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TGF-BETA SIGNALING CONTROLS THE LINEAGE CELL FATE OF HEMATOPOIETIC STEM CELLS TOWARDS ERYTHROID BRANCHING IN BETA-THALASSEMIA

Topic: 27. Thalassemias

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Maria Rosa Lidonnici[1], Giulia Chianella[1], Francesca Tiboni[1], Matteo Barcella[1], Ivan Merelli[2], Samantha Scaramuzza[1], Claudia Rossi[1], Stefania Crippa[1], Mariangela Storto[1], Maria Ester Bernardo[1, 3], Fabio Ciceri[1, 4, 5], Alessandro Aiuti[1, 3, 5], Sarah Marktel[1, 4], Giuliana Ferrari[1, 5]

[1] San Raffaele Telethon Institute for Gene Therapy, IRCCS San Raffaele Scientific Institute, Milan, Italy
[2] Institute for Biomedical Technologies, National Research Council, Segrate, Italy
[3] Pediatric Immunohematology Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
[4] Haematology and BMT Unit, IRCCS San Raffaele Scientific Institute, Milan, Italy
[5] University Vita-Salute San Raffaele, Milan, Italy

Background:

Beta-thalassemia (Bthal) is a genetic disorder due to mutations in the ß-globin gene, leading to a reduced or absent production of HbA, which interferes with erythroid cell maturation and limits normal red cell production. Patients are affected by severe anemia, hepatosplenomegaly, and skeletal abnormalities due to rapid expansion of the erythroid compartment in bone marrow (BM) caused by ineffective erythropoiesis. In a classical view of hematopoiesis, the blood cell lineages arise via a hierarchical scheme starting with multipotent stem cells that become increasingly restricted in their differentiation potential through oligopotent and then unipotent progenitors. In human, novel purification strategies based on differential expression of CD49f and CD90 enrich for long-term (49f+) and short-term (49f–) repopulating HSCs, with similar myeloid (My) and lymphoid (Ly) potential. In this view, it has been proposed that erythroid (Ery) and megakaryocytic (Mk) fates branch off directly from 49f– cell. Recently, in a new study the use of CLEC9A marker highlighted the separation between multipotent (Ery/My/Ly) long-term repopulating cells (Subset1, defined as CLEC9A^{high}CD34^{low}) and cells with only My/Ly and no Ery potential (Subset2, defined as CLEC9A^{low}CD34^{high}) within the HSC/MPP compartment.

Aims:

Recent evidence showed that the BM microenvironment is perturbed in Bthal. Our aim is to understand how the priming and kinetics of HSC undergoing lineage specification and differentiation are changed under this stress environment.

Methods:

Immunophenotype and functional analysis of the lineage commitment allowed to characterize of most primitive Bthal HSC/MPP cells. Gene expression profiling of hematopoietic subpopulations sorted from BM samples of Bthal patients and healthy donors (HDs) delineated the transcriptional networks governing hematopoiesis. We evaluated the expression levels of niche factors involved in the regulation of hematopoiesis/erythropoiesis by ddPCR on RNA purified from Bthal mesenchymal stromal cells. Their protein levels in the BM plasma were analyzed by ELISA. Confirmation experiments are ongoing *in vivo* in Bthal murine model (*Hbb$^{th3/+}$* strain).

Results:

An increased proportion of MPPs was observed in Bthal patients compared to HDs. The Subset1 compartment was actually endowed with an enhanced Ery potential. Focusing on progenitors (CD34^{+} CD38^{+}) and using a new sorting scheme that resolved My, Ery, and Mk lineage fates, we found a reduction of Ery (CD71^{+} BAH1$^{-/+}$) subsets in Bthal samples. By gene expression profiling of Bthal HSC/MPP, we observed down-regulation of TGFß signaling-induced gene expression and enrichment in erythroid signature. The levels of TGFß molecules were altered in Bthal BM micro-environment and treatment of HD Subset 1 cells demonstrates how TGFß/BMP pathway is instrumental for the erythroid cell lineage specification of HSCs. The analysis of molecular pathways driven by TGFß signaling

led to the identification of druggable targets with the potential to improve thalassemic hematopoiesis and to normalize erythropoiesis. Ongoing *in vivo* studies with specific drugs will provide cues to rescue the unbalanced hematopoietic primitive compartment

Summary/Conclusion:

Overall, these findings show that Bthal HSCs are exiting from the quiescent state towards a preferential erythroid differentiation. The drivers are likely specific niche signals, linked to the TGFß, and chronic BM stimulation or, alternatively, a specific regulation of the '*erythroid branching*', naturally present in the HSC pool, which is exacerbated by the pathophysiology of the disease.

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