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RESEARCH ARTICLE

Prognostic Significance of Caspase- 3, Bcl-2, P53 and GSTPI Expressions in Lung Adenocarcinoma

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Prognostic significance of caspase- 3, Bcl-2, P53 and GSTPI expressions in lung adenocarcinoma

SUMMARY

Lung adenocarcinoma is one of the most fatal cancers in humans, and many factors are known to contribute to its poor prognosis. The aim of this work is to investigate the prognostic value of the apoptosis-regulating markers (caspase-3, bcl-2, p53) and the important detoxification enzyme (GST pi), and analyze the relationships among these immunohistochemical (IHC) results, patients' clinical characteristics, and survival. IHC stainings were performed on 89 lung adenocarcinoma specimens obtained from 89 patients from the Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara, Turkey. The medical records of the patients were reviewed retrospectively. The correlation between the presence of protein expression and clinicopathological parameters (gender, age, tumor stage and histology) was examined by using the Pearson correlation test. Survival analysis was carried out by the Kaplan–Meier method. The prevalence of caspase 3, bcl-2, p53, and GST pi expression was 48%, 57%, 95% and 52%, respectively. The expression rate of p53 was relatively high in smokers than that in non-smokers. There were no correlations among the immunoreactivities of bcl-2 and GST pi in clinical stage, and overall survival. However, there was a significant relationship between caspase-3 and p53 expressions and overall survival. Caspase-3 expression was correlated with good prognosis, and p53 expression was correlated with poor prognosis.

Key Words: *Caspase-3; bcl-2; p53; Glutathione-S-transferase pi; Lung adenocarcinoma;*

Running Title: *Caspase- 3, bcl-2, p53, GST pi, lung cancer*

Akciğer adenokanserlerinde kaspaz-3, bcl-2, p53 ve GSTPI ekspresyonlarinin prognostik önemi

ÖZET

Akciğer adenokarsinomu insanlarda en ölümcül kanserlerden biridir ve birçok faktör onun kötü prognozuna katkıda bulunmaktadır. Bu çalışmanın amacı, apoptoz düzenleyen belirteçlerin (kaspaz-3 bcl-2, p53) ve önemli bir detoksifikasyon enziminin (GST pi) prognostik değerlerini araştırmak ve aynı zamanda immunohistokimyasal (İHK) sonuçlar ile hastaların klinik özellikleri ve sağkalım arasındaki ilişkileri analiz etmektir. Bu amaçla Atatürk Göğüs Hastalıkları ve Göğüs Cerrahisi Eğitim ve Araştırma Hastanesi, Ankara, Türkiye'den alınan toplam 89 akciğer adenokanserli hastalarda, protein ekspresyonları immunohistokimya ile değerlendirildi. Hastaların tıbbi kayıtları retrospektif olarak incelendi. Protein ekspresyonu ve klinikopatolojik parametreler (cinsiyet, yaş, tümör evresi ve histoloji) arasındaki ilişki Pearson korelasyon testi ile incelendi. Sağkalım analizi Kaplan-Meier yöntemi ile gerçekleştirildi. Kaspaz-3, bcl-2, p53, ve GST pi ekspresyonları sırasıyla, 48%, 57%, 95% ve 52% olarak saptandı. P53 ekspresyonunun, sigara içenlerde sigara içmeyenlere göre daha yüksek olduğu belirlendi. Klinik evre ve genel sağkalımda, bcl-2 ve GST pi immunoreaktivite arasında korelasyon bulunamadı. Ancak, p53 ve kaspaz 3 ekspresyonu ve genel sağkalım arasında anlamlı bir ilişki saptandı. Kaspaz-3 ekspresyonunun iyi prognoz ile, p53 ekspresyonunun ise kötü prognoz ile ilişkili olduğu saptandı.

