# *p53* Gene Mutations in Non-Small Cell Lung Cancer from Thai Patients

SONGSAK PETMITR, Ph.D.*, SIRIWAN MAKTHIENGTRONG, M.Sc.*,
PANTAP SUTINONT, M.D.**, PANATA MIGASENA, M.D., Ph.D., F.R.S.(T)*

**Abstract**

Paraffin embedded tissues from twenty-two Thai patients with non-small cell lung cancer were studied for *p53* gene mutations in exon 5 to 8 using polymerase chain reaction and single-stranded conformation polymorphism (PCR-SSCP) followed by thermal cycle sequencing. Results showed that point mutations in this region of *p53* gene were present in 3 cases. One harboured the base change from GAC to AAC at codon 281, changing amino acid from aspartic acid to asparagine, whilst the other two cases were transversion of AAA (lysine) to ACA (threonine) at codon 292. All subjects with *p53* mutation had a past history of tobacco smoking.

**Key word :** *p53* Gene, Lung Cancer, Thai Patients

Lung cancer is the largest cause of cancer death worldwide. In Thailand, the incidence of lung cancer in males is 21 per cent and in females is 3.8 per cent which is second and fourth in importance, respectively. The most common histopathological type of lung cancer in Thai patients is non-small cell lung cancer (84.6%), whereas, small cell lung cancer is found only in 8.7 per cent of these cases[1].

Recent advances in molecular biology have shown that the multi - step pathway of cancer development consists of an accumulation of adverse genetic events including activation of oncogenes and inactivation of tumor suppressor genes[2]. *p53* is a tumor suppressor gene frequently found mutated in a variety of human cancers[3,4]. *p53* gene is located on chromosome 17p13.1, spanning about 20 kb of genomic DNA and containing 11 exons. The nucleotide sequence in exon 2 to 11 encodes a nuclear phosphoprotein with molecular weight of 53 kDa[5]. In normal cells, wild type *p53* protein functions as a suppressor of cell proliferation and inhibits malignant transformation[6]. In the event of DNA damage,wild type *p53* is induced and leads either to cell cycle arrest or programmed cell death (apoptosis)[7].

* Department of Tropical Nutrition & Food Science, Faculty of Tropical Medicine, Mahidol University, Bangkok 10400.
** Department of Pathology, Faculty of Medicine, Siriraj Hospital, Mahidol University, Bangkok 10700, Thailand.

Tobacco smoking is the most important cause of lung cancer. More than half of lung cancers contain *p53* gene mutations found mostly in region of exons 5 to 8[8]. *p53* gene mutation in lung cancer caused by tobacco smoking is mainly G to T transversion[9]. DNA mutation in non smokers may occur as the result of endogenic process and environmental influence and *p53* gene mutation in this group is predominantly G to A transition[10]. The pattern of *p53* gene mutations has been used as a model for molecular epidemiology of lung cancer[11]. Mutation in *p53* gene is also associated with poor prognosis in all patients, and thus detection of *p53* gene aberration may be helpful in the selection of patients for appropriate investigational threapeutic strategies in view of improving survival and quality of life[12].

In this study, we analysed mutations in *p53* gene from twenty-two Thai patients with non-small cell lung cancer using polymerase chain reaction - single stranded conformation polymorphism (PCR-SSCP) and direct DNA sequencing.

## MATERIAL AND METHOD

### Tumor Specimens

Specimens from twenty-two Thai patients with lung cancer were obtained from the Department of Pathology, Faculty of Medicine, Siriraj Hospital, Mahidol University. Serial sections of 3 mm thickness were made of the formalin-fixed and paraffin-embedded samples. One such section was stained with hematoxylin-eosin and examined microscopically for the histological features using criteria previously described[13]. An adjacent section of 10 μm was used for DNA extraction.

### DNA Extraction

DNA was extracted from tissue specimens by proteinase K digestion followed by phenol - choloform extraction as described[14]. Tissue section was incubated in lysis buffer (10mM Tris-HCl pH 8.3, 50mM KCl, 2.5 mM $MgCl_2$, 0.45 per cent (v/v) Tween-20) containing 0.5 mg/ml proteinase K for 2 h at 60°C. Protein was removed by phenol-chloroform (1:1 v/v) solution according to standard protocol. The aqueous phase containing DNA was stored at 4°C prior to use.

