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# *In vitro* PROPERTIES OF NEURAL CREST-DERIVED MULTIPOTENT STEM CELLS FROM A BULGE REGION OF WHISKER FOLLICLE

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A culture method for multipotent neural crest-derived stem cell isolated from the bulge region of the hair follicle of whisker pad of adult mice has been described and their biological properties have been studied. It was shown that the cells possess a fibroblast-like morphology, they are nestin-positive and cytokeratin-negative, and also express the following surface markers: CD44, CD73, CD90 and Sca-1. This cell type shows the functional properties of stem cells in culture: clonogenicity, self-renewal, sphere-forming capacity and the ability to the directed multilineage differentiation. Due to these properties, neural crest-derived multipotent stem cells are promising for application in the regenerative medicine.

***Key words:*** neural crest, multipotent stem cells, hair follicle, clonogenicity, sphere-formation capacity.

*The neural crest (NC)*, a transient structure in embryo development, is unique to vertebrates. NC is derived from the ectoderm and formed at the border of neuroectoderm and somatic ectoderm. Originally NC is represented by a cell population lying in the form of epithelial cords emerging from both sides of the neural tube. At the neurulation process end the NC cells undergo an epithelial-mesenchymal transition (EMT) and migrate distantly in various tissues and organs of the developing embryo, where they give rise to the multiple differentiated cell types [1, 2].

In adult vertebrate organism the NC derivatives are represented by the following cell types: neurons and glial cells of the peripheral nervous system, the majority of sensory neurons, melanocytes, Merkel cells, odontoblasts, stromal and endothelial cells of cornea, C cells of thyroid gland, the adrenal medulla, a part of thymic stroma [1, 2]. The most plastic are the cells of cranial NC. In addition to the above mentioned differentiated cell types, they form a part of the bone, cartilage and connective tissues of the face, forehead and ventral neck [3, 4].

Unusually wide differentiation potential of the NC cells suggests an existence of multipotent stem cells. Stemple and Anderson firstly revealed the existence of multipotent stem cells in the NC in early embryonic stage of mammalian development [5]. A long time it was thought that, like NC *per se*, *neural crest-derived multipotent stem cells (NC-MSCs)* have a transient nature. Consequently, the NC-MSCs exist only at the premigratory (from the NC specification moment until the beginning of its cell migration) and migratory stages (from the NC cell delamination until their incoming into the sites of final destination), and further, within migration to sites of its final localization, undergo a process of commitment, turning into the progenitor cells with limited proliferative and/or differentiation potential [6, 7]. An example is the transition of NC-MSCs into the melanocyte precursors — melanoblasts.

In the last decades, NC-derived cells have been isolated from a number of tissues and organs of the adult mammalians. These cells demonstrate the ability to self-renewal and multilineage differentiation, at least *in vitro*, i.e., they are multipotent stem cells [8–11]. The origin of these cells from NC was proved by creating special lines of transgenic mice [12, 13], as well as by means of their transplantation in the developing chick embryo, where the NC-MSCs migrated via the

main migration pathways of the NC cells and differentiated into the appropriate cell types — NC derivatives in sites of final localization [14].

One of the NC-MSC localization sites in the adult organism is the hair follicle. Two research groups, using different methodological approaches, isolated NC-MSCs from the bulge region [15] and dermal papilla of the hair follicle [14]. It should be noted that the dermal papilla is NC-derived only in hair follicles located in the head area. Dermal papilla of hair follicles in the trunk is of mesodermal origin [16]. At the same time, NC cells were detected in a bulge region of the head and trunk hair follicles [16]. Relative accessibility of the hair follicles for cells to be isolated for further use in regenerative medicine determines the study of the bulge region NC-MSC properties as a promising trend.

The ability to be differentiated in clonal culture into the neurons, melanocytes and myofibroblasts has been shown for NC-MSCs from the bulge region of whisker follicle [15]. It was also performed directed differentiation of NC-MSCs into the chondrocytes and Schwann cells [15].