Anahtar kelimeler: *Kaspaz-3, Bcl-2, p53, GST pi, akciğer adenokanser.*

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INTRODUCTION

For lung adenocarcinoma (AC), surgery is the primary method in the treatment of patients without demonstrable metastatic disease. Staging is the most accurate means to estimate prognosis. Although surgery achieves long-term survival in early-stage patients, a significant proportion may suffer from regional or distant recurrence (1). There is a discrepancy in survival between patients within the same stage of lung cancer (2). This suggests the need to identify more molecular markers, the apoptosis-regulating markers, and detoxification enzymes (3, 4).

Although many studies have been conducted on the clinical significance of apoptosis, few studies on the clinical significance of caspase-3 expression in malignant tumors have been reported (5). In addition, most of these clinical studies on caspase-3 expression focused on hematologic tumors, such as leukemia (6) and malignant lymphoma (7). Studies on caspase-3 expression in non-small cell lung carcinoma (NSCLC) are limited and their results on survival are contradictory (8-10).

Apoptosis is important during tumorigenesis. The bcl-2 is a negative regulator of programmed cell death (apoptosis) (2). The avoidance of apoptosis via bcl-2 may lead to a survival advantage. The p53 protein may block the progression of cell growth cycle and trigger apoptosis in response to DNA damage. The mutation of p53 gene causes a loss of tumor-suppressor function, promotes cellular proliferation and inhibits apoptosis. The p53 mutation has been detected in many types of human malignancy (3). Expressions of both oncoproteins bcl-2 and p53 have been reported as a prognostic predictor in NSCLC with conflicting results in previous studies (3, 4).

Glutathione S-transferases (GSTs) are enzymes that catalyze the conjunction of glutathione to numerous exogenous and endogenous toxic compounds, such as reactive oxygen metabolites. The GST family is divided into membrane-bound and cytosolic enzymes. The cytosolic GSTs are divided into 7 classes: alpha, mu, pi, theta, sigma, omega and zeta (11). Expression differences of the GSTs are likely to influence individual susceptibility to carcinogens, including tobacco smoke (12). The GST pi and GST mu are found to regulate the mitogen-activated protein kinase pathway which participates in cellular apoptosis. GST pi is implicated in preventing apoptosis (13).

Based on their involvement of apoptosis in tumorigenesis, we investigated the relationship between caspase-3, bcl-2, p53, GST pi proteins and their correlations with clinicopathological parameters and survival in patients with lung adenocarcinoma.

MATERIALS AND METHODS

Sources of materials

The study included 89 patients with AC diagnosed and treated at the Atatürk Chest Diseases and Thoracic Surgery Training and Research Hospital, Ankara, Turkey. The patients' medical records were reviewed for clinical information and follow-up status. The median follow-up period was 34 months. Clinical staging, adequate follow-up information, and the pathology slides of the tumors were available for all patients. All patients were staged at the time of surgery by regional lymph node dissection. The disease stage was assessed based on the American Joint Committee on Cancer TNM staging system. Of the 89 patients, 75 were male and 14 were female. Their ages ranged from 35 to 77 years with an average of 57.81±9.53 years. Four (4.49%) patients were non-smoker. Among 89 surgically resected adenocarcinomas of the lung, 42 were stage I, 22 stage II, 24 stage III, and one stage IV. The clinicopathological factors considered were age, gender, tumor size, stage, and smoking status.

Immunohistochemical studies

The surgically resected specimens were fixed routinely in 10% formalin and embedded in paraffin blocks. Tissue sections were cut at 4 μm and stained with hematoxylin and eosin. For immunohistochemistry, endogenous peroxidase activity was blocked by incubating the sections in 1% hydrogen peroxide (v/v) in methanol for 10 mins at room temperature (RT). The sections were subsequently washed in distilled water for 5 mins and antigen retrieval was performed for 3 mins using 0.01 M citrate buffer (pH 6.0) in a domestic pressure cooker. The sections were transferred in 0.05 MTris-HCl (pH 7.6) containing 0.15 M sodium chloride (TBS). After washing in water, the sections were incubated at RT for 10 mins with super block (SHP125) (ScyTek Laboratories, USA) to block nonspecific background staining. The sections were then covered with the primary antibodies diluted 1:100 for anti-caspase-3, anti-GSTpi and p53; 1:50 for anti-bcl-2 in TBS at 4°C overnight. Polyclonal antibodies against caspase-3, bcl-2 and p53 raised in rabbit were purchased from Santa Cruz Biotechnology Inc., USA. After washing in TBS for 15 minutes, the sections were incubated at RT for biotinylated link antibody (SHP125) (ScyTek Laboratories, USA). Then, treatment was followed with Streptavidin/HRP complex (SHP125) (ScyTek Laboratories, USA). Diaminobenzidine was used to

