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RAPID COMMUNICATION

Human papillomavirus in esophageal squamous cell carcinoma in Colombia and Chile

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Abstract

AIM: To examine the presence of human papillomavirus (HPV) in esophageal squamous cell carcinoma (ESCC) specimens collected from Colombia and Chile located in the northern and southern ends of the continent, respectively.

METHODS: We examined 47 and 26 formalin-fixed and paraffin-embedded ESCC specimens from Colombia and Chile, respectively. HPV was detected using GP5+/GP6+ primer pair for PCR, and confirmed by Southern blot analysis. Sequencing analysis of L1 region fragment was used to identify HPV genotype. In addition, $P16^{INK4A}$ protein immunostaining of all the specimens was conducted.

RESULTS: HPV was detected in 21 ESCC specimens (29%). Sequencing analysis of L1 region fragment identified HPV-16 genome in 6 Colombian cases (13%) and in 5 Chilean cases (19%). HPV-18 was detected in 10 cases (21%) in Colombia but not in any Chilean case. Since Chilean ESCC cases had a higher prevalence of HPV-16 (without statistical significance), but a significantly lower prevalence of HPV-18 than in Colombian cases (P = 0.011) even though the two countries have similar ESCC incidence rates, the frequency of HPV-related ESCC may not be strongly affected by risk factors affecting the incidence of ESCC. HPV-16 genome was more frequently detected in p16 positive carcinomas, although the difference was not statistically significant. HPV-18 detection rate did not show any association with p16 expression. Well-differentiated tumors tended to have either HPV-16 or HPV-18 but the association was not statistically significant. HPV genotypes other than HPV-16 or 18 were not detected in either country.

CONCLUSION: HPV-16 and HPV-18 genotypes can be found in ESCC specimens collected from two South American countries. Further studies on the relationship between HPV-16 presence and p16 expression in ESCC would aid understanding of the mechanism underlying the presence of HPV in ESCC.

Key words: Human papillomavirus; Esophageal squamous cell cancer; Colombia; Chile

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INTRODUCTION

Human papillomavirus (HPV), a double-stranded DNA virus, is recognized as an etiologic agent of cervical cancer[1]. In addition, HPV is suspected of causing extragenital cancers, including cancers of the oral cavity, larynx, esophagus, and lung[2]. However, the role of HPV

infection in the pathogenesis of those malignancies is still controversial. The relationship between HPV and esophageal squamous cell carcinoma (ESCC) has been suspected since the initial reports by Syrjanen *et al* in 1982[3,4]. An extensive review by Syrjanen published in 2002 reported that HPV is positive in 22.9% of 1485 ESCC cases analyzed by *in situ* hybridization (ISH) and in 15.2% of 2020 ESCC cases analyzed by PCR[5]. HPV signals were reported to present in normal cells[6], hyperplastic and dysplastic epithelia surrounding ESCC[7,8]. Zhou *et al*[6] reported that HPV expression in normal epithelia rarely accompanies the expression of HPV E6 protein, whereas its expression is frequently observed in carcinoma cells. These findings suggest that the involvement of HPV may take place in very early stages of esophageal carcinomas, which is suspected of being a progressive multistage process[9]. As postulated in development of head and neck tumors[10], esophageal cancer may arise from the "condemned mucosa"[11], and its development is considered to involve various risk factors, including HPV.

More than 99% of cervical cancers are known to be related to HPV[1]. One of the interesting features of cervical cancer is marked overexpression of $p16^{INK4A}$ (p16), a tumor suppressor which is the cyclin-dependent kinase inhibitor[12]. P16 binds to the complex of cyclin D1 and cyclin-dependent kinase 4 and represses its ability to phosphorylate the retinoblastoma protein (pRb)[13,14]. Hyperphosphorylation of pRb promotes unbinding of pRb and E2F while the released E2F, a well-known universal transcriptional activator, stimulates p16 transcription[15]. At the same time, however, the free pRb negatively regulates p16 expression. In the presence of HPV, this negative feedback loop between pRb and p16 is disrupted by binding of HPV E7 to pRb since E7-bound pRb cannot regulate p16 expression. As a consequence, p16 overexpression is induced by the presence of HPV[15]. Therefore, the comparison of p16 expression in HPV-positive and -negative ESCC may give some insight into the etiological role of HPV in ESCC development.

