The role of EZH2 activity in the development of resistance to targeted therapy in melanoma

Scientific goal of the project

The main goal of the project is to evaluate the role of EZH2 histone methyltransferase in the progression and acquired resistance to current targeted therapies. We will investigate whether parallel inhibition of EZH2 and mutated BRAF kinase prevents the development of drug resistance, and explore EZH2-dependent mechanisms of drug resistance in melanoma.

Purpose and justification of the project

Melanoma is a malignant skin neoplasm derived from melanocytes which in the advanced stage of the disease leads to high mortality of patients. In approximately half of melanoma cases, an activating mutation in BRAF kinase ($BRAF^{V600}$) is present, which triggers constitutive activity of the MAPK pathway characterized by an increase in the phosphorylation of downstream MEK and ERK kinases responsible for the uncontrolled proliferation of melanoma cells and tumor progression. The development of specific and selective $BRAF^{V600}$ kinase inhibitors, used alone or in combination with MEK kinase inhibitors, resulted in an effective anti-tumor response and tumor reduction in the majority of treated patients. Unfortunately, after initial response tumor cells acquire resistance to targeted drugs, causing the disease relapse and patients deaths. Despite extensive research on melanoma therapy resistance, new druggable targets are still being sought to increase the efficacy of targeted drugs and to cope with drug resistance. Drug resistance mechanisms in cancer therapy are still not well understood. This resistance may arise from drug-induced mutations, reversible phenotypic plasticity of melanoma cells and transient epigenetic changes that control transcriptomic reprogramming. Enhancer of zeste homolog 2 (EZH2) histone methyltransferase is a key chromatin modifier that by methylating specific histone protein leads to target gene repression. Moreover, non-canonical EZH2 activity spans methylation of various non-histone proteins leading to their stabilization or degradation. Furthermore, EZH2 forms complexes with other proteins independent of its methyltransferase activity, which has been also attributed to the progression of various cancers. In melanoma, EZH2 is frequently overexpressed or bears activating mutations. Abnormal EZH2 activity has been linked to development of resistance to chemo- and immunotherapies in various cancers, but little is known about EZH2 involvement in resistance to targeted therapy in melanoma. Several specific EZH2 inhibitors blocking its histone methyltransferase activity are currently being tested for clinical use, however, so far they do not block non-canonical EZH2 activity. Moreover, since EZH2 requires specific long non-coding RNAs (lncRNAs) as cofactors to potentiate its activity, aberrantly modified transcriptome in drug-resistant melanoma cells might alter EZH2 affinity to its binding partners and hence promote novel responses.

Description of the research

We hypothesize that blocking both canonical and non-canonical EZH2 activity will impede melanoma progression and will prevent the emergence of drug resistance to $BRAF^{V600}$ inhibitor vemurafenib. Therefore, first we plan to evaluate transcriptional changes between drug-naïve and vemurafenib-resistant patient-derived melanoma cell lines established in our laboratory, and identify novel genes regulated by EZH2 and EZH2-lncRNA interactions unique to drug-resistant cell lines. Subsequently, we will evaluate the short-term effects of inhibition of EZH2 methyltransferase activity and EZH2 depletion in drug-naïve cells treated with vemurafenib. We will also evaluate the stability and activity of a novel EZH2 mutant of unknown functions, identified in melanoma cell lines in our laboratory. Finally, we will investigate whether blocking canonical or non-canonical EZH2 activity may prevent or postpone the emergence of drug resistance, when combined with long-term vemurafenib treatment, or revoke the already developed resistance to vemurafenib in melanoma. In this project we will use state-of-the-art molecular biology methods including next generation sequencing (RNA-seq, ChIP-seq and RIP-seq), CRISPR/Cas9 gene editing and bioinformatics analysis. The most interesting and promising results will be evaluated in *in vivo* mouse melanoma model.

The most important expected effects of the project

Successful execution of this proposal will serve multiple goals. This highly interdisciplinary project is expected to give novel insight into the mechanisms of drug resistance promoted by aberrant EZH2 activity. The results of this project will provide a new rationale for EZH2 inhibition in clinical settings of melanoma therapy, and to identify novel EZH2-lncRNAs interactions and their downstream pathways. Blocking not only EZH2 methyltransferase activity, but also non-canonical EZH2 activity, with the use of applicable inhibitor, may help to improve future melanoma treatment options.