Experimental Hematology

Radek Skoda

Department of Biomedicine
Division of Hematology
University Hospital Basel

Group Members

Dr. med. Nicola Andina* (Postdoc)
Dr. med. Serena Galli* (Postdoc)
Florian Groelly* (Intern)
Dr. Alexandre Guy (Postdoc)
Dr. Nils Hansen* (Postdoc)
Hui Hao-Shen (Technical Staff)
Julian Hilfiker* (Master/PhD Student)
Dr. Riikka Kivioja (Postdoc)
Dr. Lucia Kubovcakova* (Postdoc)
Dr. Pontus Lundberg (Postdoc)
Dr. Damien Luque Paz (Postdoc)
Dr. Julia Majewska* (Intern)
Dr. Gabriele Mild (Administrative Staff)
Dr. Ronny Nienhold* (PhD Student/ Postdoc)
Serena Petrella* (Undergraduate Student)
Sharon Pickel* (Intern)
Shivam Rai (PhD Student)
Michal Remen* (Undergraduate Student)
Margareta Rybarikova* (Intern)
Dr. Takafumi Shimizu* (Postdoc)
Fabian Spoendlin* (Intern)
Jan Stetka (PhD Student)
Dr. Rao Nageswara Tata* (Postdoc)
Marc Usart (PhD Student)
Dr. Jakub Zmajkovic* (PhD Student/ Postdoc)

*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. 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)* genes represent other frequent driver events. Despite this progress, several questions remain unsolved including what determines whether a hematopoietic stem cell (HSC) after acquiring a driver gene mutation expands to initiate MPN disease, or stays inactive and eventually disappears, what determines the progression to myelofibrosis or acute leukemia, and how a single *JAK2*-V617F mutation causes three different MPN phenotypes. 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 MPN disease initiation

The discovery of clonal hematopoiesis of indeterminate potential (CHIP) and the fact that *JAK2*-V617F is one of the most frequent CHIP mutations in healthy individuals showed that acquisition of *JAK2*-V617F not the rate-limiting step in MPN pathogenesis, as only a fraction of individuals who carry *JAK2*-V617F eventually develop MPN disease. We are examining which factors promote the expansion of the *JAK2*-mutant clone to MPN disease initiation (Fig. 1). We determined that additional mutations in epigenetic regulator genes, e. g. *Ezh2,* or *Dnmt3a,* or inflammatory signals (e. g. *IL-1β*), as well as inherited predisposition mutations can favor clonal expansion and MPN disease initiation.

Familial predisposition for MPN

Familial syndromes resembling MPN can be grouped into two classes:

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

We identified for the first time a mutation in the erythropoietin *(EPO)* gene that causes familial polyclonal erythrocytosis with elevated erythropoietin levels. This mutation, a single-nucleotide deletion (c.32delG), introduces a frameshift in exon 2 that interrupts translation of the main *EPO* messenger RNA (mRNA) transcript but initiates excess production of erythropoietin from what is normally a noncoding EPO mRNA transcribed from an alternative promoter located in intron 1. Families with "class 2" phenotype are more common than generally assumed. These germ line mutations increase the likelihood of transition from *JAK2*-V617F CHIP to MPN disease. We are using genetic methods to identify such pre-disposing mutations.

Mouse models for MPN

We generated JAK2-V617F transgenic mice that express the human *JAK2*-V617F and develop MPN phenotype. 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, Dnmt3a* promoted, while loss of *IL-1β,* the inflammatory master regulator, decreased MPN disease initiation from single HSCs. We are using our mouse models for pre-clinical screening of *Jak2* inhibitors and other potential therapeutic agents. We also found that mutant JAK2 induces metabolic reprogramming of hematopoietic cells causing increased energy requirement that can be targeted by inhibitors of metabolism (Rao et al 2019).

