RESEARCH ARTICLE

Polymorphism of p53 Gene Codon 72 in Endometrial Cancer: Correlation with Tumor Grade and Histological Type

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Abstract

Background: Endometrial cancer is the fourth most common cancer among women in developed countries. Patients with endometrial cancer may benefit from systemic chemotherapy alone or in combination with targeted therapies if the disease is clinically diagnosed prior to spread and metastasis to other organs. The aim of this study was to evaluate the prognostic role of p53 polymorphism and its correlation with tumor grade in human uterine endometrial carcinomas. Materials and Methods: A total of 75 patients with endometrial carcinomas were studied for possible mutations in exon 4 of the p53 gene using polymerase chain reaction and restricting fragment length polymorphism techniques and sequencing. Results: In recent study, The rate of homozygote genotype of pro/pro or Arg/Arg in high grade group was higher than in comparison with low grade one. In addition samples that were undigested in RFLP, showed mutation in exone 4. Conclusions: Our findings showed that high grade endometrial carcinomas are highly associated with TP53 polymorphisms in comparison with low grades.

Keywords: Endometrial carcinoma - p53 polymorphism - grade - histological type

Asian Pac J Cancer Prev, **15 (22)**, 9603-9606

Introduction

Endometrial carcinoma is known to be a kind of heterogeneous group of tumor with variable morphology, clinical behavior, ploidy, degree of differentiation and immunogenicity etc (Dobrzycka et al., 2010; Nazik et al., 2014) and it is an important health problem that leads to mortalitiy and morbidity of women. It is the most frequent malignant female genital tract with over 150,000 new cases diagnosed annually worldwide and the fourth most common cancer in women among industrialized countries (Levine et al., 1998; Hernandez et al., 2010). The American Cancer Society estimates for endometrial cancer in the United States for 2013 are about 49,560 new cases (Balik et al. 2013).

More than half of all cancer cases and deaths worldwide are potentially preventable. Although it is frequently diagnosed at an early stage, the disease-related death rate of FIGO 1-2 A is still 5-15% (Lee et al., 2010). Improvement of the ways of detection and treatment consequences in 5-year survival rate for women in developed countries to be around 80-90%, and most survivors have the opportunity to reach normal life expectancy (Garcia-Dios et al., 2013; Rowlands et al., 2013). Identifying the role of molecular factors may yield indicators for accurate triaging of the patients with different disease progression and for designing individualized treatment plans (Levine et al., 1998; Oda et al., 2005).

The most important risk factors for EC are postmenopausal status, excessive fat consumption, body mass index (BMI) of 25kg/m^2 or more, nulliparity, unovulation, and unopposed exogenous estrogen use (Jiang et al., 2011). However, only half the patients revealed the identifiable risk factors, while the other half appear to be at low risk (Levine et al., 1998).

For the last two decades, as described by Bokhman, endometrial carcinomas are divided into types 1 and 2 using both clinical and histopathological variables: estrogen-dependent endometrioid endometrial carcinomas (EECs), or type I, and nonendometrioid endometrial carcinomas (NEECs), or type II tumors (Harris et al., 1986; Salvesen et al., 2009). Approximately 90% of cases of endometrial carcinoma are sporadic, while the remaining 10% of cases are hereditary (Hernandez et al., 2010).

Type 1 tumors are demonstrated as large numbers of

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genetic changes usually associated with microsatellite instability and mutations in PTEN, CTNB1 (beta-catenin), k-RAS and PIK3CA. on the other hand, serous carcinoma as a prototype of type Π tumors ,typically shows p53 mutations (Santin et al., 2005; Hecht and Mutter 2006).

Molecular changes of both EC types are different. It is worth mentioning that, generally, cancers developed after the defect in the DNA repair, inactivation of tumor suppressor genes, and up-regulation of oncogenes (Saghir et al., 2010).

P53 is a tumor-suppressor gene that is located in 17p13.1 which encodes p53 protein , a transcription factor that induces the expression of genes necessary for cell cycle arrest and apoptosis in response to DNA damage including apoptosis, cellular transcriptional control and cell cycle regulation (Hu et al., 2010; Francisco et al., 2011; Matei et al., 2012). Tp53 gene mutations are common genetic events in cancers. The role of this gene is to preserve stability of human genome. It has a polymorphism at codon 72, with a single base change causing an amino-acid substitution inside the transactivation domain of the protein-Arg (CGC) by Pro (CCC) (Bai and Zhu 2006, Zubor et al., 2009). A common polymorphism at codon 72 of Tp53 results in either an arginine residue (Tp5372R) or a proline residue (Tp5372P) (Harris et al., 1986).

