4361 Identification of Secreted Factors with a Role in Hematopoietic Stem and Progenitor Cell Engraftment in the Developing Zebrafish

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The hematopoietic microenvironment regulates the behavior of hematopoietic stem and progenitor cells (HSPC) throughout vertebrate development. We sought to identify secreted factors that may play a role in HSPC engraftment in the caudal hematopoietic territory (CHT), an endothelial cell-rich vascular plexus that serves as the primary site of hematopoiesis in the developing zebrafish from 3-6 days post fertilization (dpf). We hypothesized that such factors would be highly expressed in endothelial cells relative to hematopoietic stem cells (HSCs). To identify these factors, endothelial cells and HSCs were purified from 3 dpf *Flk1:mcherry; Runx1:GFP* double transgenic zebrafish embryos and gene expression profiling was performed by microarray analysis. Gene set enrichment analysis of these data showed that zebrafish chemokines, cytokines, TGF-β, TNF, Notch and non-canonical WNT family members were enriched in the endothelial cell fraction with a nominal $P \leq 0.2$. Genes from the leading edge of these gene sets were then used as candidates for gain-of-function testing. Coding sequences from candidate genes were cloned downstream of the zebrafish *HSP70l* promoter and microinjected into wildtype zebrafish embryos at the single-cell stage. Gene expression was induced in F0 transgenic animals by heat shock at 36 and 48 hours post fertilization. HSPC numbers were assayed by performing whole-mount in situ hybridization to identify *runx1*- and *cmyb*-expressing cells at 3 dpf. WNT5A was found to enhance HSPC numbers in this assay ($P = 0.00046$). We conclude non-canonical WNT family members, in particular WNT5A, regulate HSPC engraftment in the developing zebrafish.

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