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Evaluation of cyclin-dependent kinase inhibitor p27 and Bcl-2 protein in non-small cell lung cancer

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ABSTRACT

Bcl-2 and p27 are apoptosis and cell cycle regulatory proteins, respectively. We studied the expression of p27 and Bcl-2 protein to investigate their association between clinicopathologic factors and prognosis in non-small cell lung cancer (NSCLC). Thirty patients with NSCLC; 15 squamous cell carcinoma (SCC), 15 adenocarcinoma (AC) were used in our study. Tumors were examined immunohistochemically. Correlation between immunoreactivity and the clinicopathologic factors were evaluated. P27 immunoreactivity was observed in 12 of 30 cases. There were 9 SCC, 3 AC. Positive Bcl-2 immunostaining were found in 8 of 30 cases; 5 SCC, 3 AC were positive. The findings of present study suggest that immunohistochemical analysis of biopsy specimens for p27 should be more useful for predicting the prognosis of SCC than AC. However, absence of statistical difference for the expression of Bcl-2 between SCC and AC does not suggest such a role for Bcl-2 for the prognosis of either SCC or AC. [Turk J Cancer 2005;35(4):166-170].

KEY WORDS:

p27, Bcl-2, NCSLC

INTRODUCTION

Lung cancer is the most common cause of cancer death in men and women. Previous studies have strongly supported a multistep mechanism of tumor initiation and progression (1). Whether a prognostic significance for expression of the tumor suppressor genes exists in resected non-small cell lung cancer (NSCLC) remains controversial.

KIP family members include p21Cip1, p27Kip1, and p57Kip2 and inhibit cyclin/cdks by binding them. In response to mitogenic and anti-mitogenic stimuli the presence of cdk inhibitors modulates the activity of cdks, thus regulates entry into and progression through the cell cycle. The balance among cyclins, cdks, and cdk inhibitors are frequently altered in cancer or disrupted secondarily by other oncogenic events (2). P27 (KIP1) is a member of cell cycle regulatory protein family that is also known as cyclin dependent kinase inhibitor (CDI), which binds to 'cyclin-CDK' complexes and cause cell cycle arrest in the G1 phase. Moreover, p27 status is also an independent prognostic factor, and loss of expression is associated with tumor progression, lymph node metastasis, early relapse, and reduced overall survival (3-6).

Bcl-2 family consists of several regulatory proteins of apoptosis, including Bcl-2 (B-cell lymphoma/leukemia-2), Bax, Bcl-Xlong and Bcl-Xshort. Bcl-2 regulates apoptosis

negatively (7). These data imply the existence of a dynamic interplay among many members of the Bcl-2 family in triggering apoptosis in this system. Bcl-2 protein is implicated in oncogenesis by its ability to prolong cell survival through the inhibition of apoptosis (8-10). High level of Bcl-2 protein occurs in many different types of solid tumors (8).

Our hypothesis is that the distribution and intensity of p27 and Bcl-2 expression in SCC and AC is likely to be important factors for the differentiation and thus may be useful for predicting the prognosis of SCC and AC. In the present study, the expression of p27 and Bcl-2 proteins was aimed to investigate using immunohistochemistry to reveal their association with clinicopathologic factors and prognosis in NSCLC.

MATERIALS AND METHODS

NSCLC patients (n=30) underwent surgical resection in the Department of Thoracic Surgery, Akdeniz University School of Medicine. All pathological materials were reviewed and analyzed by the same pathologists (two persons). Diagnosis was based on conventional morphologic examination of paraffin-embedded specimens. Tumors were classified as 15 squamous cell carcinomas (SCC) and 15 adenocarcinomas (AC) according to the cell type. Pathological staging (primary tumor size, regional lymph node involvement, occurrence of distant metastasis) was performed by correlating the operative and histological findings.

Tumors were stained immunohistochemically using streptavidin-biotin peroxidase complex staining technique. p27 antibody was purchased from Dako (Clone 5x5368 1/25 dilution) and Bcl-2 was purchased from Novacastra (Clone 100/05, 1/80 dilution).

