

HPV16-E6/E7 Oncogene Mutation and p53 Expression among Indonesian Women with Cervical Cancer

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Abstract

Objective: To characterize HPV16 E6/E7 mutation and its association with p53 expression among Indonesian women with cervical cancer. **Methods:** This is a cross-sectional study involving 31 Indonesian women with pathologically proven cervical cancer and HPV16 infection. Data about the clinical characteristics of the study population were obtained from the medical records. Biopsy specimen of the cervical cancer mass from each study participant was obtained for DNA isolation. The ORFs of E6 and E7 genes were amplified using specific primer designed according to K02718/HPV16R gene sequence obtained from GenBank. Sequencing was performed using software program MEGA10. HPV16 E6 and E7 prototype sequences for nucleotide alignment (HPV16. P, GenBank Access code: NC_001526) was selected from European variant. The sequence of nucleotide and amino acid was aligned using software program BioEdit. p53 expression was evaluated through immunohistochemistry and quantified using immunoreactivity score (IRS). **Results:** Twelve subjects (38.7%) present with E6 and E7 mutation. Median age, parity, stage and histologic type of the tumour did not associate with E6/E7 mutation. E6 and E7 mutation rate was 25.8% (8/31) and 12.9% (4/31), respectively. Seven single nucleotide changes were identified within the E6 and E7 oncogenes, including four non-synonymous and three synonymous mutations. E6 T27C was the most prevalent mutation (16.1%). Nonsynonymous mutations were more prevalent within E7 gene (9.6%) (N29T, N29S, and R77C). Median IRS did not differ between HPV16-E6/E7 variants and wildtype (p value = 0.990). There was no association between E6/E7 mutations and p53 expression in Indonesian women with cervical cancer (PR 1.4, 95% CI: 0.29-6.77, p value = 0.704). **Conclusions:** HPV16 E6 mutation was more prevalent than E7 mutation among Indonesian women. There was no association between E6/E7 mutation and p53 expression level.

Keywords: HPV16- E6 oncogene- E7 oncogene- mutation- Indonesian women

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Introduction

Cervical cancer is the fourth most frequently diagnosed cancer and the fourth leading cause of cancer death among women worldwide. It ranks as the second commonest and deadliest cancer in Indonesia. In 2020, 36,633 new cases and 21,003 deaths due to cervical cancer are reported among Indonesian women (Sung et al., 2021). Almost all cases of cervical cancer are linked to infection with high-risk human papillomavirus (HPV), particularly type 16 and 18. The major oncoproteins E5, E6, and E7 encoded by the HPV genome are the major drivers of the oncogenic cascade in cervical epithelium (Estêvão et al., 2019). The most important and well-studied role of the E6 is the degradation of the p53 protein (Martinez-Zapien et al., 2016), while E7 induces the degradation of pRB protein (Hwang et al., 2002).

The study of specific viral domains has revealed a number of intra-typic nucleotide polymorphisms within the HPV16 genome (Tommasino, 2014). The best-defined region of the viral genome, which has been used to describe distinct HPV16 variants, is the E6 oncogene (Tsakogiannis et al., 2013). A mutation from T to G at nucleotide 178 (T178G), leading to a change from aspartate to glutamate (D25E), is the most common E6 variant in Asian countries (Vaeteewoottacharn et al., 2003; Lee et al., 2011; Piao et al., 2016). In Thailand, 90% of these variants coincided with a specific type of E7 mutation, i.e. N29S (Vaeteewoottacharn et al., 2003). Other variation characterized by a T to G transition at nucleotide 350 (T350G), resulting in an amino acid change from a leucine to a valine (L83V) is commonly found in some European countries (Brady et al., 1999; Andersson et al., 2000; Kämmer et al., 2002). HPV16 E7 variants

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are less prevalent and sometimes detected together with E6 variants (Zehbe et al., 1998; Vaeteewoottacharn et al., 2003; Safaeian et al., 2010). Furthermore, the polymorphisms in the HPV16 E7 more commonly resulted in synonymous changes suggesting the highly conserved E7 sequence that is critical for HPV16-induced carcinogenesis (Safaeian et al., 2010; Mirabello et al., 2017). Studies in China and Korea reported that the most prevalent E7 mutation was A647G (N29S) (Lee et al., 2011; Zhe et al., 2019). E7 A647G has been proven to increase the risk of progression to cervical intraepithelial neoplasia 2-3 and invasive cancer (Lee et al., 2011).

