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Non-coding RNAs and human diseases, volume II: long non-coding RNAs (lncRNAs) and pathogenesis of human disease

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Non-coding RNAs and human diseases, volume II: long non-coding RNAs (lncRNAs) and pathogenesis of human disease

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Editorial: Non-coding RNAs and human diseases volume 2 -long non-coding RNAs and pathogenesis of human disease

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Editorial on the Research Topic
Non-coding RNAs and human diseases volume 2 -long non-coding RNAs and pathogenesis of human disease

Long non-coding RNAs (lncRNAs), a class of typically non-translatable transcripts with length of 200 nucleotides or longer, have attracted significant attention within the expansive realm of ncRNA research. Distinct from their smaller counterparts—such as microRNAs (miRNAs) and piwi-interacting RNAs (piRNAs)—lncRNAs exhibit a unique regulatory capacity, influencing critical biological processes and pathologies. Around 30,000 lncRNAs identified within human tissues exhibit remarkable tissue-specific expression patterns, suggesting their potential as biomarkers and therapeutic targets. The 2nd volume of our Research Topic, "Non-Coding RNAs and Human Diseases," focused on the roles of lncRNAs in various human diseases, particularly on their involvement in the pathogenesis of neurological disorders, cancers, cardiac, lung, and liver diseases.

The eight original research articles and seven comprehensive review articles in this volume explored the contribution of lncRNAs to pathogenesis and translational significance. Notably, two studies centered on lung adenocarcinoma (LUAD) addressed the importance of lncRNAs in tumorigenesis. Zeng et al., elucidated the association between lncRNAs involved in endoplasmic reticulum stress (ERS) and alterations in immune landscapes, indicating that these lncRNAs could serve as promising prognostic biomarkers and potential targets for immunotherapy. In a separate study, Luo et al., characterized specific apoptosis-related lncRNAs (ApoRLs) that correlate with LUAD prognosis, underscoring their association with malignancy-associated immunomodulatory pathways.

The exploration of lncRNAs related programmed cell death (PCD) has emerged as a promising avenue for cancer therapy. Zhong et al., investigated alteration of the pyroptosis-related lncRNAs (PRlncRNAs) in acute myeloid leukemia (AML) and revealed that their

risk scores could serve as indicator for prognosis, shedding the light for novel therapeutic strategies targeting PCD and the tumor microenvironment.

In the context of hepatocellular carcinoma (HCC), Wang et al., identified m7G-related lncRNAs, several them correlate with overall survival and response to chemotherapy, suggesting their potential for prognosis and therapeutic strategy. Similarly, Saklani et al. examined novel lncRNA-miRNA-mRNA networks in gallbladder cancer (GBC), exploring regulatory mechanisms and the potential for therapeutic strategies.

Dadyar et al., extended the potential of lncRNAs beyond cancers by focusing on T cell-related lncRNAs (tcr-lncRNAs) in multiple sclerosis (MS), demonstrating the contribution of tcr-lncRNAs dysregulation to disease progression and suggesting their utility as biomarkers or therapeutic targets.

In addition to research articles, seven review articles in this volume addressed current advances in lncRNAs and their functions across various diseases, four of which focused on cancer. Notable contributions include review of MIR31HG's multifaceted role in oncogenesis by Ruan et al., and systematic review of LINC00511 in multiple functions tumorigenesis, cell invasion, metastasis, and resistance to chemotherapy by Ghafouri-Fard et al. Xiaotong et al., discussed the potential of lncRNAs as biomarker and therapeutic strategy for osteosarcoma, while Wu et al. summarized the relevance of lncRNAs in cervical cancer radiosensitivity. Another review article by Ghafouri-Fard et al., explored the interaction between ncRNAs and SIRT1, a deacetylase involved in various disorders, emphasizing the therapeutic implications of these interactions. Furthermore, Zhipu et al. summarized the role of exosomal and endogenous miRNAs in chronic rhinosinusitis with nasal polyps (CRSwNP), and Zhang et al. reviewed ncRNAs involved in ossification of the posterior longitudinal ligament (OPLL), suggesting future research directions.

In summary, this volume presents a rich tapestry of research highlighting the significance of lncRNAs as biomarkers for diagnosis and prognosis, as well as potential therapeutic targets across a spectrum of human diseases. The findings and discussions within these articles aim to provide valuable insights for researchers and clinical physicians, fostering a deeper understanding of lncRNAs in health and disease. We hope that this compilation of knowledge serves as a useful resource for advancing research and clinical applications in the field of non-coding RNAs and their significance in human health and disease.

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SX: Writing–review and editing. LS: Writing–review and editing. CM: Writing–review and editing. Z-QT: Writing–review and editing. YL: Writing–original draft.

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Comprehensive analysis of nine m7G-related lncRNAs as prognosis factors in tumor immune microenvironment of hepatocellular carcinoma and experimental validation

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Background: Hepatocellular carcinoma (HCC) remains the most prevalent gastrointestinal malignancy worldwide, with robust drug resistance to therapy. N7-methylguanosine (m7G) mRNA modification has been significantly related to massive human diseases. Considering the effect of m7G-modified long non-coding RNAs (lncRNAs) in HCC progression is unknown, the study aims at investigating a prognostic signature to improve clinical outcomes for patients with HCC.

Methods: Two independent databases (TCGA and ICGC) were used to analyze RNAseq data of HCC patients. First, co-expression analysis was applied to obtain the m7G-related lncRNAs. Moreover, consensus clustering analysis was employed to divide HCC patients into clusters. Then, using least absolute shrinkage and selection operator-Cox regression analysis, the m7G-related lncRNA prognostic signature (m7G-LPS) was first tested in the training set and then confirmed in both the testing and ICGC sets. The expression levels of the nine lncRNAs were further confirmed *via* real-time PCR in cell lines, principal component analysis, and receiver operating characteristic curve. The m7G-LPS could divide HCC patients into two different risk groups with the optimal risk score. Then, Kaplan–Meier curves, tumor mutation burden (TMB), therapeutic effects of chemotherapy agents, and expressions of immune checkpoints were performed to further enhance the availability of immunotherapeutic treatments for HCC patients.

Results: A total of 1465 lncRNAs associated with the m7G genes were finally selected from the TCGA database, and through the univariate Cox regression, the expression levels of 22 m7G-related lncRNAs were concerning HCC patients' overall survival (OS). Then, the whole patients were grouped into two subgroups, and the OS in Cluster 1 was longer than that of patients in Cluster 2. Furthermore, nine prognostic m7G-related lncRNAs were identified

to conduct the m7G-LPS, which were further verified. A prognostic nomogram combined age, gender, HCC grade, stage, and m7G-LPS showed strong reliability and accuracy in predicting OS in HCC patients. Finally, immune checkpoint expression, TMB, and several chemotherapy agents were remarkably associated with risk scores. More importantly, the OS of the TMB-high patients was the worst among the four groups.

Conclusion: The prognostic model we established was validated by abundant algorithms, which provided a new perspective on HCC tumorigenesis and thus improved individualized treatments for patients.

KEYWORDS

hepatocellular carcinoma, N7-methylguanosine, long noncoding RNA, model, prognosis

Introduction

Hepatocellular carcinoma (HCC), accounting for nearly 90% of hepatic cancer cases (McGlynn et al., 2021), is the second leading cause of cancer-related deaths in men and the sixth in women (Kong et al., 2021). It was reported that China is a liver cancer–prevalent country. Epidemiological evidence has shown that China has had 410,000 new cases and 391,000 deaths of primary liver cancer, accounting for 45.3% and 47.1% of global cases, respectively, and bearing almost half of the global burden of liver cancer (Zeng et al., 2018), over half of patients are first diagnosed with advanced HCC, and more than 70% of patients recur within five years after treatment (Llovet et al., 2021). Given the complexity and heterogeneity of the pathogenesis of HCC, serum alpha-fetoprotein, the most used tumor biomarker in clinics, has poor sensitivity and specificity (Singal et al., 2020). Thus, there is an urgent need to explore and validate prognostic markers and therapeutic targets to guide physicians for further diagnosis and evaluate prognosis accurately.

