Observational Study

Medicine

OPEN

Expression and prognostic impact of DNA-PK in human lung cancer

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Abstract

Among all cancer patient's lung cancer is the leading cause of death. Prognostic biomarkers continue to be investigated for the detection and stratification of lung cancer for clinical use. The DNA-dependent protein kinase is involved in mechanisms of DNA damage repair. Deregulation and overexpression of DNA-dependent protein kinase is associated with poor prognosis in various tumor entities. In this study, we investigated the expression of DNA-dependent protein kinase in relation to clinicopathological features and overall survival in patients with lung cancer.

By immunohistochemistry, expression of DNA-dependent protein kinase was analyzed in 205 cases of lung cancer; 95 cases of adenocarcinoma, 83 cases of squamous cell lung carcinoma and 27 cases of small cell lung cancer and correlated with clinicopathological characteristics as well as patient's overall survival.

In patients with adenocarcinoma, a significant correlation between strong expression of DNA-dependent protein kinase and worse overall survival was found. No significant association was observed in patients with squamous cell lung carcinoma and small cell lung cancer. Strong detection of DNA-dependent protein kinase expression was most evident in small cell lung cancer (81.48 %), followed by squamous cell lung carcinoma (62.65 %) and adenocarcinoma (61.05 %).

In our study, expression of DNA-dependent protein kinase was associated with poor overall survival in patients with adenocarcinoma. DNA-dependent protein kinase could serve as a new prognostic biomarker.

Abbreviations: AC = adenocarcinoma, DNA-PK = DNA-dependent protein kinase, NSCLC = non-small cell lung cancer, SCLC = small cell lung cancer, SQCLC = squamous cell lung carcinoma.

Keywords: DNA, human lung cancer, PK

1. Introduction

Lung cancer is the leading cause of death among all cancer patients.[1] And even by 2030, lung cancer is expected to remain the leading cause of cancer-related death in the United States and Germany.[2,3] The treatment of choice for patients diagnosed with non-small cell lung cancer (NSCLC) in stage I or II (according to TNM Classification and Union for international cancer control, UICC stage) is surgical resection, which is performed in more than half of patients. In patients with stage III NSCLC, surgery is rarely performed; here, most patients receive treatment with chemotherapy and/or radiotherapy.[4]

Lung cancer is differentiated into small cell lung cancer (SCLC) and NSCLC which is further differentiated into adenocarcinoma (AC, 40%) and squamous cell lung carcinoma (SQCLC, 25%) based on histology. [5,6] In addition, lung cancer can be differentiated into many molecularly heterogeneous subgroups that require individualized therapy and are important for prognostic assessment.[7,8]

DNA-dependent protein kinase (DNA-PK) is an enzyme complex composed of the enzyme DNA-PKcs and the Ku70/Ku80 complex. The DNA-PKcs subunit is a serine/threonine protein kinase that, like the ATM and ATR kinases, belongs to the PI3K/PI4K family.[9] DNA-PK is involved in mechanisms of DNA damage repair that include nonhomologous end joining via activation of endo- and exonucleases, V(D)J recombination, class switch recombination, and telomere stability. [10,11] DNA double-strand break activates the DNA-PK. In this process, the DNA-PKcs forms the catalytic subunit and Ku70/80 binds with high affinity to free DNA ends in a sequence-independent

AT and OE contributed equally to this work.

HB and OE are supported by the Deutsche Krebshilfe Foundation (grant: 70112551). HB is supported by the University Medical center Göttingen and the Else-Kröner-Fresenius-Foundation. SY and YL are supported by the Chinese Scholarship Counsil.

The authors have no conflicts of interest to disclose.

All data generated or analyzed during this study are included in this published article [and its supplementary information files].

Supplemental Digital Content is available for this article.

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How to cite this article: Tirilomi A, Elakad O, Yao S, Li Y, Hinterthaner M, Danner BC, Ströbel P, Tirilomis T, Bohnenberger H, von Hammerstein-Equord A. Expression and prognostic impact of DNA-PK in human lung cancer. Medicine 2023;102:9(e33143).

