Original Article

Association of rs16917496 polymorphism at the miR-502 binding site in the SET8 3'UTR with the risk of Prostate Cancer and benign prostatic hyperplasia

Mahsa Haj Manochehri[1#], Faezeh Azizi[1#], Mahnoosh Rahimi[1,2], Mir Davood Omrani[1*]

[1] Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran
[2] Department of Bioinformatics and Genomics, Pharmacogenetic Research Center, Simple LIMS, San Diego, CA, USA
# These authors have the same contributions

Received: 15 April, 2017; Accepted: 5 October, 2017

Abstract

Background: MicroRNAs (miRNAs) can bind to the 3'-untranslated regions (UTRs) of messenger RNAs, where they interfere with translation and thereby regulate cell differentiation, apoptosis, and tumorigenesis. Genetic polymorphisms in the 3'-UTRs targeted by miRNAs alter the strength of miRNA binding in a manner that affects the behavior of individual miRNAs. The histone methyltransferase SET8 has been reported to be a regulator of Tumor Protein 53 (TP53) methylation, a tumor suppressor gene, and regulate genomic stability. Furthermore, an association between the TP53 and Prostate Cancer has been reported in several studies. The present study aimed to evaluate whether (rs16917496) polymorphism at the miR-502 binding site in the 3' untranslated region of the histone methyltransferase SET8 is associated with the expression of this gene in Benign Prostatic Hyperplasia (BPH) and prostate cancer (PCa) patients.

Materials and Methods: We examined whether an rs16917496 polymorphism is associated with the risk of PCa and BPH in the Iranian population. This case-control study included 40 patients with pathologically confirmed PCa, 59 patients with BPH, and 45 controls. The rs16917496 polymorphism was determined using a restriction fragment length polymorphism (RFLP).

Results: We found significant association of rs16917496 in benign prostatic hyperplasia (BPH). The most frequent genotype in the control, prostate cancer, and BPH groups were TT, TC, and CC, respectively.

Conclusion: This study demonstrates that the heterozygote genotype of the SET8 polymorphism in the mir-502 gene could be considered a risk factor for the emergence of prostate cancer.

Keywords: Prostate Cancer (PCa), Benign Prostatic Hyperplasia (BPH), Single Nucleotide Polymorphism

***Corresponding Author:** Mir Davood Omrani, Department of Medical Genetics, School of Medicine, Shahid Beheshti University of Medical Sciences, Tehran, Iran. Tel/Fax**:** (+98) 21 22721150; Email: davood_omrani@sbmu.ac.ir

Please cite this article as: Haj Manochehri M, Azizi F, Rahimi M, Omran MD. Association of rs16917496 polymorphism at the miR-502 binding site in the SET8 3'UTR with the risk of Prostate Cancer and benign prostatic hyperplasia. Novel Biomed. 2018;6(2):74-8.

Introduction

Prostate cancer (PCa) is a common solid malignancy and has high mortality rate[1]. Not only does it represent a major factor in male morbidity and mortality, but it also places an economic burden on the population[2]. The significant problem of PCa is the development and acquisition of the castrate resistant prostate cancer (CRPC) phenotype which finally leads to the development of skeletal metastasis (mCRPC), at which point it becomes an incurable disorder[1,3]. Therefore, research studies are underway to understand the

molecular basis of the mCRPC so that novel therapeutic strategies can be designed. To that end, many novel molecules are being trialed and assayed, among which microRNAs (miRNAs) are becoming an attractive area of investigation[4,5]. Another area of concern is Benign Prostatic Hyperplasia (BPH) that is one of the most common mankind disease[6].

MicroRNAs (miRNAs) with ~22 nucleotides RNA length act as posttranscriptional regulators of mRNA expression level by bounding to the "seed region"[7] of 2–8 nucleotides in the 3′ untranslated region (UTR) of mRNAs[8,9]. A perfect complement between the miRNA and its target mRNA sequence leads a reduction in protein levels due to RNA silencing[10]. Additionally, research results have indicated the evidence of the how the role of single nucleotide polymorphisms (SNPs) in the 3′ UTR can alter the targeted genes' expression and consequently affect an individual's cancer risk[6,11,12]. PR-Set7/Set8/KMT5a (SET8), located on the chromosome 12q24.31, is regulated by the miR-502 through the binding site of 3′ UTR of *SET8* mRNA, that encodes the histone H4 lysine 20 monomethyltransferase which is implicated in normal cell cycle progression[13]. Also, human PrSet7 interacts directly with the DNA replication factor PCNA and depicts specific effects at origins of replication; *SET8* depletion results in cells accumulation in S phase with increased DNA damage. Furthermore, the unsuitable SET8 expression also causes S phase defects and increased DNA damage[14]. Recent studies have revealed that the *SET8* function was vital for the p53BP1 recruitment during the DNA double-strands breaking response. Moreover, SET8 could increase the metastasis capacity of a cancer cell by promoting epithelial-mesenchymal transition[15]. It also mediates the monomethylation of p53/TP53 at Lys-382, causing repression of the p53/TP53-target genes. Recent studies have proposed that SET8 may have a link with carcinogenesis and cancer progression[7,16,17].

