

RESEARCH ARTICLE

The Polymorphism of Hypoxia-inducible Factor-1a Gene in Endometrial Cancer

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Abstract

Background: Endometrial carcinoma is the most common malignant tumor of the female genital tract and the fourth most common cancer in women after breast, colorectal and lung cancers Hypoxia-inducible factor -1 (HIF-1) is a key transcription factor that regulates cellular response to hypoxia HIF-1 plays important roles in the development and progression of cancer through activation of various genes that are involved in crucial aspects of cancer biology, including angiogenesis, energy metabolism, vasomotor function, erythropoiesis, and cell survival. In this study, we aimed to investigate the association between *HIF-1* 1772 C/T polymorphisms and endometrial cancer. **Materials and Methods:** 75 patients with endometrial carcinoma and 75 patients whose underwent hysterectomy for non tumoral indication selected for evaluation of *HIF-1* 1772 C/T polymorphisms by PCR-RFLP and sequencing. **Results:** For the 1772 C/T polymorphism, the analysis showed that the T allele and genotype TT were significantly associated with endometrial cancer risk. **Conclusions:** Our results suggest that the C1772T polymorphism of the HIF-1a may be associated with endometrial cancers.

Keywords: Endometrial carcinoma - HIF-1a polymorphism

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Introduction

Endometrial cancer is the most common gynecologic malignancy (Bansal et al., 2009; Myatt et al., 2010; Zhu et al., 2012; Wang et al., 2014) with 150,000 new cases diagnosed annually worldwide (Okuda et al., 2010). Patients often have postmenopausal uterine bleeding (Acmaaz et al., 2014) that help to diagnosis in early stages, and those with stage I EC have a survival rate of over 90% (Cetinkaya et al., 2014) however, stage III patients have a 5-year survival rate of around 60-75% (Neubauer and Lurain, 2011). Approximately 90% of endometrial cancers are sporadic, and 10% of them are hereditary (Prat et al., 2007). More than half of all cancer cases and deaths worldwide are potentially preventable (Zeng et al., 2013). 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 et al., 2001; Fader 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). Angiogenesis of gynecologic cancer is regulated by vascular endothelial growth factor (VEGF), a target gene of HIF-1a (Rasila et al., 2005). Tumors derived from cells lacking HIF-1a or HIF-1 β in comparison of parental cells have reduced vascularization rates (Kung et al., 2000). Studies showed that Polymorphisms of the gene HIF and production of HIF protein involved in different cancers (Hebert et al., 2006).

The current study revealed that, HIF 1A polymorphism is showing with different 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 type, stage and grade in north-west of Iran In fact, understanding of the HIF1A role in endometrial cancer may lead to an appropriate treatment planning and more rational targeted approaches for treating the disease.

Materials and Methods

Samples

The tissues that were used in the present study included formalin-fixed, paraffin-embedded sections (75

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endometrial carcinoma and 75 normal endometrium) 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 retrieved histologic slides were reviewed by a gyn pathologist (dr DTA) for confirmation of the previous diagnosis.

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 (catno 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://frodowimittedu/cgi-bin/primer3/primer3-www.cgi>) and were synthesized according to HIF-1a gene sequence. Moreover, 2 primer pairs were used to amplify from genomic endometrial cancer and normal DNA.

PCR

Amplification of 2 alleles of the HIF-1a gene was carried out in a total volume of 20. This volume contained 100ng of genomic DNA and a PCR reaction mix containing reaction buffer, MgCl₂, dNTPs, primers and Taq polymerase Each PCR amplification was carried out with 35 cycles of 30s at 95°C, 30s at 52°C and 30s at 72°C, with an initial denaturation of 5 min at 95°C and extension for 5 min at 72°C on a Techne Genius_PCR System We used the sense primer 5'AAGGTGTGGCCATTGTAAAACTC3' and the anti-sense primer 5'-GCACTAGTAGTTTCTTTATGTATG3' for amplification of the 333-bp PCR products.

Digestion

PCR products were digested with the restriction of endonuclease HphI 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 1772C allele was cut into two fragments of 207 and 127 bp (Figure 1) while the 1772T allele remained uncut (333bp) (Figure 2).

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.

We found a significant difference in the genotype distribution and allele frequencies between the endometrial cancer patients and control subjects(p<0001). A statistically significant difference was found for genotypic frequencies of the C1772T polymorphism between endometrial cancer patients with CT, TT, CT+TT genotypes (p<005) TT genotypes were more frequent among endometrial (13.5%) cancer patients than among controls (0%).