visualize peroxidase activity in the tissues. Nuclei were lightly counterstained with hematoxylin and then the sections were dehydrated and mounted. Both positive and negative controls were included in each run. Positive controls consisted of sections of human tonsillitis for caspase-3, bcl-2, and GSTpi, and human colon carcinoma for p53. TBS was used in place of the primary antibody for negative controls. Immunoreactivity for caspase-3 was evaluated both qualitatively and quantitatively. Immunostaining quantity was evaluated on the following scale: 0 = negative, 1 = less than 25%, 2 = 25–50%, 3 = 50–75%, 4 = more than 75% of tumor cells showing cytoplasmic positivity (Figure 1A). Immunostaining intensity of caspase-3 was evaluated as follows: 1 = weak, 2 = moderate, 3 = strong, 4 = very strong cytoplasmic staining intensity. The samples were then divided into three groups based on the combined scores as follows: 0 = no immunostaining (score 0), 1 = weak immunostaining (scores 1–4) and 2 = strong immunostaining (scores 5–8). Bcl-2 was recorded as positive if cytoplasmic staining was present in >10% tumor cells (Figure. 1B). Immunohistochemical reactivity for p53 was recorded as the percentage of stained nuclei. A specimen was considered as positive when more than 10% of the nuclei were stained (Figure. 1C). Immunostaining intensity for GST pi was evaluated on the following scale: 1 = weak, 2 = moderate, 3 = strong, 4 = very strong (Figure 1D).

Statistical analysis

Statistical analysis was performed by using SPSS software version 11.5 (SPSS Inc., Chicago, IL, USA). The correlation between the presence of protein expression and clinicopathological parameters (gender, age, tumor stage and histology) was examined by using the Spearman correlation test. Survival analysis was calculated by the Kaplan–Meier method. Three year survival rate was also calculated. A p-value <0.05 was considered as statistically significant.

