PROJECT 1

DoS: Prof. Luigi Naldini

Title: Identifying novel regulators and potential therapeutic targets of human hematopoietic stem cells engraftment using a gain-of-function screening in an in vivo model

Curriculum: Gene and Cell Therapy

Link to OSR/UniSR personal page:
https://research.hsr.it/en/institutes/san-raffaele-telethon-institute-for-gene-therapy/gene-transfer-technologies-and-new-gene-therapy-strategies/luigi-naldini.html

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

Hematopoietic stem/progenitor cell gene therapy (HSPC-GT) is successfully used in the clinic to treat patients with hematopoietic malignancies and several inherited diseases of the hematopoietic system. The HSPC-GT is based on several critical steps: HSPCs are mobilized and harvested from the patient, genetically corrected ex-vivo by gene transfer or gene editing and infused back to the patient, after administration of myeloablative conditioning to make space in the bone marrow (BM) for the modified cells. Administered HSPCs home to the BM, where they engraft and reconstitute a healthy immune system. Gene editing of human HSPCs holds the promise for precise engineering of the genome by in situ correction of the disease-causing gene. However, cell culture, electroporation and exposure to viral vectors to deliver the editing machinery hampers their long-term repopulating potential, thus leading to a significant shrinkage of the clonal repertoire, transient cell cycle arrest and apoptosis[1]. The host laboratory recently reported improvements in the ex-vivo HSPCs gene editing protocol resulting in an enhanced editing efficiency in long-term repopulating HSPCs[2]. Nevertheless, the proportion of engrafting edited HSPCs is conceivably still limiting for safely and effectively treat most diseases. Therefore, there is a clear need to improve, albeit only transiently, engraftment ability, providing a self-limiting competitive advantage to edited cells and ensure long-term repopulating capacity in vivo. While literature-based targets are valuable, there is a lack of unbiased screening approach for uncovering new players. This PhD project proposes to develop innovative schemes to increase human HSPC engraftment, which is one of the most coveted but still unaccomplished goals of HSPC-GT. In this project, the PhD candidate will exploit a gain-of-function lentiviral vector based transcriptional activation screen to identify regulators of human HSPCs engraftment in a hematochimeric mouse model, which will be then validated in the context of state-of-the-art gene editing protocols.

To identify key regulators of engraftment, the PhD candidate will use a genome-wide activation strategy based on a deactivated Cas9 (dCas9), paired with a transcriptional activation (TA) domain. The host laboratory has already set preliminary experiment showing a robust increase in 10 genes of interest involved in HSC retention, engraftment and quiescence, establishing the feasibility of this approach. The PhD candidate will first transduce HSPCs with a sgRNA library/dCas9-TA combo. HSPCs will then be sorted for the most primitive HSC compartment and transplanted via intrabone injection in the BM of immunodeficient NSGW41 mice. Cells will be collected at 1- and 12-weeks post-transplantation followed by next-generation sequencing to identify the top hits conferring a competitive engraftment advantage. The OSR bioinformatics group will help with the post-acquisition data processing. The 5 top hits (prioritized by novelty) resulting from the NGS analysis will be validated independently, using our optimized in vitro transcription platform. HSPCs transiently expressing the hits will be transplanted in immunodeficient NSG mice, followed by subsequent analysis of the transcriptional landscape, engraftment efficiency, and clonal composition to monitor an eventual increased engraftment, compared to HSPCs electroporated with a control mRNA (GFP). Final validation of the most promising hit(s) will be performed by promoting their transient and specific overexpression in edited HSPCs in order to provide them a selective advantage over the unedited counterpart.

The proposed project is based on state-of-the-art approaches and represents the follow-up of an ongoing effective line of investigation. If successful, enhanced engraftment by transient gain-of-function may be part of the next generation of HSPC-GT.

Skills to be acquired by the student:

Cellular Biology: Maintenance of human cell lines and primary cells; Generation of knock-out and knock-in cell lines; Lentiviral vector design, production and validation; Transduction.
Molecular Biology: Genome engineering-based techniques; Plasmid isolation; Cloning; HDR template design; Sequencing; DNA/RNA extraction; PCR; qPCR; ddPCR; Gel electrophoresis; Multicolor flow cytometry; In vitro transcription; Transcriptomic analysis; Off-target analysis.
Animal models: Immunocompetent and immunocompromised mice handling; Blood analysis; Transplantation of hematopoietic stem cells; Spleen, liver and bone marrow organs retrieval and processing.
Experimental design, data analysis, interpretation and presentation; Manuscript writing.

References (max. 3)

1 Schiroli et al. Precise gene editing preserves hematopoietic stem cell function following transient p53-Mediated DNA damage response. Cell Stem Cell (2019).

2 Ferrari et al. Efficient gene editing of human long-term hematopoietic stem cells validated by clonal tracking. Nature Biotechnology (2020).