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Isolation and phenotyping of cardiac-derived progenitor cells from neonatal mice

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ABSTRACT

Dysfunctions of resident progenitor cells play a significant role in the pathogenesis of decreased myocardial contractility in heart failure, so the most promising approaches to the treatment of heart disease are cardiac-derived stem/progenitor cells (CSCs).

MATERIALS AND METHODS. *Protocols for progenitor cell cultures from different parts of the heart of newborn FVB/N mice have been developed and their proliferative potential has been characterized. Comparative analysis of the expression of CD31, CD34, CD44, CD45, CD73, CD90, CD105, CD117, CD309 and troponin I by cells from native myocardial biopsies and in the obtained cultures was performed by flow cytometric immunophenotyping.*

RESULTS. *The expression of mesenchymal markers CD44 and CD90 in the absence of the hematopoietic marker CD45 was demonstrated in early passages in mouse myocardial progenitor cell cultures. Relatively high expression of CD34 and CD31 was found. The presence of a minor population of $CD44^{+}117^{+}$ cells, which correspond to the phenotype of cardiac progenitor cells, was detected. The expression of troponin I as one of the key markers of cardiomyocytes as well as the vascular endothelial growth factor receptor has been confirmed in terminally differentia-ted cultures of cells with contractile activity.*

CONCLUSIONS. *It was found that newborn mice contain more cells with the expression of markers of cardiac progenitors in the myocardial tissue than in adult animals. The relative content of such cells is higher in the atria than in the ventricles. Cardiac progenitor cells in neonatal mice derived from the atrial appendages have better proliferative potential than cell cultures isolated from the ventricles.*

KEY WORDS: *cardiac-derived progenitor cells; cardiosphere; cell culture; immunophenotyping; flow cytometry*

The cardiovascular diseases are the leading cause of disability and mortality among the working population [1]. Even state-of-the-art drug and surgical treatments do not always compensate for the progression of heart failure, and often heart transplantation or circulatory assist devices ("artificial heart") remain the only hope for such patients. Histological examination of the myocardium in patients with ischemic cardiomyopathy, accompanied by severe heart failure, confirms the morphological signs of myocardial dysfunction, which determines the progression of the clinical course of the disease. Due to the significant replacement of cardiomyocytes with connective tissue, current treatments are unable to restore hemodynamics to the required level. The development of modern cell and tissue technologies using tissue-specific stem cells aims to restore contractile function of the heart by replacing lost cardiomyocytes or activating endogenous stem cells that still remain in the myocardium [2].

In modern regenerative medicine, stem cells are of particular interest to clinicians as a new effective agent for the regeneration of damaged tissues, including the myocardium [3, 4]. Dysfunctions of resident progenitors play a significant role in the pathogenesis of decreased myocardial contractility, so the most promising approaches are the isolation, culture and transplantation of tissue-specific cardiac stem cells (CSCs) [5, 6].

The development of technologies for obtaining in a short time a required number of tissue-specific myocardial progenitors and their combination with other cell types aims to increase the regenerative potential and safety of their use in the treatment of cardiovascular diseases. The myocardial stem and progenitor cell growth protocols developed in such studies can be used to increase the contractile function of the heart and improve its perfusion in acute and chronic injuries (myocardial infarction, ischemic cardiomyopathy, etc.) accompanied by severe heart failure. Of particular interest are the prospects for cell therapy using tissue-specific cardiac-derived progenitor cells as a tissue-engineering part to the combined surgical treatment of some congenital heart defects in infants.

The **AIM** of the **STUDY** was to compare the relative number of cells with expression of cardiac progenitor markers in the heart tissue of newborn and adult mice, and to obtain cardiac-derived progenitor cell cultures from different parts of the myocardium of newborn mice.

MATERIALS AND METHODS

The study was performed using wild-type FVB/N mice, kindly provided by European Molecular Biology Laboratory (Monterotondo, Italy). Animals were kept under standard conditions of temperature and lighting with free access to water and food *ad libitum* in the vivarium of the State Institute of Genetic and Regenerative Medicine of the National Academy of Medical Sciences of Ukraine. All experiments with animals were carried out in compliance with the Law of Ukraine "On Protection of Animals from Cruelty", "European Convention for the Protection of Vertebrate Animals Used for Experimental and Other Scientific Purposes", as well as principles of bioethics and biosafety standards [7].

Obtaining primary cultures of progenitor cells from mouse myocardium. The fragments of the atrial appendages or ventricles from hearts of newborn mice (n = 10) and males aged 5 months (n = 6) were used as a source of myocardial progenitor cells. Newborn animals were euthanized by decapitation, and adults – by cervical dislocation under anesthesia with 2.5 % avertin solution (*Sigma*, USA) at a dose of 400 mg/kg intraperitoneally. The isolation of the heart in newborn mice was performed under a stereomicroscope using microsurgery technique. Under sterile conditions, the thorax was dissected, the hearts were isolated from the main vessels and transferred to 35-mm Petri dishes with RPMI-1640 medium (*Sigma*, USA), which contained 1 % of a mixture of penicillin and streptomycin antibiotics PenStrep (*Sigma*, USA).

Fragments of adipose tissue, pericardium, aorta, pulmonary arteries were removed under a stereomicroscope, and the heart cavities were washed through the ostia of the vessels with RPMI-1640 nutrient medium to remove residual blood and blood clots. Using a scalpel, the heart was cut in the frontal axis through the atria and ventricles, washed with pure RPMI-1640 medium and minced with scissors into 0.5-1 mm^3 pieces.

The fragments were transferred using Pasteur pipette into a sterile 5 mL polystyrene tube with 1 mL of DMEM nutrient medium (*HyClone*, USA) containing 0.1 % collagenase type IV (*Thermo Fisher Scientific*, USA). The tissue was fermented for 30 min at 37 ^{0}C with constant shaking at 100 rpm. After the incubation time, the collagenase was inactivated by adding 4 mL of DMEM medium and washed by centrifugation at 300 xg for 10 minutes. The resulting cell suspension was used for immunophenotyping by flow cytometry, as well as for further cultivation.