However, for the NC-MSCs from a bulge region of the hair follicle it was not revealed a capacity to be differentiated into a full range of "mesenchymal" cell types generated by the cranial NC, namely, into the osteoblasts and adipocytes. It is not also shown the ability to grow as floating spheres, which may be a general property of the NC-MSCs regardless of their tissue origin.

The aim of a study was to explore *in vitro* a phenotype, clonogenicity, self-renewal, sphere-forming capacity, and the ability to directed multilineage differentiation into the adipogenic, osteogenic, neuronal and glial lineages for the NC-MSCs from a bulge region of the whisker follicle in mice.

## Material and Methods

Experiments were performed with use of 5 cell cultures derived from FVB mice males of 4 to 6 months aged. Mice were euthanized by cervical dislocation under ether anesthesia. The experiments were performed in compliance with the principles of bioethics and biosafety standards, as confirmed by the Committee on Bioethics of State Institute of Genetic and Regenerative Medicine of NAMS.

*NC-MSCs from a bulge region of whisker follicle isolation and culturing*

NC-MSC culture was obtained from explants of a bulge region (BR) of the whisker follicle (WF) by Sieber-Blum method [15] in our modification [17]. By 3 BRs were explanted into the 35 mm Petri dish and cultured in DMEM:F12 medium supplemented with 10% fetal bovine serum (FBS), 5 ng/ml bFGF, 1% MEM vitamins, 1% Neuronal Stem Cell Supplement (PAA, Austria) and 2 mM *L*-glutamine (all from Sigma-Aldrich, USA) in a multi-gas incubator CB210 (BINDER, Germany) at 37 °C and saturating humidity, in artificial atmosphere, consisting of 90% $N_2$, 5% $O_2$ and 5% $CO_2$. Cultures were passaged with use of 0.05% trypsin in 0.53 mM $Na_2EDTA$ (Sigma-Aldrich, USA). Cell seeding density under routine culturing was $10^3$ per $cm^2$. Primary cell isolation and culturing, serial passaged cell culturing, colony-forming unit-fibroblast (CFU-F) assay and directed differentiation were performed in cell culture dishes pre-coated with collagen type I (except of neuronal differentiation assay with substrate used — poly-*L*-lysine and fibronectin (Sigma-Aldrich, USA).

*Clonogenicity (CFU-F assay) and self-renewal capacity*

To assess the ability of growing at a clonal density (at low cell seeding density, 1–50 per $cm^2$, resulting in formation of discrete colonies having a clonal origin, i.e. *clonal colonies* [18]), 100–300 NC-MSCs were seeded in collagen type I treated 100 mm Petri dishes (BD Falcon, USA) in complete growth medium. FBS concentration was increased up to 20%. Cells were cultured over 14 days. Then, they were either fixed and stained for CFU-F assay or subcloned for self-renewal capacity analysis. Plate efficiency of colony formation (PE, %) was calculated with a standard formula [18]. Reseeding (subcloning) of a clonal colony per 3 new 100 mm Petri dishes was performed by using of cloning cylinders (Sigma-Aldrich, USA). Cells were cultured over 14 days, then fixed and stained for CFU-F assay.

*Directed osteogenic and adipogenic differentiation*

NC-MSC directed differentiation into adipocytes and osteoblasts were carried out according to the standard protocols [19].

*Directed neuronal differentiation*

NC-MSCs were seeded at a concentration of $10^4$ cells per well in 96-well Imaging Plate (BD Falcon, USA) in growth medium and the following day they were switched to differentiation medium of the following composition over 7–11 days: Neuronal Base Medium P (PAA, Austria), 10% mouse brain extract, 1% Neuronal Stem Cell Supplement (PAA, Austria), 2% nutritional supplement

NeuroMix (PAA, Austria), 2 μM retinoic acid (Sigma-Aldrich, USA).

