PRC1 plays an important role in lung adenocarcinoma and is potentially targeted by fostamatinib

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Abstract. – **OBJECTIVE:** Lung adenocarcinoma (LUAD) is one of the most common cancers in the world. Protein regulator of cytokinesis 1 (PRC1) plays a role in the tumorigenesis and development of several cancers, including LUAD. The aim of the present study is to assess the characteristics of PRC1 in LUAD in order to find a potential drug that targets PRC1.

MATERIALS AND METHODS: We investigated the prognostic value of PRC1 in patients with LUAD using Cox analysis of the RNA sequencing data from The Cancer Genome Atlas (TCGA) portal. A link between PRC1 and LUAD progression, cigarette smoking mutation count, aneuploidy, and hypoxia scores was assessed. The relationship between PRC1 and tumor-infiltrating immune cells in LUAD was analyzed and Gene Set Enrichment Analysis (GSEA) was used to study the PRC1-related biological process and signal pathways. Potential drugs targeting PRC1 were identified using DrugBank database and molecular docking.

RESULTS: PRC1 expression was significantly increased in LUAD. PRC1 could be, therefore, a prognostic biomarker for predicting overall survival in LUAD. PRC1 expression was also related to cancer stage and patient's smoking history. PRC1 positively correlated with mutation count, aneuploidy and hypoxia scores. It was also significantly related to tumor-infiltrating immune cells, especially the activated mast cells. GSEA revealed that PRC1 might be correlated with cell cycle, cytokinesis and p53 signaling pathway. Additionally, fostamatinib was found to be a potential drug targeting PRC1.

CONCLUSIONS: PRC1 may have a prognostic value for patients with LUAD, and be correlated with the mutation count, aneuploidy, hypoxia and tumor-infiltrating immune cells. Fostamatinib was found to be a potential drug targeting PRC1 in LUAD.

Key Words:
   Lung adenocarcinoma, PRC1, Tumor-infiltrating immune cells, TCGA.

Introduction

Lung cancer is the most common type of cancer in the world with the highest mortality rate[1,2]. It consists of different subtypes, including small-cell lung cancer (SCLC) and non-small-cell lung cancer (NSCLC). NSCLC accounts for approximately 80% of lung cancer cases, and lung adenocarcinoma (LUAD) is the most common histological subtype of NSCLC[3,4]. Existing therapeutic methods for lung cancer, including chemotherapy, radiation and surgery, have a very limited impact on the survival rates of patients[5,6], further emphasizing the need to explore new methods and new therapeutic targets.

Microtubules are major highly dynamic components of cytoskeleton that are spread throughout the cytoplasm[7]. They play a major role in maintaining cell structure, chromosome mitosis and meiosis, intracellular transport and movement of secretory vesicles[8,9]. Microtubule-associated proteins (MAPs) participate in regulating microtubule dynamics. Recent literature has

shown that microtubules and MAPs have been found to play an important role in the immune processes, including B cell synapse formation, trafficking of B cell receptor-antigen complexes, polarization during T cell activation, and the activity of natural killer T and dendritic cells. Moreover, mutations in MAPs may promote immune cells to recognize tumors and inhibit lymph node metastasis. Therefore, microtubules and MAPs may be a good target for therapeutic immunotherapy[10,11]. Protein regulator of cytokinesis 1 (PRC1), MAP that was first discovered as a substrate for cyclin-dependent kinases (CDK) in 1998, plays an essential role in the process of cytokinesis[12]. Altering PRC1 may lead to abnormal expression of cytokines, tumorigenesis and tumor progression[13]. Knockdown of PRC1 causes aneuploidy, while strong overexpression of PRC1 can induce cytokinesis failure and chromosome instability (CIN), which are also associated with increased aneuploidy[14,15]. Previous studies[16-19] showed that PRC1 promotes proliferation and metastasis of cancer cells and is overexpressed in a variety of tumor types, such as hepatocellular, gastric and pulmonary carcinoma.

Hanselmann et al[20] found that PRC1 promoted tumorigenesis of lung cancer cell lines and mice with NSCLC. Zhan et al[18] showed that PRC1 contributes to tumorigenesis of LUAD in lung cancer cell lines and in patients (n = 90) with LUAD. However, studies with larger samples sizes are needed to investigate the correlation between PRC1 and LUAD. Therefore, the present study used gene expression data of patients with LUAD (n = 479) in The Cancer Genome Atlas (TCGA) to study the characterization of PRC1 in LUAD and to analyze the potential drug influencing PRC1 using bioinformatics.

Materials and Methods

PRC1 Transcription Analysis

The expression analysis of PRC1 mRNA between normal tissues and human cancer samples from the Cancer Genome Atlas (TCGA) was conducted by the module "exploration" in the Tumor Immune Estimation Resource database 2.0 (TIMER 2.0, available at: http://timer.cistrome.org/). Distributions of gene expression levels are displayed using box plots. The statistical significance was computed by the Wilcoxon test.

