行政院國家科學委員會專題研究計畫 成果報告

人類乳突瘤病毒16型參與肺腺癌形成及影響其臨床預後之可能機轉探討

研究成果報告(完整版)

計 畫 類 別 ：個別型
計 畫 編 號 ：NSC 100-2314-B-040-012-
執 行 期 間 ：100年08月01日至101年07月31日
執 行 單 位 ：中山醫學大學醫學研究所

計 畫 主 持 人：鄭雅文
共 同 主 持 人：李輝

報 告 附 件 ：出席國際會議研究心得報告及發表論文

公 開 資 訊 ：本計畫可公開查詢

中 華 民 國 101年10月04日

中 文 摘 要 ： 在許多種類癌組織中均可看到核糖核苷酸還原酶(ribonucleotide reductase M2； hRRM2) 和 p53-依賴型核糖核苷酸還原酶(p53-dependent RR small subunit；p53R2) 的過度表現，本研究目的在了解 p53R2/hRRM2 表現和早期非小細胞肺癌臨床因子的相關性本研究以免疫組織化學染色法分析含 92 位非小細胞肺癌患者腫瘤組織之組織晶片。分析結果發現 p53R2 蛋白表現與各臨床因子間無統計上的相關性，hRRM2 蛋白表現高者期腫瘤分化程度較差(P=0.006). p53R2+/hRRM2- 之肺癌患者有較好的臨床預後(P<0.01). 多變項分析結果發現 p53R2 (risk=0.232, 95% CI=0.086-0.626, P=0.004) 的表現不只可用來評估肺癌患者預後亦可用來評估其復發的危險性(risk=0.545, 95% CI=0.301-0.987, P =0.045) 。因此根據本研究我們認為早期肺癌患者肺腫瘤組織中的 p53R2 表現對於肺癌患者存活率及復發率評估的重要性遠高於 RRM2。

中文關鍵詞： 肺癌,存活率,核糖核苷酸還原酶

英 文 摘 要 ： Overexpression of ribonucleotide reductase M2 (hRRM2) and p53-dependent RR small subunit (p53R2) has been correlated with tumor malignancy and progression in several types of cancer. The aim of this study was to determine the association of p53R2/hRRM2 expression with clinicopathological characteristics of stage I and II non-small cell lung cancer (NSCLC). Immunohistochemistry was conducted on a tissue array that included 92 samples. Correlations between hRRM2 and p53R2 expression and clinicopathological factors, recurrence/metastasis, and outcomes were analyzed. The analyses revealed that there was no correlation between p53R2 expression and clinicopathological factors； hRRM2 was only positively related to poor tumor differentiation (P=0.006). Regarding overall survival during the follow-up period, patients with p53R2+/hRRM2- tumorshad the best outcomes (P<0.01). Multivariant Cox analysis revealed that p53R2 (risk=0.232, 95% CI=0.086-0.626, P=0.004) not only served as a prognostic biomarker to predict survival but also as an independent biomarker to predict disease-free survival (risk=0.545, 95% CI=0.301-0.987, P =0.045) of patients with NSCLC. Therefore, we consider that the expression of p53R2 can be used

not only as a biomarker for overall survival but also as an indicator for tumor recurrence. Based on our finding, p53R2 expression seems more important than that of hRRM2 in prognosis of early-stage lung cancer.

英文關鍵詞： lung cancer, survival rate, RR

Expression Status of Ribonucleotide Reductase Small Subunits hRRM2/p53R2 as Prognostic Biomarkers in Stage I and II Non-small Cell Lung Cancer

Nan-Yung Hsu[1,2], Jeng-Yuan Wu[2], Xiyong Liu[3], Yun Yen[3], Chih-Yi Chen[4], Ming-Chih Chou[1,5], Chun-Hsuan Lin[6], Huei Lee[1,7], and Ya-Wen Cheng[1,7]

[1]Institute of Medicine, Chung Shan Medical University, Taichung, Taiwan, ROC; [2]Division of Chest Surgery, Buddhist Taichung Tzu-Chi General Hospital, Taiwan, ROC; [3]Department of Medical Oncology and Therapeutics Research, City of Hope National Medical Center, Duarte, California, USA; [4] Division of Chest Surgery, China Medical University Hospital, Taichung, Taiwan, ROC; [5] Division of Chest Surgery, Chung Shan Medical University Hospital, Taichung, Taiwan, ROC; [6]Department of Biological Science and Technology, National Chiao Tung University, Hsinchu, Taiwan, ROC; [7]Department of Medical Research, Chung Shan Medical University Hospital, Taichung, Taiwan, ROC.

Running title Hsu et al: hRRM2/p53R2 Expression in Early-stage Lung Cancer

Correspondence to: Ya-Wen Cheng, PhD,

Professor, Institute of Medicine, Chung Shan Medical University, No. 110. Sec. 1,

Jianguo N. Rd., Taichung 4020. Taiwan, ROC; Tel: +886 4 24730022 ext. 11605,

Fax: +886 4 24723229, e-mail: yawen @csmu.edu.tw

Abstract. Overexpression of ribonucleotide reductase M2 (hRRM2) and p53-dependent RR small subunit (p53R2) has been correlated with tumor malignancy and progression in several types of cancer. The aim of this study was to determine the association of p53R2/hRRM2 expression with clinicopathological characteristics of stage I and II non-small cell lung cancer (NSCLC). Immunohistochemistry was conducted on a tissue array that included 92 samples. Correlations between hRRM2 and p53R2 expression and clinicopathological factors, recurrence/metastasis, and outcomes were analyzed. The analyses revealed that there was no correlation between p53R2 expression and clinicopathological factors; hRRM2 was only positively related to poor tumor differentiation (P=0.006). Regarding overall survival during the follow-up period, patients with p53R2+/hRRM2- tumorshad the best outcomes (P<0.01). Multivariant Cox analysis revealed that p53R2 (risk=0.232, 95% CI=0.086-0.626, P=0.004) not only served as a prognostic biomarker to predict survival but also as an independent biomarker to predict disease-free survival (risk=0.545, 95% CI=0.301-0.987, P =0.045) of patients with NSCLC. Therefore, we consider that the expression of p53R2 can be used not only as a biomarker for overall survival but also as an indicator for tumor recurrence. Based on our finding, p53R2 expression seems more important than that of hRRM2 in prognosis of early-stage lung cancer.

