

Prognostic Value of the Expression of p53 in Patients with Non-small Cell Lung Cancer

Biljana Ilievska Poposka¹, Snezhana Smickova², Simonida Jovanovska Crvenkovska², Beti Zafirovska Ivanovska³, Tome Stefanovski⁴, Marija Metodieva¹, Gordana Petrusavska⁵

¹Institute for Lung Diseases and Tuberculosis, Skopje, Republic of Macedonia; ²Institute for Radiology and Oncology, Faculty of Medicine, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia; ³Institute for Epidemiology, Faculty of Medicine, University "Ss Kiril and Metodij" Skopje, Republic of Macedonia; ⁴Clinic for Pneumology and Allergology, Faculty of Medicine, University "Ss Kiril and Metodij" Skopje, Republic of Macedonia; ⁵Institute for Pathology, Faculty of Medicine, University "Ss Kiril and Metodij", Skopje, Republic of Macedonia

Abstract

Key words:

Non-small cell lung cancer; immunohistochemistry; p53; prognostic factors.

Correspondence:

Biljana Ilievska Poposka, MD, PhD,
Institute for Lung Diseases and
Tuberculosis, Skopje, Republic of
Macedonia
e-mail: biljana.ilievska@yahoo.com

Received: 08-Jul-2009

Revised: 15-Oct-2009

Accepted: 16-Oct-2009

Online first: 12-Nov-2009

Background. The p53 gene is frequently mutated in non-small-cell lung cancer (NSCLC). However, the effect of p53 gene mutations on patient prognosis remains unclear.

Aim. The aim of this study is to determine the association of p53 abnormalities with clinical data and prognosis in patients with NSCLC.

Material and Methods. Tumor tissues from 80 patients with NSCLC were assessed by immunohistochemistry for expression of p53. The immunohistochemical study was performed on formalin-fixed, paraffin-embedded sections using LCAB immunoperoxidase method with mouse anti-p53 monoclonal antibody (clone DO-7; DAKO).

Results. Forty-three (53,75%) of 80 patients revealed aberrant immunostaining for p53. Except to the histological type of tumors, there was no correlation of p53 expression with the clinicopathologic features. The Kaplan-Meier survival analysis demonstrated that patients with p53 positive status had poor survival rate ($p=0,005$ by the Log Rank test= $7,914$). In Cox, regression analysis p53 and performance status emerged as independent prognostic factors ($p<0,009$ and $p<0,000$ respectively).

Conclusion. The results from this study indicated that the aberrant expression of p53 is significant and independent predictable prognostic factor for patients with NSCLC.

Introduction

Lung cancer is the leading cause of cancer death in Europe and the United States, and the same trend is seen in many other countries (1). Non-small cell lung cancers (NSCLC, s) comprise more than 75% of lung cancers, and about 70% of NSCLC cases are advanced when diagnosed and are treated with chemotherapy and radiotherapy. Despite major advances in cancer treatment in the past two decades, the prognosis for patients with NSCLC, s has improved only minimally; surgery is the treatment for the localized tumors, but even in this favorable situation only 50% of the patients survive 5 years (2). Clearly, patients at the

similar stage of disease show marked differences in probabilities of survival. This illustrates the need to identify new prognostic factors that may help clinicians to better assess the survival probability and to optimize therapeutic efforts for each individual patient (3).

Recent progress in molecular biology have allowed for the extension of the research on prognostic factors to the analyses of proteins and genes involved in cancer development. For example, factors related to growth (e.g. epithelial growth factor, erb-; B 2), cell cycle (e.g. retinoblastoma gene), or apoptosis (e.g. p53 and bcl-2) have been studied in recent publications, in order to correlate them with survival (4).

