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The Prognostic Value of Cyclin D1 Expression in Resected Non-Small-Cell Lung Cancer of Stages I–IIIA – Preliminary Report

Próba oceny prognostycznego znaczenia ekspresji cykliny D1 w operowanym niedrobnokomórkowym raku płuca w stadium I–IIIA – doniesienie wstępne

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Abstract

Background. Lung cancer is the leading cause of cancer death in the majority of developed countries and in Poland. Cyclin D1 regulates the G₁-to-S phase transition and its gene is located on chromosome 11q13.

Objectives. The aim of this study was to evaluate the prognostic significance of cyclin D1 expression in primary resected stage I–IIIA non-small-cell lung cancer (NSCLC).

Material and Methods. The expression of cyclin D1 in paraffin-embedded tumor tissue from 71 patients (53 men and 18 women, mean age: 59.27 ± 8.50 years) was measured using a monoclonal antibody to cyclin D1 (NCL-L-Cyclin D1-Gm clone P2D11F11, Novocastra).

Results. Forty-one (58%) tumor tissue specimens were positive for cyclin D1. Two distinct patterns of staining were observed, but cytoplasmic staining was found in the majority of cases. The prognostic value of cyclin D1 expression was analyzed in all the examined patients and separately in patients with squamous cell lung cancer and adenocarcinoma and for each stage, but only in stage II was there negative correlation between cyclin D1 expression and survival.

Conclusions. Expression of cyclin D1 could be negative prognostic factor in some patients with resected non-small-cell lung cancer (*Adv Clin Exp Med* 2009, 18, 1, 25–31).

Key words: cyclin D1, non-small-cell lung cancer, squamous cell lung cancer, adenocarcinoma, prognostic factor.

Streszczenie

Wprowadzenie. Rak płuca jest główną przyczyną zgonów z powodu chorób nowotworowych w większości rozwiniętych krajów i w Polsce. Cyklina D1 bierze udział w regulacji fazy G₁-S cyklu komórkowego, a jej gen jest umiejscowiony na chromosomie 11q13.

Cel pracy. Próba oceny znaczenia prognostycznego ekspresji cykliny D1 w operowanym niedrobnokomórkowym raku płuca – stadium I–IIIA.

Materiał i metody. Metodą immunohistochemiczną przeprowadzono badanie preparatów guza utrwalonych w parafinie pobranego od 71 pacjentów (w tym 53 mężczyzn i 18 kobiet, średni wiek $59,27 \pm 8,50$). Użyto przeciwciała monoklonalnego przeciw cyklinie D1: NCL-L-Cyclin D1-Gm clone P2D11F11 novocastra.

Wyniki. W 51 preparatach (58%) stwierdzono ekspresję cykliny D1, w większości przypadków była to reakcja cytoplazmatyczna. Analizowano prognostyczne znaczenie ekspresji cykliny D1 w całej badanej grupie, a następnie u chorych na raka płaskonabłonkowego i na gruczolakoraka, a także w poszczególnych stadiach zaawansowania. Stwierdzono jedynie ujemną korelację między ekspresją cykliny D1 a przeżyciem w stadium II.

Wnioski: Ekspresja cykliny D1 może być niekorzystnym czynnikiem rokowniczym u części chorych leczonych operacyjnie z powodu niedrobnokomórkowego raku płuca (*Adv Clin Exp Med* 2009, 18, 1, 25–31).

Słowa kluczowe: cyklina D1, niedrobnokomórkowy rak płuca, rak płaskonabłonkowy, gruczolakorak, czynnik prognostyczny.

Lung cancer is the leading cause of cancer death in the majority of developed countries and in Poland. Non-small-cell lung carcinoma (NSCLC) accounts for about 80% of all lung cancers [1, 2]. The overall five-year survival rates of lung cancer patients are relatively poor. In Europe it is lower than in the USA and amounts to 9.7% in males and 9.6% in females [3]. Despite many studies and improved diagnostic, therapeutic, as well as supportive care options, the prognosis remains unfavorable and long-term survival has hardly changed in recent years [2, 4]. Only patients whose tumors are surgically resectable have better prognosis, with survival ranging from 70% for stage IA to 25% for stage IIIA tumor [1]. The differing survival outcomes among patients within a particular stage suggests the existence of other tumor factors affecting prognosis. Such factors could potentially be used to further classify patients into groups according to substages that may be treated differently. The enormous potential of immunohistochemical methods in searching for prognostic factors is confirmed by a review of literature. The present authors found 462 papers and 12 reviews connected with immunochemistry and prognosis in non-small-cell lung cancer [5].

