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Hypoxia – as a possible regulator of the activity of epicardial mesothelial cells after myocardial infarction

Aim	To study the effect of hypoxia on the activity of epithelial-mesenchymal transition (EMT) in epicardial cells, which provides formation of a specialized microenvironment.
Material and methods	This study used a model of experimental myocardial infarction created by ligation of the anterior descendent coronary artery. The activity of epicardial cells after a hypoxic exposure was studied with the hypoxia marker, pimonidazole, bromodeoxyuridine, immunofluorescent staining of heart cryosections, and in vitro mesothelial cell culture.
Results	The undamaged heart maintained the quiescent condition of mesothelial cells and low levels of their proliferation, extracellular matrix protein production, and of the EMT activity. Acute ischemic injury induced moderate hypoxia in the epicardial/subepicardial region. This caused a global rearrangement of this region due to the initiation of EMT in cells, changes in the cell composition, and accumulation of extracellular matrix proteins. We found that the initiation of EMT in mesothelial cells may result in the formation of smooth muscle cell precursors, fibroblasts, and a population of Sca-1+ cardiac progenitor cells, which may both participate in construction of new blood vessels and serve as a mesenchymal link for the paracrine support of microenvironmental cells. In in vitro experiments, we showed that 72-h hypoxia facilitated activation of EMT regulatory genes, induced dissembling of intercellular contacts, cell uncoupling, and increased cell plasticity.
Conclusion	The epicardium of an adult heart serves as a "reparative reserve" that can be reactivated by a hypoxic exposure. This creates a basis for an approach to influence the epicardium to modulate its activity for regulating reparative processes.

Keywords	Epicardial cells; hypoxia; myocardial infarction; epithelial-mesenchymal transition; cardiac reparation
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Introduction

For several decades, chronic heart failure (CHF) has held one of the leading positions in the list of urgent medical and social problems. [1] Despite the high prevalence of the disease (2% in Western Europe and US, 7% in Russia), annual progressive increases in the number of cases, and a number of recent large-scale studies, the molecular and physiological mechanisms of this disease are still not entirely understood. Thus, there has been increased interest in studying the mechanisms for maintaining cardiac cellular homeostasis and regulating reparative regeneration to find new target areas in the prevention and treatment of CHF [2–4].

The epicardium, an outer layer of the heart wall closely adjacent to the myocardium and formed by epicardial mesothelial cells, collagen, and elastic fibers, is essential for coordinating physiological/reparative processes occurring in the heart [5]. During embryogenesis, epicardial cells undergo epithelial-to-mesenchymal transition (EMT), leading to the formation of endocardial progenitor cells having a prominent secretory capacity [6, 7], migratory properties, as well as the ability to differentiate into smooth muscle and endothelial cells of coronary vessels, perivascular fibroblasts, primordial mesenchymal cells of future heart valves and interstitial fibroblasts [8–11]. Mesothelial cell failure leads to serious defects in the

development of the myocardium, coronary arteries, cardiac conduction system and valves [12–16].

Although epicardial mesothelial cells gradually lose their ability to proliferate during the second half of pregnancy, transforming into a layer of resting epicardial cells and remaining so in the postnatal period [17], in acute ischemic myocardial damage, the fetal gene expression program (Wt1, Raldh^2 and Tbx18) is partially reactivated in the epicardial cells [18–20], which launches the proliferation of epicardial cells that undergo EMT and participate in the repair [21, 22]. This activation is regulated by transforming growth factor-beta, platelet-derived growth factor, retinoic acid, fibroblast growth factor, Hippo/Yap and Sonic Hedgehog signaling pathways, as well as extracellular matrix proteins [23–32], which implement cellular response to injury in combination with hypoxia. While several published works confirm the involvement of hypoxia and hypoxia-induced factors (HIF) [33, 34] in the regulation of EMT and embryonic epicardial cell differentiation, the regulation mechanisms of postnatal epicardial mesothelial cells remain little understood.

We hypothesized that the occurrence of hypoxia in epicardium/subepicardium after transmural myocardial infarction (MI) could constitute a stimulus for the activation of epicardial cells associated with the entry into EMT, switch to glycolytic metabolism, alteration of the expression profile and proliferative activity.

Objective

To study the effect of hypoxia on EMT activity in the epicardial cells contributing to the formation of a special microenvironment.

Material and methods

Animals

We used male C57BL/6 mice (12 weeks old) and Wistar rats (13 weeks old) kept in the laboratory animal breeding nursery of the Russian National Medical Research Center for Cardiology. The experiments were approved by the ethics committee of the Russian National Medical Research Center for Cardiology and carried out in accordance with the current regulations on animal testing and husbandry [Declaration of Helsinki, 2000; Order of the Ministry of Health of the USSR No. 755 «On measures on further improvements of works involving the use of experimental animals» dated 08/12/1977; Good Clinical Trial Practice in the Russian Federation (approved by the Ministry of Health of the Russian Federation on 29/12/1998)].

MI simulation and sample preparation

According to the previously described procedures, experimental MI was simulated by ligating the left anterior artery [35]. Experimental animals were eliminated (euthanasia) on days 3, 5, 7, and 14 following MI simulation, which corresponded to the different periods of reparative regeneration. Each group, including the control group undergoing placebo surgery, included from 3 to 5 animals.

Estimation of the thickness of epicardial/subepicardial area of the distribution of the derived epicardial Wt1+ cells and extracellular matrix proteins

Cryosections of the heart stained with hematoxylin and eosin were used to investigate the distribution of epicardial cells and subepicardial thickness. Cryosections were fixed for 20 minutes in 96% ethyl alcohol, washed in distilled water and stained with hematoxylin for 5 minutes. After staining, the sections were washed in running water, differentiated in 1% hydrochloric acid in alcohol and again washed in running water (10 times for 5 minutes). The sections were then placed for 5 minutes in distilled water and stained with 1% aqueous solution of eosin for 1 minute. After staining, slides were rinsed with distilled water dehydrated, and mounted in a xylene-based medium. The quantitative analysis of the subendocardial thickness was performed using the Image J software.

Immunofluorescent staining of cryosections of the heart wall was used to view extracellular matrix proteins, Wt1+ epicardial cell, and their derivatives. Frozen cryosections were fixed with 3.7% paraformaldehyde (20 minutes) or ice-cold acetone (depending on antibody specifications) and washed with phosphate-buffered saline (PBS) for 5 minutes. To assess intracellular markers, the sections were further treated with 0.1% Triton X100 solution (5 minutes). Myocardial sections were blocked with a solution containing 1% bovine serum albumin (BSA), 10% serum of the second antibody donor in PBS (30 minutes) to inhibit nonspecific binding of antibodies. Then, cryosections were stained with anti-Wt1+ and anti-fibulin activated epicardium marker antibodies, anti-extracellular matrix protein (fibronectin, collagen I, collagen III) antibodies, anti-fibroblast marker (CD90, vimentin) antibodies, anti-progenitor cell (SCA-1) antibodies, anti-endothelial cell (Pecam, von Willebrand factor) antibodies, anti-smooth muscle cell (SMA, calponin) antibodies for 1 hour, then washed in PBS, and stained with antibodies conjugated with Alexa Fluor 488/594. The cell nuclei were stained with DAPI (4', 6 diamidino-2 phenylindole).

