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СОДЕРЖАНИЕ

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КЛЕТОЧНЫЕ ТЕХНОЛОГИИ

МЕЖДУНАРОДНАЯ КОНФЕРЕНЦИЯ
“Новейшие достижения в области клеточных технологий: наука и практика. Трансляция исследований в практику”

(International Conference “Cell Technologies at The Edge,” CTERP 2018) Москва 11–13 апреля 2018 г.

Материалы конференции

На конференции рассмотрены следующие вопросы:

Фундаментальные исследования в области клеточных технологий. Клеточная терапия и регенеративная медицина сегодня. Лучшие практики в области клеточной терапии и разработки клеточных продуктов. Персонализированная медицина. Тканевая инженерия. Биобанки. Редактирование генома для клеточной терапии и научных исследований. Коммерциализация клеточной терапии, продуктов и технологий, основанных на биоматериалах. Клеточные модели заболеваний.

Конференция проведена при поддержке Отделения биологических наук РАН, ФАНО России и Российского фонда фундаментальных исследований.

* Ма

the human midbrain is a complex 3-dimensional structure composed of multiple cell types, including neurons, astrocytes and oligodendrocytes, which has been difficult to model *in vitro*, particularly in a scalable manner. Here, we describe a novel approach to obtain large numbers of high quality midbrain organoids, derived from our unique midbrain floor plate neural progenitor cells (mfNPCs) that can be differentiated with high efficiency in classical 2D cultures. Using these cells, we report the generation of midbrain-specific organoids containing astrocytes, oligodendrocytes, and midbrain dopaminergic neurons, which can be used for disease modeling. 3D organoid cultures of mfNPCs form functional neuronal networks and secrete dopamine. Importantly, midbrain organoids derived from Parkinson's disease specific stem cells reveal disease relevant phenotypes. mfNPCs, enable research to study human midbrain development, modeling of region-specific neurodegenerative diseases, and high throughput drug screening using organoids.

Keywords: iPSC, neural stem cells, midbrain dopaminergic neurons, midbrain-specific organoids, Parkinson's disease.

The Inhibition of Immunoproteasome Subunit LMP7 Reduces the Efficiency of Reprogramming Somatic Cells into Pluripotent Stem Cells

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Abstract – The ubiquitin-proteasome system plays an important role in maintaining protein homeostasis and in the regulation of many cellular processes. The proteasome is a multi-subunit protease complex, consisting of a core 20S particle and 19S regulatory particles. Under certain conditions, the constitutive catalytic subunits of the 20S particle beta1, beta2 and beta5 can be replaced by special subunits – beta1i/LMP2, beta2i/Mecl-1 and beta5i/LMP7. In this case the proteasomes, designated as immunoproteasomes (IPs), are involved in antigen presentation. On the other hand, it has been shown that there is an increased expression of the IP subunits in murine embryonic stem (ES) cells during their differentiation. Besides, there is an increased expression of IP subunits in human ES cells, while the expression of these subunits drops during their differentiation. These observations imply that IPs are involved in regulation of pluripotency and differentiation of pluripotent cells. Another intriguing issue regards a possible role of IPs in the induction of cellular pluripotency. To address the latter question, we have performed reprogramming of mouse embryonic fibroblasts (MEF) into induced pluripotent stem cells (iPSCs), using a doxycycline-activated transgene construct OSKM (polycistronic sequence of factors Oct4, Sox2, Klf4 and c-Myc). Throughout the process of reprogramming the cells were treated with a selective inhibitor of beta5i/LMP7 subunit – PR-957, and the inhibitors of the beta5, beta1i/LMP2 and beta5i/LMP7 subunits of the proteasome – MG-132. Staining for alkaline phosphatase – a known marker of undifferentiated pluripotent cells – showed a significant decrease of the efficiency of reprogramming MEF treated in the presence of the inhibitors. Our results indicate the important functions of both proteasomes and IPs in the process of cellular reprogramming.

This work was supported by RSF 16-14-10343.

Keywords: ubiquitin-proteasome system, immunoproteasome, PR-957, induced pluripotent stem cells.

