No Evidence of Correlation between Polymorphism at Codon 72 of p53 and Risk of Cervical Cancer in Japanese Patients with Human Papillomavirus 16/18 Infection

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Abstract

Human papillomavirus (HPV)-16 and -18 encode E6 oncoprotein, which binds to and induces degradation of the tumor suppressor protein p53. A common polymorphism of p53, encoding either proline or arginine at position 72, affects the susceptibility of p53 to E6-mediated degradation in vivo; Caucasian women homozygous for arginine 72 reportedly are about seven times more susceptible to HPV-associated carcinoma of the cervix than heterozygotes. To examine whether arginine 72 could be a risk factor for HPV-associated cervical carcinomas in the Japanese population, we used the same PCR-based assay to analyze p53 genotypes of HPV-positive invasive cervical carcinomas from 103 Japanese women versus 110 control samples. Inasmuch as we detected no significant difference in the frequencies of proline or arginine alleles between the two groups, p53 polymorphism at residue 72 does not seem to be involved in the development of HPV-associated cervical carcinomas in women of Japanese ethnicity.

Introduction

HPV is commonly associated with the development of cervical carcinomas, in particular types 16, 18, 31, 33, 39, 45, 51, 52, 56 and 58 (1, 2). HPV-16 and -18 are the types most frequently detected in high-grade squamous intraepithelial lesions and invasive carcinomas (2). HPV-16 and -18 each encode two viral oncoproteins, E6 and E7, that cooperatively contribute in vitro to the immortalization of human keratinocytes (3–9) and extend the life span of cultured human fibroblasts (10). E7 protein can bind and inactivate the cellular tumor suppressor protein Rb (11); E6 protein, on the other hand, binds to the tumor suppressor protein p53 and induces its degradation through the ubiquitin pathway (12, 13). Mutant p53 protein is present in most human tumors, but p53 is usually wild type in primary cervical carcinomas (14–16), indicating that inactivation of p53 by E6 probably plays a crucial role for cervical carcinogenesis.

Alan et al. (17) recently investigated whether a common p53 polymorphism, either Arg or Pro at position 72, could affect HPV-associated tumorigenesis. They reported that women who were homozygous for Arg-72 were about seven times more susceptible to development of cervical cancer than heterozygotes. To examine whether p53Arg at position 72 could represent a risk factor for HPV-associated cervical carcinomas in the Japanese population, we analyzed the p53 genotypes of 110 control samples and 103 HPV-positive invasive cervical carcinomas from Japanese women, using the PCR-based assay described by Alan’s group (17).

Materials and Methods

Tissue Specimens and DNA Extraction. Snap-frozen tumor samples from Japanese women who had undergone biopsy or surgery for cervical carcinoma at the University of Tokyo Hospital and its associated hospitals between 1993 and 1995 provided a panel of 103 HPV-positive invasive cervical carcinomas for our study. Cellular DNA was extracted by a standard SDS-protease K procedure (18). Samples for the control group consisted of DNA extracted from whole blood from 110 healthy Japanese women.

Detection and Typing of HPV. The presence and type of HPV were determined by a PCR-based assay (L1-PCR) described by Yoshikawa et al. (1), with slight modifications (19). The L1 region was amplified in 40 PCR cycles of 1.5 min at 95°C, 1.5 min at 48°C, and 2 min at 70°C, using consensus L1 primers L1C1 (5’-CTGAAAAGCTTTTCCATTTTTTTTTTTTTTTT-3’, 1 μM), L1C2 (5’TACCCCTATATCTGATTG-3’, 0.5 μM), and L1C2M (5’TACCCCTATATCTGATTG-3’, 0.5 μM). Each reaction product (10 μl) was electrophoresed through a 4% agarose gel, stained with ethidium bromide, and viewed under UV light. HSV types were identified on the basis of RFLP. Initial typing of amplified HPV DNA fragments was performed by double digestion with Ddel and RsaI, and then confirmed by digestion with at least three enzymes selected from Accl, AluI, BstXI, FokI, HaeIII, Hinfl, KpnI, Mael, MaeIII, PstI and Xbol. This assay can type at least 26 registered genital HPV types (6, 11, 16, 18, 30, 31, 33–35, 39, 42–45, 51–56, 58, 59, 61, 66, 68, and 70; Refs. 1, 20).