To obtain primary cultures, the supernatant was discarded, and complete culture medium was added to the pellet. Explants were resuspended in IMDM medium, supplemented with 20 % fetal bovine serum (FBS), 100 IU/mL penicillin, 100 µg/mL streptomycin, 2 mM/L L-glutamine (all – *Sigma*, USA) and transferred to 25 cm^2 culture flask, coated with fibronectin (*Greiner bio-one*, USA). The samples were cultured by explant method under standard conditions in a CO_2 incubator at 37 ^{0}C in a humidified atmosphere with 5 % CO_2. After reaching 80 % confluence of the monolayer, the explants were removed and the cells were subcultured using a mixture of 0.5 g/L trypsin (*Sigma*, USA) and 0.53 mmol/L EDTA (*Sigma*, USA) for 2-3 minutes at room temperature. Trypsin was inactivated with DMEM medium containing 10 % FBS.

The resulting suspension of cells from explants at $2•10^5$ cells/mL was seeded into 25 cm^2 culture flasks coated with poly-D-lysine, which contained complete growth medium for cardiospheres: 35 % IMDM medium + 65 % DMEM-F12 (*HyClone*, USA), supplemented with 10 % FBS, 2 % B-27 supplement, 0.1 mmol/L 2-mercaptoethanol, 10 ng/mL epidermal growth factor (EGF), 20 ng/mL basic fibroblast growth factor (bFGF), 40 nM/L cardiotrophin-1, 40 nM/L thrombin, 100 IU/mL penicillin, 100 µg/mL streptomycin, 2 mmol/L L-glutamine (all – *Sigma*, USA).

On day 2-3 of cultivation, cardiospheres were formed, which were collected and transferred in explant medium to fibronectin-coated flasks. After reaching 80 % confluence of the monolayer, the cells were suspended using a mixture of 0.25 % trypsin-EDTA solution and subcultured to the 3rd passage using mixtures of trypsin and EDTA [8].

Analysis of cell proliferation in vitro. Cells after the first passage in the amount of $1•10^5$ were added to a 35-mm Petri dish and cultured in DMEM-F12 medium (*Sigma-Aldrich*, USA), supplemented with 10 % FBS, penicillin 100 IU/mL, streptomycin 100 µg/mL, 1:100 non-essential amino acids, 2 mM L-glutamine (all – *Sigma-Aldrich*, USA). After reaching the confluent monolayer, the cells were detached by a mixture of 0.25 % trypsin-EDTA, the resulting cell suspension was centrifuged in DMEM-F12 medium supplemented with 10 % FBS at 380 xg for 10 min. After counting the number of cells in a Newbauer hemocytometer, $1•10^5$ cells were added to a Petri dish with a culture surface area of 9.5 cm^2 and cultured in complete nutrient medium. Subculturing was stopped when the number of cells obtained after the last passage was less than $1•10^5$. The number of passages ranged from 2 to 5.

Population doubling time (PDT) was calculated for each passage by the formula:

PDT = T / 3,31 lg (Xk / X_0), where:

T – cell culture time; Xk – the number of cells obtained after subculturing; X_0 – the number of cells at the start of the passage.

Immunophenotyping of cells by flow cytometry. The obtained primary suspensions of cells from the myocardium of mice and cultures at different passages were phenotyped by flow cytometry on markers CD31, CD34, CD44, CD45, CD73, CD90, CD105, CD117, CD309, and Troponin I using fluorochrome-conjugated monoclonal antibodies (**Table 1**) according to the standard technique and recommendations of the manufacturers [9].

Myocardial tissue after collagenase fermentation and washing was resuspended in 1 mL of DMEM medium, carefully dispersed in a homo-genizer and filtered through cellular filters with a pore diameter of 100 µm (*Falcon*, USA). The pellet was washed in 3 mL of DMEM medium by centrifugation at 350 xg for 5 min, and resuspended in pure medium. The suspension of cells from culture after washing from trypsin-EDTA was filtered through cell filters with a pore diameter of 100 µm and resuspended in DMEM medium.

$2•10^5$ cells in 50 µL of DMEM medium were transferred to 5 mL polystyrene tubes and antibodies were added at a 0.5 µg/10^6 cells. The samples were incubated for 20 minutes at +4 ^{0}C, then added 1 mL of wash buffer CellWash (*BD Bioscience*, USA), centrifuged at 350 xg for 5 minutes at +4 ^{0}C. After re-washing, the supernatant was removed, and 300 µL of CellWash buffer containing 1 % FBS was added to the tubes. Immediately before the analysis, the suspension was passed through cellular filters with a pore diameter of 70 µm. Measurements were performed on a cell sorter BD FACSAria (*Becton Dickinson*, USA) using BD FACSDiva 6.1.2 software (*Becton Dickinson*, USA). The percentage of dead and vi-

Table. 1. Panel of monoclonal antibodies for immunophenotyping of cell cultures from mouse myocardium.

MONOCLONAL ANTIBODY	MANUFACTURER	CAT. NO.	CLONE
PE rat anti-mouse CD31	BD Biosciences	553373	MEC 13.3
Alexa Fluor® 647 rat anti-mouse CD34	BD Biosciences	560230	RAM34
PE rat anti-mouse CD44	BD Biosciences	553134	IM7
PE rat anti-mouse CD45	Invitrogen	MA1-10233	EM-05
PE rat anti-mouse CD73	BD Biosciences	550741	TY/23
APC-Cy7 mouse anti-rat CD90/Mouse CD90.1	BD Biosciences	561401	OX-7
APC rat anti-mouse CD105	Invitrogen	17-1051-82	MJ7/18
APC rat anti-mouse CD117	BD Biosciences	561074	2B8
PE rat anti-mouse CD309 (FLK1)	Invitrogen	12-5821-82	Avas12a1
Alexa Fluor® 647 mouse anti-cardiac Troponin I	BD Biosciences	564409	C5

able cells was determined by the staining with 5 μL 7-Aminoactinomycin D (7-AAD) for 10 min (*BD Bioscience*, USA).

