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Status of FAS and FAS Ligand Gene Polymorphisms in Patients with Breast Cancer in Northeastern IRAN

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Abstract

Background: The First apoptosis signal (FAS) and First apoptosis signal ligand (FASL) genes initiate the apoptosis pathway, playing a central role in the tumor growth and metastasis. Gene polymorphisms including -1377 G/A in the promoter region of FAS and -844 C/T in the promoter region of FASL have shown to change the transcription activities of these genes.

Methods: In this study we evaluated association of these polymorphisms with risk of metastasis of breast cancer, in a population selected from Mashhad, Iran**.** A total of 115 patients with breast cancer and 115 controls were recruited in this case-control study. Polymerase Chain Reaction-based Restriction Fragment Length Polymorphism (PCR-RFLP) was applied for genotyping on extracted DNA from participant's blood. Unconditional logistic regression was used to estimate cancer risk by calculating odds ratios (OR) and their 95% confidence intervals (95% CIs).

Results: There was no significant association between these genetic polymorphisms and breast cancer risk. Additionally, our results showed no significant influence from the above mentioned gene polymorphisms on metastasis of breast cancer.

Conclusions: These results suggest that the FAS-1377G/A and FASL-844 C/T gene polymorphism don't have much influence on the susceptibility to metastasis of breast cancer in northeastern Iranian population. Therefore, we suggest to investigate impact of other candidate gene polymorphisms on metastasis of breast cancer for future research.

Keywords: Breast cancer, Fas Ligand, Fas receptor, Gene polymorphism, Metastasis

Introduction

Nowadays the most common cause of death due to cancers in women worldwide, breast cancer is owned (1). According to the latest World Health Organization (WHO), more than one million new cases of breast cancer are involved annually worldwide. The majority of women with this disease are diagnosed in advanced stages and of year 500,000 women die of breast cancer worldwide (2, 3). In Iran of every 105 women, 20 women with breast cancer are estimated and from ten patients, one of them has involved advanced breast cancer (4). In addition, the factors such as geographic, age, androgen hormones, lifestyle and environmental factors, genetic factors, race, family history on the development, and progression of breast cancer are considered effective (5, 6).

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Researchers believe that the study of genetic abnormalities of genes involved in programmed cell death or apoptosis, and metastases can eventually create a basis for diagnosis and appropriate medical treatment for this cancer (7, 8). Apoptosis process to prevent uncontrolled cell growth and proliferation and tumor suppression is essential to maintain homeostasis.

Adjustment disorder and inappropriate expression of single nucleotide mutations in the apoptotic pathway molecules cause tumors in tissues (9).

First apoptosis signal (FAS) and First apoptosis signal ligand (FASL) receptor molecules trigger the extrinsic pathway of apoptosis (10). FAS (CD95, APO-1) type I cell surface proteins belong to a member of the tumor necrosis factor receptor (TNFR) family. FAS gene consists of nine exons mapped on the chromosome 10q23. Several single nucleotide polymorphisms in the gene sequence of FAS are identified, but two polymorphisms of -1377 (G/A) and - 670(A/G) in the promoter region of the gene, are more important (11). The -1377G to A transition in the promoter region of FAS disrupt an Sp1 and a STAT1 transcription factor binding site, respectively, which diminish the promoter activity and consequently down-regulate the gene expression (12).

First apoptosis signal ligand (FASL, CD95L or CD178) is type II membrane protein which its gene is mapped on chromosome 1q23 in humans with four exons (13). In FASL gene in the promoter region, C to T transition at position -844(C/T) has been reported to be located in a binding motif for another transcription factor, CAAT/enhancer-binding protein ß (14). Because the effect of FAS and FASL polymorphisms on trends tumorigenesis is very important, these polymorphisms have been associated with types of cancer such as breast cancer (15-19), gastric cancer (20), esophageal cancer (21), cervical cancer (22, 23). Also meta-analysis studies have been performed to clarify the relationship between these polymorphisms and risk of lung cancer, bladder cancer, breast cancer, pancreatic cancer, uterus cancer and ovarian cancer (24, 25).

The current study aimed to investigate the association between the FAS/FASL polymorphisms with risk of metastasis of breast cancer, in a population selected from Mashhad, IRAN.

Materials and methods

Patients and Controls

This study consisted of 115 patients with breast cancer and 115 healthy controls. Patients were recruited between February 2013 - October 2014 at Omid Hospital, Mashhad University of Medical sciences, Iran. All patients with histopathological diagnosis confirmed breast cancer were enrolled. The surgical and pathological grading were performed according to The American Joint Committee on Cancer staging (AJCC 2006) and Bloom–Richardson grading system, respectively (26, 27). Moreover, the Estrogen and Progesterone receptor status was evaluated using Immunohistochemistry method (28). Patients with previous cancer, chemotherapy or radiotherapy were excluded. These patients were further divided into two groups based on the clinical presentations. These groups were 44 cases with metastases and 71 patients with non-metastases characteristics. At recruitment, informed consent was obtained from each subject and the information on demographic characteristics, such as age was collected. Control subjects were cancer-free individuals and were recruited from persons who visited the same hospital for physical examination. As shown in table 1, the selection criteria for controls included no individual history of cancer and frequency-matching to the cases by age (±5 years).

