European Journal of Obstetrics & Gynecology and Reproductive Biology 158 (2011) 330–333

Contents lists available at ScienceDirect

European Journal of Obstetrics & Gynecology and Reproductive Biology

journal homepage: www.elsevier.com/locate/ejogrb

Association between cytokine gene polymorphisms and cervical cancer in a Chinese population

Qian Wang [a], Chenhua Zhang [b], Shah Walayat [a], Hong Wei Chen [c,*], Yili Wang [a,b,**]

[a] Centre for Cancer Research, First Affiliated Hospital, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China
[b] Key Laboratory of Biomedical Information Engineering of the Ministry of Education, Institute for Cancer Research, School of Life Sciences and Technology, Xi'an Jiaotong University, Xi'an, Shaanxi 710061, China
[c] Department of Radiation Oncology, First Affiliated Hospital of Medical College, Xi'an Jiaotong University, Xi'an, Shaanxi, China

ARTICLE INFO

Article history:
Received 22 April 2010
Received in revised form 17 December 2010
Accepted 19 May 2011

Keywords:
Cervical cancer
Polymorphisms
Risk factor

ABSTRACT

Objectives: It has been hypothesized that inherited cytokine gene polymorphisms could influence susceptibility to cervical cancer. This study evaluated the association between tumour necrosis factor-alpha (TNF-α)-308, transforming growth factor-beta 1 (TGF-β1), interferon-gamma (IFN-γ)+874 and interleukin-10 (IL-10)-1082 gene polymorphisms and cervical cancer risk.
Study design: The study population included 186 histopathologically confirmed cases of cervical cancer and 200 healthy controls. TNF-α, TGF-β, IL-10 and IFN-γ gene polymorphisms were genotyped by polymerase chain reaction with sequence-specific primers.
Results: The IFN-γ+874A/A genotype was associated with high risk for the development of cervical cancer [odds ratio (OR) 2.22, $p = 0.012$], and the A allele was associated with a 1.47-fold increased risk of cervical cancer ($p = 0.009$). In contrast, no significant difference was found in the frequencies of TNF-α-308G/A, TGF-β1 codons 10 and 25 C/C-G/G and IL-10-1082G/A gene polymorphisms between patients with cervical cancer and healthy controls.
Conclusions: Homozygous IFN-γ+874A/T polymorphisms may be associated with increased risk for the development of cervical cancer.

1. Introduction

Worldwide, cervical cancer is the second most common malignancy in women, with approximately 80% of cases arising in developing countries [1]. Although the major risk factor for cervical cancer is infection with specific high-risk types of human papillomavirus (HPV), other factors including host genetic factors appear to play a role in susceptibility and the development of malignancy [2,3]. Recent data have shown that cytokines secreted by tumour cells or immune cells infiltrating and surrounding the tumour tissue affect the final outcome of the antitumour immune reaction by exerting immunomodulatory or suppressive effects [4]. The ability to produce different amounts of cytokines in response to microbe stimulation may vary between individuals, and these differences may be genetically determined [5,6].

Since the immune response plays an important role in defence against viruses and tumours, polymorphisms in genes that potentially affect the immune response are candidates to influence susceptibility to cervical cancer. Various polymorphisms, mainly single nucleotide polymorphisms (SNPs) and microsatellites, have been found to affect gene transcription, leading to individual variations that potentially affect cytokine production. These polymorphisms may be important determinants for disease risk, severity or protection against conditions in which the immune system plays a significant role, such as malignancies. Several recent investigations have focused on a possible association between cytokine gene polymorphisms and the development of certain infectious diseases, allergies, autoimmune disorders and cancers [7,8]. While some genetic polymorphisms have been shown to affect the overall expression and secretion of cytokines, others may only be useful for determining a genetic link with a particular disease-predisposing gene. The aim of the present study was to evaluate the impact of SNPs of tumour necrosis factor-alpha (TNF-α), transforming growth factor-beta (TGF-β), interleukin-10 (IL-10) and interferon-gamma (IFN-γ) genes on susceptibility to cervical cancer in a Chinese population.