It has been well understood that HPV16-E6 oncoprotein binds and targets for degradation numerous cell proteins, including the tumor suppressor p53 and several PDZ domain proteins. HPV viral E6 protein-mediated p53 degradation critically contributes to the tumorigenesis of cervical cancer. (Yang and Lu, 2015) HPV16 E6/E7 oncogene mutation could potentially alter its capacity to degrade p53 and thus, altering the expression level of p53. On the other hand, data about HPV16 E6/E7 variants among Indonesian population is still lacking (de Boer et al., 2004), particularly among women of Balinese ethnicity. Therefore, this study is aimed to characterize the HPV16 E6/E7 mutation among Indonesian women and its association with the expression of p53.

Material and Methods

The study was approved by the Ethical Committee of Faculty of Medicine, Udayana University/Prof. Dr. I.G.N.G Ngoerah General Hospital, Denpasar, Bali, Indonesia (No. 1728/UN14.2.2.VII.14/LP/2019).

Study design

This is a cross-sectional study involving Indonesian women with pathologically proven cervical cancer and HPV16 infection.

Patient selection

Women with pathologically proven cervical cancer and HPV16 infection who attended the Gynecologic Oncology Outpatient Clinic (Division of Gynecologic Oncology, Department of Obstetrics and Gynecology Faculty of Medicine Udayana University, Prof. Dr. I.G.N.G Ngoerah General Hospital, Denpasar, Bali, Indonesia), during June 2019 to December 2020 were recruited consecutively into the study population. Prof. Dr. I.G.N.G Ngoerah General Hospital is a tertiary teaching hospital in Denpasar, Bali, Indonesia, that serves as a gynecologic oncology referral centre for Bali and Nusa Tenggara regions. A written informed consent was obtained from all subjects or their legal surrogate before the commencement of the study. The inclusion criteria was women with cervical cancer who had never underwent any form of treatment prior to the commencement of this study (surgery, radiation, or chemotherapy). The exclusion criteria was women whose biopsy sample of cervical cancer were not suitable for PCR and gene sequencing, i.e. due to errors in sampling, transportation, or storage of the specimens.

Sample size calculation

The following formula was used for calculating the adequate sample size in prevalence study (Pourhoseingholi et al., 2013); $n = \frac{Z^2 P(1 - P)}{d^2}$. Where Z is the statistic corresponding to level of confidence (alpha 0.5, Z-alpha 1.960), P is expected prevalence (0.7, according to studies in Ref. (Song et al., 1997; Matsumoto et al., 2000; Vaeteewoottacharn et al., 2003)), and d is precision (0.16). According to the formula, the total number of sample required is 31.

Data collection

Clinical characteristics

Data about the clinical characteristics of the study population were obtained from the medical records. Stage are classified according to 2018 FIGO Classification. (Matsuo et al., 2019)

DNA isolation

Biopsy specimen of the cervical cancer mass from each study participant was obtained and embedded in PBS 1X/NaCl 0.9% solution. DNA was isolated using High Pure PCR Template Preparation kit (Roche®) according to the manufacturer instruction. Polymerase chain reaction (PCR) was performed to detect HPV DNA using primer My09 (5'-CGT CCM ARR GGA WAC TGA TC-3') and My11 (5'-GCM CAG GGW CAT AAY AAT GG-3') (Castle et al., 2002) with the following program: pre-denaturation at 95°C for 3 minutes, 35 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 5 minute. HPV16 DNA was amplified using specific primer (Forward: 5'-ACA AAA CGT GCA TCG GCT AC-3' and Reverse: 5'-GGA ATA TAC CCA GTG CGT CC-3') following the same amplification program as the universal HPV DNA.

