

Correlation of HPV-16/18 infection of human papillomavirus with lung squamous cell carcinomas in Western China

YAN YU, AIMIN YANG, SENKE HU and HONG YAN

Department of Public Health, Xi'an Jiaotong University School of Medicine,
No. 76, West Yanta Road, Xi'an, Shaanxi 710061, P.R. China

Received January 16, 2009; Accepted March 31, 2009

DOI: 10.3892/or_00000397

Abstract. Prevalent inconsistency of human papillomavirus (HPV) infection in lung cancer was found among recent studies from different countries with racial and geographic variations. Even in Chinese populations, the prevalent discrepancy of HPV infection in lung cancer patients was also found with the geographic variations and tumor types. To study the difference of HPV-16/18 infection in lung squamous cell carcinomas and non-cancer controls, we conducted this study to verify whether there was a similar HPV infection prevalence pattern in lung squamous cell carcinoma patients from the Western part of China. Paraffin-embedded samples (180), of 72 lung squamous cell carcinomas, 37 lung adenocarcinomas and 71 non-cancer controls, were analyzed by INNO-LIPA Genotype polymerase chain reaction (PCR) and real-time PCR analysis. The data showed that 51.4% (37/72) of lung squamous cell carcinoma samples, 16.2% (6/37) of adenocarcinoma, and 22.5% (16/71) of non-cancer controls were HPV DNA positive. The risk of lung squamous cell carcinomas was 3.5 times higher among people HPV-positive (odds ratio 3.5, 95% CI 1.6-7.3, $p < 0.001$) compared with the HPV-negative population. Adjusted by smoking status, the risk of lung squamous cell carcinomas was 3.5 times higher among people HPV-positive (odds ratio 3.5, 95% CI 1.7-7.5, $p = 0.001$) compared with the HPV-negative population. The risk of lung squamous cell carcinomas was 16.9 times higher for patients with positive HPV-16 (odds ratio 16.9, 95% CI 3.8-75.3, $p < 0.0001$) than negative HPV-16. Adjusted by smoking status, the risk of lung squamous cell carcinomas was 17.4 times higher among people HPV-16 positive (odds ratio 17.4, 95% CI 3.9-77.5, $p < 0.0001$) compared with HPV-16 negative people. INNO-LIPA Genotype analysis revealed that the frequency of high risk HPV-16 and 18 genotype in

lung squamous cell carcinoma cases appeared to be significant higher than that in the non-cancer controls ($P < 0.001$), and the most frequent genotype was HPV-16 (21 cases), followed by HPV-18 (6 cases), HPV-16/18 (4 cases) in lung squamous cell carcinomas, respectively, and the majority genotype was HPV-6 (9 cases) in controls. The HPV-16 E₂/E₆ ratio was significantly lower than the unity, suggested that the main presence of integrated form of HPV-16 genome (16/27) may contribute more to lung squamous cell carcinomas, despite its viral load estimated to be only < 1 to 2 copies per cell.

Introduction

Human papillomavirus (HPV) is a double stranded DNA virus, and is an established etiological agent causing cervix cancer. Its high-risk genotypes, including HPV-16 and -18, are associated with cervix cancer and other genital cancers (1,2). Lung cancer is a major health problem and the leading cause of cancer deaths in the world. The pathogenesis of lung cancer is complex, and is believed to be due to the interaction between environmental and genetic factors. Various evidence shows that HPV might be associated with bronchial carcinogenesis and lung cancer (3-6).