Briefly, following deparaffinization of sections, tissues were rinsed twice in phosphate buffer saline (PBS) for 10 minutes. Endogenous peroxidase activity was quenched by 3% hydrogen peroxide (H_2O_2; 0.6 ml H_2O_2 and 5.4 ml methanol) for 10 minutes and rinsed in PBS-Tween-20 (0.05 % Tween-20 in PBS, PBS-T; pH 7.3). Sections were then incubated with primary antibodies for 60 minutes at room temperature. Normal mouse antibody isotype was applied as a negative control replacing primary antibody. After several rinses in PBS-T, biotinylated horse anti-mouse IgG antibody (Vector Labs, Burlingame, CA, USA) was applied for 30 minutes. Following several PBS-T rinses, slides were incubated with streptavidin-peroxidase complex for 30 minutes (Vector Labs). Subsequently, the slides were rinsed several times in PBS-T, and then were incubated with DAB (Vector Labs) for 2 minutes. After a slight staining with hematoxylin slides were mounted with a permanent-mounting medium.

Statistical method

All slides were evaluated independently by two pathologists who were blind to slides. For the evaluation of p27 expression in cells' nuclear staining 1000 cells were counted using x40 magnification. The intensity for Bcl-2 immunoreactivity in lung tissues was semi-quantitatively evaluated as positively stained cells (%) according to immunoreactivity using the following intensity categories: - (no staining), 1+ (weak but detectable staining), 2+ (moderate or distinct staining), 3+ (intense staining). Finally, by multiplying the percent of positive cells with its intensity a histological score was obtained for each tissue used in the study.

Statistically, correlation between immunoreactivity and clinicopathologic factors were evaluated by Kruskal Wallis and Mann-Whitney Test. $P < 0.05$ represents statistical significance.

RESULTS

Specific immunoreactivity for p27 was exclusively seen in the nuclei of tumor cells and of normal cells in the lung. P27 immunoreactivity were observed in 12 (40%) of 30 cases. However, when the evaluation was carried out according to SCC and AC histological subtypes, 9 SCC (60% in total SCC cases) and 3 AC (20% in total AC cases) cases revealed immunoreactivity for p27. Moreover, in histological subtypes of NSCLC, SCC showed a higher average percentage of p27 positive nuclei then AC (Figures 1A&B and 2A&B). Statistically, these findings were found significant ($p<0.05$). Clinical and histopathologic findings of these cases were presented in table 1.

Comparing to p27 Bcl-2 immunoreactivity was observed in the cytosol. In adjacent normal respiratory epithelium, Bcl-2 was expressed only in basal cells. In tumor cells, the positive Bcl-2 immunostaining was found in 8 (26, 6%) of 30 cases. 5 SCC (33,3% in total SCC cases) and 3 AC (20% in total AC cases) were positive (Figure 3A&B). However, no significant difference was found between SCC and AC for the Bcl-2 immunoreactivity ($p > 0.05$).

There is no correlation between the level and intensity of p27 and Bcl-2 expression and clinical parameters including sex, age, histological type and clinical stage. Moreover, when tissues from patients with SCC and AC were divided into well, moderately and poorly differentiated histological grades of differentiation, no significant difference was observed among them.

Table 1
Clinical and histopathological findings

Case #	Sex	Stage	Hd	Dif	Ms (cm)	p27 (n)	Bcl-2 (c)	Survival
1	M	1b	SCC	well	8	800	-	
2	M	4	SCC	poorly	9	-	-	
3	M	1b	SCC	moderately	2,5	-	-	
4	M	3a	SCC	moderately	6	100	-	
5	M	4	SCC	well	4	200	10% md	Died
6	M	4	SCC	moderately	6	-	-	Died
7	M	3a	SCC	moderately	12	50	20% md	
8	M	3b	SCC	well	3,5	820	-	Died
9	M	4	SCC	moderately	5	-	20% md	Died
10	M	2b	SCC	well	5	300	10% md	
11	M	3a	SCC	well	3	-	-	Died
12	M	3a	SCC	moderately	3	-	-	
13	M	3a	SCC	moderately	2	50	-	
14	M	2b	SCC	well	2,5	120	70% sv	
15	M	1b	SCC	well	4	690	-	
16	F	3a	AC	moderately	2	-	-	
17	M	2b	AC	well	3	-	-	
18	F	2b	AC	well	3,2	-	-	
19	M	1b	AC	poorly	5,5	-	-	
20	M	1b	AC	well	5	80	-	
21	M	1b	AC	well	-	-	-	
22	M	4	AC	poorly	8	250	-	Died
23	F	3a	AC	well	3,5	-	-	
24	M	1b	AC	well	4	-	20% md	
25	F	3b	AC	moderately	1	-	-	Died
26	M	2b	AC	well	0,7	-	-	
27	M	1a	AC	moderately	1,5	-	-	
28	M	2b	AC	moderately	5,5	-	30% md	
29	M	3b	AC	well	1,8	300	-	
30	F	3a	AC	moderately	11	-	20% md	

