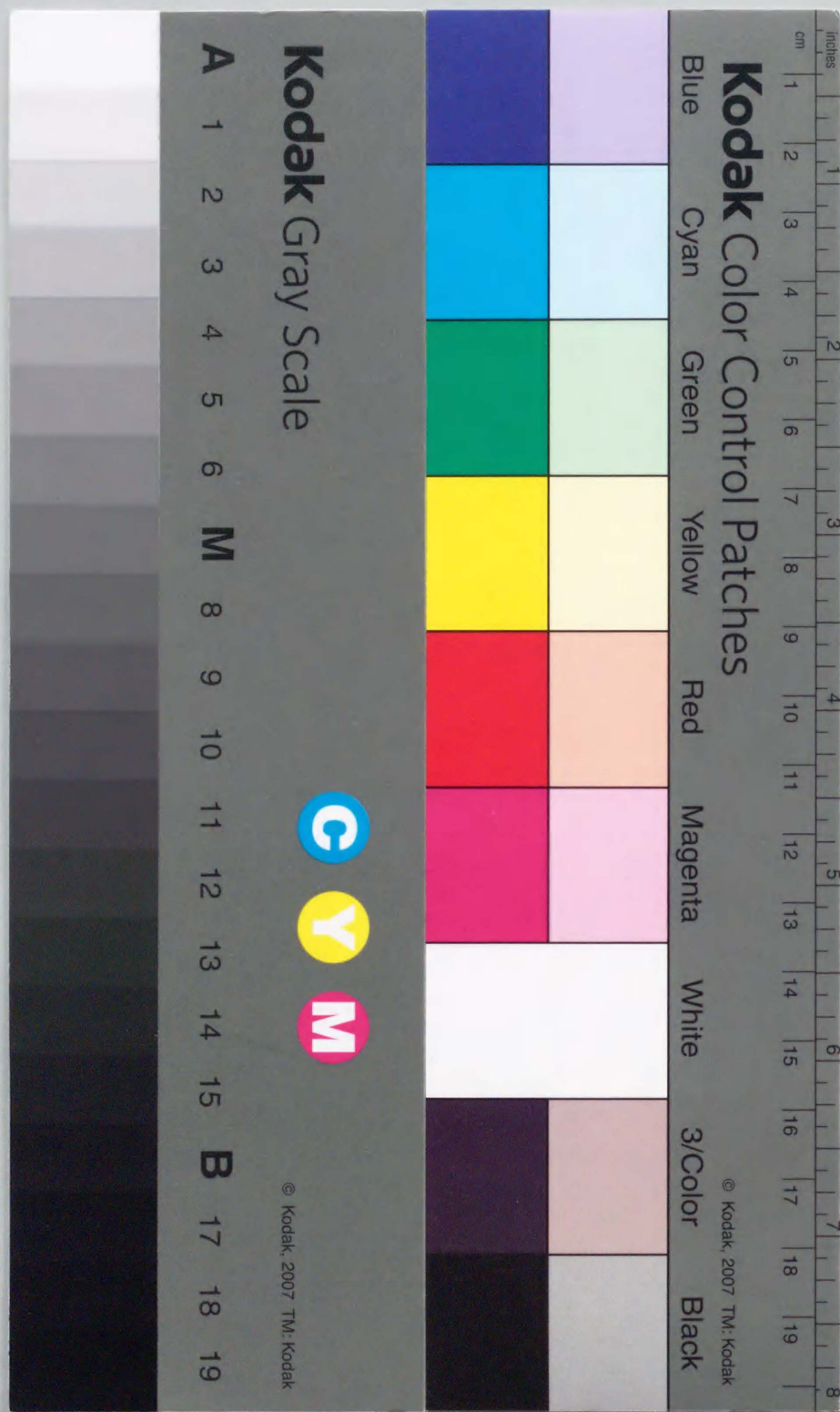


p53 Polymorphism in Human Papillomavirus-associated Esophageal Cancer

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p53 Polymorphism in Human Papillomavirus-associated Esophageal Cancer¹Hidetoshi Kawaguchi,² Shinji Ohno, Koshi Araki, Mitsuhiro Miyazaki, Hiroshi Saeki, Masayuki Watanabe, Shinji Tanaka, and Keizo Sugimachi

