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ISOLATION AND CHARACTERIZATION OF MESENCHYMAL STEM CELLS FROM HUMAN SKIN

Aralbek A.B.[1], **Ogay V.B.**[1,2*]

[1]*L. N. Gumilyov Eurasian National University, Satpayev Street, Astana 010000, Kazakhstan*
[2]*National Center for Biotechnology, Qorgalzhyn Highway 13/5, Astana 010000, Kazakhstan*
**ogay@biocenter.kz*

ABSTRACT

Skin-derived mesenchymal stem cells (sMSCs) represent a promising and minimally invasive alternative to mesenchymal stem cells obtained from bone marrow and adipose tissue. However, further studies are required to standardize isolation protocols and to comprehensively characterize their biological properties.

Primary cultures of human sMSCs were established from dermal tissue fragments using explant technique. Morphological characteristics, clonogenic potential (CFU-F assay), proliferative activity (MTT assay), immunophenotype (CD73, CD90, CD105, CD45), and multilineage differentiation capacity (osteogenic, adipogenic, and chondrogenic induction) were evaluated.

Our results showed that, cells adhering from skin explants demonstrated a progressive transition from a morphologically heterogeneous population at day 3 to a predominantly fibroblast-like monolayer by day 14. sMSCs exhibited pronounced clonogenicity with the formation of well-defined CFU-F colonies and demonstrated stable proliferative activity at early passages. Immunocytochemistry confirmed expression of characteristic MSC markers CD73, CD90, and CD105 with the absence of hematopoietic marker CD45, corresponding to ISCT criteria. Multilineage differentiation assays verified the functional plasticity of sMSCs, including lipid droplet formation during adipogenesis, calcium-rich mineralized matrix during osteogenesis, and cartilage-like microspheroid formation with metachromatic Toluidine Blue staining in chondrogenic cultures.

Thus, human skin provides a readily accessible and reliable source of mesenchymal stem cells demonstrating stable proliferation, high clonogenic potential, classical MSC immunophenotype, and preserved multipotency. These findings support the translational relevance of sMSCs and create a foundation for further development of standardized protocols and potential biomedical applications in regenerative medicine and tissue engineering.

Key words: Skin-derived mesenchymal stem cells; clonogenicity; immunophenotype; multilineage differentiation; regenerative medicine.

INTRODUCTION

Mesenchymal stem cells (MSCs) are multipotent stromal cells characterized by their ability for self-renewal and differentiation into osteogenic, chondrogenic, and adipogenic lineages, making them essential contributors to tissue repair and regeneration [1,2]. Owing to their broad differentiation potential, paracrine activity, and immunomodulatory properties, MSCs have become a central focus in regenerative medicine, cell therapy, and tissue engineering [3–5]. Beyond differentiation-driven tissue regeneration, MSCs exert significant therapeutic effects by secreting cytokines, growth factors, and extracellular vesicles that modulate inflammation, promote angiogenesis, and support wound healing [6,7].

MSCs can be isolated from various anatomical sources, including bone marrow, adipose tissue, umbilical cord blood, Wharton's jelly, and skin [8–10]. Among these, skin-derived MSCs (sMSCs) represent an especially promising population due to the minimally invasive accessibility of skin biopsies, the skin's high regenerative turnover, and the presence of well-defined stem cell niches within the dermis and epidermis [11]. Dermal MSCs exhibit high proliferative capacity, functional plasticity, and the ability to interact with the local extracellular matrix and immune microenvironment, positioning them as attractive candidates for autologous cell-based therapies [12].

According to the criteria established by the International Society for Cellular Therapy (ISCT), sMSCs display the classical MSC immunophenotype expression of CD73, CD90, and CD105 surface markers and lack of hematopoietic antigens such as CD34 and CD45 [13]. Skin-derived MSCs also demonstrate stable proliferation and viability under optimized in vitro culture conditions, especially when the culture microenvironment recapitulates physiological parameters such as oxygen tension, extracellular matrix composition, and growth factor availability [14,15].

Despite substantial progress in MSC research, several challenges remain unresolved. These include the standardization of isolation strategies, optimization of culture media to preserve multipotency and genomic stability, and evaluation of long-term safety for clinical applications [16]. Critical factors such as seeding density, serum concentration, and growth factor supplementation significantly influence cellular expansion kinetics and functional activity, underscoring the need for systematic comparative studies [17–19]. Ensuring reproducible and phenotypically stable MSC cultures is essential for their successful translational application.