Data Acquisition, Cox Analysis and Survival Analysis

We downloaded RNAseq transcriptome data of 479 LUAD samples and 54 adjacent normal lung samples from the Genomic Data Commons (GDC) data portal (available at: https://portal.gdc.cancer.gov/). The corresponding clinical data including age, stage, gender, survival time and status were obtained also from GDC. The smoking history information of patients was obtained from the University of California Santa Cruz (UCSC) Xena (available at: https://xena.ucsc.edu/). RNAseq transcriptome data were first normalized by "edgeR" package in R (The R Foundation for Statistical Computing, Vienna, Austria). Univariate and multivariate Cox regression analyses were then performed to test the prognostic value of PRC1 and other clinical information including age, stage and gender. Finally, we drew the Kaplan-Meier survival curve of PRC1 using the R packages "survival" and "survminer".

Relationships Between PRC1 and Development of LUAD as well as Smoking History Information

To test the effect of PRC1 on the development of LUAD, we compared the expression of PRC1 at different stages, and investigated the relationship between PRC1 and smoking history information obtained from UCSC Xena.

Effects of PRC1 mRNA Level on Mutation Count, Aneuploidy Score and Hypoxia Score

The connection between PRC1 and mutation count was analyzed by cBioportal database. Aneuploidy scores were acquired as described in the previous study[20] and the tumor samples were divided into high- and low-PRC1 expression groups according to the median of PRC1 expression. Comparison of aneuploidy scores in these groups was performed. Additionally, the link between PRC1 and hypoxia scores, including Buffa hypoxia score, Winter hypoxia score and Ragnum hypoxia score, was investigated by cBioportal.

Relationships Between PRC1 and Tumor-Infiltrating Immune Cells in LUAD

Comprehensive correlation analysis between PRC1 and tumor-infiltrating immune cells in LUAD was performed by CIBERSORT method in the TIMER database. Purity adjustment was performed by partial Spearman's correlation analysis. Correlations with $p<0.05$ and $r>0.20$

were considered as significantly positive, and correlations with $p<0.05$ and r < -0.20 were considered as significantly negative.

Enrichment Analysis

To investigate how PRC1 expression impacts cancer, Gene Set Enrichment Analysis (GSEA) was conducted, dividing the LUAD samples into high- and low-expression group, based on the PRC1 expression levels, and then analyzing the enrichment of signaling pathways or biological states or processes using both Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG) database. Once |NES|>1, $p<0.05$ and FDR<0.05, pathways were regarded with significant enrichment.

Potential Drugs Targeted PRC1

Previous reports[21] indicated that mutation in p53 and alterations of CDK1, PLK1 and Aurora B expression might induce PRC1 overexpression in cancer. The correlation between these factors and PRC1 in LUAD was assessed by TIMER 2.0. Related drugs targeting these factors were investigated *via* DrugBank database, and the most suitable candidates were further analyzed using molecular docking with PRC1 protein by molecular operating environment (MOE)[22].

Statistical Analysis

R software (R Foundation for Statistical Computing, Vienna, Austria) was used to process and analyze the data in the present study. For the Kaplan-Meier rates, a significant difference in two-group survival curves was assessed by a log-rank test. Spearman's rank correlation test was used to obtain the *p*-values and partial correlation values in correlation analysis. Results with $p<0.05$ were considered as statistically significant.

Results

PRC1 mRNA Levels in Different Types of Human Cancers

As shown in Figure 1, the expression levels of PRC1 mRNA in different tumor and normal tissues were analyzed by TIMER database (TCGA gene expression RNA-seq data). Compared with normal samples, the PRC1 mRNA level was significantly increased in almost all tumors, including BLCA (bladder urothelial carcinoma); BRCA (breast invasive carcinoma); CESC (cervical squamous cell carcinoma); CHOL (cholangiocarcinoma); COAD (colon adenocarcinoma); DLBC (lymphoid neoplasm diffuse large B cell lymphoma); ESCA (esophageal carcinoma); GBM (glioblastoma multiforme); HNSC (head and neck squamous cell carcinoma); KICH (kidney chromophobe); KIRC (kidney renal clear cell carcinoma); KIRP (kidney renal papillary cell carcinoma); LIHC (liver hepatocellular carcinoma); LUAD (lung adenocarcinoma); LUSC (lung
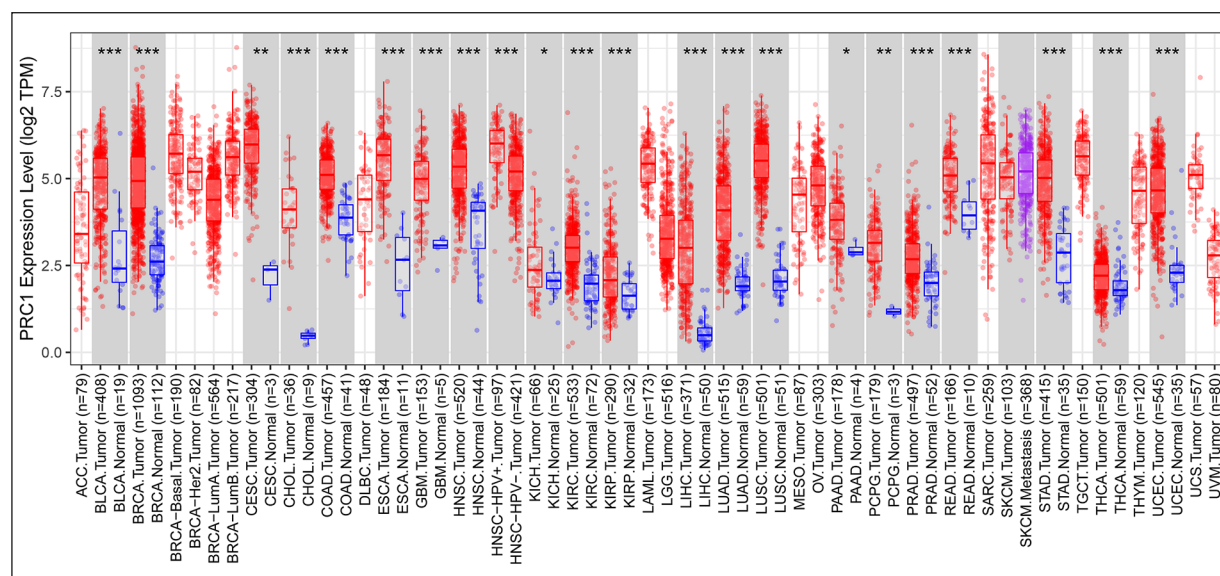

