Cytokine 76 (2015) 343–347

Contents lists available at ScienceDirect

Cytokine

journal homepage: www.journals.elsevier.com/cytokine

IL-10 gene promoter and intron polymorphisms as genetic biomarkers of cervical cancer susceptibility among Tunisians

Sabrina Zidi [a], Ezzedine Gazouani [b], Mouna Stayoussef [c], Amel Mezlini [d], Salma K. Ahmed [e], Besma Yacoubi-Loueslati [a], Wassim Y. Almawi [e,*]

[a] El Manar University, Faculty of Sciences of Tunis, Laboratory of Micro-Organisms and Active Biomolecules, 1092 Tunis, Tunisia
[b] Military Hospital of Tunis, Laboratory of Immunology, Tunis, Tunisia
[c] Research Unit of Hematological and Autoimmune Diseases, Faculty of Pharmacy, University of Monastir, Tunisia
[d] Salah Azeiz Oncology Institute, Tunis, Tunisia
[e] Department of Medical Biochemistry, Arabian Gulf University, Manama, Bahrain

ARTICLE INFO

Article history:
Received 31 March 2015
Received in revised form 27 May 2015
Accepted 31 May 2015
Available online 12 June 2015

Keywords:
Cervical cancer
Haplotypes
Interleukin-10
Polymorphisms
Serum levels

ABSTRACT

Objective: We investigated the association between polymorphisms in the promoter and intron regions of the interleukin-10 (*IL-10*) gene with the risk of cervical cancer (CC) in Tunisian patients and control women.

Methods: Study subjects comprised 86 CC cases and 126 control women. Genotyping of *IL-10* intron (rs3024491, rs3024490) and promoter (rs1800872, rs1800871, rs1800896) variants was done by real-time PCR, with defined clusters.

Results: The minor allele frequencies of the five tested *IL-10* SNPs were not significantly different between cervical cancer cases and control women. However, significantly higher frequencies of homozygous minor allele-carriers in cases was seen for rs3024490 ($P = 0.023$), rs1800872 ($P = 0.037$), and rs1800871 ($P = 0.028$). IL-10 serum levels were significantly reduced in rs3024490 T/T vs. G/G genotype carriers, and in rs1800871 T/T than C/C genotype carriers. While carriage of rs1800872 and rs3024491 minor allele was associated with reduced IL-10 secretion, this was not statistically significant. Haploview analysis demonstrated high linkage disequilibrium (LD) among the *IL10* SNPs studied, and only seven haplotypes were common, capturing 98.8% of the total possible haplotypes. Reduced frequency of haplotypes GTCCA ($P < 0.001$) and TGATG ($P < 0.001$) was seen in cervical cancer cases than in control women, thus conferring disease protection nature to these haplotype. This association remained significant for GTCCA ($Pc = 0.006$) and TGATG ($P = 0.045$) after correcting for multiple comparisons.

Conclusion: Specific *IL-10* variants (rs3024490, rs1800872, and rs1800871) and haplotype (GTCCA and TGATG) may contribute to the development of cervical cancer among Tunisian women.

1. Introduction

Worldwide, cervical cancer (CC) still continues to be a serious public health problem [1]. Although it is known that a persistent infection with an oncogenic types of human papillomavirus (HPV) is the main etiologic agent of CC [2], other factors intrinsic to the host like genetic susceptibility or alteration of the immune response, may be critical in the elimination of HP infection and progression tumoregenesis [3–5]. Consequently, host genetic differences may influence the host response against viral infection and thus modify the risk to develop CC [6,7].

Alcocer-Gonzalez et al., have determined high expression levels of *IL-10* in the cervical tumor microenvironment [8]. *IL-10* was identified only in tumor and koilocytic cells, but not in tumor infiltrating lymphocytes, suggesting that *IL-10* producing cells were those transformed by HPV [8]. IL-10 is a Th2 cytokine with both immunosuppressive and anti-angiogenic functions, and may have both tumor-promoting and tumor-inhibiting properties [9,10]. Therefore, we hypothesized that *IL-10* expression in CC can be associated with HPV infection, and this could produce an immunosuppressive microenvironment and accelerates tumor growth. It

* Corresponding author at: Department of Medical Biochemistry, College of Medicine and Medical Sciences, Arabian Gulf University, PO Box 22979, Manama, Bahrain. Tel.: +973 39717118; fax: +973 17271090.
E-mail address: wassim@agu.edu.bh (W.Y. Almawi).

http://dx.doi.org/10.1016/j.cyto.2015.05.028
1043-4666/

has been reported that several important polymorphic sites in the *IL-10* gene, appears to be responsible with modulated levels of *IL-10* [11–13]. The association of *IL-10* promoter SNPs with CC has been reported by several studies, but with inconclusive findings [14–18].

Here, we investigated the association of five common *IL-10* SNPs: −592C>A (rs1800872), −819C>T (rs1800871), −1082A>G (rs1800896), G>T (rs3024491) and G>T (rs3024490) which were linked with varied effect on IL-10 secretion as potential biomarkers for CC.