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Abstract

Human papillomavirus type 16/18 (HPV-16/18) is implicated in the pathogenesis of squamous cell carcinoma (SCC) of the cervix and esophagus. The arginine allele at codon 72 of p53 was found to be more susceptible to degradation by HPV E6 protein than is the proline allele *in vivo*, thus resulting in a high frequency of cervical SCC in individuals homozygous for arginine at the codon. There are controversial results from several clinical studies of cervical SCC. In the present study, encoding regions of p53 codon 72 and HPV-16/18 E6 were directly sequenced, using pairs of primary esophageal SCC tissue and corresponding normal mucosa, which were from 75 patients (Japanese, $n = 38$; Chinese, $n = 37$). The arginine allele alone was detected in 70.6% (12 of 17) of HPV-positive cases but only in 43.1% (25 of 58) of HPV-negative cases ($P < 0.05$). In contrast, such a significant correlation between p53 polymorphism and HPV infection was not evident in corresponding normal mucosae. Because our findings between tumor specimens and the normal mucosae differed, we suggest that the frequent loss of proline allele in HPV-associated carcinogenesis of the esophagus major plays some role. The particular type of p53 polymorphism may indicate a potential candidate for HPV-associated SCC.

Introduction

The HPV³ found in the anogenital tract can be classified as either high risk or low risk according to the association with cancer. HPV-16/18 is the most common of the high-risk group, and ~90% (1) of cervical SCC contain DNA of HPV-16/18 and 10–20% are found in esophageal SCC (2–4). HPV-16/18 encodes E6 protein, which binds to cellular tumor-suppressor protein p53 and directs degradation through the ubiquitin pathway (5). This event is mediated by another cellular protein termed E6-AP, a component of the ubiquitin pathway (6, 7). On the basis of these experiments, it is widely assumed that p53 is functionally inactivated by the viral E6 protein in HPV-associated cancer cells and that infection with high-risk HPV types leads to the same phenotype as a loss of p53 function because of *p53* gene mutations.

The association of p53 codon 72 polymorphism with HPV-associated cervical SCC risk has been studied by several groups but with inconsistent results. Storey *et al.* (8) reported that the form of the p53 protein carrying an Arg residue at this position was found to be significantly more susceptible to *in vivo* degradation by the E6 protein than was the Pro form. More importantly, the Arg allele in the codon 72 polymorphism of the *p53* gene was found to be in excess in

patients with cervical SCC. Data in the literature are controversial (9–14).

A part of esophageal SCC correlates with the presence of HPV-16/18 (2, 3). However, p53 polymorphism in esophageal SCC has apparently not been documented. We investigated the genotypic frequency of p53 codon 72 polymorphism and HPV-16/18 in esophageal SCC patients in east Asia. The data we obtained seem to be the first regarding association of this polymorphism with HPV-associated risk for cancer of the esophagus. In this study, we investigated the cause of the inconsistent frequency of p53 codon 72 polymorphism in HPV-associated SCC.

Materials and Methods

Tissue Specimens. Pairs of primary esophageal SCC tissue and corresponding normal mucosa were obtained from 38 Japanese patients who underwent surgery in the Department of Surgery II, Kyushu University Hospital, from 1993 to 1998, and from 37 Chinese patients who underwent surgery in the Chinese Academy of Medical Sciences, Beijing, China, between 1996 and 1998. No patient had been given prior treatment. In all cases, the histopathological type of the tumors was squamous cell carcinoma. Cancer tissues and well-separated normal esophageal mucosae obtained from surgically resected esophageal SCC patients were immediately snap-frozen, and these were kept in liquid nitrogen. Genomic DNA was prepared by proteinase K digestion and phenol/chloroform extraction, followed by ethanol precipitation, as described (15).

HPV Detection and Typing. Purified genomic DNA was amplified by PCR for HPV-16 and HPV-18. Oligodeoxynucleotide primers were as follows: HPV-16, forward, 5'-GAATCCATATGCTGTATGTGATAAAT-3', and reverse, 5'-GATGATCTGCAACAAGACATACATC-3'; and HPV-18, forward, 5'-ACCTGTGTATATTGCAAGACAGT-3', and reverse, 5'-TGTTTCTCTCGCTCGTTGGCT-3'. Amplified PCR products were then sequenced on a Perkin-Elmer 310 ABI automated sequencer, using each forward primer.

p53 Polymorphism Analysis. Purified genomic DNA was amplified by PCR for exon 4 of p53, using oligodeoxynucleotide primers as follows: forward, 5'-TGAGGACCTGGTCCTCTGAC-3'; and reverse, 5'-AGAGGAATCCCAAAGTTCCA-3'.

Amplified PCR products were then sequenced using the forward primer. The Arg/Pro type was scored if the area under the guanine peak was reduced to <50% of its cytosine allele or if the area under the cytosine peak was reduced to <50% of its guanine area.

