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Osteogenic potential of mesenchymal stem cells of epicardial adipose tissue in patients with coronary heart disease

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ABSTRACT

Aim. To assess the osteogenic potential of mesenchymal stem cells (MSCs) of epicardial adipose tissue (EAT) in patients with stable coronary heart disease based on obtaining gene profiles (osteogenesis markers).

Materials and methods. In EAT MSCs, the expression levels of the *RUNX2* (RUNX transcription factor encoding gene), BGLAP (osteocalcin encoding gene), SPP1 (osteopontin encoding gene), *SP7* (Osterix encoding gene) genes were determined using real-time polymerase chain reaction (PCR). Using immunofluorescence staining, the amount of RUNX2, osteocalcin, osteopontin, and Osterix proteins was determined in the supernatant of cultured MSCs.

Results. It was found that the expression of *RUNX2* in cells cultured in a medium with osteoinducers was 1.88 times higher than in undifferentiated MSCs ($p = 0.012$). The level of *RUNX2* protein was also higher in a differentiated cell culture ($p < 0.05$). Similar results were obtained regarding the level of *SPP1* mRNA expression ($p = 0.012$). *BGLAP* expression did not differ between differentiated and undifferentiated MSC cultures. The level of *SP7* gene expression did not differ in cells either with or without an osteoblastic medium. It is worth noting that using immunofluorescence staining, there were no differences detected in the expression of Osterix and OCN between cultures of differentiated and undifferentiated cells.

Conclusion. It was found that EAT MSCs have osteogenic potential, which was manifested by the expression of osteogenesis genes in both differentiated and undifferentiated MSCs. The increase in the expression level of *SSP1* and *RUNX2* mRNA on day 15 of cultivation with osteoblastic medium indicates that the studied cells are preosteoblasts and are at the stage of extracellular matrix synthesis.

Keywords: mesenchymal stem cells, adipose tissue, coronary heart disease, osteogenesis genes, calcification

Conflict of interest. The authors declare the absence of obvious or potential conflicts of interest related to the publication of this article.

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Conformity with the principles of ethics. This study was approved by the local Ethics Committee of the Research

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Остеогенный потенциал мезенхимальных стволовых клеток эпикардиальной жировой ткани у пациентов с ишемической болезнью сердца

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РЕЗЮМЕ

Цель. На основе получения профиля генов – маркеров остеогенеза – оценить остеогенный потенциал мезенхимальных стволовых клеток (МСК) эпикардиальной жировой ткани (ЭЖТ) у пациентов со стабильной ишемической болезнью сердца.

Материалы и методы. В МСК ЭЖТ методом полимеразной цепной реакции (ПЦР) в реальном времени определяли уровни экспрессии генов *RUNX2* (ген, кодирующий транскрипционный фактор RUNX), *BGLAP* (ген, кодирующий остеокальцин OCN), *SPP1* (ген, кодирующий остеопонтин OPN), *SP7* (ген, кодирующий Osterix). С помощью иммунофлуоресцентного окрашивания в супернатанте культивируемых МСК определяли количество белка RUNX2, OCN, OPN и Osterix.

Результаты. Установлено, что экспрессия RUNX2 в клетках, культивированных в среде с остеоиндукторами, была в 1,88 раза выше, чем в недифференцированных МСК ($p = 0{,}012$). Уровень белка *RUNX* также был выше в дифференцированной культуре клеток ($p < 0{,}05$). Аналогичные результаты были получены в отношении уровня экспрессии мРНК *SPP1* ($p = 0{,}012$). Экспрессия *BGLAP* не отличалась в дифференцированных и недифференцированных культурах МСК так же, как уровень экспрессии гена *SP7* в клетках с остеобластной средой и без нее. Стоит отметить, что методом иммунофлуоресцентной окраски нами не выявлено различий в экспрессии Osterix и OCN между культурами дифференцированных и недифференцированных клеток.

