Protein regulator of cytokinesis-1 expression: prognostic value in lung squamous cell carcinoma patients

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Background: Protein regulator of cytokinesis-1 (PRC1) has been shown to participate in the completion of cytokinesis, and it is dysregulated in cancer processes. However, its relevance in lung squamous cell carcinoma (SCC) remained largely unknown. We aimed to study the expression pattern of PRC1 and assess its clinical significance in lung SCC.

Methods: PRC1 protein expression in human lung SCC and adjacent normal lung tissues was detected by immunohistochemistry. PRC1 expression was assessed in association with clinicopathological features and clinical outcomes of lung SCC patients.

Results: In lung SCC tissues, PRC1 protein expression was significantly higher than those in paired normal lung tissues. The lung SCC patients with PRC1 overexpression had an advanced pathological stage (TNM stage), positive lymph node metastasis, and a shorter overall survival (OS) time more frequently than patients with low PRC1 expression. Additional, PRC1 expression was also shown to be poor as a prognostic factor for OS in patients with lung SCC.

Conclusions: Our study indicated that aberrant expression of PRC1 may point to biochemical recurrence in lung SCC. This highlights its potential as a valuable prognostic marker for lung SCC.

Keywords: Protein regulator of cytokinesis-1 (PRC1); lung squamous cell carcinoma (SCC); prognosis; proliferation

Introduction

Lung cancer, as one of the most prolific malignancies, is the leading cause of cancer death. Lung cancer caused about 21.6% of all cancer-related deaths in 2015 in China (1). Small cell lung cancer (SCLC) and non-small cell lung cancer (NSCLC) are two major histological types of lung cancers. The latter includes squamous cell carcinoma (SCC), adenocarcinoma (ADC), and large cell carcinoma (2). Lung SCC is the main type of NSCLC, and also the second-largest histologic lung cancer subtype. Even though treatment improves the patient's prognosis, the 5-year overall survival (OS) rate of all lung cancer patients is 10–15% (3,4). The high rates of distant metastasis after resection may explain the dismal prognosis of NSCLC. Therefore, identification of biomarkers and new functional genes related to tumor development could provide alternative approaches for the treatment of those suffering from lung cancer.

Table 1 Relationship of PRC1 protein levels with clinicopathological characteristics in lung SCC patients

Characteristics	Number of patients	Cytoplasmic expression		
		Low (≤6.0)	High (>6.0)	P
All patients	75	36	39	
Gender				0.203
Male	70	32	38	
Female	5	4	1	
Age				0.149
<65 y. o.	38	20	18	
≥65 y. o.	37	16	21	
Size of tumor (cm)				0.251
≤3	23	10	13	
>3	52	26	26	
Grade				0.082
Low	14	7	7	
High	61	29	32	
Lymph node metastasis (pN)				0.021*
N0	41	27	14	
N1–3	34	9	25	
p-TNM stages				0.038*
I	27	20	7	
II	19	5	14	
III	29	11	18	

*, P<0.05. PRC1, protein regulator of cytokinesis-1; SCC, squamous cell carcinoma.

Jiang *et al*. [1998] identified the gene for protein regulator of cytokinesis-1 (PRC1), which is considered a mitotic spindle associated cyclin dependent kinase (CDK) substrate (5). The PRC1 gene is located at 15q26.1, and it encodes a 620 amino acid protein with a molecular size of 71 dKa (5). The inhibition of endogenous PRC1 by siRNAS was shown to induce the dysregulation of mitosis and inhibited the process of cytokinesis, which results in two nuclei in the cells (5). In addition, accumulating evidence also suggests that PRC1 plays key roles in microtubule crosslinking (6,7).

PRC1 has been shown to overexpress in various cancers including breast cancer (8), bladder cancer (9), hepatocellular carcinoma (10,11) and pancreatic cancer (12). The previous study (13) showed that PRC1 was up-regulated in primary gastric cancers and overexpression of PRC1 in gastric cancers was associated with poor disease-specific survival and OS.

