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آموزش مهارت های کاربردی در تدوین و چاپ مقاله
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: 44-89 years) 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 DNacasy 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 (Senssoquest, 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, 5μL dNTP (2.5 mmol/L), 2 μL primer (F) (10 pmol/μL), 2 μL primer (R) (10 mmol/L), 2 μL DNA template, 1 μL Taq 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 | TCTGCCATCTCTCTCTTCCT | 141 |
|      | PTEN1 R | CCGCAGAAATGGATACAGGT |   |
| 2    | PTEN2 F | GTTGTGATTGCCATATTTCA | 217 |
|      | PTEN2 R | GGGTTAGAAATCTTTTCTAATG |   |
| 4    | PTEN4 F | GCAACATTTCTAAAGTTACCT | 237 |
|      | PTEN4 R | CATATCATTACACCGTGTCG |   |
| 5    | PTEN5 F | CATATTAAAGATTCAGCGCA | 205 |
|      | PTEN5 R | GACAGTAAGATACAGTCTATC |   |

### Table 2: Polymerase chain reaction protocol

| Exon | Denaturation (Temperature, °C / Times) | Annealing (Temperature, °C / Times) | Extension (Temperature, °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).

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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References

1. Cordon-Cardo C (2008). Molecular alterations associated with bladder cancer initiation and progression. Scand J Urol Nephrol Suppl, 218:154-65.
2. Cairns P, Evron E, Okami K, Halachmi N, Esteller M, Herman JG, Bose S, Wang SI, Parsons R, Sidransky D (1998). Point mutation and homozygous deletion of PTEN/MMAC1 in primary bladder cancers. Oncogene J, 16:3215–3218.
3. Hirao Y, Kim WJ, Fujimoto K (2009). Environmental factors promoting bladder cancer. Curr Opin Urol, 19: 494-9.
4. Borden LS Jr, Clark PE, Hall MC (2003). Bladder cancer. Curr Opin Oncol, 15: 227-33.
5. Kim TH, Jo SW, Lee YS, Kim YJ, Lee SC, Kim WJ, Yun SJ (2009). Forkhead box O-class 1 and forkhead box G1 as prognostic markers for bladder cancer. J Korean Med Sci, 24: 468-73.
6. Ha YS, Kim MJ, Yoon HY, Kang HW, Kim YJ, Yun SJ, Lee SC, Kim WJ (2010). mRNA Expression of S100A8 as a prognostic marker for

Available at: http://ijph.tums.ac.ir
progression of non-muscle-invasive bladder cancer. *Korean J Urol*, 51: 15-20.

7. Cairns P, Polascik TJ, Eby Y, Tokino K, Califano J, Merlo A, Mao L, Herath J, Jenkins R, Westra W, Rutter JL, Buckler A, Gabrielson E, Tockman M, Cho KR, Hedrick L, Bova GS, Isaacs W, Koch W, Schwab D, Sidransky D (1995). Frequency of homozygous deletion at p16/CDKN2 in primary human tumors. *Nat Genet*, 11:210–212.

8. Liedberg F, Anderson H, Chebil G, Guadjonsson S, Hoglund M, Lindgren D, Lundberg LM, Lowgren K, Ferno M, Mansson W (2008). Tissue microarray based analysis of prognostic markers in invasive bladder cancer: much effort to no avail. *Urol Oncol*, 26: 17–24.

9. Chalhoub N, Baker S (2009). PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol*, 4:127–150.

10. Furnari FB, Huang HJ, Cavenee WK (1998). Thephosphoinositol phosphatase activity of PTEN mediates a serum-sensitive G1 growth arrest in glialoma cells. *Cancer Res*, 58:5002-8.

11. Davies MA, Koul D, Dhesi H, Berman R, McDonnell TJ, McConkey D, Yung WK, Steck PA (1999). Regulation of Akt/PI3K activity, cellular growth, and apoptosis in prostate carcinoma cells by MMAC1/PTEN. *Cancer Res*, 12:2551–2556.

12. Persad S, Artwell S, Gray V, Delemotte M, Troussard A, Sanghera J, Dedic S (2000). Inhibition of integrin-linked kinase (ILK) suppresses activation of protein kinase B/Akt and induces cell cycle arrest and apoptosis of PTEN-mutant prostate cancer cells. *Proc Natl Acad Sci U.S.A.*, 97:3207-12.

13. Backman SA, Ghazanfar D, So K, Sanchez O, Wagner KU, Hennighausen L, Suzuki A, Tsao MS, Chapman WB, Stambolic V, Mak TW (2004). Early onset of neoplasia in the prostate and skin of mice with specific deletion of PTEN. *Proc Natl Acad Sci U.S.A.*, 101:1725-30.

14. Cairns P, Okami K, Halachmi S, Halachmi N, Esteller M, Herman JG, Jen J, Isaacs WB, Bova GS, Sidransky D, (1997). Frequent inactivation of PTEN/MMAC1 in primary prostate cancer. *Cancer Res*, 57: 4997-5000.

