[依頼総説]HPV infection and squamous differentiation of lung cancer cells among patients in Okinawa, Japan

HPV infection and squamous differentiation of lung cancer cells among patients in Okinawa, Japan

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ABSTRACT

We reviewed histopathological reports of lung cancer cases in Okinawa, Japan and found a strong association between human papillomavirus (HPV) infection and squamous differentiation of lung cancer cells. On the basis of these observations, we hypothesized that HPV infection induces squamous differentiation of adenocarcinoma cells. To prove our hypothesis and determine the molecular basis of the phenomenon, we conducted a series of in vitro and in vivo studies. Cultured adenocarcinoma cells were transfected with the entire HPV genome and the cells were injected into SCID mice to form tumors, which were used for subsequent histopathological and biochemical analyses. The HPV-transfected cells showed no tubular structure but solid proliferation with expression of squamous differentiation markers such as high molecular weight keratin and involucrin. In particular, cells expressing HPV E2, E6, and E7 genes exhibited more marked morphological changes with increased expression of squamous differentiation markers. These experiments demonstrated that HPV gene expression in adenocarcinoma cells induces squamous differentiation. Although the present studies are only preliminary analyses, our findings have identified a new and specific aspect of lung cancer in cases in Okinawa. More precise and thorough evaluations are required. Since the diseases in Okinawa are specific to mainland Japan, further investigations may lead to the discovery of new mechanisms of disease and thereby contribute to health promotion of people in Okinawa. *Ryukyu Med. J., 30(1~4)1~11, 2011*

Key words: lung cancer, Okinawa, squamous cell carcinoma, adenocarcinoma, HPV

1. INTRODUCTION

In Japan, the age-adjusted mortality rate of lung cancer in males and females has increased since the 1960's. Lung cancer is now the primary cause of death due to malignancies. In particular, the all age mortality rate of lung cancer in Okinawa is higher than that in mainland Japan, despite Okinawa having lower smoking rates than the rest of the country[1,2]. Although the total number of lung cancer cases has been increasing in Japan, the histopathology of lung cancer has demonstrated intriguing findings showing histological transition of lung cancer in mainland Japan. From the 1950's to the 1980's, squamous cell carcinomas and adenocarcinomas were common in Japanese males, with the distribution of squamous cell carcinomas being higher than that of adenocarcinomas. In Japanese females, adenocarcinomas have been the most frequent histological type reported since 1958[3-6]. The number of squamous cell carcinoma cases among men and women has declined since then, with increases in the number of adenocarcinoma cases[3,5,6]. A similar trend has been reported in the United States[3,7-10], with the most common histopathological type of lung cancer until the 1980's being squamous cell carcinomas, followed by adenocarcinomas[7-10]. Since the early 1990's, the number of squamous cell carcinoma cases has been decreasing, while adenocarcinomas have become the most prevalent histological type. Currently, adenocarcinomas are the most prevalent histological type in most countries[11-13]. According to histological analysis of lung cancer,

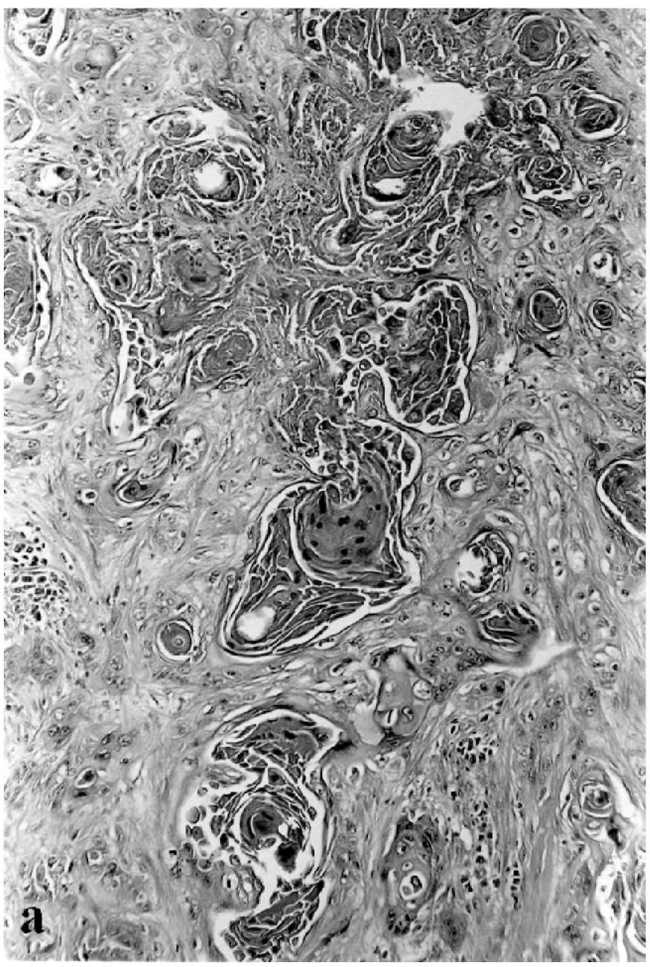

Fig. 1a **Well differentiated squamous cell carcinoma of the lung from a case in Okinawa.** Marked keratinization and many cancer pearls can be observed.

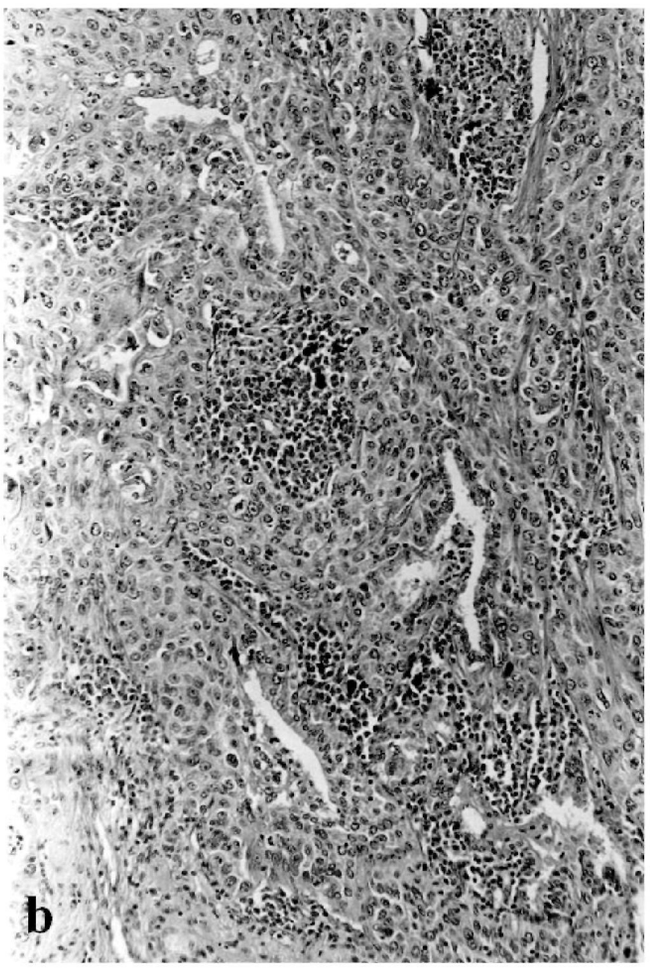

Fig. 1b **Poorly differentiated squamous cell carcinoma of the lung from a case in Niigata.** No cancer pearls are noted, but solid proliferation and a small foci of keratinization are consistent with the characteristics of a squamous cell carcinoma.

