CANCER GENOMICS & PROTEOMICS 7: 331-336 (2010)

Apoptotic Regulators: P53 and Survivin Expression in Non-small Cell Lung Cancer

IRENA PORĘBSKA[1], EWA SOBAŃSKA[2], MONIKA KOSACKA[1] and RENATA JANKOWSKA[1]

[1]*Chair and Department of Pulmonology and Lung Cancer,*
[2]*Chair and Department of Clinical Immunology, Wroclaw University of Medicine, Wroclaw, Poland*

Abstract. *Background: Survivin and p53 are proteins that take part in cell cycle and apoptosis regulation. The biological function of survivin is essential for its oncogenic potential. The p53 protein is known to be a guardian of the genome and alterations in its structure enhance resistance to apoptosis. The aim of this study was to detect survivin and p53 expression in 74 non-small cell lung cancer in relation to basic clinicopathological data including the two-year prognosis. Patients and Methods: A total of 74 patients with non-small cell lung cancer were recruited into the study. Marker presence was revealed using immunohistochemical methods on paraffin-embedded tissue. Results: The presence of p53 was visible in 52.7% of cases and its expression did not correlate with clinicopathological data. Survivin immunoreactivity was detected in 52.7% of all study cases and was statistically more often found in lung adenocarcinomas than in squamous cell subtype of lung cancer (67% and 37% respectively, p=0.03). Larger tumours, cancer with lymph node metastases and more advanced tumours according to TNM status showed higher incidence of survivin expression, but differences did not reach statistical significance. The survivin immunoreactivity did not correlate with the two-year survival. Conclusion: P53 protein expression did not appear to be a clinically important tumour marker. The clearly visible trend to more frequent survivin presence in more advanced non-small cell lung tumours needs further evaluation. The statistically significant difference in survivin immunoreactivity between two major pathological types of non-small cell lung cancer may be important for better selection of patients for specific biological therapy based on apoptosis regulation.*

Correspondence to: Irena Porębska, Chair and Department of Pulmonology and Lung Cancer, Wroclaw University of Medicine, ul. Grabiszyńska 105, 53-439 Wrocław, Poland. Tel: +48 713349558, Fax: +48 713349596, e-mail: iporebsk@poczta.onet.pl

Key Words: Apoptosis, immunohistochemistry, tumour marker, p53, survivin, non-small cell lung cancer.

Lung cancer, as the leading cause of death among all human neoplasms, remains a significant clinical challenge. Despite recent major progress in clinical and experimental oncology, the prognosis in lung cancer is unfavourable, with a five-year survival rate of only around 11% (1). Better understanding of lung cancer molecular biology, especially in relation to clinical parameters, is important for improving methods of treatment.

Apoptosis is the multistep process leading to programmed cell death. It is widely accepted that disturbances of apoptosis are very important in cancer development, progression and resistance to therapy. There are many apoptotic regulators described in the literature whose prognostic and potential therapeutic value are interesting to evaluate (2). Survivin is an inhibitor of apoptosis protein (IAP), first described in 1997 (3). This 16.5 kDa molecule of 142 amino acids is encoded by its gene located on chromosome 17 (17q25) (3). The function of survivin in the cell is strictly connected with the cell compartment and is not yet completely understood. Nuclear survivin is firstly a cell cycle regulator acting during mitosis as part of a chromosomal passenger complex (4). The mitochondria-derived survivin is a strong antiapoptotic agent due to its ability to form a heterodimer with X-linked IAP (XIAP). This complex stabilises the XIAP molecule and can inhibit caspase activity, promoting apoptosis depletion and tumour growth (5). The biological effect of survivin on programmed cell death is essential for its oncogenic function. Survivin is neither expressed nor present at low levels in differentiated tissues (3). Enhanced survivin levels have been detected in many types of cancer including breast cancer and non-small cell lung cancer (NSCLC) (3, 6-9). It is widely accepted that the key role of the p53 protein is to repair DNA damage or initiate programmed cell death if the DNA damage cannot be corrected. Thus, proper function of the p53 protein is necessary for the prevention of cell clones arising with accumulated DNA mutations, thereby providing protection from possible neoplastic transformation. The p53 molecule and survivin have a functional connection. Hoffman *et al.* (10) demonstrated that wild-type p53 protein can suppress survivin expression by inhibiting the transcription of the

Table I. *Study group characteristics*.

