RESEARCH ARTICLE

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Dysregulated TMPO-AS1/let-7b-5p/*PLK1/E2F1* Axis Associated with Poor Prognosis in Lung Adenocarcinoma

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Abstract

Objectives: This study examines the regulatory mechanisms underlying *PLK1* overexpression in lung adenocarcinoma, especially in smokers. It focuses on understanding the ceRNA network, including mRNAs, miRNAs, and lncRNAs, while also identifying potential prognostic biomarkers. **Methods:** Publicly available databases, including Oncomine, TIMER 2.0, UALCAN, OncoMX, GEPIA2, OncoDB, ENCORI, KM Plotter, CellTracer, miRNet, CancerMIRNome, and Enrichr, were extensively utilized to assess the expression profile, prognostic significance, and regulatory interactions of *PLK1* in lung adenocarcinoma (LUAD) and normal tissues. **Results:** *PLK1* expression is significantly elevated in lung adenocarcinoma (LUAD), with a hazard ratio of 1.95, indicating a correlation with poor overall survival. Among patients who smoke, elevated *PLK1* expression is associated with a higher hazard ratio of 1.64 and reduced survivability. The transcription factor *E2F1* has been identified as a potential regulator of *PLK1* expression in LUAD. Additionally, the microRNA hsa-let-7b-5p shows a negative correlation with both *E2F1* and *PLK1*, while the long non-coding RNA TMPO-AS1 is positively associated with the *E2F1*/*PLK1* axis. These findings suggest a regulatory cascade where TMPO-AS1 may function as a molecular sponge for hsa-let-7b-5p, thereby enhancing *E2F1*-mediated *PLK1* expression and promoting the progression of LUAD. **Conclusion:** The study highlights the *PLK1*/*E2F1*/hsa-let-7b-5p/TMPO-AS1 regulatory axis as a key contributor to aggressive LUAD. Targeting this ceRNA network either by inhibiting TMPO-AS1 or restoring hsa-let-7b-5p activity may offer a promising strategy for therapeutic intervention in smoking-associated LUAD.

Keywords: Adenocarcinoma- ceRNA Network- Prognosis- Smokers

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Introduction

Lung cancer, a major cause of cancer-related mortality, is primarily divided into lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC) [1]. Both subtypes have distinct genetic and molecular profiles, which are crucial for determining appropriate treatment strategies. LUAD constitutes approximately 40% of all lung cancer cases and is defined by its unique epidemiological traits, clinicopathological features, and molecular signatures [2]. Despite advancements in diagnosis and therapy, biomarkers for early detection, prediction of recurrence, mortality rates, and identification of target or immunological therapies remain unsatisfactory [3]. Developing effective biomarkers for lung cancer is challenging due to the complex and heterogeneous nature of the disease [4]. Variability in genetic mutations and molecular profiles among patients complicates the identification of universal biomarkers. Additionally, the early stages of lung cancer often present subtle or non-specific biological changes, making early detection difficult. Lung cancer has the highest incidence and mortality rates among all TCGA cancers in terms of incidence and cancer-related deaths [5]. Recent progress in genomics and transcriptomics has provided deeper insights into the molecular and regulatory networks underlying lung tumorigenesis. These advances are essential in identifying novel therapeutic targets that hold potential to enhance clinical outcomes for lung cancer patients [6]. High-throughput technologies have opened new opportunities for biomarker identification, particularly in the field of non-coding RNAs (ncRNAs), including long ncRNAs (lncRNAs) and microRNAs (miRNAs). These technologies enable the rapid sequencing and analysis of vast amounts of genetic material, facilitating the identification of potential biomarkers with high precision and efficiency. These technologies allow researchers to comprehensively profile ncRNAs, including lncRNAs and miRNAs, which play critical roles in cancer progression. These lncRNAs, which have over 200 nucleotides, participate in various biological functions, including cell differentiation, tumor growth, and metastasis [7].

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lncRNAs regulate gene expression through the competing endogenous RNA (ceRNA) mechanism. This involves binding to miRNAs, which modulates their availability to target mRNAs [8]. Disruption of this regulatory axis particularly miRNA arm-imbalance, can lead to the dysregulation of tumor suppressors and oncogenes, driving cancer progression [9]. Among key players in cell cycle regulation Polo-like kinases (PLKs) are a family of five serine/threonine kinases involved in process such as DNA replication and mitotic progression [10]. *PLK1* is closely related to cell proliferation and has been extensively studied [11, 12]. Implication of *PLK1* dysregulation has been linked to multiple malignancies, including breast [13], gastric [14], LUAD [15], colorectal [16], and lung cancer [17]. However, the molecular mechanism behind its dysregulation remains unknown. This study compared differential expression patterns of *PLK1*, miRNA-hsa-let-7b-5p, and lncRNA-TMPO-AS1 in LUAD compared to healthy lung tissues. Additionally, it explores the regulatory framework underlying *PLK1* overexpression in LUAD. Findings indicate that the transcription factor *E2F1* binds to the *PLK1* promoter region, enhancing its transcriptional activity. By disrupting normal mitotic functions, *PLK1* causes chromosomal instability, eventually leading to mitotic catastrophe and contributes to aggressive tumor progression. Furthermore, the upregulation of TMPO-AS1 leads to the downregulation of hsa-let-7b-5p by sponge formation. This in turn doesn't allow let-7b to bind to the miRNA regulatory element (MRE) present on the *PLK1* transcript, thus leading to the overexpression of *PLK1* in LUAD invasive carcinoma.

Materials and Methods

Expression Profiling of PLK1

To explore the expression profile of the PLK family members across TCGA cancers, the Oncomine [18] database was initially employed. Pan-cancer expression analysis of *PLK1* was subsequently performed using multiple databases, including OncoMX [19], TIMER 2.0 [20] and UALCAN [21]. Furthermore, the differential expression between tumor and normal tissues in LUAD and LUSC was assessed using UALCAN, and ENCORI [22] In addition, UALCAN was used to examine the association between *PLK1* and clinicopathological features such as smoking status and patient gender.

Survival Analysis

To assess the prognostic relevance of *PLK1*, survival analysis was performed using the Kaplan-Meier Plotter [23]. The analysis considered lung cancer datasets, including the histological subtypes Adenocarcinoma and Squamous cell carcinoma, and their relation to smoking history "Gene symbol, Affy id: *PLK1*, 202240_at". To minimize the biasness and the effect of outliers on the results, patients were split by median.

The transcriptional regulation analysis

The potential transcriptional factors regulating *PLK1* expression were identified through the miRNet [24] database. The correlation efficiency between selected transcription factors and *PLK1* was evaluated using TIMER, GEPIA2 [25], OncoDB [26], and ENCORI databases. The prognostic impact of key transcription factors was examined using the KM Plotter (Affy ID: 204947_at; *E2F1*), stratified by histological subtype and smoking history. The expression of *E2F1* in lung adenocarcinoma was examined using the UALCAN database based on normal vs. tumor expression level, patient smoking status, and gender.

