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HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS PROMOTE REGENERATION OF NASAL MUCOSA ATROPHY

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Mesenchymal multipotent stromal cells (mesenchymal stem cells-MSC) today are the most promising and widely used means of cell therapy [1-5]. Important biological characteristics of MSCs of various origins include their low immunogenicity, the ability to migrate to the focus of damaged tissues with further differentiation into specific cells under the influence of microenvironmental factors, stimulation of hematopoiesis and immunomodulating activity This allows considering this type of cells as potentially active inducers and regulators of regenerative processes in the recipient's body [6-11]. Common sources for their obtaining are bone marrow (as historically the first) [12] and adipose tissue [13-16] (as practically the unlimited source of autologous MSC) though this type of cells may be isolated from many tissues of adult organism, for example muscle [17], peripheral blood [18], lung [19], heart [20], dental pulp [21], endometrium [22], etc. Such tissues as placenta [23], amniotic membrane [24] and Wharton jelly [25] that are obtained after delivery (so, they are the sources of adult MSC but are formed in early embryogenesis), become more and more popular due to the fact that the cells isolated from them have several advantages comparing with cells from other sources [26-28]. Essential of them are the absence of ethical problems, non-invasive procedure of material obtaining, expression of several markers characteristic for embryonic cells [29]. This last, explains their possibility to differentiate not only into bone, cartilage and fat, but quite easy give other cell types such as myocytes and neural cells.

According to numerous publications [30-36], using of different enzymes, such as hyaluronidase, collagenase, trypsin or their combination allows obtaining of great amount of cells without methodological problems. Our results obtained in processing of more than 300 umbilical cords showed that this approach is not suitable in every case. First of all, enzymes can effect cell surface and receptors on it. That's why it's better to use the method of explants, which is also sometimes described in scientific literature [36]. Second, as has been described in our patent [37] the method for MSC isolation from hUC depends on the organometric characteristics of the cord. Taking into account the previous note about the possible damage of cell surface receptors by enzymes, in our work we have chosen cords with organometric characteristics that required MSC isolation by explants.

Chronic diseases of the upper respiratory tract, accompanied by atrophic changes of the mucous membrane, are very common and re-

quire constant attention and the implementation of appropriate preventive and curative measures. However, medical management of such conditions and clinical manifestations caused by them remains extremely conservative, symptomatically oriented and requires further scientific improvements [38].

One of the modern methods of influence on functional and morphological state of tissues in atrophic processes may be applications of regenerative medicine, including cell therapy. The discovery of stem cells and study of their properties and potential allowed obtaining new perspectives to investigate biological processes and treatment of hereditary and degenerative diseases. The most promising for use in cell therapy are multipotent mesenchymal stromal cells (MSCs).

The aim of the work was to study the possibility of WJ-MSC use for recovery of model animals with atrophic rhinitis and restoration of their nasal mucosa.

Materials and methods

MSC isolation and propagation

Method has been described earlier [39]. Shortly, umbilical cords (UCs) were received after in time delivery, minced into small fragments and cultivated in appropriate nutrient medium with all the additives known for MSC.MSC "crawled" out of the pieces, formed colonies, and after the formed 70-80% confluent layer, they were detached from the surface by usual method and replaced into new flasks. Such procedure was performed two times, and the cells at the second passage ex vivo were characterized by morphology and surface markers (positive and negative for MSC) and used for introducing into model animals.

MSC morphology vb

Cell morphology was characterized by haematoxylin staining according to standard procedures [40].

MSC phenotype

Surface markers of MSC (positive CD73, CD90, CD105 and negative CD34) were detected by FACS analysis on BD FACSAria cell sorting according to standard protocols [41] using FITC or PE-labeled primary antibodies.

Atrophic rhinitis model in mice

Atrophic rhinitis in mice was developed based on [42] report with authors' modification [43]. Shortly, 3 pathogenic strains of *Pasteurella* were used to develop atrophic rhinitis in mice, which was confirmed by clinical and morphological characteristics.

MSC transplantation into model animals

MSC (characterized, on the 2nd passage) were injected intravenously into tail vein (1×10^6 per mouse), and the experimental animals have been observed for 1 month. Each experimental group consisted of 5 animals. Animals' clinical state was examined once a week. After the time of the experiment, the post-mortal morphological study was performed.

Morphological study of nasal mucosa

The assay of general morphological picture was performed with common methods for slice staining with haematoxylin-eosin [40].