Key Words: Non-small cell lung cancer, Ribonucleotide reductase, p53R2, hRRM2.

Tumor stage is the most powerful and widely accepted parameter predictive of survival for patients with NSCLC within the stages I to IV (1). Although locoregional control of non-small cell lung cancer (NSCLC) can be achieved by curative resection, more than 30% of patients with stage I disease experience relapse within 5 years. Many prognostic molecular markers have been described for patients with NSCLC, but none are currently being used in treatment decision making (2).

Ribonucleotide reductase (RR) is a highly regulated rate-limiting enzyme which is essential for DNA synthesis as it converts ribonucleoside diphosphate to 2'-deoxyribonucleoside diphosphate (3). Mammalian RR is a multimeric enzyme comprised of the large R1 subunit and the small R2 subunit. In humans, one large subunit (M1) and two small subunits (hRRM2 and p53R2) of RR have been identified (3, 4). The two small RR subunits p53R2 and hRRM2 have an 80% similarity in protein sequence (4). RRM1 reduced the development of tumors and metastasis and it has therefore been suggested that *RRM1* is a tumor suppressor gene (5, 6). hRRM2 which contains a tyrosine free radical and a non-heme iron for enzyme activity (7) exhibits significant proliferative activity in cancer cells of both humans and other species (mice, rats, monkeys) (8). A high level of RRM2 expression correlates with cellular invasiveness (9), tumor angiogenesis (10), metastasis (11), and poor patient outcome (12). Thus, RRM2 is considered a crucial protein both in malignant progression and in gemcitabine chemoresistance. An *in vitro* assay showed that recombinant p53R2 protein, as well as hRRM2, interact with hRRM1 to form a holoenzyme with the ability to convert cytosine diphosphate (CDP) to dCDP (13, 14). p53R2 is a direct target of the tumor suppressor gene *p53*, and its induction in response to DNA damage assists in G 2 arrest and provides DNA precursors for DNA repair (15, 16). The dysfunction of p53R2 could result in failure of DNA damage repair and thus lead to gene mutation or cellular apoptotic activation (17).

Interestingly, it has been investigated that opposing regulation of hRRM2 and p53R2 in noted potential might play a critical role in determining the invasion and metastatic phenotype in cancer cells (18, 19).

For the RR subunit, clinical studies have indicated that lower RRM1 gene expression resulted in longer overall survival and a better response to gemcitabine-based chemotherapy for several types of cancer (5). But the expression of p53R2 and hRRM2, the regulatory subunits of RR, is largely unknown and warrants further investigation. The aims of this study were to examine the expressions of p53R2 and hRRM2 in surgical specimens of early-stage NSCLC and to evaluate whether such expressions are useful for predicting clinical outcomes.

Materials and Methods

Patients and samples. From January 2000 to December 2006, a total of 92 consecutive patients underwent surgical treatment for NSCLC at China Medical University Hospital in Taichung, Taiwan. Patients who had preoperative chemotherapy or radiotherapy were excluded from this study. Written informed consent for the use of the paraffin-embedded tissues and information regarding sociodemographic characteristics, as approved by the Institutional Review Board at the above hospital, was obtained from each patient before surgery. A thoracic pathologist reviewed all of the available paraffin blocks.

Patients were diagnosed with lung cancer based on the pathologic assessment of cytologic or tissue specimens under microscopic examination by a pathologist. A medical history was taken and a physical examination was performed to evaluate the general condition and cancer symptoms of each patient. A series of images, including a chest x-ray, chest computed tomography, and a whole-body bone scan were performed. A complete blood count, blood biochemistry tests, a pulmonary function test and bronchoscopy were also performed. The study population consisted of 69 men and 23 women (mean age, 64.3 years; age range, 38-78 years). All procedures, including sampling of hilar and mediastinal lymph nodes and the pathology of all specimens, confirmed stage I disease (T1-2N0M0) in 66 patients and stage II disease (T1N1, T2N1, T3N0) in 26 patients. Histological classification and grade were assessed by light microscopy according to WHO criteria (UICC 7th edition). Clinical data including gender, age (≦65 years *vs.* >65 years), smoking habit, histopathology (squamous cell carcinoma, SCC *vs.* adenocarcinoma, AD), tumor stage by TNM (T1 *vs.* T2), lymphovascular invasion, and tumor differentiation were collected from patient charts.

Postoperative follow-up was scheduled at 1, 2, and then every 3 months during the first 2 years after surgery and every 6 months thereafter, or more frequently if needed. The median duration of follow-up after curative resection was 4.8 years.

