The Mutation of DBC2 In Breast Cancer Patients From Han Ethnic Group In Eastern China

【ABSTRACT】Objective: To investigate DBC2 mutations in breast cancer patients and evaluate the relationship between the gene mutations and breast cancer susceptibility in Eastern China population. Methods: Mutation analyses of 285bp promoter sequence, coding exon 7 and its exon/intron boundaries of DBC2 were performed in thirty-two breast cancer specimens by polymerase chain reaction and direct sequencing. Eighteen benign breast tumor specimens were also analysed as control. Results: No mutation in the promoter or exon 7 was found in both group. An intronic alteration (IVS7+53C>G) was detected in 13 specimens. There was no significant difference in the rate of IVS7+53C>G alteration between study group and control (8/32 vs 5/18, P>0.05). IVS7+53C>G mutation was correlated with HER2, p53 expression (P <0.05), but not correlated with age, tumor size, lymph node metastasis, ER or PR expression (P >0.05). Conclusion: Mutation in the promoter and exon 7 is not common in Chinese population and may not contribute to the susceptibility for breast cancer in China. The intron alteration IVS7+53C>G is a common polymorphism in Chinese Han ethnic group. Further research is warranted to evaluate the relationship between IVS7+53C>G and the susceptibility for breast cancer.

【Key words】Breast cancer; DBC2;Gene Mutation

Introduction

Breast cancer is one of the most common malignancies threatening women’s healthy, more than 230,480 new cases of breast cancer will be diagnosed and 39,520 will die from the disease in the United States in 2011. The incidence rate of breast cancer in China was relatively lower before 1960’s, but it has been increasing rapidly since 1970’s. Dates from Shanghai Disease Prevention And Control Center showed that breast cancer has become the most common malignancy and ranks first among the cause of cancer death in Shanghai since 2005. Early stage breast cancer showed good outcomes. However, for most patients who were diagnosed with advanced cancer, the prognosis was very poor. Therefore, it is crucial to find new tumor markers in order to screen breast cancer susceptible population and improve the prognosis of the disease.

DBC2 gene (deleted in breast cancer 2), also known as RhoBTB2, was first cloned from breast cancer tissues by Hamaguchi and his colleagues[1]. Researches showed that DBC2 participates in varieties of cellular functions such as cell cycle control, apoptosis, cytoskeleton regulation, and protein transport[2-4]. The expression of DBC2 extinguished frequently in tumor tissues [1,5,6], and reintroduction of DBC2 gene can inhibit the growth, migration and invasion of tumor cell [1,7,8]. Therefore, DBC2 is thought to play an important role in the carcinogenesis and progression of breast cancer. However, as a newly identified tumor suppressor gene, few
researches on DBC2 in breast cancer have been reported up to now, even fewer in China. In our study, we analyzed the polymorphism in promoter and exon 7 of DBC2, as well as investigated whether the genotypic background contributes to oncogenesis of breast cancer in Chinese Han ethnic group.

Material and Method

Study subjects

Breast cancer samples were collected from Changhai Hospital. The resected specimens were immediately frozen in liquid nitrogen and stored at -80°C until DNA extraction. The diagnosis of breast cancer was confirmed by pathological examination. Informed consent was obtained from each individual recruited for this study, and the study was approved by the Ethics commission of Second Military Medical University.

Polymerase chain reaction and DNA sequencing

Genomic DNA was extracted from tumor tissue using QIAamp DNA Mini Kits, and stored at -20°C until amplification. Primers were designed by Primer 5.0 software using AF315385 as reference sequence. Primer sequences for the promoter was: 5’-CCGAAGGAAAGGGGAAAAC-3’ (Forward), and 5’-CGCACCCAAGACGACAGC-3’ (Reverse). And primer sequences for exon7 was: 5’-CTGTCCGCTCACTCCTTC-3’ (Forward), and 5’-GCACCACCCCTTCTTCACT-3’ (Reverse). The specificity of the primers were verified by blasting in GenBank database, and synthesized by Shanghai Sangon Biological Engineering Technology & Services Co., Ltd. PCR amplification for both the promoter and exon 7 were performed on the T-gradient thermocycler (Whatman Biometra, Germany) in a final volume of 60µL. Reactions were performed as recommended by the manufacturers using 35-40 cycles of denaturation, annealing (at primer specific Ta) and extension.

DNA sequencing was carried out using the PCR primers and ABI 3730XL Genetic Analyzer automated sequencer (Applied Biosystems, Foster City, CA). Any mutation-suspected sample was later confirmed by reverse sequencing.

Statistics

SPSS13.0 software was used to analyze the data. The statistical methods included Fisher's Exact Test and t-test. P values <0.05 were considered statistically significant.

Results
Patients character

32 tumor samples from primarily sporadic breast cancer patients were collected from Changhai Hospital. All patients were female, from Eastern China and of the Han ethnicity, with negative breast cancer family history. The average onset age of the disease was 51.03 years with a range of 35-77. 18 breast benign tumor samples were also analyzed as control.

Mutation analysis

No genomic variation was found in either promoter fragment or exon 7. A novel mutation (IVS7+53C>G) was detected in intron 7 in 13 specimens, including 8 breast cancer samples and 5 breast benign tumor samples (figure 1). The mutation rates in research group and control were 25% (8/32) and 27.78% (5/18) respectively, the difference was of no significance (P=1.000).

