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DNA Profiling of ING1 Gene in Triple Negative Breast Cancer

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Abstract: Breast cancer is among the most common cancers and the second in death caused from cancers in women. Triple negative breast cancer (TNBC) is a subgroup which not expressed estrogen (ER), progesterone (PR) and HER2 receptors in breast cancer. It has 15% rate of all breast cancer and has been more aggressive and has poor prognosis. For this reason, early diagnosis has a key role because of TNBC's high relapse. Investigation of DNA methylation pattern in TNBC likewise all types of breast cancer has become an important prognostic vehicle besides assessment of gene expression profile in diagnosis cancer subtypes. DNA methylation pattern of tumor suppressor genes which is one of the epigenetic modifications has highly importance. In this study, we aimed to investigate the relationship DNA methylation pattern of ING1 (inhibitor of growth family member 1), a tumor suppressor gene and TNBC. For this purpose, we searched the methylation pattern of ING1 and breast cancer patients especially TNBC. As a result, we discussed and tried to clarified the relationship between epigenetic modifications of ING1 gene and TNBC.

Keywords: Breast cancer, Triple negative breast cancer, Epigenetic factors, DNA methylation, ING1 gene.

Introduction

Breast cancer is one of the most common cancers and ranks second in cancer-related deaths in women in around the world. While the incidence of 5-year survival in early stage localized breast cancer and ductal carcinoma *in situ* is 100%; this rate decreases to 27% in metastatic breast cancers.

TNBC is a breast cancer subtype in which estrogen (ER), progesterone (PR) and HER2 receptors are not expressed. It represents 15% of all breast cancers and has a more aggressive and worse prognosis than others. Recent studies have shown that 60% of patients have a 5-year life expectancy. The lack of receptors makes TNBCs unresponsive to hormonal and anti-HER2 therapies used in other breast cancers. And unfortunately, none of the commonly used gene expression profiling tests (Oncotype DX with 21 genes, Mamma-print with 70 genes, and PAM509) are clinically useful in patients with TNBC. Therefore, looking for DNA methylation patterns in TNBC, as in other cancers, has become important prognostic tools in determining cancer subtypes as well as gene expression tests (Fackler et al., 2020).

Epigenetic changes have a high potential to be a source of innovative biomarkers in cancer with the advantages of being stable, having specific genes at high frequency and being detectable in biological fluids in a minimally

invasive way. Among the epigenetic changes, DNA methylation plays a key role in the regulation of gene expression, and abnormal methylation is implicated in many human diseases, including cancer, by disrupting normal gene expression and regulation. The possible mechanism underlying cancer development and progression is hypermethylation of CpG (cytosine-phosphate-guanine) islands located in the promoter regions of tumor suppressor genes, which causes loss of expression or hypomethylation that causes chromosome instability and increased mutation.

Since the tumor suppressor gene ING1, whose extensive methylation patterns were not studied specifically in the TNBC subtype, In this study, we investigated the effect of the methylation pattern and gene expression of the ING1 gene in triple negative breast cancer, The Cancer Genome Atlas Program – NCI-TCGA, National Center for Biotechnology Information-NCBI and The Human Protein Atlas were selected in line with the information obtained from different sources.

ING1 (Inhibitor of Growth Family Member 1) is a tumor suppressor gene involved in many processes such as cell growth, apoptosis, aging, migration and DNA repair. It has been mapped close to the telomeric region on chromosome 13q34 in the human genome (Shimada et al., 1998; Nagashima et al., 2001; He et al., 2005). Loss of ING proteins, whose expression varies greatly in different tissues, is generally correlated with cancer progression **(**Wang et al., 2010; Li et al., 2011a) and their expression is decreased /or lost in human tumors (Walzak et al., 2008).

It has been reported that loss of ING protein expression is positively correlated with tumor progression in head and neck cancers and microvessel density in ovarian cancers (Thakur et al., 2014**)**. However, it is also known that ING knockout mice spontaneously develop tumors with a high frequency (Kichina et al., 2006; Coles et al., 2007; Saito et al., 2010).

ING genes, which are frequently suppressed in cancer cells, are rarely mutated. This suggests that the mechanism of suppression of ING expression is due to epigenetic factors (Tokunaga, et al., 2000; Gunduz et al., 2002; Campos et al., 2004; Garkavtsev et al., 2004). Indeed, it has been reported that abnormally high methylation levels on the ING1 gene promoter correlate with low transcript levels in ovarian cancers **(**Shen et al., 2005).

