Summary

Introduction

Breast cancer is the most frequently diagnosed cancer both in Poland and worldwide. In recent years, more than 2 million new cases are diagnosed every year, and the mortality rate among women with breast cancer is still very high. This neoplasm is a highly differentiated disease entity, therefore various classifications are used, based mainly on clinical and pathological diagnosis, molecular profile or histological differentiation. The etiology of cancer can be multidirectional, however, oncogenesis ultimately depends on genetic and epigenetic variability. It is well known that genetic conditions modulate the response to specific therapies, therefore, during its development, diagnostics of selected genetic disorders is recommended. Although mutations of selected genes are currently at the forefront in this regard, it is reasonable that if hypermethylation or other epigenetic modifications may affect the regulation of expression, they may also affect drug resistance or sensitivity to therapeutics. Among researchers, the topic of methylation of genes and their regulatory areas is often discussed, and the impact of which on cancer pathogenesis is strongly documented in the literature. This variation is based on a reaction that produces 5-methylcytosine, typically inhibiting transcriptional activity if the promoter region is methylated. The key here is the reduced activity of proteins encoded by tumor suppressor genes, and the result is the deregulation of molecular mechanisms critical in oncogenesis, the most important of which are the control of replication, proliferation, repair of damaged DNA or cell death. An increasing number of reports confirm the prognostic value of long non-coding RNAs (lncRNAs) in breast cancer, which are regulatory molecules at various levels. Moreover, the development and progression of tumors can be regulated by the activity of genes encoding adhesion proteins.

Objectives

The aim of this study was to assess the effect of the promoter regions methylation of five genes on the breast cancer development and the effect of gene methylation and the expression level of one of them on specific clinicopathological conditions. In addition, the correlation between the experimentally tested factors was assessed.

Materials and methods

The study was conducted on a group of 106 patients with breast cancer, including triple negative breast cancer (TNBC) (N=59) and other cancer molecular groups (non-triple negative breast cancer, NTNBC) (N= 47). The control group included women with no pathological changes or

only benign changes. The research material consisted of frozen tissue or formalin-fixed and paraffin-embedded (FFPE) tissue. Most of the patients were characterized in terms of clinicopathological parameters. The methylation analysis of the promoter regions included the *MGMT, BRCA1, CDH1, ITGA4* and *MEG3* genes selected from the literature, each of which seems to be important in carcinogenesis or its progression. In addition, the *MEG3* expression level was assessed in 36 patients. The method used to assess the methylation status was a variant of the classical PCR (polymerase chain reaction) method, methyl-specific PCR (MSP). Quantitative reverse transcription PCR (RT-qPCR) was used for expression analysis.

Results

The conducted research showed several correlations, most of which are in line with the available literature. Significantly more frequent methylation of the *BRCA1* gene in the TNBC group (p=0.02509), the *ITGA4* gene in the entire breast cancer group (p=0.00321) and the *MEG3* gene in the NTNBC group (p=0.05341) was observed. Moreover, the methylation of *MGMT, ITGA4* and *MEG3* genes was more frequent in elderly patients with breast cancer, with borderline significant results for the latter two (p=0.0269, p=0.0594, p=0.0530, respectively). Considering the correlation in subgroups individually, in the NTNBC group, patients with the current *ITGA4* methylation had a higher median age (p=0.0362). The age difference was observed between the breast cancer group and the control group, the former also having a higher median age (p=0.0316). There was no effect of methylation on specific clinicopathological conditions (hitological grade, tumor size, lymph node involvement, presence of DCIS component), however, lack of *MGMT* methylation was observed more often in patients with the highest grade of malignancy (p=0.02555). Comparative analysis for the TNBC and NTNBC tumor subtypes also showed no differences in terms of the clinicopathological factors mentioned above. In turn, based on the results of expression analyses, the dependence of regional lymph node involvement on the reduced expression level was found, with the correlation being borderline significant (p=0.05704). However, there were no significant differences in the level of expression in terms of gene methylation, age, clinical parameters or molecular subtype. During the experimental selection of the reference gene for *MEG3* expression analyses, the *GAPDH* gene was found to be significantly downregulated in the TNBC group (p=0.0001), so it was rejected as the reference gene. Thus, relative *MEG3* expression was assessed against *ACTB*.

Conclusions

Based on the performed analyses, it can be concluded that methylation of promoters of *BRCA1*, *ITGA4* and *MEG3* genes is a risk factor for breast cancer or its specific subtypes. In addition, higher age is also a factor that increases the risk of cancer development and positively affects the appearance of the *MGMT*, *ITGA4* and *MEG3* genes methylation. Moreover, expression analyses revealed that downregulation of *MEG3* affects the presence of lymph node metastases, and downregulation of *GAPDH* is observed in triple negative breast cancer.