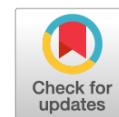


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Immunophenotyping of a population of cultured human umbilical cord cells from Wharton's jelly

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ABSTRACT

BACKGROUND: Against the background of many existing methods of defect replacement in post-traumatic injuries the methods of repair of damaged tissues based on methods using products of tissue engineering and cultures of artificially cultured human cells are becoming more and more widespread in medical practice. The literature reports weak immunogenic activity of human umbilical cord tissues, which makes these cells promising components of regenerative medicine products. Due to possible errors in explant selection and cell transformation in the process of cultivation, it is necessary to reliably determine the phenotype of cells obtained as a result of tissue explantation and their further cultivation. Thus, the specificity of obtaining multipotent mesenchymal cells from human umbilical cord Wharton's stool tissues requires reliable identification of the type of the obtained cells.

AIM: To obtain reliable features of mesenchymal stem cells in the cell population obtained from human umbilical cord stool tissue.

MATERIAL AND METHODS: Methods of cell cultivation, flow cytometry, immunocytochemistry for determination of surface and intracellular markers of mesenchymal stem cells were used in the study.

RESULTS: In the course of work on the identification of cells of the population obtained by culturing explants from human umbilical cord Wharton's stool, the heterogeneity of the type of cells constituting the cell population was established. Most of them are mesenchymal stem cells carrying fluorescent markers CD45, CD73, CD34, CD29, CD90, CD44, CD105, which agrees with the immunophenotype of mesenchymal stem cells defined by the International Society for Cell Therapy. The ratio of the applied markers allows us to refer the population of cells obtained by direct explantation of Wharton's gelatin tissue fragments to mesenchymal stem cells. Visual control confirmed the localisation of labelled antibodies on the surface of cultured cells. And also it was shown that there were no vascular muscle cells in the obtained culture.

CONCLUSION: As a result of experiments on identification of the cells obtained during explantation of Wharton's jelly tissue fragments and their further cultivation, their belonging to mesenchymal stem cells was established by immunofluorescence cytophotometry.

Keywords: culturing; cytophotometry; explant; human bud; immunofluorescence; mesenchymal stem cells; mucous connective tissue of the umbilical cord; Wharton's jelly.

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Имунофенотипирование популяции культивируемых клеток слизистой соединительной ткани (вартонова студня) пуповины человека

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АННОТАЦИЯ

Актуальность. На фоне множества способов замещения дефектов при посттравматических повреждениях все более широкое распространение в медицинской практике получают приемы репарации поврежденных тканей, основанные на методах, использующих продукты тканевой инженерии и культур искусственно культивируемых клеток человека. В литературе сообщается о слабой иммуногенной активности тканей пуповины человека, что делает эти клетки перспективными компонентами продуктов регенеративной медицины. В связи с возможной спонтанной трансформацией клеток в процессе культивирования и ошибками при отборе экспланта необходимо достоверное определение фенотипа клеток, полученных в результате эксплантации ткани и дальнейшего их культивирования. Таким образом, специфика получения мультипотентных мезенхимальных клеток из слизистой соединительной ткани пуповины человека, называемой также вартонов студень, требует достоверной идентификации типа полученных клеток.

Цель. Выявление наличия мезенхимальных стволовых клеток в клеточной популяции, полученной из слизистой соединительной ткани пуповины человека с использованием иммуноферментного маркирования.

Материал и методы. В исследовании использовали методы культивирования клеток, проточной цитофлуориметрии, иммуноцитохимии для определения поверхностных и внутриклеточных маркеров мезенхимальных стволовых клеток.

Результаты. При проведении работы по идентификации клеток популяции, полученной при культивировании эксплантов из слизистой соединительной ткани пуповины человека, установлена неоднородность типа клеток, составляющих популяцию. Большинство из них несут флуоресцентные метки CD45, CD73, CD34, CD29, CD90, CD44, CD105, что согласуется с иммунофенотипом мезенхимальных стволовых клеток, определенным Международным обществом по клеточной терапии. Соотношение примененных маркеров позволяет отнести популяцию клеток, полученную методом прямой эксплантации фрагментов слизистой соединительной ткани, к мезенхимальным стволовым клеткам. Визуальный контроль подтвердил локализацию меченных антител на поверхности культивируемых клеток. Также показано, что в полученной культуре отсутствуют мышечные клетки кровеносных сосудов.