Although smoking was the most frequent risk factor of lung cancer, the mechanism is not clear (7). Etiological involvement of HPV in development of lung cancer was originally postulated (8-10). Inconsistency in prevalence of human papillomavirus (HPV) infection in lung cancer was found among recent studies from different countries with racial and geographic variations. A large sample investigation reported that HPV DNA infection rate was 21.7% in 2468 lung carcinomas (11). HPV infection is considered a co-carcinogen of lung cancer and its contribution to pathogenesis has been evaluated (12-18). However, even in Asian populations, the prevalence discrepancy of HPV infection in lung cancer patients was also found with the geographic variations and tumor types. A study in Taiwan (13) reported that 77 (54.6%) of 141 lung tumors had HPV-16 or -18 DNA compared with 16 (26.7%) of 60 non-cancer control subjects among non-smoking Taiwanese women, and a recent study from mainland China suggested that the rates of HPV-16 and/or -18 infections in patients with 73 lung tumors were significantly higher than in 34 non-cancer control subjects (27.7 vs. 5.9%) (19). In the central part of China

Correspondence to: Dr Hong Yan, Department of Public Health, Xi'an Jiaotong University School of Medicine, No. 76, West Yanta Road, Xi'an, Shaanxi 710061, P.R. China
E-mail: hongyanpaper2008@yahoo.com

Key words: human papillomavirus, viral load, integration, lung squamous cell carcinoma, the west part of China

investigators have found that 44.1% of (138/313) NSCLC samples are positive, while 4.2% (4/96) of lung benign controls are positive (20).

To study the role of integration of HPV-16 DNA into the host genome which may contribute to lung squamous cell carcinoma development, we conducted this study to verify whether there was a similar HPV infection prevalence pattern and HPV-16 integration status in lung squamous cell carcinoma patients from Western China.

Materials and methods

Samples. The 180 paraffin-embedded samples, of 72 lung squamous cell carcinomas, 37 lung adenocarcinomas and 71 non-cancer controls were collected and diagnosed in the first affiliated hospital of Xi'an Jiaotong University, China. The 71 non-cancer controls included tuberculomas 41, hyper-trophy 19, granuloma 8, and pseudoplasm 3, respectively. All of these cases were from the West part of China during the period from 2005 to December 2007. The demographic features review and statistical analysis were approved by the Department of Public Health, Xi'an Jiaotong University School of Medicine, China. The study was approved by the Ethics Committee of Xi'an Jiaotong University, China.

DNA extraction. Formalin-fixed paraffin-embedded samples were cut into 10 μm slices and prepared according to the method described before (21). The specimens were treated with 1 ml of xylene, and then with 1 ml of ethanol. After centrifugation, the pellet was resuspended in digestion buffer (50 mM Tris-Cl pH 8.0, 1 mM EDTA pH 8.0, 0.5% Tween-20) containing 200 μg of proteinase K (Invitrogen) and incubated at 56°C overnight. After the incubation, the solution was heated at 100°C for 10 min and centrifuged. An aliquot of the supernatant was directly used for PCR.

β -globin PCR. β -globin amplification with PCO₃/PCO₄ (110 bp) primers (PCO₃ primer 5'-ACACAACTGTGTTCA CTGC-3' and PCO₄ primer 5'-CAACTTCATCCACGTTCC ACC-3') was used as the internal positive control (22). PCR condition was as follows: the initial denaturation at 95°C for 4 min, 40 cycles with the cycling profile of 95°C for 1 min, 52°C for 1 min and 72°C for 2 min and the final extension for 5 min at 72°C. DNA purified from HeLa cells containing HPV-16 was used as external positive control.

INNO-LIPA genotyping system. A 65 bp region of L1 gene of the HPV genome was amplified by PCR using short PCR fragment (SPF10) biotinylated primers. The PCR products were visualized on 4% agarose gel with ethidium bromide staining by electrophoresis and (10 μl) of the PCR product was denatured and hybridized with specific oligonucleotide probes (25 HPV type-specific probes) immobilized as parallel lines on a nitrocellulose membrane strips, following the manufacturer's instructions (INNO-LIPA HPV genotyping kit, Inno-genetics, Belgium). The 28 probes for 25 different HPV genotypes in each INNO-LIPA strip are described elsewhere (23). The strips were interpreted with a labeled acetate overlay with lines indicating the position of each probe relative to the reference mark.

