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Methylation Analysis of Promoter Region of ETS-1 Gene in Human Breast Cancer Patients

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Abstract

This study aimed to compare the methylation status of ETS1 between 18 cancerous and cancer-side tissues in breast cancer patients using matrix-assisted laser ionization-time flight mass spectrometry. A total of 46 CpG sites on FJ-1, FJ-3, and FJ-5 fragments from the promoter region of the ETS1 gene were detected for methylation levels. The CpG41/42/43 methylation level of the FJ-5 fragment in cancer tissues was significantly lower than that in adjacent tissues *(P<0.05)*. Abnormal methylation of ETS1 may lead to overexpression of ETS1-mRNA, which is related to breast cancer pathogenesis.

Keywords: Breast cancer; ETS1; DNA methylation

Introduction

The World Health Organization's International Agency for Research on Cancer (IARC) released the latest cancer burden data, which showed breast cancer was the world's most common cancer in 2020, with 2.26 million new cases. Breast cancer and its complications have become a major public health problem [1]. DNA methylation is the main mechanism of epigenetic gene regulation [2-4] which plays a key role in controlling gene activity. It is usually associated with inhibiting gene expression [5]. Many studies have shown that hypermethylation in some tumor promoter regions leads to the inactivation of tumor suppressor genes [6], and abnormal DNA methylation is a common phenotype in cancer [7]. Aberrant methylation is an important mechanism leading to tumor suppressor gene inactivation in the development and progression of breast cancer. Recent studies show that abnormal DNA methylation may be a potential biomarker for early diagnosis of breast cancer and therapeutic targets will also help improve the clinical treatment of breast cancer [8].

ETS-1 is a founding member of the ETS transcription factor family, one of which encodes the ETS transcription, factor family. The family plays a variety of roles in breast cancer development and regulates various pathways, including cancer and autoimmune disease [11-14]. Studies have found that high methylation in the ETS1 region significantly reduces the expression of ETS1 in breast cancer patients' samples, and ETS1 shows new inhibitions in breast cancer cell tumors [15].

This study aimed was to determine the expression level and DNA methylation status of ETS1 in human breast cancer tissues and explore the molecular biological mechanism of the ETS1 gene in breast cancer and provide clues for exploring new therapies for breast cancer.

Materials and methods

Human breast tissues

Breast cancer patients of various pathological types were recruited from hospitals in Ningxia from August 2017 to August 2018. After obtaining the informed consent of the patients, select patients with indications for breast cancer surgery, obtain breast cancer tissues and adjacent tissues (i.e., normal tissues 3-5 cm from the edge of the tumor) as research specimens, and store them immediately at -80°C for future experiments. And

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record the patient's medical records in detail, including the patient's basic information and clinic pathological characteristics.

Inclusion criteria: Breast cancer patients who were diagnosed with breast cancer by pathological biopsy and had indications for surgery, had never received radiotherapy or chemotherapy before surgery and had completed

Medical records are included in this study. Refer to WS338-2011, diagnostic criteria for breast cancer; breast cancer TNM staging is referenced to the International Union Against Cancer (UICC) standard; patients are diagnosed and judged by the chief physician.

Exclusion criteria: At the same time, breast cancer patients with other types of malignant tumors, breast cancer patients with severe liver and kidney damage, and breast cancer patients with incomplete clinical pathological characteristics were not included in the scope of the study.

This study was approved by the Ethics Committee of Ningxia Medical University (grant No. 2016164). Each subject signed written informed consent before the sample request.

DNA extraction

The extraction method and operation of DNA in both sets of samples are strictly DNA extraction produced by Tian gen the kit (for blood/cell/tissue genomes, TIAN amp Genomic DNA Kit, TIANGEN BIOTECH (BEIJING) CO. LTD) is operated by a guide to the experimental process. Detection of purity and concentration of the extracted DNA solution (Nanodrop 2000 Island June).

Mass ARRAY methylation assay

Experimental principle: The experimental process is essential to change the degree of methylation of the CpG site on the DNA template. This is the sequence difference of RNA digested fragments, and then the difference in molecular weight is determined by mass spectrometry. 10-per-tag was added for the forward primer and an additional T7-promoter tag for in vivo transcription.

GGCACTCCAGGGGAAGTTGGCACTTTGCGGCGAAGTGAGCGCGCTCGGGTCCCAGCCTCGCCCGCGCCGCGCCCGCTCCTCCTGCCCGGCCCTCGCCCGCTCCCTCCTCTCCGCCGGCGGCTGCCTCGTTCGCTCTCGATCTCCCGGCCGCTCTCCCTCCCTCTCGCCCTCCCGCGCTCTCCCCTCCTCTTTAGGGCGTTTCTCGCGGCGCCGCGTCTCGGCCGCTGGGTCCGCGCGCCCTGGGCCGGGCGATGTCCGCTTGGGGGAGCGAGGGGCGGGGCGTCGGGGCAGGGCGGGGAGCCGGGGGCGGGGCCGAGCACCGCGGCCAATCTCCGCCCGCGGCCCAAAGCGAAAGGAAGGGCTGGCGAGCGCGGGGCCGGGCCGCGACCGCGGAGGAGCAGTGCGTGGAGCCCCGGGCCTCGGAGCCCAGGCAGGGGCGGGAAGGGCCGGGAGCGGGTGTGCAGGCTGGGTGCACAGGC.