Uncontrolled cell proliferation is the hallmark of malignant tumors, which is why evaluation of the prognostic significance of the expressions of proteins involved in the regulation of cell proliferation remains promising. Cellular proliferation is regulated by protein complexes composed of cyclins and cyclin-dependent kinases (CDKs). Five major families of cyclins (termed A, B, C, D, and E) have been isolated and described. Cyclin A and B1 expression increase later in the cell cycle, during the S and G₂ phases, and are considered regulators of the transition to mitosis. Cyclins C, D1-3, and E reach their peak of synthesis and activity during the G₁ phase and are believed to regulate the G₁-to-S phase transition [6, 7]. The cyclin D1 gene is located on chromosome 11q13 [8]. The expressions of cyclin D1 and other cyclins have been often evaluated in many cancers and their prognostic value is disputable. In esophageal squamous cell carcinoma and hepatocellular carcinoma the expression of cyclin D1 has been reported to be associated with poor outcome [9–11]. The aim of this study was to evaluate the prognostic significance of cyclin D1 expression in primary resected stage I–IIIA non-small-cell lung cancer (NSCLC).

Material and Methods

Seventy-one patients with resected non-small-cell lung cancer (53 men and 18 women) were

evaluated. The mean age of the patients was 59.27 ± 8.50 years. All patients had undergone surgical treatment (lobectomy, bilobectomy, or pneumonectomy). The histopathological diagnosis was squamous cell carcinoma in 43 patients, adenocarcinoma in 17 patients, large-cell carcinoma in 6 patients, and non-small cell lung cancer of unspecified type in 5 patients. Based on the TNM staging system, 29 patients were in stage I (8 in IA, 21 in IB), 14 in II (2 in IIA, 12 in IIB), and 28 in IIIA. Twenty-seven patients received chemotherapy, of whom 22 received neoadjuvant chemotherapy based on cisplatin (in the majority of cases cisplatin and etoposide). Twenty-four-month survival was evaluated for all the patients; 47 (66%) patients were alive and 24 (34%) had died. The study was approved by the appropriate ethics committees related to the institution.

Immunohistochemistry

Formalin-fixed well-preserved tumor tissue blocks from surgically resected lung cancer specimens were used for the immunohistochemical study. Four- μ m sections of formalin-fixed tissues were mounted on silanized slides, deparaffinized in xylene, and rehydrated through serial baths of alcohol to water. The hydrated sections were treated in 3% hydrogen peroxide for 10 minutes to eliminate endogenous peroxidase activity and washed in phosphate-buffered saline (PBS). The primary antibody used in this study was a monoclonal antibody to cyclin D1 (NCL-L-Cyclin D1-Gm clone P2D11F11, Novocastra). The monoclonal antibody-treated slides were raised in PBS solution and incubated with a biotinylated secondary antibody (LSAB^R+ Kit DAKO). The slides were washed in PBS and then incubated with an avidin-biotin-peroxidase complex (LSAB^R+ Kit, DAKO K 0675) for 15 minutes. After washing with PBS, a chromogenic reaction was developed by incubation with 3,3-diaminobenzidine tetrahydrochloride (DAB+, Liquid K 3486 DAKO). Positive staining appeared as brown cell plasma or nucleus. Cyclin D1 accumulation was considered positive if more than 10% of the cells were stained.

Statistical Methods

Statistical analysis was performed using CSS Statistica for Windows (version 5.0). The chi-squared test was used between two or multiple groups. Differences between samples were considered significant at $p < 0.05$. Survival curves were constructed using the Kaplan-Meier method.

Results

Forty-one of the 71 (58%) tumor tissue specimens were positive for cyclin D1 and 30 (42%) were negative. Two distinct patterns of staining were observed; in the majority of cases cytoplasmic staining was observed and in only 5 cases nuclear staining. Figure 1 shows pictures of immunohistochemical staining.

The prognostic value of cyclin D1 expression in all the patients with non-small-cell lung cancer and separately in the patients with squamous cell carcinoma and adenocarcinoma and in patients in stage I, II, and IIIA was analyzed. Survival was also compared with cyclin D1 expression in the 22 patients in stage IIIA treated with chemotherapy. Only in stage II was a negative correlation between cyclin D1 expression and survival found (Tables 1–2, Figure 2).