Tissue microarray and immunohistochemical staining. The tissue microarrays of resectable early stage NSCLC were obtained from the Department of Pathology at China Medical University Hospital in Taichung, Taiwan. We constructed a tissue microarray using triplicate 2.5 mm cores from formalin-fixed and paraffin-embedded specimens of the primary tumor to analyze the p53R2 and RRM2 protein expression. Briefly, after deparaffinization, the endogenous peroxidase activity was blocked with 3% H_2O_2. The array slides were incubated with normal goat serum for 20 minutes, and then the primary antibody was applied to the slides for 20 minutes at room temperature. After 7 minutes of hydrogen peroxide treatment, the array slides were incubated with horseradish peroxidase labeled polymer conjugated with corresponding antibodies for 30 minutes. Then 3,3'-diaminobenzidine [0.05 g of 3,3'-diaminobenzidine and 100 ml of 30% H_2O_2 in 100 mL of PBS] was applied for 5 and 10 minutes, respectively. Each slide was counterstained with hematoxylin (DAKO, Carpinteria, CA, USA). PBS was used as a negative control. Two independent observers evaluated the staining intensity to maintain consistency. Negative controls were obtained by leaving out the primary antibody. A mouse polyclonal antibody against hRRM2, which is commercially produced by Convance (Princeton, NJ, USA) using recombinant hRRM2 peptide, was used for immunohistochemical staining. Rabbit antibody against p53R2 was purchased from Alexis BioChemical Company (Lausen, Switzerland) and was applied for immunohistochemical staining (1:200 dilution). After immunohistochemical staining with anti-p53R2 and anti-hRRM2 antibodies, two pathologists examined each sample

for consistency. Three observers independently evaluated the signal intensities. Negative immunostaining was defined as 0-10% positive nuclei and cases with more than 10% positive nuclei were classified as positive immunostaining.

Statistical analysis. Statistical analysis was performed using the SPSS statistical software program (Version 17.0 SPSS Inc., Chicago, IL, USA). For categorical data, we employed Fisher's exact test, or χ^2 test of proportions. Survival rates were estimated using the Kaplan and Meier method, and statistical analysis was carried out using the log-rank test for equality of the survival curves. Multivariate survival analysis, using Cox's proportional hazard model, was carried out for the variables which were significant in the univariate analysis. Results from this model are reported as relative risks with 95% confidence intervals. Statistical significance was set at $P<0.05$.

Results

Relationship of hRRM2, p53R2 expression with clinicopathological parameters. To elucidate the role of p53R2 and hRRM2 in tumor progression, 92 patients with early-stage lung cancer, 66 with stage I and 26 with stage II cancer, were collected in this study. The expression of p53R2 and hRRM2 protein in lung tumors was analyzed by immunohistochemistry in a tissue array section. There was hRRM2 expression in 25 (27.2%) patients and p53R2 was expressed in 42 (45.6%) patients. Both proteins were predominantly expressed in the cytoplasm of the lung tumor cells as shown in Figure 1A and B. As shown in Table I, positive hRRM2 expression in poorly differentiated tumor was significantly more frequently than in well and moderately differentiated tumor (P=0.006). No other correlation was found between hRRM2 expression and other clinical parameters (Table I). In addition, based on our previous report (20) there was no correlation between p53R2 and the clinical pathological parameters. We only found that p53R2 expression in well-differentiated tumor cells had a trend higher for expression than in poorly differentiated tumor cells (P =0.121).

The influence of hRRM2 and p53R2 expression on overall survival (OS) and disease free survival (DFS) of early stage lung cancer patients. We hypothesized that the expression status of p53R2 and hRRM2 contributed to tumor progression and metastasis. Therefore, we expected that p53R2 and hRRM2 expression would be associated with OS and DFS patients with early-stage lung cancer. For the 92 patients enrolled, the median follow-up was 57.6 months. Disease recurred in 32 patients (10, local recurrence; 21, distant metastasis; 1, local recurrence and distant metastasis) and 27 patients (27 of 92) died from their disease. None of the patients received adjuvant treatment before surgical therapy. Kaplan-Meier analysis showed that patients with negative p53R2 expression had a lower median OS than those with positive p53R2

expression (660 vs. 900 days, *P*=0.022; Figure 2A) (20). In addition, we observed that hRRM2-positive patients had a lower median OS than hRRM2-negative patients (660 vs. 960 days, *P*=0.044; Figure 2B). After combining the expression status of hRRM2 and p53R2, we found that the patients with p53R2-positive and hRRM2- positive expression had the best outcome (*P*=0.003) compared with the other three groups (Figure 2C).

Among the parameters, no significant correlations were found between DFS and hRRM2 protein expression (*P*=0.399; Figure 2D). Additionally, there was also no correlation between the patients' DFS and p53R2 protein expression (*P*=0.901; Figure 2E). After combining the expression status of hRRM2 and p53R2, no correlations were found between the expression of these two protein and the patient's DFS (*P*=0.362; Figure 2F).

p53R2 expression is an independent prognostic factor of OS and DFS in early-stage lung cancer. Multivariate Cox regression analysis, conducted after the parameters of age, gender, lymphovascular invasion tumor type, and tumor stage were adjusted, showed that the relative risk (RRs) for OS and DFS in patients with positive p53R2 expression were 0.232 and 0.545, respectively (95% CI=0.086-0.626, *P*=0.004 for OS; 95% CI= 0.301-0.987, *P*=0.045 for DFS; Table II). However, no statistical significance of hRRM2 expression was found. These results suggest that the loss of p53R2 may result in the inability to inhibit tumor malignancy in patients and leads to poor OS and DFS. p53R2 protein expression in malignant tumors in patients with early-stage lung cancer seems more important than that of hRRM2.

The influence of p53R2 and RRM2 expression on clinical outcome of early-stage ling cancer. The influence of p53R2 and RRM2 protein expression on the outcome of early-stage lung cancer patients was assessed by a log-rank test. Among the parameters, the patients' survival rate was significantly associated with p53R2 and/or RRM2 protein expression (Table III). Patients with positive expression (40.1%) had a significantly higher survival rate than those with negative expression (36.0%), respectively. Similarly, patients with negative RRM2 protein expression (41.8%) had a significantly higher survival rate than those with positive expression (16.0%), respectively. More interestingly, patients with the p53R2-/RRM2- or p53R2+/RRM2+ had a markedly higher survival rate than those with the p53R2+/RRM2- or p53R2-/RRM2+ (P=0.003, log-rank test; Table III).

