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人類乳突瘤病毒感染肺癌細胞及組織之可能受體研究
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行政院國家科學委員會專題計畫成果報告

人類乳突瘤病毒感染肺癌細胞及組織之可能受體研究

The putative receptor for HPV infection in lung cancer cells and lung tumors

壹、 中文摘要

本計畫原擬分三年找出HPV 進入人類肺細胞的可能受體，尤其是肺細胞，以了解HPV 感染肺癌細胞和肺癌組織之分子機轉。但只核定第一年HPV感染之肺癌細胞株之建立，此部分研究已於96年底發表於Cancer Research 因此將計劃執行內容改為『Human telomerase reverse transcriptase activated by E6 oncoprotein is required for human papillomavirus 16/18- infected lung tumorigenesis』。

過去本研究室的研究發現在肺癌患者肺腫瘤組織中可測到HPV 16/18 E6致癌蛋白且與p53蛋白的去活化有關。我們進一步推測HPV 16/18 E6致癌蛋白亦會經由啟動hTERT轉錄活性而參與肺的腫瘤化。本計畫利用免疫組織化學染色法分析135位肺癌患者肺腫瘤組織中HPV 16 E6致癌蛋白的表現，並利用即時定量反轉錄聚合酶反應定量hTERT mRNA的表現。hTERT的轉錄活性是否經由cMYC及SP1所調控則利用小片段干擾RNA、西方點墨法及CHIP分析等方式分析。hTERT活性及其致癌性則利用real-time quantitative TRAP及soft agar assay方式分析。結果發現hTERT mRNA在HPV感染、女性、不抽菸者及縣癌有較高的感染率，且hTERT在早期肺癌患者的表現量亦高於晚其患者。CHIP 分析結果亦發現cMYC及SP1協力促進hTERT的轉錄活性。在HPV感染的TL-1肺癌細胞中將hTERT及E6抑制後其致癌能力亦隨之下降。根據上述研究結果推測HPV E6致癌蛋白正調控hTERT mRNA表現可能是HPV感染相關的肺腫瘤化過程中所必須的。

關鍵字：肺癌，人類乳突瘤病毒

ABSTRACT

Purpose: Our recent report indicates that HPV16/18 E6 oncoprotein is expressed in lung tumors and is related to p53 inactivation. We further explored whether hTERT transcription could be upregulated by E6 and contributes to lung tumor development.
Experimental Design: HPV16 E6 oncoprotein of 135 lung tumors was detected by immunohistochemistry (IHC), and hTERT mRNA was evaluated by real-time RT-PCR and *in situ* hybridization, respectively. A small interference RNA (RNAi), western boltting, and CHIP analysis were used to clarify whether hTERT transcription was regulated by c-Myc and Sp1. The telomerase activity and oncogenic potential of TL-1 with or without E6- or hTERT-RNAi was determined by real-time quantitative TRAP analysis and soft agar assay, respectively.
Results: hTERT mRNA levels in E6-positive tumors, which were prevalent in female, nonsmokers, and adenocarcinomas, were significantly higher than in E6-negative tumors. In addition, hTERT mRNA levels in early tumors (stage I) were greater than levels in advanced tumors (stage II and III). CHIP assay showed that Sp1 cooperated with c-Myc to activate hTERT transcription in TL-1 cells which was similar to the SiHa cells. The telomerase activity of the TL-1 cells decreased concomitantly with the transfection of various doses of E6- or hTERT-RNAi. A soft agar assay showed that the oncogenic potential of TL-1 cells was significantly reduced after being transfected with E6 RNAi. Moreover, the colony of TL-1 cells can't be formed after transfection with hTERT RNAi.
Conclusion: Transcriptional activation of hTERT by E6 oncoprotein is required for HPV16/18-infected lung tumorigenesis..