Characteristic	Number of cases	Percentage
Gender		
Male	49	66.2
Female	25	43.8
Age		
≤60 years	36	47.3
>60 years	38	52.7
Histological type		
Adenocarcinoma	35	47.3
Squamous cell carcinoma	35	47.3
Large cell carcinoma	1	1.3
Non-small cell carcinoma§	3	4.1
Tumour		
T1	16	21.6
T2	48	64.8
T3	7	9.5
T4	3	4.1
Lymph nodes		
N0	35	47.3
N1	11	14.8
N2	26	35.1
N3	2	2.8
Metastasis		
M0	69	93.0
M1	5	7.0
Stage		
I (A i B)	31	41.9
II (A i B)	12	16.2
III A	24	32.4
IIIB i IV	7	9.5
Carcinomatous emboli		
Absent	34	53.0
Present	30	47.0

§The histopathological subtype was impossible to determine.

survivin encoding gene. The loss of proper *p53* gene can increase survivin levels *via* its promoter activity (11). This promotes cell resistance to apoptosis with many consequences particularly important in cancer cells. The diminished ability for apoptosis to take place has an important role for lung cancer carcinogenesis and the often observed resistance to chemotherapeutic agents and radiation therapy. Despite several studies of apoptotic marker expression in lung cancer, there are still unresolved questions about their usefulness in clinical practice (12). The aim of the present study was to detect the expression of p53 and survivin in lung cancer tissue in relation to basic clinicopathological data including prognosis.

Patients and Methods

Seventy-four lung cancer patients were included in the study. Cancer tissues were obtained during curative or diagnostic thoracotomy. The main characteristics of the study group are presented in Table I.

Table II. *Four different phenotypes in non-small cell lung cancer according to p53 and survivin expression*.

Phenotype	Number of phenotypes/all cases (%)
$P53^-$/$survivin^-$	16/74 (21.6%)
$P53^+$/$survivin^-$	19/74 (25.7%)
$P53^-$/$survivin^+$	19/74 (25.7%)
$P53^+$/$survivin^+$	20/74 (27.0%)

The mean age of patients was 60.5±8.3 years (range 43-77 years). The data for blood vessel cancer emboli was determined for 64 tissue sections. The two-year survival rate was available for 55 patients: 27 (49%) had died and 28 (51%) were alive. The average survival time was 19.5 months.

Patients were treated in accordance with standard procedures of the host institution, including that performing lobectomy, bilobectomy or pulmonectomy during surgery qualified patients for neoadjuvant cisplatin-based chemotherapy as deemed necessary (22 patients). Patients with inoperable lung cancer received palliative cisplatin-based therapy. The study was approved by the Ethical Committee of the host institution.

Survivin and p53 protein expression was determined immunohistochemically. Immunohistochemical staining was performed on paraffin-embedded tissue specimens using monoclonal antibodies against p53 (clone DO7; Novocastra Lab. Ltd, UK) and against survivin (clone 12C4; DakoCytomation, Denmark). Formalin-fixed, paraffin-embedded tissue samples were cut into 5 μm-thick sections. Following deparaffinisation and rehydration, antigen retrieval was performed using a 0.01 M sodium citrate buffer (pH 6.0) heated by microwave oven (800 W, 2×15 min). The study was carried out using a standard EnVision technique [EnVision+ System-HRP (DAB); Dako, Denmark]. After endogenous peroxidase blocking, sections were incubated with the primary antibodies (both at 1:50 dilution) against p53 at room temperature for one hour and against survivin overnight at 4˚C. Visualisation was performed with 3,3'-diaminobenzidine (DAB) as a chromogen. Finally, preparations were counterstained with Mayer's hematoxylin and coverslipped. Negative controls for each specimen were performed by omitting the primary antibodies.

The results of the immunostaining were evaluated using light microscopy. A case was scored as positive if more than 10% cells had nuclear accumulation of p53 protein. The staining for survivin was considered positive in cases where more than 20% of cells exhibited cytoplasmic or nuclear staining. In addition, the intensity of the p53 and survivin staining was also graded as: + (low), ++ (medium) and +++ (high). The result of immunostaining was evaluated using a double-headed BHS microscope (Olympus, Japan) by two independent observers (IP, ES). The clinical data of the analysed cases were not disclosed during microscopic evaluation.

