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Comprehensive Analysis of Chromobox 1 Expression, DNA Methylation and Non-Coding RNA Interactions in Lung Adenocarcinoma

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Chromobox 1 (CBX1), an epigenetic regulator involved in chromatin remodeling, has been implicated in various cancers; however, its role in lung adenocarcinoma (LUAD) remains underexplored. In the present study, a multi-omics bioinformatics approach with datasets from TIMER2.0, GEPIA2, OSlihc, OncoDB, MethSurv, miR-Net, and ENCORI was used to investigate *CBX1* expression patterns, epigenetic regulation, and non-coding RNA networks in LUAD. *CBX1* was shown to be significantly overexpressed in LUAD tissues, with high expression levels correlating with poor prognosis. DNA methylation analysis revealed that hypermethylation of specific CpG sites near *CBX1* was associated with elevated expression and adverse clinical outcomes. Notably, *hsa-miR-29b-3p* and *hsa-miR-29c-3p* were significantly upregulated in LUAD and were negatively correlated with *CBX1* expression, indicating a potential regulatory relationship. Further analysis identified a network of long non-coding RNAs (lncRNAs) and pseudogenes that interact with these micro RNAs (miRNAs), several of which are linked to tumor progression and poor prognosis. These findings highlight the *CBX1*-miRNA-lncRNA axis as a promising target for diagnostic and therapeutic strategies in LUAD, provide system-level insights into its regulatory environment, and support its potential role in precision medicine.

Keywords: *Chromobox 1*, Lung adenocarcinoma, Prognostic biomarker, DNA methylation, Non-coding RNA network

Introduction

Lung adenocarcinoma (LUAD) is the most common histological subtype of non-small cell lung cancer (NSCLC) and remains a leading cause of cancer-related deaths worldwide [1]. Despite advancements in targeted therapies and immunotherapies, the overall prognosis of patients with LUAD remains poor due to late-stage diagnosis, high recurrence rates, and therapeutic resistance [2]. Therefore, the identification of novel molecular biomarkers and therapeutic targets is crucial for improving clinical outcomes and advancing precision oncology strategies.

Epigenetic alterations, particularly DNA methylation, have been shown to contribute significantly to cancer development by regulating gene expression [3]. Aberrant promoter hypermethylation often silences tumor suppressor genes, whereas hypomethylation may activate oncogenes, thereby disrupting cellular homeostasis [4]. Several studies have reported distinct methylation patterns in LUAD tissues that correlate with tumor progression and prognosis, indicating that DNA methylation signatures may serve as valuable diagnostic and prognostic biomarkers [5].

Chromobox 1 (CBX1), a key component of heterochromatin protein 1, plays

a role in chromatin organization and transcriptional repression. Recent evidence has shown that *CBX1* is aberrantly expressed in various cancers, and may promote tumorigenesis by influencing the epigenetic landscape [6]. However, the specific mechanisms that regulate *CBX1* expression in LUAD tissues and their functional implications remain largely unexplored.

Simultaneously, non-coding RNAs (ncRNAs), particularly micro RNAs (miRNAs), long non-coding RNAs (lncRNAs), circular RNAs (circRNAs), and pseudogenes, have emerged as critical regulators of gene expression [7]. MiRNAs function post-transcriptionally by targeting mRNAs for degradation or translational repression. They also serve as central components of competing endogenous RNA (ceRNA) networks, wherein lncRNAs, circRNAs, and pseudogenes compete for shared miRNAs, thereby modulating mRNA stability and translation. Notably, disruptions in these ceRNA networks have been implicated in cancer pathogenesis, metastasis, and drug resistance [8].

In this study, we aimed to elucidate the regulatory landscape of *CBX1* in LUAD tissues through comprehensive multi-omics analysis, including mRNA expression profiling, DNA methylation assessment, and ncRNA-based regulatory network construction. We hypothesized that *CBX1* is subject to multi-layered regulation through both epigenetic modifications and ceRNA-mediated interactions and that such dysregulation may contribute to LUAD progression and poor prognosis.

Through integrative bioinformatics analysis, we identified key miRNAs, including *hsa-miR-29b-3p* and *hsa-miR-29c-3p*, that were predicted to target *CBX1* and were associated with adverse clinical outcomes. Furthermore, we mapped extensive regulatory networks involving these miRNAs and their interactions with lncRNAs and pseudogenes, indicating that a complex ncRNA-mediated mechanism underlies *CBX1* dysregulation.

This study specifically focused on LUAD rather than on lung squamous cell carcinoma (LUSC), although both are NSCLC subtypes. LUAD and LUSC differ significantly in terms of histopathology, molecular alterations, and therapeutic responses [9]. The inclusion of both could introduce heterogeneity that obscures subtype-specific mechanisms. Therefore, focusing solely on LUAD allowed us to characterize the regulatory dynamics of *CBX1* in this biologically distinct tumor type more accurately.

Collectively, our findings highlight *CBX1* as a potential oncogenic driver in LUAD tissues and indicate that its expression is tightly regulated through a coordinated interplay between

DNA methylation and ncRNA networks. These results indicate that *CBX1* and its associated pathways may serve as promising diagnostic markers and therapeutic targets for LUAD.

Methods

mRNA expression analysis of *chromobox 1* in lung adenocarcinoma tissues

To evaluate the mRNA expression levels of *CBX1* in LUAD tissues, multiple publicly accessible bioinformatics platforms, including TIMER2.0 (<http://timer.cistrome.org/>) and GEPIA2 (<http://gepia2.cancer-pku.cn/>), were used. These databases integrate multi-omics data derived from The Cancer Genome Atlas (TCGA) to facilitate comprehensive cancer analysis. Differential expression between tumor and normal tissues was assessed using TIMER2.0, which offers standardized gene expression profiles across various cancer types [10]. GEPIA2 was used to compare *CBX1* expression in tumors and normal tissues across datasets [11].

Prognostic Analysis of *chromobox 1* in lung adenocarcinoma tissues

To evaluate the prognostic relevance of *CBX1* expression in LUAD tissues, Kaplan–Meier (KM) survival analyses were conducted using the OSlihc platform (<https://bioinfo.henu.edu.cn/LIHC/LIHCList.jsp>) [12]. The survival endpoints analyzed included overall survival (OS), disease-free interval (DFI), progression-free interval (PFI), and disease-specific survival (DSS).

DNA methylation and prognostic analysis of *chromobox 1* in lung adenocarcinoma tissues

The methylation of *CBX1* was assessed using a combination of publicly available bioinformatics platforms including OncoDB (<https://oncodb.org/>) and MethSurv (<https://biit.cs.ut.ee/methsurv/>) [13]. OncoDB was used to evaluate the methylation patterns of *CBX1* across normal and tumor tissues. In addition, MethSurv facilitated analysis of the association between methylation and patient survival, thereby enabling a comprehensive evaluation of the epigenetic regulation and prognostic significance of *CBX1* in LUAD tissues.