Received: 17 December 2022 / Received in final form: 8 February 2023 / Accepted: 9 February 2023

http://dx.doi.org/10.1097/MD.0000000000033143

manner.[12] This activates other substances, including the histone variant γ-H2AX in response to DNA damage.

Interestingly, DNA-PK plays a critical role in basic cellular processes in cancer. Deregulation and overexpression of DNA-PK is often detectable, which is associated with poor prognosis in tumors.[13] Effect of DNA-PK deregulation in cancer has been demonstrated in various tumor entities, including nasopharyngeal cancer, colorectal carcinoma, serous ovarian tumors and lymphoid malignancies.[14–17] The important role of DNA-PK as a prognostic factor in NSCLC has been investigated.[18,19] An increasing overexpression of DNA-PK was detected with increasing degree of differentiation of NSCLC. This may be a cause of radioresistance in NSCLC.[18] In addition, increased expression of DNA-PK is associated with poor prognosis in NSCLC patients.[19] Since DNA-PK plays an important role in carcinogenesis and progression, this kinase is already being further investigated as a therapeutic target by inhibition in preclinical and clinical trials, such as in uveal melanoma.[20,21]

In this study, expression of DNA-PK was investigated in relation to clinicopathological features as well as overall survival in patients with non-small cell AC and SQCLC and SCLC.

2. Methods

Tissue samples from patients who underwent lung cancer surgery at the Department of Thoracic and Cardiovascular Surgery, University Medical Center Göttingen, Germany, were analyzed for DNA-PK expression. Informed consent was obtained from all patients. The study was performed according to the Declaration of Helsinki (October 2013 version) and institutional, state, and federal guidelines and after approval by the Ethics Committee of the University Medical Center Göttingen (#1-2-08). Adult patients (over 18 years of age) with a histology of AC, SQCLC, or SCLC were included. Patients with other histology, with unresectable tumor or those with neoadjuvant treatment were excluded.

2.1. Immunohistochemical staining

Tissue microarrays were immunohistochemically stained as previously described.[22] First, the 2-mm tissue sections were incubated in En Vision Flex Target Retrieval Solution at low PH (Dako/agilent, Santa Clara, CA) followed by incubation with the primary antibody against DNA-PK (Novus Bio, #NPB2-33995, 1:500) for 20 minutes at room temperature. Visualization was performed with a polymeric secondary antibody coupled to horseradish peroxidase (EnVision Flex+, Dako) and DAB (Dako). Counterstaining was performed with Meyer hematoxylin and finally the tissue samples could be analyzed by light microscopy. According to the intensity of the staining, findings were classified to; Negative; Weak expression, and; Strong expression.

2.2. Statistical analysis

Data were collected using Microsoft Excel 2010 and GraphPad Prism was used (version 7.00 for Windows, GraphPad Software, La Jolla, CA, www.graphpad.com) for statistical analysis. Chi-square test and Student *t* test were used to analyze the correlation between clinicopathologic characteristics and protein expression of DNA-PK. Patient's survival in relation to DNA-PK expression was examined using Kaplan–Meier estimators. Mantel-Cox log rank test was used to calculate the differences. *P* values < .05 were considered statistically significant.

3. Results

3.1. Patient characteristics

A total of 205 patients with lung cancer (139 men and 66 women) were included in this study. On average, patients were 67 years old (range 34–85 years), with 1-third of patients younger than 60 years. The leading histology was non-small cell lung cancer (NSCLC); 95 patients had adenocarcinoma and 83 patients had SQCLC. Significantly fewer patients (27 patients in total) had SCLC. Resection status R0 was achieved in over 90% of patients.

Lymph node metastasis was detected in <50% of patients. Detailed data on patient's characteristics, clinical status, and degree of tumor differentiation are shown in Table 1 and Table S1, Supplemental Digital Content, http://links.lww.com/MD/I572.

3.2. Impact of DNA-PK expression and survival

Strong detection of DNA-PK expression was most evident in 22 patients (81.48 %) of SCLC detected, followed by 52 patients

Table 1

Patient characteristics.

