Expression of VEGF-A Gene in Normal Retina and in Proliferative Vitreoretinopathy Retina

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Introduction. Vascular Endothelial Growth Factor-A, VEGF-A, is a key angiogenesis protein in embryonic development and in adult tissues. It is also involved in the pathogenesis of different human diseases including eye disorders, like proliferative vitreoretinopathy (PVR). Both in normal tissue expression and in PVR VEGF-A are regulated by p53 family members. This protein induces cellular differentiation regulating division in staminal cells, leading to asymmetric division, while its loss of function leads to the symmetric division, resulting in cellular growth.

Material and methods. The study was performed on nine retinal PVR samples, taken during necessary retinectomies, and one healthy human retina as control.

Quantitative real-time reverse transcriptase PCR (qRT-PCR): expression levels of p53, p63, p73 and VEGF-A genes were evaluated through quantitative Real-time reverse-transcriptase PCR analysis qRT-PCR which was performed with TaqMan technology using the ABI Prism 7000 apparatus (Applied Biosystems, Foster City, CA, USA). Gene expression analysis was performed using TaqMan® Assays-on-Demand containing primers and fluorescent probe mix (Applied Biosystems, Foster City, CA, USA). All reactions were in triplicate, and the negative control was obtained by performing qRT-PCR without cDNA. Normal human retina was used as control to normalize gene expression levels in the relative quantitative analysis, using the comparative cycle threshold (ΔCt) method. The cycle threshold (Ct) was determined as the initial increase in probe fluorescence above background, and the ΔCt was the difference between amplification cycles (Ct) of the target and endogenous control. Further, statistical analysis to individuate possible relationship between genes under study was performed.

Results. Expression of all genes in normal tissue was revealed in both samples affected by PVR. Controls showed basal levels of VEGF-A expression; however, the study indicated VEGF-A over-expression in samples affected by a marked proliferative vitreoretinopathy compared with healthy human retina. P53, p63 and p73 gene seems to have a significant relationship with VEGF-A expression.

Conclusions. The increase of VEGF-A levels in PVR tissues promotes differentiation and cell survival and decreases apoptosis suggesting its role in proliferation of retinal cells. The analysis could be critical for the studies aimed to novel therapies for retinal diseases.