UDK 612.173:576.343

CELL THERAPY OF HEART PATHOLOGIES

L. L. LUKASH

Institute of Molecular Biology and Genetics
of National Academy of Sciences of Ukraine, Kyiv
E-mail: lukash@imbg.org.ua

The review article is devoted to cellular cardiomyoplasty as a novel technology of treatment of cardiac insufficiency. The experiments on animals and the first clinical trials have shown the possibility to improve the contractile function of diseased heart after transplantation of different types of donor cells: cardiomyocytes, bone marrow enriched by hematopoietic stem cells and mesenchymal stem cells. The problems of cellular cardiomyoplasty are discussed.

Key words: Cellular cardiomyoplasty, stem cell, cardiomyocyte, differentiation, tissue engineering in vitro, experimental models of myocardial infarction in vivo, autologeous, allogenic and xenogenic transplantation.

The heart failure (HF) is one of the main health problems of all economically developed countries. According to the recent survey data, its spreading among the population increases; for this reason there is a necessity for development in essence new, accessible and effective methods of HF correction. New cell technologies in cardiology are integrated by the term «cellular cardiomyoplasty» similar to different techniques of «cardiomyoplasty» which are intended to change the processes of remodeling of the myocardium and to influence its regeneration. So that all of them lead to the improvement of functions of a cardiac muscle [1].

The most often cause of HF is acute myocardium infarction. The miscellaneous pathological processes in the myocardium are accompanied by its complex adaptive — compensator rearrangement — «remodeling». Remodeling of the myocardium is a process of serious adaptive structurally functional changes of a cardiac muscle as a result of its damage. The processes of remodeling take part both in the zone of damage, and in able-bodied myocardium. The zone of infarct is step-by-step expanded and punched, and the unimpaired sites are hypertrophied and later are dilated, adapting to new conditions of functioning [2, 3].

The two-decade-old field of tissue engineering relies on the body's own repair mechanisms. It was considered that the damages of a cardiac muscle are irreversible and they result in remodeling under the scenario described above and are often finished by development of a heart failure within several months or years [4]. J. Kajstura et al. [5] have demonstrated that the mitosises of cardiomyocytes are possible, but they are very rare terminated by cytokinesis and are located, mainly, in periinfarct area and, as a consequent, can not substitute a defect which appeared after the damage of the myocardium. It is determined that a mitotic index of cardiomyocytes in pathological heart (cardiomyopathy) is approximately 10 times higher than in normal myocardium, that is, the proliferation of cardiac cells plays the relevant role in compensator processes of the myocardium.

Although a population of self-renewing stem cells in the hearts of mammals (human, mouse, dog) has been discovered, it is not enough to restore the cardiac tissue after massive myocardial infarction which leads quickly to cardiomyocytes death and the loss of tissue. As a result, replacement of defect of a cardiac muscle occurs, mainly, due to a proliferation of stromal cells (fibroblasts) [6].

The development of biotechnology, molecular and cell biology has made a cell not only the main object of influences, but also a tool for treatment of many diseases. Cell transplantation has a number of relevant advantages in comparison with the replacement of the whole organ. One of these advantages is that the cell therapy permits to obtain cell populations saved of an impurity of extraanti-