Recently, Chen *et al*. (11) found that PRC1 had an oncogenic function in promoting cancer proliferation, metastasis and tumorigenesis of hepatocellular carcinoma. Currently, the expression profile of PRC1 and the prognostic value of PRC1 in lung SCC have not been determined.

Methods

Patients

Commercial lung cancer tissue microarrays (TMAs) were obtained from the National Engineering Center for BioChips in Shanghai, China. All tissues were confirmed as lung SCC or adjacent normal lung tissue using histopathological methods. The clinicopathologic characteristics of the lung SCC patients are summarized in *Table 1*. The research protocol was reviewed and approved by the Ethical

Committee and Institutional Review Board of the Nanjing University (2017NZHX-018), and written informed consent was obtained from each patient included in the study.

Immunohistochemical staining and scoring in TMAs

To evaluate PRC1 expression, 75 lung SCC samples and paired adjacent normal lung tissues were used in commercial TMAs (Hlug-squ150sur-01, Shanghai Outdo Biotech, Shanghai, China). Antigen retrieval was performed using high pressure about 5 minutes and citrate buffer for pH 6.0. Sections were incubated with the primary anti-PRC1 antibody for overnight at 4 ℃ (Rabbit monoclonal anti-PRC1 antibody, ab51248, Abcam). PBS replacement of the primary antibody was used for negative control analysis. The samples were then independently analyzed by two pathologists who did not have any clinical information about the patients. The scores for protein expression were determined as previously described (14,15). Staining intensity was scored as the following: 0 (negative), 1 (weak), 2 (medium) or 3 (strong). The level of staining was taken as the percentage of areas with positive stained tumor cells in relation to the entire tumor area. Areas were scored using the following scale: 0 (0%), 1 (1–25%), 2 (26–50%), 3 (51–75%) and 4 (76–100%). Multiplying the positivity and intensity scores gave the total score of PRC1 protein expression (overall scores, 12).

Statistical analysis

GraphPad Prism 6.00 (GraphPad Software, La Jolla California, USA) was used for all statistical analyses. A paired *t*-test was performed to determine the difference in the level of protein expression of PRC1 in lung SCC and adjacent matched normal tissues. A Mann-Whitney U-test was performed to assess associations between PRC1 expression and the patient clinicopathological characteristics. The day of surgery to the date of the last follow-up or the date of death was considered the OS value and survival at the last follow-up was censored. The Kaplan-Meier method was used to calculate the post-operative survival curves. A log-rank test was used to determine differences in the survival rates. Statistical significance was set at P<0.05.

Results

PRC1 is upregulated in lung SCC tissues

We first examined PRC1 protein levels and distribution in

75 pairs of lung SCC tissues and matched adjacent normal tissues using immunohistochemical staining (*Figure 1A*). PRC1 protein was predominantly located in cytoplasm. The scores of PRC1 protein expression were calculated by multiplying the positivity and intensity scores. Cytoplasmic PRC1 protein levels were also higher in the 75 lung SCC tissues than in the matched adjacent normal tissues (P<0.001), as shown in *Figure 1*. These data showed PRC1 expression is up-regulated in lung SCC tissues.

Correlations between PRC1 levels and clinicopathological factors in lung SCC patients

The 75 lung SCC patients were broken into two groups according to mean expression level (staining scores) to ascertain the clinical importance of cytoplasmic PRC1 levels. Correlations between the clinicopathological factors and PRC1 levels in patients with lung SCC are shown in *Table 1*. Cytoplasmic PRC1 staining scores of ≤6 were defined as low level and scores >6 were defined as high level (*Figure 2A*). High cytoplasmic PRC1 levels were associated with tumor size (P=0.021) and p-TNM stage (P=0.038). There was no significant association between gender, age, size of tumor, grade and PRC1 expression.