Discussion

As is known from previous studies, patients with lower *RRM1* and *RRM2* gene expression live longer and exhibit a better response to chemotherapy for several types of cancer (5, 21-25). Zheng *et al*. indicated that analyzing RRM1 protein in NSCLC specimens is difficult because of technical limitations (26). Therefore, most previous reports discuss the correlation between *RRM1* mRNA expression and gemcitabine/cisplatin therapy response. In addition, Månsson *et al*. 2003 indicated that *p53R2* may have a splice variant (27). Therefore, most studies discuss the correlation between p53R2 and clinicopathological characteristics with the focus on protein expression (28). In this study, we focused on p53R2 and RRM2 protein expression and the clinical outcome.

Most reports have discussed RRM2 expression with response to chemotherapy in lung cancer (6, 29). Studies have shown that alterations in the balance of R1 and R2 expression can significantly modify transformation, tumorigenicity, and metastatic potential (30). A recent report indicated that overexpression of RRM2 and p53R2, but not RRM1, in mice specifically induces lung neoplasma (31). Only one paper indicated that the p53R2 immunocytochemical marker alone plays an important prognostic role in NSCLC, and the DNA repair pathway mediated by p53R2 may be responsible for controlling the growth of lung cancer (32). To the best of our knowledge, ours is the first report to help us understand the role of RRM2 and p53R2 protein expression in lung tumor tissues and their clinical significance in early-stage lung cancer. In our study, patients with p53R2+ or hRRM2- had a significantly higher median OS rate compared with patients with p53R2- or hRRM2+ (Figure 2; Table II). Additionally, patients with p53R2+/hRRM2- had a significantly higher median survival rate (960 days) than the other three groups of patients with (p53R2-/hRRM2-,

810 days; p53R2-/hRRM2+, 600 days; p53R2+/hRRM2+, 840 days; *P*=0.003; Table III). When adjusted with the other clinical factors, only p53R2 but not hRRM2 was used as an independent prognostic factor (Table II; *P*=0.004, 95% CI=0.086 –0.626). We also found that p53R2 expression was used as an independent factor for DFS (Table II; *P*=0.045, 95% CI=0.301–0.987). These results suggest that the role of p53R2 in early stage lung tumor progression is more important than that of hRRM2. Piao *et al*. indicated that p53R2 negatively modulates serum-induced MAPK/ERK kinase /extracellular signal-regulated kinase (MEK–ERK) activity and inhibits the MEK–ERK-mediated malignant potential of human cancer cells (33). Therefore, we considered that the expression of p53R2 protein in NSCLC tumors to be not only a biomarker for chemotherapy response in late stage lung cancer but also an independent prognostic factor in patients with early-stage lung cancer.

p53R2 has been found to have malignancy-suppressing activity (19). In our previous study, we found that p53R2 protein expression correlated negatively with tumor cell differentiation in early-stage NSCLC (20). Positive p53R2 expression confered significantly better overall survival (*P*=0.022). These findings appear to be inconsistent with a previous report. Uramato *et al*. (2006) reported that p53R2 was higher in patients with stage II/III, pathological T3-4, and N1-3 NSCLC (32). However, it was not possible to use p53R2 expression as an independent prognostic marker in NSCLC (32). Additionally, previous reports have shown that p53R2 expression correlated with tumor invasion, lymph node metastasis, and tumor size in esophageal and oral cancer (34, 35). p53R2 expression in patients with late stage cancer was higher than in patients with early-stage esophageal cancer (34). Therefore, we suggest that p53R2 expression may play a different role in early and advanced stages of lung cancer.

Recent reports have indicated that overexpression of RRM2 and p53R2, but not RRM1 in mice, specifically induces lung neoplasms (31),which might be independent of RR enzyme activity because there is lung tumor induction in RRM2 and p53R2 but not in RRM1 transgenic mice (31). Cell model experiments have demonstrated that RRM2 interacts with a variety of oncogenes to promote cell transformation and tumorigenesis (36). Both human and mouse cell models have demonstrated that RRM1 has malignancy-suppressing activity (6), whereas hRRM2 has been proven to play a critical role in enhancing invasive potential (35, 37). In the present study, although the relationship of hRRM2 protein expression and lymphovascular invasion did not reach statistical significance (P=0.358), the frequency of hRRM2 expression in the lymphovascular invasion group (6 of 16; 37.5%) was higher than in the non-invasion group (19 of 76; 25.0%). Furthermore, the hRRM2 expression in poorly differentiated tumor was significantly more frequently expressed in well-differentiated tumor (P=0.006). Therefore, we consider that the expression of hRRM2 in patients with early-stage lung cancer may be involved in enhancing tumor cell malignancy.

In conclusion, we showed that expression of p53R2 protein is a favorable prognostic factor and biomarker of relapse in early stage lung cancer. The role of p53R2 in early-stage lung cancer seems more important than that of hRRM2. The expression of p53R2 can be used not only as a independent biomarker for OS but also as an indicator for tumor recurrence.

Acknowledgment

This work was supported by grants from the National Science Council (NSC 97-2314-B-040 -010 -MY3) of Taiwan, Republic of China.

Conflict of Interest

We declare that we have no proprietary, financial, professional or other personal interest of any kind in any product, service and/or company that could be constructed as influencing the position presented in, or the review of this manuscript.