The sequence of fragment fj-5 is GCCTTCATGGTGCCAGGAGTGGGGGACGTACGGGATGGTAGCAAGTTTGCAGTTACTGTTGTTTTTCTTTTTAATGAGGATTAGTAACAGGGGGAAGGGGACGGGGGAAATCCGACTTTCTTCCCAAAAATCTCAAATTCCCGCTGCCTTTCTTTCCCCCGCGCCCGGACGGTGCGCGCCCGGCACTCCAGGGGAAGTTGGCACTT.

The sequence of fragment fj-1 is GGGGAAGAAGTCCAGGGGACCCCCGGCCTCTGGCCGAGAGCTTGGGTGGGGGCCTCGGCCGTCGCCACTCACCCGGGGAGGGGAAAAGCTCCAGATCGACTTTTTCCGTCTTGATGATGGTGAGAGTCGGCTTGAGATCGACGGCCGCCTTCATGGTGC CAGGAGTGGGG.

Statistical analysis

SPSS 26.0 statistical software was used for statistical analysis, and $P<0.05$ was considered statistically significant. Paired t-test and Wilcoxon test were used to compare differences in ETS1 and methylation levels between the two groups.

Results

Test results for methylation levels

We compared global methylation levels of six matched breast cancer samples with control samples. The methylation levels Detection results in the six samples showed that the CpG sites in the detected fragment FJ-1 had CpG 1/2, CpG3/4/5, CpG39, CpG41 / 42,43, CpG44, CpG45/46/47, CpG48 in CpG1/2, CpG6, CpG8, CpG9, CpG11/12; FJ-3 CpG1/2, CpG3/4/5, CpG39, CpG41/42,43, CpG44, CpG45/46/47, CpG48, CpG2, CpG3, CpG4, CpG9/10/11/12; FJ-5 fragment 49. CpG50/51/52, CpG54, CpG55/56/57/58/59, CpG60/61, CpG62, CpG6364, CpG65, CpG66, CpG67; Cancerous tissue and corresponding cancer-side tissue. The above three fragments between mean methylation levels were shown to be of no statistical significance after matching rank and test ($P>0.05$); see Table 1; FJ-1, FJ-3. The difference in methylation level comparisons between all sites in the fragment and the corresponding cancer-side tissue was also not statistically significant (*P>0.05*), see Table 2, but FJ-5 in the fragment CpG41/42/43 found differences in the rank matching and the test methylation levels between the cancerous tissue and the corresponding cancer-side tissue found significant differences ($P<0.05$). The mean method of cancerous tissue at this site is 0.02 (0.01,0.02), and cancer-side tissue is 0.03 (0.03,0.04), see Table 3.

Table 1: Different degrees of methylation of different fragments (M, (Q25, Q75)).

fragments	groups		*P*
	Cancerous tissue	cancer-side tissue	
FJ-1	0.03(0.03,0.06)	0.03(0.03,0.08)	1
FJ-3	0.01(0.01,0.02)	0.01(0.00,0.03)	0.79
FJ-5	0.02(0.02,0.05)	0.02(0.02,0.02)	0.56

Table 2: Comparison of methylation levels on FJ-1 and FJ-3 fragments (M, (Q25, Q75)).

FJ-1 CPG unit	Group		*P*	FJ-3 CPG unit	Group		*P*
	Cancerous tissue	cancer-side tissue			Cancerous tissue	cancer-side tissue	
CpG1/2	0.04(0.04,0.05)	0.03(0.02,0.04)	0.14	CpG2	0.00(0.00,0.01)	0.00(0.00,0.02)	0.37
CpG6	0.02(0.02,0.,02)	0.02(0.02,0.02)	0.59	CpG3	0.00(0.00,0.00)	0.00(0.00,0.00)	1
CpG8	0.03(0.03,0.07)	0.04(0.03,0.12)	0.36	CpG4	0.00(0.00,0.00)	0.00(0.00,0.00)	-
CpG9	0.03(0.03,0.09)	0.03(0.02,0.10)	1	CpG9/10/11/12	0.03(0.02,0.05)	0.02(0.02,0.09)	0.79
CpG11/12	0.04(0.03,0.10)	0.03(0.03,0.20)	0.59				

Table 3: Comparison of methylation levels on FJ-5 fragments (M, (Q25, Q75)).

