DOI: 293-301. DOI: 10.20514/2226-6704-2022-12-4-293-301
EDN: DXUZOX
УДК 616.12-008.46-085.361

И.С. Долгополов[1], М.Ю. Рыков*[1,2], В.А. Осадчий[1,3]

[1] — ФГБОУ ВО «Тверской государственный медицинский университет» Минздрава России, Тверь, Россия
[2] — Медицинская клиника «НАКФФ», Москва, Россия
[3] — ГБУЗ ТО «Клиническая больница скорой медицинской помощи», Тверь, Россия

РЕГЕНЕРАТИВНАЯ ТЕРАПИЯ ПРИ ХРОНИЧЕСКОЙ СЕРДЕЧНОЙ НЕДОСТАТОЧНОСТИ: ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ КЛЕТОЧНЫХ И БЕСКЛЕТОЧНЫХ ТЕХНОЛОГИЙ

I.S. Dolgopolov[1], M.Yu. Rykov*[1,2], V.V. Osadchij[1,3]

[1] — Tver State Medical University, Tver, Russia
[2] — Medical clinic "NAKFF", Moscow, Russia
[3] — Clinical Emergency Hospital, Tver, Russia

Regenerative Therapy for Chronic Heart Failure: Prospects for the Use of Cellular and Acellular Technologies

Резюме

Хроническая сердечная недостаточность (ХСН) является одной из наиболее распространенных и тяжелых форм ишемической болезни сердца (ИБС), на фоне которой существенно снижается продолжительность и качество жизни пациентов. Применяемые в настоящее время фармакологические и немедикаментозные методы ~~ее~~ лечения недостаточно эффективны, а трансплантация сердца ограничена организационными и техническими сложностями, возникающими при выполнении этого оперативного вмешательства, а также недостаточной доступностью донорских органов. Известно, что потенциал клеток миокарда к репарации невелик, поэтому регенеративная терапия может быть востребована, как новое перспективное направление лечения ХСН.

Существует несколько направлений клеточной терапии, способствующей улучшению процессов репарации миокарда. Одним из них является трансплантация соматических стволовых клеток, которая считается безопасной и несколько улучшает сократимость миокарда, преимущественно за счет паракринных механизмов регуляции клеточного цикла. В качестве альтернативы этой методики, для трансплантации непосредственно в поврежденные участки миокарда могут быть использованы кардиомиоциты, полученные из индуцированных плюрипотентных стволовых клеток (iPSC). Однако до начала применения таких клеток у лиц, страдающих ХСН, предстоит решить проблемы их потенциальной онкогенности и недостаточно хорошей выживаемости в условиях редукции кровотока на фоне тяжелого коронарного атеросклероза. В ряде исследований рассматривались и другие направления клеточной терапии, в частности бесклеточный подход к прямому перепрограммированию, заключавшийся в преобразовании эндогенных сердечных фибробластов в индуцированные кардиомиоцитоподобные клетки. В обзоре рассматривается текущая ситуация и перспективы использования регенеративных клеточных и бесклеточных технологий при ХСН, которые могут быть введены в клиническую практику в ближайшем будущем.

Ключевые слова: *хроническая сердечная недостаточность, регенеративная клеточная терапия, клеточные и бесклеточные технологии, кардиомиоциты, фибробласты*

*Контакты: Максим Юрьевич Рыков, e-mail: wordex2006@rambler.ru
*Contacts: Maksim Yu. Rykov, e-mail: wordex2006@rambler.ru
ORCID ID: https://orcid.org/0000-0002-8398-7001

Конфликт интересов

Авторы заявляют, что данная работа, её тема, предмет и содержание не затрагивают конкурирующих интересов

Источники финансирования

Авторы заявляют об отсутствии финансирования при проведении исследования

Статья получена 07.02.2022 г.

Принята к публикации 05.04.2022 г.