In addition, Previous studies show that SNP rs16917496-T/C located in the 3′UTR of the SET8 mRNA was associated with the risk of the early onset of breast cancer, and this SNP region was foretold as a potential binding site of *miR-502*[17,18]. This SNP was afterward shown by others to be a susceptibility factor for some cancers, such as epithelial ovarian cancer and cervical cancer[17]. This broad spectrum of association proposes that this SNP is a robust genetic regulatory factor fundamental to cells. Nevertheless, the role of the SET8 3′-UTR SNP in PCa prognosis has remained unknown and has not been reported, which is the main motivation of this survey[17,19]. In this study, we genotyped rs16917496 in Pca and BPH patients in a case-control study to assess its relationships to cancer risk and outcome.

Methods

Patients and controls: The 40-PCa and 59 BPH cases were collected from the Urology Center of Labafinejad Hospital, Tehran, Iran. This study included patients with newly diagnosed and histologically confirmed PCa and BPH. The 44 control samples of genetically unrelated men were collected during the same time. Control samples were selected based on a normal result of DRE (Digital Rectal Examination), and prostate specific antigen (PSA) levels less than four ng/milt. The criteria for selecting participants in the study included a diagnosis by a urologist and having no sign of other genetic or congenital disease or genital malformations. The ages ranged from 50 to 80 for all the samples. Peripheral blood samples from patient and control groups were taken using Venoject tubes containing EDTA (0.5 M). DNA samples were extracted using DNA extraction kits according to the manufacturer's protocol (Qiagen, Valencia, California).

Genotyping of rs16917496 polymorphisms: The *SET8 3'UTR* was analyzed for rs16917496 polymorphisms using a restriction fragment length polymorphism (RFLP) for both case and control groups. The amplification conditions were: 95°C for 2 min, 35 cycles of 95°C for 30 s, 59°C for 30 s, and 72°C for 45 s, and a final extension step at 72°C for 5 min. The primers on the *miR-502* binding site were 5′-GGCCTCACGACGGTGCTAC -3′ and 5′-GTTCCCCAGGAGGATGCTTAC-3′. A 308-bp DNA fragment was produced in this process, and then digested the DNA produced by SWAI (New England BioLabs, Inc.) overnight at 25°C. Allele T produced two bands including 149-bp and 159-bp, TC heterozygote produces three bands (149-bp, 159-bp, and 308-bp) and allele C lacks the SWAI restriction site and only produced a 308-bp band.

Statistical analysis: All SNPs data were evaluated for

the Hardy-Weinberg equilibrium. The unprocessed data were analyzed with SNPStats software, T-test, chi-square and SPSS software (version 20.0). An association between each of the genotypes and prostate cancer risk was assessed by calculating a P-value with 95% confidence intervals. A P-value<0.05 was considered significant.

Bioinformatic miRNA target prediction and analysis: The miRWalk 2.0 database was used to predict the potential target genes for miR-502, which combines the information with a comparison of binding sites resulting from other three existing miRNA-target prediction programs: miRNAMap, Targetscan6.2, miRanda-rel2010.

Results

We found significant associations of rs16917496 [OR 5.8; 95% CI 1.30-26.51; p 0.001 < 0.05] and [OR 5.13; 95% CI 0.92-28.57; p 0.001 < 0.05] in BPH with the risk of PCa and BPH in our population, respectively (Table 1). The phenotype data and frequency distribution of case groups are classified as shown in (Table 1). The results of the rs16917496 genotype analysis revealed the highest frequency of the TT genotype in the control group, and the highest frequency of the CT genotype in the both PCa and BPH samples. Higher frequency of C allele was in the BPH cases (n=59) compared with controls. However, the prostate cancer patients showed the same frequency of C allele as the healthy population (Table 2). We performed a Hardy–Weinberg equilibrium (HWE) test for studied polymorphism (rs16917496) in the three group-studied populations where the genotypic distributions of this polymorphism showed a p-value less than 0.05 considered in the H-W equilibrium. However, the rs16917496 revealed a significant p-value in the PCa and BPH groups patients, indicating a deviation from (HWE; Table 2). To determine the correlation between rs16917496 polymorphism with the incidence of PCa and BPH, genotypic information from the three groups of "control / PCa," " PCa / BPH" and " BPH / control " was used (Table 1). A comparison between the three groups shows that the genotype CT in Iranian population has a strong correlation with the incidence rate of PCa (OR = 6.19, CI = 1.99-19.28) and BPH (OR = 4.52, CI = 1.83-11.18). Both the control/ PCa and control / BPH groups revealed a significant *p* value. On the contrary, the p value PCa / BPH was not significant (Table 1).