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Evaluation of proliferative cell activity in the epicardial/subepicardial area

Bromodeoxyuridine (BrdU), a synthetic nucleoside analog of thymidine, which enables the visualization of proliferating cells in the myocardial sections stained with antibodies, was used to evaluate the proliferative activity of epicardial cells following ischemic damage. BrdU was injected intraperitoneally (once a day, 100 mg/kg body weight, for 5 days before euthanasia). The insertion of BrdU into the DNA of dividing cells (its presence in the cell nuclei) was determined by immunofluorescent staining with anti-BrdU antibodies and anti-Alexa Fluor 488 secondary antibodies. Before staining, sections were fixed in formalin. Nonspecific antibody binding was inhibited with a solution containing 1% of BSA and 10% of second antibody donor serum in PBS (30 minutes). Additional sections were treated with 2N hydrochloric acid for 30 minutes (37°C) to denature DNA. The acid was neutralized with 0.1 M borate buffer (2 times for 5 minutes). Activated epicardial cells were visualized using anti-fibulin-5 antibodies and anti-Alexa Fluor 488 secondary antibodies.

Evaluation of cell hypoxia in the epicardial/subepicardial area

Pimonidazol, which is a part of the commercial Hypoxyprobe™ Kit, was used to assess the hypoxic status of myocardial cells. Pimonidazol was injected intraperitoneally (60 mg/kg of body weight) into Wistar rats two hours before euthanasia. Pimonidazol was injected into intact animals and rats after MI simulation (5 and 14 days after surgery); the animals were divided into groups of three individuals. After the elimination of animals from the experiment, hearts were removed from the chest cavity, washed with PBS, then immersed in a cuvette with a freezing medium and frozen in liquid nitrogen vapor. Serial 7 μm microns sections were made in every 250 μm on a cryostat. Heart sections were fixed in formalin, washed with PBS and incubated in 1% BSA solution with 10% second antibody donor serum for 30 minutes. Anti-pimonidazole antibodies diluted by 1:100 were applied on the sections, which were incubated at 4°C for 8 hours. The sections were washed in the PBS solution 3 times for 5 minutes, then stained with anti-Alexa Fluor 594 secondary antibodies.

Isolation of mouse epicardial cells and hypoxia simulation

To obtain epicardial cells, hearts of mice (2–3 days old) were removed from the chest cavities and washed in heparinized Krebs–Ringer solution, than transferred into 5 ml of trypsin solution (for 6 hearts) and incubated three times for 5 minutes at 37°C in a Hybaid incubator shaker. After that, the hearts were removed. 5 ml of an enzyme inactivation medium (IMDM medium containing 20% fetal bovine serum (FBS)) was added to the resulting cells suspension and centrifuged at 300g for 10 minutes. After resuspending the sediment in the IMDM medium for epicardial cells, containing 1% FBS, 10 μM SB431542 inhibitor and antibiotics, it was transferred to gelatin-coated plates. The medium was changed every two days. Epicardial cell clones obtained using cloning cylinders and having confirmed morphology and immunophenotype were used for the experiments. A New Brunswick Scientific™ incubator was used to simulate hypoxia. Epicardial cell culture with 21% and 3% oxygen was carried out for 24 and 72 hours.

Evaluation of morphology and tight junctions in epicardial cells cultured with 21% and 3% oxygen

The morphology of cells was determined using phase-contrast microscopy. Tight junctions in epicardial cells cultured with 21% and 3% oxygen were examined using immunocytochemistry. Cells were permeabilized with 0.1% Triton X-100 solution (5 minutes) and stained with anti-ZO-1 marker antibodies for 1 hour. The cells were then washed and stained with antibodies conjugated with Alexa Fluor 488 (1:800, at 37°C for 60 minutes). The cell nuclei were stained with DAPI.

Microscopy and image analysis

Cells and myocardial cryosections were analyzed using an Axiovert 200 M fluorescence microscope and AxioVision 4.7 software.

Sample preparation and real-time polymerase chain reaction

Ribonucleic acids were isolated from the cells using a Qiagen kit. The reverse transcription reaction was performed using a Maxima First Strand cDNA Synthesis Kit reagents. The real-time polymerase chain reaction was performed in a Step One Plus Real-Time PCR System amplifier under the standard protocol using the following primers: SNAI1 (for: ACATCCGAAGCCACACG; rev: GTCAGCAAAAGCACGGTTG), SNAI2 (for: ACACATTAGAACTCACACTGGG; rev: TGGAGAAGGTTTTGGAGCAG), Wt1 (for: AGCACGGTCACTTTCGACG; rev: GTTTGAAGGAATGGTTGGGGAA), Ly6a (Sca-1) (for: GAGGCAGCAGTTATTGTGGAT; rev: CGTTGACCTTAGTACCCAGGA).

The data obtained were analyzed using the Statistica 8.0 suite. The data are expressed as mean and standard deviation (M±SD). The comparisons were made using the Mann–Whitney U-test and Wilcoxon test. The differences were statistically significant with $p<0.05$.

Results

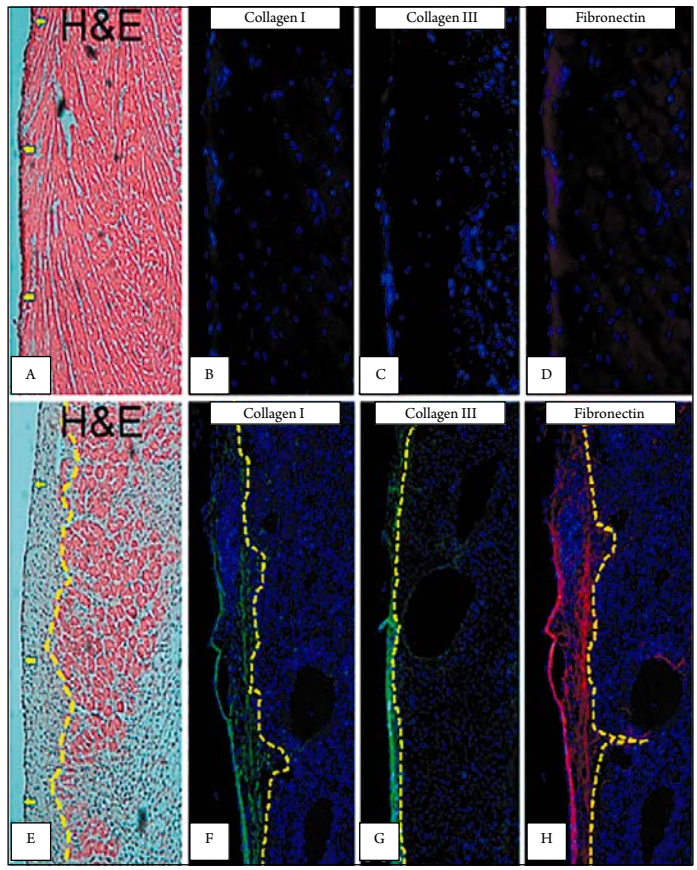
A key pathophysiological component of MI, hypoxia activates the HIF signaling pathway [36] and launches a wide range of cellular responses, including regulation of gene expression and post-translational modification of proteins. Animal heart sections obtained after MI simulation were examined to study the effect of tissue hypoxia on the activity of epicardial cells. Comparative histological analysis of intact rat heart sections and post-MI heart sections showed significant differences in the organization of the epicardial microenvironment. Epicardium of the intact heart is a thin layer composed of 2–4 layers of cells (Figure 1, A – D), which is located on the basal membrane consisting of extracellular matrix proteins. In acute ischemic damage to the heart, the thickness of the epicardial area, which is structurally divided into two parts (epicardium with high cell density and less compact subepicardium) increases 8–12 fold (Figure 1, E). After MI, a significant amount of extracellular matrix proteins, collagen I, III and fibronectin are visualized in the subepicardium (Figure 1, F-H), which may be due to the EMT activation in the epicardial cells and the formation of the mesenchymal derivatives actively producing extracellular matrix proteins.