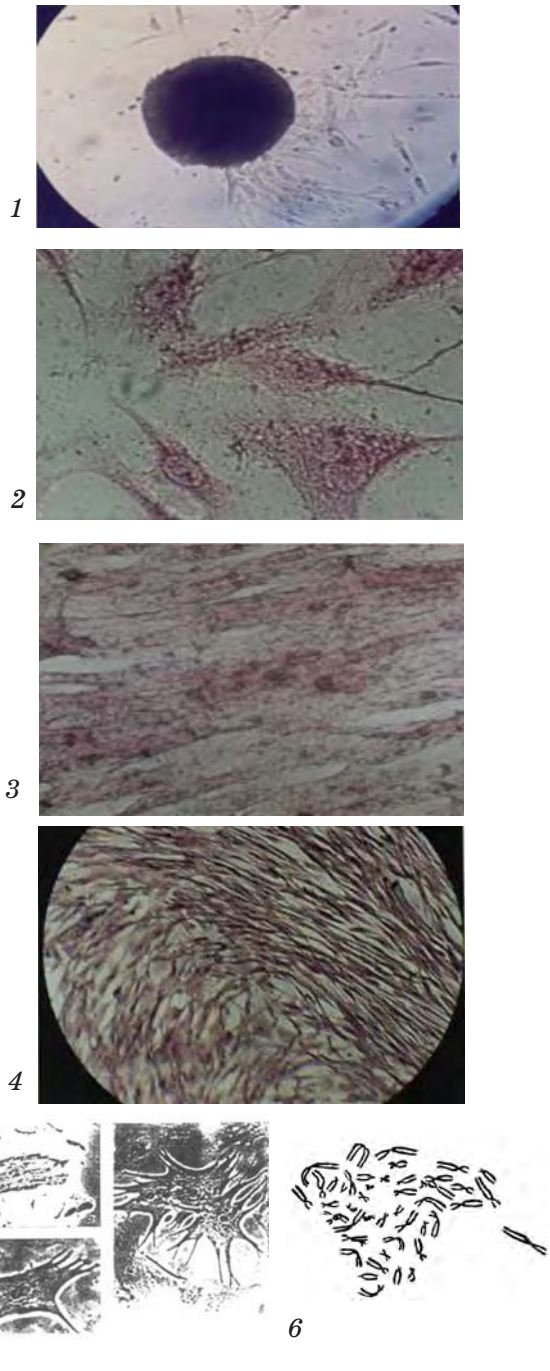
genic donor cells, which first of all participate in the process of immune rejection by using of cultural methods. Another advantage is that in comparison with organ transplantation, such operations are more accessible and cheaper for the patients. There is a capability to realize repeated cell transplantations.

Nowadays polipotent stem cells become a new source of cellular material for transplantation because they are capable to differentiate into different types of cells under the influence of microenvironment and specific growth factors ensuring information conditioning [7].

For the first time in 2001 [8], the results which testified a capability of human embryonic stem cells to differentiate into cardiomyocytes were published. In 2003 it was reported [9] that cardiomyocytes might be derived from embryonic stem cells, embryonic germ cells, cord blood cells and peripheral blood under the influence of microenvironment and specific growth factors in vitro.

The strategy of cardiomyogenic differentiation of mesenchymal stem cells using the composition of growth factors and the media conditioned by primary cardiomyocytes has been developed in the Institute of Molecular Biology and Genetics of NAS of Ukraine [10]. We obtained adult mesenchymal stem cell lines (CB-1, 4BL and SK-1) originated from cord blood, peripheral blood and skin of adult donors by cultural methods and cultivated the cells in vitro during 40–100 passages. Then the human mesenchyme-like cells have been passed in regular DMEM medium with 10 % of fetal bovine serum or in the same medium with addition of special growth factors which induce cardiomyogenic differentiation [11, 12]. The parental and differentiated cells have been characterized by cytogenetical method and immunophenotyping (Fig.). After long time cultivation in differentiation medium the studied cell populations contained more than 90% of myocytes which carried anti-A-smooth muscle marker. Practically this approach allows us to obtain and to keep cryopreserved unlimited amount of cells for research in different fields. We think that one of the most important and attractive field for application of the human cell lines obtained by us is the development of technology for cellular cardiomyoplasty which could be realized within hours after myocardial infarction.