Competitive Endogenous RNA (ceRNA) Network Analysis

To elucidate the ceRNA-mediated regulation of *PLK1*, miRNet databases were used to identify a network of miRNAs associated with both *PLK1* and *E2F1*. The correlation between *PLK1*, *E2F1* and candidate miRNAs was determined using ENCORI database and validated using the CancerMIRNome. UALCAN provided insights into pathological attributes such as gender and smoking status in LUAD samples. The prognostic implications of *PLK1*-related miRNAs were assessed using KM Plotter. To identify lncRNAs potentially regulating *PLK1*, Enrichr [27] and UALCAN were utilized, with further correlation validation conducted using ENCORI.

PLK1 Associated Biological Process and Proliferation Analysis

The functional role of *PLK1* in lung cancer was explored using the CellTracer database [28], focusing on its involvement in various biological pathways. Correlation analysis between *PLK1* and the proliferation marker MKI67 was performed using TIMER2.0, OncoDB, and ENCORI. Furthermore, the regulatory control of MKI67 was investigated using ENCORI database to determine factors contributing to MKI67 regulation.

Statistical Analysis

PLK1 gene expression was conducted using unpaired t-tests and integrated statistical models provided by respective databases. Associations between *PLK1* expression and clinical prognosis were examined through cox-proportional hazard models employed by the survival analysis tools, and a p-value < 0.05 was considered statistically significant.

Results

Polo-like Kinase: Pan-cancer Expression and Prognostic Relevance of PLK1

To evaluate the expression profile of Polo-like kinase (PLK) family members (*PLK1*–PLK5) across various cancers, a comprehensive pan-cancer analysis was performed where the oncomine database analysis revealed that *PLK1* and PLK4 were overexpressed in multiple tumor types, with *PLK1* showed the highest expression in lung cancer cases, as shown in Figure 1A. Similarly, the OncoMX database confirmed *PLK1* overexpression in lung cancer with a fold change of 3.73 as listed in Table 1. Consistent findings were observed across TIMER 2.0 and UALCAN databases, demonstrating upregulation of *PLK1* in both LUAD and LUSC conditions as shown in Figures 1B and C. Differential expression analysis

Table 1. Pan-Cancer Analysis of *PLK1*

Gene Symbol	Log2 F.C.	P-value	Adj. P-value	Significant	Expression Trend	TCGA Study	Patient Freq.	Source
PLK1	3.75	5.96E-106	1.99E-102	Yes	up	uterine cancer	16	TCGA
	3.73	2.47E-278	5.22E-275	Yes	up	lung cancer	78	
	3.51	3.02E-70	1.92E-67	Yes	up	liver cancer	29	
	3.41	2.71E-189	2.19E-186	Yes	up	breast cancer	97	
	2.35	2.95E-97	3.5E-95	Yes	up	kidney cancer	82	
	2.26	4.11E-20	5.73E-18	Yes	up	bladder cancer	13	
	1.9	2.93E-13	5.35E-11	Yes	up	esophageal cancer	7	
	1.61	8.99E-43	1.54E-40	Yes	up	head_and_neck cancer	36	
	1.52	2.38E-18	1.18E-16	Yes	up	stomach cancer	24	
	1.44	2.14E-37	5E-36	Yes	up	colorectal cancer	45	
	1.38	5.6E-19	1.27E-17	Yes	up	prostate cancer	42	

using UALCAN further confirmed significant *PLK1* overexpression in LUAD (FC = 8.7; normal = 59, tumor = 515) and LUSC (FC = 9.6; normal = 52, tumor = 503), as depicted in Figures 2A–B. Further differential expression analysis using ENCORI database supported this observation in LUAD (P = 6.1e-62, FC = 10.07, FDR = 2.4e-59) and LUSC (P = 7.5e-151, FC = 17.16, FDR = 6.1e-147) as shown in Figure 2C-D. Analysis of *PLK1* expression in relation to clinicopathological factors revealed higher expression among male patients and smokers in both LUAD and LUSC subtypes (Supplementary Figure 1A–D). Notably, *PLK1* expression was more elevated in LUSC compared to LUAD. Kaplan–Meier survival analysis using KM Plotter indicated that high *PLK1* expression was significantly associated with poor overall survival in LUAD patients (HR = 1.95, CI = 1.64–2.33, P = 3.3–14) and less significantly with LUSC patients (HR = 1.25, CI = 1.02-1.52, P = 0.027), as shown in Figure 2E-F. Importantly, LUAD patients with a smoking history exhibited significantly worse survival outcomes with *PLK1* overexpression (HR = 1.64, 95% CI = 1.25–2.13, P = 0.00025) compared to LUSC smokers, as shown in Figure 2G-H. Collectively, these results suggest that *PLK1* may serve as a poor prognostic marker for LUAD-smoking patients.

Expression Regulation of PLK1 By Transcription Factors

To explore the transcriptional regulation of *PLK1*, we employed the miRNet database to identify transcription factors associated with *PLK1*. The analysis revealed that *E2F1*, *E2F3, E2F4,* and *TP53* are key transcriptional regulators potentially involved in *PLK1* expression, as illustrated in Figure 3A. Further correlation analysis using the TIMER database showed a strong positive correlation between *PLK1* and *E2F1* (R = 0.74), moderate correlations with E2F3 (R = 0.513) and E2F4 (R = 0.416), and a weak negative correlation with TP53 (R = -0.027), as shown in Figures 3B–E. The study validated these observations using GEPIA2, OncoDB, and ENCORI databases. The results consistently demonstrated a strong positive correlation between *E2F1* and *PLK1* expression (R = 0.65, 0.73, and 0.73, respectively), while E2F3, E2F4, and TP53 showed relatively weaker associations, as shown in Figures 3F–Q. This suggests that *E2F1* may serve as a major transcriptional regulator of *PLK1*. Moreover, *E2F1* may be a potential therapeutic target for *PLK1*-related diseases. Thus, we further analyzed *E2F1* expression in LUAD using the UALCAN database and found that *E2F1* was significantly overexpressed in the tumor compared with the normal *** = <1e-12, smoker compared with non-smoker *** = 1.6e-12, and male compared with female *** = <1e-12, respectively, as shown in Supplementary Figure 2A-C. The subsequent survival analysis using the KM Plotter database also indicated *E2F1* overexpression to be extremely associated with adenocarcinoma (HR = 1.63, 95% CI =1.37 to 1.94, P = 2.2e-08) and smoker (HR = 1.49, 95% CI = 1.14 to 1.93, P = 0.0028) patients as shown in Supplementary Figure 2D-E. According to the data above, *E2F1* may function as a regulator that regulates *PLK1* expression in the surroundings of LUAD.