Заключение. В результате экспериментов по идентификации клеток, полученных при эксплантации фрагментов слизистой соединительной ткани, и дальнейшего их культивирования, методом иммунофлуоресцентной цитометрии установлена их принадлежность к мезенхимальным стволовым клеткам.

Ключевые слова: вартонов студень; иммунофлуоресценция; культивирование; мезенхимальные стволовые клетки; пуповина человека; слизистая соединительная ткань пуповины; цитометрия; эксплант.

Как цитировать

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培养的人脐带粘膜结缔组织（Vartonov）细胞群的免疫表型

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摘要

论证。在创伤后缺损替代方法层出不穷的背景下，利用组织工程产品和人工培养的人体细胞修复受损组织的方法在医疗实践中越来越广泛。文献报道，人类脐带组织的免疫原性很弱，这使得这些细胞成为再生医学产品中很有前途的成分。由于细胞在培养过程中可能发生自发转化，以及外植体选择过程中的误差，因此有必要对组织外植体及其进一步培养过程中获得的细胞表型进行可靠的鉴定。因此，从人体脐带粘膜结缔组织（也称为Vartons果冻）中获得多能间充质细胞的特异性需要对获得的细胞类型进行可靠的鉴定。

目的 利用免疫酶标记法检测从人类脐带粘膜结缔组织中获取的细胞群中是否存在间充质干细胞。

材料和方法。该研究采用细胞培养、流式细胞荧光测定法和免疫细胞化学法来确定间充质干细胞的表面和细胞内标志物。

结果。在鉴定从人体脐带粘膜结缔组织培养中获得的群体细胞的工作中，确定了构成群体的细胞类型的异质性。它们中的大多数都带有荧光标记 CD45、CD73、CD34、CD29、CD90、CD44、CD105，这与国际细胞治疗学会（International Society for Cell Therapy）定义的间充质干细胞的免疫表型一致。根据应用标记物的比例，我们可以将粘膜结缔组织片段直接外植获得的细胞群归属于间充质干细胞。肉眼观察证实了标记抗体在培养细胞表面的定位。结果还表明，培养物中没有血管肌肉细胞。

结论。通过对粘膜结缔组织碎片外植并进一步培养获得的细胞进行鉴定实验，通过免疫荧光细胞光度法确定其属于间充质干细胞。

关键词：Vartonov果冻；免疫荧光；培养；间充质干细胞；人脐带；脐带粘膜结缔组织；细胞光度法；外植体。

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Currently, various methods for isolation and cultivation of mesenchymal stem cells (MSCs) and their use in clinics have been reported in the literature [1]. MSC transplantation stimulates regeneration of bone tissue, skin, myocardium, peripheral nervous system, skeletal muscle, and liver tissue, and MSCs serve as a source of growth factors and cytokines [2]. The decisive aspect that plays an important role in the selection of artificial biomedical materials is the influence of the implant on the regeneration processes of the recipient's damaged tissues, its immunomodulatory properties [3]. Among the techniques for replacing defects in posttraumatic injuries, those involving the use of tissue engineering products and artificially cultured human cells are becoming increasingly prevalent in medical practice. In such cases, the use of the patient's own tissues or tissues with low immunogenicity is advantageous. Several publications have reported the weak immunogenic activity of human umbilical cord tissues [5]. In this regard, human umbilical cord Wharton's jelly stem cells (HWJSCs) are a promising source of MSCs. There are various studies on the application of HWJSCs and other umbilical cord tissues. However, there is a need for a systematic approach to organize and select the optimal variants of laboratory and technological solutions for reproducible MSC cultivation [6, 7]. Many researchers have proposed the use of human umbilical cord mucosa as an alternative source of MSCs. Given that HWJSC is the tissue of the provisional organ — the umbilical cord — procedures involving it is subject to minimal ethical restrictions. Furthermore, obtaining explants from HWJSC is performed using a non-invasive method. The process of acquiring such explants includes dissecting the surrounding tissue, including the vein and artery of the umbilical cord. This may cause explanting of tissue fragments of other types along with HWJSCs, resulting in the acquisition of cells with a different status than that of MSCs. Owing to possible errors in explant selection and cell transformation during the cultivation process, the phenotype of cells obtained as a result of tissue explantation and during their further cultivation should be determined. The International Society for Cell Therapy has defined the immunophenotype of MSCs as CD90+, CD105+, CD44+, CD29+, CD105+, CD73+, CD166+, CD45–, and CD34– [9]. The most effective method in determining characteristic cell surface proteins is the fluorescence method [10]. In the present study, the surface markers CD45, CD73, CD34, CD29, CD90, CD44, and CD105 were identified and the presence of vascular muscle tissue cells was assessed using the specific cytoskeletal marker alpha-actin in the population of cells derived from HWJSCs.

