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Relationship of common vascular endothelial growth factor polymorphisms and haplotypes with the risk of cervical cancer in Tunisians

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ABSTRACT

Objective: We investigated the association between common vascular endothelial growth factor (*VEGF*) single nucleotide polymorphisms (SNPs) and the risk of cervical cancer (CC) in Tunisian patients and control women.
Methods: Study subjects comprised 86 CC cases and 124 control women. Genotyping of *VEGF* rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068, rs833070, rs3025039 SNPs was done by real-time PCR.
Results: Higher minor allele frequencies (MAF) of rs699947 (−2578C/A) [$P = 0.04$; OR (95% CI) = 1.52 (1.02–2.29)], and rs1570360 (−1154G/A) [$P = 0.04$; OR (95% CI) = 1.58 (1.01–2.47)] were seen in CC cases compared to control women. Marked differences in the distribution of rs699947 ($P = 9 \times 10^{-4}$) and rs1570360 ($P = 0.03$) genotypes were seen between CC cases and control groups; the distribution of the remaining SNPs was comparable between CC cases and control women. The association of rs699947 and rs1570360 with heightened CC risk with was seen in the heterozygous, and more so in the homozygous states. Haploview analysis revealed high LD between rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068 and rs833070 but weak or no LD between rs3025039 and the other SNPs. Seven-locus (rs699947/rs833061/rs1570360/rs2010963/rs25648/rs833068/ rs833070) haploview analysis identified only CTGCCAG haplotype to be positively associated with CC [$P = 0.022$; OR(95% CI) = 1.74 (1.08–2.79)].
Conclusion: Specific *VEGF* variants (rs699947, rs1570360) and haplotype (CTGCCAG) may contribute to the development of CC among Tunisian women.

1. Introduction

Cervical cancer (CC) rates as the third most common malignancy, and the fourth leading cause of cancer-related deaths in women worldwide [1]. Several causative agents contribute to the pathogenesis of CC, of which infection with human papillomavirus (HPV) constitutes the greatest risk factor for CC [2,3]. While HPV infection is necessary for induction of malignancy, it is not sufficient to induce cellular abnormalities, and thus the development of invasive cancer. This was highlighted by the detection of HPV genotypes in cytologically normal women, and in the diagnosis of CC in a fraction of HPV-infected women [4]. Collectively, this suggested that inter-individual genetic variations significantly affect on the susceptibility to CC.

Angiogenesis was shown to be essential for the development, growth, invasion, and metastasis of malignant tumors [5]. Several factors modulate angiogenesis, including vascular endothelial growth factor (*VEGF*), which was shown to be an important initiator and regulator of angiogenesis [6]. VEGF contributes to the progression and prognosis of malignancy through a variety of mechanisms, including modulation of endothelial cell proliferation, survival, and migration [6,7]. Heightened VEGF expression was associated with tumor progression, increased micro-vessel

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density, and with poor prognosis for various solid tumors, including CC [8,9]. This was supported by the finding that HPV oncoproteins enhance tumor angiogenesis by increasing expression of VEGF in CC [10,11].

The human *VEGF* gene maps to chromosome 6p21.3, and consists of eight exons separated by seven introns [12]. Several single nucleotide polymorphisms (SNPs) were reported in the *VEGF* gene, some of which were functional, affecting VEGF secretion [13,14], and thus diseases susceptibility, in particular to conditions linked with altered angiogenesis, such as CC [15]. Of these, two promoter [−1154G/A (rs1573060), −2578C/A (rs699947)], one 5′-untranslated region (UTR) [−634G/C (rs2010963)], and one 3′-UTR [+936C/T (rs3025039)] SNPs have minor alleles correlating with a decreased level of VEGF [14,16,17], whereas −460T/C (rs833061) and −7C/T (rs25648) appear to be associated with higher VEGF expression [14,18]. Associations of these and other *VEGF* polymorphisms [398G/A(rs833068),497G/A(rs833070)] were reported in various types of cancers [13,19], but with inconsistent findings. Our aim was to investigate the association of those eight common *VEGF* SNPs as potential host immune modifiers with CC in Tunisian women.

2. Patients and methods

2.1. Study subjects

From October 2010 to August 2012, 86 women with histologically confirmed cervical carcinoma were recruited from Salah Azeiz Oncology Institute (SAI, Tunis, Tunisia). Cancer diagnosis was established by clinical examination and biopsy findings, and confirmed by two senior SAI pathologists. Clinical data were collected through self-reported questionnaires; review of case records, and from personal interviews. Tumor staging was according to International Federation of Gynecology and Obstetrics (FIGO) classification (www.figo.org). Peripheral blood EDTA anti-coagulated blood specimens were collected from CC patients shortly before radiation therapy or chemotherapy. Controls comprised 124 healthy women unrelated to either cases or controls, and were free of malignancy, drug allergy, hypertension, diabetes, or cardiovascular disease. Genomic DNA was extracted using QIAamp® DNA blood Mini Kit, according to manufacturer's instruction (Qiagen GmbH, Hilden, Germany). Study subjects were from different zones of Tunisia, and were asked to sign a consent form agreeing to participate in the study; all institutional ethics requirements were met.