To adjust the overlap compensation of fluorochrome emission spectra in multiparameter analysis, control samples of cells without the antibodies (unstaining control), samples with each of the antibodies alone (single staining control) and samples with a combination of several antibodies without one (fluorescence minus one – FMO control) were used.

Statistical data processing. Statistical analysis was performed using the nonparametric Wilcoxon-Mann-Whitney U-test using Statistica 7.0 software (*StatSoft Inc.*, USA) [10]. Sampling of data in *in vitro* experiments included the results obtained from 3 experiments. The results are presented as the mean in each experimental group ± standard error of the mean (Mean ± SEM). Differences were considered statistically significant at $p < 0.05$.

RESULTS AND DISCUSSION

MORPHOLOGICAL CHARACTERISTICS OF PRIMARY CULTURES OF PROGENITOR CELLS FROM THE MYOCARDIUM OF NEWBORN MICE

On day 3-5 of cultivation, fibroblast-like cells began to migrate from mouse myocardial explants attached to the surface of the fibronectin culture flask, and round cells were also present. After the formation of a monolayer of fibroblast-like cells from explants of heart tissue, the formation of a population of small rounded phase-contrast cells floating in the nutrient medium was observed (**Fig. 1**).

Tissue explants were removed from the primary culture after 2 weeks. At 4-5 weeks, the cells acquired a homogeneous morphology, expanded at a moderate rate and after reaching 90 % confluence they were subcultured. The obtained cell cultures of subsequent passages were characterized by stable morphology. Cells with fibroblast-like morphology were present in the culture, as well as a population of polygonal cells raisen above the surface of the flask (like a "coble stone").

Then the resulting population of explant cells was grown in culture flasks with Poly-D-Lysine in a medium that promoted the formation of cardiospheres. Within 24-72 hours after the start of cultivation, the spheroids increased in size and had a diameter of 20 to 150 μm (**Fig. 2**).

Under selected optimal conditions *in vitro* cells expanded, divided, some of them detached and formed free-floating aggregates – cardiospheres. The cardiosphere forms a three-dimensional structure, the center of which is presented by undifferentiated proliferating c-kit$^+$ cells. The cardiosphere consists of progenitor cells of various types, which express

Fig. 1. Micrograph of the primary culture of progenitor cells from the mouse myocardium, the arrows indicate the primary spheroids. Light microscopy, phase contrast, scale – 50 μm.

certain hematopoietic, endothelial and mesenchymal markers, which will be discussed below [11].

On the 2nd day after the formation of cardiospheres, they showed spontaneous contractile activity, which remained until the 2nd passage. Because the cardiospheres are quite large, they cannot be used, for example, by intracoronary infusion due to the risk of microvascular embolism. Therefore, an additional stage involves the dissociation of cardiospheres or their re-cultivation on surfaces covered with fibronectin [12, 13].

On day 7, the cardiospheres were isolated and added to fibronectin-coated flask in complete IMDM medium. After three days, the culture of cells isolated from newborn mice reached approximately 70 % confluence (**Fig. 3**).

The determination of cell growth kinetics in *in vitro* culture showed that cell culture derived from ventricles proliferated three times slower compared to cell cultures derived from atrial appendages ($p < 0.001$) (**Fig. 4**).

It is known that cells isolated from cardiospheres have a high proliferative potential and are able to differentiate into endothelial, smooth muscle cells, as well as cardiomyocytes. Immunofluorescence study with confocal microscopy by Smith R. et al. confirms the high proliferative activity of the cardiosphere in the expression of Ki-67. In this case, proliferating cells express connexin-43 and α-sarcomeric actin, which indicates a high potential for integration of electrical conductivity between

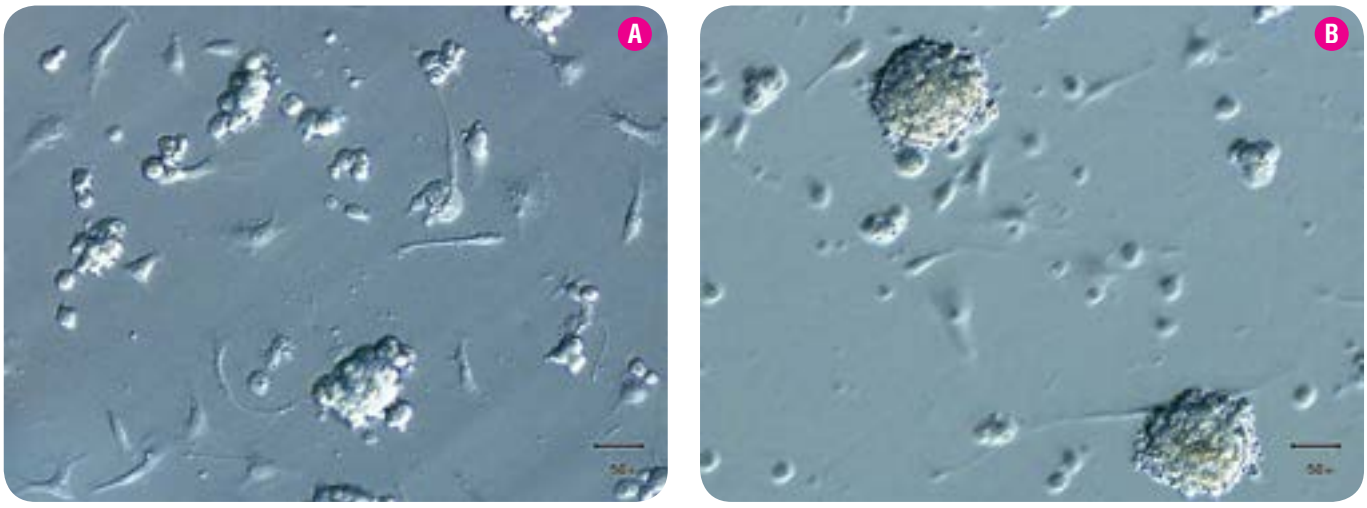

Fig. 2. Micrographs of the culture of progenitor cells from mouse myocardium on the surface of the flask with poly-D-lysine in the medium for cardiospheres; passage 1: **A** – 1st day of cultivation; **B** – 3rd day of cultivation. Light microscopy, phase contrast, scale – 50 μm.

