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Short communication

An investigation of relationships between hypoxia-inducible factor-1α gene polymorphisms and ovarian, cervical and endometrial cancers

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Abstract

Background: DNA sequence variations in HIF-1α gene might yield changes both in the production outcomes and in the activities of the gene. Overexpression of the HIF-1α subunit, resulting from intratumoral hypoxia and genetic alterations, has been demonstrated in common human cancers and is correlated with tumor angiogenesis and patient mortality. In this study, we aimed to determine how the three single nucleotide polymorphisms (SNPs, C1772T and G1790A exon 12, C111A exon 2) in the HIF-1α gene coding regions affect the ovarian, cervical and endometrial cancer patients in the Turkish population. A study on this relationship has not been conducted to date. *Method:* 102 gynecologic cancer patients and 107 healthy controls were studied. Genotypes of the three polymorphisms were analyzed by PCR-RFLP. *Results:* There was no significant difference between ovarian cancer patients and controls in terms of the distribution of C1772T genotypes and alleles ($P > 0.05$). However, there was a highly significant increase in the frequency of both CT 1772 and TT 1772 genotypes in patients with cervical and endometrial cancers compared with healthy controls. In fact, 1772T allele-carriers (CT + TT genotypes) showed an association with the risk of cervical and endometrial cancers compared to the wild type (OR = 3.84, 95% CI: 1.65–8.93; OR = 7.41, 95% CI: 2.33–23.59, respectively). C1772T polymorphism was not associated with family history concerning gynecologic and/or other cancer types, stages (I–IV) and grades of tumor, smoking habits and existence of other diseases that generate a hypoxic microenvironment even after multivariable logistic regression analysis. As for HIF-1α G1790A genotypes, the frequencies of G alleles were 98% in ovarian patients and 100% in the control group. We found no significant difference in the genotype distribution and allele frequencies between the ovarian patients and healthy control subjects. There were no GA and AA genotypes among the cervical and endometrial cancer patients. As for HIF-1α C111A polymorphism, we did not find CA and AA variants of the gene in controls or in any of the three types of patients. *Conclusion:* Our results suggest that the C1772T polymorphism of the HIF-1α may be associated with cervical and endometrial cancers.

Keywords: HIF-1α; Gynecologic cancers; Polymorphism; Risk factors; Turkish population; Family history; Genomic DNA; Hardy-Weinberg equilibrium

1. Introduction

Tumor vascularization supplies nutrition and oxygen to proliferating cells, cellular adaptation to hypoxia, and strongly correlates with the risk of invasion and metastasis [1]. An important mediator of such cellular adaptation is hypoxia-inducible factor-1 (HIF-1), a critical transcription factor [2]. HIF-1 consists of a constitutively expressed HIF-1ß subunit and one of three subunits (HIF-1α–HIF-3α). HIF-1α forms a heterodimeric complex with HIF-1ß on hypoxic responsive elements and activates transcription of a wide variety of genes which are particularly relevant to cancer [3–6]. Tumors derived from cells lacking HIF-1α or HIF-1ß show significantly reduced vascularization and growth rates compared to parental cells [7,8]. In addition, enhanced

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expression levels of HIF-1α have been reported in human malignancies including colon, breast, stomach, pancreas, prostate, kidney, and esophagus [9–11].

The determination of SNPs is a new means to study the etiology of polygenic disorders with complex inheritance patterns, such as cancer [12]. All regions of the HIF-1α were examined and a total of 35 SNPs in the gene were found [13]. Polymorphisms of the gene have not been associated with some diseases [14], however, some of them were associated with production of HIF protein and reported to be involved in susceptibility to several disorders [14–18].