Staining of heart sections with anti-Wt1+ activated epicardium marker antibodies showed that cells expressing this transcription factor are formed in the epicardium and subepicardium after MI. Most of such cells are formed on days 7–14 after MI (Figure 2). Wt1+ cells were not detected in the intact heart epicardium (Figure 2, A), which suggests that their appearance is an important indicator of the epicardial activation.

Experimental tests were performed using pimonidazole [37], which was administered intraperitoneally both to control specimens and rats after MI simulation, to assess epicardial cell activation and epicardial/subepicardial hypoxia. Pimonidazol is metabolized by cells in the state of hypoxia; its metabolites can be identified using specific antibodies (Figure 3) [38, 39].

Histological analysis showed that specific staining for pimonidazole metabolites, which is present only in samples taken from animals with MI, is localized mainly in the myocardium and only in single epicardial cells. As shown in Figure 3, the area of

Figure 1. Representative images of histological and immunofluorescent staining of intact heart sections (A–D) and in 7 days (G–H) after myocardial infarction simulation. Photos of heart tissue



A, G – hematoxylin and eosin staining; staining with anti-collagen I antibodies (B, F – green), anti-collagen III antibodies (C, G – green); anti-fibronectin antibodies (D, H – red). Cell nuclei are stained with DAPI (blue). Yellow arrows show the epicardial line. The yellow dotted line separates the epicardial/subepicardial area from the muscle tissue.

positive staining for pimonidazole metabolites travels from the epicardium deeper into the heart wall on day 14 after MI compared with day 5. Given that pimonidazole allows registering metabolic activity of cells subjected to high-degree hypoxia (O_2 <1%), it can be assumed that severe hypoxia develops in the event of transmural MI in only a small part of cells, while the overwhelming majority exists in the area of moderate oxygen deficiency.

The in vitro experiments were carried out by the simulation of moderate hypoxia (O_2 3%) to confirm that hypoxia is engaged in the regulation of epicardial activity (Figure 4). It was shown that 24-hour hypoxic epicardial cell culture was not accompanied by the increased expression of EMT regulator genes (SNAI1, SNAI2, Wt1). A longer (72 hours) culture in a hypoxic environment showed changes in the morphology of epicardial cells, which was manifested by a loss of cubical form, ZO1+ intercellular contact destruction, and

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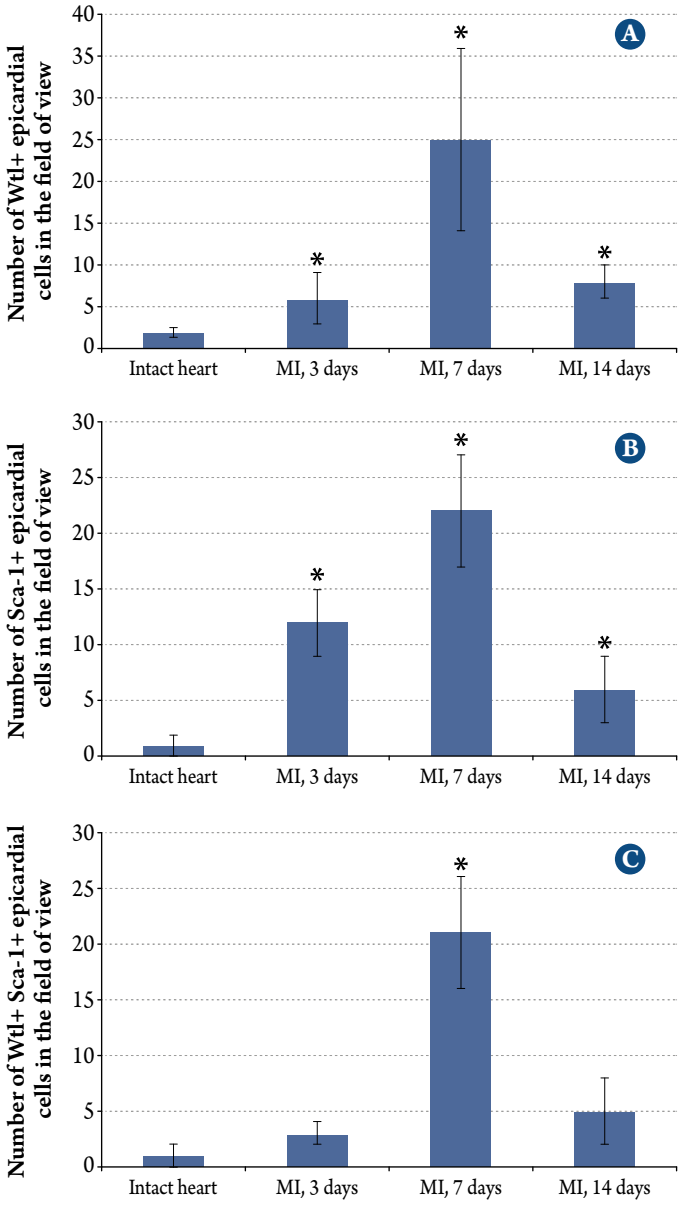
Figure 2. Diagrams of quantitative evaluation of epicardial cells expressing Wt1+ and Sca-1+ in the intact heart at different time points after MI (days 3, 7, and 14): the number of cells in the field of view in the epicardial/subepicardial area

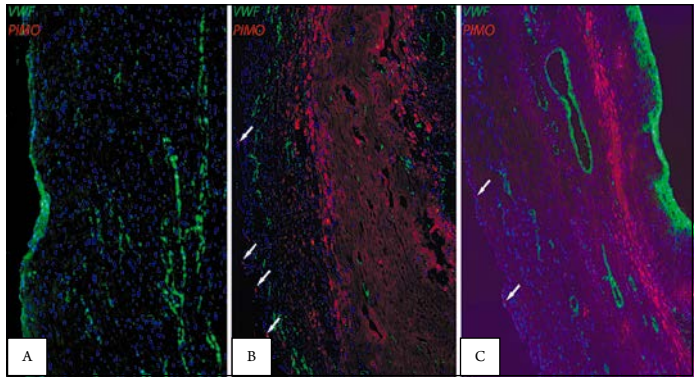


A – Wt1+ cells; B – Sca1+ cells;
C – Wt1+Sca1+ cells; MI – myocardial infarction.

adopting a fibroblast-like phenotype. Those changes were combined with the increased expression of EMT and Ly6a regulator genes (Sca-1), indicating the signs of epithelial-to-mesenchymal transition.