Analyzing the competitive endogenous RNA regulatory network

The dysregulation of *PLK1* and *E2F1* mRNA has been associated with poor prognosis, tumor progression, and metastasis in LUAD, probably influenced by microRNAs. To investigate this, a miRNA-mRNA network was constructed using the miRNet database, and the study revealed 12 miRNAs (hsa-mir-34a-5p, hsa-mir-196a-5p, hsa-mir-126-3p, hsa-mir-7-5p, hsa-let-7b-5p, hsa-mir-26a-5p, hsa-mir-29c-3p, hsa-mir-17-5p, hsa-mir-125a-3p, hsa-mir-1-3p, hsa-mir-16-5p, hsa-mir-155-5p) implicated in LUAD as shown in Figure 4A-B. The miRNet topology, set to "concentric circle," was found to be most suitable for miRNA associated with the gene, with hsa-let-7b-5p being in proximity with *PLK1*. A negative correlation between hsa-let-7b-5p and *PLK1* was observed in the CancerMIRNome (R = -0.467, P = 7.14e-30) and ENCORI (R = -0.382, P = 3.14e-19) databases, as shown in Figure 4C-D. Additionally, a significant negative correlation between hsa-let-7b-5p and *E2F1* was identified (R = -0.365, P = 1.33e-17) using the ENCORI database, as shown in Supplementary Figure 3A. The correlation of hsa-let-7b-5p with *PLK1* and *E2F1* indicates a strong negative association, suggesting that the hsa-let-7b-5p down expression in LUAD may lead to a poor prognosis. The study employed the CancerMIRNome

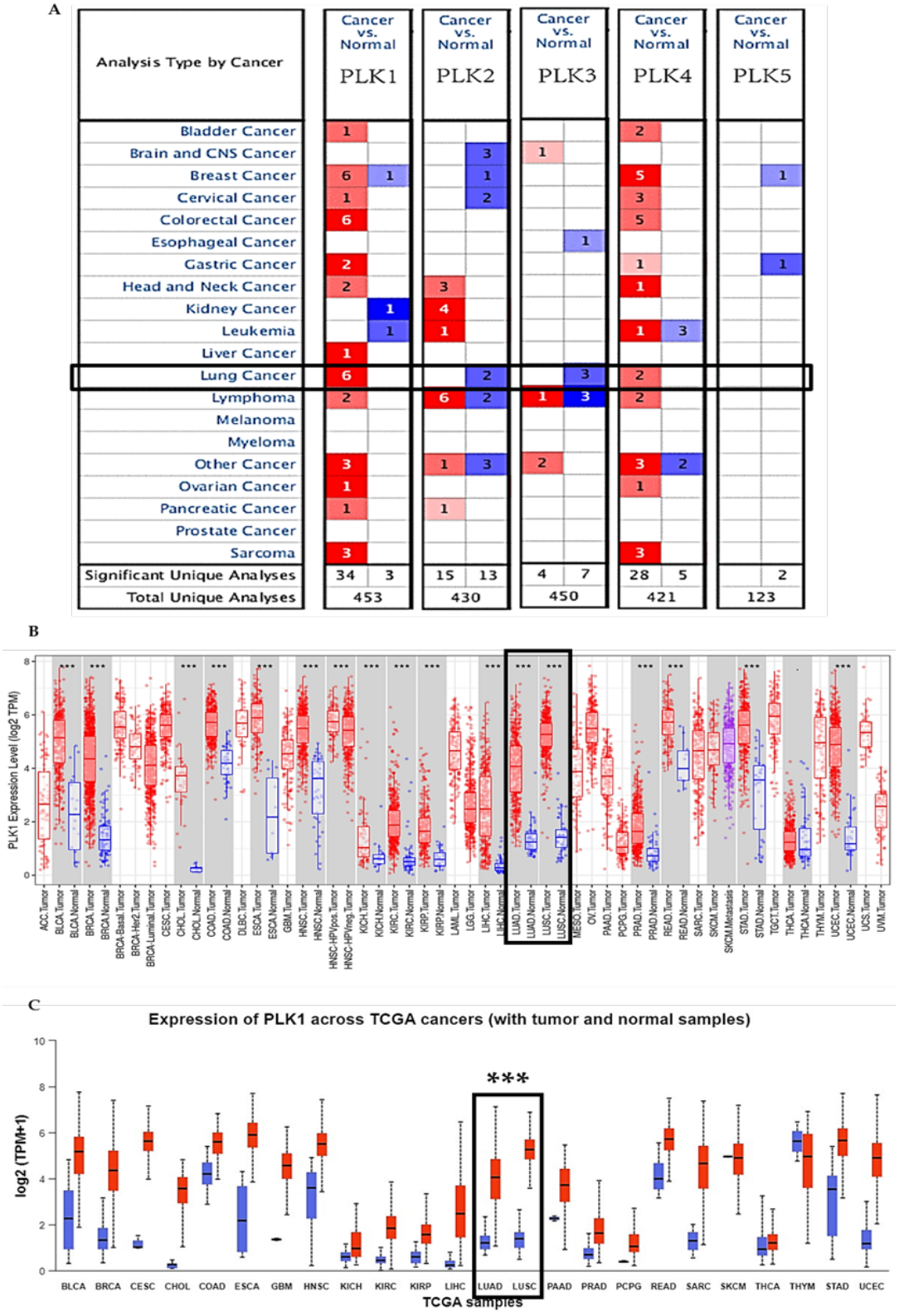

Figure 1. Expression Pattern of *PLK* Across Pan-Cancer. (A) The expression profile of the PLK family was determined by the Oncomine database; the red boxes indicate their expression level in cancer, and the blue boxes indicate their expression level in normal. (B) TIMER 2.0 database for tumor versus normal samples; the red bar-dot plot indicates tumor, and the blue bar-dot plot corresponds to normal tissue. Error bars represent the SD (***p < 0.001). (C) expression of PLK1 in pan-cancer by UALCAN database, tumors compared with matched normal samples, red bars = tumor, blue bars corresponding to normal tissue.

and ENCORI databases to analyze the expression level of let-7b-5p in normal and tumor conditions. Significant downregulation was observed and downregulated let-7b significantly affected the survival of LUAD patients (P = 3.82e-18, P = 2.1e-15, respectively), as shown in Figure 4E-F. Furthermore, the study validated the expression of

