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POSTER ABSTRACTS

101.RED CELLS AND ERYTHROPOIESIS, EXCLUDING IRON

Disruption of the Malate-Aspartate Shuttle Leads to Anemia

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Erythropoiesis, the process of differentiating multipotent hematopoietic stem cells (HSCs) into mature enucleated red blood cells (RBCs), is a metabolically intensive process, resulting in the generation of over 2 million new RBCs per second. Although anemia due to impaired erythropoiesis is a common condition, therapeutic options are often limited to RBC transfusions. However, the emergence of effective metabolite therapies, such as glutamine supplementation for sickle cell disease, under-scores the potential of understanding metabolism for developing additional treatments.

Two key enzymes in the malate-aspartate shuttle (MAS), glutamic-oxaloacetic transaminase 1 (GOT1) and 2 (GOT2), play cru-cial roles in transferring energy in the form of NADH from the cytosol into the mitochondria for oxidative phosphorylation. NADH cannot directly cross the mitochondrial membrane, so its electrons are transported on malate, which enters the mi-tochondria through a specific transporter. Within this shuttle, mitochondrial GOT2 utilizes glutamine to generate aspartate, which is then transported back into the cytoplasm to be metabolized by GOT1. Previous research has shown that aspartate-dependent nucleotide biosynthesis is essential for HSC regeneration.

To investigate the roles of GOT1 and GOT2 in erythropoiesis, we crossed mice expressing tamoxifen-inducible erythroid-specific Cre recombinase (*Gata1-Cre* [ERT2] BAC transgenic mice) to *Got1* or *Got2* floxed lines, generating *Got1* [flox/flox];*Gata1-Cre* [ERT2](*Got1* CKO) or *Got2* [flox/flox];*Gata1-Cre* [ERT2](*Got2* CKO) mice, respectively. Upon tamoxifen administration to these mice, conditional *Got1* or *Got2* deletion occurs in megakaryocytic-erythrocytic progenitor (MEP) cells, which give rise to both RBCs and platelets.

Complete blood count analysis revealed decreased hemoglobin levels and RBC counts, along with a reciprocal increase in platelet count in both *Got1* and *Got2* CKO mice. These results were unexpected, as previous studies showed that hematopoi-etic *Got1* loss led to increased aspartate-driven nucleotide biosynthesis and HSC regeneration, while the opposite effects were observed in mice with hematopoietic *Got2* loss. Although these enzymes are expected to have opposite effects on aspartate levels, the deletion of either enzyme disrupts the transfer of reducing equivalents between the cytosol and mito-chondria. This results in NADH accumulation and reductive stress, which in turn leads to metabolic dysfunction, affecting glycolysis and oxidative phosphorylation. Therefore, the occurrence of anemia in mice with either *Got1* or *Got2* deletion in the erythroid compartment suggests that impaired redox balance, rather than aspartate-driven nucleotide biosynthesis, is likely the cause of the anemia.

To further understand the basis of anemia in *Got1* and *Got2* CKO mice, we harvested bone marrow cells and used conventional cell surface markers to quantify MEPs, burst-forming unit erythroid cells (BFU-Es), colony-forming unit erythroid cells (CFU-Es), and terminally differentiated erythroblast populations. Compared to littermate controls with no deletion of *Got1* and *Got2*, the absolute numbers of pre-CFU-Es and CFU-Es were higher in both *Got1* and *Got2* CKO mice, while the absolute numbers of differentiated erythroblasts were lower in both mutant mice. These findings indicate a block in erythroid differentiation,

which is currently under further investigation. Collectively, these preliminary data suggest a novel role for MAS in regulating erythropoiesis. Ongoing studies are actively exploring the mechanisms by which dysfunctional MAS leads to anemia.

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