Real-time PCR. Real-time PCR was performed with the ABI Prism 7000 Sequence Detection System and SYBR-Green PCR master mix (PE, Applied Biosystems). The amplification conditions were 15 cycles at 95°C and a two-step cycle of 95°C for 30 sec and 60°C for 60 sec for a total of 40 cycles. The used primer sets were as follows: E₆F: GAGAACTG CAATGTTTCAGGACC and E₆R: TGTATAGTTGTTTGC AGCTCTGTGC; E₂F: AACGAAGTATCCTCTCCTGAA ATTATTAG, and E₂R: CCAAGGCGACGGCTTTG. Those primers amplify a fragment of E₆ (81 bp) and E₂ (76 bp) ORFs, respectively (24). The final concentration of primers was 0.5 μM . All the experiments were made in duplicate. The specificity of amplification was confirmed using dissociation analysis starting at 60°C and agarose gel electrophoresis of amplified products.

Statistical analysis. The central focus of our analysis was to examine the independent associations between HPV infection and lung squamous cell carcinomas, modeled as categorical variable. Significant differences in demographic characteristics and smoking status across case and control were assessed using χ^2 test or Fisher's exact test for the equality of proportions. The associations between HPV status, HPV-16 status and lung squamous cell carcinomas were assessed using logistic regression and were expressed as the odds ratio (OR) and its 95% CI. We evaluated possible effect modification by smoking status by adding product terms to the regression models. Differences were considered significant at $P \leq 0.05$. We used Stata version 9.2 (Stata /SE 9.2 StataCorp, College Station, TX) for all analyses.

Results

Demographic features of lung carcinoma cases and controls are summarized. Male patients accounted for 66.7% (48 patients) of the 72 lung squamous cell carcinomas cases, 64.9% (24 patients) in 37 lung adenocarcinomas and 63.4% (45 patients) in 71 non-cancer control cases. The mean age of the cases and controls was 63.6 \pm 11.7 years and 63.3 \pm 12.4, respectively (data not shown).

The β -globin gene was successfully amplified by PCR in all samples. The HPV-positive signal was not only detected in the agarose-gel electrophoresis but was confirmed by INNO-LIPA HPV genotyping analysis kit. Thirty-seven cases were detected in the lung squamous cell carcinoma group, 6 cases were detected in 37 lung adenocarcinomas, while 16 cases were detected in non-cancer control group. The frequency of HPV-positive cases (51.4%) was higher in lung squamous cell carcinomas than in adenocarcinomas and non-cancer control group (16.2 and 22.5%) ($P < 0.001$) (Table I).

Statistical analyses revealed that HPV infection was not correlated with gender, age, tumor stage and grades, but the positive rate of HPV is 42.5% (48/113) and 15.8% (3/19) in the smoker and non-smoker, respectively, and the frequency of HPV-positive in the cases was significantly associated with smoking habit ($p = 0.034$) (Table II).

The risk of lung squamous cell carcinomas was 3.5 times higher among people HPV-positive (odds ratio 3.5, 95% CI 1.6-7.3, $p < 0.001$) compared with HPV-negative people.



Difference of HPV DNA positive between lung cancer and non-cancer control cases.

	HPV-positive	HPV-negative	Total	χ^2	P
SQCs	37	35	72		
Adenocarcinomas	6	31	37		
Non-cancer control	16	55	71		
Total	59	121	180	19.304	<0.001

Table II. Clinicopathological parameter features of HPV DNA detection by PCR in lung squamous cell carcinomas and non-cancer control group.