References

1. Montain CF: Revison in the international system for staging lung cancer. Chest 111(6): 1710-1717, 1977.
2. WHO: The World Health Organization. Histological typing of lung tumors. Am J Clin Pathol 77: 123-136, 1982.
3. Reichard P: Ribonucleotide reductases: the evolution of allosteric regulation, Arch Biochem Biophy 397(2): 149-155, 2002.
4. Tanaka H, Arakawa H, Yamaguchi T, Shiraishi K, Fukuda S, Matsui K, Takei Y, and Nakamura Y: Ribonucleotide reductase gene involved in a p53-dependent cell-cycle checkpoint for DNA damage. Nature 404(6773): 42-49, 2000.
5. Jorderin LP, Seve P, Tredon O, and Dumontet C: The ribonucleotide reductase large subunit (RRM1) as a predictive factor in patients with cancer. Lancet Oncol 14, 2010.
6. Fan H, Huang A, Villegas C, and Wright JA: The R1 component of mammalian ribonucleotide reductase has malignancy-suppressing activity as demonstrated by gene transfer experiments. Proc Natl Acad Sci USA. 94(24):13181-13186, 1997.
7. Chang CH, and Cheng YC: Substrate specificity of human ribonucleotide reductase from Molt-4F cells. Cancer Res 39(12): 5081–5086, 1979.
8. Heidel JD, Liu JYC, Yen Y, Zhou B, Heale BS, Rossi JJ, Bartlett DW, and Davis ME: Potent siRNA inhibitors of ribonucleotide reductase subunit RRM2 reduce cell proliferation in vitro and in vivo. Clin Cancer Res 13(7): 2207-2205, 2007.
9. Duxbury MS, Whang EE: RRM2 induces NF-kappaB-dependent MMP-9 activation and enhances cellular invasiveness. Biochem Biophys Res Commun 354(1): 190–196, 2007.

10. Zhang K, Hu S, Wu J, Lu J, Wang X, Liu X, Zhou B, and Yen Y: Overexpression of RRM2 decreases thrombspondin-1 and increases VEGF production in human cancer cells in vitro and in vivo: implication of RRM2 in angiogenesis. Mol Cancer 8: 11, 2009.

11. Liu X, Zhou B, Xue L, Yen F, Chu P, Un F, and Yen Y: Ribonucleotide reductase subunits M2 and p53R2 are potential biomarkers for metastasis of colon cancer. Clin Colorectal Cancer 6(5): 374–381, 2007.

12. Souglakos J, Boukovinas I, Taron M, Mendez P, Mavroudis D, Tripaki M, Hatzidaki D, Koutsopoulos A, Stathopoulos E, Georgoulias V, and Rosell R: Ribonucleotide reductase subunits M1 and M2 mRNA expression levels and clinical outcome of lung adenocarcinoma patients treated with docetaxel gemcitabine. Br J Cancer 98(10); 1710–1715, 2008.

13. Shao J, Zhou B, Zhu L, Qiu W, Yuan YC, Xi B, and Yen Y: *In vitro* characterization of enzymatic properties and inhibition of the p53R2 subunit of human ribonucleotide reductase. Cancer Res 64(1): 1-6, 2004.

14. Guittet O, Hakansson P, Voevodskaya N, Fridd S, Gräslund A, Arakawa H, Nakamura Y, and Thelander L: Mammalian p53R2 protein forms an active ribonucleotide reductase *in vitro* with the R1 protein, which is expressed both in resting cells in response to DNA damage and in proliferating cells. J Biol Chem 276(44): 40647-40651, 2001.

15. Nakan OK, Balint E, Ashcroft M, and Vousden KH: A ribonucleotide reductase gene is a transcriptional target of p53 and p73. Oncogene 19(37): 4283–4289, 2000.

16. Yamaguchi T, Matsuda K, Sagiya Y, Iwadate M, Fujino MA, Nakamura Y, and Arakawa H: p53R2-dependent pathway for DNA synthesis in a p53-regulated cell cycle checkpoint. Cancer Res 61(22): 8256–8262, 2001.

17. Devlin HL, Mack PC, Burich RA, Gumerlock PH, Kung HJ, Mudryj M, and deVere White RW: Impairment of the DNA repair and growth arrest pathways by p53R2 silencing enhances DNA damage-induced apoptosis in a p53-dependent manner in prostate cancer cells. Mol Cancer Res 6 (5): 808-818, 2008.

18. Fan H, Villegas C, and Wright JA: Ribonucleotide reductase R2 component is a novel malignancy determinant that cooperates with activated oncogenes to determine transformation and malignant potential. Proc Natl Acad Sci USA 93(24):14036-13040, 1996.

19. Liu X, Zhou B, Xue L, Shih J, Tye K, Lin W, Qi C, Chu P, Un F, Wen W, and Yen Y: Metastasis-suppressing potential of ribonucleotide reductase small subunit p53R2 in human cancer cells. Clin Cancer Res 12(21): 6337-6344, 2006.

20. Hsu NY, Wu JY, Liu X, Yen Y, Chen CY, Chou MC, Lee H and Cheng YW: p53R2 expression as a prognostic biomarker in early stage non-small cell lung cancer. Oncol Lett 1: 609-614, 2010.

21. Bepler G, Olaussen KA, Vataire AL, Soria JC, Zheng Z, Dunant A, Pignon JP, Schell MJ, Fouret P, Pirker R, Filipits M, and Brambilla E: ERCC1 and RRM1 in the International Adjuvant Lung Trial by automated quantitative *in situ* analysis. Am J Pathol 178(1): 69-78, 2011.

22. Kretschmer C, Sterner-Kock A, Siedentopf F, Schoenegg W, Schlag PM, and Kemmner W: Identification of early molecular markers for breast cancer. Mol Cancer 10(1):15, 2011.

23. Morikawa T, Maeda D, Kume H, Homma Y, and Fukayama M: Ribonucleotide reductase M2 subunit is a novel diagnostic marker and a potential therapeutic target in bladder cancer. Histopathology 57(6): 885-892, 2010.

