MOLECULAR HAEMOPOIESIS 28

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ABSTRACT SUBMISSION FORM

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Abstract title	Novel insights on the role of CXCL12-CXCR4 signaling in hematopoietic development
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Abstract

Embryogenesis is a highly regulated process driven by the tight coordination of multiple signaling pathways, controlling blood vessel morphogenesis with the spatiotemporally associated emergence of hematopoietic progenitors. Before the generation of hematopoietic stem cells (HSCs), an early wave of fetal-restricted multipotent progenitors (eMPPs) emerges within the major arteries and contributes to blood formation *in utero* as well as postnatally. In this context, the role of CXCL12-CXCR4 axis, which is essential for adult hematopoiesis and inflammation, is not yet fully understood. The aim of this project is to dissect the specific requirements of CXCL12-HMGB1-CXCR4 signaling in the generation, expansion and migration of distinct subsets of embryonic hematopoietic progenitors.

Using the reporter CXCL12-eGFP mouse strain, we evaluated the expression of CXCL12 in primary and secondary hematopoietic sites at embryonic day (E) 10.5, detecting strong expression in the vitelline artery, which we previously observed to give rise to fetal-restricted hematopoietic progenitors. Moreover, CXCL12-null embryos exhibit a significant reduction in the number of fetal liver (FL) erythro-myeloid progenitors (EMPs; $Kit^{+}CD41^{lo}CD16/32^{+}$) and a delayed erythroid maturation.

To dissect the temporal requirement of the CXCL12-CXCR4 axis during fetal hematopoiesis, we took advantage of a conditional knockout strategy employing Cdh5-$CreER^{T2}$;$R26^{LSL\text{-}tdTomato}$;$Cxcr4^{fl/fl}$ transgenic mice. Preliminary data show that deleting CXCR4 during the window of emergence of the hematopoietic stem cell (HSC)-dependent wave tends to impair immunophenotypic HSC frequency starting from E12.5, while deleting the receptor during HSC-independent wave leads to a defect in erythroid maturation.

Moreover, we tested the role of High Mobility Group Box 1 (HMGB1) in embryonic hematopoiesis. HMGB1 is an additional ligand which binds CXCR4 as a heterodimer with CXCL12. HMGB1 knockout mice showed a phenotype similar to what we observed in CXCL12-null embryos, with a significant reduction in $Kit^{+}CD41^{lo}$ frequency in the peripheral blood and a defect in FL MPPs ($LSK^{+}CD48^{+}CD150^{-}$). Interestingly, the phenotype was more severe at E12.5 than E14.5, suggesting that HMGB1 is especially needed during the early stage of FL colonization.

Notably, hematopoietic colony-forming unit (CFU) assays across all the above models revealed diminished multilineage colony output. Furthermore, HMGB1-null mice displayed a broad reduction in the individual counts of all clonogenic subsets.

These findings underscore stage-specific functions of CXCL12, CXCR4 and HMGB1 in early blood development. Although, as expected, our data suggest common roles for these players, at the same time we highlight their specific requirements for distinct embryonic hematopoietic progenitors, including the early-arising subsets of eMPPs and EMPs, or definitive HSCs. By continuing to explore this pathway through *in vivo* transplants as well as transwell migration assays and single-cell RNA-seq, we will uncover insights with potential implications for congenital hematologic disorders, regenerative therapies and pediatric leukemias.