Results

MSCs isolated from WJ-UCs by their morphological characteristics fully corresponded the demands for this type of cells: they had typical spindle-shaped morphology (Figure 1).

Evaluation of surface markers' expression also confirmed that the isolated from WJ cells belonged to MSC-they matched minimal criteria for defining MSC [44]. they showed high – expression level of positive surface markers typical for MSC (CD73, CD90 and CD105) and almost no expression of CD34, which is characteristic for hematopoietic cells (Figure 2). The data confirmed the adequacy of method used for MSC isolation and propagation in culture, and their suitability for transplantation into model animals.

The model of atrophic rhinitis was developed in mice. It has infectious genesis, so to prevent infection of the researchers and to stop the disease in animals, injection of antibiotic during 3 days was performed.

Examination of clinical condition of the animals after 1 month showed that experimental animals demonstrated lethargy, slowing of movement, poor appetite, accumulation of secretions in the nose, formation of crusts, and dryness of mucous membranes. The decreasing average body weight (12.3±0.6 g compared to 16.7±0.7 g in the control) was observed. Thus, clinical observations suggest general toxic effect of *Pasteurella multocida* on mice and the development of pathological process in the mu-

cosa of the upper respiratory tract symptoms, which in one month may indicate the development of atrophy.

Morphological study of nose mucosa of model animals compared with controls (uninfected mice), confirmed this assumption (Figure 3). Control mice were characterized with the presence of typical picture of a thin layer of multiciliated epithelium with a thin layer of connective tissue of the lamina propria of mucosa vessels of different calibre (Figure 3A). As for nasal mucosa of experimental animals, destructive-dystrophic changes with signs of epithelial desquamation and covert polymorphic cellular infiltrations and expansion of blood vessels are present (Figure 3B).

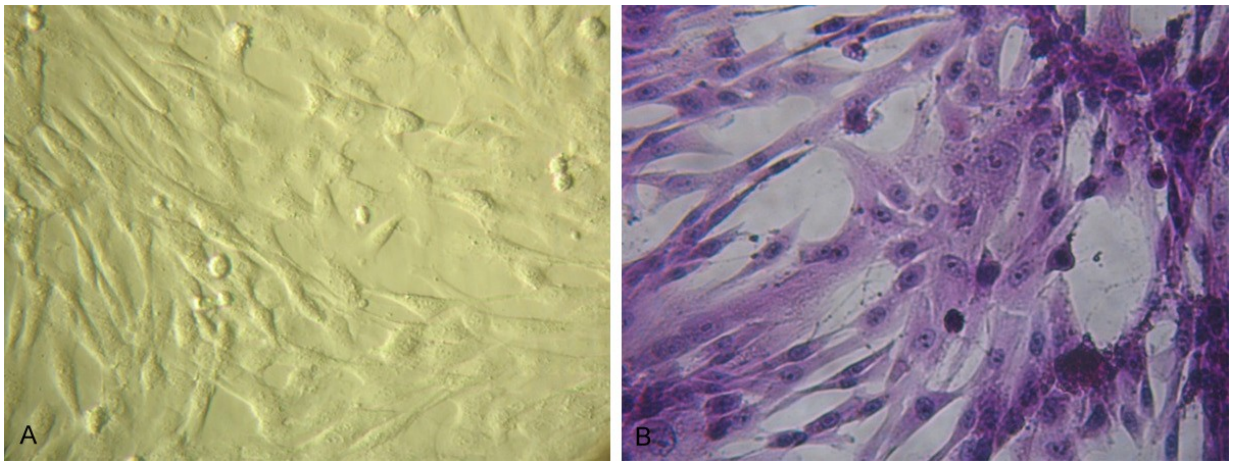

Figure 1. Morphology of MSC, 2nd passage *ex vivo*. A. Typical morphology of 2nd passage MSC used for transplantation in animals, unstained culture ×100. B. Haematoxylin stained cell culture (2nd passage), ×100.

To determine the therapeutic effect of MSC on model animals with atrophic rhinitis, 30 days after infection with *Pasteurella* and further treatment with antibiotics, mice were injected MSCs from human umbilical cord into the tail vein. Within one week, the animals seemed more active, agile, with the usual gusto and adequate nasal breathing. Meanwhile, small amount of clear mucus around the nose vestibule was observed in 33.0% of them. One month after the MSC injection average mice weight was 14.5±0.6 g. Animals maintained normal activity, ate well, their airways were free. The mucous membrane of the pharynx looked pink and moist. Without treatment with MSC mice looked more lethargic, inactive, poorly fed, that is markedly different from the animals of experimental and control groups. The average weight of these mice was 12.6±0.5 g. Accumulation of thick mucus and crusts near nasal vestibule of all the animals significantly influenced free breathing. Oropharyngeal mucosa was pale pink, poorly hydrated. At the same time, the weight of intact animals in the control group was 16.6±0.7 g and no peculiarities in this group of animals were found.

