

Prognostic relevance of mdm2 protein expression in non-small cell lung cancer

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Objectives. In this study we investigated the prognostic value of mdm2 protein expression (in correlation to previously assessed p53 status) in non-small cell lung cancer (NSCLC) patients.

Material and methods. The study group included 123 NSCLC patients who underwent pulmonary resection. Expression of mdm2 protein was assessed immunohistochemically with the use of monoclonal antibody (clone IF2, Oncogene Science).

Results. Expression of mdm2 protein was found in 40 samples (33%), whereas mdm2 and p53 co-expression - in 19 samples (15%). There was no relationship between the expression of mdm2 and major clinicopathological factors and neither there was an impact of mdm2 protein expression (considered separately or jointly with p53) on disease-free and overall survival. In uni- and multivariate analysis stage of disease and tumor grade were independent prognostic factors.

Conclusions. These results suggest the lack of prognostic relevance of mdm2 expression in surgically treated NSCLC patients.

Key words: mdm2, NSCLC, prognosis

Introduction

In spite of significant progress in therapeutic methods, the prognosis in non-small cell lung cancer (NSCLC) is still unsatisfactory [1]. It is believed that a better understanding of lung cancer biology and in particular the molecular nature of the tumor may result in more efficient cancer management.

One of the most extensively studied genetic alterations in NSCLC are *P53* gene abnormalities. The *P53* gene, called the “guardian of the genome”, is located in 17p13 and encodes for a 53-kDa nuclear phospho-

protein (393 amino acids). The *P53* gene is involved in cell cycle control, DNA repair, cellular differentiation, senescence, angiogenesis and particularly in apoptosis. The protein product of the *P53* gene causes cell cycle arrest in response to DNA damage by inducing GADD45, p21^{WAF1}, PCNA and mdm2 expression [2]. Apart from mutation, the function of this gene may be inactivated in human tumors by several other mechanisms, including altered subcellular localization of p53, association of p53 protein with viral proteins or binding of p53 protein to mdm2 cellular oncoprotein [2, 3].

MDM2/HDM2 (mouse/human double minute) gene is an evolutionary conserved oncogene located in 12q13-14. It was originally identified in a tumorigenic derivative of the mouse Balb/c cell line called 3T3DM, in which *MDM2* was amplified and overexpressed [4]. *MDM2* gene tumorigenic activity results from mdm2 protein overexpression, which is possible due to three mechanisms: *MDM2* gene amplification, *MDM2*mRNA increased transcription or enhanced mdm2 protein translation. Currently little is known about the role of *MDM2* in physiological conditions [5].

A protein product of *MDM2* is a 90-kDa oncoprotein, physically associated with p53 and inhibiting wt-p53 transactivation function. This protein has also transforming activity when it is overexpressed in murine fibroblasts [3]. Mdm2 and p53 proteins form an autoregulatory feedback-loop in which p53 positively regulates mdm2 levels, whereas mdm2 inhibits p53 expression and

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activity [3, 6, 7]. Mdm2 plays important functions not only in the nucleus (by forming a complex with wt- or mt-p53) [3, 5], but also via transcellular effects [8].

The prognostic relevance of mdm2 protein expression in surgically treated NSCLC patients remains unclear, especially in relation to p53 protein expression. Higashiyama et al. [9] suggested that overexpression of mdm2 protein (in mdm2+/p53- phenotype) was paradoxically associated with better prognosis. In other studies, however, the presence of mdm2 protein was positively correlated with lymph node involvement [10] or had no impact on survival [11]. Since the prognostic value of mdm2 proteins expression in NSCLC is still a matter of controversy, we decided to assess this issue in a consecutive series of surgically treated NSCLC patients.

Material and methods

The study group included 123 NSCLC patients who underwent curative pulmonary resection at the Department of Thoracic Surgery, Medical University of Gdańsk, Poland, between 1994 and 1998. Tumor samples were formalin-fixed and paraffin-embedded. Three independent pathologists (A.K., K.H.W. and C.B.) assessed tumor type and grade using haematoxylin-eosin stained sections. Stage of disease (pTNM) was determined, after pathological examination of primary tumor and regional lymph nodes.

