Polymorphic Genotypes of Enzymes Involved in Carcinogen Metabolism as Risk Factors for the Development of Human Cervical Cancer

Moritoshi Kinoshita*, Tamiko Seno*, Sadahito Shin*, Toshihiro Aono**

Summary Polymorphisms of the genes for enzymes participating in the metabolism of several carcinogens have been identified. These polymorphisms are associated with varieties in the enzymatic activity or substrate specificity, and thus additive or multiplicative risks for development of various human cancers. In this study, we compared the genotype frequencies of four enzymes (CYP1A1, CYP2D6, N-acetyltransferase and glutathione S-transferase) from cervical cancer patients and healthy control individuals, to estimate the importance of polymorphisms in human papillomavirus infection and development of cervical cancer. The frequency of the Slow acetylator N-acetyltransferase genotype differed significantly between cervical cancer patients and healthy controls (P < 0.05, \( \chi^2 = 4.24 \)). No significant difference was found between these two groups in the frequency of any of the other genotypes studied. These findings suggest that the arylamine compounds metabolized by N-acetylation play an important role in human cervical carcinogenesis.

Key words: arylamine N-acetyltransferase, cervical cancer, polymorphism, carcinogen metabolism

Introduction

Human papillomavirus (HPV) is detected in most patients with cervical cancer, and a close association has been found between the infectivity of HPV and the characteristics of epithelial lesions of carcinoma of the uterine cervix*. Since not all HPV-infected patients necessarily develop carcinoma of the uterine cervix, as shown by recent epidemiological studies, it is essential that the molecular mechanisms of tumorigenesis following infection with HPV is determined**. Cigarette smoking and sexual intercourse with many and unspecified partners have been suspected as two of the causes of cervical cancer. Recently, polymorphisms of several enzyme genes involved in the metabolism of carcinogens have been identified. These polymorphisms have profound effects on the enzymatic activity or substrate specificity of these enzymes, and are associated with additive or multiplicative risks in the development of bronchogenic carcinoma in cigarette smokers* and of bladder cancer*. In this study, we estimated the importance of polymorphisms of relevant genes for enzymes playing roles in the metabolism of carcinogens as host risk factors for the development of invasive carcinoma of the uterine cervix following HPV infection. We focused our research interest on poly-
morphisms of the following enzyme genes: 1) CYP1A1 (cytochrome P450 IA1), involved in oxidation of polycyclic hydrocarbons, and induced by foreign chemicals such as benzo [a]-pyrene; 2) CYP2D6 (cytochrome P450 IID6), involved in debrisoquine/sparteine metabolism, for which two distinct phenotypes due to genetic polymorphism, an extensive metabolizer (EM) and a poor metabolizer (PM), have been defined; 3) arylamine N-acetyltransferase (NAT), involved in metabolism of primary aromatic amines or hydrazine, for which there are three polymorphic genotypes, "Rapid acetylator", "Intermediate" and "Slow acetylator" and 4) glutathione S-transferase class μ (GST1), involved in the detoxification of electrophilic compounds through conjugation to glutathione.

Identification of CYP1A1, CYP2D6, NAT genotypes and the GST1 gene

We developed simple assays using the polymerase chain reaction (PCR) to determine the genotypes of CYP1A1, CYP2D6 and NAT, as well as the presence of the GST1 gene. All PCR reactions were performed in a DNA thermal cycler (Techne) over 40 cycles with 1 μM primers and a Gene Amp kit (Perkin-Elmer Cetus) under the conditions recommended by the kit supplier. Each reaction cycle included denaturation at 94°C for 1 min, primer annealing at 55°C for 1.5 min, and primer extension at 72°C for 1.5 min.

(a) CYP1A1

To determine polymorphic mutations of either ATT (Ile) or GTT (Val) in exon 7 of the CYP1A1 gene, genomic DNA was amplified by PCR using a 'Forward' primer and mismatched 'Reverse' primer, as shown in Fig. 1. This procedure formed a new Ncol restriction site (CCATGG) from the Ile type CYP1A1 gene. Following PCR, amplified DNA fragments were digested with the restriction endonuclease Ncol and resolved by electrophoresis in a 3% agarose gel. Since the Ile-type fragment could be digested with Ncol, while the Val-type fragment was retained, the CYP1A1 genotype was identified as Ile/Ile, Ile/Val or Val/Val.

