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Research Article

Identification of Different Allelic Forms of MTNR1A Gene and its Association with Gastric Adenocarcinoma

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Abstract

Objectives: It has been shown that MTs receptors are associated with cancer development in different tissues and single nucleotide polymorphisms (rs2119882 in melatonin receptor 1, (MTNR1A) gene) are an active area of investigation. The aim of the present study was to determine whether or not the mentioned polymorphisms are associated with a risk of gastric cancer in an Iranian population.

Methods: After DNA extraction, genotyping of MTNR1A gene in 40 patients and 40 controls was done by RFL-P PCR technique. Then, Logistic regression model was carried out for statistical analysis using SAS 9.1 software.

Results: We found that homozygous CC genotype in rs2119882 in MTNR1A gene was highly associated with a decreased risk compered to TT genotype by 5-fold. The genotypes and allelic frequencies distributions for the rs2119882 SNPs are significantly different between case and control groups.

Conclusion: TT genotype of rs2119882 SNP of MTNR1A gene was associated with susceptibility of gastric cancer. Moreover, this polymorphism may have effect on gene expression. Taken together, these findings suggest an eminent role for MT1 receptor in gastric cancer.

Keywords: *Melatonin Receptor 1 A; Polymorphism; rs2119882; Gastric Carcinoma*

Introduction

Gastrointestinal cancer is one of the diseases with high morbidity and mortality and its incidence is increasing, worldwide especially in developing countries [1]. Based on previous reports, Gastric cancer lead to 10% of total cancer deaths worldwide [1]. Finding an effective drug therapy for the treatment of gastric cancer is an important interest of research. Melatonin is a multifunctional molecule that mainly is secreted in the pineal gland. Researches has reported melatonin could be synthesized in other organs such as eyes, skin, gastrointestinal tract, bone marrow and lymphocytes [2]. Intracellular regulators, Metallothioneins, play a notable role in several crucial processes in carcinogenesis such as proliferation, metastasis, differentiation, angiogenesis and apoptosis [3,4].

To determine the role of melatonin in the treatment of different cancer types, several experimental studies have been conducted, and findings have shown that melatonin inhibit the growth of gastric [5,6], colon [7,8], pancreatic [9-11], liver [12-15], breast [16-19], prostate [20-22], oral [23,24] cancers. MT proteins are encoded by a family of genes in humans [25]. So far, two distinct melatonin receptors

have been characterized: MT1 (known as MTNR1A) and MT2 (known as MTNR1B) [26,27], which are found in different part of human body [28].

Previous studies have indicated an association between altered levels of MTs mRNA in various tumors including lung, kidney, ovarian, tongue squamous cell, laryngeal, prostate, cervical cancers, hepatocellular, gastric, colorectal, and thyroid cancers [25,29-34].

Recently, individual genetic variation was identified in MTs, and its polymorphic form lead to altering signaling pathways during tumor development and growth, cell proliferation, apoptosis [35-37]. A number of studies have demonstrated a significant association between MT1/2 gene polymorphisms (SNPs) and various type of tumors such as oral cancer [38], squamous cell laryngeal cancer [39], prostate cancer [40,41] and etc.

Nowadays, identification of different allelic forms in MT genes and their association with various cancers especially gastric cancer has been an increasing area of interest in experimental studies.

Aim of the Study

The aim of this study was to evaluate an association between the risk of gastric cancer in an Iranian population-based case-control study and the presence of mentioned SNP: at loci C/T (rs2119882) in MT1 gene. (MTNR1A).

Material and Methods

Patients and samples

The study involved 40 patients diagnosed with gastric cancer in Tooba expert clinic (Sari, Iran) from 2011 to end of 2015. All the malignant cases were classified based on the WHO classification of gastric tumor. Additionally, 40 healthy individuals were considered as normal control group. For both patient and control group, 1.5 ml whole blood sample was taken from each individual and stored at -20°C. Written informed consent was signed by each subject and the study design was approved by the Ethical Committee of Medical Science University, Mazandaran, Iran number 909. Also, the sheets including patients informations and informed consents have been obtained from participants.

DNA extraction

DNA from blood samples was extracted with DynaBio DNA extraction Kit (Cat: KI0015), following the manufacturer's instructions. The quality and quantity of extracted DNA was measured by spectrophotometer. Then, DNA samples were stored at -20°C.

