Medical University of Graz

The role of RAS-signaling aberrations in the development of myeloid sarcoma

Summary

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Supervisor:	Prof. Dr. Armin Zebisch
Availability:	This position is available.
Offered by:	Medical University of Graz
Application deadline:	Applications are accepted between August 03, 2022 00:00 and September 20, 2022 23:59 (Europe/Zurich)

Description

Background:

Acute myeloid leukemia (AML) is an aggressive hematopoietic malignancy caused by the malignant transformation of hematopoietic stem and progenitor cells. Myeloid sarcoma (MS) is a subtype of acute myeloid leukemia (AML), in which leukemic cells invade extramedullary tissues and form solid tumors. MS may manifest as an isolated event or with concomitant involvement of leukemic bone marrow (BM), the latter affecting up to 20% to 30% of all AML cases. MS may occur at any point during the disease course, and almost every site of the body can be affected. Very frequently, the clearing of extramedullary leukemic blasts by conventional chemotherapy and allogeneic hematopoietic stem cell transplantation is insufficient. Consequently, residual leukemic cells in MS sites frequently cause systemic AML relapses. Deciphering the mechanisms behind the tissue infiltration of leukemic blasts will increase our knowledge about the formation of MS and enable the design of novel therapeutic approaches for AML in general.

Recently, next-generation sequencing and transcriptomic analyses shed more light on the molecular landscape of MS and revealed an enrichment of aberrations within the *RAS*- signaling cascades. However, validation in larger patient cohorts is missing, and these lesions' functional relevance of these lesions in the tissue invasion of leukemic cells and MS formation is largely unexplored.

Hypothesis and Objectives:

Based on previous data from our group, we hypothesize that the tissue invasion of leukemic cells and MS formation is caused by aberrations affecting the *RAS*-signaling cascades.1, 2 The knowledge gained in this thesis will help to pave the way for the development of targeted treatment approaches for this AML subtype.

Methodology:

Within preliminary experiments of this project, we have already identified enrichment of *RAS*- signaling mutations in a cohort of primary MS specimens. We will validate these data by

analyzing a larger collection of primary human MS specimens from the MedUni Graz Leukemia Biobank with targeted next-generation sequencing covering more than 40 leukemia-relevant genes. This panel will also detect mutations in *NRAS* and *KRAS*, as well as in a series of *RAS*- signaling genes.1 This project will be performed in the course of international collaboration within the TRANSCAN-3 ERA-NET program, allowing us to extend these data by whole genome-wide sequencing and transcriptomic analyses within these samples. We will use these large-scale mutational and expression data to comprehensively analyze signaling-related genes and strengthen a potential correlation with aberrant *RAS* signaling.

In the second part of this thesis, we will delineate the functional relevance of these aberrations by employing state-of-the-art functional MS assays in leukemic cell lines and primary MS patient specimens. This approach will enable us to decipher the role of aberrant intracellular signaling in MS pathogenesis. In addition, we will create the basis for the development and preclinical testing of targeted therapeutic approaches. In more detail, this project phase will initially focus on creating *RAS*-signaling aberrations in AML cell lines and primary hematopoietic cells. We have already created an isogenic AML cell line model carrying the most prevalent $NRAS^{G12D}$mutation by CRISPR/Cas9 knock-

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in technologies. The PhD student will use this technique to engineer additional *RAS*-signaling aberrations in AML cell lines and healthy CD34+ hematopoietic stem and progenitor cells. Subsequently, she/he will study the effects of these aberrations on leukemic tissue infiltration in state-of-the-art in-vitro and in-vivo approaches. This will include in-vitro invasion and migration assays and the in vivo chorioallantoic membrane (CAM) model, which was successfully established in our laboratory and allows functional characterization of specific genetic lesions in the process of MS formation.2 Although the CAM assay is not routinely used for engraftment assays of myeloid leukemias, it represents an ideal tool for testing the tissue infiltration of malignant cells. This is because leukemic cells can be placed externally on the CAM in a ring, and the invasion and migration into the CAM can be ideally assessed.

References:

1. Kashofer K, Gornicec M, Lind K, et al. Detection of prognostically relevant mutations and translocations in myeloid sarcoma by next generation sequencing. Leuk Lymphoma. 2018;59(2):501-50410.1080/10428194.2017.1339879 [doi].

1. Caraffini V, Perfler B, Berg JL, et al. Loss of RKIP is a frequent event in myeloid sarcoma and promotes leukemic tissue infiltration. Blood. 2018;131(7):826-83010.1182/blood-2017- 09-804906 [doi].

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