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Response of Human Retinal Pigment Epithelial Cells to the Effect of the Conditioned Media of Newt Retinal Regenerates

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The development of the retina and signaling pathways involved in this process are conserved within vertebrates. Therefore it was hypothesized that active factors stimulating the retinal regeneration in newts can cause transdifferentiation of human retinal pigment epithelial (RPE) cells. To verify this, we studied the effect of conditioned media (CM) obtained from *in vitro* regenerating retinas of newts on the phenotype, expression of a number of genes, and localization of cell proteins of human RPE cells of the ARPE-19 line. Within 120 h of cultivation, a change in the morphology of human RPE cells was observed, which manifested itself in a change in shape and an increase in cell size, which was accompanied by a decrease in proliferative activity. 24 hours after exposure to CM, ARPE-19 showed a short-term increase in the expression of *TUBB3* (a panneuronal marker) and a decrease in the expression of *BMP2* and *BMP4* neural differentiation inhibitors. Using immunocytochemical (ICC) methods, during the period between 24 and 72 h after exposure, we observed an increase in the intensity of staining of cells with antibodies to βIII-tubulin protein and a decrease in the intensity of staining with antibodies to the Cx43 protein of gap junctions (compared to the control). At the same time, we observed a decrease in the levels of mRNA expression of the RPE markers *OTX2* and *KRT18*, as well as the *KLF4* gene, which is responsible for proliferation and differentiation. Additionally, the shift in the distribution of β-catenin to cytoplasmic localization was recorded using the ICC method. To exclude mesenchymal (and osteogenic in particular) differentiation under the effect of CM, we studied qPCR expression of the *SPP1* and *RUNX2* genes. No *SPP1* transcription was found in ARPE-19 cells in the control and under the effect of CM, and a decrease in the expression level of *RUNX2* mRNA under the effect of CM was observed. Therefore, an attempt to shift to neuronal differentiation was observed in ARPE-19 cells during the period between 24 and 72 h after exposure to the CM of newt retinal grafts, as indicated by an increase in transcription and translation of βIII-tubulin, weakening of intercellular adhesion and a decrease in the expression of inhibitors of neural differentiation *BMP2* and *BMP4*, as well as RPE markers *OTX2* and *KRT18*. However, after that, due to the short-term effect of CM, during the period between 72 and 120 h after exposure, the cells returned to the initial differentiation, as evidenced by a decrease in the expression of neuronal differentiation markers (*NES*, *TUBB3*, *PAX6*) and an increase in the expression of RPE markers (*OTX2*, *MITF*). Abbreviations: CM – conditioned medium, RPE – retinal pigment epithelium, ESC – embryonic stem cells, EMT – epithelial-mesenchymal transition.

Keywords: retinal pigment epithelium, ARPE-19, conditioned medium, newt retina grafts, bFGF, proliferation, differentiation, gene expression, *KLF4*