Although seroepidemiological evidence is important in evaluating the etiological role of HPV in ESCC, studies have reported inconsistent results. A study in Finland showed that ESCC patients have elevated serum antibody against HPV-16 when compared to blood bank donors[16]. Whereas a study in Norway has confirmed the Finnish study[17], a study in Sweden could not confirm it[18]. On the other hand, a study conducted in Shaanxi Province, China, where esophageal cancer risk is known to be high, ESCC patients have elevated serum antibodies against HPV-16[19]. However, none of these studies has tried to confirm the presence of HPV in esophageal carcinoma cells.

HPV has been found in variable proportions of ESCC, depending on the methodology and study areas[5]. Interestingly, studies from Asian countries, especially from China, have reported relatively high percentages of HPV-positive ESCC cases when compared to reports from Western European countries. To our knowledge, there is no study comparing the HPV infection rates in the South American Continent. In the present study, we examined ESCC in Colombia and Chile, located in the northern and southern ends of the South American Continent, and compared the detection rate of HPV in ESCC in the two countries. In addition, the association of HPV presence with p16 expression was also examined in order to evaluate the possible etiological role of HPV in ESCC development.

MATERIALS AND METHODS

Patients

We examined 47 formalin-fixed and paraffin-embedded ESCC specimens from Hospital Universitario del Valle in Cali, Colombia, from 1996 to 2001, and 26 paraffin-embedded ESCC specimens from Hospital San Camilo, San Felipe, Chile, in 1996-2000. Institutional Review Board of the Faculty of Medicine, Kagoshima University, Japan, approved the present study.

DNA extraction

Each formalin-fixed and paraffin-embedded sample was cut into 10-μm thick sections. The specimens were treated with 1 mL of xylene and then 1 mL of ethanol. After centrifugation, the pellet was resuspended in digestion buffer (50 mmol/L Tris-Cl pH 8.0, 1 mmol/L EDTA pH 8.0, 0.5% Tween 20) containing 200 μg of proteinase K (Invitrogen) and incubated at 56℃ for 24 h. After incubation, the solution was heated at 100℃ for 10 min and centrifuged. An aliquot of the supernatant was directly used for PCR.

PCR, Southern blot hybridization and sequencing

HPV amplification with *GP5+/GP6+* primer pair[20,21] was made in a reaction mix containing 2.5 μL of template DNA, 200-μmol/L dNTP, 0.5 μmol/L of each primer and 1.0 U Taq DNA polymerase (Takara, Japan) in a total volume of 25 μL reaction buffer (50 mmol/L KCl, 20 mmol/L Tris-Cl, pH 8.3). The conditions of amplification were as follows: an initial denaturation at 95℃ for 4 min followed by 45 cycles at 95℃ for 1 min, at 40℃ for 2 min, at 72℃ for 1.5 min, and a final extension at 72℃ for 5 min. Beta-globin amplification with *PCO3/PCO4* (110 bp) primers was used as the internal positive control. PCR conditions were as follows: an initial denaturation at 95℃ for 4 min followed by 40 cycles with the cycling profile at 95℃ for 1 min, at 52℃ for 1 min, at 72℃ for 2 min, and a final extension at 72℃ for 5 min. DNA purified from Hela cells containing HPV-18 was used as external positive control.

