RESEARCH ARTICLE

The MMP-2 -735 C Allele is a Risk Factor for Susceptibility to Breast Cancer

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Abstract

Background: The expression of MMP genes has been demonstrated to be associated with tumor invasion, metastasis and survival rate for a variety of cancers. The functional promoter polymorphism MMP-2 C-735T is associated with decreased expression of the MMP-2 gene. The aim of present study was to detect any association between MMP-2 C-735T and susceptibility to breast cancer. Materials and Methods: The MMP-2 C-735T polymorphism was studied in 233 women (98 with breast cancer and 135 healthy controls). All studied women were from Kermanshah and Ilam provinces of Western Iran. The MMP-2 C-735T polymorphism was detected using a polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Results: The frequencies of MMP-2 CC, CT and TT genotypes in healthy individuals were 59.3, 38.5 and 2.2%, respectively. However, in breast cancer patients, only CC (71.4%) and CT (28.6%) genotypes were observed (p=0.077). In patients the frequency of the MMP-2 C allele was significantly higher (85.7%) compared to that in controls (78.5 %, p=0.048). The presence of C allele of MMP-2 increased the risk of breast cancer by 1.64-fold [OR=1.64 (95%CI 1.01-2.7, p=0.049)]. The frequency of MMP-2 C allele was also higher in patients ≤40 years (88.9%) than those aged ≥41 years (67.5%, p=0.07). In addition, the frequency of MMP-2 C allele tended to be higher in patients with a family history of cancer in first –degree relatives (76.6%) compared to that without a family history of cancer (67.3%, p=0.31). Conclusions: Our findings indicate that the C allele of MMP-2 C-735T polymorphism is associated with increased risk of breast cancer. Also, the MMP-2 C allele might increase the risk of young onset breast cancer in our population.

Keywords: Breast cancer - MMP-2 C-735T - gene polymorphism - Western Iran

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Introduction

Breast cancer is the most common cancer in women that is associated with an enormous amount of morbidity and mortality throughout the world (Hughes et al., 2007). Its incidence is increasing in developing countries in a rate of 3-4% (Babu et al., 2011).

Due to high incidence rate of breast cancer, it is considered as one of the common health problems among various populations. Interaction between genetic background and different environmental risk factors is involved in the pathogenesis of the disease. Reports from different populations suggest that genetic factors such as single nucleotide polymorphisms, insertion or deletion mutations and rearrangements might strongly influence on individual's susceptibility to breast cancer (Boroujeni et al., 2013).

Matrix metalloproteinases (MMPs) are zinc-dependent endopeptidase that degrade and restructure the extracellular matrix components and basal lamina, the two physical barriers for cancer cells (Rahimi et al., 2013, Rahimi et al., 2014). The extracellular proteins such as collagen type I-VIII, gelatin, elastin, laminin and also myelin basic protein and several growth factors are substrates of MMPs (Ke et al., 2013). These enzymes are generally present at lower level in healthy individuals, but their up-expression has been reported in human cancers in all stages of carcinogenesis (Zhou et al., 2011). Over-expression of MMPs genes has also been significantly correlated with tumor grade and tumor cell invasion for a variety of cancers. So, it might the high expression of MMPs genes allow the remodeling of extracellular matrix to increase the invasive potential of cancer cells (Gonzalez-Arriaga et al., 2012).

MMP-2 is known as gelatinase A that degrades the type IV collagen and gelatin, which are the main constituents of basal lamina and might affect early events in carcinogenesis and tumor invasion and/or metastasis (Sanii et al., 2012). The MMP-2 gene locates on chromosome 16 at q13-21 and has thirteen exons (Saeed et al., 2013). Numerous studies have demonstrated that breast cancer disease is associated with elevated level of MMP-2 gene expression (Zhou et al., 2011; Srivastava et al., 2013a). Alteration in the gene transcription could be an

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important mechanism influences on the various individual susceptibility to breast cancer (Gonzalez-Arriaga et al., 2012; Srivastava et al., 2013b). A functional single nucleotide polymorphism in the cis-acting regulatory element in the promoter region of MMP2 may influence the expression of this gene (Wang et al., 2011; Zhang et al., 2013). A C→T transition at nucleotide -735 (rs2285053) has allele specific effects on the regulation of MMP2 gene transcription (Wang et al., 2011).