Numerous studies have been conducted to investigate the direct modulator effect of genetic polymorphisms on the risk of, or prognosis and survival in endometrial cancer, however they have almost focused on the negative results, and, are about gene-environment interaction (Harris et al., 1986; Matei et al., 2012). Nevertheless, there is a rare number of studies which are carried out about tumor grade. The current study reveald that, P53 polymorphysm at codon 72 is showing with equal frequency in endometrial cancer cells in comparison with nontumoral cells in control groups. we also re-evaluated the role of this SNP in development of gynecological malignancies which was in correlation with tumor grade in north-west of Iran. In fact, understanding of the p53 suppresion role in endometrial cancer may lead to an appropriate treatment planning and more rational targeted approaches for treating the disease.

Materials and Methods

Samples

After proposal approved by ethical board of AL-Zahra Educational and Medical Hospital, Tabriz, Iran The tissues that were used in the present study included formalin-fixed, paraffin-embedded sections (75 endometrial carcinoma) from patients who had been admitted to the Department of Obstetrics and Gynecology from 2011-2013 at AL-Zahra Educational and Medical Hospital, Tabriz, Iran. All retrived histologic slides were reviewed by a gyn pathologist (dr DT.A) for confirmation of the previous diagnosis.

Methods

DNA extraction

At first, The paraffin blocks which contain representative tissue were selected. Then, DNA was extracted by using the QIAamp DNA FFPE Tissue kit (cat.no 56404). The extracted genomic DNA was quantified by using nano-drop 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/primer 3/primer 3-www.cgi) and were synthesized according to P53 gene sequence. Moreover,2 primer pairs were used to amplify exon 4 from genomic endometrial cancer and normal DNA.

PCR

Amplification of 2 alleles of the p53 gene was carried out in a total volume of 20. This volume contained 100 ng of genomic DNA and a PCR reaction mix containing reaction buffer, MgCl 2, dNTPs, primers and Taq polymerase. Each PCR amplification was carried out with 35cycles of 30s at 95°C, 30s min at 65°C and 30min at 72°C, with an initial denaturation of 2min at 95°C and extension for 10min at 72°C on a Techne Genius_PCR System. We used the sense primer 5'TCTACAGTCCCCCTTGCCGTCC-3' and the anti-sense primer 5'-TGTCCCAGAATGCAAGAAGCC-3' for amplification of the 259-bp PCR products.

Digestion

PCR products were digested with the restriction of endonuclease Bst UI (NEB) at 60°C for 2h. Digested DNA fragments were separated by 8% acryl amid gel stained with silver nitrate and analyzed by an ultraviolet source using an image analysis system. The homozygous genotype Pro/Pro was detected by 110-and 149-bp

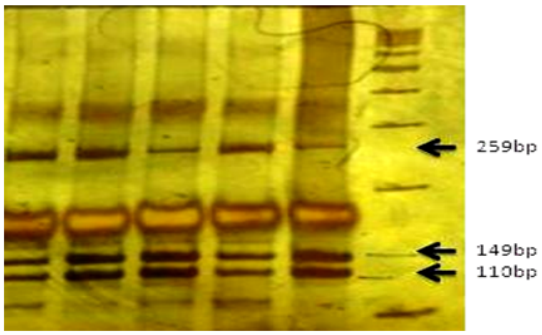

Figure 1. Digestion of Codon 72 of p53 Gene

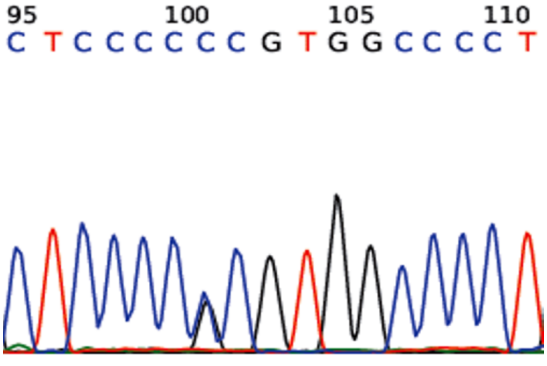

Figure 2. Sequensing of Undigested of Samples for P53 Gene

fragments, the genotype Arg/Arg by 259-bp fragments and Arg/Pro heterozygote by the presence of 3 bands (Figure 1).

