov, and Yu. B. Lishmanov, *Vopr. Med. Khimii*, **46**, No. 2, 127-134 (2000).
13. L. M. Nepomnyashchikh, *Byull. Eksp. Biol. Med.*, **121**, No. 1, 4-13 (1996).
14. L. M. Nepomnyashchikh, *Ibid*., **131**, No. 1, 11-21 (2001).
15. L. M. Nepomnyashchikh, E. L. Lushnikova, and G. I. Nepomnyashchikh, *Morphometry and Stereology and Heart Hypertrophy* [in Russian], Novosibirsk (1986).
16. L. M. Nepomnyashchikh, E. L. Lushnikova, and D. E. Semenov, *Regenerative and Plastic Cardiac Insufficiency: Morphological Bases and Molecular Mechanisms* [in Russian], Moscow (2003).
17. L. V. Polezhaev, *Uspekhi Sovrem. Biol*., **3**, 196-211 (1994).
18. I. V. Potapov, M. E. Krasheninnikov, and N. A. Onishchenko, *Vestn. Transplantol. Iskusstv. Organov*, No. 3, 52-61 (2001).
19. V. S. Repin, *Klin. Gerontol*., No. 12, 29-36 (2001).
20. V. S. Repin, *Patol. Fiziol. Eksp. Ter*., No. 2, 3-7 (2001).
21. V. S. Repin and G. T. Sukhikh, *Medical Cell Biology* [in Russian], Moscow (1998).
22. V. D. Rozenberg and L. M. Nepomnyashchikh, *Pathological Anatomy of Postinfarction Heart Remodeling* [in Russian], Moscow (2002).
23. V. D. Rozenberg and L. M. Nepomnyashchikh, *Dilated Cardiomyopathy: General Pathology and Pathophysiology* [in Russian], Moscow (2004).
24. P. P. Rumyantsev, *Cardiomyocytes in Reproduction, Differentiation, and Regeneration Processes* [in Rusasian], Leningrad (1982).
25. D. S. Sarkisov, *Structural Bases of Adaptation and Compensation of Impaired Functions* [in Russian], Moscow (1987).
26. G. T. Sukhikh, V. V. Malaitsev, I. M. Bogdanova, and I. V. Dubrovina, *Byull. Eksp. Biol. Med*., **133**, No. 2, 124-131 (2002).
27. I. V. Uryvaeva, *Ibid*., **124**, No. 10, 364-368 (1997).
28. I. V. Uryvaeva, *Isv. Akad. Nauk, Ser. Biol*., No. 6, 728-737 (2001).
29. I. L. Chertkov and N. N. Drize, *Ter. Arkhiv*, No. 7, 5-11 (2004).
30. I. L. Chertkov and A. Ya. Fridenshtein, *Cellular Bases of Hemopoiesis (Hemopoietic Stem Cells)* [in Russian], Moscow (1977).