According to literature there is no available report to examine the possible association between MMP-2 C-735T polymorphism with breast cancer susceptibility. The aim of present study was to investigate the frequency of MMP-2 C-735T variants and their possible association with breast cancer risk in an Iranian population with Kurdish ethnic background.

Materials and Methods

Sample

In a case-control study 98 breast cancer patients including 97 females and 1 male with the mean age of 49.5±10.2 years (29-79 years) and 135 healthy individuals consisted of 129 females and 6 males with the mean age of 38.7±9.4 years (22-68 years) as controls were investigated. The breast cancer diagnosis was according to standard clinical, radiological and histological parameters. All patients and controls were from Kermanshah and Ilam provinces of Iran with Kurdish ethnic background who admitted to the Kermanshah University of Medical Sciences clinics. Demographic, histopathology and medical characteristics of patients including age, sex, family history of cancer, tumor stage, lymph node metastasis and the status of estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor (HER2), P53 and KI67 were obtained from the files of patients. All procedures of the study were approved by the Ethics Committee of Kermanshah University of Medical Sciences, Iran. All individuals contributed in this research agreed to participate in the study and signed an informed consent in accordance with the principles of the Helsinki II declarations. All subjects were informed about their disease and the aim and procedures of the research.

Genotyping

From each subject 5 ml whole blood sample was collected into tubes containing EDTA. Genomic DNA was extracted from peripheral blood leukocytes according to a standard phenol-chloroform method as previously described (Rahimi et al., 2010; Rahimi et al., 2006). Extracted DNA was verified by electrophoresis on 1% agarose gel. The concentration and purity of DNA was assessed with a Nanodrop spectrophotometer (Thermo).

The MMP-2 C-735T polymorphism was identified using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP). The PCR was conducted by the forward primer of 5'-ATAGGGTAAACCTCCCCACAT T-3' and the reverse primer of 5'-GGTAAAATGAGGCTGAGACCT G-3' using Master Cycler Gradient-Eppendorf (Germany). The PCR reagents consisted of 20 pmol of each primer, 300 ng of extracted DNA, 200 µM dNTPs, 1.5 mM MgCl2, 1 U Taq polymerase and 2.5 µl of 10X PCR buffer with final volume of 25 µl. The PCR thermal cycling parameters were: 1 cycle at 94°C for 5 min, 35 cycles by 94°C for 60s, 62°C for 60s and 72°C for 60s followed with final extension for 10 min at 72°C. The obtained 300-bp PCR product was digested with HinfI restriction enzyme. In the presence of C allele, the PCR product remained intact while in the presence of T allele two fragments of 254- and 46-bp were produced (Gai et al., 2010).

Statistics

The allelic frequencies were calculated by the chromosome counting method. The significance of the difference of alleles and genotype frequencies between the groups was tested using the chi-square method. Data on quantitative characteristics are expressed as means±standard deviations. Odds ratios (OR) were calculated as estimates of relative risk for disease and 95% confidence intervals were measured by logistic regression using SPSS software. A two-tailed Student's t test analysis was used to compare quantitative data. Statistical significance was assumed at the $p<0.05$ level. All of the statistical analyses were performed using SPSS statistical software package version 16.0.

Results

The demographic, biochemical, and clinical parameters of studied women are demonstrated in Table 1. Eighty nine percent of patients were with invasive ductal carcinoma, 9.8% of patients had invasive lobular carcinoma and 1.2% of patients were with in situ type. Based on available immunohistochemical status, there was 64 patients (73.6%) with ER+ and 23 (26.4%) with ER-. The PR+ was detected in 66 patients (75.9%) and 21 patients were PR- (24.1%). The frequency of other immunohistochemical markers were HER2+ (60.5%), P53+ (42.9%), and Ki67+ (95.6%) (Table 1).

Agarose gel electrophoresis pattern of some of RFLP products of the MMP-2 gene digested with HinfI is demonstrated in Figure 1.

The frequencies of MMP-2 genotypes were in Hardy–Weinberg equilibrium in both patients and controls ($\chi^2=2.72$, $p>0.1$).

