13. Streszczenie pracy doktorskiej w języku angielskim

Title of the thesis: *"Overcoming multidrug resistance in cancer at the genome level: development of a method to prevent overexpression of ABC transporters in cancer cells based on inhibitors of chromatin remodeling enzymes"*

Despite years of research and scientists' efforts to develop novel therapies, cancer is still the second most common cause of death in Poland, and chemotherapy is the most widely used treatment. Nearly half of patients with triple-negative breast cancer experience a relapse after treatment with this type of therapy. This phenomenon is due to the acquisition of multidrug resistance (MDR) as a result of stress factors such as hypoxia, radio- and chemotherapy. Cells with MDR are characterized by decreased sensitivity to cytostatics through, among other things, overexpression of ABC transporters and polynucleation. In a previous work by our team, it was shown that induction of cisplatin resistance in the A549 and MDA-MB-231 cell lines led to the appearance of p300 acetyltransferase on the promoter of the *ABCC10* gene. Therefore, in the first part of the study, the effect of the CBP/p300 bromodomain inhibitor I-CBP112 on the down-regulation of ABC transporter expression in baseline and clinically relevant, induced resistance lines MDA-MB-231 and A549 was described. In addition, the safety profile of the test compound against normal lines was verified, as well as the ability of I-CBP112 to induce and enhance poly(aneu)ploidy, which is among the mechanisms of cancer cell resistance. Complementary to the present experiments, we prepared two review papers on the phenomenon of polynucleation in cancer, as well as a comparison of pharmacokinetic properties, ADMET parameters and collateral targets of commercially available CBP/p300 inhibitors differing in their mechanism of action. Considering that PARP1 protein is a correlator of CBP/p300 action, in the second part of the thesis I focused my attention on the effect of PARP1/HPF1 complex on the regulation of ABC transporter expression, as well as identification of the transcription factor responsible for PARP1-dependent transcription of *ABCC3*, *ABCC4* and *ABCC5* genes.

This paper presents results from two published experimental papers, which clearly demonstrate the significant effect of the bromodomain inhibitor I-CBP112 on breaking multidrug resistance of baselines and resistant MDA-MB-231 and A549. I-CBP112 sensitizes cells by down-regulating the expression of ABC transporters, increasing the accumulation of anticancer drugs, resulting in a lower IC50 of selected cytostatics. Moreover, the tested CBP/p300 bromodomain inhibitor has desirable ADMET properties, does not show toxicity to healthy cell lines, and does not induce the process of polynucleation. I was also able to show that I-CBP112 acts on the same

regulatory pathway as the commonly used pan-ABCC inhibitor, and its action is dependent on LSD1 demethylase.

In the second part of the work, I demonstrated the essential role of PARP1 protein in regulating ABC gene transcription. Applications of PARP inhibitors and silencing of components of the PARP1/HPF1 complex resulted in decreased expression of resistance-relevant genes and enhanced accumulation of selected cytostatic agents in 2D cultures and in spheroids. Importantly, PARP1 activity itself was higher in the doxorubicin-resistant lineage, which could be due to the higher tolerance of resistant cells to DNA-damaging agents, as well as increased levels of post-translational modifications of this protein such as methylation, acetylation and phosphorylation. The stage of identifying the transcription factor responsible for PARP1-dependent expression of ABCC3 and ABCC4 transporters has identified SMARCA1 as a potential candidate for further study. Its significant effect on doxorubicin-induced cell resistance was confirmed by a decrease in the expression of selected ABCCs in SMARCA1-deficient cells, accompanied by an increase in the accumulation of selected anticancer drugs and enhanced cytotoxicity.

Considering the findings obtained in the initial section of the paper, I can conclude that the p300/CBP inhibitor I-CBP112 is an attractive candidate to complement standard chemotherapy, as it enhances its efficacy in lines with generated multidrug resistance. Moreover, it is not toxic to healthy cells, has a desirable ADMET profile, and does not induce the formation of poly(aneu)ploidy cells. PARP1 is a cofactor of p300, acting in regulatory regions of certain ABC genes, and the mediator of this enzyme's activity is SMARCA1, which physically interacts with PARP1, HPF1 and p300.

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