Table 1: Correlation analysis of rs16917496 polymorphism with prostate cancer and BPH disease in the Iranian population.

Groups	Genotype	OR (CI 95%)	p value
Control-Prostate Cancer	TT	1.00	
	CT	6.19 (1.99-19.28)	0.0021
	CC	5.88 (1.30-26.51)	
BPH-Prostate Cancer	TT	1.00	
	CT	1.37 (0.43-4.36)	0.12
	CC	5.13 (0.92-28.57)	
Control-BPH	TT	1.00	
	CT	4.52 (1.83-11.18)	0.0018
	CC	1.15 (0.23-5.71)	

Discussion

In the present study, we investigated the association between rs16917496 polymorphisms in the SET8 gene with Prostate cancer disease and BPH in the Iranian population. We found a significant higher frequency T allele in both the control/ prostate cancer and control/ BPH. However, the similar frequency rate of the T allele in our studied patients with PCa might be a result of having a smaller number of samples of PCa compared to BPH and healthy subjects (Table 2). The results of the allele frequency of the rs16917496 polymorphism in our population indicate that the C allele is less common compared to other populations in previous studies[19,20]. We found a strong association between the heterozygous (CT) genotype of the rs16917496 variant and risk of BPH and PCa in our population; however, in the control group, the TT genotype had higher frequency (Table 1). The results of the analysis of the polymorphism rs16917496 genotype and allele frequencies in our groups indicate that despite the fact that the T allele could reduce the P53 gene expression, a high allele frequency in a healthy control population and population of BPH could also be indicated. It seems though the majority of the Iranian male population have an allele predisposed to cancer, may numerous genetic and environmental factors play an important role in the prevalence of PCa. Our results also showed a deviation from the Hardy-Weinberg equilibrium for this polymorphism in our patients. Although the rs16917496 polymorphism in the control group was in the Hardy-Weinberg equilibrium, the results from the patients with PCa and BPH show a deviation from the equilibrium. This might be a result of the role of this polymorphism in a susceptibility factor for cancer and BPH, such as age onset of cancer, tumor size, and blood PSA levels. On the contrary, the results of studies on PCa show inconsistent results. We can conclude that the differing results may be due to the low number of research samples on breast cancer. The problem can be solved by having more samples and examining more closely correlation of the rs16917496 polymorphism with PCa. Of course, different genetic and environmental factors in breast and PCa, as well as people with gender differences, can also be a reason for the differences observed in the results of the two studies. In 2012, a study on the relationship between the rs16917496 polymorphism and hepatocellular carcinoma (HCC) had shown that a C to T in the rs16917496 polymorphism could cause the loss of miR-502 binding site and SET8. The inability to connect the miR-502 gene to SET8 can cause the enhanced expression of SET8 and ultimately reduce the P53 gene expression. The results of this study suggest that the miR-502 gene polymorphism junction in SET8 could affect the outcome of HCC. In fact, genotype CC in SET8 causes a lower expression of RNA and protein production, and that is associated with more survival time in patients. Univariate and multivariate analyses suggest that single nucleotide polymorphisms at the junction of miRNA 502 can be used as a diagnostic factor in patients with HCC[20].

Conclusion

Our findings suggest the rs16917496 polymorphisms in

Table 2: Polymorphism rs16917496 genotype and allele frequencies in control population, patients with prostate cancer and BPH.

Groups	rs16917496 polymorphism					
	Allele frequency		Genotype frequency			p value (H-W)
	C	T	CC	TT	TC	
Control	0.32	0.68	0.11	0.47	0.42	0.75
Prostate cancer	0.52	0.48	0.18	0.12	0.7	0.025
BPH	0.43	0.57	0.05	0.19	0.76	<0.0001

SET8 3'UTR may harbor a susceptibility to PCa and BPH. Our findings may contribute to provide new insight into the molecular genetics of PCa and BPH. In addition, the observed SRD5A2 mutations indicate this gene is one of the most effective causes of PCa that need to be studied in other populations with larger sample size. Further investigations with diverse ethnicities and larger sample sizes are required to verify these results.