A number of research works demonstrates a perspective development of cellular car diomyoplasty [13]. T. Reffelmann et al. [13] have shown in 2003 that the transplantation of neonatal cardiomyocytes induces angiogenesis



The morphology of differentiated and expanded *in vitro* cardiomyocytes [10,11]:

1 — Embryoid body with contractile area, formed by human embryonic germ cells;
2 — Growing individual cells, isolated from contractile area, after staining;
3 — Intercellular contacts between growing cells;
4 — Monolayer of stained cardiomyocytes;
5 — Scheme images of early differentiated cardiomyocytes;
6 — Stained chromosomes of individual cardiomyocyte

in zones of acclimation of cellular transplanted body. J. Leor et al. [14] have observed that cardiomyocytes grown in polymer carriers reshape a teleorganic tissue and stimulate neovascularizations. T. McDevitt et al. [15] have shown that elastomeric polyurethane film, which is biodegraded material, can serve as the optimal carrier to support the stability of 3D-culture of cardiomyocytes, that is the new technology of transplantation of organized heart tissue constructions with the purpose of myocardium reparation.

Morphological, physiological, immunophenotypical and molecular-genetic features of populations of cardiomyocytes, which are cultivated in vitro, have been studied with the purpose of their practical usage [16, 17]. It is known that postnatal cardiomyocytes have very poor growth potential in vitro and in vivo. At the same time, fetal cells have more potential to grow in culture and in an organism of the recipient comparing with postnatal ones. Thus, they excrete biologically active compounds — inducers of cardiomyocytes regeneration and myocardium remodeling, that is an information signal for implementation of definite biochemical process and a component of biochemical reaction of this process in an ambient tissue.

J. Wang et al. [18] offered and confirmed experimentally the hypothesis that microenvironment of the myocardium creates certain conditions and signals for cardiomyogenic differentiation. In their research muscle stem cells of rat after cultivation and removing the majority of hematopoietic stem cells, were grafted into myocardium and were differentiated into cardiomyocytes, created slot-hole contacts. B. Leobon et al. [19] found that grafted myoblasts are differentiated into specific myofibriles with completely independent contractile activity in relation to adjacent cardiomyocytes.

S. Makino et al. [20] have obtained cardiomyogenic cell line from murine stromal cells of the bone marrow. After treatment with 5-azacytidine in vitro, the processes of cardiomyogenic differentiation were induced. The obtained cells expressed a lot of genes specific for cardiomyocytes, had phenotype of fetal cardiomyocytes of heart ventricle. Cardiomyocytes differentiated by this way contracted spontaneously, were connected among themselves by plug-in disks, formed myotubes, had a metastructure, characterictic for cardiomyocytes. The myotubes generated action potential, which resembled those in ventricular cells.

L. Klug et al. [21] modified pluripotent embryonic stem cells genetically with the purpose that cardiomyogenic way of their differentiation would dominate in cellular population. They generated a line of stem cells, which carried a gene for heavy chain of myosin. Immunohistological and ultrastructural analysis showed, that cardiomyocytes obtained by this way were highly differentiated (99 % of cells contained myosin, myofibriles, the plug-in disks) and formed grafts in the hearts of adult mice during 7 weeks after implantation. It has been observed the appearance of all components of plug-in disks between recipient and donor cardiomyocytes in the myocardium of dogs, which had inheritable muscle dystrophia, in 10 weeks after transplantation of fetal cardiomyocytes [22].

R. Li et al. [23] were engaged in transplantation of fetal cardiomyocytes and cardiomyocytes of newborn rats. The authors of this work observed that the cardiomyocytes grown up in vitro formed a heart-like tissue both the structure as well as the functions. Cardiomyocytes contained the organized sarcomeres and were joined by intercellular contacts, which actuated desmosomes and fascia adherences. Cardiomyocytes were regularly, spontaneously and synchronously contracted in vitro. The comparative analysis had shown that the transplantation was more successful when introducing fetal cells rather than the neonatal cell material of 5-day's rats. In the first case up to 92 % of cells survived, in the other case it was only 50 % of survival. The cells isolated from young 22-day's and adult 32-day's animals did not survive in adult heart after a transplantation. The same authors have shown that the transplantation of autologous cardiomyocytes from mature animals into cryodamaged myocardium permitted only to persist them in the tissues during 5 weeks (duration of research). However, grafted cells modified the process of the myocardium remodeling: rendered assistance to avoid its dilatation and expansion of cicatrix, to preserve the depth of its wall.

