Rozprawa doktorska:

„Rozwój metod i modeli badawczych umożliwiających selekcję oraz potwierdzenie specyficzności i mechanizmu działania innowacyjnych związków małocząsteczkowych celujących w białka kompleksu SWI/SNF”

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„Development of research methods and models enabling novel SWI/SNF-targeting small molecule inhibitors selection, specificity confirmation and mechanism of action determination”

One of the mechanisms responsible for gene expression are chromatin remodeling complexes, such as SWI/SNF (SWitch/Sucrose Non-Fermentable chromatin remodeling complex). As the result of the complex interaction, nucleosomes can slide along the DNA, causing chromatin relaxation or condensation, which in turn can affect both the activation and repression of gene expression. Previous studies of the cancer cells and patient samples genome have detected a high percentage of SWI/SNF subunits alterations, which also included mutations in the *SMARCA4* gene (SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily A, member 4), encoding the key catalytic protein of the complex - BRG1 (Brahma-related gene 1). According to the preliminary findings, the lack of BRG1 in cancer cells made them dependent on the presence of other regulators, including homologous catalytic protein - BRM (Brahma), encoded by the *SMARCA2* gene. Such observed phenomenon, called synthetic lethality, is now a widely used mechanism for the development of new anti-cancer therapies and became the main focus of Ryvu Therapeutics' efforts.

The aim of the study conducted within this doctoral dissertation was to develop the methodology that could be used in the process of new drugs development, which can prove effective in patients with neoplasms characterized by non-functional BRG1 protein.

In the first step, the sensitivity of cells with *SMARCA4* mutations to *SMARCA2* silencing has been confirmed by bioinformatic analysis of data published on the DepMap and cBioPortal portals. Moreover, the BRM+BRG1- non-small cell lung cancer cells were identified as the most sensitive tumor type. According to the literature reports, a therapy with selective BRM inhibitors should not cause systemic toxicity, numerous side effects were however observed for compounds with dual specificity for BRM and BRG1 proteins. The development of BRM small molecule inhibitors was challenging, as the amino acid sequences of BRM and BRG1 are identical in 90%. Nevertheless, Ryvu Therapeutics decided to take the risk approach and create a cascade of screening tests for the selection and characterization of the new small molecule compounds. This doctoral dissertation mainly focused on the optimization of cellular tests, as well as biophysical and in vivo assays. The developed Thermal Shift-based assays, optimized for BRM or BRG1 protein, created a unique set of methods which enabled confirmation of the interaction of the compound with a target protein. As part of the doctoral dissertation, four isogenic cell lines have been created to study compounds' activity and selectivity. In addition, an extended cell lines panel, representing therapeutic potential, has been selected and optimized to perform long-term viability studies. The key step in the new drug development process includes the determination of cellular biomarkers, which can be defined with the use of RNAseq and ChIPseq methods. As a result of such analyses, two direct biomarkers were selected (*KRT80* – measured by qPCR and the confidential Biomarker 2 – evaluated by AlphaLISA). Further in vivo studies have shown that both *KRT80* and Biomarker 2 could also act as pharmacodynamic biomarkers in the A549 xenograft model in Athymic nude mice. All optimized methods used in the dissertation work were included in the test cascade created by the Ryvu Therapeutics, which emphasizes their high application potential.

Due to the encountered challenges in the process of the selective BRM inhibitors development, the high-throughput phenotypic screening test has been developed in parallel. For assay optimization, the isogenic cell lines with and without loss of function mutations in the *SMARCA4* gene were used in the study. As the result, it was intended to identify compounds, which will be able active only in cells lacking the BRG1 protein, and, will represent a new mechanism of action.

In conclusion, the project performed within this doctoral dissertation led to the development of a novel method for small molecule compound libraries testing in a high-throughput format which brings new hope for patients with BRG1-loss tumors.