

RESEARCH ARTICLE

Role of exon 7 PTEN Gene in Endometrial Carcinoma

Leila Kafshdooz^{1,2}, Taiebeh Kafshdooz^{1,2}, Ali Dastranj Tabrizi^{1,2*}, Seyyed Mojtaba Mohaddes Ardabili², Abolfazl Akbarzadeh^{3*}, Jalal Gharesouran², Morteza Ghojzadeh², Safar Farajnia⁴

Abstract

Background: Endometrial carcinoma is the most common malignant tumor of the female genital tract and the fourth most common cancer in Iranian women after breast, colorectal and lung cancers. Various genetic alterations appear to be early events in the pathogenesis of endometrial carcinoma and it seems that PTEN is the most commonly mutated gene in the endometrioid subtype. The aim of the present study was to investigate the correlation between mutations in exon 7 of PTEN gene and endometrial carcinoma. **Materials and Methods:** Seventy-five patients with endometrial carcinoma and 75 females whose underwent hysterectomy for non tumoral indication were selected for evaluation of PTEN mutations in exon 7 by PCR-SSCP and sequencing. Correlations between the frequency and type of mutation and the pathologic findings of the cancer (tumor subtype, stage and grade) were assessed. **Results:** All of the samples were obtained from Iranian patients. 60 % (45 cases) of the tumors were endometrioid and 40% (30 cases) were of serous type. The grade distributions of the 75 cases according to the FIGO staging system were as follows: low grade, 20 cases; high grade 55 cases, low stage, 41 cases; high stage 34 cases. For exon 7 of the PTEN gene, the analysis showed that there were no mutations in our cases. **Conclusions:** Our findings in the present study suggest that exon 7 of PTEN does not play any significant role in the development of endometrial carcinoma in Iranian cases.

Keywords: Endometrial carcinoma - PTEN - exon 7 - mutations

Asian Pac J Cancer Prev, 16 (11), 4521-4524

Introduction

Endometrial cancer is the most common gynecologic malignancy (Bansal, Yendluri et al., 2009; Myatt et al., 2010; Zhu et al., 2012; Wang et al., 2014). It is an important health problem that leads to mortality and morbidity of women (Hernandez et al., 2010) with 150,000 new cases diagnosed annually worldwide (Okuda et al., 2010). Approximately 90% of endometrial cancers are sporadic, and 10% of them are hereditary (Prat, Gallardo et al., 2007). Endometrial carcinoma is the most common invasive malignant neoplasm of the female genital tract, with an estimated 46,470 diagnosed cases and 8,120 deaths in 2011 in the United States (Cancer Statistics, 2011). Two major types of endometrial carcinoma include type I or endometrioid endometrial carcinoma (EEC) and type II including uterine serous and clear cell carcinomas (Sun, Enomoto et al., 2001; Fader, Arriba et al., 2009). Cancer is one of the leading causes of death in the world (Zhao et al., 2009). The cause of endometrial cancer remains unclear (Jiang et al., 2014) and numerous genetic alterations have been found in cancer cells (Liu, 2007). Some of the genes are important in development of endometrial carcinoma including P53, Kras, Catenin and PTEN (Zhao

et al., 2009). PTEN is the most frequently mutated gene in endometrial carcinoma (Konopka et al., 2007). The PTEN gene appears to be the second most frequently mutated tumor suppressor gene in human cancers after TP53 (Ashton et al., 2009). It is a tumor suppressor gene on human chromosome 10q23, a locus that is highly susceptible to mutation in primary human cancers (Smuc et al., 2006). The PTEN gene encodes a single 403 amino acid protein (Prat et al., 2007). It also encodes a phosphatase that antagonizes the PI3K/AKT pathway by dephosphorylating PIP3, the product of PI3K (Gadducci et al., 2011). Moreover, decreased PTEN activity causes an increased cell proliferation (Chow and Baker, 2006). PTEN germline mutations are also associated with Cowden disease (Seeber et al., 2010). PTEN is composed of two major structural domains: the phosphatase and C2 domains (Gericke et al., 2006). Genetic inactivation of PTEN is frequently found in the glioblastomas, melanomas, endometrial, prostate, colon, and bladder cancers, and reduced PTEN expression has been observed in the lung and breast cancers (Ortega-Molina and Serrano, 2013). In the endometrial cancer, PTEN mutations are detected in complex endometrial atypical hyperplasia and endometrial intraepithelial carcinoma (EIN) (pre-malignant