The present study aims to isolate and comprehensively characterize primary mesenchymal stem cells derived from human dermis. Particular emphasis is placed on optimizing culture conditions, including medium composition and initial cell seeding density, as well as performing detailed morphological, phenotypic, and functional assessments. These analyses are complemented by tri-lineage differentiation assays and expression profiling of lineage-specific markers to confirm the multipotency of isolated sMSCs.

MATERIALS AND METHODS

Isolation and culture of skin-derived MSCs

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Human facial skin samples were obtained from three patients (1 male and 2 females; 35-48 years old) who had undergone head and face plastic surgery. All experiments were authorized by the National Center for Biotechnology Local Ethic Committee, and the patients gave their informed consent to tissue donation. Fresh human skin samples were transported to the laboratory of stem cells, and MSCs were isolated in aseptic conditions. Briefly, all hairs and subcutaneous fat tissues were removed, and the samples were then cut into 1-2 mm^2 explants containing the epidermis and dermis. Skin explants were attached to the 6-well culture plate, and 2 mL of α-MEM with ribonucleosides (Thermo Fisher Scientific, USA) supplemented with 15 % fetal bovine serum (FBS; Thermo Fisher Scientific, USA), 10 ng/mL epidermal growth factor (EGF; Abcam, UK), 10 ng/mL basic fibroblast growth factor (bFGF; Abcam, UK), 100 U/mL antibiotic-antimycotic (Thermo Fisher Scientific, USA) were added. The culture plates were incubated in a CO_2-incubator at 37°C and 5% CO_2 for 5 days. After the remaining skin tissue fragments were removed, the adherent cells were cultured in vitro, with the growth medium refreshed twice per week. Once the cultures reached confluence, the cells were detached using TrypLE Express (Thermo Fisher Scientific, USA) and centrifuged at 200 × g for 5 minutes. The resulting cell pellet was subsequently reseeded and maintained in culture until passage 3.

Immunocytochemical analysis

Human skin-derived MSCs were fixed with a freshly prepared 4% paraformaldehyde solution in PBS (pH 7.2) for 15 min. After a 5-minute treatment with Triton X-100, the cells were washed three times with PBS and incubated with 1% bovine serum albumin (BSA; Sigma, USA) for 30 min. The cells were then incubated with primary antibodies against CD90, CD105, CD73, and CD45 (all from Abcam, UK). To obtain the required working concentrations, the antibodies were diluted in a solution containing 1% BSA and 0.2% Tween-20 in phosphate buffer.

The primary antibodies were diluted as follows: mouse monoclonal antibodies against CD90, CD105, and CD45 (1:100), and rabbit polyclonal antibodies against CD73 (1:200). Incubation with primary antibodies was carried out at 37°C for 1 hour. After three 5-minute washes in 0.2% Tween-20 in PBS, the cells were incubated with goat anti-rabbit and anti-mouse secondary antibodies (1:500) conjugated with Alexa Fluor 488 (Invitrogen, USA) for 45 min at 37°C in the dark.

The cells were washed three times for 5 min with 0.2% Tween-20, air-dried, and then 20 µL of an anti-fade mounting medium containing DAPI (Thermo Fisher Scientific, USA) was applied to each slide. The samples were examined using an Axio Observer A1 inverted fluorescence microscope (Carl Zeiss, Germany) equipped with Zen 2011 software. Image processing was performed using ImageJ software.

Fibroblast Colony-Forming Unit (CFU-F) Assay

Cells isolated from human skin were seeded into T25 culture flasks at 10 cells per cm^2 and cultured in complete growth medium for 14 days at 37°C and 5% CO_2. Upon termination of the culture period, cells were washed with PBS and stained with a 0.5% Crystal Violet solution for 5 minutes at room temperature. After washing twice with PBS, colonies were counted with SZ61 stereomicroscope (Olympus, Germany). The images were taken by an SC-100 CCD camera (Olympus, Germany).

Morphological Analysis

The cell monolayer was washed twice with PBS, then fixed for 2 minutes using ethanol:PBS (1:1) solution. After the treatment, the cells were incubated for 10 minutes in freshly prepared ethanol. The Petri dishes with cells were air dried after removing the ethanol and stained for 25 minutes with 0.5% Crystal Violet. The Petri dishes containing stained cells were rinsed in running water, followed by deionized water, air-dried, and analyzed using Axio Observer A1 inverted microscope (Carl Zeiss, Germany).