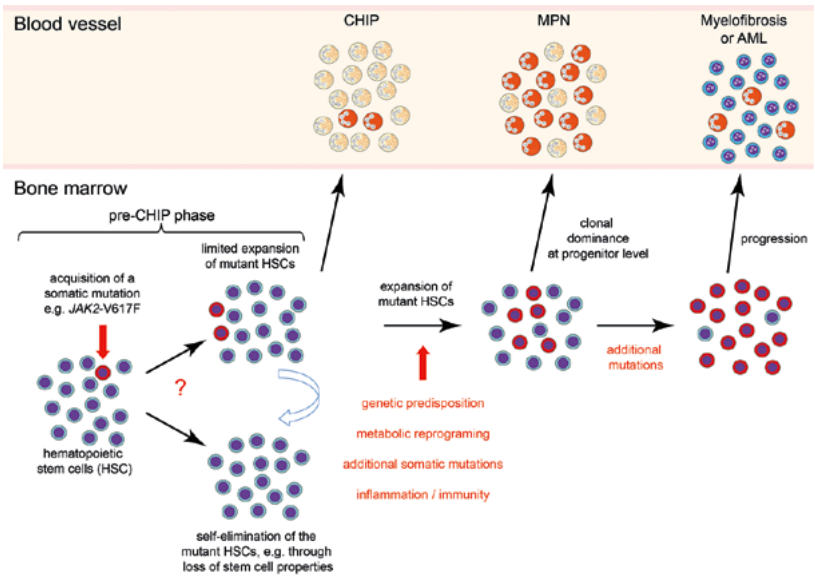

Fig. 1: Model of MPN disease initiation. Somatic mutations can occur in all cells including hematopoietic stem cells (HSCs). A single HSC in bone marrow that acquired a driver gene mutation (marked in red), e. g. *JAK2*-V617F, can either divide and increase the number of mutant HSCs, or divide and differentiate to mature cells of limited life span, thereby eliminating the mutant clone. This "pre-CHIP" phase is not yet detectable in peripheral blood. The *JAK2*-mutant clone has to expand to become self-sustaining and to generate sufficient numbers of mature blood cells to become detectable in peripheral blood (CHIP-phase). Further expansion and clonal dominance of the *JAK2*-mutant HSCs is required to initiate MPN disease characterized by increased numbers of erythrocytes and/or platelets (MPN-phase). Several factors (marked in red) can favor the transition from CHIP to MPN and the progression to myelofibrosis or acute leukemia (AML).

Connection to Clinical Practice

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

New therapeutic approaches to treat MPN: From bench to bedside

A collaborative study with Dr. S. Mendez-Ferrer showed that a β3-adrenergic receptor agonist restored Nestin-positive cells within the stem cell niche, and thereby normalized blood counts and improved myelofibrosis in our *JAK2*-V617F MPN mouse model (Arranz *et al., Nature* 512:78-81, 2014). Based on these observations, we performed a clinical phase-II study with the β-3 sympathomimetic agonist Mirabegron in 39 *JAK2*-V617F-positive MPN patients (Drexler *et al.,* 2019). While the primary end point (reduction of *JAK2*-V617F allele burden of ≥50 %) was not reached, we observed increase in Nestin-positive niche cells and decrease in reticulin fibrosis in Mirabegron-treated patients. These results are encouraging and show that Mirabegron can modify the microenvironment where the *JAK2*-mutant stem cells are maintained and thereby diminish the fibrotic manifestations of MPN.

Selected Publications

Nienhold R, Ashcroft P, Zmajkovic J, Rai S, Rao NR, Drexler B, Meyer SC, Lundberg P, Passweg JR, Leković D, Čokić VP, Bonhoeffer S, Skoda RC. MPN patients with low mutant JAK2 allele burden show late expansion restricted to erythroid and megakaryocytic lineages. (2020) Blood, 136: 2591–95.

Rao TN, Hansen N, Hilfiker J, Rai S, Majewska JM, Leković D, Gezer D, Andina N, Galli S, Cassel T, Geier F, Delezie J, Nienhold R, Hao-Shen H, Beisel C, Di Palma S, Dimeloe S, Trebicka J, Wolf D, Gassmann M, Fan TWM, Lane AN, Handschin C, Dirnhofer S, Kröger N, Hess C, Radimerski T, Koschmieder S, Čokić VP, Skoda RC. JAK2 mutant hematopoietic cells display metabolic alterations that can be targeted to treat myeloproliferative neoplasms. (2019) Blood 134:1832–1846.

Drexler B, Passweg JR, Tzankov A, Bigler M, Theochari-des APA, Cantoni N, Keller P, Stussi G, Ruefer A, Benz R, Favre G, Lundberg P, Nienhold R, Fuhrer A, Biaggi A, Manz MG, Bargetzi M, Mendez-Ferrer S and Skoda RC, on behalf of the Swiss Group for Clinical Cancer Research (SAKK). The sympathomimetic agonist mirabegron did not lower JAK2-V617F allele burden, but restored nestin-positive cells and reduced reticulin fibrosis in patients with myeloproliferative neoplasms: results of phase 2 study SAKK 33/14. (2019) Haematologica, 104: 710–716.

Zmajkovic J, Lundberg P, Nienhold R, Lyngaas Torgersen M, Sundan A, Waage A Skoda RC. A gain-of-function mutation in EPO in familial erythrocytosis. (2018) N. Engl. J. Med., 378:924–930.

Shimizu T, Kubovcakova L, Nienhold R, Zmajkovic J, Meyer S, Hao-Shen H, Geier F, Dirnhofer S, Guglielmelli P, Vannucchi AM, Milosevic J, Kralovics R, Orkin SH, Skoda RC. Loss of Ezh2 synergizes with JAK2-V617F in initiating myeloproliferative neoplasms and promoting myelofibrosis. (2016) J. Exp. Med. 213: 1479–96.