

## Short Communication

# Risk of cervical cancer is not increased in Chinese carrying homozygous arginine at codon 72 of p53

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**Summary** Homozygous arginine at codon 72 (HA72) of p53 was found in 22% of normal cervixes and 30.0% of cervical cancers and no significant difference was detected between normal and cervical cancer with or without HPV 16/18. There was no correlation between HA72 and risk of cervical cancer in Chinese.

**Keywords:** p53 codon 72 polymorphism; cervical cancer; Chinese

Cervical cancer is the commonest lower genital tract cancer found among Hong Kong Chinese. Human papillomavirus (HPV) infection has been found in over 80% of cervical cancers locally (Ngan et al, 1997). HPV plays an important role in cervical cancer due to degradation of p53 by the HPV E6 protein via the ubiquitine pathway (Werness et al, 1990). Storey et al (1998) found that women with homozygous arginine-72 (HA72) in p53 have a sevenfold increase in the risk of cervical cancer, perhaps because HA72 is more susceptible to E6-mediated degradation. However, subsequent studies in three Caucasian populations and one Japanese population have failed to support their findings (Hayes et al, 1998; Lanham et al, 1998; Minaguchi et al, 1998; Rosenthal et al, 1998). Since the frequency of p53 polymorphism varies amongst populations dwelling at different latitudes (Beckman et al, 1994), and Hong Kong is nearer the equator than the four reported studies, this study aims to determine the risk of cervical cancer in women with HA72 in p53 in Hong Kong Chinese.

## MATERIALS AND METHODS

### DNA samples

One hundred and two patients with cervical cancer and 68 women with normal cervixes were studied. DNA samples (stored at  $-70^{\circ}\text{C}$ ) left from a previous study on p53 and HPV in cervical cancer (Ngan et al, 1997) were used in this study.

### PCR amplification of p53 codon 72 polymorphic alleles

p53 arginine and proline sequences were separately amplified from each sample with primers as described by Storey et al (1998). Polymerase chain reaction (PCR) was done in a volume of 25  $\mu\text{l}$  containing 100 ng total cellular DNA, 200  $\mu\text{M}$  of each deoxy-nucleotide triphosphate, 0.625 U of Ampli *Taq* DNA polymerase

(Perkins-Elmer Cetus), 1  $\times$  reaction buffer containing 1.25 mM magnesium ion. PCR was carried out in a Thermal Cycler (Perkins-Elmer Cetus) under conditions as follows: 40 cycles at  $94^{\circ}\text{C}$  for 30 s,  $60^{\circ}\text{C}$  for 30 s and  $72^{\circ}\text{C}$  for 30 s. The resulting PCR products were run in a 3% agarose gel and made visible under UV by ethidium bromide staining. The arginine PCR product was 141 bp and the proline PCR product was 177 bp.

### Detection of HPV

Results of HPV 16 and 18 from a previous study (Ngan et al, 1997) were used. The procedure used to detect HPV 16 and 18 E6 was essentially that described previously (Ngan et al, 1994). Briefly, DNA was extracted and subjected to two PCR assays using specific primers for HPV 16 and HPV 18 E6. PCR products were run on 4% agarose gels and blotted onto nylon membranes and hybridized with specific probes to HPV 16 and 18 E6 products. DNA from Caski and Hela were used as positive controls for HPV 16 and 18 respectively, and DNA derived from the C33 cell line and water were used as negative controls.

### Statistical analysis

Chi-square test was used to analyse nominal data. A *P*-value of  $< 0.05$  was considered significant.

## RESULTS

HPV 16 or 18 E6 was detected in 78 samples (76.5%) of cervical cancer. Frequencies of arginine and proline p53 alleles in normal cervixes and cervical cancer are shown in Table 1. In normal cervical tissue, 22% had HA72. This was not significantly different from cervical cancer with or without HPV infection having 32% and 25% of HA72 respectively ( $\chi^2$  test,  $P > 0.05$ ).

## DISCUSSION

The rate of arginine homozygosity in codon 72 polymorphism of p53 in normal cervical tissue of the local Chinese population was 0.22. This was much lower than that reported in other populations

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**Table 1** Frequency of arginine (Arg) and proline (Pro) p53 alleles in normal cervixes and in cervical cancer

	No.	Pro	Arg	Pro/Arg
Cervical cancer				
HPV-positive	78	15 (0.19)	25 (0.32)	38 (0.49)
HPV-negative	24	6 (0.25)	6 (0.25)	12 (0.50)
Normal cervixes	68	8 (0.12)	15 (0.22)	45 (0.66)

in higher latitudes (Hayes et al, 1998; Lanham et al, 1998; Minaguchi et al, 1998; Rosenthal et al, 1998). In fact, Beckman et al (1994) found an increasing frequency of A2 allele (i.e. arginine) p53 polymorphism with increasing latitude and suggested that it may be ethnically related. Both Lanham et al (1998) and Rosenthal et al (1998) reported on UK population at a latitude of 52° where the HA72 rate was 0.57–0.68. Hayes et al (1998) reported on a population in Northern Holland living at a latitude of 52° where the HA72 rate was 0.57, a figure close to that in the UK. Minaguchi et al (1998) reported on a Japanese population living at a latitude of 36° where the HA72 rate was 0.36. Though both Chinese and Japanese are Orientals, the Japanese in that study were residing at higher latitude than the Hong Kong Chinese. In our study, Hong Kong is at a latitude of 23° and has 0.22, the lowest HA72 rate of any study. Combining the data from the above reports, our resulting observation is consistent with that of Beckman et al (1994). However, the biological significance of these findings is not apparent.

A recent study by Storey et al (1998) showed an increased susceptibility to degradation of p53 with HA72 by the HPV E6 protein and hence an increased risk of developing cervical cancer. In their study, a sevenfold increase in the risk of cervical cancer

was found in HA72 carriers compared to heterozygous carriers. However, in our study as well as in other studies (Hayes et al, 1998; Lanham et al, 1998; Minaguchi et al, 1998; Rosenthal et al, 1998), no increase in the risk of cervical cancer in HA72 carriers was found. This may be the reason why cervical cancer is still quite common in our population despite the low HA72 rate. The clinical significance of HA72 in cervical cancer needs further exploration.

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