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P1397 CHRONICALLY REDUCED LEVELS OF THROMBOPOIETIN IMPAIR HEMATOPOIETIC STEM CELL FUNCTION AND MEGAKARYOCYTE BONE MARROW NICHE

Topic: 23. Hematopoiesis, stem cells and microenvironment

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Background: In the last decade many studies unraveled the regulation of the bone marrow (BM) niche and hematopoietic stem cells (HSC) by using transgenic knock-out or reporter mice, in steady state or upon acute stimulation. However, HSC-niche interactions are still underexplored in disease condition associated to chronic stress. Beta-thalassemia (BT) is a severe congenital anemia with ineffective erythropoiesis and multi-organ secondary complications and may represent an ideal model to study HSC in a chronically altered BM microenvironment. We recently demonstrated an impaired function of HSC due to the defective crosstalk with stromal BM niche in BT mice (*Aprile et al., Blood 2020*). In addition to the BM stroma, we found altered levels of multiple local and systemic factors, including reduction of systemic thrombopoietin (TPO).

Aims: Further investigation is pivotal to define the role of chronically reduced stimulation of TPO signaling on HSC and BM microenvironment.

Methods: Gene expression profiling of HSC, megakaryocytes (Mk) and spleen macrophages from *Hbb*$^{th3/+}$ (*th3*) BT mice was assessed by RNAseq analysis. Flow cytometry characterization, *in vitro* Mk maturation, histological analysis on the BM of BT mice and patients, *in vivo* platelet (Plt) biogenesis, half-life and phagocytosis were performed. *In vivo* stimulation of TPO was evaluated.

Results:

Since TPO is a key regulator of both HSC and Mk, we investigated the dual role of TPO defect in the disease model of BT. RNAseq profiling revealed a downregulation of TPO signaling and target stemness genes in *th3* HSC, including *Cdkn1a*, *Hoxa9* and *Hoxb4*, negatively affecting HSC function. The decreased TPO causes a reduced commitment of HSC towards the Mk lineage, with under-expression of Mk-biased genes and lower frequency of CD41^{+}CD9^{high} HSC. *In vivo* stimulation of TPO axis in *th3* mice restored the pool of quiescent HSC, thus demonstrating the contribution of defective TPO signaling in altering BT HSC.

Consistently, histopathological analyses of *th3* mice showed dysmegakaryopoiesis and this defect was confirmed in BM sections from BT patients. The decreased maturation of *th3* Mk, with loss of the mature polyploid profile, correlated with a reduced *in vivo* Plt biogenesis and impaired *in vitro* differentiation of *th3* Mk. Sorted BT Mk showed the downregulation of niche factors, as *Pf4*, *Cxcl12*, *TnC*, relevant for HSC maintenance and reduced expression of extracellular matrix molecules, contributing to the impaired HSC-niche crosstalk.

We explored the origin of TPO defect: TPO levels fluctuate in response to Plt number and its reduced production by hepatocytes in *th3* mice is associated to the increased count of Plt. A negative correlation between Plt and TPO was

confirmed in BT patients. Consistently, acute Plt depletion in *th3* animals is sufficient to restore normal TPO levels, thus excluding an intrinsic defect. *In vivo* labeling demonstrated a higher half-life of BT Plt, which accumulate in the circulation. Phagocytosis assays proved a reduced Plt clearance by *th3* spleen macrophages, whose activity is impaired by iron overload and increased erythrophagocytosis associated to the disease.

Summary/Conclusion: Our results unraveled the dual role of TPO on HSC and Mk BM niche in a disease condition of chronically reduced TPO stimulation. This research elucidates the multiple mechanisms of the BM niche regulation in a model of chronic hematopoietic stress, with a potential relevance in improving HSC transplantation approaches for hematological diseases.