PROGETTO

Supervisore:	APRILE ANNAMARIA
Titolo/*Title*:	Targeting the hematopoietic stem cell niche to improve gene therapy for hemoglobinopathies
Curriculum:	GENE AND CELL THERAPY (GCT)
Link alla pagina personale del sito web di Ateneo o del polo ospedaliero di riferimento:	https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/gene-transfer-into-stem-cells.html

Descrizione del progetto (max 3.000 caratteri spazi inclusi)

Background/gap of knowledge

The hematopoietic stem cells (HSC) lie at the apex of the hierarchical process of hematopoiesis and reside in a specialized bone marrow (BM) microenvironment, termed niche[1]. The complex regulation of HSC and the BM niche remains underexplored in inherited disorders, as hemoglobinopathies. Hemoglobinopathies, β-thalassemia (BThal)[2] and sickle cell disease (SCD)[3], are globally widespread genetic disorders of hemoglobin. Despite differences in etiology, in both BThal and SCD, anemia causes chronic stress in different organs, including in the BM[4]. We provided the first demonstration of impaired HSC function caused by an altered BM stromal niche in BThal[5,6] and we have recently identified fibroblast growth factor-23 (FGF23)[7] as the molecular link connecting anemia, bone and the HSC niche[8]. FGF23 is high in BThal patients and *in vivo* inhibition of FGF23 signaling rescues bone defects and the BM niche, thus restoring HSC function. Alterations of HSC and BM niche have been described also in SCD and our preliminary data showed increased levels of FGF23 in SCD patients.

Rationale and hypothesis

Definitive cure for hemoglobinopathies is achieved by HSC transplantation from healthy donors or gene therapy transplantation of autologous HSC from patients[9]. The quality and the engraftment of HSC depend on the BM environment, thus studying the molecular basis and developing strategies targeting the HSC-niche crosstalk might be relevant for transplant outcome. How FGF23 is involved in BM niche regulation still need to be elucidated. Our hypothesis

is that FGF23 is the missing link at the crossroads of anemia and HSC niche defects in hemoglobinopathies through common and disease-specific mechanisms and that pharmacological inhibition of FGF23 might restore the HSC-BM niche crosstalk, thus contributing to a better clinical outcome of transplantation and gene therapy.

Objectives and specific aims

The main objectives of the project are to investigate the molecular mechanisms of the altered BM niche in BThal and SCD, focusing on the role of FGF23, and to target the identified defects to ameliorate the outcome of gene therapy transplantation. The specific aims are (1) to investigate the molecular mechanisms regulating the role of FGF23 in BThal and SCD HSC niche by single-cell transcriptomics, barcoding[10] and imaging approaches[11], (2) to test our FGF23 inhibition strategy[8] in the mouse model of SCD, (3) to perform preliminary studies on the combination of FGF23 inhibition with transplantation and gene therapy for hemoglobinopathies.

Expected outcomes

The expected outcomes of the project include the identification of the role of FGF23 in the BM niche by integrating single-cell RNA sequencing and *in vivo* barcoding strategies in the murine models of BThal and SCD, the evaluation of the safety and efficacy of FGF23 inhibition in SCD and the assessment of a protocol to combine FGF23 inhibition with gene therapy approaches.

Competenze che deve acquisire lo studente (Max 600 caratteri spazi inclusi):

These studies will be performed by using *Hbb*$^{th3/+}$ BThal and Townes SCD mice, as well as *in vivo* tracing and reporter strains, i.e. the CARLIN and α-catulin^{GFP} mice. The student will acquire both cellular and molecular techniques from immunophenotype analyses to *in vitro* and *in vivo* functional assays, histological, barcoding and single cell-RNA sequencing analyses. The student will be followed in acquiring specific skills for the analysis and critical interpretation of research data.

Bibliografia (max. 15)

(1) Crane, G.M., et. al. *Adult haematopoietic stem cell niches* (2017) Nat Rev Immunol. doi: 10.1038/nri.2017.53

(2) Taher, A.T., et. al. *β-Thalassemias* (2021) N Engl J Med. doi:10.1056/NEJMra2021838

(3) Ware, R.E., et al. *Sickle cell disease* (2017) Lancet. doi: 10.1016/S0140-6736(17)30193-9

(4) Aprile A., et al. *Targeting the Hematopoietic Stem Cell Niche in beta-Thalassemia and Sickle Cell Disease* (2022) Pharmaceuticals (Basel). doi: 10.3390/ph15050592

(5) Aprile A., et al. *Hematopoietic stem cell function in beta-thalassemia is impaired and is rescued by targeting the bone marrow niche* (2020) Blood. doi: 10.1182/blood.2019002721

(6) Crippa S., et al. *Bone marrow stromal cells from beta-thalassemia patients have impaired hematopoietic supportive capacity* (2019) J Clin Invest. doi: 10.1172/JCI123191

(7) Edmonston D., et al. *FGF23 at the crossroads of phosphate, iron economy and erythropoiesis* (2020) Nat Rev Nephrol. doi: 10.1038/s41581-019-0189-5

(8) Aprile A., et al. *Inhibition of FGF23 is a therapeutic strategy to target hematopoietic stem cell niche defects in beta-thalassemia* (2023) Sci Transl Med. doi: 10.1126/scitranslmed.abq3679

(9) Marktel S. et al. *Intrabone hematopoietic stem cell gene therapy for adult and pediatric patients affected by transfusion-dependent β-thalassemia* (2019) Nat Med. doi: 10.1038/s41591-018-0301-6

(10) Bowling S., et al. *An engineered CRISPR-Cas9 mouse line for simultaneous readout of lineage histories and gene expression profiles in single cells* (2020) Cell. doi: 10.1016/j.cell.2020.06.018

(11) Acar M., et al. *Deep imaging of bone marrow shows non-dividing stem cells are mainly perisinusoidal* (2015) Nature. doi: 10.1038/nature15250