Cytoskeletal feedback control of mechanotransduction and cell motility by YAP/TAZ
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Cell motility is critical to developmental morphogenesis and is influenced by physical cues from both the extracellular matrix and from other cells. Generally, cells move away from one another (termed contact inhibition) and from softer to stiffer regions of a tissue (durotaxis). My laboratory is interested in understanding how cells sense and respond to these mechanical cues, and how these signals influence development.

Here, we tested the hypothesis that the transcriptional regulators Yes-associated protein (YAP) and transcriptional co-activator with PDZ-motif (TAZ) mediate the mechanical regulation of vasculogenesis using vasculogenic, cord blood-circulating endothelial colony forming cells (ECFCs). We first observed that YAP/TAZ depletion by RNAi completely abrogated ECFC motility. We initially hypothesized that this was through transcriptional control of canonical angiocrine factor expression (i.e., Cyr61). Surprisingly, however, this effect was instead cell-autonomous, demonstrated both by candidate-based rescue attempts and conditioned media swap experiments. Our subsequent data reveal a feedback mechanism that allows cells to establish cytoskeletal equilibrium and enables motility in response to mechanical signals communicated at cell-cell and cell-matrix interfaces, critical features of vascular morphogenesis.

We observed profound changes in ECFC morphology and cytoskeletal organization on soft (E = 1.85 kPa) vs. stiff (29 kPa) polyacrylamide substrates, which mechanocyto embryonic and mature soft tissues, respectively. YAP and TAZ were mechanosensitive in ECFCs, and were both stabilized and activated in a manner dependent on motility status. While YAP and TAZ were dispensable for microtubule polarization, meaning YAP/TAZ-depleted cells could still sense a migratory stimulus from physical cell-cell interactions, these cells were effectively tethered in place, illustrated here by live-migration sparklines: siControl: , siYAP/TAZ: (25µm scale-bar: ).

Cell motility requires acto-myosin force production and new focal adhesion formation, but this must be coordinated with cytoskeletal remodeling and focal adhesion disassembly to enable forward motion. We found that YAP/TAZ depletion increased stress fiber formation through filamentous F-actin polymerization from monomeric G-actin, resulting in a 1.8-fold increase in migration-dependent cell stiffness compared to control cells, measured by microindentation at the cell apex (Fig. 1). YAP/TAZ interference also increased focal adhesion number, density, and maturity, though this was not due to defective focal adhesion disassembly, as both β1-integrin internalization and recycling by Rab7+ endosomes were not impaired by YAP/TAZ depletion.

Rather, YAP/TAZ depletion increased myosin light-chain phosphorylation, driving tension-dependent focal adhesion growth, but not formation of tension-independent focal adhesions. Inhibition of myosin-II (Blebb) or the myosin activator ROCK (Y-27632) significantly rescued stress fiber overgrowth, focal adhesion formation, and cell motility. We therefore searched for putative YAP/TAZ-regulated genes that could control myosin activation. We found that mRNA expression of the myosin phosphatase de-activator, NUAK2, was upregulated in YAP/TAZ-depleted cells, and YAP/TAZ/NUAK2 co-depletion significantly normalized the cytoskeleton, focal adhesions, and cell motility. Functional assays confirmed the necessity of this pathway in vasculogenesis both in vitro and ex vivo.

Taken together, we have uncovered an intracellular feedback mechanism by which YAP/TAZ, activated in response to motile stimuli or mechanical properties of the ECM, regulate cytoskeletal and focal adhesion dynamics through NUAK2 regulation of myosin, to control ECFC contractility, motility, and vasculogenesis.

Figure 1. YAP/TAZ depletion inhibits migration and increases cell stiffness in ECFCs.