„Mechanisms of action of usnic acid derivative against pancreatic cancer cells"
Mariola Gimła, MSc

Cancer is currently one of the most serious health challenges worldwide. Pancreatic cancer is the third most deadly neoplasia, after lung and colon cancer. In 2022, almost half a million people died from this disease. The increase in morbidity and mortality due to malignant tumors, including pancreatic cancer, is the reason for the continuous search and development of new drugs, also based on naturally occurring compounds.

Usnic acid (UA 2,6-diacetyl-7,9-dihydroxy-8,9b-dimethyldibenzo[b,d]furan 1,3(2H,9bH)-dione) belongs to the benzofuran secondary metabolites of lichens and exhibits many biological activities, including anticancer activity. Numerous studies indicate that UA has antiproliferative potential by arresting the cell cycle at the G0/G1, S or G2/M phase, and also causes the death of cancer cells through apoptosis or necrosis. Despite its promising activity, UA also has disadvantages. First of all, it must be used in high concentrations to exhibit anticancer activity, and it is also characterized by hepatotoxicity. To improve its properties, including increasing its activity and selectivity towards cancer cells, as well as enhancing its water solubility and bioavailability, researchers are designing its synthetic derivatives.

One of such derivatives, which more strongly than UA reduces the viability of various types of cancer cells, is (R)-8-acetyl-5,7-dihydroxy-3,4a,6-trimethyl-2,4a-dihydro-4H benzofuro[3,2-f]indazol-4-one, a pyrazole derivative of UA, designated as **5**.

This study aimed to compare activity of derivative **5** with the parent compound against human pancreatic cancer cells and to understand the mechanisms of its action.

The obtained results indicate that derivative **5** acts multidirectionally in pancreatic cancer cells of the Mia PaCa-2 and PANC-1 lines. It exhibits stronger antiproliferative and cytotoxic potential than UA by inhibiting the cell cycle at the G0/G1 phase and inducing cell death. The basis of these processes is endoplasmic reticulum (ER) stress. Importantly, this effect is also visible *in vivo*. In mice transplanted with human pancreatic cancer cells, derivative **5** effectively inhibits tumor growth and causes no visible side effects.

Moreover, derivative **5** effectively limits the migration and invasion of pancreatic cancer cells. This is accompanied by modulation of the levels of epithelial-mesenchymal

transition (EMT) markers, especially the increase in the level of Claudin-1 protein, suggesting that this compound, by maintaining the integrity of the epithelium, could prevent or reverse the EMT process and thus reduce the metastatic potential of cancer cells. Derivative **5** also normalizes mitochondrial morphology, which is associated with a decrease in ATP levels in cancer cells. Combination of derivative **5** with a glycolysis inhibitor, 3-BrPA, decreases ATP levels in a synergistic way, which affects the intensity and type of cell death.

In summary, by affecting processes such as the cell cycle, cell death, migration, invasion, EMT and energy metabolism, derivative **5** limits many key stages in the progression of pancreatic cancer. The fact that it is effective and safe in the in vivo model encourages further research on its use in the therapy of this cancer.