Pathol. Oncol. Res. (2010) 16:55–59
DOI 10.1007/s12253-009-9187-4

Comparison of Osteopontin, β-catenin and hnRNP B1 Expression in Lung Carcinomas

Muhammet Emin Guldur · Yasemin Kıbar · Hale Denız · Kemal Bakır

Received: 15 December 2008 / Accepted: 7 July 2009 / Published online: 17 July 2009

Abstract This study was performed to compare osteopontin (OPN), β-catenin and heterogeneous nuclear ribonucleoprotein B1 (hnRNP B1) immunreactivities in small cell lung carcinomas (SCLC) and non-small cell lung carcinomas (NSCLC). Correlation of these three antibodies with grade and clinicopathologic stage of the tumor in NSCLC was also investigated. Twenty-nine SCLC, 6 large cell carcinoma, 36 adenocarcinoma and 30 squamous cell carcinoma (SCC), totally 101 cases, were included in this study. OPN, β-catenin and hnRNP B1 expressions were immunohistochemically evaluated. OPN positivity was 6.9% in SCLC and 67% in NSCLC. When NSCLC types were individually considered, OPN positivity was 66.7% in large cell carcinoma, 80% in SCC and 55.6% in adenocarcinomas. β-catenin positivity was observed in 48.6% of NSCLC and none of SCLC cases. These results were statistically significant ($p<0.05$). Neither grade nor stage of NSCLC was correlated with osteopontin, β-catenin or hnRNP B1 immunreactivity. We observed that OPN and β-catenin are useful in differentiating SCLC from NSCLC. This may be helpful in small lung biopsies where morphology is obscured by crush artifacts.

Keywords Osteopontin · Beta-catenin · Heterogeneous nuclear ribonucleoprotein B1 · Lung cancer

M. E. Guldur
Department of Pathology, Harran University Faculty of Medicine, 63100 Sanliurfa, Turkey
e-mail: sevimemin@mynet.com

Y. Kıbar
Department of Pathology, Sehitkamil Hospital, Gaziantep, Turkey

H. Denız (✉)
Gaziantep Children Hospital, Department of Pathology, Sehitkamil, Gaziantep, Turkey
e-mail: haleden2000@yahoo.com

K. Bakır
Department of Pathology, Gaziantep University Faculty of Medicine, Gaziantep, Turkey

Introduction

Lung cancer is the most important cause of cancer-related death throughout the world. It's known that environmental factors, most importantly smoking, are the major causes of lung cancer. Osteopontin (OPN) is an integrin-binding protein which is overexpressed in many malignancies. It's suggested that OPN overexpression is related to prognosis and stage in non-small cell lung carcinomas (NSCLC) [1, 2]. Beta catenin is an important structural component in normal epithelium and malignant cells [3]. Its reduced expression is suggested to be related to poor differentiation and more aggressive behavior in various tumor types [4]. Heterogeneous nuclear ribonucleoprotein B1 (hnRNP B1) is an RNA binding protein which is overexpressed in lung carcino-

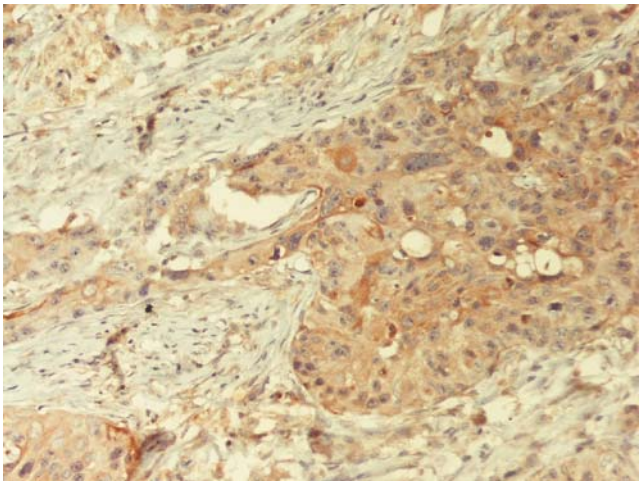

Fig. 1 Cytoplasmic osteopontin immunreactivity in SCC (X200)

