**Abstract**

**Introduction:** The occurrence of the phenomenon, where loss of an adequate response to anti-cancer treatment is observed, or drug resistance, is connected with, among other things: the occurrence of new DNA mutations; metabolic changes in cancer cells; drug inactivation; inhibition of cancer cell apoptosis; the epithelial-mesenchymal transition (EMT); heterogeneity of the cells constituting the tumor mass; the influence of epigenetic factors; as well as any combination of the listed factors.

The role of epigenetic mechanisms engaged in the loss of an adequate response to treatment should be remembered, including the regulation of gene expression through miRNA molecules.

A promising drug in anti-cancer therapy seems to be salinomycin, a mechanism of which is related to the induction of an improper ion balance between sodium and potassium in cell membranes, which consequently leads to cancer cell apoptosis.

Semaphorins (SEMA) belong to a group of membrane proteins which participate in the regulation of such cell processes like: proliferation; cancer cell migration; angiogenesis. They also participate in the process of the drug resistance phenomenon occurring.

Despite research into the role of SEMA3B in cancer formation, based on the current available literature, the relationship between the expression of SEMA3B and the overall survival rate in endometrial cancer patients has as of yet, not been established.

**Aim:** The main aim of this study was to determine the expression profile of mRNA and miRNA related with the drug resistance phenomenon as well as the signaling pathways dependent on caspases in Ishikawa line endometrial cancer cells treated with salinomycin compared to the control culture.

**Materials and Methods:** Ishikawa line endometrial cancer cells were exposed to salinomycin at a concentration of 1μM over a period of 12,24 and 48 hours, compared to a control culture, which was formed of cells untreated by the drug.

The study group consisted of 45 patients diagnosed with endometrial cancer, above 45 years of age, including 17 patients with a cancer at a histopathological differentiation grade of G1, 15 patients with a cancer at a grade of G2, and 13 patients with a cancer at a grade of G3. Samples were obtained during a hysterectomy and afterwards were assessed in histopathological terms, which allowed for the separation of the samples based on the endometrial cancer histopathological differentiation grade.

The control group consisted of 15 patients, in which endometrial samples were taken during hysterectomy, conducted due to non-oncological reasons.

Molecular analysis included the extraction of whole RNA from endometrial cancer cells, assessing the quality and quantity of the extracts, determining an expression profile of the mRNA related with the occurrence of the drug resistance phenomenon and the caspase pathway through the oligonucleotide microarray technique as well as determining the micro-RNA (miRNA) potentially regulating the expression of the assessed transcripts using miRNA microarrays. In the clinical material, the expression of SEMA3B was determined using immunohistochemical staining.

**Results:** Assessing the microarray expression profile of genes related to drug resistance, it was observed that the number of mRNA differentiating the culture incubated with the drug from the control, depending on the exposition time of the cells to salinomycin was the following: H_12 vs C – 9 mRNA; H_24 vs C - 7 mRNA; H_48 vs C - 1 mRNA. The largest changes in gene expression were determined for: TUFT1; ABCB1; MTMR11; SLC30A5.

On the basis of the predictive analysis carried out, the strongest relationship was noted between: *TUFT1* and hsa- miR-3188 (FC + 2.48); *MTMR11* and has-miR-16 (FC - 1.74); as well as between *SLC30A5* and hsa-miR-30d (FC -2.01).

In turn, for the caspase pathway, it was observed that the number of mRNA differentiating the endometrial cancer cells exposed to salinomycin compared with the control culture for each incubation time of the cells with the drug were: H_12 vs C - 8 mRNA; H_24 vs C - 10 mRNA; H_48 vs C - 3 mRNA. Furthermore, it was observed that 5 out of the 14 differentiating mRNA differentiated the Ishikawa line endometrial cancer cell culture regardless of the incubation time of the cells with the drug and corresponded to the genes: *CASP3*; *CASP8*; *CASP9*.

The highest probability of influencing the *CASP3* mRNA expression was observed for hsa-miR-30d (average FC value – 2.01). In turn, the highest probability of influencing the expression profile of the CAS*P8* mRNA was noted for hsa-miR-21 (average FC value +1.31), while the expression of the *CASP9* mRNA seems to be potentially regulated by hsa-miR-1271 (average FC value +1.71).

Based on the conducted research, the expression of SEMA3B was noted in endometrial cancer, vascular endothelium, and tumor stroma cells, of which, the highest expression determined in endometrial cancer cells was at a G1 grade, in tumor stroma cell clippings it was G2, while in endothelial cells in cancer samples, the grade of differentiation was G3.

**Conclusions:** Based on the conducted research as part of this study, it was confirmed that salinomycin added to the Ishikawa line endometrial cancer cell culture indices changes in the mRNA and miRNA transcriptome related to the drug resistance phenomenon and caspase pathway. Furthermore, we observed that salinomycin induces apoptosis in endometrial cancer cells, mainly through the mitochondrial pathway. It was also determined that during endometrial cancer, the expression of SEMA3B is different than under physiological conditions. A close relationship between the metastatic potential and the intensity of angiogenesis was confirmed as well as the occurrence of an interaction between the signaling pathways in the neoplastic transformation process.

**Keywords:** endometrial cancer; drug resistance; caspases; miRNA; SEMA3B