The amplified products were revealed by electrophoresis with 3.0% agarose gels at 100 volts for 30 min. After electrophoresis, the DNA was transferred onto Hybond N+ nylon transfer membrane (Amersham, UK) by capillary blotting using 0.4 N NaOH. The generic *GP5+/GP6* + PCR products amplified from HPV-18, -16, -11 and -6 were purified from agarose gels with QIAEX Ⅱ extraction kits (Qiagen GmbH and Qiagen Inc., Hilden, Germany) and used as probes. The hybridization was performed at 42℃ overnight and then the membranes were washed at 42℃ with solution containing 6 mol/L urea, 0.4% SDS and 0.5 × SSC buffer. For the detection of the HPV band, hybridization was carried out using the

Table 1 Clinico-pathological distribution of ESCC cases in Colombia and Chile

	Colombia (%)	Chile (%)	*P*
Age (yr)	63.6 ± 12.9	72.2 ± 8.9	0.003
Sex			1.000
Female	22 (47)	12 (46)	
Male	25 (53)	14 (54)	
Differentiation of tumors			0.071
Well	17 (36)	4 (15)	
Moderate	23 (49)	13 (50)	
Poor	7 (15)	9 (35)	
p16 expression[1]			0.625
Negative	21 (46)	14 (54)	
Positive[2]	25 (54)	12 (46)	

[1]p16 expression was not examined in 1 Colombian case because of inappropriate condition for immunostaining; [2]Positive p16 expression: p16 immunostaning was observed in 10% or higher of observed carcinoma cells.

Table 2 HPV detection in 73 cases of Colombia and Chile ESCC

	n	HPV16 (%)	*P*	HPV18 (%)	*p*
Country			0.506		0.011
Colombia	47	6 (13)		10 (21)	
Chile	26	5 (19)		0 (0)	
Sex			0.745		0.499
Female	34	6 (18)		6 (18)	
Male	39	5 (13)		4 (10)	
Age (yr)			0.316		0.212
< 60	19	1 (5)		5 (26)	
60-	22	3 (14)		2 (9)	
70-	32	7 (22)		3 (9)	
Differentiation of tumors			0.834		0.682
Well	21	4 (19)		4 (19)	
Moderate	36	5 (14)		4 (11)	
Poor	16	2 (13)		2 (13)	
p16 expression			0.190		0.515
Negative	35	3 (9)		6 (17)	
Positive[1]	37	8 (22)		4 (11)	

[1]Positive p16 expression: p16 immunostaning was observed in 10% or higher of observed carcinoma cells.

ECL direct labeling and detection kit (Amersham, UK) according to the manufacturers instructions. Amplified PCR products that appeared as a visible band after ethidium bromide staining were purified using QIAGEN PCR purification kit and directly sequenced by fluorescent dye-labeled dideoxynucleotides and cycle sequencing methods using the "Big DyeTerminator cycle sequencing kit" (PE Applied Biosystems, New Jersey, USA). In the samples where positive signal was seen only after Southern blot analysis but not in the agarose-gel electrophoresis, a second round of PCR was conducted. Sequence analysis was performed on the ABI PRISM 310 genetic analyzer (PE Applied Biosystems, New Jersey, USA). The nucleotide sequences were aligned and compared with those of known HPV types available from the GenBank database (NCBI, National Institute of health, Bethesda, MD, USA) by using BLAST 2.2 (http://www.ncbi.nih.gov/BLAST/).

P16^{INK4A} protein immunostaining

Sections of paraffin-embedded tissue with a thickness of 2-3 μm were placed on silane-coated glass slides, and deparaffinized by passage through xylene. After the endogenous peroxidase activity was blocked with 0.3% H_2O_2/methanol, the slides were then rehydrated with 0.01 mol/L sodium phosphate/citrate buffer, pH 8.0. For antigen retrieval, the slides were heated in 0.01 mol/L-citrate buffer, pH 6.0, at 95℃ for 30 min, and left to cool for 30 min. After rinsed in 0.01 mol/L phosphate-buffered saline (PBS), pH 7.4, nonspecific antibody binding was reduced by incubating the sections with 10% fetal bovine serum in PBS for 30 min. Then, the sections were incubated overnight at 4℃ with a mouse monoclonal antibody of p16 protein (1:200 dilution, GST-p16^{INK4A}, PharMingen International). After washing thoroughly with PBS, the slides were incubated with biotinylated horse anti-mouse IgG for 30 min followed by 1:100 dilution of the avidin-biotin-peroxidase complex (Vectastain elite ABC kit, Vector Laboratories, Burlingame, CA) for an additional 30 min. The peroxidase signal was visualized by treatment with DAB substrate-chromogen system (DAKO) for 8 min. Finally the sections were stained lightly with hematoxylin. In statistical analysis, those having less than 10% cells stained positive were classified as negative cases and the others were regarded as positive cases[22].