Genotyping and Polymorphism Analysis

About 5 mL of peripheral blood samples were collected from the patients and healthy controls and drawn in ethylenediaminetetraacetic acid (EDTA) tubes for genotyping. Genomic DNA was then extracted from whole blood samples of all controls and patients, using a routine salting out method (29). Genomic DNA concentration was determined using BioPhotometer (Eppendorf-Germany). Genotyping for FAS-1377 G/A and FASL -844 C/T polymorphisms was performed by polymerase chain reaction–based restriction fragment length polymorphism (PCR–RFLP)

The PCR primers for amplification of the FAS and FASL promoter variants, specificities, restriction enzymes and digestion patterns are shown in Table 2. PCR amplifications were amplified by Personal Cycler™ amplificator (Biometra, Germany) under conditions as

previously described (30). The digested PCR products were separated on 3% agarose gel containing ethidium bromide and visualized under the UV transilluminator.

Table 1. Distribution of selected characteristics of patients and controls

		Case N=115	Control =115	P value
Characteristics	Metastasis	44(38.2%)		
	Non metastasis	71(61.7%)		
Age range		24-79	24-86	
Age (mean± SD)		45±12	46±11	0.14
Menopausal status	Pre Menopause	92(80%)		
	Post-menopausal	23(20%)		
Tumor Stage (AJCC 2006)	Stage 1	57(49.5%)		
	Stage 2	14(12.1%)		
	Stage 3	44(38.2%)		
Lymph node involvement	Negative	27(23.4%)		
	Positive	79(68.6%)		
	Missing	9(7.8%)		
Histological Grade (Bloom–Richardson grading system)	Grade one	65(56.5%)		
	Grade two	44(38.2%)		
	Missing	6(5.2%)		
Estrogen Receptor	Negative	37(32.1%)		
	Positive	50(43.4%)		
	Unknown	28(24.3%)		
Progesterone receptor	Negative	52(45.2%)		
	Positive	40(34.7%)		
	Unknown	23(20%)		

Table 2. The characteristics of primers and amplified sequences of FASL and FAS polymorphisms genotype

Gene Location	Primer	Primer Sequences	Base Pair	Restriction Enzyme	Genotype
Fas -1377 G/A	F	5′-TGTGTGCACAAGGCTGGCGC-3′	122	BstUI	GG: 104 + 18 bp; GA: 122 + 104 + 18 bp; AA: 122 bp
	R	5′-TGCATCTGTCACTGCACTTACCACCA-3			
Fas-L -844 T/C	F	5’-CAGCTACTCGGAGGCCAAG-3	401	BsrDI	CC: 233 + 168 bp; CT: 401 + 233 + 168 bp; TT: 401 bp
	R	5’-GCTCTGAGGGGAGAGACCAT-3’			

Statistical Analysis

Statistical analysis was performed by SPSS software version 18. Genotype and allele frequency differences of FAS and FASL promoter polymorphisms were analyzed between the cases and controls, and into two patient groups using Chi-squared test. Logistic regression analysis was used to assess the association of haplotype and combined genotype effects of these polymorphisms between the cases and controls. P values less than 0.05 were regarded statistically significant. The genetic trait association between the groups was measured by odds ratio (OR) and the exact confidence intervals (CI) of 95% were obtained. To assess the consistency of genotype distribution with the Hardy-Weinberg equilibrium, Chi-squared test was used.

Results

The frequencies of FAS-1377 G/A and FASL-844C/T were shown in Table 3. As shown in Table 3, distribution of genotype and allele frequencies for FAS and FASL polymorphisms was not statistically different between patients with breast cancer and the controls.

Table 3. Genotype and Allele Frequencies of FAS-1377 and FASL-844

Genotypes and Alleles	Controls (n = 115)	Patients With Breast Cancer (n = 115)	P-Value	OR[1]	CI 95%[2]
FAS -1377 G/A					
Genotype					
GG	30 (26.1%)	35 (30.4%)		1.00	
GA	58 (50.4%)	57 (49.6%)	0.7	0.84	(0.46-1.55)
AA	27 (23.5%)	23 (20%)		0.73	(0.35-1.53)
GA+AA	85 (73.9%)	80 (69.6%)		0.81	(0.45-1.43)
Allele					
G	118(51%)	127(0.55)	0.45	0.845	(0.592-1.23)
A	112(49%)	103(0.45)			
FASL -844 C/T					
Genotype					
CC	42 (36.5%)	33 (28.7%)		1.00	
CT	53 (46.1%)	50 (43.5%)	0.14	1.20	(0.66-2.18)
TT	20 (17.4%)	32 (27.8%)		2.04	(0.99-4.19)
CT+TT	73 (63.5%)	82 (71.3%)		1.43	(0.82-2.49)
Allele					
C	137(0.6%)	116(0.5)	0.06	1.448	1.001-2.094)(
T	93(0.4%)	114(0.5)			

1. OR: Odds ratio
2. Confidence interval 95%

Furthermore, as shown in table 4, the distribution of genotype and allele frequencies of both FAS and FASL polymorphisms were compared among patients with metastasis and non metastasis breast cancer. (Table 4).