J. Leor et al. [24] carried out a transplantation of allogenic fetal heart tissue to rats at different terms after a myocardial infarction and observed that the cells persisted up to 65 days, saved fetal phenotype and did not differentiated in mature cardiomyocytes.

At the same time, applications of allogenic fetal cardiomyocytes in clinical practice is limited by ethical standards and by the necessity to use the immunosupression as well, which by itself does not guarantee long-term existence of grafted material and reduces efficiency of transplantation. Therefore, for clinical usage advantage returns to an autologous material, such as the populations of mesenchymal stem cells of the bone marrow, in which it is possi-

ble to induce cardiomyogenic differentiation.

It has been shown that the injection of bone marrow cells into infarcted myocardium in mice resulted in some improvement in cardiac function and a decrease of infarcted zone [25-28]. However, in prior studies the investigators have used hemopoietic stem cells rather than mesenchymal cell precursors which are much less immunogenic [2,3].

An approach is developed for direct intravenous injection of allogenic bone marrow-derived mesenchymal stem cells for improvement of left ventricular function in the porcine model of myocardial infarction [29]. Direct intramyocardial injection of mesenchymal stem cells results in successful intramyocardial engraftment and differentiation into cardiomyocytes and endothelial cells and preserves left ventricular function after myocardial infarction in pigs [30].

S. Fukuhara et al. [31] have demonstrated, that the direct intercellular interaction with cardiomyocytes plays a key role in differentiation of stromal cells of the bone marrow into cardiomyocytes. The culture obtained is suitable for recovery of cardiomicroenvironment in vitro with the purpose of investigation of cell transplantation in cardiology.

Clinical trials of the methods of cell therapy are under way now. They have been started with using mostly autologous mixed cell populations from the bone marrow or cell populations enriched in hemopoietic $CD34^+$ stem cells or mesenchymal stem cells (only in some cases). The results of clinical trials vary in different medical centers [32]. In general, clinicians pointed out that all the studies were done safe and that some patients fared better after the trials. At the same time, they show a modest improvement (6,0–8,5 %) in heart function.

One of the main problems of cellular cardiomyoplasty is poor efficiency. Difficulties in the implementation of this progressive method can be connected with the limited cell source, an absence of designed criteria for estimation of quality and quantity of cell material prepared for transplantation. Another problem is that the patients after acute myocardium infarction need help immediately. In such a situation it is better to keep a cellular bank of standardized allogenic cells prepared for cell therapy.

It is considered now that probably the optimal cell type for cardiomyoplasty is cardiomyocyte progenitor cell. Cardiac stem cells or progenitors of cardiomyocytes with marker isl-1^+ have been identified in the hearts of different mammals. It has been shown that these cells participate in the regeneration of damaged myocardium [33–37]. So, in principal, it is possible to get biopsy from cardiac tissue of a patient and to isolate isl-1^+ cells for expansion in vitro. Autologous cells should be harvested, separated and then grown up in culture before injection. But it is obvious that the procedure of their preparing for practical application will take a long time.

Another possibility is to use allogenic mesenchymal stem cells differentiated into progenitors of cardiomyocytes in vitro. It is known that the prominent characteristic feature of terminally differentiated cardiomyocytes is their capacity to spontaneous automatic contractions. The experiments on obtaining the cardiomyocytes in culture and registration of their images conducted at the Institute of Molecular Biology and Genetics and Institute of Cybernetics of NAS of Ukraine, Kiev, have shown that the cells, grown up by conventional technique, have slower rhythm of contractions (from 20 up to 60 beats per minute) compared to a normal rhythm of human heart [11]. According to the data of other authors cardiomyocytes, which are cultured in vitro, sometimes reveal higher rhythm of spontaneous contractions, than the normal heart [7]. The mechanisms of rhythmogenesis are intensively investigated in experimental models [38].