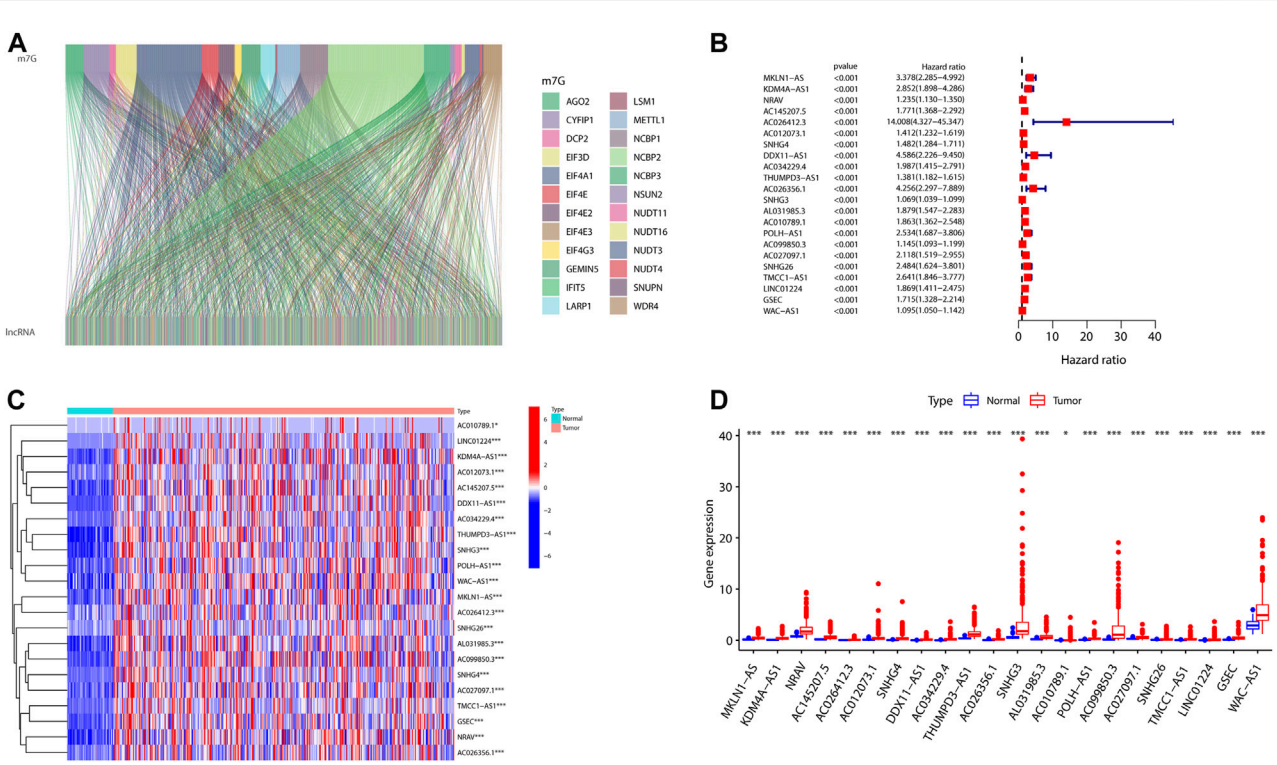

FIGURE 1
Expression profiles of m7G-related genes in patients with HCC. **(A)** Sankey for m7G-related genes and m7G-related lncRNAs. **(B)** Forest plot of 22 prognostic m7G-related lncRNAs. **(C,D)** Heatmap **(C)** and expression **(D)** of 22 m7G-related lncRNAs in the TCGA set. (*$p < 0.05$; ***$p < 0.001$). m7G, N7-methylguanosine; HCC, hepatocellular carcinoma; lncRNAs, long non-coding RNAs.

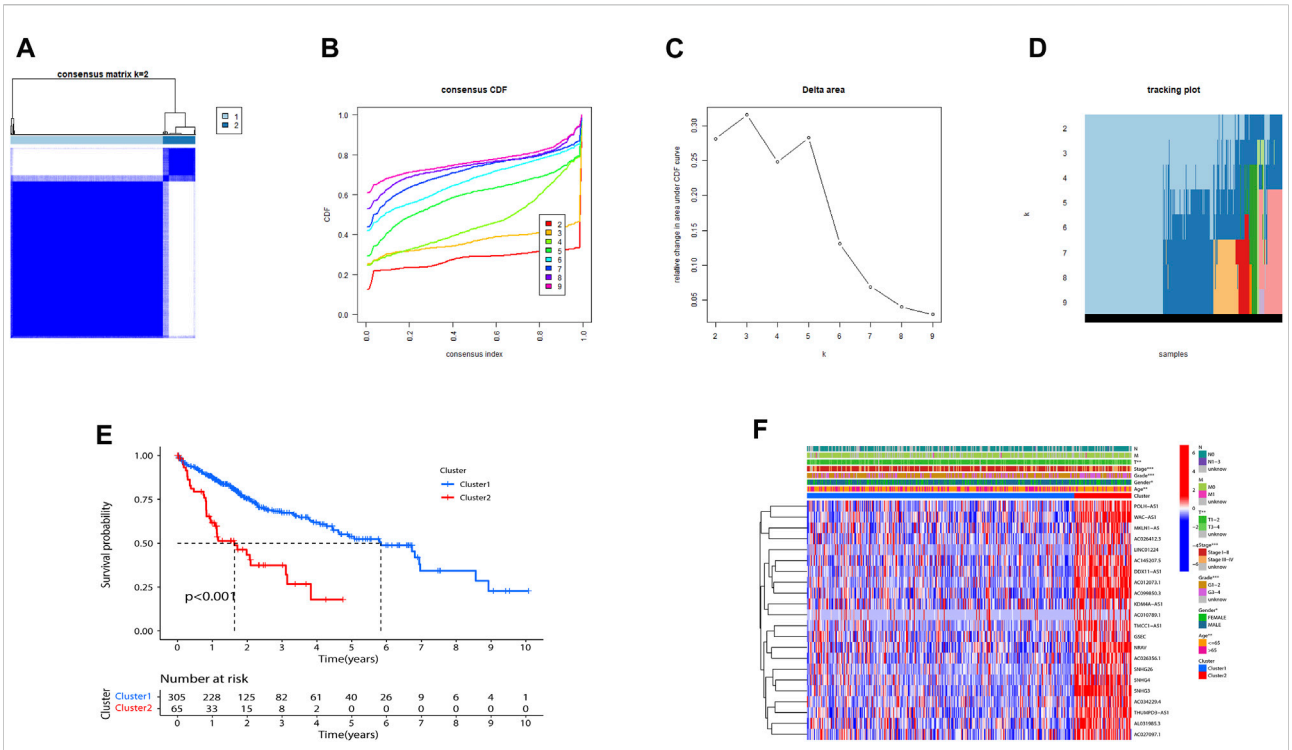

FIGURE 2
Consensus matrix of prognostic m7G-related lncRNAs. **(A–D)** Consensus clustering matrix for k = 2. **(E)** Kaplan–Meier of overall survival for the two different clusters of HCC patients. **(F)** Heatmap of Cluster1 and Cluster2 with clinicopathological features and expressions of 22 m7G-related lncRNAs in each cluster. (**$p < 0.01$; ***$p < 0.001$). m7G, N7-methylguanosine; lncRNAs, long non-coding RNAs.

With the leap forward in high-throughput sequencing technology, the great potential of long non-coding RNAs (lncRNAs) in various human diseases and critical biological mechanisms has attracted the attention of researchers (Chen K. et al., 2021). Studies showed that lncRNAs played a variety of roles in cancers, including epigenetics, DNA damage and cell cycle regulation, regulation of microRNAs, involvement in signaling pathways, and mediating hormone-induced cancers (Winkle et al., 2021). Among the RNA modifications, N7-methylguanosine (m7G) modification is ubiquitous in eubacteria, eukaryotes, and a few archaea. As the most abundant apparent modification in the 5' cap of mRNA (Lin et al., 2018), changes in the expression of m7G modifying enzymes regulate downstream oncogenes or by altering mRNA methylation level of tumor-related genes, suggesting that studying RNA epigenetic modifications will greatly help us to unravel the unknown mysteries of tumors and open up new avenues of tumor therapy (Katsara and Schneider, 2021). Various studies showed that the aberrant m7G modifications were linked to the development and progression of numerous human cancers such as bladder cancer, lung cancer, HCC, and gastrointestinal cancers (Ying et al., 2021; Teng et al., 2021; Xia et al., 2021; Xie et al., 2020).

Accumulative work demonstrated that the dysfunctional expression of lncRNA was engaged in tumorigenesis, recurrence, and metastasis, suggesting the great potential for outcome predicting and personalized treatment guidance (Huo et al., 2017; Wei et al., 2019). Tang et al. found that three novel lncRNAs (RP11-160H22.5, XLOC-014172, and LOC149086) could act as prognostic markers for HCC metastasis (Tang et al., 2015). Shuai et al. reported that lncRNA MNX1-AS1 led to a rather poor prognosis in patients with gastric cancer (Shuai et al., 2020). Recently, Wang et al. found that m7G-related lncRNAs (LOC102554730 and LOC102555374) were significantly upregulated and aggravated disease progression in a mouse model of hypoxia-induced pulmonary hypertension (Wang et al., 2022). Nevertheless, whether and how m7G modification-related lncRNAs affect the pathogenesis of HCC remains to be elucidated. As a result, it was imperative to identify m7G-associated lncRNAs biomarkers for unraveling the mechanism and driving events of hepatocarcinogenesis from a multi-dimensional, multi-omics, and multi-systemic perspective and to develop new precision treatment strategies. Here, we reported the establishment of m7G-related lncRNA prognostic signatures (m7G-LPS) by bioinformatic and statistical analysis to predict survival outcomes in HCC patients.

Material and methods

Acquisition and preprocessing of hepatocellular carcinoma data

HCC samples with clinical data and normalized gene sequencing were downloaded from the publicly available databases: The Cancer Genome Atlas (TCGA; https://portal.gdc.cancer.gov) and the International Cancer Genome Consortium (ICGC) website (https://dcc.icgc.org/releases/current/Projects/LIRI-JP). In total, 374 and 230 malignant tissues of primary HCC from TCGA and ICGC with their clinical information were acquired (Supplementary Table S1). A total of 29 m7G regulators based on the published literature (Tomikawa.2018; Chen et al., 2022) and the Molecular Signatures Database (MSigDB) Team (http://www.broad.mit.edu/gsea/msigdb/) are shown in Supplementary Table S2.

Bioinformatic analysis

Pearson correlation analysis (|coefficients| >0.4, and $p < 0.001$) and univariate Cox regression analysis were used to select lncRNAs associated with m7G genes as m7G-related lncRNAs.