Construction of the micro RNA–long non-coding RNA–mRNA network and prognostic analysis of *chromobox 1* in lung adenocarcinoma tissues

To identify candidate miRNAs targeting *CBX1*, the miRNet database (<https://www.mirnet.ca>) was used [14]. This integra-

tive platform enabled the prediction of miRNA–mRNA interactions and identification of lncRNA interactions associated with the selected miRNAs. To further explore the regulatory networks involving *CBX1*-associated miRNAs, we used the ENCORI (starBase) database (<https://rnasysu.com/encori/index.php>). ENCORI enabled a comprehensive analysis of miRNA–lncRNA and miRNA–pseudogene correlations specific to LUAD. The expression levels and prognostic significance of *CBX1*-associated miRNAs, lncRNAs, and pseudogenes were systematically analyzed using the integrated ENCORI.

Statistical analysis

Gene expression data and corresponding clinical information for tumor and normal tissue samples were obtained from publicly accessible online databases, including TIMER2.0, GEPIA2, Biomedical Informatics Institute, and OncoDB. These platforms integrate gene expression profiles from TCGA and GTEx, and provide normalized and curated expression values. The prognostic value of the genes was assessed using KM survival curves and log-rank tests. Survival analyses were conducted using multiple platforms including the Biomedical Informatics Institute, MethSurv, and ENCORI. Pearson's correlation analysis was used to evaluate the co-expression relationships between the variables. To address the issue of multiple hypothesis testing, false discovery rate (FDR) correction was applied. Statistical significance was set at $p < 0.05$ or $FDR < 0.05$, where applicable.

Results

mRNA expression of *chromobox 1* in lung adenocarcinoma tissues

To evaluate the differential expression of *CBX1* between tumors and normal tissues across various cancer types, including LUAD, we analyzed data using the TIMER2.0 database. The results demonstrated a marked upregulation of *CBX1* expression in LUAD tissues, as well as in several other cancers, such as bladder urothelial carcinoma, breast invasive carcinoma, cholangiocarcinoma, colon adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, liver hepatocellular carcinoma, LUSC, pheochromocytoma and paraganglioma, and stomach adenocarcinoma (Fig. 1A). The GEPIA2 database was used to further validate the findings specific to LUAD, confirming the significant overexpression of *CBX1* in LUAD tissues compared to that in normal tissues (Fig. 1B).

Prognostic value of *chromobox 1* expression in lung adenocarcinoma tissues

To assess the prognostic significance of *CBX1* expression in LUAD tissues, KM survival analyses were conducted using the Biomedical Informatics Institute database. The evaluated survival endpoints included OS, DFI, PFI, and DSS. Elevated *CBX1* expression was significantly associated with worse clinical outcomes including OS (hazard ratio [HR] = 1.542, $p = 0.0254$), DFI (HR = 1.809, $p = 0.0348$), PFI (HR = 1.419, p

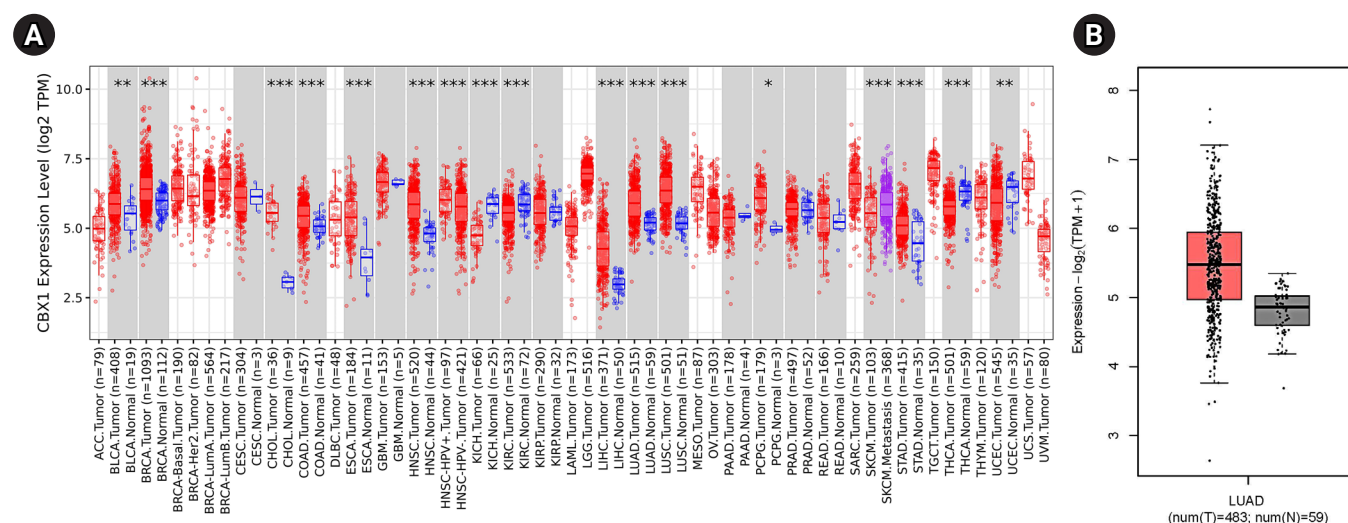


Fig. 1. mRNA expression of *CBX1* in LUAD tissues. (A) Comparison of *CBX1* expression between tumorous and normal tissues. (B) Comparison of *CBX1* expression between LUAD and normal tissues. * $p < 0.05$, ** $p < 0.01$, and *** $p < 0.001$. *CBX1*, *chromobox 1*; LUAD, lung adenocarcinoma.

= 0.0544), and DSS (HR = 1.625, p = 0.0541; Fig. 2). These findings indicate that high *CBX1* expression is a potential indicator of poor prognosis in patients with LUAD.

Correlation of *chromobox 1* expression with DNA methylation in lung adenocarcinoma tissues

To explore the potential regulatory role of DNA methylation in *CBX1* expression in LUAD tissues, analyses were performed using the OncoDB database. *CBX1* expression was significantly correlated with DNA methylation at specific probes located within both the promoter and exon regions in LUAD and nor-

mal tissues (Fig. 3, Table 1). In the generated heat map, hypermethylated regions are shown in red, whereas hypomethylated regions are shown in blue. In particular, the cg17778721 probe was located adjacent to a hypermethylated region (Fig. 4A). To assess the prognostic significance of this methylation-associated probe, survival analysis was conducted using the MethSurv database. The hypermethylated probe cg17778721 (HR = 1.446, p = 0.052) was associated with poor prognosis in patients with LUAD, although the association was not statistically significant (Fig. 4B).