### Identification of *p53* gene mutation

The gene mutation in exons 5 to 8 of *p53*, was identified by PCR-SSCP and thermal sequencing. For PCR-SSCP, 10 μl reaction volume contained 100 ng DNA, 50 mM KCl, 10 mM Tris-HCl pH 8.3, 1 per cent (v/v) Triton X-100, 25 μM of each dNTP, 1μCi of ($\alpha$-$^{32}$P) dCTP (3,000 Ci/mmol, Amersham, Inc., UK), 1μM of each primers and 1 U *Taq* DNA polymerase (Perkin Elmer, U.S.A.). The sequences of primers used are shown in Table 1. PCR was performed in DNA Thermal Cycler 480 (Perkin Elmer, U.S.A.), for 30 cycles (each cycle consisting of 95°C for 1 min; 57°C for 1 min for exons 5 and 7 and 55°C for 1 min for exons 6 and 8; and 72°C for 1 min). The PCR products were electrophoresed in 6 per cent polyacrylamide gel containing 5 per cent glycerol at 400 V for 7 h. The migration of DNA strands in the gel were detected

**Table 1. PCR primers used for the amplification of *p53* genes in exons 5 to 8.**

| Exon | Primer | Nucleotide position |
|---|---|---|
| 5 | F 5' TTCCTACAGTACTCCCCTGC 3'<br>R 5' AGCTGCTCACCATCGCTATC 3' | 13046-13248 |
| 6 | F 5' CCTCTGATTCCTCACTGATT 3'<br>R 5' TTGCAAACCAGACCTCAGGC 3' | 13292-13445 |
| 7 | F 5' GTGTTATCTCCTAGGTTGGC 3'<br>R 5' TCCTGACCTGGAGAGTCTTCCA 3' | 13986-14116 |
| 8 | F 5' CCTGAGTAGTGGTAATCTAC 3'<br>R 5' GCTTGCTTACCTCGCTTAGT 3' | 14443-14598 |

Note : F = sense strand primer
R = antisense strand primer

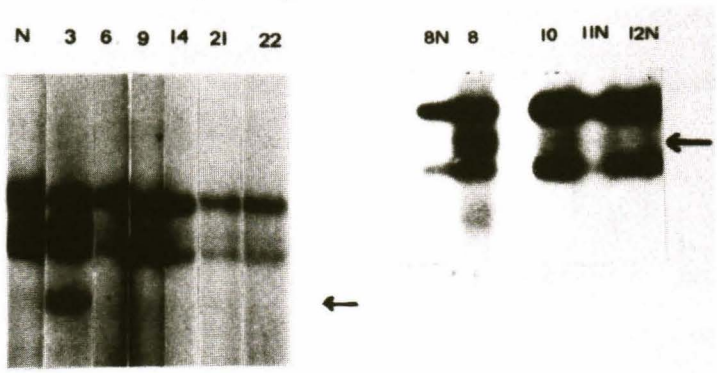


**Fig. 1. PCR-SSCP analysis for *p53* gene mutation in exon 8 of DNA extracted from tissue of lung cancer. N = leukocyte DNA from normal individual. The arrow indicates an abnormal mobility shift.**

by autoradiography. The nucleotide sequences of exons 5 to 8 of *p53* from all patients were determined by AmpliCycle™ Sequencing Kit (Perkin Elmer, U.S.A.) using sense or antisense primer as the sequencing primer. The reactions were performed according to the instructions provided by the manufacturer.

## RESULTS

Of the 22 patients with lung cancer, 12 had a past history of tobacco smoking and 10 were non-smokers. Histological features of these patients sample revealed 12 adenocarcinomas and 10 squamous cell carcinomas. Mutation in exon 5 to 8 of *p53* gene were screened by PCR-SSCP which showed that DNA of 3 patients exhibited an abnormal mobility shift in exon 8 (Fig. 1). The nucleotide sequences of this exon indicated a base change in codon 281, from GAC (aspartic acid) to AAC (asparagine) in one patient (Fig. 2A) and a nucleotide change in codon 292, from AAA (lysine) to ACA (threonine) in the other two cases (Fig. 2B). Histological features of these patients were 2 adenocarcinomas and one squamous cell carcinoma (Table 2). All the subjects had a past history of tobacco smoking.

## DISCUSSION

We examined 22 paraffin embedded non-small cell lung cancer tissues for mutations in exons 5 through 8 of *p53* gene by PCR-SSCP and direct DNA sequencing. Alterations were detected in 3 cases (14%). One case of adenocarcinoma associated with smoking exhibited G to A transition in codon 281, whereas, the other two cases, adenocarcinoma and squamous cell carcinoma associated with smoking, showed A to C transversion in codon 292. The latter mutation has not previously been reported in any human lung neoplasm.

