

Prognostic Significance of Cyclin E Overexpression in Resected Non-Small Cell Lung Cancer

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Abstract

Cyclin E plays a pivotal role in the regulation of G₁-S transition and relates to malignant transformation of the cells. However, the clinical significance of cyclin E expression in patients with non-small cell lung cancer remains unknown. We examined the expression of cyclin E in 242 resected non-small cell lung cancer in pathological stages I-IIIa and analyzed its relation to clinicopathological factors. Cyclin E overexpressions were observed frequently in deeply invasive tumors. Multivariate analysis revealed that complete resection, pathological stage, and cyclin E expression were independent prognostic indicators. When cyclin E and proliferating cell nuclear antigen are combined, the cases negative for both had a significantly better prognosis than the other cases. We concluded that cyclin E overexpression relates to deeply invasive tumors and is correlated with poor prognosis. New therapeutic options may be provided by combination of cyclin E expression and proliferating cell nuclear antigen overexpression.

Introduction

Lung cancer has become one of the leading causes of cancer death in the world. Lung cancer consists of a heterogeneous group of tumors with distinct biological and clinical characteristics. The cell cycle is governed by a family of Cdks² (1). Cyclins are prime cell cycle regulators and play a central role in the control of cell proliferation by forming a complex with different Cdks (1-3). Cyclins D1 and E are known to cooperate with Cdk2 and function in G₁ and in the G₁-S transition (1). Abnormalities in cell cycle regulators and subsequent deregulation of the G₁-S transition may be one of the most important biological events in malignant cell transformation (4-6). Overexpression of cyclin D1 has been described as a negative prognostic factor in lung cancer and several other carcinomas (7, 8). However, to our knowledge, the prognostic role of cyclin E has not been reported in lung cancer. Moreover, Keyomarsi *et al.* (4) have reported that cyclin E expression increased with increasing grade and stage of the tumor and that these alterations were more consistent than c-erb B2 or cyclin D1 overexpression in breast cancer. Proliferation activity is a potent biological marker that estimates the growth of neoplasms quantitatively and can aid in determining the prognosis of patients with carcinomas (9). PCNA is a nonhistone nuclear protein with a molecular weight of 36 kDa and functions as an auxiliary factor of DNA polymerase δ , an enzyme playing a key role in DNA replication (10). This protein is expressed specifically in the cell nucleus from late G₁ to S (11). We have previously demonstrated that PCNA expression is a significant prognostic determinant in NSCLC with intrapulmonary

metastases (12). Several authors also reported that PCNA expression is a significant prognostic determinant in NSCLC (10, 13). We examined the expression of cyclin E immunohistochemically and analyzed its relation to clinicopathological factors including PCNA expression and the prognostic value of cyclin E and PCNA.

Patients and Methods

Of the consecutive 276 NSCLC patients with pathological stage I-IIIa who were hospitalized in our department and underwent operations between 1988 and 1993, we chose those who met the following conditions as our subjects: (a) those who had not undergone induction chemotherapy or radiation therapy before operation; (b) those whose primary focus of the tumor was completely resected macroscopically and underwent mediastinal lymphadenectomy; and (c) cyclin E and PCNA expressions in the primary tumors could be evaluated. Distant metastasis was evaluated using computed tomography of the head and abdomen and bone scinti. The subjects were 242 patients consisting of 164 men and 78 women. Their average age was 62.3 ± 9.2 years. Their histological types were adenocarcinomas in 148 cases, squamous cell carcinomas in 85 cases, large cell carcinomas in 6 cases, and adenosquamous carcinomas in 3 cases. Their pathological stages were stage I in 98 cases, stage II in 30 cases, and stage IIIa in 114 cases (Table 1). Their operations were segmental resections in 2 cases, lobectomy in 206 cases, bilobectomy in 19 cases, and pneumonectomy in 15 cases. Two hundred twenty-nine patients (94.6%) underwent complete resection. A complete resection was defined as a surgical procedure in which all gross tumor was removed, the microscopic margins were histologically normal, and the highest node sampled was free of tumor. The tumor-node-metastasis classification was determined according to the International Union against Cancer staging system (14). Staining by immunohistochemistry was performed on formalin-fixed and paraffin-embedded resected specimens with labeled streptavidin-biotin method using DAKO LSAB Kit (DAKOPATTS AB, Copenhagen, Denmark), as we reported before (12, 15). The resected specimens were sliced into 4- μ m sections, deparaffinized, and hydrated with ethanol. Microwave treatment in distilled water was performed for 30 min on the specimens for cyclin E stain. Endogenous peroxidase activity was inhibited using 0.3% H₂O₂ containing methanol. Mouse monoclonal antibody against cyclin E (HE12 diluted to 1:50; Santa Cruz Biotechnology, Inc., Santa Cruz, CA) and PCNA (PC10 diluted to 1:500) (DAKOPATTS AB) were used as primary antibodies and they were made to react at 4°C for 12 h. They were made to react with secondary antibodies and finally made to react using peroxidase labeled streptavidin as an enzyme reagent. Diaminobenzidine (DAKOPATTS AB) was used for color development.