	HPV-positive	HPV-negative	χ^2	P
Case			18.551	<0.001
Lung squamous cell carcinomas	37	35	0.410	0.814
I	6	4		
II	18	17		
III	13	14		
Non-cancer control	16	55		0.062
Tuberculosis	5	36		
Hypertrophy	7	12		
Granuloma	3	5		
Pseudoplasm	1	2		
Gender			0.028	0.865
Male	34	59		
Female	19	31		
Ages (years)			1.794	0.408
<55	18	26		
55-64	25	38		
≥65	10	26		
Smoking			6.787	0.034
Non	3	16		
Smoker	48	65		
Unknown	2	9		
Total	53	90		

Adjusted by smoking status, the risk of lung squamous cell carcinomas was 3.5 times higher among people HPV-positive (odds ratio 3.5, 95% CI 1.7-7.5, $p=0.001$) compared with HPV-negative people.

The risk of lung squamous cell carcinomas was 16.9 times higher for people with positive HPV-16 (odds ratio 16.9, 95% CI 3.8-75.3, $p<0.0001$) than those with negative HPV-16. Adjusted by smoking status, the risk of lung squamous cell carcinomas was 17.4 times higher for people with positive HPV-16 (odds ratio 17.4, 95% CI 3.9-77.5, $p<0.0001$) than that with negative HPV-16. There was no association between smoking status and lung squamous cell carcinomas (Table III).

Analysis of HPV genotypes revealed that the predominant genotype was HPV-16 (21 cases), followed by

HPV-18 (6 cases), HPV-16/18 (4 cases), HPV-6 (4 cases) and other genotype (2 cases) in lung squamous cell carcinomas cases, but the major genotype was HPV-6 (9 cases), followed by other genotype (3 cases), HPV-16 (2 cases), HPV-6/18 (1 cases) and HPV-18 (1 case) in non-cancer control cases. The high risk HPV genotypes 16 and/or 18 were detected in 43.06% (31/72) and 5.63% (4/71) in lung SQC cases and non-cancer control cases, respectively ($P<0.001$) (Table IV).

Results of real-time PCR analysis are summarized in Table V. The quantity of HPV-16 DNA ranged from 0.003 to 44.942 copies per nanogram of genomic DNA. Results from repeated analyses showed a difference less than 5% in all the cases. E_2/E_6 ratio was determined by real-time PCR. In

Table III. Difference of HPV-positive and HPV-16 positive in lung squamous cell carcinomas and non-cancer control group associated with smoking habit.

	Lung squamous cell carcinomas		Non-cancer control	
	HPV-positive	HPV-negative	HPV-positive	HPV-negative
Non-smoker	4	7	2	6
Smoker	31	26	13	43
Total	35	33	15	49

	Lung squamous cell carcinomas		Non-cancer control	
	HPV-16 positive	HPV-16 negative	HPV-16 positive	HPV-16 negative
Non-smoker	2	9	1	7
Smoker	22	35	1	55
Total	24	44	2	62

Table IV. HPV DNA genotypes detected by INNO-LIPA genotyping analysis.

HPV genotypes	Lung squamous cell carcinomas	Non-cancer control	P
HPV-16	21	2	<0.001
HPV-18	6	1	
HPV-6	4	9	
HPV-16/HPV-18	4	0	
HPV-6/HPV-18	0	1	
Other HPV	2	3	
Total	37	16	

16 cases (59.26%), E₂ DNA was not detected. In the rest of the cases, E₂ DNA was detected but the E₂/E₆ ratio was less than the unity.

Discussion

The frequency of lung carcinomas with HPV genomic sequences showed a wide range variation, from none to almost 80% (11,25,26). A review by Syrjanen (11) showed that HPV was detected in 536 of 2468 (22%) bronchial carcinomas. Meanwhile a study from Okinawa, Japan found positive HPV in 79% of lung squamous cell carcinomas (12).

In our study, HPV was detected not only using SPF10 primer PCR, but was confirmed by INNO-LIPA HPV genotyping analysis. Thirty-seven cases were detected in the 72 lung squamous cell carcinoma group, 6 cases were detected in 37 lung adenocarcinomas, while 16 cases were detected in 71 non-cancer controls. The frequency of HPV-positive cases (51.4%) was higher in lung squamous cell carcinomas than in non-cancer control group (22.5%). The positive rate of HPV is 42.5% (48/113) and 15.8% (3/19) in the smoker and non-smoker, respectively. It seemed that the frequency of HPV-positivity is significantly associated to smoking habit (P=0.034), and HPV detection rate was not associated with gender or age. This percentage was higher than the value of 22% reported by Syrjanen in bronchial carcinomas (11), and lower than 79% reported from Okinawa of Japan in lung squamous cell carcinomas (12).