Заключение. МСК ЭЖТ имеют остеогенный потенциал, что проявилось экспрессией генов остеогенеза как дифференцированных, так и недифференцированных МСК. Увеличение уровня экспрессии мРНК *SSP1* и *RUNX2* на 15-е сут культивирования с остеобластной средой свидетельствует о том, что изучаемые нами клетки являются преостеобластами и находятся на стадии синтеза внеклеточного матрикса.

Ключевые слова: мезенхимальные стволовые клетки, жировая ткань, ишемическая болезнь сердца, гены остеогенеза, кальцификация

Конфликт интересов. Авторы заявляют об отсутствии явных и потенциальных конфликтов интересов, связанных с публикацией настоящей статьи.

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развития болезней системы кровообращения с учетом коморбидности на основе изучения фундаментальных, клинических, эпидемиологических механизмов и организационных технологий медицинской помощи в условиях промышленного региона Сибири».

Соответствие принципам этики. Все пациенты подписали информированное согласие на использование биологического материала в исследовании. Исследование одобрено локальным этическим комитетом НИИ КПССЗ (протокол № 12 от 20.03.2023).

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INTRODUCTION

Atherosclerosis and its associated pathology – coronary heart disease (CHD) are leading causes of mortality in economically developed countries, despite significant advancements in treatment. A common complication accompanying CHD is calcification of the coronary arteries [1]. Although vascular calcification was recognized as a form of extra-skeletal ossification over a century ago, it is now understood to be a strictly regulated process. Its stages are similar to bone morphogenesis, including the expression of major pro-osteogenic factors such as osteocalcin, osteoprotegerin, osteopontin, and other markers [2]. The exact mechanisms underlying the pathogenesis of coronary artery calcification remain unclear.

For a long time, it was believed that at least 4 types of cells can lead to vascular calcification: 1) pericytes in microvessels; 2) pericyte-like calcifying vascular cells in the intima of the aorta; 3) smooth muscle cells in the media; and 4) myofibroblasts in adventitia. Along with resident cells, circulating cells, including bone marrow-derived mesenchymal stem cellss, can migrate into the vessel wall and contribute to calcification [3, 4]. However, adipose tissue (AT) surrounding the heart and blood vessels may also be the source of mesenchymal stem cells (MSCs). [5]. It is assumed that the main function of AT MSCs is to regenerate damaged areas surrounding the organ, as well as to produce biologically active substances, including anti-apoptotic, growth, and immunomodulatory factors. At the same time, AT MSCs are multipotent cells, possessing the ability to differentiate into osteogenic, chondrogenic, and adipogenic lines [6]. Information regarding the function of AT MSCs surrounding the heart and blood vessels is limited. Existing literature on this topic indicates that the AT surrounding the heart and blood vessels is a source of MSCs that are capable of differentiating into various cell types [7, 8]. It is also known that the paracrine activity of AT MSCs, which involves the production of growth factors and cytokines, has an effect on inducing angiogenesis and increasing the survival of cardiomyocytes [5].

The ability of AT MSCs to differentiate into osteoblasts secreting calcium salts into the extracellular space may be a link in the pathogenesis of vascular wall calcification. Currently, the role of AT MSCs in the formation of vascular calcification remains understudied. However, this area holds significant promise from both fundamental and clinical perspectives, since MSCs may not only be one of the key participants in the development of pathological calcification but also serve as a therapeutic target for regulating osteoblastic potential. Existing literature presents contradictory information regarding the osteogenic ability of AT MSCs. For example, A.B. Malashicheva et al.compared subcutaneous fat MSCs from healthy donors and patients with aortic valve calcification, revealing a reduced osteogenic potential in the MSCs of patients with aortic valve calcification [9]. Conversely, there is evidence that the ability of AT MSCs to undergo osteogenic differentiation depends on the tissue localization [10].

The aim of this study is to evaluate the osteogenic potential of MSCs derived from epicardial adipose tissue by assessing the expression levels of osteoblastic differentiation genes and their corresponding proteins in patients with stable coronary heart disease.

