The SNP rs3746444 within mir-499a is associated with breast cancer risk in Iranian population

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Abstract

Objective: Our study aimed to evaluate the possible association between hsa-mir-499a (rs3746444 A>G) polymorphism and susceptibility to breast cancer in an Isfahanian population.

Materials & methods: In this case–control study we enrolled 91 healthy subjects and patients with breast cancer. Allele-specific primer PCR was applied for genotyping the SNP.

Results: Our study showed that the hsa-mir-499a rs3746444 G allele increased the risk of breast cancer regarding to allele frequency differences (OR: 1.922; 95% CI: 1.064–3.470; p = 0.02952) and Armitage's trend test (OR: 1.722; p = 0.04732) in comparison with the A allele. In addition, an in silico attempt to find functional consequences of A>G substitution suggested that the G allele may decrease hsa-mir-499a stability based on calculated free energy differences between A and G alleles.

Conclusion: Our findings illustrated that the mir-499a rs3746444 G polymorphism is associated with higher risk of developing breast cancer in Isfahanian population.

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Keywords: Breast cancer; Bioinformatics; Functional SNP; mir-499a

1. Introduction

MicroRNAs (miRNAs) are small non-coding single stranded RNAs (21–25 nucleotides) which negatively regulate gene expression by binding to imperfect complementary sites in the 3′ untranslated regions (3′ UTRs) of their mRNA targets [1]. Bioinformatics studies point out that a single miRNA can bind to numerous mRNA targets and therefore small alteration in expression of a specific miRNA may have pathological and physiological outcome [2]. The connection between miRNAs and cancer was firstly recognized in 2006 [3] among chronic lymphocytic leukemia patients. MiRNAs has a key role in cancer biology due to their function in the regulation of cellular processes, including growth, differentiation and apoptosis [4]. Point mutations in miRNA and mRNA sequences, loss or mutation in the promoter regions for specific miRNA clusters, and epigenetic changes may disrupt miRNA activity in cancer cells [5]. In addition, single nucleotide polymorphisms (SNPs) at the miRNA gene region (miR-SNPs) may alter miRNA expression and/or maturation [6–8]. In spite of existence of various reports for correlation between the SNPs and physiological events [9], few association studies have shown the miRNA related SNPs in complex disease such as cancer.
Breast cancer (BC) is the second most commonly diagnosed malignancy worldwide and the most common kind of cancer among women in developed countries [10]. Recently, we identified genetic variants in the target site of mRNA and precursor miRNA sequence as possible biomarkers in BC [11,12]. In this study, we have investigated the possible association between hsa-mir-499a rs3746444 polymorphism (n.73A>G) and BC risks.

2. Materials and methods

2.1. Bioinformatics analysis

MiRNASNP V1.0 bioinformatics online tool (http://bioguo.org/miRNASNP/index.php) [13] was used to predict the effect of the rs3746444 polymorphism (n.73A>G) within hsa-mir-499a stem-loop. Predicted free energy hairpin structure of mir-499a stem-loop with the A and G allele was assumed to address SNPs impact on the mature miRNA biogenesis procedure.

2.2. Study subjects

91 blood samples from women with BC (43 samples) and healthy controls (48 samples) from August 2013 and August 2014 were collected from the Sayed-ol-Shohada Hospital, Isfahan, Iran for this study. BC samples were definite after clinical, mammography, and serological examination. In this study, control samples with any history of cancer were excluded. This study protocol was approved by Payame Noor University, Yazd, Iran, and written informed consents were acquired from all participants.