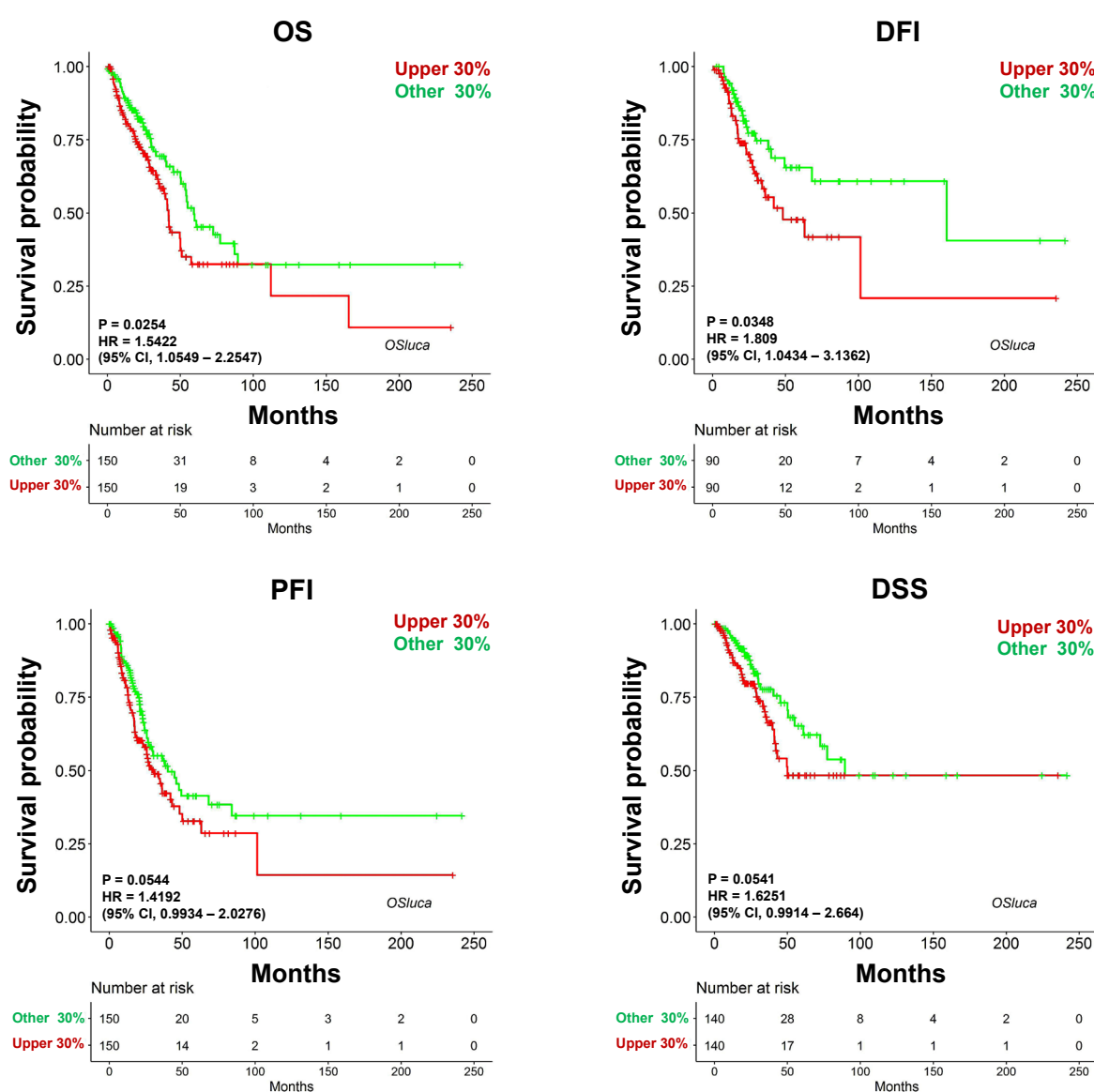


Fig. 2. The prognostic significance of *CBX1* expression in LUAD tissues. OS, overall survival; HR, hazard ratio; CI, confidence interval; DFI, disease-free interval; PFI, progression-free interval; DSS, disease-specific survival; *CBX1*, *chromobox 1*; LUAD, lung adenocarcinoma.

Prediction of target micro RNAs and construction of the *chromobox 1*-associated co-expression network

To identify potential miRNAs targeting *CBX1*, a predictive analysis was conducted using the miRNet database, a comprehensive platform for investigating miRNA-target interactions. This analysis identified 26 miRNAs potentially associated with *CBX1* (Fig. 5, Table 2). These findings indicate that a

complex regulatory network of *CBX1*-associated miRNAs is involved in lung disease pathogenesis and key cancer-related processes, highlighting their potential significance as biomarkers and therapeutic targets in LUAD.

Expression and prognostic significance of *chromobox 1*-associated micro RNAs in lung adenocarcinoma tissues

To assess the expression and potential prognostic value of *CBX1*-targeting miRNAs in LUAD tissues, data from the ENCORI database were systematically analyzed. Among the predicted candidates, *hsa-miR-29b-3p* and *hsa-miR-29c-3p* were significantly upregulated in LUAD tissues compared to normal tissues (Fig. 6A). *hsa-miR-29b-3p* ($r = -0.381$, $p < 0.001$) and *hsa-miR-29c-3p* ($r = -0.362$, $p < 0.001$) exhibited a significant negative correlation with *CBX1* expression in LUAD tissues (Fig. 6B). Survival analysis revealed that lower expression levels of both *hsa-miR-29b-3p* and *hsa-miR-29c-3p* were significantly associated with poor prognosis in LUAD tissues (Fig. 6C). These findings indicate that both *hsa-miR-29b-3p* and *hsa-miR-29c-3p* may serve as potential prognostic indicators and play functional roles in the regulatory network involving *CBX1* in LUAD tissues.

Correlation of long non-coding RNA genes associated with *chromobox 1*-targeting micro RNAs in lung adenocarcinoma tissues

To investigate the potential regulatory relationships be-

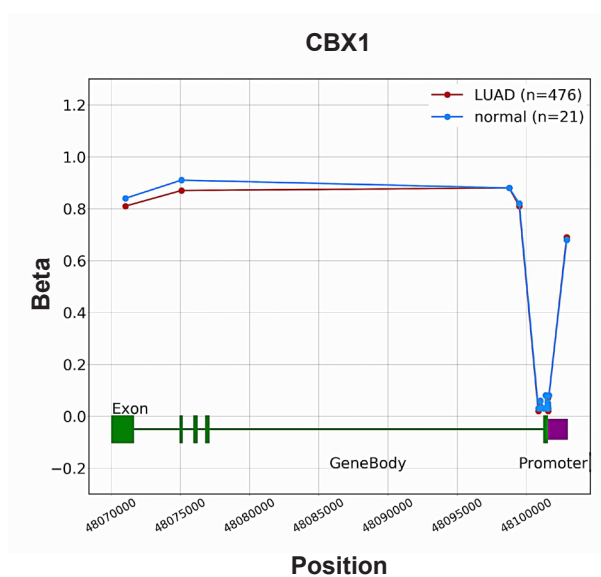


Fig. 3. Variations of DNA methylation of *CBX1* between LUAD and normal tissues. *CBX1*, *chromobox 1*; LUAD, lung adenocarcinoma.