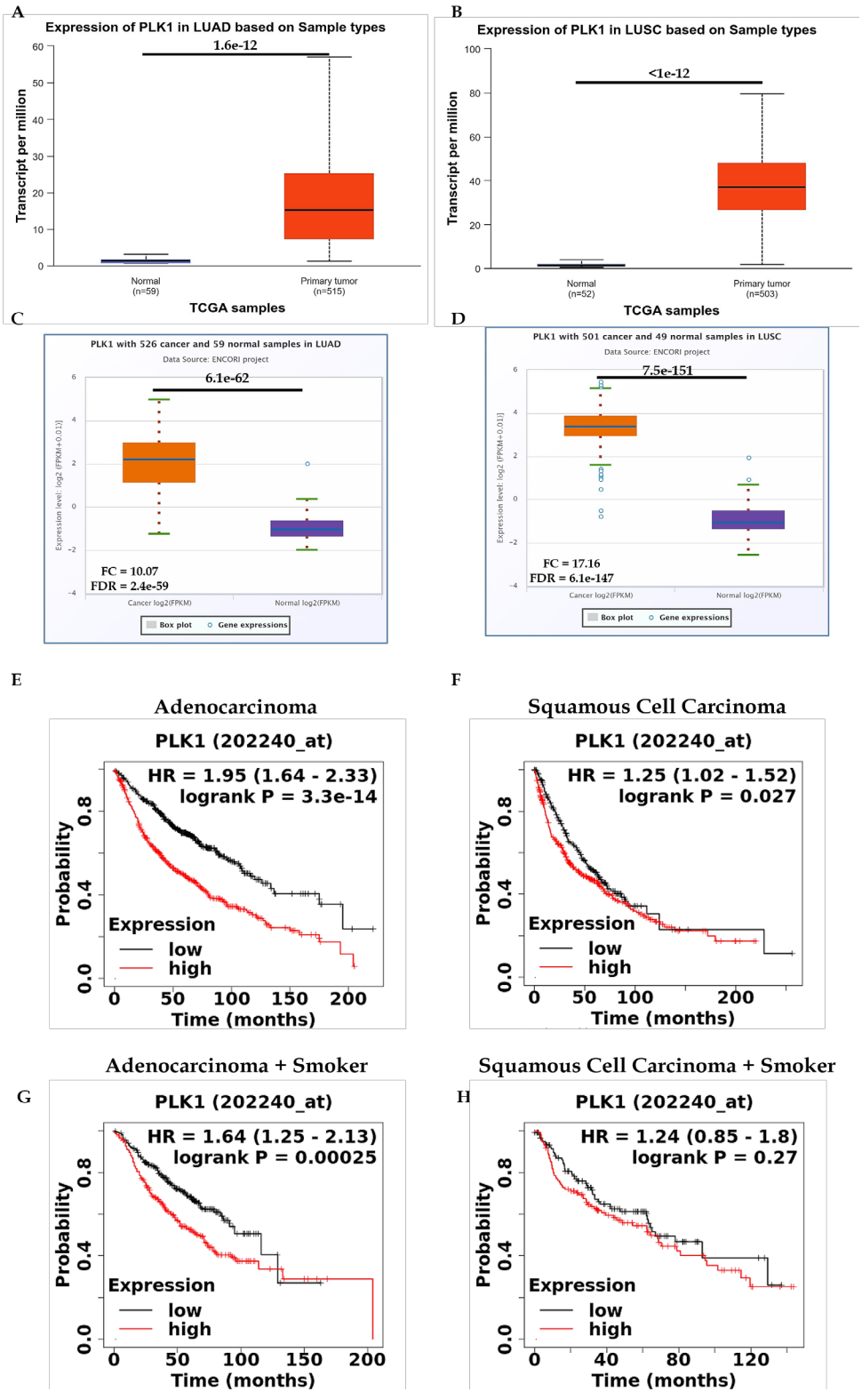

Figure 2. Differential Expression of *PLK1* in Lung Cancer. (A–D) mRNA expression was analyzed in normal lung tissue and primary tumors from the publicly available database, such as UALCAN (A) LUAD (normal n = 59, tumor n = 515), (B) LUSC (normal n = 52, tumor n = 503), and ENCORI (C) LUAD (normal n = 59, tumor n = 526), and (D) LUSC (normal n = 49, tumor n = 501). (E-H) Prognostic role of mRNA expression of PLK1 in lung cancer patients. Kaplan-Meier survival curves were plotted for (E) adenocarcinoma (n = 1161), (F) squamous cell carcinoma (n = 780), (G) adenocarcinoma + smoker (n = 546), and (H) squamous cell carcinoma + smoker (n = 244).

hsa-let-7b-5p correlated with smoking history and poor prognosis in LUAD patients using UALCAN and KM Plotter and found downregulated let-7b significantly affected the survival of the patients (HR = 0.71, CI = 0.53-0.95, P = 0.021), as shown in Figure 4G-H. This suggests that controlling the expression level of hsa-let-7b-5p can

