Accelerated maturation of ARPE-19 cells for the translational assessment of gene therapy

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Conclusion

Our study demonstrates that the combination of NAM with LN-521 accelerated the expression of RPE signature genes, reduced expression of EMT (epithelial to mesenchymal transition) genes, accompanied with improved epithelial cell morphology and cytoskeletal organization. This allows the dedifferentiated ARPE-19 to be closer to their *in vivo* phenotype. Moreover, viral-mediated RPE-specific gene expression demonstrates the amenability of the combinatorial cultures for translational assessment of RPE gene therapies.

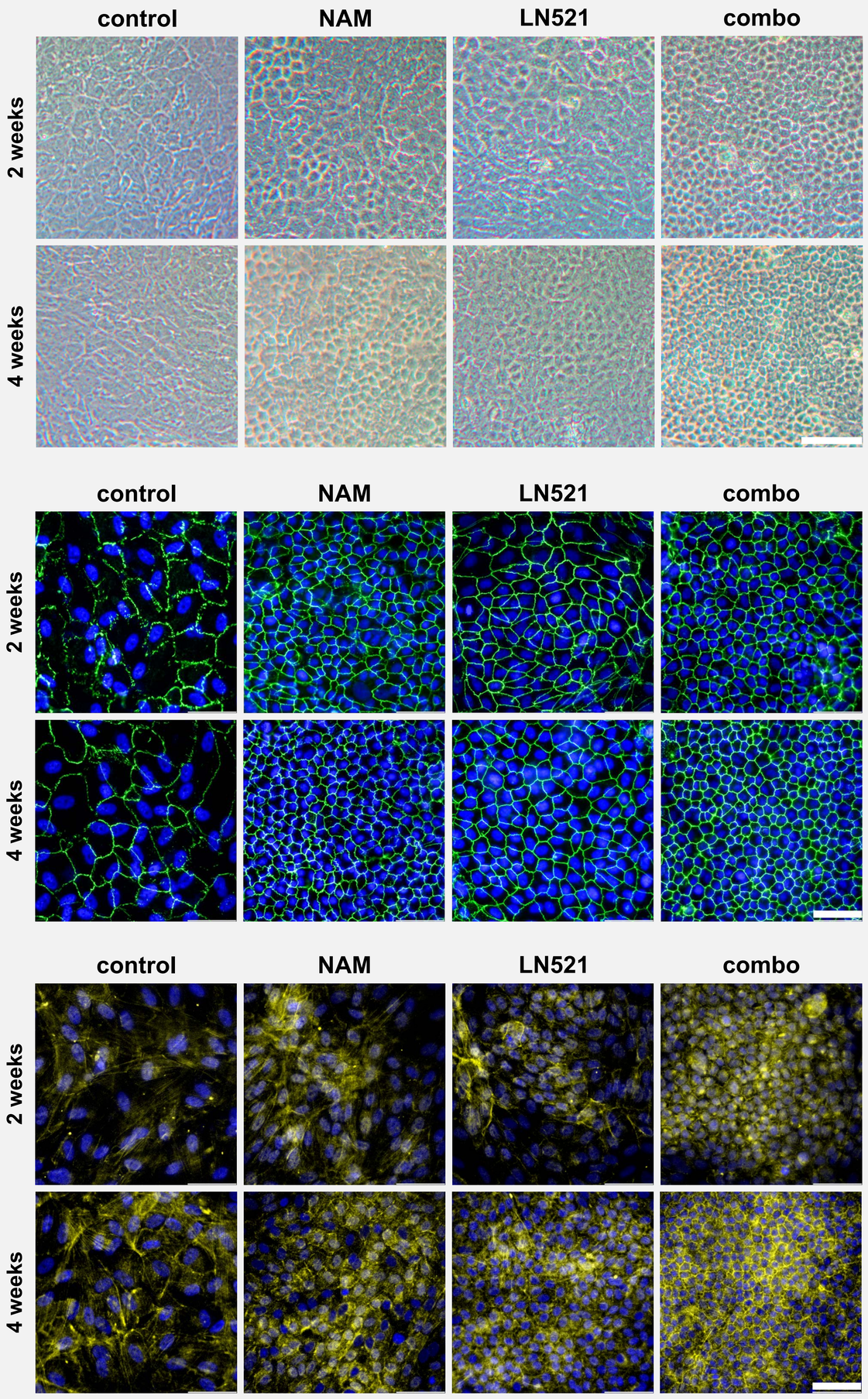
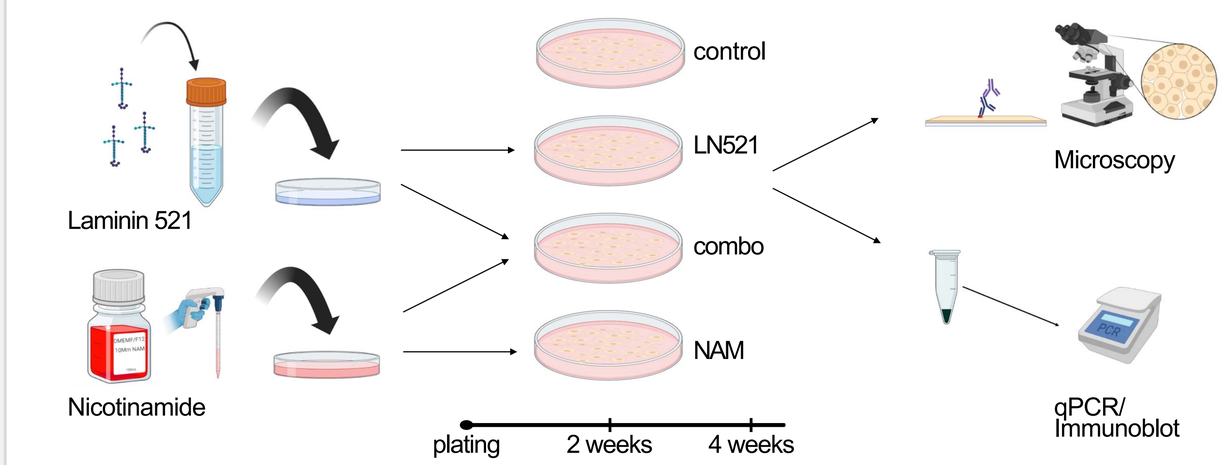