Lung squamous cell carcinoma development was strongly associated to smoking habit because more than 90% of individuals undergoing squamous cell carcinomas were heavy smokers (27). To find the correlation between the smoking status and the risk of lung squamous cell carcinomas in HPV-positive cases, the associations between HPV status, HPV-16 status and lung squamous cell carcinomas were assessed using a logistic regression model. We evaluated the possible effect modification by smoking status by adding product terms to the regression models.

We found that the risk of lung squamous cell carcinomas was 3.5 times higher among people HPV-positive (odds ratio 3.5, 95% CI 1.6-7.3, p<0.001) compared with HPV-negative people. Adjusted by smoking status, the risk of lung squamous cell carcinomas was 3.5 times higher among people HPV-positive (odds ratio 3.5, 95% CI 1.7-7.5, p=0.001) compared with HPV-negative people. The risk of lung squamous cell carcinomas was 16.9 times higher among people HPV-16 positive (odds ratio 16.9, 95% CI 3.8-75.3, p<0.0001) compared with HPV-16 negative people. Adjusted by smoking status, the risk of lung squamous cell carcinomas was 17.4 times higher among people HPV-16 positive (odds ratio 17.4, 95% CI 3.9-77.5, p<0.0001) compared with HPV-16 negative people. Interestingly, there was no association between smoking status and lung squamous cell carcinomas, while HPV-16 positive had a predominantly higher risk than HPV-16 negative for the development of lung squamous cell carcinomas in Western China.

Cheng *et al* reported that lung carcinomas with high risk HPV was much more frequently found among females than males (4,13). The study from Japan (17) also reported that HPV-16 was more frequently found among female than male cases, and the frequency of HPV-16 positive cases among all the HPV-positive cases differed among the sexes. But in our study, no difference was found in gender and age. It was similar to the report from Noutsou *et al* (28) that no correlation was found between HPV infection with sex, age and clinical outcome of the patient, or the histological type and the differentiation grade of the tumor.

Meanwhile, some reports revealed that the higher risk of HPV-16 and HPV-18 were more strongly associated with non-small cell lung cancer (14,15,29). But inconsistent



SPANDIDOS HPV genotypes by INNO LIPA and HPV-16 viral loaded by RT-PCR.

Sample	Group	Genotype	E ₂ copies/ng	E ₆ copies/ng	E ₂ /E ₆	Status
1	SQCs	16	0	0.087	0	Integrated
2	SQCs	16	0	0.089	0	Integrated
3	SQCs	16	0	0.003	0	Integrated
4	SQCs	16	0	0.004	0	Integrated
5	SQCs	16	0	0.014	0	Integrated
6	SQCs	16	0	0.084	0	Integrated
7	SQCs	16	0	0.013	0	Integrated
8	SQCs	16	0.651	1.165	0.559	Mix
9	SQCs	16	0.210	1.972	0.107	Mix
10	SQCs	16	0	0.036	0	Integrated
11	SQCs	16/18	0.140	14.320	0.010	Mix
12	SQCs	16	0	0.013	0	Integrated
13	SQCs	16	0	0.005	0	Integrated
14	SQCs	16/18	0.005	1.223	0.004	Mix
15	SQCs	16	1.866	14.123	0.132	Mix
16	SQCs	16	0.074	1.035	0.071	Mix
17	SQCs	16	0	0.004	0	Integrated
18	SQCs	16	12.126	44.9422	0.270	Mix
19	SQCs	16	0	0.011	0	Integrated
20	SQCs	16/18	0.371	5.242	0.071	Mix
21	SQCs	16	0	0.003	0	Integrated
22	SQCs	16	1.613	7.210	0.224	Mix
23	SQCs	16	0	0.033	0	Integrated
24	SQCs	16	0	0.051	0	Integrated
25	SQCs	16/18	0.480	10.928	0.044	Mix
26	control	16	0	0.224	0	Integrated
27	control	16	0.003	0.0326	0.089	Mix

