Differentiated retinal pigment epithelial cells as a model for uncovering sigma-2 receptor functions and novel therapeutics for dry AMD

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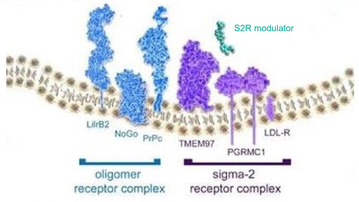
INTRODUCTION

In dry age-related macular degeneration (dry AMD), dysfunctional retinal pigmented epithelial (RPE) cell trafficking of lipids and photoreceptor outer segments is followed by RPE degeneration. Currently, there is an ongoing clinical trial assessing the effect of the sigma-2 receptor (S2R, TMEM97) modulator CT1812 in dry AMD(NCT05893537). The S2R has been linked to dry AMD in genome-wide association studies[1,2], and small molecule modulators of S2R rescue RPE functional deficits[3]. S2R interacts with proteins involved in lipid trafficking, such as low-density lipoprotein receptor (LDLR) and Niemann-Pick Protein 1 (NPC1)[4]. Given that disruption in lipid trafficking is a key factor in dry AMD[5,6], we hypothesize that S2R plays a functional role in this process and measured the effects of S2R modulation on LDL transport in a model of human, mature RPE cells.

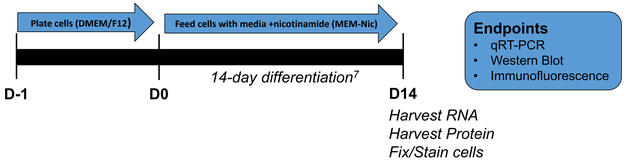


Schema 1. The sigma-2 receptor is comprised of TMEM97 and PGRMC1 (purple). This receptor complex closely interacts with the LDL receptor (LDLR, grey) as well as other co-receptors such as prion protein (PrP, blue).

METHODS

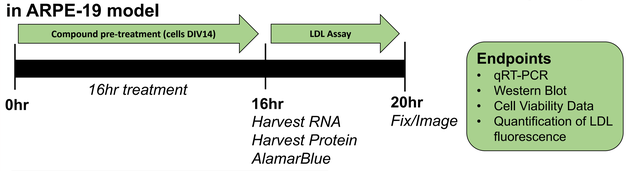
Goal: Uncover the mechanism of S2R involvement in LDL trafficking at the retinal pigment epithelium *in vitro*

Aim 1: Characterize TMEM97/S2R levels in differentiated ARPE-19s



Endpoints
- qRT-PCR
- Western Blot
- Immunofluorescence

Aim 2: Determine effects of S2R modulators on the LDL transport system in ARPE-19 model



Endpoints
- qRT-PCR
- Western Blot
- Cell Viability Data
- Quantification of LDL fluorescence

Treatments			
Compound	**Doses Tested**	**Compound Details**	**S1R/S2R K_i**
U18666A[8]	1-30μM	Inhibits LDL biosynthesis and lysosomal transport	---
Z4857158944	1-10μM	S2R modulator	S2R: 3.4nM
CT2074	1-10μM	S2R modulator	S2R: 21nM
PRE084	1-10μM	S1R agonist	S1R: 2.2nM

Culturing Medias		
Media	**Abbreviation**	**Purpose**
DMEM/F12 Complete Growth Medium	DMEM/F12	Plating/ Expanding cells
MEM Complete Growth Medium +Nicotinamide (10mM)[5]	MEM-Nic	Feeding cells/ Differentiation

Differentiated ARPE-19s demonstrate hallmark morphology and markers of mature RPE