*Directed glial (Schwann cells) differentiation*

NC-MSCs were seeded at a concentration of $10^4$ cells per well in growth medium and the following day they were switched to differentiation medium of the following composition over 7–11 days: Neuronal Base Medium P (PAA, Austria), 10% of mouse brain extract, 1% Neuronal Stem Cell Supplement (PAA, Austria), 2 μM retinoic acid (Sigma-Aldrich, USA), 100 nM isoproterenol (Sigma-Aldrich, USA).

*Sphere-forming capacity under serum-free cell culture conditions*

NC-MSCs were washed twice from FBS residues by centrifugation (200g, 5 min), then seeded in a concentration of $10^4$ cells per ml in 35 mm Petri dish for suspension cell culture in a growth medium of the following composition: DMEM:F12, 2% Neuronal Stem Cell Supplement (PAA, Austria), 2 mM *L*-glutamine, 20 ng/ml bFGF, 40 ng/ml EGF. Growth factors were added every 48 hours. Change of half of the medium was carried out twice a week. Cells were cultured for 14–21 days.

*Cell surface and intracellular marker analysis by flow cytometry*

NC-MSC immunophenotype was determined at P3 cell culture with use of a flow cytometer / cell sorter BD FACSAria (Becton Dickinson, USA) and fluorochrome-conjugated anti-mouse monoclonal antibody to CD44 (PE), CD45 (PE-Cy-7), CD73 (PE), CD90 (biotinylated primary antibody, streptavidin-PE), CD117 (PE-Cy-7), Sca-1 (PE) and nestin (PE) (BD Pharmingen, USA). Analysis was performed using software BD FACS Diva 6.1 (Becton Dickinson, USA).

*Cytochemistry analysis*

To evaluate cell morphology and to determine the CFU-F type, cell cultures were fixed for 20 minutes with cold ethanol and stained by Romanowsky-Giemsa method (Macrochem, Ukraine) for other 20 min. To detect the osteogenic and adipogenic differentiation, the cells were fixed for 20 min in a 10% formalin (Macrochem, Ukraine), rinsed with phosphate buffered saline (PBS) and stained for 20 minutes with 2% Alizarin Red S (pH 4.1; for detecting calcified extracellular matrix) or 0.5% Oil Red O (for neutral lipid staining) (Sigma-Aldrich, USA).

*Immunocytochemistry analysis*

To assess the purity and homogeneity of a cell population obtained by explant method, a primary NC-MSC culture was stained with murine FITC-conjugated anti-pancytokeratin antibodies (1:50, Sigma-Aldrich, USA) and the primary rabbit anti-nestin antibodies (1:100, Sigma-Aldrich, USA), followed by further detection with goat anti-rabbit Alexa 594-conjugated antibodies (1:2000, Abcam, UK). For neuronal differentiation detection the primary mouse anti-βIII-tubulin (TU-20) monoclonal antibodies (1:500, Abcam, UK) and secondary goat anti-mouse FITC-conjugated antibodies (1:2000, Abcam, UK) have been used. To detect the differentiation into Schwann cells the primary mouse anti-S-100 monoclonal antibodies (1:500, Abcam, UK) and secondary goat anti-mouse FITC-conjugated antibodies (1:2000, Abcam, UK) have been used.

Staining was performed using the following protocol: cell fixation with cold Cytofix (Becton Dickinson, USA) for 30 min, cell permeabilization (0.1% Triton X-100, Sigma-Aldrich, USA) in PBS for 30 min, incubation with blocking solution (0.1% Triton X-100 and 0.5% BSA (PAA, Austria) in PBS) for 30 min, incubation for 12 hours at +4 °C with primary antibodies and 1 hour at room temperature with secondary antibodies, for 15 min at room temperature with a Hoechst 33342 solution (2 μg/ml) or propidium iodide solution (PI, 2 μg/ml) to visualize cell nuclei.