Morphological studies performed one month after administration of MSCs into mice with experimental atrophic rhinitis showed active restoration of nose mucosa structure. Prevalence of productive processes with the growth of blood vessels in the mucosa lamina propria and restoration of epithelial cover structure.

Discussion

Taking into account the results obtained, it could be concluded the following. Clinical and morphological data showed positive effect of human UC-MSC transplantation for restoration of nasal mucosa in mice comparing with untreated control. The mechanism of this action is not fully clear. Presumably, such results were gained due to the release of many growth and nutrient factors present in MSC [45]. Considering that human UC-MSC are

xenogeneic for mice they are destroyed by the animal immune system in several days and only biologically active substances can have a positive impact on the disease passing. But this hypothesis should be proved and requires additional study.

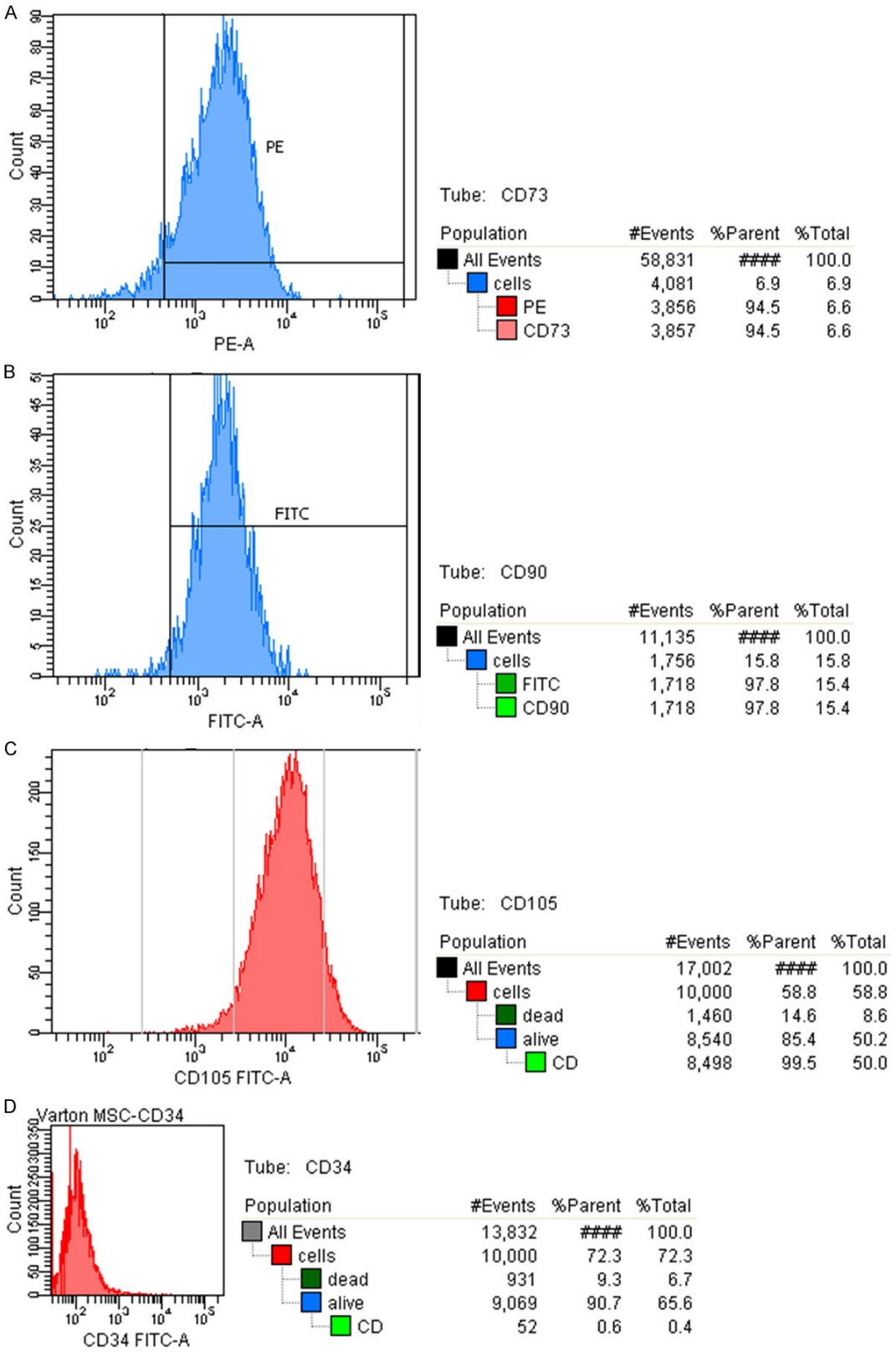