Table 1. Correlation of *CBX1* Expression with DNA Methylation in LUAD

Gene	Probe	Chr	Position	Average of cancer sample	Average of normal sample	p-value
<i>CBX1</i>	cg24458315	Chr17	48071045	0.81	0.84	1.5e-06
	cg26932693	Chr17	48075087	0.87	0.91	1.9e-07
	cg21215337	Chr17	48098755	0.88	0.88	8.5e-01
	cg18929316	Chr17	48099486	0.81	0.82	3.8e-01
	cg06150642	Chr17	48100857	0.02	0.03	2.7e-02
	cg20440414	Chr17	48100984	0.04	0.03	9.4e-02
	cg11194725	Chr17	48100998	0.06	0.06	6.7e-01
	cg12245530	Chr17	48101256	0.03	0.03	2.6e-01
	cg11729481	Chr17	48101375	0.03	0.03	9.6e-01
	cg17778721	Chr17	48101383	0.08	0.08	8.0e-01
	cg04864609	Chr17	48101553	0.07	0.05	3.8e-05
	cg02835499	Chr17	48101556	0.04	0.04	2.3e-02
	cg21511817	Chr17	48101565	0.02	0.03	6.7e-01
	cg13342109	Chr17	48101569	0.03	0.03	8.2e-01
	cg01553295	Chr17	48101633	0.08	0.08	7.7e-03
	cg01544580	Chr17	48102907	0.69	0.68	4.1e-01

CBX1, *chromobox 1*; LUAD, lung adenocarcinoma.

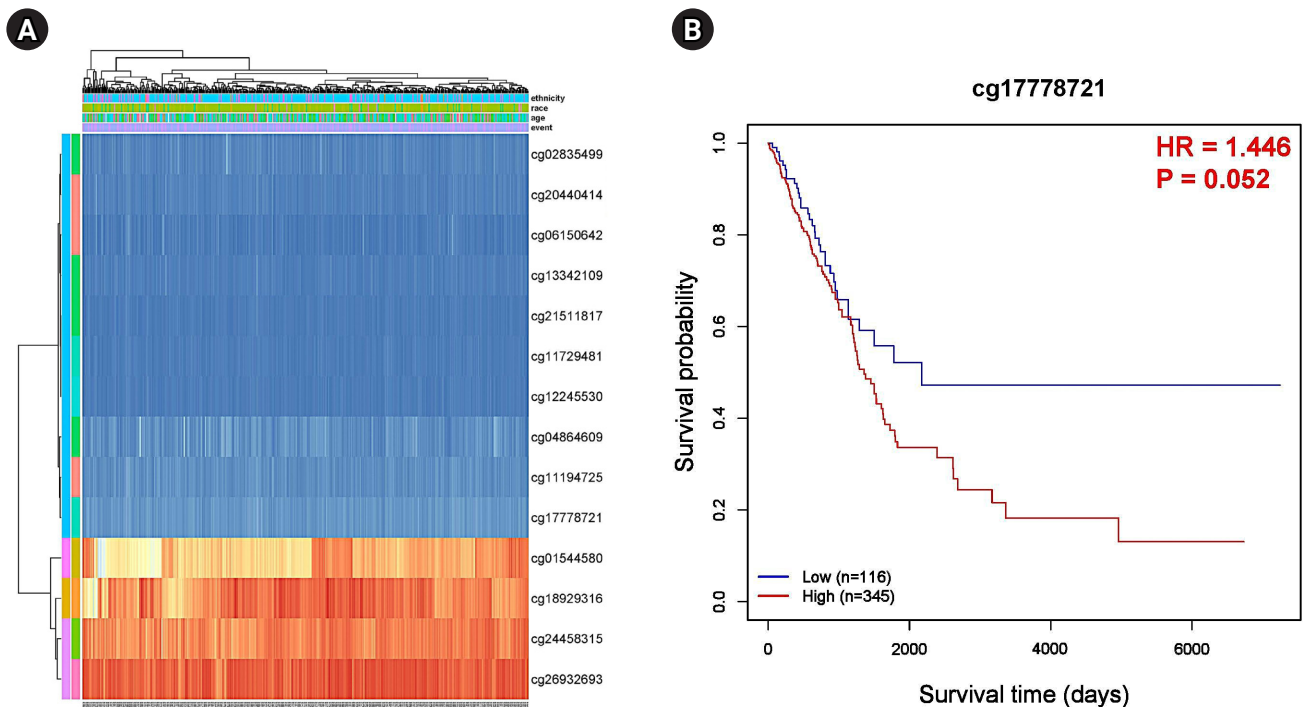


Fig. 4. Prognostic significance on promoter methylation of *CBX1* in LUAD tissues. (A) Heatmap showing promoter methylation of *CBX1*. (B) KM plotter showing promoter methylation of *CBX1*. HR, hazard ratio; *CBX1*, *chromobox 1*; LUAD, lung adenocarcinoma; KM, Kaplan-Meier.

tween lncRNAs and *CBX1*-targeting miRNAs in LUAD tissues, correlation analyses were performed using the ENCORI database. The analysis focused on two miRNAs, *hsa-miR-29b-3p* and *hsa-miR-29c-3p*, which were previously shown to be upregulated in LUAD tissues. The expression of *hsa-miR-29b-3p* exhibited a positive correlation with several lncRNAs, including *NEAT1* ($r = 0.367, p < 0.001$), *THUMP3* ($r = 0.326, p < 0.001$), *GAS5* ($r = 0.185, p < 0.001$), *AC092747.4* ($r = 0.133, p = 0.00252$), *SLX1A-SULT1A3* ($r = 0.194, p < 0.001$), *AC009078.3* ($r = 0.229, p < 0.001$), *AC109460.3* ($r = 0.328, p < 0.001$), *PSMA3-AS1* ($r = 0.222, p < 0.001$), *AL137129.1* ($r = 0.123, p = 0.005$), *MIR29B2CHG* ($r = 0.380, p < 0.001$), *VASH1-AS1* ($r = 0.191, p < 0.001$), and *AL645608.1* ($r = 0.139, p = 0.002$). In contrast, it was negatively correlated with *OIP5-AS1* expression ($r = -0.122, p = 0.006$; Fig. 7A). Similarly, *hsa-miR-29c-3p* exhibited significant positive correlations with *NEAT1* ($r = 0.184, p < 0.001$), *OIP-AS1* ($r = 0.141, p = 0.001$), *THUMP3-AS1* ($r = 0.118, p = 0.008$), *KCNQ1OT1* ($r = 0.131, p = 0.003$), *AC092747.4* ($r = 0.190, p < 0.001$), *AL031282.2* ($r = 0.166, p < 0.001$), *AC009078.3* ($r = 0.130, p = 0.003$), *AC005154.1* ($r = 0.112, p = 0.011$), *PSMA3-AS1* ($r = 0.269, p < 0.001$), *VIM-AS1* ($r = 0.206, p < 0.001$), *VASH1-AS1* ($r = 0.164, p < 0.001$) and *AC104964.4* (r

$= 0.176, p < 0.001$; Fig. 7B).

