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Polymorphisms of the FAS and FASL genes and risk of breast cancer

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Abstract. FAS and its ligand FASL are crucial in apoptotic cell death. Loss of FAS and gain of aberrant FASL expression are common features of malignant transformation. This study was designed to investigate whether the functional polymorphisms of FAS -1377G/A (rs2234767) and FASL -844T/C (rs763110) affect the risk of developing breast cancer. Genotypes were analyzed by a polymerase chain reaction-restriction fragment length polymorphism assay in 436 breast cancer patients and 496 healthy controls. In this study, as compared to the wild-type homozygote and heterozygote, the distribution of the FAS -1377GG, GA and AA genotypes among breast cancer patients were significantly different from those among healthy controls (P=0.011), with the AA genotype being more prevalent among patients than the controls (P=0.003). Similarly, the frequencies of the FASL -844TT, TC and CC genotypes also significantly differed among breast cancer patients and healthy controls (P<0.001), with the CC genotype being significantly over-represented in breast cancer patients compared with the controls (P<0.001). In the unconditional logistic regression model following adjustment for age, the subjects carrying the FAS -1377AA genotype had a 1.75-fold increased risk [95% confidence interval (CI), 1.13-2.69] for development of breast cancer compared with patients carrying the GG genotype. Similarly, in the recessive model, the FASL -844*CC* genotype significantly increased the risk of breast cancer with an odds ratio (OR) of 1.92 (95% CI 1.46-2.54) compared with the *TT* or *TT* + *TC* genotypes. Our results suggest that functional polymorphisms in the death pathway genes FAS and FASL significantly contribute to the occurrence of breast cancer.

Introduction

Malignancy formation is not only associated with unlimited proliferation, but is also associated with the suppression of apoptosis. Apoptosis, a complex process in which cells neatly commit suicide, exerts critical roles in not only the development, but also the homeostasis and normal functioning of adult multi-cellular organisms (1). The over-functioning of apoptosis during development may result in abortion or abnormalities, while failure of inducing apoptosis of DNA-damaged cells may lead to tumor development (2). Accumulating evidence suggests that the acquired ability to resist apoptosis is a common hallmark of various types of malignant diseases, and that the regulatory defects of components of the apoptosis pathway contribute to tumorigenesis, tumor cell invasion and metastasis (1,2).

FAS (also known as Apo-1 or CD95), a potent member of the death receptor family, plays a key role in apoptotic signaling in many cell types (3). FAS interacts with its FAS-ligand (FASL) to trigger the death signal cascade, and subsequently induces apoptotic cell death (4). The interactions between FAS and FASL have been shown to be involved in the establishment of an immune privileged status of the tumor by inducing FAS-mediated apoptosis in tumor-specific lymphocytes (5). Evidence has shown that a number of tumors exhibit downregulation of FAS or loss-of-function conferring resistance to death signals induced by the immune system, as well as an increased expression of FASL-mediated immune privilege, inducing peripheral tolerance to antigens of normal organ environments via the apoptosis of FAS-positive lymphocytes (5-8). Consequently, a decreased expression of FAS and/or increased expression of FASL may have promoted malignant transformation and progression (9). In addition, the functional mutations in FAS and FASL genes that impair apoptotic signal transduction have been shown to be associated with an increased risk of various types of cancers (10). Thus, the FAS/FASL system may be significant in cancer initiation, development and progression, and single nucleotide polymorphisms (SNPs), which possess the potential to alter the expression of FAS and/or FASL, have been proposed to be significant in the genetic susceptibility to cancer.

G to A transition at position -1377 (-1377G/A, rs2234767) in the promoter region of FAS has been found to diminish the affinity for binding to transcriptional factor stimulatory protein 1 (Sp1), resulting in a decreased expression of FAS (11). Nevertheless, the FASL gene also has a functional polymorphism in its promoter, a T to C change at position -844 (FASL -844T/C, rs763110), which is located in a binding motif for the transcription factor CAAT/enhancer-binding

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Table I. Distribution of selected characteristics.

