

The Role of Cross-linked Actin Networks in the Proliferation and Senescence of Primary Human Trabecular Meshwork Cells

Authors: **Joseph M. Dalloul**¹, Devon Harvey¹, Jiannong Dai¹, Chenna Kesavulu Sugali¹, Weiming Mao^{1,2}

Affiliations: ¹Indiana University School of Medicine, Department of Ophthalmology; ²Indiana University School of Medicine, Department of Biochemistry and Molecular Biology

Purpose: TGF β 2 has been found to be elevated in the trabecular meshwork (TM) of patients with primary open-angle glaucoma. TM cells treated with TGF β 2 form cross-linked actin networks (CLANs). These CLANs are a restructuring of the cells' actin cytoskeleton that appear as web-like (2D)/spherical (3D) structures. In this study, we used TGF β 2 treated primary human TM (pHTM) cells to illuminate the relationship between CLAN formation and cell proliferation and senescence.

Methods: A characterized pHTM cell strain was seeded into a 96-well plate. Some cells were treated with 5ng/mL TGF β 2 for 1 week while the others served as a control. The cells were then fixed and immunostained for cell proliferation (Ki-67) and senescence (p21, H2AX, and β -galactosidase) markers. DAPI was also used to stain nuclei and phalloidin-Alexa-488 to stain F-actin/CLANs. Analysis was conducted through student's t-tests.

Results: TGF β 2-treated pHTM cells formed significantly more CLANs compared to control. The cell proliferation marker Ki-67 was rarely expressed in both groups (not statistically significant) but was mostly observed in CLAN- cells and seen only in the control. Ki-67 was almost completely absent in CLAN+ cells. Among senescence markers, none were expressed significantly differently between the two groups, however TGF β 2 led to an increase in p21 and β -gal. Most CLAN+ cells expressed p21. No cells expressed H2AX.

Conclusions: TGF β 2 increases the formation of CLANs in pHTM cells. It seems that CLAN formation inhibits cell proliferation. However, the effect of CLAN formation on cell senescence is not clear. More investigation is needed to help further understand the relationship between CLAN formation and cell proliferation and senescence.