RELEVANCE BETWEEN FGFR - 2 GENE POLYMORPHISM AND BREAST CANCER

YANG WANG[1,2], HAIYU ZHANG[3], YONGSHENG WANG[1,4*]
[1]Shandong Cancer Hospital Affiliated to Shandong University, Jiyan Road No. 440, Jinan, Shandong, 250117, China - [2]Department of Galactophore Surgery, Weifang People's Hospital, Guangwen Street No. 151, Weifang, Shandong , 261041, China - [3]Department of Antenatal Diagnosis, Weifang People's Hospital, Guangwen Street No. 151, Weifang, Shandong , 261041, China - [4]Shandong Academy of Medical Sciences, Jinan, Shandong 250062, China

ABSTRACT

Objective*: To investigate the relevance between the second intron single nucleotide polymorphism of fibroblast growth factor receptor 2 (FGFR2) gene and the onset of breast cancer in women.*

Methods*: The 132 cases of female breast cancer (breast cancer group) and 145 normal women (control group) were detected by combining polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method with agarose gel electrophoresis. The polymorphism distribution of two single nucleotide polymorphisms (rs2981579) and (rs2981582) of the second intron of FGFR2 gene was analyzed statistically.*

Results*: For FGFR2 gene single nucleotide polymorphism rs2981579, the frequencies of genotype (CC, CT, TT) in breast cancer group and the control group were 31.06%, 43.18%,25.76% and 26.90%, 45.52%,27.58% respectively. The frequencies of C allele in breast cancer group and control group were 68.94% and 75.17% respectively, and the frequencies of T allele in the two groups were 31.06% and 24.83% respectively. There was no statistically significant difference in genotype and allele frequency distribution between the two groups. For FGFR2 gene single nucleotide polymorphism s2981582, the frequencies of genotype (CC, CT, TT) in breast cancer group and the control group were 37.12%, 46.97%, 15.91% and 35.86% .45.52% .18.62% respectively. The frequencies of C allele in breast cancer group and control group were 72.73% and 71.72% respectively, and the frequencies of T allele were 27.27% and 31.72% respectively. The distribution of genotype frequency and allele frequency were compared between the two groups and there was no statistically significant difference (p>0. 05).*

Conclusion*: There is no significant correlation between the 2 polymorphism sites of FGFR2 intron and breast cancer.*

Keywords*: Breast cancer, fibroblast growth factor receptor 2, Gene polymorphism.*

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Introduction

Breast cancer is the most common malignancy in women and is the leading cause of cancer deaths in women worldwide. The incidence is increasing year by year with patients tending to be younger[(1)]. This trend is more obvious in sporadic and non-hereditary breast cancer[(2)]. FGFR2 (fibroblast growth factor receptor 2 gene is located in 10q26, a zone often with structural abnormalities and decreased expression in transitional cell carcinoma. A large number of studies have shown that FGFR2 can inhibit onset and development of a variety of tumors. FGFR2 gene mutation can also induce tumorigenesis. The absent expression of the gene in the tumor is likely to be the result of mutation inactivation, though the specific mechanism is not yet clear. Foreign studies have shown that FGFR2 single nucleotide polymorphism has a correlation with breast cancer[(3)]. There are already a number of studies on relevance between single nucleotide site rs2981582 and rs2981579 polymorphisms of FGFR2 gene and breast cancer at home and abroad, but the results vary with geographical differences. There is no consensus on the relevance between FGFR2 gene polymorphism and breast cancer onset risk in Shandong population. This study was to determine the relationship between FG-FR2 gene polymorphism and breast cancer onset risk in Shandong population.

Materials and methods

Specimen source

The 132 cases of breast cancer patients admitted in Weifang City People's Hospital during 2016-06-30 -2017-04-30 were enrolled as breast cancer group. Aged 26-70 years, the patients were in mean age of 45.2 years and median age of 48 years. All patients were diagnosed by pathology after surgery. The control group was 145 age-matched healthy women in physical examination. Aged 25-68 years, the group had a mean age of 44.6 years and median age of 45 years. The two groups were from Weifang.

Methods

DNA extraction and standardization

The 5mL fasting venous blood was extracted from breast cancer and control group, followed by anticoagulation with disodium edetate dehydrate (EDTA), to be saved in -80 °C refrigerator after separately packed. Use whole genomic DNA extraction system kit (TIANGEN Tiangen Company) to extract genomic DNA in strict accordance with instructions. After TE dissolved (pH8.0) DNA, measure with DU640 protein nucleic acid quantitative analyzer (Beckman, USA) and calculate DNA content and purity. Each specimen was diluted to about 100 gn / μL application solution.

Genotyping Use PCR-RFLP method for genotyping

Use Primer Premier 5 software for primer design: primer sequence of rs2981582: upstream primer5′-GAGAACCTCCAAGTAT-CACA-3′; downstream primer5′-TCCTTCAATGACAAGT-GCC-3′; primer sequence of rs2981579: S 5′-GTGACTCCCTTCATCGTG-3′;A 5′-GGCT CCTGGTCTATTTCTC-3′(primer was synthesized by Beijing AuGCT Biological Company).