Feature	Cases	AC	SQCLC	SCLC
Total	205	92	83	27
Age median (range)	67 (39–85)	68 (34–85)	67 (49–83)	67 (54–81)
Gender				
Male	139 (67.8%)	51 (53.7%)	66 (79.5%)	22 (81.5%)
Female	66 (32.2%)	444 (46.3%)	17 (20.5%)	5 (18.5%)
Age				
≤60	68 (32.2%)	29 (30.5%)	28 (33.7%)	9 (33.3%)
>60	139 (67.8%)	66 (69.5%)	55 (66.3%)	18 (66.7%)
Degree of differentiation				
I + II	133 (65.5%)	67 (71.3%)	66 (79.5%)	0 (0%)
III	70 (34.5%)	27 (28.7%)	17 (20.5%)	32 (100%)
Lymph node metastasis				
No	113 (73.2%)	55 (29.1%)	44 (53.0%)	14 (63.6%)
Yes	85 (42.9%)	38 (40.9%)	39 (47.0%)	8 (34.6%)
Clinical stage				
I + II	145 (73.2%)	70 (75.3%)	56 (67.5%)	19 (86.4%)
III + IV	53 (26.8%)	23 (24.7%)	27 (32.5%)	3 (13.6%)
Resection status				
R0	179 (91.8%)	84 (93.3%)	74 (90.2%)	23 (91.3%)
R1 + 2	16 (8.2%)	6 (6.7%)	8 (9.8%)	2 (8.7%)

AC = adenocarcinoma, SCLC = small cell lung cancer, SQCLC = squamous cell lung cancer.

(62.65 %) of SQCLC and 58 patients (61.05 %) of AC. Weak immunostaining of DNA-PK was seen in 27 patients (32.53 %) from SQCLC, 22 patients (23.16 %) from AC and 3 patients (11.11 %) from SCLC. Negative result was most frequently observed in 15 patients (15.79 %) from AC, followed by 2 patients (7.41 %) from SCLC and 4 patients (4.82 %) from SQCLC (Fig. 1).

In patients with AC, SQCLC, and SCLC, the correlation between DNA-PK expression level and overall survival was assessed using the Kaplan-Meyer-Estimation. The median follow-up time was 23 months (range 1–125). In patients with AC, a significant correlation between strong expression of DNA-PK and worse overall survival (median survival 32.5 vs 20 months, hazard ratio = 0.2163, 95% CI of ratio = 0.0951 to 0.4922, and P = .0200) was observed (Fig. 2A). No significant association was observed in patients with SQCLC and SCLC (Fig. 2B and C).

3.3. Protein expression of DNA-PK in lung cancer patients and correlation with clinicopathological characteristics

Within the AC subgroup, there were no statistically significant correlation between expression of DNA-PK and any clinicopathological features of patients (Table 2).

4. Discussion

High mortality rate of lung cancer is often related to late diagnosis at an advanced stage of the disease.[23] Patients with lung cancer are treated based on TNM classification and UICC stage of the cancer. Surgical resection plays an important role in stage I and II of NSCLC, with adjuvant therapy increasing in importance from stage II onwards. Lung cancer is characterized by molecularly heterogeneous subgroups and requires individualized therapy.[4,24]

DNA-PK play an important role in DNA damage repair.[10] Overexpression of DNA-PK in cancer cells reflects increased DNA repair in cancer cells due to the unstable genome, resulting in resistance of cancer cells to chemotherapy and radiotherapy.[25,26] For DNA-PK, a therapeutic approach by inhibition in combination with chemotherapy or radiotherapy is already being explored in various tumor entities.[27,28]

In glioblastoma, DNA-PK was shown to regulate the central transcription factor SOX2 and interact with SOX2. Inhibition of DNA-PKcs impaired tumor growth in glioblastoma and resulted in sensitization to radiotherapy.[29] A better response to radio chemotherapy by inhibiting DNA-PK was also observed in rectal cancer models.[30] Wang et al[28] demonstrated increased expression of DNA-PK in human tissue from NSCLC, which was associated in patients with adverse outcome. Combined therapy of a DNA-PK inhibitor with chemotherapy in NSCLC was investigated. This showed an enhanced effect of chemotherapy when combined with a DNA-PK inhibitor in NSCLC, resulting in tumor regression.[28]

Therefore, in patients with lung cancer, the expression of DNA-PK and the possible importance of this kinase as a prognostic biomarker were investigated in this present study.