Key words: lung cancer, Human papillomavirus

Introduction

Telomerase activity is detected in more than 90% of immortalized and cancer cells but is absent in most normal somatic cells, suggesting that telomerase activation is an important event in the transformation process of malignancy (1-3). The key determinant of human telomerase activity is considered to be human telomerase reverse transcriptase (hTERT) (1,2). Although various post-transcriptional and post-translational modifications can regulate hTERT function, transcriptional control of the gene is a major contributor to the regulation of telomerase activity in many human cancers, including lung cancer (1-3). The activation of hTERT transcription has been found in early genetic abnormalities of bronchial carcinogenesis (4). Additionally, elevated hTERT mRNA is associated with an increased relative risk in the prevalence and incidence of bronchial squamous cell carcinoma (5). hTERT overexpression has also been observed in 77% of high-grade atypical alveolar hyperplasia representing the lung adenocarcinoma precursor lesion in 97% of nonmucinous bronchioloalveolar hyperplasia, but in only 27% of low-grade atypical alveolar hyperplasia (6). These results suggest that the activation of hTERT transcription may play a role in the initiation of squamous cell carcinomas of the lung and atypical alveolar lung

adenocarcinomas.

Lung cancer is the most common malignancy worldwide and also in Taiwan (8,9). Cigarette smoking is the major cause of lung cancer; however, about 50% of Taiwanese lung cancer cases cannot be explained by active cigarette smoking, especially among Taiwanese women, 90% of whom have never smoked (8,9). Thus, different etiological factor(s) may be involved in lung carcinogenesis in Taiwan. We have previously shown that an HPV16/18 infection might be associated with lung cancer development in Taiwanese women (9,10). In fact, our most recent study showed that the HPV 16/18 E6 protein is indeed expressed in about half of HPV 16/18 DNA-positive lung tumors, and leads to p53 inactivation (11). p53 has been reported to repress hTERT expression by binding to Sp1 and preventing its access to the hTERT promoter (12). Human keratinocytes, transformed by HPV16 E6, are partially mediated through hTERT transcriptional activation by promoting the binding of c-Myc and Sp1 to GC-rich sequences within the hTERT promoter (1,13-16). These results have led us to elucidate whether transcriptional activation of hTERT by E6 oncoprotein could play a crucial role in HPV16/18-infected lung tumorigenesis.

PATIENTS and METHODS

Study subjects

Lung tumor specimens were collected from 135 patients with primary lung cancer. All of these patients, including 63 females and 72 males who were admitted to the Department of Thoracic Surgery, Taichung Veteran's General Hospital (TVGH), Taichung, Taiwan, between 2000 and 2003, were asked to submit a written informed consent based on a biology study approved by the Institutional Review Board. None of the subjects had received radiation therapy or chemotherapy prior to surgery. Tumor types and stages were histologically determined according to the WHO classification system (World Health Organization, 1981). Pathology samples were processed for conventional histological procedures.

Preparation of RNA and real-time quantitative RT-PCR

Total RNA from the lung tumors (100 mg) and from the tumor cell lines (1×10^5 cells) was extracted by homogenization in 1 ml TRIzol reagent followed by chloroform re-extraction and isopropanol precipitation. Total RNA (3 μg) was reverse transcribed using SuperScript II Reverse Transcriptase (Invitrogen, CA, USA) and oligo d(T)15 primer. Real-time quantitative PCR was performed in a final volume of 25 μl containing 1 μl of each cDNA template, 10 pmol of hTERT gene-specific primer and 12.5 μl of a SYBR-Green master mix. The primers were designed using ABI Prism 7500 SDS Software. Quantification was carried out using the comparative threshold cycle (CT) method, and water was used as the negative control. An arbitrary threshold was chosen on the basis of the variability of the baseline. CT values were calculated by determining the cycle number at which the fluorescence exceeded the threshold limit. The average CT values for the target gene were normalized to an endogenous housekeeping gene encoding 18S rRNA.