There is basis to consider that this is the divergence in rhythms which creates informational noncompatibility of tissues. The divergence in rhythms, apparently, results in incompatibility of physiological processes, which descend in recipient organ particularly, and in an organism as a whole. Probably, with realization of operations on cell transplantation in the case, for example, of myocardial infarction, one of the causes of unsatisfactory functional outcomes can be an arrhythmia or noncompatibility of rhythms of contractions of transplanted biological tissue and a recipient organ. The authors [19] have observed that the grafted myoblasts are differentiated in specific myofibriles with completely independent contractile activity in relation to adjacent cardiomyocytes. So, another problem which should be resolved in future is biological sychronization of the cardiomyocytes of a patient and injected cells.

Nowadays cell transplantation is esteemed by the majority of the explorers as the alternative to organ transplantation. Apparently, it will be applied in clinical practice for improvement of the forecast in patients with a heart failure of different genesis (myocardial infarction, wound of heart, dilatation cardiomyopathy etc.). From the established facts, it is possible to

draw a conclusion that selection of stem cell type (fetal or postnatal), direction and conditions of differentiation, culture conditions and information compatibility of biological objects influence the success of transplantation.

LITERATURE

1. *Cleland J. F. G., McGowan J.* Heart Failure due to Ischaemic Heart Disease: Epidemiology, Pathophysiology and Progression // J. Cardiovasc. Pharmacol. — 1999. — V. 33, N3. — P. 17–29.
2. *Jackson K. A., Majka S. M., Wang H. et al.* Regeneration of ischemic cardiac muscle and vascular endothelium by adult stem cells // J. Clin. Invest. — 2001. — V. 107, N 11. — P. 1395–1356.
3. *Orlic D., Kajstura J., Chimenti S. et al.* Mobilized bone marrow cells repair the infarcted heart, improving function and survival // Proc. Natl. Acad. Sci USA. — 2001. — V. 98, N 18. — P. 10344–10349.
4. *Потапов И. В., Крашенинников М. Е., Онищенко Н. А.* Клеточная кардиомиопластика // Вестник трансплантологии и искусственных органов. — 2001. — N2. — С. 53–62.
5. *Kajstura J., Leri A., Finato N. et al.* Myocyte proliferation in endstage cardiac failure in humans. Proc. Natl. Acad. Sci. USA. — 1998. — V. 95. — P. 8801–8805.
6. *Kim W. H., Joo C. U., Ku J. H. et al.* Cell cycle regulators during human atrial development // Korean J. Intern. Med. — 1998. — V. 13, N2. — P. 77–82.
7. *Itskovitz-Eldor J. et al.* Differentiation of human embryonic stem cells can differentiate into embryoid bodies compromising the three embryonic germ layers // Mol. Med. — 2000. — V. 6. — P. 88–95.
8. *Kehat I., Kenyagin-Karsenti D., Snir M. et al.* Human embryonic stem cells can differentiate into myocytes with structural and functional properties of cardiomyocytes // J. Clin. Invest. — 2001. — V. 108, N3. — P. 407–414.
