PROJECT 1

DoS: MARIA ESTER BERNARDO

Title: COMPREHENSIVE GENE PROFILING AND FUNCTIONAL CHARACTERIZATION OF DIFFERENT STROMAL CELL SUBTYPES TO IMPROVE THEIR SUPPORTIVE CAPACITY IN HEMATOPOIETIC STEM CELL TRANSPLANTS

Curriculum: Cellular and Molecular Physiopathology

Link to OSR/UniSR personal page:

http://www.unisr.it/medicina-chirurgia/ddr-internazionale-in-medicina-molecolare/maria-ester-bernardo/

http://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/pathogenesis-and-therapy-of-primary-immunodeficiencies/maria-ester-bernardo.html

Project description (*Number of characters, including spaces*: 2.000 - 3.000):

In the context of gene therapy using hematopoietic stem cells (HSCs), the maintenance/expansion of primitive HSCs represents a fundamental requirement to sustain long term (LT) engraftment of genetically corrected HSCs. Stress signals are activated by *in vitro* genetic procedures (transduction/editing), which induce HSCs to exit the quiescent state and differentiate. In our laboratory, we study the biology of mesenchymal stromal cells (MSCs) as a tool to preserve primitive HSCs *in vitro* and facilitate their rapid and LT engraftment in vivo, using different models of humanized bone marrow (BM) niche. MSCs are a rare population of stromal cells resident in the human BM. They are easily isolated and expanded *in vitro* thanks to their ability to adhere to plastic. They are characterized by 1) expression of specific surface markers; 2) ability to differentiate into adipocytes, osteocytes and chondrocytes; 2) capacity to sense several inflammatory signals and modulate innate and adaptive immunity. Most importantly, MSCs offer structural support to HSCs and regulate HSC homeostasis in the human BM by cell contact and releasing specific HSC supportive factors. Co-infusion of *in vitro* expanded MSCs has been shown to improve HSC engraftment and accelerate hematopoietic recovery in different clinical trials of HSC transplantation. Based on these data, we intend to use MSCs as a tool to ameliorate the outcome of gene corrected HSC transplantation. However, several aspects of MSC biology and molecular mechanisms underlying their hematopoietic supportive capacity are still largely unclear. In particular, when expanded in vitro MSCs become a heterogeneous population containing different subtypes of cells characterized by specific stemness and functional properties and may loose their primary properties upon plastic adherence and exposure to culture medium. With this project, we intend to exploit the hematopoietic supportive function of MSCs by:

A) Comprehensive genome-wide transcriptome analysis of different *in vitro* expanded MSC subpopulations to globally define stem cell properties, metabolic energy requirements, capacity to sustain expansion/maintenance of primitive HSCs. Functional studies (clonogenic assay, differentiation capacity, metabolic assays) will be performed to validate in silico data. 2D and 3D co-culture systems will be adopted to evaluate the supportive capacity of different MSC populations in addition to transplantation and humanized BM niche models in vivo.
B) Comparative global gene profiling of *in vitro* expanded and freshly isolated MSCs to define which modifications are culture-induced and in what extent, and to gain insights into primary MSC associated genes and pathways, whose expression can be modulated transiently or using lentiviral vectors to reprogram cultured MSCs to primary state. This will allow us to control culture-induced modifications and preserve MSC functional characteristics.

Skills to be acquired by the student:

Cell culture techniques for MSC and HSC expansion and manipulation

Clonogenic, proliferation and differentiation assays

2D and 3D (bioreactor) co-culture systems

Flow cytometry and cell sorting

Molecular biology techniques for RNA isolation, retrotranscription, quantitative PCR, PCR, RNA sequencing.

Mouse handling: intra tail vein transplantation, scaffold implantation, peripheral blood sampling

References (max. 3)

1. Beyer Nardi N and da Silva Meirelles L. Mesenchymal stem cells: isolation, in vitro expansion and characterization. *Handb Exp Pharmacol.* 2006

2. S. J. Morrison, D. T. Scadden, The bone marrow niche for haematopoietic stem cells. *Nature.* 2014

3. Passaro D Bioengineering of humanized bone marrow microenvironments in mouse and their visualization by live imaging. *J Vis Exp*. 2017