characteristics of lung cancer in Okinawa are distinct from those in other areas[14,15]. During the 1980's, squamous cell carcinoma was the primary histological type in Okinawa, with a higher prevalence than other types. Similar to mainland Japan and the United States, the number of adenocarcinoma cases in Okinawa has been increasing, with it becoming the most prevalent histological type of lung cancer in men and women in the region. This increase in the number of adenocarcinoma cases has also been reported in European countries[11-13]. Thus, there seems to be a worldwide trend of transition in the main histological type of lung cancer from a squamous cell carcinoma to an adenocarcinoma. Although this trend is observed in many developed countries, the reason for this transition remains to be clarified.

The human papillomavirus (HPV) is a well-known causative viral agent of uterine cervical cancer and benign warts. Of more than 100 types of HPV, HPV16 and HPV18 are typical high-risk HPV strains; E6 and E7 genes of these strains cause degradation of the tumor suppressor genes p53 and RB, respectively[16,17]. In contrast, low-risk HPV strains such as HPV6 and HPV11 do not cause malignant transformation, but instead induce benign tumors characterized by papillary proliferation of the squamous epithelium. In 1980, Syrjänen first suggested the possible involvement of HPV in bronchial squamous cell lesions, including squamous cell papillomas and invasive squamous cell carcinomas, on the basis of morphological analysis[18]. Since then, an association between

bronchial squamous cell carcinomas and HPV has been reported by many researchers[14,15,19-22].

In this article, we describe the association between HPV infection and squamous differentiation of lung cancer cells in cases in Okinawa. We determined this relationship by reviewing lung cancer cases in Okinawa and confirmed our findings by conducting an in vitro study.

2. Morphological changes in lung cancer in Okinawa

As described previously, a trend of transition in the histopathological type of lung cancer has been observed in mainland Japan, where the frequency of adenocarcinomas has been increasing, while that of squamous cell carcinomas has been decreasing[3,5,6]. On the basis of histopathological diagnoses, we noticed a high frequency of lung squamous cell carcinomas in Okinawa, and therefore assumed squamous cell carcinomas to be the most prevalent type of lung cancer in Okinawa. We also noticed that the differentiation status of squamous cell carcinomas between Okinawa and mainland Japan was quite different. However, no report showing annual histopathological analyses of lung cancer in Okinawa existed. Therefore we first attempted to determine the difference in histopathological characteristics of lung cancer between Okinawa and mainland Japan. On comparing, we found that Okinawa had a higher prevalence of squamous cell carcinomas than Niigata (mainland Japan), but this difference was not statistically significant (Table 1a). We next compared the differentiation status of lung squamous cell carcinomas between the two regions. The representative histopathology of lung cancer in the two regions is shown in Fig. 1. As shown in Table 1b, the frequency of well differentiated squamous cell carcinomas in Okinawa was considerably higher than that in mainland Japan (65.1% versus 10%). Furthermore, 95.3% of total squamous cell carcinoma cases in Okinawa consisted of well and moderately differentiated squamous cell carcinomas, whereas the prevalence of these types of carcinoma was only about 50% in mainland Japan (Table 1b)[14]. On the basis of these data, we concluded that squamous cell carcinoma cases in Okinawa had a more differentiated histopathology than similar cases in mainland Japan.

We next examined the annual change in histological prevalence of lung cancer in Okinawa. As shown in Fig. 2a and 2b, the frequency of squamous cell carcinomas decreased, whereas that of adenocarcinomas increased, thereby adenocarcinomas becoming the most prevalent histological type. We also examined the annual change in the differentiation status of squamous cell carcinomas in Okinawa, and found that the total number and frequency of well differentiated squamous cell carcinomas decreased (Fig. 2c). In contrast to this reduction, an increase was observed in the prevalence of moderately and poorly differentiated squamous cell carcinomas, comprising approximately 80% of all cases.

Table 1a **Histological classification of lung carcinoma cases in Okinawa and Niigata**

	Lung carcinoma from Okinawa	Lung carcinoma from Niigata
Adenocarcinoma	**39 (41.4%)**	**43 (45.9%)**
Squamous cell carcinoma	**43 (45.7%)**	**30 (34.5%)**
Large cell carcinoma	**3 (3.2%)**	**5 (5.7%)**
Small cell carcinoma	**3 (3.2%)**	**5 (5.7%)**
Adenosquamous carcinoma	**5 (5.3%)**	**2 (2.3%)**
Mucoepidermoid carcinoma	**1 (1.1%)**	**2 (2.3%)**
	Total: 94	**Total: 87**

Table 1b **Histological differentiation of squamous cell carcinoma of the lung cases Okinawa and Niigata**

Squamous cell carcinoma from Okinawa		Squamous cell carcinoma from Niigata	
Well	n=28 (65.1%)	Well	n=3 (10%)
Moderately	n=13 (30.2%)	Moderately	n=12 (40%)
Poorly	n=2 (4.7%)	Poorly	n=15 (50%)
	Total: 43		Total: 30

Table 1c **HPV detection of squamous cell carcinoma of the lung from Okinawa and Niigata**

Squamous cell carcinoma from Okinawa			Squamous cell carcinoma from Niigata		
		HPV positivity			**HPV positivity**
Well	: 28	23/28 (82.1%)	Well	: 3	0/3 (0%)
Moderately	: 13	11/13 (84.6%)	Moderately	: 12	3/12 (25%)
Poorly	: 2	0/2 (0%)	Poorly	: 15	6/15 (40%)
		Total: 34/43 (79.1%)			Total: 9/30 (30%)

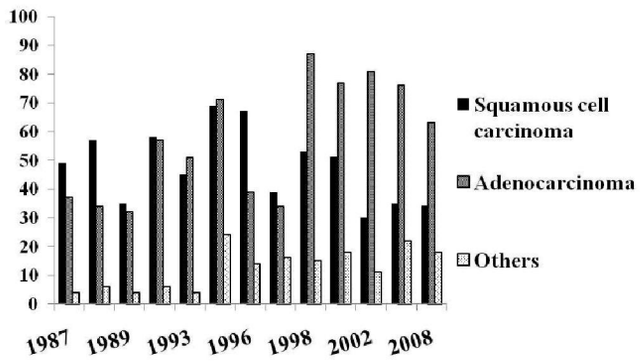

Fig. 2a **Histological prevalence of lung cancer in Okinawa.** Each bar represents the number of cases. Black bars, squamous cell carcinoma: shaded bars, adenocarcinoma: dot bars, other histological types. The number of adenocarcinoma cases increased in recent years.

