

Regulation of RPE phenotype by Annexin A8 and Wnt signalling

Lueck K, Greenwood J, Moss SE.

Department of Cell Biology, UCL Institute of Ophthalmology, 11-43 Bath Street, London EC1V 9EL, United Kingdom

Purpose.

Fenretinide (FR), a retinoic acid derivative, is capable of trans-differentiating retinal pigment epithelial (RPE) cells into a neuronal-like phenotype in culture. Microarray analysis pre- and post-FR treatment revealed down-regulation of Annexin (Anx) A8 and various proteins involved in Wnt signalling in trans-differentiated cells. AnxA8, a member of a superfamily of calcium-dependent phospholipid-binding proteins, is expressed in RPE cells and involved in membrane and cytoskeletal organisation and cell proliferation. The purpose of this study was to analyse the role of AnxA8 and its relationship with Wnt signalling in epithelial trans-differentiation.

Methods.

At 10% confluence, human RPE cells were treated with 3% charcoal dextran-treated foetal bovine serum (FBS) for 24 h. 3 μ M FR or vehicle (0.1% dimethylsulfoxide) was added to the cells every day for 7 days. As a second approach, AnxA8 was suppressed in RPE cells using short interfering RNA (siRNA). Cells were then analysed for expression of AnxA8, neuronal markers (Calbindin, Calretinin) and Wnt signalling proteins (β -Catenin, Frizzled-1, Frizzled-4, Wnt2b, Wnt3a) using immunofluorescence staining, qPCR and western blot analysis.

Results.

FR and AnxA8 siRNA treatment both induced a decrease in AnxA8 expression and inhibited cell proliferation. FR also led to trans-differentiation of ARPE-19 cells into neuron-like cells and a concomitant up-regulation of neuronal markers. Additionally, expression of proteins involved in Wnt signalling was decreased. The effect of FR was partially reversible by activating Wnt signalling using recombinant Wnt3a or SB216763, a glycogen synthase kinase-3 β inhibitor.

Conclusions.

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.