results were found among these studies. A study in Taiwan (13) reported that 77 (54.6%) of 141 lung tumors had HPV-16 or -18 DNA compared with 16 (26.7%) of 60 non-cancer control subjects among non-smoking Taiwanese women, and a report from Central China found that the rates of HPV-16 and/or -18 infections in patients with 73 lung tumors were significantly higher than in 34 non-cancer control subjects (27.7 vs. 5.9%) (19). Also they found that 44.1% of (138/313) NSCLC samples were positive, while 4.2% (4/96) of lung benign controls were positive (20). Our data showed that the high risk HPV genotypes 16/18 were 43.1% and 5.6% in lung squamous cell carcinoma cases and non-cancer control cases, respectively ($P < 0.001$). It is very similar to the study from the central part of China in NSCLC (20), but our study focused on lung squamous cell carcinomas cases.

In the majority of carcinomas associated with high-risk HPV and some advanced HPV-associated precancerous lesions, the whole viral genome or its fragment is integrated into the chromosomal DNA of the host cell whereas the HPV genome was retained as episomal molecules in early dysplastic low-grade lesions (17). Although the mechanism involved in switching from episomal state to viral integration was yet unclear, it was suspected that methylation pattern on HPV genome was different in episomal and integrated states. Kim *et al* reported that the overall methylation status of the LCR was greater than that seen in the more highly differentiated cells (30).

Since HPV integration may result in deletion of the E₂ gene, the status of HPV in the host cells was determined on the basis of the E₂/E₆ ratio (24). When the ratio was equal to or higher than 1, all the HPV genome was considered to be in an episomal form and not integrated. The lack of amplified HPV E₂ genome was considered to indicate the integration of all the HPV genome into the host genome. When the E₂/E₆ ratio was larger than zero and smaller than 1, the condition was considered as the mixture of episomal and integrated forms, where a portion of HPV genome was integrated into the host genome. In our study, real-time PCR was used in 27 samples to confirm the presence of HPV-16. E₂ was not found in 59.3% (16/27) of the samples, suggesting that at least a partial HPV-16 genome was integrated into the host genome, which is similar to the 66.7% reported in Chile (31).

Although the viral load was low in our study, ranging from 0.003 to 44.942 copies per nanogram of genomic DNA, which corresponds to <1 to 2 copies/cell, our data were very similar to the reports from a Chinese study on esophageal cancer tissues (<1 to 157 copies/cell) (32) and a Finnish study on head and neck SCC (4.6 to 49 copies/cell) (33). The low viral load may be sufficient to promote carcinogenesis especially if viral DNA is integrated into the cell (34). Our data emphasize the importance of high-risk HPV DNA integration into the host genome contributing to lung squamous cell carcinogenesis.

In summary, there is a significant difference of HPV-positivity between lung squamous cell carcinomas and non-cancer controls, and the HPV-16 positive had higher risk than HPV-16 negative in the development of lung squamous cell carcinomas. A possible mechanism is the integration of HPV-16 into the host genome.

Acknowledgements

We thank Dr Suminori Akiba, Kagoshima University, Japan, for proving assistance in analyzing the data. We also thank Dr Zhang Guanjun for his contribution in the pathological diagnosis, and assistance in the preparation of paraffin-embedded slices.