MATERIALS AND METHODS

The study included 5 patients with CHD, all under the age of 75,who signed a voluntary informed consent to participate. All patients had indications for open-heart surgery, specifically direct myocardial revascularization via coronary artery bypass grafting (CABG). The study did not include patients over the age of 75 and those with clinically

significant concomitant pathologies (such as type 1 and type 2 diabetes mellitus, myocardial infarction (MI), anemia, renal and liver failure, oncological and infectious-inflammatory diseases during exacerbation, autoimmune diseases). Epicardial adipose tissue (EAT) stem cells were isolated from biopsies of epicardial localization, with each biopsy weighing from 3 to 5 grams.The source of EAT was the right parts of the heart, particularly the regions with the highest presence, namely the right atrium and right ventricle. The obtained EAT samples were thoroughly washed with a sterile phosphate-buffered saline (PBS) (Gibco, USA) to remove erythrocytes, blood clots, and local anesthetics from the surface. Subsequently, the EAT was cut into small, irregularly shaped pieces (1–3 mm^2) using scissors, with an average weight of about 4 g per piece. The tissue pieces were then placed in 20 ml of PBS supplemented with penicillin (600 U/ml) (Gibco, USA) and streptomycin (300 mg/ml, Gibco, USA) in a 50 ml test tube for 5–10 minutes at room temperature to remove remnants of connective tissue and/or dermis, blood vessels. Following this, the small pieces of adipose tissue were pipetted into 25 cm^2 culture vials (Biologix, Germany). The cells were incubated in a CO_2 incubator (5% CO_2, 95% air, 37 °C), in a medium supporting the growth of MSCs (MesenCult Proliferation Kit, STEMCELL Technologies, Canada) with the addition of 100 U/ml penicillin and 100 U/ml streptomycin (Gibco, USA). When the primary cells reached 80–90% confluence, they were treated with 0.25% trypsin solution (Trypsin, PanEco, Russia) and transferred into 75 cm^2 culture vials (Biologix, Germany). The cells were then cultured to 80–90% cell fusion.

IMMUNOPHENOTYPING OF CELLS (FLOW CYTOMETRY)

To confirm that the resulting cell culture consisted of mesenchymal stem cells, their phenotype was assessed using a combination of conjugated monoclonal antibodies: CD90 FITC (BC, IM1839U), CD 34 APC (BC, PN IM2472U), CD73 APCCy7 (Biolegend, 344022), CD 105 PE (Biolegend, 323206). The phenotype CD34-, CD73+, CD 90+, CD 105+ was regarded as corresponding to the AT MSC according to the definition of the International Society for Cell Therapy (ISCT). The results are presented as a percentage of the number of cells exhibiting the corresponding antigens.

The gating strategy included the removal of duplicates on the FSC-H/FSC-A histogram, followed by the isolation of the studied population based on FSC/SSC parameters. According to the expression level of CD90 and CD73 receptors, the main cell population was isolated: CD90+CD73+ (79.47%). This population was subsequently examined for the presence of CD105 and CD34 membrane antigens. CD105 and CD90 were detected in 79.71% of the cells, while CD105 antigenic marker was present in 17.54% of the cells, and CD34 was found in only 3.76% of the cells. Thus, the phenotype of the cell culture obtained from the EAT was determined to be CD73+CD 90+CD105+CD34-/+

OSTEOGENIC DIFFERENTIATION OF MSCS OF EPICARDIAL ADIPOSE TISSUE

To perform osteogenic differentiation, cells from the 3rd passage of EAT were transferred into two T-75 vials. At the preparatory stage, human fibronectin (PanEco, Russia) was diluted to a concentration of 20 μg/ml in a single-use PBS and introduced into a 6-well tablet (for RNA isolation) and an 8-well Ibidi chamber (Germany) to ensure that the protein covered the culture plastic. Following this, the fibronectin was removed, and the PBS wells were washed (Gibco, China), cells were added in an appropriate volume. To start osteogenic cell differentiation, the MesenCult™ Osteogenic Differentiation Kit (Human, STEMCELL Technologies, Canada) was used. Cell differentiation process was conducted over a period of 15 days, with the osteogenic medium being replaced every 2–3 days. Cells that did not undergo osteogenic differentiation served as control samples.