![DNA sequencing reveals a IVS7+53C>G mutation](image)

Figure 1 Mutation detection in intron 7 of DBC2 gene. DNA sequencing reveals a IVS7+53C>G mutation (mutant, arrowed).

Relationship between DBC2 mutation and clinicopathological characteristics

In patients with breast cancer, the average onset age of the patients carried IVS7+53C>G mutant was 51.13±11.23 years, and 51±8.57 years for the IVS7+53 wild-type patients. No statistical significant difference was noted (P=0.974). Expression of HER2 and p53 was much higher in patients carried IVS7+53C>G mutant than those carried IVS7+53 wild-type DBC2. While other characteristics, including tumor size, lymph node metastasis, ER and PR expression were similar between the two groups (table 1), indicating that IVS7+53C>G mutation was correlated with HER2, p53 expression (P <0.05), but not correlated with age, tumor size, lymph node metastasis, ER or PR expression (P >0.05).

Table 1 Correlation of DBC2 IVS7+53C>G mutation and clinicopathological characteristics of breast cancer (n=32)

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Comment [F3]: One can clearly see the G as well as C peaks can the author also provide figure of reverse sequencing also?

Comment [F4]: No method has been given how this information was obtained?
## Discussion

*DBC2*, located in chromosome 8p21, is a newly identified tumor suppressor gene [1]. Regulated by E2F1, DBC2 plays a key role in inducing cell apoptosis and inhibiting tumor growth, migration an invasion through interaction with CCND1, BRMS1 and Cul3 ubiquitin ligase complexes [2,7,9-11]. DBC2 mutation is correlated to the oncogenesis and development of varieties of malignant tumor, including breast cancer, gastric cancer and bladder cancer [1,5,6,12-14]. Recently, Ohadi et al. reported two mutation sites (-238G>A and -121C>T) in DBC2 promoter in breast cancer tissues, which abolised binding site for the transcription factors Sp1-1 and E2F respectively [12]. Knowles et al. discovered a missense mutation (1681G>A, causing protein 561G>S) in DBC2 7 exon in a bladder cancer sample [14]. Both the deletion of transcription factor binding site and missense mutation could alter gene expression. In our study, we conducted gene
mutation analysis in the promoter and exon 7 of DBC2 gene to confirm its role in breast cancer.

However, similar results was not repeated in our study. No mutation was found in DBC2 promoter or exon 7 in patients with breast cancer. Possible explanations for the discrepancy could be: 1) The samples obtained in our study were from Han ethnic group. There might be a difference in genetic predisposition between Han ethnicity and European or Middle-eastern population in Knowles and Ohadi study; 2) DBC2 was found to participate in varieties of cellular functions such as cell cycle control, apoptosis, cytoskeleton regulation, and protein transport. However, despite mutation found in 7 exon in bladder cancer, whether 7 exon mutation in DBC2 plays a role in the carcinogenesis and progression of breast cancer is still not clear. Genomic sites other than promoters and exon 7 could be of interest for future investigation. In addition, since the frequency of the mutation in both Ohadi et al and Knowles et al is low (less than 1%). Our study sampling might not be big enough to detect the mutation.

In our study, we detected 13 cases of IVS7+53C>G mutation, including 8 cases of malignant tumor and 5 cases of benign tumor. The mutation frequency of IVS7+53C>G in research group and control one is 25% (8/32) and 27.78% (5/18) respectively. The difference between two groups is of no significance. By analyzing the correlation of IVS7+53C>G mutation and the clinicopathological factors, we found IVS7+53C>G mutation was correlated with HER2, p53 expression \( P < 0.05 \), but not correlated with age, tumor size, lymph node metastasis, ER or PR expression \( P > 0.05 \). Because both HER2 and p53 mutant have the potential activity of promoting carcinogenicity, it remained uncertain whether IVS7+53C>G mutation effect breast cancer susceptibility in Chinese Han population at present.

In summary, mutation in the promoter and exon 7 of DBC2 was not common in Chinese population, and may not contribute to the susceptibility for breast cancer in this region. IVS7+53C>G was a common polymorphism in Chinese Han ethnicity. Our data showed IVS7+53C>G is correlation to the expression of HER2 and p53, further study is warranted to investigate whether IVS7+53C>G is a independence risk factor for breast cancer in a large population.

Previously studies showed DBC2 variations in expression is very frequent, while the gene mutation is rather infrequent\[16, 16\], indicated that epigenetics events may play much more important role in loss of DBC2 expression. Recently, Shi and his colleagues analyzed promoter methylation and DBC2 gene expression in a bladder cancer research by MS-PCR and RT-PCR. They reported the frequency of methylation in DBC2 promoter was significantly higher in bladder tumor samples than that of corresponding normal tissues, and hyper-methylation was highly correlated to the gene inactive. They speculated that DBC2 inactivation by hyper-methylation may
be a crucial step in the process of cell carcinogenicity. Therefore, research on hyper-methylation at 
CpG island may be a new way for revealing the relationship between the inactivation of DBC2 
and susceptibility for breast cancer, hence provide a novel molecular target for diagnosis, 
treatment and prognosis of the disease.

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[References]