References

- Bosch FX, Lorincz A, Munoz N, Meijer C and Shah KV: The causal relationship between human papillomavirus and cervical cancer. *J Clin Pathol* 55: 244-265, 2002.
- Yanagawa N, Osakabe M, Hayashi M, Tamura G and Motoyama T: Detection of HPV-DNA, p53 alterations, and methylation in penile squamous cell carcinoma in Japanese men. *Pathol Int* 58: 477-482, 2008.
- Clavel CE, Nawrocki B and Bosseaux B: Detection of human papillomavirus DNA in bronchopulmonary carcinomas by hybrid capture II: a study of 185 tumors. *Cancer* 88: 1347-1352, 2000.
- Chiou HL, Wu MF and Liaw YC: The presence of human papillomavirus type 16/18 DNA in blood circulation may act as a risk marker of lung cancer in Taiwan. *Cancer* 97: 1558-1563, 2003.
- Snijders P, van den Brule A, Meijer C and Walboomers J: HPV and cancer of the aerodigestive tract. *Papillomavirus Rep* 6: 157-162, 1995.
- Nadji S, Mokhtari-Azad T, Mahmoodi M, Yahyapour Y, Naghshvar F, Torabizadeh J, Ziaee A and Nategh R: Relationship between lung cancer and human papillomavirus in north of Iran, Mazandaran province. *Cancer Lett* 248: 41-46, 2007.
- Wen Cheng Y and Lee H: Environmental exposure and lung cancer among nonsmokers: an example of Taiwanese female lung cancer. *J Environ Sci Health C Environ Carcinog Ecotoxicol Rev* 21: 1-28, 2003.
- Syrjanen KJ: Condylomatous changes in neoplastic bronchial epithelium. Report of a case. *Respiration* 38: 229-304, 1979.
- Syrjanen KJ: Epithelial lesions suggestive of a condylomatous origin found closely associated with invasive bronchial squamous cell carcinoma. *Respiration* 49: 150-160, 1980.
- Hirayasu T, Twamasa T and Kamada Y: Human papillomavirus DNA in squamous cell carcinoma of the lung. *J Clin Pathol* 49: 810-817, 1996.
- Syrjanen KJ: HPV infections and lung cancer. *J Clin Pathol* 55: 885-891, 2002.
- Miyagi J, Tshako K, Kinjo T, Iwamawa T and Hirayasu T: Recent striking changes in histological differentiation and rate of human papillomavirus infection in squamous cell carcinoma of the lung cancer in Okinawa: a subtropical island in Southern Japan. *J Clin Pathol* 53: 676-684, 2000.
- Cheng YW, Chiou HL, Sheu GT, Hsieh LL, Chen JT, Chen CY, Su JM and Lee H: The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking Taiwanese women. *Cancer Res* 61: 2799-2803, 2001.
- Cheng YW, Lee H, Shiau MY, Wu TC, Huang TT and Chang YH: Human papillomavirus type 16/18 up-regulates the expression of interleukin-6 and antiapoptotic Mcl-1 in non-small cell lung cancer. *Clin Cancer Res* 14: 4705-4712, 2008.
- Cheng YW, Wu MF, Wang J, Yeh KT, Goan YG, Chiou HL, Chen CY and LH: Human papillomavirus 16/18 E6 oncoprotein is expressed in lung cancer and related with p53 inactivation. *Cancer Res* 67: 10686-10693, 2007.
- Aguayo F, Meneses M, Corvalan A, Munoz M, Koriyama C, Eizuru Y and Akiba S: Identification of human papillomavirus 16 (HPV 16) in keratinizing lung carcinoma. *Rev Chil Enferm Respir* 18: 83-89, 2002.
- Castillo A, Aguayo F, Koriyama C, Shuyama K and Akiba S: Human papillomavirus in lung carcinomas among three Latin American countries. *Cancer Sci* 15: 883-888, 2006.
- Park MS, Chang YS and Shin JH: The prevalence of human papillomavirus infection in Korean non-small cell lung cancer patients. *Yonsei Med J* 48: 69-77, 2007.