Multilineage Differentiation Assay

For differentiation into chondrocytes, cells were resuspended in differentiation medium consisting of high-glucose DMEM, 1% ITS solution, 100 µM Ascorbate-2-phosphate, 10^{-7} M Dexamethasone, 10 ng/ml TGF-β1 , at a concentration of 1.25×10^6 cells/ml. To produce chondrogenic pellets (microbeads), (2.5×10^5 cells) were placed in each V-shaped well of a 96-well polypropylene plate (Phoenix, USA) and then centrifuged at 500 × g and transferred to a CO_2 incubator at 37°C and 5% CO_2. On the 21st day of differentiation, those pellets were collected and fixed in a 4% paraformaldehyde solution (pH 7.2). Samples were embedded in paraffin, cut with a microtome, and processed for Toluidine Blue staining.

For osteogenic differentiation of the cells, induction medium including 10^{-7} M Dexamethasone, 10 µM β-glycerol phosphate, and 50 µM Ascorbate-2-phosphate was used. Culturing was conducted for 3 weeks, after which the cells were stained with Alizarin Red S.

Adipogenic differentiation was achieved by culturing the obtained cells in an induction medium containing: 10^{-6} M Dexamethasone, 0.5 µM 3-isobutyl-1-methylxanthine, (10 ng/ml) Insulin for 3 weeks. The cells were dyed with Oil Red O dye at the end of the culture period.

Statistical analysis

All values of the counted and calculated cell numbers and intensities of immunostainings were statistically analyzed by the ANOVA test. Data were expressed as mean±SD. Differences were considered to be significant when $P<0.05$.

RESULTS AND DISCUSSION

The primary culture of mesenchymal stem cells derived from human skin (sMSCs) was successfully obtained from dermal tissue fragments using the explant culture technique. By day 3 of cultivation, migrating spindle-shaped and polygonal cells forming a morphologically heterogeneous monolayer were observed in the explant adhesion zone. By day 7, the number of adherent cells increased, with the formation of local clusters and a gradual predominance of fibroblast-like cells. By day 14, the culture became predominantly homogeneous, consisting of elongated spindle-shaped cells exhibiting dense adhesive growth in a monolayer (Fig. 1).

This dynamic pattern of morphological changes is consistent with previously reported characteristics of dermal MSCs and multipotent skin-derived precursors obtained using explant or enzymatic methods [2,11]. In particular, Toma et al.

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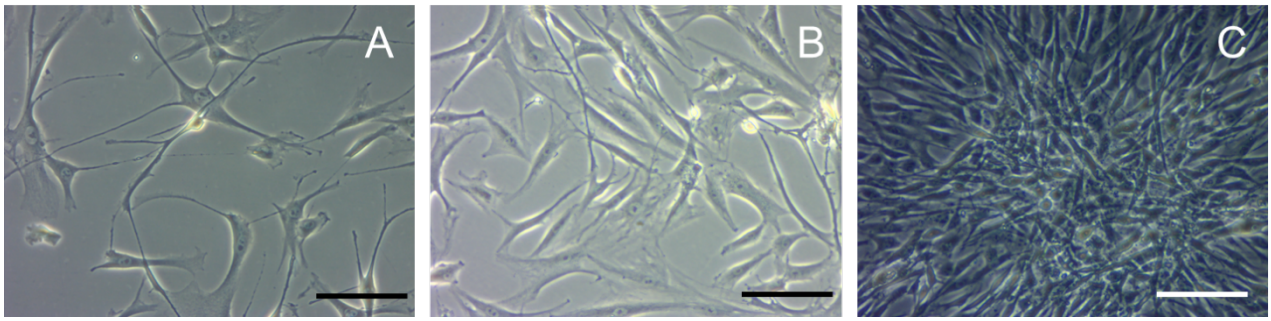

Figure 1 - Primary culture of human skin-derived MSCs (scale bar = 50 μm). A–C. Primary culture (P0) of human skin-derived MSCs observed on days 3 (A), 7 (B), and 14 (C).
A) On day 3, irregular and morphologically heterogeneous hSMSCs originating from a skin fragment were visible in the culture plates. B) By day 7 of P0, actively proliferating hSMSCs with irregular shapes were observed. C) After approximately 2 weeks of P0, the culture consisted of adherent, fibroblast-like cells with a predominantly homogeneous morphology.