Epidemiological studies have clearly shown that most human lung cancers are caused by tobacco smoke[15]. Tobacco smoke contains a large number of carcinogens and cocarcinogens which could be involved in the developmental steps of lung cancer carcinogenesis[16]. Carcinogens in tobacco smoke and mutational events in cellular genes have been investigated. Mutation caused by benzo(a) pyrene, a potent carcinogen in tobacco smoke is mostly, G to T transversion, whereas, A to C transversion is found only in 4 per cent of the cases[17]. The other carcinogen in cigarette smoke is 4-methylnitrosoamino -1- (3-pyridyl)-1-butanone (NNK) which induces G to A transition in animal model[18]. The type of mutations found in this study is consistent with an exposure to benzo(a) pyrene and NNK in tobacco smoke.

The association of the nature of *p53* mutations with lung cancer type according to histological features has also been reported[3]. G to A transition is commonly found in both adenocarcinoma (30%) and squamous cell carcinoma (28%), whereas, A to C transversion is rarely found in adenocarcinoma (2%) and squamous cell carcinoma

(1%). Nevertheless, in this report 2 out of 3 cases harboured A to C transversion. The reason for this is not clear and may just reflect the small sample size.

Three cases with *p53* gene mutation in this report did not harbour K- *ras* gene mutation (unpublish data). This indicates that *p53* and K-*ras* gene mutations may have occured independently during the molecular events in the development of lung cancer[19].

In this report, mutations of *p53* gene were detected in 3 out of 22 patients (14%) with non-

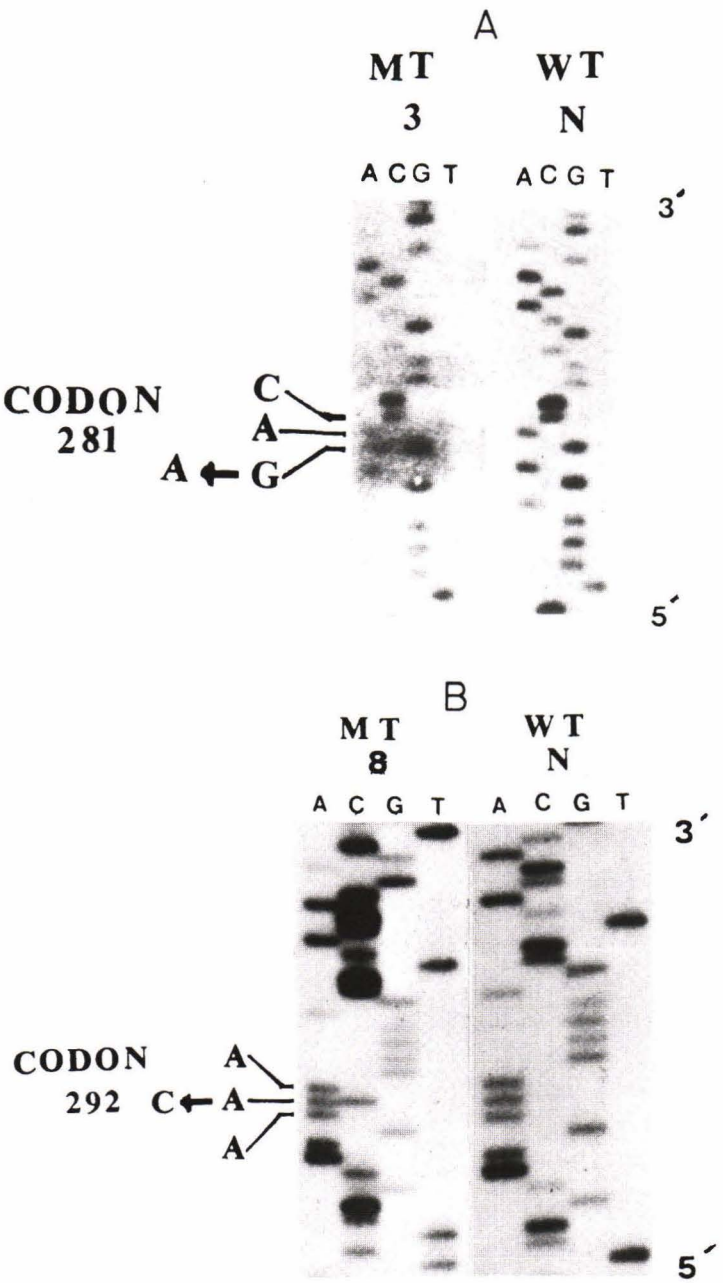


**Fig. 2. Autoradiogram of nucleotide sequencing gel of *p53* gene mutation. (A) The nucleotide sequence of *p53* gene at codon 281 of patient no. 3. (B) The nucleotide sequence at codon 292 of patient no. 8.**

