

비인강암에서 중합효소반응을 이용한 유두종 바이러스의 검출

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Detection of Human Papillomavirus using Polymerase Chain Reaction(PCR)
in Nasopharyngeal Carcinoma

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-ABSTRACT-

Background and Objectives : Nasopharyngeal carcinoma (NPC) is an unique epithelial malignancy which occurs at a high frequency in certain regions of Southeast Asia. Epstein-Barr virus (EBV) has been proposed to be closely associated with NPC. Human papillomavirus (HPV) is associated with a proportion of upper aerodigestive tract carcinomas. We hypothesized that HPV might also contribute to the pathogenesis of NPC. This study attempted to detect HPV DNA in nasopharyngeal carcinoma by polymerase chain reaction (PCR). **Materials and Methods** : We reviewed thirty patients with nasopharyngeal carcinoma from 1995 to 2000 at the Department of Otolaryngology, Pusan National University Hospital. Thirty paraffin-embedded tissues were selected to PCR using type-specific primer pairs. Control group was selected twenty normal adenoid tissue. **Results** : HPV DNA was detected in 3/30 (10%) of NPC specimens, and in none of control specimens. WHO class I nasopharyngeal carcinomas (three cases) were all HPV type 6 positive. **Conclusion** : These results suggest that HPV type 6 may also be involved in the carcinogenesis of EBV-negative squamous cell nasopharyngeal carcinoma. (J Clinical Otolaryngol 2004;15:103-108)

KEY WORDS : Nasopharyngeal carcinoma · PCR · HPV.

서 론

(glandular epithelium),

10

1

가 .

,

0.4%

.

, 가

가

: 2004 4 11

: 2004 5 13

: , 602 - 739

1가

. EBV

¹⁾

가

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²⁾³⁾

WHO
(keratinizing squamous cell carcinoma), 2
(non - keratinizing squamous cell carcinoma), 3
(undifferentiated carcinoma)
WHO 1, 2 60% 가 WHO
3 40% 가 5)
EBV 2 3 20
6)
(HPV) 24 74
46.1 18
12 1.5 : 1
7)
PCR을 이용한 HPV 검출
HPV
Oligonucleotide primer
HPV 6,11,16,18,31,33 oli-
gonucleotide primer Queen Korn
DNA synthesizer . HPV
base pair 6 280, 11

Table 1. Sequences of oligonucleotide primers

Primers	Nucleotide sequences	Amplimer length
-globin	+5' TGACGGGGTCACCCACACTGTGCC 3' - 5' CTAGAACCATTGGGGTGGACGATG 3'	267 bp
HPV 6	+5' TAGTGGGCCTATGGCTCGTC 3' - 5' TCCATTAGCCTCCACGGGTG 3'	287 bp
HPV 11	+5' GGAATACATGCGCCATGTGG 3' - 5' CGAGCAGACGTCCTCCTCG 3'	360 bp
HPV 16	+5' TGCTAGTGCTTATGCAGCAA 3' - 5' ATTTACTGCAACATTGGTAC 3'	152 bp
HPV 18	+5' AAGGATGCTGCACCGGCTGA 3' - 5' CACGCACACGCTTGGCAGGT 3'	216 bp
HPV 31	+5' CTACAGTAAGCATTGTGCTATGC 3' - 5' ACGTAATGGAGAGGTGCAATAACCC 3'	153 bp
HPV 33	+5' ATGATAGATGATGTAACGCC 3' - 5' GCACACTCCATGCGTATCAG 3'	455 bp

360, 16 152, 18 216, 31 153, 33 455
(Table 1).

DNA

10 µm
1.5 ml xylene 1 ml
5 12,000 rpm
5 ethanol 1 ml 가
90% ethanol 75% ethanol
ethanol 10
1.5 ml TEN buffer 300 µl,
10% SDS 25 µl proteinase - K(10 mg/ml) 20 µl
37 14 400 µl phenol/
chloroform/isoamyl alcohol(25 : 24 : 1) 가
5 12,000 rpm chlo-
roform/isoamyl alcohol (24 : 1) 400 µl
1 5 M NaCl 10 µl, cold
absolute ethanol 1 ml -20 2

12,000 rpm 20
. DNA pellet 70% ethanol 1 ml
12,000 rpm 5
30 30 µl PCR
. DNA -
globin test 267 bp
single band가 DNA HPV

. - globin test
DNA DNA

(PCR)
PCR 25 µl primer(10 pmole/µl)
2.5 µl, deoxynucleotide (2.5 mM) 2 µl, 10X
buffer 2.5 µl, MgCl₂(25 mM) 1.5 µl AmpliTag
Gold™(Perkin - Elmer, Norwalk, USA) 1 unit DNA
template 4 µl
PCR Programmable thermal cycler(Perkin -
Elmer 2400, Norwalk, USA) 95

:

10 Tag polymerase 95
30 denaturation, 55 30 anealing,
72 1 extension
40 , 72 10
post - extension .

PCR

PCR 10 µl
6X loading buffer(0.25% bromphenol blue, 0.25%
xylene cyanol, 30% glycerol in water) 2 µl
2% (agarose gel) 100 volt, 20
ethidium bromide

통계처리

Pearson's ² - test
p<0.05 .