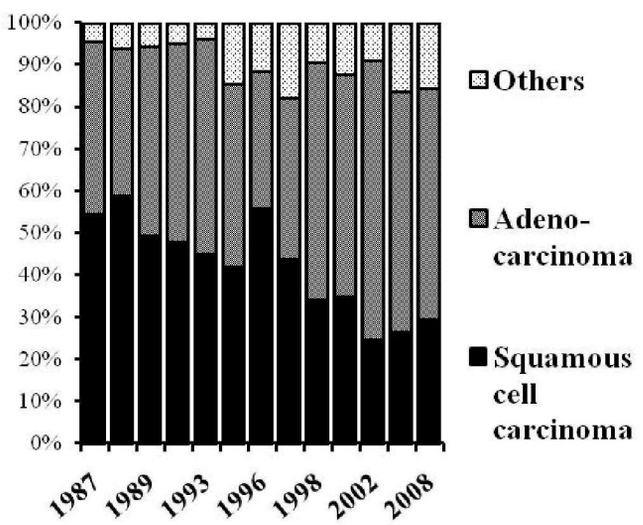

Fig. 2b **Histological prevalence of lung cancer in Okinawa.** Each bar represents the proportion of histological types of lung cancer. Black bars, squamous cell carcinoma: shaded bars, adenocarcinoma: dot bars, other histological types. The proportion of squamous cell carcinoma decreased, whereas adenocarcinoma showed a small annual increase.

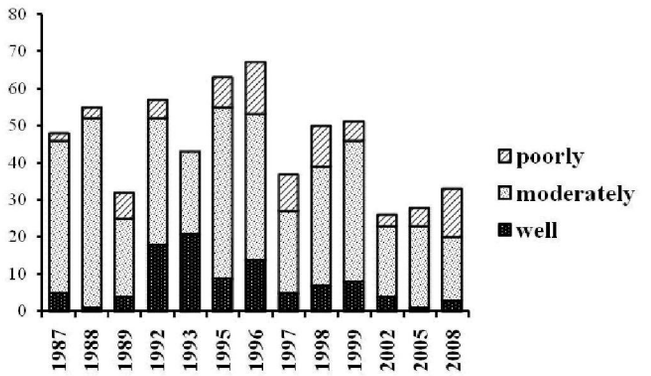

Fig. 2c **Histological differentiation status of squamous cell carcinoma of the lung in Okinawa.** Solid bar, number of cases of well differentiated squamous cell carcinoma: shaded bar, moderately differentiated squamous cell carcinoma: hatched bar, poorly differentiated squamous cell carcinoma. The number of cases of well differentiated squamous cell carcinoma diminished compared to the early 1990's.

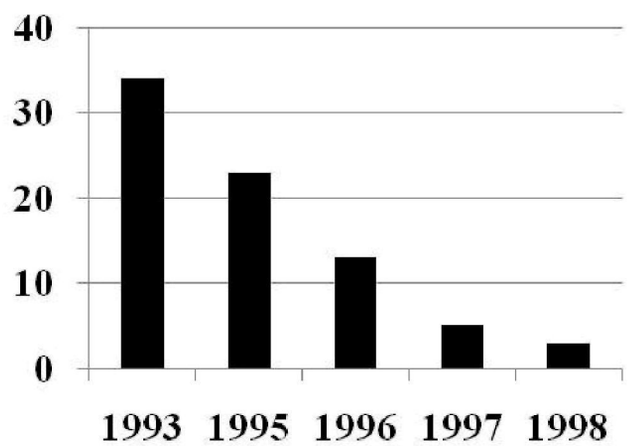

Fig. 2d **Changes in the incidence of human papillomavirus (HPV) positive cases in squamous cell carcinoma of the lung from Okinawa.**

Table 2 **Number of HPV positive cases in squamous cell carcinomas of lung from Okinawa**

	HPV6	HPV11	HPV16	HPV18	Two types	Three types	Total
1993	2	0	5	5	17	5	34
1995	5	6	5	3	4	0	23
1996	3	3	1	4	2	0	13
1997	0	2	1	1	1	0	5
1998	1	3	0	1	0	0	3

3. Lung cancer in Okinawa and HPV infection

In 1993 and 1995, two different Japanese research groups reported approximately 10% prevalence of HPV infection in lung cancer[23,24]. We attempted to detect HPV infection by PCR using viral gene-specific primers rather than using consensus. Surprisingly, HPV infections were detected in over 80% of well and moderately differentiated squamous cell carcinoma cases in Okinawa. On the other hand, only 30% of squamous cell carcinoma cases in mainland Japan were associated with HPV infection (Table 1c)[14].

These results suggested that the high prevalence of differentiated type of squamous cell carcinomas in Okinawa may be related to HPV infection.

Intriguingly, since 1993, the number of HPV infections has diminished in parallel with a reduction in squamous cell carcinomas (Fig. 2d and Table 2). The change in histopathology and reduction in HPV infections in lung cancer cases in Okinawa implies that differentiation of squamous cell carcinomas may be associated with the prevalence of HPV infection. To further investigate the association between histopathological changes and HPV infection in lung cancer cases in Okinawa, we analyzed adenosquamous carcinomas using morphological and molecular biological methods. Morphologically, these adenosquamous carcinomas contained both a squamous cell carcinoma component and an adenocarcinoma component. These analyses showed that cancer cells in the adenocarcinoma component adjacent to the squamous cell carcinoma component exhibited enlarged and eosinophilic cytoplasm that morphologically resembled squamous cell carcinomas (Fig. 3a and 3b). These findings implied a morphological transition from an adenocarcinoma to a squamous cell carcinoma. Immunohistochemical analysis showed that the adenocarcinoma component adjacent to the squamous cell carcinoma component expressed high molecular weight keratin and involucrin, both of which are squamous differentiation markers (Fig. 3c and 3d). PCR analysis detected HPV in approximately 80% of the adenosquamous carcinomas, while in situ hybridization analysis demonstrated HPV DNA in both the squamous cell carcinoma components and the adenocarcinoma component adjacent to the squamous cell carcinoma component (Fig. 3e and 3f)[25]. These results suggested that HPV infection induces morphological changes in lung cancer cells, resulting in a transition from an adenocarcinoma to a squamous cell carcinoma.