Immunohistochemistry

Formalin fixed and paraffin embedded specimens were sectioned at a thickness of 3 μm. All sections were then deparaffinised in xylene, rehydrated through serial dilutions of alcohol, and washed in PBS (pH 7.2), the buffer which was used for all subsequent washes. For HPV 16 E6 detection, sections were heated in a microwave oven twice for 5 min in citrate buffer (pH 6.0) and then incubated with polyclonal anti-HPV 16 E6 antibody (Santa Cruz, California, USA) for 90 min at 25°C. The conventional streptavidin peroxidase method (DAKO, LSAB Kit K675, Copenhagen, Denmark) was performed to develop signals, and the cells were counter-stained with hematoxylin. Negative controls were obtained by leaving out the primary antibody. Three observers independently evaluated the intensities of the signals. Negative immunostaining was defined as having 0-10% positive nuclei and cases with more than 10% positive nuclei were specified as positive immunostaining. Positive control slides for p53 protein detection were purchased from DAKO (Denmark) and the cervical cancer tumor tissues with HPV 16/18 were used as a positive control for HPV 16/18 E6. The antibody dilution buffer was used to replace antibodies to serve as a negative control.

In situ-hybridization (ISH)

ISH, for the detection of hTERT mRNA, was performed using digenoxenin-labeled (DIG-labeled) oligonucleotide probes and a commercially available hybridization kit (Boehringer Mannheim, Indianapolis, IN). Briefly, the hybridizing probe was prepared by PCR amplification using hTERT specific primer (UP: 5' GCGGAAGAC -AGTGGTGAACT3' and DN: 5' AGCTGGAGTAGTCGCTCTGC3') with DIG-ddUTP as the substrate following the manufacturer's instructions. The deparaffinised and rehydrated 5 μm sections were digested with proteinase K and DNase, rinsed with PBS, and dehydrated. The hybridization was performed in a humidified chamber at 48℃ for 16 hrs followed by a washing with sodium chloride-sodium citrate (SSC). Thereafter, the detection reagent anti-DIG-HRP was applied to the sections and then the sections were incubated with DAB solution (DAKO, Denmark) to allow the signals to develop. After the signal development, the sections were counterstained with hematoxylin, rinsed briefly in absolute ethanol, mounted, and observed for

signals under a microscope.

Establishment of HPV16-infected and non-infected lung cancer cell lines from patients' pleural effusions

Lung tumor cells were isolated from the pleural effusion of lung cancer patients by the Ficoll-Paque method. The clinical characteristics of these patients and the identification of these cell lines according to the lung adenocarcinoma cell type are recorded as described previously (11).

Protein extraction and Western blotting

Total protein was extracted from cells with a lysis buffer (100 mM Tris, pH 8.0, 1% SDS), and recovered protein concentrations were determined using the Bio-Rad protein assay kit followed by separation with SDS-PAGE (12.5% gel). After the electrophoretic transfer to a PVDF membrane, nonspecific binding sites were blocked with 5% nonfat milk in TBS containing 0.1% Tween-20. HPV 16 E6, c-Myc, SP-1 and β-actin were detected by incubating the membrane with anti-HPV16 E6 (Santa Cruz Biotechnology, Santa Cruz, CA, USA, and CHEMICON International, Inc., CA, USA) or anti-c-Myc (Santa Cruz Biotechnology) or anti-SP1 (Santa Cruz Biotechnology) or anti-β-actin (DAKO, Copenhagen, Denmark) for 60 min at room temperature, followed by a subsequent incubation with a peroxidase-conjugated secondary antibody (1:5000 dilution). After each antibody incubation step, extensive washing with TBS/Tween-20 was performed to remove non-specific binding. The protein bands were observed using enhanced chemiluminescence (NEN Life Science Products Inc., Boston MA, USA).

Silencing of endogenous HPV 16 E6 or hTERT expression by RNAi

The target sequences for RNA interference (RNAi) for HPV16 E6 and hTERT have been previously verified (17,18) and the sequence of the hTERT-RNAi sense strand-directed small interfering RNA (siRNA) was 5,-UCAGACAGCACUUGAA -GAdTdT-3'. To suppress transcription of the endogenous HPV16 E6 *or hTERT* gene, TL-1 cells were transiently transfected with synthetic siRNAs against HPV16 E6 using Oligofectamine reagent (Invitrogen, USA) according to the manufacturer's instructions. The detailed procedures were performed as described previously (11).