P53 has been the topic of numerous publications in lung cancer patients. It is a 53 kD nuclear phosphoprotein, produced by the p53 tumor suppressor gene, which is localized on human chromosome 17p13. It binds to double-stranded deoxyribonucleic acid (DNA) and has three main physiological functions: cell cycle regulation, induction of apoptosis and stabilization of the genome (5). p53 gene could induce cell arrest in G1 phase and apoptosis. Mutations in the p53 gene appear to be the most frequent targets among the genetic abnormalities identified in NSCLC (6-9). Despite the large number of studies, the relation between the 53 alterations and survival in NSCLC has been controversial. p53 overexpression has been variously correlated with worse outcome, better prognosis or no influence on patient's survival (10-15).

The aim of the present study was to investigate the predictive value of p53 in patients with NSCLC.

Materials and Methods

Patients and tissue samples

Our study population consisted of 80 patients with non-small cell lung carcinoma (NSCLC) diagnosed between January 2004 and December 2006 in the Institute for Lung Diseases and Tuberculosis, Skopje, Republic of Macedonia. Eligible patients included those with histologic diagnosis of NSCLC by bronchial biopsy and treated with chemotherapy and/or radiotherapy after diagnosis. Patients included 73 men and 7 women. According to the age, 57 patients (71.25%) were above 64 years old, and only 23 (28.75%) were older than 65 years. Smokers were 64 patients (80.00%). The diagnosis of NSCLC in all patients was established by histological examination of tissue samples obtained during the bronchoscopy.

All histological analysis of the tumor tissue and immunohistochemistry (ICH) were performed in the Institute for Pathology, Medical Faculty, Skopje. Histological type and degree of differentiation were defined according to World Health Organization criteria (16). Tumors included 18 adenocarcinomas (22.50%), 56 squamous cell carcinomas (70.00%) and 6 tumors (7.50%) defined as "others" (bronchoalveolare, large cell carcinomas, nondifferentiated carcinomas). For histological differentiation, well, moderately and poorly differentiated tumors were graded as grade 1, 2 and 3 respectively. According to the International Staging System for Lung Cancer, 80 NSCLC patients were divided in: 8 patients in stage IIB (10.00%), 22 patients in stage IIIA (27.50%), 34 patients in stage IIIB (42.50%)

and 16 patients in stage IV (20.00%) (17). The staging procedure for the majority of patients was standardized including fiber-optic bronchoscopy, routine laboratory parameters, chest CT, abdomen CT and bone scan. The patients with stage I to IIIA, did not undergo surgery because of poor respiratory or cardiac function, pulmonary complication, or patient refusal.

So, all patients included in the study were treated with chemotherapy and/or radiotherapy in the Institute for Radiotherapy and Oncology, Medical Faculty, Skopje. Patients received combined chemotherapy: the first drug was based on platinum, and the second drug was from the group Etoposide, from the group Taxani, or Gemcitabine. The dose was determined according to body surface and kreatinin klirens. All patients were followed up regularly in a time frame of 2 to 3 months. The patients were followed up at least 24 months (from 1 to 48 months). At the time of the last follow-up 75 patients (93.75%) had died and 5 patients (6.25%) were still alive. The survival time was calculated from the date of histological diagnosis to the date of death or the end of follow-up.

Immunohistochemical analyses

The immunohistochemical studies were performed on formalin-fixed, paraffin-embedded sections using LCAB immunoperoxidase method with mouse anti-p53 monoclonal antibody (clone DO-7; DAKO, Denmark).

Analyses were done with semiquantitative grading system. P53 expression was observed in the nuclei. Negative indicated no immunoreaction or <10% of the tumor cells stained; (+) 10% to 25% staining; (++) 25% to 50% staining, and (+++) over 50% staining.

Statistical Analysis

The associations between expression of p53 and characteristics of patients except in the stage of diseases were analyzed by use of the χ^2 test or Fisher's exact test as appropriate (stage was analysed by employing Mann-Whitney U test and histologic type was analysed by Kolmogor-Smirno (K-S) test). The associations between individual clinical and pathologic variables such as age, sex, performance status, pathologic grade, T, N, M stage, p53 status and survival were assessed using the Cox proportional hazards regression model. Survival curves were estimated using the Kaplan-Meier method. The difference was considered to be statistically significant at $p < 0.05$.