Variable	Patients (n=436)		Controls (n=496)		P-value[a]
	n	%	n	%	
Age (years)					0.536
≤40	23	5.3	18	3.6	
41-50	50	11.5	64	12.9	
51-60	132	30.3	166	33.5	
61-70	160	36.7	178	35.9	
>70	71	16.3	70	14.1	
Metastasis at diagnosis					
Stage I	131	30.0			
Stage II	162	37.2			
Stage III	90	20.6			
Stage IV	53	12.2			
Histological differentiation					
Low	56	12.8			
Intermediate	166	38.1			
High	214	49.1			

[a]Two-sided χ^2 test.

protein β (12). A higher basal expression of FASL is significantly associated with the -844C allele compared with the -844T allele (12). The role of these two functional polymorphisms in carcinogenesis has been extensively studied, and have been shown to be associated with an increased risk of various types of cancer (13,14).

Breast cancer is one of the most common fatal malignant tumors worldwide (15). Downregulation of FAS has been observed in certain carcinomas including breast cancer (16), while FASL is occasionally overexpressed in numerous human tumors, including breast cancer (17,18). Therefore, we hypothesized that the functional polymorphisms, FAS -1377G/A and FASL -844T/C, may increase the risk of breast cancer in a Chinese population owing to the reduced expression of FAS and/or increased expression of FASL. To test this hypothesis, we conducted a hospital-based, case-control study of breast cancer in a Chinese population including 436 breast cancer patients and 496 healthy controls.

Materials and methods

Study population. This study consisted of 436 patients with breast cancer and 496 healthy controls. Patients were recruited between January 2000 and June 2011 at Taizhou Hospital, Wenzhou Medical College (China). All patients with histopathologically confirmed breast cancer were enrolled. Patients with previous cancer, previous chemotherapy or radiotherapy were excluded. Control subjects were cancer-free individuals and were recruited from persons who visited the same hospital for physical examination. The selection criteria for controls included no individual history of cancer and frequency-matching to the cases by age (±5 years). The subjects were unrelated, ethnic Han Chinese. At recruitment, informed consent was obtained from each subject and the information on demographic characteristics, such as age was collected. This study was approved by the institutional review board of Taizhou Hospital, Wenzhou Medical College.

Polymorphism analysis. Genomic DNA was extracted from blood samples of all controls and cases collected at recruitment. FAS -1377G/A and FASL -844T/C genotypes were determined using PCR-based restriction fragment length polymorphism (PCR-RFLP) methods as previously described (19,20). A 10% masked, random sample of subjects was tested twice by various investigators, and the results were concordant for all masked duplicate sets.

Statistical analysis. The Hardy-Weinberg Equilibrium analysis was performed by comparing the observed and expected genotype frequencies in controls using the χ^2 test. Pearson's χ^2 test was used to estimate the differences in demographic variables and genotype distribution of FAS -1377G/A and FASL -844T/C between patients and controls. The associations between the polymorphisms and risk of breast cancer were estimated using odds ratios (ORs) and their 95% confidence intervals (CIs), which were calculated by unconditional logistic regression models following adjustment for age. Data were analyzed by SPSS 16.0 software. $P<0.05$ was considered to indicate a statistically significant difference.

Results

Subject characteristics. The analysis included 436 breast cancer patients and 496 controls. The distribution of selected characteristics is shown in Table I. No significant differences were found between cases and controls in terms of median age

Table II Genotype and allele frequencies of FAS and FASL in breast cancer patients, and their contribution to breast cancer risk.

Genotype	Patients (n=436)		Controls (n=496)		OR[a] (95% CI)
	n	%	n	%	
FAS -1377G/A					
GG	138	36.8	197	39.7	1.00
GA	171	45.6	246	49.6	0.96 (0.71-1.30)
AA	66	17.6	53	10.7	1.75 (1.13-2.69)[b]
GG + GA	309	82.4	443	89.3	1.00
A allele frequency	0.404	0.355			
FASL -844T/C					
TT	32	8.5	48	9.7	1.00
TC	120	32.0	237	47.8	0.72 (0.43-1.19)
TT + TC	152	59.5	285	57.5	1.00
CC	268	61.5	211	42.5	1.92 (1.46-2.54)[c]
C allele frequency	0.755	0.664			

[a]ORs and 95% CIs were calculated by the unconditional logistic regression model adjusted for age and other genotypes where appropriate. [b]P=0.011, compared with the GG genotype; [c]P<0.001, compared with the *TT* or *TT + TC* genotypes.

(P=0.536). Of the 436 patients, 131 (30.0%) had stage I, 162 (37.2%) had stage II, 90 (20.6%) had stage III and 53 patients (12.2%) had stage IV breast cancer. In terms of histological differentiation, 56 (12.8%) patients had low-differentiated tumors (Grade I), 166 (38.1%) patients had intermediate-differentiated tumors (Grade II) and 214 (49.1%) patients had high-differentiated tumors (Grade III).