PCR Amplification of Polymorphic p53 Sequences. Analysis of the p53 genotype at position 72 was performed as described by Alan et al. (17) with some modifications. Pro sequences were detected by PCR using primers p53Pro+/p53− and Arg with primers p53+/p53Arg−; 500 pmol of each primer was end-labeled with 3P by polynucleotide kinase in a total volume of 50 μl containing 2.5 μCi [α-3P]dATP. PCR amplification was performed in 25-μl reaction volumes containing 50 ng of cellular DNA, 20 pmol of each labeled primer, 1.5 mm dNTP, 67 mM Tris-HCl (pH 8.8), 16.6 mM (NH4)2SO4, 10 mM β-mercaptoethanol, 6.7 mM EDTA, 3 mM MgCl2, and 1.25 units of EX Taq DNA polymerase (TaKaRa). PCR conditions were as follows: 25 cycles of 30 s at 94°C, 30 s at 55°C (for p53Pro+/p53−) or 60°C (for p53+/p53Arg−), and 30 s at 72°C. Reaction products (3 μl each) were fractionated on an 8% polyacrylamide gel, dried, and exposed to X-ray film.

Results and Discussion

The PCR-based assay that we used to analyze p53 genotypes specifically detects either the Pro or Arg allele (Fig. 1). Table 1 summarizes the results of our analysis. The allele frequencies in our Japanese control group were slightly different from those in the Caucasian controls reported by Alan et al. (17); homozygosity for p53Pro in our control group was slightly more common (0.17 versus 0.05), and Pro/Arg heterozygosity slightly less common (0.46 versus 0.58). When we compared the frequencies of the two alleles in HPV-positive invasive carcinomas with those in the control group, we failed to detect any difference between the two groups (Table 1). We also found no difference even when we selected carcinomas that were positive for only HPV-16 or -18 (Table 1).

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3 The abbreviations used are: HPV, human papillomavirus; LOH, loss of heterozygosity.
NO RELATION BETWEEN p53 POLYMORPHISM AND CERVICAL CANCER

Because LOH on chromosome 17p has been reported in 15–22% of cervical cancers (21, 22), the frequency of our cervical cancer patients homozygous for p53Pro or Arg may be overestimated. We performed PCR genotyping for LOH status of the tumor DNAs that were homozygous for p53Pro or Arg using three microsatellite markers, D17S1520 (17p12: Ref. 23), TP53 (17p13.1: Ref. 24), and D17S513 (17p13.3: Ref. 25). Of the 52 samples, 6 tumors (all homozygous for p53Arg) showed homozygosity for all of the three markers, suggesting in linkage disequilibrium with it. In HPV-associated cervical carcinomas other than Arg/Pro at codon 72 may influence the ability of E6 of Pro versus Arg alleles between healthy women and individuals with HPV-positive cervical carcinomas, even the high-risk HPV-16 or 18. Of 103 HPV-positive carcinoma samples, 51 (49.5%) had HPV-16 and 23 (22.3%) had HPV-18. The fact that we detected no significant difference in the frequencies of Pro versus Arg alleles between healthy women and individuals with HPV-positive cervical carcinomas, even the high-risk HPV-16 or -18 types, could be explained in several ways: (a) the p53 sequence needed for E6 to induce degradation might be influenced by factors that vary according to ethnic background; for example, polymorphisms other than Arg/Pro at codon 72 may influence the ability of E6 to degrade p53 in Japanese patients; (b) the sampled groups in both studies might have been too small to determine accurately the role of the codon-72 polymorphism, and/or other polymorphic sites that are in linkage disequilibrium with it, in HPV-associated cervical carcinomas; and (c) this p53 polymorphism may not be related to HPV-associated tumorigenesis at all. Larger epidemiological studies should be undertaken among a variety of populations.

References


17. Fig. 1. PCR amplification of polymorphic p53 sequences from cellular DNA of controls and cervical carcinomas. The primer pair Pro+/p53– amplifies a product of 177-bp (Pro allele), and the primer pair p53+/Arg– amplifies a product of 144-bp (Arg allele). Positive controls were amplified using as templates cellular DNAs whose p53 polymorphic sequence had been determined by direct sequencing. In the negative control, distilled water served as the template.
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