Discussion

Cyclin D1 was expressed in 58% of the examined specimens. In the majority of studies, cyclin D1 expression is detected in about 50% of cases (Ayeda et al. 49%, Betticher et al. 47%, Nguyen et al. 51%) [12–14], but the differences in the degree of expression could be significant. Radovic et al. did not find expression of cyclin D1 in 14 cases of non-small-cell lung cancer [15], Mishina et al. observed it in only 11.7% of cases [16], and Volm et al. showed expression in as much as 72% of cases [17]. These authors describe cyclin D1 as predominantly present in the cytoplasm of lung carcinoma cells [13, 14], which the present findings confirm.

The present study found that cyclin D1 expression was an unfavorable prognostic factor only in stage II disease (Cox-Mantel test, $p = 0.00018$). In the other groups there was no correlation between cyclin D1 expression and survival. Correlation between cyclin D1 expression and clinicopathological findings as well as prognosis remains disputable. Mishina et al. showed that five-year survival was longer in patients with cyclin D1-positive tumors (89% vs. 64%) and cyclin D1 expression tended to be a favorable prognostic factor in univariate analysis ($p = 0.08$) [16]. Ayeda et al. observed in 98 patients with resected stage I and II NSCLC that patients with cyclin D1-positive tumors had shorter survival than those with negative expression (five-year survival rates of 48% vs. 74%, $p = 0.006$). These authors also indicated a correlation between cyclin D1 and unfavorable clinicopathological findings. Cyclin D1 expression was significantly higher in patients with poorly differentiated carcinoma with presence of vascular invasion and visceral pleural invasion [12]. Other research showed that negative cyclin D1 expression could be a favorable prognostic factor only in combination with other markers. The following combinations of markers may have favorable prognostic value: p53 positivity and low Ki-67 expression, p53 positivity and lack of cyclin D1 expression, bcl-2 positivity and low Ki-67 expression, and lack of cyclin D1 expression and low Ki-67 expression [13]. Experimental studies indirectly confirmed the oncogenic potential of cyclin D1. The overexpression of D-type cyclin accelerated the G₁ phase in rodent cells and microinjection of cyclin D1 antibodies into dividing cells blocked them in the G₁ phase [18].

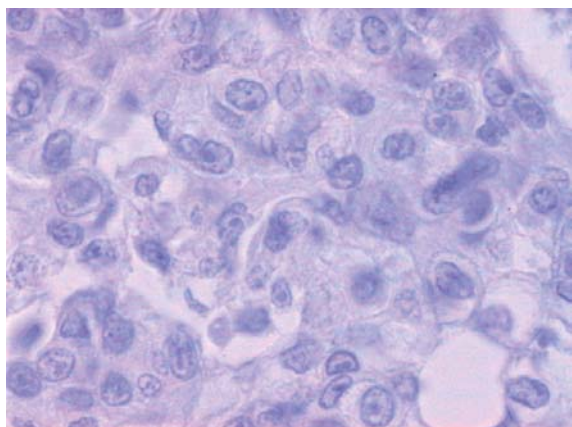
Table 1. Comparison of 24-month survival depending on histological type

Tabela 1. Porównanie 24-miesięcznego przeżycia z ekspresją cykliny D1 w zależności od typu histologicznego

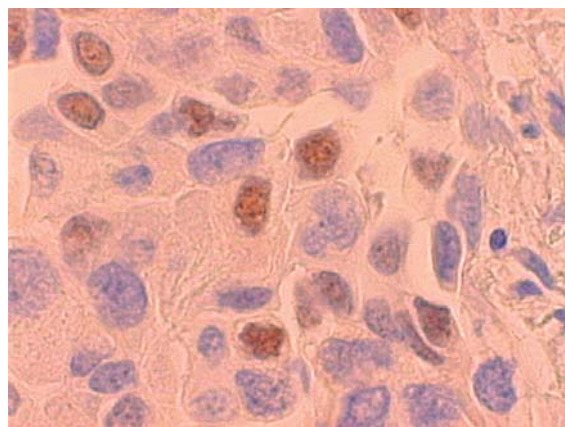
Survival (Przeżycie)	Cyclin D1 positive; n (%) (Dodatnia ekspresja cykliny D1)	Cyclin D1 negative; n (%) (Brak ekspresji cykliny D1)	Chi ²	<i>p</i>	Cox- Mantel
All patients with NSCLC (Wszyscy badani pacjenci)					
< 24 months (mies.)	16 (39.02%)	8 (26.67%)	1.18	0.28	0.40
> 24 months (mies.)	25 (60.98%)	22 (73.33%)			
Patients with squamous cell carcinoma (Chorzy na raka płaskonabłonkowego)					
< 24 months (mies.)	9 (39.13%)	7 (35%)	0.08	0.78	0.83
> 24 months (mies.)	14 (60.87%)	13 (65%)			
Patients with adenocarcinoma (Chorzy na gruczolakoraka)					
< 24 months (mies.)	6 (50%)	1 (20%)	1.31	0.25	0.22
> 24 months (mies.)	6 (50%)	4 (80%)			