Statistical analysis

Fisher's exact test was used to examine the association between HPV status and each clinico-pathological factor including p16 expression. We used Wilcoxon rank-sum test for the comparison of age distribution. All the *P* values presented in the present study were two-sided.

RESULTS

We examined 47 Colombian cases and 26 Chilean cases. Their clinico-pahological features are summarized in Table 1. Chilean patients were older than Colombian patients, and Chilean ESCC tended to be more poorly-differentiated than Colombian tumors.

HPV was detected using GP5+/GP6+ primer pair by PCR and its presence was confirmed by Southern blot analysis using HPV-16, -18, -11 and -6 specific probes. In all HPV-negative samples, beta-globin gene was successfully amplified by PCR. Table 2 summarizes the results of HPV detection analysis. HPV-16 and -18 were detected in 11 and 10 cases, respectively. No other HPV genotypes were detected in either country. We confirmed the absence of HPV-6 and -11 by Southern blot analysis using the probes specific for HPV-6 and -11. HPV genotype was determined by sequencing analysis of L1 region fragment.

HPV-16 was detected in 6 Colombian cases (13%) and 5 Chilean cases (19%). The five positive samples from Chile were detected only after Southern blot analysis, and a second round of PCR was necessary for sequence analysis. The difference in HPV detection rate of the two countries was not statistically significant ($P = 0.181$). HPV-16 detection rate did not show any statistically significant association with sex or age. The presence of

HPV-16 genome was more frequent in well-differentiated carcinoma and p16-positive tumors but their association was not statistically significant.

HPV-18 was detected in 10 Colombia cases(21%) but not in any Chilean cases. The observed difference was statistically significant ($P = 0.011$, Fisher's exact test). HPV-18 detection rate did not show any statistically significant association with sex, age, or with p16 expression. Its detection rate was higher in well-differentiated tumors as was the case for HPV-16 but the association was not statistically significant.

DISCUSSION

In the present study, we detected HPV in 29% of ESCC specimens using PCR and Southern blot analysis. The observed frequency is similar to that obtained from the meta-analysis by Syrjanen[4]. HPV-16 and -18 were detected in 15% and 14% of ESCC specimens, respectively. HPV-16 positive and negative tumors did not show any differences in demographic and pathological features.

The etiological role of HPV in development of ESCC is as yet unclear. There is, however, evidence that HPV plays an etiological role in development of ESCC. For example, high risk HPV E6/E7 genes have been shown to be capable of inducing immortalization in established cell lines of fetal esophageal epithelial cells and primary culture of human esophageal keratinocytes[22,23]. HPV E6 proteins are known to bind to p53 *in vivo* and to abrogate p53-mediated repression of transcription[24]. The mode of p53 activation at a cancer site may differ. It is suggested that the major mode of p53 inactivation may be through interaction with E6 in HPV-infected cervical tissue while both E6-p53 interaction and mutation of the p53 take place in upper aero-digestive tract carcinomas[11].

The carcinogenic mechanism of HPV involves the binding and inactivation of pRb by HPV E7 products as well, and the expression of HPV-16 E7 protein correlates with reduced pRb levels in cervical biopsies[25]. However, such a relationship has not been confirmed in esophageal carcinomas. On the other hand, the loss of this protein causes overexpression of p16, which is an inhibitor of cyclin-dependent kinase. This event is very frequent in cervical cancer, where HPV has been demonstrated to play an etiological role[12]. In the present study, positive HPV-16 was more frequently detected among p16 positive cases, supporting the hypothesis that HPV may have an etiologic role in esophageal carcinogenesis. The observed association was, however, not statistically significant. On the other hand, the frequency of HPV-18 did not differ in p16-positive and -negative carcinomas. Further studies on this association seem warranted.