Statistical Analysis. χ^2 test was used to examine the correlation between the p53 codon 72 polymorphism of the esophageal SCC patients and the presence of HPV-16/18; odds ratio were also calculated.

Results and Discussion

Frequency of HPV-16/18 among Esophageal SCC Patients. The same 75 DNA samples (Japan, $n = 38$; China; $n = 37$) were analyzed for the presence of oncogenic HPV, using two independent, PCR-based methods and type-specific (HPV-16 or -18) oligonucleotide PCR primers. HPV DNA was detected in 17 of 75 cases (22.6%). We found no differences in the frequency and types of HPV infection between patients from Japan and China. These results are similar to previous reports in Japan and China (2–4).

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³ The abbreviations used are: HPV, human papillomavirus; SCC, squamous cell carcinoma; LOH, loss of heterozygosity; E6-AP, E6-associated protein.

Arg Allele at the Codon 72 in HPV-associated Esophageal SCC.

Direct sequencing was done on 75 DNA samples from the tumor specimens to analyze the association between codon 72 polymorphism and HPV-associated esophageal SCC (Fig. 1).

Frequency of the two alleles in a series of samples of HPV-positive, esophageal SCC was compared with frequency in the HPV-negative

Table 1 Arg and Pro alleles of p53 in SCCs of esophagus and breast carcinoma

	Pro	Pro/Arg	Arg
Esophageal SCC (<i>n</i> = 75) ^a	19 (25.3)	19 (25.3)	37 (49.3)
HPV negative (<i>n</i> = 58)	16 (27.6)	17 (29.3)	25 (43.1)
HPV positive (<i>n</i> = 17)	3 (17.6)	2 (11.8)	12 (70.6) ^b
Breast carcinoma (<i>n</i> = 17)	5 (29.4)	7 (41.2)	5 (29.4)

^a Numbers in parentheses are percentages.

^b *P* < 0.05.

Table 2 Arg and Pro alleles of p53 in normal mucosa of the esophagus

	Pro/Pro	Pro/Arg	Arg/Arg
Normal mucosa (<i>n</i> = 75) ^a	15 (20.0)	37 (49.3)	23 (30.7)
HPV negative (<i>n</i> = 58)	12 (20.7)	29 (50.0)	17 (29.3)
HPV positive (<i>n</i> = 17)	3 (17.6)	8 (47.1)	6 (35.3)

^a Numbers in parentheses are percentages.

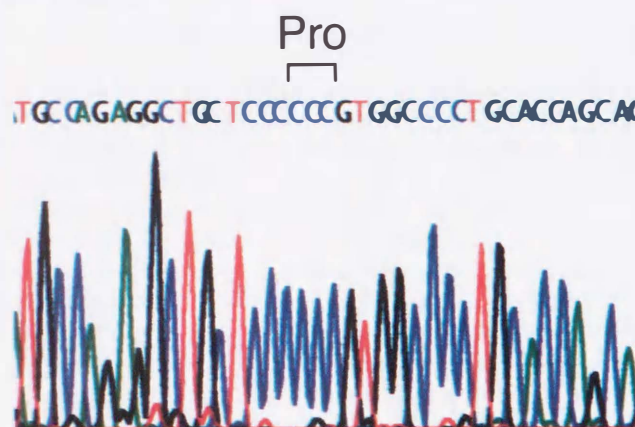
group. There was a marked difference in the frequency of Pro/Arg alleles between HPV-positive and HPV-negative groups. The p53 Arg allele alone was detected in 12 of 17 (70.6%) of the HPV-positive group, compared with 25 of 58 (43.1%) of the HPV-negative group. The Pro allele alone genotype or Pro/Arg genotype was found in 5 of 17 (29.4%) of the HPV-positive group, compared with 32 of 58 (55.2%) of the HPV-negative group. Consequently, an individual homozygous for p53 Arg would be more likely to develop HPV-associated esophageal SCC than would a Pro/Arg heterozygote or a Pro homozygote (*P* < 0.05; odds ratio, 3.17; 95% confidence interval, 1.02–9.85).