Distribution of MMP2 genotypes and alleles in breast cancer patients and controls is depicted in Table 2. All three genotypes of CC (59.3%), CT (38.5%) and TT (2.2%) were observed in healthy individuals, but in breast cancer patients, only CC genotype with a frequency of 71.4% and CT genotype with a frequency of 28.6% were found. A borderline significant difference in the distribution of MMP-2 C-735T genotypes was observed between controls and breast cancer patients ($p=0.077$). In patients the frequency of MMP-2 C allele was significantly higher (85.7%) compared to that in controls (78.5 %, $p=0.048$). Also, the presence of C allele of MMP-2 increased the risk of breast cancer by 1.64 fold [OR=1.64 (95%CI: 1.01 -2.7, $p=0.049$)].

A statistical analysis was carried out to investigate

Table 1. Characteristics of Breast Cancer Patients and Controls

Variable		Patients (n=98)	Controls (n=135)
Gender	Female	97 (99%)	129 (95.6%)
	Male	1 (1%)	6 (4.4%), p=0.13
Age (years)		49.5±10.2 (range: 29-79)	38.7±9.4 (range: 28-70) p<0.001
Family history of cancer			
	No	59 (60.2%)	-
	Yes (first-degree relatives)	39 (39.8%)	
Stage	I	13 (19.7%)	-
	II	42 (63.6%)	
	III	11 (16.7%)	
Lymph node metastasis			
	No	15 (22.7%)	-
	Yes	51 (77.3%)	
Histological types			
	In situ	1 (1.2%)	-
	Invasive ductal carcinoma	73 (89.0%)	
	Invasive lobular carcinoma	8 (9.8%)	
ER	Negative	23 (26.4%)	-
	Positive	64 (73.6%)	
PR	Negative	21 (24.1%)	-
	Positive	66 (75.9%)	
HER2	Negative	34 (39.5%)	-
	Positive	52 (60.5%)	
P53	Negative	36 (57.1%)	-
	Positive	27 (42.9%)	
KI67	Negative	3 (4.4%)	-
	Positive	65 (95.6%)	

Table 2. Distribution of MMP-2 C-735T Genotypes and Alleles in Patients and Controls

Genotype		Patients, n=98 n (%)	Controls, n=135 n (%)
MMP-2 C-735T	CC	70 (71.4)	80 (59.3)
	CT	28 (28.6)	52 (38.5)
	TT	0 (0)	3 (2.2)
			χ^2=5.12, p=0.077
MMP-2 alleles	C	168 (85.7)	212 (78.5)
	T	28 (14.3)	58 (21.5)
			χ^2=3.9, p=0.048
		OR=1.64 (95%CI 1.01 -2.7, p=0.049)	

any possible association between MMP-2 alleles and clinical parameters such as family history of cancer, breast cancer stage, lymph node metastasis, histological type and immunohistochemical data of the cancer. As indicated in Table 3, the frequency of MMP-2 C allele was tended to be higher in patients with a family history of cancer in first –degree relatives (76.6%) compared to that without a family history of cancer (67.3%, p=0.31). The frequency of this allele was the highest in patients with the stage III of the disease (80%), was the modest in patients with the stage II of the disease (75%) and was the lowest in patients with the stage I of the disease (69.2%, p=0.83). Also, a higher frequency of MMP-2 C allele was detected in ER- (77.3%) than ER+ (69.5%), in PR- (75%) compared to PR+ (70.5%), and in P53- (73.5%) than P53+ (60%) patients that did not reach to a statistical significance (Table 3).

Furthermore, the frequency of MMP-2 C allele was higher in patients ≤40 years (88.9%) than those patients aged ≥ 41 years (67.5%, p=0.07).

Table 3. MMP-2 C-735T Polymorphism and Clinical Parameters of Patients

Clinical parameters		MMP-2 C n (%)	MMP-2 T n (%)
Family history of cancer			
No		33 (67.3)	16 (32.7)
Yes (first-degree relatives)		36 (76.6)	11 (23.4), p=0.31
Stage	I	9 (69.2)	4 (30.8)
	II	30 (75)	10 (25)
	III	8 (80)	2 (20.0), p=0.83
Lymph node metastasis			
No		17 (70.8)	7 (29.2)
Yes		21 (61.8)	13 (38.2), p=0.47
Histological types			
In situ		1 (100)	0 (0)
Invasive ductal carcinoma		58 (71.6)	19 (28.4)
Invasive lobular carcinoma		6 (85.7)	1 (14.3), p=0.6
ER	Negative	17 (77.3)	5 (22.7)
	Positive	41 (69.5)	18 (30.5), p=0.49
PR	Negative	15 (75)	5 (25)
	Positive	43 (70.5)	18 (29.5), p=0.69
HER2	Negative	22 (66.7)	11 (33.3)
	Positive	34 (73.9)	12 (26.1), p=0.48
P53	Negative	25 (73.5)	9 (26.5)
	Positive	15 (60)	10 (40.0)
KI67	Negative	3 (100)	0 (0)
	Positive	41 (68.3)	19 (31.7) , p=0.27