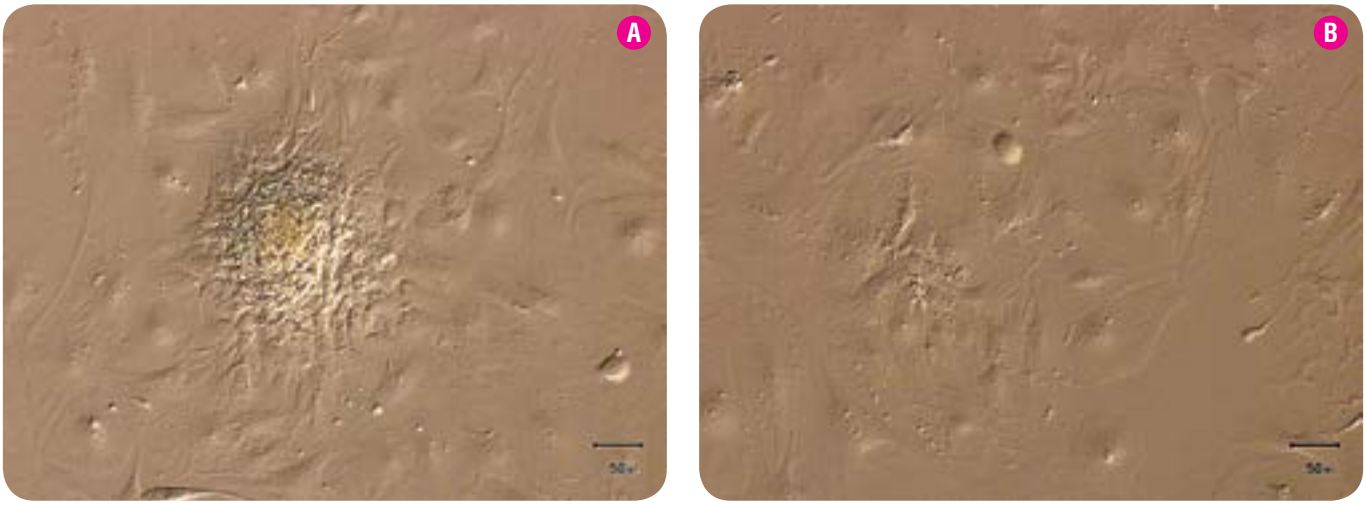

Fig. 3. Micrographs of the culture of progenitor cells from mouse myocardium on the fibronectin-coated surface in the medium for cardiospheres; passage 1: **A** – 1st day after subculturing of cardiospheres; **B** – 3rd day of cultivation. Light microscopy, phase contrast, scale – 50 μm.

cells. When co-cultured with newborn rat cardiomyocytes, human and porcine CSCs exhibit biophysical characteristics of cardiomyocytes, including synchronization of calcium transfer between neighboring cells. It is important that after 3 or more passages (6 population doublings, 60 days in culture) do not show deviations in their karyotype [14].

Thus, to obtain cardiospheres and populations of cardiosphere-derived cardiac progenitors, a necessary condition is the presence of additional exogenous factors and components of the extracellular matrix – Poly-D-Lysine and fibronectin [15]. Poly-D-Lysine contains polymers with a molecular weight of 50-150 kDa. Fibronectin is a high-molecular weight glycoprotein (~500-600 kDa) of the extracellular matrix that binds to membrane-spanning receptor proteins called integrins. Both cell expansion and differentiation depend on the surface properties of the culture substrate. In particular, the polycationic properties of the Poly-D-Lysine molecule allow it to interact with the anionic sites of cells, promoting effective attachment to the growth surface. The fibronectin molecule contains the RGD amino acid sequence (Arg-Gly-Asp), which is the site of cell attachment via α5β1 and αVβ3 integrins on the cell surface. Fibronectin also binds to other extracellular matrix proteins such as collagen, fibrin, and heparan sulfate proteoglycans. That is, the main function of these components is to improve cell adhesion to the culture surface, promote cell proliferation, growth, morphogenesis, differentiation, migration and increase the contact area with the substrate [25, 26].

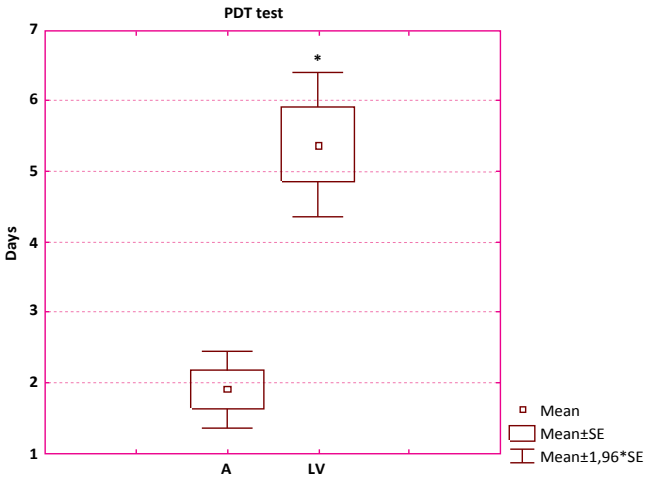

Fig. 4. Population doubling time in the culture of progenitor cells isolated from the ventricles (LV) and atrial appendages (A) of the myocardium of neonatal mouse (Mean ± SEM).
*Note: * – p < 0.001 compared to the group of cells derived from atrial appendages.*

IMMUNOPHENOTYPE OF PROGENITOR CELLS FROM NATIVE MOUSE MYOCARDIUM

According to the scientific data available, the key markers for progenitor cells from the myocardium are CD90, CD105, CD117 (c-kit) [11]. In addition, CSCs can moderately express the hematopoietic stem cell marker CD34, the endothelial marker CD31, and the vascular endothelial growth factor (VEGF) receptor CD309 [11, 16]. However, they are negative for markers MDR1, CD133 and CD45, which significantly distinguishes them from bone marrow and circulating endothelial progenitors, which can populate the heart [17].