결 과

30 WHO 1 3 (10%), 2 12
(40%) 3 15 (50%) 3 가 . 30
3 (10%) HPV
20
HPV . HPV
3 WHO 1 , WHO 2 3
HPV가
(p<0.001)(Table 2). HPV
3 HPV 6 (Fig. 1).

고 찰

Table 2. Number of nasopharyngeal carcinoma according to WHO classification (N=30)

	Cases (%)	HPV positive (%)
WHO type I	3 (10)	3 (100)*
WHO type II	12 (40)	0 (0)
WHO type III	15 (50)	0 (0)

* : p<0.001

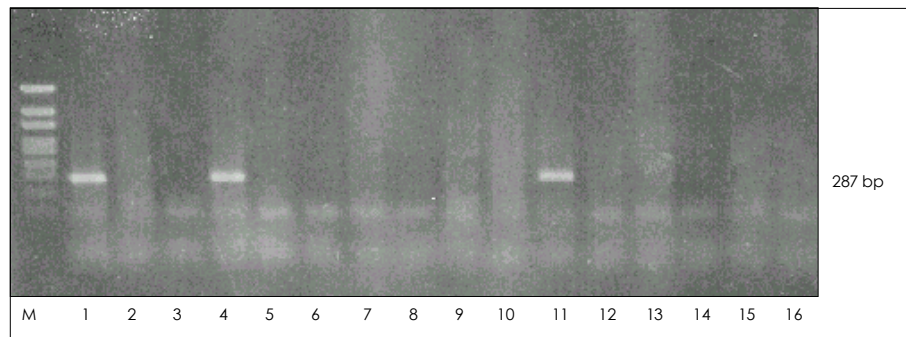


Fig. 1. HPV expression analyzed by PCR. DNA molecular mass markers (100 bp) are indicated in lane M.

1 , DNA , 가 가 .
10 30 가 .⁸⁾ HPV
 , HPV 6 11 HPV 16 , 18 , 31
가 , EBV , 33 , 35 , 51
Burkitt 가 EBV .¹²⁾ HPV
가 EBV .
50~94% EBV .
Tsay⁹⁾ WHO 1 80%, 2 3 97% Selacek¹³⁾ HPV 47% HPV
EBV , Pathmanathan⁶⁾ HPV가 .
WHO 1 , 2 , 3 100% EBV
Niedobitek¹⁰⁾ WHO 1 , ,
0%, 2 50%, 3 88% EBV Brandsma Abramson,⁷⁾ Ishihashi¹⁴⁾
WHO 1 HPV 16
WHO 1 EBV 18%, 29%, 13%, 5%
가 EBV .
2 3 Tyan³⁾ 30 14 (46%)
 ,⁶⁾ in situ hybridization HPV 16 WHO 2
EBV RNA 18 3 Hording¹⁵⁾ 15 WHO 1
17 (94%) WHO 2 3 (20%) HPV 16 , 1 HPV 11
3 .¹¹⁾ 2 3 HPV
HPV HPV 11 16 WHO 1
80 . Giannoudis¹⁶⁾ 63
8000 base pairs 12 (19%) HPV WHO 1

18 8 (44%) HPV , EBV
EBV WHO 1 HPV
가 , HPV
Rassekh 17) HPV EBV WHO
1 3
Lizarraga 18) 81%
HPV 31

Southern blot hybridization, in situ hybridization,
polymerase chain reaction(PCR)
HPV southern
blot hybridization

in situ hybridization
HPV DNA가

가 PCR
PCR

가 가

EBV가 WHO 2 3
WHO 1 EBV,

HPV 11 , 16 , 31 , EBV HPV
가 가 WHO

1 HPV 6 HPV 6
WHO 1 가 HPV

6 HPV 6
Judd 19)

가 HPV 6

가 가

HPV 가 가

결론

WHO HPV 6 가

중심 단어 :

2004 (2004 -
20)

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