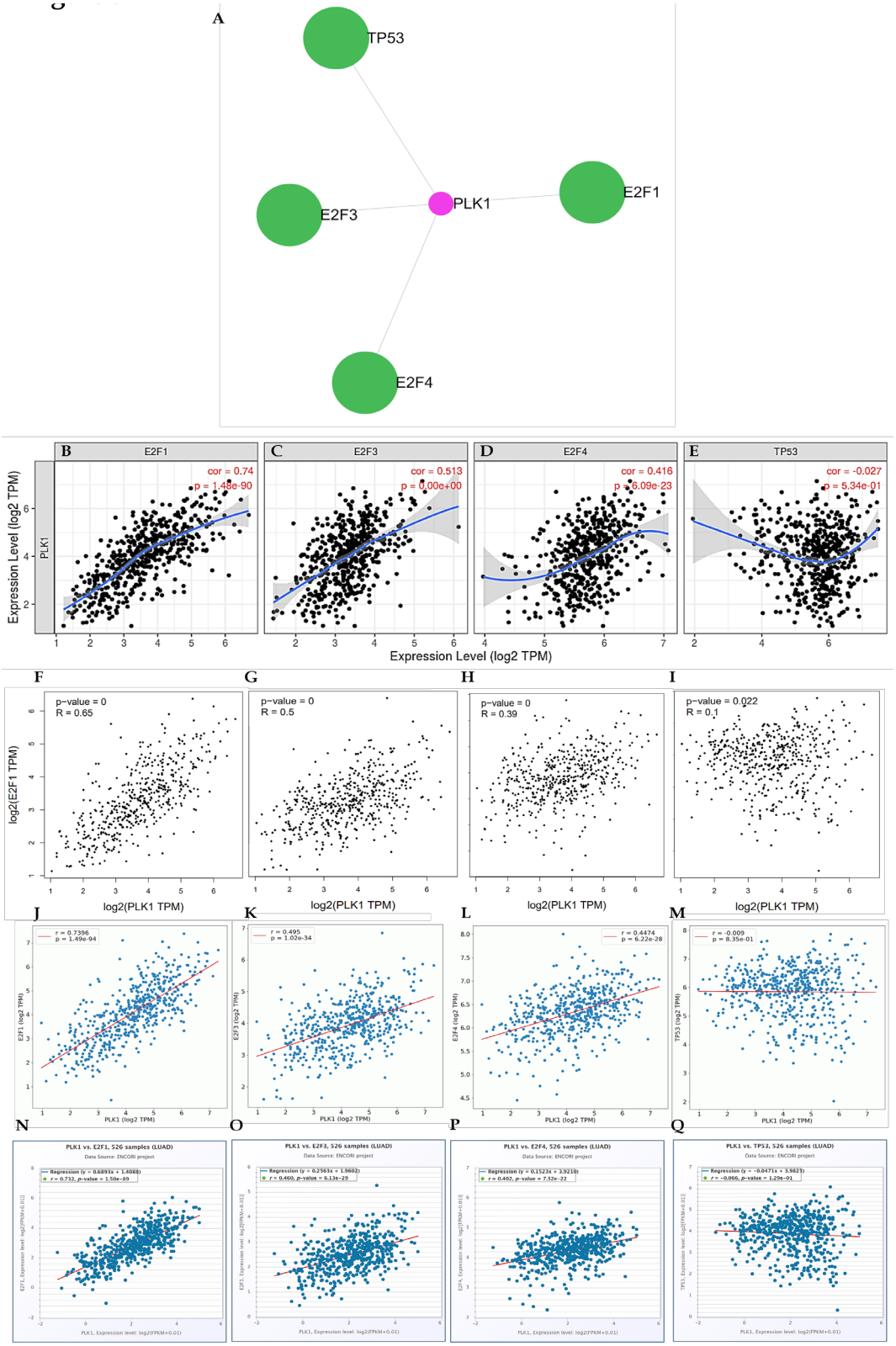

Figure 3. Expression Correlation Analysis between *PLK1* and Transcription Factors. (A) Network showing E2F1, E2F3, E2F4, and TP53 transcription factors associated with PLK1 in lung cancer by using the miRNet database. Further, correlation values between PLK1 and transcriptional factor in lung adenocarcinoma were found using the B-E. TIMER database, the F-I GEPIA2 database, the J-M OncoDB database, and the N-Q ENCORI database.

regulate *PLK1*'s expression, making let-7b-5p a prognostic biomarker for LUAD smoker patients. Also, controlling let-7b-5p expression can potentially improve prognosis in LUAD patients.

Next, the work will explore how *PLK1*, hsa-let-7b-5p, and associated lncRNA interact with miRNA stability and regulation to determine their sponge effect. Several lncRNAs associated with *PLK1* have been identified

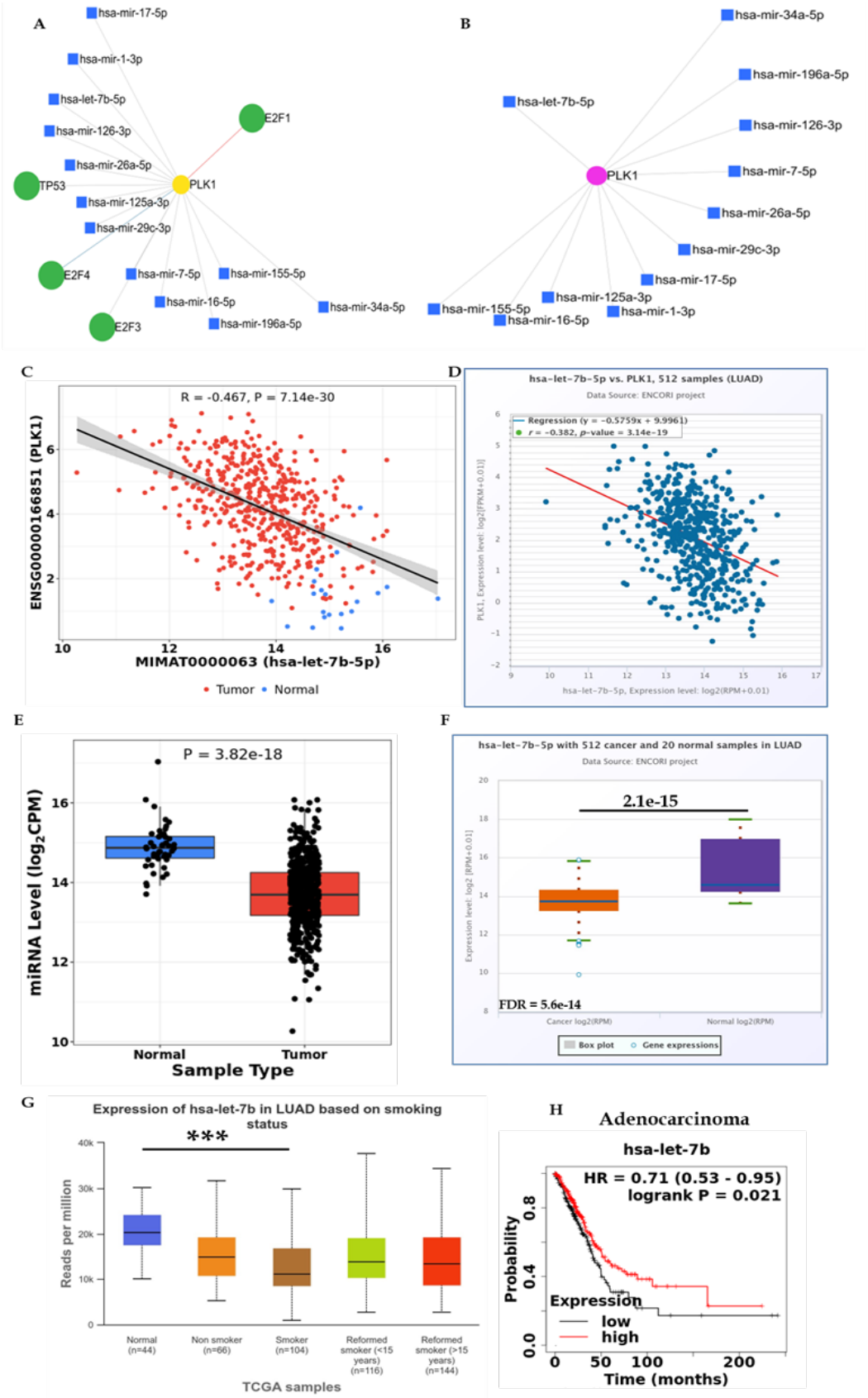

Figure 4. miRNA Correlation, Expression, and Survival Status in Tumor Tissues from Lung Cancer Patients by Using Various Databases. Network analysis between (A) PLK1, transcription factors, and miRNAs, and (B) PLK1 and miRNAs using the miRNet database. Correlation analysis between PLK1 and hsa-let-7b-5p using (C) CancerMIRNome and (D) ENCORI databases. Differential expression analysis of hsa-let-7b-5p in lung adenocarninoma patients using (E) CancerMIRNome and (F) ENCORI. Expression of hsa-let-7b in LUAD based on smoking status was determined using the (G) UALCAN database. Survival analysis of hsa-let-7b-5p down expression in LUAD patients by using (H) KM Plotter.

