SUMMARY

Breast cancer is one of the most frequently detected malignant tumors among women around the world. According to a report by the World Health Organization in 2012, breast cancer was diagnosed in nearly 1.7 million patients and was the cause of deaths of over half a million people.

Despite many studies, the etiology of breast cancer remains not well understood.

Taking into account the histological features of breast cancer, one can distinguish between pre-invasive and invasive breast cancers. When analyzing changes in the gene expression and immunohistochemical traits, breast cancer is divided into particular types: luminal A, luminal B with / without HER2, HER2 (+), triple-negative breast cancer.

The most important problem regarding cancer is the effectiveness of the therapy. It is associated with the induction of multidrug resistance (MDR) in cancer cells. The phenomenon of MDR is manifested by the disappearance or complete lack of sensitivity of tumor cells to cytostatic drugs. This is often related to the inhibition of the process of tumor cell apoptosis.

The aim of this study was:

1. Analysis of the changes in the expression of 92 genes associated with the apoptosis process using the TLDA microarray method in the fragments of tumor tissue collected from patients with triple-negative breast cancer compared to the normal tissue surrounding the tumor.
2. Analysis of the changes in the expression of 40 genes from the ABC family (including 14 genes related to the induction of multidrug resistance) using the TLDA microarray method in the fragments of tumor tissue collected from patients with triple-negative breast cancer compared to the normal tissue surrounding the tumor.
3. Denotation of mutual correlations between the levels of gene expression associated with the process of apoptosis.
4. Denotation of mutual correlations between the levels of gene expression belonging to the ABC family (including 14 genes related to the induction of multidrug resistance).
5. Denotation of mutual correlations between the levels of gene expression associated with the process of apoptosis and genes associated with the induction of multidrug resistance.

The studies involved 32 patients diagnosed with triple-negative breast cancer who were treated at the Center of Oncology of the Lublin Region. Patients did not receive neoadjuvant chemotherapy before taking the material.

During the surgical procedure, from the patients with diagnosed triple-negative breast cancer, a fragment of the tumor tissue (test sample) and a piece of tissue surrounding the tumor (control sample) were collected.

In subsequent stages homogenization of the obtained tissues and RNA isolation were performed. RNA isolation was performed using the modified method of Chomczyński and Sacchi using the TriReagent reagent.

In the next step, isolated RNA was used to carry out the reverse transcription process, resulting in cDNA on the mRNA template.

The cDNA preparations obtained after the reverse transcription process were amplified using the semi-quantitative gene analysis technique, i.e. real time PCR. Analysis of gene expression levels associated with multidrug resistance and apoptosis was performed using TaqMan®Human ABC Transporter Array and TaqMan®Human Apoptosis Array.

Based on the available literature sources, I can say that the studies I have presented have not been carried out so far. For the first time, I have described the changes in the expression profile of 92 genes associated with the process of apoptosis and 40 genes belonging to the ABC family (including 14 genes associated with the induction of multidrug resistance) in tumor tissue obtained from patients with triple negative breast cancer, not undergoing neoadjuvant therapy.

The results of my research have shown that the average expression levels of all genes tested, with the exception of the *BIRC5* gene, are reduced when compared to the surrounding tissue. I also showed an increased expression of the *BIRC5* gene in the TNBC patients, which is in line with the results obtained by Zhang and co-workers.

Confirmation of the reduced expression of the *ABCB1* gene allows to support the hypothesis given by Delou that the decrease in the expression of this gene is associated with the initial stage of disease progression (before diagnosing and implementing pharmacological treatment). Such gene regulation promotes the proliferation and inhibition of mechanisms controlling apoptosis and autophagy processes. The average lowered level of expression of all genes involved in the process of multidrug resistance in TNBC tumor cells is probable, as it is likely to indicate similar mechanisms of gene regulation, which results in increased tumor cell proliferation and progression of the disease.

In the group of patients diagnosed with triple-negative breast cancer described herein, none of the women underwent neoadjuvant chemotherapy. Literature data indicate that in patients after therapy there is an increase in expression of genes from the ABC family, which results in the induction of multidrug resistance. Based on the above data, it can be concluded

that a different regulation of gene expression related to the induction of multidrug resistance, occurring in patients without chemotherapy and after chemotherapy, induces the same end result (i.e., increased proliferation and progression of cancer).

As a result of the r-Spearman analysis, I showed that among the patients with TNBC three clusters characterized by different gene regulation can be distinguished, both in the case of genes related to the induction of multidrug resistance as well as genes related to the apoptosis process. Cluster No. 1 included patients who were characterized by a positive (elevated) expression value of the examined genes. Cluster No. 2 included patients who were characterized by a negative (decreased) expression value of the examined genes. Cluster No. 3 included patients who were characterized by low variability of the expression values of the examined genes, oscillating around zero. Both in the studied genes related to the process of apoptosis and genes related to the induction of multidrug resistance, individual clusters included most frequently the same patients.

I suppose that the reduction in the expression level of all the genes involved in the apoptosis process is probably due to the fact that the cancer cell disables all signaling pathways leading to the induction of the apoptosis process, promoting pathways associated with proliferation and survival.

The genes with the most strongly reduced expression among the genes involved in the apoptosis process include the following ones: *CASP5, HRK, BOK, BCL2, CARD6, BIRC8, BAK1*. The *CASP5* gene regulates the Myc/Max/Mad transcription pathway. The reduced level of *CASP5* gene expression promotes the growth of tumor cells, inhibiting their differentiation and apoptosis. The other genes mentioned above have pro-apoptotic functions and the reduction in their expression may be a factor promoting tumor development.