31. Yu. L. Shevchenko, *Vestn. Ros. Akad. Med. Nauk*, No. 11, 6-9 (2003).
32. J. D. Abbott, Y. Huang, D. Liu, *et al., Circulation,* **110**, 3300-3305 (2004).
33. Y. Adachi, J. Imagawa, Y. Suzuki, *et al., J. Mol. Cell. Cardiol.*, **36**, 707-710 (2004)
34. P. Anversa, B. Hiler, R. Ricci, *et al., J. Am. Coll. Cardiol*., **8**, 1441-1448. (1986).
35. P. Anversa and J. Kajstura, *Circ. Res*., **83**, 1-14 (1998).
36. P. Anversa, B. Nadal-Ginard, *Nature*, **415**, 240-243 (2002).
37. I. M. Barbash, P. Chouraqui, J. Baron, et al., Circulation, **108**, 863-868 (2003).
38. A. Behfar, L. V. Zingman, D. M. Hodgson, *et al., FASEB J*., **16**, 1558-1566 (2002).
39. A. P. Beltrami, L. Barlucchi, D. Torella, *et al., Cell*, **114**, 763-776 (2003).
40. A. P. Beltrami, K. Urbanek, J. Kajsrura, *et al., N. Engl. J. Med*., **344**, 1750-1757 (2001).
41. D. W. Benson, G. M. Silberbach, A. Kavanaugh-McHugh, *et al., J. Clin. Invest.*, **104**, 1567-1573 (1999).
42. D. M. Bodine, N. E. Seidel, M. S. Gale, *et al., Blood*, **84**, 1482-1491 (1994)
43. T. Brazelton, F. M. Rossi, G. I. Keshet, and H. M. Blau, Science, 290, 1775-1779 (2000).
44. W. Brenner, A. Aicher, T. Eckey, *et al., J. Nucl. Med*., **45**, 512-518 (2004).
45. C. Chimenti, J. Kajstura, D. Torella, *et al., Circ. Res*., **93**, 604-613 (2003).
46. M. A. Eglitis and E. Mezey, *Proc. Natl. Acad. Sci. USA*, **94**, 4080-4085 (1997).
47. G. Ferrari, G. Cusella-De Angelis, M. Coletta, *et al., Science*, **279**, 1528-1530 (1998).
48. G. D. Fischbach and R. L. Fischbach, *J. Clin. Invest.,* **114**, 1364-1370 (2004).
49. N. G. Frangogiannis, J. L. Perrard, L. H. Mendoza, *et al., Circulation*, **98**, 687-698 (1998).
50. S. Fukuhara, S. Tomita, S. Yamashiro, *et al., J. Thorac. Cardiovasc. Surg.*, **125**, 1470-1480 (2003).
51. A. M. Gewirtz, D. L. Sokol, and M. Z. Ratajczak, *Blood*, **92**, 712-736 (1998).
52. C. Grepin, L. Robitaille, T. Antakly, and M. Nemer, *Mol. Cell. Biol.,* **15**, 4095-4102 (1995).
53. J.-Q. He, Y. Ma, Y. Lee, *et al., Circ. Res*., **93**, 32-39 (2003).
54. E. D. Israels and L. G. Israels, *Stem Cells*, **19**, 88-91 (2001).
55. K. A. Jackson, S. M. Majka, H. Wang, *et al., J. Clin Invest.,* **107**, 1395-1402 (2001).
56. M. Jankowski, B. Danalache, D. Wang, *et al., Proc. Natl. Acad. Sci. USA*, **101**, 13 074-13 079 (2004).
57. J. Kajstura, A. Leri, N. Finato, *et al., Ibid.,* **95**, 8801-8805 (1998).
58. J. Kajstura, B. Pertoldi, A. Leri, *et al., Am. J. Pathol*., **156**, 813-819 (2000).
59. M. Kobling and Z. Estrov, *N. Engl. J. Med*., **349**, 570-582 (2003).
60. T. Kofidis, J. L. de Bruin, T. Yamane, *et al., Stem Cells,* **22**, 1239-1245 (2004).

