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POSTER ABSTRACTS

501.HEMATOPOIETIC STEM AND PROGENITOR CELLS AND HEMATOPOIESIS: BASIC AND TRANSLATIONAL

Blood Flow Directs Yap/Taz-Mediated Transcriptional Regulation of Self-Renewal Programs to Control Developmental HSPC Expansion By Mechanical Stimulation of Piezo1

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Hematopoietic stem and progenitor cells (HSPCs) emerge from artery-derived hemogenic endothelium (HE) in the vertebrate embryo during development. This process entails a cellular reprogramming from endothelial to hematopoietic gene signatures driven by the Runx1 transcription factor (TF), which is referred to as the endothelial-to-hematopoietic transition (EHT). A major goal of cellular therapeutics is to derive patient-specific HSPCs from iPSCs for clinical use, yet current differentiation protocols largely fail to recapitulate EHT to produce or expand long-lived multi-potent HSPCs *in vitro* suggesting an incomplete understanding of the *in vivo* regulatory cues. Physical forces of wall shear stress (WSS) and cyclic stretch (CS) produced by hemodynamic blood flow in embryos are one such cue required during EHT to generate HSPCs from HE, however the mechanisms by which these forces are sensed and converted into a "stemness" regulatory module remain undefined.

Our previous work implicated the Hippo pathway TF YAP as essential for the maintenance, but not initiation, of the hematopoietic program in newly specified HE. Here, using scRNA-sequencing of sorted zebrafish trunk endothelial cells to capture all stages of EHT progression, we find that overexpression of YAP drives lipid metabolism, cell cycling, and propagation of a hematopoietic gene regulatory network (GRN) in the earliest specified HE cells. By employing a heat shock-inducible dominant negative YAP zebrafish line, we unmask a latent role for the YAP paralogue TAZ in hematopoiesis, which can promote CD41 $^{+}$ and Flk $^{+}$/Myb $^{+}$ HSPC production upon reduced YAP function. YAP and TAZ initiate transcriptional responses downstream of mechanical stimuli and require DNA binding cofactors to regulate target genes. Surprisingly, luciferase assays in HEK293 cells demonstrated a potent synergistic effect of TAZ/RUNX1, but not YAP/RUNX1, in transcriptional regulation at RUNX enhancers.

Finally, by pharmacologic and genetic manipulation, we identify the stretch-gated membrane ion channel Piezo1 as a potent regulator of CS-induced YAP/TAZ mechanotransduction in HE. Stimulation of zebrafish embryos with the Piezo1 small molecule agonist Yoda1 significantly increases HSPC number and YAP target gene expression in a YAP-dependent fashion. A similar modulation of blood and YAP target genes in human iPSC-derived CD34+ HE cells is seen with Yoda1, suggesting that this stretch-Piezo1-YAP axis can be chemically tuned *in vitro* to enhance HSPC differentiation. These results illuminate molecular details of how YAP/TAZ directs the production of HSPCs from HE in response to mechanical cues, and have broader implications for alternate regulatory effects of mechanically-stimulated Hippo TFs depending on the transcriptional milieu in cell-type specific contexts.

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