Arg Allele at the Codon 72 in the Surrounding Normal Mucosa in HPV-associated Esophageal SCC. The frequency of the presence of Arg allele alone from the tumor specimen was high 12 of 17 (70.6%; Table 1) compared with the frequency found in cases of lung cancer (16, 17). This means there is the possibility of a frequent LOH in this allele, although Story *et al.* (8) hypothesized that the rate of LOH at this locus was low. To examine this hypothesis, we analyzed normal mucosa to exclude the influence of LOH (Table 2). Differences in p53 polymorphism in the corresponding normal mucosa were nil between HPV-positive and -negative tissues (*P* = 0.64; odds ratio, 1.32; 95% confidence interval, 0.42–4.13). In the HPV-positive group, six of eight cases displayed LOH in the eight patients in whom the Pro/Arg type was detected using the corresponding normal mucosa (Tables 1 and 2). Interestingly, all of the six LOH cases had lost the Pro allele, yet the Arg allele was never lost (Fig. 2).

The p53 tumor-suppressor protein accumulates rapidly through posttranscriptional mechanisms and is also activated as a transcriptional factor, thus leading to growth arrest or apoptosis when DNA damage has occurred (18). The ubiquitin-dependent proteolytic pathway plays a major role in selective protein deregulation. The E6 oncoprotein of oncogenic HPV-16/18 uses this cellular proteolytic system to target the p53 protein (5). The E6 oncoprotein binds to a cellular protein of E6-AP, and the E6-E6-AP complex interacts with p53, resulting in the rapid ubiquitin-dependent degradation of p53 (19). The level and half-life of p53 in E6 immortalized cell lines or in HPV-positive cervical carcinoma cells are generally decreased (20). Certain HPV types such as HPV-16/18 found in the SCC of cervix and esophagus suggest a model by which E6 degrades cell growth control by elimination of the p53 tumor suppressor protein and leads to HPV-associated cervix and esophageal SCC.

Our studies revealed the potential role of the loss of the Pro allele in HPV-associated carcinogenesis of the esophagus. The relationship between the targeted LOH of the p53 gene and HPV infection warrants ongoing studies.

Case 1



Case 2



Case 3

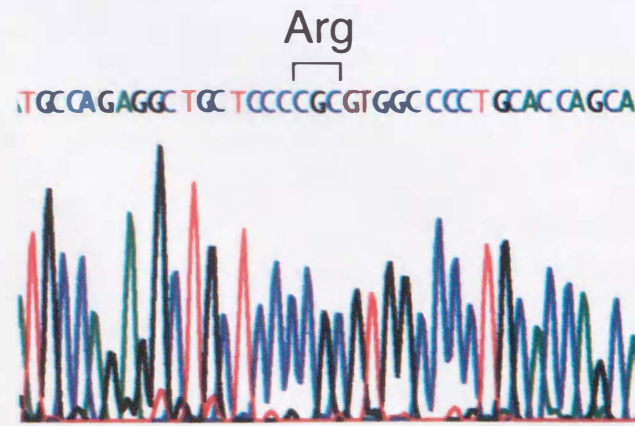


Fig. 1. Direct sequencing of codon 72. Direct sequencing of PCR products. Case 1, Pro/LOH or Pro/Pro; Case 2, Arg/Pro; Case 3, Arg/LOH or Arg/Arg.

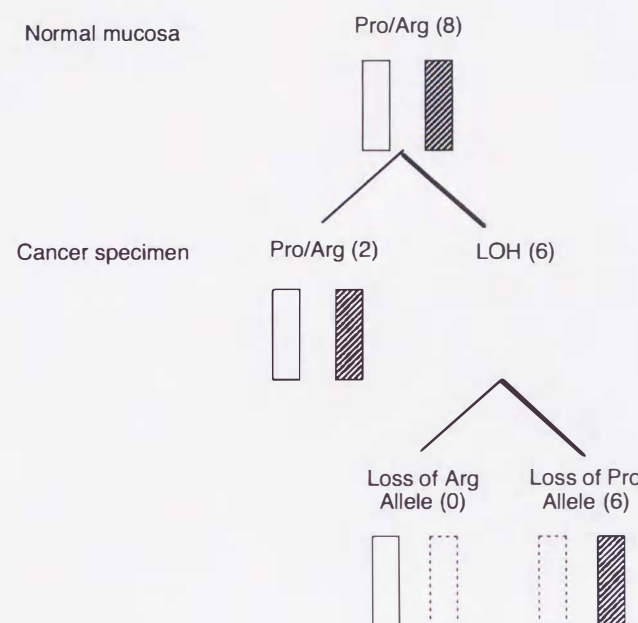


Fig. 2. Schema polymorphism of codon 72 in HPV-associated esophageal SCC. Direct sequencing detected eight cases of the Arg/Pro type of codon 72 polymorphism in the normal mucosa in HPV-positive patients. All six with LOH had lost the Pro allele, in cases of esophageal SCC.

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