¹Women's Reproductive Health Research Center, ²Faculty of Medicine, ⁴Drug Applied Research Center, ³Department of Medical Nanotechnology, Faculty of Advanced Medical Sciences, Tabriz University of Medical Sciences, Tabriz, Iran *For correspondence: dastranjt@gmail.com, akbarzadehab@tbzmed.ac.ir

lesions in the progression of endometrial neoplasia) and , suggesting an initiation role for endometrial carcinomas (Chalhoub and Baker, 2009).

The current study conducted for investigation of mutations of the PTEN gene in exon 7 and the prognostic significance of mutated exon 7 in endometrial carcinomas in Iranian patients.

Materials and Methods

The tissues used in the present study were formalin-fixed and paraffin-embedded sections (75 endometrial carcinomas and 75 normal endometrium) from patients who had been admitted to the Department of Obstetrics and Gynecology from 2011-2013 in the AL-Zahra educational-treatment hospital, Tabriz, IRAN. A pathologist reviewed and confirmed the histological diagnosis of all cases. All cases underwent for grading and staging by FIGO 2009 staging and grading protocol.

DNA extraction

DNA was extracted from the tumor paraffin-embedded tissues. Tissues were removed from micro-dissection slides. This slide was used to separate the relevant tissues from the other slides. DNA was extracted by using the QIAamp DNA FFPE Tissue kit (cat. No.56404). Before beginning the procedure, deparaffinization was carried out by the xylen. The extracted genomic DNA was quantified by using nanodrop spectrophotometer.

Primer design

Primers for polymerase chain reaction (PCR) amplification and sequencing were designed using the primer 3 program (<http://frodo.wi.mit.edu/cgi-bin/primer3/primer3-www.cgi>) and were synthesized according to exon 7 of PTEN gene sequence. Two primer pairs were used to amplify from genomic endometrial cancer and normal DNA.

PCR

The conditions for the PCR reaction were denaturation at 95°C for 4 min followed by 35 cycles of denaturation at 95°C for 30 s, annealing at 51°C for 30s, and extension at 72°C for 30 s, followed by a final extension at 72°C for 10 min. The forward primer was 5' TTG ACA GTT AAA GGC ATT TC-3' and the reverse primer was 5'-CCT ATT TTG GAT ATT TCT CCC-3'.

Each 20µl reaction mixture for PTEN amplification contained 100 ng of genomic DNA, 250_M of each deoxynucleotide triphosphate, 1 x_PCR Buffer II (Perkin-Elmer), 2.5 mM MgCl₂, 0-10% DMSO, 0.5 unit of AmpliTaq Gold (Perkin-Elmer), and 1 µM of each primer. PCR amplifications were performed in a Perkin-Elmer Cetus 9600 thermocycler with denaturation at 94°C for 10 min, followed by 35 cycles of 94°C for 30s, 44-55°C for 30s, 72°C for 30s, and final extension at 72°C for 10 min.

Single Strand Conformation Polymorphism (SSCP) and sequencing

SSCP analysis for point mutations was performed under the following conditions: a mixture of 5 µl of a

35-cycle PCR product and 12 µl of loading buffer were denatured for 10 min at 95°C, cooled rapidly on ice and separated on an non-denaturing 10% polyacrylamide gel in 1xTris borate-EDTA buffer at 4-8°C for 16-20h at 100 V. Bands were revealed by silver staining.

The PCR products showed a mobility shift by SSCP analysis, which suggested the presence of mutation. The products submitted for direct sequencing.

DNA sequencing

Samples that displayed abnormal SSCP patterns were sequenced. Each sample was amplified in a new 25 µl PCR reaction and sequenced.

Statistical analysis

The present study was a descriptive analysis and calculations were performed using SPSS version 16.00.

Results

Patients and tumors

Seventy-five endometrial carcinomas and seventy-five normal endometrium were investigated. All of the samples were obtained from Iranian patients. 60 % (45 cases) of the tumors were endometrioid and 40% (30 cases) of the tumors were serous type.

