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ISOLATION AND CHARACTERIZATION OF RODENT NERVOUS TISSUE-DERIVED CELL CULTURE

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Tissue-derived cell culture has been widely used in medical research. The ability to produce *in vitro* cultures of neuronal cells has been fundamental to advancing our understanding of nervous system functions. The perspective direction in the treatment of socially significant diseases is the application of cell replacement therapy. Therefore the development of methodological approaches to establishment of viable cell cultures is currently important.

Keywords: cell culture, nervous tissue, morphology, phenotype.

The development of methods of obtaining viable cell cultures of nervous tissue, which can be further used for modeling of pathogenic mechanisms of neurodegenerative diseases, new therapeutic protocol testing and the evaluation of treatment effectiveness are very important and actual up to date. The complexity of neuronal cell culture isolation is determined by the heterogeneity of nervous tissue (neuronal cells, astrocytes, oligodendrocytes, radial glia, ependymal cells, and microglia) and the lack of the standardized protocol of the establishment of cell culture. Therefore the development of methodological approaches to produce the cultures of neuronal cells *in vitro* is currently important and has been fundamental to advancing our understanding of nervous system functions.

Aim. The optimization of the neuronal cell culture isolation method and the estimation of its morpho-phenotypic specific features *in vitro*.

Materials and methods. Neuronal cells were isolated from the rodent brain (n=6) using three protocols: only the mechanical disaggregation of the tissue with the subsequent cultivation of the explants (1) and the combination of mechanical disaggregation with the treatment of the tissue with collagenase I type (2) or trypsin (3). The cell suspension was plated in the concentration of 10^6 cells per well of a 24-well plate, covered partly with fibronectin for improvement of cellular adhesiveness. The cells were cultivated in DMEM-F12 supplemented by 10 % fetal bovine serum, 1 % antibiotic-antimycotic and 1 % L-glutamine at 37 °C under 5 % CO_2 condition. The monitoring of cell cultures morphology and growth *in vitro* was done by a phase contrast microscopy method. The phenotype of the cell cultures was determined using the immunocytochemistry with the immunoperoxidase visualization of nestin, vimentin and nerve growth factor receptor (NGFR).

Results. It was established that the neuronal cell cultures isolated using the mechanical tissue disaggregation protocol formed the multicellular aggregates attached to a substrate on the 4–6th day, with the proceeded cellular growth. The majority of the nerve tissue cells (95 (90÷96) %) prone to the formation of sprouts and further intercellular contacts were observed after a mechanical tissue disaggregation combined with enzymatic treatment with trypsin solution. However, the neuronal cell cultures cultivated in the presence or absence of the special adhesive fibronectin cover did not significantly differ in the adhesive capacity.

It was shown, that the cell cultures obtained from the rodent brain via the mechanical and enzymatic treatment of nervous tissue had higher proliferative activity and colonial growth compared to the cell culture obtained via only the mechanical method. On the 10–14th day of cultivation, the cells acquired the typical morphology of nerve cells, characterized by the formation of neuritis, the tendency to form intercellular contacts, and also the expression of specific markers (vimentin, nestin, and NGFR) after a mechanical tissue disaggregation combined with enzymatic treatment with trypsin solution. The cell cultures were characterized by certain heterogeneity and can include stem / progenitor nerve cells, mature neuronal and glial cells. It was established that the cells isolated from the brain tissue of rodents via mechanical and enzymatic disaggregation revealed the expression of vimentin (93(89÷95) %), nestin (90(87÷91) %) and NGFR (91(89÷93) %) and there were no statistically significant differences in their expression in different isolation protocols.

Conclusion. The obtained results allowed us to determine the optimal method of the separation of rodent nervous tissue-derived cell culture, which can be further used for modeling of pathogenic mechanisms of neurodegenerative diseases and for the evaluation of treatment protocol effectiveness including cell therapy.

EXOSOMES AS BIOMARKERS IN PATHOLOGY

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Exosomes can be used as biological markers for both negative and positive changes in the environment that surrounds the object under the study. It could be done through tracking the amount of the exosomes, which are secreted by cells before and after changes in the environment. It is also possible to determine the changes in the qualitative composition of exosomes.

Keywords: Exosomes, molecular organization, functions, exosome detecting methods, exosome content, applications of exosomes.

Environmental factors constantly affect biological objects. Consequently, cells secrete special vesicles, as a specific way of communication. Communication between cells is the most important way of regulating of vital activity of all multicellular organisms [1].

Most eukaryotic cells secrete membrane vesicles (exosomes) that can affect both neighboring and distant cells. Exosomes formed inside secretory cells in endosomal compartments are called multivesicular bodies. Exosomes are produced by a number of cell types: reticulocytes, platelets, B and T cells, mast cells, dendritic cells, macrophages, Schwann cells, astrocytes, neurons, melanocytes, mesothelioma cells, intestinal epithelial cells, adipocytes, fibroblasts, and tumor cells. Exosomes have been described to exist in many biological fluids (for example, breast milk, blood, urine, amniotic fluid, saliva, cerebrospinal fluid etc.). An exosome can contain up to 4563 proteins, 194 lipids, 1639 mRNA and 764 miRNA [2].

Exosomes have multiple functions and can be considered as an alternative method of intercellular communication. The physiological functions of exosomes include the participation in intercellular communication, the transportation of various molecules from the donor cell to the recipient cell, the stimulation of the immune system, the presentation of the antigen, immunosuppressive effects on immune and tumor cells.

The role that exosomes play in malignant and virus-infected cells is widely known. For instance, pathogenic organisms can use exosomes for intercellular communication. The pathological role of exosomes in the development of such diseases as Burkitt's lymphoma, Glioblastoma, Creutzfeldt-Jakob disease, Systemic amyloidosis, Alzheimer's disease, Huntington's disease is widely known as well [4].

Exosomes can be studied both *in vitro* and *in vivo*. The main approaches for isolating exosomes are the use of monoclonal and polyclonal antibodies, a western blot analysis, a FACS analysis, electron microscopy.

In addition to the application in clinical therapy, exosomes are utilized as a cancer vaccine (Sipuleucel-T became the first immunotherapeutic vaccine that functions using the antigen presentation that involves dendritic cells); diagnostic biomarkers, and drug delivery vesicles.

Thus, exosomes can be used as biological markers for both negative and positive changes in the environment that surrounds the object under the study. It could be done through the tracking of the amount of exosomes, which are secreted by cells before and after changes in the environment. Moreover, it is also possible to determine the changes in the qualitative composition of exosomes.

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