c: cytoplasmic; Dif: differentiation; F: Female; Hd: histopathologic diagnosis; M: Male; md: mild; Ms: macroscopic size; n: nuclear; sv: severe

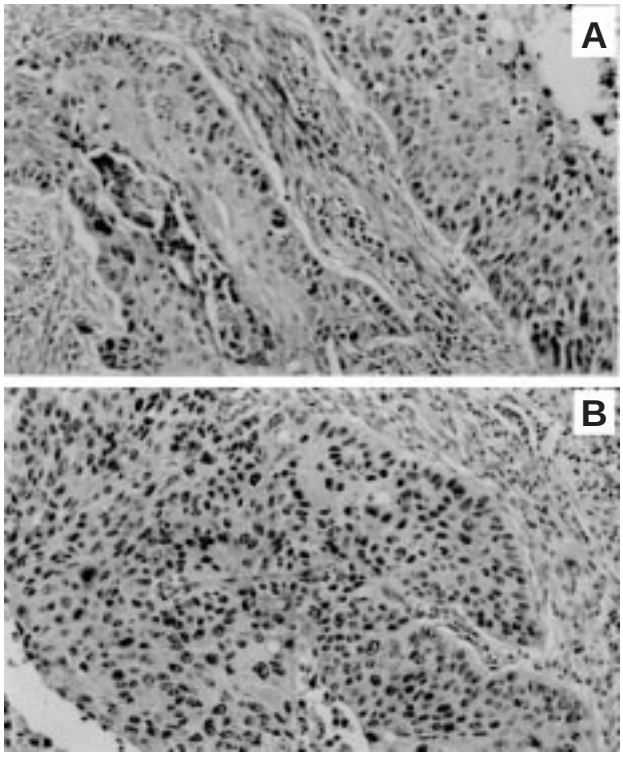

Fig 1 (A&B). (A): Representative microscopic appearance of the SCC case (H&E, x100); (B): P27 immunoreactivity is observed in the nucleus of SCC cells (x200)

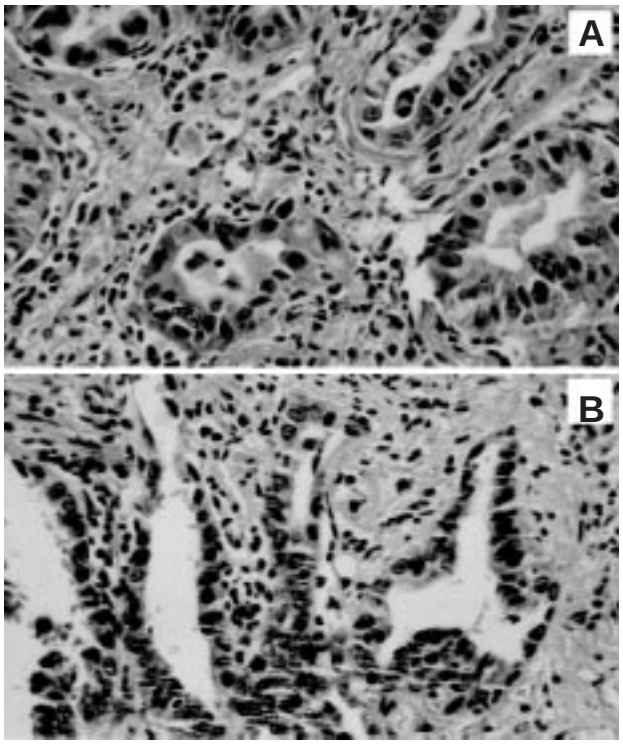

Fig 2 (A&B). (A): Representative microscopic appearance of the AC case (H&E, x400); (B): P27 immunoreactivity is observed in the nucleus of AC cells (x400)