E6 and E7 gene amplification

The ORFs of E6 and E7 genes were amplified using specific primer designed according to K02718/HPV16R gene sequence obtained from GenBank (E6 Forward: 5'-GAA ACC GGT TAG TAT AAA AGC AGA C-3', Reverse: 5'-AGC TGG GTT TCT CTA CGT GTT CT-3'; E7 Forward: 5'-GAT GAA ATA GAT GGT CCA GC-3', Reverse: 5'-GCT TTG TAC GCA CAA CCG AAG C-3'). The mixture for amplification (25 µl) was as follows: 2.5 µl DNA template, 0.5 µM of each primer, 12.5 µl of MasterMix (Go2green Master Mix-Promega), and water. DNA was amplified using Thermal Cycler (MiniAmp-BioSystems) with the following program: pre-denaturation at 95°C for 3 minutes, 35 cycles of denaturation at 95°C for 1 minute, annealing at 55°C for 1 minute, extension at 72°C for 1 minute, and final extension at 72°C for 5 minutes. After PCR was done, sample was analysed on electrophoresis using 1.5% agarose gel.

E6 and E7 Gene sequencing

Sequencing was performed using software program MEGA10. HPV16 E6 and E7 prototype sequences for nucleotide alignment (HPV16. P, GenBank Access code: NC_001526) was selected from European variant. The

sequence of nucleotide and amino acid was aligned using software program BioEdit.

p53 expression

Immunohistochemical staining of p53 was performed using the DakoEnVision[®] Dual Link System-HRP (DAB+) (Dako, Denmark) and monoclonal anti-p53 antibody (BIOS, USA). Other materials use materials from Sigma-Aldrich (USA). The tissue was cut 3–5 m thick using a microtome. Before painting, the slides will go through a process of deparaffinization and rehydration, including immersion in xylene solution for 2x5 minutes, ethanol 100% for 2 minutes, ethanol 96% for 2x2 minutes, ethanol 70% for 2 minutes in 70% ethanol and PBS for 2 minutes. Antigen retrieval was performed by immersing the slides in trisodium citrate buffer and microwave-heated for 5 minutes (700 watt) and 15 minutes (280 watt) and left at room temperature for 20 minutes, followed by rinsing the PBS 2x buffer for 5 minutes. Endogenous peroxidase blocking was performed within the plastic box with dual endogenous enzyme block for 10 minutes. The slides were then rinsed with PBS 1x for 5 minutes, twice. A 100 uL 5% FBS solution was applied for 30 minutes. The slides were again rinsed with PBS 1x for 5 minutes, twice, before incubated overnight with primary antibody. Labelled polymer HRP was applied to the slides for 30 minutes before rinsing in PBS 1x solution for four times, 3 minutes each. DAB was applied subsequently and the slides were rinse again with PBS 1x solution. Hematoxyllin-Gill was applied for 5 minutes before irrigated with water. The slides were then immersed within ethanol and xylene solution for 5 minutes, twice. The slides were finally mounted and covered with glass.

The IHC staining of mutant p53 was assessed according to the immunoreactive score (IRS), which is based on the percentage of positive cells and the staining intensity (Fedchenko and Reifenrath, 2014). The cells were considered positive for p53 antigen when there was an intranuclear DAB staining (brown color). The percentage of positive cells were assessed with the help of labeling index (P53 Labeling index = Number of IHC Positive Cells X 100/total number of cells observed). The two scores were multiplied to get IRS score, ranging from 0 to 12 and corresponded to ≤6 as low and >6 as high groups of p53 expression. The counting was done by two observers and the mean was taken as a final count.

Statistical analysis

All samples were coded and analysed by individuals who had no knowledge of the subject's disease status. Numeric data were tested for normality using the Shapiro-Wilk test. Normally distributed continuous variables were presented in mean ± SD and analysed using the independent t-test while non-normally distributed continuous variables were presented in median (interquartile range/IQR) and analysed using the Mann-Whitney U test. Categorical variables were presented in percentage (%) and analysed using the Chi-square test. All tests were two-sided and p<0.05 was considered statistically significant. Data were analysed using SPSS version 28.0.

