# GDCB Seminar

**Tuesday, Feb. 22, 2022 — 4:10 p.m. 1414 Molecular Biology Building**

# 'Ccl44 is essential for normal embryonic zebrafish hematopoiesis'

**Abstract:** Hematopoiesis is the process of blood formation dependent on the continual need for blood cell replenishment throughout the life of the organism. Capable of self-renewal and differentiation into daughter cells, hematopoietic stem and progenitor cells (HSPCs) differentiate into all the mature blood cells in the body. By studying the molecular pathways responsible for this differentiation, we can better understand and treat a multitude of blood diseases, which often arise from defects in these processes. The zebrafish (Danio rerio) is an ideal model organism because their blood development is similar to humans and they have high fecundity which allows us to look at a larger sample pool in a shorter time frame. In addition, the embryos develop outside the body making manipulation and observation easier. Through RNA sequencing, our laboratory identified the top 100 genes integral to HSPC maintenance identified from zebrafish supportive stromal cell lines. One such gene was chemokine (C-C motif) ligand 44, ccl44. Investigation with morpholinos showed a reduction in erythroid and myeloid cells which were then successfully rescued with co-injection of mRNA. To validate these findings, we also performed knockout experiments using the CRISPR/Cas9 system. Zebrafish with fluorescently labeled myeloid and erythroid cells were injected with ccl44 guide RNA along with Cas9 protein. The removal of ccl44 was confirmed with Sanger sequencing and RT-PCR. In mutant animals, a decrease in myeloid and erythroid cells was observed with fluorescent microscopy, flow cytometry, and a methylcellulose assay. Many phenotypic defects were also seen in the mutated embryos, including shortened tail length and spinal curvature. Elucidating the role of ccl44 in hematopoiesis and mesoderm formation should help us understand how the vertebrate hematopoietic system evolved and could have clinical importance for the treatment of human blood diseases.

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**Host:** Raquel Espin Palazon, genetics, development and cell biology assistant professor