Results

Detection of p53 protein expression

P53 protein positive expression was detected in 53.75% (43/80) of the patients with NSCLC. A correlation analysis was performed with the clinicopathologic parameters including age, gender, tumor differentiation, performance status, histology, pathologic TNM parameters and stages (Table 1).

Table 1. Relationship between expression of the p53 and clinicopathological features in NSCLC patients

Features (n)	p 53 + n (%)	p Value
Total (80)	43 (53.75%)	
Sex		
Male (73)	41 (56.16%)	
Female (7)	2 (28.57%)	NS
Age		
< 65 yr (57)	30 (52.63%)	
> 65 yr (23)	13 (56.52%)	NS
Smoking history		
Smokers (64)	34 (53.12%)	
Nonsmokers (16)	9 (56.25%)	NS
Histology		
Squamous cell carcinoma (56)	32 (47.14%)	
Adenocarcinoma (18)	11 (61.11%)	
Others (6)	0 (0%)	P<0.05*
Performance status		
WHO 0 (12)	7 (58.33%)	
WHO 1 (53)	26 (49.05%)	
WHO 2 (8)	4 (50.00%)	
WHO 3 (7)	6 (85.71%)	
WHO 4 (0)	0 (0%)	NS
Differentiation		
Well (3)	3 (100.00%)	
Moderate (43)	23 (53.48%)	
Poorly (34)	17 (50.00%)	NS
Stage (NSCLC)		
II B (8)	6 (75.00%)	
III A (22)	8 (36.36%)	
III B (34)	18 (52.94%)	
IV (16)	11 (68.75%)	NS
T stage		
1 (1)	1 (100.00%)	
2 (49)	22 (44.89%)	
3 (15)	11 (73.73%)	
4 (15)	9 (60.00%)	NS
N stage		
0 (3)	1 (33.33%)	
1 (10)	8 (80.00%)	
2 (37)	16 (43.24%)	
3 (30)	18 (60.00%)	NS
M stage		
0 (64)	32 (50.00%)	
1 (16)	11 (68.75%)	NS

Note: statistical analysis was performed using the χ^2 test, the Mann-Whitney U test and K-S test.

We did not find any correlation among p53 protein expression and clinicopathological parameters such as sex, age, performance status (classified according to WHO 0-IV), tumour differentiation and TNM stages of the disease. A unique correlation was between p53 protein expression and the histologic type of tumors: p53 expression was the most frequent in the patients with squamous cell carcinoma, contrary to the patients with adenocarcinoma or other NSCLC.

Analysis of survival

All 80 patients were included in the analyses of overall survival.

In a univariate analysis (Table 2), a limited number of associations within the curable patients were found regarding the probability of survival. p53 immunoreactivity was statistically significantly associated with decreased overall survival (HR = 1.65; 95% CI = 1.025-2.669; p=0.039). Overall survival was statistically significantly decreased in patients with

Table 2: Cox regression analyses of various prognostic factors in NSCLC patient.

Variables	HR	95% CI	p
Univariate analysis			
Age			
< 65 yr			
> 65 yr	1.71	1.03-2.86	0.038
Sex			
	0.68	0.29-1.58	0.376
Smoking History			
	0.76	0.43-1.33	0.338
Performance status			
WHO 0			0.000
WHO 1	2.03	1.02-4.02	0.043
WHO 2	2.30	0.87-6.12	0.092
WHO 3	12.97	4.55-36.93	0.000
Differentiation			
Well	0.17		
Moderate	0.36	0.10-1.22	0.102
Poorly	0.49	0.14-1.63	0.248
TNM status			
IIB	0.24		
IIIA	1.05	0.44-2.50	0.906
IIIB	1.33	0.58-3.04	0.490
IV	1.99	0.81-4.88	0.129
P53 positive status	1.85	1.16-2.95	0.009
Multivariate analysis			
Age			
	1.16	0.66-2.05	0.586
Performance status			
WHO 1			0.001
WHO 2	2.06	1.01-4.17	0.044
WHO 3	2.33	0.88-6.19	0.089
WHO 4	10.07	3.23-31.36	0.000
P53 positive status	1.54	1.94-2.54	0.048