Для цитирования: Долгополов И.С., Рыков М.Ю., Осадчий В.А. РЕГЕНЕРАТИВНАЯ ТЕРАПИЯ ПРИ ХРОНИЧЕСКОЙ СЕРДЕЧНОЙ НЕДОСТАТОЧНОСТИ: ПЕРСПЕКТИВЫ ИСПОЛЬЗОВАНИЯ КЛЕТОЧНЫХ И БЕСКЛЕТОЧНЫХ ТЕХНОЛОГИЙ. Архивъ внутренней медицины. 2022; 12(4): 293-301. DOI: 10.20514/2226-6704-2022-12-4-293-301. EDN: DXUZOX

Abstract

Cardiovascular diseases are the second leading cause of death and disability worldwide after malignancies. Heart failure (HF) has a large impact not only on the economics of healthcare but also on the quality of life, functionality and life expectancy of patients. Pharmacological and non-pharmacological therapies have been developed, but these medical therapies have limited effects to cure patients with severe CH. Heart transplantation is limited due to the low number of donor organs. Human cardiac potential for spontaneous repair is insignificant, so regenerative therapy is in great demand as a new treatment strategy. Currently, there are several strategies for heart regeneration. Transplantation of somatic stem cells was safe and modestly improved cardiac function after myocardial infarction and in patients with CF mainly through paracrine mechanisms. Alternatively, new cardiomyocytes could be generated from induced pluripotent stem cells (iPSCs) to transplant into injured hearts. However, several issues remain to be resolved prior to using iPSC-derived cardiomyocytes, such as a potential risk of tumorigenesis and poor survival of transplanted cells in the injured heart. Recently, direct cardiac cell-free reprogramming has emerged as a novel technology to regenerate damaged myocardium by directly converting endogenous cardiac fibroblasts into induced cardiomyocyte-like cells to restore cardiac function.

Many researchers have reported direct reprogramming of the heart in vivo in animal and human cells. In this review, we review the current status of cardiac cell-based and cell-free regenerative technology, a great hope to treat cardiovascular diseases in clinical practice.

Key words: *chronic heart failure, regenerative cell therapy, cell and cell-free technologies, cardiomyocytes, fibroblasts*

Conflict of interests

The authors declare no conflict of interests

Sources of funding

The authors declare no funding for this study

Article received on 07.02.2022

Accepted for publication on 05.04.2022

For citation: Dolgopolov I.S., Rykov M.Yu., Osadchij V.V. Regenerative Therapy for Chronic Heart Failure: Prospects for the Use of Cellular and Acellular Technologies. The Russian Archives of Internal Medicine. 2022; 12(4): 293-301. DOI: 10.20514/2226-6704-2022-12-4-293-301. EDN: DXUZOX

CHF — chronic heart failure, CMPCs — cardiomyocyte progenitor cells, DCM — dilated cardiomyopathy, EF — ejection fraction, ESCs — embryonic stem cells, IHD — ishemic heart disease, iPSCs — induced pluripotent stem cells, LV — left ventricle, MNCs — mononuclear cells

Introduction

Ischemic heart disease (IHD) is reasonably considered the leading cause of disability and death in most countries of the world. IHD leads to the development of chronic heart failure (CHF) that is a global problem of present-day society, dramatically reduces the duration and quality of life of the population, and increases medical care economic burden. More than 40 million adults worldwide suffer from CHF. By 2030, its prevalence is supposed to increase by no less than 45-50 %. The incidence of detection of circulatory failure increases with population aging and is approaching a critical value of 10 per 1,000 individuals in the age group 65+.

The pathogenetic basis of CHF is primarily impaired systolic function of the myocardium of left and right ventricles. As a result, the volume of intercellular fluid increases, congestion develops in pulmonary and systemic circulation, perfusion of organs and tissues worsens, and multiple organ failure gradually develops. Classifications of circulatory failure that are currently widely used in clinical practice define the need for mechanical support of blood circulation and heart transplantation as the basic criteria for assessing disease severity, along with the degree of limitation of patient's functional activity, severity of congestive changes and their resistance to ongoing therapy. This need is due to a sharp decrease in the left ventricle (LV) ejection fraction (EF) in the vast majority of patients hospitalized for decompensated CHF. Moreover, the analysis of the prevalence of decreased LV contractile function reveals a number of significant racial and gender differences. Thus, the highest incidence of CHF is observed in African American male patients; it is accompanied by significantly decreased EF in approximately 70 % of cases. On the contrary, in Caucasian female patients, EF in 60 % of cases is slightly reduced or within normal [1, 2].