Then, different HCC patients were clustered *via* the Consensus ClusterPlus package. CIBERSORT is a tool for providing gene expression feature sets for 22 immune cell subtypes. The ESTIMATE package uses the single sample gene set enrichment analysis (ssGSEA) algorithm to score individual samples of both stromal and immune gene sets in the tumor expression matrix and, thus, the content of these two types of cells, and finally, calculate the tumor purity.

The m7G-LPS was established *via* the least absolute shrinkage and selection operator (LASSO)-Cox regression. The LASSO is extensively utilized in clinical studies (Chen T. et al., 2021; Liu et al., 2021; Liu et al., 2022a; Liu et al., 2022b; Liu et al., 2022c). The risk score was calculated as Risk score = $\sum$ni = 1Coefi×Xi (Coef i represents the coefficient). Thus, high- and low-risk groups were divided according to the median risk score. The Kaplan–Meier (K-M) survival curve, receiver characteristic (ROC) curve, and principal component analysis (PCA) analyses were all used to comprehensively validate and compare the accuracy of the m7G-LPS model. Finally, a nomogram was established to demonstrate clinicopathological factors and risk values at 1-, 3-, and 5-years.

The Gene Set Enrichment Analysis (GSEA) displayed the biological function of the high- and low-risk groups. We analyzed the degree of immune cell infiltration and enrichment in each sample by ssGSEA. In addition, we used the pRRophetic and ggplot2 packages to assess the sensitivity to chemotherapy.

Quantitative real-time polymerase chain reaction analysis

Normal human liver cell (L02) and HCC cell lines (Hu-7) were purchased from the Cell Bank of the Chinese Academy of Sciences (Shanghai, China). All cells were cultured in high fructose DMEM (gibco) at 37°C in a 5% CO_2-containing humidified incubator. Total RNA of cultured cells was extracted using TRIzol reagent (Invitrogen), and cDNA was synthesized using a reverse transcription master kit (Invitrogen). Quantitative real-time polymerase chain reaction (qRT-PCR) analysis was quantified using SYBR-Green (Takara). The primer sequence is listed in Supplementary Table S3. Expression values were calculated using the $2^{-\Delta\Delta Ct}$ method. β-Actin was used as endogenous control.

Statistical analysis

All biological statistical analyses in the study were performed using the R software version 4.1.2; unless otherwise specified, $p < 0.05$ was considered statistically significant.

Results

Expression profiles of m7G-Related in patients with hepatocellular carcinoma

In the view of our flowchart (Supplementary Figure S1), 1465 lncRNAs were shown to be significantly corrected with 29 m7G (Figure 1A). A univariate Cox regression analysis was implemented that 22 of 1465 were defined as prognostic m7G-related lncRNAs in HCC patients (Figure 1B). We then analyzed the levels of the 22 lncRNAs in HCC patients and found that all of them were overexpressed lncRNAs (Figures 1C–D).

Consensus clustering of prognostic m7G-Related lncRNAs

Consensus clustering was utilized to classify HCC subgroups according to the expression of prognostic m7G-related lncRNAs. Finally, we selected k = 2 to minimize interference between subgroups (Figures 2A–D). Thus, two HCC subtypes: Cluster 1 (n = 305) and Cluster 2 (n = 65) were identified. Notably, the K-M curve analysis showed that Cluster1 had a longer overall survival (OS) than Cluster 2 (Figure 2E). As illustrated in Figure 2F, expressions of the 22 lncRNAs in Cluster 2 were substantially higher than that in Cluster 1. Not surprisingly, patients in Cluster 2 were more markedly related to an advanced clinical stage and grade than those in Cluster 1.

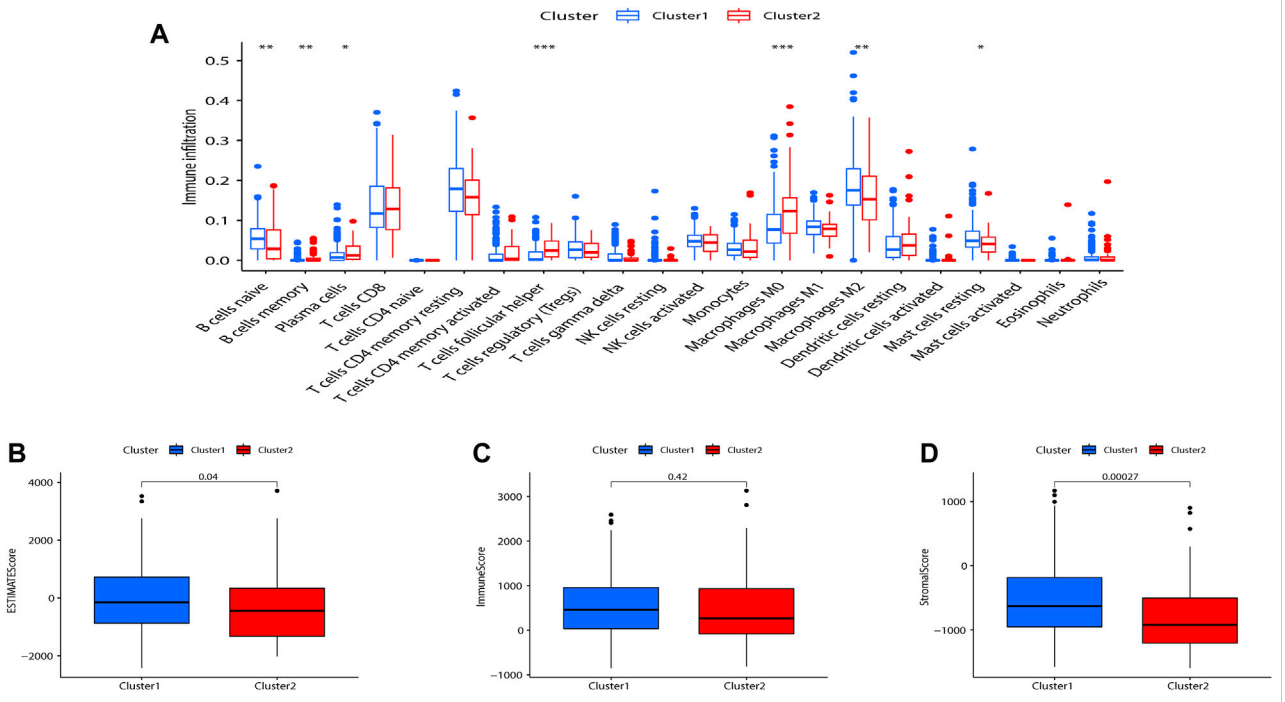

FIGURE 3
Consensus clustering correlated with immune infiltration. **(A)** Differences in the level of infiltration of 22 immune cells in the two clusters. The expression of the comprehensive score **(B)**, immune score **(C)**, and stromal cell score **(D)** between the two clusters. (*$p < 0.05$; **$p < 0.01$).

Consensus clustering correlated with immune infiltration

Tumor-infiltrating immune cells are instructive in evaluating tumor prognosis and immunotherapy efficacy. CIBERSORT analysis showed that compared with Cluster 2, B cells memory ($p < 0.01$), plasma cells ($p < 0.05$), T cells follicular helper ($p < 0.001$), M2 macrophages ($p < 0.001$), and mast cells resting ($p < 0.05$) were significantly upregulated in Cluster 1 (Figure 3A). The immune score, stromal score, and ESTIMATE score between the two indicated that Cluster 1 was more enriched in immune-related cells and with lower tumor cell purity compared to Cluster 2 (Figures 3B–D).

Establishment of m7G-related lncRNA prognostic signature

Nine lncRNAs were finally selected to frame a prognostic signature m7G-LPS for predicting HCC patients' OS through LASSO-Cox regression analysis (Figures 4A–C). The m7G-LPS were constructed based on FPKM value and corresponding coefficients: risk score = (0.366825372367671 × expression of MKLN1-AS) + (0.146426813107297 × expression of KDM4A-AS1) + (0.172062395362062 × expression of AC026412.3) + (0.00427733397199361 × expression of SNHG4) + (0.228579214185232 × expression of AC026356.1) + (0.158465677466972 × expression of AL031985.3) + (0.354660948654385 × expression of POLH-AS1) + (0.353144896760336 × expression of TMCC1-AS1) + (0.213408781976956 × expression of LINC01224).

Prognostic value of the m7G-LPS

To explore the robustness and accuracy of the m7G-LPS, we categorized 370 malignant tissues into a training set ($n = 278$) and a testing set (n = 92) according to the median risk score. The nine lncRNAs were overexpressed in high-risk groups (Figures 4D–I), and the same results were observed in the testing set (Figures 4G–I). In addition, real-time PCR results on the expression of the nine m7G-related lncRNAs were in line with our prediction results (Supplementary Figure S2). Furthermore, K-M curves showed that HCC patients with high-risk scores had worse OS than those with low-risk scores in both the training and testing sets (Figures 4J and K). Considering the heterogeneity of HCC, we choose ICGC data as an independent validation to further verify the accuracy of m7G-LPS. As expected, the result in the ICGC set including 230 HCC patients was in line with the TCGA (Figure 4L). Hence the risk score had a strong capacity to forecast the OS of HCC patients.