9. *The International* Journal of Artificial Organs. Abstracts of the XXX ESAO Congress «High Tech and Medicine», Aachen, Germany, 3–6 September 2003. — 2003. — V. 26, N7.
10. *Lukash L. L.* Directed differentiation of stem cells to cardiomyocytes for replacement therapy of heart pathologies // Ibid. — P. 612.
11. *Lukash L., Kovalenko O., Pidpala O., Yatsishina A.* Cardiomyogenic differentiation of mammalian stem cells in vitro // 4th Annual Meeting of the European Tissue Engineering Society. — 2005. — N146. — P. 38.
12. *Lukash L., Bazika D., Belyaeva N. et al.* Myogenic differentiation of adult human mesenchyme stem cells in vitro // J. Artific. Organs. — 2006. — V. 29, N5. — P. 509.
13. *Reffelmann T., Dow J. S., Dai W. et al.* Transplantation of neonatal cardiomyocytes after permanent coronary artery occlusion increases regional blood flow of infracted myocardium // J. Mol. Cell Cardiol. — 2003. — V. 35, N 6. — P. 607–613.
14. *Leor J., Aboulafia-Etzion S., Dar A. et al.* Bioengineered Grafts to Repair the Infarcted Myocardium. A New Approach to Repair the Infarcted Myocardium? // Circulation. — 2000. — V. 102, N3. — P. 56–61.
15. *McDevitt T. C., Woodhouse K. A., Hauschka S. D., Stayton P. S.* Spatially organized layers of cardiomyocytes on biodegradable polyurethane films for myocardial repair // J. Biomed. Mater Res. — 2003. — V. 66A, N3. — P. 586–595.
16. *Hughes S.* Cardiac stem cells // J. Pathol. — 2002. — V. 197. — P. 468–478.
17. *Anversa P., Leri A., Kajstura J., Nadal-Ginard B.* Myocyte growth and cardiac repair // J. Mol. Cell Cardiol. — 2002. — V. 34. — P. 91–105.
18. *Wang J. S., Shum-Tim D., Galipeau J. et al.* Marrow stromal cells for cellular cardiomyoplasty: feasibility and potential clinical advantages // J. Thorac. Cardiovasc. Surg. — 2000. — V. 120. — P. 999–1006.
19. *Leobon B., Garcin I., Menasche P. et al.* Myoblasts transplanted into rat infarcted myocardium are functionally isolated from their host // Proc. Natl. Acad. Sci. USA. — 2003. — V. 100, N13. — P. 7808–7811.
20. *Makino S., Fukuda K., Miyoshi S. et al.* Cardiomyocytes can be generated from marrow stromal cells in vitro // J. Clin. Invest. — 1999. — V. 103. — P. 697–705.
21. *Klug M. G., Soonpaa M. H., Koh G. Y. et al.* Genetically Selected Cardiomyocytes from Differentiating Embryonic Stem Cells Form Stable Intracardiac Grafts // J. Clin. Invest. — 1996. — V. 98. — P. 216–224.
22. *Koh G. Y., Soonpaa M. H., Klug M. G. et al.* Stable fetal cardiomyocyte grafts in the hearts of dystrophic mice and dogs // J. Clin. Invest. — 1995. — V. 96, N4. — P. 2034–42.
23. *Li R. K., Jia Z. Q., Weisel R. D. et al.* Cardiomyocyte transplantation improves heart function // Ann. Thorac. Surg. — 1996. — V. 62, N3. — P. 654–60.
24. *Leor J., Patterson M., Qumones M. J. et al.* Transplantation of Fetal Myocardial Tissue Into the Infarcted Myocardium of Rat. A Potential Method for Repair of Infarcted Myocardium // Circulation. — 1996. — V. 94, N2. — P. 332–336.
25. *Kocher A. A., Schuster M. D., Szaboles M. J. et al.* Neovascularization of ischemic myocardium by human bone-marrow-derived angioblasts prevents cardiomyocyte apoptosis,