*Microscopy*

For cell culture and stained cell slide visualization the inverted fluorescence microscope IX71 (Olympus, Japan) equipped with a digital camera DP-20 and software QucikPHOTO MICRO, as well as an inverted fluorescence microscope Axio Observer A1, equipped with a digital camera AxioCam ERc 5s and software ZEN 2012, have been used (Carl Zeiss, Germany).

*Statistics*

Quantitative characteristics of random variables are presented as mean values and their standard deviations ($M \pm m$). Significance of the differences was evaluated using Student's $t$-test.

## Results and Discussion

*NC-MSCs' isolation and culturing; their functional (clonogenicity, self-renewal) and phenotypic properties*

Lifelong, a hair follicle of the mammalian is in a state of dynamic cycle in which the growth phase (anagen) is replaced by a short transitional phase (catagen), during which the regression of some structures of a hair follicle

occurs. A resting phase (telogen) followed by a catagen [20]. The ability of such cyclic changes is provided by the existence of a pool of stem cells of various types. BR is a thickening of the outer root sheath and is a permanent part of the hair follicle, persisting in all phases of its life cycle [21]. Previously, it was shown that just a BR is a site of localization of epidermal stem cells responsible for the generation of the hair shaft and capable of forming interfollicular epidermis during healing of skin wounds [22]. BR has been successfully used to obtain a cell culture of epidermal keratinocytes [21]. Thus, to obtain a pure NC-MSC culture from WF BR explants the selective culture conditions are required. Initially, this problem was solved by using as a culture supplement the chicken embryo extract [15], which has several drawbacks, in particular the difficulty of obtaining and variable poorly characterized composition. We modified the protocol, replacing the chicken embryo extract by combination of the bFGF and commercial nutritional supplement with a defined chemical composition — Neuronal Stem Cell Supplement (PAA, Austria).

Using our protocol at Day 3–5 of culturing from all explanted WF BRs the emigration and active proliferation of fibroblast-like cells have begun (Fig. 1, *A, B*). Immunocytochemistry analysis of a given cell population showed their positive staining for nestin (a marker of neural stem cells and NC-MSCs) and negative staining for cytokeratins. It confirms the absence of keratinocyte contamination of NC-MSC culture (Fig. 1, *B*).

To prevent spontaneous differentiation at high cell density and to maintain the cells in an undifferentiated state, the explants were removed and the cells were reseeded into T-25 flask at Day 7–10. Upon reaching P1-culture confluent state [cell culture Day 7; average cell number — $(1.034\pm0.211)\times10^6$; average cell density — $(41.36\pm8.4)\times10^3$ cells per $cm^2$] the cells were collected and used to perform CFU-F assay. It was shown an ability of WF BR fibroblast-like cells to grow at clonal density (Fig. 2, *A, B*). The PE value in such culture system was $72.58\pm12.35\%$. It is noteworthy that clonal colonies were heterogeneous by a cell number and morphology. All clonal colonies could be divided into small (up to 20 large morphologically distinct differentiated cells), medium (50–100 cells of variable morphology) and large ones (more than 500 cells, mostly small in size, with any morphological signs of differentiation). Such a cell heterogeneity for proliferation potential in a culture under clonal density may have two explanations: 1) the culture contains several various cell types; 2) the culture is composed of one cell type, but the cell population possess an hierarchical structure (differentiated, committed progenitors and stem cells), as it was previously shown for the epidermal keratinocytes [23] and $LNHFR^+THY\text{-}1^+$ ($CD271^+CD90^+$) population of bone marrow mesenchymal stem cells [24]. One method of verification of these hypotheses is a colony subcloning for further analysis of secondary clonal colonies.