Figure 2. Expression of typical surface markers by 2nd passage MSC. A-C. CD73, CD90, CD105 (positive); D. CD34 (negative).

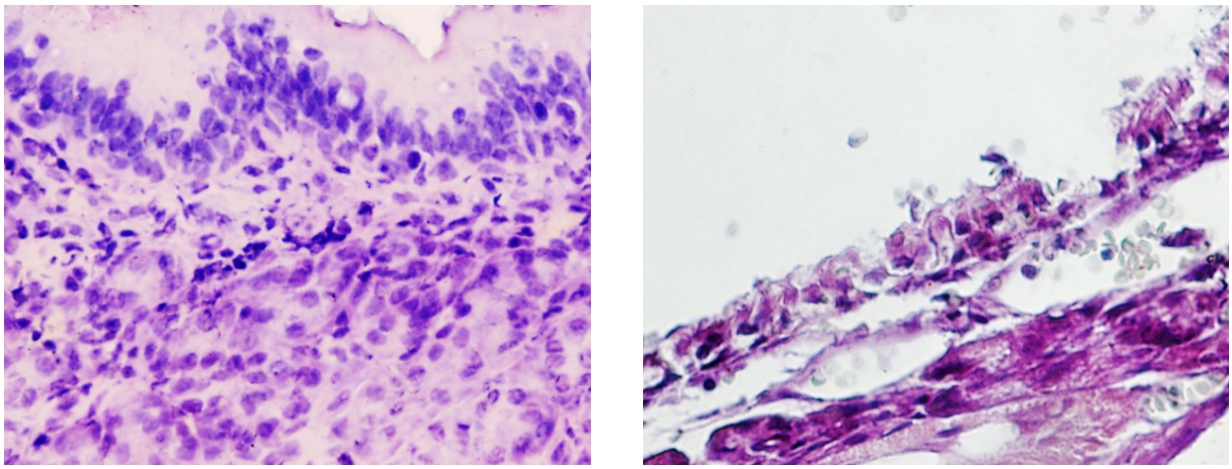

Figure 3. Morphology of the nasal mucosa: a) respiratory epithelium and own lamina (lamina propria) of the mucous membrane with vessels und glandular formations in an intact animal; b) atrophically altered and thinned nasal mucosa in an animal 14 days after the procedure for reproducing experimental atrophy. Staining with hematoxylin and eosin. Ob. 40, ey. 10.

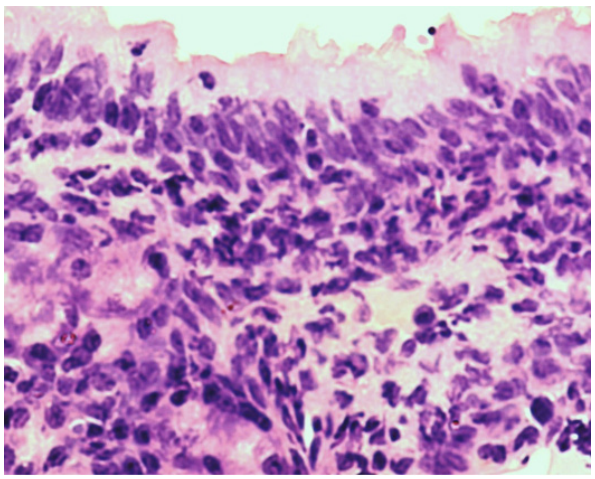

Figure 4. Proliferative processes and restoration of the morphological structure of the nasal mucosa of mice 2 months after the development of an atrophic state and its correction with mesenchymal stem cells (MSC). Staining with hematoxylin and eosin. Ob. 40, ey. 10.

Conclusions

Main conclusions can be formulated as: UC-MSCs possess strong therapeutical potential for the treatment of nasal mucosa atrophy restoration in model animals; The developed method of UC-MSC obtaining corresponds well for this purpose.