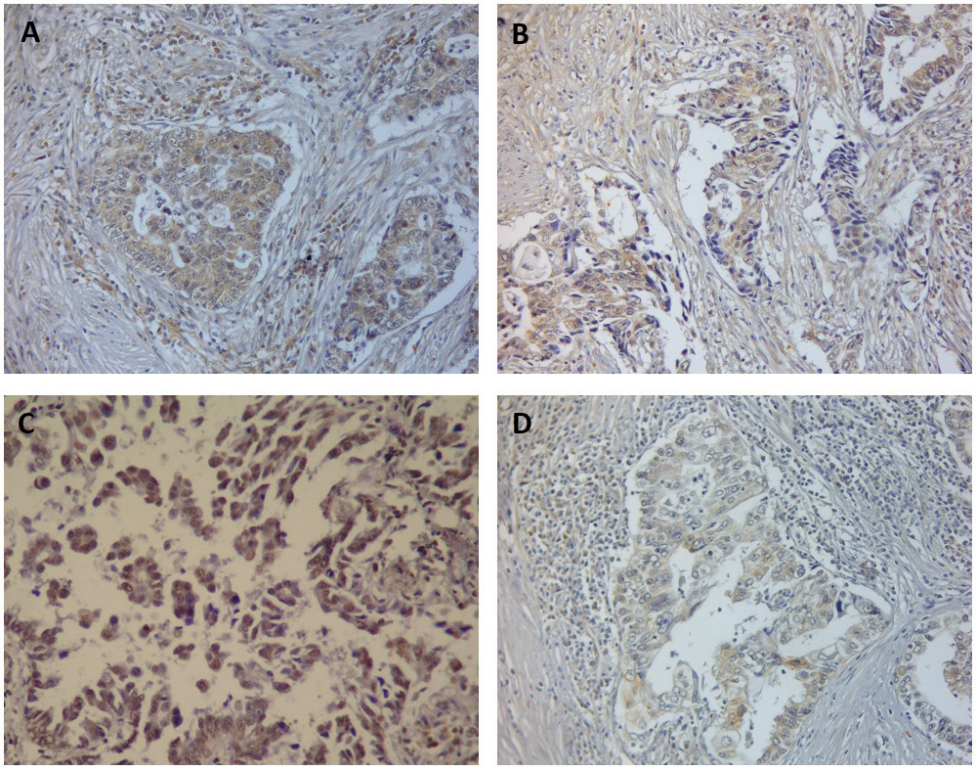

Figure 1. Composite figures of immunohistochemical stains of caspase-3 (**A**), bcl-2 (**B**) p-53 (**C**) and GST-pi **(D)**. **A:** lung adenocarcinoma cells moderate staining for caspase-3 immunoreactivity; original magnification, 40X. **B:** bcl-2 positive immunoreactivity in cancer cells showing cytoplasmic staining. Immunoperoxidase stain; original magnification, 40X. **C:** cancer cells staining positively for p53 immunoreactivity; original magnification, 40X. **D:** cytoplasmic staining of GST-pi protein in cancer cells. Immunoperoxidase stain; original magnification, 40X.

RESULTS

Caspase-3 expression has been analysed in tumor tissues of patients with lung adenocarcinoma. Among the 89 cases, 85 (95.50%) showed positive staining for caspase-3. The immunostaining of caspase-3 revealed diffuse cytoplasmic positivity restricted to the tumor areas (Fig. 1A). Among the 89 cases, 51 (57.30%) showed cytoplasmic staining for bcl-2 (Fig. 1B). Nuclear staining for p53 was positive in 42/89 (47.19%) cases (Fig. 1C). All samples from lung adenocarcinoma cases had positive staining with the GST pi protein being expressed in the cytoplasm, the nucleus, or both (Fig. 1D).

The expression of caspase-3, bcl-2, p53 and GST pi markers were not significantly associated with any of the prognostic factors (sex, tumor stage and tumor size) (p>0.05) (Table 1). When the staining scores were taken into account, there was a statistically significant difference in p53 expression between smoker and nonsmoker patients. The p53 expression scores in smoker patients was significantly higher than nonsmoker patients (p=0.028) (Table 1).

To determine whether the expression of caspase-3, bcl-2, p53 and GST pi markers have prognostic value, we evaluated the survival rates of 89 patients. In the whole study population, caspase-3 expression was correlated with better outcome. The estimated 3-year survival rate