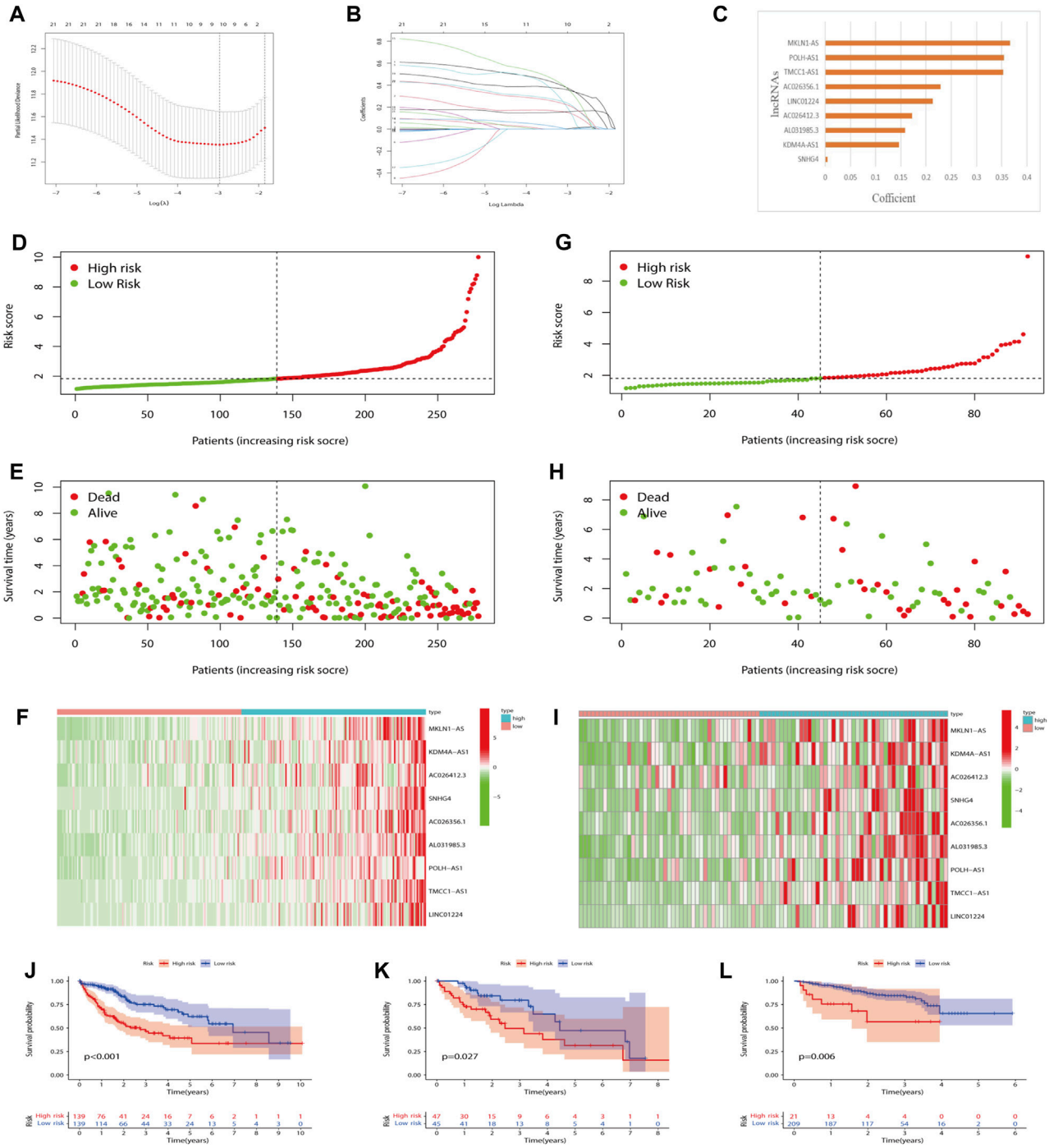

FIGURE 4
Prognostic value of the m7G-LPS. **(A–C)** LASSO regression was performed **(A)**, and the optimal criteria **(B)** and coefficients **(C)** were calculated. **(D–I)** Risk plots, survival state, and heatmap of HCC patients at low- and high-risk scores in training **(D–F)** and testing set **(G–I)**. **(J–L)** Kaplan–Meier analysis in training, testing and ICGC set. m7G, N7-methylguanosine; lncRNAs, long non-coding RNAs. m7G-LPS, m7G-related lncRNA prognostic signature; LASSO, Least absolute shrinkage and selection operator; HCC, hepatocellular carcinoma.

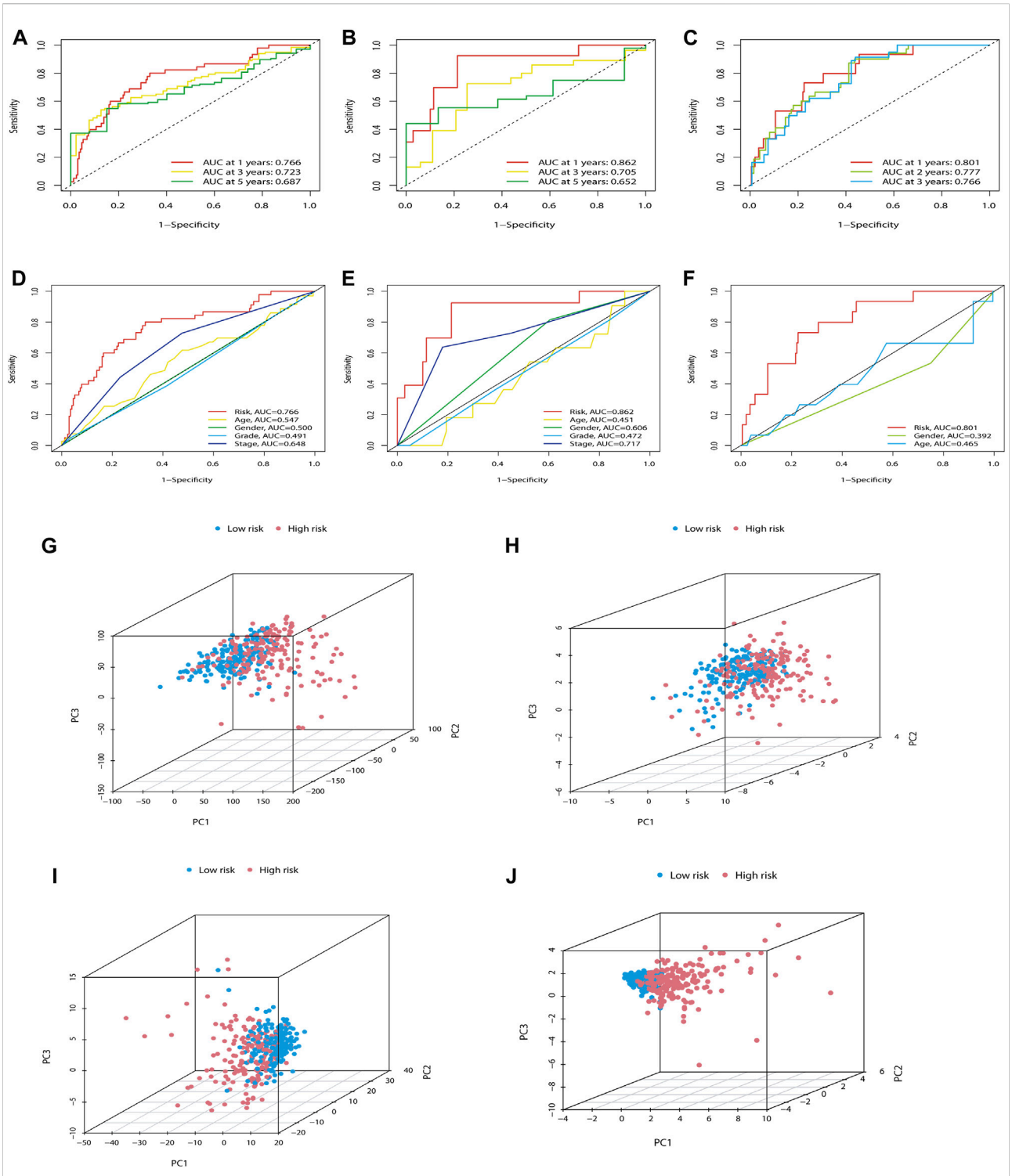

FIGURE 5
Further verification of the ability of the m7G-LPS. **(A–C)** ROC curves of m7G-PLS to predict the sensitivity and specificity of 1-, 3-, and 5-years survival in training **(A)**, testing **(B)**, and ICGC sets **(C)**. **(D–F)** The time-dependent AUC value with different clinical characteristics in the training, testing, and ICGC set. **(G–J)** PCA between the high- and low-risk groups based on whole gene expression profiles **(G)**, 29 m7G genes **(H)**, 22 prognostic m7G-related lncRNAs **(I)**, and m7G-LPS **(J)**. m7G, N7-methylguanosine; lncRNAs, long non-coding RNAs; m7G-LPS, m7G-related lncRNA prognostic signature; ROC, Receiver operating characteristic curve.