Clonal proliferating CSCs also express stem cell antigen Sca-1 and a number of NKX2-5, NOTCH1, NUMB proteins associated with pluripotency [18, 19]. Sca-1+ progenitors in the heart have high telomerase activity, the ability to migrate to the damaged myocardium and differentiate into cardiomyocytes [20]. Koninckx et al. for the first time identified a population of ALDH+ myocardial stem cells that expressed marker islet-1 and aldehyde dehydrogenase, which increased their survival in ischemia, and had a greater ability to differentiate into cardiomyocytes than previously known CSCs [21]. Bolli R. et al. confirmed that c-Kit+ CSCs are capable of differentiation into all cardiac cell lines. In terminal *in vitro* or *in vivo* differentiation, this cell type begins to express cardiomyocyte-specific markers troponin I, troponin T, myosin heavy chains, and connexin-43. Some of the transplanted CSCs are also able to form vascular structures and express smooth muscle α-actin [22].

After the analysis of the scientific data, the necessary monoclonal antibodies were selected and panels for multiparametric immunophenotyping of progenitor cells from mouse myocardium were developed (**Table 1**).

Significantly higher ($p \leq 0.05$) expression levels of markers CD90, CD73 and CD105 in cell suspension from atrial appendages compared with ventricular samples were found by immunophenotyping of freshly isolated cells from native myocardium of newborn mice (**Figs. 5, 6**). The expression levels of CD34 and CD309 were higher in the cell suspension from the ventricles than from the atria ($p \leq 0.05$).

Immunophenotyping of cell suspension from native myocardium in adult mice at 5 months of age showed significantly lower ($p \leq 0.05$) expression of markers CD90, CD73, CD105 and CD34 in both ventricular and atrial samples compared to newborn animals (**Figs. 7, 8**). At the same time, no significant difference was found in these indicators for the

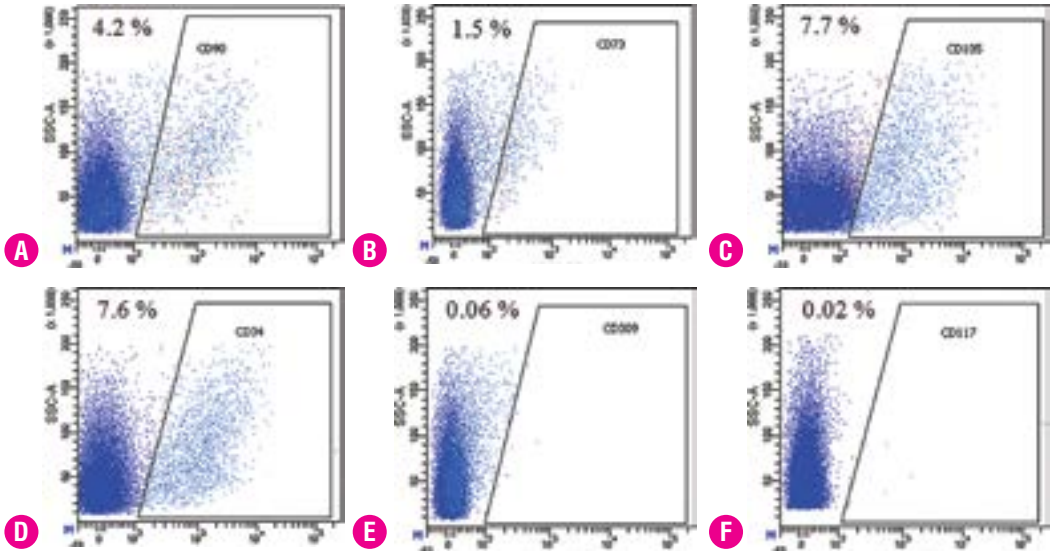

Fig. 5. Histograms of the expression of markers CD90 (**A**), CD73 (**B**), CD105 (**C**), CD34 (**D**), CD309 (**E**), CD117 (**F**) in a suspension of cells from the ventricles of the native myocardium of newborn mice (n = 10); immunophenotyping by flow cytometry.

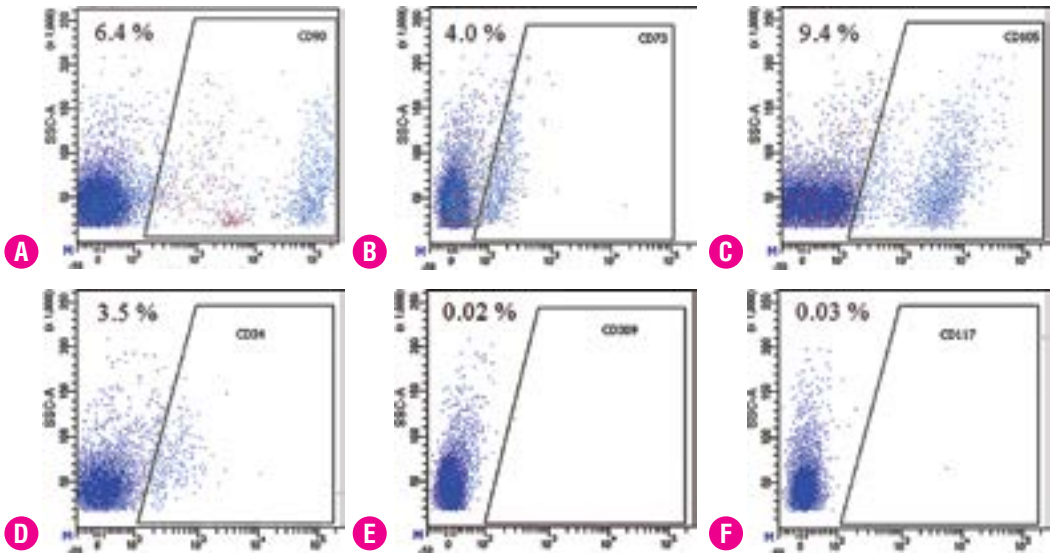

Fig. 6. Histograms of the expression of markers CD90 (**A**), CD73 (**B**), CD105 (**C**), CD34 (**D**), CD309 (**E**), CD117 (**F**) in suspension of cells from the atria of the native myocardium of newborn mice (n = 10); immunophenotyping by flow cytometry.