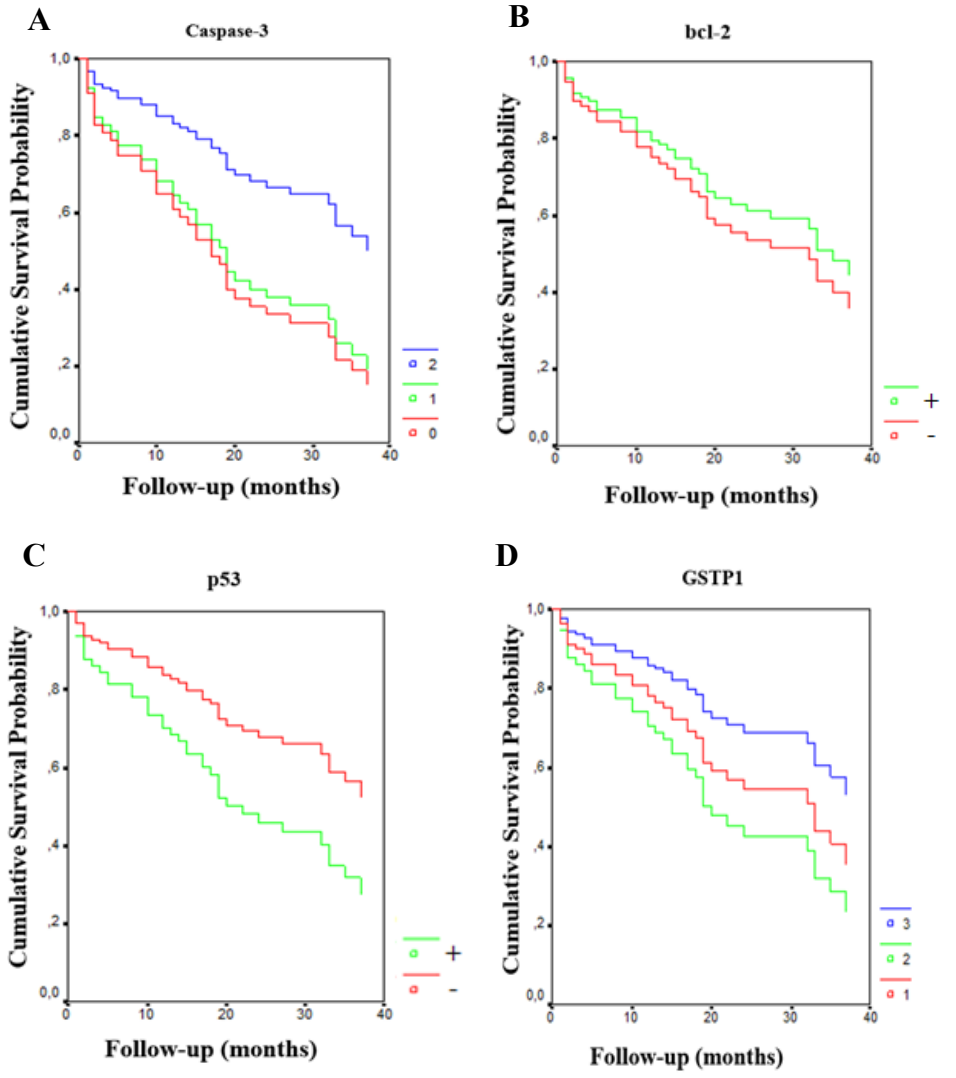

Figure 2. Overall survival of 89 lung cancer patients by caspase-3 (p=0.007<0.05) (A), bcl-2 (p=0.481>0.05) (B), p53 (p=0.030<0.05) (C) and GSTpi (GSTP1) (p=0.086>0.05) (D) staining, Kaplan-Meier function.

Table 1. The Relationships between the staining scores of the tumour markers and clinical parameters of lung adenocarcinoma patients.

		Gender		Smoking Status		Tumor Stage				Tumor Size (n=89)	Age (n=89)
		M (n=75)	F (n=14)	S (n=85)	NS (n=4)	TS1 (n=42)	TS2 (n=22)	TS3 (n=24)	TS4 (n=1)	4.6±2.02[e]	57.81±9.53[f]
Caspase-3[a]	*$n_{positive}$/%n	72/96.00	13/92.85	81/95.30	4/100	41/97.62	22/100	21/87.50	1/100	>0.05	>0.05
	$n_{negative}$/%n	3/4.00	1/7.14	4/4.70	0/0	1/2.38	0/0	3/12.50	0/0		
	p Value	>0.05		>0.05		>0.05					
bcl-2[b]	$n_{positive}$/%n	45/60.00	6/42.86	49/57.65	2/50.00	25/59.52	10/45.45	16/66.67	0/0	>0.05	>0.05
	$n_{negative}$/%n	30/40.00	8/57.14	36/42.35	2/50.00	17/40.48	12/54.55	8/33.33	1/100		
	p Value	>0.05		>0.05		>0.05					
p53[c]	$n_{positive}$/%n	36/48.00	6/42.86	42/49.41	2/50	19/45.24	11/50.00	11/45.83	1/100	>0.05	>0.05
	$n_{negative}$/%n	39/52.00	8/57.14	43/50.59	2/50	23/54.76	11/50.00	13/54.17	0/0		
	p Value	>0.05		***0.028***		>0.05					
GSTP1[d]	$n_{positive}$/%n	75/100	14/100	85/100	4/100	42/100	22/100	24/100	1/100	>0.05	***0.006***
	$n_{negative}$/%n	0/0	0/0	0/0	0/0	0/0	0/0	0/0	0/0		
	p Value	>0,05		>0,05		>0,05					