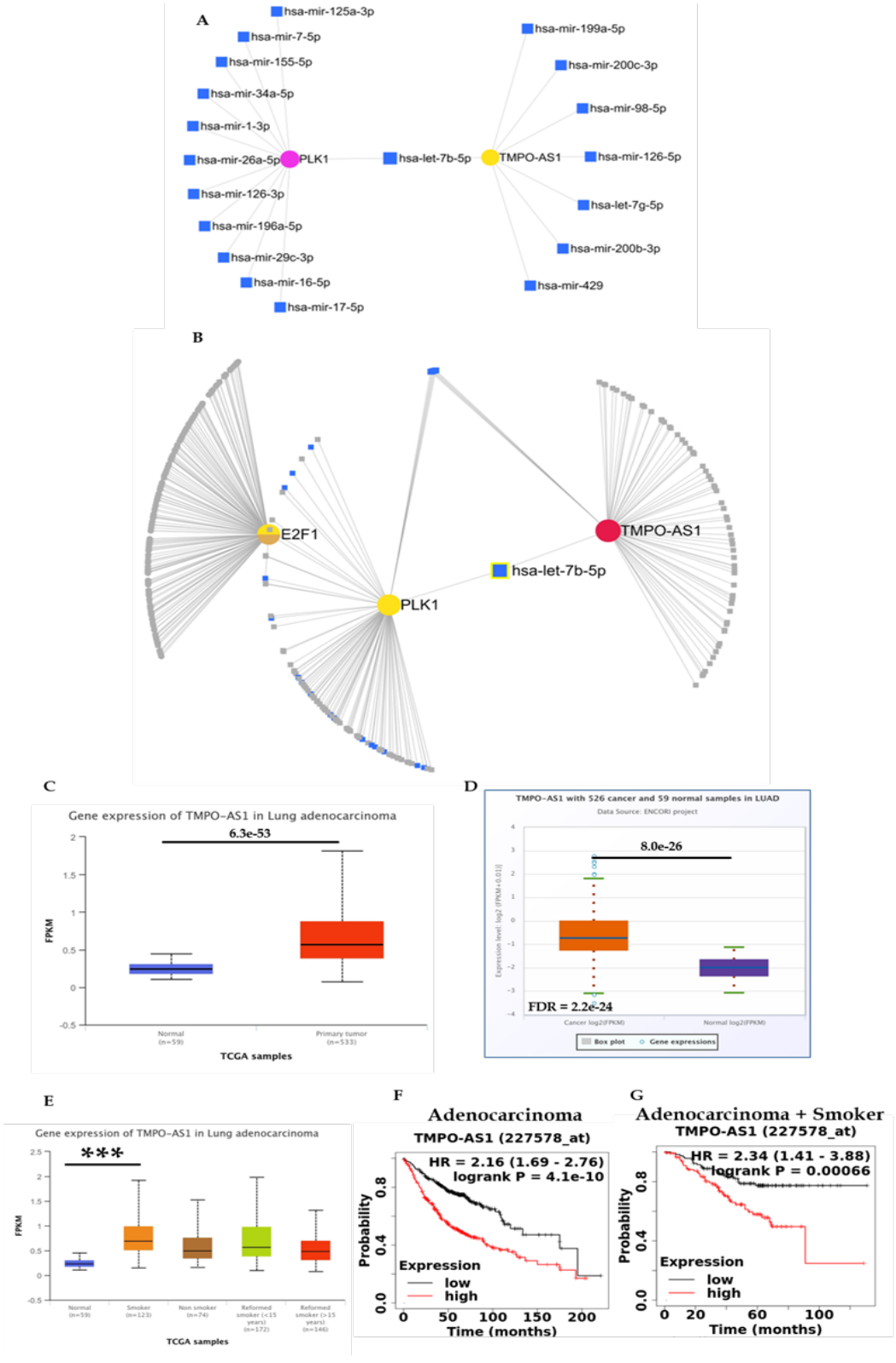

Figure 5. Analysis of lncRNA Associated with *PLK1*. Using the miRNet database to create a network between (A) PLK1, hsa-let-7b-5p, and TMPO-AS1, and (B) PLK1, hsa-let-7b-5p, TMPO-AS1, and E2F1. Differential expression analysis of TMPO-AS1 in lung adenocarcinoma using (C) UALCAN (Normal = 59, Tumor = 533) and (D) ENCORI (Normal = 59, Tumor = 526) databases. (E) Expression of TMPO-AS1 in lung adenocarcinoma based on smoking status using the UALCAN database. Survival analysis of TMPO-AS1 overexpression by using KM Plotter in cases of (F) adenocarcinoma (n = 672) and (G) adenocarcinoma + smoker (n = 231).

using the Enrichr database, including PRC1-AS1, CSRP3-AS1, H2AZ1-DT, DEPDC1-DT, DDX11-DT, LINC01775, APOBEC3B-AS1, RRM1-AS1, HMMR-AS1, LINC00618, LIX1L-AS1, LINC01096, LINC01224, PRMT5-AS1, SGO1-AS1, CDKN2A-DT, TMPO-AS1, FOXD2-AS1, LINC01842, UBL7-AS1 and further their correlation with *PLK1* was studied using UALCAN and ENCORI as presented in Supplementary Table 1-3.