DETERMINATION OF OSTEOGENIC POTENTIAL OF AT MSCS BY GENE EXPRESSION LEVEL VIA PCR

Total ribonucleic acid (RNA) extraction from MSCs was performed on day 15 of osteogenic differentiation to assess expression levels of a screening panel of osteogenic differentiation genes (quantitative polymerase chain reaction (PCR) after reverse transcription), including *RUNX2* (which encodes the transcription factor of the same name), *SP7* (which encodes Osterix transcription factor), *BGLAP* (which encodes osteocalcin), and *SPP1* (which encodes osteopontin). To do this, the medium was removed from the 6-well tablet, and each well was washed with 1 ml of single-use PBS.

Subsequently, 1 ml of Trizol was added to each well for 5 minutes and the MSC lysate was collected using a scraper. In total, there were three wells designated for osteogenically differentiated MSCs, while two wells were allocated for undifferentiated MSCs.

To isolate the RNA, the samples were placed in a cooled Trizol (*Extract RNA Reagent*, Eurogene, Russia) for 5 minutes. RNA was then extracted using guanidine-thiocyanate-chloroform extraction with Trizol (*Extract RNA Reagent*, Eurogene, Russia) according to the manufacturer's instructions. The amount and purity of the isolated RNA were determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA), while its quality was determined on a Qubit 4 fluorimeter (Invitrogen, USA) by evaluating the RIQ index (RNA Integrity and Quality) using a set of reagents Qubit RNA IQ Assay Kit (Invitrogen, USA). Reverse transcription and synthesis of complementary DNA (cDNA) from the isolated RNA were conducted using the OT-M-MuLV-RH kit (Biolabmix, Russia). The amount of synthesized cDNA was determined using a NanoDrop 2000 spectrophotometer (Thermo Scientific, USA). All samples were diluted in RNase and DNase-free water (*Sterile water treated with diethylpyrocarbonate (DEPC, DEPC), without RNase and DNase*, Biolabmix, Russia) to a volume of 1.5 ml, achieving a cDNA concentration of 20 ng / l.

The results of the analysis are presented as a relative expression values. To calculate these values, the ΔCt method (a variant of the Livak method) was used. This method is based on determining the difference between the Ct values of the reference genes and the target Ct values for each sample. Normalization of PCR results was performed relative to the geometric mean of the Ct values of three reference genes: *ACTB* (β-actin), *GAPDH* (glyceraldehyde-3-phosphatedehydrogenase), and *B2M* (beta-2-microglobulin).

Table 1

Primers used to evaluate the expression of osteogenic differentiation genes

Gene name	Primer	
	Forward	Reverse
Genes used for normalization in calculating expression levels		
GAPDH	AGCCACATCGCTCAGACAC	GCCCAATACGACCAAATCC
B2M	TCCATCCGACATTGAAGTTG	CGGCAGGCATACTCATCTT
ACTB	CATCGAGCACGGCATCGTCA	TAGCACAGCCTGGACAGCAAC
Genes of interest		
RUNX2	AGATGGACCTCGGGAACCCA	TGAGGCGGGACACCTACTCT
SP7	TGCTTGAGGAGGAAGTTCAC	AGGTCACTGCCCACAGAGTA
BGLAP	TCACACTCCTCGCCCTATTG	TAGCGCCTGGGTCTCTTCAC
SPP1	CATCACCTGTGCCATACCAGTT	TTGGAAGGGTCTGTGGGGCTA

Note: ACTB – β-actin, GAPDH-glyceraldehyde-3-phosphatedehydrogenase, B2M-beta-2-microglobulin, *RUNX2* (encodes the transcription factor of the same name), *SP7* (encodes Osterix transcription factor), BGLAP (encodes osteocalcin), SPP1 (encodes osteopontin).

IMMUNOFLUORESCENCE STAINING OF AT MSCS AND OSTEOBLASTS

For immunofluorescence staining, rabbit and mouse antibodies were selected as primary antibodies, including RUNX2, osteopontin, osteocalcin, Osterix, (Abcam, UK). Cells were stained with a blue fluorescent dye for nucleic acids (DAPI) (dilution 1:100), the detection of the results of the study was performed using a confocal microscope. Fluorescence intensity was analyzed using the ImageJ software. Ten visual fields were analyzed from each sample, and the results are presented as conventional units of fluorescence intensity.