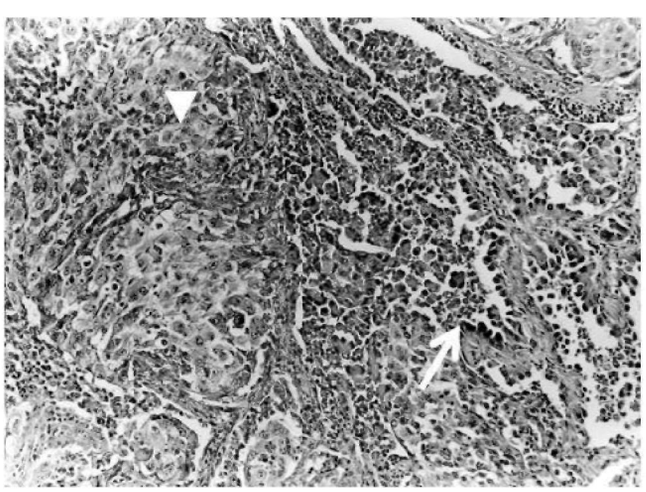

Fig. 3a **Adenosquamous carcinoma of the lung from a case in Okinawa.** Both the adenocarcinoma component (arrow) and squamous cell carcinoma component (arrowhead) are identified.

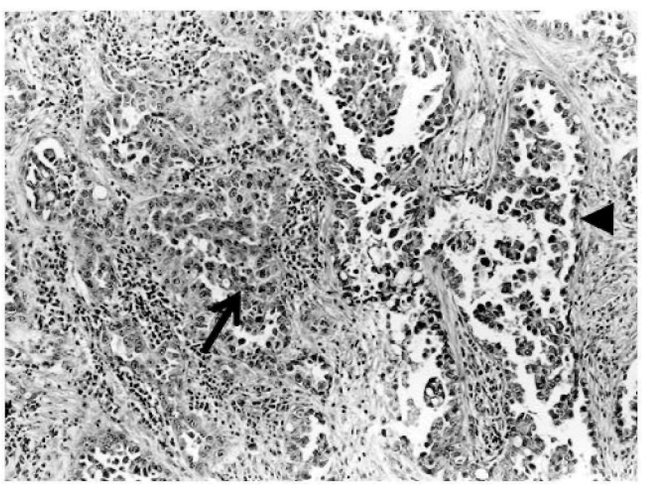

Fig. 3b **Adenocarcinoma component adjacent to a squamous cell carcinoma component in adenosquamous carcinoma.** In contrast to the well differentiated adenocarcinoma component (arrowhead), enlarged adenocarcinoma cells (arrow) with similar appearance to squamous cell carcinoma cells can be observed.

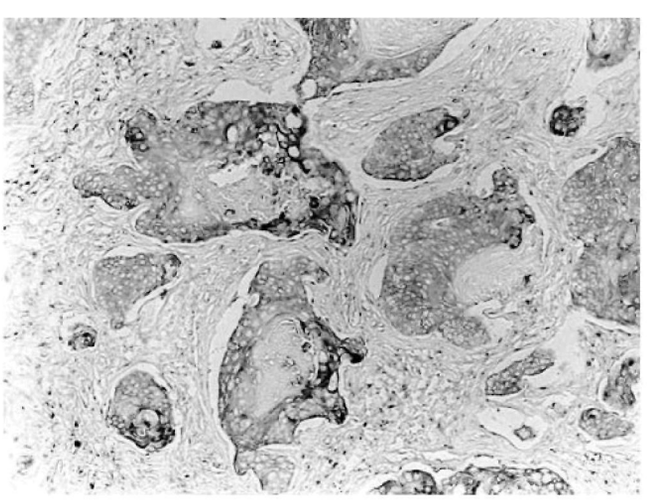

Fig. 3c **Immunohistochemical demonstration of high molecular weight keratin in a squamous cell carcinoma component.**

4. Squamous differentiation and HPV

Given the results of the above epidemiological and histopathological analyses, we assumed that HPV infections are associated with differentiation of the squamous epithelium. To evaluate the correlation between HPV infections and squamous differentiation of adenocarcinoma cells at a molecular level, we transfected the HPV genome into cultured adenocarcinoma cell lines[26]. After the HPV-expressing clones were selected and purified, they were injected separately into SCID mice. Two weeks after injection, tumors appeared on the backs of mice and these tumors were removed for morphological examination and biochemical analysis. HPV genome integration into the host nucleus was observed by in situ hybridization, and RT-PCR revealed expression of HPV genes such as E6, E7, and E2 (Fig 4a). Histology showed that tumors from the HPV-transfected adenocarcinoma cells showed solid proliferation of cancer cells with larger cytoplasm, which was quite different from the original histological findings of adenocarcinoma cells (Fig. 4b and 4c). The protein expression profile of the HPV-transfected cells was similar to that of squamous cell carcinomas. Squamous differentiation markers, such as involucrin, high molecular weight keratin (Moll's No. 1, 5, 10, and 14), and transglutaminase I, were also detected in the HPV-transfected cells by immunohistochemistry (Fig. 4d), Western blotting with two-dimensional electrophoresis (Fig. 4e), and RT-PCR (Fig. 4a). Ultrastructural analysis by electron microscopy revealed the formation of desmosomes between HPV-transfected adenocarcinoma cells[26,27]. Furthermore, the increased expression of E2, E6, and E7 genes was associated with strong induction of squamous differentiation[26,27]. These data suggested that HPV induces a morphological change from an adenocarcinoma to a squamous cell carcinoma. With reference to the correlation between cell differentiation and HPV genes, Wilson and Laimins demonstrated HPV gene expression in organotypic

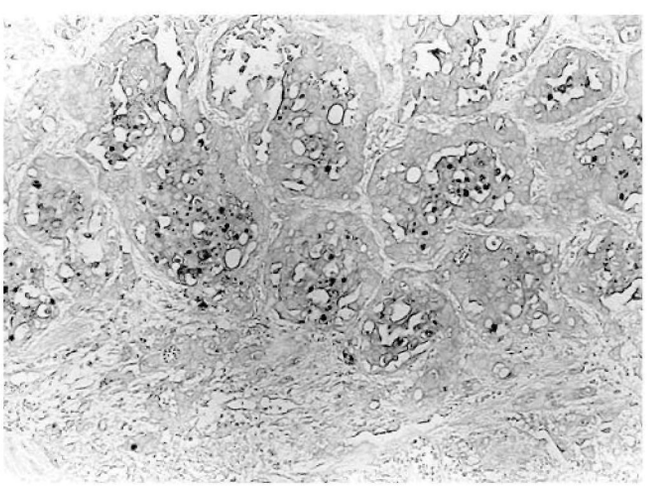

Fig. 3e **Demonstration of human papillomavirus DNA in the squamous cell carcinoma component by in situ hybridization.** Signals of HPV 16 DNA are present in the nuclei of squamous cell carcinoma components.