To assess the NC-MSC self-renewal capacity and discriminate the above two hypotheses we realized a subcloning of different type clonal colonies. As a result, reseeding of small ($n = 5$) and medium ($n = 5$) clonal colonies did not lead to the formation of secondary colonies. Subcloning of large clonal colonies ($n = 5$) was successful and led to the formation of secondary clonal colonies, and it was observed the formation of colonies of all the above types, including large ones (Fig. 2, *B, D*). Thus, we conclude that NC-MSCs possess a self-renewal capacity (stem cell property) and such cell

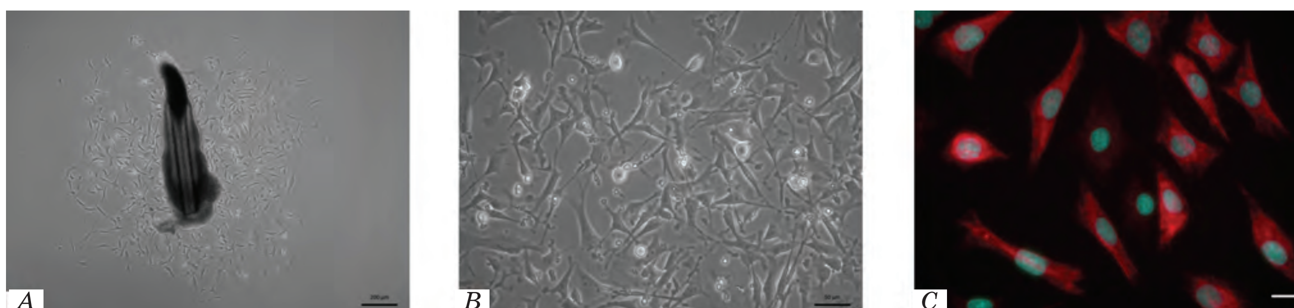


*Fig. 1.* **NC-MSC morphology *in vitro*:**
*A* — NC-MSC emigration and proliferation from bulge region explants of whisker follicle: Day 5 cell culture, phase contrast, 200 μm scale;
*B* — NC-MSC morphology in a primary culture: phase contrast, 50 μm scale;
*C* — nestin+/cytokeratin- NC-MSCs in a primary culture: fluorescence microscopy, 20 μm scale

population in a culture has a hierarchical structure that is comprised of cells at various differentiation stages.

Further study of NC-MSC phenotype at P3-culture showed cells express the markers characteristic for stem cells (Sca-1 and nestin), multipotent mesenchymal stromal cells (MSCs) (CD44, CD73 and CD90) and the culture lacks $CD45^+$ cells indicating the absence of hematopoietic cell contamination (Table). A substantial part of cells in P3-culture expresses CD117 (*c-kit*, receptor for SCF, stem cell factor), which distinguishes NC-MSCs from the bone marrow- and adipose-derived MSCs. It is known the mutations in *c-kit* gene or in its ligand SCF gene lead to development of some serious pathologies like anemia, infertility, pigmentation disorders and various development defects of sensory neurons (degree of damage to sensory neurons depends on the animal species) [25]. Pigmentation disorders caused by mutations in *c-kit* gene are associated with impaired development of melanocytes and have no significant impact on the development of other cell types — NC derivatives [26]. On the basis of these data, it was previously thought that among NC-derived cells, CD117 was a marker of the melanoblasts. It was obtained an experimental evidence the injection of blocking *c-kit* antibodies into the developing mouse embryo [27], as well as an addition of these antibodies to culture of mouse embryonic NC cells [28], led to the elimination of melanoblasts/melanocytes from an organism or a cell culture. However, CD117 has been successfully used recently as a marker for NC-MSC selection from a heterogeneous population of cells obtained during the differentiation of mouse embryonic stem cells [29]. Selected $CD117^+$ cells had all the properties of NC-MSCs and were able to differentiate into the neurons, glial cells and melanocytes.

Then an unexpected multipotency of $CD117^+$ melanoblasts isolated from the adult mice skin has been demonstrated. *In vitro* they all have self-renewal and multilineage differentiation capability into the neurons, glial cells, and melanocytes [30]. However, the functional significance of CD117 expression in the culture of NC-MSCs remains unclear and requires a range of cell sorting experiments as well as comparing the properties of $CD117^+$ and $CD117^-$ populations of NC-MSCs with use of other animal models.

