

Human papillomavirus 16/18 and nasopharyngeal carcinoma

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Abstract

Sixteen cases of nasopharyngeal carcinoma (eight anaplastic and eight well differentiated squamous types) were examined for the presence of human papillomavirus types 16 and 18 genomes using the polymerase chain reaction on paraffin wax embedded biopsy specimens. Although nasopharyngeal carcinoma, particularly the anaplastic type, is strongly associated with Epstein-Barr virus, other factors may be involved in its pathogenesis. No DNA of either human papillomavirus subtype was detected. It is concluded, therefore, that these two "high risk" types of human papillomavirus are not implicated in the pathogenesis of nasopharyngeal carcinoma. The number of cases in this series was small, however, and further studies are warranted using fresh biopsy material and including other viral subtypes.

Using molecular biology techniques, human papillomavirus (HPV), particularly type 16 has been found to be associated with some types of squamous carcinomas of the upper aerodigestive tract.¹⁻³ No attempt has been made thus far, however, to detect the presence of the HPV genome in nasopharyngeal carcinoma, common in Hong Kong Chinese people. We therefore decided to analyse a small series of cases of nasopharyngeal carcinoma for HPV 16 and 18, the types most commonly associated with malignancy in humans,⁴ using the polymerase chain reaction (PCR). This method was selected because of its sensitivity, specificity, and its suitability for use with small, paraffin wax embedded biopsy specimens.

Methods and results

Sixteen cases of nasopharyngeal carcinoma, eight anaplastic (WHO type 1) and eight well differentiated squamous (WHO type 3), were selected from the files of the Department of

Pathology, University of Hong Kong. The slides were reviewed and the original diagnoses confirmed. DNA was extracted from dewaxed sections of the biopsy material. The oligonucleotide primers used in the PCR for HPV 16 were complementary to the upstream regulatory region⁵ of the viral DNA, while for HPV 18 they were complementary to sequences in the E6 region⁶ (table). These regions were chosen because they are almost always retained even if the HPV DNA is integrated in tumours. In each case three 16 µm thick sections were cut from the paraffin wax embedded tissues and were dewaxed with sequential washes of xylene, 95% ethanol, and absolute ethanol and then vacuum dried. The sections were resuspended in 63.5 µl of 0.1 mm EDTA, boiled for 11 minutes, and snap cooled to 0°C to release and denature the DNA before PCR analysis. Amplifications of HPV 16 or 18 target DNA sequences were carried out using a DNA amplification kit (Perkin Elmer Cetus) and programmable thermal controller under the following conditions: initial template denaturation at 94°C for 1.5 minutes followed by annealing at 37°C for two minutes, extension at 72°C for three minutes, and denaturation at 94°C for one minute. This sequence was repeated for a total of 39 cycles. For the fortieth cycle annealing was followed by prolonged extension for 10 minutes at 72°C. The amplified products were heated at 68°C for five minutes, snap cooled at 0°C, and were analysed by gel electrophoresis in 4% agarose. The gels were subsequently stained with ethidium bromide and photographed under ultraviolet light. No HPV DNA of types 16 or 18 was noted in any of the 16 cases or the negative controls, only in the positive control. CaSki and HeLa cell lines DNA were used as positive controls for HPV 16 and 18, respectively. The negative control consisted of a typical PCR reaction mix without the template DNA. Control primers were successfully amplified in these PCR reactions, verifying that there were no inhibitory elements present in the extracted DNA. The amplified products were further analysed by Southern blotting of the gel followed by probing of the membrane

