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Research article

MiR-608 rs4919510 C>G polymorphism increased the risk of bladder cancer in an Iranian population

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Abstract: MicroRNAs (miRNAs) participate in diverse biological pathways and may act as oncogenes or tumor suppressors. The single nucleotide polymorphisms (SNPs) in miRNAs potentially can alter miRNA-binding sites on target genes as well as affecting miRNAs expression. The present study aimed to evaluate the impact of miR-608 rs4919510 C>G variant on bladder cancer risk. This case-control study conducted on 233 bladder cancer patients and 252 healthy subjects. Genotyping of miR-608 rs4919510 was done using polymerase chain reaction-restriction fragment length polymorphism (PCR-RFLP) method. Our findings showed that CG as well as CG+GG genotypes significantly increased the risk of bladder cancer (OR = 1.94, 95% CI = 1.28–2.94, p = 0.002, and OR = 1.90, 95% CI = 1.26–2.86, p = 0.002, respectively) compared to CC genotype. The G allele significantly increased the risk of bladder cancer compared to C allele (OR = 1.69, 95% CI = 1.17–2.45, p = 0.005). Our findings proposed that miR-608 polymorphism might be associated with increased risk of bladder cancer in a sample of Iranian population. Further large-scale studies with different ethnicities are needed to verify our findings.

Keywords: miR-608; bladder cancer; polymorphism

1. Introduction

Bladder cancer (BC) is one of the most frequently-diagnosed malignancies worldwide, with an estimated 430,000 new cases diagnosed in 2012 [1,2]. In the United States, there are about 69,000 newly diagnosed and approximately 15,000 deaths occurred of BC in 2014 [3]. It has been proposed that in addition to the environmental factors such as smoking, obesity, and physical inactivity [4-6], genetic factors can also play an important role in the BC development [7-10].

MicroRNAs (miRNAs) are a highly conserved single-stranded non-coding RNAs of ~22 nucleotides that regulate posttranscriptional gene expression by binding to the 3`-UTR of their target messenger RNAs (mRNAs) and inducing either translational repression or mRNA degradation [11,12]. Single nucleotide polymorphisms (SNPs) in miRNA genes, including primary miRNAs (pri-miRNAs), precursor miRNAs (pre-miRNAs), and mature miRNAs can affect biogenesis, processing, and target binding affinity and specificity [13,14].

The mir-608 gene is located on chromosome 10 (10q24.31) within an intron of the SEMA4G gene. It has been suggested that rs4919510 variant of mir-608 affecting the mature miR-608 sequence [15]. The predicted targets of miR-608 comprise insulin receptor (INSR), interleukin-1 alpha (IL1A), growth hormone receptor (GHR), and TP53 [15].

Several studies inspected the impact of miR-608 rs4919510 C>G polymorphism on the risk as well as outcome in different types of cancer, but the findings were inconsistent [16-27]. To the best of our knowledge, there is no report regarding the impact of miR-608 rs4919510 polymorphism on BC risk. Therefore, this case-control study was designed to assess the possible association between miR-608 rs4919510 polymorphism and susceptibility to BC in an Iranian population.

2. Materials and Methods

2.1. Patients

This case-control study was done on 233 histologically confirmed bladder cancer patients and 252 sex and age matched healthy subjects with no history of cancer of any type (as the control group). All the subjects were enrolled from the Shahid Labbafinejad Medical Center at the Shahid Beheshti University of Medical Sciences, Tehran, Iran. Ethical approvals for recruitment were taken from local Ethics Committee of Zahedan University of Medical Sciences, and informed consent was obtained from all patients and healthy individuals.