**Table 2. Characteristic of lung cancer patients with *p53* gene mutation (n=22).**

| Patient no. | Age(yr)/sex | Histological type | *p53* gene mutation | | |
|---|---|---|---|---|---|
| | | | codon | base change | amino acid change |
| 3 | 60/M | Sq | 281 | GAC to AAC | Asp to Asn |
| 7 | 62/M | Ad | 292 | AAA to ACA | Lys to Thr |
| 8 | 58/M | Sq | 292 | AAA to ACA | Lys to Thr |

Note: Sq = Squamous cell carcinoma
Ad = Adenocarcinoma.

small cell lung cancer, a lower frequency than that previously reported[8,20]. This may be attributed to variation in genetic background of the subject group and/or environmental carcinogen exposure [11]. The therapeutic application using *p53* gene for gene therapy is approaching[21] and the identification of *p53* gene mutations in Thai patients with lung cancer may be helpful in the selection of patients for future treatment by this strategy.

## SUMMARY

*P53* gene mutations have been analyzed in 22 Thai patients with non-small cell lung cancer using PCR-SSCP and nucleotide sequencing. Three out of 22 cases (14%) harboured point mutation, all of the subjects had a past history of tobacco smoking. The frequency of *p53* gene mutation is lower than that reported in other ethnic groups indicating the variation in genetic background of the subjects group and/or environmental carcinogen exposure.

## ACKNOWLEDGEMENTS

This work was supported by the China Medical Board of New York, Inc. (U.S.A.). The authors wish to thank Prof. Thira Limsila for his encouragement. We wish to thank Ms. Patay Sirivaidyapong for the tissue preparation, Ms. Supa Werawat for providing the clinical information and Prof. Prapon Wilairat for his criticism of the paper.

(Received for publication on September 28, 1998)

## REFERENCES

1. Vatanasapt V, Martin N, Sriplug H, et al. Cancer in Thailand 1988-1991. IACR Technical report no. 16 1996; 164.
2. Weinberg RA. Molecular mechanisms of carcinogenesis. In: Leder P, et al. eds. Introduction to molecular medicine, Scientific American Inc, New York 1994; 277-305.
3. Hollstein M, Sidransky D, Vogelstein B, Harris CC. *p53* mutations in human cancers. Science 1991; 253: 49-53.
4. Greenblatt MS, Bennett WP, Hollstein M, Harris CC. Mutations in the *p53* tumor suppressor gene: clues to cancer epidemiology and molecular pathogenesis. Cancer Res 1994; 54: 4855-78.
5. Murakami Y, Hayaki K, Sekiya T. Detection of aberations of the *p53* alleles and the gene transcript in human tumor cell lines by single-strand conformation polymorphism analysis. Cancer Res 1991; 51: 3356-61.
6. Soussi T, Cardon de Fromentel, May P. Structure aspects of the p53 protein in relation to gene evoluation. Oncogene 1990; 5: 945-52.
7. Canman CE, Chen C-Y, Lee M-H, Kastan MB. DNA damage response : p53 induction, cell cycle perturbations, and apoptosis. Cold Spring Harbor Sym Quant Biol 1994; 59: 277-86.
8. Ryberg D, Kure E, Lystad S, et al. *p53* Mutations in lung tumors : relationship to putative susceptibility markers for cancer. Cancer Res 1994; 54: 1551-5.
9. Ronnai A, Gradia S, Peterson A, Hecht S. G to A transitions and G to T transversions in codon 12 of the Ki-ras oncogene isolated from mouse lung tumors induced by 4-methylnitrosoamino-1-(3-pyridyl)-1-butanone (NNK) and related DNA methylating and pyridylxobutylatiog agents. Carcinogenesis 1993; 14: 2419-22.