In the present study, the prevalence of HPV-18 was significantly lower in Chilean ESCC cases than in Colombian cases, whereas HPV-16 detection rate did not show any differences between the two countries. Regarding this observation, the prevalence of HPV in the two countries may give some insights. A study on cervical smears collected from women aged 15-69 years in Santiago, Chile[26], showed that the most common HPV genotype is HPV-16 followed by HPV-56 and -58, while HPV-18 is detected in 0.5 % of subjects and HPV-16 in 2.6% of subjects. In Bogota, Colombia, a study on cervical smears[27] showed that HPV-16 prevalence is 16.1% among women aged 35 or older and HPV genotypes of 18, 39, 45, 59, and 68 are present in 13.6% of women of the same age group. Although comparison of the results obtained from these studies is difficult because of the differences in their study designs, their results suggest that HPV 18 is much less common than HPV-16 in the study areas.

The mortality of esophageal cancer is estimated to be 7.3 per 100 000 men and 3.5 per 100 000 women and the fifth leading cause of cancer death in men and the seventh in women[28]. Interestingly, its frequency evidently varies worldwide[29]. The high-risk areas include the so-called Asian esophageal cancer belt from eastern Turkey, Iraq, Iran, and western and northern China[30]. The wide geographic variations in the risk of esophageal cancer suggest that environmental and genetic factors are involved in development of esophageal carcinomas, and HPV is suspected of a potential factor contributing to the high incidence of ESCC in areas of Iran, China and other countries[31,32]. Latin America is not known to be a region where esophageal cancer risk is high. In Cali, Colombia, the local cancer registry office reported that esophageal cancer incidence (crude rate) is 6.5 per 100 000 men and 2.0 per 100 000 women, respectively, in 1995-1998[33]. In Chile, cancer registry data are not available. However, cancer mortality, which is assessable even if cancer registry is not present, is expected to be not much different from cancer incidence because its prognosis is poor as is the case in other countries[34]. In this country, esophageal cancer is the fifth leading cause of death from neoplasic diseases in men (5.8/100 000) and the twelfth in women (3.5/100 000)[35]. The Hospital San Camilo, which participated in the present study, is the major medical institution in San Felipe city, Aconcagua Province. In this area, esophageal cancer mortality rate is estimated to be 6.5/100 000 persons, not evidently different from the rate in the entire nation. Thus, we can conclude that the ESCC risk is not much different in the two countries. Since Chilean ESCC cases had a higher prevalence of HPV-16 (without statistical significance), but a significantly lower prevalence of HPV-18 than in Colombian cases in the present study. The frequency of HPV-related ESCC may not be related to factors that play an important role in the incidence of ESCC in the two countries. However, the small number of cases examined makes it difficult to draw any definitive conclusion.

In conclusion, HPV-16 and -18 genotypes can be found in ESCC patients from Colombia and Chile. Further studies on the relationship between HPV-16 and -18 presence and p16 expression are needed for understanding the mechanism underlying the presence of HPV in ESCC.

REFERENCES

1 **Bosch FX**, Manos MM, Muñoz N, Sherman M, Jansen AM, Peto J, Schiffman MH, Moreno V, Kurman R, Shah KV. Prevalence of human papillomavirus in cervical cancer: a worldwide perspective. International biological study on cervical cancer (IBSCC) Study Group. *J Natl Cancer Inst* 1995; **87**: 796-802

2 **Gillison ML**, Shah KV. Chapter 9: Role of mucosal human papillomavirus in nongenital cancers. *J Natl Cancer Inst Monogr* 2003; 57-65

3 **Syrjänen K,** Pyrhönen S, Aukee S, Koskela E. Squamous cell papilloma of the esophagus: a tumour probably caused by human papilloma virus (HPV). *Diagn Histopathol* 1982; **5**: 291-296

4 **Syrjänen KJ**. Histological changes identical to those of condylomatous lesions found in esophageal squamous cell carcinomas. *Arch Geschwulstforsch* 1982; **52**: 283-292

5 **Syrjänen KJ**. HPV infections and oesophageal cancer. *J Clin Pathol* 2002; **55**: 721-728

6 **Zhou XB**, Guo M, Quan LP, Zhang W, Lu ZM, Wang QH, Ke Y, Xu NZ. Detection of human papillomavirus in Chinese esophageal squamous cell carcinoma and its adjacent normal epithelium. *World J Gastroenterol* 2003; **9**: 1170-1173

