Exploring Relationships between RhoA Signaling and YAP in Multiple Cell Types

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Introduction:

To be able to repair tissues, one must understand the intracellular proteins and pathways that direct cell growth and movement. Transcriptional coregulator Yes-associated protein (YAP) is one protein that upregulates cell proliferation. RhoA, a GTPase involved in reorienting the cytoskeleton to facilitate cell movement, is known to regulate YAP, but little is known about how it does this. To better understand the interactions between these proteins, we utilized optogenetics, which involves modifications of proteins to become light sensitive. To this effect, genes for an Opto-RhoA tool have been created and experimented with. We used this tool to observe what occurs when RhoA signaling is activated, and to better understand the relationship between RhoA, YAP, and cell proliferation and movement.

Materials and Methods:

Opto-RhoA is created by combining RhoA with a photoreceptor protein (BcLOV4) and a staining protein (mCherry), so that the tool can be light activated and seen with fluorescence microscopy. Using DNA isolated from bacterial plasmids for Opto-RhoA, human embryonic kidney cells (HEKs) and UMR-106 cells were transfected with Opto-RhoA and first observed live using fluorescence microscopy. Cells were quickly exposed to EGFP light and observed to note changes in morphology and Opto-RhoA localization. After live imaging, cells were fixed and immunostained to locate YAP, nuclei, and actin in the cells. Using images of these fixed cells, we compared cells with and without induced RhoA activation to see if YAP localized in different locations.

Results and Discussion:

From live imaging, we found that activation of RhoA causes membrane localization of RhoA, as well as breaking of cell-cell and cell-ECM adhesions, which facilitated contraction in these cells. Light activated cells without Opto-RhoA demonstrated no movement. From the fixed-cell images, we noted that RhoA activated cells had higher concentrations of nuclear YAP and lower concentrations of cytoplasmic YAP (see Figure 1). Higher nuclear YAP leads to the transcription of proliferative proteins, and therefore higher probability of cell proliferation. These findings show that activation of RhoA signaling induces cell contraction and proliferation.

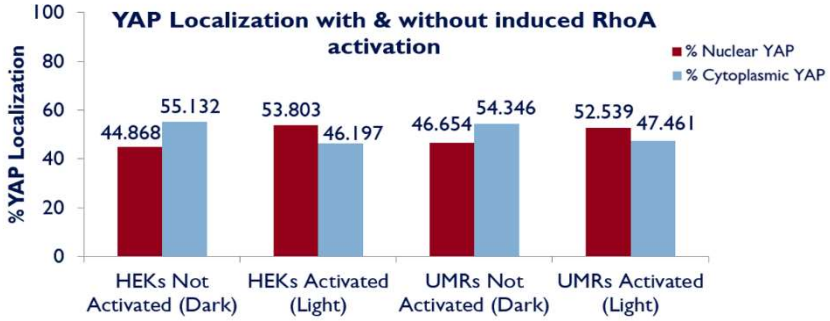

Figure 1. Graph of YAP localization by area (Nuclear YAP in maroon, Cytoplasmic YAP in blue) in HEK cells (left) and UMR cells (right) with and without RhoA activation. RhoA activated cells were found to have higher nuclear YAP and lower cytoplasmic YAP.

Conclusion:

These experiments demonstrated that RhoA activation seems to facilitate YAP acting as a transcriptional coregulator by helping localize YAP to the nucleus. We also observed that RhoA activation promotes cell contraction and may help regulate proliferation. These findings help solidify a relationship between RhoA and YAP and confirm these proteins as key factors in the proliferative and movement processes of the cell. The applications for these proteins in wound healing and bone fracture repair increase notably with these findings.

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