CHIP assay

TL-1, TL-4 and SiHa cells (~$4.0x10^6$) cultured in 10 cm plates were transfected with or without HPV 16 E6 RNAi. After 48 h, cells were treated with 1% formaldehyde (cross-linker) for 7.5 minutes, harvested, sonicated (into 200 to 1000 bp fragments) in media to achieve a final concentration of 1%, and incubated at 37°C for 30 min. Formaldehyde was quenched with 0.125M of glycine. Chromatin-protein complexes were immunoprecipitated using anti-myc (Santa Cruz Biotechnology) or anti-SP1 (Santa Cruz Biotechnology) DNA was extracted from proteins using phenol/chloroform/isoamyl alcohol (25:24:1), precipitated with ammonium acetate, resuspended in water, and subsequently analyzed by PCR using primers specific for the human hTERT promoter as follows: S-1, 5'-CGGGCTCCCACTGGGATTC-3' and AS-1, 5'-ACGTGCGCAGCAGGACG -CAG-3'. The PCR product was amplified within 25 cycles and analyzed by ethidium bromide agarose gel electrophoresis using standard protocols.

Real time quantitative –TRAP analysis

The Real –time quantitative –TRAP method was used to detect telomerase activity according to a previous report (19). Briefly, the SYBR Green RQ-TRAP assay was conducted with cell extracts (1000 cells), 0.1 µg of telomerase primer TS, and 0.05 µg of anchored return primer ACX, in 25 µl of SYBR Green PCR Master Mix (Applied Biosystems). We used the primer sequences as described by Kim and Wu (20). Using the ABI Prism 7500 thermal cycler (Applied Biosystems), samples were incubated for 20 min at 25°C and amplified in 35 PCR cycles for 30 s at 95°C and 90 s at 60°C (two-step PCR). The threshold cycle values (C_t) were determined from semi-log amplification plots (log increase in fluorescence versus cycle number). The default setting for the amplification threshold was 10 standard deviations above the mean background fluorescence. Standards, inactivated samples, and the lysis buffer were assayed anew on every plate. Each sample was analyzed in duplicate. Telomerase activity was expressed relative to TL-1 without E6-RNAi or hTERT-RNAi, i.e. the percentage of telomerase activity compared with TL-1 without E6-RNAi or hTERTRNAi.

Statistical analysis

The $\chi 2$ test, Fisher's exact test (two-tailed), and the Mann-Whitney U test were used for statistical analysis. All analyses were performed using the SPSS Version 11.0 statistical package.

RESULTS

hTERT mRNA expression levels were markedly higher in HPV16/18 E6-positive tumors compared with those of E6-negative tumors

Real-time RT-PCR analysis indicated that hTERT mRNA levels in HPV16 E6 positive tumors were significantly higher than in HPV16 E6 negative tumors ($P < 0.0001$), but not in HPV18 tumors (P

= 0.1000); however, a difference between HPV 16 or 18 E6-positive and –negative tumors (P = 0.001; Table 1) was noted. hTERT mRNA levels from tumors of females, nonsmokers, adenocarcinomas, and stage I tumors were higher than those of males, smokers, squamous cell carcinomas, and stage II+III tumors, respectively (Table 2). This association was not apparent for other clinical parameters including age, tumor size (T value), and nodal micrometastasis (N value) (Table 2). Additionally, immunohistochemistry and *in situ* hybridization were used respectively to detect the HPV16/18 E6 oncoprotein and hTERT mRNA expression in serial sections of lung tumors. As shown in Fig. 1, hTERT mRNA was predominately expressed in the E6-positive tumor area, but to a lesser degree in the E6-negative tumor area, suggesting that the HPV16/18 E6 oncoprotein may contribute to the activation of hTERT transcription in HPV-infected lung tumors.