This study aimed to identify MSCs in the cell population obtained from HWJSCs using immunoenzymatic labeling.

Objectives of the study:

1) To demonstrate the absence of vascular smooth muscle cells in the obtained culture and perform immunohistochemical staining for the presence of smooth muscle actin (alpha-smooth muscle actin, α -SMA) in the cytoskeleton of the cells of the studied population

2) To examine the obtained cell population for the presence of MSCs using the surface markers CD45, CD73, CD34, CD29, CD90, CD44, and CD105 by flow cytometry and observe fluorescence on the cell surface during microscopy

MATERIALS AND METHODS

Obtaining a population of HWJSCs

The primary human umbilical cord material was obtained by the staff of the Department of Obstetrics and Gynecology of the Kirov Military Medical Academy and acquired in the course of planned cesarean section. The medical history of the primary material donors was recorded and stored by the staff of the department. Informed consent was obtained from the donor. The selection of primary material for further processing and harvesting of tissue fragments for explantation were performed in the delivery room under aseptic conditions. Cord material without exsanguination was placed in the transport medium immediately after separation from the placenta. Standard sterile 0.9 % sodium chloride solution in a sterile container was used as the transport medium [11]. The material was transported at ambient temperature. The umbilical cord material was transferred to the sterile room of the Cell Technology Research Laboratory of the Research Department of Biomedical Research of the Research Center, where HWJSCs were extracted and primary explants were obtained under sterile conditions.

DMEM F/12 medium, which was utilized in our previous studies to obtain cell culture from human umbilical cord tissue [11], was used to culture explants.

Immunophenotyping of the obtained cell population and immunohistochemical detection of SMA

Immunohistochemical staining for the presence of SMA in the cytoskeleton was performed to detect vascular smooth muscle cells in the obtained culture. Monoclonal Mouse Anti-Human Smooth Muscle Actin primary antibodies (Dako, Denmark), secondary antibodies conjugated to the fluorochrome Alexa Fluor 488 (Abcam, England), were used, and nuclei were stained with Slow-Fade Glass Soft-set Antifade Mountant with DAPI-Nuclear (Invitrogen, USA). The study was performed using an LSM 880 (Carl Zeiss, Germany) confocal microscope. Fluorescence was excited by a 405 nm laser with detection at 410–495 nm and an argon laser at 488 nm with detection at 495–605 nm.

The expression of stem cell surface markers in the umbilical cord MSC was investigated. Immunophenotyping was performed using a CytoFlex flow cytometer (Beckman Coulter, USA). Antibodies labeled with phycoerythrin (PE) or fluorescein (FITC) were used as labels: CD45-FITC, CD73-PE, CD34-PE, CD29-FITC, CD90-FITC, CD44-FITC, and CD105-FITC (Bio Rad, USA). Thus, cells were stripped from the surface of the culture plate by the conventional method (trypsin-serum solution at 1:3 ratio), washed three times with phosphate buffer solution (pH 7.2–7.4), and mixed with a diluted ($\times 20$) antibody dilution solution consisting of 20 μL fetal bovine serum (Gibco), 10 mg NaN_3 , and 50 mL phosphate buffer. The cell suspension was injected into measuring tubes of the cytofluorimeter; then, the tubes were placed in the cytofluorimeter autosampler, and further fluorescence measurements and processing of the obtained signals were performed automatically. An Axio Imager M2 fluorescence microscope (Carl Zeiss, Germany) was used to observe the cell surface for fluorescence.