performance status 3 (HR = 12.97; 95% CI = 4.55-36.93; $p=0.000$) versus performance status 1, 2. There was also a statistical difference in survival based on the age of the patients (HR = 1.71; 95% CI = 1.03-2.86; $p = 0.032$). Sex, smoking history, tumor grade, TNM status did not affect survival in this group of patients.

To determine which of the factors are independent prognostic factors to predict survival in the patients with NSCLC, a multivariate analysis using the Cox proportional hazards regression model was performed. Factors in the analysis included age of the patients, performance status and p53 protein expression. As a result, p53 expression and performance status emerged as independent prognostic factors (Table 2).

As shown in Fig. 1, the Kaplan-Meier survival curve demonstrated that the patients with p53 positive status survived for a shorter period of time ($p = 0.027$ by the Log Rank test = 4.88, $df = 1$). The median survival for patients with p53 positive status was 9 months, while that in the patients with p53 negative status was 12 months. At the time of the last follow-up only 5 patients (6.25%) were still alive: two of them were p53 positive, and three were p53 negative.

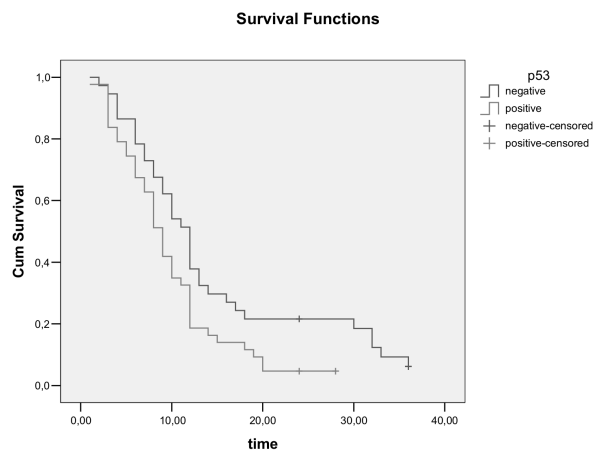


Figure 1: Kaplan-Meier survival curves based on the p53 expression ($p = 0.027$ by the Log Rank test = 4.88), Log Rank = 4.88, $df=1$, $p=0.027$.

Discussion

Mutations of the p53 gene were first implicated in tumorigenesis in 1985 when Jenkis et al. discovered that the transforming activity of p53 in the murine system was enhanced following mutation of its amino acid sequence (18). Subsequently it has been declared the most frequently mutated tumor suppressor gene in human tumors. Dysfunction of the p53 gene allows the

inappropriate survival of genetically damaged cells, setting the stage for the accumulation of multiple mutations and subsequent evolution of a cancer cell, and it plays a critical role in lung cancer (19). Mutation of p53 gene often result in the production of a p53 protein with an increased stability that explains which, generally are not stained due to the shorter life of the wild p53 protein (20). The wild-type p53 is found in the nucleus as a low-abundance protein, whereas mutant forms have longer half-lives and accumulate in the nucleus. Specifically, the wild type protein has a half-life of only 5-10 minutes, but mutant forms can extend up to 30-fold in transformed cells (11). Although the mutations resulting in p53 immunostaining are frequent enough to allow this technique to be of some practical value, a negative immunostaining does not rule out p53 mutations. (8, 21). Immunohistochemistry (IHC) is the most practical method of assessing protein expression changes in histopathology. IHC not only provides a semiquantitative assessment of protein abundance but also defines the cellular localization of expression (22). In our current immunohistochemical study, overexpression of p53 protein was identified in 43 (53.75%) of 80 NSCLC patients, and it was not related to patient or some tumor characteristics (except histologic type); p53 overexpression was significantly higher in patients with squamous cell carcinoma. These immunohistochemical results on p53 overexpression are compatible with previously published reports using p53 antibody (10, 11, 21, 22). Other studies have reported p53 abnormalities in 45-55% of NSCLC,s based on a variety of different techniques: sequencing, SSCP, and immunohistochemistry on fresh cell lines or frozen materials (6, 9, 12, 16, 23-25). The overall incidence was 46% in the meta-analysis by Tammemagi and colleagues (26).