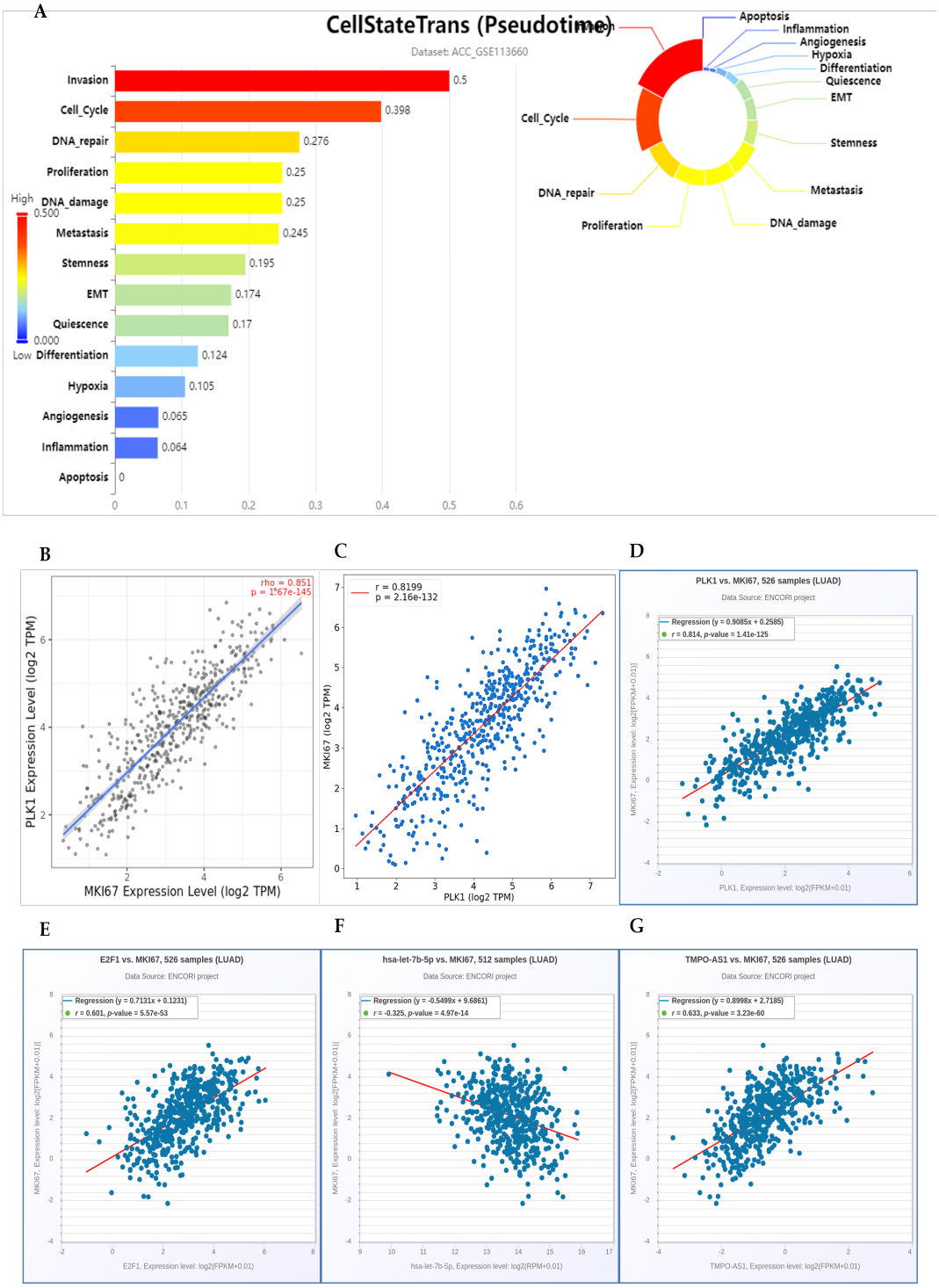

Figure 6. Biological Processes and Proliferation Mechanism Analysis. (A) Using the CellTracer database to find out the role of PLK1 in various biological processes. Correlation analysis between PLK1 and MKI67 by using (B) TIMER 2.0, (C) OncoDB, and (D) ENCORI databases. Using the ENCORI database to analyze the correlation between (E) E2F1 vs. MKI67, (F) hsa-let-7b-5p vs. MKI67, and (G) TMPO-AS1 vs. MKI67.

We found that three lncRNAs, namely TMPO-AS1, LINC01775 and UBL7-AS1, were strongly correlated with *PLK1*, but on further analysis of their prognostic significance, only TMPO-AS1 was consistent. As shown in Supplementary Figure 3 B-D, TMPO-AS1 strongly correlated with *PLK1*, with a significant positive correlation (R = 0.638, P = 1.65e-61), along with *E2F1* (R = 0.581, P = 1.01e-48). A negative correlation between TMPO-AS1 and hsa-let-7b-5p (R = -0.2777, P = 1.90e-10) was observed, suggesting the formation of sponges. Further analysis of the association between *PLK1*, *E2F1*, hsa-let-7b-5p, and TMPO-AS1 was done using a network visual analytics platform database, miRNet, and the network showed the proximity between these

genes as shown in Figures 5A–B. Differential expression analysis of TMPO-AS1 in LUAD using UALCAN (P = 6.3e-53) and ENCORI (P = 8.0e-26) showed significant upregulation in its expression in tumors as compared to normal samples (Figure 5C-D). Also, the gene expression level of TMPO-AS1 was associated with poor prognosis in adenocarcinoma (HR = 2.16, CI = 1.69–2.76, P = 4.1e–10) and adenocarcinoma + smokers (HR = 2.34, CI = 1.41-3.88, P = 0.00066) using UALCAN and KM Plotter, as shown in Figure 5E–G. Considering the available data, it has been concluded that the TMPO-AS1/has-let-7c-5p/*PLK1*/*E2F1* feedback loop may play a role in the progression of lung adenocarcinoma, based on the available data.

PLK1's Role in Biological Processes and Proliferation

Understanding the role of *PLK1* in cancer progression is essential for identifying potential therapeutic targets. The CellTracer database, which is based on single-cell RNA sequencing data, offers valuable insights into the involvement of *PLK1* in various biological processes. Analysis of the data reveals that *PLK1* overexpression in lung cancer is significantly associated with key processes such as cell invasion, proliferation, metastasis, and epithelial-mesenchymal transition. These findings suggest that *PLK1* could serve as a promising biomarker for lung cancer progression, as illustrated in Figure 6A. The correlation between *PLK1* gene expression in LUAD and MKI67, a gold standard proliferation maker, was evaluated using the TIMER2.0, OncoDB, and ENCORI databases. The results showed a strong positive correlation, with values of R = 0.851, P = 1.67e-145 (TIMER 2.0), R = 0.8199, P = 2.16e-132 (OncoDB), and R = 0.814, P = 1.41e-125 (ENCORI), as shown in Figure 6B-D. Further, the ENCORI database suggests that *PLK1* overexpression may be associated with increased proliferation activity in LUAD. The above-mentioned studies have studied the regulatory mechanisms of *PLK1* associated with transcription factors and the ceRNA network. The correlation of MKI67 vs. *E2F1*, hsa-let-7b-5p, and TMPO-AS1 was positive, suggesting a positive correlation between *E2F1* and TMPO-AS1 with MKI67 and a negative correlation between hsa-let-7b-5p and MKI67 in LUAD, as shown in Figure 6E-G. The study suggests that hsa-let-7b-5p may inhibit MKI67 and *E2F1* in LUAD, while TMPO-AS1 may activate these genes. *PLK1* may play a crucial role in LUAD's regulatory mechanisms and serve as a potential therapeutic target. *PLK1* inhibitors could be a promising treatment option, but further research is needed to understand its role in LUAD pathogenesis and develop effective *PLK1* inhibitors for clinical use.