CPG unit	Group		*p*	CPG unit	Group		*p*
	Cancerous tissue	cancer-side tissue			Cancerous tissue	cancer-side tissue	
CpG1/2	0.02(0.01,0.07)	0.02(0.01,0.13)	0.53	CpG54	0.00(0.00,0.03)	0.01(0.00,0.06)	0.46
CpG3/4/5	0.07(0.06,0.11)	0.07(0.05,0.17)	0.59	CpG55/56/57/58/59	0.08(0.06,0.11)	0.06(0.06,0.13)	1
CpG39	0.01(0.00,0.05)	0.02(0.00,0.05)	0.89	CpG60/61	0.01(0.00,0.07)	0.00(0.00,0.10)	0.58
CpG41/42/43	0.02(0.01,0.02)	0.03(0.03,0.04)	0.03	CpG62	0.02(0.01,0.07)	0.02(0.01,0.11)	0.68
CpG44	0.00(0.00,0.00)	0.00(0.00,0.02)	1	CpG63/64	0.03(0.01,0.05)	0.03(0.01,0.05)	0.59
CpG45/46/47	0.08(0.07,0.09)	0.08(0.07,0.10)	1	CpG65	0.00(0.00,0.00)	0.00(0.00,0.02)	1
CpG48/49	0.03(0.02,0.03)	0.03(0.02,0.04)	0.37	CpG66	0.02(0.1,0.03)	0.02(0.02,0.14)	0.42
CpG50/51/52	0.05(0.03,0.07)	0.03(0.02,0.10)	0.84	CpG67	0.02(0.02,0.02)	0.01(0.01,0.03)	0.58

Discussion

The mechanism of ETS1 in breast cancer has not yet been fully elucidated. In this study, we tested the expression and methylation status of the ETS1 gene in breast cancer tissues and adjacent normal tissues. The results showed that the expression level of ETS1 mRNA in cancer tissues was higher than that in normal tissues. Compared to adjacent tissues, CPG in cancerous tissues is highly methylated.

We observed that the methylation level of cancer tissue was only lower in CpG41/42/43 of the FJ-5 fragment than in adjacent tissues (*P<0.05*). Previous studies have shown that the transcription factor ETS1 is expressed in breast cancer tissues and para-cancerous tissues, and its expression level is increased in cancer tissues, and participates in the regulation of the expression of invasion-related molecules in breast cancer cells [15], suggesting that the abnormality of ETS1 expression may be closely related to the occurrence and development of cancer.

Genome-wide studies have shown that DNA methylation is an important regulator of gene expression and an early event in the development of various tumors, including breast cancer. Its dynamic regulation is the key epigenetic mechanism of cancer occurrence, maintenance, and progression [16-18]. Abnormal DNA methylation is a common phenotype in cancer. Abnormal DNA methylation has been linked to breast cancer. Cytosine-related methylation in DNA occurs mainly in the CpG island structure of mammals [19,20]. Therefore, three fragments of the ETS1 promoter region were selected for methylation detection, and the results showed that CpG islands have abnormal methylation sites.

To sum up, DNA methylation plays an important role in the discovery, diagnosis, treatment, and genetic mechanism of breast cancer. Further study of DNA methylation is needed. In tumorigenesis, abnormal hypermethylation is usually observed in promoters of key regulatory genes involved in preventing tumor changes, including cell cycle processes, DNA repair, and apoptotic pathways. Many studies have shown that DNA methylation can be used as a potential biomarker for the early detection of breast cancer [18,22]. This study also fully demonstrates that the ETS1 gene can also be used as an indicator of a breast cancer diagnosis. However, there is still a lack of reliable evidence for the potential relationship between DNA methylation and breast cancer risk [22]. Therefore, abnormal methylation of CpG loci can be identified by studying whole genome DNA methylation, which is conducive to the clinical diagnosis and prognosis of breast cancer. Although the results of this study are good, the sample size is too small to represent the population well. We can try to expand the sample size in future studies.

In conclusion, this study revealed the expression and methylation level of the ETS1 gene in breast cancer and provided clues for the etiology of breast cancer based on CpG DNA methylation. This research provides important molecular markers for early diagnosis and prevention of breast cancer. Methylation of the ETS1 gene may be a potential biomarker for breast cancer diagnosis and prognosis.

Conclusion

This study found that the methylation level of CpG41/42/43 in the FJ-5 fragment of the ETS1 gene is lower than that in adjacent cancer tissues. ETS1 methyl is expected to become a biomarker for early diagnosis and prevention of breast cancer. Methylation sites may become targets for breast cancer treatment, so that breast cancer patients can benefit.

Declarations

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Ethics statement: This study was approved by the Ethics Committee of Ningxia Medical University (grant No. 2016164). Each subject signed written informed consent prior to the sample request.

Declaration of competing interest: The authors have declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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