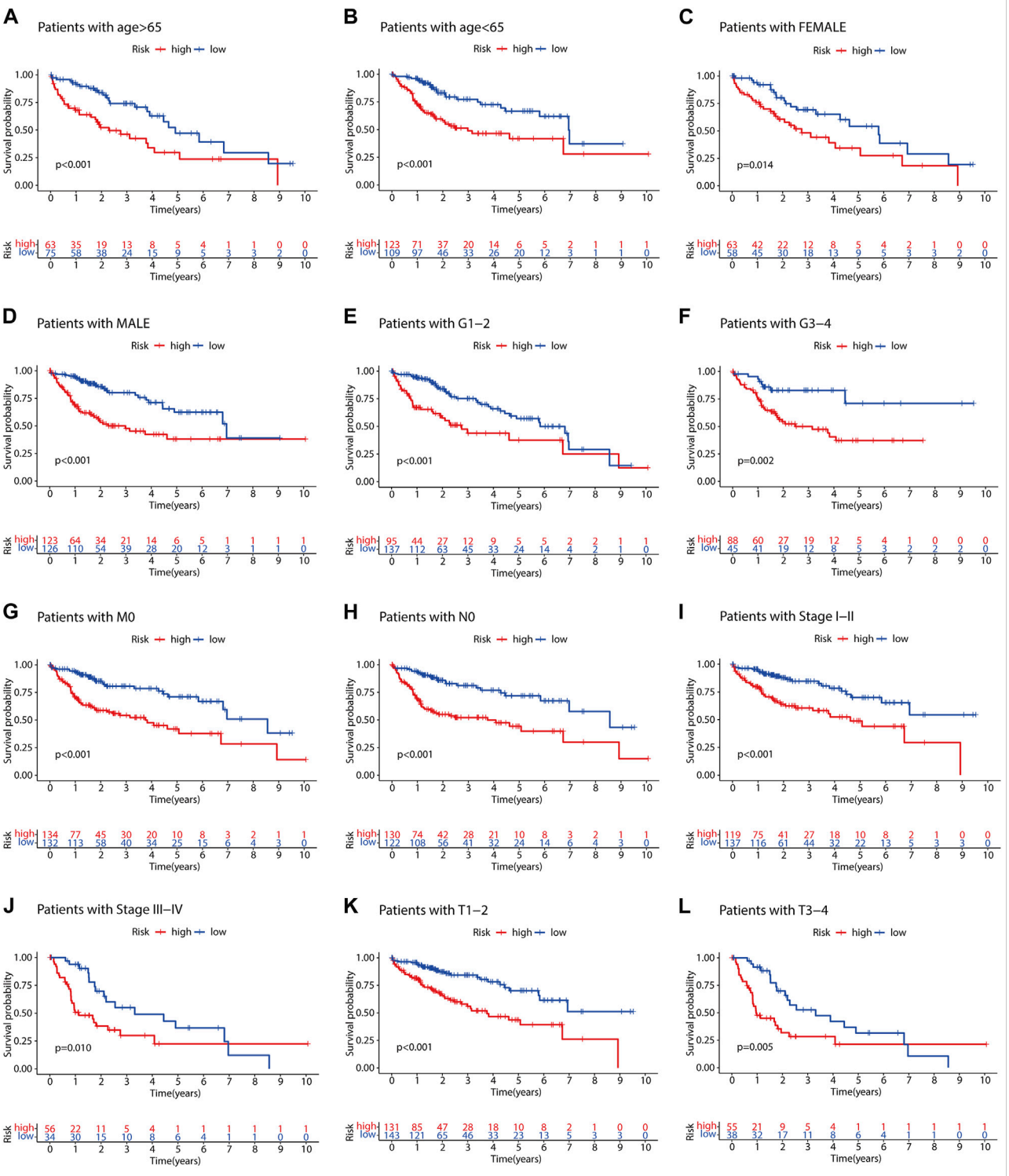

FIGURE 6
(A–L) Kaplan–Meier analyses of patients with AJCC-T, tumor grade, stage, gender, and age.

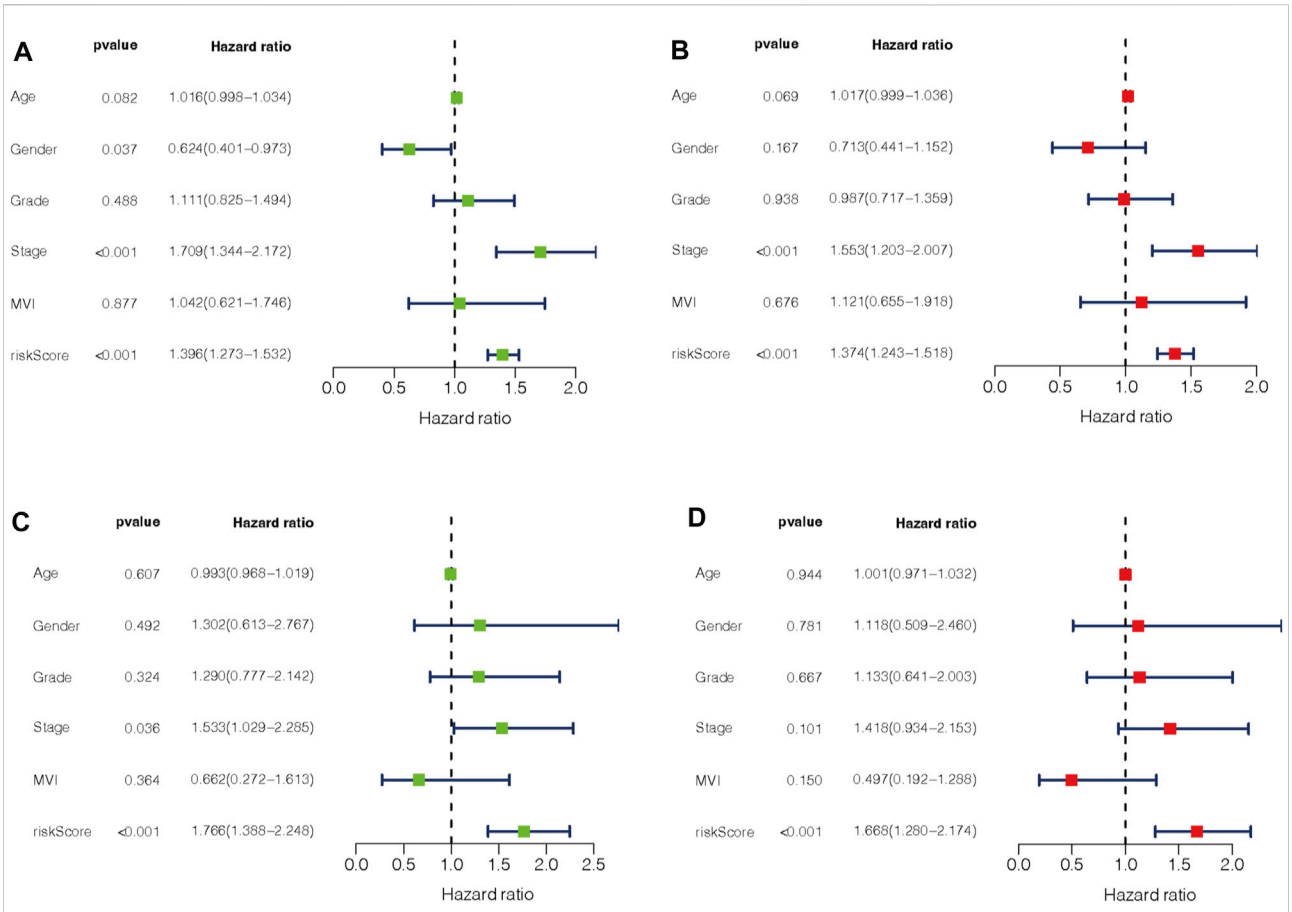

FIGURE 7
(A–D) Univariate and multivariate Cox regression analysis in training **(A,B)** and testing set **(C,D)**.

Further verifies the ability of the m7G-LPS

In addition, we also predicted the capability of the established m7G-LPS in the training, testing, and ICGC sets. The model showed excellent predictive accuracy (Figures 5A–C). In addition, the predictive power of the m7G-LPS was far higher than that of the clinical data across the training and testing sets, as evidenced by time-dependent ROC analysis (Figures 5D–F). PCA analysis revealed different whole gene expression profiles (Figure 5G), 29 m7G genes (Figure 5H), 22 prognostic m7G-related lncRNAs (Figure 5I), and m7G-LPS (Figure 5J). However, the results obtained based on our model illustrated a high separation between high- and low-risk groups (Figure 5J). More importantly, compared with the HCC prognostic models constructed by Zhou et al. (2021), Zhang et al. (2022a), and Jin et al. (2021), our model has a stronger predictive power (Supplementary Figures S2A–S2D). All these details certificated an excellent precision of the m7G-LPS.