* Corresponding author. Tel.: +86 29 82655499; fax: +86 29 82655499.
** Co-corresponding author.
E-mail addresses: chenhwdr@hotmail.com (H.W. Chen), wangyili@mail.xjtu.edu.cn (Y.L. Wang).

0301-2115/$ – see front matter © 2011 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.ejogrb.2011.05.019

2. Materials and methods

2.1. Study population

One hundred and eighty-six patients from the First Affiliated Hospital, Medical College of Xi'an Jiaotong University with squamous cell carcinoma of the cervix were enrolled in this study. The control group comprised 200 healthy blood donor volunteers. Informed consent was obtained from all participants, and the study was approved by the ethics committee of the First Affiliated Hospital, Medical College of Xi'an Jiaotong University.

2.2. Extraction of genomic DNA

Genomic DNA was extracted from granulocyte pellets using the phenol-chloroform extraction method [9]. Briefly, granulocyte pellets were digested by the addition of 300–400 μl lysis buffer (1 M Tris–HCl solution, 0.5 M EDTA, 1% SDS) and 20 μl proteinase K (15.6 mg/ml Roche Diagnostics Mannheim, Germany) at 65 °C for 1 h in a water bath. Lysates were extracted with phenol:chloroform:isopropanol (25:24:1). The DNA in the aqueous phase was precipitated with ice-cold ethanol and 3 M sodium acetate, washed with 70% alcohol, and resuspended in TE buffer (100 mM Tris–HCl, pH 8.0, 1 mM EDTA) after complete air drying at room temperature.

2.3. Cytokine gene polymorphism

All polymerase chain reaction (PCR) amplifications were performed with ready-to-use PCR mix (Tiangen, Beijing, China) by adding DNA (25–100 ng) and 0.1 mM solution of each of the specific primers (Table 1). The final volume was made up to 20 μl with sterile water. SNPs were determined by PCR with sequence-specific primers [10,11]. The reaction conditions were as follows: 95 °C for 1 min; 10 cycles at 95 °C for 15 s, 60 °C for 50 s and 72 °C for 40 s; and 25 cycles at 95 °C for 20 s, 56 °C for 50 s and 72 °C for 50 s, with a final extension of 5 min at 72 °C in the last cycle. The PCR products were separated by electrophoresis on a 2% agarose gel stained with ethidium bromide (0.5 mg/ml), and visualized with ultraviolet light. Internal control primers, by which a 426-base pair human growth hormone sequence was amplified, were used to confirm successful PCR amplification.

Table 1
Primer sequences used in polymerase chain reactions with specific sequence primers for the detection of cytokine gene polymorphisms.

Cytokine gene polymorphisms	Sequence
Tumour necrosis factor-alpha (promoter-308)	Common primer 5′-tct cgg ttt ctt ctc cat cg-3′ G allele primer 5′-ata ggt ttt gag ggg cat gg-3′ A allele primer 5′-ata ggt ttt gag ggg cat ga-3′
Transforming growth factor-beta 1 (codons 10 and 25) Codon 10	Common primer, 5′-tcc gtg gga tac tga gac ac-3′; C allele primer 5′-tcc ggg ctg cgg ctg ctg cc-3′; T allele primer 5′-tcc ggg ctg cgg ctg ctg ct-3′;
Codon 25	Common primer, 5′-ggc tcc ggt tct gca ctc-3′; G allele primer, 5′-gtg ctg acg cct ggc cg-3′; C allele primer, 5′-gtg ctg acg cct ggc cc-3′
Interleukin-10 (promoter −1082)	Common primer 5′-cag tgc caa ctg aga att tgg-3′ G allele primer 5′-cta cta agg ctt ctt tgg gag-3′ A allele primer 5′-cta cta agg ctt ctt tgg gaa-3′
Interferon-gamma (intron 1 +874)	Common primer, 5′-tca aca aag ctg ata ctc ca-3′ T allele primer 5′-ttc tta caa cac aaa atc aaa tct-3′ A allele primer 5′-ttc tta caa cac aaa atc aaa tca-3′
Internal control	Primer 1 5′-gcc ttc cca acc att ccc tta-3′ Primer 2 5′-tca cgg att tct gtt gtg ttt c-3