Immunohistochemically, the strongest detection of DNA-PK occurred most in SCLC, followed by SQCLC and AC. Regarding overall survival, a correlation between poorer overall survival and DNA-PK expression was found in the AC subgroup.

Hsu et al[11] demonstrated that overexpression of DNA-PK leads to therapy resistance to chemotherapy and radiotherapy

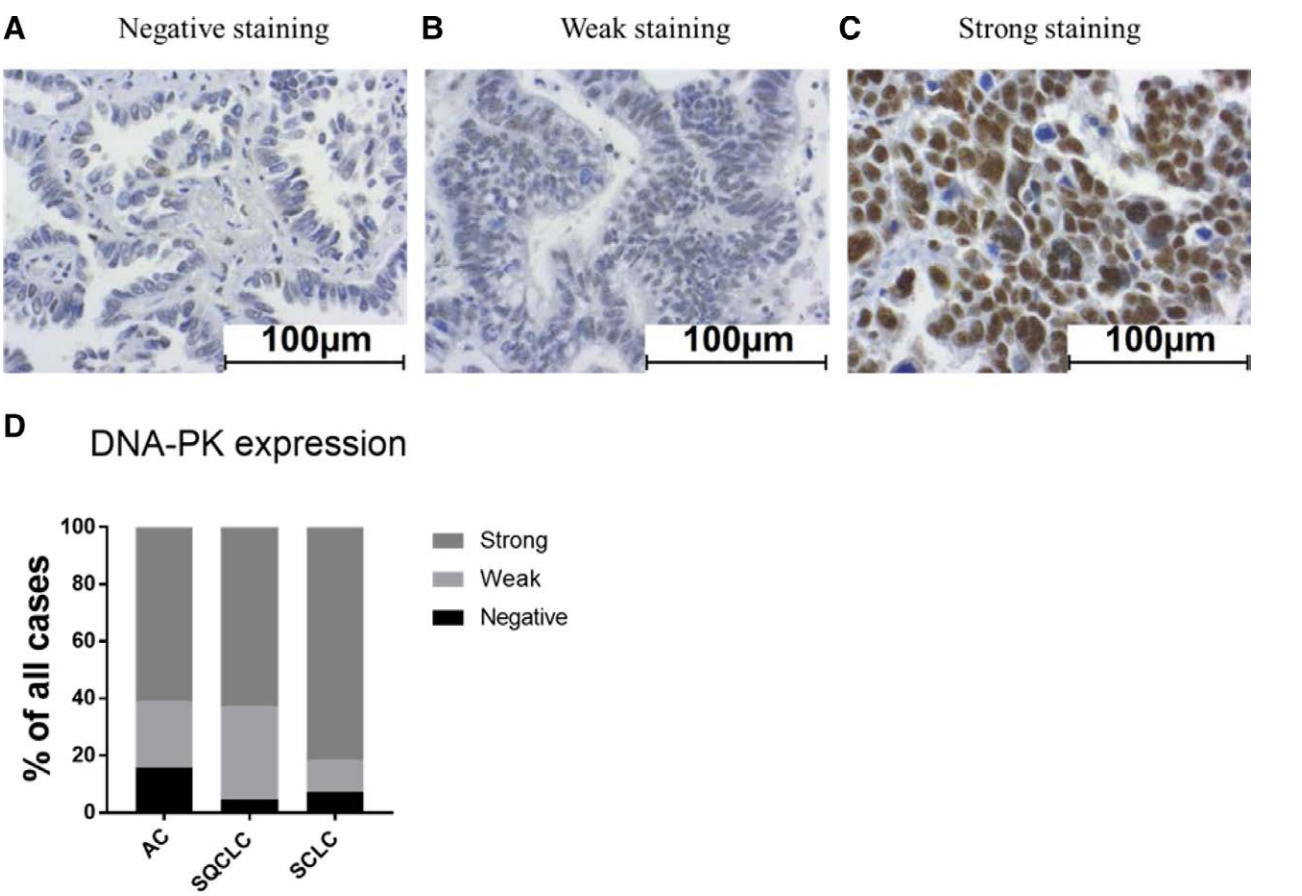

Figure 1. Representative immunohistochemical staining with negative, (A) weak, (B) or strong, (C) immunostaining of DNA-PK in lung cancer samples. Scale bar: 100 µm, and (D) DNA-PK expression sorted by entity and categorized as negative, weak and strong expression. DNA-PK = DNA-dependent protein kinase.