Figure 1. PRC1 mRNA levels in various types of human cancers form TIMER database. The statistical significance computed by the Wilcoxon test is annotated by the number of stars (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).
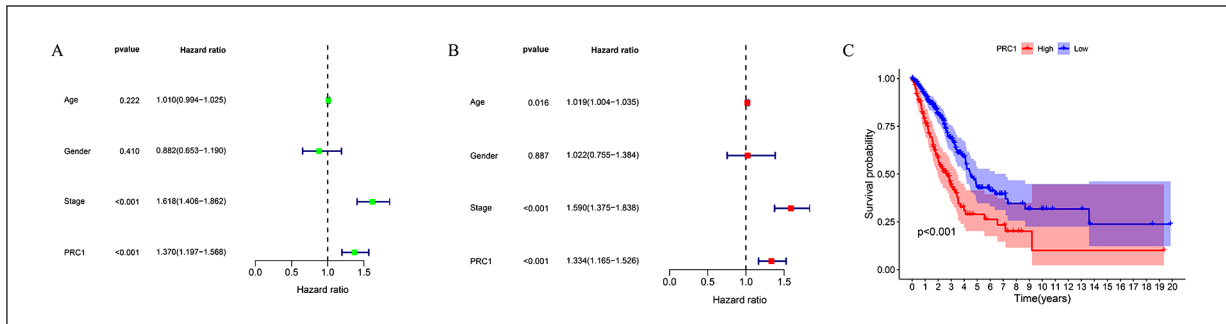
Figure 2. Independent prognostic values of PRC1 in patients with LUAD. Univariate (**A**) and multivariate (**B**) Cox regression analysis for PRC1 and key clinical parameters in LUAD. The overall survival curves of PRC1 in LUAD (**C**).

squamous cell carcinoma), PAAD (pancreatic adenocarcinoma); PCPG (pheochromocytoma and paraganglioma); PRAD (prostate adenocarcinoma); READ (rectum adenocarcinoma); STAD (stomach adenocarcinoma); THCA (thyroid carcinoma); and UCEC (uterine corpus endometrial carcinoma).

Prognostic Value of PRC1 in LUAD

To test whether PRC1 mRNA could be a prognostic biomarker in LUAD, we conducted the univariate and multivariate Cox regression analysis. The results showed that, among the clinical features and PRC1 mRNA, stage and PRC1 could both be independent prognostic factors for LUAD (Figure 2A and 2B; HR>1, $p<0.001$). The Kaplan-Meier survival curve of PRC1 showed that patients with high PRC1 mRNA level have poorer overall survival compared with patients with low PRC1 level (Figure 2C; $p<0.001$).

Correlation Analysis Between PRC1 and Development of LUAD, as well as Smoking History Information

To investigate whether the PRC1 could influence the development of LUAD, comparison analysis of PRC1 mRNA in different stages was performed. As shown in Figure 3A, compared with earlier stages (I and II), PRC1 mRNA expression was significantly increased in later stages (III and IV) in LUAD ($p<0.01$). In addition, the association of PRC1 and smoking history was also investigated (Figure 3B). Compared with lifelong non-smokers or current reformed smokers for > 15 years, PRC1 mRNA was significantly increased in current smokers.

Effects of PRC1 Overexpression on Mutation Count, Aneuploidy Score and Hypoxia Score