Validation of the m7G-LPS in clinical features of hepatocellular carcinoma

To evaluate the impact of our prognostic risk model on the clinical characteristics of HCC patients, we conducted a hierarchical analysis based on their universal clinicopathological characteristics. The finding confirmed that according to the risk score, the prognosis of HCC patients could be distinguished well (Figures 6A–L). In addition, the ability of m7G-LPS was also verified by clinical features, as shown in Supplementary Figure S3. After further bioinformatics statistics, the risk score was determined as an independent prognostic symbol for HCC, irrespective of other clinical features (Figures 7A–D). The heatmap showed that grade, cluster, and stage were significant factors between the two different risk groups (Figure 8A). Cluster 2 had a high proportion in the high-risk group, indicating an unfavorable prognosis in the high-risk group. To assess the possible impact of different interventional treatment options on patients, we

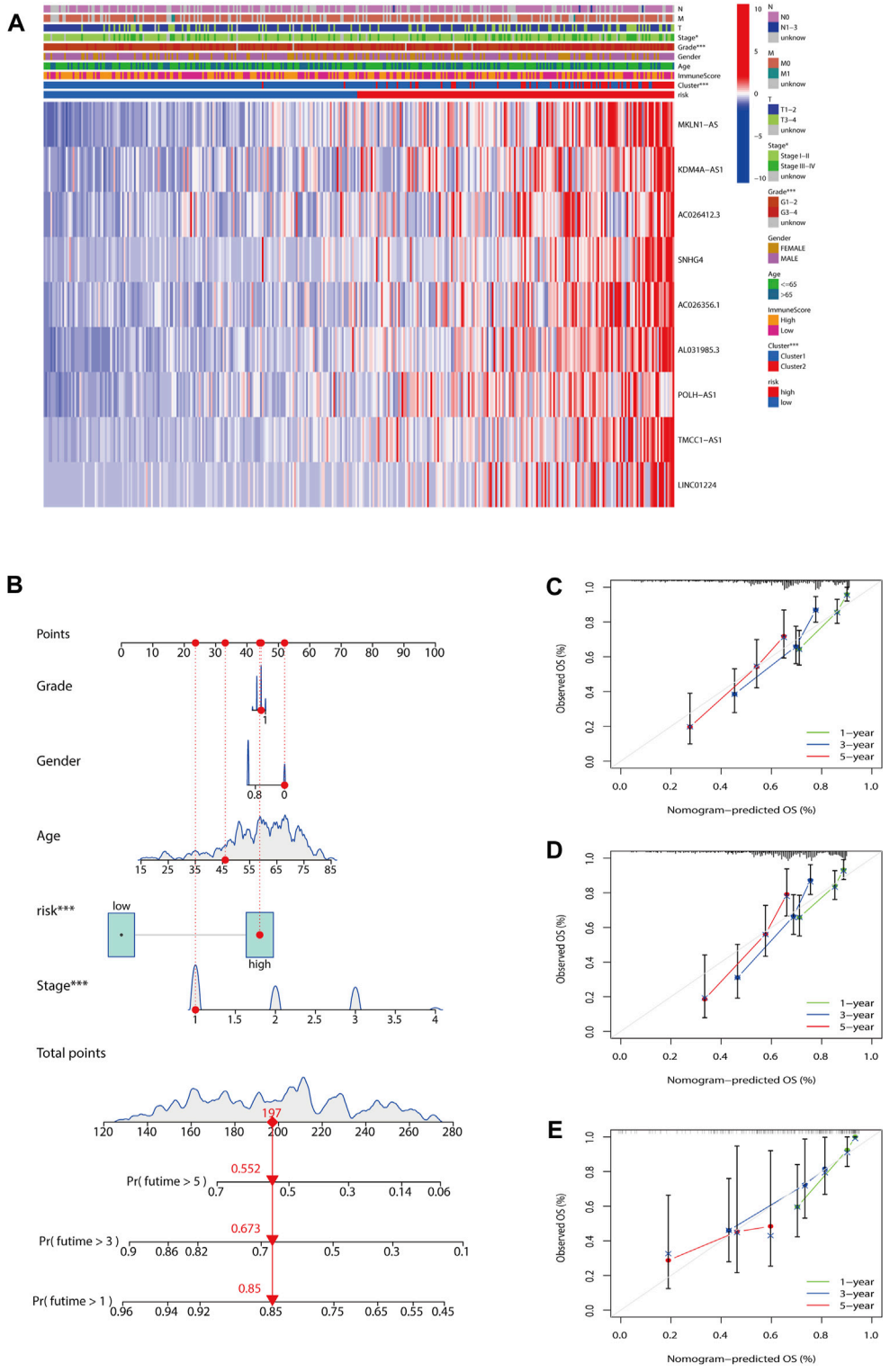

FIGURE 8
Establishment of nomogram. **(A)** The heatmap included the expression of nine m7G-LPS, clustering characteristics, immune score, and TMN stage. **(B)** Nomogram based on grade, gender, age, and risk score. **(C–E)** Calibration curves of the nomogram for predicting and observing 1-, 3-, and 5-years OS (*$p < 0.05$; ***$p < 0.001$). m7G-LPS, m7G-related lncRNA prognostic signature. m7G, N7-methylguanosine; lncRNAs, long non-coding RNAs.

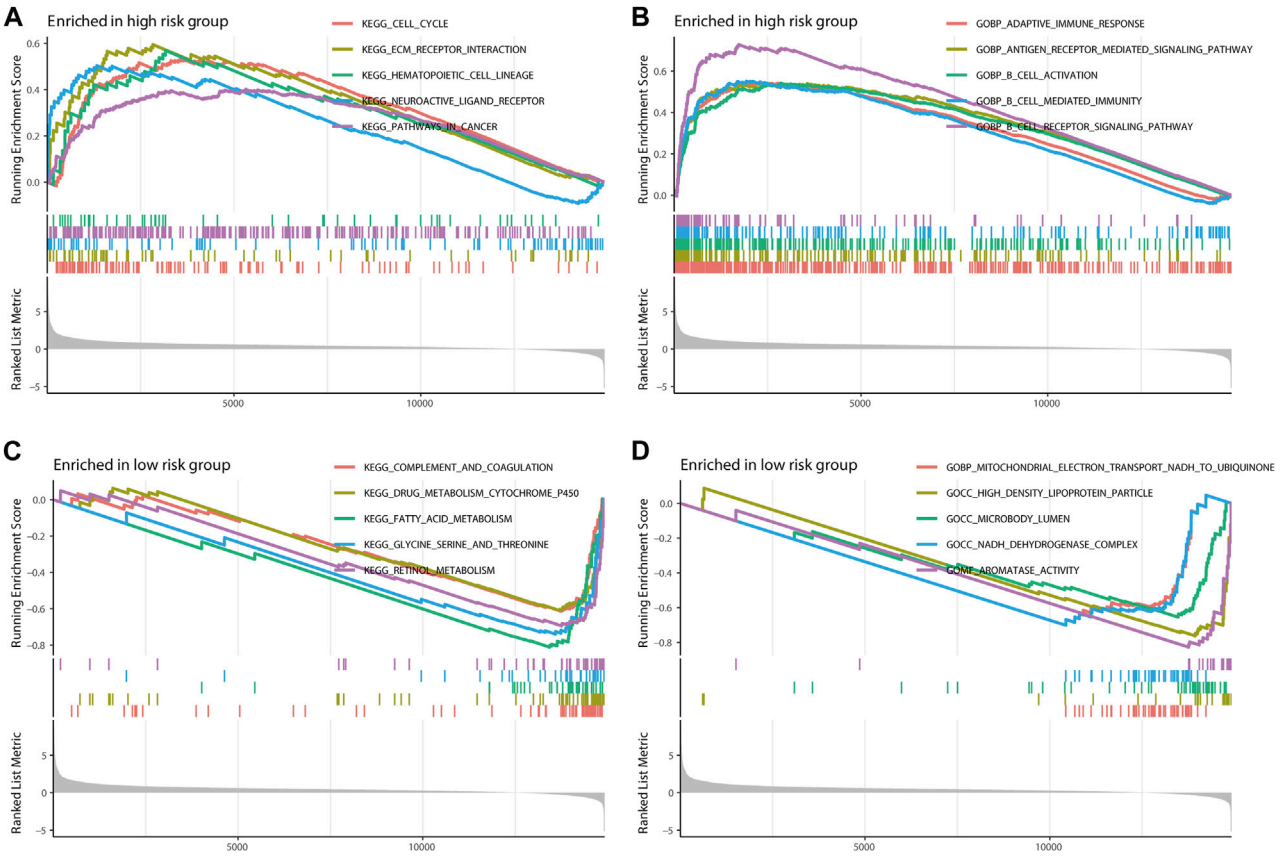

FIGURE 9
Gene Set Enrichment Analysis. The top five pathways in two different risk groups by KEGG enrichment analysis **(A,C)** and GO enrichment analysis **(B,D)**. KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology.

established a nomogram incorporating the risk score and clinical features (Figure 8B). Additionally, the results of the calibration curve analysis showed that the predicted survival probability by the nomogram was consistent with the actual one (Figures 8C–E).

Gene set enrichment analysis

The Kyoto Encyclopedia of Genes and Genomes (KEGG) and the Gene Ontology (GO) enrichment analysis demonstrated that m7G-LPS was enriched in immune-, metabolism-, and tumor-related pathways. In the KEGG enrichment analysis, the cell cycle, extracellular matrix (ECM)-interaction, hematopoietic-cell-lineage, neuroactive-ligand receptor interaction, and pathways in cancer were significantly enriched in the high-risk group (Figure 9A). The significantly enriched pathways in the low-risk group were various metabolic processes, including drug metabolism-cytochrome-P450, fatty acid metabolism, glycine metabolism, serine metabolism, threonine metabolism, and retinal metabolism (Figure 9B). In GO enrichment analysis, the significantly enriched pathways in the high-risk group were various immune processes such as adaptive-immune response, B-cell activation, B cell-mediated immunity, and B-cell receptor signaling pathway (Figure 9C), while the GO function enrichment of low-risk score demonstrated enrichment in mitochondrial-electron-transport-NADH-to-ubiquinone, high-density-lipoprotein-particle, microbody-lumen, NADH-dehydrogenase-complex, and protein–lipid-complex (Figure 9D).