Statistical analysis. Statistical data processing and graphical representation of the results were conducted using the standard package of statistical methods of IBM SPSS Statistics 27. The data are presented as median values along with the 25th and 75th percentiles: *Me* [25%; 75%]. The nonparametric Mann – Whitney U-test was used to evaluate differences in quantitative characteristics when comparing two independent groups with distributions that deviate from normality. A critical significance level of p is <0.05.

RESULTS

Evaluation of the immunophenotype of the EAT cell culture

The analysis revealed that CD105 and CD90 were expressed in 79.71% of the cells in the EAT of a patient with CHD. Notably, the surface marker CD105 was present in 17.54% of the cells (Fig. 1). Antigenic markers CD73 and CD90 were found in 79.47% of the cells, with CD73 being expressed on the surface of 18.26% of the cells. In contrast, CD34 was present only in 3.76% of the cells. Thus, the phenotype of the main cell culture derived from the EAT was CD34-/+, CD73+, CD 90+, CD 105+, which corresponds to one of the criteria of the AT MSC [11]. In addition to the main cell population, two minor populations were identified within the EAT culture: 1. CD90+,CD34+,CD73+,CD105 – presumably representing an endothelial population, 2. CD90+ CD105-CD34-CD73 – the smallest cell population.

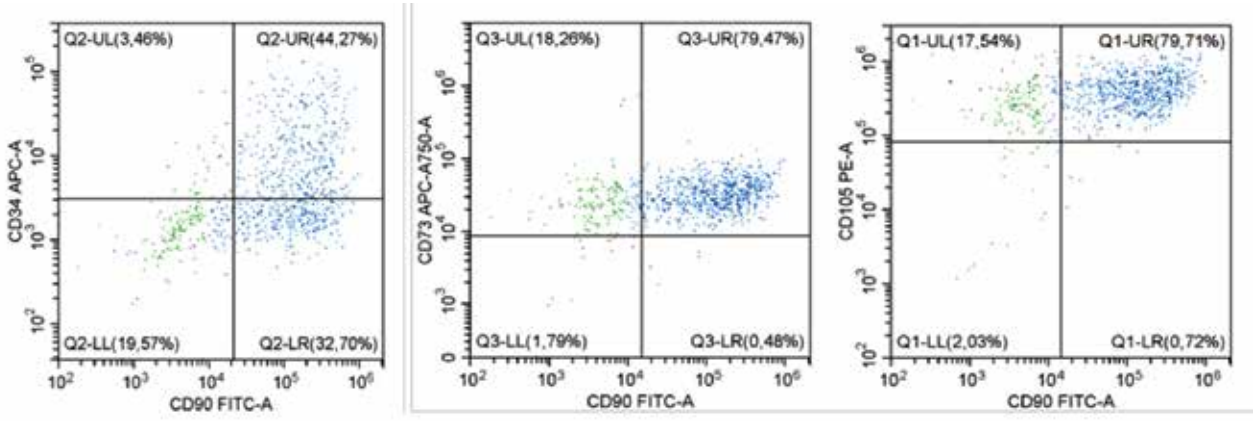

Fig. 1. Flow cytometry of cells derived from epicardial adipose tissue of a patient with coronary heart disease.
Note: Blue color in the figure indicates the largest cell population according to the immunophenotype belonging to the MSC, green and pink are two minor cell populations.

Expression of osteoblastic differentiation genes

The activation of transcription of genes involved in osteogenic induction was assessed on day 15 of culture, since during this period MSCs acquire specific properties of preosteoblasts and actively synthesize bone matrix proteins. Real-time PCR analysis revealed that the expression of the key osteogenic factor, the *RUNX2* gene, in cells cultured in a medium with osteoinducers was 1.88 times higher than in undifferentiated MSCs (Fig. 2).