PRC1 contributes to poor prognosis in lung SCC patients

Seventy-five patients were broken into two groups according to the mean expression level to ascertain the importance of PRC1 overexpression in predicting lung SCC clinical outcomes. It is interesting that patients with higher levels of PRC1 protein had a shorter survival time than patients with lower PRC1 levels (P=0.021, log-rank test, *Figure 2B*). Notably, multivariate Cox regression analysis showed that N stage, p-TNM Stage and high expression of PRC1 protein were independent prognostic factors for lung SCC patients (*Table 2*).

Discussion

Lung SCC is a prevalent subtype of NSCLC that is often fatal. SCC is mainly diagnosed in people with a history of smoking and is responsible for approximately 20–30% of new lung cancer cases (1). Until recently, the various subtypes of NSCLC were grouped together for the purpose of treatment, but it is now widely known that different histologic subtypes should be treated as separate disease entities (16). SCC is the second most prevalent type of lung
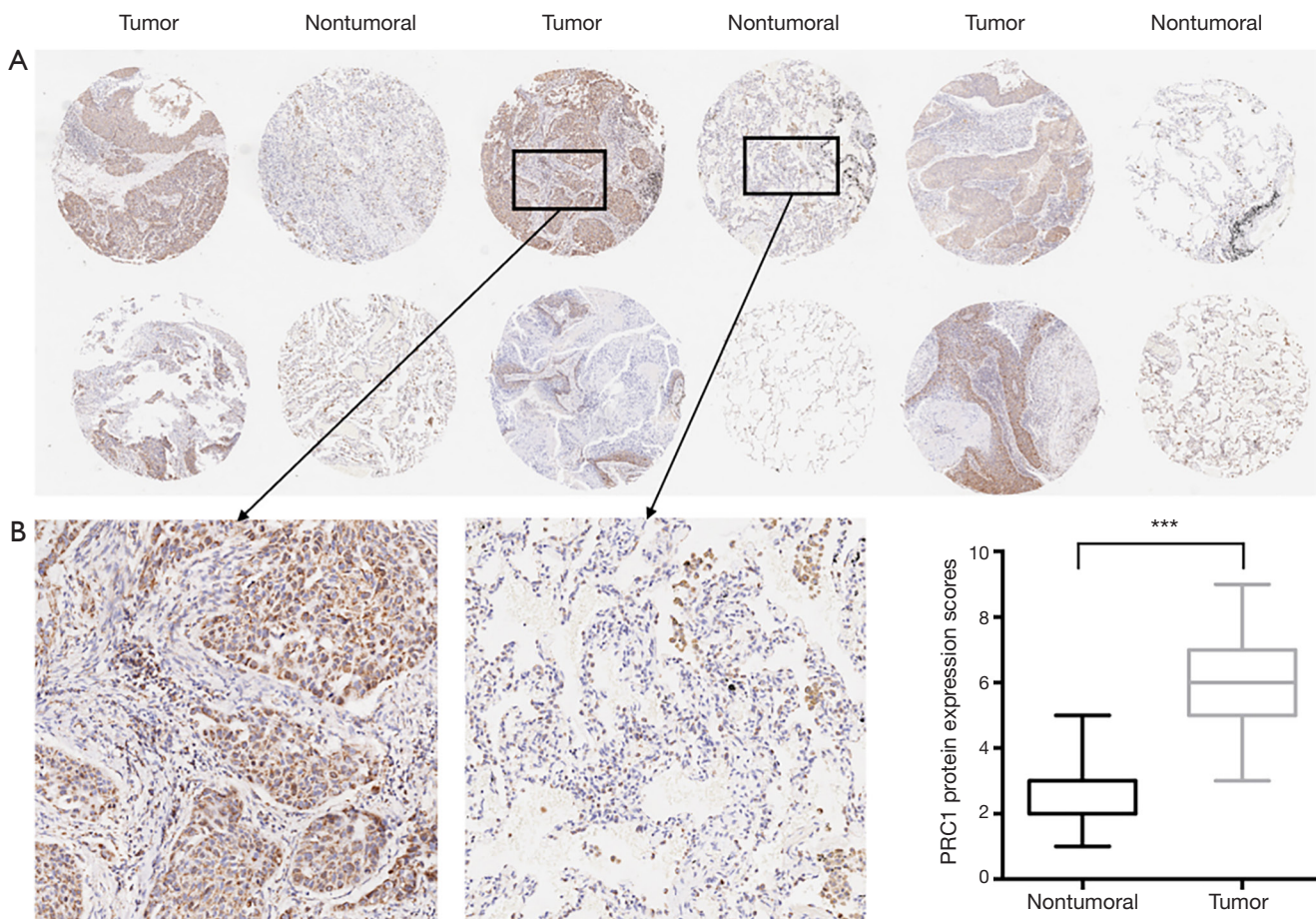
Figure 1 PRC1 is upregulated in primary lung PRC1 tissues. (A) PRC1 protein levels in TMAs (six paired lung SCC tissues and adjacent normal lung tissues) were measure with IHC (×10); (B) representative examples of PRC1 staining in lung SCC tissues and normal lung tissues are represented. The right panel indicated the quantitative analysis of relative expression density (×200). ***, P<0.005. PRC1, protein regulator of cytokinesis-1; SCC, squamous cell carcinoma; TMAs, tissue microarrays; IHC, immunohistochemistry.