Correlation of pseudogenes associated with *chromobox 1*-targeting micro RNAs in lung adenocarcinoma tissues

Correlation analyses were performed using the ENCORI database to examine the potential involvement of pseudogenes in regulatory networks of *CBX1*-targeting miRNAs in LUAD tissues. Expression analysis revealed that *hsa-miR-29b-3p* was negatively correlated with several pseudogenes, including *HSP90AB3P* ($r = -0.160, p < 0.001$), *CHCHD3P3* ($r = -0.118, p = 0.008$), *TPI1P1* ($r = -0.111, p = 0.012$), *BZW1P2* ($r = -0.117, p = 0.008$), *AC067904.2* ($r = -0.094, p = 0.034$), *SPCS2P4* ($r = -0.188, p < 0.001$), *LYPLA1P3* ($r = -0.105, p = 0.018$), *AL596087.1* ($r = -0.118, p = 0.008$), *RALGAPA1P1* ($r = -0.094, p = 0.033$), *HSP90AB2P* ($r = -0.198, p < 0.001$) and *LAPTM4BP1* ($r = -0.123, p = 0.005$). In contrast, it positively correlated with *AL138785.1* ($r = 0.122, p = 0.006$; Fig. 8A). Similarly, *hsa-miR-29c-3p* exhibited significant negative correlations with *HSP90AB3P* ($r = -0.089, p = 0.045$), *CCT5P1* ($r = -0.196, p < 0.001$), *CHCHD3P3* ($r = -0.148, p < 0.001$), *AL138785.1* ($r = -0.091, p = 0.039$), *BZW1P2* ($r = -0.130, p = 0.003$), *TPI1P1* ($r = -0.186, p < 0.001$) and *LAPTM4BP1* ($r =$

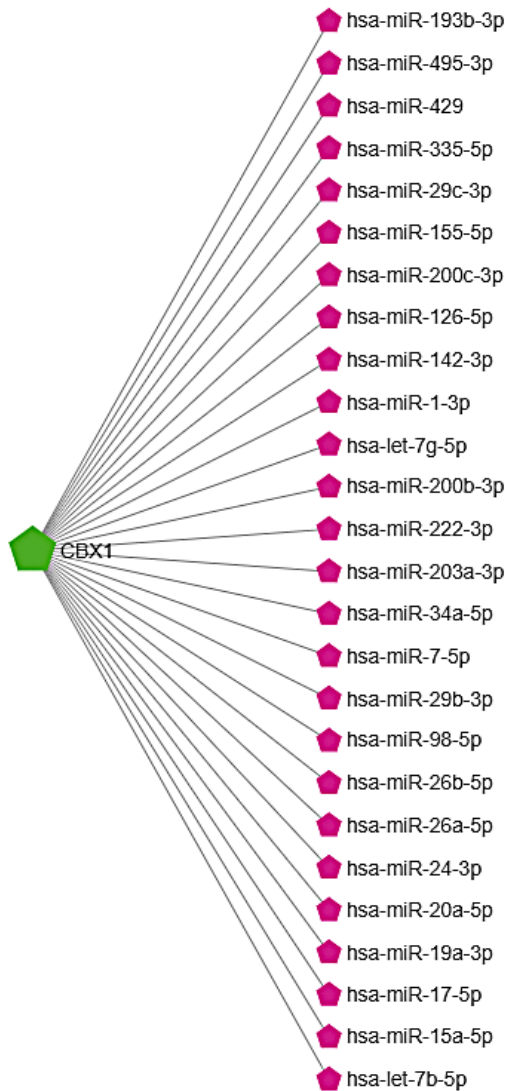


Fig. 5. miRNA network associated with *CBX1*. *CBX1*, *chromobox 1*; miRNA, micro RNA.

-0.134, $p = 0.002$). In contrast, it was positively correlated with *AL672207.1* ($r = 0.117$, $p = 0.008$) and *SUCLG2P2* ($r = 0.119$, $p = 0.007$; Fig. 8B). These findings highlight the potential role of pseudogenes as components of the *CBX1*-associated miRNA regulatory network, and show their possible contribution to LUAD progression.

Discussion

In the present study, we comprehensively analyzed the expression patterns, epigenetic modifications, and ncRNA regulatory networks associated with *CBX1* in LUAD. Our findings demonstrate that *CBX1* is significantly overexpressed in LUAD tissues, consistent with previous reports indicating its onco-

Table 2. *CBX1*-associated co-expressed miRNAs in LUAD

Gene	miRNA	
<i>CBX1</i>	<i>hsa-let-7b-5p</i>	<i>hsa-miR-222-3p</i>
	<i>hsa-miR-15a-5p</i>	<i>hsa-miR-200b-3p</i>
	<i>hsa-miR-17-5p</i>	<i>hsa-let-7g-5p</i>
	<i>hsa-miR-19a-3p</i>	<i>hsa-miR-1-3p</i>
	<i>hsa-miR-20a-5p</i>	<i>hsa-miR-142-3p</i>
	<i>hsa-miR-24-3p</i>	<i>hsa-miR-126-5p</i>
	<i>hsa-miR-26a-5p</i>	<i>hsa-miR-200c-3p</i>
	<i>hsa-miR-26b-5p</i>	<i>hsa-miR-155-5p</i>
	<i>hsa-miR-98-5p</i>	<i>hsa-miR-29c-3p</i>
	<i>hsa-miR-29b-3p</i>	<i>hsa-miR-335-5p</i>
	<i>hsa-miR-7-5p</i>	<i>hsa-miR-429</i>
	<i>hsa-miR-34a-5p</i>	<i>hsa-miR-495-3p</i>
	<i>hsa-miR-203a-3p</i>	<i>hsa-miR-193b-3p</i>

CBX1, *chromobox 1*; miRNA, micro RNA; LUAD, lung adenocarcinoma.

genic role through heterochromatin remodeling and transcriptional repression in multiple cancers, including LUAD [15]. Elevated *CBX1* levels were found to correlate with poor prognosis in patients with LUAD, in line with findings from hepatocellular carcinoma and breast cancer, where *CBX1* promotes proliferation and metastasis [16].