(b) CYP2D6

We determined the polymorphic phenotypes corresponding to the EM and PM genotypes of the CYP2D6 gene, by means of PCR-restriction fragment length polymorphism (RFLP) with a sense primer at positions 428-452 (5'-GCCTGGGCAA-GAAAGTCGCTGGAGCA-3') and an antisense primer at positions 650-669 (5'-ACCTCGCGCAGAAAGCC-CA-3') of the CYP2D6 gene. The resulting 242 bp fragment was digested with restriction endonuclease BstN1. The fragment from the EM gene allele was digested, whereas that from the PM gene allele lacking the first guanine base in exon 4 was not.

Materials and Methods

Materials

Tissue and blood specimens were obtained from patients with cervical cancer at the Department of Obstetrics and Gynecology, Tokushima University Hospital. Prior to this study, tissue specimens had been obtained by single punch biopsy of the cervix in the colposcopy clinic. These specimens were histological in diagnosis using the Papanicolaou smear. Patients studied by biopsy included 22 with invasive carcinoma between 32 and 77 years of age. Peripheral leukocytes were obtained from the blood, (to which EDTA-2Na was added) of 79 healthy volunteers in Otsuka Pharmaceutical Co., Ltd., Tokushima, and of the 22 patients with the tumor.

DNA isolation

DNA was isolated and purified from tissue specimens (wet weight, 40-80 mg each) or peripheral leukocytes using conventional methods.

HPV detection

HPV was detected and its type identified by Southern blot hybridization with DNA probes as described.
Fig. 1 Determination of the CYP1A1 genotype using the method shown in (a), and electrophoretic profiles in 3% agarose gel (b)

(a) PCR was performed using a sense oligonucleotide primer (dGTTAAGTGAGAAGGTGATTA) and a mismatched antisense primer (dGGATAGCCAGGAAGAGAAAGACCTCCCAGCGGGCCA). For the Ile allele, the resulting fragment contained the Ncol restriction site. (b) The amplified fragments were incubated with (D) or without (N) NcoI. Panel 1 shows an example of the Ile/Ile genotype; both fragments from two alleles were completely digested. Panels 2 and 3 show that amplified fragments from two heterozygous alleles (Ile/Val) and a homozygous allele (Val/Val) were digested partly and not at all, respectively.

(c) NAT

Figure 2 shows the identification of the NAT phenotypes, which include the Rapid, Intermediate and Slow acetylators, from polymorphic NAT genotypes. Gene 1 of NAT has restriction sites for both TaqI and BamHI in the PCR product, while the BamHI site is lost in Gene 2 and the TaqI site in Gene 3. The Rapid acetylator phenotype had the genotype Gene 1/Gene 1, that of the Intermediate acetylator for the heterozygous genotypes Gene 1/Gene 2 or Gene 1/Gene 3, and that of Slow acetylator for homozygous or heterozygous combinations of Genes 2 and 3.

(d) GST1

The presence or absence of the GST1 gene was determined using a sense primer from the 5' end of exon 4 (5'-CTGCCCTACTTTGATTGATGGG-3') and an antisense primer from the 3' end of the
The oligonucleotide primers used in the PCR for determination of NAT genotypes are shown in (a), and the genotype analysis of the cervical cancer patient MA is shown in (b).

(a) PCR was performed using N1 (sense) and N2 (antisense) primers for TaqI digestion, and N3 (sense) and N4 (antisense) primers for BamHI digestion. (b) Panel 1 shows that the amplified fragments were incubated with (D) or without (N) TaqI, revealing complete digestion. Panel 2 shows that two alleles were present in patient MA (No. 8 in Table 1). Patient MA was considered to have an Intermediate acetylator genotype (Gene1/Gene2).

exon 5 (5'-CTGGATTGTAGCATCATC-3') as described. The absence of a band corresponding to 273 bp indicated the homozygous deletion of the GST1 gene. In this study, a homozygous deletion was considered to be present when PCR fragments of other enzyme genes appeared in the same DNA specimen.
Table I  Clinical characteristics of cervical cancer patients

<table>
<thead>
<tr>
<th>No.</th>
<th>Name</th>
<th>Age (year)</th>
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<th>Clinical stage</th>
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<th>CYP2D6</th>
<th>NAT</th>
<th>GST1</th>
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</table>

* Kinoshita et al., 199310.