PCR-restriction fragment length polymorphism analysis of MT1 polymorphism

Genotyping of the rs2119882 SNP was determined by PBR technique.

SNP acc.no	Primer Sequences	Enzyme	Gene	TM (°C)	Alleles (bp)	Ref.
rs2119882	5-CCGTTCATTGTGTTTCCT-3 5-AGACAGTCCTTGGTTTTTTC-3	*AvaII*	MT1	56.5	C: 154, 61 T: 206	[42]

Table 1: *Primer sequences for amplify the MT1 gene.*

Primers were designed using Oligo 7 software according to evaluate of rs2119882 SNP for MT1 gene (Table 1). In each 25 µl reaction, 2 µl genomic DNA (100 ng/µl) was amplified by 12.5 µl of Master Mix (EmeraldAmp® MAX HS PCR Master Mix cat.no: RR330A) and 0.8 µl of each primer. The PCR conditions were set as follows: 94°C for 5 minutes, 36 cycles of 94°C for 30s, 56.5 or 61.5°C for 30s and 72°C for 30s and a final extension step of 72°C for 10 minutes then the quality of PCR product from each sample was evaluated by 2% agarose

gel electrophoresis. For genotyping, 10 µl PCR product was digested by restriction Enzyme (*AvaII*) following manufactures instruction. After electrophoresis on 2% agarose gel, photographs were taken by GelDoc instrument (BioDoc-It with Fluor Cam 220 serial Number: 97-0183-01).

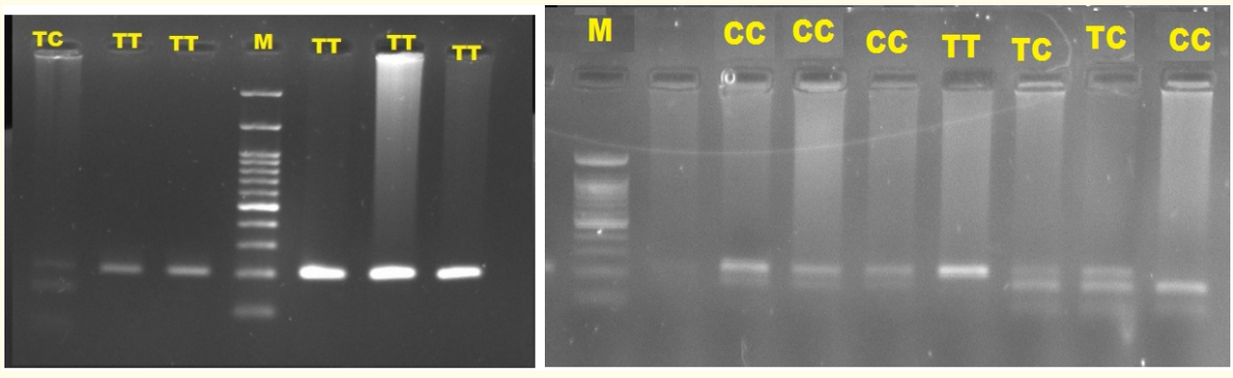
Statistical analysis

The genotype and allele frequency of SNP were analysed using the PopGene 32 software for Hardy Weinberg equilibrium (HWE). To evaluate the distribution of the mentioned polymorphisms in case and control groups, Logistic regression model was carried out. All statistical tests were considered significant with a level of $p < 0.05$ and were carried out with SAS 9.1 software.

Results

Amplification of fragments and genotyping

Polymerase chain reactions were carried out using specific primers for amplification of fragments in MT1 gene. All extracted DNAs from both case and control samples have yielded a specific single band PCR product without any non-specific band. For genotyping and screening of different alleles of MTI gene, the PCR-RFLP procedure developed using *AvaII* restriction enzyme. Then, products were analyzed by electrophoresis on a 2% agarose gel (Figure 1).



A *B*

Figure 1A and 1B: The results of MT1 genotyping in several samples by PBR technique. Undigested 206 bp products show TT genotype, and totally digested 154 and 61 bp products represent CC genotype; TC heterozygous genotype shows both the undigested 206 bp band and digested 154 and 61 bp bands.

The rs2119882 SNP genotype distribution

The results of genotyping in MT1 gene in healthy group showed three genotypes including TT, TC and CC with frequencies 10, 80 and 10 percent, respectively, while among the patients, the frequencies of TT, TC and CC genotypes were 50, 40 and 10, respectively (Table 2). To determine whether the subjects met the Hardy-Weinberg equilibrium, Chi-square test was used. We confirmed that only case group was compatible with the HWE. There is a significant difference between genotypes distribution in two groups ($P < 0.05$).