24. Fujita H, Ohuchida K, Mizumoto K, Itaba S, Ito T, Nakata K, Yu J, Kayashima T,

Souzaki R, Tajiri T, Manabe T, Ohtsuka T, and Tanaka M: Gene expression levels as predictive markers of outcome in pancreatic cancer after gemcitabine-based adjuvant chemotherapy. Neoplasia 12(10): 807-817, 2010.

25. Toffalorio F, Giovannetti E, De Pas T, Radice D, Pelosi G, Manzotti M, Minocci D, Spaggiari L, Spitaleri G, Noberasco C, Catania C, Boselli S, Danesi R, and de Braud F: Expression of gemcitabine- and cisplatin-related genes in non-small-cell lung cancer. Pharmacogenomics J 10(3): 180-190, 2010.
26. Zheng Z, Chen T, Li X, Haura E, Sharma A, and Bepler G: DNA synthesis and repair genes *RRM1* and *ERCC1* in lung cancer. N Engl J Med 356(8): 800-808, 2007.
27. Månsson E, Flordal E, Liliemark J, Spasokoukotskaja T, Elford H, Lagercrantz S, Eriksson S, and Albertioni F: Down-regulation of deoxycytidine kinase in human leukemic cell lines resistant to cladribine and clofarabine and increased ribonucleotide reductase activity contributes to fludarabine resistance. Biochem Pharmacol 65 (2): 237-47, 2003.
28. Wang X, Zhenchuk A, Wiman KG, and Albertioni F: Regulation of p53R2 and its role as potential target for cancer therapy. Cancer Lett 276(1): 1-7, 2009.
29. Boukovinas I, Papadaki C, Mendez P, Taron M, Mavroudis D, Koutsopoulos A, Sanchez-Ronco M, Sanchez JJ, Trypaki M, Staphopoulos E, Georgoulias V, Rosell R, and Souglakos J: Tumor *BRCA1*, *RRM*1 and *RRM2* mRNA expression levels and clinical response to first-line gemcitabine plus docetaxel in non-small cell lung cancer patients. PLoS One 3(11): e3695, 2008.
30. Furuta E, Okuda H, Kobayashi A, and Watabe K: Metabolic genes in cancer: their roles in tumor progression and clinical implications. Biochim Biophys Acta 1805(2): 141-152, 2010.
31. Xu X, Page JL, Surtees JA, Liu H, Lagedrost S, Lu Y, Bronson R, Alani E, Nikitin

AY, and Weiss RS: Weiss, Broad overexpression of ribonucleotide reductase genes in mice specificy induces lung neoplasms. Cancer Res 68(8): 2652-2560, 2008.

32. Uramoto H, Sugio K, Oyama T, Hanagiri T, and Yasumoto K: P53R2, p53-inducible ribonucleotide reductase gene, correlated with tumor progression of non-small cell lung cancer. Anticancer Res 26(2A): 983-988, 2006.

33. Piao C, Lin M, Kim HB, Lee SM, Amatya PN, Hyun JW, Chang IY, and You HJ: Ribouncleotide reductase small subunit p53R2 suppresses MEK-ERK activity by binding to ERK kinase 2. Oncogene; 28(21): 2173-2184, 2009.

34. Okumura H, Natsugoe S, Yokomalura N, Kita Y, Matsumoto M, Uchikado Y, Setoyama T, Owaki T, Ishigami S, and Aikou T: Expression of p53R2 is related to prognosis in patients with esophageal squamous cell carcinoma. Clin Cancer Res 12(12): 3740-3744, 2006.

35. Yanamoto S, Kawasaki G, Yamada S, Yoshitomi I, Yoshida H, and Mizuno A: Ribonucleotide reductase small subunit p53R2 promotes oral cancer invasion via the E-cadherin/beta-catenin pathway. Oral Oncol 45(6): 521-525, 2009.

36. Fan H, Villegas C, Huang A, and Wright JA: The mammalian ribonucleotide reductase R2 compnent cooperates with a variety of oncogenes in mechanisms of cellular transformation. Cancer Res 58(8): 1650-1653, 1998.

37. Zhou BS, Tsai P, Ker R, Tsai J, Ho R, Yu J, Shih J, and Yen Y: Overexpression of transfected human ribonucleotide reductase M2 subunit in human cancer cells enhances their invasive potential. Clin Exp Metastasis 16(1): 43-49, 1998.

Legends

Figure 1. Immunohistochemical analysis of hRRM2 and p53R2 protein in lung tumors. A: A negative immunostaining result in tumor cells X100; B: hRRM2 protein expressed in tumors X400; and C: p53R2 protein expressed in tumors X400.

Figure 2. Overall survival (OS) and disease-free survival (DFS) curves for all studied patients with hRRM2 (A and D), p53R2 expression (B and E), and combined hRRM2 and p53R2 protein expression (C and F). A to C are OS and D to F are DFS.

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

101 年 10 月 03 日

報告人姓名	鄭雅文	服務機構	台北醫學大學一幀生物學與藥物研發博士學位學程	職稱	教授
會議正式名稱	中文：第 6 屆亞洲毒物學會				
	英文：The 6th International Congress of Asia Society of Toxicology				
會 議 時 間	自 101 年 07 月 17 日至 101 年 07 月 20 日	地點（國、州、城市）	日本仙台		

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日本仙台，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「Epigenetic effects of XRCC3 and XRCC5 induced by E6 oncoprotein may enhance Benzo[a]pyrene-induced chromosome instability in lung cancer cells」屬於 poster discussion section，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

