DoS: Maria Rosa Lidonnici

PhD Program in Gene and Cell Therapy

Project Title: Definition of molecular networks of erythropoiesis and hematopoiesis in beta-thalassemia

Link to OSR/UniSR personal page:
https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/gene-transfer-into-stem-cell/maria-rosa-lidonnici.html

Project description

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. The aim of this project is definition of a model of hematopoiesis and erythropoiesis in this disease, that has never been investigated and it is relevant to better understand the complex pathophysiology and to guide development of combined gene therapy strategies. In human hematopoiesis, a new model has been recently proposed with megakaryocytes, erythroid-myeloid and lympho-myeloid divergence initiating at the level of the long-term hematopoietic stem cell (LT-HSC) compartment and not at the multipotent progenitor (MPP) stage, as previously postulated. Since Bthal is an erythropoietic disorder, it could be considered as a relevant model to study the '*erythroid branching*' in the hematopoietic hierarchy. Indeed, our preliminary studies provide an initial overview of the early human hematopoiesis in Bthal *(1)* and we recently discovered that the general perturbed and stress condition present in the thalassemic BM microenvironment impacts HSC function *(2).* Transcriptional profile analysis in Bthal HSC/MPP cells highlighted that several pathways, involved in the maintenance of primitive cells in a quiescent state and in their lineage commitment, were deregulated. We will investigate how these processes are linked to the disease and affect HSC and MPP molecular and functional properties. Understanding how the cellular and molecular composition of the HSC/progenitor compartment changes in stress conditions could have important implications for regenerative medicine and treatment of this pathology. Indeed, understanding the differentiation road map of the HSC is crucial to the optimization of gene therapy approaches for Bthal. To this aim we will study at molecular level the lineage commitment of primitive cells in patients' cells and/or in mutant thalassemic mice. Furthermore, we will perform scRNA analysis on hematopoietic stem and progenitor cells isolated from thalassemic patients treated with gene therapy *(3),* in order to study the shape of reconstituted hemato/erythropoiesis by gene-modified transplanted $CD34^+$ cells. Overall, these studies will lead to better define the steps towards the erythroid commitment during hematopoietic differentiation in a diseased background, and the impact of gene therapy

Skills to be acquired by the student:

These studies will be conducted by using both human cells derived from patients and mutant thalassemic mice. The student will acquire both cellular and molecular techniques from multicolor flow cytometry analyses to *in vitro* and *in vivo* functional assays for hematopoietic stem and progenitor cells, molecular analyses, gene expression profiling and computational skills for transcriptome analysis. The student will be followed in acquiring specific skills in analysis and critical interpretation of research data.

References (max. 3)

(1) Lidonnici, M.R., Scaramuzza, S., Rossi, C., Tiboni, F., Ciceri, F., Aiuti, A., Marktel, S. and Ferrari, G. Reconstitution of Hematopoiesis in Patients Treated with Gene Therapy for Beta-Thalassemia. Molecular Therapy (2018)a 26, (5, Supplement 1):5

(2) Aprile A., Gulino A., Villa I., Beretta S., Merelli I., Rubinacci A., Ponzoni M., Marktel S., Tripodo C., Lidonnici M. R., Ferrari G. Hematopoietic Stem Cell Function in β-Thalassemia Is Impaired and Is Rescued By Targeting the Bone Marrow Niche. Blood Blood. 2020 Jul 30;136(5):610-622.

(3) Marktel S, Scaramuzza S, Cicalese MP, Giglio F, Galimberti S, Lidonnici MR, Calbi V, Assanelli A, Bernardo ME, Rossi C, Calabria A, Milani R, Gattillo S, Benedicenti F, Spinozzi G, Aprile A, Bergami A, Casiraghi M, Consiglieri G, Masera N, D'Angelo E, Mirra N, Origa R, Tartaglione I, Perrotta S, Winter R, Coppola M, Viarengo G, Santoleri L, Graziadei G, Gabaldo M, Valsecchi MG, Montini E, Naldini L, Cappellini MD, Ciceri F, Aiuti A, Ferrari G. Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent ß-thalassemia. Nat Med. 2019 Feb;25(2):234-241