Genotype	Patient	Control	OR	P-Value
TT	22 (55%)	12 (30%)	-	
TC	11 (27.5%)	21 (52.5%)	0.2857 (0.103 - 0.787)	0.0154
CC	7 (17.5%)	7 (17.5%)	0.5455 (0.154 - 1.926)	0.3465
TC+CC	18 (45%)	28 (70%)	0.4835 (0.224 - 1.04)	0.0631
TT+CC	29 (72.5%)	19 (47.5%)	-	
TC	11 (27.5%)	21 (52.5%)	0.465 (0.211 - 1.024)	0.057

Table 2: *The MT1 (rs2119882 SNP) genotype distribution in patients and controls.*

The probability for HWE of case and control group is 0.017 and 0.732, respectively.

As shown in table 1, in the logistic regression model, genotype TC was associated with a decreased risk for gastric cancer by 0.28-fold compared with the homozygote TT, and genotype TT was associated with an increased risk for gastric cancer compared with the combined genotype TC+CC and TC. Thus, this polymorphism may be an appropriate marker for gastric cancer.

Chi-square test was used to determine whether the subjects met the Hardy-Weinberg equilibrium. We confirmed that only control group was compatible with the HWE, for in control and case groups the value was 0.732 and 0.017 respectively. Moreover, in this region, we observed a polymorphism site which could be useful to understand deeply the mechanism of this gene in gastric cancer.

Discussion

Foregoing studies confirmed that there are high concentrations of melatonin in the serum and lower melatonin concentrations in the ovarian follicles of patients with polycystic ovary syndrome [43]. Additionally, our previous studies have been interestingly shown the high expression for MT1 and MT2 receptors in cancer and marginal cancer groups comparing with normal group [1] which these results for MT2 receptors expression in gastric adenocarcinoma tissues are consistent and in parallel with breast and colon cancer studies and high expression of this receptors in the marginal tissues indicate refractory mechanism which shows the defending role of melatonin in the GI system [1]. melatonin receptors such as melatonin receptor 1A (MTNR1A) and MTNR1B mediate the melatonin function [44]. Both MTNR1A and MTNR1B is mainly expressed in a-cells and b-cells, respectively [45], which is up-regulated in patients with gastric cancer, suggesting that these receptors may have a key role in gastric cancer. Based on our knowledge, in human, there have been few reports about polymorphisms of MTR1A and gene. In the present study, rs2119882, the MTNR1A promoter polymorphism was evaluated among Iranian population with gastric carcinoma.

rs2119882 SNP of MTNR1A

According to ensemble database, one splicing variant and mRNA transcript of MTNR1A gene has been reported in the human (www.ensembl.org). Due to rs2119882 is located on CpG island of the promoter region of MTNR1A gene, Thus, it could be having an eminent effect on activity of the MTNR1A gene promoter and also its expression profile. In addition, previous studies reported that the T allele of the rs2119882 could bind with POU2F1 transcription factor, and the C allele could also bind with C/EBPD and SP1 [46]. Thus, the different allelic forms in this site could be effective in regulation of MTR1A gene transcription based on the above-mentioned transcription factors. Moreover, the exact mechanism of effect of these transcription factors on MTR1A and its association with gastric cancer needs further investigations.

Our results have shown that the genotypes and allelic frequencies distributions for the rs2119882 SNPs are significantly different between case and control groups. Moreover, TT genotype frequency was higher among patients compared to control group, suggesting that this polymorphism is associated with an increased risk of gastric cancer. Park., *et al*. (2011) have shown a significant association between the rs2119882 polymorphism and schizophrenia in the Korean population. In the present study, we found a significant association between rs2119882 SNP and gastric cancer in Iranian population. Moreover, these results provide evidence that the MTNR1A gene is a major gene and may have an eminent role in the gastric cancer development.

Conclusion

Based on our knowledge, this is one of the first studies, which evaluated rs2119882 SNP of MTNR1A genes among Iranian population with gastric cancer which shown rs2119882 SNP of MTNR1A gene had a significant association with gastric cancer. So, this finding could suggest an eminent role for MT1 receptor in gastric cancer. Moreover, some complementary studies with more papulations are necessary to evaluate better these correlations.

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