Statistics. Statistical analysis was performed using CSS Statistica for Windows version 5.0 (StatSoft Ltd, Poland). The expression of survivin and p53 in different groups of NSCLC, as well as mutual relations of the studied proteins were compared using the chi-square test. Survival curves were constructed with the Kaplan-Meier method. Differences were regarded as statistically significant at $p<0.05$.

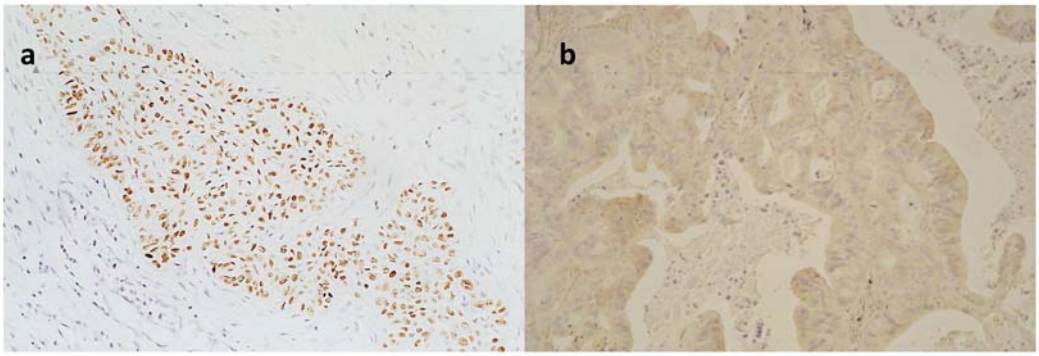

Figure 1. *Representative immunostaining of p53 and survivin: a: Positive p53 nuclear expression in lung cancer (original magnification ×200); b: Cytoplasmatic immunoreactivity of survivin in lung cancer (original magnification ×200).*

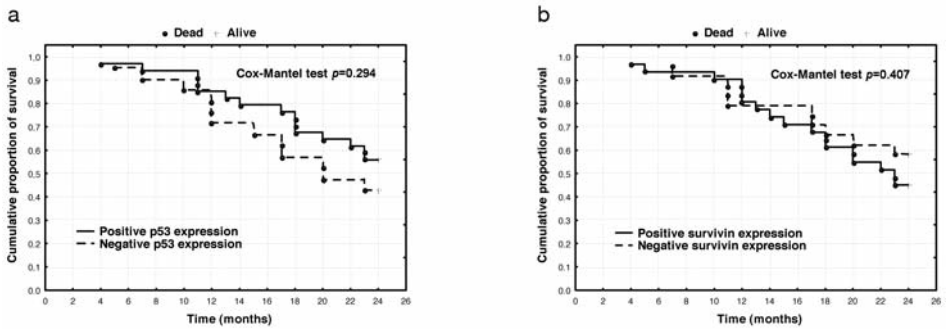

Figure 2. *Cumulative proportion of survival by Kaplan-Meier analysis according to: p53 expression (a) and survivin expression (b).*

Results

P53 and survivin expression in lung cancer tissue. Representative cases of survivin and p53 protein reactivity are demonstrated in Figure 1. The p53 protein was detected only in cancer nuclei. A total of 39 (52.7%) cases were p53 positive. The percentage of positive cells ranged from 10 to 100%, although in most tissue specimens, the majority (more than 50%) of cancer nuclei were stained. Survivin expression was revealed in 39 (52.7%) cases. In all survivin-positive cells, cytoplasmatic reactivity was observed. Accessory nuclear expression was observed only in two cases. The weak (+) and moderate (++) pattern of survivin staining intensity was predominant. Both p53 and survivin expression patterns were heterogeneous according to the extent and intensity of staining.