Similar results were obtained for the expression level of *SPP1* mRNA (OPN, osteopontin), which, like *RUNX2*, is expressed at the early stages of mesenchymal cells differentiation into osteoblasts. Thus, the expression of the *SPP1* gene was found to be 1.35 times higher in MSCs cultured in the presence of an osteogenic medium compared to the control sample.

The expression of *BGLAP*, which encodes osteocalcin (OSN) and is responsible for the formation of mature osteoblasts, did not differ between differentiated and undifferentiated MSC cultures. The expression of the *SP7* (Osterix) gene, responsible for the differentiation of cells into mature osteoblasts and finally into osteocytes during bone formation, did not differ between cells cultured in osteoblastic medium and those without it.

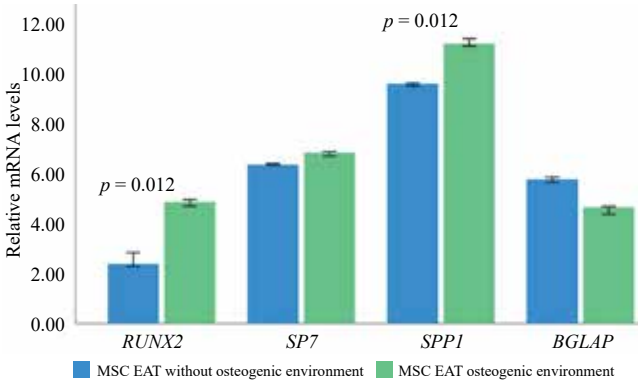

Fig. 2. Expression levels of osteogenic genes in differentiated and undifferentiated mesenchymal stem cells of epicardial adipose tissue of patients with coronary heart disease on day 15 of culture. Note: MSC – Mesenchymal stem cells, EAT – epicardial adipose tissue, *RUNX2* – encodes the transcription factor of the same name, *SP7* – encodes the transcription factor Osterix, *BGLAP* – encodes osteocalcin, *SPP1* – encodes osteopontin

Immunofluorescence staining

Based on the results of staining cells with specific antibodies, an analysis was conducted to evaluate the effect of directed osteogenic differentiation on the culture of MSCs derived from EAT. It was shown that a significant ($p < 0.05$) increase in half of the studied markers was observed in the culture of MSCs of EAT obtained on day 15 after incubation with an osteoblastic medium compared to control MSCs (Fig. 3, 4). Thus, the key regulator and marker of osteogenic differentiation – RUNX2 – was 1.6 times higher in osteogenic-induced MSCs than in intact (control) cell cultures. Another marker of early osteogenic differentiation – OPN – was also increased by 1.6 times in differentiated MSCs. It is worth noting that by the method of immunofluorescence staining, there were no differences detected in the expression of Osterix and OCN between cultures of differentiated and undifferentiated cells (Fig. 3, 4).

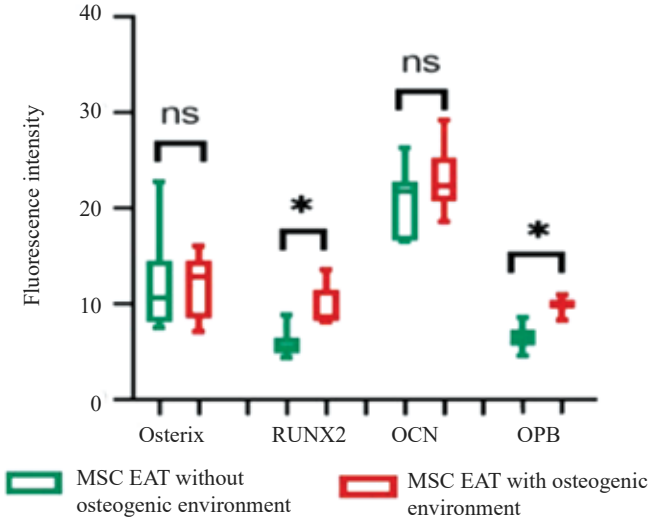

Fig. 3. Quantitative analysis of osteogenic differentiation markers of MSCs of EAT in the 3rd passage (day 15) by immunofluorescence staining: MSC – Mesenchymal stem cells, EAT – epicardial adipose tissue, OCN – osteocalcin, OPN – osteopontin