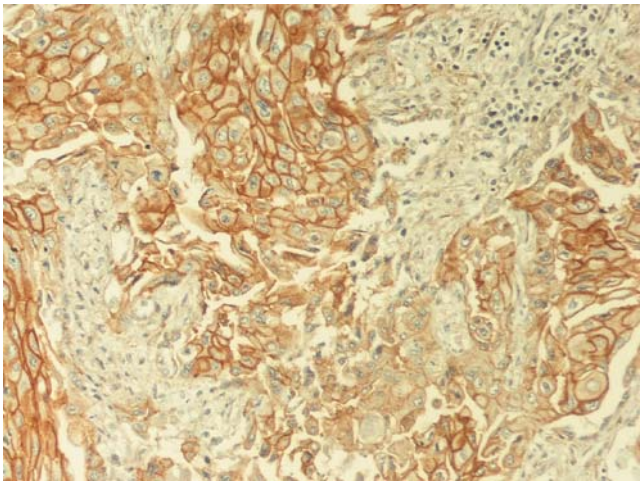

Fig. 3 Strong Membranous β-catenin immunreactivity in SCC (X200)

mas, particularly squamous cell lung carcinomas (SCC) even in very early stages [5, 6]. This study was performed to compare OPN, β-catenin and hnRNP B1 immunoreactivities in SCLC and NSCLC. Correlation of these three antibodies with grade and stage in NSCLC was also investigated.

Material and Method

A total of 101 lung carcinoma cases (29 SCLC, 30 SCC, 36 adenocarcinoma and six large cell carcinoma) from the archive of Gaziantep University, School of Medicine, Department of Pathology, were included in this retrospective study. A representative slide was chosen for each case and 4 μm-thick sections from formalin-fixed paraffin embedded tissue was prepared to perform immunohistochemistry (IHC). Ostepontin (Cat No. RB-9097-P1, Neomarkers), β-catenin (Cat.No.RB-9035-P1, Neomarkers) and hnRNP B1 (Cat No. MS-179 -P1, Neomarkers) primary antibodies were used. Avidin-biotin-peroxidase complex method for OPN and beta-catenin, UltraVision LP Value/HRP (DAB) polymer method for hnRNP B1 were used. After heat induced antigen retrieval using Tris-EDTA, pH9.0 (20 min) for β-catenin and boiling in 10 mM citrate buffer, pH:6.0

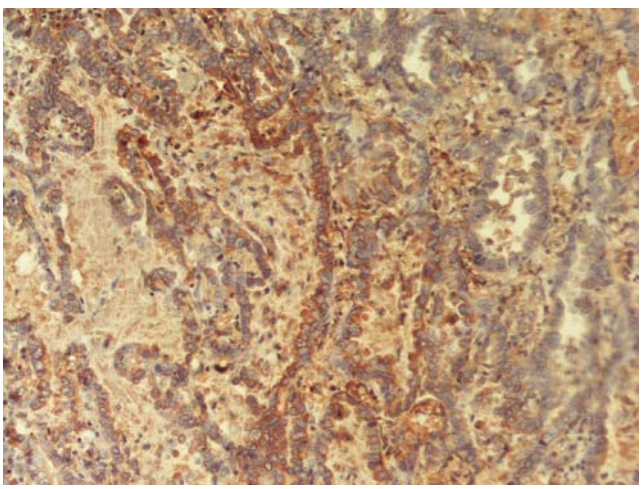

Fig. 2 Cytoplasmic osteopontin immunreactivity in adenocarcinoma (X20)

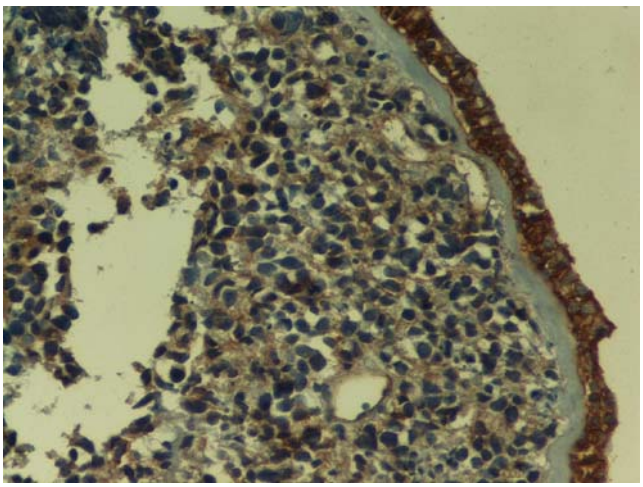

Fig. 4 Reduced β-catenin expression is observed in tumour cells of SCLC. Respiratory epithelium on the right shows strong staining with β-catenin (X200)

