

HPV 16/18 E6/E7 Ki- 67

=Abstract=

A study on expression of Human Papillomavirus 16/18, E6/E7 and Ki- 67 in the cervical cancer

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The squamous cell carcinoma of the uterine cervix is the most common malignant tumor among women in Korea. Since 1976, when a research result that human papillomavirus(HPV) infection played some role in the carcinogenesis of the uterine cervical carcinoma had been published, numerous reports supporting the result have been released. Among the types of the HPV, type 16 and type 18 are classified as high risk types because they are frequently found in cervical lesions with high grade dysplasia and invasive carcinomas. However, it is impossible to ascertain by host histologic or cellular changes which type of HPV is infected. The HPV genome is composed of six open reading frames (ORF's) named as E1, E2, E4, E5, E6 and E7 in the early region. Among these oncoproteins HPV E6/E7 have been strongly suggested to be important in carcinogenesis. When HPV infects the epithelial cells, it promotes cellular proliferation. The cellular proliferation can be evaluated by immunohistochemistry with the antibodies for proliferating cell nuclear antigen(PCNA) and Ki-67. Because PCNA has long half-life, and can be detected 48 hours after completion of mitosis, an estimation of proliferating cells by PCNA could be inaccurate. The expression of Ki-67 antigen is more correct than PCNA for the evaluation of proliferation cells due to its short half-life and rapid degradation after completion of the mitosis.

In this study, immunohistochemical staining was conducted to determine the rate of expression of HPV E6, E7 and Ki-67, correlation with relationship in carcinoma in situ and invasive uterine cervical cancer. Fifty cases of carcinoma of in situ(CIS) and invasive carcinoma were immunohistochemically stained, and the results obtained were as follows:

1) E6 protein of HPV type 16/18 was expressed in 5 of 14 cases(35.7%) of carcinoma in situ, in 3 of 7 cases of microinvasive carcinoma (42.8%) and in 12 of 20 cases of invasive carcinoma(60%) but there was no significant difference in expression between the carcinoma in situ and invasive cancer group (p=0.138).

2) E7 protein of HPV type 16/18 was expressed in 10 of 14 cases(71.4%) of carcinoma in situ, in 6 of 7 cases of microinvasive carcinoma (85.7%), and in 18 of 20 cases of invasive carcinoma(90%) but there was no significant difference in expression between the carcinoma in situ and invasive cancer group (p=0.138).

3) The cell fraction expressing Ki-67 was expressed in 5 of 14 cases(35.7%) of carcinoma in situ, in 5 of 7 cases of microinvasive carcinoma (71.4%), and in 18 of 20 cases of invasive carcinoma.(90%) The cell fraction expressing Ki-67 increased according to the progress of cervical cancer.

4) There was no statistical significance between HPV type 16/18 E6 protein and the cell fraction expressing Ki-67(p=0.09).

5) There was no statistical significance between HPV type 16/18 E7 protein and the cell fraction expressing Ki-67(p=0.17).

The above results suggest that the cell fraction expressing Ki-67 increases according to the invasiveness of cervical cancer and E6/E7 protein seem to play a role in the progression of cervical cancer. However we were not able to reveal a relation between E6/E7 protein and the cell fraction expressing Ki-67 in progress of cervical carcinoma, and it is recommended that further studies should be undertaken.

Keywords: Human papilloma virus(HPV), E6/E7 protein, Ki-67

I. (cis regulatory sequences)
가 400 bp upstream regulatory region
(URR), 6 open reading frame(E1, E2, E4,
E5, E6, E7) (ORF) ,
L1, L2
ORF E6, E7
ORF가 HPV
HPV 23
89,10
80 90
Polyma virus, SV40 virus Papovaviri-
dae 45-55 nm, 7800-7900 base pairs(II).
bp), 가 DNA , (species)
가 2.
(Cell line) HPV DNA가
type HPV
HPV가 가
E1 E2 ORF DNA E6, E7, ORF DNA
(repressor)
type HPV HPV type 6, 11 가 12 E6, E7
(condyloma accumi-
nata)
(HPV)
, HPV type 16, 18, 31, 33, 35
3456. mRNA
HPV DNA
CIN I HPV

, CIN II, III 5% ,

13).

HPV 6/11 100%가 episome ,

HPV 18 100% episo-

me , 100%

6. HPV 16 18d E6

E7

14) E7 E6 15)

c-Ha-ras-1

1617) .

가

(fraction)

18).

(proliferating cell nuclear antigen: PCNA)

Ki-67

PCNA 40%가 G0

49

1921) Ki-67 (G1, S

G2)

2224)

in situ hybridization(ISH)

HPV16,

18 50

HPV E6, E7 Ki-67

.

1.

1994 1997

.

10%

8 24

hematoxylin-eosin

(carcinoma in

situ), (microinvasive carcinoma)

(invasive carcinoma)

(squamous cell ca), ,

(adenocarcinoma) (adenosquamous ca-

rcinoma) (Table 1).