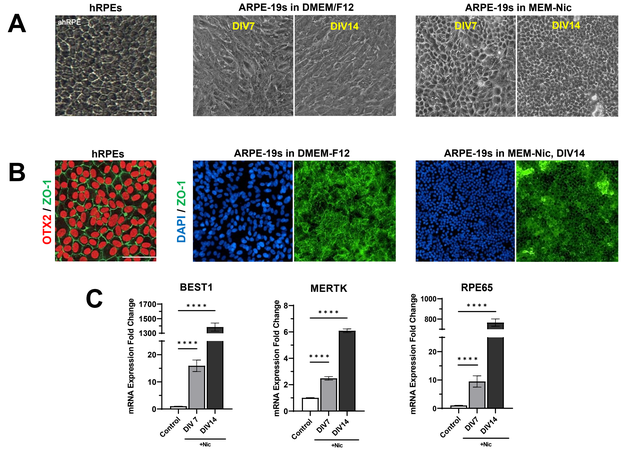


Figure 1. A) Brightfield images of adult human RPE cells[9] (left), and ARPE-19 cells at different stages of differentiation (MEM-Nic) compared to a control culture (DMEM/F12). B) Immunofluorescence staining showing expression pattern of ZO-1 in adult human RPE cells[6] (left), and ARPE-19 cells (right) with or without differentiation in MEM-Nic. C) qRT-PCR analysis of RPE-specific gene expression after 7 and 14 days of differentiation in MEM-Nic (control conditions n=14, DIV7 conditions n=4, DIV14 conditions n=10); normalized to EIF4A2 + control, mean +/- SEM, unpaired t-test. **** p<0.0001

TMEM97 and co-receptors are differentially expressed during RPE maturation

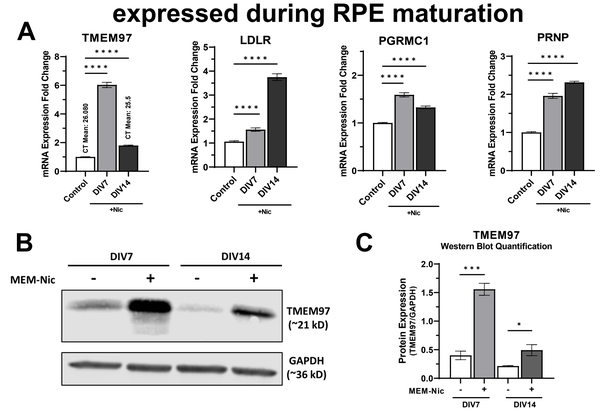


Figure 2. A) qRT-PCR analysis of TMEM97 and co-receptors' expression after 7 and 14 days of differentiation (medium), control (DMEM/F12) conditions n=14, DIV7 conditions n=4, DIV14 conditions n=10, normalized to EIF4A2 + control, mean +/- SEM, unpaired t-test, **** p<0.0001. B) Western blot showing the expression of TMEM97 after 7 and 14 days of differentiation, compared to control (DMEM/F12), DIV7 samples N=2, DIV14 samples N=4. C) Quantification of the western blot in B, TMEM97 normalized to GAPDH, DIV7 samples N=2, DIV14 samples N=4, mean +/- SEM, unpaired t-test, * p< 0.0332 *** p<0.0002.

U18666A treatment induces a dose-dependent increase in tagged LDL fluorescence

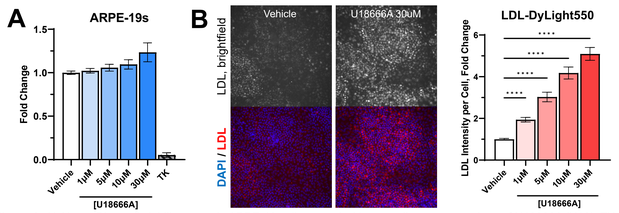


Figure 3. A) AlamarBlue cell viability assay in ARPE-19 cells treated with ascending concentrations of U18666A (U18), TK = total kill control, (N=4), normalized to vehicle, mean +/- SEM. B) LDL-Dylight assay fluorescence representative images +/- U18666A and quantification of a concentration response experiment assessed by CX7 Spot Detector. Spot Total Intensity per Cell after treatment with increasing concentrations of U18, N=4, normalized to vehicle; mean +/- SEM, ordinary one-way ANOVA.**** p<0.0001

