RPV4 is involved in myofibroblast transdifferentiation of retinal pigment epithelial cells

Posterboard#: A0451

Abstract Number: 3164 - A0451

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DisclosureBlock: Shigeo Tamiya, None; Siham Hourani, None; Ganapathy Jagatheesan, None;

Purpose
Myofibroblast plays an essential role in fibrosis. Myofibroblast transdifferentiation of retinal pigment epithelial (RPE) cells have been implicated in retinal fibrotic complications such as proliferative vitreoretinopathy and exudative age-related macular degeneration. However, the detailed mechanisms as well as key molecules involved is yet to be clarified. In this study, we examined the role of TRPV4 channel on myofibroblast transdifferentiation of RPE cell in vitro.

Methods
Porcine RPE cells were isolated from freshly obtained eyes using dispase as previously reported . Cells were routinely cultured in DMEM supplemented with FBS, and used for experiments at 2nd or 3rd passage. QRT-PCR was used to detect expression of several TRP channels as well as cell phenotype markers. Ca imaging was performed using cells loaded with Cal-520 AM dye and stimulated with TRPV4 specific agonist GSK1016790A in the presence or absence of TRPV4 antagonist HC067047. Myofibroblast function was assessed by collagen hydrogel contraction assay, and western blot analyses were performed to determine myofibroblast marker protein alpha-SMA (aSMA).

Results
QRT-PCR showed RPE cells expressing TRPV4 but not another family member TRPV1. GSK1016790A, TRPV4 agonist, increased intracellular calcium, detected as increased Cal-520 fluorescence, was blocked by preincubation in HC067047, TRPV4 antagonist, confirming the presence of functional TRPV4 channels on cultured RPE cells. HC067047 significantly inhibited TGFbeta2-induced collagen hydrogel contraction by RPE cells, and this was accompanied by reduced expression of aSMA protein.

Conclusions
Functional TRPV4 channels expressed on RPE cells plays a key role in myofibroblast transdifferentiation.