DNA methylation analysis has revealed both promoter hypermethylation and gene-body hypomethylation, which are dual regulatory patterns increasingly recognized in cancer epigenetics [17]. Specifically, the cg17778721 probe in the promoter region showed hypermethylation associated with increased *CBX1* expression and poor survival, albeit with borderline significance. This indicates that *CBX1* may be epigenetically activated through noncanonical methylation dynamics, as described for other chromatin-modifying genes [18]. Specific histone modification patterns may also have co-occurred with altered methylation, thereby reinforcing *CBX1* overexpression [19].

Recent studies have demonstrated that epigenetic alterations initiate tumorigenesis and drive cancer progression and staging [20]. In particular, promoter hypermethylation of tumor suppressor genes is frequently observed in early stage cancers, whereas gene body and global DNA hypomethylation is more prevalent in advanced stages, contributing to genomic instability and aggressive phenotypes [21]. Moreover, high-stage LUAD is often characterized by diverse and widespread methylation changes, especially at the loci associated with metastasis, epithelial-to-mesenchymal transition, and therapeutic resistance [22]. These stage-specific methylation patterns may reflect the evolving regulatory landscape of key

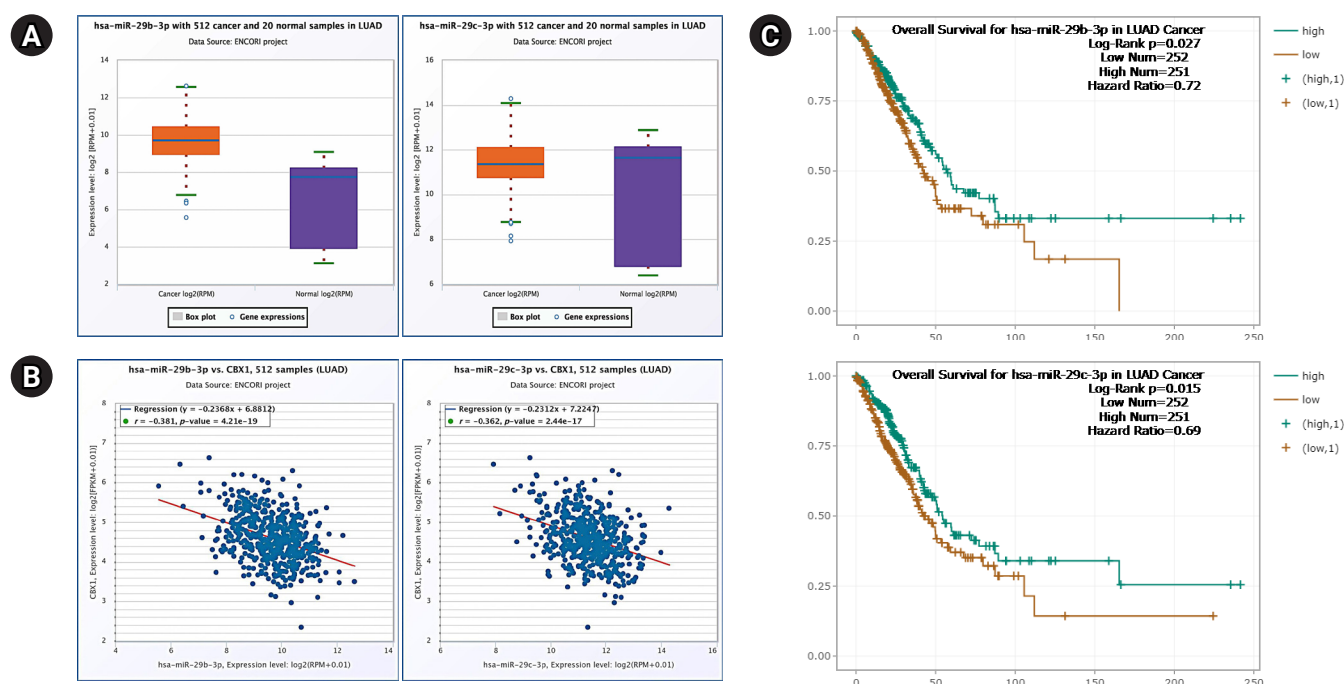


Fig. 6. Expression and prognostic significance of CBX1-associated miRNAs in LUAD tissues. (A) Expression of hsa-miR-29b-3p and hsa-miR-29c-3p in LUAD compared to normal tissues. (B) Expression and correlation between CBX1 and hsa-miR-29b-3p and hsa-miR-29c-3p in LUAD tissues. (C) KM plotter of hsa-miR-29b-3p and hsa-miR-29c-3p in LUAD tissues. LUAD, lung adenocarcinoma; CBX1, chromobox 1; miRNA, micro RNA; KM, Kaplan-Meier.

oncogenes such as *CBX1*, highlighting the potential of methylation profiling as a prognostic tool and stratification biomarker for LUAD.

Furthermore, integrative network analysis identified *hsa-miR-29b-3p* and *hsa-miR-29c-3p* as key miRNAs negatively correlated with *CBX1* expression. These miRNAs have been reported to suppress oncogenic targets and predict a favorable prognosis in patients with LUAD and other NSCLCs [23]. Surprisingly, despite its upregulation, *CBX1* expression remained high, prompting the investigation of ceRNA-mediated regulatory mechanisms.

Our ceRNA analysis revealed that lncRNAs, such as *NEAT1* and *THUMP3-AS1*, were positively correlated with miR-29b/c expression. *NEAT1* has previously been shown to function as an oncogenic sponge for miR-204 family members in LUAD tissues, thereby enhancing expression of target oncogenes [24]. Similarly, *THUMP3-AS1* has been reported to promote tumor progression by modulating miRNA availability in various cancers, including lung cancer [25].

This regulatory scenario indicates that *CBX1* overexpression in LUAD tissues may arise from miRNA sequestration through overexpressed ceRNAs and not from the loss of inhibitory miRNAs. This buffering system, mediated by In-

cRNAs and pseudogenes, neutralizes the repressive functions of miR-29 family members. In addition, *CBX1* may form feedback loops with other chromatin modifiers or oncogenic transcription factors that stabilize its expression after epigenetic or post-transcriptional activation. For example, *CBX1* recruits PRC1-like complexes and interacts with histone methyltransferases [26], which could further reinforce chromatin compaction and oncogenic transcriptional programs.