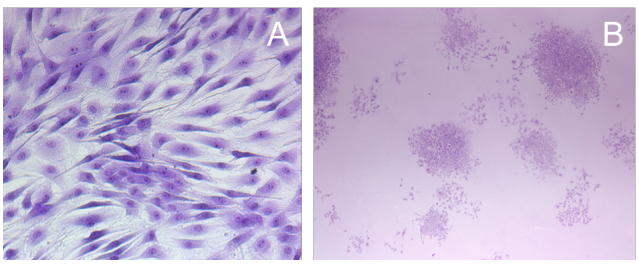

Figure 2 – Morphological and clonogenic characteristics of human skin-derived MSCs. A) Morphology of MSCs. Crystal violet staining. B) A Colony formation of MSCs isolated from human skin.

demonstrated a similar transition from a heterogeneous population to a relatively uniform fibroblast-like culture during the isolation of multipotent skin-derived precursors [12], confirming the methodological comparability of our approach with established protocols.

The clonogenic potential assessed using the CFU-F assay demonstrated the ability of sMSCs to form well-defined colonies of various sizes (Fig. 2). The presence of pronounced colony-forming activity indicates retention of self-renewal capacity and the presence of functionally active progenitor subpopulations within the culture.

Similar to the findings reported for dermal and adipose-derived MSCs by Riekstina et al. and Lindroos et al., our results confirm the high clonogenicity of sMSCs, comparable to MSCs from other anatomical sources [11,18]. This is particularly important given the observations of Baxter et al., who showed that prolonged *in vitro* expansion may lead to accelerated cellular ageing and reduced regenerative potential [17]. In the present study, analysis was performed at early passages, minimizing the risk of replicative senescence and ensuring the relevance of the obtained data for further biotechnological applications.

Immunocytochemical analysis confirmed that the obtained cell population meets the minimal criteria of the International Society for Cellular Therapy (ISCT) for mesenchymal stromal cells [13]. The cells expressed the classical mesenchymal markers CD73, CD90, and CD105 and did not express the hematopoietic marker CD45 (Fig. 3).

A similar immunophenotypic profile has been described for MSCs derived from bone marrow, adipose tissue, and dermal stromal cells [8,11,18]. Our findings are consistent with these reports and confirm that skin-derived MSCs can be reproducibly characterized in accordance with international standards, which is crucial for their translational and laboratory use [13,16].

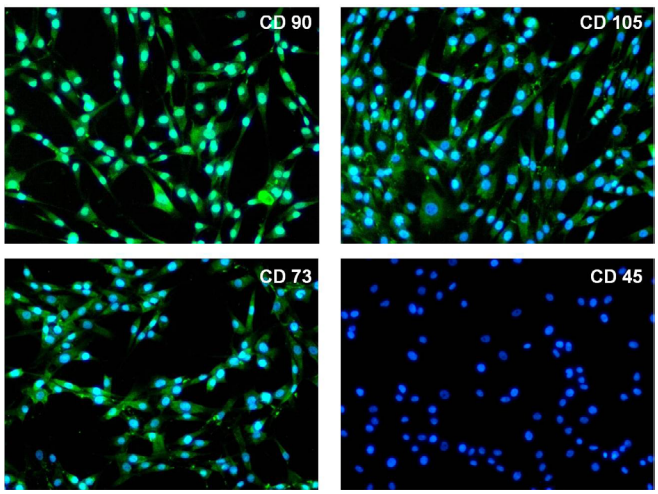

Figure 3 – Immunocytochemistry of human skin-derived MSCs. SMSCs displayed typical MSC morphology, expressed the mesenchymal stem cell marker CD73, CD90, CD105 (green), and were negative for the hematopoietic stem cell marker CD45. Nuclei are stained with DAPI (blue).