2.2. VEGF genotyping

Genotyping of *VEGF* rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068, rs833070, and rs3025039 SNPs was performed by the allelic (VIC and FAM-labelled) discrimination method. TaqMan assays, as assay-on-demand, were ordered from Applied Biosystems (Applied Biosystems; Foster City, NJ, USA). The reaction was performed in 6 μl volumes on StepOne/StepOne Plus real-time PCR systems, as recommended by the manufacturer (Applied Biosystems). Replicate, blinded, quality control samples were included to assess reproducibility of the genotyping procedure; concordance was >99%.

2.3. Statistical analysis

Statistical analysis was performed on SPSS v. 20.0 (IBM, Chicago, IL, USA). Data were expressed as a percentage of total (categorical variables). Pearson χ^2 test was used in assessing inter-group significance. Allele frequencies were calculated by the gene-counting method; each polymorphism was tested for Hardy–Weinberg equilibrium using χ^2 goodness-of-fit test using HPlus 2.5 software (http://qge.fhcrc.org/hplus). All analyses were conducted assuming an additive genetic effect, as this is the most conservative mode. Linkage disequilibrium (LD) analysis and haplotype reconstruction was performed using Haploview 4.1 (http://www.broad.mit.edu/mpg/haploview). Logistic regression analysis was performed in order to determine the odds ratios (OR) and 95% confidence intervals (95%CI).

3. Results

3.1. Study design, patients, and controls

Baseline and demographic characteristics of the study population are described in Table 1. The median age was 52 years for patients and healthy controls (range: 30–70 years), with most CC cases being in the 51–60 years category. Among CC patients, 85 (98.80%) were married, 81 (94.20%) used hormonal contraceptives, 20 (23.25%) reported positive family history of cancer, and 24 (28.00%) were pre-menopausal and 62 (72.00%) patients were post-menopausal. Diagnoses of squamous cell carcinoma confirmed by histology as per FIGO revealed: stage I, 27 (31.40%), stage II 30 (34.90%), stage III 25 (29.10%), and stage IV 4 (4.60%). Furthermore, three histological types were identified: squamous cell carcinoma 71 (82.55%), adenocarcinoma 13 (15.12%) and sarcoma 2 (2.33%).

3.2. VEGF alleles

Eight *VEGFA* SNPs were selected for this study based on their minor allele frequency (MAF) of >5% in Tunisians. The allele distributions of rs699947, rs833061, rs1570360, rs2010963, rs25648, rs833068, rs833070, rs3025039 *VEGF* SNPs between CC patients and controls are summarized in Table 2. The genotype distributions of the tested *VEGF* variants did not deviate from Hardy–Weinberg equilibrium ($P > 0.05$). Significantly higher MAF of rs699947 (−2578C/A) [$P = 0.04$; OR (95% CI) = 1.52 (1.02–2.29)],

Table 1
Characteristics of study participants.

Characteristic	Cases ($n = 86$)	Controls ($n = 124$)	P[a]
Age (mean ± SD)	51.3 ± 0.7	44.8 ± 10.6	<0.001
30–40 yr	10 (11.6)[b]	42 (33.8)	<0.001
41–50 yr	30 (35.0)	51(41.2)	0.220
51–60 yr	34 (39.5)	18 (14.5)	<0.001
61–70 yr	12 (13.9)	13 (10.5)	0.290
Marital status			
Married	85 (98.8)	120 (96.8)	0.318
Oral contraceptive use	81 (94.2)	112 (90.4)	0.228
Family history of cancer	20 (23.3)	8 (6.5)	0.000
Menopause status			
Pre-menopausal	24 (27.9)	27 (21.7)	0.195
Post-menopausal	62 (72.1)	97 (78.3)	
FIGIO staging			
Stage I	27 (31.4)	N/A[c]	N/A
Stage II	30 (34.9)	N/A	N/A
Stage III	25 (29.1)	N/A	N/A
Stage VI	4 (4.6)	N/A	N/A
Histology			
Squamous cell carcinoma	71 (82.6)	N/A	N/A
Adenocarcinoma	13 (15.1)	N/A	N/A
Sarcoma	2 (2.3)	N/A	N/A

[a] Pearson's chi square/Fisher's exact test for categorical variables, *t*-test for continuous variables.
[b] Number of subjects (percent total).
[c] N/A = not applicable.