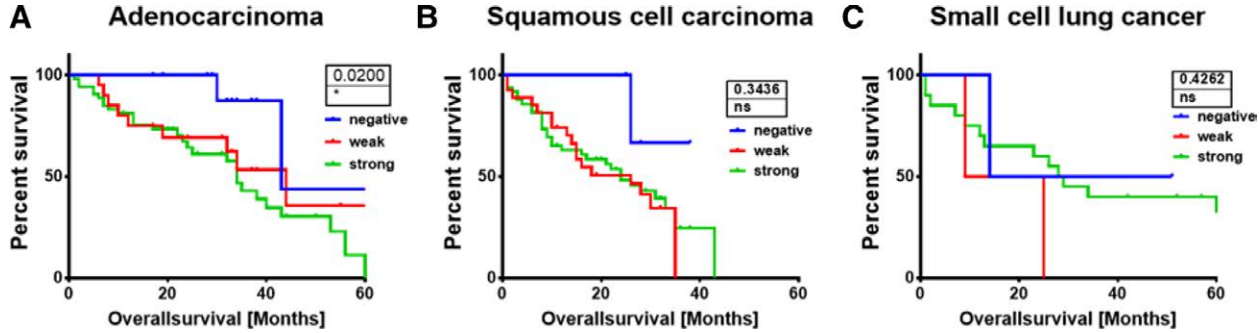

Figure 2. Kaplan–Meier analysis of overall survival in patients with AC, (A) SQCLC, (B) SCLC, and (C) The *P* value is from a log-rank test. AC = adenocarcinoma, SCLC = small cell lung cancer, SQCLC = squamous cell lung carcinoma.

Table 2

DNA-PK expression in Adenocarcinoma sorted by clinical features.

		DANN-PK		
Feature	**Cases**	**-**	**+**	**p**
Gender				
Male	51	7 (13.7%)	44 (59%)	0.55
Female	44	8 (18.2%)	36 (81.8%)	NS
Age				
≤60	29	6 (20.7%)	23 (79.3%)	0.39
>60	66	9 (13.6%)	57 (86.4%)	NS
Degree of differentiation				
I + II	67	11 (16.4%)	56 (83.6%)	0.51
III	27	3 (11.1%)	24 (89.9%)	NS
Lymph node metastasis				
No	55	10 (18.2%)	45 (81.8%)	0.31
Yes	38	4 (10.5%)	34 (89.5%)	NS
Clinical stage				
I + II	70	11 (15.7%)	59 (84.3%)	0.76
III + IV	23	3 (13.0%)	20 (87.0%)	NS
Resection status				
R0	84	12 (14.3%)	72 (85.7%)	0.21
R1 + 2	6	2 (33.3%)	4 (66.7%)	NS

P values are calculated according to chi-square test.
DNA-PK = DNA-dependent protein kinase.

in cancer cells. A context between DNA-PK and natural killer cell activation and the release of proinflammatory cytokines is suspected. Further studies are needed to further investigate the function of DNA-PK in the regulation of macrophages and natural killer cells.[11] Furthermore, overexpression leads to poorer survival in patients with cervical cancer, and lung cancer, among others.[19,25,26] In addition, analysis of DNA-PK can serve as a prognostic biomarker for solid cancer.[19]

Several clinical trials are investigating various DNA-PK inhibitors as potential anticancer drugs.[31] DNA-PK can be used as a prognostic biomarker in lung cancer patients, especially in patients diagnosed with adenocarcinoma. The mechanistic role that DNA-PK is playing in tumorigenesis of AC should be investigated as well. In the long term, the importance of DNA-PK in lung cancer as a therapeutic target should be further investigated.

The single-center design of the current study limits the results. Therefore, a multi-center study with a larger patient cohort is proposed.

Acknowledgments

We thank Jennifer Appelhans for her technical support. The authors acknowledge support by the Open Access Publication Funds of Göttingen University.

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