*: Number of positive stained samples with varying staining scores.

a: The cytoplasmic staining scores for Caspase-3 were calculated based on the sum of the staining intensity and the percentage of positively stained neoplastic cells. Staining intensity was graded as: 0 for no, 1 for weak, 2 for moderate and 3 for strong stainings. Percentage of stained cells was evaluated as: 0 if no positively stained cells, 1 if 1– 25% of the cells stained, 2 if 25– 50% of the cells stained, 3 if 50–75% of the cells stained and 4 if >75% of the cells stained. Combined scores of Caspase-3 were calculated as: 0 if the sum of staining intensity and percentage was 0, 1 if the sum of staining intensity and percentage was 1-4 and 2 if the sum of staining intensity and percentage was 5-8.

b: The cytoplasmic staining scores for bcl-2 were calculated based on the staining percentage of positively stained neoplastic cells. Percentage of stained cells was evaluated as: 0 if <10% positively stained cells and 1 if >10% of the cells stained.

c: The nuclear staining scores for p53 were calculated based on the staining percentage of positively stained neoplastic cells. Percentage of stained cells was evaluated as: 0 if <10% positively stained cells and 1 if >10% of the cells stained.

d: The cytoplasmic staining scores for GSTP1were calculated based on the staining intensity of positively stained neoplastic cells. Staining intensity was graded as: 0 for no, 1 for weak, 2 for moderate and 3 for strong stainings.

e: Mean±SE of Tumor Size in 89 patients.

f: Mean±SE of age of patients. M: Male, F: Female, TS: Tumor Stage. The Relationships between the staining scores of the tumour markers and clinical parameters of lung adenocarcinoma patients were investigated bySpearman's Rank Correlation Test. The results were considered significant for p-value less than 0.05.

was more in patients with caspase-3 negative tumors than with patients with caspase-3 positive tumors (p = 0.007) (Figure 2A). On the other hand, in patients with p53 positive tumors, the 3-year survival rate was more than patients with p53 negative tumors. The p53 expression tended to have an adverse impact on survival (p=0.030) (Figure 2C). The expressions of bcl-2 and GST pi (GSTP1) proteins had no statistically significant impact on survival (p=0.481 and p=0.086 respectively) (Figures 2B and 2D).

DISCUSSION

In the present study, the expression of apoptosis related markers, namely, caspase-3, bcl-2, p53, and GST pi were evaluated immunohistochemically. The immunohistochemical results showed that lung adenocarcinomas exhibit varying expressions of caspase-3, bcl-2, p53 and GST pi proteins.

We have found that positive caspase-3 expression was correlated with a good prognosis. This result confirms Koomagi and Volm (8, 9), who reported positive caspase-3 expression, as determined immunohistochemically, showed a significantly good prognosis as compared to those with negative caspase-3 expression. However, Takata et al. (10) reported that patients with positive caspase-3 expression were associated with a poor prognosis. It is plausible to suggest that survival is improved by accelerated apoptotic cancer cell death when caspase-3 is expressed strongly.

Martin et al. (14) reported that NSCLC patients with bcl-2 positive tumors had significantly better survival than those with Bcl-2-negative tumors. On the other hand, Yang et al. (15) and Yoo et al. (16) did not find any statistically significant association between bcl-2 and overall survival in lung adenocarcinoma. Also in this study, the expression of bcl-2 had no statistically significant impact on survival. The contradictory results could be due to the different histological types of lung cancers in previous studies.