At least five visual fields of the immunohistochemically stained sample were observed at random at a magnification of $\times 100$ or 400. Over 1000 tumor cells were counted, and two investigators (T. F. and T. H.) who were blinded to the clinical data analyzed the rate of positivity. If the rate of positive cells was 20% or more, the case was diagnosed as positive. Survival rates were calculated with Kaplan-Meier method, and the differences between survival rates were tested with log-rank test. The Cox proportional hazards models were used for multivariate analysis. *P* values less than 0.05 were considered statistically significant.

Results

Survival Curve for All of the Cases. In the 242 cases, the 3-year survival rate was 66.3%, and the 5-year survival rate was 61.0%.

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² The abbreviations used are: Cdk, cyclin-dependent kinase; NSCLC, non-small cell lung cancer; PCNA, proliferating cell nuclear antigen.

Expression of Cyclin E and PCNA. Expression of cyclin E (Fig. 1A) was heterogeneous in most tumors. Immunoreaction to cyclin E was localized in the nuclei of neoplastic cells. 47.1% (114 of 242 cases) were determined to be cyclin E positive. For PCNA (Fig. 1B), nuclear staining was detected in 166 of 242 cases (68.6%).

Cyclin E overexpression was observed frequently in deeply inva-

Table 1 Characteristics and cyclin E expressions of cases with stage I-IIIa

Variable	Total	Cyclin E expression		P
		Negative	Positive	
Sex				NS ^a
Male	164	88	76	
Female	78	40	38	
Age (mean ± SD)	62.3 ± 10.4			NS
Histology				NS
Adenocarcinoma	148	85	63	
Squamous cell carcinoma	85	41	44	
Large cell carcinoma	6	2	4	
Adenosquamous cell carcinoma	3		3	
P stage				NS
I	98	56	42	
II	30	15	15	
IIIa	114	57	57	
pT factor				0.043
T ₁	86	53	33	
T ₂	110	54	56	
T ₃	46	21	25	
pN factor				NS
N ₀	121	69	52	
N ₁	32	16	16	
N ₂	89	43	46	

^a NS, not significant.

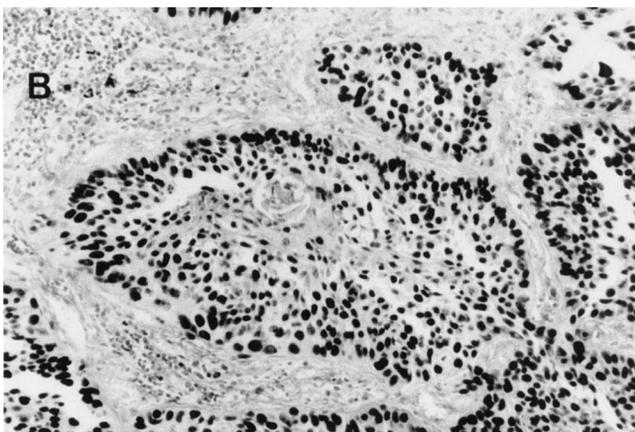
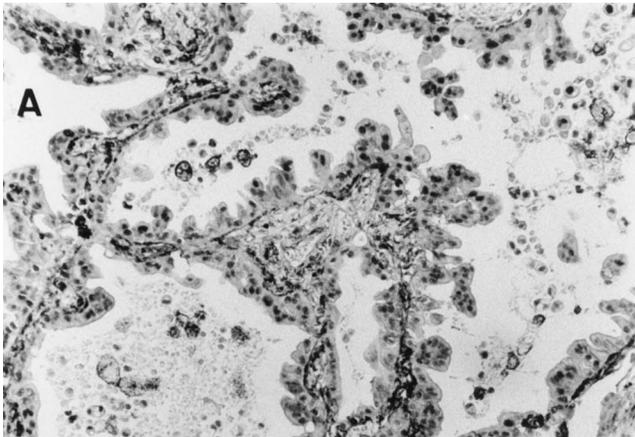


Fig. 1. A, immunohistochemical staining of cyclin E in NSCLC. Most carcinoma cells were positive for cyclin E. × 100. B, immunohistochemical staining of PCNA in NSCLC. Most carcinoma cells were positive for PCNA. × 100.