The present study found no significant relationship between clinicopathological characteristics and C1772T polymorphism in cancer patients and controls(p>0.005).

Statistical analysis

Data of the present underwent for statical analysis using spss software (version16) for identifying of correlation between HIF-1a polymorphism in one hand and other type, tumor grade and tumor stage on the other hand.

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).

HIF-1a is the target of novel anti-cancer drugs that regulates tumor angiogenesis (Hurwitz et al., 2004).

Histologically, The endometrioid endometrial

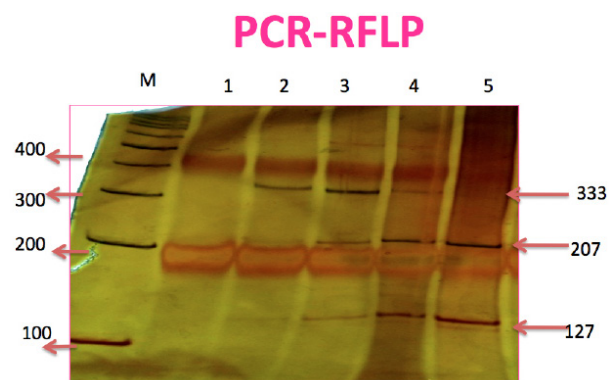


Figure 1 Digestion of PCR Product HIF1A by HphI

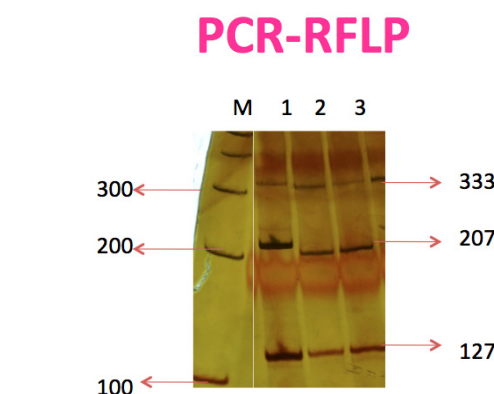


Figure 2 Hetrozygous Type of C1772T HIF1A

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 (Garcia-Dios et al., 2013).

Proline 564 (P564) and proline 402 (P402) are key amino acids in the oxygen-dependent destruction of the HIF-1 α subunit by the 26S proteasome (Epstein et al., 2001). Amino acid changes within the ODD of the subunit (proline to serine at position 582) have been described in relation to patients with RCC (Clifford et al., 2001), and head and neck squamous cell carcinoma (HNSCC) (Tanimoto et al., 2003).

It is believed that there are two different pathogenetic types of endometrial carcinomas: estrogen-dependent type I and estrogen-independent type II (Thanappapasr and Thanappapasr, 2013).

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 (Minaguchi et al., 2001).

Therefore, we investigated the polymorphic effects of the changes in the C1772T of the HIF-1 α gene on endometrial cancer patients.

C1772T polymorphisms have been reported in relation to patients with head and neck squamous cell carcinoma (HNSCC) (Tanimoto et al., 2003) and renal cell carcinoma (RCC) (Clifford et al., 2001). Tanimoto et al. (2003) found CC and CT genotypes of C1772T polymorphism, but none of the subjects had the homozygous genotype TT.

In the present study, the distribution of C1772T genotypes in control groups was approximately similar to those reported in previous studies (Clifford et al., 2001; Tanimoto et al., 2003; Ling et al., 2005). We found no evidence of a relationship between clinicopathological characteristics and C1772T polymorphism in endometrial cancer patients.

On the other hand, evaluation of angiogenesis intensity in endometrial cancer also seems to be an independent prognostic factor and statistically correlates with FIGO stage of disease, histological type and grade of tumor, depth of myometrial invasion and metastasis (Mazurek and Kuc, 2005).

The variable results of these studies do not necessarily contradict the findings presented here, as the aetiology and progression of malignant diseases is multifarious. A limitation of this study is its retrospective design, whereby a survival bias cannot be excluded. The strengths of the study are its relatively high number of participants, as well as the clinically validated phenotypes. Prospective studies are needed to learn more about the role of HIF1A polymorphisms in cancer. This would lead to a better understanding of tumour biology and behaviour and would possibly clarify the inconsistency of data found in recent studies.

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 HIF-1 α increase the risk of endometrial cancer. Thus, further studies with larger samples need to be carried out in order to clarify new mutation sites and the role of this SNP in the oncogenesis of endometrial cancer among Iranian women.

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