Experimental Hematology

Radek Skoda
Department of Biomedicine
Division of Hematology
University Hospital Basel

Group Members

Dr. Jean Grisouard* (Postdoc)
Dr. Nils Hansen (Postdoc)
Hui Hao-Shen (Technician)
Dr. Morgane Hilpert (Postdoc)
Dr. med. Axel Karow* (Postdoc)
Dr. Lucia Kubovcakova (Postdoc)
Renate Looser* (Technician)
Dr. Pontus Lundberg* (Postdoc)
Dr. Julia Majewska (Guest Scientist)
Dr. Hélène Mereau (Technician)
Dr. Gabriele Mild-Schneider (Administrative Assistant)
Ronny Nienhold (PhD Student)
Shivam Rai (PhD Student)
Silvia Sendelov-Plattner* (Technician)
Dr. Takafumi Shimizu (Postdoc)
Yukiko Shimizu* (External Collaborator)
Barbara Szczerba* (PhD Student)
Dr. Nageswara Rao Tata (Postdoc)
Jakub Zmajkovic (PhD Student)

*left during report period

Molecular pathogenesis of myeloproliferative neoplasms

Myeloproliferative neoplasms (MPN) are a group of blood diseases characterized by aberrant proliferation of precursors of the myeloid, erythroid and megakaryocytic lineages. They represent clonal stem cell disorders with a tendency towards leukemic transformation. Currently, no curative therapy is available. MPNs comprise 3 entities: polycythemia vera (PV), essential thrombocythemia (ET) and primary myelofibrosis (PMF). The goal of our studies is to advance the understanding of the molecular events that initiate MPN and influence its progression to leukemia. A recurrent mutation Janus kinase 2 (JAK2) gene that substitutes a valine to phenylalanine at position 617 (JAK2-V617F) is present in a majority of patients with MPN, in particular PV. This mutation leads to activation of the Jak2 tyrosine kinase and represents a driver for the proliferation of hematopoietic cells. Activating mutations calreticulin (CALR) and the thrombopoietin receptor (MPL) represent other frequent driver events. Despite this progress, several questions remain unsolved including how a single JAK2 mutation causes three different MPN phenotypes, what other genes might be involved and what determines the progression to acute leukemia. We are examining these questions by combining three approaches: molecular studies in patients with sporadic MPN, genetic analysis of familial MPN and transgenic mouse models that mimic the human disease.

Analysis of clonal progression in MPN

Using next generation sequencing, we determined the mutational profiles of MPN patients and analyzed correlations with clinical outcome and prognosis. We found that increasing number of somatic mutations per patient was associated with higher risk of transformation to acute leukemia and with reduced survival (Fig. 1). Some gene mutations, e.g. TP53 had a particularly bad prognostic impact. By examining individual colonies grown from patient's peripheral blood, the clonal architecture can be precisely determined. In a subset of patients with sporadic MPN additional somatic mutations can either precede or occur after the acquisition of JAK2-V617F. We are examining the impact of the clonal architecture on outcome and response to therapy.

Familial predisposition for MPN

Familial syndromes resembling MPN can be grouped into two classes:

1. Inherited disorders with high penetrance and polyclonal hematopoiesis.
2. Hereditary predisposition to true MPN, with low penetrance, clonal hematopoiesis and occurrence of somatic mutations, e.g. in JAK2-V617F.

We identified mutations in the thrombopoietin (THPO) gene as the cause for an inherited form of thrombocythemia in several families with a "class 1" phenotype. In another family we found a previously described mutation in MPL. However, in the majority of families neither THPO nor MPL is mutated. The search for these disease genes is ongoing. Families with "class 2" phenotype are more common than generally assumed. These germ line mutations increase the likelihood of acquiring a somatic JAK2-V617F mutation. We are using genetic methods to map the locus for these pre-disposing mutations.

Mouse models for MPN

We generated JAK2-V617F transgenic mice that express the human JAK2-V617F. This conditional construct can be activated by Cre-recombinase. Depending on the mode of Cre-mediated activation, these mice developed a phenotype resembling ET with strongly elevated platelet counts or a PV-like phenotype with increased hemoglobin, thrombocytosis and neutrophilia. A major focus of our research is to examine the nature of the MPN initiating stem cells and their interactions with the bone marrow microenvironment. We demonstrated that MPN can be initiated by transplanting single hematopoietic stem cells that carry JAK2-V617F as the sole genetic alteration and that loss of function mutations in Ezh2 promoted disease initiation from single cells and accelerated disease towards myelofibosis. We are dissecting the contributions of additional genes to the pathogenesis using conditional knockout models. We are also using our mouse models for pre-clinical screening of Jak2 inhibitors and other potential therapeutic agents.