The mean age of the patients was 57.6 and the mean age of control group was 55.75.

The tumors were divided into high grade and low grade, The grade distributions of the 75 cases according to the FIGO staging system were as follows :low grade, 20 cases; high grade 55 cases, low stage, 41 cases; high stage 34 cases.

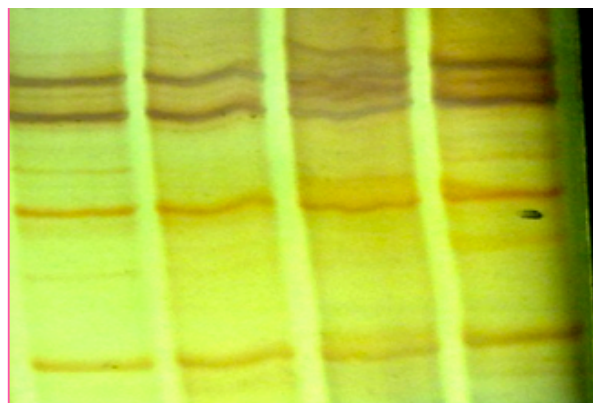


Figure 1. PTEN Exon 7 Mutation Analyzed by PCR-SSCP

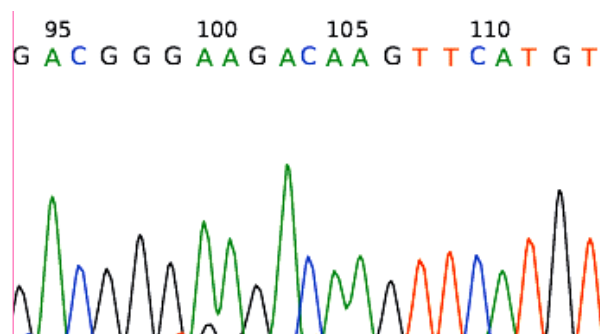


Figure 2. Direct Sequencing of PTEN Exon 7

PCR-SSCP and sequence analysis results

We screened exon 7 of the PTEN gene by PCR-SSCP analysis for the presence of mutation in 75 endometrial carcinomas and 75 normal endometrium. Examples of the cases that showed mobility shifts by SSCP analysis are shown in Figure 1. No mutations were subsequently confirmed by sequencing. All sequence variants were compared with the PTEN pseudogene sequence to exclude the possibility of pseudogene coamplification (Figure 2).

In a series of 75 endometrial carcinomas and 75 normal endometrium, no mutations were detected in exon 7 in case and control groups.

Discussion

Endometrial carcinoma is the most common gynecological cancer. It is a disease which can be prevented and cured when treated right. It is fourth common cancer after breast, lung and colon cancers in women. Most malignancy cases are found in women aged 50 and over, with more than half of all endometrial cancer cases diagnosed in the 50-69 age group (Balik et al., 2013).

Lee et al. (1999) showed that PTEN crystal structure consists of a phosphatase domain and a C2 domain, which are both important for tumor suppressor function. Exon 7 consists of CBR3 loop, which is thought to play an essential role in phospholipid membrane binding of the C2 domain. The mutation on the CBR3 loop decreases the affinity for membranes *in vitro* (Lee et al., 1999). Ali et al. (1999) showed that most mutations in exon 7 are frameshifts or nonsense mutations.

PTEN mutation was detected in 37 (55%) out of 67 endometrial carcinomas from Japanese patients. Among them, 7 mutations were observed in exon 7 and their mutation frequency and distribution were consistent with some findings (Risinger et al., 1997; Tashiro et al., 1997) on caucasian patients.

In the study by Hongbo Sun, they found that in 57 endometrial carcinoma, 7 mutations were observed in exon 7 but they found no significant association between these mutations and FIGO staging.

In the current study we assessed relationship between the mutation in exon 7 and endometrial tumors, however no mutation was found in this context. These findings suggest that mutation outside the exon 7 of PTEN might be a molecular predictor of tumors. In the present study, the molecular findings in exon 7 of PTEN in two groups (case and control) were similar.