The multilineage differentiation potential of sMSCs was confirmed in three classical mesodermal directions — adipogenic, osteogenic, and chondrogenic (Fig. 4). Under adipogenic induction, cells accumulated cytoplasmic lipid droplets detected by Oil Red O staining, indicating adipocyte differentiation. Under osteogenic conditions, mineralized deposits intensely stained with Alizarin Red S were observed, indicating calcium accumulation and the formation of osteoblast-like cells. In chondrogenic culture, microspheroids exhibiting intense metachromatic Toluidine Blue staining were formed, reflecting the accumulation of sulfated glycosaminoglycans in the cartilage-like matrix. This spectrum of differentiation potential corresponds to the classical results reported by Pittenger et al., Zuk et al., and subsequent studies of MSCs from various sources [1, 8]. Thus, our results demonstrate that skin-derived MSCs do not differ functionally from MSCs derived from bone marrow or adipose tissue, while offering the important advantage of minimally invasive tissue sampling.

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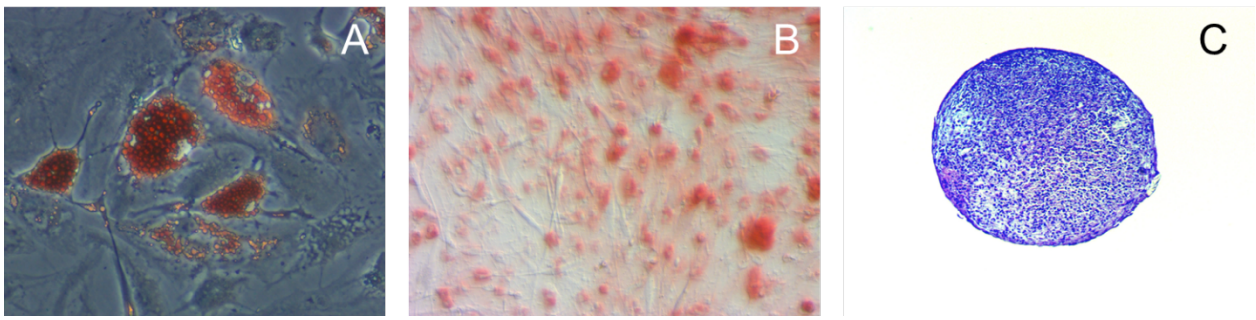

Figure 4 – Multilineage potential of human skin-derived MSCs. A) Adipocytes containing lipid vacuoles stained with Oil Red O; B) Osteoblasts after Alizarin Red staining, showing orange-red mineralized deposits indicating substantial calcium accumulation; C) Chondrogenic microsphere stained with Toluidine blue.

The subsequent stageof the study was aimed at determining the optimal initial seeding density for the *in vitro* expansion of MSC cultures. Four seeding densities were evaluated: 1×10^3, 2×10^3, 3.5×10^3, and 5×10^3 cells/cm². Cell viability and total cell number were assessed using trypan blue exclusion and quantified with a TC20 automated cell counter (Bio-Rad, USA). The proliferation dynamics and cumulative cell yield across different seeding densities are summarized in Figure 5.

The highest total cell number was recorded at the seeding density of 5×10^3 cells/cm², where the culture reached approximately 600,000 cells by Day 7. However, the density of 3.5×10^3 cells/cm² resulted in a total yield of ~460,000 cells and was identified as the most advantageous condition, providing a favorable balance between proliferation efficiency and economical use of culture resources.

Taking into account both growth dynamics and practicality of culture maintenance, the seeding density of 3.5×10^3 cells/cm² was determined to be optimal. This condition ensures efficient cell expansion while minimizing the consumption of culture plasticware, reagents, and incubation surface area, thereby offering the best balance between proliferation rate and cost-effectiveness of cell production.

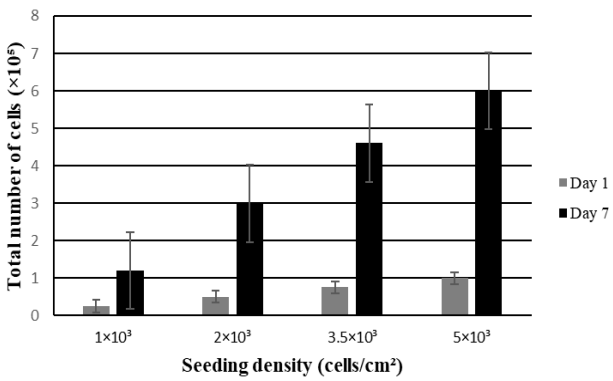

Figure 5 — Growth of human skin-derived MSCs at Different Seeding Densities. The bar chart illustrates the total number of cells on Days 1 and 7 at the initial seeding densities of 1×10^3, 2×10^3, 3.5×10^3, and 5×10^3 cells/cm². Error bars indicate standard deviation (n = 3).