reduces remodeling and improves cardiac function // Nat. Med. — 2001. — V. 7. — P. 430–436.
26. *Toma C., Pittenger M. F., Cahill K. S. et al.* Human mesenchymal stem cells differentiate to a cardiomyocyte phenotype in the adult murine heart // Circulation. — 2002. — V. 105. — P. 93–98.
27. *Tomita S., Mickle D. A., Weisel R. D. et al.* Improved heart function with myogenesis and angiogenesis after autologous porcine bone marrow stromal cell transplantation // J. Thorac. Cardiovasc. Surg. — 2002. — V. 123. — P.1132–1140.
28. *Shake J.G., Gruber P.J., Baumgartner W.A. et al.* Mesenchymal stem cell implantation in a swine myocardial infarct model: Engraftment and functional effects // Ann. Thorac. Surg. — 2002. — V. 73. — P. 1919–1925.
29. *Price M. J., Chou C-C., Frantzen M. et al.* Intravenous mesenchymal stem cell therapy early after reperfused acute myocardial infarction improves left ventricular function and alters electrophysiological properties // Inter. J. Cardiol. — 2005. — P. 1–9.
30. *Makkar R. R., Price M. J., Lill M. et al.* // J. Cardiovasc. Pharmacol. Therapeut. — 2005. — V. 10, N4. — P. 1–9.
31. *Fukuhara S., Tomita S., Yamashiro S. et al.* Direct cell-cell interaction of cardiomyocytes is key for bone marrow stromal cells to go into cardiac lineage in vitro // J. Thorac. Cardiovasc. surg. — 2003. — V. 125, N6. — P. 1470–1480.
32. *Scott C. T.* Stem cell now. — 2005. — 243 p.
33. *Urbanek K. et al.* Intense myocyte formation from cardiac stem cells in human cardiac hypertrophy // PNAS. — 2003. — V. 100. — P. 10440.
34. *Beltrami A. P. et al.* Adult cardiac stem cells are multipotent and support myocardial regeneration // Cell. — 2003. — V. 114. — P. 763.
35. *Laugwitz K-L. et al.* Postnatal isl-1^{+} cardioblasts enter fully differentiated cardiomyocyte lineages // Nature. — 2005. — V. 433. — P. 647.
36. *Linke 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., USA. — 2005. — V. 102, N25. — P. 8966.
37. Penn M.S. et al. Role of stem cell homing in myocardial regeneration // Int. Cardiol. — 2004. — V. 95, N1. — P. 23.
38. *Li J., Patel V. V., Kostetskii I. et al.* Cardiac-specific loss of N-cadherin leads to alteration in connexins with conduction slowing and arrhythmogenesis // Circ. Res. — 2005. — V. 97, N2(5). — P. 474–481.

КЛІТИННА ТЕРАПІЯ СЕРЦЕВОЇ ПАТОЛОГІЇ

Л. Л. Лукаш

Інститут молекулярної біології і генетики Національної академії наук України, Київ
E-mail: lukash@imbg.org.ua

Оглядова стаття присвячена клітинній кардіоміопластиці як новій технології лікування серцевої недостатності. Експерименти на тваринах і перші клінічні випробування показали можливість поліпшення скорочувальної функції ураженого міокарда після трансплантації різних типів клітин: кардіоміоцитів, кісткового мозку, збагаченого гемопоетичними стовбуровими клітинами, а також мезенхімальних стовбурових клітин. Обговорюються проблеми клітинної кардіоміопластики.

Ключові слова: клітинна кардіоміопластика, стовбурові клітини, кардіоміоцити, тканинна інженерія в культурі, експериментальні моделі інфаркту міокарда in vivo, аутологічна, алогенна, ксеногенна трансплантація.

КЛЕТОЧНАЯ ТЕРАПИЯ СЕРДЕЧНОЙ ПАТОЛОГИИ

Л. Л. Лукаш

Институт молекулярной биологии и генетики Национальной академии наук Украины, Киев
E-mail: lukash@imbg.org.ua

Обзорная статья посвящена клеточной кардиомиопластике как новой технологии лечения сердечной недостаточности. Эксперименты на животных и первые клинические испытания новой технологии показали возможность улучшения сократительной функции пораженного миокарда после трансплантации различных типов клеток: кардиомиоцитов, костного мозга, обогащенного гемопоэтическими стволовыми клетками, а также мезенхимальных стволовых клеток. Обсуждаются проблемы клеточной кардиомиопластики.

Ключевые слова: клеточная кардиомиопластика, стволовые клетки, кардиомиоциты, тканевая инженерия в культуре, экспериментальные модели инфаркта миокарда in vivo, аутологичная, аллогенная, ксеногенная трансплантация.