Tumor immune cell infiltration and gene expression

Immune cell-related functions, including cytolytic activity, major histocompatibility complex-class-I, type-I-interferon (IFN)-response, and type-II-IFN-response were remarkably different between the two risk groups (Figure 10A). We further found that cytolytic activity, type-I-IFN-response, and

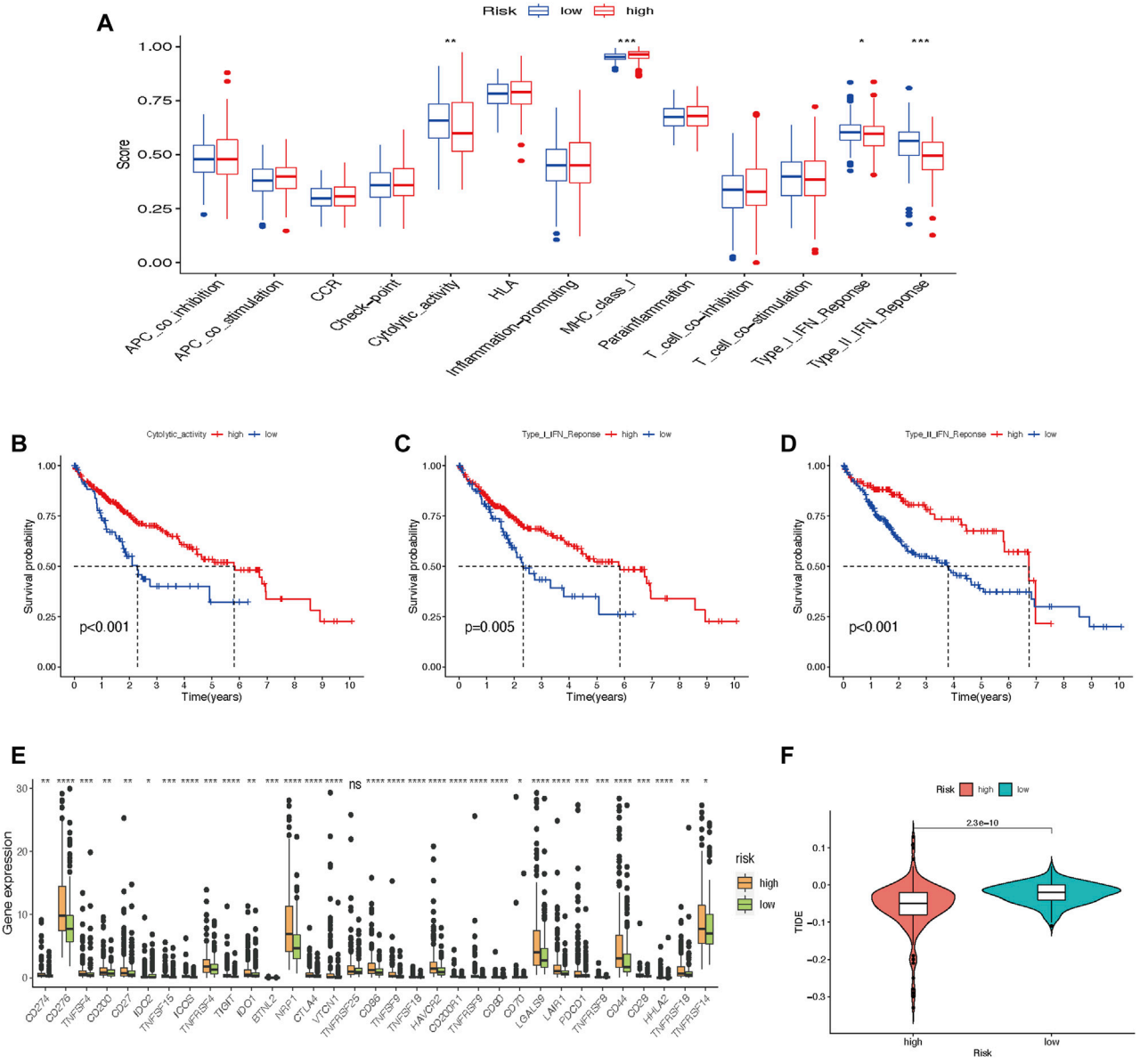

FIGURE 10
Tumor immune cell infiltration and gene expression. **(A)** Box plot of immunologic function analysis. **(B–D)** Relationship between cytolytic-activity, type-I-IFN-response, and type-II-IFN-response and survival of HCC patients in high- and low-risk groups. **(E)** Box plot of 33 immune checkpoints. **(F)** TIDE analysis shows different immune responses in high- and low-risk groups (*$p < 0.05$; **$p < 0.01$; ***$p < 0.001$). HCC, hepatocellular carcinoma; TIDE, tumor immune dysfunction and exclusion.

type-II-IFN-response were closely related to the survival of HCC patients (Figures 10B–D). In comparison with the low-risk group, immune checkpoint expressions in the high-risk group were higher (Figure 10E). Furthermore, the score of Tumor Immune Dysfunction and Exclusion (TIDE) was significantly lower in the high-risk group than in the low-risk group (Figure 10F), which suggested that patients in the high-risk subtype may have a better prospect for immunotherapy.

Risk-related drug-sensitivity prediction

Based on the pRRophetic package, some representative and commonly used clinical drugs were predicted to have different effectiveness in HCC patients with different risk scores. The results indicated that axitinib, dasatinib, erlotinib, and sorafenib were more effective in the low-risk group, while sunitinib may be more effective in the high-risk group (Figures 11A–E).

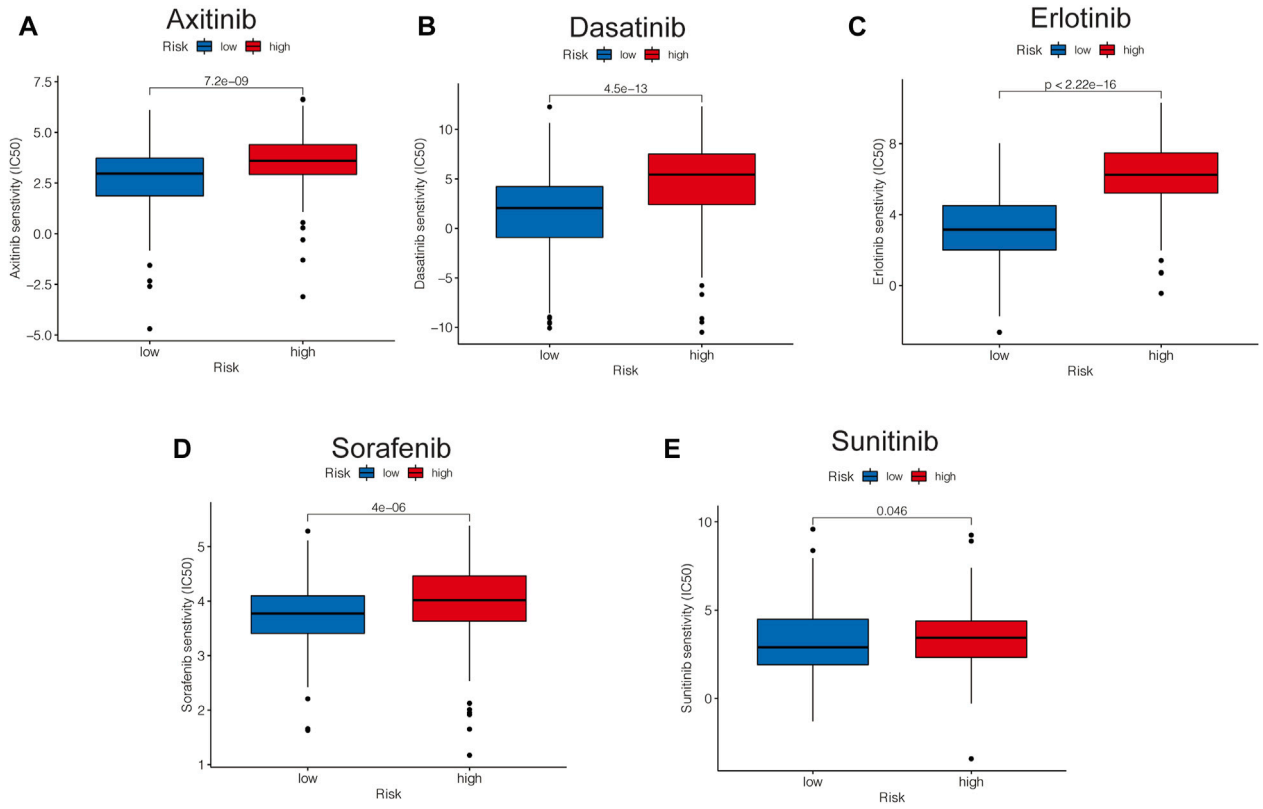

FIGURE 11
Risk-related drug-sensitivity prediction. **(A–E)** Relationship between risk score and chemotherapeutic sensitivity.

Correlation between the m7G-LPS and tumor mutation burden

An oncoplot showed the topmost 20 mutated HCC genes in the two different risk groups (Figures 12A and B). The outcomes revealed that TP53, CTNNB1, and MUC16 were more frequently mutated in the high-risk group, while TTN, ALB, and APOB were more frequently mutated genes in the low-risk group. TMB-high HCC patients had a shorter OS ($p < 0.001$; Figure 12C). Moreover, patients with both the high TMB and the high-risk score had the worst OS among the four groups ($p < 0.001$; Figure 12D).