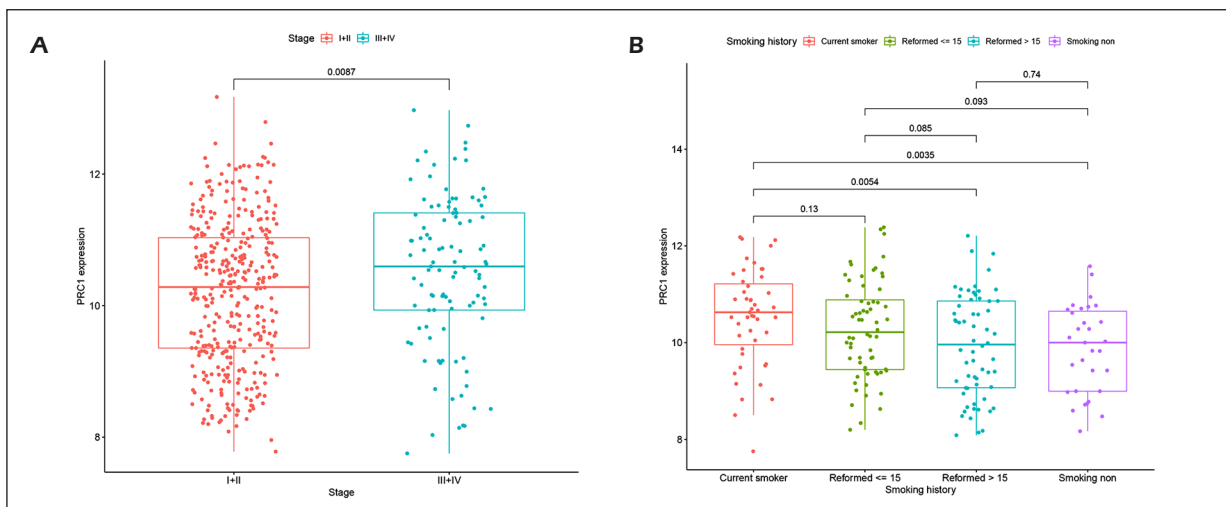As shown in Figure 4, PRC1 was positively related to mutation count (r>0.4, $p<0.001$) and aneu-

Figure 3. Relationship between PRC1 and development of LUAD (**A**) and smoking history information (**B**). Reformed ≤ 15 means current reformed smoker ≤15, reformed > 15 means current reformed smoker for > 15 years.
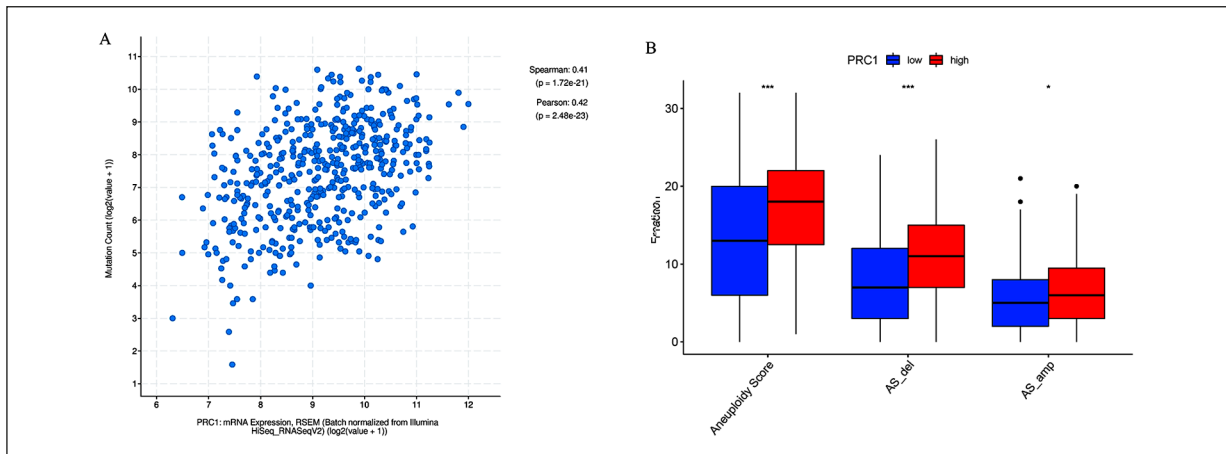
Figure 4. Effects of PRC1 mRNA expression on mutation count (**A**) and aneuploidy score (**B**). AS_del: deletion aneuploidy score; AS_amp: amplification aneuploidy score. (*: $p < 0.05$; **: $p < 0.01$; ***: $p < 0.001$).

ploidy score. Deletion and amplification score were also significantly higher in high PRC1 expression group compared to low expression group ($p<0.05$). In addition, PRC1 was also positively related to hypoxia scores including Buffa, Winter and Ragnum hypoxia scores (r>0.6, $p<0.001$; Figure 5).

Correlation of PRC1 and Tumor-Infiltrating Immune Cells in LUAD

The relationship between PRC1 and tumor-infiltrating immune cells was firstly investigated by TIMER database. The results indicated that PRC1 was positively related to activated memory CD4 T cells, resting mast cells, M0 macrophages and M1 macrophages, but negatively related to resting memory CD4 T cells, monocytes, resting myeloid dendritic cells, memory B cells and activated mast cells (Figure 6).

