REGULATION OF RPE PHENOTYPE BY ANNEXIN A8 AND WNT SIGNALLING

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Fenretinide (FR), a retinoic acid derivative, is capable of trans-differentiating retinal pigment epithelial (RPE) cells towards a neuron-like phenotype in culture. Microarray analysis post-FR treatment revealed down-regulation of Annexin (Anx) A8 as well as proteins involved in Wnt signalling in trans-differentiated cells. AnxA8, a calcium-dependent phospholipid-binding protein, is expressed in the RPE cell cytosol, where it is believed to be involved in membrane and cytoskeletal organisation and cell proliferation. The aim here was to analyse the role of AnxA8 and its interaction with Wnt signalling in RPE cell trans-differentiation. Therefore, human RPE cells were seeded at a concentration of 2,200/cm^2 and treated with 3% charcoal dextran-treated foetal bovine serum (FBS) for 24h. 1 µM FR or vehicle (0.1% dimethylsulfoxide) was added every day for 7 days. As a second approach, AnxA8 was suppressed in RPE cells using short interfering RNA (siRNA). FR and AnxA8 siRNA treatment both induced a decrease in AnxA8 expression and inhibited cell proliferation. It further led to trans-differentiation of ARPE-19 cells into neuron-like cells and a concomitant up-regulation of the neuronal markers Calbindin and Calretinin analysed by qPCR and immunofluorescence. Additionally, expression of Wnt signalling proteins such as β-Catenin, Frizzled-1, Frizzled-4, Wnt2b and Wnt3a was decreased. The effect of FR was partially reversible by activation of Wnt signalling using recombinant Wnt3a or SB216763, a glycogen synthase kinase-3β inhibitor. Wnt inhibitors, such as Dickkopf-1 and DAPT were not able to reverse the FR effect. These data imply an important role for AnxA8 in maintaining RPE phenotype. Down-regulation of AnxA8 appears to be sufficient for neuronal trans-differentiation of RPE cells and the expression of neuronal markers. Further, the interdependence of AnxA8 and Wnt proteins suggests that AnxA8 might be an important regulator in Wnt signalling.

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