While the mRNA measured in the normal Hs578Bst mammary epithelial cell line is quite high, the steady-state levels of all ING1 transcript isoforms were decreased 10 to 100-fold in most breast cancer cell lines examined (Walzak et al., 2008). It is also known that ING1 expression is frequently lost in familial and sporadic breast cancers, and loss of ING1 is associated with poor prognosis in breast cancer (Toyama et al., 1999; Tokunaga, et al., 2000; Ythier et al., 2008). In addition, there is information in researches that changing ING1 levels affects the expression of genes known to be altered in breast cancer **(**Thakur et al., 2014).

The Purpose

Considering that the ING1 gene is active in normal tissues and inactive as a result of hypermethylation in the promoter regions in cancerous tissues, it is thought to be used as a marker in the prediction of TBNC. Therefore, in this study plan, it is aimed to discuss the usability of the promoter methylation pattern of the ING1 gene as a biomarker in TNBC with an aggressive course.

Results and Discussion

The ING1, ING2, ING3, ING4, and ING5 members of the growth inhibitor (ING) family of type II tumor suppressors have a variety of roles in biological processes, including growth, proliferation, DNA repair, invasion, migration, and cell death (Smolle et al., 2019**)**. Reduced ING3 expression in the nucleus has been related in research to being a stand-alone prognostic factor in breast cancer. The study provided the first in-depth description of ING3 expression in breast cancer and showed that it was a reliable predictor of breast cancer (Wu et al., 2021). While the ING2 protein is a chromatin reader and a stable member of the mSin3A/HDAC complex, other studies have connected it to lung cancer. ING2 expression was lost in various cancer subtypes, but it was still discovered to have a variety of tumor-suppressive effects in cancer cell lines (Blondel et al., 2019).

While different research discovered unmistakable proof that certain cancers exhibit diminished or even absent nuclear expression of p33ING1b in comparison to their normal counterparts, the potential utility of the ING1 gene for vaccine-based diagnostics and treatment rose when the immunogenicity of the gene was observed in breast cancer patients. ING1 is a gene therapy that can target particularly tumor cells since it plays a significant role in promoting apoptosis in tumor cells, which guards against harm to healthy cells. Because tumor suppressor genes exist in cancer cells as non-mutated wild-type alleles, they aid in the discovery of treatments. This study concluded that ING1 gene mutations are an uncommon event in cancer (Nouman et al., 2003).

Another two studies revealed ING1 as a gene whose deletion promotes and accelerates tumor development. This means that in many distinct tumor types, its expression is lost or significantly diminished (Ythier et al., 2008; Li et al., 2011b). According to a study, the epigenetic regulator ING1 may engage in new cell functions when expressed at high levels **(**Thakur et al., 2012), and a study on the ING1 gene has offered strong support for its function as a tumor suppressor in the development of cancer (Bányai et al., 2021).

Low levels of ING1 in breast cancer enhance metastasis, according to different research studies. ING1 is a suppressed gene in breast cancer cell lines that contributes to the control of gene expression, and it was demonstrated in this study that altering ING1 levels modifies the expression of genes known to be altered in breast cancer. Increased levels of ING1 are also strongly linked to both overall disease-specific survival and survival free of illness, and overexpression of ING1 helps to prevent cell migration (Thakur et al., 2014).

An initial report for the examination of ING1 mutations and expression in 452 groups of cancer samples was submitted. Breast tumors (58%) with low ING1 expression contained metastatic spread to local lymph nodes, compared to just 9% of tumors with increased ING1 expression relative to the surrounding normal tissue. Although the study found that ING1 mutagens are extremely uncommon in breast or ovarian cancer, ING1 expression was commonly decreased concurrently with breast cancer progression (Toyama et al., 1999).

According to a study, this gene's suppression is directly related to the aggressive character of TNBC, and its activation improves anti-tumor activity (Vasilatos et al., 2013). In our work, we look forward to directly linking the ING1 gene to triple-negative breast cancer, as you will see an accurate investigation of the gene and its comparison between healthy cells and precancerous cells in this type of cancer.

Conclusion

Research has found that conventional treatment methods are not completely effective in treating BC, especially TNBC, due to the poor prognosis of this cancer and its lack of response to current therapies. It has a poor prognosis and fewer treatment options, as evidenced by its higher mortality rate when compared to other subtypes of breast cancer **(**Mahmoud et al., 2022).

The research reached the possibility of using gene therapies to combat this disease, and our work was based on the possibility of using the ING1 gene to predict the event of cancer, as this gene, when over expressed, indicates that cancer does not occur, and the reverse occurs when it is underexpressed, which is a distinctive feature of the occurrence of TNBC **(**Eastlack & Alahar, 2015). In our paper, we aimed to find out that ING1 gene oligomerization is a characteristic.

Recommendations

We recommend that to investigate ING1 gene and TNBC relationship epigenetically and the other cancer types.

Scientific Ethics Declaration

The authors declare that the scientific ethical and legal responsibility of this article published in EPSTEM journal belongs to the authors.

Acknowledgements or Notes

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