Enrichment Analysis of High and Low PRC1 Expressed Tumor Samples in LUAD

GSEA was conducted on the high- and low PRC1 expression groups. A total of 555 data sets met the requirement of |NES|>1, $p<0.05$ and FDR<0.05 for GO analysis, and a total of 8 data sets met the above requirement for KEGG analysis. Among these enriched terms, we selected the first four most relevant terms with cancer for GO and KEGG analysis respectively (Figure 7). For GO analysis, enriched terms associated with cancer were the cell cycle G2 phase to M phase, chromosome segregation, cytokinesis and p53 signaling pathway (Figure 7A). For KEGG analysis, enriched terms are cell cycle, mismatch repair, oocyte meiosis and p53 signaling pathway (Figure 7B).
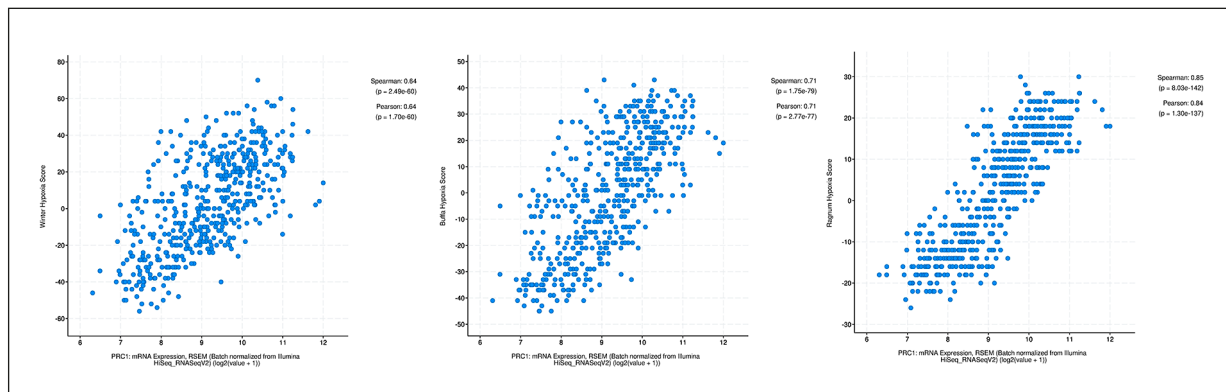

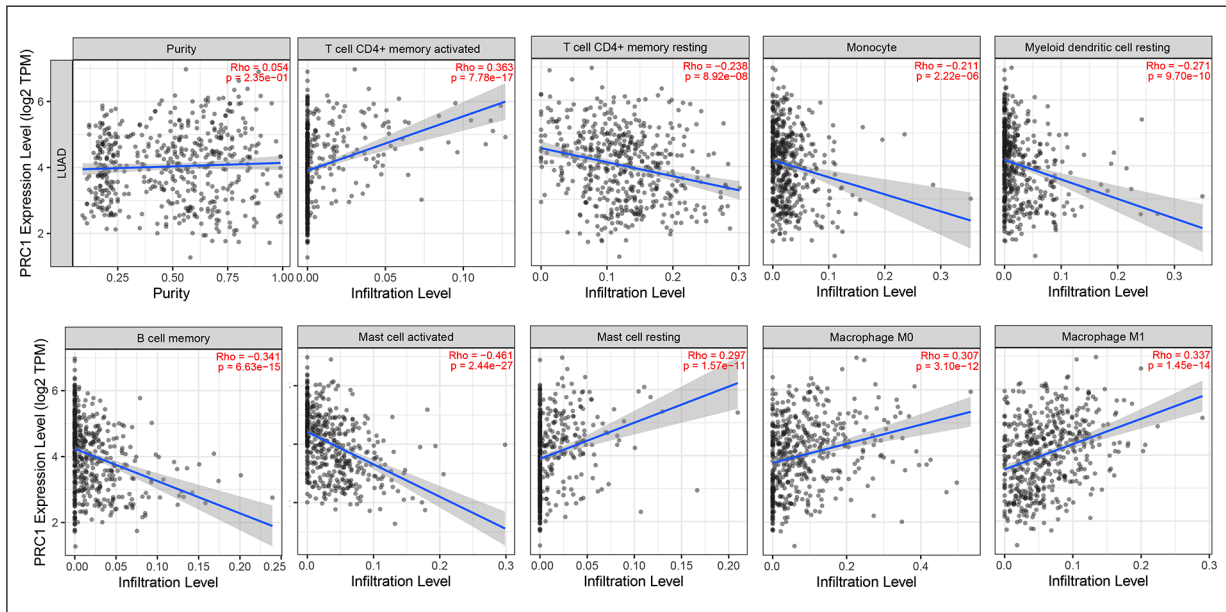**Figure 5.** Effects of PRC1 mRNA expression on hypoxia score.

Figure 6. Correlation of PRC1 and tumor infiltrating immune cells in LUAD. Scatter plots were generated with partial Spearman's correlation and statistical significance.

Potential Drugs Targeted PRC1

Figure 8A showed that PRC1 was significantly increased in tumor samples with p53 mutation, compared with samples with the wild type p53 gene. Additionally, PRC1 mRNA levels positively correlated with MYBL2, FOXM1, CDK1, PLK1 and Aurora B (r>0.8, p<0.001; Figure 8B-F). Subsequently, we acquired the approved drugs targeted p53, CDK1, PLK1 or Aurora B from DrugBank database, and found that fostamatinib targeted CDK1, PLK1 and Aurora B as an inhibitor (Table I).