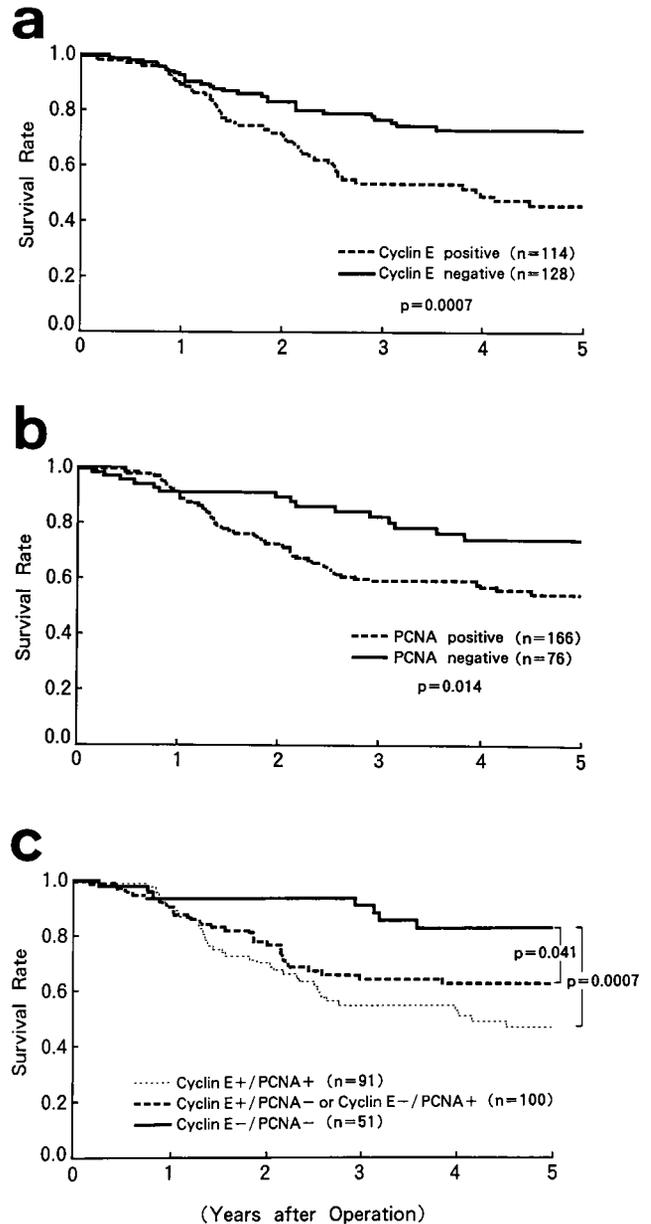


Fig. 2. A, survival curves of cases with NSCLC according to cyclin E expression. B, survival curves of cases with NSCLC according to PCNA expression. C, survival curves of cases with NSCLC according to cyclin E and PCNA expression status.

sive tumors (T₁ versus T₂ and T₃; $P = 0.043$). The rate of cyclin E positive cells had no significant correlation with lymph node metastases, histology, differentiation, or sex (Table 1).

Ninety-one patients had both cyclin E- and PCNA-positive tumors, 75 had cyclin E-negative and PCNA-positive tumors, 25 had cyclin E-positive and PCNA-negative tumors, and 51 had both cyclin E- and PCNA-negative tumors. No significant correlation was observed between cyclin E expression and PCNA expression (Pearson correlation coefficient, 0.16; $P = 0.038$).

Prognostic Significance of Cyclin E and PCNA. Tumors that were cyclin E positive had significantly worse prognosis than those that were cyclin E negative ($P = 0.0007$; Fig. 2a). Tumors that were PCNA positive had significantly worse prognosis than those that were PCNA negative ($P = 0.014$; Fig. 2b). When cyclin E and PCNA were combined, cases negative for both had significantly better prognosis than cases positive for either or both ($P = 0.041$ and 0.0007 , respectively; Fig. 2c).

