P1563 INTRACELLULAR IRON OVERLOAD IMPAIRS HSC FUNCTION BY AFFECTING MITOCHONDRIAL FITNESS IN BETA-THALASSEMIA

Topic: 29. Iron metabolism, deficiency and overload

Silvia Sighinolfi1,2, Annamaria Aprile1, Laura Cassina3, Mariacarla Panzeri4, Stefano Beretta1, Mariangela Storto1, Alessandra Boletta3, Ivan Merelli1,5, Giuliana Ferrari1,2

1 San Raffaele-Telethon Institute for Gene Therapy (SR-Tiget), IRCCS San Raffaele Scientific Institute, Milan, Italy, Milan, Italy; 2 Vita-Salute San Raffaele University, Milan, Italy, Milan, Italy; 3 Division of Genetics and Cell Biology, Dibit, San Raffaele Scientific Institute, Milan, Italy, Milan, Italy; 4 Advanced Light and Electron Microscopy BioImaging Center (ALEMBIC), San Raffaele, Milan, Italy, Milan, Italy; 5 Institute for Biomedical Technologies, National Research Council, Segrate, Milan, Italy

Background:

β-Thalassemia (BT) is a genetic hemolytic anemia caused by reduced or absent synthesis of hemoglobin β-chains. Iron overload (IO), associated with ineffective erythropoiesis and therapeutic blood transfusions, is one of the main complications, leading to morbidities in various organs. Despite the improvement in chelation therapies over the past years, IO toxicity is an important issue for a successful therapeutic outcome. Recently, we demonstrated an impaired function and activated transcriptional responses to stress in HSCs from thalassemic th3 mice. We hypothesized that IO and the resulting oxidative stress might impair the bone marrow (BM) niche, thus interfering with the maintenance of HSCs. We also reported that IO reduces the hematopoietic supportive capacity of BM mesenchymal stromal cells in BT patients. However, there is no evidence of the direct effect of IO on HSCs in BT.

Aims:

To understand the key factors affecting BT HSC function it is essential to gain novel insight into BT pathophysiology. To this aim, we investigated the role of iron on HSCs.

Methods:

Gene expression profiling of HSCs from th3 mice was assessed by RNAseq. IO and oxidative stress were analyzed by flow cytometry. Mitochondria were evaluated by flow cytometry and transmission electron microscopy. We studied HSC metabolism by ATP analysis after in vitro inhibition of the oxidative phosphorylation (OXPHOS) using oligomycin and glucose uptake assay by flow cytometry. HSC function was evaluated following in vivo treatment with the mitochondria-targeted antioxidant MitoQ.

Results:

We found a positive enrichment of genes involved in iron homeostasis, such as Tfr1, Steap3, Fth1 and Ftl1, in th3 HSC transcriptome suggesting iron uptake and intracellular storage. These findings are corroborated by high levels of free reactive iron (Fe2+) in th3 HSCs. Interestingly, we reported an accumulation of Fe2+ in the mitochondria, correlating with a 2-fold increase in mitochondrial reactive oxygen species (ROS) content. As a result, mitochondria in th3 HSCs are impaired, with low mass and activity, as assessed by decreased mitochondrial membrane potential. Moreover, th3 HSCs showed a reduced expression of mitochondrial biogenesis and mitophagy genes, thus suggesting an accumulation of damaged mitochondria.

In line with mitochondrial dysfunction, in vitro inhibition of OXPHOS did not affect ATP levels in th3 HSCs. Interestingly, they preferentially rely on glycolysis rather than OXPHOS for their energy demands, as shown by the positive enrichment of glycolytic genes and by the 1.4-fold higher glucose uptake of th3 HSCs.
Finally, in vivo reduction of mitochondrial ROS by MitoQ administration rescues mitochondrial activity and th3 HSC quiescence. Ongoing studies on iron chelation will reveal how HSCs sense iron and the molecular mechanism leading to intracellular IO, providing clues for improving BT HSC function.

**Summary/Conclusion:**

Our study unveils that IO directly affects HSCs in BT. IO promotes oxidative stress, which in turn induces mitochondrial dysfunction. Alterations in mitochondrial activity and metabolic profile, in response to IO, are expected to negatively impact HSC function.

This research will add novel contribution to the field of iron regulation and HSC biology by unraveling how intracellular iron and ROS affect the metabolic and transcriptional programs underlying HSC function and fate.