7 **Chang F**, Syrjänen S, Shen Q, Cintorino M, Santopietro R, Tosi P, Syrjänen K. Human papillomavirus involvement in esophageal carcinogenesis in the high-incidence area of China. A study of 700 cases by screening and type-specific in situ hybridization. *Scand J Gastroenterol* 2000; **35**: 123-130

8 **Astori G**, Merluzzi S, Arzese A, Brosolo P, de Pretis G, Maieron R, Pipan C, Botta GA. Detection of human papillomavirus DNA and p53 gene mutations in esophageal cancer samples and adjacent normal mucosa. *Digestion* 2001; **64**: 9-14

9 **Shen ZY**, Xu LY, Li EM, Cai WJ, Shen J, Chen MH, Cen S, Tsao SW, Zeng Y. The multistage process of carcinogenesis in human esophageal epithelial cells induced by human papillomavirus. *Oncol Rep* 2004; **11**: 647-654

10 **Hunter KD**, Parkinson EK, Harrison PR. Profiling early head and neck cancer. *Nat Rev Cancer* 2005; **5**: 127-135

11 **Pillai MR**, Nair MK. Development of a condemned mucosa syndrome and pathogenesis of human papillomavirus-associated upper aerodigestive tract and uterine cervical tumors. *Exp Mol Pathol* 2000; **69**: 233-241

12 **Sano T**, Oyama T, Kashiwabara K, Fukuda T, Nakajima T. Expression status of p16 protein is associated with human papillomavirus oncogenic potential in cervical and genital lesions. *Am J Pathol* 1998; **153**: 1741-1748

13 **Serrano M**, Hannon GJ, Beach D. A new regulatory motif in cell-cycle control causing specific inhibition of cyclin D/CDK4. *Nature* 1993; **366**: 704-707

14 **Lukas J**, Parry D, Aagaard L, Mann DJ, Bartkova J, Strauss M, Peters G, Bartek J. Retinoblastoma-protein-dependent cell-cycle inhibition by the tumour suppressor p16. *Nature* 1995; **375**: 503-506

15 **Khleif SN**, DeGregori J, Yee CL, Otterson GA, Kaye FJ, Nevins JR, Howley PM. Inhibition of cyclin D-CDK4/CDK6 activity is associated with an E2F-mediated induction of cyclin kinase inhibitor activity. *Proc Natl Acad Sci USA* 1996; **93**: 4350-4354

16 **Dillner J**, Knekt P, Schiller JT, Hakulinen T. Prospective seroepidemiological evidence that human papillomavirus type 16 infection is a risk factor for oesophageal squamous cell carcinoma. *BMJ* 1995; **311**: 1346

17 **Bjørge T**, Hakulinen T, Engeland A, Jellum E, Koskela P, Lehtinen M, Luostarinen T, Paavonen J, Sapp M, Schiller J, Thoresen S, Wang Z, Youngman L, Dillner J. A prospective, seroepidemiological study of the role of human papillomavirus in esophageal cancer in Norway. *Cancer Res* 1997; **57**: 3989-3992

18 **Lagergren J**, Wang Z, Bergström R, Dillner J, Nyrén O. Human papillomavirus infection and esophageal cancer: a nationwide seroepidemiologic case-control study in Sweden. *J Natl Cancer Inst* 1999; **91**: 156-162

19 **Han C**, Qiao G, Hubbert NL, Li L, Sun C, Wang Y, Yan M, Xu D, Li Y, Lowy DR, Schiller JT. Serologic association between human papillomavirus type 16 infection and esophageal cancer in Shaanxi Province, China. *J Natl Cancer Inst* 1996; **88**: 1467-1471

20 **Snijders PJ**, van den Brule AJ, Schrijnemakers HF, Snow G, Meijer CJ, Walboomers JM. The use of general primers in the polymerase chain reaction permits the detection of a broad spectrum of human papillomavirus genotypes. *J Gen Virol* 1990; **71** (Pt 1): 173-181