Culture conditions and medium composition are of particular relevance in the context of translational use. In this study, α-MEM supplemented with 15% FBS and a combination of EGF and bFGF supported efficient adhesion and expansion of cells. This is in line with the findings of Mohammadi et al., who emphasized the importance of medium composition and seeding density for maintaining proliferative activity and phenotypic stability of MSCs [19]. At the same time, accumulating evidence highlights the contribution of microenvironmental factors such as oxygen tension and signaling molecules to the maintenance of MSC stemness [15]. Although hypoxic conditions were not specifically evaluated in our experiments, the obtained data indicate that under standard normoxic conditions sMSCs retain key stem-cell properties at early passages. Future studies incorporating controlled hypoxia and more refined medium optimization may further enhance the functional performance of skin-derived MSCs, as demonstrated by Han et al. and Kim et al. [14,15].

From a practical perspective, skin-derived MSCs represent a promising resource for regenerative medicine, tissue engineering, and the development of cell-based products for wound healing and reconstructive surgery. Their high proliferative activity, pronounced clonogenicity, and confirmed multipotency make them an attractive alternative to bone-marrow-derived MSCs, particularly in the context of autologous therapies [3,9,10]. Nonetheless, unresolved challenges remain regarding the standardization of isolation protocols and long-term culture conditions, genomic stability control, and development of GMP-compliant manufacturing workflows — aspects emphasized by Sensebé et al. and Galipeau & Sensébé [5,16]. The results of this study provide a solid basis for further investigations, including scale-up of cultures, evaluation of immunomodulatory properties of sMSCs, and the development of both cell-based and cell-free (secretome, exosome-based) therapeutic approaches.

CONCLUSION

Overall, the results demonstrate that human skin is a readily accessible and highly promising source of mesenchymal stem cells. These cells exhibit morphological, phenotypic, and functional characteristics comparable to MSCs from traditional sources and fully comply with ISCT standards. This creates the prerequisites for the development of standardized protocols for obtaining sMSCs and their subsequent application in biomedical research and clinically oriented regenerative technologies.

REFERENCES

1. Pittenger MF, Mackay AM, Beck SC, Jaiswal RK, Douglas R, Mosca JD, et al. Multilineage potential of adult

DOI: 10.11134/btp.4.2025.18

human mesenchymal stem cells // Science. 1999. – Vol. 284, № 5411. – P. 143–147.

2. Caplan AI. Mesenchymal stem cells // J Cell Biochem. 1991. – Vol. 47, № 3. – P. 256–264.

3. Uccelli A, Moretta L, Pistoia V. Mesenchymal stem cells in health and disease // Nat Rev Immunol. 2008. – Vol. 8, № 9. – P. 726–736.

4. Squillaro T, Peluso G, Galderisi U. Clinical trials with mesenchymal stem cells: An update // Cell Transplant. 2016. – Vol. 25, № 5. – P. 829–848.

5. Galipeau J, Sensébé L. Mesenchymal stromal cells: Clinical challenges and therapeutic opportunities // Cytotherapy. 2018. – Vol. 22, № 6. – P. 824–833.

6. Shi Y, Wang Y, Li Q, Liu K, Hou J, Shao C, et al. Immunoregulatory mechanisms of mesenchymal stem and stromal cells in inflammatory diseases // Nat Rev Immunol. 2018. – Vol. 14, № 8. – P. 493–507.

7. Vizoso FJ, Eiro N, Cid S, Schneider J, Perez-Fernandez R. Mesenchymal stem cell secretome: Toward cell-free therapeutic strategies in regenerative medicine // Int J Mol Sci. 2017. – Vol. 18, № 9. – P. 1852.

8. Zuk PA, Zhu M, Mizuno H, Huang J, Futrell JW, Katz AJ, et al. Multilineage cells from human adipose tissue: Implications for cell-based therapies // Tissue Eng. 2001. – Vol. 7, № 2. – P. 211–228.

9. Mason C, Dunnill P. A brief definition of regenerative medicine // Regen Med. 2008. – Vol. 3, № 1. – P. 1–5.

10. Huang GTJ, Gronthos S, Shi S. Mesenchymal stem cells derived from dental tissues vs. those from other sources: Their biology and role in regenerative medicine // J Dent Res. 2009. – Vol. 88, № 9. – P. 792–806.