According to Lee et al. (1999), the cells expressing PTEN mutant of the phosphatase active site showed an extensive proliferation similar to the control cells, whereas CBR3 and C2 mutants showed intermediate growth suppressive activities. Most mutations in exon 7 were reportedly frameshifts or nonsense mutations (Ali et al., 1999).

Those mutations in exon 7 resulted in truncation of both the CBR3 loop in exon 7 and the C2 element in exon 8. It is possible that loss of both the CBR3 and C2 elements in the C2 domain may be required to disrupt the tumor suppressor function to the extent comparable with mutation of the phosphatase active site.

It is believed that there are two different pathogenetic types of endometrial carcinomas: estrogen-dependent type I and estrogen-independent type II (Voss, Ganesan et al., 2012; Thanappapasr and Thanappapasr, 2013). Exposure to unopposed estrogen, either endogenous or exogenous, is thought to contribute to the development of type I tumors (Tangjitgamol et al., 2010). Type I tumors, most of which are histologically low-grade endometrial adenocarcinomas, tend to occur in younger premenopausal women and have a favorable prognosis. Type II is histologically composed of high-grade serous carcinoma and clear cell carcinomas. In contrast to type I, type II is likely to occur in older postmenopausal women and has a poor prognosis. A few recent studies (Risinger, Hayes et al., 1998; Minaguchi et al., 2001) have indicated that PTEN mutation is associated with low-grade endometrioid histology and favorable prognosis of patients.

However due to controversial and challenging aspects of this background, other studies with describing detail molecular findings in different types of endometrial carcinoma is necessary.

In conclusion numerous studies suggest that mutation in this gene could be involved in development of type I endometrial tumors. Although our study failed to find any PTEN mutation in this type of tumors. Larger epidemiological studies and further molecular research are required to clarify this issue.

Acknowledgements

We would like to thank authorities of Tabriz University of Medical Sciences for the scientific and ethical approval and financial support of this research. This study has been done as a msc thesis of L kafshdooz (Thesis No:5/89/1891) in Women's Reproductive Health Research Center, Tabriz University of Medical Sciences.

References

- Ali IU, Schriml LM, Dean M (1999). Mutational spectra of PTEN/MMAC1 gene: a tumor suppressor with lipid phosphatase activity. *JNCI J Natl Cancer Inst*, **91**, 1922-32.
- Ashton K, Proietto A, Otton G, et al (2009). Estrogen receptor polymorphisms and the risk of endometrial cancer. *BJOG*, **116**, 1053-61.
- Balik G, Kagitci M, Ustuner I, et al (2013). Which endometrial pathologies need intraoperative frozen sections? *Asian Pac J Cancer Prev*, **14**, 6121-5.
- Bansal N, Yendluri V, Wenham RM (2009). The molecular biology of endometrial cancers and the implications for pathogenesis, classification, and targeted therapies. *Cancer Control J Moffitt Cancer Cente*, **16**, 8.
- Chalhoub N, Baker SJ (2009). PTEN and the PI3-kinase pathway in cancer. *Annu Rev Pathol*, **4**, 127.
- Chow LM, Baker SJ (2006). PTEN function in normal and neoplastic growth. *Cancer Lett*, **241**, 184-96.
- Fader AN, Arriba LN, Frasure HE, von Gruenigen VE (2009). Endometrial cancer and obesity: epidemiology, biomarkers, prevention and survivorship. *Gynecol Oncol*, **114**, 121-7.
- Gadducci A, Cosio S, Genazzani AR (2011). Tissue and serum biomarkers as prognostic variables in endometrioid-type endometrial cancer. *Crit Rev Oncol Hematol*, **80**, 181-92.
- Gericke A, Munson M, Ross AH (2006). Regulation of the PTEN

