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ORIGINAL ARTICLE

The MIF rs755622 Variant may Increase Susceptibility of Breast Cancer but not Gastrointestinal Cancer in a Turkish Population

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OBJECTIVE

An increasing number of epidemiological and molecular evidence proposes that inflammation is a significant factor in the etiology of cancers. Macrophage Migration Inhibitory Factor (MIF) encodes a lymphokine involved in cell-mediated immunity, immunoregulation, and inflammation. It has been reported that MIF is linked with a higher risk of several cancer types. In the present study, we investigated the association of MIF rs755622 variant with the risk of breast cancer (BC) and gastrointestinal cancer in a Turkish cohort.

METHODS

The present study included a total of 153 subjects, which consisted of 33 BC patients, 53 gastrointestinal cancer patients and 67 healthy controls. Genomic DNA extracted from peripheral venous blood. The rs755622 variant of the MIF gene was genotyped using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. The results were statistically analyzed by calculating the odds ratios (OR) and 95% confidence intervals (CI) using the χ^2 test.

RESULTS

There was a statistical difference between the BC patients and controls for the MIF rs755622 variant. MIF rs755622 GG genotype and G allele were increased in BC patients compared to controls (p=0.016, p=0.017, respectively). No significant difference was observed between gastrointestinal cancer patients and controls for the MIF rs755622 variant (p>0.05).

CONCLUSION

Our results showed that the MIF rs755622 variant might play a potential role in BC physiopathology.

Keywords: Breast cancer; gastrointestinal cancer; MIF gene; PCR-RFLP; variant.

Introduction

Breast cancer (BC) is the most common cancer and the leading cause of cancer mortality among women in the world.[1] Despite the decreased BC death rates owing to earlier diagnosis and better treatment modalities, the incidence of BC continues to increase in developing countries. BC is a multifactorial disease, and many-

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factors were linked with BC development and progress, such as polygenic inheritance, lifestyle, and exposure to radiation. Human immune responses include initiate and adaptive immune reactions, and their alterations may result in increased susceptibility to several diseases, such as cancer. Recent studies report strong evidence that cytokines and cells in the immune response are related to BC risk and prognosis.[2]

Gastrointestinal cancer is among the most common causes of mortality in the world. Many patients cannot be cured mainly due to late diagnosis, hence requiring palliative medical care. Patients' nutritional status, such as weight loss, muscle wasting known as sarcopenia, and inflammation, should be considered.[3] Systemic inflammation that arises from the tumor development and progression has been reported to play major roles in these adverse effects, and recent studies have found that some genetic polymorphisms involved in immune or inflammatory processes may have an impact on patient outcomes, including weight loss or survival, using the modulation of these pathways.[4]

Macrophage migration inhibitory factor (MIF) belongs to the transforming growth factor-β (TGF-β) superfamily, which is considered a pleiotropic cytokine that is a key regulator of innate immunity. MIF serves as an upstream regulator of several other inflammatory cytokines.[5] The MIF gene, found on chromosome 22q11.2, belongs to the transforming growth factor-b (TGF-b) superfamily. It is synthesized by T-lymphocytes.[6] MIF is involved in inducing tumor growth, regulating immune responses, and facilitating tumor-associated angiogenesis.[7] It bears a single nucleotide polymorphism (SNP; G to C transition) located in the 5'-flanking region at position -173,which affects MIF gene expression.[8] Recent studies show that MIF variants play crucial roles in cancer susceptibility, including acute myeloid leukemia, colorectal cancer, bladder cancer, cervical cancer, acute lymphoblastic leukemia, gastric cancer, and prostate cancer.[9] However, to our knowledge, there have been no studies focusing on the MIF variants and the BC, gastrointestinal cancer risk in the Turkish population. In this study, we carried out this case-control research to evaluate the possible link between a common MIF rs755622 variant and BC, gastrointestinal cancer susceptibility in a Turkish population.

Materials and Methods

This study included a total 153 subjects,which consisted of 33 BC patients and 53 gastrointestinal cancer (consisting of gastric and colorectal cancers) patients and 67 healthy controls. All BC and gastrointestinal cancer tumors were histopathologically confirmed and the blood samples were recruited from the Department of Medical Oncology of of Dr. Sadi Konuk Training and Research Hospital (Istanbul, Turkey). The healthy control group was similar concerning age and sex distribution; subjects in this group did not have any evidence of any malignancy. Informed written consent was obtained from all participants. The study protocol was approved by the Local Ethics Committees in accordance with the ethical standard for human experimentation established by the Declaration of Helsinki.