Management of CHF mainly includes pathogenesis-targeted combined drug therapy. The optimal

treatment, first of all, contributes to maintaining LV myocardium contractile function, reducing pulmonary hypertension, eliminating congestive changes, suppressing excessive activity of humoral regulatory systems, in particular, renin-angiotensin-aldosterone system that causes generalized disorders in electrolyte balance. However, the options of drug treatment, especially after the development of severe congestive circulatory failure, are often limited what results in high mortality rate in such patients. One of the most important reasons for the low efficiency of conservative treatment is the constant degradation and death of cardiomyocytes, their gradual replacement with fibroblasts that are not able to ensure the proper functional activity of ventricles. Surgical treatment can be considered as an alternative to the drug therapy of terminal CHF, the options are coronary artery bypass grafting, atrioventricular annuloplasty, valve replacement, aneurysmectomy, etc. However, their practicability and effectiveness are ambiguously assessed by different authors. Heart transplantation is considered to be the most effective procedure, however, it is limited by severe shortage of donors, stringent criteria for selecting patients, and high risk of surgical intervention. Thus, currently available pharmacological and surgical methods of CHF treatment are in some cases not effective enough and require further improvement.

One of the promising areas for the treatment of CHF patients can be considered regenerative cell-based and cell-free therapy that allows potentiating the processes of myocardial repair and thereby increasing the duration and quality of life of patients. It is generally assumed that mammalian cardiomyocytes are terminally-differentiated cells. As a result, mammals are not able to spontaneously restore the myocardium damaged by one or another pathological factor, unlike, for example, amphibians or fish that demonstrate stable regenerative reactions of myocardial after traumatic injury. However, neonatal mice demonstrated their ability to regenerate significant parts of heart muscle after its partial surgical resection [3]. Results of studies conducted by a group of researchers from Karolinska Institute revealed that the pool of cardiomyocytes is renewed during life, in humans at a rate of 0.5–1 % of the entire population per year [4]. However, the regenerative capacity of human cardiomyocytes is not sufficient enough and is not able to ensure the restoration of a more or less significant area of myocardium. At the initial stage, there were attempts of heart muscle regeneration using bone marrow mononuclear cells (MNCs). Although early clinical trials have demonstrated improvement in myocardial contractility, results of subsequent studies were less encouraging [5]. With the development of cellular technologies and the ability to obtain *in vitro* cardiomyocyte progenitor cells (CMPCs) that are able to proliferate and differentiate into mature specialized myocardial cells, there was a start of new stage of regenerative cell therapy [6]. Use of autologous CPC culture resulted in some improvement in myocardial contractile function and appeared to be safe. However, the survival rate of transplanted cells remains low, and their ability to differentiate into mature cardiomyocytes is very limited. Positive effects observed with the use of MNC and CPC cell therapy are most likely associated with paracrine effects on functioning cardiomyocytes than with their regeneration [7]. Use of cardiomyocytes derived from allogeneic pluripotent stem cells, such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), has also demonstrated its effectiveness. However, the use of these cell sources is limited due to the low rate of engraftment, as well as their potential oncogenicity, risk of graft rejection, and ethical reasons. Currently, cell-free regenerative methods are also developed. Stem cell therapy and reprogramming of resident fibroblasts into cardiomyocyte-like cells performed directly *in vivo* by transduction of certain cardiac-specific factors is one of the newest technologies aimed at the regeneration of cardiomyocytes and the restoration of myocardial functional performance [8, 9].

This review summarized achievements of present-day medical science and practice in investigating the options for regeneration of highly specific heart cells, as well as possibilities and issues of the use of cell-based and cell-free technologies in the clinical practice of CHF management in the near future.