Relation between p53 and survivin expression and clinicopathological variables. Survivin expression appeared to be statistically more frequent in lung adenocarcinomas than in squamous cell lung cancer (23 cases, 67%, and 13 cases, 37%, respectively, $p=0.03$). Larger tumours, those with lymph node metastases and more advanced tumours according to TNM classification showed a higher incidence of survivin expression, however the differences did not reach statistical significance (6/16, 37% for T1 and 24/48, 50% for T2; 14/35, 40% for N0 and 24/39, 61% for N+; 19/43, 44% for I and II stages *versus* 19/31, 61% for III and IV stages). There were no associations between survivin expression and gender, age, cancer cell emboli in blood vessels and grade of tumour. p53 presence appeared to be independent of clinicopathological features. The survivin and p53 expression did not correlate with survival over a two-year observation period. The survival curves are shown in Figure 2.

Relationship between p53 and survivin. Taking into account the coexpression of the studied markers, four different phenotypes were observed (Table II). There was no relationship between p53 and survivin expression in individual cases. There were also no dominant phenotype visible in the entire NSCLC group. The detailed analysis of different phenotypes in lung cancer tissues revealed that one

or two studied marker expressions (phenotypes: $p53^+$/$survivin^+$; $p53^+$/$survivin^-$ or $p53^-$/$survivin^+$) were observed more often in cases with lymph node metastases than without (35/39, 90% *vs*. 22/35, 63%). This clearly visible trend did not reach statistical significance ($p>0.5$). There was no correlation between the p53 and survivin phenotypes and survival.

Discussion

The results of survivin expression studies in lung cancer remain unclear. Different expression of survivin in NSCLC have been reported, varying from 53% (11) to 95% (7) of cases. In the present study, the percentage of survivin-positive lung cancer was relatively small and close to the results achieved by Kren *et al*. (13). The cytoplasmatic pattern of survivin immunostaining in the present study is in agreement with that of Han *et al*. (14) but many other authors observed mainly nuclear survivin expression or both cytoplasmatic and nuclear localisation (8, 15). The difference can be explained by the antibodies used or the cases studied. Based on the role of survivin in cancer cells, this protein may be an attractive therapeutic target (16, 17). The aim of an anti-survivin strategy is to restore capability for programmed cell death and to enhance the sensitivity to chemo- and radiotherapy (9). The evaluation of survivin expression can distinguish patients who will be more susceptible to anti-survivin treatment, although the clinical value of immunohistochemical evaluation of survivin in that therapeutic goal is not yet known.

This study analysed the p53 protein presence in cancer cells because of the relation between survivin and mutated p53 protein in the multistep course of apoptosis. The p53 protein was revealed in cancer cell nuclei and this appearance was typical for mutant p53 by immunohistochemical evaluation. As previously reported, altered p53 protein appeared in almost 60% of studied NSCLC cases and its expression was higher than in small cell lung cancer (18). The present study confirmed that p53 protein expression is a common feature in NSCLC. Taking into account that the majority of NSCLC cases have impaired p53 molecules, the probability of deregulation of proteins functionally related to p53, including survivin, is high. It is possible that this specific molecular profile of lung cancer is connected with the long-term exposure to various carcinogens from cigarette smoke and accumulation of genetic changes in bronchial mucosa tissue. Moreover Dasgupta *et al*. (19) revealed that nicotine can diminish the effect of cytototoxic agents through up-regulation of the survivin and XIAP function.

There are only few reports on survivin expression according to histopathology in NSCLC and the results are often contradictory. Han *et al*. (14) observed more frequent survivin expression in squamous cell lung cancer than in adenocarcinomas. There are also reports confirming that there is no relationship of survivin with the squamous or adenocarcinoma type of lung cancer (13). In the present study, the expression of survivin was observed statistically more often in adenocarcinomas than in squamous cell carcinomas and this observation is close to the results described by Vischioni *et al*. (8). Expression of the p53 protein was not related to the histological type of NSCLC in the present study, confirming the results of Greatens *et al*. (20), although there are some reports regarding differences of p53 presence in histopathological subtypes of NSCLC (21). The lack of a clearly defined p53 and survivin correlation with two major NSCLC types suggest that the evaluation of apoptosis inhibitors should be performed separately for each individual patient. The correlation of survivin expression and clinical stage in lung cancer is controversial. Kren *et al*. (13) described a positive correlation of survivin expression and stage but only for squamous cell lung cancer. Ulukus *et al*. (7) reported that nuclear but not cytoplasmatic survivin correlate with clinical stage. Finally, Shinohara *et al*. (15) demonstrated that nuclear survivin presence did not correlate with clinical stage. The trend for a higher percentage of survivin-positive cases among more advanced NSCLC observed among the tissue specimens in the present study needs further evaluation. The prognostic significance of survivin in lung cancer also remains unresolved. The majority of reports suggest that nuclear survivin immunoreactivity corresponds with poor prognosis (15, 22, 23), but other reports do not confirm this observation (13, 5). In the present study, there was no correlation between survivin expression and prognosis of NSCLC. Cytoplasmatic immunoreactivity was seen in all survivin-positive cases and only in two cases was accessory nuclear survivin present. The prognostic value of immunohistochemically detected p53 protein expression in lung cancer was not firmly established according to a meta-analysis in 2006 by Zhu *et al*. (24). Ulukus *et al*. (7) did not find a correlation between p53 expression and clinical lung cancer stage. In the present study, there was no correlation between p53 and clinical stage or prognosis.