Sp1 cooperates with c-Myc to activate hTERT transcription in HPV E6-positive lung cancer cells

The core promoter of the hTERT gene contains multiple E-boxes which are bound with c-Myc, and Sp1 binding sites are responsible for hTERT transcription (13-16, 21). To examine whether c-Myc and Sp1 are involved to activate hTERT transcription in HPV E6-positive lung cancer cells, Western blotting was used to evaluate E6, Sp1, and c-Myc protein expression in HPV16-infected TL1 lung cancer cells, SiHa cervical cancer cells, HPV-non-infected TL4 lung cancer cells, and A549 lung cancer cells. As shown in Fig. 2, the c-Myc expression levels in E6-positive TL1 and SiHa cells were higher than in E6-negative TL4 and A549 cells, but a similar Sp1 expression level was observed among the four cells (Fig. 2A). To clarify whether hTERT mRNA was upregulated by E6 through the induction of c-Myc, E6 of TL-1 and SiHa cells were respectively knockdown by E6-RNAi. Western blotting showed that c-Myc expression decreased significantly in the E6-knockdown TL1 and SiHa cells compared those with RNAi negative controls and parental cells (Fig. 2B). Consequently, hTERT mRNA expression levels reduced significantly in both E6-knockdown cells (Fig. 2C).

To elucidate whether the binding activity of c-Myc and Sp1 on the hTERT promoter is regulated by E6, CHIP analysis showed that the binding activity of c-Myc and Sp1 in E6-knockdown TL-1 and SiHa cells was almost eliminated compared with those of RNAi negative controls and parental cells, respectively (Fig. 2D). These results were consistent with previous studies showing that c-Myc and Sp1 are involved in the transcriptional activation of the hTERT gene in HPV16 E6-transfected keratinocytes (13,22-24), cervical cancer cells (25), and HPV16 E6 positive TL-1 lung cancer cells.

Telomerase activity is related with hTERT mRNA upregulated by E6 to confer oncogenic potential of TL-1 cells

Our recent study indicated that HPV16/18 E6 was involved in p53 inactivation and contributes to lung tumor development (11). Telomerase activity is determined by the transcriptional activation of hTERT, and may play a role in lung tumorigenesis (26,27). To elucidate whether hTERT mRNA upregulated by E6 was associated with the elevated telomerase activity, a real-time quantitative TRAP analysis was performed to evaluate telomerase activity in E6- and hTERT-knockdown TL-1 cells. Our data showed that E6 and hTERT levels in TL-1 cells were gradually reduced by the transfection with various doses of E6- and hTERT-RNAi. The telomerase activity of the TL-1 cells after transfection with E6- or hTERT-RNAi for 96 hr, decreased about 30~40% compared with the RNAi negative control cells (Fig. 3). A soft agar assay showed that the largest colonies were formed in TL-1 cells as compared with E6-negative TL-4 and A549 lung cancer cells, revealing that oncogenic potential of TL-1 cells was higher than that of TL-4 and A549 cells. As expected, the colony of TL-1 cells formed in the soft agar decreased significantly after being transfected with E6 RNAi (Fig. 4B). However, the colonies were not formed when the TL-1 cells were transfected with hTERT RNAi (Fig. 4C). These results clearly indicate that the transcriptional activation of the hTERT gene by E6 plays an essential role in HPV16/18-infected lung tumorigenesis, at least in TL-1 lung cancer cells.

DISCUSSION

A previous study showed that c-Myc transcription was suppressed by p53 to cause G1 cell cycle arrest (12), and thus c-Myc up-regulation to promote hTERT transcription could be indirectly mediated through p53 inactivated by E6. This observation is consistent with our present study which indicates that c-Myc expression in TL-1 and SiHa cells decreases significantly when the cells are transfected with E6 RNAi (Fig. 2B). Recently, c-Myc activation has been associated with the integration of HPV DNA at the c-Myc locus in genital tumors, implying that c-Myc activation by HPV DNA integration may play an important role in cervical carcinogenesis (28). A genome-wide analysis revealed that a higher prevalence of c-Myc allelic gain at the 8q24 locus in HPV-positive lung tumors compared to HPV-negative tumors suggests that c-Myc over-expression in HPV-positive lung cancer could be related to HPV DNA integration (unpublished data). Therefore, transcriptional activation of the hTERT gene by c-Myc, which is through p53 inactivated by E6 or HPV DNA integration, may play a role in HPV-associated lung tumorigenesis.