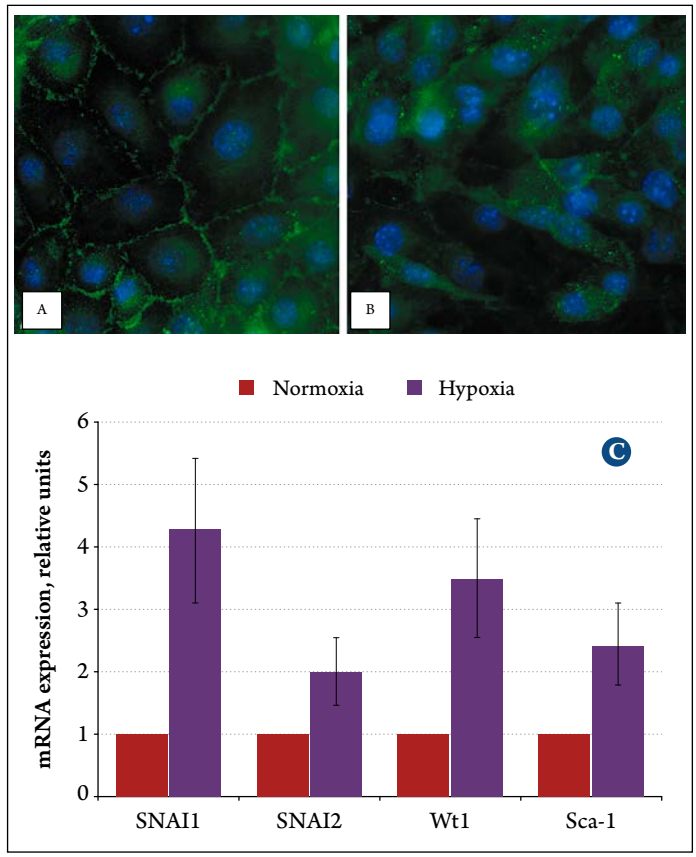
Given the increased EMT gene expression, greater number of Wt1+ cells and significant subepicardial thickening after ischemic damage, we studied the proliferative activity of epicardial cells. Epicardial activation is known to be accompanied by the increased proliferation activity of epicardial cells during embryogenesis [40]. BrdU was used to assess the level of epicardial cell proliferation and

Figure 3. Representative images of the vessels and pimonidazole metabolite distribution in the rat's intact myocardium sections (A) and after myocardial infarction (shown by arrows) on days 5 (B) and 14 (C)



Images of immunofluorescent staining of blood vessels (von Willebrand factor – green), pimonidazole, and metabolites (red) in the heart wall are presented.

Figure 4. Changes in the intercellular junctions and expression profile in the epicardial cells after exposure to hypoxia

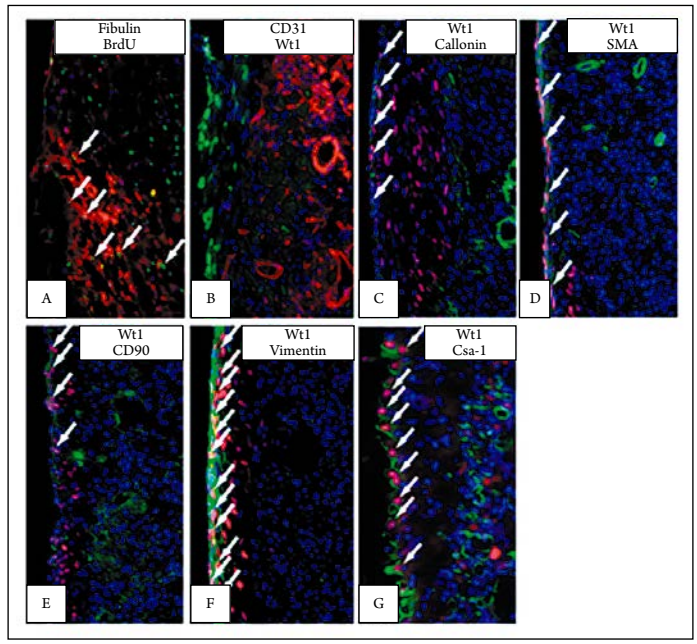


Representative images of immunofluorescence staining of epicardial cells for the tight intercellular junction marker (ZO1 – green) after the culture under normoxia (A) and hypoxia (B) are presented; C – a diagram of the assessment of the regulator gene expression of epithelial-to-mesenchymal transition in the epicardial cells after culture under normoxia (red columns) and hypoxia (O_2 3%; purple columns). The level of gene expression in cells cultured under normoxia is taken equal to one.

visualize the proliferating cells. It was found that the number of proliferating cells increases 8-fold in the epicardial/subepicardial 5 days after MI compared to their numbers in the intact heart (5±2 cells and 40±12 cells in the field of view, respectively). The number of proliferating cells continued to increase to reach 102±16 BrdU+ cells within the field of view, which corresponds to the changes in the subepicardial thickness after MI. We showed that the newly formed post-MI subepicardial microenvironment includes not only the extracellular matrix proteins (collagen and fibronectin), but also various types of cells. A significant accumulation of the activated epicardial cells and their derivatives (Wt1+ cells with smooth muscle cell markers and fibroblasts) was found in this area (Figure 5).

We also found an accumulation of Wt1+ cells co-expressing Sca-1 cardiac progenitor cells marker [41, 42] in the subepicardial area (see Figure 2, B, C; see Figure 5, G), which may indicate the origin of these epicardial cells by entering EMT. The intact subepicardial area contains single Sca-1+ cells in the field of view; their quantity increased significantly after IM (12±3 cells in the field of view on day 3, 22±5 cells in the field of view on day 7).

Figure 5. Representative images of immunofluorescent staining of the rat heart sections on day 7 after myocardial infarction simulation with anti-epicardial mesothelial cell marker antibodies and anti-vascular pronunciation cell marker antibodies



A – staining with an anti-fibulin marker of activated epicardial cells antibodies (red) and BrdU (green, nuclear staining); B – staining with anti-Wt1 antibodies (green) and anti-Pecam antibodies (endothelial cells, red); C – staining with anti-Wt1 antibodies (red) and anti-calponin antibodies (green); D – staining with anti-Wt1 antibodies (red) and anti-SMA antibodies (green); E – staining with anti-Wt1 antibodies (red) and anti-CD90 antibodies (green); F – staining with anti-Wt1 antibodies (red) and anti-vimentin antibodies (green); G – staining with anti-Wt1 antibodies (red) and anti-Sca-1 antibodies (green). The arrows show the colocalization of stain signals.

Discussion

These data indicate that low oxygen tension (hypoxia) in the heart is an important factor in post-MI remodeling of the epicardial microenvironment. Resting mesothelium cells, a low level of proliferation and the ability to produce extracellular matrix proteins are maintained in the intact heart along with EMT activity. Conversely, acute ischemic damage causes global epicardial remodeling and changes in the cellular composition. In the in vitro experiments, we showed that 72-hour hypoxia promotes the EMT regulator gene activation, causing intercellular junction disassembly, cell disintegration and increased cell flexibility. We found that the mesothelium cells entering EMT may result in the formation of the smooth muscle cell and fibroblast precursors, as well as the population of Sca-1+ cardiac progenitor cells that may participate in the construction of new vessels and serve as a mesenchymal link for the paracrine support of the microenvironment cells [43, 44]. The surprising accumulation of extracellular matrix proteins in the epicardial/subepicardial space creates unique conditions for the derived epicardial cells, providing isolation from the underlying tissue and inflammatory cells. However, epicardial cells are not subjected to severe hypoxic stress during the development of transmural MI. It can be assumed that pericardial fluid, a plasma ultrafiltrate that comprises many different protein factors along with dissolved oxygen, produces a certain tissue-protecting effect on the epicardium. It was shown earlier that patients with cardiac tamponade had lower oxygen pressure in the pericardial fluid than in the venous bed, ranging between 5 to 24 mm Hg at an average of 14 mmHg [45]. In another study, oxygen pressure in the pericardial fluid and venous bed did not differ in dogs with pericardial exudate and was about 35 mm Hg [46]. These values were higher than in the hypoxic conditions (7 mm Hg in O_2 1%). Given that the blood is supplied to the pericardium (as opposed to the epicardium and myocardium) by the pericardiacophrenic artery and pericardial branches of the thoracic aorta, these data suggest that a relatively high concentration of oxygen remains in the pericardial fluid during MI. By preventing the development of severe hypoxic conditions, the diffusion of oxygen from the pericardial fluid into the epicardium/subepicardium may protect this area from damage and help to maintain the regenerative potential of epicardial cells. The formation of a zone of moderate

