Live cell imaging of F-actin in dexamethasone treated trabecular meshwork cells
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Purpose: Regulation of actin cytoskeleton in trabecular meshwork (TM) and Schlemm’s canal endothelial (SCE) cells is important for the control of aqueous humor outflow. Some reports showed administration of dexamethasone increased aqueous humor outflow resistance and induced unusual actin structure, such as cross-linked actin networks (CLANs), in TM cells. However, the mechanism of the phenomenon has not fully clarified, partly because actin stress fibers were observed only in fixed cells. To dissolve this limitation, we conducted live-cell imaging of actin dynamics in TM cells with or without dexamethasone treatment.

Methods: The GFP-actin was transfected with Cell Light® Actin GFP (Invitrogen) in porcine TM cells. The GFP-actin expressed cells were fixed and stained with phalloidin-TRITC. Time-lapse imaging for live TM cells treated with 100 nM dexamethasone or vehicle (DMSO) was performed using inverted fluorescence microscope. Fluorescent and phase contrast images were recorded every 30 minutes for 72 hours after dexamethasone treatment.

Results: The GFP-actin expressed about 22.6 ± 10.0 % of transfected TM cells. The distribution of GFP corresponded to the distribution of phalloidin labeled F-actin in TM cells. In live TM cells, many actin stress fibers were observed before treatment. The vehicle treated cells showed ameboid movement during observation. Parts of dexamethasone treated cells presented CLAN formation within 72 hours after treatment, and showed less ameboid movement after CLAN formation.

Conclusions: CLAN formation after dexamethasone treatment was correlated with ameboid movement in TM cells. The live cell imaging of actin cytoskeleton may provide valuable information on the actin dynamics in TM cells.