11. Riekstina U, Cakstina I, Parfejevs V, Hoogduijn M, Jankovskis G, Muiznieks I, et al. Characterization of human skin-derived mesenchymal stem cell proliferation rate in vitro // Stem Cells. 2008. – Vol. 26, № 11. – P. 2875–2883.

12. Toma JG, McKenzie IA, Bagli D, Miller FD. Isolation and Characterization of Multipotent Skin-Derived Precursors from Human Skin // Stem Cells. 2005. – Vol. 23, № 6. – P. 727–737.

13. Dominici M, Le Blanc K, Mueller I, Slaper-Cortenbach I, Marini FC, Krause DS, et al. Minimal criteria for defining multipotent mesenchymal stromal cells: The International Society for Cellular Therapy position statement // Cytotherapy. 2006. – Vol. 8, № 4. – P. 315–317.

14. Han S, Kim J, Lee G, Na J, Kang Y. Enhanced viability and functionality of mesenchymal stem cells under hypoxic conditions // Stem Cell Res Ther. 2017. – Vol. 8, № 1. – P. 18.

15. Kim M, Kim C, Choi YS, Park HS, Lee S, Lee WJ, et al. Maintenance and regulation of stemness in mesenchymal stem cells by low oxygen concentration // Cell Physiol Biochem. 2018. – Vol. 49, № 2. – P. 842–854.

16. Sensebé L, Bourin P, Tarte K. Good manufacturing practices for mesenchymal stromal cells: Issues and challenges // Cytotherapy. 2013. – Vol. 15, № 4. – P. 362–366.

17. Baxter MA, Wynn RF, Jowitt SN, Wraith JE, Fairbairn LJ, Bellantuono I. Study of telomere length reveals rapid aging of human marrow stromal cells following in vitro expansion // Stem Cells. 2004. – Vol. 22, № 5. – P. 675–682.

18. Lindroos B, Suuronen R, Miettinen S. The potential of adipose stem cells in regenerative medicine // Stem Cell Rev Rep. 2011. – Vol. 4, № 2. – P. 269–291.

19. Mohammadi S, Nikbakht M, Tavakolifar Y, Gholami M, Taheri M, Vahidinia A, et al. Optimization of culture conditions for human mesenchymal stem cells using different media and seeding densities // Stem Cell Res Ther. 2016. – Vol. 7, № 1. – P. 97.

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ПОЛУЧЕНИЕ И ХАРАКТЕРИСТИКА МЕЗЕНХИМАЛЬНЫХ СТВОЛОВЫХ КЛЕТОК КОЖИ ЧЕЛОВЕКА

Аралбек А.Б.[1], Огай В.Б.[1,2*]

Евразийский национальный университет им. Л.Н. Гумилева, ул. Сатпаева, 2, г. Астана, Казахстан
ТОО «Национальный центр биотехнологии», Кургальжинское шоссе 13/5, Астана, 010000, Казахстан
**ogay@biocenter.kz*

АБСТРАКТ

Мезенхимальные стволовые клетки кожи (МСКК) представляют собой многообещающую и минимально инвазивную альтернативу мезенхимальным стволовым клеткам, полученным из костного мозга и жировой ткани. Однако необходимы дальнейшие исследования для стандартизации протоколов выделения и всесторонней характеристики их биологических свойств.

Первичные культуры человеческих МСКК были получены из фрагментов дермальной ткани с использованием эксплантационной техники. Были оценены морфологические характеристики, клоногенный потенциал (анализ КОЕ-Ф), пролиферативная активность (анализ МТТ), иммунофенотип (CD73, CD90, CD105, CD45) и способность к мультипотентной дифференцировке (остеогенная, адипогенная и хондрогенная индукция).

Наши результаты показали, что клетки, прикрепившиеся к эксплантатам кожи, демонстрировали постепенный переход от морфологически гетерогенной популяции на 3 день к преимущественно фибробластоподобному монослою к 14 дню. МСКК проявляли выраженную клоногенность с образованием четко выраженных колоний CFU-F и демонстрировали стабильную пролиферативную активность на ранних пассажах. Иммуноцитохимия подтвердила экспрессию характерных маркеров МСК CD73, CD90 и CD105 при отсутствии гематопоэтического маркера CD45, что соответствует критериям ISCT. Многолинейные дифференцировочные анализы подтвердили функциональную пластичность МСКК, включая образование липидных капель во время адипогенеза, образование богатой кальцием минерализованной матрицы во время остеогенеза и образование хрящеподобных микросфероидов с метахроматическим окрашиванием толуидиновым синим в хондрогенных культурах.