hTERT mRNA detected by real-time RT-PCR and *in situ* hybridization showed that E6-positive tumors had higher hTERT mRNA expression than

in E6-negative tumors. The transcriptional activation of hTERT gene by E6 in lung cancer cells was similar to previous studies of HPV16 E6-immortalized keratinocytes showing that c-Myc expression induced by E6 promotes its binding onto the hTERT promoter to activate hTERT transcription (13-16). Although Sp1 expression was not affected by HPV16 E6 in SiHa and TL-1 cells (Fig. 2C), the binding activity of Sp1 on the hTERT promoter decreased significantly in the E6-knockdown SiHa and TL-1 cells (Fig. 2D). These results are consistent with previous reports indicating that Sp1 cooperates with c-Myc to activate transcription of the hTERT gene. In addition, the Sp1 protein expression level in HPV16-infected TL-1 lung cancer cells was relatively higher than in HPV16-infected SiHa cervical cancer cells (Fig2B), suggesting that the transcriptional activation of the hTERT gene by Sp1 in cooperation with c-Myc may be more notable in TL-1 cells than in SiHa cells. This observation in TL-1 cells seems to support the finding that E6-positive tumors have higher hTERT mRNA levels than E6-negative tumors. Higher hTERT mRNA levels in tumors of females, nonsmokers, and adenocarcinomas was due to the E6 oncoprotein commonly expressed in these categorized tumors (Table 2), even though E6 expression was not associated with tumor stage of lung tumors (data not shown). More interestingly, hTERT mRNA levels in early tumors (stage I) was markedly higher than in those of advanced tumors (stage II and III). Early hTERT mRNA expression has been reported in lung carcinogenesis, including preneoplastic bronchial lesions (5), high-grade atypical alveolar hyperplasia (AAH) (6), and nonmucinous bronchioloalveolar carcinomas (7). A significant increase in hTERT mRNA expression has been observed with increasing degrees of cervical dysplasia including ASCUS, LGSIL and HGSIL (29-31). We thus suggest that transcriptional activation of the hTERT gene by E6 may play more important role in early stage of lung tumor development.

The telomerase activity of TL-1 cells decreased gradually with various doses of RNAi of E6 and hTERT during a 96 hr treatment, suggesting that hTERT transcription upregulated by E6 may activate telomerase activity and contribute to lung tumor development (Figs. 2, 3). Moreover, the colony of hTERT knockdown TL-1 cells was not observed in a soft agar assay (Fig. 4), and the stable clones of E6-knockdown TL-1 cells can't be established. These observations strongly suggest that transcriptional activation of the hTERT gene by E6 is required for HPV-infected lung tumorigenesis. In summary, we analyzed a panel of tumors from Taiwanese lung cancer patients and identified a marked increase in hTERT mRNA expression in lung cancer cells and in lung tumor tissues expressing HPV16/18 E6. The transcriptional activation of hTERT gene by E6 is required for the oncogenic potential of HPV16 E6-positive TL-1 lung cancer cells. We thus strongly suggest that hTERT gene may be as a molecular target for HPV-infected lung cancer therapy using specific telomerase inhibitors (32).

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Table 1. Association of HPV 16/18 E6 protein with hTERT mRNA levels in lung tumors.

E6	hTERT mRNA ($Ct/10^4$)	p value
HPV 16		
Negative (n = 96)	309.86 ± 915.37	
Positive (n = 39)	931.99 ± 1084.50	<0.0001
HPV 18		
Negative (n = 97)	370.21 ± 633.70	
Positive (n = 38)	794.30 ± 1138.27	0.100
HPV 16 or 18		
Negative (n = 72)	227.76 ± 434.52	
Positive (n = 63)	788.81 ± 1042.79	0.001

Table 2. Relationships between hTERT mRNA levels and tumors' clinical parameters.