Table 2. Comparison of 24-month survival depending on stage**Tabela 2.** Porównanie 24-miesięcznego przeżycia z ekspresją cykliny D1 w zależności od stopnia zaawansowania

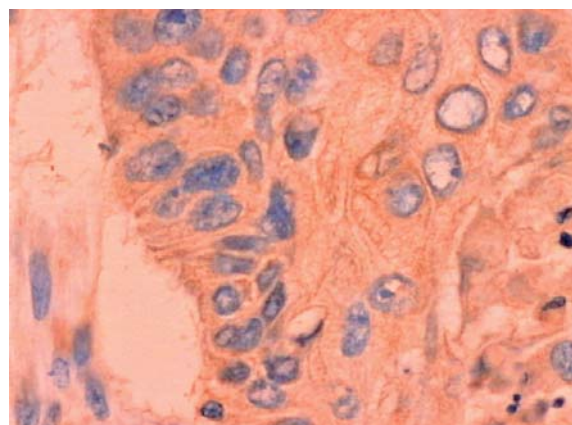
Survival (Przeżycie)	Cyclin D1 positive; n (%) (Dodatnia ekspresja cykliny D1)	Cyclin D1 negative; n (%) (Brak ekspresji cykliny D1)	Chi ²	<i>p</i>	Cox- Mantel
Patients in stage I (Pacjenci w stadium I)					
< 24 months (mies.)	3 (17.65%)	1 (8.33%)	0.51	0.47	0.47
> 24 months (mies.)	14 (82.35%)	11 (91.67%)			
Patients in stage II (Pacjenci w stadium II)					
< 24 months (mies.)	5 (100%)	1 (11.11%)	10.37	0.0013	0.00018
> 24 months (mies.)	0 (0%)	8 (88.89%)			
Patients in stage IIIA (Pacjenci w stadium IIIA)					
< 24 months (mies.)	8 (42.11%)	6 (66.67%)	1.47	0.22	0.22
> 24 months (mies.)	11 (57.89%)	3 (33.33%)			
Patients in stage IIIA treated with chemotherapy (Pacjenci w stadium IIIA leczeni chemioterapią)					
< 24 months (mies.)	8 (57.14%)	5 (62.50%)	0.06	0.80	0.81
> 24 months (mies.)	6 (42.86%)	3 (37.53%)			



A



B



C

Fig. 1. A – Negative immunostaining for cyclin D1, B – Positive nuclear immunostaining for cyclin D1, C – Positive cytoplasmic immunostaining for cyclin D1

Ryc. 1. A – Brak ekspresji cykliny D1, B – Jądrowa ekspresja cykliny D1, C – Cytoplazmatyczna ekspresja cykliny D1

The correlation between cyclin D1 expression and smoking or other chronic pulmonary diseases could influence analyses. It has been shown that the carcinomas of smokers often expressed cyclin D1 (in 77% of the cases, in carcinomas of non-smok-

ers 57%) [17] and the expression of cyclin D1 is higher in patients with obstructive pulmonary disease [12]. Some authors suggest that cyclin D1 gene polymorphism could modulate smoking-induced cancer risk and response to platinum-