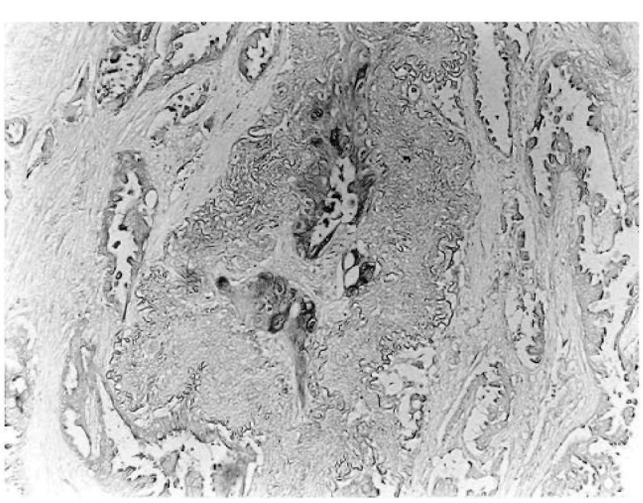

Fig. 3d **Immunohistochemical demonstration of high molecular weight keratin in a adenocarcinoma component.** The expression of high molecular weight keratin is also seen in the adenocarcinoma component.

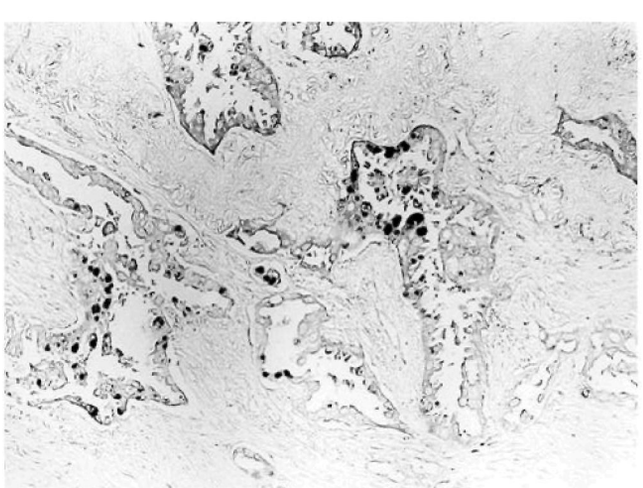

Fig. 3f **Human papillomavirus DNA in an adenocarcinoma component demonstrated by in situ hybridization.** Signals of HPV 16 DNA are present in the nuclei of enlarged adenocarcinoma components.

raft cultures and cultures with methylcellulose induced cell differentiation[28]. On the other hand, expression of HPV18 E6 and E7 in normal human keratinocytes has been reported to stimulate cell proliferation and delay differentiation[29]. In our investigations, the HPV-transfected cells showed squamous differentiation as well as cell cycle arrest and apoptosis. The HPV-transfected cells showed an increase in the G0-G1 fraction and a decrease in the G2-M fraction (Fig. 4f), along with decreased expression of the cell cycle progression-associated genes cdk4 and cyclin D1 (Fig. 4a). These findings indicated down-regulation of the cell cycle[26,27]. TUNEL showed many apoptotic sig-

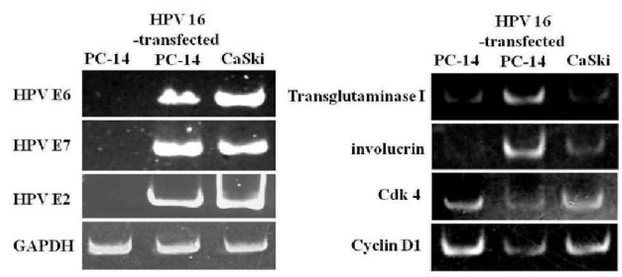

Fig. 4a **The results of RT-PCR in HPV 16-transfected PC-14 cells.** The expression of HPV E6, E7, and E2 was demonstrated in HPV 16-transfected PC-14 cells. These cells exhibited higher expression of squamous differentiation markers such as transglutaminase I and involucrine, and also had lower expression of cell cycle progression genes.

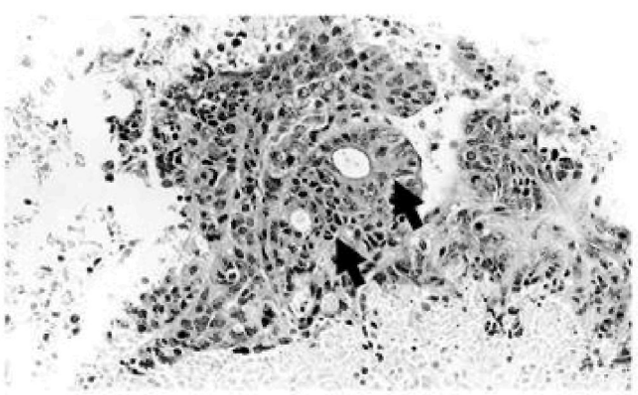

Fig. 4b **Control DLD-1 cells without HPV transfection.** The arrow indicates the tubular structure.

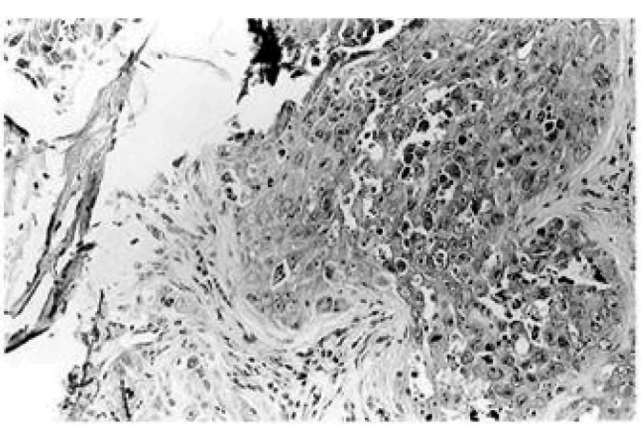

Fig. 4c **HPV 16-transfected DLD-1 cells. Squamous differentiation is demonstrated.**