- Fei Y, Yang J, Hsieh WC, Wu JY, Wu TC, Goan YG, Lee H and Cheng YW: Different human papillomavirus 16/18 infection in Chinese non-small cell lung cancer patients living in Wuhan, China. *Jpn J Clin Oncol* 36: 274-279, 2006.
- Wang YP, Wang AF, Jiang R, Pan HX, Huang BX, Lu Y and Wu CH: Human papillomavirus type 16 and 18 infection is associated with lung cancer patients from the central part of China. *Oncol Rep* 20: 333-339, 2008.
- Greer CE, Wheeler CM and Manos MM: PCR amplification from paraffin-embedded tissues: sample preparation and the effects of fixation. In *PCR Primer: a laboratory manual*. Carl WD and Gabriella SD (eds). Cold Spring Harbor Laboratory Press, New York, pp99-112, 1995.
- Gillison ML and Shah KV: Role of mucosal human papillomavirus in non-genital cancers. *J Natl Cancer Inst Monogr* 31: 57-65, 2003.
- Ketler B, van Doorn LJ, Schrauwen L, Molijn A, Sastrowijoto S, ter Schegget J, Lindeman J, ter Harmsel B, Burger M and Quint W: Development and clinical evaluation of a highly sensitive PCR-reverse hybridization line probe assay for detection and identification of anogenital human papillomavirus. *J Clin Microbiol* 137: 2508-2517, 1999.
- Peitsaro P, Johansson B and Syrjanen S: Integrated human papillomavirus type 16 is frequently found in cervical cancer precursors as demonstrated by a novel quantitative real-time PCR technique. *J Clin Microbiol* 140: 886-891, 2002.
- Lele SM, Pou AM and Ventura K: Molecular events in the progression of recurrent respiratory papillomatosis to carcinoma. *Arch Pathol Lab Med* 126: 1184-1188, 2002.
- Zafer E, Ergun MA and Alver G: Detection and typing of human papillomavirus in non-small cell lung cancer. *Respiration* 71: 88-90, 2004.
- Steckley SL, Pickworth WB and Haverkos HW: Cigarette smoking and cervical cancer. Part II: a geographic variability study. *Biomed Pharmacother* 57: 78-83, 2003.
- Noutsou A, Koffa M, Ergazaki M, Siafakas NM and Spandidos DA: Detection of human papillomavirus (HPV) and K-ras mutations in human lung carcinomas. *Int J Oncol* 8: 1089-1093, 1996.
- Hsu NY, Cheng YW, Lin MH and Chou MC: Association of expression of human papillomavirus 16/18 E6 oncoprotein and survival for pathological stage I non-small cell lung cancer. *Proc Am Assoc Cancer Res* 49: 1124, 2008.
- Kim K, Garner-Hamrick PA, Fisher C, Lee D and Lambert PF: Methylation pattern of papillomavirus DNA its influence on E2 function, and implication in viral infection. *J Virol* 77: 12350-12459, 2003.
- Aguayo F, Castillo A, Koriyama C, Higashi M, Itoh T, Capetillo M, Shuyama K, Corvalan A, Eizuru Y and Akiba S: Human papillomavirus-16 is integrated in lung carcinomas: a study in Chile. *Cancer Res UK* 97: 85-91, 2007.
- Si HX, Tsao SW, Poon CS, Wang LD, Wong YC and Cheung AL: Viral load of HPV in esophageal squamous cell carcinoma. *Int J Cancer* 103: 496-500, 2003.
- Koskinen W, Chen RW, Leivo I, Makitie A, Back L, Kontio R, Suuronen R, Lindqvist C, Auvinen E, Molijn A, Quint WG, Vaheri A and Aaltonen LM: Prevalence and physical status of human papillomavirus in squamous cell carcinomas of the head and neck. *Int J Cancer* 107: 401-406, 2003.
- Si HX, Tsao SW, Poon CS, Wong YC and Cheung AL: Physical status of HPV-16 in esophageal squamous cell carcinoma. *J Clin Virol* 32: 19-23, 2005.