Discussion

Lung adenocarcinoma (LUAD), a major subtype of non-small cell lung cancer (NSCLC), remains one of the most commonly diagnosed cancers worldwide and is associated with poor five year survival rate and high mortality [29]. Despite extensive research on molecular biomarkers and tumorigenic mechanisms, LUAD incidence and associated deaths continue to escalate, largely due to the complex genetic makeup. NSCLC represents approximately 85% of all lung cancer cases and is often driven by specific oncogenic mutations [30]. Among these, the T790M mutation in the EGFR gene is a well-recognized mechanism of acquired resistance to first- and second-generation EGFR tyrosine kinase Inhibitors (TKIs). However, the emergence of third-generation TKIs has provided a promising therapeutic avenue, as they are capable of targeting the T790M mutation, which accounts for resistance in approximately 50-60% of NSCLC patients [31]. The study aimed to identify key kinesins influencing LUAD prognosis using bioinformatics analysis. For which, expression and survival plot analyses of PLKs across cancer genome atlas (TCGA)-LUAD patient samples were performed, identifying only significant ones. We then performed overall survival analysis, mutational enrichment and obtained *PLK1* as the final prognostic biomarker responsible for LUAD pathogenesis. The study focuses on determining the regulation of *PLK1* and its associated ceRNA, specifically focusing on upregulation for lung cancer treatment.

PLK1, a protein kinase, controls cell division in eukaryotic cells and is crucial for DNA replication and p53 control [32]. Aberrant overexpression of *PLK1* has been consistently associated with poor prognosis in NSCLC. Clinical investigations of small-molecule *PLK1* inhibitors, particularly in Phase II trials, have demonstrated favorable anti-tumor activity in NSCLC patients [33]. *PLK1*'s capacity to impair cancer cell survival, promote apoptosis, and enhance the efficacy of chemotherapeutic agents underscores its therapeutic relevance. Strategies to inhibit *PLK1*, either through RNA interference (RNAi) or small-molecule inhibitors, have shown significant promise in suppressing tumor cell proliferation [34, 35]. However, considering the instability of RNA-based approaches due to nuclease degradation and delivery challenges, small-molecule inhibitors offer a more feasible and safer modality for clinical targeting of *PLK1*. Overexpression of *PLK1* has been documented across multiple malignancies, including esophageal carcinoma [36], triple negative breast cancer (TNBC) [37], papillary thyroid cancer [38], and prominently in both lung adenocarcinoma, and lung squamous cell carcinoma [39, 40]. While *PLK1* is recognized for its involvement in mitotic progression and oncogenic transformation, its precise biological and regulatory functions in lung cancer remain to be fully elucidated [41].

In the present study, we explored the regulatory landscape governing PLK1 dysregulation in LUAD, aiming to establish its prognostic relevance, particularly among smoker patients. Differential expression analysis demonstrated that *PLK1* is significantly upregulated in both LUAD and LUSC, with a stronger expression correlation observed in LUSC. Despite this, survival analysis revealed that elevated *PLK1* expression is significantly associated with poor overall survival specifically in LUAD patients with a history of smoking, underscoring its potential as a prognostic biomarker in this subgroup. Transcription factor analysis identified *E2F1* as a potential upstream regulator of *PLK1*, suggesting a transcriptional

axis that may contribute to its aberrant expression in LUAD. Furthermore, the study also identified the role of microRNAs and long non-coding RNAs in regulating *PLK1* expression post-transcriptionally through the ceRNA network. Specifically, we identified 12 miRNAs associated with *PLK1* as shown in Figure 4B, among which hsa-let-7b-5p emerged as a key negative regulator, displaying a significant negative correlation with *PLK1* expression. Importantly, downexpression of hsa-let-7b-5p was also linked to poor prognosis in LUAD, reinforcing its role in modulating oncogenic pathways through *PLK1* repression.

Among the 20 identified lncRNAs, TMPO-AS1, exhibited a strong positive correlation with *PLK1*/*E2F1* expression (as mentioned in Supplementary Table 1) and a negative correlation with hsa-let-7b-5p, as shown in Supplementary Figure 3C-D. This expression pattern suggests a functional ceRNA interaction, where TMPO-AS1 may act as a molecular sponge for hsa-let-7b-5p, thereby relieving its suppressive effect on *PLK1* and *E2F1*. Dysregulation of this TMPO-AS1/let-7b-5p/*PLK1*/*E2F1* axis was significantly associated with adverse clinical outcomes, particularly in smoker subgroups, highlighting its prognostic and potential therapeutic significance. Functional enrichment analysis of biological processes revealed that *PLK1* overexpression is closely linked to aggressive tumor phenotypes, including enhanced cellular proliferation, invasion, and metastatic potential. Notably, a strong positive correlation between *PLK1* and the proliferation marker MKI67 further substantiates the role of *PLK1* in driving proliferative signaling in LUAD, potentially via the same upstream regulatory circuit involving *E2F1*, hsa-let-7b-5p, and TMPO-AS1. However, we acknowledge that this study relies on In-silico analyses of public datasets, which may be affected by data variability and limited clinical detail. The proposed TMPO-AS1–let-7b-5p–*PLK1*/*E2F1* regulatory axis lacks experimental validation, and smoking-specific effects require further confirmation in cell line models. However, we believe this In-silico study provides a strong foundation for future experimental validation and mechanistic investigations.

In conclusion, this study indicates that high levels of *PLK1* expression are linked to poor prognosis in lung adenocarcinoma patients, especially those with a history of smoking. *PLK1* is positively regulated by the transcription factor *E2F1* and is negatively correlated with the tumor-suppressive microRNA hsa-let-7b-5p. Additionally, *PLK1* is positively associated with the oncogenic long non-coding RNA TMPO-AS1. Together, the *PLK1*/*E2F1*/hsa-let-7b-5p/TMPO-AS1 axis may serve as a promising prognostic biomarker for lung adenocarcinoma in smokers and presents a potential target for future therapeutic interventions.

Author Contribution Statement

RN: Conception, study design, critical reading, and intellectual assessment of the manuscript and preparation of the manuscript. PV: Study design, and preparation of the manuscript. BB: Preparation of the manuscript, critical review. CS: Preparation of the manuscript, critical review.

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Ethics Statement

Since this study exclusively utilized publicly available online databases for data extraction and analysis, ethical clearance was not required as per institutional guidelines.

Data Availability

The data for this in-silico study were sourced from publicly accessible databases. The respective links and references for these datasets are provided with the methodology section for transparency and reproducibility.

Conflict of Interest

The authors declare that they have no competing interests.

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