Available online at www.sciencedirect.com
SCIENCE DIRECT
Cancer Letters 218 (2005) 63–68
CANCER Letters
www.elsevier.com/locate/canlet

# *Lymphotoxin-α* polymorphism and the risk of cervical cancer in Japanese subjects

Yoshimitsu Niwa[a,*], Kaoru Hirose[b], Keitaro Matsuo[b], Kazuo Tajima[b], Yoko Ikoma[c], Toru Nakanishi[c], Akihiro Nawa[c], Kazuo Kuzuya[c], Akiko Tamakoshi[a], Nobuyuki Hamajima[a]

[a]*Department of Preventive Medicine/Biostatistics and Medical Decision Making, Nagoya University Graduate School of Medicine, 65 Tsurumai-cho, Showa-ku, Nagoya 466-8550, Japan*
[b]*Department of Epidemiology and Prevention, Aichi Cancer Center Research Institute, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan*
[c]*Department of Gynecology, Aichi Cancer Center Hospital, 1-1 Kanokoden, Chikusa-ku, Nagoya 464-8681, Japan*

Received 4 June 2004; received in revised form 27 August 2004; accepted 7 September 2004

**Abstract**

To examine the possible association between cervical cancer and *Lymphotoxin-α* (*LTα*) polymorphisms, C804A and A252G, an incident case–control study was conducted in Japanese. The cases were 131 cervical cancer patients. Controls were 320 healthy women. Risk estimation was conducted by an unconditional logistic model. Complete linkage disequilibrium was seen between *LTα* C804A and *LTα* A252G. We found that, compared with the *804CC* genotype, *804CA* and *804AA* were associated with a decreased risk of cervical cancer (OR = 0.64, 95% CI = 0.40–1.02; and OR = 0.45, 95% CI = 0.21–0.95, respectively), especially of SCC (OR = 0.54, 95% CI = 0.32–0.91; and OR = 0.39, 95% CI = 0.16–0.92, respectively).


*Keywords:* *LTα* C804A polymorphism; *LTα* A252G polymorphism; Cervical cancer; Polymerase chain reaction with confronting two-pair primers

## 1. Introduction

There are many studies suggesting that human papillomavirus (HPV) plays an important role in the pathogenesis of cervical cancer [1,2]. In HPV-related cervical cancer, the two viral oncoproteins (E6 and E7) expressed by cancer cells impair the control of cell replication. The E7 protein of HPV-16, an oncogenic HPV, expressed by human uterine cervical cancer cells is also released in the extracellular compartment where it induces immune suppression [3].

It is not known what is decisive for the progression and regression of HPV-induced cervical lesions, but there is some evidence that host immune surveillance is of great importance to the limited growth and regression of these lesions. Immunosuppressed women with renal allografts were found to be at

* Corresponding author. Tel.: +81 52 744 2133; fax: +81 52 744 2971.
*E-mail address:* ytt-niwa@xg7.so-net.ne.jp (Y. Niwa).

0304-3835/$ - see front matter © 2004 Elsevier Ireland Ltd. All rights reserved.
doi:10.1016/j.canlet.2004.09.021

greater risk of developing cervical intraepithelial neoplasia and HPV infection [4].

It has been suggested that there is stromal and epithelial infiltration by T-lymphocytes in high-grade squamous intraepithelial lesions (SIL) [5], but not in low-grade SIL [6]. The leukocyte migration process is controlled by adhesion molecules expressed on vascular endothelial cells, which include members of the selectin family, such as E-selectin, P-selectin, members of the immunoglobulin superfamily such as intercellular adhesion molecule 1 (ICAM1) and vascular cell-adhesion molecule 1 (VCAM1), and their ligands expressed on lymphocytes [7]. The upregulation of these adhesion molecules is important to controlling the migration of lymphocytes to sites of inflammatory or immunologic activity [8]. Coleman and Stanley reported an enhanced expression of vascular adhesion molecules in high-grade SIL, but not in low-grade SIL [9]. Their findings suggest that the expression level of adhesion molecules is functionally important in enabling the local recruitment of immunocompetent cells in high-grade cervical disease.

Ozaki et al. reported that functional single-nucleotide polymorphism in the *lymphotoxin-α* (*LTα*) gene on chromosome 6p21 in the HLA class III region was related to myocardial infarction susceptibility [10]. The polymorphism is from 804C to 804A in exon 3, resulting in the amino-acid substitution Thr26Asn. In their report, although both wild-type (26Thr) and variant (26Asn) LTα stimulated mRNA expression of *vascular cell-adhesion molecule 1* (*VCAM1*), LTα 26Asn had a two-fold higher level of transcriptional activity for *VCAM1* than did LTα 26Thr. In addition, LTα 26Asn exhibited significantly higher mRNA expression of *E-selectin* mRNA in human artery smooth-muscle cells, whereas LTα 26Thr did not affect its expression. Ozaki et al. have also reported that the *LTα* A252G polymorphism in intron 1 was associated with its transcriptional activity.

In analysis of loss of heterozygosity in invasive cervical cancer and cervical intraepithelial neoplasia (CIN) at chromosome 6p21, the genetic deletions at the site have been reported frequently in high-grade CIN and low-grade CIN as well as in invasive cervical cancer [11]. These data suggest that the 6p21 genetic alterations in tumor cells occur early in cervical carcinogenesis and that the chromosome site may be critical to the development of cervical cancer.

We hypothesized that the *LTα* genetic polymorphism was associated with cervical cancer risk, since enhanced expression of VCAM1 and E-selectin in a local cervical environment can encourage the cervical stromal endothelial cells to recruit lymphocytes and to support the local antineoplastic immune response in high-grade cervical intraepithelial lesions. To investigate whether *LTα* C804A and *LTα* A252G polymorphisms influence the likelihood of cervical cancer, we conducted an incident case–control study in Japanese subjects.

## 2. Materials and methods

### 2.1. Study subjects

The cases comprised 131 cervical cancer patients who were diagnosed at Aichi Cancer Center Hospital between October 2001 and November 2003, and they were enrolled in the Hospital-based Epidemiologic Research Program at Aichi Cancer Center II (HERPACC-II) [12]. All were histologically confirmed to have cervical cancer, including 87 cases with primary squamous cell carcinoma (SCC) and 44 with adenocarcinoma (ADC) or adenosquamous carcinoma (ADSC). Controls consisted of examinees, who attended a health checkup provided by the Nagoya municipal government on 3 days during 2000. Written informed consent for the anonymous use of their residual blood for research purposes was obtained from 468 of 489 (95.7%) examinees. After excluding males and those with a past history of cancer, 320 blood samples were analyzed. All these subjects were asked to complete an informed consent form for genotyping as well as a self-administered questionnaire. The Aichi Cancer Center has an ethical committee to review studies related to genomic tests, and this committee approved our genetic polymorphism examinations (approval numbers 11-12 and 41-2).

### 2.2. Laboratory methods

DNA was extracted from buffy-coat fraction, using a QIAamp DNA Blood Mini Kit (Qiagen, Valencia,