Discussion

The present study in a homogenous population of breast cancer patients from Western Iran reports a significantly higher frequency of MMP-2 -735 C allele compared to that in healthy individuals that increased the risk of breast cancer 1.64 times. Also, the frequency of this allele tended to be higher in patients with a family history of the disease than those without a family history of the cancer and there was a higher prevalence of MMP-2 C allele in higher stages than lower stages of the disease. The MMP-2 C allele was more prevalent in young breast cancer patients than older breast cancer patients. Furthermore, the frequency of this allele was tended to be higher in ER-, PR- and P53- breast cancer patients than those patients that were positive for ER, PR, and P53.

Breast cancer is the most frequent malignancy diagnosed in women worldwide and it is the third cause of death among Iranians after coronary heart diseases and accidents (Taghavi et al., 2012; Zheng et al., 2014). Epidemiologic studies have reported numerous risk factors for breast cancer, including genetic and environmental factors (Zhou et al., 2011; Yadav et al., 2014). A number of polymorphisms have been reported in the promoter regions of MMPs genes including MMP-2 gene that some of them are associated with the increased risk of breast cancer (Shagisultanova et al., 2004). Some of these polymorphisms have allele-specific effects on the regulation of MMP-2 gene expression and are associated with the development and progression of many diseases including cancers, coronary heart disease, ischemic stroke and adenomyosis (Wang et al., 2011; Saeed et al., 2013). Because the human MMP-2 promoter contains a number of cis-acting regulatory elements, the constitutive and

inducible expression level of MMP-2 is likely regulated by transcription factors. Among these regulatory elements, the Sp1 binding site is particularly interesting. Among the well studied polymorphisms locate at promoter region of MMP-2, a C to T transition at -1306 and -735 disrupts a SP1 binding element results in decreased promoter activity and reduces the MMP-2 expression (Hajihoseini et al., 2011; Guo et al., 2012; Sharma et al., 2012). There is no data available to demonstrate the role of MMP-2 C-735T variants in susceptibility to breast cancer. However, the role of this polymorphism has been investigated in other cancers. Zhou et. al. observed that the variant allele of MMP- 2 -1306T was a protective factor for the development of breast cancer disease in Chinese women (Zhou et al., 2004). Also, Hajihoseini et al. reported that the frequency of MMP-2 C allele was more common in head and neck squamous cell carcinoma patients (Hajihoseini et al., 2011). Further, Yu et al. indicated that in patients carriers for -735CC genotype there was an increased risk of developing esophageal squamous cell carcinoma (Yu et al., 2004; Srivastava et al., 2013a). In contrast, the MMP-2 TT genotype increased the risk of bladder cancer when combined with CT genotype in Northern Indian population (Srivastava et al., 2013a). However, in a study from Indian population Srivastava et al. reported no significant association between MMP-2 C-735T gene polymorphism and the risk of developing cervical cancer (Srivastava et al., 2013b).

The absence of Sp1 consensus sequence results in a lower promoter activity and decreased MMP-2 expression in individuals carrying the TT than CC genotype. The benefit of lower expression of T allele might results in reduced excessive degradation of fibrillar collagens and other extracellular matrix components and development of breast cancer (Zhou et al., 2004; Wang et al., 2011). So, it seems the higher expression of MMP-2 C allele than the T allele that was more prevalent in our studied patients compared to healthy individuals not only increased the risk of breast cancer but also might be a risk factor for younger onset of the disease, higher stages of the tumor and familial breast cancer disease.

In conclusion, the present study indicates that the C allele of MMP-2 is a risk factor for susceptibility to breast cancer. Also, the MMP-2 C allele might increase the risk of young onset breast cancer in population of Western Iran that could be confirmed in more studies with larger sample size.

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