While the importance of p53 mutations in the carcinogenesis of human cancer, including lung cancer is obvious, it is still not clear whether p53 alterations affect patient's survival (27). Our results showed that there was statistical significant correlation between the expression of p53 protein and patient survival. As shown in Fig. 1, the Kaplan-Meier survival curve demonstrated that the patients with positive p53 status survived for a shorter period of time ($p < 0.027$ by the log rank test). In the multivariate analysis it was found that p53 immunoreactivity is a statistically significant predictor of poor outcome in patients with NSCLC. Similarly, in some other studies survival has been adversely affected by p53 gene mutation and/or protein overexpression (7, 11, 14, 15, 21, 27). Some authors found that p53 alterations were favorable to increase disease-free survival, which was explained by the

inheritance of the wild-type tumor suppressor function (28, 29). In another studies there was no relationship between p53 alterations and survival (8,13). These controversial results are difficult to interpret: they may significantly vary depending on the different method used, such as the use of nucleic acid-based screening versus immunohistochemistry, and in immunohistochemistry, the use of different antibodies, different procedures and different cut of value. Furthermore, adequate patient numbers need to be included in these studies for meaningful conclusions.

Harada in his work has found that p53 expression is associated with response to chemotherapy in patients with NSCLC (30). Several mechanisms are involved in drug resistance, including those related to apoptosis, drug transportation and drug detoxification (31). p53 is involved as a transcription factor in the expression of many cell cycle-regulating and apoptosis-related genes (32). Although the effect of p53 on response to chemotherapy in NSCLC, s is controversial, according to the results of Harada immunostaining of p53 may be a useful marker for stratifying NSCLC patients into chemoresponsive and chemoresistant groups.

In conclusion, these findings are potentially important for prognostic reasons. Using immunohistochemical staining of p53 as clinical biological marker, it may be possible to predict patients with NSCLC with poorer or better prognosis. Our results suggest that p53 is a useful clinical tool for stratifying patients with NSCLC into more accurate prognostic group. Furthermore, immunohistochemical staining of p53 may identify patients with worse prognosis and more aggressive additional adjuvant therapy may be of benefit to these patients.

