ABSTRACT IN ENGLISH LANGUAGE

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Title of the doctoral thesis: Resistance mechanisms in therapy of acute myeloid leukemia

Acute myeloid leukemia (AML) is a hematologic cancer known for its extensive heterogeneity, poor treatment outcomes and high relapse rate. Therapy outcome is often compromised by highly resistant leukemic clones present at diagnosis, which evade chemotherapy and continue to spread the disease. Identification of their cellular features is, therefore, a key in successful targeting and eliminating of these resistant leukemic cells. AML cells can, however, develop drug resistance even overtime due to prolonged drug exposure. Extremely high adaptability of leukemic cells enables them to survive whenever therapeutic stress stimuli occur. Uncovering molecular mechanisms that cells utilize to activate their survival mode is crucial in selection of treatment leading to maximal efficacy.

Based on these grounds, two main aims of this thesis were set. First, to determine clinical relevance of ABC efflux transporters in AML and to evaluate the effect of targeted agents on chemotherapy. The focus was put on agents belonging to either FLT3 inhibitors (midostaurin) or CDK4/6 inhibitors (abemaciclib, palbociclib, ribociclib). Second aim was to evaluate transcriptome and proteome changes in AML cell line after having acquired resistance to gilteritinib, another drug from the group of FLT3 inhibitors. Lysosomes were further explored as mediators of gilteritinib resistance in more detail.

In the first part, we focused on ABCB1 transporter widely recognized for its role in cancer resistance. In peripheral blood mononuclear cells isolated from patients *de novo* diagnosed with AML, increased *ABCB1* gene expression was identified in patients not responding to anthracycline-based induction therapy, and therefore, not achieving complete remission. Patients highly expressing *ABCB1* were predominantly CD34 positive and belonged to a patient group with adverse cytogenetic risk. Activity of ABCB1 was diminished by all tested kinase inhibitors (midostaurin, abemaciclib, palbociclib, ribociclib), which was reflected in elevated intracellular anthracycline concentrations while at the same time providing evidence of ABCB1 being their off-target. Moreover, exposure of ABCB1-overexpressing leukemic cells to midostaurin and anthracycline led to induction of apoptosis. We also found a direct linkage

between ABCB1 efflux activity and miR-9 expression, which post-transcriptionally regulates ABCB1 in AML. Collectively, we provide evidence that ABCB1 gene expression and function is highly related to resistant AML phenotype and that miR-9, if used as a biomarker, could be helpful in identifying such patients.

The second part elaborated on gilteritinib-resistant HL-60 G75 cell line developed in our lab. Cells developed resistance, which was transient, and upon gilteritinib withdrawal, it was completely reversed only after four weeks. Distinct transcriptome and proteome profiles were revealed in HL-60 G75 when compared to gilteritinib-sensitive HL-60 WT. Although the resistance appeared to be resulting from modification of multiple cellular processes, some stood out more than the others. Lysosomes-related processes as one of the most deregulated ones were explored further. Lysosomes-specific staining revealed increased number of lysosomes in HL-60 G75, however, gilteritinib withdrawal led to immediate decrease. Sunitinib, a drug with similar mechanism of action and physicochemical properties as gilteritinib, but fluorescent, was utilized to detect sequestering capacity of lysosomes. Sunitinib was fully sequestered in lysosomes in HL-60 G75 but seemed to be predominantly spread within cytosol upon gilteritinib withdrawal. Fluctuation of lysosomal mass and/or activity appeared to be highly dependent on gilteritinib presence. Although the exact mechanism is not yet known, we suppose that gilteritinib might have a direct or indirect impact on lysosomal biogenesis.

In summary, data presented in this thesis brought new insights into mechanisms of resistance, which to this day presents one of the most challenging obstacles in AML pharmacotherapy.