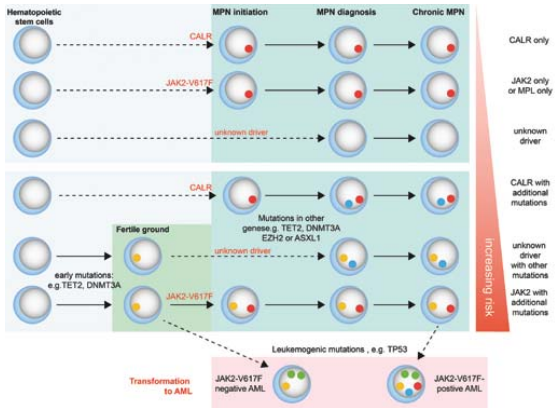

Fig. 1: Model of MPN disease evolution and risk stratification in correlation to mutational events.

Selected Publications

Shimizu T, Kubovcakova L, Nienhold R, Zmajkovic J, Meyer SC, Hao-Shen H, Geier F, Dirnhofer S, Guglielmelli P, Vannucchi AM, Feenstra JD, Kralovics R, Orkin SH and Skoda RC. (2016) Loss of Ezh2 synergizes with JAK2-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. The Journal of Experimental Medicine 213, 1479–1496

Grisouard J, Li S, Kubovcakova L, Rao TN, Meyer SC, Lundberg P, Hao-Shen H, Romanet V, Murakami M, Radimerski T, Dirnhofer S and Skoda RC. (2016) JAK2 exon 12 mutant mice display isolated erythrocytosis and changes in iron metabolism favoring increased erythropoiesis. Blood 128, 839–851

Grisouard J, Shimizu T, Duek A, Kubovcakova L, Hao-Shen H, Dirnhofer S and Skoda RC. (2015) Deletion of Stat3 in hematopoietic cells enhances thrombocytosis and shortens survival in a JAK2-V617F mouse model of MPN. Blood 125, 2131–2140

Lundberg P, Takizawa H, Kubovcakova L, Guo G, Hao-Shen H, Dirnhofer S, Orkin SH, Manz MG and Skoda RC. (2014) Myeloproliferative neoplasms can be initiated from a single hematopoietic stem cell expressing JAK2-V617F. The Journal of Experimental Medicine 211, 2213–2230

Lundberg P, Karow A, Nienhold R, Looser R, Hao-Shen H, Nissen I, Girsberger S, Lehmann T, Passweg J, Stern M, Beisel C, Kralovics R and Skoda RC. (2014) Clonal evolution and clinical correlates of somatic mutations in myeloproliferative neoplasms. Blood 123, 2220–2228

Duek A, Lundberg P, Shimizu T, Grisouard J, Karow A, Kubovcakova L, Hao-Shen H, Dirnhofer S and Skoda RC. (2014) Loss of Stat1 decreases megakaryopoiesis and favors erythropoiesis in a JAK2-V617F-driven mouse model of MPNs. Blood 123, 3943–3950

Connection to Clinical Practice

Prof. Jakob Passweg and
Dr. Pontus Lundberg
Division of Hematoloy, University Hospital Basel

Improved diagnostics of MPN and new therapeutic approaches: From bench to bedside

The detection of somatic driver gene mutations in JAK2, CALR and MPL is now a key step in the diagnostic approach to MPN in accordance with the revised WHO criteria. Furthermore, mutations in genes that can modify the course of the disease, such as ASXL1, EZH2, or TP53 are now in the focus interest and are likely to become increasingly important in clinical decision making. To cover the increasing need for the comprehensive molecular diagnostics of MPN, we developed a next generation sequencing (NGS) based gene panel that allows a molecular classification of patients with myeloid neoplasm. This panel is based on our NGS study (Lundberg et al, Blood 2014), and is now being used for the routine diagnostics of MPN patients.

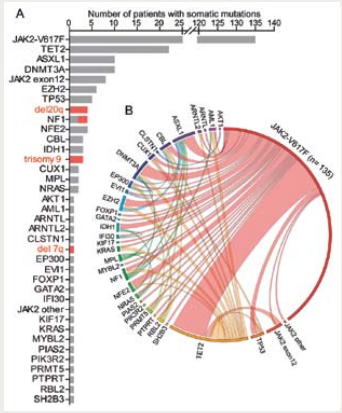

Fig. 2: Frequency and distribution of mutations in patients with MPN. (A) Number of patients with mutations in the genes as indicated. Red bars and red text indicate chromosomal aberrations. (B) Circos plot illustrating co-occurrence of somatic mutations in the same individual. The length of the arc corresponds to the frequency of the mutation, while the width of the ribbon corresponds to the relative frequency of co-occurrence of two mutations in the same patient.