Table 1. Materials for study

Diagnosis	No. of cases
Squamous cell ca	
In situ	14
Keratinizing	1
Large cell nonkeratinizing	13
Microinvasive	7
Keratinizing	1
Large cell nonkeratinizing	6
Invasive	20
Keratinizing	12
Large cell nonkeratinizing	8
Adenocarcinoma	4
Adenosquamous carcinoma	5
In situ	2
Invasive	3
Total	50

2. Human papillomavirus (HPV)

nonisotope in situ hybridization (NISH)

DNA

Alu

biotin probe(Research Genetics, USA)

DNA

.

slide 4x

ethanol . 0.2N

HCl 45'C 10 0.01N HCl

pepsin 5 mg/ml 0.01N HCl

15

0.1M NaCl 0.1M Tri HCl(pH 7.4)

100% ethanol 5

. Alu probe

95 5

HPV 16/18 E6/E7 Ki- 67

37 15 60% 3,3 diaminobenzidine 가 10

nucleotide 0.1M NaCl Hams' hematoxylin

0.1M Tris HCl(pH7.4) strep-

tavidin alkaline phosphatase 30

5-bromo-4-chloro-3-indolyl

phosphate(BCIP)/nitro blue tetrazolim(NBT) 4. Ki- 67

3% methyl green 4 μ m

, xylene 0.1M citrate

HPV NISH buffer(pH6.0) microwave oven 10

HPV 0.1M phosphate

biotin pan HPV probe(Kreatech, 0.3% peroxide methanol

Netherland) 30

pan HPV HPV

probe(HPV16, 18) 가 30

(MIB-1;

BioGenex, USA) 1

PBS 3 biotinylated antimouse

rabbit serum(BioGenex, USA)

PBS streptavidin labeled

peroxidase(BioGenex, USA) 30

PBS diaminobenzidine(DAB)

xylene , 100% hematoxyline

95%, 80%, 70%

10

citrate (sodium citrate 10mM, pH

6.0) 3L 가

(103kPa)

2

HPV16-E7 4

16-E7

(ZYMED, 28-0006, 1:2) HPV16/18-E6(Calbiochem,

DP12, 1:10) 1

tris (Tris 5mM, sodium

chloride 140mM, pH 7.4) biotin

2 가 40

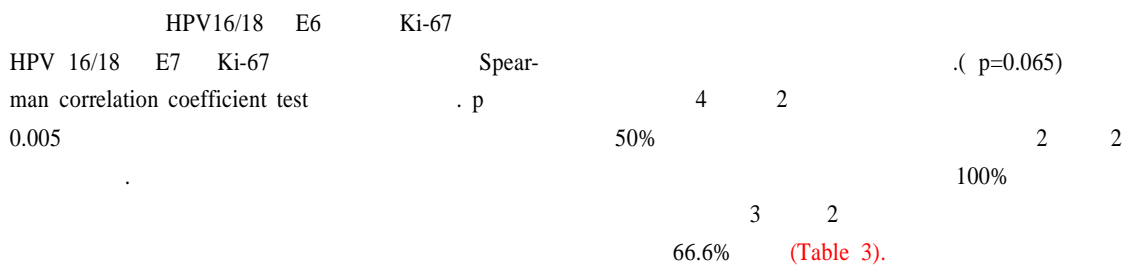
tri peroxidase가

streptavidin(BioGenex, USA) 30

tris

Ki-67, E6, E7

Chi-square test



1. HPV 16/18 E6

E6	+	-	No. of Positive	(%)
In situ	14	5	19	35.7%
Microinvasive	7	3	10	42.8%
Invasive	20	12	32	60%
Adenocarcinoma	4	1	5	25%
Adenosquamous carcinoma	2	1	3	33.3%
Invasive	3	1	4	50%

Table 3. E7 Protein Positivity

	+	-	No. of Positive	(%)
Squamous cell ca				
In situ	10	4	10/14	71.4*
Microinvasive	6	1	6/7	85.7*
Invasive	18	2	18/20	90*
Adenocarcinoma	2	2	2/4	50
Adenosquamous carcinoma				
In situ	2	0	2/2	100.0
Invasive	2	1	2/3	66.6
	40	10		

* P = 0.065

Table 2. E6 Protein Positivity

	+	-	No. of Positive	(%)
Squamous cell ca				
In situ	5	9	5/14	35.7*
Microinvasive	3	4	3/7	42.8*
Invasive	12	8	12/20	60*
Adenocarcinoma	1	3	1/4	25
Adenosquamous carcinoma				
In situ	1	1	1/2	50
Invasive	1	2	1/3	33.3
	23	27		

* P = 0.138

2. HPV 16/18 E7

E7	+	-	No. of Positive	(%)
In situ	14	5	19	71.4%
Microinvasive	7	3	10	85.7%
Invasive	20	12	32	90%

3. Ki-67

Ki-67

Fig. 1 Focal and moderate cytoplasmic and nuclear staining for E6 in invasive squamous cell carcinoma(Peroxidase: DAB; x200)

