P1519 EXPLORING THE ROLE OF FGF23 IN BETA-THALASSEMIA: A NOVEL THERAPEUTIC TARGET TO AMELIORATE ALTERED INTERACTIONS BETWEEN HSC AND BONE MARROW NICHE

Topic: 27. Thalassemias

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Background:

Beta-thalassemia (BT) is a congenital blood disorder resulting in severe anemia and ineffective erythropoiesis. Therapeutical approach for BT is represented by the correction of the erythropoietic defect by allogeneic hematopoietic stem cells (HSC) transplantation from healthy donors or by autologous HSC upon gene therapy. In both settings bone marrow (BM) niche and functional HSC are key elements for successful engraftment and reconstitution. Our group showed that chronic condition of hematopoietic stress and secondary alterations associated to BT interfered with HSC-niche interaction having a negative impact on HSC function. HSC defect is reversible by targeting the BM stromal niche (Aprile et al., Blood 2020). We showed a reduced bone mineral density (BMD) in th3 mice, associated to lower levels of parathyroid hormone (PTH), consistent with osteoporosis and hypoparathyroidism in BT patients.

To investigate the molecular causes of bone defect, we focused on fibroblast growth factor 23 (FGF23), a phosphaturic hormone negatively affecting BMD and PTH production. Bone and BM erythroid cells are the primary sources of FGF23, which is secreted as active intact protein (iFGF23) and is inactivated by cleavage in c-terminal fragment (cFGF23). Recent publications showed erythropoietin (EPO) as a novel inducer of FGF23 production. In this scenario, we hypothesized that high EPO levels, characteristic of BT, contribute to stimulate FGF23 synthesis, thus negatively affecting bone and BM niche homeostasis.

Aims:

We investigate the molecular mechanisms and the role of EPO in enhancing FGF23 levels, focusing on the impact of high FGF23 levels on bone and HSC-BM niche crosstalk in th3 mice.

Methods:

We measured FGF23 levels in th3 mice and BT patients by ELISA. We blocked EPO pathway by monoclonal anti-EPO antibody. We performed in vitro stimulation and inhibition of EPO signaling. We inhibited FGF23 signals by cFGF23 administration in BT mice and we evaluated bone, BM niche and HSC function upon treatment.

Results:

We found increased systemic levels of FGF23 and an unbalanced iFGF23/cFGF23 ratio in favor of cFGF23. FGF23 is also enhanced in th3 BM fluids associated to its increased expression by bone and BM erythroid cells. In vivo neutralization of EPO pathway normalized FGF23 levels demonstrating that EPO acts upstream of FGF23. To dissect the molecular mechanism linking EPO to FGF23, we modelled in vitro EPO stimulation on bone and BM
erythroid cells, showing an increase of both Fgf23 transcription and post-translational cleavage with decreased expression of Galnt3, involved in stabilizing iFGF23. Inhibition of specific EPO signaling showed that Erk1/2 and Stat5 pathways are involved in enhancing Fgf23 and decreasing Galnt3 transcription in EPO-stimulated bone and BM erythroid cells, suggesting a transcriptional regulation.

FGF23 inhibition strategy in th3 mice rescued bone mineralization and bone deposition following 38h and 13 days, respectively. FGF23 inhibition was efficacious in normalizing the expression of the niche factors osteopontin, Jagged1 and CXCL12, and restored HSC function. Further analysis will unravel the impact of high FGF23 levels on other BM niche populations involved in the crosstalk with HSC.

**Summary/Conclusion:**

Our findings unveil FGF23 as the missing link between hematopoiesis and bone homeostasis in a congenital hemolytic anemia, as a condition of chronic EPO stimulation. FGF23 inhibition provides a novel strategy to target bone defects and HSC-BM niche interactions and could be a valuable strategy to improve HSC transplantation and gene therapy approaches for BT.