Regenerative cell therapy for myocardial damage

Use of adult somatic stem cells and embryonic cells

At the early stages of regenerative medical trials, bone marrow MNCs generated considerable interest, as they demonstrated cardiogenic potential *in vitro*, as well as effectiveness in rodent models of myocardial infarction [5, 10]. Results of small clinical trials of various routes of administration of MNCs to humans have demonstrated moderately increased ejection fraction and several positive changes in the area of focal scar myocardial changes. However, subsequent numerous, randomized and double-blind clinical trials resulted in failure to reproduce previously obtained results [5, 11]. Bone marrow mesenchymal stem cells (MSCs) have also demonstrated *in vitro* cardiogenic potential and improved cardiac function in animal models of myocardial infarction. However, results of multicentre clinical trials such as POSEIDON revealed only moderate improvement in cardiac function, and further studies demonstrated that MSCs have no ability to differentiate into fully mature cardiomyocytes [12, 13].

Interest of researchers in cardiomyocyte progenitor cells (CMPCs) was driven by the report that these

cells are able to differentiate in three types required for the regeneration of myocardial structures, that is, cardiomyocytes, smooth muscle cells, and endothelial cells. *In vitro* experiments revealed the role of one of the hematopoietic markers on the surface of CMPCs. In several preclinical trials, *c-kit* (the gene encoding kit protein tyrosine kinase receptor) positive CMPCs demonstrated regenerative potential in small and large animal models [14]. First clinical trials, SCIPIO, when patients with ischemic cardiomyopathy received intracoronary autologous c-kit (+) CMPCs, revealed a slight increase in ejection fraction and a decrease in the size of cardiosclerosis area [15]. In the following study, CADUCEUS, a mixed CPC population, with c-kit (+) cells and cardiospheres, was used [7]. It was found that intracoronary infusion of autologous CMPCs during post-infarction period is safe, technically realizable and effective. In particular, there was a significant reduction in scar mass, increase in viable heart mass, and improvement in local contractility compared to the control group. However, there was no significant difference in changes in ejection fraction and end systolic and diastolic volume of LV during treatment with autologous CMPCs in treatment and control groups. More recent animal studies demonstrated that only small amount of c-kit (+) CMPCs is transformed into cardiomyocytes, they were mainly transformed in endothelial cells [16].

According to the results of preclinical studies of cell cultures, skeletal muscle precursor cells localized under the basal lamina of muscle fibers were also considered as a source for myocardial regenerative therapy. However, experiments in animal models and small clinical trials in humans revealed high incidence of ventricular arrhythmias that increased the possibility of developing sudden coronary death [17]. Pathophysiological basis for abnormal ventricular extrasystoles and episodes of paroxysmal ventricular tachycardia was the absence of electromechanical connection between transplanted cells and host cells [18]. The results of MAGIC study demonstrated no effectiveness of the use of skeletal muscle cells in ischemic cardiomyopathy, either in the near or in the long term [19, 20]. A meta-analysis of 667 patients from 11 studies who received autologous MNCs for non-ischemic dilated cardiomyopathy demonstrated good results, mainly for increasing LV ejection fraction and reducing LV end-diastolic volume. Moreover, the group of patients after MNC transplantation demonstrated improvement in the results of six-minute walk test compared with the control group [21].

A number of studies have reported the successful use of CD34+ hematopoietic autologous stem cells derived from peripheral blood along with granulocyte colony-stimulating factor mobilization in patients with dilated cardiomyopathy (DCM). Thus, according to the results of a randomized study that involved 110 patients with DCM, in treatment group there was a significant increase in LVEF, increase in walking distance in 6-minute walking test, and decreased level of N-terminal pro B-type natriuretic peptide that is one of the reliable markers of hypervolemia in CHF. Duration of follow-up for such patients was at least 5 years. Their five-year survival was 2.3-fold higher than in the control group. The increased LV EF directly correlated with the dose of CD34+ cells transplanted into myocardium [22]. However, in contrast to the fairly good results of cell therapy in DCM, its effectiveness in the management of myocardial infarction, focal or diffuse cardiosclerosis is highly controversial. In particular, method of administration of stem cells and their doses require further investigation. It also seems that there is a careful selection of patients in treatment groups considering rigorous criteria for inclusion in protocols [23].