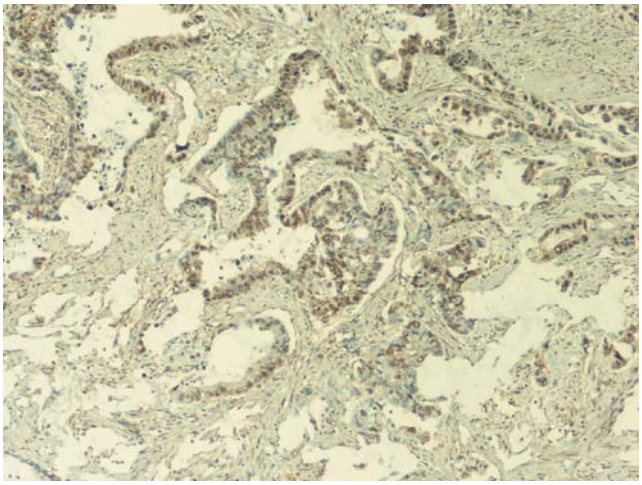

Fig. 5 Nuclear hnRNP B1 staining in well-differantiated adenocarcinoma (X100)

(20 min) for OPN and hnRNP B1, the primary antibodies were incubated as follows: OPN and hnRNP B1: 30 min; dilution 1:50, β-catenin: 20 minutes, dilution:1:125. Both staining intensity (0=negative, 1=weak, 2=intermediate, 3=strong) and percentage of positive cells (0=none or <1%, 1=1–10%, 2=11–30%, 3>30%) were used for scoring the cytoplasmic OPN expression. OPN was defined as positive if the sum of the two scores was more than 3, while others were considered as negative. For β-catenin, membranous staining in more than 70% of cells was defined as positive. Pure cytoplasmic staining and membranous staining in less than 70% was considered as reduced expression. For hnRNP B1, staining in more than 30% of tumor cells—either cytoplasmic or nuclear—was accepted as positive, while staining in less than 30% was negative. Immunoreactivities of these three antibodies were investigated. In NSCLC, these immunoreactivities were also compared with grade, clinicopathologic stage and subtypes.

Chi-square and Kolmogorov-Smirnov tests were used for statistical analysis. $P<0.05$ was considered statistically significant.

Table 1 Comparison of Osteopontin, β-catenin and hnRNP B1 immunoreactivities between SCLC and NSCLC

	Osteopontin		Beta-catenin		hnRNP B1		Total
	+	−	+	−	+	−	
SCLC	2	27	0	29	18	11	29
NSCLC	48	24	35	37	56	16	72

SCLC small cell lung carcinoma, *NSCLC* non-small cell lung carcinoma

Results

OPN positivity was 6.9% in SCLC and 67% in NSCLC (Figs. 1, 2). Some of the macrophages adjacent to the tumor cells were also positive. Reduced β-catenin expression was observed in 51.4% of NSCLC and all of SCLC cases (Figs. 3, 4). hnRNP B1 was positive in 62% of SCLC and 78% of NSCLC (Figs. 5, 6). Comparison of OPN, β-catenin and hnRNP B1 immunreactivities in SCLC and NSCLC is shown in Table 1.

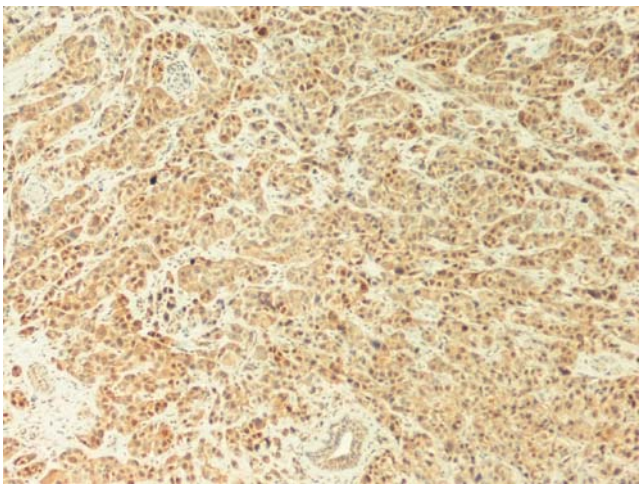

Fig. 6 Nuclear and cytoplasmic hnRNP B1 staining in SCC (X100)