These results indicated that fostamatinib possibly decreases PRC1 expression by inhibiting CDK1, PLK1 and Aurora B. To test whether fostamatinib potentially interacts with PRC1 protein directly, the molecular docking was performed by MOE software (Figure 9). The dock binding free energy of fostamatinib and PRC1 was -7.428 kcal/mol. The 3D image is shown in Figure 9A, and protein-ligand interaction is shown in Figure 9B. There were four interaction forces of PRC1 and fostamatinib during docking (Table II).
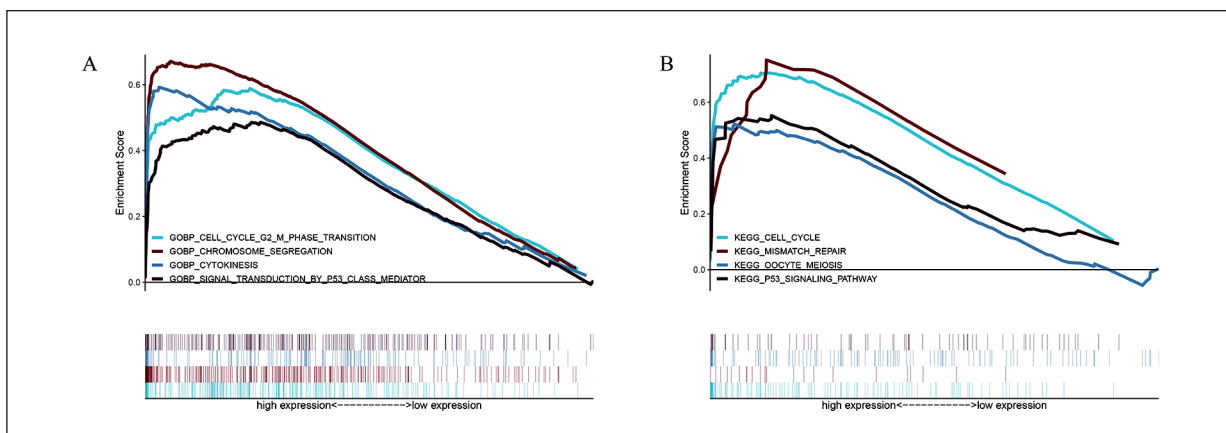

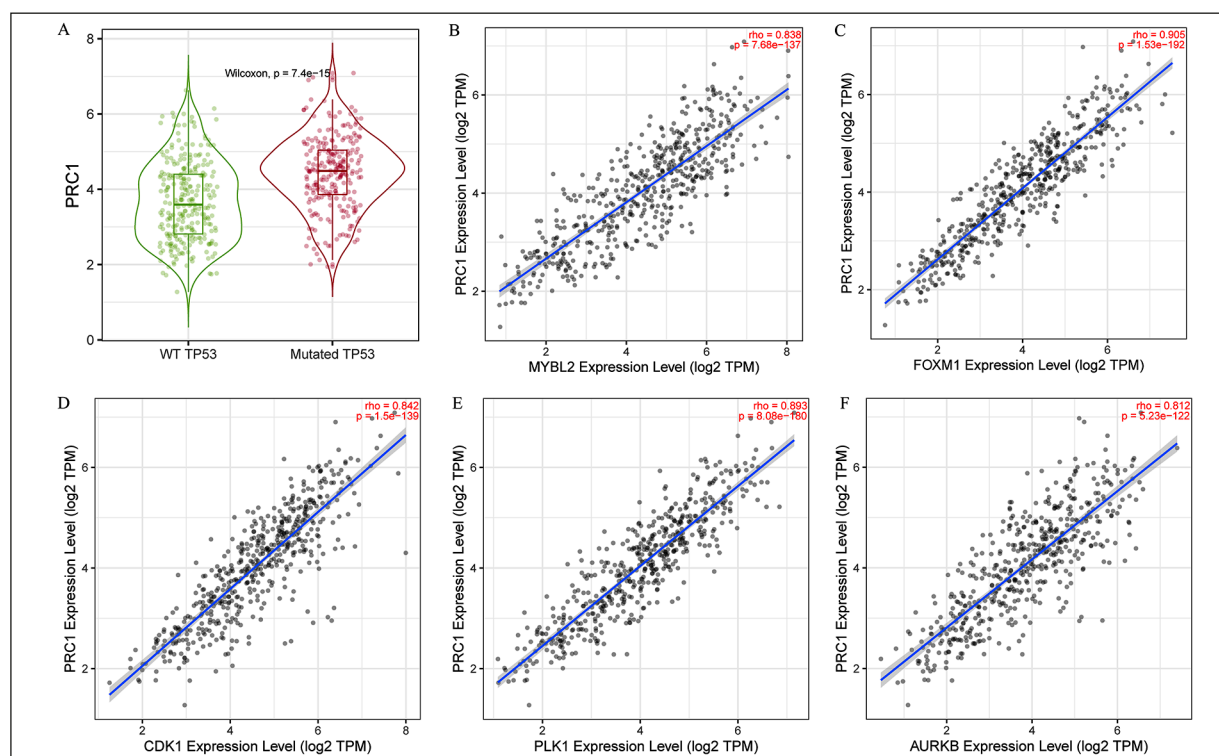**Figure 7.** GSEA analysis of PRC1 related biological process (**A**) and signal pathway (**B**) in GO or KEGG databases.