This multilayered regulatory landscape highlights the complexity of *CBX1* dysregulation in LUAD tissues, which involves both epigenetic and post-transcriptional mechanisms. Nonetheless, our study has some limitations. First, all analyses were based on publicly available datasets and require experimental validation in independent LUAD cohorts. Second, although the focus was on miR-29b-3p and miR-29c-3p, additional miRNAs and regulatory ncRNAs may also modulate *CBX1* and should be considered in future studies. Thirdly, we did not perform functional experiments to validate the predicted ceRNA interactions, which are important for confirming causality.

From a clinical perspective, our findings highlight the potential of *CBX1* as a prognostic biomarker as well as a therapeutic target. The *CBX1*-miR-29-lncRNA axis represents a

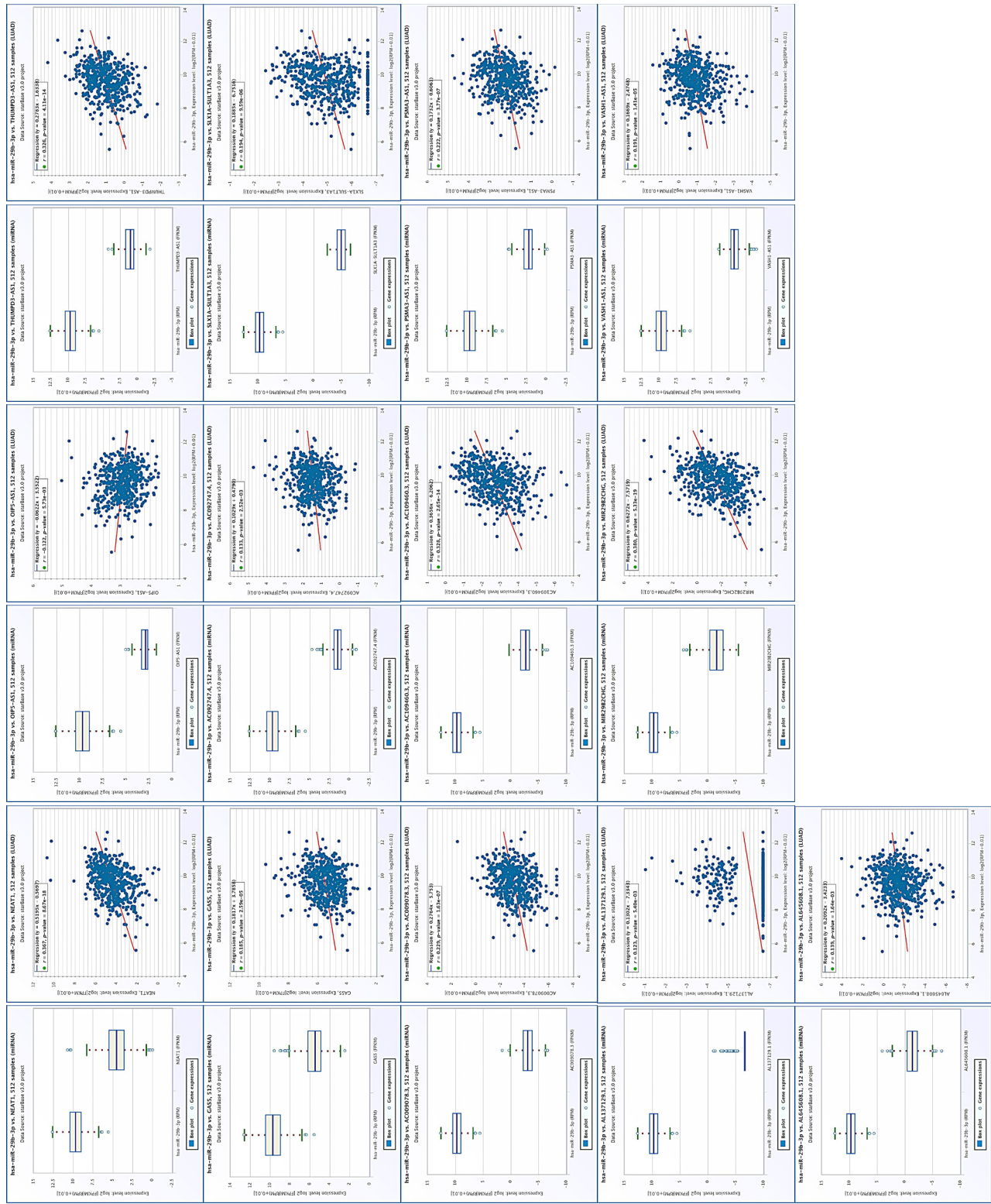


Fig. 7. IncRNA interaction of CBX1-associated miRNA in LUAD tissues. (A) Expression and correlation of *hsa-miR-29b-3p*. (B) Expression and correlation of *hsa-miR-29c-3p*. IncRNA, long non-coding RNA; CBX1, chromobox 1; miRNA, micro RNA; LUAD, lung adenocarcinoma. (Continued to the next page)