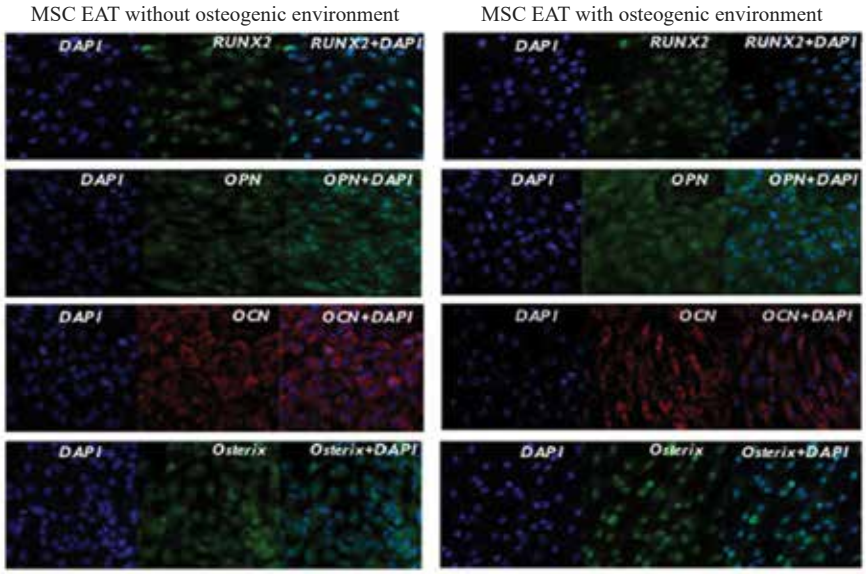

Fig. 4. Immunofluorescence staining of the studied osteoblastic differentiation markers. Note: DAPI – nuclear dye (4',6-diamidino-2-phenylindole), OCN – osteocalcin, OPN – osteopontin.

DISCUSSION

The study of the osteo-cardiovascular continuum represents a relatively new and promising area of contemporary scientific research. Recent studies have shown that during vascular calcification, various signaling pathways associated with bone formation and repair are activated in cells. For example, G. Fadini et al. have shown that cells derived from bone marrow can migrate from blood circulation to blood vessels, transform into osteogenic cells, and then contribute to the development of vascular calcification [12]. With the potential for osteogenic differentiation and recruitment of damaged vessels,

MSCs play a crucial role in the "circulating calcifying cell theory," suggesting that they may serve as a source of osteoblast-like cells. However, the role of MSC in the process of vascular calcification remains unclear and controversial. The question of whether MSCs contribute to or inhibit the development of vascular calcification is still to be determined.

In this study, we focused on the activation of transcription genes involved in osteogenic induction on day 15 of culturing MSCs derived from EAT, since it is known that multipotent cells acquire specific properties of preosteoblasts and begin to actively synthesize bone matrix proteins during this period [13, 14].

It is known that the maturation and function of osteoblasts are directly related to the expression of two main transcription factors of osteogenesis: *RUNX2* and *SP7* transcription factor (Osterix). The *RUNX2* transcription factor is an important regulator of bone formation and the osteogenic differentiation of MSCs; it initiates differentiation of MSCs into preosteoblasts and suppresses adipogenic and chondrogenic differentiation [15]. During osteoblast differentiation, *RUNX2* expression increases in preosteoblasts, reaches a maximum level in progenitor cells, and decreases in mature osteoblasts [16]. *RUNX2* activates the expression of calcification-related proteins such as osteopontin, bone sialoprotein II, and osteocalcin, thus inducing extracellular bone matrix synthesis and mineralization.

According to the literature, *RUNX2* is slightly expressed in undifferentiated MSCs and increases during the proliferation of preosteoblasts, which corresponds to day 7 of culture in an osteogenic medium. Its expression level is maintained at a relatively low level throughout the entire period of osteocyte differentiation. RT-PCR analysis has shown that the expression of the *RUNX2* gene increases after the day 7 of cultivation and reaches peak values on day 21 [17]. In this study, it was shown that the level of *RUNX2* mRNA in cells cultured in an osteogenic medium was 66% higher than in undifferentiated MSCs. According to the results of the immunofluorescence study, it was also shown that RUNX2 protein levels were higher in differentiated cell culture.