RESULTS AND DISCUSSION

Immunocytochemical examination for the presence of the specific cytoskeletal marker alpha-actin in the cytoplasm did not show autofluorescence. No specific staining of the cytoskeleton characteristic of smooth muscle cells was obtained in the cell populations studied from HWJSCs (Fig. 1*B*). Rat myometrium cells was stained with SMA as a positive control (Fig. 1*Г*).

The expression of mesenchymal cell surface markers was examined by immunophenotyping with a CytoFlex

flow cytometer (Beckman Coulter, USA). To check the conjugation efficiency of the antibodies, phycoerythrin-labeled CD73-PE and CD34-PE and fluorescein-labeled CD45-FITC, CD29-FITC, CD90-FITC, and CD105-FITC were used. The cells removed from the surface of the culture plastic and conjugated with antibodies were placed on slides and observed in an epifluorescence microscope using FITC light filters (green region of the spectrum for antibodies), and TRITC (red spectral region for phycoerythrin-labeled antibodies) at $\times 400$ magnification and photofixation was performed in additive mode. The results are presented in Figure 2.

Finding cells in solutions for cell removal from the culture surface, conjugating cells with labeled antibodies, and obtaining preparations for microscopy did not result in destruction of most HWJSCs studied. Figure 2*A* shows the original unlabeled cell in suspension and cells positively conjugated with fluorescently labeled antibodies (Fig. 2*B* and *Г*) and negatively conjugated (non-conjugated) with labeled antibodies (Fig. 2*Б*). The localization of the fluorescently labeled antibodies was observed on the surface of the tested cells. To obtain images of cells negatively conjugated with labeled antibodies, they were contrasted with DAPI after antibody conjugation.

Then, the suspension of labeled cells was placed into the cytofluorimeter dispenser tubes, and the distributions of surface markers in the evaluated cell population were recorded. Figure 3 presents the results of surface marker distribution of the obtained MSC culture.

A positive reaction was detected in the markers of 5-nucleotidase CD73 (55.61 %), protein integrins CD29 (41.11 %), stem cells and axonal processes in mature

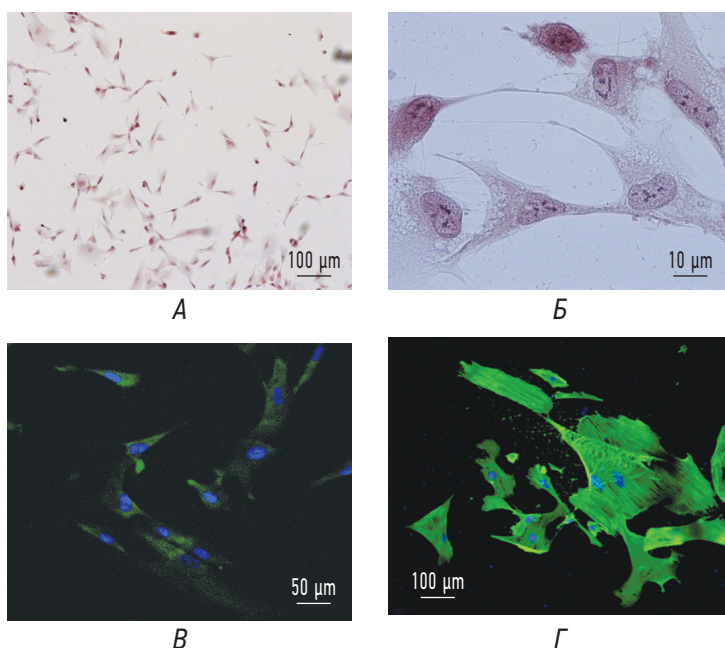


Fig. 1. MSC culture: *A, Б* — staining with haematoxylin and eosin; *Б* — immunocytochemical staining for the presence of smooth muscle α -actin in the cytoskeleton of cells obtained from umbilical cord, *Г* — stained rat myometrial cells

Рис. 1. Культура МСК: *A, Б* — окрашивание гематоксилином и эозином; *Б* — иммуноцитохимическое окрашивание на наличие гладкомышечного альфа-актина в цитоскелете клеток, полученных из пуповины; *Г* — окрашенные клетки миометрия крысы