Association between FAS and FASL polymorphisms and risk of breast cancer. The allele frequencies and genotype distributions of FAS -1377G/A and FASL -844T/C in breast cancer patients and controls are shown in Table II. The allele frequencies for FAS -1377A and FASL -844C were 0.355 and 0.664, respectively, in the controls compared with 0.404 and 0.755, respectively, in patients. The genotype frequencies of FAS -1377G/A and FASL -844T/C in the healthy controls conformed to the Hardy-Weinberg equilibrium (P=0.064 and P=0.112, respectively). The distribution of the FAS -1377GG, GA and AA genotypes among breast cancer patients were significantly different from those among the controls (χ^2=8.658; P=0.011), with the AA genotype being more prevalent among the patients than the controls (17.6 versus 10.7%, P=0.003). Similarly, the frequencies of the FASL -844TT, TC, CC genotypes also significantly differed between patients and controls (χ^2=25.560; P<0.001), with the CC genotype being significantly over-represented in breast cancer patients compared to the controls (61.5 versus 42.5%, P<0.001). In the unconditional logistic regression model following adjustment for age, the subjects carrying the FAS -1377AA genotype had a 1.75-fold increased risk (95% CI 1.13-2.69) for the development of breast cancer compared to patients with the GG genotype. Similarly, in the recessive model, the FASL -844*CC* genotype was associated with a significantly increased risk of breast cancer with an OR of 1.92 (95% CI 1.46-2.54) compared with the *TT* or *TT + TC* genotypes.

Discussion

This study investigated whether genetic polymorphisms in genes for the death receptor, FAS and its ligand, FASL, have an effect on the risk of developing breast cancer in a Chinese population. Our results revealed that the polymorphisms in the promoter regions of FAS -1377G/A and FASL -844T/C have substantial effects on the risk of breast cancer. The FAS -1377AA and FASL -844CC genotypes were significantly associated with an increased risk for developing breast cancer.

There has been accumulating evidence that functional germline polymorphisms of FAS genes are associated with a high risk of cancer (21-24). Recently, two meta-analyses evaluated the relationship between the FAS -1377 G/A polymorphism and cancer risk. Qiu *et al* analyzed 10,564 cancer cases and 12,075 controls from 17 studies (25). Zhang *et al* analyzed 11,461 cases and 12,708 controls from 34 case-control studies (14). The results obtained by these authors were consistent in that the FAS -1377AA genotype was associated with a significantly increased cancer risk compared to the G/G genotype (14,25). Another meta-analysis of the FASL -844T/C polymorphism in relation to cancer risk was conducted by Zhang *et al*. These authors reported 11,105 cancer cases and 11,372 controls from 19 published studies and concluded that the FASL -844T variant allele was associated with a significantly reduced cancer risk (13). Consistent with these results, our study demonstrated that the individuals carrying the FAS -1377AA or FASL -844CC genotype were at an increased risk for developing breast cancer, which is in agreement with the biological plausibility. The FAS -1377 G to A change disrupts the crucial transcriptional activator Sp1 binding site and reduces promoter activity (11), thus a decrease in FAS expression associated with the -1377 genotype is expected. The FASL -844T/C polymorphism is also located in the promoter region of the gene, and the C allele has been shown to create

a binding site for the CAAT/enhancer-binding protein β transcription factor, resulting in a significantly higher basal FASL expression in luciferase reporter assay peripheral blood fibrocytes (12). Furthermore, a large body of evidence has demonstrated that the downregulated expression of FAS and heightened expression of FASL are common features of malignant transformation and oncogenic events in the evolution of the majority of types of human cancer (6,9,26). According to these lines of evidence, individuals carrying the FAS-1377AA and/or FASL-844 variant genotypes exhibited an aberrant expression of FAS and FASL and presented a higher risk for developing breast cancer. Furthermore, this current result was also consistent with findings of recent studies of other types of cancer, including cervical (24), pancreatic (27) and renal cancer (28).

In conclusion, this study provides the first evidence that polymorphisms in promoter regions of FAS and FASL are significant in the risk of occurrence of breast cancer in a Chinese population. These results confirm the hypothesis that FAS -1377G/A and FASL -844T/C polymorphisms are the susceptible factors for the development of breast cancer, although further independent studies are required to confirm our results.

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