21 **de Roda Husman AM**, Walboomers JM, van den Brule AJ, Meijer CJ, Snijders PJ. The use of general primers GP5 and GP6 elongated at their 3' ends with adjacent highly conserved sequences improves human papillomavirus detection by PCR. *J Gen Virol* 1995; **76** (Pt 4): 1057-1062

22 **Ralhan R**, Mathew R, Arora S, Bahl R, Shukla NK, Mathur M. Frequent alterations in the expression of tumor suppressor genes p16INK4A and pRb in esophageal squamous cell carcinoma in the Indian population. *J Cancer Res Clin Oncol* 2000; **126**: 655-660

23 **Sashiyama H**, Shino Y, Kawamata Y, Tomita Y, Ogawa N, Shimada H, Kobayashi S, Asano T, Ochiai T, Shirasawa H. Immortalization of human esophageal keratinocytes by E6 and E7 of human papillomavirus type 16. *Int J Oncol* 2001; **19**: 97-103

24 **Lechner MS**, Mack DH, Finicle AB, Crook T, Vousden KH, Laimins LA. Human papillomavirus E6 proteins bind p53 in vivo and abrogate p53-mediated repression of transcription. *EMBO J* 1992; **11**: 3045-3052

25 **Fiedler M**, Müller-Holzner E, Viertler HP, Widschwendter A, Laich A, Pfister G, Spoden GA, Jansen-Dürr P, Zwerschke W. High level HPV-16 E7 oncoprotein expression correlates with reduced pRb-levels in cervical biopsies. *FASEB J* 2004; **18**: 1120-1122

26 **Ferreccio C**, Prado RB, Luzoro AV, Ampuero SL, Snijders PJ, Meijer CJ, Vaccarella SV, Jara AT, Puschel KI, Robles SC, Herrero R, Franceschi SF, Ojeda JM. Population-based prevalence and age distribution of human papillomavirus among women in Santiago, Chile. *Cancer Epidemiol Biomarkers Prev* 2004; **13**: 2271-2276

27 **Molano M**, Van den Brule A, Plummer M, Weiderpass E, Posso H, Arslan A, Meijer CJ, Muñoz N, Franceschi S. Determinants of clearance of human papillomavirus infections in Colombian women with normal cytology: a population-based, 5-year follow-up study. *Am J Epidemiol* 2003; **158**: 486-494

28 **Pisani P**, Parkin DM, Bray F, Ferlay J. Estimates of the worldwide mortality from 25 cancers in 1990. *Int J Cancer* 1999; **83**: 18-29

29 **Muñoz N**. Epidemiological aspects of oesophageal cancer. *Endoscopy* 1993; **25**: 609-612

30 **Lam AK**. Molecular biology of esophageal squamous cell carcinoma. *Crit Rev Oncol Hematol* 2000; **33**: 71-90

31 **Li T**, Lu ZM, Chen KN, Guo M, Xing HP, Mei Q, Yang HH, Lechner JF, Ke Y. Human papillomavirus type 16 is an important infectious factor in the high incidence of esophageal cancer in Anyang area of China. *Carcinogenesis* 2001; **22**: 929-934

32 **Farhadi M**, Tahmasebi Z, Merat S, Kamangar F, Nasrollahzadeh D, Malekzadeh R. Human papillomavirus in squamous cell carcinoma of esophagus in a high-risk population. *World J Gastroenterol* 2005; **11**: 1200-1203

33 **Carrascal E.** In: Parkin DM, Whelan SL, Ferlay J, Teppo L, Thomas DB, eds. Cancer Incidence in Five Continents, Vol. VII. Cali, Colombia: IARC Scientific publications, 2002: 114-115

34 **Braghetto I,** Csendes A, Cornejo A, Amat J, Cardemil G, Burdiles P, Korn O. [Survival of patients with esophageal cancer subjected to total thoracic esophagectomy]. *Rev Med Chil* 2000; **128**: 64-74

35 **Medina E,** Kaempffer AM. [Cancer mortality in Chile: epidemiological considerations]. *Rev Med Chil* 2001; **129**: 1195-1202

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