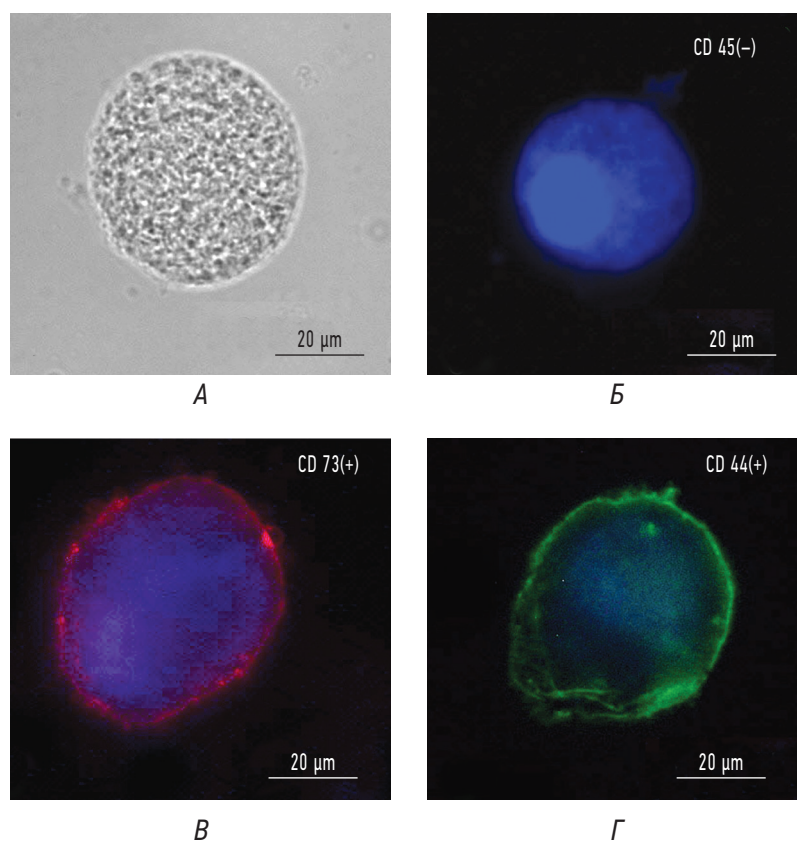


Fig. 2. Results of immunofluorescence labelling of surface antigens (SD) of mesenchymal cells of human umbilical cord Warton's stool tissue. *A* — mesenchymal cell before labelling; *Б, В, Г* — images of cells labelled with different antibodies: *Б* — absence of conjugation with antibody (DAPI contrasting); *В, Г* — presence of conjugation with labelled antibody

Рис. 2. Результаты иммунофлуоресцентного мечения поверхностных антигенов (CD) мезенхимных клеток слизистой соединительной ткани пуповины человека. *А* — мезенхимальная клетка до мечения; *Б, В, Г* — имиджи клеток, меченых различными антителами: *Б* — отсутствие конъюгации с антителом (контрастирование DAPI); *В, Г* — наличие конъюгации с меченым антителом

neurons CD90 (6.45 %), hyaluronic acid receptor CD44 (55.62 %), and endoglin CD105 (0.52 %). A negative reaction was noted in the marker of total leukocyte antigen CD45 (24.59 %).

Studies have reported that flow cytometry, immunofluorescence, and qRT-PCR analysis show predominant expression of the surface markers CD29, CD44, CD73, CD90, CD105, and CD166 in umbilical cord MSCs and that markers of endothelial cells and hematopoietic cells are absent [9, 10]. CD29, CD90, CD105, and CD166 persist in MSCs even after adipogenic, osteogenic, and chondrogenic induction [9]. Additionally, decreased CD44 and CD73 expressions in response to induction of trilineage differentiation has been demonstrated [10]. This reveals that they are the most reliable markers of stem cells, which are observed only in undifferentiated cord MSCs [8, 9]. Positive surface markers CD73 and CD44 indicate that stem cells predominated in the culture obtained by explantation. Moreover, the expression of the marker of hematopoietic cell adhesion proteins CD34 (27.19 %) confirms the presence of hematopoietic cells in the obtained population.