Figure 8. Potential reasons for PRC1 overexpression in LUAD. The correlation analysis for PRC1 and p53 mutation (**A**), MYBL2 (**B**), FOXM1 (**C**), CDK1 (**D**), PLK1 (**E**) and Aurora B (**F**).

Table I. Approved drugs targeted p53, CDK1, PLK1 and Aurora B in DrugBank database.

Targeted genes	Drugbank ID	Name	Actions
p53	DB00945	Acetylsalicylic acid	Inducer
	DB01593	Zinc	-
	DB14487	Zinc acetate	-
	DB14533	Zinc chloride	Chaperone
	DB14548	Zinc sulfate, unspecified form	Chaperone
CDK1	DB12010	Fostamatinib	Inhibitor
PLK1	DB12010	Fostamatinib	Inhibitor
Aurora kinase B	DB04703	Hesperidin	-
	DB12010	Fostamatinib	Inhibitor

Discussion

LUAD is the most common primary lung cancer in the United States and has a strong association with a smoking history[23]. LUAD of-ten occurs in the lung periphery, in scar tissue or areas of chronic inflammation[23]. MAPs and tumor-infiltrating immune cells in the tumor microenvironment (TME) can both influence the oncogenesis and development of LUAD. PRC1,

Table II. Detailed PRC1-fostamatinib interactions.

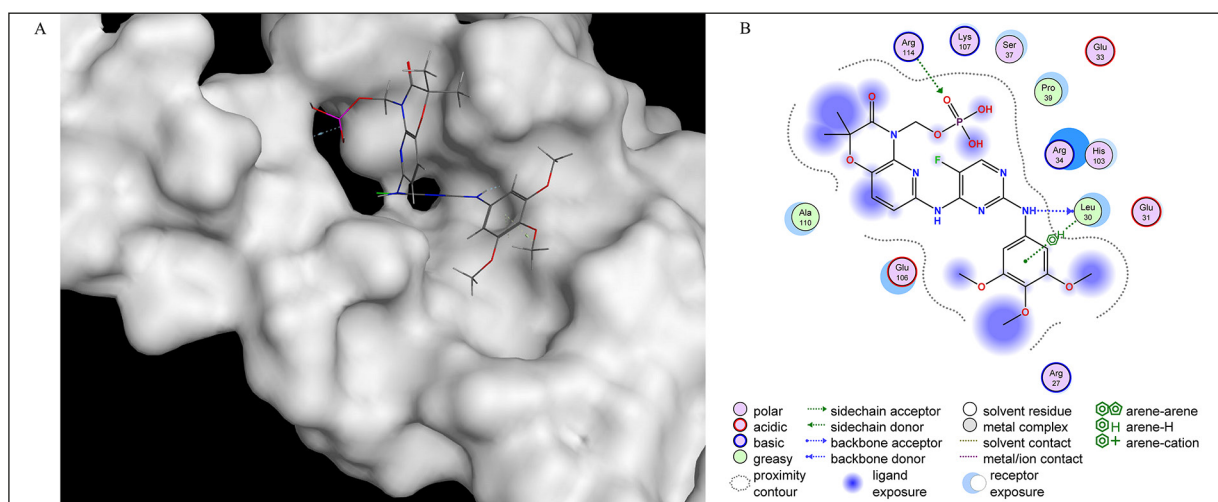| Ligand	Receptor		Interaction	Distance	E (kcal/mol)
N 10	O	LEU 30	H-donor	3.21	-1.8
O 35	NH2	ARG 114	H-acceptor	3.14	-1.3
6-ring	CD1	LEU 30	pi-H	4.25	-0.7
6-ring	CD2	LEU 30	pi-H	4.17	-0.5

Figure 9. Molecular docking for PRC1 and fostamatinib. **A,** 3D docking image. **B**, Protein-ligand interaction.

as one of the MAPs, promotes cell proliferation in various types of cancer including LUAD[24]. In the present study, we used bioinformatic approach based on tumor-infiltrating immune cells to investigate the effect of PRC1 on formation and development of LUAD.

Our study shows that PRC1 could be an independent prognostic biomarker to predict overall survival for patients with LUAD, as indicated by the Cox regression analysis. Patients with high PRC1 expression have poorer overall survival compared the low expression group. These results are in agreement with other previous studies[19,20]. In addition, Zhan et al[18] indicated that PRC1 is overexpressed in patients with lung squamous cell carcinoma (LSCC) and indicates poorer prognosis in LSCC. We found that, compared with earlier stages, PRC1 level was significantly raised in later stages of LUAD, indicating that PRC1 may play a partial role in the development of LUAD. We also found that PRC1 level in current smokers was significantly higher than in nonsmokers or currently reformed (for > 15 years) smokers with LUAD, which indicated that PRC1 was associated with smoking in LUAD patients. Previous studies[25] linking PRC1 and smoking history in LUAD patients are limited. A recent study by Cao et al[26] reported that long-term smokers have a high detection rate of lung cancer with the main type being adenocarcinoma, suggesting that LUAD is closely related to cigarette smoking. In our study, we inferred that cigarette smoking could induce PRC1 upregulation in LUAD patients and lead to a poor overall survival.

