Regulation of epithelial cell phenotype by annexin A8

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Background
The retinoic acid derivative Fenretinide (Fr) is capable of trans-differentiating retinal pigment epithelial (RPE) cells towards a neuron-like phenotype in culture. Microarray analysis of Fr-treated ARPE-19 cells revealed down-regulation of annexin (anx) A8 in trans-differentiated cells. AnxA8, a calcium-dependent phospholipid-binding protein, is expressed in RPE cells, where it may be involved in membrane and cytoskeletal organisation and cell proliferation.

Objectives
The purpose of this study was to analyse the role of anxA8 in maintaining the RPE cell phenotype.

Methods
RPE cells were seeded at 2,200 cells/cm^2 and treated with 3% charcoal dextran-treated foetal bovine serum (FBS) for 24 h. 1µM 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). Further, an anxA8-GFP construct was used to overexpress anxA8 and to restore the anxA8 loss derived from Fr or anxA8 suppression. Cells were analysed for anxA8 and the neuronal markers Calbindin and Calretinin using immunofluorescence staining and qPCR.

Results
Fr and anxA8 siRNA treatment both induced a decrease in anxA8 expression and inhibited cell proliferation. They also led to RPE trans-differentiation into neuron-like cells and a concomitant up-regulation of neuronal markers. Overexpression of anxA8 led to a recovery of the anxA8 loss-induced neuron-like cell phenotype.

Conclusions
These data reveal an important role for anxA8 in maintaining RPE phenotype. Down-regulation of anxA8 appears not only to be sufficient, but also to be necessary for neuronal trans-differentiation of RPE cells and the expression of neuronal markers.

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