Angiogenesis of gynecologic cancer is regulated by vascular endothelial growth factor (VEGF), a target gene of HIF-1α [4]. In studies of ovarian cancer, VEGF expression and microvessel density (MVD) have been correlated with poor survival [19]. Birner et al. found that the combination of HIF-1α protein overexpression with nonfunctional p53 indicates a dismal prognosis of ovarian cancer [20]. It has been reported that HIF-1α expression was an independent prognostic marker in early stage cervical carcinoma [21]. Another study suggests that the increase of proliferating cell nuclear antigen (PCNA) index and MVD may enhance development of endometrial carcinoma [22]. Indeed, the role of HIF-1α in the development of ovarian, cervical, and endometrial cancers and their transition to metastases remain to be elucidated.

On the basis of these facts, HIF-1α polymorphisms might contribute to the development and progression of ovarian, cervical, and endometrial cancers. Nevertheless, there have been no published studies on any population so far, as regards to their relationship. Therefore, this study was designed to examine the role of three SNPs, which were located in coding regions of the gene- one in exon 2 (S28Y), and two in exon 12 (P582S, A588T) in the development and progress of ovarian, cervical and endometrial cancers in the Turkish population.

2. Materials and methods

We studied a total of 102 gynecologic cancer patients who were selected from patients admitted to the Departments of Obstetrics and Gynecology, Faculty of Medicine, Gazi University and the Ankara Oncology Education and Research Hospital. A total of 107 subjects were enrolled as healthy controls (mean ages of menarche: 13 ± 0.25 and mean age: 48 ± 0.2 years, range 25–65). The study was approved by the Committee of Ethics of the Gazi University. All cases and controls were of Caucasian origin and became subjects of this study after submitting their written consents. Age of menarche and first diagnosis, history of gynecologic and/or other cancer types in the family, stages (I–IV) and grades of tumor (based on the International Federation of Gynecology and Obstetrics (FIGO) stage method), information on smoking habits and existence of other diseases such as stroke and cardiovascular diseases that generate a hypoxic microenvironment were obtained from the patient files. Gynecologic or other cancers, smoking habits and existence of other diseases were not encountered in the family history of the control individuals. Clinicopathological characteristics of the patients are summarized in Table 1.

Genomic DNA was isolated from peripheral blood by using a DNA extraction kit (Heliosis®, Metis Biotechnology, Turkey) according to the manufacturer's instructions. Amplifications of the C1772T and G1790A regions of the HIF-1α gene were carried out by placing in a Mastercycler gradient (Eppendorf, Germany) thermal cycler, a total volume of 50 μL PCR mixture containing 50 ng genomic DNA, 2.5 mM $MgCl_2$, 100 μM dNTP, 50 pmol/μl of each primer and 1.0 U/μL Taq DNA polymerase.

For the C1772T, the following pair of primers produced a PCR product of 346 bp: forward 5′-AAG GTG TGG CCA TTG TAA AAA CTC-3′, reverse 5′-GCA CTA GTA GTT TCT TTA TGT ATG-3′ (Genbank accession no. AH006957; nucleotides 425–770). We set the PCR cycling conditions for the gene as explained by Ollerenshaw [16]. The

Table 1
Clinicopathological characteristics of the patients

	Cancer		
	Ovarian (%)	Cervical (%)	Endometrial (%)
Number of cases	49 (48)	32 (31)	21 (21)
Mean ages of menarche (years)	13 ± 0.43	13 ± 0.18	13 ± 0.14
Mean ages of first diagnosis (years)	48 ± 0.71	49 ± 0.46	50 ± 0.52
Range (years)	16–74	25–68	39–81
History of gynecologic and/or other cancer types in the family	9 (18.37)	7 (21.8)	9 (42.8)
Stages of tumor[a]			
I	2 (4.08)	5 (15.6)	10 (47.6)
II	5 (10.20)	16 (50.0)	5 (23.8)
III	41 (83.67)	10 (31.3)	5 (23.8)
IV	1 (2.05)	1 (3.1)	1 (4.8)
Histological grade (G1/G2 and G3)	2/47	5/27	10/11
Smoking habits	8 (16.33)	4 (12.5)	1 (4.76)
Existence of other diseases	11 (22.45)	5 (15.6)	5 (23.8)

[a] Stages of tumor (staging was performed according to the current classification of the International Federation of Gynecology and Obstetrics (FIGO)).