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КРАТКАЯ ИНСТРУКЦИЯ ПО МЕДИЦИНСКОМУ ПРИМЕНЕНИЮ ЛЕКАРСТВЕННОГО ПРЕПАРАТА ФОРСИГА®: РЕГИСТРАЦИОННЫЙ НОМЕР: ЛП-002596 **ТОРГОВОЕ НАЗВАНИЕ:** ФОРСИГА (FORXIGA)®. **МЕЖДУНАРОДНОЕ НЕПАТЕНТОВАННОЕ НАЗВАНИЕ:** ДАПАГЛИФЛОЗИН. **ЛЕКАРСТВЕННАЯ ФОРМА:** таблетки, покрытые плёночной оболочкой. **ПОКАЗАНИЯ К ПРИМЕНЕНИЮ: САХАРНЫЙ ДИАБЕТ 2 ТИПА** у взрослых пациентов в дополнение к диете и физическим упражнениям для улучшения гликемического контроля в качестве: монотерапии, когда применение метформина невозможно ввиду непереносимости; комбинированной терапии с метформином, производными сульфонилмочевины (в том числе, в комбинации с метформином), тиазолидиндионами, ингибиторами дипептидилпептидазы 4 (ДПП-4) (в том числе, в комбинации с метформином); агонистом рецепторов глюкагоноподобного полипептида-1 (ГПП-1) эксенатидом пролонгированного действия в комбинации с метформином; препаратами инсулина (в том числе, в комбинации с одним или двумя гипогликемическими препаратами для перорального применения) при отсутствии адекватного гликемического контроля на данной терапии; стартовой комбинированной терапии с метформином, при целесообразности данной терапии. Сахарный диабет 2 типа у взрослых пациентов с установленным диагнозом сердечно-сосудистого заболевания или двумя и более факторами сердечно-сосудистого риска (возраст у мужчин ≥ 55 лет или ≥ 60 лет у женщин и наличие не менее одного фактора риска: дислипидемия, артериальная гипертензия, курение) для снижения риска госпитализации по поводу сердечной недостаточности. **СЕРДЕЧНАЯ НЕДОСТАТОЧНОСТЬ (II-IV функциональный класс по классификации NYHA) со сниженной фракцией выброса у взрослых пациентов для снижения риска сердечно-сосудистой смерти и госпитализации по поводу сердечной недостаточности. ПРОТИВОПОКАЗАНИЯ:** повышенная индивидуальная чувствительность к любому компоненту препарата; сахарный диабет 1-го типа; диабетический кетоацидоз; нарушение функции почек при расчетной СКФ (рСКФ) стабильно менее 45 мл/мин/1,73 м², включая нарушение функции почек тяжелой степени и терминальную стадию почечной недостаточности, при применении по показанию «сахарный диабет 2 типа»; нарушение функции почек тяжелой степени и терминальная стадия почечной недостаточности (рСКФ < 30 мл/мин/1,73 м²), при применении по показанию «сердечная недостаточность»(в связи с ограниченным опытом применения в клинических исследованиях); наследственная непереносимость лактозы, дефицит лактазы или глюкозо-галактозная мальабсорбция; беременность и период грудного вскармливания; детский возраст до 18 лет (безопасность и эффективность не изучены). **С ОСТОРОЖНОСТЬЮ:** печеночная недостаточность тяжелой степени, инфекции мочевыделительной системы, повышенное значение гематокрита. **ПРИМЕНЕНИЕ В ПЕРИОД БЕРЕМЕННОСТИ И ГРУДНОГО ВСКАРМЛИВАНИЯ** В связи с тем, что применение дапаглифлозина в период беременности не изучено, препарат противопоказан в период беременности. В случае диагностирования беременности терапия дапаглифлозином должна быть прекращена. Неизвестно, проникает ли дапаглифлозин и/или его неактивные метаболиты в грудное молоко. Нельзя исключить риск для новорожденных/младенцев. Дапаглифлозин противопоказан в период грудного вскармливания. **СПОСОБ ПРИМЕНЕНИЯ И ДОЗЫ:** Внутрь, независимо от приёма пищи, не разжевывая. Сахарный диабет 2 типа. Монотерапия: рекомендуемая доза препарата Форсига составляет 10 мг один раз в сутки. Комбинированная терапия: рекомендуемая доза препарата Форсига составляет 10 мг один раз в сутки в комбинации с метформином, производными сульфонилмочевины (в том числе, в комбинации с метформином), тиазолидиндионами, ингибиторами ДПП-4 (в том числе, в комбинации с метформином); агонистом рецепторов ГПП-1 – эксенатидом пролонгированного действия, в комбинации с метформином; препаратами инсулина (в том числе, в комбинации с одним или двумя гипогликемическими препаратами для перорального применения). С целью снижения риска гипогликемии при совместном назначении препарата Форсига с препаратами инсулина или препаратами, повышающими секрецию инсулина (например, с производным сульфонилмочевины), может потребоваться снижение дозы препаратов инсулина или препаратов, повышающих секрецию инсулина. Стартовая комбинированная терапия с метформином: рекомендуемая доза препарата Форсига составляет 10 мг один раз в сутки, доза метформина – 500 мг один раз в сутки. В случае неадекватного гликемического контроля дозу метформина следует увеличить. СД2 у взрослых пациентов с установленным диагнозом сердечно-сосудистого заболевания или двумя и более факторами сердечно-сосудистого риска для снижения риска госпитализации по поводу сердечной недостаточности: рекомендуемая доза препарата Форсига составляет 10 мг один раз в сутки. **Сердечная недостаточность: рекомендуемая доза препарата Форсига составляет 10 мг один раз в сутки. ПОБОЧНОЕ ДЕЙСТВИЕ.** Краткий обзор профиля безопасности. В клинических исследованиях СД2 более 15000 пациентов получали терапию дапаглифлозином. Первичная оценка безопасности и переносимости проводилась в заранее запланированном анализе объединенных данных 13 краткосрочных (до 24 недель) плацебо-контролируемых исследований, в которых 2360 пациентов принимали дапаглифлозин в дозе 10 мг и 2,295 пациентов получали плацебо. В исследовании дапаглифлозина в отношении сердечно-сосудистых исходов при СД2 (DECLARE) 8574 пациента получали дапаглифлозин 10 мг и 8569 получали плацебо (медиана воздействия 48 месяцев). В общей сложности экспозиция дапаглифлозина составила 30623 пациенто-лет. В исследовании дапаглифлозина в отношении сердечно-сосудистых исходов у пациентов с сердечной недостаточностью со сниженной фракцией выброса (DAPA-HF) 2368 пациентов получали дапаглифлозин 10 мг и 2368 получали плацебо (медиана воздействия 18 месяцев). Популяция включала пациентов с СД2 и без него, и пациентов с рСКФ ≥ 30 мл/мин/1,73 м². Профиль безопасности дапаглифлозина в исследованиях был в целом схожим по изучаемым показаниям. Тяжелую гипогликемию и диабетический кетоацидоз отмечали только у пациентов с сахарным диабетом. Ниже представлены НР, отмечавшиеся в плацебо-контролируемых клинических исследованиях и при пострегистрационном применении. Ни одна из них не зависела от дозы препарата. НР классифицированы по частоте и классу систем и органов. Частота НР представлена в виде следующей градации: очень часто (≥ 1/10), часто (≥ 1/100, < 1/10), нечасто (≥ 1/1000, < 1/100), редко (≥ 1/10000, < 1/1000), очень редко (< 1/10000) и неуточненной частоты (невозможно оценить по полученным данным). Инфекционные и паразитарные заболевания: часто – вульвовагинит, баланит и связанные с ними генитальные инфекции, инфекция мочевыводящих путей; нечасто – вульвовагинальный зуд, грибковые инфекционные заболевания; очень редко – некротизирующий фасциит промежности (гангрена Фурнье). Нарушения со стороны обмена веществ и питания: очень часто – гипогликемия (при применении в комбинации с производным сульфонилмочевины или инсулином); нечасто – снижение ОЦК, жажда; редко – диабетический кетоацидоз (при применении при СД2). Нарушения со стороны нервной системы: часто – головокружение. Нарушения со стороны желудочно-кишечного тракта: нечасто – запор, сухость во рту. Нарушения со стороны кожи и подкожных тканей: часто – сыпь; очень редко – ангионевротический отек. Нарушения со стороны костно-мышечной системы и соединительной ткани: часто – боль в спине. Нарушения со стороны почек и мочевыводящих путей: часто – дизурия, полиурия; нечасто – никтурия. Лабораторные и инструментальные данные: часто – дислипидемия, повышение значения гематокрита, снижение почечного клиренса креатинина на начальном этапе терапии; нечасто – повышение концентрации мочевины в крови, повышение концентрации креатинина в крови на начальном этапе терапии.

