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Original Article

Effect of PTEN Gene Mutations and Environmental Risk Factors on the Progression and Prognosis of Bladder Cancer

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Abstract

Background: Bladder cancer is the most frequent genitourinary malignancy in Iran. Environmental and genetic factors are the two factors linked with bladder cancer expansion. The aim of this study was to investigate the role of *PTEN* gene and environmental risk factors on the progression and prognosis of bladder cancer.

Methods: We evaluated 55 tumor specimens and 66 bladder mucosa samples of non-cancerous patients between 2011 and 2013. All samples were analyzed for *PTEN* mutations using PCR and direct DNA sequencing methods. Demographic data collected, were analyzed using SPSS version 19.0 software and a *P value* of < 0.05 was considered statistically significant.

Results: Of the 55 patients examined, tumor stage was T1, T2 (T2a, T2b) in 34 (61.8%) and 21 (38.2%) and tumor grade was high, low in 34 (61.8%) and 21 (38.2%), respectively. No mutations in the *PTEN* gene were found in patients with bladder cancer and control. Among the risk factors studied, only the occupation and history of urinary tract stones, were significantly associated with bladder cancer (*P value*<0.05). However, other risk factors did not show such a relationship.

Conclusion: No mutation was found in *PTEN* gene of patients with bladder cancer. Therefore, mutations in this gene cannot predict the prognosis and progression of urothelial bladder cancer. On the other hand, significant relationship was found between occupation and urinary stones with bladder cancer. This communication reflects the impact of these factors on the risk of bladder cancer.

Keywords: PTEN, Chromosome 10q, Mutation, DNA sequencing, Risk factors, Bladder cancer, Iran

Introduction

Bladder cancer is the most prevalent malignancy of the urinary tract, and the second most frequently diagnosed genitourinary malignancy among people living in the United States (1). About 90% of malignant tumors arising in the bladder are urothelial cell carcinomas (1). Urothelial bladder cancer constitutes two distinct clinical phenotypes. The common tumors are low grade and non-invasive which may relapse locally but development infrequently; other tumors which are muscle invasive often develop rapidly and have a poor prognosis (2).

Environmental and genetic factors are the two factors associated with bladder cancer develop-

ment. Understanding the etiology and identifying the risk factors are essential for the primary prevention of this deadly disease. The relationship between bladder cancer (BC) and smoking, occupational exposure to aromatic hydrocarbons, family history of cancer, chemotherapy and radiotherapy is quite likely (3). These factors result in uncontrolled growth of cell population, decreased cell death, invasion and metastasis, and may influence the patient's prognosis. Recognition of the aggressive features of BC is very essential for suitable management of this disease (4). Although several risk factors for relapse and progression of BC have been identified, their limited value has demonstrated the need for new molecular markers of BC outcomes (5, 6). While several people are exposed to bladder cancer risk factors, BC develops in only a fraction of these individuals; therefore, environmental or dietary factors or genetic backgrounds can be involved in susceptibility to bladder carcinogenesis (3).

Accumulation of multiple genetic events leads to adult sporadic cancers. Many of these genetic events have been recognized in BC while others remain to be identified. The p53 gene on chromosomal arm 17p, the *Rb* gene on chromosomal arm 13q, and the *CDKN2a* gene on chromosomal arm 9p are genetic factors known to contribute to bladder cancer. The recognition of chromosomal deletions suggest that additional suppressor loci are important in bladder carcinogenesis (7,8).

PTEN/MMAC1 is a tumor suppressor gene located on human chromosome 10q23.3 that is found to be inactivated by homozygous deletion or point mutation in endometrial cancer, malignant gliomas and with a lower rate in prostate and breast cancer. Germ-line mutations in *PTEN/MMAC1* have been associated with Cowden disease, an autosomal dominant cancer predisposition syndrome that increased the risk of skin, breast and thyroid tumors and occasional cases of other cancers including bladder cancer. The *PTEN/MMAC1* gene contains 9 exons and encodes a 403-aa protein. *PTEN* gene acts as a phospholipid and phosphoprotein phosphatase. The tumor suppressor activity of *PTEN* is due to the action of its phosphatase and also its ability to negatively regulate phosphatidylinositol 3-kinase pathway. Following the expression of PTEN, cell cycle progression can be slowed down, cell migration is reduced, and cell cycle arrest and apoptosis are induced.(9-12) Loss of *PTEN* activity leads to increased cell proliferation and reduced cell death (11).

PTEN mutations have been observed in glioblastomas (12, 13), carcinomas of the prostate (13) and breast (14), endometrial carcinoma (15) and melanoma (16) in different studies; hence, *PTEN* can probably be a proper target of deletion in these cases. A similar trend has lately been found in bladder cancer cases (17).

The rate of *PTEN* mutation in bladder cancer has not been adequately studied in Asia. We analyzed bladder cancers of 55 Iranian patients to study the role of *PTEN* mutation in tumor progression.

Materials and Methods

Tumor Specimens

Bladder tumor samples were obtained from individuals who underwent surgery at the Sina Hospital. A small piece of the surgical specimen was removed for molecular analysis and stored at -80 °C until DNA extraction. Of the 55 patients, 52 were males and 3 were females with the mean age of 64.5 years ranging from 42 to 87 years with bladder cancer whose samples were obtained by cystectomy and TURBT procedures; 66 tissue samples were also taken from 66 male (66.5 years, range: 44-89years) patients with normal tissue; the study was conducted at the Urology Research Center of Sina Hospital.