References

1. Cancer Facts & Figures-1995. Atlanta, GA; American Cancer Society, 1995.
2. Ginsberg RJ, Vokes E, Rosenzweig K. Cancer of the lung. In: De Vita VT, Helman S, Rosenberg SA, editors. Cancer: principles and practice of oncology. 6th ed. Philadelphia: Lippincott, 2001:925-83.
3. Sorensen JB, Osterlind K. Prognostic factors: from clinical parameters to new biological markers. In: Van Houtte P, Klastersky J, Rocmans JP. Progress and perspectives in the treatment of lung cancer. Berlin: Springer, 1999:1-21.
4. Steels E, Peasmans M, Berghmans T, Branle F, Lemaitre F, Mascaux C, Meert AP, Vallot F, Lafitte JJ, and Sculier JP. Role of p53 as a prognostic factor for survival in lung cancer: a systematic review of the literature with a meta-analysis. *Eur Resp J.* 2001;18(4):705-19. doi:10.1183/09031936.01.00062201 PMID:11716177
5. Sidransky D and Hollstein M. Clinical application of the p53 gene. *Annu Rev Med.* 1996;47:285-301. doi:10.1146/annurev.med.47.1.285 PMID:8712782
6. Chiba I, Takahashi T, Nau MM, D,Amico D, Curiel DT, Mitsudomi T, Butchhagen DL, Carbone D. Mutations in the p53 gene are frequent in primary, resected non-small cell lung cancer. Lung Cancer Study Group. *Oncogene.* 1990;5:1603-10.
7. Ebina M, Steinberg SM, Mulshine JL, Linnoila RI. Relationship of p53 overexpression and up-regulation of proliferating cell nuclear antigen with the clinical course of non-small cell lung cancer. *Cancer Res.* 1994;54(9):2496-503. PMID:7909277
8. Moldvay J, Scheid P, Wild P, Nabil K, Siat J, Borrelly J et al. Predictive survival markers in patients with surgically resected non-small cell lung carcinoma. *Clin Cancer Res.* 2000;6(3):1125-34. PMID:10741743
9. Carvalho OPE, Ongelo AL, Fabiola delCarlo B, Eduardo L, Leao LE, Rodrigues OL, Capelozzi VL. Useful prognostic panel markers to express the biological tumor status in resected lung adenocarcinoma. *Japanese Journal of Clin Oncology.* 2000;30:478-86. doi:10.1093/jjco/hyd128
10. McLaren R, Kuzu I, Dunnill M, Harris A, Lane D, Gatter KC. The relationship of p53 immunostaining to survival in carcinoma of the lung. *Br J Cancer.* 1992;66:735-8. PMID:1329907
11. Quinlan D, Davidson A, Summers C, Warden HE, Doshi HM. Accumulation of p53 protein correlates with poor prognosis in human lung cancer. *Cancer Res.* 1992;52:4828-31. PMID:1324796
12. Iggo R, Gatter K, Bartek J, David L, Harris A. Increased expression of mutant forms of p53 oncogene in primary lung cancer. *Lancet.* 1990;335(8691):675-9. doi:10.1016/0140-6736(90)90801-B PMID:1969059
13. Schiller HJ, Adak S, Feins HR, Keller MS, Fry AW, Livingstone BR, Hammond ME, Wolf B, Sabatini L, Jett J, Kohman L, Johnson DH. Lack of prognostic significance of p53 and K-ras mutations in primary resected non-small-cell lung cancer on E4592: a Laboratory Ancillary Study on an Eastern Cooperative Oncology Group Prospective Randomized Trial of Postoperative Adjuvant Therapy. *J Clin Oncol.* 2001;19(2):448-57. PMID:11208838
14. Horio Y, Takahashi T, Kuroishi T, Hibi K, Suyama M, Niimi T, Shiokata K, Yamakawa K, Nakamura Y, Ueda R, Takahashi T. Prognostic significance of p53 mutations and 3p deletions in primary resected non-small cell lung cancer. *Cancer Res.* 1993;53(1):1-4. PMID:8380124
15. Gebitekin C, Bayram AS, Tunca B, Balaban SA. Clinical

significance of p53 gene mutation in T1-2 N0 Non-Small cell lung cancer. *Asian Cardiovasc Thorac Ann*. 2007;15(1):35-8. [PMID:17244920](#)

16. The World Health Organization histological typing of lung tumours. Second edition. *Am J Clin Pathol*. 1982;77(2):123-36. [PMID:7064914](#)

17. Mountain CF. Revision in the international system for staging lung cancer. *Chest*. 1997;111(6):1710-7. [doi:10.1378/chest.111.6.1710](#) [PMID:9187198](#)

18. Jenkins JR, Rudge K, Chumakov P and Currie GA. The cellular oncogene p53 can be activated by mutagenesis. *Nature*. 1985;317(6040):816-8. [doi:10.1038/317816a0](#) [PMID:3903515](#)

19. Bennet WP, Hussain SP, Vahakangas KH, Khan MA, Shields PG, Harris CC. Molecular epidemiology of human cancer risk: gene-environment interactions and p53 mutation spectrum in human lung cancer. *J Pathol*. 1999;187(1):8-18. [3.0.CO:2-Y" target= blankdoi:10.1002/\(SICI\)1096-9896\(199901\)187:1<8::AID-PATH232>3.0.CO:2-Y](#) [PMID:10341702](#)