Mdm2 protein expression [12] and p53 protein [13] expression was assessed with the use of immunohistochemistry, as previously described. Monoclonal antibodies: IF2 clone – against mdm2 and Pab 1801 – against p53 were purchased from Oncogene Science. Two independent observers (K.H.W. and C.B.) assessed the immunostaining. Any nuclear staining for mdm2 was regarded positive ($\geq 1\%$). Data base included the following characteristics: age, sex, smoking habit, tumor histology and degree of differentiation, stage of disease, pTNM designations, date of surgery, adjuvant treatment, date and site of recurrence, survival status including last follow-up or date of death, as well as mdm2 and p53 expression.

Statistics

Chi² and Fisher tests were used to assess the relation between mdm2 and p53 expression, and clinical characteristics. Disease-free survival (DFS) was calculated from the date of surgery to the date of relapse or to the date of death, whichever occurred first. Patient overall survival (OS) was calculated from the day of surgery to the date of last follow-up or date of death. All deceased patients were included in the survival analysis, irrespective of cause of death. Survival curves were calculated according to the Kaplan-Meier method and compared with the use of the log-rank test. Multivariate analysis was based on the Cox regression model with p values lower than 0.05 considered as statistically significant.

Results

Nuclear expression of mdm2 protein was detected in 40 samples (33%). Co-expression of mdm2 and p53 was found in 19 cases (15%). There was no correlation between mdm2 and p53 protein expression even if various cut-off values were taken.

In the chi² analysis there was no correlation between mdm2 expression (analyzed separately or jointly with

p53) and the clinical variables, including patient age and sex, tumor type, grade and stage of disease (Table I). Since only 6 patients (5%) in this series were never smokers, the correlation between mdm2 protein expression and smoking habit was not performed.

Table I. Correlation between mdm2 expression and clinicopathological variables (Chi² test)

Variable	mdm2 (-)	mdm2 (+)	p
Age			
>60 years	41	15	0.29
≤60 years	42	25	
Sex			
female	20	11	0.85
male	63	29	
Stage			
I	39	17	0.77
II	6	5	
IIIA	35	16	
IIIB + IV	3	2	
Histology			
squamous	41	23	0.48
adenous	24	3	
large cell	10	2	
adeno-squamous	8	2	
Grade			
G1	13	7	0.86
G2	50	22	
G3	20	11	
p53			
negative	39	21	0.70
positive	44	19	

At the time of this analysis (June 2005) 29 patients (24%) remained free of disease. Median DFS for the entire group was 28 months, and one- and five-year DFS probability was 61% and 31%, respectively. Median DFS for patients with and without mdm2 protein expression was 12 and 31 months, respectively, and five-year DFS probability was 25% and 35%, respectively ($p=0.22$). There were no differences in DFS between four possible mdm2/p53 phenotypes (mdm2-/p53-, mdm2-/p53+, mdm2+/p53-, mdm2+/p53+) (Table II).

At the time of this analysis, with a median follow-up of 71 months (range 52 to 113), 31 patients were alive (25%). Median OS for the entire group was 34 months and one- and five-year survival probability was 70% and 35%, respectively. Median survival for patients with and without mdm2 expression was 16 months and 38 months, respectively and 5-year survival probability was 24% and 40%, respectively ($p=0.17$).

There was no significant difference in overall survival between particular groups of patients with four possible mdm2/p53 phenotypes (Table II). There was a trend though for increased overall and disease-free survival in patients with mdm2-/p53+ phenotype (Figures 1A. and 1B.).