DNA Extraction

High molecular weight DNA was obtained form the tissues using DNeasy blood & tissue kit (QIAGENE, Cat. No. 69504) according to the manufacturer's instructions and stored at -20°C.

Polymerase Chain Reaction Analysis

To determine the *PTEN / MMAC1* gene mutation in samples, all samples were screened by polymerase chain reaction (PCR) application (Sensoquest, Labcycler, Germany), using genomic pri-

mers for four exons (1,2,4 and 5 exons). Primer sequences for *PTEN* are shown in Table 1. PCR system is composed of 5 µL PCR buffer solution, 5µLdNTP (2.5 mmol/L), 2 µL primer (F) (10 pmol/µL), 2 µL primer (R) (10 mmol/L), 2 µL DNA template, 1 µLTaq DNA polymerase (5 units/µL), 33 µL ddH2O.

The PCR protocol was carried out as outlined in Table 2. Five µL of the PCR amplified product was put on a 2% agarose gel containing Gel red, 100 bp DNA ladder as a standard reference, electrophoresed for 45 min at 100 V. The results were observed with an ultraviolet transmission reflect analysis instrument and photo was taken with an automatic gel documentation system.

Table 1: Primers used for Polymerase chain reaction

Exon	Primer	Sequence	Product size (bp)
1	PTEN1 F	TCTGCCATCTCTCTCCTCCT	141
	PTEN1 R	CCGCAGAAATGGATACAGGT	
2	PTEN2 F	GTTTGATTGCCATATTTCAG	217
	PTEN2 R	GGCTTAGAAATCTTTTCTAAATG	
4	PTEN4 F	GCAACATTTCTAAAGTTACCTACTTG	237
	PTEN4 R	CATATCATTACACCAGTTCG	
5	PTEN5 F	CATTATAAAGATTCAGGCAATG	205
	PTEN5 R	GACAGTAAGATACAGTCTATC	

Table 2: Polymerase chain reaction protocol

Exon	Denaturation (Temperature, ^{0}C / Times)	Annealing (Temperature, ^{0}C / Times)	Extension (Temperature, ^{0}C / Times)
Exon 1	95/60	62/45	72/15
Exon 2	95/30	57/45	72/60
Exon 4	95/40	62/60	72/60
Exon 5	95/40	60/45	72/60

DNA Sequencing

PCR products was purified by QIAquick pcr purification kit (cat.no.28104) then Sequencing reactions were carried out using cyclic sequencing reaction using BigDye™ terminator and the products were analysed on ABI 3730XL DNA Analyzer. Sequencing of both strands was carried out using the initial PCR primers.

Sequencing results were compared with the genome sequence by Software of Chromas 2.3 and BioEdit 7.

Statistical Analyses

Data analyses were performed by the SPSS software (Statistical Package for the Social Sciences, version 19.0, SPSS, (Chicago, ILL, USA).

Result

Totally, 55 patients with bladder cancer and 66 healthy controls were studied. The average age of the subjects was 65.5 years (64.5 and 66.5 years in patients and control group, respectively). The age range was 42-89 years. All controls and 52 patients were male.

Detection of the *PTEN* gene exons 1,2,4,5 of genomic DNA in subjects indicated that the amplified PCR product had no mutations.

Evaluation of risk factors showed that there was a significant association between occupation (exposure to chemicals such as aromatic amines) and urinary stone with bladder cancer (P<0.05).

Discussion

Bladder cancer can be influenced by environmental and genetic factors. In order to prevent bladder cancer in early stages, BC risk factors and its etiology should be determined. Numerous factors including environmental and genetic risk factors can cause tumorigenesis in bladder (3).

In our study, occupation and urinary tract stones had a significant relationship with bladder cancer among the studied environmental risk factors. Thus, these two factors can be considered as BC risk factors.

Genetic factors including tumor suppressor gene mutation are also significant factors in studying cancers (18, 19).

As an effective tumor suppressor, PTEN plays a considerable role in several cancers (20, 21); as a result, its mutation has been evaluated in several studies carried out on tumors (22-24). *PTEN* alteration can perhaps be an etiological factor in the said tumors, because it is reported in sporadic tumor types frequently (23).

The association between *PTEN* and liver (25), bladder (26, 28) and lung (29) cancers was studied through generating tissue-specific and/or inducible homozygous deletions of *PTEN* in mice in various studies (24).

Consequently, *PTEN* acts as tumor suppressor gene and is mutated in many cancers including urothelial BC (30,31). The difference in *PTEN* mutation frequency in studies might be due to variations in sample size, tumor grade and stage.

No *PTEN* mutations were, however, detected in this study; this finding is in line with previous reports of a low *PTEN* mutation frequency in BC (2%, n = 88) (31, 32) and no *PTEN* mutation in urothelial BC (33). Therefore, mutations in this gene cannot predict the prognosis and progression of urothelial bladder cancer. Yet, the significance of *PTEN* as an important factor in BC development cannot be ignored, since *PTEN* has frequently demonstrated reduced expression (32) as well as homozygous deletion in Urothelial BC (31, 33).

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Conclusion

No mutation was found in *PTEN* gene of patients with bladder cancer. Therefore, mutations in this gene cannot predict the prognosis and progression of urothelial bladder cancer.

Ethical considerations

Ethical issues (Including plagiarism, Informed Consent, misconduct, data fabrication and/or falsification, double publication and/or submission, redundancy, etc.) have been completely observed by the authors.

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