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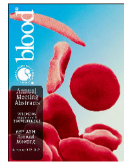

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POSTER ABSTRACTS

102.IRON HOMEOSTASIS AND BIOLOGY

Intracellular Iron Controls HSC Metabolism By Affecting Mitochondrial Fitness

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Iron is an important source of reactive oxygen species (ROS) within the cell and recent evidence highlighted its role in regulating HSC self-renewal and differentiation. However, whether and how iron influences HSC metabolism is still undetermined. Cellular metabolism is a key regulator of HSC maintenance and HSCs adapt their metabolic state to their function. Quiescent HSCs have low energy requirements and depend on anaerobic glycolysis, whereas active HSCs require high energy for proliferation and differentiation thus enhancing oxidative phosphorylation (OXPHOS), glycolysis and fatty acid oxidation (FAO).

To explore which metabolic pathways are triggered by intracellular iron levels, we took advantage of the thalassemic Hbb $^{th3/+}$ mice (*th3*), characterized by chronic iron overload (IO). We previously demonstrated an impaired function of *th3* HSCs due to persistence into altered BM niche (*Aprile et al., 2020*).

Th3 HSCs showed higher levels of intracellular free reactive iron (1.7±0.2 calcein MFI fold to wt, p<0.0001) and interestingly iron specifically accumulated in the mitochondria. As a result, mitochondria are impaired, with low mass and activity, as assessed by decreased mitochondrial membrane potential (*th3* 1068±167 vs. wt 3215±686 MFI TMRE, p<0.05). These data, together with a reduced expression of mitochondrial biogenesis and mitophagy genes, indicate an accumulation of damaged mitochondria. To demonstrate a direct effect of iron on mitochondrial fitness in HSCs, we treated wt mice with iron dextran. Consistently, IO wt HSCs showed reduced mitochondrial activity following intracellular iron accumulation.

Since mitochondria are the major site of cellular ATP production mostly through OXPHOS, we measured the metabolic state of HSCs in ß-thalassemia. ATP levels in sorted *th3* HSCs was halved than the one in wt mice (*th3* 3.7±0.3 vs. wt 9.8±2.6 ATP nM, p<0.01). Inhibition of OXPHOS by oligomycin lowered the ATP content in wt cells with a minor effect in *th3* HSCs, thus indicating a reduced OXPHOS-dependent metabolism, despite their active cycling profile. Since mitochondria are damaged and OXPHOS and glycolysis are the two main metabolic pathways involved in cellular ATP generation, we hypothesized increased glycolytic dependency to compensate for reduced OXPHOS in *th3* HSCs. Indeed, in these cells we observed a positive enrichment of glycolytic genes, such as *Tpi1*, *Gapdh* and *Pklr*, and a 1.4-fold higher glucose uptake (*th3* 2401±85 vs. wt 1639±163 MFI 2NBDG, p<0.05). *In vivo* administration of the iron chelator DFO normalized intracellular iron content and rescued mitochondrial dysfunction in *th3* mice, indicating the reversibility of mitochondrial defects. Moreover, HSCs from DFO-treated mice showed reduced glucose uptake, thus demonstrating that IO causes mitochondrial defects and metabolic adaptations of HSCs.

Excess iron is expected to give rise to ROS and, consistently, *th3* HSCs displayed high mitochondrial ROS (mtROS) levels (*th3* 5205±459 vs. wt 2507±756 MFI MitoSOX, p<0.01). *I n vivo* reduction of mtROS by MitoQ administration restored mitochondrial activity and decreased glucose uptake. Remarkably, MitoQ rescued the quiescence and self-renewal ability of *th3* HSCs, as shown by superior chimerism in secondary transplantation of HSCs from MitoQ-treated *th3* donors in lethally irradiated recipients (*th3* HSC+MitoQ 81.7±1.6 vs. untreated *th3* HSC 34.9±14.6 vs. % of chimerism, p<0.05), thus indicating that IO-derived ROS impair HSC function by affecting mitochondrial fitness and bioenergetics.

Preliminary results confirmed intracellular IO and mitochondrial defects also in HSCs from Townes mice, a model of sickle cell anemia characterized by IO.

Collectively, our study revealed that increased intracellular iron and ROS levels alter HSC metabolic programs by inducing mitochondrial dysfunction. Since mitochondria are defective, IO HSCs are unable to produce appropriate levels of ATP through OXPHOS and rely on glycolysis, despite their active cycling activity. Finally, the rescue of mitochondrial activity can restore the functional defects of *th3* HSCs.

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