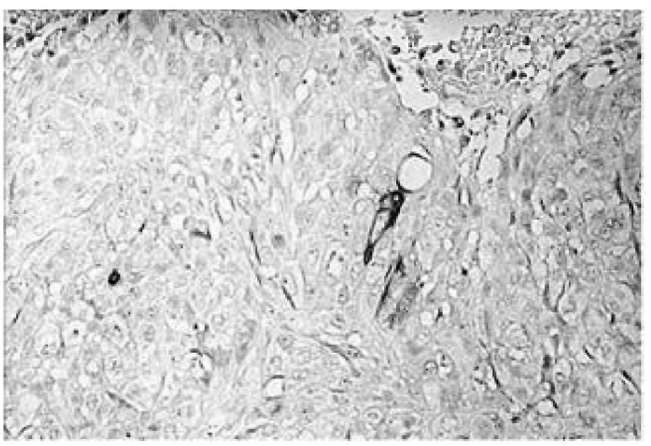

Fig. 4d **Immunohistochemical demonstration of high molecular weight keratin in HPV 16-transfected DLD-1 cells.**

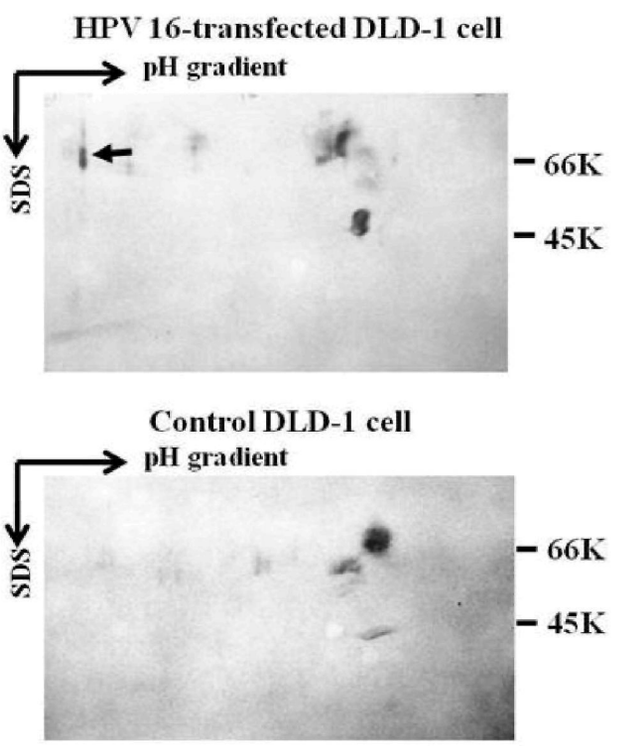

Fig. 4e **Western blot analysis of high molecular weight keratin in HPV 16-transfected DLD-1 cells.** Moll's number 1 keratin was demonstrated by two dimensional electrophoresis (arrow).

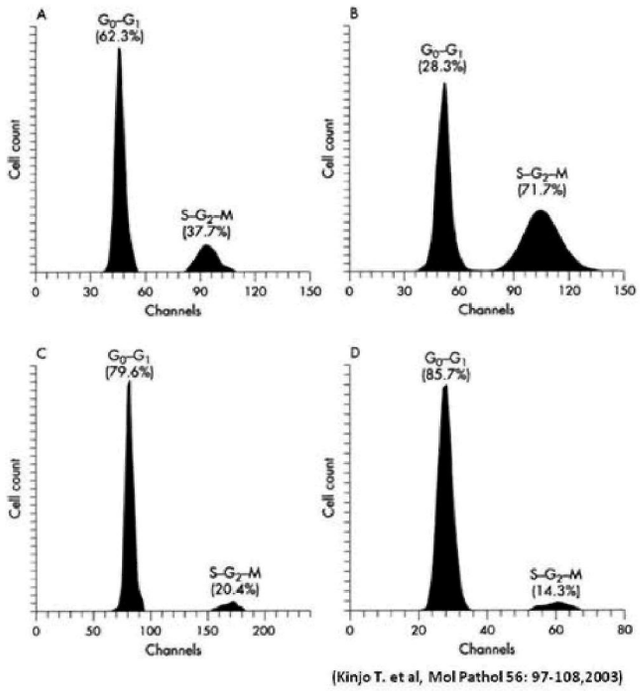

Fig. 4f **Flowcytometric analysis of cell cycle population in HPV 16-transfected DLD-1 and PC-14 cells.** Compared with DLD-1 (upper left) and PC-14 (upper right), HPV 16-transfected DLD-1 (lower left) and HPV 16-transfected PC-14 (lower right) showed smaller population of S-G2-M phase cells. (From Kinjo T et al Mol Pathol 56; 97-108, 2003.)

nals in cells expressing the HPV gene and increased expression of apoptosis-related genes such as those for the apoptosis-inducing factor and caspase 8[26]. In general, differentiating cells cease cell cycle progression[30], and therefore, we concluded that squamous differentiation induced down-regulation of the cell cycle, while terminal differentiation incited apoptosis. To identify which HPV gene was the main contributor to squamous differentiation, we constructed three types of HPV single gene-expressing cells that included E2, E6, and E7 expressing cells. HPV single gene-expressing cells showed increased expression of genes for squamous differentiation markers and cell adhesion molecules, and decreased expression of genes for cell cycle and apoptosis. However, the morphological changes in these cells was not as distinct as those in cells transfected with the entire HPV genome[31]. These experimental data suggested that multiple HPV gene expression induces clear morphological and biochemical transition from an adenocarcinoma to a squamous cell carcinoma.

Lung squamous cell carcinomas are assumed to be the result of multi-step accumulation of genetic and/or epigenetic alterations, and a morphological stepwise process is also believed to occur in the bronchial epithelium as cells progress from normal to basal cell hyperplasia, squamous metaplasia, squamous dysplasia (mild, moderate, and severe), and subsequently carcinoma in situ[32,33]. In terms of the molecular mechanisms of preneoplastic bronchial lesions, Lamy et al. reported aberrant methylation of the p16 gene promoter region[34], and Sousa et al. suggested intense expression of EGFR and increase in EGFR gene copy number due to polysomy[33], however, the precise molecular mechanism of squamous carcinogenesis remains to be clarified. Although we found an association between HPV infection and squamous cell carcinomas in Okinawa, we observed squamous differentiation rather than transformation of the bronchial epithelium by HPV infection. Since Syrjänen et al. first described an HPV infection in bronchial squamous cell carcinomas[18], many investigators reported an association between lung cancer and HPV[35-39]. Recently, Klein et al. evaluated 53 reports and showed that the mean incidence of HPV in lung cancer was 24.5% and that a geographic variation existed with a high incidence observed in Asia (35.7%) compared with Europe (17%) and the United States (15%)[40]. With regards to carcinogenesis, some researchers reported that HPV is unlikely to be an etiological agent for lung cancer[41,42], whereas other studies suggested that HPV is associated with both squamous cell carcinomas[36,43,44] and adenocarcinomas[37,45]. In addition, controversy exists regarding carcinogenesis and the route of transmission of HPV. Kountouri et al. suggested that HPV positivity in lung cancer may reflect an epiphenomenon rather than the cause of the tumor, because HPV may be integrated more easily into the tumor cell genome compared with the normal cell genome[46]. Our findings suggested that HPV induces squamous differentiation in lung cancer in Okinawa, which might be a new aspect of HPV pathogenesis. However, we must be cautious in our hypothesis, because the phenomenon was noted only in Okinawa, and further studies are required to confirm our results.