Despite the functional connection between the p53 and survivin, their expressions appeared to be independent of each other in the present study. There are only few published analyses of survivin expression in relation to p53 protein. The nuclear but not cytoplasmic expression of survivin correlated with p53 expression in the study by Ulukus *et al*. (7). Contrary to this result, Akyürek *et al*. (25) observed no correlation between p53 and survivin presence in lung cancer cells. Taking into account the controversy in the clinical value of single markers, the present study compared the four different cancer phenotypes according to p53 and survivin presence with clinical parameters and survival, but did not observe any correlation, with the

exception of a trend for more frequent p53- and/or survivin-positive phenotype in cancer with lymph node involvement. However, at present, this observation remains unverified due to a lack of relevant literature reports. A large proportion of cases with accumulated antiapoptotic protein expression, as revealed in the present study, can indirectly confirm that in NSCLC, there are deep and multidirectional apoptosis alterations, determining specific biological properties of this cancer, especially the often observed resistance to chemo- or radiotherapy. Expression of p53 and survivin were not useful as prognostic markers in the present study.

References

1 Verdecchia A, Francisci S, Brenner H, Gatta G, Micheli A, Mangone L and Kunkler I: EUROCARE-4 Working Group: Recent cancer survival in Europe: a 2000-02 period analysis of EUROCARE-4 data. Lancet Oncol *8*: 784-796, 2007.

2 Shivapurkar N, Reddy J, Chaudhary MP and Gazdar FA: Apoptosis and lung cancer: a review. J Cellular Biochem *88*: 885-898, 2003.

3 Ambrosini G, Adida C and Altieri DC: A novel anti-apoptosis gene, survivin, expressed in cancer and lymphoma. Nat Med *3*: 917-921, 1997.

4 Altieri DC: New writings in the survivin networks. Oncogene *27*: 6276-6284, 2008.

5 Dohi T and Altieri DC: Mitochondrial dynamics of survivin and 'four-dimensional' control of tumor cell apoptosis. Cell Cycle *4*: 21-23, 2005.

6 Barnes N, Haywood P, Flint P, Knox WF and Bundred NJ: Survivin expression in *in situ* and invasive breast cancer relates to COX-2 expression and DCIS recurrence. Br J Cancer *94*: 253-258, 2006.

7 Ulukus EC, Kargi HA, Sis B, Lebe B, Oztop I, Akkoclu A, Onen A and Sanli A: Survivin expression in non-small cell lung carcinomas: correlation with apoptosis and other apoptosis-related proteins, clinicopathologic prognostic factors and prognosis. Appl Immunohistochem Mol Morphol *15*: 31-37, 2007.

8 Vischioni B, van der Valk P, Span SW, Kruyt FAE, Rodrigez JA and Giaccone G: Nuclear localization of surviving is a positive prognostic factor for survival in advanced non-small cell lung cancer. Ann Oncol *15*: 1654-1660, 2004.

9 Yang C-T, Li J-M, Weng H-H, Li Y-C, Chen H-C and Chen M-F: Adenovirus-mediated transfer of siRNA against survivin enhances the radiosensitivity of human non-small cell lung cancer cells. Cancer Gene Therapy *17*: 120-130, 2010.

10 Hoffman WH, Biade S, Zilfou JT, Chen J and Murphy M: Transcriptional repression of the anti-apoptotic survivin gene by wild-type p53. J Biol Chem *277*: 3247-3257, 2002.