61. R. Kronenwett, S. Martin, and R. Haas, *Ibid*., **18**, 320-330 (2000).
62. T. Kunisada, H. Yoshida, H. Yamazaki, *et al., Development*, **125**, 2915-2923 (1998).
63. M. A. Laflamme, D. Myerson, J. E. Saffitz, C. E. Murry, *Circ. Res.*, **90**, 634-640 (2002).
64. X. Lagasse, H. Connors, M. Al Dhalimy, *et al., Nat. Med*., **6**, 1229-1234 (2000).
65. M. S. Lee and R. R. Makkar, *Ann. Intern. Med*., **140**, 729-737 (2004).
66. A. Leri, L. Barlucchi, F. Limana, *et al., Proc. Natl. Acad. Sci. USA*, **98**, 8626-8631 (2001).
67. J. L. Liesveld, K. Rosell, and N. Panoskaltsis, *et al., J. Hematother. Stem Cell Res*., **10**, 643-655 (2001).
68. K. Liu, M. M. Schoonmaker, B. L. Levine, *et al., Proc. Natl. Acad. Sci. USA*, **96**, 5147-5152 (1999).
69. S. Makino, K. Fukuda, S. Miyoshi, *et al., J. Clin. Invest.*, **103**, 697-705 (1999).
70. N. Malouf, W.B. Coleman, and J. Grisham, *et al., Am. J. Pathol*., **158**, 1929-1935 (2001).
71. V. A. Maltsev, J. Rohwedel, J. Hescheler, and A. M. Wobus, *Mech. Dev.*, **44**, 41-50 (1993).
72. L. Martin-Rivera, E. Herrera, J. P. Albar, M. A. Blasco, *Proc. Natl. Acad. Sci. USA*, **95**, 10 471-10 476 (1998).
73. Y. Matsui, K. M. Zsebo, and B. L. Hogan, *Nature*, **347**, 667-669 (1990).
74. K. Matsuura, T. Nagai, N. Nishigaki, *et al., J. Biol. Chem*., **279**, 11 384-11 391 (2004).
75. E. Messina, L. De Angelis, G. Frati, *et al., Circ. Res.*, **95**, 911-921 (2004).
76. E. Mezey, K. J. Chandrosss, G. Harta, *et al., Science*, **290**, 1779-1782 (2000).
77. G. K. Michalopoulos and M. C. de Frances, *Ibid*., **276**, 60-66 (1997).
78. S. Miyagawa, Y. Sawa, S. Taketani, *et al., Circulation*, **105**, 2556-2561 (2002).
79. S. J. Morrison, N. M. Shah, and D. J. Anderson, *Cell*, **88**, 286-298 (1997)
80. J. S. Odorico, D. S. Kaufman, J. A. Thomson, *Stem Cells*, **19**, 193-204 (2001).
81. S. Oparil, S.P. Bishop, and F.J. Clubb, *Hypertension*, **6**, Suppl. III, III-38-III-43 (1984).
82. D. Orlic, J. M. Hill, and A. E. Arai, *Circ. Res.*, **91**, 1092-1102 (2002).
83. D. Orlic, J. Kajstura, S. Chimenti, *et al., Nature,* **410**, 701-705 (2001).
84. D. Orlic, J. Kajstura, S. Chimenti, *et al., Ann. N.Y. Acad. Sci*., **938**, 221-230 (2001).
85. D. Orlic, J. Kajstura, S. Chimenti, *et al., Proc. Natl. Acad. Sci. USA*, **98**, 10 344-10 349 (2001).
86. H. N. Pak, M. Qayyum, D. T. Kim, *et al., J. Cardiovasc. Electrophysiol.,* **14**, 841-848 (2003).
87. T. Papayannopoulou and B., Nakamoto *Proc. Natl. Acad. Sci. USA*, **90**, 9374-9378 (1993).
88. V. Patella, I. Marino, E. Arbustini, *et al., Circulation*, **97**, 971-978 (1998).
89. D. G. Penney, M. S. Baylerian, J. E. Thill, *et al., Am. J. Physiol*., **244**, H289-H297 (1983).
90. E. C. Perin, H. F. R. Dohmann, R. Borojevic, *et al., Circulation*, **107**, 2294-2302 (2003).
91. B. E. Petersen, W. C. Bowen, K. D. Patrene, *et al., Science,* **284**, 1168-1170 (1999).
92. R.O. Petersen and R. Baserga, *Exp. Cell. Res*., **40**, 340-352 (1965).
93. M.F. Pittenger and B.J. Martin, *Circ. Res*., **95**, 9-20 (2004).
94. F. Prosper, D. Stroncek, J.B. McCarthy, *et al., J. Clin. Invest.*, **101**, 2456-2467 (1998).
95. F. Quaini, K. Urbanek, A.P. Beltrami, *et al., N. Engl. J. Med*., **346**, 5-15 (2002).
96. S. Rangappa, J. W. C. Entwistle, A. S. Wechsler, and Y. Kresh, *J. Thorac. Cardiovasc. Surg*., **126**, 124-132 (2003).
97. T. Saito, J.-Q. Kuang, C. C. H. Lin, and R. C.-J. Chiu, *Ibid*, 114-122.
98. R. Sasaki, Y. Watanabe, T. Morishita, and S. Yamagata, *Tohoku J. Exp. Med*., **95**, 177-184 (1968).
99. M. Sata, A. Saiura, A. Kunisato, *et al., Nat. Med*., **8**, 403-409 (2002).
100. R. E. Schwartz, M. Reyes, L. Koodie, *et al., J. Clin. Invest*., **101**, 1291-1302 (2002).
101. J. G. Shake, P. J. Gruber, W. A. Baumgartner, *et al., Ann. Thorac. Surg*., **73**, 1919-1925 (2002).
102. S. Shintani, T. Murohara, H. Ikeda, *et al., Circulation*, **103**, 2776-2779 (2001).
103. B. E. Strauer, M. Brehm, T. Zeus, *et al., Ibid.*, **106**, 1913-1918 (2002).
104. B. Swynghedauw, *Physiol. Rev*., **79**, 215-262 (1999).
105. T. Takahashi, B. Lord, P. C. Schulze, *et al., Circulation,* **107**, 1912-1916 (2003).
106. M. Teyssier-Le Discorde, S. Prost, E. Nandrot, and M. Kirszanbaum, *Br. J. Haematol*., **107**, 247-253 (1999).
107. J. A. Thomson, J. Itskovitz-Eldor, S. S. Shapiro, *et al., Science*, **282**, 1145-1147 (1998).
108. C. Toma, M. F. Pittenger, K. S. Cahill, *et al., Circulation*, **105**, 93-98 (2002).
109. K. Urbanek, F. Quaini, G. Tasca, *et al., Proc. Natl. Acad. Sci. USA*, **100**, 10 440-10 445 (2003).
110. T. B. van Dijk, E. van den Akker, M. Parren-van Amelsvoort, *et al., Blood,* **96**, 3406-3413 (2000).
111. C. Ventura and M. Maioli, *Circ. Res.*, **87**, 189-194 (2000).
112. C. M. Verfaillie, R. Hurley, R. Bhatia, *et al., Crit. Rev. Oncol. Hematol.,* **16**, 201-224 (1994).
113. I. L. Weissman, *Cell*, **100**, 157-168 (2000).
114. A. M. Wobus, G. Kaomei, J. Shan, *et al., J. Mol. Cell. Cardiol*., **29**, 1525-1539 (1997).
115. C. Xu, S. Police, N. Rao, M. K. Carpenter, *Circ. Res*., **91**, 501-508 (2002).
116. M. Xu, M. Wani, Y.-S. Dai, *et al., Circulation*, **110**, 2658-2665 (2004).
117. W. Xu, X. Zhang, H. Qian, *et al., Exp. Biol. Med.*, **229**, 623-631 (2004).
118. Y.-S. Yoon, J.-S. Park, T. Tkebuchava, *et al., Circulation*, **109**, 3154-3157 (2004).