Results

E6/E7 mutation

We identified 12 subjects (38.7%) with E6/E7 mutation. Median age, parity and proportion of subjects with advance stage did not differ between the two groups (Table 1). Histologic type also did not differ between the two groups. E6 mutation rate was 25.8% (8/31), while E7 mutation rate was only 12.9% (4/31) (Table 2). Seven single nucleotide changes were identified within the E6 and E7 oncogenes, including four non-synonymous (E6 G371A and E7 A86C, A86G, C229T) and three synonymous mutations (E6 T27C, A360G and E7 T285C). E6 T27C was the most prevalent mutation in this population (16.1%). G371A/R124K was the only non-synonymous mutation found within E6 gene (3.2%). Nonsynonymous mutations were more prevalent within E7 gene (9.6%) (N29T, N29S, and R77C). None of the E6 mutation coexisted with the E7 mutation. One subject has two co-existing E7 mutation (N29S and S95S).

Association between E6/E7 mutations and p53 expression

Median IRS of p53 expression did not differ between the two groups [mutant vs. wildtype: 2 (IQR:4) vs 2 (IQR: 6), p value 0.990]. There was no association between E6/E7 mutations and p53 expression in Indonesian women with cervical cancer (PR 1.4, 95% CI: 0.29-6.77, p value = 0.704) (Table 3).

Table 1. Clinical Characteristics of the Study Population

Characteristics	All subjects (N=31)	Wild type E6 and E7 (N=19)	Mutant E6/E7 (N=12)	p value
Age, median (IQR), years	51.0 (16)	50.0 (16.0)	54.5 (19.7)	0.734
Parity, median (IQR)	2.0 (1.0)	2.0 (1.0)	2.5 (2.5)	0.326
Stadium, n (%)				
IA1-IIA2	6 (19.4)	4 (21.1)	2 (16.7)	0.574
IIB-IVB	25 (80.6)	15 (78.9)	10 (83.3)	
Histology				
Squamous cell carcinoma	28 (90.3)	17 (89.5)	11 (91.7)	0.690
Adenocarcinoma	2 (6.4)	1 (5.3)	1 (8.3)	
Other type (neuroendocrine)	1 (3.3)	1 (5.3)	0 (0)	

Table 2. Mutation of HPV16 E6 and E7 Gene

Nucleotide position	Prototype	Variant	N (%)*	Amino acid position	Prototype	Variant	N (%)
E6							
27	T	C	5 (16.1)	9	F	F	-
360	A	G	2 (6.4)	120	E	E	-
371	G	A	1 (3.2)	124	R	K	1 (3.2)
E7							
86	A	C	1 (3.2)	29	N	T	1 (3.2)
86	A	G	1 (3.2)	29	N	S	1 (3.2)
229	C	T	1 (3.2)	77	R	C	1 (3.2)
285	T	C	1 (3.2)	95	S	S	-

*Mutation rate was among all subjects with HPV-16 positive cervical cancer

Table 3. The Association between HPV16-E6/E7 Mutation and p53 Expression Level

HPV-E6/E7	P53 expression level		PR (95% CI)	p value
	High	Low		
Variant	4	8	1.4 (0.29-6.77)	0.704
Prototype	5	14		

Discussion

In this study, we identified 38.7% mutation rate of HPV16 E6/E7 oncogene among Indonesia women of Balinese ethnicity. The rate of E6/E7 mutation may vary according to race, ethnicity and geographic location. The first study of HPV variants in Indonesia was conducted by de Boer et al. (2004) who reported high rate of E6/E7 mutation among Indonesian women of Javanese ethnicity, which they referred to as Javanese variant (de Boer et al., 2004). The prototype variant (HPV 16-R) was detected in none of the Indonesian samples. The Javanese variant, i.e. L1 C6826T and E7 G666A covariation, was found in 73% of Indonesian samples. In 56% of these variants, a third mutation was seen in the E6 ORF (A276G) that gives the N58S amino acid change. A study in Thailand reported a higher rate of E6 mutation than our study, i.e. 87% (27/31 samples) (Vaeteewoottacharn et al., 2003). In China, one study reported a lower rate of HPV E6/E7 mutation than our study, i.e. 25.3% (19/75 samples) (Zhe et al., 2019). A study in UK found that the rate of E6 prototype was 38% (Brady et al., 1999).

