

## **Study of neuroregenerative effects of progenitor cells *in vitro***

Due to the limited capabilities of endogenous stem/progenitor cells (SCs/PCs) for effective neurogenesis in the affected central nervous system (CNS), the possible ways to its enhancing are considered. The use of different types of SCs/PCs remains at the forefront of the latest developments in technologies for restoring the damaged CNS. To date, more than 470 clinical randomized controlled trials on the safety and efficacy of SCs/PCs application in CNS diseases have been registered, most of which involve the use of neurogenic SCs/PCs (NSCs/NPCs) or mesenchymal multipotent SCs (MMSCs). But the mechanism of the therapeutic effect of SCs/PCs is far from being understood. Previously, preference was given to the concept of cell replacement of affected CNS areas, however, recent studies support the mechanism of SCs/PCs paracrine influence. Paracrine effects of SCs/PCs contribute to neurotrophic support (neuroprotection) or immunomodulation due to the ability to produce a wide range of biologically active signaling molecules (secretome) — cytokines, growth and neurotrophic factors, microRNAs, etc., which are involved in the key cell signaling processes.

**The aim** — to study the effects of conditioned media (CM) from cultures of fetal neurogenic cells (FNCs, as a source of NSCs/NPCs) and adipose-derived mononuclear cells (adMCs, as a source of MMSCs) in model of nerve tissue regeneration *in vitro*.

**Methods.** Fetal rat brain cells (14th day of gestation, E14) were cultured until reaching a confluent monolayer with main cell types of nervous tissue, then the scratch assay was performed and the DMEM with 10% fetal calf serum (standard culture conditions, control), or the FNCs-CM or adMCs-CM in concentrations 0.1, 0.2 or 0.3 mg/ml (according to the total protein amount) were added. CM were obtained from 24-h cell cultures of rat FNCs (E14) or adMCs (adult). At the 4th and 8th day after scratch assay immunocytochemical and morphometric studies were performed.

**Results.** In 24-h FNCs cultures the number of Nestin+ cells (immunopositive for type VI intermediate filament protein, one of the NSCs/NPCs markers) was  $66,9 \pm 0.9\%$ ; cells had the ability to form “spheroids”, the potency upon cultivation to differentiate into neurons and astrocytes, confirming the presence of the predominant NSCs/NPCs fraction. In 24-h adMCs cultures the number of CD105+ cells (immunopositive for endoglin, one of the MMSCs markers) was  $82.8 \pm 0.7\%$ ; cells had the ability to form “spheroids”, the potency upon cultivation in adhesive surface

to differentiate into at least three cell types, evidencing the presence of the predominant MMSCs fraction.

FNCs-CM as well as adMCs-CM in dose-dependent manner stimulated migration processes in culture of rat neural cells, cell differentiation into GFAP+ astrocytes and beta-tubulin III+ neurons, branching of processes (including dendrites and axonal branching) and forming a network, thus contributing to overgrowing of the scratched area. After addition of FNCs-CM the overgrown zone reached 70.5% of full length of the scratched area; after addition of adMCs-CM — 97,4–100%. The addition of FNCs-CM and adMCs-CM resulted in beta-catenin translocation into nucleus of cells in rat neural cell cultures, which correlated with the overgrowth of the scratched area, evidencing the involving of beta-catenin signaling pathway in stimulating of rat neural cells migration.

**Conclusion.** FNCs-CM and adMCs-CM are a source of signaling molecules which modulate the microenvironment and activate endogenous repair mechanisms, enhancing migration and differentiation processes in neural cell culture (*in vitro* model of nerve tissue regeneration).