5. CONCLUSIONS

Histological analysis of lung cancer in Okinawa revealed a change in the most prevalent histopathological type, seen as a transition from a squamous cell carcinoma to an adenocarcinoma. This change occurred in parallel with a decrease in the incidence of HPV infection. Because the smoking rate had not changed drastically over the past few years, the main cause of this transition in the histological type of lung cancer was unknown. Thus we identified a correlation between lung squamous cell carcinomas in Okinawa and HPV infection[14,15,25-27,31,47]. Using both morphological and molecular biological analyses, we demonstrated that HPV gene expression in adenocarcinoma cells induced squamous differentiation[26,27,31]. As our report is the first to suggest that HPV may induce phenotypic changes in lung cancer, further studies are necessary to confirm this possibility. Stem cell properties are required for both carcinogenesis[48] and metaplastic changes[49]. To understand the molecular mechanism behind these events, stem cell properties in the host cell must be considered when examining phenotypic changes and carcinogenesis associated with HPV gene expression.

6. ACKNOWLEDGMENTS

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7. REFERENCES

1) Center for Cancer Control and Information Services, N.C.C., Japan.: Vital Statics Japan. Ministry of Health, Labour and Welfare.
2) Okinawa prefecture government.: Annual statics report of health and environment 1986-1995. Okinawa prefectual government, 1995.
3) Morita, T.: A statistical study of lung cancer in the annual of pathological autopsy cases in Japan, from 1958 to 1997, with reference to time trends of lung cancer in the world. Jpn J Cancer Res 93; 15-23, 2002.
4) Watanabe S, Tsugane S, Arimoto H, Shimosato Y, Suemasu K, Arai H, and Urano Y.: Trend of lung cancers in the National Cancer Center of Japan and comparison with that of Japanese pathological autopsy records. Jpn J Cancer Res 78; 460-466, 1987.
5) Toyoda, Y., Nakayama, T., Ioka, A. and Tsukuma, H.: Trends in lung cancer incidence by histological type in Osaka, Japan. Jpn J Clin Oncol 38; 534-539, 2008.
6) Yoshimi, I., Ohshima, A., Ajiki, W., Tsukuma, H. and Sobue, T.: A comparison of trends in the incidence rate of lung cancer by histological type in the Osaka Cancer Registry, Japan and in the Surveillance, Epidemiology and End Results Program, USA. Jpn J Clin Oncol 33; 98-104, 2003.
7) Alberg, A.J. and Samet, J.M.: Epidemiology of lung cancer. Chest 123; 21S-49S, 2003.
8) Gazdar, A.F. and Linnoila, R.I.: The pathology of lung cancer--changing concepts and newer diagnostic techniques. Semin Oncol 15; 215-225, 1988.
9) Travis, W.D., Lubin, J., Ries, L. and Devesa, S.: United States lung carcinoma incidence trends: declining for most histologic types among males, increasing among females. Cancer 77; 2464-2470, 1996.
10) Vincent, R.G., Pickren, J.W., Lane, W.W., Bross, I., Takita, H., Houten, L., Gutierrez, A.C., and Rzepka, T.: The changing histopathology of lung cancer: a review of 1682 cases. Cancer 39; 1647-1655, 1977.
11) Janssen-Heijnen, M.L., Nab, H.W., van Reek, J., van der Heijden, L.H., Schipper, R., and Coebergh JW.: Striking changes in smoking behaviour and lung cancer incidence by histological type in south-east Netherlands, 1960-1991. Eur J Cancer 31A; 949-952, 1995.
12) Skuladottir, H., Hirsch, F.R., Hansen, H.H. and Olsen, J.H.: Pulmonary neuroendocrine tumors: incidence and prognosis of histological subtypes. A population-based study in Denmark. Lung Cancer 37; 127-135, 2002.
13) Charloux, A., Rossignol, M., Purohit, A., Small, D., Wolkove, N., Pauli, G., Quoix, E., and Kreisman, H.: International differences in epidemiology of lung adenocarcinoma. Lung Cancer 16; 133-143, 1997.