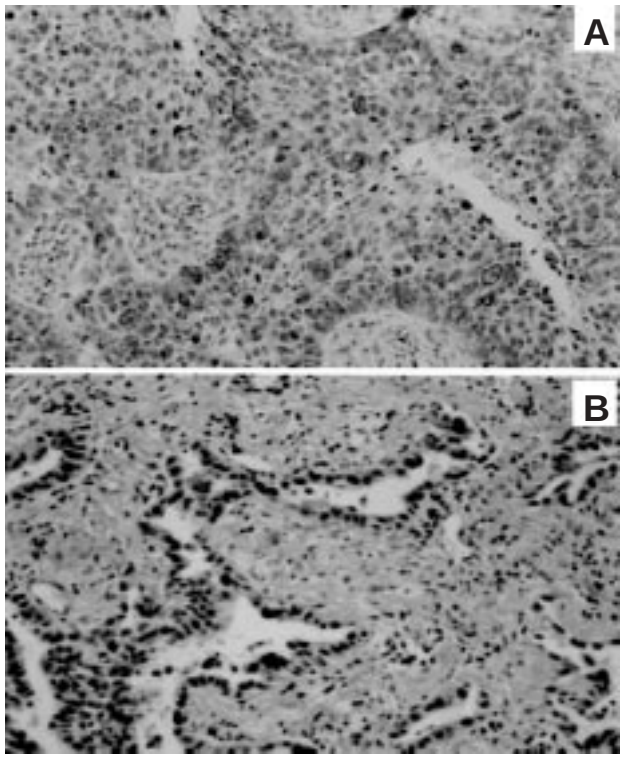

Fig 3 (A&B). (A): Representative micrograph for Bcl-2 immunoreactivity in SCC; (B): Representative micrograph for Bcl-2 immunoreactivity in AC tissues. Bcl-2 immunoreactivity is seen with a cytoplasmic localization (x200)

CONCLUSIONS

In general, several parameters, such as clinic stage, performance status and histopathologic diagnosis have been known among clinicians as good prognostic factors for patients with NSCLC. Recent advances in molecular biology have provided several new prognostic factors for NSCLC. Among many oncogenes and tumor suppressor genes, p27 may the candidates for new prognostic factors for NSCLC.

There are many conflicting results in the previous studies. Esposito et al. (6) and Yatabe et al. (11) showed that high level of p27 expression was associated with good prognosis in NSCLC patients with early stage. Ishihara et al. (5) suggested p27 expression as an independent prognostic factor in advanced NSCLC patients. Another study by Hirabayashi et al. (4) described that abnormal p27 expression might be a useful indicator to predict postoperative prognosis, especially in patients with early stage NSCLC, as compared to other tumor suppressor gene products that is examined in the study. Hayashi et al. (12) indicated that reduced p27 expression was associated with tumor progression and may play a role in progression during the development of pulmonary AC. In our study,

among the subtypes, we detected that in SCC, p27 expression was higher than AC and this finding was found statistically significant suggesting a modulatory role for p27 in SCC.

Bcl-2 expression would be associated with a worse outcome because it implies an abnormal accumulation of neoplasm cells protected from the programmed cell death. Studies did not show a significant effect of Bcl-2 expression on survival in patients with NSCLC (10) which is similar to previous reports that either showed no survival benefit or failed to find a better prognosis in Bcl-2 positive patients (13,14). O'Neill et al. (15) suggested that apoptosis in NSCLC occured independently and is not modulated primarily by Bcl-2. Ritter et al. (16) showed no significant difference in survival of 5 years comparison of all cases with Bcl-2 expression and those without, but Pezzella et al. (17) concluded that Bcl-2 is abnormally expressed in some lung carcinomas and its expression may have prognostic importance. In our study, Bcl-2 immunostaining was observed in 33,3% of SCC and 20% of AC, a difference that was not significant statistically suggesting that Bcl-2 protein immunoreactivity has no significant prognostic value in the pathological evaluation of NSCLC.

In conclusion, although our cases are limited in number with heterogenous stage, the findings of present study suggest that immunohistochemical analysis of biopsy specimens for p27 should be more useful for predicting the prognosis of SCC than AC. However, absence of statistical difference for the expression of Bcl-2 between SCC and AC does not suggest such a role for Bcl-2 for the prognosis of either SCC or AC.

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