Sequencing

Genotypes of selected participants were confirmed by direct sequencing of amplified PCR fragments (Figure 2).

Results

Clinical and histopathological findings

The studied population consisted of 75 women with histologically aproved endometrial carcinoma (mean age 56.2 years) and 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.

As for the genotype frequency, the presence of homozygous form of p53 gene (Arg/Arg) was recorded in 8.3% of high grade tumors in comparison with 6.5% hemozygosity in low grade ones. Based on these findings, our results indicate for higher frequency of homozygote genotype of Arg/Arg allele among high grade tumors compared with low grade ones. As an important finding, we did not find any homozygote allele for Arg in non tumoral control group (14.8% *vs* 0.0%).

Statistical analysis

Data of the present underwent for statical analysis using spss software (version16) for identifying of correlation between p53 polymorphsm in one hand and other type, tumor grade and tumor stage on the other hand.

Discussion

Endometrial carcinoma is the most common gynecological malignancy. It is a disease which can be prevented and cured when treated right. After breast, lung and colon cancer, its the fourth cancer most common cancer in women. There are numerous studies during several decades to evaluate effective carcinogenic effects on the endometrium. 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).

Moreover and throughout the present study, the association between p53 alteration and clinicopathologic characteristics of tumor also was investigated. Genetic factors, like polymorphism were included as parts of these studies. Clinical outcomes, including progression-free and disease-specific survival, were distinctly different in tumors. It is mentionable that, about 85 single-nucleotide polymorphisms have been compiled in the International Agency for Research on Cancer TP53 Mutation Database (Hu et al., 2010) (http://www-p53.iarc.fr/PolymorphismView.asp). Actually, Codon 72 of exon 4 was the first polymorphism that was detected in the p53 gene, and it was suggested that the two alleles of codon 72 might have different oncogenic properties with p53 overexpression compared with those without p53 overexpression. Ethnicity or genetic make-up is important risk factors for women to develop for endometrial cancer (Baloch et al., 2012).

Histologically, The endometrioid endometrial carcinoma(EEC) shows, it seems that,the genetic alteration may act as a significant factor in development of cancer in different individuals. Polymorphisms have different forms. One of them is codon changes and the other is the changes in different types of protein synthesis.

p53 mutations occur in 90% of non-endometrioid endometrial carcinoma, it is only seen in 10-20% of endometrioid endometrial carcinoma ,which are mostly high-grade. Overexpression of p53 is associated with high histological grade and more advanced stage as well as unfavorable prognosis (Niwa et al., 2005; Garcia-Dios et al., 2013).

In the current study, we found a higher frequency of homozygote genotype Arg/Arg or Arg allele among high grade tumors compared with low grade ones. In addition, the presence of 14.8% of homozygozity in both low and high grade tumors as a one group in comparison with 0% of this finding in non tumoral group promptly confirm the role of this type of polymorphism in endometrial carcinogenesis. However due to lower number of cases we could not calculate p value. This suggests that preferential retention of the Arg-allele may be as a result of an antiapoptotic advantage conferred by the TP53 mutation. It is considered to be necessary to include larger number of subjects, to allow the generalization of the results for the female population of Northwest Iran, the issue that can be carried out concurrently by development in DNA collection processes.

Ueda et al (2013) reported an increased risk of endometrial cancer in Japanese patients harboring the Arg/Arg genotype compared to those with Arg/Pro and Pro/Pro genotypes combined that our findings are in line with them. Niwa et al. (Niwa et al., 2005), however, did not find such association. By contrast, it is conferred that, having Pro allele increased risk in Korean women (Garcia-Dios et al., 2013). Ashton et al. (Ashton et al., 2009) showed no effect of the TP53 polymorphism and MDM2 SNP309 alone or in combination with endometrial cancer risk. The status of the TP53 gene (wild-type or mutant type) is critical when determining the relationship between grade and a TP53 polymorphism (Hui et al., 2005; Oda et al., 2005; Ueda et al., 2006).

Howevere 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, it can be said that endometrial cancer is characterized by numerous genetic alterations, including those in p53, K-ras, PTEN and β-catenin. The study demonstrated that polymorphisms of TP53 increase the risk of endometrial cancer, Thus, further studies with larger samples need to be carried out in order to clarify new mutation site and the role of this SNP in the oncogenesis of endometrial cancer among Iranian women.

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 taiebeh kafshdooz in Women's Reproductive Health Research Center, Tabriz University of Medical Sciences.

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