Good results were obtained with intramyocardial use of CD34+ MNCs in patients with exertional angina of 3–4 functional class that is resistant to combined antianginal therapy. A meta-analysis of randomized, double-blind, phase 1 and phase 2 ACT-34 study and phase 3 RENEW study during long-term follow-up demonstrated improved exercise tolerance, decreased intensity and incidence of chest pain, as well as significantly reduced incidence of myocardial infarction and clinically significant CHF in patients who received cell therapy [24]. In regard to the pathogenetic basis of such a good therapeutic effect, the authors mention that cells with CD34+ receptor on their surface are able to trigger the processes of angiogenesis and neovascularization of heart tissues via several mechanisms. First, CD34+ MNCs differentiate into smooth muscle cells and endothelial cells that are the basic structural components of the inner walls of blood vessels. This, in turn, leads to vascular re-endothelialization and myocardial revascularization [25]. Secondly, these cells perform paracrine regulation by producing factors that stimulate angiogenesis and suppress apoptosis of endothelial cells and cardiomyocytes. In addition, factors released by CD34+ MNCs contribute to extracellular matrix remodeling and mobilization of additional progenitor cells [25, 26]. The pro-angiogenic mechanism of cell therapy with CD34+ MNCs is also mediated by the production of so-called exosomes, i.e. membrane-bound nanobubbles. Exosomes carry pro-angiogenic miRNAs that activate the processes of division and differentiation of stem cells [27].

The concept of using embryonic stem cells (ESCs) is attractive due to their pluripotency and ability to differentiate in any type, including functional cardiomyocytes [28]. However, the clinical use of ESCs is limited by ethical issues, as well as by the fact that they are highly immunogenic and can cause rejection reactions. In addition, there is an open question concerning the potential genetic instability of these cells and the development of benign, and possibly malignant neoplasms from them [29, 30].

Cardiomyocytes derived from induced pluripotent stem cells (iPSCs)

Another therapeutic approach involves the use of functional cardiomyocytes obtained *in vitro* from autologous or allogeneic iPSCs [31]. This treatment method was developed after the publication of the results of studies conducted by Takahashi K. et al. [32, 33] on the potential to directly reprogram mouse and human fibroblasts using a combination of four transcription factors: Oct 4, Sox 2, Klf 4 and c-Myc, also known as Yamanaka factors. iPSCs obtained that way share basic morphological and functional characteristics with ESCs and demonstrate the expression of genes of the same type; all this makes them a good alternative to embryonic cells [32–34]. Thus, the discovery of iPSCs helped to solve the existing ethical issues and has great potential for the development of cell regenerative therapy in the time of personalized medicine. Treatment with autologous fibroblasts reprogrammed into cardiac-specific iPSCs also seems to be promising. A group of Japanese researchers reported that transplanted cardiomyocytes derived from allogeneic iPSCs were able to persist in heart tissues in immunosuppressed monkeys for up to 12 weeks. Upon that, improved myocardial contractile function and increased LV EF were observed [35]. During evaluation of adverse effects, the researchers observed high incidence of ventricular arrhythmias that were most likely associated with different degrees of maturity and functional activity of the transplanted cardiomyocytes. Despite the fact that the results of further studies have demonstrated the possibility of obtaining a more mature and homogeneous cell population, the heterogeneity of cardiomyocytes obtained from iPSCs can be one of the significant obstacles to the implementation of this technique in clinical practice [36]. Another challenge is that iPSCs demonstrate pronounced genetic instability and the ability to develop teratomas *in vivo* [37].

Direct reprogramming of myocardial cells

Direct reprogramming of resident scar-forming fibroblasts into cardiomyocytes can change approach to cell therapy for cardiovascular diseases, primarily, myocardial infarction (Figure 1).