Table 4. Genotype and allele frequencies of FAS-1377 and FASL-844 gene polymorphisms among metastasis and non-metastasis breast cancer patients.

Genotypes and Alleles	Non-metastasis breast cancer N=71	Metastasis breast cancer N=44	P-Value	OR[1]	CI 95%[2]
FAS -1377 G/A					
Genotype					
GG	18 (25.4%)	17(38.6%)		1.00	
GA	38(53.5%)	19(43.2%)	0.32	1.772	(0.549-5.714)
AA	15(21.1%)	8(18.2%)		1.464	(0.415-5.162)
Allele					
G	74(52.11%)	53(60.22)	0.275	0.718	(0.419-1. 232)
A	68(47.89%)	35(39.78)			
FASL -844 C/T					
Genotype					
CC	21 (29.6%)	12 (27.3%)		1.00	
CT	35 (49.3%)	15 (34.1%)	0.106	0.691	(0.262-1.822)
TT	15 (21.1%)	17 (38.6%)		2.339	(0.828-6.605)
Allele					
C	77(54.23%)	39(44.31%)	0.174	1.488	(0.871-2.541)
T	65(45.77%)	49(55.69%)			

1. OR: Odds ratio
2. Confidence interval 95%

Moreover, in genetic studies linkage disequilibrium, means a non-random association of alleles at two or more loci on chromosomes is ancestral. Linkage disequilibrium exists in populations that the combination of alleles or genotypes inheritance is consistent with the ratio expected (23). In this study, we focused on two polymorphisms FAS-1377G/ A and FASL-844C/T and the results showed that there were no linkage disequilibrium associations between mentioned polymorphisms (P value: 0.45). Moreover, haplotype analysis revealed that there was not significant relationship between the two mentioned loci haplotypes with breast cancer (Table 5).

Table 5. Haplotype association of FAS and FASL polymorphisms with breast cancer

FAS -1377	FASL -844	Total	Control	Patient	P-value	OR	CI 95%
G	C	0.335	0.349	0.376	—	1.00	
G	T	0.276	0.2675	0.329	0.8	1.09	(0.57 - 2.07)
A	C	0.266	0.2564	0.1347	0.098	0.51	(0.23 - 1.13)
A	T	0.132	0.130	0.161	0.95	0.98	(0.51 - 1.87)

Discussion

Apoptosis or programmed cell death, a process to remove the cancer cells or virus -infected, control the number of cells and inhibit excessive cell proliferation (8). First apoptosis signal (FAS) and First apoptosis signal ligand (FASL) receptor molecules trigger the extrinsic pathway of apoptosis. which are responsible for the tumor suppressive (31). Any change in the gene of these molecules affect on the performance of apoptosis because of their important role in tumor development, malignancies, and tumors escape immune cells (32).

The current study showed that FAS-1377 and FASL -844C/T polymorphisms were not associated with breast cancer. Also there were not association between patient groups metastasis and non-metastasis. Moreover, no significant relation between mentioned polymorphisms and estrogen receptor and progesterone, the number of pregnancy in women, menopausal status, grades and different stages in patients were found. Results of previous studies has shown no significant association with breast cancer and polymorphisms and cervical cancer (23, 33).

Also in previous studies lack of relevance of each of these polymorphisms with various cancers such as polymorphism FAS-1377 G / A with breast cancer (16, 18), gastric cancer (20) and cancer Neuroblastoma (34), and polymorphism-844 C / T with breast cancer (18) has been proven. in return in other studies between -844C / T polymorphism was significantly associated with risk of breast cancer has been demonstrated (16).

However, in the latest meta-analysis study the relationship between these two polymorphisms and risk of 52 types of cancer was studied (25). The mentioned meta-analysis study reported that in patient with -1377AA and -844CC genotypes, the risk of cancers, such as breast cancer, gastric cancer and esophageal cancer especially in the Asian population, have significantly increased (25). One of the inconsistent results of previous studies is that the mentioned polymorphisms may have different roles in different body sites play in face of cancer (15).

Although even in the same location of the tumor in case of small volume samples caused different results. Another cause can be selected control group of healthy people or those who have simply admitted to hospital and in terms of pathological and laboratory tests approved health which are not a symbol of the community. Also the genetic differences of the various geographic areas and different nationalities and life styles may be the cause of the different results of the studies.

Finally, more studies with larger sample size are needed on various population and races to evaluate the association of cell death pathway receptors polymorphisms and risk of cancers.

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