Genotyping

Genomic DNA was extracted from peripheral blood leucocytes by the standard salting-out method.[10] PCR was performed using a forward (5'-ACTAAGAAAGACCCGAGGC-3') and reverse (5'-GGGGCACGTTGGTGTTTAC-3') primers. For MIF (-173), a 330 bp fragment was amplified, which was then digested with AluI restriction enzyme (Fermentas), overnight at 37ºC. The products were then separated on 3% agarose gel. The 330 bp PCR products had a consistent restriction site resulting in 62 and 268 bp fragments. The GG genotype did not have a second cutting site for AluI. The CC genotype had a second cutting site, resulting in three fragments of size 205, 62 and 63 bp. As an internal quality control, to avoid sample or reading errors, the experiment was duplicated in 20% of the samples.[11]

Statistical Analysis

All statistical analyses were performed using the Statistical Package for the Social Sciencefor Windows (version 18.0; SPSS Inc, Chicago, IL, U.S.A.).The genotype distribution and allele frequency of the MIF gene rs755622 variant in control and patient groups were compared using the chi-square and Fisher's exact tests. Odds ratios (ORs) and 95% confidence intervals (95% CIs) were obtained using logistic regressions to investigate associations between genotype, allele distribution of MIF rs755622 variant and susceptibility of BC/Gastrointestinal cancer. The Hardy-Weinberg equilibrium (HWE) was calculated using the de Finetti program (Online HWE and Association Testing-InstitutfürHumangenetik, Munich, Germany). P value≤0.05 was accepted as statistically significant.

Results

A case-control study was performed with a total of 153 subjects, inducting 86 cancer patients (33 BC, 53 gastrointestinal cancer) and 67 healthy controls. Clinical and demographical characteristics of BC patients (gender, age, weight, length, family history, smoking, menapause status, and molecular subtypes) are shown in Table 1.

The genotypic and allelic frequencies of MIF rs755622 are shown in Table 2. The genotype distribution of MIF rs755622 between BC patients and the control group had a significant difference. MIF rs755622 GG genotype was higher in BC patients than controls, while GC genotype was higher in control group compared to BC patients (p=0.016, and p=0.030, respectively). MIF rs755622 G allele was more prevalent in BC patients compared to the control group (p=0.017). The genotype and allele frequencies of MIF rs755622 showed no statistically significant difference between gastrointestinal cancer patients and controls (p>0.05). The genotypic frequencies for MIF rs755622 among patients and controls were in HWE (p=0.71, andp=0.52, respectively).

We investigated the association between BC molecular subtypes and MIF rs755622 genotype distribution. The patients with BC had Luminal A, Luminal B and HER2(+) molecular subtypes. There was no statistically significant association between BC molecular subtypes and genotype distribution (Table 3).

Table 1 Baseline clinical and demographics features of the BC patients

Characteristics	Study group (n=33)
Gender, female, n (%)	33 (100)
Age, mean years	60.33
Weight, mean	76.78
Length, mean	160.51
Family history	9 (27.27)
Smoking	9 (27.27)
Menopause	26 (78.78)
Molecular subtypes	
Luminal A	21 (63.63)
Luminal B	5 (15.15)
HER2 (+)	2 (6.07)
Unknown	5 (15.15)

Discussion

Cancer remains to be a major cause of death worldwide. Various components of the innate immunity are induced in cancer pathogenesis to diminish cancer-mediated inflammation.[12] This process also induces adaptive immune responses for targeting cancer through more specific immune mechanisms. Macrophage migration inhibitory factor (MIF) was originally identified as a substance isolated from the supernatants of activated T lymphocyte culture and was manifested as a cytokine that can hinder the random migration of macrophages, being one of the first to be described.[13] Today, MIF is believed to be a multifunctional substance that induces the synthesis of inflammatory cytokines including tumor necrosis factor-α

Table 2 The distribution of genotypes and alleles of MIF rs755622variant in groups

MIF rs755622	BC patients n^a=33 (%)	Gastrointestinal cancer patients n^b=53 (%)	Controls n=67 (%)	p
Genotypes				
GG	29 (87.87)	43 (81.14)	44 (65.67)	0.016[a] 0.059[b]
GC	4 (12.13)	9 (16.98)	21 (31.34)	0.030[a] 0.064[b]
CC	0 (0)	1 (1.88)	2 (2.98)	0.255[a] 0.583[b]
Alleles				
G	62 (93.93)	95 (89.63)	109 (81.34)	0.017[a] 0.074[b]
C	4 (6.07)	11 (10.37)	25 (18.65)	
HWEp	0.71	0.52	0.78	

[a]: BC patients versus controls group; [b]: Gastrointestinal cancer patients versus control group; &Fisher's Exact Test; *OR (95%CI) was adjusted by age and sex; HWE: Hardy-Weinberg equilibrium. The results that are statistically significant are typed in bold

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Table 3 MIF genotype distribution according to BC molecular subtypes