It was demonstrated that a combination of several cardiac-specific factors, such as *Gata4* (the gene encoding proteins for "zinc fingers" binding to the "GATA" DNA sequence and playing a role in the differentiation of myocardial cells), *Mef2c* (the gene encoding myocyte-specific enhancer binding factor 2C), and *Tbx5* (the gene encoding T-box transcription factor 5), is able to directly convert fibroblasts into heart muscle cells without passing through stem cell stage [9, 38]. This method allows circumventing the limitations associated with the requirements for the number of transplanted cells, their survival rate and significantly reducing the risk of teratoma development. During the experiment on animals, reprogrammed cardiomyocytes demonstrated good intercellular interaction and structural organization, had global gene expression profiling similar to natural heart muscle cells, as well as electrophysiological potentials and spontaneous contractions. Unfortunately, this combination of factors was found to be insufficient in the studies with human cells [39]. However, despite the low efficiency of reprogramming and the absence of spontaneous beating, these cells demonstrated the ability to mature and contract synchronously when co-growing with mouse cardiomyocytes. Cao N. et al in their papers have demonstrated that human fibroblasts were able to transform into cardiomyocyte-like cells using a combination of nine different chemical compounds [40].

During direct reprogramming of cardiac cells, the following different signaling pathways interact with each other: transforming growth factor-β (TGF-β), Rho-associated kinase (ROCK), WNT pathway proteins (Wnt signaling pathway is one of the mammalian intracellular signaling pathways that regulates embryogenesis and differentiation cells), Notch (transmembrane proteins that regulate cell differentiation and interaction of adjacent cells), and Akt (protein kinase B family proteins). Influence on these pathways at different stages can alter the effectiveness of therapy. It is notable that TGF-β pathway is one of the active pathways in fibroblasts as well. Inhibition of TGF-β and WNT pathways was shown to increase the efficiency of reprogramming [41, 42]. Cellular signals that ensure the normal functioning of fibroblasts probably act as a barrier during attempts to transform them into other cell types and should be suppressed for successful reprogramming. Epigenetic barriers are another obstacle to the direct reprogramming process, in addition to the typical for fibroblasts signaling pathways. For successful reprogramming, cells should be able to use genes that are inactive in a given cell population. Epigenetic factors control their activity with the help of histone methylation, acetylation, and ubiquitination [9]. Research group of Zhou Y. et al. obtained Bmi1 (B cell-specific Moloney murine leukemia virus integration site 1), a protein from the group of proteins that can remodel chromatin that is a critical epigenetic barrier for direct reprogramming of fibroblasts into cardiomyocytes [43]. The authors demonstrated that Bmi1 regulates key cardiogenic genes through direct binding of these loci in fibroblasts, and inhibition of Bmi1 contributes to their activation.

The overall goal of direct reprogramming is to restore damaged myocardium and to improve its functional state by converting endogenous fibroblasts into cardiomyocytes. The authors of several studies reported *in vivo* direct reprogramming by delivering a