14) Hirayasu, T., Iwamasa, T., Kamada, Y., Koyanagi, Y., Usuda, H., and Genka, K.: Human papillomavirus DNA in squamous cell carcinoma of the lung. J Clin Pathol 49; 810-817, 1996.
15) Miyagi, J., Tsuhako, K., Kinjo, T., Iwamasa, T. and Hirayasu, T.: Recent striking changes in histological differentiation and rate of human papillomavirus infection in squamous cell carcinoma of the lung in Okinawa, a subtropical island in southern Japan. J Clin Pathol 53; 676-684, 2000.
16) Dyson, N., Howley, P.M., Munger, K. and Harlow, E.: The human papilloma virus-16 E7 oncoprotein is able to bind to the retinoblastoma gene product. Science 243; 934-937, 1989.
17) Werness, B.A., Levine, A.J. and Howley, P.M.: Association of human papillomavirus types 16 and 18 E6 proteins with p53. Science 248; 76-79, 1990.
18) Syrjanen, K.J.: Epithelial lesions suggestive of a condylomatous origin found closely associated with invasive bronchial squamous cell carcinomas. Respiration 40; 150-160, 1980.
19) Cheng, Y.W., Chiou, H.L., Sheu, G.T., Hsieh, L.L., Chen, J.T., Chen, C.Y., Su, J.M., and Lee, H.: The association of human papillomavirus 16/18 infection with lung cancer among nonsmoking Taiwanese women. Cancer Res 61; 2799-2803, 2001.
20) Syrjanen, K., Syrjanen, S., Kellokoski, J., Karja, J. and Mantyjarvi, R.: Human papillomavirus (HPV) type 6 and 16 DNA sequences in bronchial squamous cell carcinomas demonstrated by in situ DNA hybridization. Lung 167; 33-42, 1989.
21) Yousem, S.A., Ohori, N.P. and Sonmez-Alpan, E.: Occurrence of human papillomavirus DNA in primary lung neoplasms. Cancer 69; 693-697, 1992.
22) Nuorva, K., Soini, Y., Kamel, D., Pöllänen, R., Bloigu, R., Vähäkangas, K., and Pääkkö, P.: p53 protein accumulation and the presence of human papillomavirus DNA in bronchioloalveolar carcinoma correlate with poor prognosis. Int J Cancer 64; 424-429, 1995.
23) Ogura, H., Watanabe, S., Fukushima, K., Masuda, Y., Fujiwara, T., and Yabe, Y.: Human papillomavirus DNA in squamous cell carcinomas of the respiratory and upper digestive tracts. Jpn J Clin Oncol 23; 221-225, 1993.
24) Kinoshita, I., Dosaka-Akita, H., Shindoh, M., Fujino, M., Akie, K., Kato, M., Fujinaga, K., and Kawakami, Y.: Human papillomavirus type 18 DNA and E6-E7 mRNA are detected in squamous cell carcinoma and adenocarcinoma of the lung. Br J Cancer 71; 344-349, 1995.
25) Tsuhako, K., Nakazato, I., Hirayasu, T., Sunakawa, H. and Iwamasa, T.: Human papillomavirus DNA in adenosquamous carcinoma of the lung. J Clin Pathol 51; 741-749, 1998.
26) Kinjo, T., Kamiyama, K., Chinen, K., Iwamasa, T., Kurihara, K., and Hamada, T.: Squamous metaplasia induced by transfection of human papillomavirus DNA into cultured adenocarcinoma cells. Mol Pathol 56; 97-108, 2003.
27) Kinjo, T., Kamiyama, K., Chinen, K., Arasaki, A. and Iwamasa, T.: Possible factors related to phenotype change from adenocarcinoma to squamous cell carcinoma. Acta Histochem. Cytochem. 37; 7-14, 2004.
28) Wilson, R. and Laimins, L.A.: Differentiation of HPV-containing cells using organotypic "raft" culture or methylcellulose. Methods Mol Med 119; 157-169, 2005.
29) Woodworth, C.D., Cheng, S., Simpson, S., Hamacher, L., Chow, L.T., Broker, T.R., and DiPaolo, J.A.: Recombinant retroviruses encoding human papillomavirus type 18 E6 and E7 genes stimulate proliferation and delay differentiation of human keratinocytes early after infection. Oncogene 7; 619-626, 1992.
30) Kim, D.W. and Hirth, F.: Genetic mechanisms regulating stem cell self-renewal and differentiation in the central nervous system of Drosophila. Cell Adh Migr 3; 402-411, 2009.
31) Kinjo, T.: "Correlation between squamous cell carcinoma and HPV infection" implies another effect of HPV genes. Curr. Top. Virol. 7; 89-98, 2008.
32) Ishizumi, T., McWilliams, A., MacAulay, C., Gazdar, A. and Lam, S.: Natural history of bronchial preinvasive lesions. Cancer Metastasis Rev 29; 5-14, 2010.
33) Sousa, V., Santo, J.E., Silva, M., Cabral, T., Alarcão, A.M., Gomes, A., Couceiro, P., and

Carvalho, L.: EGFR/erB-1, HER2/erB-2, CK7, LP34, Ki67 and P53 expression in preneoplastic lesions of bronchial epithelium: an immunohistochemical and genetic study. Virchows Arch [Epub ahead of print], 2011.
34) Lamy, A., Sesboüé, R., Bourguignon, J., Dautréaux, B., Métayer, J., Frébourg, T., and Thiberville, L.: Aberrant methylation of the CDKN2a/p16INK4a gene promoter region in preinvasive bronchial lesions: a prospective study in high-risk patients without invasive cancer. Int J Cancer 100; 189-193, 2002.
35) Rezazadeh, A., Laber, D.A., Ghim, S.J., Jenson, A.B. and Kloecker, G.: The role of human papilloma virus in lung cancer: a review of the evidence. Am J Med Sci 338; 64-67, 2009.
36) Zhao, X., Rasmussen, S., Perry, J. and Kiev, J.: The human papillomavirus as a possible cause of squamous cell carcinoma: a case study with a review of the medical literature. Am Surg 72; 49-50, 2006.
37) Chen, Y.C., Chen, J.H., Richard, K., Chen, P.Y. and Christiani, D.C.: Lung adenocarcinoma and human papillomavirus infection. Cancer 101; 1428-1436, 2004.
38) Miasko, A., Nikliñska, W., Nikliński, J., Chyczewska, E., Naumnik, W., and Chyczewski, L.: Detection of human papillomavirus in non-small cell lung carcinoma by polymerase chain reaction. Folia Histochem Cytobiol 39; 127-128, 2001.
39) Giuliani, L., Favalli, C., Syrjanen, K. and Ciotti, M.: Human papillomavirus infections in lung cancer. Detection of E6 and E7 transcripts and review of the literature. Anticancer Res 27; 2697-2704, 2007.
40) Klein, F., Amin Kotb, W.F. and Petersen, I.: Incidence of human papilloma virus in lung cancer. Lung Cancer 65; 13-18, 2009.
41) Coissard, C.J., Besson, G., Polette, M.C., Monteau, M., Birembaut, P.L., and Clavel, CE.: Prevalence of human papillomaviruses in lung carcinomas: a study of 218 cases. Mod Pathol 18; 1606-1609, 2005.
42) Lim, W.T., Chuah, K.L., Leong, S.S., Tan, E.H. and Toh, C.K.: Assessment of human papillomavirus and Epstein-Barr virus in lung adenocarcinoma. Oncol Rep 21; 971-975, 2009.
43) Nakazato, I., Hirayasu, T., Kamada, Y., Tsuhako, K. and Iwamasa, T.: Carcinoma of the lung in Okinawa, Japan: with special reference to squamous cell carcinoma and squamous metaplasia. Pathol Int 47; 659-672, 1997.
44) Syrjanen, K.J.: HPV infections and lung cancer. J Clin Pathol 55; 885-891, 2002.
45) Li, Y.J., Tsai, Y.C., Chen, Y.C. and Christiani, D.C.: Human papilloma virus and female lung adenocarcinoma. Semin Oncol 36; 542-552, 2009.
46) Kountouri, M.P., Mammas, I.N. and Spandidos, D.A.: Human papilloma virus (HPV) in lung cancer: unanswered questions. Lung Cancer 67; 125, 2010.
47) Iwamasa, T., Miyagi, J., Tsuhako, K., Kinjo, T., Kamada, Y., Hirayasu, T., and Genka, K.: Prognostic implication of human papillomavirus infection in squamous cell carcinoma of the lung. Pathol Res Pract 196; 209-218, 2000.
48) Kratz, J.R., Yagui-Beltran, A. and Jablons, D.M.: Cancer stem cells in lung tumorigenesis. Ann Thorac Surg 89; S2090-2095, 2010.
49) Colman, A. and Dreesen, O.: Induced pluripotent stem cells and the stability of the differentiated state. EMBO Rep 10; 714-721, 2009.