The p53 protein is encoded by *TP53* tumor suppressor gene that is inactivated by mutations in more than 50% of lung adenocarcinoma patients (17, 18). Many retrospective studies have examined the prognostic role of *TP53* gene mutations in lung adenocarcinoma (19). However, the relationship between p53 expression and prognosis in lung adenocarcinoma is not well known. In this study we have found that p53 expression had an adverse impact on survival. In line with our results, Ishida et al. (20) and Moldvay et al. (3) reported that p53 positive staining is significantly associated with shorter survival. Overexpression of p53 protein determined by immunostaining may contribute to the adverse outcome due to the ability of *TP53* to act as a dominant oncogene (17). Also in smoker patients we have found that p53 expression was significantly higher than nonsmoker patients showing the unfavorable effect of smoking on survival via p53 expression.

The effect of GST pi expression on survival has been studied in different cancer types namely colorectal (21), laryngeal (22) and breast (23) cancers, however significant associations have not been found. Likewise, the expression of GST pi had no statistically significant impact on overall survival in our study.

In conclusion, the overexpression of caspase-3 has good prognostic value in patients with lung adenocarcinoma, while p53 has poor prognostic value for survival of the same patients. Although, prediction of survival using these markers seems uncertain, the observed correlations with apoptotic proteins reinforce the potential usefulness of caspase-3 and p53 in the development of therapeutic strategies for lung cancer in future. The exact clinical role of these markers should be defined through further investigations.

CONFLICT OF INTEREST STATEMENT

The authors report no conflicts of interest.

REFERENCES

1. Martini N, Bains MS, Burt ME, Zakowski MF, McCormack P, Rusch VW, Ginsberg RJ. Incidence of local recurrence and secondary primary tumors in resected stage I lung cancer. *J Thorac Cardiovasc Surg* 109: 120-129, 1995.
2. Chowdry RP, Sica GL, Kim S, Chen Z, Goodman A, Alexis D, Deng X, Owonikoko TK. Phosphorylated Bcl-2 and Mcl-1 as prognostic markers in small cell lung cancer. *Oncotarget* doi: 10.18632/oncotarget.7485. [Epub ahead of print], 2016
3. Moldvay J, Scheid P, Wild P, Nabil K, Siat J, Borrelly J, Marie B, Farré G, Labib T, Pottier G, Sesboüé R, Bronner C, Vignaud JM, Martinet Y, Martinet N. Predictive survival markers in patients with surgically resected non-small cell lung cancer. *Clin Cancer Res* 6: 1125-1134, 2000.
4. Nguyen VN, Mirejovsky P, Mirejovsky T, Meliova L, Mandys V. Expression of cyclin D1, Ki-67 and PCNA in non-small cell lung cancer: prognostic significance and comparison with p53 and bcl-2. *Acta Histochem* 1102: 323-328, 2000.