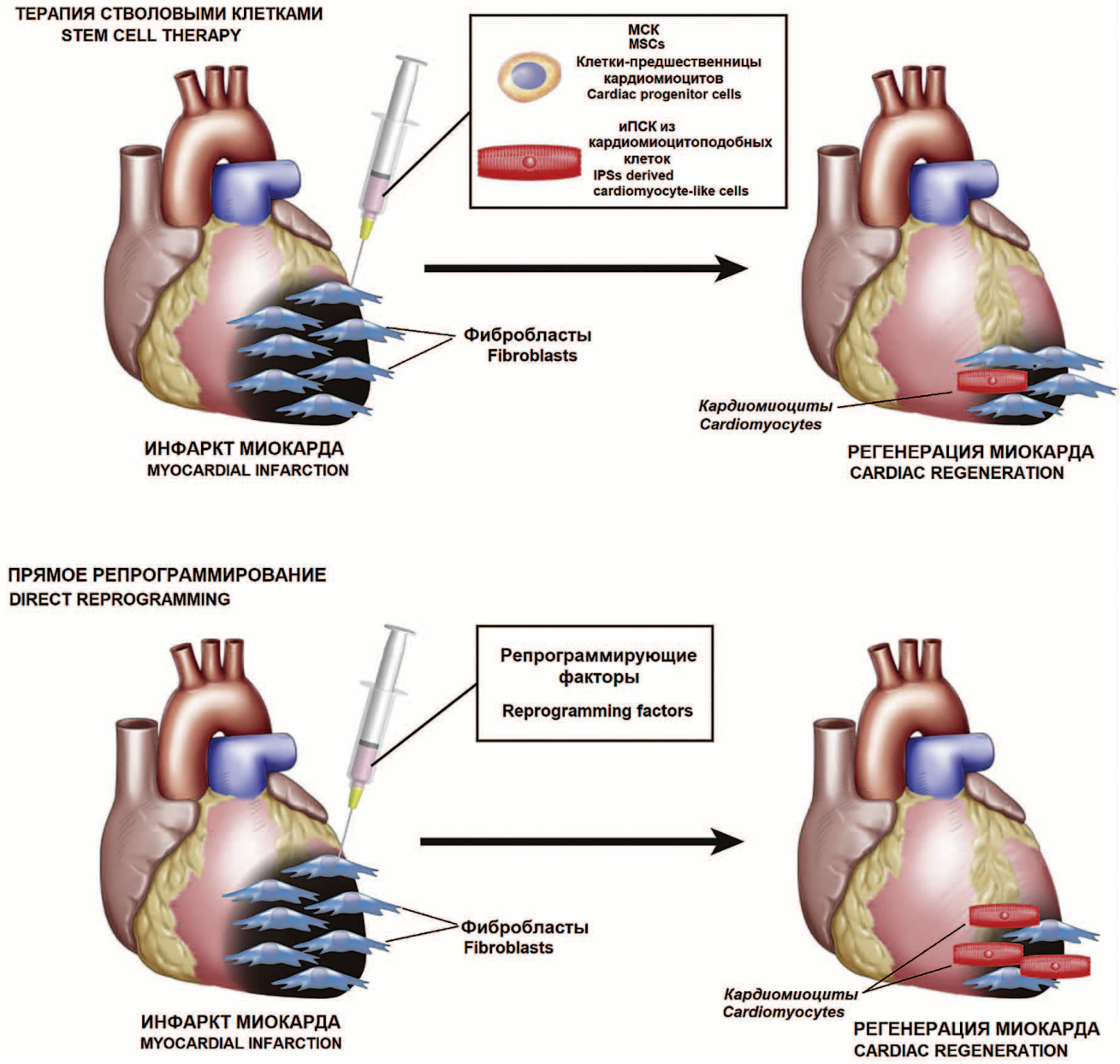

Figure 1. *Strategies for regeneration of damaged myocardium*
Note: MSC-mesenchymal stem cells, iPSCs — induced pluripotent stem cells

set of required factors to the ischemic myocardium of mice [44, 45]. Clone tracing was carried out to demonstrate the origin of these cardiomyocytes from resident cardiac fibroblasts; it allowed to confirm that the obtained cell population was not the result of a fusion with existing myocardial cells. Results of these studies demonstrated that induced cardiomyocytes developed *in vivo* are more similar in regard to their morphological and physiological parameters to endogenous cardiomyocytes in comparison with those obtained *in vitro*. This may be the result of exposure to factors of natural microenvironment, such as extracellular matrix, secreted proteins, and intercellular interactions. Although in vivo reprogramming may improve cardiac function and reduce the severity of fibrotic changes after myocardial infarction, the use of retroviral and lentiviral vectors for the delivery of cardiospecific factors to cells has prevented large-scale clinical trials. Viral vectors can randomly insert in DNA, change its sequence and contribute to insertional mutagenesis. Before the implementation of the methods of direct cardiac cell reprogramming in clinical practice, one should develop methods for controlling these cells that exclude viral integration into DNA. An interesting approach was proposed by a group of Japanese researchers who developed the Sendai polycistronic viral vector (mouse parainfluenza virus) that expressed the cardiospecific factors Gata4, Mef2c and Tbx5 (SeV-GMT). Effectiveness of SeV-GMT was demonstrated in experiments *in vitro* and *in vivo* on

animal models [46]. Sendai virus is a non-segmented RNA virus of the paramyxovirus family that replicates only in cytoplasm and does not integrate into the host genome. It should be mentioned that the use of the new technology resulted in a significant improvement in the contractile function of heart and decreased severity of myocardial scar changes in mice during post-infarction period in comparison with the group of animals that received conventional retrovirus-based treatment.