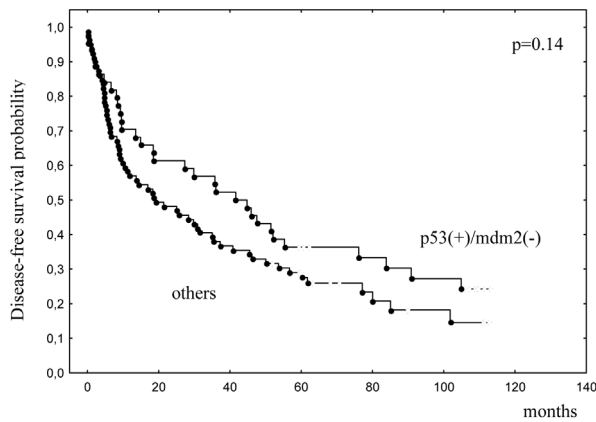


Figure 1A. Kaplan-Meier survival curves for p53(+)/mdm2(-) phenotype vs others - disease-free survival

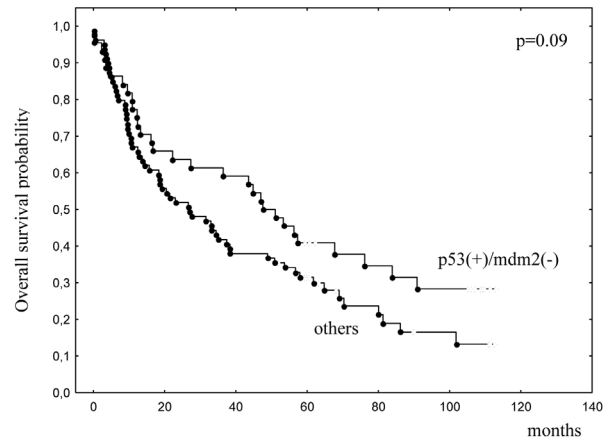


Figure 1B. Kaplan-Meier survival curves for p53(+)/mdm2(-) phenotype vs others – overall survival

Table II. The impact of joint mdm2 and p53 expression on DFS and OS

Patient subgroup	No. (%)	Disease-free survival		Overall survival	
		p	Hazard ratio (95% CI)	p	Hazard ratio (95% CI)
mdm2-/p53- vs. others	39 (32%)	0.72	1.08 (0.64-1.52)	0.67	1.10 (0.66-1.54)
mdm2-/p53+ vs. others	44 (36%)	0.14	0.72 (0.29-1.15)	0.09	0.69 (0.24-1.12)
mdm2+/p53- vs. others	21 (17%)	0.37	1.27 (0.75-1.79)	0.28	1.34 (0.83-1.86)
mdm2+/p53+ vs. others	19 (15%)	0.52	1.20 (0.66-1.76)	0.51	1.20 (0.65-1.76)

Table III. Multivariate Cox proportional hazard model of DFS and OS

Variable	Disease-free survival		Overall survival	
	p	HR (95% CI)	p	HR (95% CI)
Sex	0.77	0.94 (0.53-1.35)	0.93	0.98 (0.57-1.39)
Age	0.07	1.40 (1.03-1.76)	0.06	1.50 (1.12-1.87)
Histology	0.42	1.06 (0.92-1.21)	0.50	1.05 (0.90-1.20)
Stage of disease	<0.001	1.52 (1.36-1.69)	<0.001	1.58 (1.40-1.74)
Grade	0.01	1.47 (1.17-1.77)	0.003	1.60 (1.30-1.92)
mdm2 status	0.56	0.84 (0.51-1.27)	0.75	0.94 (0.56-1.32)
p53 status	0.64	0.92 (0.58-1.27)	0.67	0.93 (0.58-1.28)

HR – hazard ratio, 95% CI – 95% confidence interval

In uni- and multivariate analysis stage of disease and tumor grade were independent prognostic factors for DFS and OS (Table III).

