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

Topic: 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 Hbbth3/+ (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+CD9high 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 Plt4, 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.