-	HPV 16/18	E6/E7	Ki- 67	-
	14	5	7	5
	20	16		
		가	가	(Table 4).
	HPV 16/18	E6		
Ki-67			23	10

Table 5. Relationship between E6 and Ki-67 Antigen

Ki-67	-	+	++	+++	No. of Positive(%)	
E6	-	6	12	6	3	21/27(77.8)
	+	13	5	4	1	10/23(43.4)
Total		19	17	10	4	31/50(62.0)

Spearman coefficient = 0.24(P = 0.09)

Fig. 2 Diffuse and strong cytoplasmic and nuclear staining for E7 in invasive squamous cell carcinoma.(Peroxidase: DAB; x200)

Table 4. Epithelial distribution of Ki-67 Antigen Expression

	No. of Cases	No. of Positive	Distribution		
			basal	middle	superficial
Squamous cell ca					
In situ	14	5	+	+	+
Microinvasive	7	5	+	+	+
Invasive	20	16	+	+	+
Adenocarcinoma	4	2	+	+	+
Adenosquamous carcinoma					
In situ	2	1	+	+	+
Invasive	3	2	+	+	+
Total	50	31			

43.4% E6 가
27 Ki-67
가 21 77.8%
(p=0.09 Table 5).
5. HPV 16/18 E7
Ki- 67
HPV 16/18 E7
Ki-67
40 25 62.5%
E7 가 10 Ki-67
가 6
60.0%

(p=0.17

Table 6).

Table 6. Relationship between E7 and Ki-67 Antigen

Ki-67	-	+	++	+++	No. of Positive(%)	
E7	-	4	4	1	1	6/10(60.0)
	+	15	14	7	4	25/40(62.5)
Total		19	18	8	5	31/50(62.0)

Spearman coefficient = 0.18(P = 0.17)

Fig. 3 Ki-67 is expressed in some cells of carcinoma in situ (immunohistochemistry: peroxidase : DAB)

4. HPV 16/18 E6 Ki-67

Ki-67

Fig. 4 Ki-67 is expressed in the most of the nuclei of the invasive squamous cell carcinoma (immunohistochemistry: peroxidase : DAB)

가 가

. 10

(HPV)가

가 .25)

HPV 8,000bp double-stranded circular DNA

virus 80 .26)

, , ,

27,28,29).

,

30,31).

20

(HPV 16,18,31,45),

(HPV 33, 35, 39, 51, 56, 58,

59, 68), (HPV 6, 11,

26, 42, 43, 44, 53, 54, 55, 62, 66)

25,32,33).

immunohistochemistry: peroxidase : DAB HPV

34),

DNA 50% 가

.

DNA open reading frames

(ORFs: i.e., potential coding regions)

. Early gene (E) DNA

(E1),

DNA ,

(E2),

(E4), epidermal growth factor (EGF)

colony stimulator factor (CSF) receptor

(E5),

(E6, E7) . E6, E7

가 35), 100-150

conserved C-terminal zinc binding cysteine

motifs 가 . 가

가

E1 E2 open reading frame

(ORF) E6 E7 가

E6, E7

. E7 E6

adenovirus E1A conseved domain 1

2 sequence

polyoma virus large T myc

16). E6 p53

36,37), E7 retinoblastoma(Rb)

38,39).

E6

35.7%, 42.8%

60%, E7 71.4%,

85.7%, 90%

E6 E7

가

.

Polymerase chain reaction(PCR), In situ hybridization

(ISH), Southern blot, immunohistochemistry

- HPV 16/18 E6/E7 Ki-67 -

, HPV Ki-67

가 가 PCR

가 localization , Ki-67

가 nonisotope ISH(NISH)가 가

가 가 p53 44,46

가 E6 ,E7 44,46

Ki-67 E6/E7 가

nuclear organizer regions

(silver stain) (AgNORs) V.

DNA precursor incorporation, flow cytometry image analysis

50

HPV 16/18 E6,

(PCNA) Ki67 E7 Ki-67

40%가 Go 48

1) HPV 16/18 E6 35.7%(5/14), 42.8%(3/7), 60%(12/20)

Ki-67 (G1,S G2) 19,20,21.

22,23,24, Ki-67 .(p=0.138) 25%(1/4), 33.3%(1/3)

Ki-67 2) HPV 16/18 E7 71.4%(10/14), 85.7%(6/7), 90%(18/20)

thymidine incorporation 3H- 5,40 Ki-67 .(p=0.065) 50%(2/4), 66.6%(2/3)

reserve cell 3) HPV 16/18 Ki-67 35.7%(5/14), 71.4% (5/7), 80%(16/20)

37.5%, 가 50%(2/4), 71.4% 80%, 66.6%(2/3)

Ki-67 가 가 4) HPV 16/18 Ki-67 E6

가 가

41,42,43 .(p=0.09)

5) HPV 16/18 Ki-67 E7

(p=0.17)

Ki-67

가

, E6, E7

Ki-67

E6, E7

가

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