2.3. SNP genotyping

DNA was isolated from whole blood samples by using PrimePrep Genomic DNA Isolation kit (GeNetBio, Chongnam, South Korea), according to the manufacturer's protocol, and the DNA purity was measured by spectrophotometer (NanoDrop 1000; Thermo Fisher Scientific Inc., Wilmington, DE, USA). Allele-specific primer polymerase chain reaction (ASP-PCR) assay was applied to genotype SNP [14]. SNP genotyping was performed by using A allele specific forward: 5’AGCACAGACTTGCTGTGTT3’, G allele specific forward: 5’AGCACAGACTTGCTGTGTC3’, and common reverse: 5’GGTGAAAGAGAAGCGTAAGA3’ primers. Standard cycling was performed in a thermocycler (ASTEC PC-818; ASTEC, Fukuoka, Japan) under the following conditions: Initial denaturation at 96 °C for 2 min followed by 35 cycles of 94 °C for 30 s, 52.5 °C for 30 s, and 72 °C for 30 s, and finally 72 °C for 7 min. PCR reactions with A allele specific forward and G allele specific forward primers were performed separately (two reactions are needed for each sample). The allele specific PCR product was electrophoresed by 1.5% agarose gel electrophoresis in 1X Tris-Borate-EDTA buffer at 100 V and stained with RedSafe Nucleic Acid Staining solution (Boca Scientific, Inc., Boca Raton, FL, USA) for visualization. The amplicon sizes for hsa-mir-499a rs3746444 polymorphism (A>G) were 214 bp for both alleles (Fig. 1).

2.4. Statistical tests

Statistical analysis was assessed by comparing case and control samples using the DefiniNetti program (https://ihg.gsfd.de/cgi-bin/hw/hwa1.pl) to evaluate the association between the SNP and BC. Odds ratios (ORs) with 95% confidence intervals (CIs), and the Armitage's trend test were estimated using Pearson test. In addition, Logistic regression models were used to determine if odds ratios (OR) are associated with 95% confidence intervals (95% CI). P < 0.05 was considered as statistically significant criteria. Moreover, statistical power calculation was performed by OSSE online tool (http://osse.bii.a-star.edu.sg/index.php).

3. Results

3.1. Bioinformatics analysis

In silico predictions suggested that rs3746444 G allele may decrease hsa-mir-499a stability based on calculated free energy difference between A and G alleles. Free energy for A and G allele was −62.32 and −61.92 kcal/mol, respectively.

3.2. Statistical tests

The frequency distribution of hsa-miR-499a rs3746444 polymorphism (A>G) alleles in BC patients and healthy subjects are shown in Table 1. A significant difference was observed between case and control cohorts. The G allele increased the risk of BC regarding to allele frequency difference (OR: 1.922; 95% CI: 1.064–3.470; p = 0.02952) and Armitage's trend test (OR: 1.722; p = 0.04732) in comparison with the A allele (Table 1). In addition, the G allele by dominant model was associated with BC (A/G + G/G vs A/A; OR: 2.567; 95% CI: 1.034–6.368; p = 0.03945).

![Fig. 1. Representative PCR amplicons of allele-specific primer PCR resolved by 1.5% agarose gel electrophoresis to detect the pre-miRNA-499 rs3746444 A>G polymorphism.](image-url)
4. Discussion

In recent years, it has been revealed that miRNAs are critical members of complex regulatory systems. SNPs or mutations occurring in the miRNA gene region may disturb the properties of miRNAs and result in defect in protein translation of target mRNAs [15]. The hsa-mir-499a rs3746444 polymorphism contains A>G nucleotide substitution, that results in an alteration from an A:U pair to a G:U mismatch in the stem-loop structure of the mir-499a precursor (ΔAG = 0.4 kcal/mol; G vs A allele). In the current study, it has been found the hsa-mir-499a rs3746444 G allele and G allele carriers (individuals with G/G and A/G genotypes) are associated with the BC incidence in Isfahanian population. Growing evidence has shown that rs3746444 SNP has different effects on different kinds of cancers and population. For instance, Wang et al. reviewed eleven investigations of Asian and four studies of Caucasian ethnics, and found that the mir-499a rs3746444 G allele has different effects in various populations [16]. In summary, Wang et al. utilized meta-analysis of 7188 cases and 8548 controls and found a significant correlation between rs3746444 polymorphism and increased BC risk in the subgroup of Asian population [16]. Similarly, the present investigation and studies performed by Hu et al. and Omrani et al. suggested that the presence of G allele significantly increase BC risk [17,18].

Generally, in this case-control study of BC in Isfahan, Iran, it has been demonstrated that the genetic polymorphism in the hsa-mir-499a (rs3746444 A>G) gene is associated with increased risk of BC incidence. One of the limitations of this study is comparatively small sample size (statistical power: 33%). Large group and diverse ethnicity studies are needed for more proofs.

Conflict of interests

There is no conflict of interest to declare.

References