Таким образом, кожа человека представляет собой легкодоступный и надежный источник мезенхимальных стволовых клеток, демонстрирующих стабильную пролиферацию, высокий клоногенный потенциал, классический иммунофенотип МСК и сохраненную мультипотентность. Эти результаты подтверждают трансляционную значимость мезенхимальных стволовых клеток кожи и создают основу для дальнейшего развития стандартизированных протоколов и потенциальных биомедицинских применений в регенеративной медицине и тканевой инженерии.

Ключевые слова: Мезенхимальные стволовые клетки кожи; клоногенность; иммунофенотип; мультипотентная дифференцировка; регенеративная медицина.

АДАМ ТЕРІСІНІҢ МЕЗЕНХИМАЛЫҚ БАҒАНАЛЫ ЖАСУШАЛАРЫН БӨЛІП АЛУ ЖӘНЕ СИПАТТАУ

Аралбек А.Б.[1], Огай В.Б.[1,2*]

[1]Л.Н. Гумилев атындағы Еуразия ұлттық университеті, Сәтпаев көшесі, 2, Астана, 010000, Қазақстан
[2]Ұлттық биотехнология орталығы, Қорғалжын тас жолы, 13/5, Астана, 010000, Қазақстан
**ogay@biocenter.kz*

АБСТРАКТ

Тері мезенхималық бағаналы жасушалары (ТМБЖ) сүйек кемігінен және май тінінен алынған мезенхималық бағаналы жасушаларға перспективалы және минималды инвазивті балама болып табылады. Дегенмен, оқшаулау хаттамаларын стандарттау және олардың биологиялық қасиеттерін жан-жақты сипаттау үшін қосымша зерттеулер қажет.

Адам ТМБЖ-ларының бастапқы дақылдары эксплантация әдісін қолдана отырып, тері тінінің фрагменттерінен алынды. Морфологиялық сипаттамалары, клоногендік потенциалы (CFU-F талдауы), пролиферативті белсенділігі (МТТ талдауы), иммунофенотипі (CD73, CD90, CD105, CD45) және мультипотентті дифференциациялау қабілеті (остеогендік, адипогендік және хондрогендік индукция) бағаланды.

Біздің нәтижелеріміз тері эксплантттарына жабысқан жасушалардың 3-ші күні морфологиялық тұрғыдан гетерогенді популяциядан 14-ші күні негізінен фибробласт тәрізді моноқабатқа біртіндеп ауысқанын көрсетті. ТМБЖ айқын клоногенділік танытты, айқын КФУ-F колонияларын құрады және ерте өтулерде тұрақты пролиферативті белсенділік көрсетті. Иммуноцитохимия CD45 гемопоэтикалық маркері болмаған кезде CD73, CD90 және CD105 тән МБЖ маркерлерінің экспрессиясын растады, бұл ISCT критерийлеріне сәйкес келеді. Көпжіпті дифференциациялық талдаулар МБЖ функционалдық пластикасын, соның ішінде адипогенез кезінде липид тамшыларының пайда болуын, остеогенез кезінде кальцийге бай минералданған матрицаның пайда болуын және хондрогенді дақылдарда метакроматика-

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лық толуидин көк бояуы бар шеміршек тәрізді микросфероидтардың пайда болуын растады.

Осылайша, адам терісі тұрақты пролиферацияны, жоғары клоногендік потенциалды, классикалық МБЖ иммунофенотипін және сақталған мультипотенттілікті көрсететін мезенхималық бағаналы жасушалардың оңай қолжетімді және сенімді көзі болып табылады. Бұл нәтижелер тері мезенхималық бағаналы жасушаларының трансляциялық маңыздылығын растайды және стандартталған хаттамаларды және регенеративті медицина мен тін инженериясындағы әлеуетті биомедициналық қолдануды одан әрі дамыту үшін негіз болады.

Кілт сөздер: Тері мезенхималық бағаналы жасушалары; клоногенділік; иммунофенотип; мультипотентті дифференциация; регенеративті медицина.