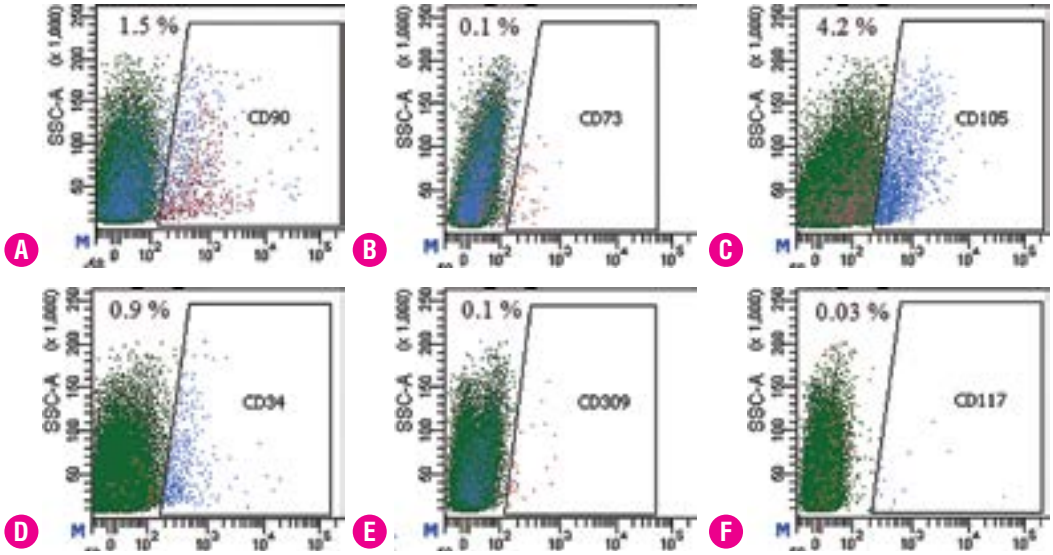

Fig. 7. Histograms of the expression of markers CD90 (**A**), CD73 (**B**), CD105 (**C**), CD34 (**D**), CD309 (**E**), CD117 (**F**) in a suspension of cells from the ventricles of the native myocardium of 5-month-old mice (n = 4); immunophenotyping by flow cytometry.

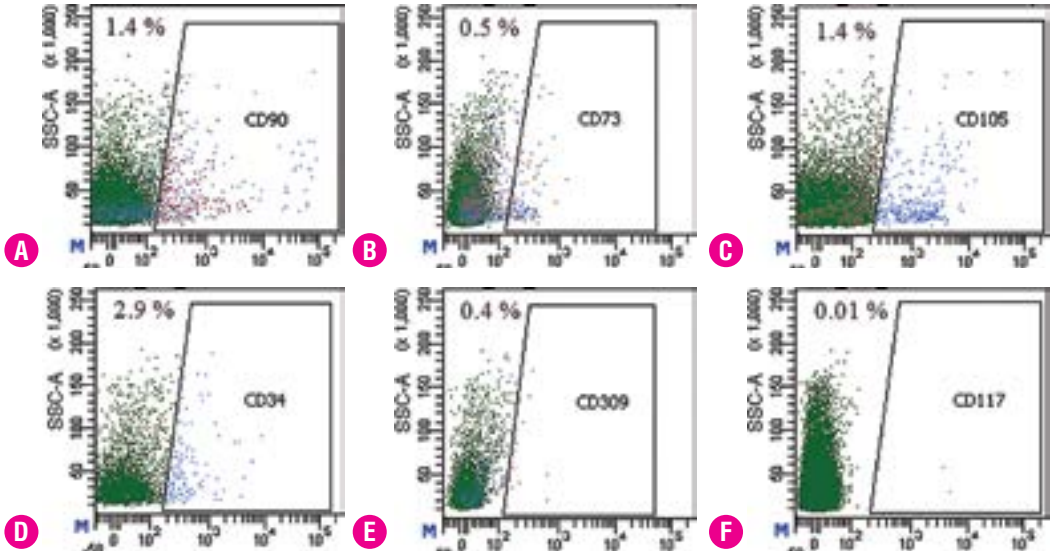

Fig. 8. Histograms of the expression of markers CD90 (**A**), CD73 (**B**), CD105 (**C**), CD34 (**D**), CD309 (**E**), CD117 (**F**) in a suspension of cells from the atrial appendages of the native myocardium of mice aged 5 months (n = 4); immunophenotyping by flow cytometry.