Table 2 Distribution of osteopontin, β-catenin and hnRNP B1 expression among subtypes of non-small cell lung carcinoma

	Osteopontin		Beta-catenin		hnRNP B1		Total
	+	−	+	−	+	−	
SCC							
n	24	6	16	14	22	8	30
%	80	20	53.4	46.6	73.3	26.7	
Adenocarcinoma							
n	20	16	15	21	29	7	36
%	55.6	44.4	42	58	80.5	19.5	
LCC							
n	4	2	4	2	5	1	6
%	66.7	33.3	66.7	33.3	83.3	16.7	

SCC squamous cell carcinoma, *LCC* large cell carcinoma

Table 3 Relation of OPN, β-catenin and hnRNP B1 immunoreactivities with grade in Squamous cell carcinoma and adenocarcinomas

Grade	Osteopontin		Beta-catenin		hnRNP B1		Total
	+	−	+	−	+	−	
SCC							
Well	3	0	2	1	2	1	3
Intermediate	19	5	12	12	18	6	24
Poor	2	1	1	2	2	1	3
Adenocarcinoma							
Well	6	5	4	7	10	1	11
Intermedite	8	6	10	4	11	3	14
Poor	6	5	2	9	9	2	11

There was statistically significant difference in both OPN and β-catenin expressions between SCLC and NSCLC ($p<0.05$). No significant difference was found in hnRNP B1 immunostaning ($p>0.05$). Immunreactivities of three antibodies among subtypes of NSCLC is shown in Table 2. When subgroups of NSCLC were compared, there was no statistically significant difference in any of the three antibodies.

Neither grade nor stage of NSCLC was correlated with osteopontin, β-catenin or hnRNP B1 immunreactivities (Tables 3, 4).

Discussion

In this study, we evaluated OPN, β-catenin and hnRNP B1 immunreactivities in SCLC and NSCLC. Fourty eight of 72 NSCLC (67%) and two of 29 SCLC (6.9%) were stained positively with OPN. Some of the macrophages adjacent to the tumor cells were also positive. OPN immunoreactivity was higher in SCC than other NSCLC subgroups (80%). These results are in concordance with Zhang et al [7]. They found overexpression of OPN in 37.5% of NSCLC (69% in SCC), and 11% of SCLC. Hu et al [8] reported 64.5% positivity (205 of 318) in NSCLC.

In our study reduced expression of beta catenin was observed in 37 of 72 NSCLC (51.4%), while no expression was observed in 29 SCLC cases (100%). Kase et al [9] reported reduced β-catenin expression in 37% of NSCLC. The difference in OPN and β-catenin immunoreactivities between SCLC and NSCLC was found to be statistically significant ($p<0.05$).

In our study hnRNP B1 was positive in 62% of SCLC and 78% of NSCLC. No significant difference was found in RNP immunostaning between SCLC and NSCLC ($p>0.05$). When subtypes of NSCLC were individually evaluated, positivity of RNP was 73.3% in SCC, 80.5% in adenocarcinoma and 83.3% in large cell carcinoma. Our results are in concordance with Wu et al [10] who investigated hnRNP B1 expression in occult carcinoma, NSCLC and dysplastic lesions. They reported 71% and 64.3% positivity in SCC and adenocarcinoma respectively. In another study Veronika et al [11] found 47% positivity of hnRNP B1 in both SCC and adenocarcinoma, which is lower than our results. The difference in large cell carcinoma was even greater. They found only 3% positivity in this group.

In this study, grade and stage of NSCLC were not found to be correlated with OPN, β-catenin and hnRNP B1. In some studies OPN overexpression was found to be strongly correlated with stage and grade [1, 2], however no such association was found in another study [12]. Kase et al [9] found no correlation between β-catenin expression and tumor stage or grade. Choi et al [13] suggested that reduced β-catenin expression indicates tumor cell dedifferentiation and poor prognosis. In the literature we couldn't find any study comparing hnRNP B1 immunoreactivities between tumor grades. Veronika et al [11] reported that hnRNP B1 expression was higher in stage III/IV than stage I/II cases.

The distinction between SCLC and NSCLC is still predominantly based on morphologic criteria as seen in conventionally stained sections. But in small biopsies, some technical problems such as crush artifacts may cause serious difficulties in interpretation. In such cases, osteopontin and β-catenin may be useful because of the significant difference between SCLC and NSCLC.

