Abstract

Tumor heterogeneity has been recognized for decades. The molecular mechanisms impacting clonal heterogeneity in hematological diseases, specifically myeloproliferative neoplasms (MPN) and mantle cell lymphoma, with the focus on several inherited genetic factors, inflammation, the protective mechanisms of DNA damage response (DDR) in the leukemic transformation and the treatment strategies are the focus of this thesis.

Firstly, I focus on studying germline *JAK2* variants and how these may influence the initiation and progression of MPN diseases, and even contribute to further genomic alterations in the mutated clone. A study performed by our cooperating lab in Utah, USA,[1] analyzing the mutational landscape of 31 *JAK2* V617F-positive polycythemia vera (PV) patients identified two novel germline mutations in *JAK2* gene, *JAK2* T108A and *JAK2* L393V. Another study[2], performed by our cooperating lab in Olomouc, Czech Republic, characterized two germline *JAK2* mutations, E846D and R1063H, in a case of hereditary erythrocytosis accompanied by megakaryocytic atypia. The *JAK2* R1063H variant was initially described in 3 out of 93 PV patients that were *JAK2* V617F-positive.[3] Our aim was to identify the role of selected inherited mutations in *JAK2* gene in the initiation and progression of myeloproliferative neoplasms. We show that the mutations *JAK2* T108A and L393V are weakly activating, they give rise to a kinase hypersensitive to erythropoietin, which could predispose cells carrying these mutations to proliferate at higher rate in physiological conditions. It is therefore possible that these two variants could precede the acquisition of *JAK2* V617F mutation in the course of the disease and contribute to further genomic alterations in the mutated clone and perhaps even leukemic transformation. We further characterized the double mutant *JAK2* V617F/R1063H in a cohort of MPN patients from Belgium and Romania. The two mutations are shown to cooperate to further increase JAK-STAT signaling characteristic of the single mutant JAK2 V617F. MPN patients with these two mutations present with higher amounts of white blood cells, and consequently higher amounts of neutrophils, which could be linked to increased biochemical association of the JAK2 R1063H mutant to granulocyte colony-stimulating factor receptor (G-CSFR).

Secondly, I focus on deciphering the protective role of KAP1 protein in the progression of myeloproliferative neoplasms. We hypothesize that a similar process of activating DRR components as described in solid tumors underlines the course of MPNs and that the activation of

a tumor suppressor barrier counteracts the progression to acute leukemia. In order to characterize the role of KAP1 protein in DDR of *JAK2* V617F-positive cells we generated HEL cell line (human erythroleukemia, *JAK2* V617F-positive) carrying either *JAK2* WT or *JAK2* V617F allele and consequently we knocked-out *KAP1* gene in these cells. KAP1 will further be studied in this setting, its role in cell proliferation upon induced DNA damage, differentiation and its impact on genomic instability will be characterized.

Thirdly, I focus on identifying the role of prolyl hydroxylase 1 (*EGLN2*/PHD1) and FOXO3A transcription factor in mantle cell lymphoma (MCL). It has been previously[4] reported that an inability of PHD1 to hydroxylate FOXO3A promotes its accumulation in cells, which in turn suppresses *cyclin D1* expression by a yet unknown mechanism. Cyclin D1 is overexpressed in MCL and it is therefore of interest whether iron chelation, which inhibits the function of PHD1, would be beneficial in downregulating cyclin D1 in MCL cells. It was indeed shown that iron chelation decreases cyclin D1 mRNA and protein levels in MCL cell lines[5], the molecular mechanism, however, remains unknown. Iron chelation has been previously reported to promote cyclin D1 proteasomal degradation by a ubiquitin-independent mechanism.[6] In our study we show that in MCL cell lines iron chelation is effective in inhibiting proliferation, inducing apoptosis and cell cycle arrest by means of regulating cyclin D1 levels. We also show that the overexpression of cyclin D1 in these cells makes them more susceptible to chelation treatment. We found out that cyclin D1 in MCL cells escapes PHD1 – FOXO3A regulation circuit and cyclin D1 downregulation upon iron depletion is regulated by a different yet unknown mechanism. We further studied the role of NQO1, previously shown to be involved in ubiquitin-independent proteasomal degradation, by employing NQO1 inhibitor dicoumarol to determine whether its unavailability could lead to destabilization of cyclin D1 protein in MCL cell lines. DIC-treated cells exhibited downregulation of *cyclin D1* mRNA and protein levels. It remains unclear whether the observed cyclin D1 repression is an outcome of NQO1 having a specific protective role in protein degradation or is a result of unspecific inhibition of its oxidoreductase activity.

1. Wang L, Swierczek SI, Drummond J, et al. Whole-exome sequencing of polycythemia vera revealed novel driver genes and somatic mutation shared by T cells and granulocytes. *Leukemia*. 2014;28(4):935–938.
2. Kapralova K, Horvathova M, Pecquet C, et al. Cooperation of germ line JAK2 mutations E846D and R1063H in hereditary erythrocytosis with megakaryocytic atypia. *Blood*. 2016;128(10):1418–23.
3. Levine RL, Wadleigh M, Cools J, et al. Activating mutation in the tyrosine kinase JAK2 in polycythemia vera, essential thrombocythemia, and myeloid metaplasia with myelofibrosis. *Cancer Cell*. 2005;7(4):387–397.
4. Zhang Q, Gu J, Li L, et al. Control of Cyclin D1 and Breast Tumorigenesis by the EglN2 Prolyl Hydroxylase. *Cancer Cell*. 2009;16(5):413–424.
5. Vazana-Barad L, Granot G, Mor-Tzuntz R, et al. Mechanism of the antitumoral activity of deferasirox, an iron chelation agent, on mantle cell lymphoma. *Leuk. Lymphoma*. 2013;54(4):851–9.
6. Nurtjahja-Tjendraputra E, Fu D, Phang JM, Richardson DR. Iron chelation regulates cyclin D1 expression via the proteasome: a link to iron deficiency-mediated growth suppression. *Blood*. 2007;109(9):4045–54.