本人第二次參加亞洲毒物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。

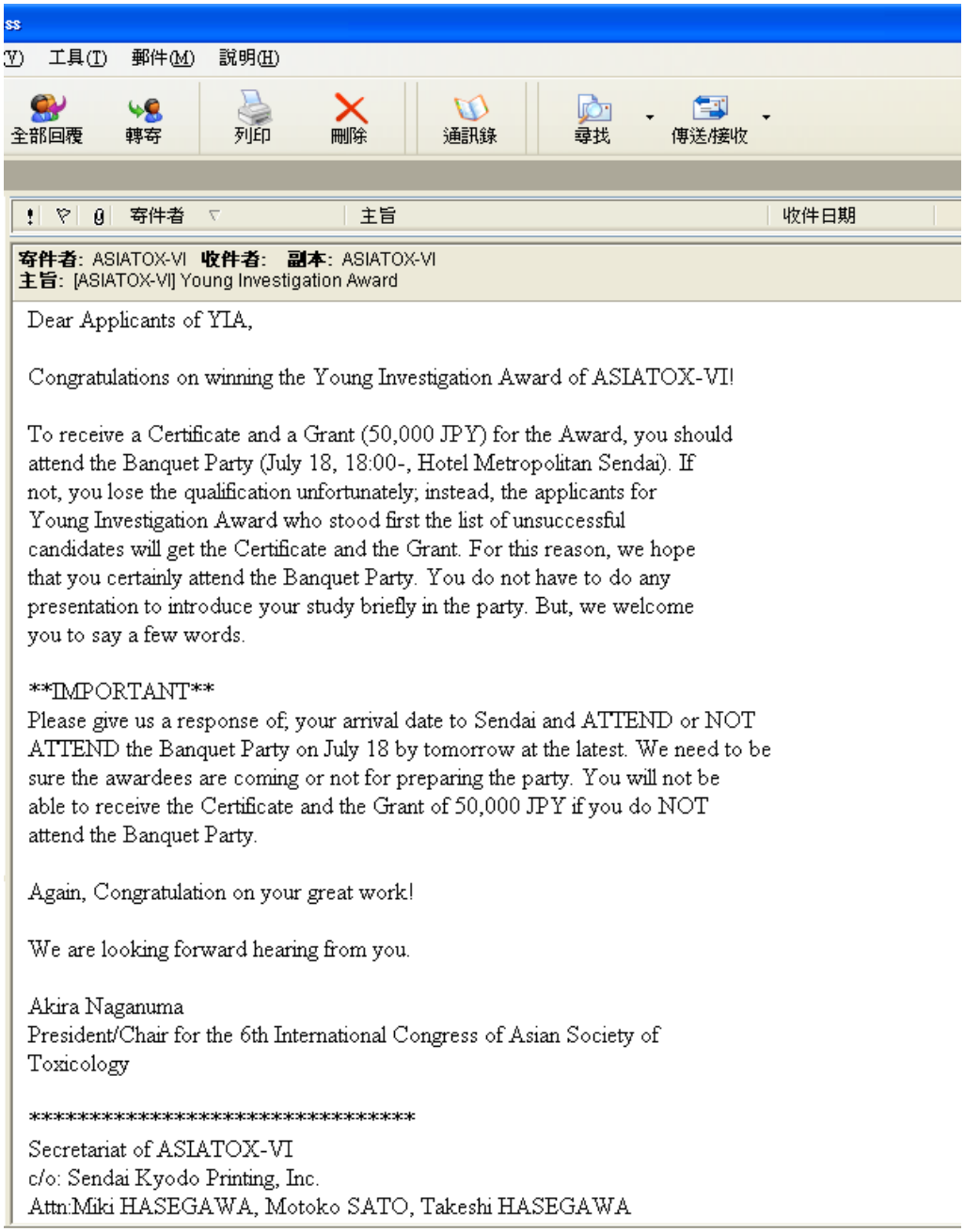
三、考察參觀活動（無是項活動者省略）

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

附件：



ss

(V) 工具(T) 郵件(M) 說明(H)

全部回覆 轉寄 列印 刪除 通訊錄 尋找 傳送/接收

! 寄件者 主旨 收件日期

寄件者: ASIATOX-VI **收件者:** **副本:** ASIATOX-VI
主旨: [ASIATOX-VI] Young Investigation Award

Dear Applicants of YIA,

Congratulations on winning the Young Investigation Award of ASIATOX-VI!

To receive a Certificate and a Grant (50,000 JPY) for the Award, you should attend the Banquet Party (July 18, 18:00-, Hotel Metropolitan Sendai). If not, you lose the qualification unfortunately; instead, the applicants for Young Investigation Award who stood first the list of unsuccessful candidates will get the Certificate and the Grant. For this reason, we hope that you certainly attend the Banquet Party. You do not have to do any presentation to introduce your study briefly in the party. But, we welcome you to say a few words.

IMPORTANT
Please give us a response of; your arrival date to Sendai and ATTEND or NOT ATTEND the Banquet Party on July 18 by tomorrow at the latest. We need to be sure the awardees are coming or not for preparing the party. You will not be able to receive the Certificate and the Grant of 50,000 JPY if you do NOT attend the Banquet Party.

Again, Congratulation on your great work!

We are looking forward hearing from you.