Conclusion

Known therapeutic approaches to the management of myocardial damage that leads to CHF development cannot completely prevent the development of fibrotic changes in ischemic areas of cardiac muscle and restore their normal functional activity. Cell therapy was proposed for clinical practice as a promising approach to heart muscle regeneration. However, the results of clinical trials in regard to somatic stem cells revealed their moderate effect on contractile function. One of the reasons for this result may be associated to the low engraftment of transplanted cells. Further work on the determination of the optimal doses of cells and the time of their transplantation, routes of administration, as well as the development of new technologies, such as biomatrix-based cell delivery systems and tissue engineering methods, can probably help to overcome these issues. Direct reprogramming of myocardial cells can become one of the main directions of regenerative treatment in chronic circulatory failure. Since the discovery of cardiac-specific factors, the technologies of direct reprogramming of cardiac cells have made a significant progress towards its clinical use. However, several issues should be addressed prior to start clinical trials. First, the efficiency of reprogramming remains low, and generated cardiomyocytes demonstrate heterogeneous maturity. Reprogramming efficiency can be increased by identifying additional transcription factors, miRNAs, finding new active chemical compounds, and developing methods for modifying epigenetic mechanisms of gene regulation. Second, there is a need to develop a standard optimal protocol for cardiomyocyte generation that will allow obtaining comparable research results in this area. Finally, experiments should be conducted directly on CHF models. Almost all experiments and preclinical studies on reprogramming of cardiac cells *in vivo* were carried out in the acute period of myocardial infarction. We do not know whether *in vivo* reprogramming can be applied to CHF models when there is a high demand for regenerative technologies. Regenerative medicine is a high-potential method for the management of chronic circulatory failure, and the widespread use of various types of cell therapy could significantly improve its short- and long-term results and reduce the mortality rate associated with this disease.

Вклад авторов:

Все авторы внесли существенный вклад в подготовку работы, прочли и одобрили финальную версию статьи перед публикацией

Долгополов И.С. (ORCID ID: https://orcid.org/0000-0001-9777-1220): формулирование идеи, целей и задач, анализ и интерпретация полученных данных, подготовка рукописи с внесением ценного интеллектуального содержания, участие в научном дизайне работы

Рыков М.Ю. (ORCID ID: https://orcid.org/0000-0002-8398-7001): участие в разработке концепции, формулировка и развитие ключевых целей и задач, анализ и интерпретация полученных данных, подготовка и редактирование текста, его критический пересмотр с внесением ценного интеллектуального содержания

Осадчий В.А. (ORCID ID: https://orcid.org/0000-0001-9099-1351): сбор данных, участие в составлении черновика рукописи с внесением ценного интеллектуального содержания, статистическая обработка результатов

Author Contribution:

All the authors contributed significantly to the study and the article, read and approved the final version of the article before publication

Dolgopolov I.S. (ORCID ID: https://orcid.org/0000-0001-9777-1220): idea formation, formulation and development of key goals and objectives, analysis and interpretation of data, preparation of a manuscript with the introduction of valuable intellectual content, participation in the scientific design of the work

Rykov M.Yu. (ORCID ID: https://orcid.org/0000-0002-8398-7001): participation in the development of the concept, formulation and development of key goals and objectives, data collection, analysis and interpretation of results, preparation and editing of the text, its critical revision with the introduction of valuable intellectual content

Osadchiy V.A. (ORCID ID: https://orcid.org/0000-0001-9099-1351): data collecting, participating in the drafting of the manuscript with the introduction of valuable comments of intellectual content, statistical processing of the results

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