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HUMAN UMBILICAL CORD-DERIVED MESENCHYMAL STEM CELLS PROMOTE REGENERATION OF NASAL MUCOSA ATROPHY

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A b s t r a c t

Introduction: Mesenchymal multipotent stromal cells (mesenchymal stem cells-MSCs) are currently the most promising and widely used means of cell therapy.

Common sources for obtaining them are bone marrow and adipose tissue, but now the umbilical cord and placenta are gaining more and more popularity, since the cells isolated from them have a number of advantages over other sources.

Aim: To study the peculiarities of human umbilical cord MSCs influence of on the regeneration of the mucous membrane of the nasal cavity in experimentally induced atrophy.

Materials and methods: 30 laboratory mice were observed, which divided into two experimental groups (10 animals each) and control group (10 mice). Clinical and morphological studies were perfoemed 1 and 2 months after the development of atrophic rhinitis.

Results: Umbilical cords were obtained after timely delivery, chopped into small fragments and cultured in the appropriate nutrient medium with all supplements known for MSCs. MSCs migrated from the pieces, formed colonies, and after the formation of a 70-80% confluent layer, they were detached from the surface in the usual way and transferred to new vials. This procedure was performed twice, after which the cells were characterized by surface markers (positive and negative for MSCs) and used for administration to model animals. Atrophic rhinitis in mice was developed using 3 pathogenic strains of Pasteruella and confirmed by clinical and morphological characteristics. MSCs (characterized, at the 2nd passage) were injected intravenously (1×10^6 per mouse) and experimental animals were observed for 2 months. The clinical condition of the animals was examined once a week. After the end of the experiment, a postmortem morphological study was performed. Clinical and morphological data showed positive effect of transplantation of human umbilical cord MSCs for nasal mucosa regeneration in mice compared to untreated controls.

Key words: Mesenchymal stromal cells, multipotent stromal cells, human umbilical cord, atrophic rhinitis, mucous membrane of the nasal cavity.

ВПЛИВ МЕЗЕНХІМАЛЬНИХ СТОВБУРОВИХ КЛІТИН ПУПОВИНИ ЛЮДИНИ НА СТАН СЛИЗОВОЇ ОБОЛОНКИ ПОРОЖНИНИ НОСА ПРИ ІНДУКОВАНІЙ АТРОФІЇ У ЕКСПЕРИМЕНТАЛЬНИХ ТВАРИН

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А н о т а ц і я

Мета: Вивчення особливостей впливу МСК пуповини людини на регенерацію слизової оболонки порожнини носа при індукованій в експерименті атрофії.

Матеріали та методи: Під наглядом перебували 30 лабораторних мишей, які були розподілені на дві дослідні групи (по 10 тварин) та контрольну групу (10 мишей). Після формування атрофічного риніту через 1 та 2 місяці були проведені клінічні та морфологічні дослідження.

Результати: Мезенхімальні мультипотентні стромальні клітини (мезенхімальні стовбурові клітини-МСК) на сьогодні є найбільш перспективним і широко використовуваним засобом клітинної терапії. Поширеними джерелами для їх отримання є кістковий мозок і жирова тканина, але зараз все більшої популярності набувають пуповина і плацента, оскільки виділені з них клітини мають ряд переваг перед іншими джерелами.

Пуповини отримували після своєчасних пологів, подрібнювали на невеликі фрагменти та культивували у відповідному живильному середовищі з усіма добавками, відомими для МСК. МСК мігрували зі шматочків, утворювали колонії, і, після формування 70-80% конфлюентного шару, їх звичайним способом відкріпляли від поверхні і переміщували в нові флакони. Таку процедуру проводили двічі, після чого клітини характеризували поверхневими маркерами (позитивними та негативними для МСК) і використовували для введення модельним тваринам. Атрофічний риніт у мишей був розвинений з використанням 3 патогенних штамів Pasteruella та підтверджений клініко-морфологічною характеристикою. МСК (охарактеризовані, на 2-му пасажі) вводили внутрішньовенно (1×106 на мишу) і спостерігали за дослідними тваринами протягом 2 місяців. Клінічний стан тварин досліджували 1 раз на тиждень. Після закінчення часу експерименту проводили постмортальне морфологічне дослідження. Клінічні та морфологічні дані показали позитивний ефект трансплантації МСК пуповини людини для відновлення слизової оболонки носа у мишей порівняно з необробленим контролем.

Ключові слова: Мезенхімальні стромальні клітини, мультипотентні стромальні клітини, пуповина людини, атрофічний риніт, слизова оболонка порожнини носа.