The second point concerns the content of nestin-positive cells. In a primary culture all the cells are positive for this marker,

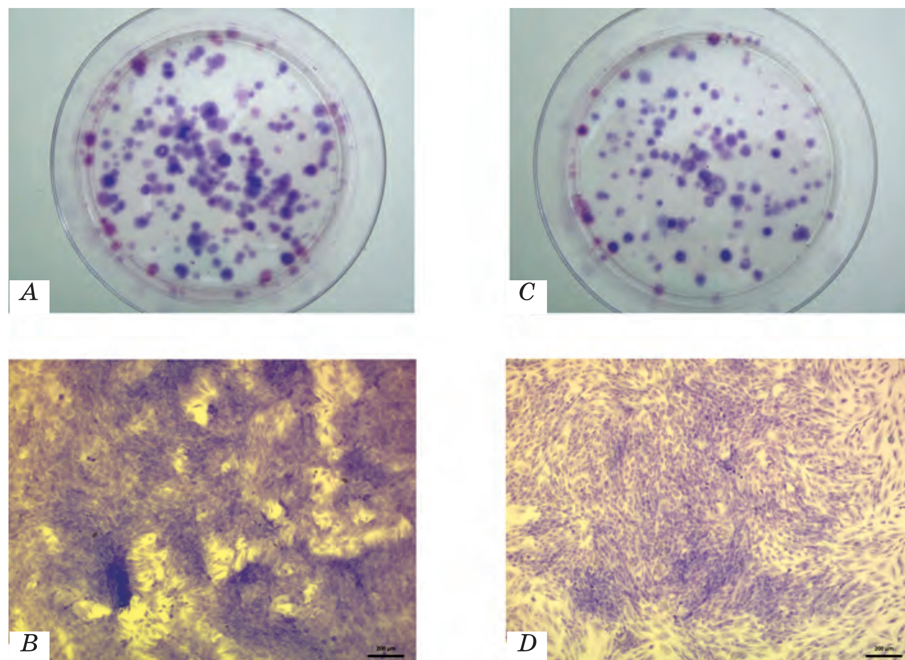

*Fig. 2*. **NC-MSC clonogenicity and self-renewal capacity:**

*A* — CFU-F assay;
*B* — a large NC-MSC clonal colony: a tendency to the formation of three-dimensional cell structures — spheroids;
*C* — a large clonal colony subcloning leads to the formation of secondary clonal colonies;
*D* — a large secondary clonal colony formation after subcloning of a large primary clonal colony: Romanowsky-Giemsa stain; 200 μm scale on *B*, *D*

**Immunophenotype of NC-MSCs from a bulge region of whisker follicle at cell culture P3 (M±m)**

| Marker | CD44 | CD73 | CD90 | CD117 (*c-kit*) | Sca-1 | Nestin | CD45 |
|---|---|---|---|---|---|---|---|
| Positive cells, % of total cell number | 98.92 ± 0.78 | 86.52 ± 4.78 | 97.24 ± 3.24 | 42.43 ± 11.86 | 95.52 ± 2.81 | 85.61 ± 13.23 | 0.94 ± 0.49 |

whereas in P3-culture nestin+ cells constitute 85.61±13.23%. Nestin is a marker of stem/progenitor state for various cell types [31, 32]. Thus, reduced number of nestin-positive cells indicates spontaneous differentiation processes taking place in the NC-MSC culture. On the other hand, with given cell culture split ratio (about 1:40), we can conclude that we have developed a culture system, which greatly contributes to NC-MSC self-renewal and maintains the cells in an undifferentiated state.