***Figure* – Structural reorganization of ARPE-19 cells in supplemented culture conditions.** Cobblestone morphology can be appreciated after 2 weeks of culture in the combo condition. Tight junctions organization was studied by ZO-1 immunolabelling (green). The RPE-specific BEST-1 expression was assayed by immunolabelling (yellow). Hoechst was used as fluorescence counterstain for the nuclei (blue). Scale bar = 100µm.

Experimental design



Background

The human retinal pigment epithelium (RPE) cell-line ARPE-19 is widely used as an alternative to primary RPE. However, these cells undergo epithelial-mesenchymal transition losing many features of primary RPE such as polarity, pigmentation, and expression of RPE signature markers, limiting their usefulness as an in vitro model of RPE cells.

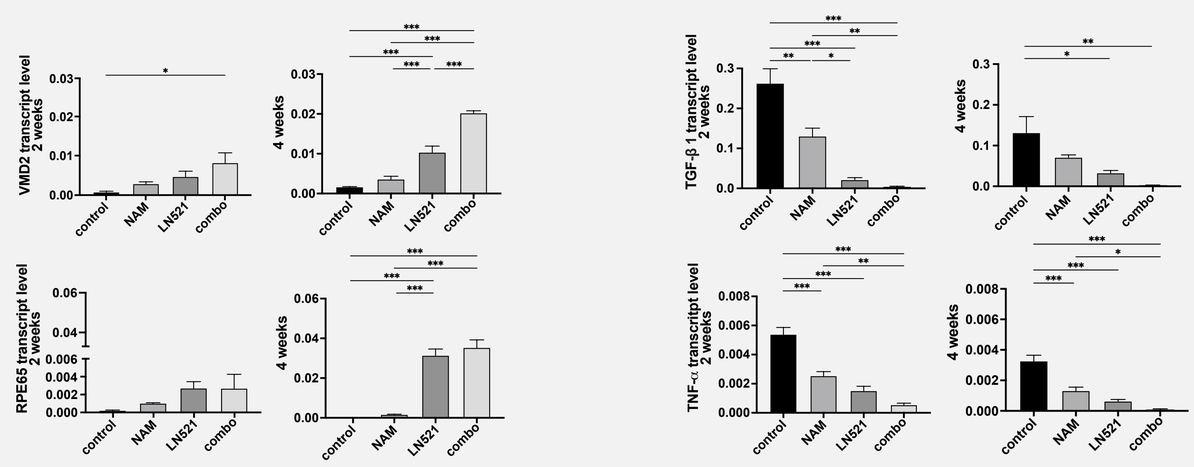


***Figure* – Transcriptional regulation of RPE signature, differentiation and EMT genes.** qPCR analysis of RPE signature and EMT related genes was assayed in ARPE-19 cells in the different culturing conditions at 2-week and 1-month.

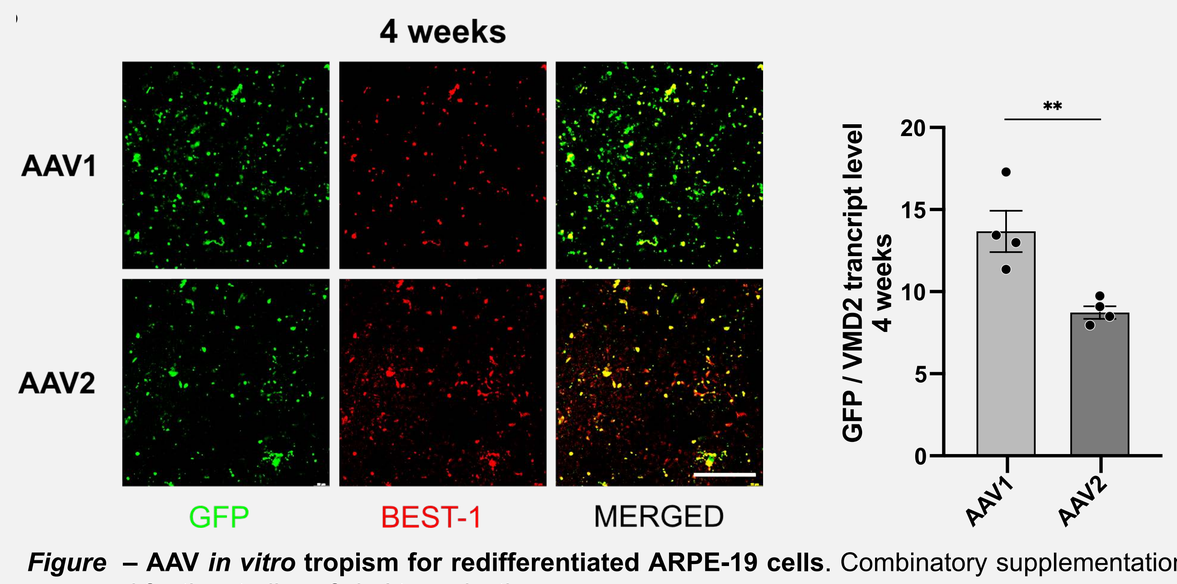


***Figure* – AAV *in vitro* tropism for redifferentiated ARPE-19 cells**. Combinatory supplementation was used for the studies of viral transduction.

Perspective

The establishment of a fast and cost-effective cell model opens the opportunity for *in vitro* gene therapy screening assays. This allows to understand molecular mechanisms in human RPE on a cellular level.

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