Parameters	hTERT mRNA ($Ct/10^4$)	p value
Age		
≦65 (n = 66)	579.40±875.99	
>65 (n = 69)	403.68±771.57	0.163
Gender		
Female (n = 63)	709.14±968.88	
Male (n = 72)	297.45±622.41	0.003
Smoking status		
- (n = 95)	622.31±934.65	
+ (n = 40)	174.37±305.43	0.002
Tumor type		
AD (n = 87)	604.04±875.06	
SQ (n = 48)	282.14±689.50	0.011
Tumor stage		
Early (n = 52)	568.56±806.21	
Late (n = 38)	440.11±839.02	0.030
T factor		
1+2 (n = 109)	539.41±880.27	

3+4 (n = 21)	280.71±502.42	0.085
N factor		
0 (n = 58)	560.59±822.16	
1+2+3 (n = 77)	436.10±830.03	0.093

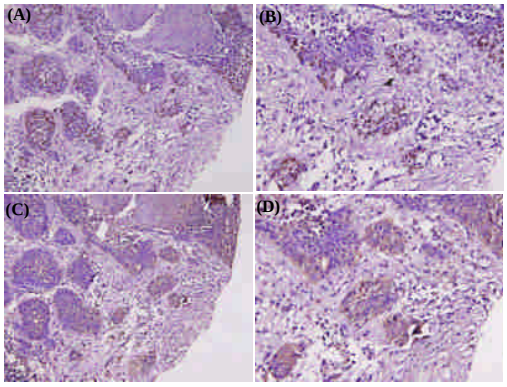

Fig. 1. The representative reciprocal relationships between HPV 16 E6 protein and hTERT mRNA expression in serial tissue array paraffin sections of lung tumors analyzed by immunohistochemistry and *in-situ* hybridization. (A) and (C) (100X), (B) and (D) (200X) were two serial sections from the same lung tumors, respectively. (A) and (C) were HPV 16 E6 protein positive and (B) and (D) were hTERT mRNA positive.

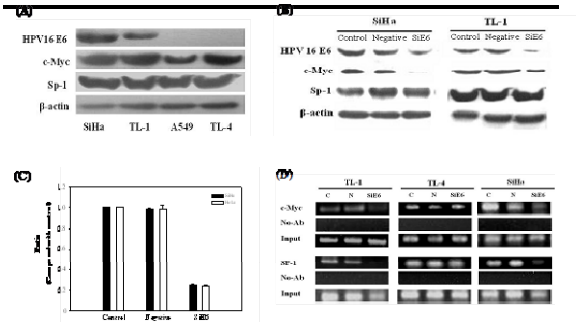

Fig. 2. (A) HPV16 E6, c-Myc, and Sp-1 protein expressions in HPV 16-infected TL-1 lung cancer cells. SiHa cervical cancer cells were used as a positive control, and A549 and TL-4 lung cancer cells were used as HPV non-infected controls. β-actin protein was as a protein loading control. (B) HPV 16 E6 of TL-1 lung cancer cells were knocked down by E6 RNAi. c-Myc, Sp1, and HPV 16 E6 protein in E6 knockdown TL-1 cells were evaluated by western blotting to verify the efficiency of RNAi for E6 as compared with TL-1 parental cells. (C) hTERT mRNA levels in E6 knockdown TL-1 cells evaluated by real-time RT-PCR were compared with that of TL-1 parental cells. (D) Binding activity of c-Myc and Sp-1 to the hTERT promoter evaluated by CHIP analysis in HPV-positive (TL-1) and HPV-negative (TL-4) cells. Chromatin was isolated and immunoprecipitated with an antibody specific for c-Myc and Sp-1. SiHa cells were used as a positive control. P: parental control, N: negative control, and SiE6: the cells were transfected with E6 RNAi.