Ссылка на полную инструкцию: Инструкция по медицинскому применению лекарственного препарата Форсига ® (таблетки, покрытые пленочной оболочкой, 5 мг, 10 мг). Регистрационное удостоверение ЛП-002596 от 21.08.2014

ХСНнФВ – хроническая сердечная недостаточность со сниженной фракцией выброса; СС – сердечно-сосудистый; СН – сердечная недостаточность.

* Включая неотложные обращения по причине СН. # Снижение относительного риска сердечно-сосудистой смерти и смерти от всех причин в группе дапаглифлозина по сравнению с плацебо в исследовании DAPA HF.

1. Инструкция по медицинскому применению лекарственного препарата Форсига® (таблетки, покрытые пленочной оболочкой, 5 мг, 10 мг). Регистрационное удостоверение № ЛП 002596 от 21.08.2014.
2. Клинические рекомендации Хроническая сердечная недостаточность 2020. https://scardio.ru/content/Guidelines/2020/Clinic_rekom_HSN.pdf (дата обращения 04.05.2021)
3. McMurray JJV et al., N Engl J Med. 2019;381(21):1995–2008.
4. Перечень жизненно необходимых и важнейших лекарственных препаратов для медицинского применения.
5. Перечень лекарств для обеспечения отдельных граждан.

Материал предназначен для специалистов здравоохранения. Имеются противопоказания. Перед назначением ознакомьтесь, пожалуйста, с полной инструкцией по медицинскому применению лекарственного препарата.

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hypoxia (2–3%) in the epicardial area contributes to the remodeling of the epicardial microenvironment. The results of numerous studies show that hypoxia can control the cellular microenvironment by activating the HIF (hypoxia-induced factor) signaling pathway [47]. In normoxic conditions, prolyl hydroxylase enzymes 1, 2, and 3 (PHD1/2/3) and Von Hippel-Lindau protein have a complex effect on HIF-1 and HIF-2 factors, causing their proteasomal degradation [48]. Under hypoxic conditions, HIF-1 and HIF-2 also stabilize and co-ordinate cellular responses to hypoxia by activating gene expression programs for cellular adaptation to oxygen deficiency [48]. This is achieved due to HIF-mediated EMT by the increased secretion of TGFb and activation SNAIL1, SNAIL2, TWIST, SIP1 and ZEB1 transcription factors. This mechanism of EMT activity regulation was demonstrated in different cell lines following exposure to hypoxia or constitutive HIF expression. EMT induced by HIF may comprise a mechanism for the formation of an epicardial cellular niche by forming the original stem/progenitor cell Sca-1+, which mainly utilizes cytoplasmic glycolysis and resists hypoxic stress. However, hypoxia promotes the activation of the epicardial cell pool, with the formation of epicardial precursor cells providing cellular heterogeneity of the microenvironment. This may be necessary to provide various cells in the damaged area for the rapid scarring and the de novo blood vessel formation [49], as well as multidirectional repair paracrine signals. The follistatin-like protein (Fstl-1) secreted by the epicardial cells is a perfect example of such regulation [50]. Although Fstl-1 production is maintained in embryonic hearts at a high level, it almost completely disappears in the adult heart. However, the activated myocardial cells begin to re-produce this protein in response to acute ischemic damage [51], which increases the survival of cardiomyocytes in the ischemic area, stimulating their entry into the cell cycle, activating arteriogenesis, as well as providing other cardioprotective effects.

Conclusion

The epicardium of the adult heart is shown to serve as a cell depot capable of reactivating its own repair potential under the influence of hypoxia. This provides the basis for the development of approaches to targeting the epicardium aimed at modulating its activity for the regulation of repair processes.