We observed that E6 mutation was more prevalent than E7 mutation (25.8% vs. 12.9%). Other studies also noticed similar observation that E7 sequences are more conserved than E6 sequences (Zehbe et al., 1998; Vaeteewoottacharn et al., 2003; Safaeian et al., 2010; Zhe et al., 2019). In a large case-control analysis by Mirabello et al., it was suggested that strict conservation of the 98 amino acids of E7, which disrupts Rb function, is critical for HPV16 carcinogenesis (Mirabello et al., 2017). E7 is less constrained in benign infections, and genetic variation in E7 reduces HPV16 carcinogenicity. Furthermore, HPV16 E7 has been suggested to be more hypovaryable than HPV31 E7 and HPV73 E7, and this was thought to be a possible clue to HPV16's greater carcinogenicity (Safaeian et al., 2010). E7 hypovariation is also consistent

in different geographic locations and racial groups and suggests, in summary, that E7 variation greatly decreases the risk of invasive cancer (Mirabello et al., 2017).

T27C, which is a synonymous mutation, was the most common E6 mutation in our study. A study in Thailand reported that E6 T178G mutation leading to a change from aspartate to glutamate (D25E) was the most common mutation and 90% of these mutation coincided with a specific type of E7 mutation, N29S (Vaeteewoottacharn et al., 2003). In China, E6 T350G and T178G mutation and E7 A647G mutation were the most common mutation (Zhe et al., 2019). In our study, none of the subjects had E6 T178G or T350G mutation. After thorough and careful review of previous studies investigating HPV E6 mutation in Asian women, none of the E6 mutations in our study was found in those studies (Vaeteewoottacharn et al., 2003; de Boer et al., 2004; Ishizaki et al., 2013; Yang et al., 2014; Zhe et al., 2019). E6 T27C might be a synonymous mutation unique to our population.

We observed E7 N29S mutation in one subject (3.2%). E7 N29S is the most common variant of E7. One study reported that E7 N29S were prevalent in Japanese (48.1%) and Vietnamese strains (40.4%) and less prevalent in Philippine strains (11.5%) (Ishizaki et al., 2013). A study in China reported the rate of E7 N29S mutation was 68.4% (Yang et al., 2014). Other E7 mutations in this study (R77C and S95S) were also observed in other studies conducted among Chinese women (Yang et al., 2014; Zhe et al., 2019). One subject in our study had coexisting E7 mutation between N29S and S95S. E7 S95S is also another hotspot for E7 mutation. In one study by Yang et al., they reported that all subjects with E7 N29S mutation also had S95S mutation (Yang et al., 2014). They also suggested that E6 D25E and E7 N29S-S95S were linkage mutations, which is the common variant.

Interestingly, we observed that nonsynonymous mutations were more prevalent within E7 oncogene (75% or 3 in 4 nucleotide mutations), as compared to E6 oncogene (33.3% or 1 in 3 nucleotide mutations). A study by Yang et al., (2016) found higher rate of non-synonymous mutation within E6 oncogene (72.2% or 13 in 18 mutations) as compared to E7 oncogene (30% or 3 in 10 mutations). A study by Zhe et al., (2019) also reported higher prevalence of nonsynonymous mutation within the E6 oncogene. Thus, our observation may be

unique to Indonesian women.

E6 and E7 variants can induce molecular mechanisms that lead to more aggressive HPV 16 phenotypes in cervical cancer. Studies have reported that E6 variants contained mutations that were identified in areas likely to be important for protein-protein interaction with p53 or in areas of immunological significance (Zehbe et al., 1998; Hang et al., 2014; Rodríguez-Ruiz et al., 2019; Hadami et al., 2021). These variations have been proven to change the oncogenic potency of E6. For example, the L83V variants displayed more efficient degradation of Bax and binding to E6BP (Lichtig et al., 2006). In comparison with E6 prototype, E6 variants have been found to alter the gene expression profiles in in vitro model, including genes involved in cellular processes related to cervical carcinogenesis, such as adhesion, angiogenesis, apoptosis, differentiation, cell cycle, proliferation, transcription and protein translation (Jang et al., 2011; Hochmann et al., 2016; Zacapala-Gómez et al., 2016) as well as cellular metabolism (Richard et al., 2010; Garibay-Cerdenares et al., 2021). In one in vitro study, primary human foreskin keratinocytes (PHFKs) expressing E6 variants (Q14H/H78Y/L83V) were significantly faster dividing, developed larger cells in monolayer cultures, showed double the epithelial thickness and expressed cytokeratin 10 when grown as organotypic raft cultures, as compared to PHFKs expressing prototype E6. A study by Lee et al, suggested that N95S can predict progression to cervical intraepithelial neoplasia 2-3 and invasive cancer (Lee et al., 2011). A recent study by Bello-Rios et al, proposed that N29S mutation has a significant influence on the 3D structure of the E7 protein of HPV16, which could be related to the oncogenic capacity of this protein (Bello-Rios et al., 2021).

