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ORAL ABSTRACTS

102.IRON HOMEOSTASIS AND BIOLOGY

Irf5 Expression in Macrophages Contributes to Iron Regulation within Erythromyeloblastic Islands

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Erythroblastic islands (EBIs) classically consist of a central macrophage supporting surrounding erythroid precursors as they develop from CFU-Es to reticulocytes. More recently, it has been shown that, in the bone marrow, these structures also support granulopoiesis, expanding our understanding of the niche, and leading to the concept of erythromyeloblastic islands (EMBI). Under certain inflammatory conditions, there is an imbalance between erythropoiesis and granulopoiesis, eventually leading to anemia and increased neutrophil counts. The central macrophage has been proposed to play a role in regulating this balanced blood production; however, the molecular mechanisms remain unknown. Recently, we demonstrated that mice lacking the transcription factor interferon regulatory factor 5 (*Irf5* $^{-/-}$) present iron deficiency anemia as they age, along with altered structures in the bone marrow EMBIs. Further, our CITEseq studies showed that Irf5 is specifically expressed by macrophages in the EMBIs. Therefore, we concluded that Irf5 is a regulator of the balance between erythropoiesis and granulopoiesis in the bone marrow erythromyeloblastic niche.

To understand the mechanisms leading to iron deficiency anemia, as well as changes in the island, we first undertook a comprehensive analysis of hematopoietic progenitors. We measured the relative amounts of megakaryocytic-erythroid progenitors (MEP), granulocyte-monocyte progenitors (GMP) and common myeloid progenitors (CMP) in the bone marrow and observed decreased MEP (WT: 82.81% vs KO: 76.28%, p-value 0.011), increased GMP (WT: 14.94% vs KO: 22.16%, p-value 0.002) and unchanged CMP compared to wild-type (WT) as the mice aged. Given that Irf5 is expressed in both hematopoietic and non-hematopoietic cells, we generated Irf5fl/fl-Vav-iCre+ mice to evaluate the contribution of non-hematopoietic Irf5 to EMBI formation and composition. We observed a significant reduction in the number of EMBI, with overrepresentation of myeloid cells, suggesting that the phenotype observed in *Irf5* $^{-/-}$ is mostly due to hematopoietic Irf5. However, when we measured the relative amounts of MEP and GMP in the Irf5fl/fl-Vav-iCre+ mice, these remained unchanged, suggesting that cell non-autonomous functions contribute to EMBI composition.

scRNAseq analyses of EMBIs from 3 and 9 months-old littermate-matched WT and *Irf5* $^{-/-}$mice revealed that heme synthesis was the only downregulated pathway. In addition, central macrophages from *Irf5* $^{-/-}$ EMBIs presented decreased expression of major iron regulatory proteins, including heme transporter Slc48a1 and Heme-Oxygenase-1 (HMOX1). Functional analyses using Calcein-AM staining and flow cytometry of freshly isolated central macrophages from enriched EMBIs of WT and *Irf5* $^{-/-}$mice revealed that the labile iron pool (LIP) of the central macrophage of *Irf5* $^{-/-}$ EMBIs was significantly reduced (mean 14.0) compared to WT (mean 49.0, p-value 0.004). This apparent decrease in intracellular iron was concomitantly supported by significant decreases in serum iron (WT: 67.27 uM/L vs KO: 39.67 uM/L, p-value: 0.0059), increased serum EPO (WT: 108 pg/dL vs KO: 163.8 pg/dL, p-value: .0075), and Prussian blue staining of *Irf5* $^{-/-}$spleen indicating iron deposition in aging *Irf5* $^{-/-}$ mice. Finally, these findings were conserved in Irf5fl/fl-Vav-iCre+ mice, highlighting a cell autonomous function for Irf5 in iron regulation within the island.

Together, these data suggest that Irf5 may be a new regulator of iron scavenging, trafficking, and/or utilization within the central macrophage, potentially playing a major role in the red-cell production capacity of the EMBI.

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