TMEM97 expression correlates with U18666A-mediated LDL fluorescence increase

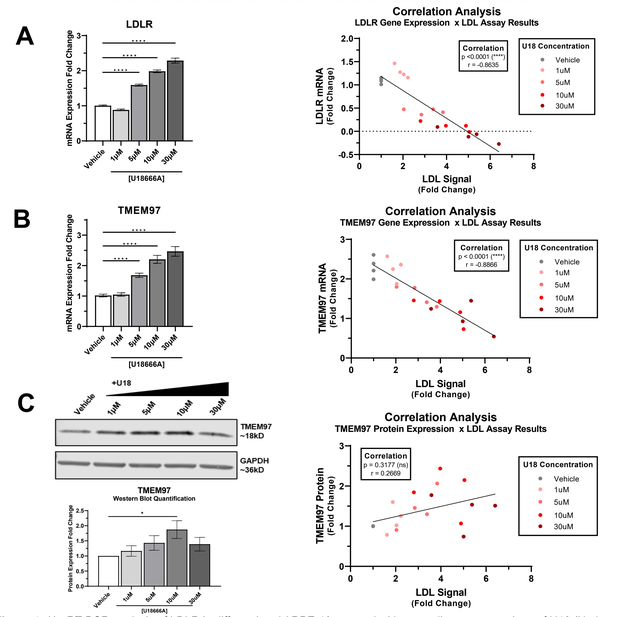


Figure 4. A) qRT-PCR analysis of LDLR in differentiated ARPE-19s treated with ascending concentrations of U18 (N=4, normalized to EIF4A2 and vehicle, mean +/- SEM, ordinary one-way ANOVA) with corresponding correlation analysis comparing the expression of mRNA (ΔCT, normalized to EIF4A2) with U18 effect size (fold change, normalized to vehicle) in the LDL assay, **** p<0.0001. B) qRT-PCR analysis of TMEM97 in differentiated ARPE-19s treated with ascending concentrations of U18 (N=4, normalized to EIF4A2 and vehicle mean +/- SEM, ordinary one-way ANOVA) with corresponding correlation analysis as in A, **** p<0.0001. C) Western blot and quantification of differentiated ARPE-19s treated with ascending concentrations of U18 (N=4, normalized to GAPDH and vehicle, mean +/- SEM, ordinary one-way ANOVA) with corresponding correlation analysis of protein (fold change, normalized to GAPDH + vehicle) with U18 effect size as in A, * p< 0.0332. Lines shown on correlation analyses are simple linear regressions.

S2R modulators exhibit concentration-dependent increase in LDL fluorescence

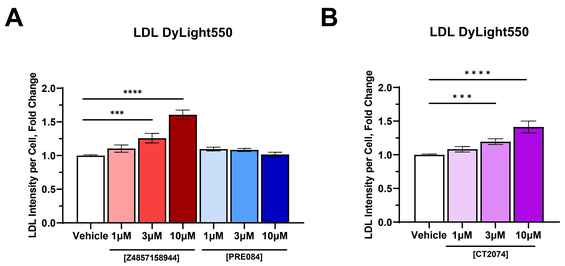


Figure 5. A) LDL-Dylight assay fluorescence quantification assessed by CX7 Spot Detector, Spot Total Intensity per Cell shown after treatment with increasing concentrations of S2R receptor modulator (Z4857158944) or S1R modulator (PRE084); data normalized to vehicle, N=5-12, mean +/- SEM, ordinary one-way ANOVA. *** p<0.0002, **** p<0.0001. B) LDL-Dylight assay fluorescence quantification assessed by CX7 Spot Detector, Spot Total Intensity per Cell shown after treatment with increasing concentrations of proprietary S2R receptor modulator (CT2074); data normalized to vehicle, N=7, mean +/- SEM, ordinary one-way ANOVA. *** p<0.0002, **** p<0.0001. These data combined with Fig.4 suggest that TMEM97 expression is involved with LDL transport at lysosomes in the RPE, and that TMEM97 modulation can affect the receptor's activity. Experiments aimed at uncovering the mechanisms behind the correlations found in this study are ongoing.

CONCLUSIONS

- S2R and its co-receptor expression increase during ARPE-19 differentiation, making this a useful model for investigating S2R function in RPE
- LDL fluorescence increases with U18 treatment, which correlates with increased TMEM97 expression, making it a useful tool for the development of a S2R-driven functional assay
- An increase in LDL fluorescence occurred after treatment with S2R, but not S1R, modulators, indicating that the S2R plays a functional role in LDL transport at the RPE
- Results warrant continued effort towards assay development and target validation studies to uncover mechanisms underlying S2R modulator-mediated LDL trafficking using this model

These findings support the further investigation of the role of S2R in the RPE, and the continued development of S2R modulators for restoring RPE functions

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