Retina

IDENTIFICATION AND ACTIVATION IN VITRO OF HIDDEN CELL SOURCES FOR REINAL REGENERATION IN LOWER AND HIGHER VERTEBRATES

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In our study we applied the novel method of 3D, long-term, rotary organotypic culturing of eye tissues and their sandwiches. The method was to enable to give an opportunity to maintain tissue viability and induce regenerative responses of some cells.

In vitro experiments we used isolated neural retina (NR) and retinal pigmented epithelium (RPE) in the content of posterior eye cup. Eye tissues were obtained from the adult newt (Pl. waltl.) and albino rat (Wistar). After cultivation of NR alone we applied methods of morphology, BrdU or PCNA immuno-assays, and cell-type specific proteins’ immunohistochemistry. We found that in both newt and rat NR spheroids formed in 1 week in vitro there were populations of cells in G1, S- and M phases. In newt NR proliferating cells were localized in retinal growth zone and nuclear layers, as well. The presence of the pool of blast cells was confirmed by the expression of nucleostemin and βII-tubulin identified by PCR. Later, in 1 month we registered many undifferentiated cells which divided, migrated and replaced of some lost retinal cells, including recoverin+ photoreceptors. In rat NR we found outer nuclear layer cells translocation and entering of macroglial, vimentin/GFAP+ cells and macrophages into M phase. However, neither changes of cell differentiation nor cell growth from retinal periphery were observed in the rat NR in vitro.

The RPE cells of the newt demonstrated in vitro different behavior: from long term keeping their initial morphology to producing of retinal anlage, NF-200+ cells. Number of BrdU+ cells could be increased by a removal of RPE underling coats and FGF2 addition to the medium. RPE cells of the rats demonstrated in vitro rare mitoses, withdrawal from the layer, phenotype change and expression of macrophage antigen. These events were very similar to those well known in PVR and other eye diseases. Interestingly, that some of RPE cells both of the newt and rat lowered a level of their differentiation and expressed pan-neuronal proteins (NF-200, β-tubulin).

In order to increase the number of cells that could respond by proliferation and pro-neuronal differentiation we added in vitro the antioxidant SkQ1 at the beginning of culturing. In the newt NR SkQ1 increased a pool of undifferentiated cells up to 80% of the total number of NR cells. In the rat RPE it remarkably reduced cell death and inhibited transformation of RPE cells towards macrophage phenotype. In present in vitro-like in vivo models we developed are used for revealing of genes and signal molecules responsible for an induction of NR and RPE cells regenerative responses.