In this study, we did not find any association between HPV16 variants and p53 expression. Variants found in our study might have similar ability to degrade p53, as compared to the prototype. However, other studies have reported varied observation. Hadami, et al., reported significant dissimilarities in p53 degradation activities of HPV16-E6 prototype and variants (Hadami et al., 2021). They reported that as compared to the prototype, the highest p53 degradation were exhibited by the African variants Af2-a/r, Af1-d/G295 and Af2-a/G285 ($p < 0.001$), followed by the European variants E- C442/G350 and E-G350/r ($p < 0.01$), then, the North American variant NA1-b/r ($p < 0.05$). Asadurian et al., (2007) reported that the L83V variants showed significant activity, comparable to that of the E6 prototype, in reducing p53 levels. Meanwhile, in a study by Hang et al., (2014) a similar ability to degrade p53 was observed among EUR E6, As E6, EUR E6-L83V and As E6-E113D. A rare variation, EUR E6-R10G, was found to shorten the half-life of p53 more efficiently than the other variations. Chopjitt et al., (2016) reported that E6D25E and E6 Prototype oncoproteins were comparable for their abilities to degrade p53. Ristriani et al., (2009) reported that HPV16 E6 F47R mutant is defective for polyubiquitination and subsequent degradation of p53.

Our study has several limitations. First, this study

is a single center study focusing the characterization of E6/E7 mutation among women of Balinese ethnicity. Nation-wide multicenter study is still required to fully understand the nature of HPV16 E6/E7 mutation among larger population of Indonesian women. Second, we used semiquantitative method (i.e. IHC with IRS Scoring system) to evaluate the p53 expression levels, which limits the accuracy. We propose using quantitative methods (e.g. ELISA or Western blot analysis) to more accurately quantify the expression level of p53 in future study. Third, the proportion of E6 mutants was low in our study. We propose expanding sample size in future study to better understand the nature of association between E6 mutants and p53 expression level. However, as a pilot study, our results give insight into the molecular epidemiology of HPV16 and its oncogene expression among Indonesian women, from which the data are still extremely lacking.

In conclusion, HPV16 E6 mutation was more prevalent than HPV16 E7 mutation in Indonesian women. However, nonsynonymous mutation was more prevalent within the HPV16 E7 oncogene. T27C is a synonymous mutation within the E6 oncogene which may be unique to Indonesian population. There was no association found between HPV16 variants and p53 expression level.

Author Contribution Statement

I Nyoman Bayu Mahendra: Conceptualization, methodology, resources, writing-review and editing, project administration, funding. Pande Kadek Aditya Prayudi: Software, formal analysis, investigation, data curation, writing-original draft, visualization. Ida Bagus Nyoman Putra Dwija: methodology, resources, project administration. Ketut Suwiyoga: Conceptualization, validation, writing-review and editing, supervision.

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Disclosure

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Data availability

Data are available upon reasonable request. All data relevant to the study are included in the article or uploaded as supplementary information.

Conflict of Interest

All the authors declare no conflict of interests.

References

- Andersson S, Alemi M, Rylander E, et al (2000). Uneven distribution of HPV 16 E6 prototype and variant (L83V) oncoprotein in cervical neoplastic lesions. *Br J Cancer*, **83**, 307-10.
- Asadurian Y, Kurilin H, Lichtig H, et al (2007). Activities of human papillomavirus 16 E6 natural variants in human keratinocytes. *J Med Virol*, **79**, 1751-60.