Discussion

In our study, mdm2 protein expression was found in 33% of NSCLC cases. The frequency of mdm2 expression in other lung cancer series ranged widely from 6 to 78% [9-11, 14-16]. These large discrepancies may be due to several factors. One of them seems to be the type of

primary antibody and detection system. We used the APAAP (*alkaline phosphatase anti-alkaline phosphatase*) technique, whereas other authors used the ABC complex (*avidin-biotin-peroxidase*) [9, 14] or SAB (*streptavidin-biotin-peroxidase*) system [11, 17]. Similarly to other authors [9, 10, 14, 15, 17], in this study IF2 clone was used to detect mdm2 protein. This antibody recognizes –NH₂ terminal region of mdm2. Other authors used 1B10 clone recognizing –COOH terminal region of mdm2 protein [10, 15], or SMP-14 clone recognizing mdm2 epitopes located between 154-167 amino acids of mdm2

[11]. There is no commercially available p53 antibody able to distinguish the exact form of accumulated p53 protein (wild or mutated type). Therefore, studies addressing p53 expression in NSCLC can not define the mechanism of p53 accumulation. Similarly, the cause of mdm2 overexpression is unknown, especially in the mdm2-p53 auto-regulatory feedback-loop context.

In this study, we considered as positive samples showing only nuclear expression of mdm2 protein. Some authors included also cases with protein located outside the nucleus e.g. in the cytoplasm [14]. There were also differences in cut-off values. Some authors considered as positive samples showing >0% [14], >5% [18] or >10% of positive cells [9, 10, 15]. Marchetti et al. [14] did not report the criterion of mdm2 positivity, whereas the cut-off value of >50% for p53 protein was taken. All these factors preclude meaningful joint analysis of the results obtained in particular studies.

There have been only a few studies addressing co-expression of mdm2 and p53. In five studies [9, 10, 15, 16, 19] there was no correlation between expression of both proteins and in one the presence of mdm2 coincided with the high expression of p53 [14]. In our series, the co-expression of mdm2 and p53 protein was found in 15% of cases. There was no correlation between the expressions of both proteins, even if the highest expression of p53 was considered (>50%). Some authors suggested that the function of mdm2 is not limited to p53 regulation [10]. It is likely that mdm2 may act in carcinogenesis independently of p53, and that overexpression of this proto-oncogene could lead to neoplastic transformation, also in the absence of other genetic disorders [8].

In this study, similarly to Ko et al. [11], no correlation between expression of mdm2 and p53 and clinicopathological features was observed (for both proteins analyzed jointly or separately). Some studies demonstrated increased mdm2 expression in lung adenocarcinomas [9, 14], whereas others (including ours) failed to show this relation [11, 16, 17]. Similarly to other authors [9, 19], we did not find correlation between mdm2 and tumor grade or stage of disease. However, Gorgoulis et al. [10] reported increased involvement of lymph nodes in tumors with both mdm2 overexpression ($p < 0.005$) and mdm2+/p53+ phenotype ($p < 0.001$). Akiawa et al. [16] demonstrated that the presence of mdm2 was correlated with p21^{WAF1/CIP1} protein expression and stage of disease, but not with p53.

In this series, we did not find any influence of mdm2 expression on DFS and OS. To our knowledge, there have been only five published studies addressing mdm2 protein expression in NSCLC, of which only three analyzed the prognostic value of mdm2.

Joint analysis of mdm2 and p53 expression in this series failed to identify any phenotype related to prognosis. This finding should however be interpreted cautiously due to relatively small patient samples in particular subpopulations. Akiawa et al. [16] reported no prognostic value of mdm2/p53 protein expression in 112

NSCLC patients, despite more frequent mdm2 overexpression in advanced disease. Ko et al. [11] observed no influence of mdm2, p53 protein expression and P53 gene mutation on survival, but the presence of MDM2 mRNA was correlated with better prognosis. Higashiyama et al. [9] suggested that expression of mdm2 and p53 analyzed separately had no impact on survival ($p = 0.62$), however the mdm2+/p53- phenotype was correlated with better prognosis ($p = 0.039$). Gorgoulis et al. [10] demonstrated worse prognosis and more frequent lymph node involvement related to mdm2+/p53+ phenotype, but that study did not include survival analysis.

In summary, our study suggests that the clinical relevance of mdm2 expression be it with or without accompanying p53 expression, remains questionable.

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