Akira Naganuma
President/Chair for the 6th International Congress of Asian Society of Toxicology

****
Secretariat of ASIATOX-VI
c/o: Sendai Kyodo Printing, Inc.
Attn:Miki HASEGAWA, Motoko SATO, Takeshi HASEGAWA

AP-110

Epigenetic defects of XRCC3 and XRCC5 induced by E6 oncoprotein may enhance benzo[a]pyrene-induced chromosome instability in lung cancer cells

Ya Wen CHENG, Huei LEE

Institute of Medicine, Chung Shan Medical University, Taiwan

Majority of investigation is focused on lung tumorigenesis in smokers, little in nonsmokers. Previously, Benzo[a]pyrene (B[a]P)-DNA adducts levels in nonsmoking female patients were higher than in nonsmoking male patients. However, p53 mutation rate in female patients did not differ from male patients. Moreover, HPV16/18 infection rate in female patients was much higher than in male patients. We therefore hypothesized that HPV infection could synergistically increased chromosomal instability (CIN) induced by B[a]P-DNA adducts and might contribute to lung tumorigenesis. Herein, three HPV-positive and five HPV-negative lung cancer cells were enrolled to test the hypothesis. FISH analysis was used to determine the micronuclei formation when these cells were treated with various concentrations of B[a]P for different time-intervals. The efficacy of micronuclei and DNA adduct formation induced by B[a]P in HPV-positive cells were significantly higher than HPV-negative cells. Mechanistically, the micronuclei and DNA adduct formation are dependent on HPV E6 oncoprotein expression. We next questioned whether B[a]P-induced CIN enhanced by E6 could be through altering DNA repair gene expressions. The promoter hypermethylation and mRNA expression of six DNA repair genes including hMLH1, hMSH2, BRCA1, and BRCA2, and XRCC3, and XRCC5 were evaluated by MSP and real-time RT-PCR, and data indicated that XRCC3 and XRCC5 expressions in E6-positive cells were markedly lower than in E6-negative cells and the reduction of both genes was caused by promoter hypermethylation. Collectively, the promoter hypermethylation of XRCC3 and XRCC5 induced by E6 may increase B[a]P-induced CIN and contribute to lung tumorigenesis in nonsmokers.

ASIATOX VI

Certificate

The 6th International Congress of Asian Society of Toxicology
(ASIATOX-VI)

presents the

Young Investigators' Award

for Excellent Presentation
at the 6th International Congress of Asian Society of Toxicology
to

Ya Wen Cheng

July 17 - 20, 2012
Sendai International Center, Sendai, Japan

Akira Naganuma, PhD
Congress President/Chair,
ASIATOX-VI

Yoshito Kumagai, PhD
Secretary General
ASIATOX-VI

國科會補助計畫衍生研發成果推廣資料表

日期:2012/10/04

國科會補助計畫	計畫名稱: 人類乳突瘤病毒16型參與肺腺癌形成及影響其臨床預後之可能機轉探討
	計畫主持人: 鄭雅文
	計畫編號: 100-2314-B-040-012- 學門領域: 公共衛生及環境醫學

無研發成果推廣資料

100 年度專題研究計畫研究成果彙整表

計畫主持人：鄭雅文	計畫編號：100-2314-B-040-012-
計畫名稱：人類乳突瘤病毒 16 型參與肺腺癌形成及影響其臨床預後之可能機轉探討	

成果項目			量化			單位	備註（質化說明：如數個計畫共同成果、成果列為該期刊之封面故事...等）
			實際已達成數（被接受或已發表）	預期總達成數(含實際已達成數)	本計畫實際貢獻百分比		
國內	論文著作	期刊論文	0	0	100%	篇	
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%		
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（本國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
國外	論文著作	期刊論文	2	0	100%	篇	Hsu NY, Cheng YW*, Chan IP, Ho HC, Chen CY, Hsu CP, Lin MH, Chou MC. Association between expression of human papillomavirus 16/18 E6 oncoprotein and survival in patients with stage I non-small cell lung cancer.

							Lee H and Cheng YW*. Expression Status of Ribonucleotide Reductase Small subunits hRRM2/p53R2 as prognostic biomarkers in Stage I and II Non-small Cell Lung Cancer. Anticancer Res, 31(10):3475-81, 2011.
		研究報告/技術報告	0	0	100%		
		研討會論文	0	0	100%		
		專書	0	0	100%	章/本	
	專利	申請中件數	0	0	100%	件	
		已獲得件數	0	0	100%		
	技術移轉	件數	0	0	100%	件	
		權利金	0	0	100%	千元	
	參與計畫人力（外國籍）	碩士生	0	0	100%	人次	
		博士生	0	0	100%		
		博士後研究員	0	0	100%		
		專任助理	0	0	100%		
其他成果 (無法以量化表達之成果如辦理學術活動、獲得獎項、重要國際合作、研究成果國際影響力及其他協助產業技術發展之具體效益事項等，請以文字敘述填列。)		榮獲 2012 亞洲毒物學會年輕研究學者獎					

	成果項目	量化	名稱或內容性質簡述
科教處計畫加填項	測驗工具(含質性與量性)	0	
	課程/模組	0	
	電腦及網路系統或工具	0	
	教材	0	
	舉辦之活動/競賽	0	
	研討會/工作坊	0	
	電子報、網站	0	

目	計畫成果推廣之參與（閱聽）人數	0	

國科會補助專題研究計畫成果報告自評表

請就研究內容與原計畫相符程度、達成預期目標情況、研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）、是否適合在學術期刊發表或申請專利、主要發現或其他有關價值等，作一綜合評估。

1. 請就研究內容與原計畫相符程度、達成預期目標情況作一綜合評估

■達成目標

□未達成目標（請說明，以 100 字為限）

□實驗失敗

□因故實驗中斷

□其他原因

說明：

2. 研究成果在學術期刊發表或申請專利等情形：

論文：■已發表 □未發表之文稿 □撰寫中 □無

專利：□已獲得 □申請中 ■無

技轉：□已技轉 □洽談中 ■無

其他：(以 100 字為限)

3. 請依學術成就、技術創新、社會影響等方面，評估研究成果之學術或應用價值（簡要敘述成果所代表之意義、價值、影響或進一步發展之可能性）(以 500 字為限)

We considered that the expression of p53R2 can be used not only as a biomarker for overall survival but also as an indicator for tumor recurrence. Base on our finding, p53R2 expression seems more important than hRRM2 in early stage lung cancer prognosis.