5. Krajewska M, Wong H, Krajewski S, Zapata JM, Shabaik A, Gascoyne R, Reed JC. Immunohistochemical analysis of in vivo patterns of expression of CPP32 (caspase-3), a cell death protease. *Cancer Res* 57: 1605-1613, 1997.
6. Faderl S, Thall PF, Kantarjian HM, Talpaz M, Harris D, Van Q, Beran M, Kornblau SM, Pierce S, Estrov Z. Caspase 2 and caspase 3 as predictors of complete remission and survival in adults with acute lymphoblastic leukemia. *Clin Cancer Res* 5: 4041-4047, 1999.
7. Donoghue S, Baden HS, Lauder I, Sobolewski S, Pringle JH. Immunohistochemical localization of caspase-3 correlates with clinical outcome in B-cell diffuse-cell lymphoma. *Cancer Res* 59: 5386-5391, 1999.
8. Koomagi R, Volm M. Relationship between the expression of caspase-3 and the clinical outcome of patients with non-small cell lung cancer. *Anticancer Res* 20: 493-496, 2000.
9. Volm M, Koomagi R. Prognostic relevance of c-myc and caspase-3 for patients with non-small cell lung cancer. *Oncol Rep* 7: 95-98, 2000.
10. TakataT, Tanaka F, Yamada T, Yanagihara K, Otake Y, Kawano Y, Nakagawa T, Miyahara R, Oyanagi H, Inui K, Wada H. Clinical significance of caspase-3 expression in pathologic-stage I, nonsmall-cell lung cancer. *Int J Cancer* 96: 54-60, 2001.
11. Hayes JD, Flanagan, JU, Jowsey, IR. Glutathione Transferases. *Annu Rev Pharmacol Toxicol* 45: 51-88, 2005.
12. Cote ML, Kardia SL, Wenzlaff AS, Land SJ, Schwartz AG. Combinations of glutathione S-transferase genotypes and risk of early-onset lung cancer in Caucasians and African Americans: a population-based study. *Carcinogenesis* 26: 811-819, 2005.
13. Townsend DM, Tew KD. The role of glutathione-S-transferase in anti-cancer drug resistance. *Oncogene* 22: 7369-7375, 2003.
14. Martin B, Paesmans M, Berghmans T, Branle F, Ghisdal L, Mascaux C, Meert AP, Steels E, Vallot F, Verdebout JM, Lafitte JJ, Sculier JP. Role of Bcl-2 as a prognostic factor for survival in lung cancer: a systematic review of the literature with meta-analysis. *Brit J Cancer* 89: 55-64, 2003.
15. Yang X, Xue L, Guo L, Wen P, Lin D. Clinicopathological and prognostic significance of a panel of tumor biomarkers in lung adenocarcinoma: a tissue microarray study. *Zhongguo Fei Ai Za Zhi* 17: 243-253, 2014.
16. Yoo J, Jung JH, Choi HJ, Kang SJ, Kang CS. The expression of c-myc, bcl-2 and p53 proteins in adenocarcinomas of lung. *Cancer Res Treat* 36: 146-150, 2004.
17. Ahrendt SA, Chow JT, Yang SC, Wu L, Zhang MJ, Jen J, Sidransky D. Cigarette smoking and alcohol consumption increase the frequency of p53 gene mutations in non-small cell lung cancer. *Cancer Res* 60: 3155-3159, 2000.
18. Skaug V, Ryberg D, Kure EH, Arab MO, Stangeland L, Myking AO, Haugen A. p53 mutations in defined structural and functional domains are related to poor clinical outcome in non-small cell lung cancer patients. *Clin Cancer Res* 6: 1031-1037, 2000.
19. Mitsudomi T, Hamajima N, Ogawa M, Takahashi T. Prognostic significance of p53 alterations in patients with non-small cell lung cancer: a meta-analysis. *Clin Cancer Res* 6: 4055-4063, 2000.
20. Ishida H, Irie K, Itoh T, Furukawa T, Tokunaga O. The prognostic significance of p53 and bcl-2 expression in lung adenocarcinoma and its correlation with Ki-67 growth fraction. *Cancer* 80: 1034-1045, 1997.
21. Kim SH, Kwon HC, Oh SY, Lee DM, Lee S, Lee JH, Roh MS, Kim DC, Park KJ, Choi HJ, Kim HJ. Prognostic value of ERCC1, thymidylate synthase, and glutathione S-transferase pi for 5-FU/oxaliplatin chemotherapy in advanced colorectal cancer. *Am J Clin Oncol* 32: 38-43, 2009.
22. Mao ZP, Zhao LJ, Zhou SH, Liu MQ, Tan WF, Yao HT. Expression and significance of glucose transporter-1, P-glycoprotein, multidrug resistance-associated protein and glutathione S-transferase-π in laryngeal carcinoma. *Oncol Lett* 9: 806-810, 2015.
23. Franco RL, Schenka NG, Schenka AA, Rezende LF, Gurgel MS. Glutathione S-transferase Pi expression in invasive breast cancer and its relation with the clinical outcome. *J BUON* 17: 259-264, 2012.