FINDINGS

1. The ratio of the applied markers allows for attributing the population of cells obtained by direct explantation of HWJSC fragments to the population of MSCs.
2. Visual inspection and flow cytometry confirmed the localization of labeled antibodies on the surface of cultured cells.
3. Immunohistochemical staining for SMA did not reveal vascular smooth muscle cells in the resulting cell culture.

CONCLUSIONS

As a result, of experiments on identification of cells obtained by explantation of fragments of HWJSCs and their further cultivation, their affiliation to MSCs was established by immunofluorescence cytophotometry. No vascular muscle cells were observed in the obtained culture. The obtained population contained hematopoietic cells (cells of the hematopoietic system); however, undifferentiated stem cells predominated. Establishing a human cell culture with a preponderance of identifiable stem cells, using donor organ tissue, enables MSC

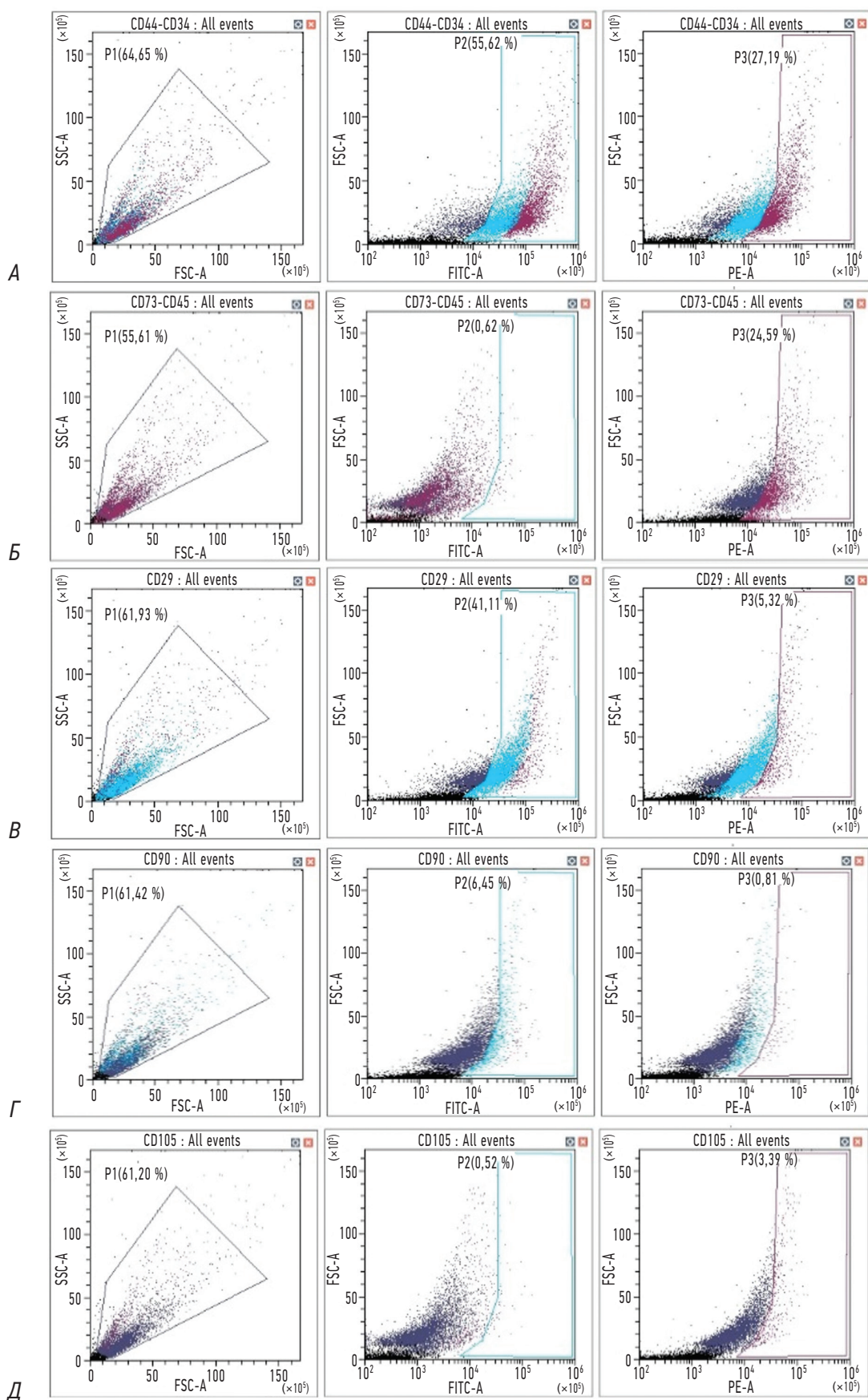


Fig. 3. Distribution of surface markers in the obtained population of human umbilical cord MSCs: A — CD34, CD44; B — CD45, CD73; B — CD29, Г — CD90; Д — CD105

Рис. 3. Распределение поверхностных маркеров в полученной популяции МСК пуповины человека: А — CD34, CD44; Б — CD45, CD73; В — CD29, Г — CD90; Д — CD105

extraction and allows for the use of the extracted cells in a wide range of studies of human physiology and biochemistry with minimal ethical restrictions.

ADDITIONAL INFORMATION

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Conflict of interest: The authors declare that there is no conflict of interest.

Ethical review: The conduct of the study was approved by the local ethical committee of the S.M. Kirov Military Medical

Academy of the Ministry of Defense of the Russian Federation (minutes no. 230; dated December 17, 2019).

Authors' contribution: V.E. Chernov, obtaining, maintenance of cell culture, writing the article; M.O. Sokolova, obtaining the results of experiments on the distribution of surface CD markers of cell population, writing the article; A.A. Kokorina, immunocytochemical evaluation of the presence of specific cytoskeleton marker alpha-actin in cell population by confocal microscopy; G.I. Pendinen, visual evaluation and photofixation of immunofluorescence labeling of surface antigens (SD) of mesenchymal cells of Wharton's jelly tissue by fluorescence microscopy, editing the text of the article.

REFERENCES