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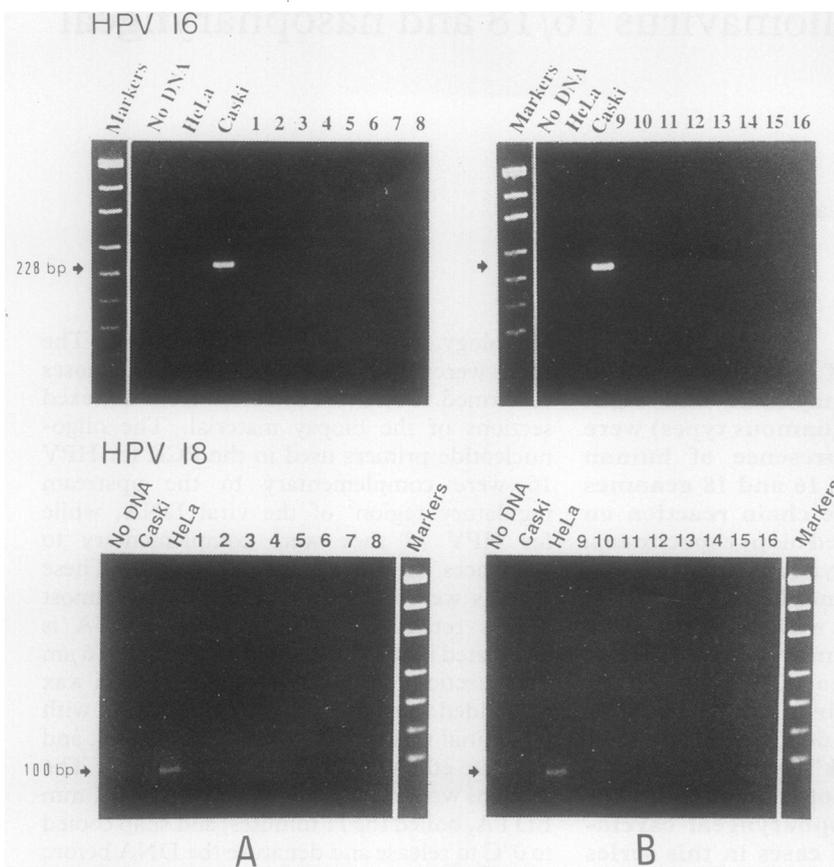
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Sequences of oligonucleotide primers

HPV type	Sequence (5' → 3')	Genomic location	Size of amplified product (base pairs)
HPV 16	A: AAG GCC AAC TAA ATG TCA C (19-mers)	7763-7781	228
	B: GCG GAT CCT GTC TGC TTT TAT ACT AA (26-mers)	61-86	
HPV 18	A: ACC TTA ATG AAA AAC CAC GA (20-mers)	371-390	100
	B: CGT CGT TGG AGT CGT TCC TG (20-mers)	451-470	



4% agarose gels stained with ethidium bromide showing absence of PCR amplified DNA products of HPV 16 and HPV 18 in cases 1–8 from anaplastic (A) and in cases 9–16 from well differentiated squamous (B) nasopharyngeal carcinoma. *HpaII* digested pUC19 DNA was used as marker.

with ^{32}P -labelled genomic probes specific for HPV 16 and 18 DNA which confirmed the above results. The hybridisation and washing conditions were identical with those previously reported by our laboratory.⁷

Discussion

Nasopharyngeal carcinoma is a major health problem among Hong Kong Chinese, and in 1987, the most recent year for which official figures are available, 1099 new cases were notified to the Hong Kong Cancer Registry, from a population of about 5.5 million, making it the second commonest malignancy reported that year. The common anaplastic form ("lymphoepithelioma" type, WHO type 3) has long been known to have a strong association with Epstein-Barr virus (EBV), and viral DNA has recently been demonstrated in the tumour cells by in situ hybridisation techniques.^{8,9} The much less common, well differentiated

squamous variety (WHO type 1), however, which accounts for about 2% of the cases seen in our department, has a less clearly defined association with EBV and might also, theoretically, be more likely to be related to HPV infection because of its differentiation. Moreover, other factors have been postulated as aetiological agents—carcinogens and mutagens in salted fish consumed in childhood.¹⁰ It would not be unreasonable to postulate, therefore, that the "high risk" human papillomaviruses might be involved. As is clearly shown in the figure, however, no HPV DNA of types 16 or 18 was detectable in any of our 16 cases of nasopharyngeal carcinoma studied. The PCR is an extremely sensitive method for detecting specific DNA sequences, and in theory requires only a single copy of the target sequence. In our laboratory we have had no difficulty detecting the EBV genome using this method in cases of anaplastic nasopharyngeal carcinoma on paraffin wax embedded material (Dickens P, *et al* unpublished observations). We conclude that HPV types 16 and 18 do not seem to be implicated in the pathogenesis of nasopharyngeal carcinoma of anaplastic or well differentiated squamous types, although the number of cases in this series is small. It would be interesting, however, to study a larger number of cases using fresh biopsy material and also look for a wider range of HPV subtypes.

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