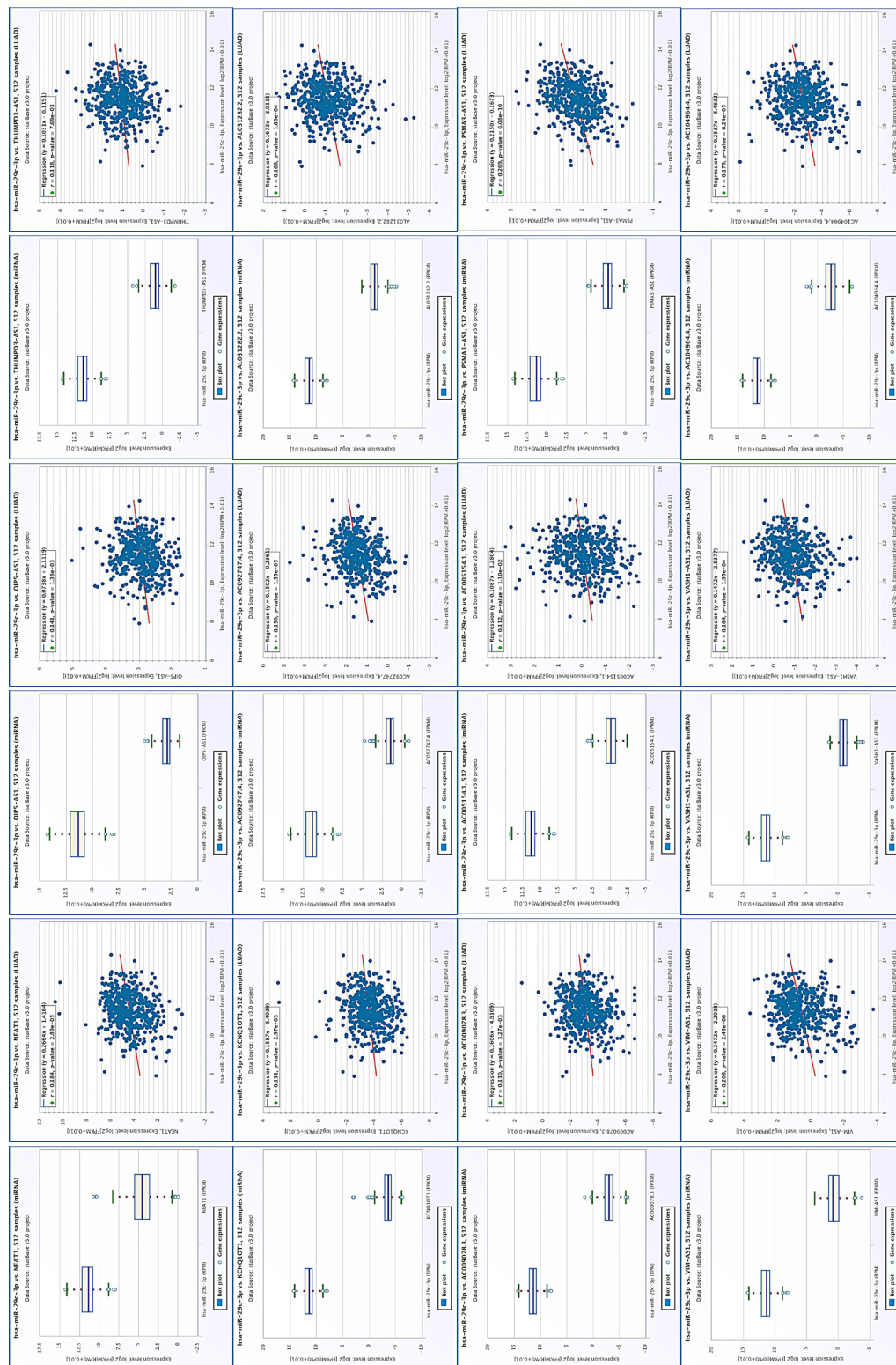
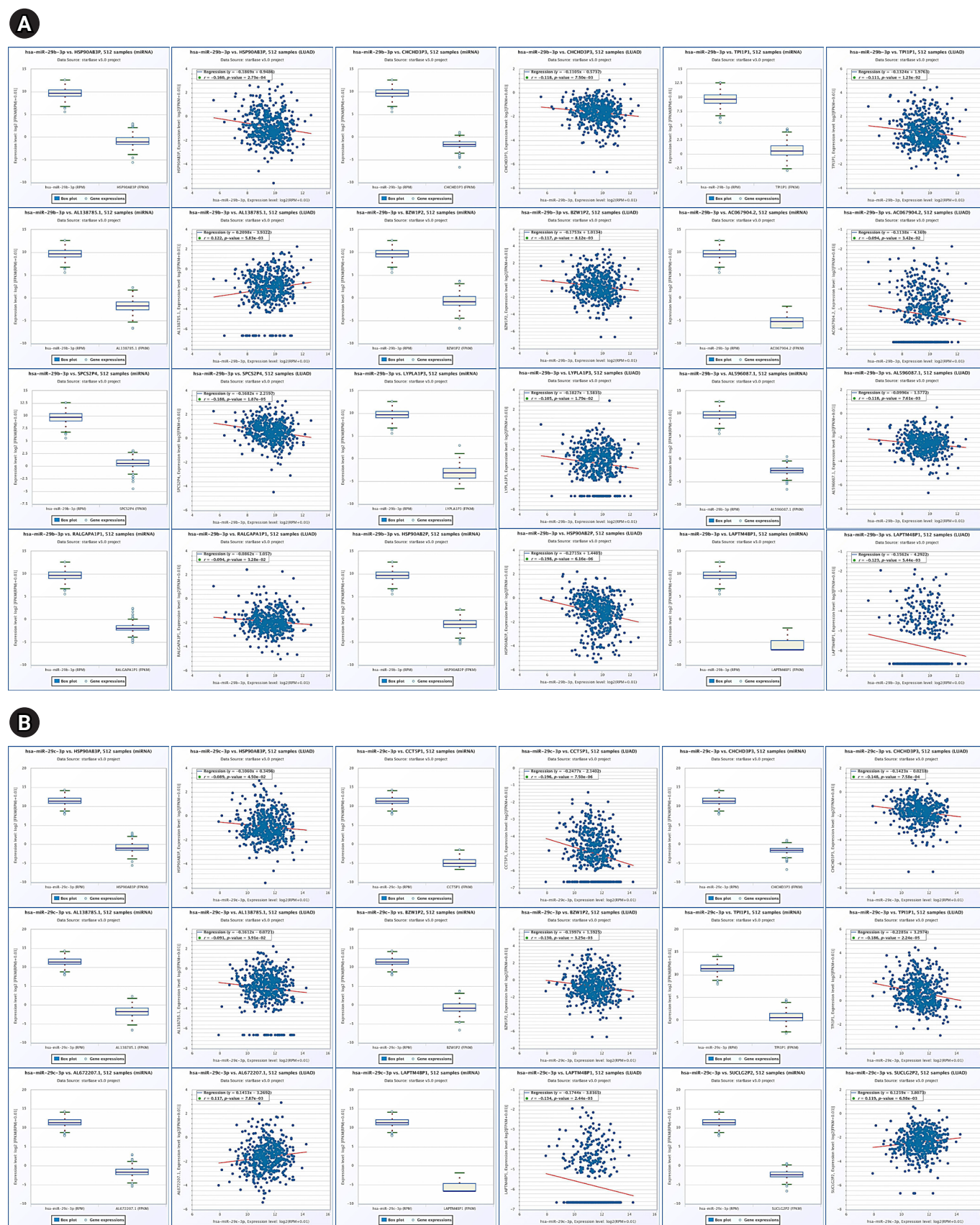


Fig. 7. Continued.



promising framework for developing RNA-based therapeutics aimed at disrupting ceRNA-mediated oncogene activation. Moreover, epigenetic drugs targeting DNA methylation machinery can be investigated in tandem to suppress aberrant *CBX1* activity. Ultimately, a dual-targeting strategy that addresses both methylation status and ncRNA interactions may improve therapeutic precision in patients with LUAD.

In conclusion, our findings indicate that *CBX1* and its associated ncRNA network are potential biomarkers and therapeutic targets for LUAD. Targeted disruption of the *CBX1*–ceRNA axis, including strategies using miR-29 mimics or lncRNA inhibitors, may hold promise for enhancing the clinical outcomes in patients with LUAD [27,28].

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Ethical approval

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Conflict of interest

The authors have nothing to disclose.

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