10. Rideout WM, Coetzee GA, Olumi AF, Lones PA. 5-Methyl cytosine as an endogenous mutagent in the human LDL receptor and *p53* gene. Science 1990; 249: 1288-90.
11. Soussi T. The *p53* tumor suppressor gene: a model for molecular epidemiology of human cancer. Mol Med Today 1996; 1: 32-7.
12. Dennis Q, Ann D, Carol S, Herbert W, Himansh D. Accumulation of p53 protein correlates with a poor prognosis in human lung cancer. Cancer Res 1992; 52: 4828-31.
13. World Health Organization. Histological typing of lung tumors. 2nd ed. International histological classification of tumors, Geneva: WHO, 1981.
14. Tada M, Omata M, Ohto M. Analysis of *ras* gene mutation in human hepatic malignant tumours by polymerase chain reaction and direct sequencing. Cancer Res 1990; 50: 1122-4.
15. Shopland DR, Eyre HJ, Pechacek TF. Smoking-attributable cancer mortality in 1991: Is lung cancer now the leading cause of death among smoker in the United States? J Natl Cancer Inst 1991; 83: 1142-8.
16. Hoffmann D, Rivenson A, Murphy E, et al. Cigarette smoking and adenocarcinoma of the lung: the relevance of nicotine-derived N-nitrosamines. J Smoking Rel Disord 1993; 4: 165-90.
17. Eric E, Jane W, Janin P, et al. Carcinogenic epoxides of benzo(a)pyrene and cyclopental[cd] pyrene induce base substitutions via specific transversions. Proc Natl Acad Sci USA 1982; 79: 1945-9.
18. Horsfall MJ, Gardon AJ, Burns PA, et al. Mutational specificity of alkylating agents and the influence of DNA repair. Environ Mol Mutagen 1990; 15: 1107-12.
19. Fukuyama Y, Mitsudomi T, Sugio T, Akazawz K, Sugimachi K. K-ras and *p53* mutations are an independent unfavourable prognostic indicator in patients with non-small cell lung cancer. Br J Cancer 1997; 75: 1125-30.
20. Sukuki H, Takahashi T, Kuroishi T, et al. *p53* mutation in non-small cell lung cancer in cancer in Japan : association between mutations and smoking. Cancer Res 1992; 52: 734-6.
21. Arthur B, Gary B, Timothy S, Paul T, Michael S. Tumor suppressor genes : prospects for cancer therapies. Bio Tech 1995; 13: 127-31.

## การผ่าเหล่าของยีน *p53* ในผู้ป่วยมะเร็งปอดชนิด Non-small cell carcinoma

*ทรงศักดิ์ เพ็ชรมิตร, ปร.ด.*, ศิริวรรณ มักเที่ยงตรง, วท.ม.*,*
*ปานเทพ สุทธินนท์, พ.บ. ว.ว.**, ปณต มิคะเสน, พ.บ., Ph.D., ราชบัณฑิต**

การตรวจหาสภาพการผ่าเหล่าของยีน *p53* บริเวณเอกซอน 5 ถึง 8 ในตัวอย่างชิ้นเนื้อที่ได้รับการตรวจทางพยาธิแสดงว่าเป็นมะเร็งปอดแบบ non-small cell carcinoma จำนวน 22 ราย โดยใช้วิธี PCR-SSCP และการหาลำดับนิวคลีโอไทด์ พบว่า 3 ตัวอย่างมีการผ่าเหล่าในยีน *p53* สภาพการผ่าเหล่าที่พบแบ่งออกเป็นแบบนิวคลีโอไทด์เปลี่ยนแปลงจากเดิม GAC เป็น AAC ที่ตำแหน่งโคดอน 281 ทำให้มีการเปลี่ยนแปลงกรดอะมิโนจากเดิม กรดแอสปาร์ติคเป็นแอสปาราจีนหนึ่งตัวอย่าง ส่วนอีกสองตัวอย่างมีการเปลี่ยนแปลงนิวคลีโอไทด์จากเดิม AAA เป็น ACA ที่โคดอน 292 ทำให้มีการเปลี่ยนแปลงกรดอะมิโนจากเดิม ไลซีน เป็น ทรีโอนีน ผู้ป่วยมะเร็งปอดทั้ง 3 รายนี้มีประวัติการสูบบุหรี่มาก่อน

**คำสำคัญ** : ยีน พี 53, มะเร็งปอด

* ภาควิชาโภชนศาสตร์เขตร้อนและวิทยาศาสตร์อาหาร, คณะเวชศาสตร์เขตร้อน, มหาวิทยาลัยมหิดล, กรุงเทพฯ 10400
** ภาควิชาพยาธิวิทยา, คณะแพทยศาสตร์ศิริราชพยาบาล, มหาวิทยาลัยมหิดล, กรุงเทพฯ 10700