cancer. However, because the actionable gene mutations of this disease are not well understood, no approved targeted therapeutics is available for its treatment up to date (17). Therefore, performing comparative analyses of lung SCCs and identifying potential therapeutic targets would lead to significant growth in cancer treatments.

The PRC1 gene was initially thought to be a CDK substrate according to an *in vitro* phosphorylation screen. It was subsequently shown to be a midzone-associated protein that was required for completion of cytokinesis (5). PRC1 is phosphorylated by CDK1 (Cdc2/Cyclin B) in early mitosis. PRC1 is then changed to an inactive and monomeric state (6). It is dephosphorylated during the metaphase-anaphase transition and interacts with KIF4, a kinesin motor that translocates PRC1 towards the plus end of antiparallel interdigitating microtubules along mitotic spindles. The dephosphorylated PRC1 protein parcels the antiparallel interdigitating microtubules to establish the midzone, which is necessary for cytokinesis (18).

Cytokinesis is a complex process, involving sophisticated biochemical and cellular dynamics factors. Abnormal cytokinesis has been shown to lead to cell chromosome instability and malignant transformation (19,20). Dysregulated expression of PRC1, as a critical protein of cytokinesis, can cause disorders of cytokinesis. Previous studies (8,9,11) have implicated PRC1 as an oncogenic factor in tumorigenesis of bladder cancer, breast, and hepatocellular carcinoma. Here, we showed that PRC1protein expression was upregulated in lung SCC compared to paired normal lung tissues. We then found overexpression of PRC1
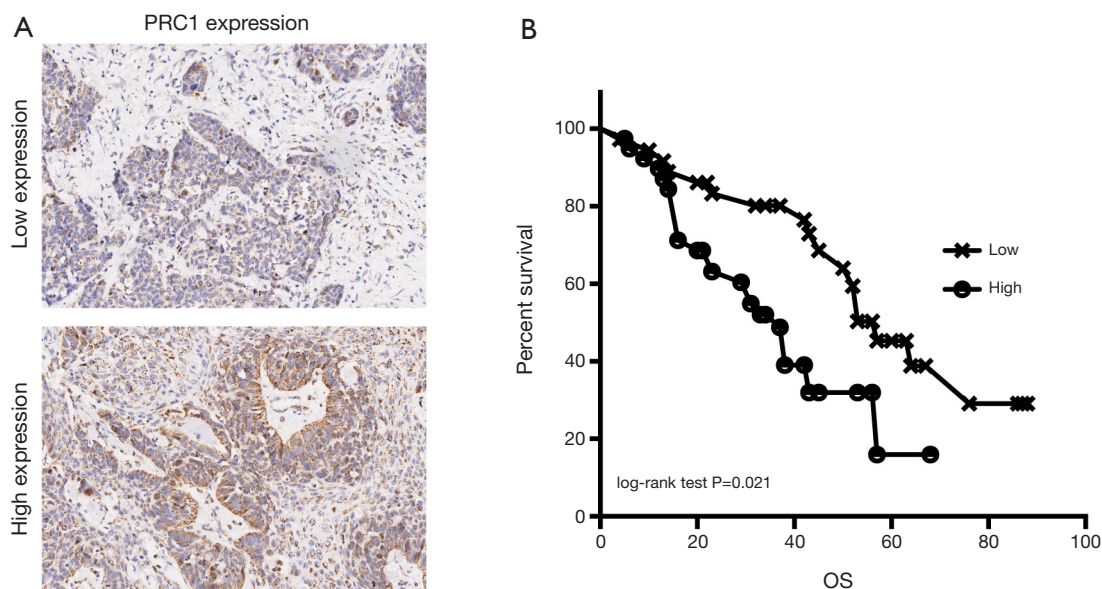
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Figure 2 PRC1 overexpression indicated an unfavorable prognosis in lung SCC patients. (A) IHC staining of high expression (down) and low expression (up) PRC1 in lung SCC tissues (×200); (B) Kaplan-Meier curves indicating the OS rate in 75 patients with lung SCC according to the expression status of PRC1. The median staining score served as a cutoff for high and low PRC1 expression. High level of PRC1 protein expression correlated with significantly poor OS. PRC1, protein regulator of cytokinesis-1; SCC, squamous cell carcinoma; IHC, immunohistochemistry; OS, overall survival.

