Medical University of Graz

Identification of interactions between leukemic stem cells and the bone marrow microenvironment

Summary

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Supervisor:	Dr. Andreas Reinisch
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 14, 2020 00:00 and October 10, 2020 23:59 (Europe/Zurich)

Description

Background:

Normal hematopoiesis is maintained by hematopoietic stem cells (HSCs) and is organized in a hierarchical fashion. AML displays a similar hierarchical organization with leukemic stem cells (LSCs) at the top of the hierarchy. LSCs emerge from HSCs, accumulate mutations with specific patterns, and are considered resistant to chemotherapy and responsible for relapse of disease after treatment.[1] Potential mechanisms underlying the enhanced chemotherapy-resistance are protective LSC interactions within the bone marrow (BM) microenvironment (aka BM niche). Therefore, identification of novel treatment strategies eradicating LSCs may have a significant impact on the survival of leukemia patients.

LSC biology and the molecular mechanism mediating LSC interaction with the BM niche are still poorly understood. Studies using xenotransplantation models indicate that LSCs preferentially localize in specific areas within the BM and provide evidence that unique molecular and cellular interactions of LSCs with different niche cells may be targets to improve treatment of leukemia.[2]

Hypothesis and Objectives:

The central hypothesis of this project is that LSCs are sustained by unique molecular interactions within the BM niche, which can be targeted for leukemia therapy. The general objective of the proposal is to identify the molecular and cellular determinants of a normal and a leukemic stem cell niche employing a novel synthetic Notch (synNotch) system.[3]

Our goal is to eliminate leukemic stem cells (LSCs) in acute myeloid leukemia (AML), by targeting their interaction with their supportive BM niche.

Methodology:

The successful applicant will be an integral part in the development of a unique synthetic Notch reporter system (SynNotch) that allows tracking and isolation of cells that experienced direct cell-cell (LSC with BM stroma) contact. You will adapt this system for studying LSC-niche interactions.

You will learn and apply key technologies such cell culture technology, molecular biology techniques, flow cytometry and CRISPR/Cas9 genome engineering. Single cell gene expression profiling (scRNAseq) will be employed to identify the unique molecular determinants of the leukemic stem cell niche.

- Isolation and culture of primary human hematopoietic cells (including HSCs and LSCs) and leukemic cell lines
- Isolation and culture of primary human mesenchymal stromal cells (MSCs)
- Polychromatic flow cytometry: analysis, sorting
- Molecular biology: PCR, qRT-PCR, ddPCR, molecular cloning, Western blotting,
- CRISPR/Cas9 genome engineering in cell lines and primary cells (knock in)
- Adeno-Associated Virus (AAV) production
- Single cell RNAseq

References:

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1 Reinisch, A., Chan, S. M., Thomas, D. & Majeti, R. Biology and Clinical Relevance of Acute Myeloid Leukemia Stem Cells. *Seminars in hematology* **52**, 150-164, doi:10.1053/j.seminhematol.2015.03.008 (2015).

2 Ishikawa, F. *et al.* Chemotherapy-resistant human AML stem cells home to and engraft within the bone-marrow endosteal region. *Nat Biotechnol* **25**, 1315-1321, doi:10.1038/nbt1350 (2007).

3 Morsut, L. *et al.* Engineering Customized Cell Sensing and Response Behaviors Using Synthetic Notch Receptors. *Cell* **164**, 780-791, doi:10.1016/j.cell.2016.01.012 (2016).