The total volume of PCR amplification was 25 μL, including: 10 × PCR buffer 2.5 μL, 25 mmol / L MgCl 21.5 μL, 2.5 mmol / L dNTP 2.0 μL, 10 μmol / L 1.0 μL of upstream and downstream primers, Taq DNA polymerase (2.5 U / μL, Beijing Tiangen Biochemical Technology Co., Ltd.) 0.4μL, 1μL DNA template, sterile ddH2O 15.6μL. rs2981582PCR cycle conditions: 95 °C pre-denaturation 5min, 95 °C denaturation 45s, 66.2 °C annealing 40s, 72 °C extension 1min, after 30 cycles, 72 °C extension 7min and 4 °C preservation. rs2981579PCR cycle conditions: 95 °C pre-denaturation 5min, 95 °C denaturation 45s, 56.9 °C annealing 40s, 72 °C extension 1min, after 30 cycles, 72 °C extension 7min and 4 °C preservation.

Identification of PCR products by 2% agarose gel electrophoresis

Prepare 2% agarose gel (containing EB 5μg / mL), add each hole with PCR product 2μL and 2SL loading buffer solution, and add a hole with DNAMarker for control. The buffer was 0.5 × TBE, 10v / cm 25min and was photographed and recorded on UVP gel imager (BIO-RAD, USA).

Configure enzyme digestion system with HhaI restriction endonuclease 1μL, PCR product 10μL, and detect the digestion result with 3% agarose gel. Compare and identify the genotype with DNAMAN 6.0.3.99 and FG-FR2 gene sequences in Genebank. Read the genotype from the sequencing peak map, with homozygous peak as single peak and heterozygous peak as double peak.

Statistical methods

SAS 9.4 software was used for statistical processing. Genotype and allele frequencies were calculated by direct counting method. The genotype and allele frequencies of each group were compared by $\chi 2$ test. The level of test $p < 0.05$ indicates statistically significant difference.

Result

FGFR2 rs2981582 test results

There was no significant difference in FGFR2 rs2981582 gene and genotype frequencies between breast cancer group and control group p>0.05) (Table1).

Electrophoresis results of rs2981582 PCR products and enzyme digestion products are shown in Figure 1 (HhaI enzyme electrophoresis of FGFR2rs2981582 gene amplification products).

Group	n	Genotype frequency*			gene frequency**	
		CC	CT	TT	C	T
Breast cancer group	132	49(37.12)	62(46.97)	21(15.91)	192(72.73)	72(27.27)
Control group	145	52(35.86)	66(45.52)	27(18.62)	208(71.72)	92(31.72)
Total	277	101(36.46)	128(46.21)	48(17.33)	361(65.16)	193(34.84)

Table 1: Relevance between FGFR2 rs2981582 genotype and gene frequency distribution and breast cancer [n (%)].

*x^2 =0.355 P>0.05; **x^2 =0.784 P>0.05*

The electrophoresis fragments of the PCR products were 141bp, the fragments after digestion were 122bp and 19bp, which were divided into three genotypes: homozygous mutant (CC), heterozygous mutant (CT) and wild type (TT).

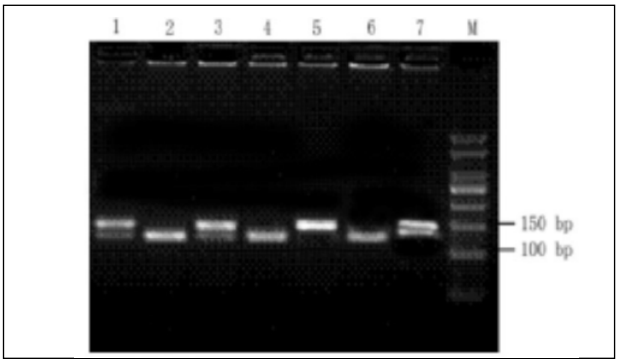

Fig. 1: The amplification of FGFR2 rs2981582 and products resolved by agarose gel electrophoresis.

2.4.6marker 1.3homozygous mutant(CC) 5.wild type(TT) 7.heterozygous mutant(CT). Figure1 The amplification of FGFR2 rs2981582 and products resolved by agarose gel electrophoresis.

FGFR2 rs2981579 test results

There was no significant difference in FGFR2 rs2981579 gene and genotype frequencies between breast cancer group and control group ($p > 0.05$). (See Table 2).

Group	n	Genotype frequency*			gene frequency**	
		CC	CT	TT	C	T
Breast cancer group	132	41(31.06)	57(43.18)	34(25.76)	182(68.94)	82(31.06)
Control group	145	39(26.90)	66(45.52)	40(27.58)	218(75.17)	72(24.83)
Total	277	80(28.88)	123(44.40)	74(26.72)	400(72.20)	154(27.80)

Table 2: Relevance between FGFR2 rs2981579 genotype and gene frequency distribution and breast cancer [n (%)].