The second most important factor (after RUNX2) inducing osteoblast differentiation and the synthesis of bone-specific proteins is Osterix [18]. Both of these factors regulate the activation cascade of genes encoding bone-specific proteins that form bone tissue. [19]. The expression of *SP7* is necessary for the differentiation of preosteoblasts into mature and functional osteoblasts. However, one of the most important functions of this protein is its ability to inhibit the differentiation of chondrocytes in RUNX2-expressing osteoblast precursors [20]. In this study, the expression level of the *SP7* gene did not differ between differentiated and undifferentiated MSCs. Immunofluorescence staining of this osteogenesis marker also revealed no significant difference in Osterix levels between cultures of differentiated and undifferentiated cells. At the same time, the expression levels of *SP7* mRNA and Osterix protein were relatively high in MSCs incubated both with and without osteoblastic medium. It is possible that the absence of a significant difference between differentiated and undifferentiated MSCs may be attributed to the fact that, according to the literature, an increase in *SP7* gene expression is noted at a later stage, approximately on day 16-21 of osteoblast formation (the phase of extracellular matrix synthesis) [21].Osteopontin (OPN) is one of the main non-collagenous bone proteins and plays an important role in bone remodeling. OPN not only mediates the early differentiation of osteoblasts but also activates the function of osteoclasts during resorption. A high level of expression of the *SPP1* gene encoding OPN synthesis indicates an active process of bone extracellular matrix formation, since OPN is the main non-collagenous bone protein. The maximum activity of OPN corresponds to the stage of mineralization in the process of osteogenesis [22]. The results of this study are consistent with existing data indicating that the peak of *SPP1* expression during osteoblast differentiation is achieved twice: during proliferation and mineralization, which corresponds to days 3 and 14 of differentiation. Using confocal microscopy on day 15 of osteoblastic differentiation, it was also found that OPN levels were higher in differentiated cells. In addition, the *SSP1* gene expression and the OPN level obtained by immunofluorescence test were higher than other studied osteogenic markers. These data may indicate that the process of osteogenesis in cell cultures is in the initial stage of extracellular matrix synthesis.

The next step was to evaluate the expression of the *BGLAP* gene encoding OCN. Its level was lower than that of other studied markers, which is consistent with the literature. OCN performs a mechanical

function in the bone matrix due to its ability to firmly bind hydroxyapatite and form a complex with collagen through the osteopontin matrix protein [23]. OCN is used as a late marker of bone formation, as it is expressed at the later stages of extracellular matrix mineralization by mature osteoblasts, which corresponds to day 16-21 of differentiation. This may explain why, in this study, we did not observe an increase in the levels of the *BGLAP* gene and OCN protein in cultures of differentiated MSCs.

CONCLUSION

It was found that MSCs derived from EAT have osteogenic potential, which is manifested by the expression of osteogenic differentiation genes in both differentiated and undifferentiated MSC cultures. These data can be the basis for further study of EAT-derived MSCs from the perspective of their role in the formation of vascular calcification in patients with coronary heart disease. The high level of *SPP1* expression, along with relatively low levels of *RUNX2* and *BGLAP* in differentiated cultures, may indicate that on day 15 of incubation, EAT-derived MSCs are preosteoblasts and are at the initial stage of extracellular matrix synthesis.

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Authors' contribution

Uchasova E.G., Gruzdeva O.V., Dyleva Yu.A. – development of the concept and design of the study. Uchasova E.G., Slesareva T.A., Ponasenko A.V., Velikanova E.A., Matveeva V.G., Dvadtsatov I.V. – data analysis and interpretation. Uchasova E.G, Dyleva Yu.A., Belik E.V., Gruzdeva O.V. – substantiation of the manuscript or critical revision of the manuscript for important intellectual content. Uchasova E.G., Slesareva T.A., Tarasova O.L., Gruzdeva O.V. – final approval of the manuscript for publication.

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