Table 2 Multivariate prognostic factor analysis

Factors	RR ^a	95% CI	P
Sex	0.661	0.398–1.099	0.110
Male/female			
Histology	1.001	0.713–1.400	0.992
Ad/Sq/Lc/Adsq			
Differentiation	0.899	0.681–1.187	0.453
Poorly/moderate/well			
P-stage	1.457	1.008–2.105	0.045 ^b
I/II/IIIa			
Complete resection	1.907	1.366–2.663	0.0002 ^b
Yes/no			
Cyclin E expression	1.923	1.190–3.109	0.0076 ^b
Negative/positive			
PCNA expression	1.706	0.957–3.04	0.0702
Negative/positive			

^a RR, relative risk; CI, confidence interval.

^b $P < 0.05$, considered statistically significant.

Multivariate Analysis Using the Cox Proportional Hazards Models. Of sex, histological type, cyclin E expression, PCNA expression, complete resection, and pathological stage, the significant factors were complete resection, pathological stage, and cyclin E expression; PCNA expression was not a significant factor (Table 2).

Relationship between Cyclin E and PCNA Expressions and Prognosis According to Pathological Stages. In pathological stage I (Ia and Ib), with univariate analyses, cyclin E-positive cases had a significantly worse prognosis than cyclin E negative cases ($P = 0.041$). However, with multivariate analyses, pathological T factor was the only significant prognostic factor ($P = 0.022$). No significant difference was observed between PCNA expressions and prognosis. In stage II (IIa and IIb), neither cyclin E expression nor PCNA expression was a significant prognostic factor. In stage IIIa, both cyclin E expression and PCNA expression were significant prognostic factors with both univariate and multivariate analyses.

Discussion

Alterations in multiple cyclins in several cancers have been reported, and abnormal expression may cause proliferation and may contribute to the development and progression of cancer (6, 16, 17). Abnormalities in cyclin E have been reported in several cancers. It is reported that cyclin E overexpression was observed in 27% of breast carcinoma (6), 40–60% of gastric cancer (16), and 92% of colorectal cancer (17). No report was available on NSCLC, but our study has revealed cyclin E overexpression in 47.1% of the cases. It has been reported that cyclin E overexpression relates to deeply invasive tumors, lymph node metastasis, and advanced stage (4, 16). In this study also, a correlation was observed between T factor and cyclin E overexpression, but no correlation was observed between cyclin E overexpression and histological type, differentiation, or N factor. We also analyzed PCNA overexpression, which is often used as a marker for proliferate activity. The univariate analysis revealed that PCNA overexpression was a significant prognostic factor. However, multivariate analysis, including cyclin E, revealed that PCNA overexpression was not an independent prognostic indicator. No correlation was observed between PCNA overexpression and cyclin E overexpression. A previous study by Keyomarsi *et al.* (4) also revealed no correlation between cyclin E and PCNA overexpressions in breast cancer. Studies by Dutta *et al.* (5) and Porter *et al.* (18), however, revealed a correlation between cyclin E overexpression and Ki-67 overexpression, which is one of the proliferation factors. These studies reported that cyclin E is superior to PCNA or Ki-67 in that it works as a marker for oncogenesis and not just for proliferation and that it selectively detects tumor cells committed to cell division (late G₁ and beyond), possibly enabling prediction of responsiveness to chemotherapy targeted at cells in S and M phases. In this study, also, cyclin E overexpression was proved to be a prognostic indicator with both univariate and multivariate analyses, which may

suggest that cyclin E is more sensitive as a prognostic factor than PCNA. Also, the facts that there was no correlation between PCNA overexpression and cyclin E overexpression and that cases negative for both have a significantly better prognosis than those positive for either or both suggests more specific therapeutic options if these two factors are combined.

In this study, neither cyclin E overexpression nor PCNA overexpression was a significant prognostic factor in stage I, but both were significant prognostic factors in stage IIIa cases. These results were consistent with our previous studies in which we demonstrated that PCNA expression was a significant prognostic factor in cases with intrapulmonary metastases but not in cases with stage I (12, 15). Thus, in the advanced stage, in which there are massive metastases, proliferation activity may be an important prognostic factor, but in the early stage, angiogenesis factors or adhesive factors may be more important than proliferation factors. Recently, Bergers *et al.* (19) also proved that antiangiogenic drugs are most effective in specific stages of cancer. In the future, prognostic factors dominant in specific stages or therapeutic options might be elucidated.

Our study is considered significant in that more than 200 resected NSCLC cases are involved and that it clearly indicates the correlation between cyclin E and prognosis in NSCLC.

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