Acknowledgement We thank Huseyin Kizilirmak for his skillful technical assistance and Dr. Neriman Aydin for her expert statistical analyses.

Table 4 Relation of OPN, β-catenin and hnRNP B1 immunoreactivities with stage in NSCLC

	Osteopontin		Beta-catenin		hn RNP B1		Total
	+	−	+	−	+	−	
Stage 1	10	2	4	8	11	1	12
Stage 2	13	8	12	9	17	4	21
Stage 3	18	8	14	12	17	9	26
Stage 4	1	1	1	1	1	1	11

References

1. Coppola D, Szabo M, Boulware D, Muraca P, Alsarraj M, Chambers AF, Yeatman TJ (2004) Correlation of osteopontin protein expression and pathological stage across a wide variety of tumor histologies. Clin Cancer Res 10:184–190
2. Donati V, Boldrini L, Dell'Omodarme M, Prati MC, Faviana P, Camacci T, Lucchi M, Mussi A, Santoro M, Basolo F, Fontanini G (2005) Osteopontin expression and prognostic significance in non-small cell lung cancer. Clin Cancer Res 11:6459–6465
3. Nozawa N, Hashimoto S, Nakashima Y, Matsuo Y, Koga T, Sugio K, Niho Y, Harada M, Sueishi K et al (2006) Immunohistochemical α and β-catenin and E-cadherin expression and their clinocopathological significance in human lung adenocarcinoma. Pathol Res Pract 202:639–650
4. Prinen RT, Hirvikoski P, Johansson RT, Hollmen S, Kosma VM (2001) Reduced expression of α-catenin, β-catenin is associated with high cell proliferative activity and poor differentiation in non-small cell lung cancer. J Clin Pathol 54:391–395
5. Sueoka E, Sueoka N, Goto Y, Matsuyama S, Nishimura H, Sato M, Fujimura S, Chiba H, Fujiki H (2001) Heterogeneous nuclear ribonucleoprotein B1 as early cancer biomarker for occult cancer of human lungs and bronchial dysplasia. Cancer Res 61:1896–1902
6. Zhou J, Mulshine JL, Unsworth EJ, Scott FM, Avis IM, Vos MD, Treston AM (1996) Purification and characterization of a protein that permits early detection of lung cancer. Identification of heterogeneous nuclear ribonucleoprotein-A2/B1 as the antigen for monoclonal antibody 703D4. J Biol Chem 271:10760–10766
7. Zhang J, Takahashi K, Takahashi F, Shimizu K, Ohshita F, Kameda Y, Maeda K, Nishio K, Fukuchi Y (2001) Differential osteopontin expression in lung cancer. Cancer Lett 171:215–222
8. Hu Z, Lin D, Yuan J, Xiao T, Zhang H, Sun W, Han N, Ma Y, Di X, Gao M, Ma J, Zhang J, Cheng S, Gao Y (2005) Overexpression of osteopontin is associated with more aggressive phenotypes in human non-small cell lung cancer. Clin Cancer Res 11:4646–4652
9. Kase S, Sugio K, Yamazaki K, Okamoto T, Yano T, Sugimachi K (2000) Expression of E-cadherin and β-Catenin in human non-small cell lung cancer and the clinical significance. Clin Cancer Res 6:4789–4796
10. Wu S, Sato M, Endo C, Sakurada A, Dong B, Aikawa H, Chen Y, Okada Y, Matsumura Y, Sueoka E, Kondo T (2003) HnRNP B1 protein may be a possible prognostic factor in squamous cell carcinoma of the lung. Lung Cancer 41:179–186
11. Zech VF, Dlaska M, Tzankov A, Hilbe W (2006) Prognostic and diagnostic relevance of hnRNP A2/B1, hnRNP B1 and S100 in non- small cell lung cancer. Cancer Detect Prev 30:395–402
12. Schneider S, Yochim J, Brabender J, Uchida K, Danenberg KD, Metzger R, Schneider PM, Salonga D, Hölscher AH, Danenberg PV (2004) Osteopontin but not osteonectin messenger RNA expression is a prognostic marker in curatively resected non-small cell lung cancer. Clin Cancer Res 10:1588–1596
13. Choi YS, Shim YM, Kim SH, Son DS, Lee HS, Kim GY, Han J, Kim J (2003) Prognostic significance of E-cadherin and β-catenin in resected stage 1 non-small cell lung cancer. Eur J Cardiothorac Surg 24:441–449