1. Pal'tsev MA. Stem cells and cell technologies: the present and the future. *Remedium*. 2006;(8):6–13. (In Russ.) EDN: HOCEWV
2. Tolar J, Le Blanc K, Keating A, Blazar BR. Concise review: hitting the right spot with mesenchymal stromal cells (MSCs). *Stem Cells*. 2010;28(8):1446–1455. doi: 10.1002/stem.459
3. Meleshina AV, Bystrova AS, Rogovaya OS, et al. Skin tissue-engineered constructs and stem cells application for the skin equivalents creation (review). *Modern technologies in medicine*. 2017;9(1): 198–218. EDN: YIZWKF doi: 10.17691/stm2017.9.1.24
4. Payushina OV, Domarackaya EI, Sheveleva ON. Participation of mesenchymal stromal cells in muscle tissue regeneration. *Zhurnal obshchey biologii*. 2019;80(1):3–13. (In Russ.) EDN: YWYFRZ doi: 10.1134/S0044459619010044
5. Kalyuzhnaya LI, Kharkevich ON, Schmidt AA, Protasov OV. Regenerative properties of human extraembryonal organs in tissue engineering. *Bulletin of the Russian Military Medical Academy*. 2018;(4(64)):192–198. (In Russ.) EDN: VMOCMF
6. Shamanskaya TV, Osipova EYu, Rummyantsev SA. Mesenchymal stem cells ex vivo cultivation technologies for clinical use. *Onkogematologia*. 2009;4(3):69–76. (In Russ.) EDN: MNICAJ doi: 10.17650/1818–8346–2009–0–3–69–76
7. Aleksandrov VN, Kamilova TA, Martynov BV, Kalyuzhnaya LI. Cell therapy in ischemic stroke. *Bulletin of the Russian Military Medical Academy* 2013;(3(43)):199–205. (In Russ.) EDN: RCLBMV
8. Aisenstadt AA, Erukashvili NI, Zolina TL, et al. Comparison of proliferation and immunophenotype of MSC, obtained from bone marrow, adipose tissue and umbilical cord. *Herald of north-western state medical university named after I.I. Mechnikov*. 2015;7(2):14–22. EDN: UZAGDP
9. Ali H, Al-Yatama MK, Abu-Farna M, et al. Multi-lineage differentiation of human umbilical cord Wharton's Jelly Mesenchymal Stromal Cells mediates changes in the expression profile of stemness markers. *PLoS One*. 2015;10(4):e0122465. doi: 10.1371/journal.pone.0122465
10. Ramos TL, Sánchez-Abarca LI, Muntión S, et al. MSC surface markers (CD44, CD73, and CD90) can identify human MSC-derived extracellular vesicles by conventional flow cytometry. *Cell Commun Signal*. 2016;14:2. doi: 10.1186/s12964-015-0124-8
11. Chernov VE, Sokolova MO, Ivanova AK, et al. Initiation and cultivation of multipotent mesenchymal human umbilical stroma cells in a laboratory experiment. *Russian Military Medical Academy Reports*. 2022;41(3):283–291. (In Russ.) EDN: AEJEXW doi: 10.17816/rmmar104363