2. Subjects and methods

2.1. Subjects

This retrospective case–control study was performed from October 2010 to August 2012. Study subjects comprised 86 women with histological confirmed CC patients recruited from Salah Azeiz Oncology Institute (SAI, Tunis, Tunisia) and 126 healthy women free of malignancy, drug allergy, hypertension, diabetes, or cardiovascular disease. Among patients, 15 (17.4%) had family history of cancer, of whom 6 had a positive family history of cervical cancer. Clinical data were collected through self-reported questionnaires, and tumor staging was according to International Federation of Gynecology and Obstetrics (FIGO) classification (www.figo.org). 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.

Blood samples were taken from all participants in EDTA-containing tube for total genomic DNA extraction shortly before to radiation therapy or chemotherapy. Genomic DNA was extracted using QIAamp® DNA blood Mini Kit, according to manufacturer's instruction (Qiagen GmbH, Hilden, Germany).

2.2. IL-10 serum levels

Serum was prepared by centrifuging coagulated blood tubes at 2000*g* for 10 min at room temperature. Samples were tested for IL-10 using human IL-10 sandwich enzyme-linked immunosorbent assay (catalogue number D1000B; R&D Systems, Minneapolis, MN). Assay sensitivity was 3.9 pg/ml, and inter-assay and intra-assay precision (CV%) ranged from 5.9% to 7.5% and 1.7% to 5.0%, respectively.

2.3. IL-10 Genotyping

We selected five polymorphisms in the *IL-10* gene (two intronic and three in promoter region) with a minor allele frequency (MAF) of >5% in Caucasians. *IL-10* genotyping was performed by the allelic (VIC- and FAM-labeled) discrimination method. TaqMan assays, as assay-on-demand, were ordered from Applied Biosystems (Foster City, CA): C_15983669_10 (rs3024491), C_15983670_10 (rs3024490), C_1747363_10 (rs1800872; −592C>A), C_1747362_10 (rs1800871; −819C>T) and C_1747360_10 (rs1800896; −1082A>G). The reaction was performed in 6 µl volume on StepOne/StepOne Plus real-time PCR systems, according to manufacturer's instructions (Applied Biosystems). Replicate blinded quality control samples were included to assess reproducibility of the genotyping reaction and concordance was >99%. Additional quality control measures comprised direct DNA re-sequencing of patient ($n = 40$) and control ($n = 40$) specimens (ABI 3130×l Genetic Analyzer; Applied Biosystems). Genotyping call rate exceeded 99%, with no significant differences between cases and control samples.

2.4. Statistical analysis

Statistical analysis was performed on SPSS v. 21.0 (SPSS Inc., Chicago, IL). Data were expressed as percentages of total (categorical variables) or as mean ± SD (continuous variables). Student's *t*-test was used to determine differences in means, and Pearson χ^2 or Fisher's exact test was used to assess inter-group significance. Allele frequencies were calculated by the gene-counting method, genotypes were tested for departures from Hardy–Weinberg equilibrium (HWE) in the control population using Haploview version 4.2 (http://www.broad.mit.edu/mpg/haploview).

All analyses were conducted under additive genetic effect, as it is the conservative model, using SNPStats software (bioinfo.iconcologia.net/snpstats/). Linkage disequilibrium analysis was performed using Haploview 4.2, and haplotype reconstruction (linear arrangements of alleles on the same chromosome inherited as a unit) was performed by the expectation maximization method (Haploview 4.2). Logistic regression analysis was performed in order to determine the odds ratios (OR) and 95% confidence intervals (95%CI) associated with the cervical cancer risk, taking the control as the reference group. Statistical significance was set at $P < 0.05$; statistically significant differences being designated as boldface in the tables.

2.5. Ethics

The study protocol was approved by the Ethics Committee at Salah Azeiz Oncology Institute in Tunis, Tunisia.

3. Results

3.1. Study subjects

Demographics and clinical characteristics of cases and control groups are described in Table 1. The median age was 51.26 ± 0.70 years for patients and 44.84 ± 10.60 healthy controls.

Of 86 women with CC, 27 (31.40%) women had cervical tumor in stage I, 30 (34.90%) had CC in stage II, 25 (29.10%) in stage III and 4 (4.60%) women had tumor in stage IV. The major histological type were squamous cell carcinoma 71 (82.55%) and adenocarcinoma 13 (15.12%) however, sarcoma was the minor histological type 2 (2.33%).

3.2. Association studies

Genotype distributions of rs3024491 ($P = 0.31$), rs3024490 ($P = 0.23$), rs1800872 ($P = 0.19$), rs1800871 ($P = 0.23$), and rs1800896 ($P = 0.08$) were in Hardy–Weinberg equilibrium among

Table 1
Demographics and clinical characteristics of study population.

Characteristic	Cases ($n = 86$)	Controls ($n = 126$)
Age	51.26 ± 0.70	44.84 ± 10.60
30–40	10 (11.60%)	44 (34.92%)
41–50	30 (35.00%)	51(40.50%)
51–60	34 (39.50%)	18 (14.28%)
61–70	12 (13.90%)	13 (10.30%)
FIGIO staging: Stage I	27 (31.4%)	N/A
Stage II	30 (34.9)	N/A
Stage III	25 (29.1)	N/A
Stage VI	4 (4.6)	N/A
Histology: squamous cell carcinoma	71 (82.55%)	N/A
Adenocarcinoma	13 (15.12%)	N/A
Sarcoma	2 (2.33%)	N/A

FIGO = International Federation of Gynecology and Obstetrics.