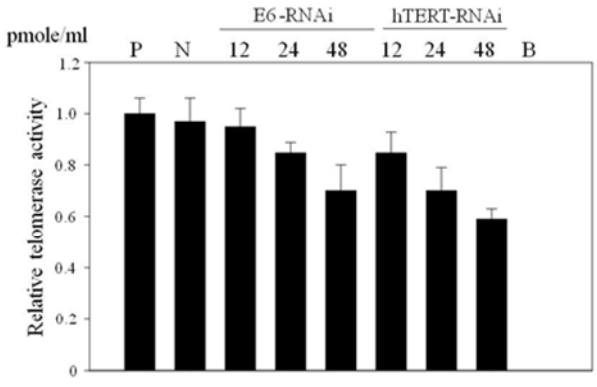

Fig. 3. The human telomerase activity of TL-1 cells after transfection with various doses of the transfection of E6- and hTERT-RNAi compared with negative RNAi controls (N) and parental cells (P). The telomerase activity was evaluated by real-time quantitative-PCR analysis after the cells were transfected with RNAi for 96 hr. B: blank.

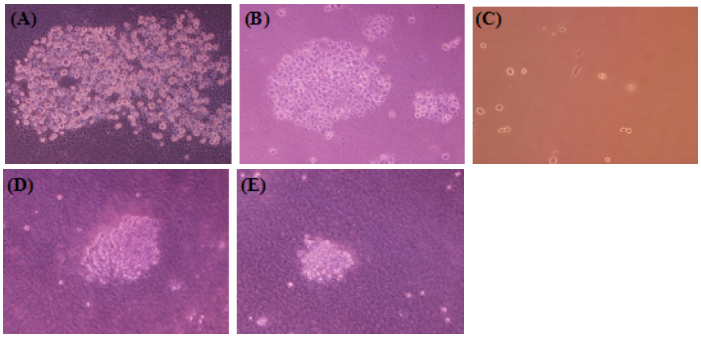

Fig. 4. Soft agar assays were used to evaluate the oncogenic potential of TL-1 cells after transfected with or without HPV16 E6 RNAi or hTERT RNAi. Large colonies of TL-1 cells were formed in soft agar (A), small colonies were observed in TL-1 cells with HPV16 E6 RNAi transfection (B), but the colony was not formed in TL-1 cells with hTERT RNAi transfection (C). HPV-non-infected TL-4 (D), and A549 cells (E) were as negative control. Photos are magnified as 200X.

行政院國家科學委員會補助團隊參與國際學術組織會議報告

年　月　日

報告人姓名	鄭雅文	服務機構	中山醫學大學 醫學研究所	職稱	副教授
會議正式名稱	中文：第 12 屆世界肺癌學會				
	英文：12th World Conference on Lung Cancer				
會　議　時　間	自 96 年 09 月 02 日至 96 年 09 月 06 日	地點（國、州、城市）	韓國首爾		

報告內容應包括下列各項：

一、參加會議經過

本人於開會當天抵達韓國首爾，熟悉該城市之大眾運輸系統之使用方式及找尋開會之會議中心的所在位置，會議開始當天即前往會場辦理報到手續，隨即參加大會所舉辦之各場演講，並於海報展示區與各國學者進行討論，得到許多寶貴的資訊。本人此次參與的報告題目為「HPV 16/18 E6 protein involvement in p53 inactivation to HPV-infected lung tumorigenesis is correlated with p53 codon 72 genotypes」屬於，引起與會學者的熱烈討論，對本研究之進行有極大助益。

二、與會心得

本人第三次參加世界肺癌學會所舉辦之研討會，對國外學者參與國際會議之精神甚為佩服。每天從早到晚的各場演講均擠滿了聽取新知的學者，於海報討論區更處處是討論的景況，每個人都像海綿吸水般努力吸收新知，以便增加國際競爭力，與會期間認識不少知名學者，並得到許多有利於未來研究之建議，參與此會議收穫甚多。

三、考察參觀活動（無是項活動者省略）

四、建議事項

希望國科會能大力提倡國內學者、博士班學生或博士後研究員出席國際會議，此舉將有助於提高國內培養之科技人才之國際競爭力，並應積極爭取國際性會議之主辦權，已提高台灣之國際競爭力。

五、其他