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REFERENCES

1. Bueno H, Moura B, Lancellotti P, Bauersachs J. The year in cardiovascular medicine 2020: heart failure and cardiomyopathies. European Heart Journal. 2021;42(6):657–70. DOI: 10.1093/eurheartj/ehaa1061
2. Lafuse WP, Wozniak DJ, Rajaram MVS. Role of Cardiac Macrophages on Cardiac Inflammation, Fibrosis and Tissue Repair. Cells. 2020;10(1):51. DOI: 10.3390/cells10010051
3. Dergilev K.V., Vasilets Yu.D., Tsokolaeva Z.I., Zubkova E.S., Parfenova E.V. Perspectives of cell therapy for myocardial infarction and heart failure based on cardiosphere cells. Therapeutic Archive. 2020;92(4):111–20. [Russian: Дергилев К.В., Василец Ю.Д., Цоколаева З.И., Зубкова Е.С., Парфенова Е.В. Перспективы клеточной терапии инфаркта миокарда и сердечной недостаточности на основе клеток кардиосфер. Терапевтический Архив. 2020;92(4):111-20]. DOI: 10.26442/00403660.2020.04.000634
4. Dergilev KV, Shevchenko EK, Tsokolaeva ZI, Beloglazova IB, Zubkova ES, Boldyreva MA et al. Cell Sheet Comprised of Mesenchymal Stromal Cells Overexpressing Stem Cell Factor Promotes Epicardium Activation and Heart Function Improvement in a Rat Model of Myocardium Infarction. International Journal of Molecular Sciences. 2020;21(24):9603. DOI: 10.3390/ijms21249603
5. Dergilev K.V., Komova A.V., Tsokolaeva Z.I., Beloglazova I.B., Parfenova E.V. Epicardium as a new target for regenerative technologies in cardiology. Genes and cells. 2020;15(2):33–40. [Russian: Дергилев К.В., Комова А.В., Цоколаева З.И., Белоглазова И.Б., Парфенова Е.В. Эпикард как новая мишень для регенеративных технологий в кардиологии. Гены и клетки. 2020;15(2):33-40]. DOI: 10.23868/202004016
6. Christoffels VM, Grieskamp T, Norden J, Mommersteeg MTM, Rudat C, Kispert A. Tbx18 and the fate of epicardial progenitors. Nature. 2009;458(7240):E8–9. DOI: 10.1038/nature07916
7. Rudat C, Kispert A. Wt1 and Epicardial Fate Mapping. Circulation Research. 2012;111(2):165–9. DOI: 10.1161/CIRCRESAHA.112.273946
8. Mikawa T, Gourdie RG. Pericardial Mesoderm Generates a Population of Coronary Smooth Muscle Cells Migrating into the Heart along with Ingrowth of the Epicardial Organ. Developmental Biology. 1996;174(2):221–32. DOI: 10.1006/dbio.1996.0068
9. Dettman RW, Denetclaw W, Ordahl CP, Bristow J. Common Epicardial Origin of Coronary Vascular Smooth Muscle, Perivascular Fibroblasts, and Intermyocardial Fibroblasts in the Avian Heart. Developmental Biology. 1998;193(2):169–81. DOI: 10.1006/dbio.1997.8801
10. Männer J. Does the subepicardial mesenchyme contribute myocardioblasts to the myocardium of the chick embryo heart? A quail-chick chimera study tracing the fate of the epicardial primordium. The Anatomical Record. 1999;255(2):212–26. PMID: 10359522
11. Gittenberger-de Groot AC, Vrancken Peeters M-PFM, Mentink MMT, Gourdie RG, Poelmann RE. Epicardium-Derived Cells Contribute a Novel Population to the Myocardial Wall and the Atrioventricular Cushions. Circulation Research. 1998;82(10):1043–52. DOI: 10.1161/01.RES.82.10.1043

ISSN 0022-9040. Kardiologiia. 2021;61(6). DOI: 10.18087/cardio.2021.6.n1476

12. Gittenberger-de Groot AC, Vrancken Peeters M-PFM, Bergwerff M, Mentink MMT, Poelmann RE. Epicardial Outgrowth Inhibition Leads to Compensatory Mesothelial Outflow Tract Collar and Abnormal Cardiac Septation and Coronary Formation. Circulation Research. 2000;87(11):969–71. DOI: 10.1161/01.RES.87.11.969
13. Pérez-Pomares J-M, Carmona R, González-Iriarte M, Atencia G, Wessels A, Muñoz-Chápuli R. Origin of coronary endothelial cells from epicardial mesothelium in avian embryos. The International Journal of Developmental Biology. 2002;46(8):1005–13. PMID: 12533024
14. Poelmann RE, Lie-Venema H, Gittenberger-de Groot AC. The role of the epicardium and neural crest as extracardiac contributors to coronary vascular development. Texas Heart Institute Journal. 2002;29(4):255–61. PMID: 12484609
15. Rothenberg F, Hitomi M, Fisher SA, Watanabe M. Initiation of apoptosis in the developing avian outflow tract myocardium. Developmental Dynamics. 2002;223(4):469–82. DOI: 10.1002/dvdy.10077
16. Schaefer KS, Doughman YQ, Fisher SA, Watanabe M. Dynamic patterns of apoptosis in the developing chicken heart. Developmental Dynamics. 2004;229(3):489–99. DOI: 10.1002/dvdy.10463
17. Chen TH-P, Chang T-C, Kang J-O, Choudhary B, Makita T, Tran CM et al. Epicardial Induction of Fetal Cardiomyocyte Proliferation via a Retinoic Acid-Inducible Trophic Factor. Developmental Biology. 2002;250(1):198–207. DOI: 10.1006/dbio.2002.0796
18. Zhou B, Honor LB, He H, Ma Q, Oh J-H, Butterfield C et al. Adult mouse epicardium modulates myocardial injury by secreting paracrine factors. Journal of Clinical Investigation. 2011;121(5):1894–904. DOI: 10.1172/JCI45529
19. Limana F, Bertolami C, Mangoni A, Di Carlo A, Avitabile D, Mocini D et al. Myocardial infarction induces embryonic reprogramming of epicardial c-kit+ cells: Role of the pericardial fluid. Journal of Molecular and Cellular Cardiology. 2010;48(4):609–18. DOI: 10.1016/j.yjmcc.2009.11.008
20. Qian L, Huang Y, Spencer CI, Foley A, Vedantham V, Liu L et al. In vivo reprogramming of murine cardiac fibroblasts into induced cardiomyocytes. Nature. 2012;485(7400):593–8. DOI: 10.1038/nature11044
21. von Gise A, Pu WT. Endocardial and epicardial epithelial to mesenchymal transitions in heart development and disease. Circulation Research. 2012;110(12):1628–45. DOI: 10.1161/CIRCRESAHA.111.259960
22. Smits A, Riley P. Epicardium-Derived Heart Repair. Journal of Developmental Biology. 2014;2(2):84–100. DOI: 10.3390/jdb2020084
23. Missinato MA, Tobita K, Romano N, Carroll JA, Tsang M. Extracellular component hyaluronic acid and its receptor Hmmr are required for epicardial EMT during heart regeneration. Cardiovascular Research. 2015;107(4):487–98. DOI: 10.1093/cvr/cvv190
24. Singh A, Ramesh S, Cibi DM, Yun LS, Li J, Li L et al. Hippo Signaling Mediators Yap and Taz Are Required in the Epicardium for Coronary Vasculature Development. Cell Reports. 2016;15(7):1384–93. DOI: 10.1016/j.celrep.2016.04.027
25. Wada AM, Smith TK, Osler ME, Reese DE, Bader DM. Epicardial/Mesothelial Cell Line Retains Vasculogenic Potential of Embryonic Epicardium. Circulation Research. 2003;92(5):525–31. DOI: 10.1161/01.RES.0000060484.11032.0B
26. Lu J, Landerholm TE, Wei JS, Dong X-R, Wu S-P, Liu X et al. Coronary Smooth Muscle Differentiation from Proepicardial Cells Requires RhoA-Mediated Actin Reorganization and p160 Rho-Kinase Activity. Developmental Biology. 2001;240(2):404–18. DOI: 10.1006/dbio.2001.0403
27. Merki E, Zamora M, Raya A, Kawakami Y, Wang J, Zhang X et al. Epicardial retinoid X receptor alpha is required for myocardial growth and coronary artery formation. Proceedings of the National Academy of Sciences of the USA. 2005;102(51):18455–60. DOI: 10.1073/pnas.0504343102
28. Lavine KJ, White AC, Park C, Smith CS, Choi K, Long F et al. Fibroblast growth factor signals regulate a wave of Hedgehog activation that is essential for coronary vascular development. Genes & Development. 2006;20(12):1651–66. DOI: 10.1101/gad.1411406
29. Morabito CJ, Dettman RW, Kattan J, Collier JM, Bristow J. Positive and negative regulation of epicardial-mesenchymal transformation during avian heart development. Developmental Biology. 2001;234(1):204–15. DOI: 10.1006/dbio.2001.0254
30. Vega-Hernandez M, Kovacs A, De Langhe S, Ornitz DM. FGF10/FGFR2b signaling is essential for cardiac fibroblast development and growth of the myocardium. Development. 2011;138(15):3331–40. DOI: 10.1242/dev.064410
31. Zamora M, Manner J, Ruiz-Lozano P. Epicardium-derived progenitor cells require -catenin for coronary artery formation. Proceedings of the National Academy of Sciences USA. 2007;104(46):18109–14. DOI: 10.1073/pnas.0702415104
32. Austin AF, Compton LA, Love JD, Brown CB, Barnett JV. Primary and immortalized mouse epicardial cells undergo differentiation in response to TGFβ. Developmental Dynamics. 2008;237(2):366–76. DOI: 10.1002/dvdy.21421
33. Tao J, Barnett J, Watanabe M, Ramírez-Bergeron D. Hypoxia Supports Epicardial Cell Differentiation in Vascular Smooth Muscle Cells through the Activation of the TGFβ Pathway. Journal of Cardiovascular Development and Disease. 2018;5(2):19. DOI: 10.3390/jcdd5020019
34. Tao J, Doughman Y, Yang K, Ramirez-Bergeron D, Watanabe M. Epicardial HIF signaling regulates vascular precursor cell invasion into the myocardium. Developmental Biology. 2013;376(2):136–49. DOI: 10.1016/j.ydbio.2013.01.026
35. Dergilev K.V., Tsokolaeva Z.I., Beloglazova I.B., Ratner E.I., Molokotina Yu.D., Parfenova E.V. Characteristics of angiogenic properties of c-Kit+cells of the myocardium. Genes and Cells. 2018;13(3):82–8. [Russian: Дергилев К.В., Цоколаева З.И., Белоглазова И.Б., Ратнер Е.И., Молокотина Ю.Д., Парфенова Е.В. Характеристика ангиогенных свойств с-Kit+-клеток миокарда. Гены и клетки. 2018;13(3):82-8]. DOI: 10.23868/201811038
36. Kindrick JD, Mole DR. Hypoxic Regulation of Gene Transcription and Chromatin: Cause and Effect. International Journal of Molecular Sciences. 2020;21(21):8320. DOI: 10.3390/ijms21218320
37. Krohn KA, Link JM, Mason RP. Molecular Imaging of Hypoxia. Journal of Nuclear Medicine. 2008;49(Suppl 2):129S-148S. DOI: 10.2967/jnumed.107.045914
38. Nordsmark M, Loncaster J, Aquino-Parsons C, Chou S-C, Ladekarl M, Havsteen H et al. Measurements of hypoxia using pimonidazole and polarographic oxygen-sensitive electrodes in human cervix carcinomas. Radiotherapy and Oncology. 2003;67(1):35–44. DOI: 10.1016/S0167-8140(03)00010-0
39. Sanada F, Kim J, Czarna A, Chan NY-K, Signore S, Ogórek B et al. c-Kit–Positive Cardiac Stem Cells Nested in Hypoxic Niches Are Activated by Stem Cell Factor Reversing the Aging Myopathy. Circulation Research. 2014;114(1):41–55. DOI: 10.1161/CIRCRESAHA.114.302500
40. Romano N, Ceci M. The face of epicardial and endocardial derived cells in zebrafish. Experimental Cell Research. 2018;369(1):166–75. DOI: 10.1016/j.yexcr.2018.05.022
41. Dergilev K.V., Rubina K.A., Parfenova E.V. Resident cardiac stem cells. Kardiologiia. 2011;51(4):84–92. [Russian: Дергилев К.В., Рубина К.А., Парфенова Е.В. Резидентные стволовые клетки сердца. Кардиология. 2011;51(4):84-92. PMID: 21623726]
42. Iancu CB, Iancu D, Renţea I, Hostiuc S, Dermengiu D, Rusu MC. Molecular signatures of cardiac stem cells. Romanian Journal of Morphology and Embryology. 2015;56(4):1255–62. PMID: 26743269
43. Scalise M, Marino F, Cianflone E, Mancuso T, Marotta P, Aquila I et al. Heterogeneity of Adult Cardiac Stem Cells. P. 141-178. DOI: 10.1007/978-3-030-24108-7_8. In: Stem Cells Heterogeneity in Different Organs. [ISBN: 978-3-030-24107-0, 978-3-030-24108-7. Series Title: Advances in Experimental Medicine and Biology]. Birbrair A, editor -Cham: Springer International Publishing;2019.
44. Samal R, Sappa PK, Gesell Salazar M, Wenzel K, Reinke Y, Völker U et al. Global secretome analysis of resident cardiac progenitor cells from wild-type and transgenic heart failure mice: Why ambience matters. Journal of Cellular Physiology. 2019;234(7):10111–22. DOI: 10.1002/jcp.27677