*Sphere-forming and directed multilineage differentiation capacity*

One of the characteristic of NC-MSCs isolated from various tissues and organs of postnatal mammalian body, is the ability to grow with the formation of floating spheres when cultured under certain conditions (serum-free medium, low adhesion substrate and a high concentration of growth factors) [9, 10, 13, 14]. These spheres by morphology, mechanism and condition formation are similar to neurospheres derived from neural stem cells of the CNS [33]. For NC-MSCs from WF BR the sphere-forming capacity has not been shown to date. Basically, it is believed, the NC-MSC growth as floating spheres is provided by their ability to reversible mesenchymal-epithelial and epithelial-mesenchymal transitions (MET and EMT, respectively), which NC-MSCs are also *in vivo* undergo during embryonic development of the organism [2]. So, sphere-forming capacity must be a general characteristic of NC-MSCs regardless of their tissue origin.

In our study, when we switching of NC-MSCs under serum-free culture conditions, a part of cells died within the initial four culture days. Surviving cells underwent morphological changes and began to proliferate with a formation of small clusters of rounded cells at culture Day 7–10 (Fig. 3, *A*). Further cell proliferation in clusters led to the formation of large floating spheres over 2-3 culture weeks (Fig. 3, *B*). Thus, WF BR NC-MSCs are able to sphere formation.

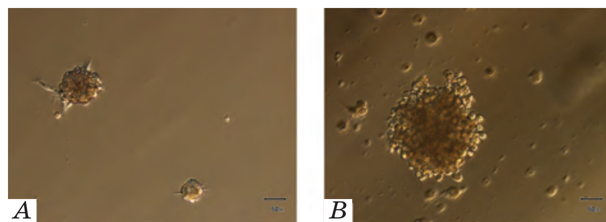


*Fig. 3.* **Sphere formation by cultured NC-MSCs under specific serum-free conditions:**
*A* — Day 7 cell culture; *B* — Day 21 cell culture; relief contrast; 50 μm scale

When NC-MSCs cultured in appropriate inductive medium, the cells undergo morphological and functional changes specific to given direction of differentiation. When cultured in adipoinductive medium the cells acquired a rounded-polygonal shape, small lipid vacuoles in the cytoplasm, which increased in size over differentiation time and they were stained with specific for neutral lipids dye Oil Red O (Fig. 4, *A*). When cultured in osteoinductive medium the cells were of polygonal form and began to produce calcified extracellular matrix, which is stained with Alizarin Red S (Fig. 4, *B*). Directed differentiation into the "neural" cell types also leads to characteristic morphological changes and expression of specific neuronal and glial (Schwann cells) proteins. During directed differentiation into the neurons, fibroblast-like cells transformed into the cells with a rounded refractory soma and long processes and positively stained for β-III-tubulin in immunocytochemical reaction with specific antibodies (Fig. 4, *C*). When cultured in inductive medium for glial differentiation, NC-MSCs acquired characteristic glial cell morphology and expressed specific glial protein S-100 (Fig. 4, *D*).

Thus, the bulge region of a whisker follicle of adult mice contains a population of cells which by phenotypic (nestin+/cytokeratin-) and functional (clonogenicity, self-renewal, sphere-forming and multilineage differentiation capacities) characteristics satisfies the criteria for neural crest-derived multipotent stem cells. NC-MSCs are of a great interest for regenerative medicine. A number of experimental studies produced positive results in on NC-MSC application in models of spinal cord [34] and peripheral nerve injuries [35, 36], etc.

Ability to obtain osteoblasts from the NC-MSCs can be of practical importance in the field of regenerative medicine and tissue engineering of bone. NC-MSCs could