Aneuploidy is a hallmark of cancer, with approximately 90% of tumor displaying some degree of whole-chromosome aneuploidy, probably as a result of chromosomal instability (CIN)[27]. Deregulation of PRC1 could result in aneuploidy by inducing cytokinesis defects[21]. Our results showed that aneuploidy score was significantly higher in high PRC1 expression group, which was consistent with previous literature.

Hypoxia is found in many solid tumors and is a consequence of rapid growth of tumor cells. In normal tissues, oxygen homeostasis is maintained by a balance of oxygen supply and demand. However, in proliferating tumors oxygen demand exceeds supply due to metabolic reprogramming[28]. Our results showed that PRC1 is positively related to hypoxia score, which might indicate that increased PRC1 reflects hypoxia and rapid growth of tumor cells.

Previous studies[29] indicated that 90-95% of all cancer cases could be attributed to environment and lifestyle. Low tumor-infiltrating lymphocytes in the tumor microenvironment (TME) has been known as a poor prognostic biomarker in early-stage NSCLC[30]. Tumor-associated macrophages are abundant in the TME of most cancer and generally related to poor clinical outcomes in cancer patients[31,32]. Our results showed that PRC1 expression had a strong association with immune infiltrating levels of multiple immune cells in general and activated mast cells in particular (strongest association in our study). After activation, mast cells release inflammatory mediators to regulate immune response and to promote the recruitment of immune cells to

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the site of inflammation[33]. The impacts of mast cells in LUAD are complicated and the results of the related studies are contradictory[34]. Baram et al[35] showed that mast cells promote tumorigenesis and indicate poor survival in patients with stage I LUAD. However, Kurebayashi et al[36] suggested that mast cells may be associated with prolonged survival in LUAD. Another study revealed that high level infiltration of mast cells correlates with the better survival in patients with LUAD. Our results showed that activated mast cells correlated with better outcomes in LUAD patients. However, whether mast cells activation induces decreased PRC1 expression, or downregulated PRC1 induces activated mast cells infiltration to prolong survival in LUAD remains unclear.

GSEA results showed that differentially expressed genes in high- and low-PRC1 expression groups of LUAD samples were mainly enriched in cell cycle, cytokinesis and p53 signal pathways. These results suggest that these pathways may provide a mechanism by which PRC1 expression influence patients with LUAD. Cell cycle is orchestrated by sequential activation of cyclin-dependent kinases (CDKs)[37]. Since PRC1 is a CDK substrate[24], the PRC1 expression is closely related to cell cycle. Cytokinesis failure contributes to cancer development[38], and it is possible that PRC1 dysregulation can induce cytokinesis to promote cancer[21]. The protein p53 is a tumor suppressor that correlated with mitosis, and its dysregulation can induce cancer by causing chromosome instability[39]. The p53 signal pathway alteration can lead to PRC1 expression dysregulation[40]. In agreement with previous studies, our results show that PRC1 is closely related to cell cycle, cytokinesis and p53 signaling pathway.

While there are no drugs available that can directly target PRC1, drugs targeting p53, CDK1, PLK1 and Aurora B could possibly influence PRC1 expression[21]. Activation of p53 directly decreases PRC1 transcription in various cancers, and p53 also can indirectly decrease PRC1 *via* inhibiting MYBL2-MuvB (MMB)-FOXM1 complex that is essential for cell cycle progression[41]. Our results indicated that p53 mutation might induce PRC1 overexpression, and PRC1 is positively related to MYBL2, as well as FOXM1 expression in LUAD, which is consistent with literature. Similarly, in agreement with previous studies[21] that showed that the deregulation of CDK1, PLK1 and Aurora B causes abnormal activation of PRC1, our results confirm that PRC1 positively correlates with CDK1, PLK1 and Aurora B. We hypothesize that drugs targeting p53, CDK1, PLK1 and Aurora B potentially regulate PRC1 expression and may be used in treatment of LUAD. A search for the approved drugs that target the above genes from DrugBank database identified fostamatinib that targets CDK1, PLK1 and Aurora B. Fostamatinib is a potent Spleen tyrosine kinase (SYK) inhibitor approved for treatment of immune thrombocytopenia (ITP) in United states, Canada and Europe[42]. However, there are no studies that report the effects of fostamatinib on LUAD. Our results indicated that fostamatinib could inhibit CDK1, PLK1 and Aurora B, and may potentially inhibit PRC1. To investigate whether fostamatinib could bind PRC1 directly, molecular docking was performed. The results showed that binding-free energy of fostamatinib and PRC1 was -7.428 kcal/mol and four forces of interaction, which indicates that fostamatinib possibly binds PRC1 directly.

Conclusions

We confirmed that PRC1 has independent prognostic value in LUAD. By analyzing a large number of human LUAD samples in TCGA, we showed that tobacco smoking increases PRC1 expression to accelerate development of LUAD. We found the PRC1 positively correlates with aneuploidy score, mutation count and hypoxia score, and is related to the level of immune cells in TME. Finally, fostamatinib could be a potential drug targeted PRC1 for LUAD treatment.

Conflict of Interest
The Authors declare that they have no conflict of interests.

Ethics Approval
The ethics approval is exempted since the data involved in this study are freely available in the public domain.

Informed Consent
Not applicable.

Availability of Data and Materials
All materials are available by the corresponding author.

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