Streszczenie pracy doktorskiej w języku angielskim

Title of the thesis: The role of SWI/SNF-EP300 complex in the development of cancer cell resistance to chemotherapy

Cancer is a significant public health issue worldwide. According to the most recent data from the GLOBOCAN project, cancer incidence was approximately 20 million in 2022, with cancer mortality at 9.7 million, ranking cancer as the second leading cause of death globally. Furthermore, statistics predict an increase in cancer incidence in the coming decades. It is estimated that 32.6 million new cases will be diagnosed in 2045.

A significant proportion of cancers are characterised by high mortality rates despite the availability of various treatment methods, such as surgery, radiotherapy, chemotherapy and targeted therapies. Combination therapies are the standard of current cancer treatments and apply to all treatment regimens. The combination of therapies is attractive from a clinical point of view for several reasons: combination therapy improves treatment outcomes and provides better therapeutic effects, especially when synergistic anticancer effects are achieved; the combination approach overcomes clonal heterogeneity, which is further associated with improved response rates; combination treatment regimens reduce the toxicity of therapy, as they allow individual drugs to be used in reduced doses while maintaining therapeutic efficacy; combination therapies reduce the incidence of drug resistance by eliminating cellular mechanisms associated with adaptive resistance. Since resistance to therapies is accompanied by genetic and epigenetic changes in cancer cells, the use of combination therapies involving epigenetic regulators appears to be a promising solution. Epigenetic modifications are flexible and dynamic, making them attractive therapeutic targets that can help achieve the desired transcriptome profile in cancer cells.

Histone acetyltransferase p300 (encoded by the *EP300* gene) is one of the most frequently dysregulated acetyltransferases in cancer. The activity of this protein is closely related to H3K27 acetylation at enhancer sites. A correlation has been demonstrated between a decreased overall survival rate in patients with high EP300 expression, which may be related to reduced treatment efficacy in these patients. Increasing experimental evidence indicates that drug resistance in tumours may be determined by p300 activity. The promoter sequences of ABC transporters, which are overexpressed in cisplatin-resistant breast and lung cancer cell lines and are responsible for the removal of drugs from these cells, are characterised by higher levels of nucleosome acetylation catalysed by p300. p300 also plays an important role in DNA repair, as it is recruited to DNA break sites, where it facilitates damage repair by acting as a cofactor and binding module for many proteins involved in DNA repair pathways.

Acetylation of histone H3 tails, regulated among others by p300, increases the affinity of the SWI/SNF complex to nucleosomes. The SWI/SNF complex participates in the mobilisation of nucleosomes, thereby promoting or inhibiting the expression of specific genes. The main components of the complex include ATPases, BRG1 and BRM. The ATPase domains of BRG1 and BRM mediate the binding of nucleosomes and enable their movement, removal and insertion through ATP hydrolysis. Structurally, BRG1 and BRM are very similar, but may exhibit tissue-specific functions. Changes in the expression of specific SWI/SNF subunits may

be considered prognostic markers of survival. In addition, a link between certain components of the SWI/SNF complex and resistance to chemotherapy and targeted therapies has been demonstrated.

The aim of the research carried out as thesis project was to investigate the role of the SWI/SNF-p300 complex in the development of cancer resistance associated with:

1) overexpression of ABC transporter fractions, which increases in lysosomes, causing increased accumulation of drugs in these organelles

2) an increase in the expression of genes functionally related to the response to DNA damage, caused by the activation of the ATM/ATR-Chk1/Chk2-p53 pathway

In the first part of my thesis, I focused on the problem of increased expression of ABC transporters, which contribute to treatment failure by removing or sequestering drugs in organelles. I presented experimental evidence for the involvement of BRG1 and p300 in the transcriptional control of ABCC genes, which are overexpressed in paclitaxel-resistant cell lines. In particular, I focused on the influence of chromatin remodelling enzymes on the transcription of ABCC3, ABCC5 and ABCC10 genes, as their products are enriched in the lysosomes of paclitaxel-resistant cells. Functional analysis of these three lysosomal proteins revealed their role in the sequestration of doxorubicin and paclitaxel (OregonGreen), and thus these membrane transporters are capable of reducing the cytotoxicity of at least some drugs used in chemotherapy. The active promoters of these genes are characterised by H3K4 trimethylation and the presence of BRG1, p300 and their co-regulator HIF1A. I also demonstrated that inhibition of SWI/SNF using PFI3 or degradation of SWI/SNF ATPases using PROTAC, inhibition of p300 using C646, and silencing of HIF1A significantly increase drug toxicity through the simultaneous reduction of several ABC transporters in paclitaxel-resistant phenotypes. Based on data deposited in the TCGA/GTEx databases, I provided evidence that high expression of EP300, SMARCA4, and HIF1A may serve as a prognostic marker of response to taxane-based chemotherapy in breast cancer patients. At the same time, high transcription of HIF1A, EP300 and SMARCA4 may be clinically relevant and affect treatment outcomes. Therefore, quantitative assessment of their expression profile may help in deciding on a treatment regimen, replacing chemotherapy or combining chemotherapeutic drugs with ABCC inhibitors or BRG1, p300 or HIF1A inhibitors.

In the second part of my work, I focused on the mechanism related to resistance to cisplatin, a drug that causes DNA damage. I demonstrated that the activation of the ATM/ATR-Chk1/Chk2-p53 pathway by cisplatin does not significantly affect the level of p300 inside the cell, but intensifies the interaction of p300 with chromatin, especially with the promoters of genes controlled by E2F1, in which p53 acts as a transcription repressor. Promoters silenced by p53 in proliferating cells were characterised by relatively high nucleosome density, low H3K27 acetylation and H3K4 trimethylation. Induction of the ATM/ATR-Chek1/2-p53 pathway by sublethal doses of cisplatin caused the release of p53 from the promoters of the described genes, which was also accompanied by the release of KDM5B and an increase in histone acetylation, enabling p300- and SWI/SNF-dependent transcription. In our experiments, both inhibition of p300 with C646 and inhibition of SWI/SNF complex ATPase with PFI3 reduced the expression

of cisplatin-activated genes and sensitised cancer cells to low concentrations of cisplatin. The disassociation of p53 under the influence of cisplatin was also accompanied by the release of KDM5B and the activation of gene expression. KDM5B and p53 therefore acted as repressors of these genes. It is worth considering the reduction in expression and inactivating mutations of TP53 and KDM5B as biomarkers of increased expression of these genes, which may predispose to resistance to DNA-damaging drugs. Inactivation or low expression of these proteins may be an indication for a therapeutic approach other than DNA-damaging therapy or the use of SWI/SNF or p300 inhibitors as chemotherapeutic sensitizers.

The results presented in this study suggest that the use of SWI/SNF inhibitors may sensitise cells to chemotherapy and reverse an existing resistant phenotype by regulating the transcription of certain ABC family genes and genes associated with the response to DNA damage. The use of an inhibitor + drug combination increases the cytotoxicity of the drug, allowing lower doses to be used, which could potentially increase the effectiveness of the therapy and reduce side effects. However, further studies are needed to verify the potential of such combination therapies *in vivo*.

Karolina Gronkowska