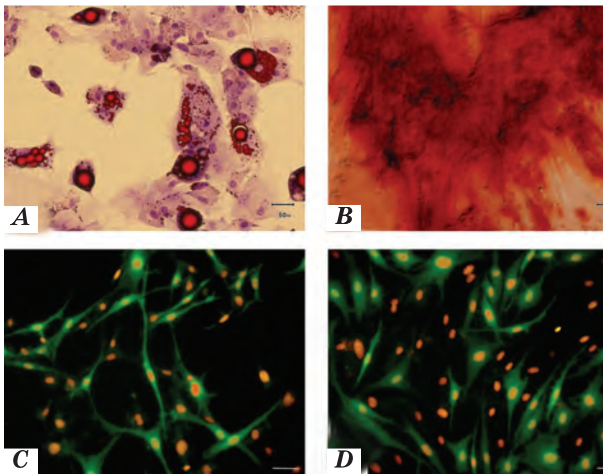


*Fig. 4.* **NC-MSC directed multilineage differentiation:**

*A* — adipogenic differentiation, Oil Red O stain contrasted with Romanowsky-Giemsa stains; 50 μm scale;

*B* — osteogenic differentiation, Alizarin Red S stain; 50 μm scale;

*C* — neuronal differentiation, fluorescent staining with anti-β-III-tubulin antibodies (secondary FITC-conjugated), fluorescence microscopy; 50 μm scale;

*D* — glial differentiation (Schwann cells), fluorescent staining with anti-S-100 antibodies (secondary FITC-conjugated), fluorescence microscopy, 50 μm scale

potentially be used to repair injured cranial bones, which are formed in ontogenesis from the NC cells via mechanisms of intramembranous ossification. So, therapeutically used cells must have correct positional information for cranial bone recovery [37].

We have developed a cell culture system that allows NC-MSCs efficient expansion with preservation most of the cells in a stem/progenitor state (85.61±13.23% nestin+ cells at P3-culture).

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## ВЛАСТИВОСТІ МУЛЬТИПОТЕНТНИХ СТОВБУРОВИХ КЛІТИН — ПОХІДНИХ НЕРВОВОГО ГРЕБЕНЯ З БУЛЬБАРНОГО РЕГІОНУ ВОЛОСЯНОГО ФОЛІКУЛА *in vitro*

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Описано спосіб одержання культури мультипотентних стовбурових клітин — похідних нервового гребеня — з бульбарного регіону волосяного фолікула вібрис дорослих мишей та досліджено їхні біологічні властивості. Виявлено, що клітини мають фібробластоподібну морфологію, є нестин-позитивними та цитокератин-негативними, експресують поверхневі маркери CD44, CD73, CD90 і Sca-1. Цей клітинний тип виявляє в культурі функціональні властивості стовбурових клітин: клоногенність, здатність до самовідновлення, ріст у вигляді флотуючих сфер та здатність до спрямованого мультилінійного диференціювання. Завдяки цим властивостям мультипотентні стовбурові клітини — похідні нервового гребеня — є перспективними для застосування в галузі регенеративної медицини.

***Ключові слова:*** нервовий гребінь, мультипотентні стовбурові клітини, волосяний фолікул, клоногенність, сфероґенез.

## СВОЙСТВА МУЛЬТИПОТЕНТНЫХ СТВОЛОВЫХ КЛЕТОК — ПРОИЗВОДНЫХ НЕРВНОГО ГРЕБНЯ БУЛЬБАРНОГО РЕГИОНА ВОЛОСЯНОГО ФОЛЛИКУЛА *in vitro*

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Описан способ получения культуры мультипотентных стволовых клеток — производных нервного гребня из бульбарного региона волосяного фолликула вибрисс взрослых мышей и исследованы их биологические свойства. Установлено, что клетки имеют фибробластоидную морфологию, являются нестин-позитивными и цитокератин-негативными, экспрессируют поверхностные маркеры CD44, CD73, CD90 и Sca-1. Данный клеточный тип в культуре проявляет функциональные признаки стволовых клеток: клоногенность, самообновление, сферогенез и способность к направленной мультилинейной дифференциации. Благодаря этим свойствам мультипотентные стволовые клетки — производные нервного гребня — являются перспективными для применения в области регенеративной медицины.

***Ключевые слова:*** нервный гребень, мультипотентные стволовые клетки, волосяной фолликул, клоногенность, сферогенез.