2.2. Genotyping

Genotyping of miR-608 rs4919510 C>G was done by PCR-RFLP methods as described previously [28]. The forward and reverse primers were 5′-TCTGGCTAGGTAATGGCTCC-3′ and 5′-GCATCTGTGGCCTTCCATGA-3′, respectively. Into 0.20 mL reaction solution, 1 μL genomic DNA (~100 ng/mL), 1 μL (10 μM) forward and reverse primers, 10 μL 2X Prime Taq Premix (Genet Bio, Korea), and 7 μL ddH2O were added. The PCR conditions were as follows: 5 min preheating at

95 °C, 30 cycles of 95 °C for 30 s, 65 °C for 30 s, and 72 °C for 30 s followed by a final extension step for 10 min at 72 °C. Then, 10 μL of PCR product was digested by PvuII restriction enzyme (Fermentas) according to the manufacturer's procedure. The G allele digested and produced two fragments (242-bp and 117-bp), while the C allele undigested (359-bp fragment) (Figure 1).

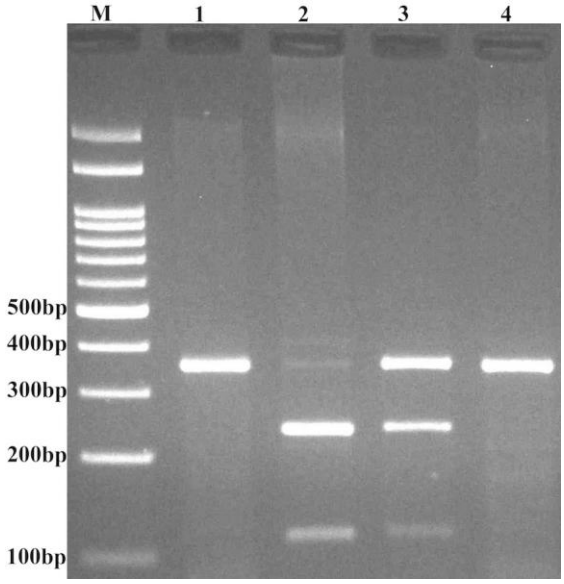

Figure 1. Photograph of miR608 rs4919510 C>G variant using PCR-RFLP method. The G allele digested by PvuII restriction enzyme and produced 242-bp and 117-bp pattern, while the C allele was undigested (359-bp fragment). M: DNA marker; Lanes 1 and 4: CC; Lane 2: GG; Lane 3: CG.

2.3. Statistical analysis

Statistical analysis was done using statistical package SPSS 22 software. The categorical and continuous data were analyzed using $\chi 2$ and t-test, respectively. The association between genotypes and BC were assessed by computing the odds ratio (OR) and 95% confidence intervals (95% CI) from logistic regression analyses. A p-value of <0.05 was considered to be statistically significant.

3. Results

The study group consisted of 233 bladder cancer patients (193 male, 40 female; age 63.4 ± 12.1 years) and 252 hospital-based healthy subjects (210 male, 42 female; age 62.3 ± 10.7 years). No significant difference was observed between the groups regarding sex and age ($p = 0.904$ and $p = 0.316$, respectively).

The genotype and allele frequencies of miR-608 rs4919510 C>G polymorphism in bladder cancer patients and healthy subjects are shown in Table 1. Our findings showed that rs4919510 CG genotype as well as CG + GG genotype significantly increased the risk of bladder cancer (OR = 1.94, 95% CI = 1.28–2.94, $p = 0.002$ and OR = 1.90, 95% CI = 1.26–2.86, $p = 0.002$, respectively) compared to CC genotype. The G allele was significantly associated with increased the risk of bladder cancer (OR = 1.69, 95% CI = 1.17–2.45, $p = 0,005$) compared to C allele.