*$x^2 = 0.5862$ $P>0.05$; **$x^2 = 2.675$ $P>0.05$

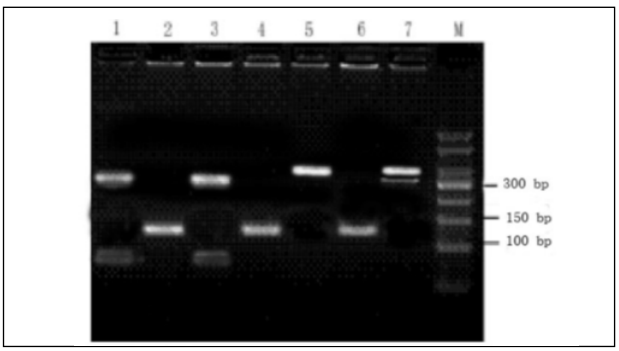

Fig. 2: The amplification of FGFR2 rs2981579 and products resolved by agarose gel electrophoresis.

Electrophoresis results of rs2981579 PCR products and enzyme digestion products are shown in Figure 2 (HhaI enzyme electrophoresis of FGFR2rs2981579 gene amplification products). The electrophoresis fragments of the PCR products were 437bp, the fragments after digestion were 350bp and 87bp, which were divided into three genotypes: homozygous mutant (CC), heterozygous mutant (CT) and wild type (TT).

Marker 1.3homozygous mutant(CC) 5.wild type(TT) 7.heterozygous mutant(CT)

Discussions

The onset and development of breast cancer means a very complex process. The current view is that environment and genes play an important role in onset and development of breast cancer[4-5]. FGFR2 gene is located on chromosome 10 (10q26) from base pair 123237843 to base pair 123357971, which belongs to the FG-FR gene family and contains 22 exons[6]. The expression of FGFR2 gene can promote tumor cell growth, differentiation, invasion, metastasis and tumor angiogenesis[7-8]. Gene polymorphism of different sites plays an important role in the process of cell proliferation, differentiation, angiogenesis and skeletal development. FGFR2 gene mutations and / or overexpression can lead to changes in protein tyrosine kinase activity, which then strongly increases tyrosine kinase phosphorylation and activity downstream effect, starting FGFR2-mediated breast cancer pathogenesis[9]. FGFR2 plays an important role in the growth of breast cancer tumors and can provide a new approach to breast cancer treatment. Easton et al.[10] discovered five susceptible regions of breast cancer in 2007 using Genome-wide Association Studies (GWAS), and Michailidou et al.[11] identified 15 new susceptible regions in 2015 through study on 120 000 specimens. Despite the huge number of such studies, what's most closely related to pathogenesis of breast cancer is polymorphism site located on FGFR2 (rs2981582) and TNRC90122 (rs3803662) gene[12].

Afterwards, a number of studies on the correlation between FGFR2 and breast cancer onset risk were conducted at home and abroad. In the study by Hunter et al.[13], the selected 1145 cases in patient group and 1142 cases in healthy control group showed that FGFR2 gene polymorphism was closely related to breast cancer incidence. Liang et al.[14] analyzed thousands of breast cancer patients in

China, screened three susceptible sites, rs2981582, rs1219648 and rs2420946 of FGFR2, and demonstrated that the patients with these three mutation sites at the same time had the highest incidence. Tapper et al.[15] also screened two SNPs most relevant with breast cancer (ts2981582, rs1219648).

In this study, two SNP sites of rs2981582 and rs2981579 were detected in the second intron of FGFR2 gene of Weifang population. The results showed that there was no statistically significant difference in genotype distribution of two detected SNP sites between control group and breast cancer group, indicating that there may be no correlation between these two sites and onset of breast cancer in the Han population. However, the study of the European population by Udler et al.[16] found that rs2981582 is the site significantly correlated with breast cancer.

For reason of this result, this study speculated that:

• There may be racial differences in correlation between SNP site and tumor susceptibility. The study by Udler et al. on rs2981582 targeted at German Jews and Jews in Spain or Portugal, but this study was on the Han population, so the racial differences may lead to different results.

• Onset of the tumor is a result of multiple factors, as a variety of biological activity factors in vivo form a complex network with mutual inhibition or promotion.

• The difference in sample size may also lead to different results. Sample size in the study by Udler et al. has reached more than 10,000 cases, while this study had a sample size of about 200 cases. The differences in sample size are very likely the reason for different results. At present, the mechanism of FGFR2 gene-induced tumor susceptibility or inhibition is still unknown and controversial. Therefore, it is necessary to increase the sample size and conduct a large number of functional studies to further explore the relationship between FGFR2 gene and the tumor.

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Corresponding author
Yongsheng Wang
Breast Cancer Center, Shandong Cancer Hospital Affiliated to Shandong University, Jiyan Road No. 440, Jinan, Shandong, China, Shandong Academy of Medical Sciences, Jinan Shandong
(China)