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POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Atg4a Promotes Mitochondrial Quality Control and Hemoglobin Production *In Vivo*

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Mitochondrial quality control is essential for maintaining cellular homeostasis and contributes to the regenerative capacity of hematopoietic stem cells and the differentiation of lineage-committed progeny. During red blood cell (RBC) production, mitochondria contribute to the production of hemoglobin and are the site for heme synthesis. Mitochondrial dysfunction is found in patients with mtDNA mutations, sickle cell disease, and myelodysplastic syndromes; therefore, it is critical to delineate the molecular mediators of mitochondrial quality control during erythropoiesis. While it is established that autophagy contributes to mitochondrial clearance during reticulocyte maturation, less is known about the regulation of mitochondrial quality control in erythroid progenitors. Our previous study identified an erythroid-specific role for the autophagy-related protease ATG4A in human erythropoiesis (Stolla MC et al., Blood Advances, 2022). To better understand the contribution of Atg4a-dependent autophagy to erythropoiesis *in vivo*, we have developed a new genetically modified mouse model .

To evaluate the role of Atg4a *in vivo*, we disrupted the expression of *Atg4a* by targeting exons 5/6 using CRISPR/Cas9 in C57/BL6 embryos. Analysis of the peripheral blood of adult *Atg4a-/-* (KO) and *Atg4a+/+* (WT) mice revealed a normal hematocrit (47.5% WT vs. 47% KO), but elevated numbers of red blood cells (10.7 M/μL WT vs. 11.2 M/μL KO, p=0.012) and a compensatory 5.8 % decrease in RBC volume (MCV: 44.3 fl WT vs. 41.7 fl KO, p=0.0004). Consistent with a reduction in RBC size, we observed a modest reduction in total hemoglobin (16.8 g/dl WT vs. 16.0 g/dl KO, p=0.054) and a profound decrease in the mean corpuscular hemoglobin concentration (MCHC: 35.39 g/dl WT vs. 34.17 g/dl KO, p=0.038) in *Atg4a -/-* (KO) mice.

To determine if *Atg4a-/-* mice are iron deficient, we evaluated iron parameters in peripheral blood. While *Atg4a-/-* mice had slightly lower values of serum iron (152 μg/dl WT vs. 128.7 μg/dl KO, p=0.21) and total-iron binding capacity (511 μg/dl WT vs. 456 μg/dl KO, p=0.04) they had comparable levels of transferrin saturation (29.9% WT vs. 28.1% KO, p=0.65). Furthermore, we found similar amounts of EPO in the serum of *Atg4a-/-* and *Atg4a+/+* mice (314 pg/ml WT vs. 345 pg/ml KO, p=0.78). Thus, loss of Atg4a leads to a reduction in hemoglobin production and RBC size without overt iron deficiency.

To determine if alterations in erythroid maturation contributed to the decrease in MCV and MCHC, we used flow cytometry to quantify bone marrow erythroid progenitors (I), basophilic erythroblasts (II), polychromatic and orthochromatic erythroblasts (III), and reticulocytes (IV) using CD44, Ter119, CD71, and lineage markers (CD11b, B220, Gr-1) in *Atg4a-/-* mice. Similar numbers of erythroid populations (I-IV) were observed in the bone marrow and spleen of *Atg4a-/-* and *Atg4a+/+* mice suggesting that erythroid maturation was not significantly altered *in vivo*. Since Atg4a is known to facilitate mitochondrial clearance during human erythropoiesis, we next evaluated mitochondrial dynamics in murine erythroid populations I-IV using dyes for active (TMRM) and total mitochondria (Mitotracker Green, MTG). Compared to *Atg4a+/+* mice, *Atg4a-/-* mice had increased MTG fluorescence in erythroid populations I and II and a 25% decrease in TMRM fluorescence. Therefore, loss of Atg4a significantly reduces the ratio of active-to-total mitochondria in erythroid progenitors and basophilic erythroblasts, indicating that elimination of inactive mitochondria promotes mitochondrial function early in erythropoiesis. Since mitochondria are the site for heme synthesis, we sorted erythroid populations from *Atg4a-/-* and *Atg4a+/+* mice and measured total heme content. While in *Atg4a +/+* mice heme content increased by 3-fold between erythroid populations III and IV, heme content did not increase between erythroid populations III and IV from *Atg4a-/-* mice resulting in significantly reduced heme content in reticulocytes. Our study highlights an important protective role for autophagy in erythropoiesis where it mediates the elimination of dysfunctional mitochondria. Loss of mitochondrial quality control restricts heme production reducing the hemoglobin concentration of circulating RBCs. Further investigation will determine if the absence of Atg4a compromises the response to stress erythropoiesis.

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