- phosphatase. *Gene*, **374**, 1-9.
- Hernandez E, Obermair A, Hoskins PJ, Magrina JF, McLellan R (2010). Controversies in the management of endometrial cancer. *Obstet Gynecol Int*. [Epub ahead of print].
- Jiang X-F, Tang Q-L, Zou Y, et al (2014). Does HBV infection increase risk of endometrial carcinoma? *Asian Pac J Cancer Prev*, **15**, 713-6.
- Konopka B, Janiec-Jankowska A, Czapczak D, et al (2007). Molecular genetic defects in endometrial carcinomas: microsatellite instability, PTEN and beta-catenin (CTNNB1) genes mutations. *J Cancer Res Clin Oncol*, **133**, 361-71.
- Lee J-O, Yang H, Georgescu M-M, et al (1999). Crystal structure of the PTEN tumor suppressor: implications for its phosphoinositide phosphatase activity and membrane association. *Cell*, **99**, 323-34.
- Liu FS (2007). Molecular carcinogenesis of endometrial cancer. *Taiwanese J Obstet Gynecol*, **46**, 26-32.
- Minaguchi T, Yoshikawa H, Oda K, et al (2001). PTEN mutation located only outside exons 5, 6, and 7 is an independent predictor of favorable survival in endometrial carcinomas. *Clinical Cancer Res*, **7**, 2636-42.
- Myatt SS, Wang J, Monteiro LJ, et al (2010) Definition of microRNAs that repress expression of the tumor suppressor gene FOXO1 in endometrial cancer. *Cancer Res*, **70**, 67-377.
- Okuda T, Sekizawa A, Purwosunu Y, et al (2010). Genetics of endometrial cancers. *Obstet Gynecol Internl*, 2010.
- Ortega-Molina A, Serrano M (2013). PTEN in cancer, metabolism, and aging. *Trends Endocrinol Meta*, **24**, 184-9.
- Prat J, Gallardo A, Cuatrecasas M, Catusus L (2007). Endometrial carcinoma: pathology and genetics. *Pathol*, **39**, 72-87.
- Risinger JI, Hayes AK, Berchuck A, Barrett JC (1997). PTEN/MMAC1 mutations in endometrial cancers. *Cancer Res*, **57**, 4736-8.
- Risinger JI, Hayes K, Maxwell GL, Carney ME, Dodge RK, Barrett JC, Berchuck A (1998). PTEN mutation in endometrial cancers is associated with favorable clinical and pathologic characteristics. *Clin Cancer Res*, **4**, 3005-10.
- Seeber L, Zweemer RP, Verheijen RH, van Diest PJ (2010). Hypoxia-inducible factor-1 as a therapeutic target in endometrial cancer management. *Obstet Gynecol Internl*, 2010.
- Smuc T, Ruprecht R, Sinkovec J, Adamski J, Rižner TL (2006). Expression analysis of estrogen-metabolizing enzymes in human endometrial cancer. *Mol Cell Endocrinol*, **248**, 114-7.
- Sun H, Enomoto T, Fujita M, et al (2001). Mutational analysis of the PTEN gene in endometrial carcinoma and hyperplasia. *Am J Clin Pathol*, **115**, 32-8.
- Tangjitgamol S, Manusirivithaya S, Srijaipracharoen S, et al (2010). Endometrial cancer in Thai women: clinicopathological presentation and survival. *Asian Pac J Cancer Prev*, **11**, 1267-72.
- Tashiro H, Blazes MS, Wu R, et al (1997). Mutations in PTEN are frequent in endometrial carcinoma but rare in other common gynecological malignancies. *Cancer Res*, **57**, 3935-40.
- Thanappapasr D, Thanappapasr K (2013). Molecular therapy as a future strategy in endometrial cancer. *Asian Pac J Cancer Prev*, **14**, 3419-23.
- Voss MA, Ganesan R, Ludeman L, et al (2012). Should grade 3 endometrioid endometrial carcinoma be considered a type 2 cancer-a clinical and pathological evaluation. *Gynecol Oncol*, **124**, 15-20.
- Wang H-L, Liu M-M, Ma X, et al (2014). Expression and effects of JMJD2A histone demethylase in endometrial carcinoma. *Asian Pac J Cancer Prev*, **15**, 3051.
- Zhao T, Lv J, Zhao J, Nzekebaloudou M (2009). Hypoxia-inducible factor-1 α gene polymorphisms and cancer risk: a meta-analysis. *J Exp Clin Cancer Res*, **28**, 159.
- Zhu X-L, Ai Z-H, Wang J, et al (2012). Weighted gene co-expression network analysis in identification of endometrial cancer prognosis markers. *Asian Pac J Cancer Prev*, **13**, 4607-11.