Results

The findings obtained from the 22 patients with cervical carcinoma are summarized in Table 1. Ten patients with invasive carcinoma were positive for HPV16, while two were positive for HPV18 and one for HPV31. HPV-DNA was undetectable in nine patients. We compared the enzyme genotype frequencies between cervical cancer patients and healthy control subjects.

(a) CYP1A1 (Table 2)

The frequencies of various CYP1A1 genotypes among healthy controls were 60.8% for Ile/Ile, 32.9% for Ile/Val, and 6.3% for Val/Val. The corresponding frequencies among cervical cancer patients were 54.5%, 45.5%, and 0%, respectively. No significant difference was found between the cervical cancer and healthy control groups in the frequency of the Ile/Ile or other CYP1A1 genotypes ($\chi^2 = 0.28$).
(b) CYP2D6

All of the cervical cancer patients and healthy controls were positive by EM. In this study, neither the PM allele nor the intermediate phenotype was detected in any individual.

(c) NAT (Table 3)

The frequencies of NAT phenotypes identified were as follows: 57.0% Rapid acetylator, 32.9% Intermediate acetylator and 10.1% Slow acetylator in the group of healthy controls and 59.1, 13.6 and 27.3% respectively in the group of cervical cancer patients. The frequency of Slow acetylators among cervical cancer patients differed significantly from that among the healthy controls ($P < 0.05, \chi^2 = 4.24$).

(d) GST1 (Table 4)

The presence of the 273 bp band was considered to indicate the presence of at least one GST1 gene. Among the 79 healthy volunteers, 40 (50.6%) were positive for this band, while 63.6% of the cervical cancer patients was positive. There was no significant difference in the incidence of the 273 bp band between these two groups ($\chi^2 = 1.21$).

Discussion

Genetic polymorphism of CYP1A1 has been reported, and is accompanied by a
variation in activity or substrate specificity. Although the Val/Val phenotype was considered to indicate a high risk for lung cancers associated with cigarette smoking, our data failed to associate it with cervical cancer.

Individuals with a complete deletion of the GST1 gene are reportedly at high risk for lung cancer. In this study, no significant difference was found in the frequency of a homozygous deletion of the GST1 gene between the cervical cancer and healthy control groups. This finding suggests that GST1 plays no role in the pathogenesis of cervical cancer.

No polymorphism of the CYP2D6 gene was identified in the first guanine base of exon 4. It may be necessary to determine other polymorphic sites in the coding region of the CYP2D6 gene.

N-acetylation polymorphism has been implicated in susceptibility to bladder cancer, systemic lupus erythematosus, and other diseases. In this study, a significant difference was found between cervical cancer patients and healthy controls in the frequency of the Slow acetylator genotype. This finding suggests that HPV-infected patients with the Slow acetylator genotype may be disposed to the progression to cervical cancer.

In our study, HPV-DNA was detected in 13 out of 22 patients with invasive cervical carcinoma. These included HPV16, HPV18 and HPV31. Among these patients, no correlation was found between enzyme polymorphisms and any of the clinical stages, histological diagnosis, types of HPV, oncogenes (c-myc, N-myc, c-erbB-2, H-ras, N-ras) amplification or chromosomal integration of HPV-DNA (data not shown). We showed that in almost all patients with invasive carcinoma of the uterine cervix, HPV-DNA was integrated into the host cell genome, but not, in any individual with benign diseases such as dysplasia or condyloma acuminatum. Concerning the relationship of HPV infection to tumorigenesis, we propose that arylamine compounds that cannot be metabolized by Slow acetylators promotes progression to cervical cancer by increasing, the chromosomal integration of HPV-DNA. Although the mechanism of this promotion is unknown, the Slow acetylator NAT genotype appears to be an indication of risk for progression to cervical cancer in humans.

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