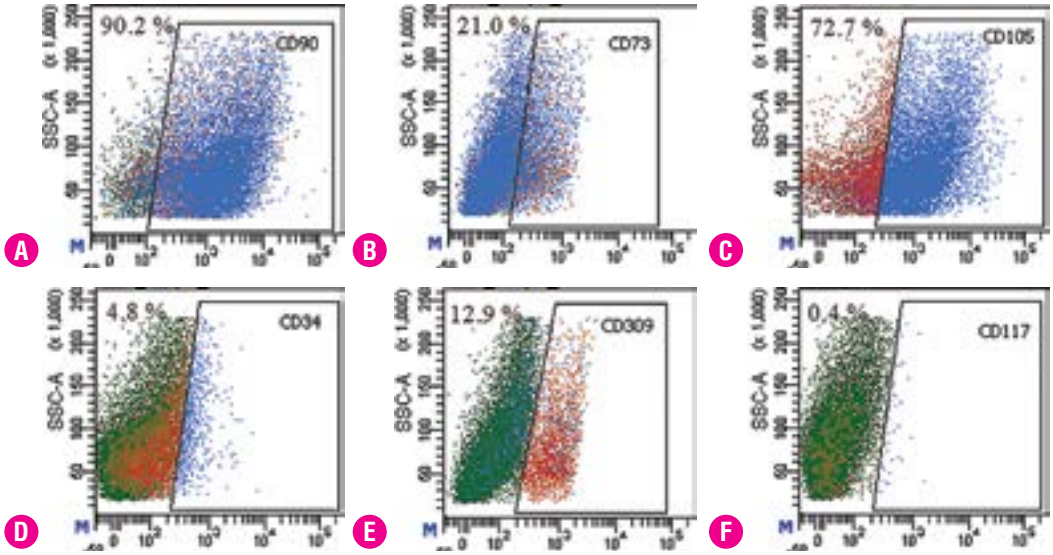

Fig. 9. Histograms of the expression of markers CD90 (**A**), CD73 (**B**), CD105 (**C**), CD34 (**D**), CD309 (**E**), CD117 (**F**) in the culture of cardioshere-derived progenitor cells from the ventricles of newborn mice (passage 3); immunophenotyping by flow cytometry.

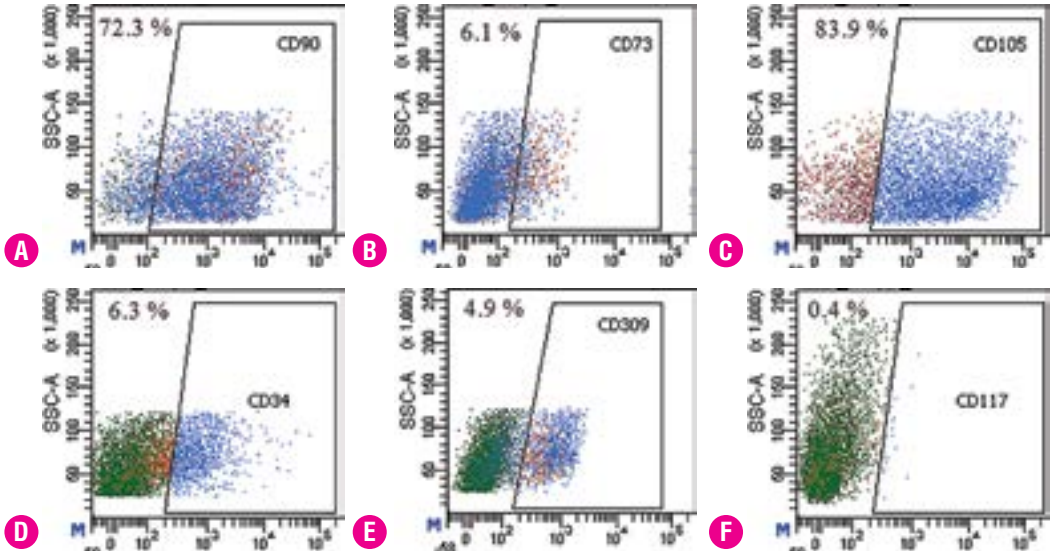

Fig. 10. Histograms of the expression of markers CD90 (**A**), CD73 (**B**), CD105 (**C**), CD34 (**D**), CD309 (**E**), CD117 (**F**) in the culture of cardioshere-derived progenitor cells from the atrial appendages of newborn mice (passage 3); immunophenotyping by flow cytometry.

samples from different parts of the heart. At the same time, the expression level of the vascular endothelial growth factor receptor CD309 was higher in both samples compared to cells from newborn animals ($p \leq 0.05$). The expression level of CD117 did not differ significantly between the respective comparison groups of adult and newborn mice.

Thus, in newborn mice, myocardial tissue contains more cells expressing markers of cardiac progenitors than in adult animals. The relative content of such cells is higher in the atria than in the ventricles. Therefore, the priority of further research is the isolation and cultivation of cells from the myocardium of newborn mice, in particular – from the ears of the atrial appendages.

IMMUNOPHENOTYPE OF CARDIOSPHERE-DERIVED CELL CULTURE FROM NEONATAL MICE

The cultivation of the isolated suspension of myocardial cells from newborn mice up to 3 passages resulted in a morphologically homogeneous population that expressed high levels of CD90 and CD105 markers with low CD73 expression, which is typical for myocardial progenitors compared to multipotent mesenchymal stromal cells (**Fig. 9, 10**).

There was also a population of cells with relatively high levels of expression of CD34 and CD309 markers. In the culture of cardioshere-derived progenitors from ventricles, the expression of vascular growth factor receptor was significantly higher (12.9 %) than in atrial culture (4.9 %) ($p \leq 0.05$). The expression of the CD117 marker remained quite low (0.4 %) in both groups.

The presence of a minor population of cells with phenotype $CD44^{+}117^{+}$ (3.7 ± 1.3% in the first passage), which may belong to cardiac progenitor cells, was detected (**Fig. 11**). At the same time, a rather high expression was found in the CD34 marker (up to 47.6 %), although the population of cells with the endothelial precursor phenotype $CD34^{+}CD31^{+}$ at the first passage remained low (up to 2.4 %).

Subsequent cultivation yielded a homogeneous population that expressed a high level of the CD105 marker, as well as CD90 and CD73 in the absence of the hematopoietic marker CD45, which could be similar to the phenotype of multipotent mesenchymal stromal cells (MMSCs) [23]. However, the obtained cultures were characterized by the presence of a population of CD34-positive cells, which is not typical for MMSCs, and terminal differentiation showed high expression of the cardiac troponin I. There were differences in troponin I expression: in the culture of terminally differentiated cells from ventricles this marker was 27.1 %, while in atrial appendages-derived culture – 69.8 % (**Fig. 12**).

It can be assumed that the culture of progenitor cells from the atrial appendages quickly reaches the terminal phenotype typical of cardiomyocytes, which correlates with the data of proliferative potential and contractile activity in morphological examination. The results confirm the data of Chan et al., which also showed differences in the proliferative potential and expression of markers of cell culture from the atria and ventricles [24].

