Synthesis and study of biological activity of isothiocyanates against bladder cancer cell lines

Bladder cancer is the 10th most common cancer worldwide. Bladder cancers are widely recognized as chemoresistant, and the failure of first-line therapy, which is mainly based on cisplatin, leads to even more resistant, life-threatening malignancies that are difficult to treat, even with the latest drugs (such as vinflunine). Therefore, it is necessary to search for new compounds that could be potential anticancer drugs. A group of such compounds that could be new drugs in the future are isothiocyanates. These compounds are found in cruciferous vegetables such as broccoli, cauliflower, and Brussels sprouts. They are formed as a result of the hydrolysis of glucosinolates with the participation of myrosinase. Isothiocyanates have chemopreventive, anticancer and bactericidal properties. They affect many different processes including the: cell cycle, apoptosis and angiogenesis. They accumulate in the bladder, therefore bladder cancer becomes a natural target for therapy involving these compounds.

This PhD thesis has been divided into two main parts. The aim of the first part was to generate bladder cancer cell lines resistant to cytostatics currently used in therapy, i.e. cisplatin, vinblastine, gemcitabine and both cisplatin and gemcitabine, and then performing their molecular characterization. The aim of the second part of the study was to synthesize a set of structurally diverse isothiocyanates derived from amino acid esters and to evaluate the biological activity of the synthesized compounds against bladder cancer cells, characterized during the first part of the study.

Based on the results obtained, it was found that the drug-resistant cells, derived from various parental cell lines, despite the fact that they are resistant to the same cytostatics, developed their resistance through different mechanisms, which was found based on the level of the genes and proteins associated with resistance. This resulted in completely different cross-resistance profiles of bladder cancer-resistant cells to other compounds.

During the second part of the research, 29 novel isothiocyanates, derived from amino acid esters were synthesized. Compounds were synthesized as two series. The first one included 18 compounds: methyl, butyl, ethyl, benzyl, isopropyl and cyclohexyl esters of alanine, phenylalanine or phenylglycine. On the basis the results of the *in vitro* antiproliferative activity of these compounds, it was found that isothiocyanates, which are derivatives of methyl esters, are more active than the others. Therefore, the second series included 11 isothiocyanates containing methyl esters group. These compounds were also subjected to *in vitro* antiproliferative tests in order to select the most active compounds and investigate the mechanisms responsible for their biological activity, and then try to answer the question of how the structure of isothiocyanates affects their antiproliferative activity. The results obtained indicate that the synthesized compounds demonstrate antiproliferative activity against both, sensitive and drug-resistant bladder cancer cells. Mechanisms of its activity include: increased activity of caspase 3/7, inhibition of the cell cycle in the G2/M phase, reduced clonogenic potential of cells, and inhibition of tubulin polymerization. It has been shown that the chemical structure of isothiocyanates has an impact on their biological activity, i.e. the simpler the side chain of the isothiocyanate amino acid and the shorter the aliphatic chain of the ester, the greater antiproliferative activity of the compound.

In summary, during research for this doctoral dissertation, four new drug-resistant bladder cancer cell lines were developed and their molecular characterization was performed. 29 isothiocyanates derived from amino acid esters were synthesized, which exhibit antiproliferative activity against cells of the sensitive and drug-resistant bladder cancer cell lines. It has been proven that the chemical structure of the synthesized isothiocyanates affects their antiproliferative properties.