IL-6 Orchestrates the Migration of Urokinase Receptor-Deficient Neuroblastoma Cells

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Abstract—Relevance. Neuroblastoma is a extracranial tumor of the nervous system with a highly invasive phenotype. Previously it was shown that activation of urokinase and its receptor (uPAR) has a stimulating effect on migration and invasion of most cancer cells. Here we show a new mechanism

of neuroblastoma migration involving IL-6 upregulation.

Purpose of the research. To determine the role of the uPAR in the migration of neuroblastoma cells.

Research methods. Downregulation of uPAR (Neuro2a-ΔuPAR) in Neuro2a murine neuroblastoma cell line was carried out using CRISPR/Cas9. Overexpression of uPAR (Neuro2a-uPAR) was performed using plasmid transfection with cDNA of murine uPAR. uPAR and IL-6 content was evaluated by Western blot. Wound scratch assay was performed to evaluate the migration capacity of cells. Results were analyzed within 24 h using Leica AF6000LX detection system and data counting by Wound Healing Tool (ImageJ software).

Results. We investigated the effect of uPAR downregulation on migration of Neuro2a cells in the presence of its genuine ligand uPA. We demonstrated that the uPA-mediated Neuro2a migration inversely depend on uPAR expression: administration of uPA within 12 h of the experiment resulted in a 1.5-fold increase in Neuro2a-ΔuPAR migration to the scratch wound compared to control and Neuro2a-uPAR cells. After 24 h Neuro2a-ΔuPAR cells almost completely (up to 79%, $p < 0.05$) recolonized the scratch wound, while control and Neuro2a-uPAR cells recolonized it to a significantly less extent (9 and 12%, correspondingly). Furthermore in Neuro2a-ΔuPAR cell we detected a significant upregulation of IL-6 expression – a highly potent migratory and metastatic cytokine for neuroblastomas.

Conclusions. These results allow us to propose a novel mechanism of uPAR-dependent neuroblastoma cell migration involving IL-6.

Source of funding: grant no. 14-24-00086 of the Russian Science Foundation and grant no. 14-50-00029 of the Russian Foundation of Basic Research.

Keywords: uPAR, IL-6, neuroblastoma, cell migration, CRISPR/Cas9.

Evaluation of the Influence of Superparamagnetic Iron Oxide Nanoparticles on the Properties of Bone Marrow Mesenchymal Cells under in vitro Conditions

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Abstract—Integration of nanoparticles into medicine as therapeutic or diagnostic tools is a rapidly developing field, with a great potential for use in regenerative medicine. Currently, superparamagnetic iron oxide nanoparticles (SPIONs) are the most used and approved by the FDA for clinical use (enhanced contrast in MRI, targeted drug delivery, tumor therapy). The use of nanoparticles in cell therapy makes it possible to trace the further fate of cells implanted in the body. Despite the fact that SPIONs are the only metal oxides approved for medical use, their influence on cell functions is still being discussed.

Objective. To investigate the effects of SPIONs on the functions of cultured cells, in particular on the expression of cytoplasmic proteins under in vitro conditions.

Materials and methods. Human mesenchymal bone marrow cells (MSCs FET) were used in the work. The cells were first incubated with SPIONs (less than 50 nm in size) at a concentration of 150 μg/mL for 24 hours, then the cells were re-introduced in a ratio of 1 : 3, after which they were incubated at different times (1, 3, 6, 10, 24 and 48 hours) and fixed by freezing. Using the methods of protein electrophoresis and immunoblotting, a cytoplasmic cell extract was analyzed.

Results and conclusions. Quantitative differences in the content of cytoplasmic proteins in the experimental variant and in control cells that did not contain nanoparticles were found. The inclusion of SPIONs by cells effectively influences the expression of cytoplasmic proteins with molecular masses of 55 kDa and 70 kDa (presumably, tubulin and HTS-70) at terms of 3, 6, 10, 24 h. In addition, immunoblotting at the same time was the difference in the content of cytoplasmic G-actin is revealed, which implies the influence of nanoparticles on the nature of the organization of the cytoskeleton and the ability of cells to migrate.

The work was carried out with the financial support of the RNF Grant no. 14-50-00068.

Keywords: nanoparticles, regenerative medicine, human mesenchymal bone marrow cells.