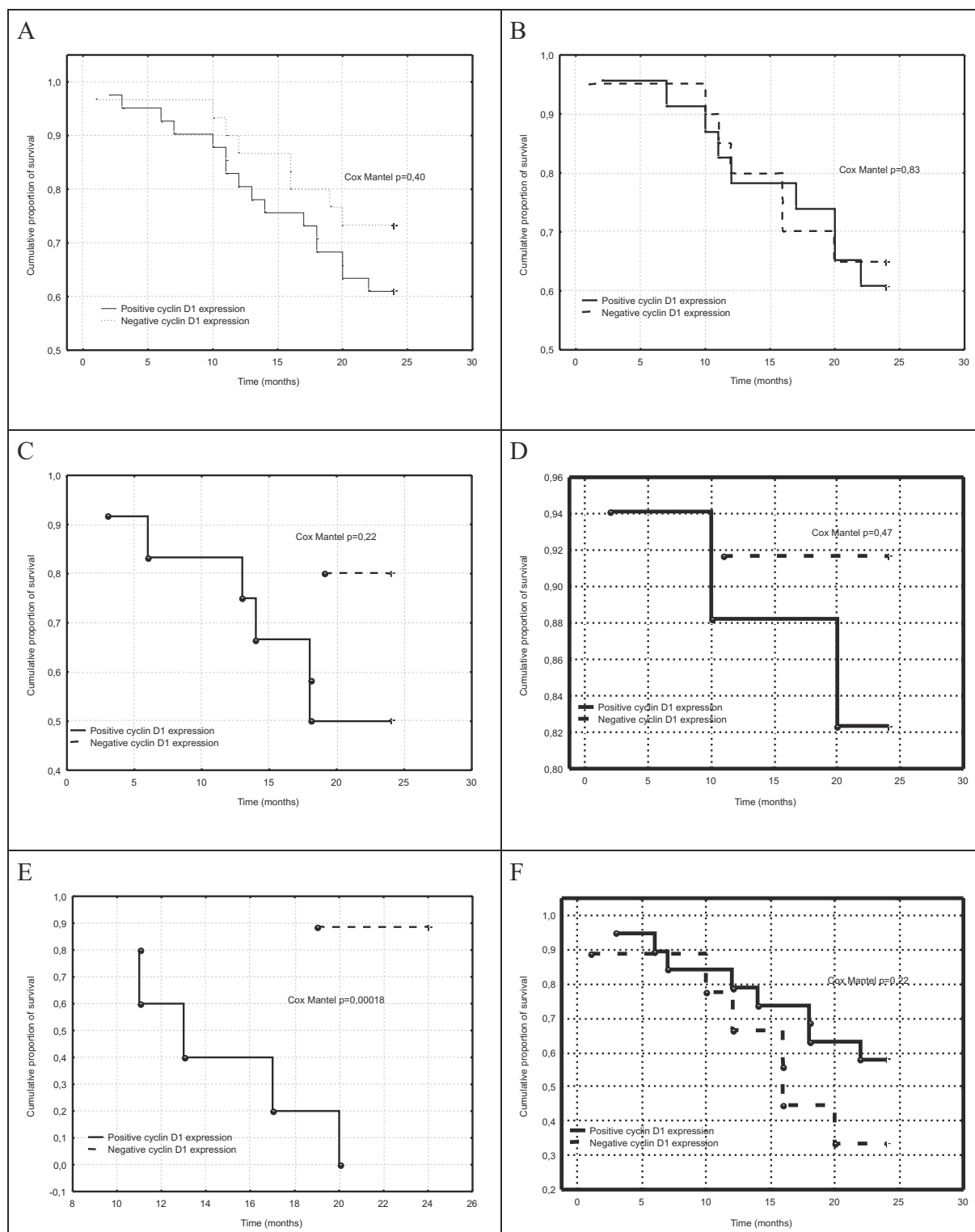


Fig. 2. Cumulative proportion of Kaplan-Meier survival according to cyclin D1 expression: A – in all patients with non-small-cell lung cancer; B – in patients with squamous cell lung cancer; C – in patients with adenocarcinoma; D – in patients in stage I; E – in patients in stage II; F – in patients in stage IIIA

Ryc. 2. Skumulowana proporcja przeżyjących Kaplana-Meiera w zależności od ekspresji cykliny D1:

A – u wszystkich chorych na raka niedrobnokomórkowego; B – u chorych na raka płaskonabłonkowego;

C – u chorych na gruczolakoraka; D – u pacjentów w stadium I; E – u pacjentów w stadium II; F – u pacjentów w stadium IIIA

based chemotherapy [19]. In the present study only 22 patients of the 71 patients received chemotherapy and no correlation between survival and cyclin D1 expression in this group was observed.

The latest studies indicate that glycogen synthase kinase-3 β (GSK-3 β), a protein kinase which plays an important role in the determination

of cyclin D1 expression level by regulating mRNA transcription and protein degradation, could be a potential new target for chemotherapy [20].

The authors concluded that the expression of cyclin D1 could be a negative prognostic factor in some patients with resected non-small-cell lung cancer.

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