- Bello-Rios C, Montaña S, Garibay-Cerdenares OL, et al (2021). Modeling and molecular dynamics of the 3D structure of the HPV16 E7 protein and its variants. *Int J Mol Sci*, **22**.
- Brady CS, Duggan-Keen MF, Davidson JA, et al (1999). Human papillomavirus type 16 E6 variants in cervical carcinoma: relationship to host genetic factors and clinical parameters. *J Gen Virol*, **80**, 3233-40.
- Castle PE, Schiffman M, Gravitt PE, et al (2002). Comparisons of HPV DNA detection by MY09/11 PCR methods. *J Med Virol*, **68**, 417-23.
- Chopjitt P, Pientong C, Sunthamala N, et al (2016). E6D25E, HPV16 Asian variant shows specific proteomic pattern correlating in cells transformation and suppressive innate immune response. *Biochem Biophys Res Commun*, **478**, 417-23.
- de Boer MA, Peters LA, Aziz MF, et al (2004). Human papillomavirus type 16 E6, E7, and L1 variants in cervical cancer in Indonesia, Suriname, and The Netherlands. *Gynecol Oncol*, **94**, 488-94.
- Estêvão D, Costa NR, Gil da Costa RM, et al (2019). Hallmarks of HPV carcinogenesis: The role of E6, E7 and E5 oncoproteins in cellular malignancy. *Biochim Biophys Acta Gene Regul Mech*, **1862**, 153-62.
- Fedchenko N, Reifenrath J (2014). Different approaches for interpretation and reporting of immunohistochemistry analysis results in the bone tissue - a review. *Diagn Pathol*, **9**, 221.
- Garibay-Cerdenares OL, Sánchez-Meza LV, Encarnación-Guevara S, et al (2021). Effect of HPV 16 E6 oncoprotein variants on the alterations of the proteome of C33A cells. *Cancer Genomics Proteomics*, **18**, 273-83.
- Hadami K, Saby C, Dakka N, et al (2021). Degradation of p53 by HPV16-E6 variants isolated from cervical cancer specimens of Moroccan women. *Gene*, **791**, 145709.
- Hang D, Gao L, Sun M, et al (2014). Functional effects of sequence variations in the E6 and E2 genes of human papillomavirus 16 European and Asian variants. *J Med Virol*, **86**, 618-26.
- Hochmann J, Sobrinho JS, Villa LL, et al (2016). The Asian-American variant of human papillomavirus type 16 exhibits higher activation of MAPK and PI3K/AKT signaling pathways, transformation, migration and invasion of primary human keratinocytes. *Virology*, **492**, 145-54.
- Hwang SG, Lee D, Kim J, et al (2002). Human papillomavirus type 16 E7 binds to E2F1 and activates E2F1-driven transcription in a retinoblastoma protein-independent manner. *J Biol Chem*, **277**, 2923-30.
- Ishizaki A, Matsushita K, Hoang HT, et al (2013). E6 and E7 variants of human papillomavirus-16 and -52 in Japan, the Philippines, and Vietnam. *J Med Virol*, **85**, 1069-76.
- Jang M, Rhee JE, Jang DH, et al (2011). Gene expression profiles are altered in human papillomavirus-16 E6 D25E-expressing cell lines. *Virol J*, **8**, 453.
- Kämmer C, Tommasino M, Syrjänen S, et al (2002). Variants of the long control region and the E6 oncogene in European human papillomavirus type 16 isolates: implications for cervical disease. *Br J Cancer*, **86**, 269-73.
- Lee CW, Bae JH, Lee SJ, et al (2011). Distribution of human papillomavirus type 16 E6 and E7 gene variants in the progression of cervical dysplasia in Korean women. *J Obstet Gynaecol Res*, **37**, 1320-6.
- Lichtig H, Algrisi M, Botzer LE, et al (2006). HPV16 E6 natural variants exhibit different activities in functional assays relevant to the carcinogenic potential of E6. *Virology*, **350**, 216-27.
- Martinez-Zapien D, Ruiz FX, Poirson J, et al (2016). Structure of the E6/E6AP/p53 complex required for HPV-mediated degradation of p53. *Nature*, **529**, 541-5.
- Matsumoto K, Yoshikawa H, Nakagawa S, et al (2000). Enhanced oncogenicity of human papillomavirus type 16 (HPV16) variants in Japanese population. *Cancer Lett*, **156**, 159-65.