Thus, the expression of mesenchymal markers CD44, CD105 and CD90 increased in early passages in cell cultures from myocardial explants of newborn mice. The presence of a minor

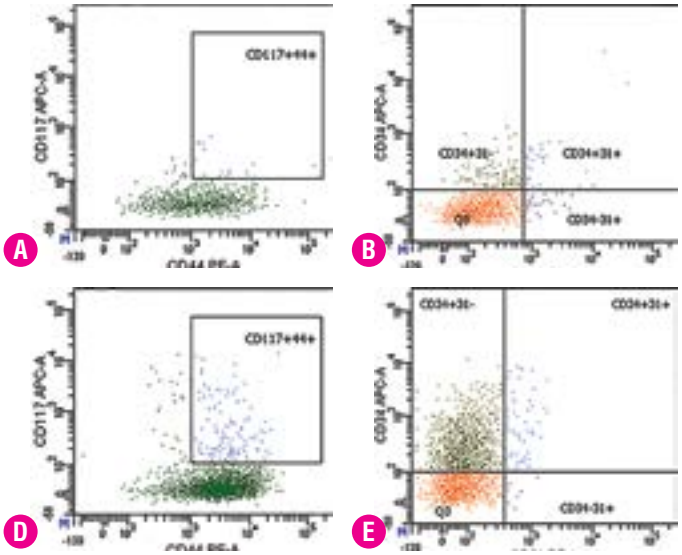

Fig. 11. Dot-plot histograms of subpopulations with the combination of markers CD44/CD117 (**A, C**) and CD34/CD31 (**B, D**) in cardiosphere-derived cell culture from newborn mice according to flow cytometry, passage 0 (**A, B**) and 1 (**C, D**).

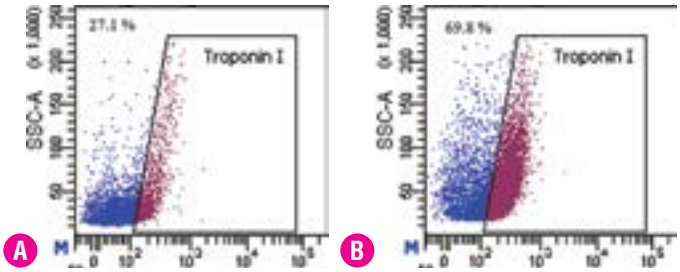

Fig. 12. Histograms of troponin I expression in the culture of terminally differentiated cardiosphere-derived progenitor cells from the ventricles (**A**) and atrial appendages (**B**) of newborn mice (passage 3) according to immunophenotyping by flow cytometry.

population of $CD44^{+}117^{+}$ cells which correspond to the phenotype of cardiac progenitor cells, was detected. The expression of troponin I and VEGFR (CD309) has been confirmed in terminally differentiated cultures of progenitor cells from the myocardium.

Developed protocols for culture of cardiac-derived progenitor cells can be used in further preclinical studies to increase contractile function and improve perfusion in ischemic heart disease associated with severe heart failure in both adult and neonates with congenital heart disease. As a result, the development of new high-tech approaches to cell therapy of cardiovascular diseases and their translation to the clinic will increase the efficiency of medical care, as well as reduce disability and mortality in a population.

CONCLUSION

1. ***It was found that newborn mice contain more cells with the expression of markers of cardiac progenitors in the myocardial tissue than adult animals. The relative content of such cells is higher in the atria than in the ventricles.***
2. ***It was found that cardiac progenitor cells in newborn mice derived from the atrial appendages have better proliferative potential than cell cultures isolated from the ventricles, and also differ in immunophenotypic characteristics.***

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Виділення та фенотипування прогеніторних клітин з міокарда новонароджених мишей

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РЕЗЮМЕ

В патогенезі зниження скоротливої здатності міокарда при серцевій недостатності значну роль відводять дисфункції резидентних клітин-прогеніторів, тому найбільш перспективними для клітинної терапії захворювань серця вважають підходи з виділення, нарощування та застосування тканиноспецифічних стовбурових/прогеніторних клітин міокарда – cardiac-derived stem/progenitor cells (CSCs).

***МАТЕРІАЛИ ТА МЕТОДИ**. Розроблено протоколи отримання культур прогеніторних клітин з різних відділів серця новонароджених мишей лінії FVB/N та охарактеризовано їх проліферативний потенціал. За допомогою мультипараметричного імунофенотипування методом проточної цитометрії проведено порівняльний аналіз експресії маркерів CD31, CD34, CD44, CD45, CD73, CD90, CD105, CD117, CD309 та тропонін I клітинами з біоптатів нативного міокарда та в отриманих культурах.*

***РЕЗУЛЬТАТИ**. Продемонстровано, що на ранніх пасажах в культурах прогеніторних клітин міокарда мишей наростала експресія мезенхімальних маркерів CD44 та CD90 за відсутності гемопоетичного маркера CD45. При цьому встановлено відносно високу експресію CD34 та CD31. Відмічено присутність мінорної популяції клітин, які за фенотипом $CD44^{+}117^{+}$ відповідають кардіальним прогеніторним клітинам. У термінально диференційованих культурах з клітин із скоротливою активністю підтверджено експресію тропоніну I, як одного з ключових маркерів кардіоміоцитів, а також рецептора фактора росту ендотелію судин VEGFR.*

***ВИСНОВКИ**. Встановлено, що у новонароджених мишей в тканині міокарда міститься більша кількість клітин з експресією маркерів кардіальних прогеніторів, ніж у дорослих тварин. При цьому відносний вміст таких клітин є більшим у передсердях, ніж у шлуночках. Кардіальні прогеніторні клітини у новонароджених мишей, отримані з вушка передсердя, володіють кращим проліферативним потенціалом, ніж культури клітин, виділені зі стінок шлуночків.*

***КЛЮЧОВІ СЛОВА**: прогеніторні клітини з міокарда; кардіосфери; культура клітин; імунофенотипування*