Table 2 Multivariate analyses of PRC1 protein levels and the other clinical prognostic markers related to OS in lung SCC

Item	OS	
	HR (95% CI)	P
Age (≥65/<65 years)	2.346 (0.85–3.446)	0.216
Sex (female/male)	1.458 (0.735–4.249)	0.148
Size of tumor (>3/≤3 cm)	1.482 (0.567–4.215)	0.257
Grade (high/low)	0.832 (0.358–2.362)	0.472
Lymph node stage (N1-3/N0)	1.841 (1.359–4.057)	0.025*
p-TNM stage (III vs. I+II)	2.042 (1.321–3.921)	0.036*
PRC1 level (high/low)	1.532 (1.251–3.325)	0.021*

*, P<0.05.

protein associated with metastasis of lymph node and was an independent poor prognostic marker for lung SCC patients.

There is sufficient evidence that p53 enhances the sensitivity of tumor cells to chemotherapy and radiotherapy, induces cell cycle arrest and inhibits cell growth and the proliferation of tumor cells (21-23). Li *et al.* (24) showed that p53 directly suppresses PRC1 gene transcription. In this study, they showed p53 interacts with the PRC1 gene promoter in breast cancer cells. This indicated that PRC1 is a common downstream target of p53. PRC1 was also confirmed as a downstream target of heat shock transcription factor 2 (HSF2) and is involved regulating cytokinesis (25). Moreover, our previous study (13) indicated that knockdown expression of PRC1 suppressed the proliferation and invasion of lung gastric carcinoma *in vitro* and *in vivo*. Collectively, these results indicate that silencing of PRC1 could inhibit tumorigenesis by blocking cytokinesis in a wide assortment of cancer cells. PRC1 is expected to become a new target for antitumor gene therapy.

In summary, we first identified that the expression pattern and the clinicopathological characteristics of PRC1 in lung SCC. Our results supply a foundation for the concept that PRC1 is upregulated in human SCC tissues and overexpression of PRC1 in human lung SCC could be vital in the attainment of an aggressive and poor prognostic phenotype. Further study is required to achieve a full understanding of the underlying molecular mechanisms.

Acknowledgements

Footnote

Conflicts of Interest: The authors have no conflicts of interest to declare.

Ethical Statement: The research protocol was reviewed and approved by the Ethical Committee and Institutional Review Board of the Nanjing University (2017NZHX-018), and written informed consent was obtained from each patient included in the study.

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