20. Chang F, Syrjanen S, Tervahauta A et al. Tumorigenesis associated with the p53 tumor suppressor gene. *Br J Cancer*. 1993;68(4):653-61. [PMID:8398688](#)

21. Han H, Landeneau RJ, Santucci TS, Tung MY, Macherey RS, Shackney SE, Sturgis CD, Raab SS, Silverman JF. Prognostic value of immunohistochemical expression of p53, HER-2/neu, and bcl-2 in stage I non-small-cell lung cancer. *Hum Pathol*. 2002;33(1):105-10. [doi:10.1053/hupa.2002.30183](#) [PMID:11823980](#)

22. Cheng YL, Lee SC, Harn HJ, Chen CJ, Chang YC, Chen JC, et al. Prognostic prediction of the immunohistochemical expression of p53 and p16 in resected non-small cell lung cancer. *Eur J Cardiothorac Surg*. 2003;23(2):221-8. [doi:10.1016/S1010-7940\(02\)00749-2](#) [PMID:12559346](#)

23. Zhu CQ, Shih W, Ling CH, Tsao MS. Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation. *J Clin Pathol*. 2006;59(8):790-800. [doi:10.1136/jcp.2005.031351](#) [PMID:16873561](#)

24. Ahrendt AS, Hu Y, Buta M, McDermott PM, Benoit N, Yang SC, Wu L, Sidransky D. p53 mutations and survival in stage I Non-Small-Cell Lung Cancer: results of a prospective study. *J Natl Cancer Inst*. 2003;13:961-70. [PMID:12837832](#)

25. Laudanski J, Niklinska W, Burzykowski T, Chyczewski L, Niklinski J. Prognostic significance of p53 and bcl-2 abnormalities in operable nonsmall cell lung cancer. *Eur Respir J*. 2001;17(4):660-6. [doi:10.1183/09031936.01.17406600](#) [PMID:11401061](#)

26. Tammemagi MC, McLaughlin JR, Bull SB. Meta-analysis of p53 tumor suppressor gene alterations and clinicopathological features in resected lung cancers. *Cancer Epidemiol Biomarkers Prev*. 1999;8(7):625-34. [PMID:10428201](#)

27. Mitsudomi T, Hamajima N, Ogawa M, and Takahashi T. Prognostic significance of p53 alterations in patients with non-small cell lung cancer: A meta-analysis. *Clinical Cancer Research*. 2000;6(10):4055-63. [PMID:11051256](#)

28. Passlick B, Izbicki JR, Haussinger K, Thetter O, Pantel K. Immunohistochemical detection of p53 protein is not associated with poor prognosis in non-small cell lung cancer. *J Thorac Cardiovasc Surg*. 1995;109(6):1205-11. [doi:10.1016/S0022-5223\(95\)70204-0](#) [PMID:7776684](#)

29. Lee JS, Yoon A, Kalapuracal SK. Expression of p53 oncoprotein in non-small-cell lung cancer: A favorable prognostic factor. *J Clin Oncol*. 1995;13(8):1893-903. [PMID:7636531](#)

30. Harada T, Ogura S, Yamazaki K, Kinoshita I, Itoh T, Isobe H, Yamashiro K, Doska-Akita H, Nishimura M. Predictive value of expression of p53, Bcl-2 and lung resistance-related protein for response to chemotherapy in non-small cell lung cancers. *Cancer Sci*. 2003;94(4):394-9. [doi:10.1111/j.1349-7006.2003.tb01453.x](#) [PMID:12824911](#)

31. Pastan I, Gottesman. Multiple-drug resistance in human cancer. *N Engl J Med*. 1987;316:1388-93. [PMID:3553950](#)

32. Levine A.J. p53, the cellular gatekeeper for growth and division. *Cell*. 1997;88(3):323-31. [doi:10.1016/S0092-8674\(00\)81871-1](#) [PMID:9039259](#)