45. Balakrishnan S, Hartman CW, Grinnan GL, Bartel AG, Crisler C, Brickman RD. Pericardial fluid gas analysis in hemorrhagic pericardial tamponade. The Annals of Thoracic Surgery. 1979;27(1):55–8. DOI: 10.1016/s0003-4975(10)62971-2
46. De Laforcade AM, Freeman LM, Rozanski EA, Rush JE. Biochemical analysis of pericardial fluid and whole blood in dogs with pericardial effusion. Journal of Veterinary Internal Medicine. 2005;19(6):833–6. PMID: 16355677
47. Li T, Mao C, Wang X, Shi Y, Tao Y. Epigenetic crosstalk between hypoxia and tumor driven by HIF regulation. Journal of Experimental & Clinical Cancer Research. 2020;39(1):224. DOI: 10.1186/s13046-020-01733-5
48. Higgins DF, Kimura K, Iwano M, Haase VH. Hypoxia-inducible factor signaling in the development of tissue fibrosis. Cell Cycle. 2008;7(9):1128–32. DOI: 10.4161/cc.7.9.5804
49. Dergilev K.V., Tsokolaeva Z.I., Beloglazova I.B., Traktuev D.O., Gorelova A.V., Zubko A.V. et al. Intramyocardial Injection of Plasmid Encoding Platelet Growth Factor Increases Epicardial-Mediated Post Infarction Myocardial Vascularization (Experimental Study). General Reanimatology. 2020;16(6):54–64. [Russian: Дергилев К.В., Цоколаева З.И., Белоглазова И.Б., Трактуев Д.О., Горелова А.В., Зубко А.В. и др. Интрамиокардиальное введение плазмиды, кодирующей фактор роста тромбоцитов, способствует увеличению эпикардопосредованной васкуляризации постинфарктного сердца (экспериментальное исследование). Общая реаниматология. 2020;16(6):54-64]. DOI: 10.15360/1813-9779-2020-6-54-64
50. Wei K, Serpooshan V, Hurtado C, Diez-Cuñado M, Zhao M, Maruyama S et al. Epicardial FSTL1 reconstitution regenerates the adult mammalian heart. Nature. 2015;525(7570):479–85. DOI: 10.1038/nature15372
51. Widera C, Horn-Wichmann R, Kempf T, Bethmann K, Fiedler B, Sharma S et al. Circulating Concentrations of Follistatin-Like 1 in Healthy Individuals and Patients with Acute Coronary Syndrome as Assessed by an Immunoluminometric Sandwich Assay. Clinical Chemistry. 2009;55(10):1794–800. DOI: 10.1373/clinchem.2009.129411

ISSN 0022-9040. Kardiologiia. 2021;61(6). DOI: 10.18087/cardio.2021.6.n1476