Molecular subtypes	MIF rs755622		Total	p*
	GG n (%)	GC n (%)		
Luminal A				
Yes	20 (95.24)	1 (4.76)	21	0.125
No	9 (75)	3 (25)	12	
Luminal B				
Yes	5 (100)	0 (0)	5	1
No	23 (82.14)	5 (17.86)	28	
HER2 (+)				
Yes	2 (100)	0 (0)	2	1
No	27 (87.10)	4 (12.90)	31	

*: Fisher's exact test

(TNF-α), interleukin-1β (IL-1β), interleukin-6 (IL-6), and interferon (IFN-γ).[13] Studies conducted since the discovery of MIF have supported its role in inflammation as well as in the innate and adaptive immune responses.[14] Therefore, because of its important role in the regulation of the inflammatory and immune responses, MIF has been considered to be the link that connects inflammatory response to cancer.[14] MIF also inhibits the pro-apoptotic and growth inhibitory function of the p53 tumor suppressor in neoplastic cell lines.[15] Breast, colon and lung-derived tumors all have significantly higher levels of MIF mRNA and/or protein compared to their noncancerous, normal tissue counterparts.[16] Notably, MIF overexpression in the serum of cancer patients and in tumor biopsies has been associated with increased tumor progression and metastasis.[17,18]

Polymorphisms with potential functional role have been described in the MIF gene promoter. SNP of the nucleotide position -173 (G to C) has been found to be related to modified levels of the MIF gene transcription in vitro.[19] Previous studies showed that the MIF rs755622 was linked with the risk of peptic ulcer disease, systemic lupus erythematosus, polycystic ovary syndrome and rheumatoid arthritis.[20] In addition, a growing number of evidence implied that MIF rs755622 was involved in the pathogenesis of cancer. Tong et al. found the MIF rs755622 could increase the risk of cancer among Asians but not in Caucasians.[20] Also, in a meta-analysis, findings showed that there is a significant relationship between having any C allele at the -173 site within the MIF promoter and cancer.[21] Ramireddy et al. reported that MIF rs755622 was related to acute myelocytic leukemia susceptibility in Taiwan.[22] Yuan and colleagues reported that the MIF rs755622 could increase the risk of bladder cancer in southeast China.[23] It was reported that the MIF rs755622 might be associated with a higher risk of prostate cancer in Chinese.[24] Lin et al. reported that CG, CC, and CG-CC genotype carriers in MIF rs755622 have a significantly increased risk of BC in Chinese females.[9] In the present study, we found MIF rs755622 GG genotype and G allele were more prevalent in BC patients compared to the control group. However, GC genotype increased in control group than the BC patient group (Table 2). Because our findings are not compatible with other results, this may be due to the difference in lifestyles, as well as ethnic differences.We also evaluated the association of MIF rs755622 variant and molecular subtypes with the development of BC (Table 3). Investigating whether there is an association between MIF rs755622 and molecular subtypes in the BC patient group, we found no significant association ($p>0.05$). Although it was not statistically significant, the MIF rs755622 GG genotype was more prevalent in all molecular subtypes.

It was reported that expression of the MIF was significantly linked with the location of gastric tumor. However, this expression has no statistically significant correlation with variables, including age, gender histological subtypes, distant metastasis, and lymph node involvement, stage and grade of the tumor.[25] He et al. analyzed tissue microarray containing 117 samples of gastric cancer and investigated adjacent non-cancer normal tissues for MIF expression by immunohistochemistry.[26] They found that MIF expression in gastric cancer tissues were higher than that in adjacent non-cancer normal tissues ($p<0.001$), and high level of

MIF was associated with poor tumor differentiation, advanced tumor stage, lymph node metastasis, and poor patient survival ($p<0.05$ for all). There are not many studies examining the relationship between MIF rs755622 variant and gastrointestinal cancer.Li et al. found that subjects with MIF rs755622 GC genotype and C allele have an increased risk of severe chronic atrophic gastritis.[27] Also, they observed that the MIF rs755622 CC genotype and C allele increased intestinal metaplasia. It was found that MIF rs755622 was associated with colorectal cancer susceptibility in Taiwan. [28] In this study, there was no significant difference between genotype-allele frequencies of MIF rs755622 and gastrointestinal cancer risk in our population.

There are several limitations in our case-control analysis. The first limitationis that the study group is scarce in number and recruited from the same region. Secondly, other variations of the MIF gene have not been studied. Finally, MIF expression level was not evaluated.

Conclusion

Cancer is a multifactorial disease. Breast cancer (BC) and gastrointestinal cancer in the population probably result from complex interactions between many genetic and environmental factors over time. However, every finding obtained about this issue will help us to understand the molecular structure of the disease. In conclusion, this study suggested the MIF rs755622 may play a role in the etiology of BC in a Turkish population. Further studies with larger populations are needed to confirm our findings.

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