2.4. Statistical analysis

Statistical analysis was performed using Statistical Package for the Social Sciences (SPSS) Version 11.5 (SPSS Inc., Chicago, IL, USA). The expected numbers were calculated in accordance with the Hardy–Weinberg equilibrium theory. The distribution of cytokine gene polymorphisms in patients with cervical cancer and healthy controls was compared using Chi-squared test or Yates' correction. A p-value <0.05 was considered to be statistically significant.

3. Results

The median age of the 186 patients with cervical cancer was 54 years, with a range of 30–75 years. Diagnoses of squamous cell carcinoma were confirmed by histopathological examination as International Federation of Gynecology and Obstetrics stage I (1%), II (50%) and III (49%). The median age of the 200 healthy controls was 42 years, with a range of 18–55 years.

Genotyping for TNF-α-308G/A, TGF-β1 codons 10 and 25 C/C-G/G, IL-10-1082G/A and IFN-γ+874T/A gene polymorphisms revealed the distribution of these cytokine genotypes between the patients with cervical cancer and healthy controls (Table 2). Examination of these four gene polymorphisms at genotype level confirmed that almost all allele frequencies fall in Hardy–Weinberg equilibrium: the only exception was TGF-β1 codons 10 and 25 C/C-G/G ($\chi 2 = 24.57$; $P < 0.01$). No significant difference was found in genotype frequencies of TNF-α-308G/A, TGF-β1 codons 10 and

Table 2
Cytokine gene polymorphisms in patients with cervical cancer and healthy controls.

	Patients		Controls		p-Value	OR (95% CI)
	n	%	n	%		
TNF-α-308						
Genotype frequency						
G/G: low producer	149	80.1	144	72		ref
G/A + A/A: high producer	37	19.9	56	28	0.063	0.64 (0.39–1.03)
Allelic frequency						
G allele	328	88.1	334	83.5		ref
A allele	44	11.9	66	16.5	0.064	0.68 (0.45–1.02)
TGF-β codons 10 and 25						
Genotype frequency						
T/T-G/G: high producer T/C-G/G: high producer T/C-G/C: high producer	137	73.7	150	75	0.640	ref
C/C-G/G: intermediate producer T/T-G/C: intermediate producer	40	21.5	37	18.5		1.18 (0.72–1.96)
C/C-G/C: low producer C/C-C/C: low producer T/T-C/C: low producer T/C-C/C: low producer	9	4.8	13	6.5		0.76 (0.31–1.83)
IL-10-1082						
Genotype frequency						
G/G: high producer	24	12.9	21	10.5		ref
G/A: intermediate producer	85	45.7	76	38.0		0.98 (0.50–1.90)
A/A: low producer	77	41.4	103	51.5		0.65 (0.34–1.26)
Allelic frequency						
G allele	133	35.8	118	29.5		ref
A allele	239	65.2	282	70.5	0.064	0.75 (0.55–1.02)
IFN-γ+874						
Genotype frequency						
T/T: high producer	21	11.3	38	19.0		ref
T/A: intermediate producer	84	45.2	96	48.0		1.58 (0.86–2.91)
A/A: low producer	81	43.6	66	33.0	0.012	2.22 (1.19–4.15)
Allelic frequency						
T allele	126	33.9	172	43.0		ref
A allele	246	55.1	228	57.0	0.009	1.47 (1.10–1.97)

OR, odds ratio; CI, confidence interval.