Acknowledgment

We would like to thank our patients and their families for their participation. This study was funded by Shahid Beheshti University of Medical Sciences.

References

1. Eidelman E, Twum-Ampofo J, Ansari J, Siddiqui MM. The Metabolic Phenotype of Prostate Cancer. Frontiers in oncology. 2017;7:131.
2. Board PDQCGE. Genetics of Prostate Cancer (PDQ(R)): Health Professional Version. PDQ Cancer Information Summaries. Bethesda (MD): National Cancer Institute (US); 2002.
3. Jadvar H. Multimodal Imaging in Focal Therapy Planning and Assessment in Primary Prostate Cancer. Clinical and translational imaging. 2017;5(3):199-208.
4. Peng X, Guo W, Liu T, et al. Identification of miRs-143 and -145 that is associated with bone metastasis of prostate cancer and involved in the regulation of EMT. PLoS One. 2011;6(5):e20341.
5. Hassan O, Ahmad A, Sethi S, Sarkar FH. Recent updates on the role of microRNAs in prostate cancer. Journal of hematology & oncology. 2012;5:9.
6. Dai X, Fang X, Ma Y, Xianyu J. Benign Prostatic Hyperplasia and the Risk of Prostate Cancer and Bladder Cancer: A Meta-Analysis of Observational Studies. Medicine. 2016;95(18):e3493.
7. Diao L, Su H, Wei G, et al. Prognostic value of microRNA 502 binding site SNP in the 3'-untranslated region of the SET8 gene in patients with non-Hodgkin's lymphoma. Tumori. 2014;100(5):553-8.
8. Bartel DP. MicroRNAs: genomics, biogenesis, mechanism, and function. Cell. 2004;116(2):281-97.
9. Ambros V. The functions of animal microRNAs. Nature. 2004;431(7006):350-5.
10. Chin LJ, Ratner E, Leng S, et al. A SNP in a let-7 microRNA complementary site in the KRAS 3' untranslated region increases non-small cell lung cancer risk. Cancer research. 2008;68(20):8535-40.
11. Saetrom P, Biesinger J, Li SM, et al. A risk variant in an miR-125b binding site in BMPR1B is associated with breast cancer pathogenesis. Cancer research. 2009;69(18):7459-65.
12. Martinez E, Silvy F, Fina F, et al. Rs488087 single nucleotide polymorphism as predictive risk factor for pancreatic cancers. Oncotarget. 2015;6(37):39855-64.
13. Wu S, Wang W, Kong X, et al. Dynamic regulation of the PR-Set7 histone methyltransferase is required for normal cell cycle progression. Genes & development. Nov 15 2010;24(22):2531-2542.
14. Jorgensen S, Elvers I, Trelle MB, et al. The histone methyltransferase SET8 is required for S-phase progression. The Journal of cell biology. Dec 31 2007;179(7):1337-1345.
15. Wang C, Wu J, Zhao Y, Guo Z. miR-502 medaited histone methyltransferase SET8 expression is associated with outcome of esophageal squamous cell carcinoma. Scientific reports. 2016;6:32921.
16. Hou L, Li Q, Yu Y, Li M, Zhang D. SET8 induces epithelialmesenchymal transition and enhances prostate cancer cell metastasis by cooperating with ZEB1. Molecular medicine reports. 2016;13(2):1681-8.
17. Wang C, Guo Z, Wu C, Li Y, Kang S. A polymorphism at the miR-502 binding site in the 3' untranslated region of the SET8 gene is associated with the risk of epithelial ovarian cancer. Cancer genetics. 2012;205(7-8):373-6.
18. Yang S, Guo H, Wei B, et al. Association of miR-502-binding site single nucleotide polymorphism in the 3'-untranslated region of SET8 and TP53 codon 72 polymorphism with non-small cell lung cancer in Chinese population. Acta biochimica et biophysica Sinica. 2014;46(2):149-54.
19. Song F, Zheng H, Liu B. An miR-502-binding site single-nucleotide polymorphism in the 3'-untranslated region of the SET8 gene is associated with early age of breast cancer onset. Clinical cancer research : an official journal of the American Association for Cancer Research. 2009;15(19):6292-6300.
20. Guo Z, Wu C, Wang X, Wang C, Zhang R, Shan B. A polymorphism at the miR-502 binding site in the 3'-untranslated region of the histone methyltransferase SET8 is associated with hepatocellular carcinoma outcome. Int J Cancer. 2012;131(6):1318-22.