11 Altieri DC: Validating survivin as cancer therapeutic target. Nat Rev Cancer *3*: 46-54, 2003.

12 Zhu C-Q,ShihW, Ling C-H and Tsao M-S: Immunohistochemical markers of prognosis in non-small cell lung cancer: a review and proposal for a multiphase approach to marker evaluation. J Clin Pathol *59*: 790-800, 2006.

13 Kren L, Brazdil J, Hermanova M, Goncharuk VN, Kallakury BV, Kaur P and Ross JS: Prognostic significance of anti-apoptosis proteins survivin and bcl-2 in non-small cell lung carcinomas: a clinicopathologic study of 102 cases. Appl Immunohistochem Mol Morphol *12*: 44-49, 2004.

14 Han PH, Li XJ, Qin H, Yao J, DU N and Ren H: Up-regulation of survivin in non-small cell lung cancer and its clinicopathological correlation with p53 and Bcl-2. Xi Bao Yu Fen Zi Mian Yi Xue Za Zhi *25*: 710-713, 2009.

15 Shinohara ET, Gonzales A, Massion PP, Chen H, Li Ming, Freyer SA, Olson JS, Andersen JJ, Shyr Y, Carbone DP, Johson DH, Hallahan DE and Lu B: Nuclear survivin predicts recurrence and poor survival in non-small cell carcinoma. Cancer *15*: 1685-1692, 2005.

16 Pennati M, Folini M and Zaffaroni N: Targeting survivin in cancer therapy: fulfilled promises and open questions. Carcinogenesis *28*: 1133-1139, 2007.

17 Giaccone G, Zatloukal P, Roubec J, Floor K, Musil J, Kuta M, van Klaveren RJ, Chaudhary S, Gunther A and Shamsili S: Multicenter phase II trial of YM155, a small-molecule suppressor of survivin, in patients with advanced, refractory, non-small cell lung cancer. J Clin Oncol *27*: 4481-4482, 2009.

18 Porebska I, Wyrodek E, Kosacka M, Adamiak J, Jankowska R and Harłozińska-Szmyrka A: Apoptotic markers p53, Bcl-2 and Bax in primary lung cancer. In Vivo *2*: 599-604, 2006.

19 Dasgupta P, Kinkade R, Joshi B, DeCook Ch, Haura E and Chellappan S: Nicotine inhibits apoptosis induced by chemotherapeutic drugs by up-regulating XIAP and survivin. PNAS *103*: 6332-6337, 2006.

20 Greatens TM, Niehans GA, Rubins JB, Jessurun J, Kratzke RA, Maddaus MA and Niewoehner DE: Do molecular markers predict survival in non-small cell lung cancer? Am J Respir Crit Care Med *157*: 1093-1097, 1998.

21 Gregorc V, Ludovini V, Pistola L Darwish S, Floriani I, Bellezza G, Sidoni A, Cavaliere A, Scheibel M, De Angelis V, Bucciarelli E and Tonato M: Relevance of p53, bcl-2 and Rb expression on resistance to cisplatin-based chemotherapy in advanced non-small cell lung cancer. Lung Cancer *39*: 41-48, 2003.

22 Mohamed S., Yasufuku K, Nakajima T, Hiroshima K, Chiyo M, Yoshida S, Suzuki M, Sekine Y, Shibuya K, Agamy G, El-Shahhat H, Fujisawa T and Yoshino I: Nuclear survivin in pN2 non-small cell lung cancer: prognostic and clinical implications. Eur Respir J *33*: 127-133, 2009.

23 Lu B, Gonzales A, Massion PP, Shyr Y and Shaktour B: Carbone DP Hallahan DE: Nuclear survivin as a biomarker for non-small cell lung cancer. Br J Cancer *91*: 537-540, 2004.

24 Zhu CQ, Shih W, Ling CH and 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 *59*: 790-800, 2006.

25 Akyürek N, Memiş L, Ekinci O, Köktürk N and Oztürk C: Survivin expression in pre-invasive lesions and non-small cell lung carcinoma. Virchows Arch *449*: 164-170, 2006.

Received July 22, 2010
Revised October 3, 2010
Accepted October 4, 2010