- Matsuo K, Machida H, Mandelbaum RS, et al (2019). Validation of the 2018 FIGO cervical cancer staging system. *Gynecol Oncol*, **152**, 87-93.
- Mirabello L, Yeager M, Yu K, et al (2017). HPV16 E7 genetic conservation is critical to carcinogenesis. *Cell*, **170**, 1164-74.e6.
- Piao J, You K, Guo Y, et al (2016). HPV16 E6 mutations and p53 codon72 polymorphism among women with cervical intraepithelial neoplasia 2 and 3 in China. *Eur J Gynaecol Oncol*, **37**, 649-52.
- Pourhoseingholi MA, Vahedi M, Rahimzadeh M (2013). Sample size calculation in medical studies. *Gastroenterol Hepatol Bed Bench*, **6**, 14-7.
- Richard C, Lanner C, Naryzhny SN, et al (2010). The immortalizing and transforming ability of two common human papillomavirus 16 E6 variants with different prevalences in cervical cancer. *Oncogene*, **29**, 3435-45.
- Ristriani T, Fournane S, Orfanoudakis G, et al (2009). A single-codon mutation converts HPV16 E6 oncoprotein into a potential tumor suppressor, which induces p53-dependent senescence of HPV-positive HeLa cervical cancer cells. *Oncogene*, **28**, 762-72.
- Rodríguez-Ruiz HA, Garibay-Cerdenares OL, Illades-Aguir B, et al (2019). In silico prediction of structural changes in human papillomavirus type 16 (HPV16) E6 oncoprotein and its variants. *BMC Mol Cell Biol*, **20**, 35.
- Safaeian M, van Doorslaer K, Schiffman M, et al (2010). Lack of heterogeneity of HPV16 E7 sequence compared with HPV31 and HPV73 may be related to its unique carcinogenic properties. *Arch Virol*, **155**, 367-70.
- Song YS, Kee SH, Kim JW, et al (1997). Major sequence variants in E7 gene of human papillomavirus type 16 from cervical cancerous and noncancerous lesions of Korean women. *Gynecol Oncol*, **66**, 275-81.
- Sung H, Ferlay J, Siegel RL, et al (2021). Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. *CA A Cancer J Clin*, **71**, 209-49.
- Tommasino M (2014). The human papillomavirus family and its role in carcinogenesis. *Semin Cancer Biol*, **26**, 13-21.
- Tsakogiannis D, Kyriakopoulou Z, Amoutzias G, et al (2013). Identification of novel E6-E7 sequence variants of human papillomavirus 16. *Arch Virol*, **158**, 821-8.
- Vaeteewoottacharn K, Jearanaikoon P, Ponglikitmongkol M (2003). Co-mutation of HPV16 E6 and E7 genes in Thai squamous cervical carcinomas. *Anticancer Res*, **23**, 1927-31.
- Yang L, Yang H, Wu K, et al (2014). Prevalence of HPV and variation of HPV 16/HPV 18 E6/E7 genes in cervical cancer in women in South West China. *J Med Virol*, **86**, 1926-36.
- Yang X, Lu L (2015). Expression of HPV-16 E6 protein and p53 inactivation increases the uterine cervical cancer invasion. *Drug Res (Stuttg)*, **65**, 70-3.
- Yang Y, Ren J, Zhang Q (2016). Distribution of human papilloma virus type 16 E6/E7 gene mutation in cervical precancer or cancer: A case control study in Guizhou Province, China. *J Med Virol*, **88**, 345-50.
- Zacapala-Gómez AE, Del Moral-Hernández O, Villegas-Sepúlveda N, et al (2016). Changes in global gene expression profiles induced by HPV 16 E6 oncoprotein variants in cervical carcinoma C33-A cells. *Virology*, **488**, 187-95.
- Zehbe I, Wilander E, Delius H, et al (1998). Human papillomavirus 16 E6 variants are more prevalent in invasive cervical

carcinoma than the prototype. *Cancer Res*, **58**, 829-33.
Zhe X, Xin H, Pan Z, et al (2019). Genetic variations in E6, E7 and the long control region of human papillomavirus type 16 among patients with cervical lesions in Xinjiang, China. *Cancer Cell Int*, **19**, 65.



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