СПИСОК ЛИТЕРАТУРЫ

1. Пальцев М.А. Стволовые клетки и клеточные технологии: настоящее и будущее // Ремедиум. 2006. № 8. С. 6–13. EDN: HOCEWV
2. Tolar J., Le Blanc K., Keating A., Blazar B.R. Concise review: hitting the right spot with mesenchymal stromal cells (MSCs) // Stem Cells. 2010. Vol. 28, N 8. P. 1446–1455. doi: 10.1002/stem.459
3. Малешина А.В., Быстрова А.С., Роговая О.С., и др. Тканеинженерные конструкции кожи и использование стволовых клеток для создания кожных эквивалентов (обзор) // Современные технологии в медицине. 2017. Т. 9, № 1. С. 198–202. EDN: YIZWKF doi: 10.17691/stm2017.9.1.24
4. Паюшина О.В., Домарацкая Е.И., Шевелева О.Н. Участие мезенхимных стромальных клеток в регенерации мышечной ткани // Журнал общей биологии. 2019. Т. 80, № 1. С. 3–13. EDN: YWYFRZ doi: 10.1134/S0044459619010044
5. Калюжная Л.И., Харкевич О.Н., Шмидт А.А., Протасов О.В. Регенераторные свойства внеэмбриональных органов человека в тканевой инженерии // Вестник Российской военно-медицинской академии. 2018. № 4 (64). С. 192–198. EDN: VMOCMF
6. Шаманская Т.В., Осипова Е.Ю., Румянцев С.А. Технологии культивирования мезенхимальных стволовых клеток ex vivo для клинического использования // Онкогематология. 2009. Т. 4, № 3. С. 69–76. EDN: MNICAJ doi: 10.17650/1818–8346–2009–0–3–69–76
7. Александров В.Н., Камилова Т.А., Мартынов Б.В., Калюжная Л.И. Клеточная терапия при ишемическом инсульте // Вестник российской военно-медицинской академии. 2013. № 3 (43). С. 199–205. EDN: RCLBMV
8. Aisenstadt A.A., Erukashvili N.I., Zolina T.L. et al. Comparison of proliferation and immunophenotype of MSC, obtained from bone marrow, adipose tissue and umbilical cord // Herald of North-Western State Medical University named after I.I. Mechnikov. 2015. Vol. 7, N 2. С. 14–22. EDN: UZAGDP

9. Ali H., Al-Yatama M.K., Abu-Farna M., et al. Multi-lineage differentiation of human umbilical cord Wharton's Jelly Mesenchymal Stromal Cells mediates changes in the expression profile of stemness markers // PLoS One. 2015. Vol. 10, N 4. Art. e0122465. doi: 10.1371/journal.pone.0122465

10. Ramos T.L., Sánchez-Abarca L.I., Muntión S., et al. MSC surface markers (CD44, CD73, and CD90) can identify human MSC-derived

extracellular vesicles by conventional flow cytometry // Cell Commun. Signal. 2016. Vol. 14. P. 2. doi: 10.1186/s12964-015-0124-8

11. Чернов В.Е., Соколова М.О., Иванова А.К., и др. Получение и культивирование мультипотентных мезенхимальных клеток стромы пуповины человека в лабораторном эксперименте // Известия Российской военно-медицинской академии. 2022. Т. 41, № 3. С. 283–291. EDN: AEJEXW doi: 10.17816/rmmar104363

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