15. Steck PA, Pershouse MA, Jasser SA, Yung WK, Lin H, Ligon AH, Langford LA, Baumgard ML, Hattier T, Davis T, Frye C, Hu R, Svedlund B, Teng DH, Tavtigian SV, (1997). Identification of a candidate tumour suppressor gene, MMAC1, at chromosome 10q23.3 that is mutated in multiple advanced cancers. *Nat Gen*, 15:356-362.1

16. Rhei E, Kang L, Bogomolniy F, Federici MG, Borgen PI and Boyd J (1997). Mutation analysis of the putative tumor suppressor gene PTEN/MMAC1 in primary breast carcinomas. *Cancer Res*, 57: 3657–3659.

17. Risinger JI, Hayes AK, Berchuck A and Barrett JC (1997). PTEN/MMAC1 mutations in endometrial cancers. *Cancer Res*, 57: 4736–4738.

18. Guldberg P, thorStraten P, Birek A, Ahrenkel V, Kirkin AF and Zeuthen J (1997). Disruption of the MMAC1/PTEN gene by deletion or mutation is a frequentevent in malignant melanoma. *Cancer Res*, 57: 3660–3663.

19. Liaw D, Marsh DJ, Li J, Dahia PL, Wang SI, Zheng Z, Bose S, Call KM, Tsou HC, Peacocke M, Eng C, Parsons R, (1997). Germline mutations of the PTEN gene in Cowden disease, an inherited breast and thyroid cancer syndrome. *Nat Genet*, 16: 64–67.

20. Li J, Yen C, Liaw D, Podsyspanina K, Bose S, Wang SI, Pue J, Miliarese C, Rodgers L, McCombie R et al. (1997). PTEN, a putative protein tyrosine phosphatase gene mutated in human brain, breast, and prostate cancer. *Science*, 275: 1943–1947.

21. Ali I U, Schriml LM, Dean M (1999). Mutational spectra of PTEN/MMAC1 in human tumours suppressor with lipid phosphatase activity. *J Natl Cancer Inst*, 91: 1922–1932.

22. Eng C (2003). PTEN: one gene, many syndromes. *Hum Mutat*, 22: 183–198.

23. Hollander MC, Blumenthal GM, Dennis PA (2011). PTEN loss in the continuum of common cancers, rare syndromes and mouse models. *Nat Rev Cancer*,11:289–301.

24. Horie Y, Suzuki A, Kataoka Ei, et al (2004). Hepatocyte-specific PTEN deficiency results in steatohepatitis and hepatocellular carcinomas. *J Clin Invest*, 113: 1774–1783.

25. Tsuruta, H. Kishimoto H, Sasaki T (2006). Hypoplasia and carcinomas in Ptene-deficient mice and reduced PTEN protein in human bladder cancer patients. *Cancer Res*, 66: 8389–8396.

26. Marsh DJ, Dahia PL, Zheng Z, Liaw D, Parsons R, Gorlin RJ and Eng C (1997). Germline mu-
mutations in PTEN are present in Bannayan–Zonana syndrome. Nat Genet, 16: 333–334.
27. Nelen MR, van Staveren WC, Peeters EA, Hassel MB, Gorlin RJ (1997). Germline mutations in the PTEN/MMAC1 gene inpatients with Cowden disease. Hum Mol Genet, 6: 1383–1387.
28. Yanagi S, Kishimoto H, Kawahara K, et al. (2007). Pten controls lung morphogenesis, bronchioalveolar stem cells, and onset of lung adenocarcinomas in mice. J Clin Invest, 117: 2929–2940.
29. Jaiswal BS, Janakiraman V, Kljavin NM, Chaudhuri S, Stern HM, et al. (2009). Somatic mutations in p85alpha promote tumorigenesis through class IA PI3K activation. Cancer Cell, 8;16(6): 463–74.
30. Aveyard JS, Skilleter A, Habuchi T, Knowles MA (1999). Somatic mutation of PTEN in bladder carcinoma. Br J Cancer, 80(5–6): 904–8.
31. Puzio-Kuter AM, Castillo-Martin M, Kinkade CW, Wang X, Shen TH, et al. (2009). Inactivation of p53 and Pten promotes invasive bladder cancer. Genes Dev, 15:23(6): 675–80.
32. Sjodahl G, Lauss M, Gudjonsson S, Liedberg F, Hallden C, et al. (2011). A systematic study of gene mutations in urothelial carcinoma; inactivating mutations in TSC2 and PIK3R1. PLoS One, 6: e18583.
33. Han KS, Jeong IG, Jung JY, Yang SO, Chung J, Seo HK, Kwon KS, Park WS, Lee KH (2008). Clinical value of PTEN in patients with superficial bladder cancer. UrolInt, 80: 264–269.
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