Table 1. Association of miR-608 rs4919510 C>G polymorphism and risk of bladder cancer.

miR608 rs4919510 C>G	Case n (%)	Control n (%)	OR (95%CI)	*p*
Genotype				
CC	156 (67.0)	200 (79.4)	1.00	-
CG	74 (31.7)	49 (19.4)	1.94 (1.28–2.94)	0.002
GG	3 (1.3)	3 (1.2)	1.28 (0.26–6.44)	0.986
CG+GG	77 (33.0)	52 (20.6)	1.90 (1.26–2.86)	0.002
Allele				-
C	386 (82.8)	449 (89.1)	1.00	-
G	80 (17.2)	55 (10.9)	1.69 (1.17–2.45)	0.005

Hardy-Weinberg equilibrium (HWE) was assessed by $\chi 2$ test. The genotype of miR-608 rs4919510 polymorphism in both controls and cases were in HWE ($\chi 2 = 4.13$, $p = 0.999$ and $\chi 2 = 3.17$, $p = 0.074$, respectively).

No significant association between miR-608 rs4919510 genotypes and clinicopathological characteristics (age, sex, histologic type, stage) of BC patients was found (data not showed).

4. Conclusion

For the first time, we examined the impact of miR-608 rs4919510 variant on the risk of BC in a sample of Iranian population. The findings indicated that CG, CG + GG genotypes as well as G allele significantly increased the risk of BC in our population. We found no significant association between miR-608 rs4919510 genotypes and clinicopathological characteristics (age, sex, histologic type, stage of BC patients (data not showed).

Several studies inspected the association between mir-608 rs4919510 variant and development of various cancer, but the results are inconsistent. Qiu et al. [29] have found that miR-608 rs4919510 polymorphism is associated with an increased risk of nasopharyngeal carcinoma in southern China (GC + GG vs CC genotype, OR = 1.36, 95% CI = 1.10–1.70) and eastern China (GC + GG vs CC, OR = 1.37, 95% CI = 1.08–1.74). After they merged the two populations the ORs and 95%CI were 1.38 and 1.18 to 1.62, respectively.

No significant association were found between rs4919510 variant and risk of thyroid cancer [16], colorectal cancer [18,20], hepatocellular carcinoma [21], esophageal squamous cell carcinoma (ESCC) [22], breast cancer [23,30,31], lung cancer [32,33], and gastric cancer [34]. We have shown previously that mir-608 rs4919510 variant significantly decreased the risk of breast cancer [28]. Jiao et al. [17] have found no significant association between miR-608 rs4919510 polymorphism and breast cancer survival in Chinese population. Huang et al. [30] have observed that miR-608 rs4919510 variant is associated with increased risk of HER2-positive breast cancer. It has been proposed that mir-608 rs4919510 polymorphism may be a potential marker of hepatocellular carcinoma (HCC) [24,35], and colorectal cancer (CRC) [20,36,37]. A meta-analysis performed by Liu et al. [38] revealed that miR-608 rs4919510 variant significantly decreased cancer risk in recessive model (OR = 0.89, 95% CI = 0.82–0.97, $p = 0.009$, CC vs GG + GC).

Aberrant expression of miR-608 has been reported in cases of esophageal squamous cell carcinoma [27], breast cancer [30], colorectal cancer [36,37], ovarian cancer [39], lung cancer [35,40], and cancer lines of chordoma [41]. Dysregulation expression of miR-608 could affect the risk of cancer by altering the expression of mRNA targets.

It cannot currently clarify the precise functional mechanism through which miR-608 rs4919510 affect bladder cancer risk. Although the variant does not lie within the seed sequence of miR-608, it lies within the mature sequence of miR-608 and is located at the junction between the stem and canonical hairpin loop [20]. As this rigid secondary structure is a required for recognition and processing of pre-miRNA, it is possible that disruption of structure at this critical point might affect recognition or subsequent processing. Since each miRNA has many different targets, deregulation of miRNA expression has been associated with many pathological conditions, including various cancers [15,42,43].

In summary, this study provides the first evidence that miR-608 rs4919510 variant increased the risk of bladder cancer in a sample of Iranian population. However, we found no significant association between this variant and the clinicopathological characteristics of BC patients. Further studies with larger sample sizes with different ethnicities are essential to confirm our finding.

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Conflict of interests

The Authors declare that there is no conflict of interest to disclose.

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