Discussion

Despite some success in diagnosis and development of vaccines, the surge in HCC prevalence and short overall survival has spurred investigators to discover new prognostic signatures for HCC patients, which will enable stratification of patients and precise treatment. Since 2018, there has been a mushrooming of research studies on the processes of m6A in the proliferation, differentiation, prognosis, and apoptosis of various tumors (Yi et al., 2020; Zuo et al., 2020). Precise prognostic models help physicians make better clinical decisions and allow health authorities to allocate health care resources more rationally (Ferraro et al., 2022). However, no m7G-related lncRNAs signature has been reported in tumor research. Therefore, it is crucial to explore the m7G-related lncRNAs at the cellular and molecular levels in tumors. Based on the public databases, 22 m7G-related lncRNAs were screened out by bioinformatics and statistical analysis of HCC patients' clinical data. Interestingly, all selected lncRNAs were with high expressions in HCC patients as risk factors. Then, HCC patients were divided into two clusters according to different expressions of lncRNAs. Patients in Cluster 2 had advanced clinical features and worse OS than Cluster 1. CIBERSORT analysis also showed that the effect of immunotherapy might be better in Cluster 1. All these findings indicated that the tumor immune microenvironment has enhanced the oncogenesis of HCC.

In our study, a total of nine out of 22 m7G-related lncRNAs, which included MKLN1-AS, KDM4A-AS1, AC026412.3, SNHG4, AC026356.1, AL031985.3, POLH-AS1, TMCC1-AS1,

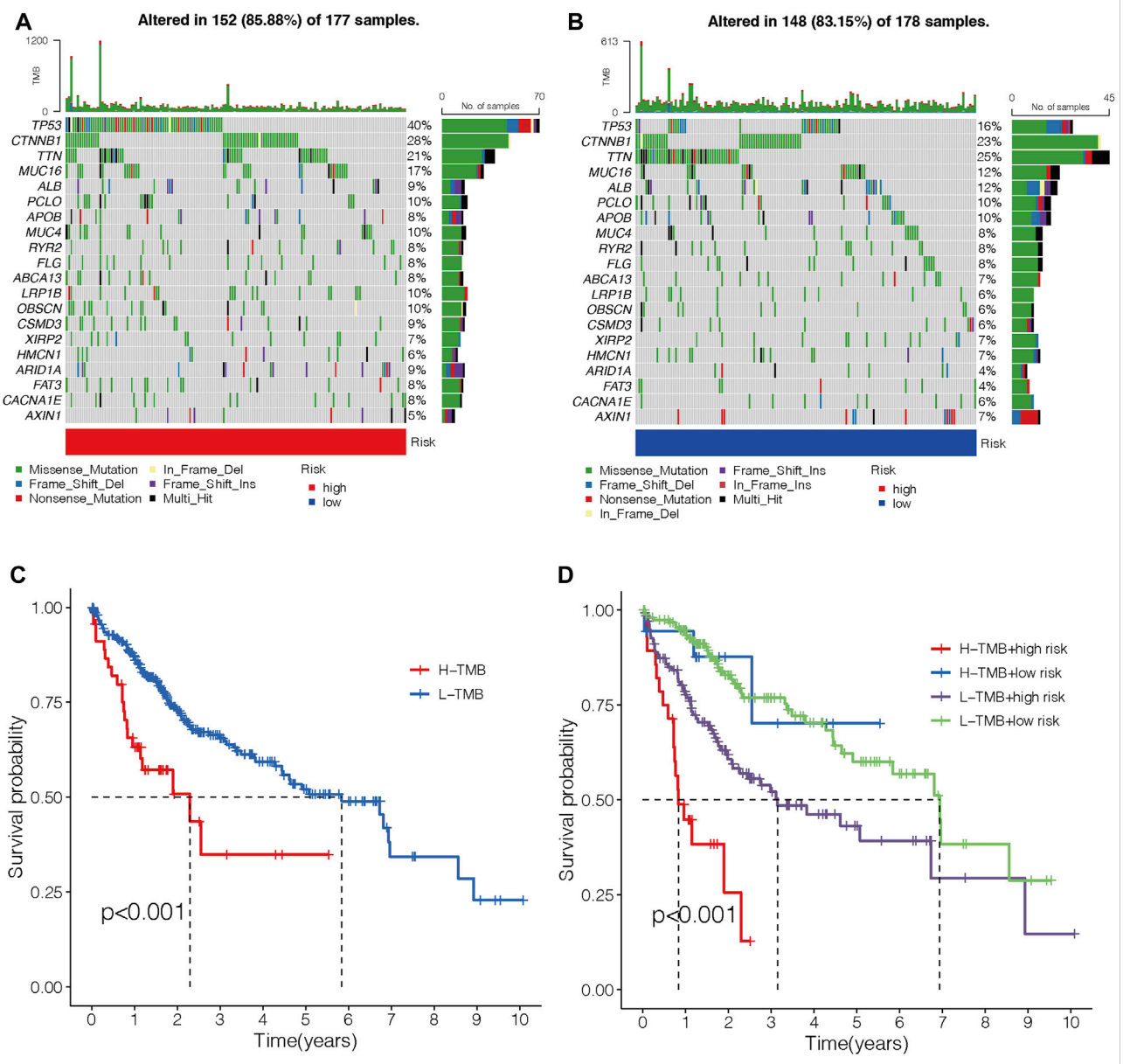

FIGURE 12
Correlations between the risk model and TMB. **(A–B)** Waterfall diagram shows the top 20 mutated genes in the high- **(A)** and low-risk groups **(B)**. **(C)** OS curves for high- and low-TMB sets. **(D)** OS curves for patients are divided into both TMB and risk scores. TMB, tumor mutation burden. OS, overall survival.

and LINC01224, were identified as key lncRNAs with HCC patients' OS. Among them, high MKLN1-AS expression was previously reported to aggravate hepatocellular carcinoma progression and was associated with shorter overall survival and disease-free survival in patients with HCC (Gao et al., 2020). Of these lncRNAs, POLH-AS1 has not been studied but was linked to the development and occurrence of diseases (Zhang et al., 2022b). It is worth noting that the coefficient value of POLH-AS1 levels was the second highest in our constructed model, after MKLN1-AS. The study by Chen al. showed that KDMAS-1 was significantly increased in hepatocellular carcinoma tissues, and the higher the expression of KDMAS-1 was, the worse the prognosis HCC patients had (Bray et al., 2018). AC026412.3 was a member of the prognostic model of m6A-associated lncRNA conducted by Wang, which was significantly correlated with tumor grade, stage, and T stage (Wang et al., 2021). Several diseases are associated with overexpression of SNHG4, and silencing SNHG4 is expected

to be a new therapeutic target for human diseases (Chu et al., 2021). Investigators found that overexpressed SNHG4 was closely related to advanced tumor grade, stage, and OS (Zhu et al., 2019; Jiao et al., 2020). According to Wang, SNHG4 was one of the two lncRNAs highly associated with HCC prognosis in a prognostic model (Wang et al., 2021). An autophagy-related and immune-related lncRNA, AL031985.3, had been shown to predict HCC prognosis (Jia et al., 2020; Kong et al., 2020). Similar to TMCC1-AS1, LINC01224 has been implemented in multiple prognostic models for HCC (Zhao et al., 2018; Deng et al., 2020; Xu et al., 2021). After HCC patients were classified into two risk groups (high-risk and low-risk) based on the constructed risk model, the signature we established could predict the OS of HCC patients in the training, testing, and ICGC sets with high accuracy, according to the survival analysis. Univariate and multivariate Cox regression analyses showed that the m7G-LPS was closely related to the OS of HCC. Additionally, we examined the m7G-LPS stratification in clinicopathological features, and the results indicated that m7G-LPS might be useful for the evaluation of clinical prognosis. In summary, based on the rich biological statistics and experimental validation, we have sufficient reasons to believe that the above nine lncRNAs can predict the survival of HCC patients.

Hepatocellular carcinoma is a typical inflammation-associated tumor, and, thus, there may be complex interactions between cancer cells and multiple other cells in the tumor microenvironment. We then performed GSEA to better realize the biological behaviors mediated by m7G-LPS. The GO function enrichment of high-risk score focusing on adaptive immune functions and cell cycle and ECM receptor interaction was enriched in KEGG analysis, while the low-risk group may be significantly enriched in lipid metabolism, indicating that m7G-LPS may affect the prognosis of patients through immune mechanisms. Increasing evidence has confirmed that the cellular and non-cellular components of the tumor microenvironment may impact the prognosis and treatment of HCC. A previous study of pathological sections of HCC patients showed that differential gene expression in tumor cells and their stroma has important implications for tumor survival and molecular subtypes (Hu and Huang, 2022). Recently, immunotherapy provided advanced HCC patients with more options beyond targeted drugs. Immune checkpoint inhibitors clear tumor cells by altering the tumor immune microenvironment so that tumor cells are specifically recognized. In the high-risk group, 33 immune checkpoints were significantly overexpressed compared with the low-risk group. The TIDE algorithm can predict clinical response to immune checkpoint blockade using tumor transcriptomic signatures. Our research discovered that high-risk patients had lower TIDE scores in contrast to those with low-risk scores. All these results suggested that high-risk HCC patients may have a better prospect for immunotherapy.

Hepatocellular carcinoma, due to its heterogeneity, is recognized as one of the most chemotherapy-resistant tumors (Huang et al., 2020; Xu et al., 2020), indicating that different patients may show different responses to conventional treatments, so it becomes crucial to predict the effectiveness of different interventional regimens on HCC patients. Among the five common chemotherapy drugs, we found the high-risk group was more sensitive to axitinib, dasatinib, erlotinib, and sorafenib, while sunitinib may have a better effect in patients in the high-risk group. This suggested that different treatment strategies could be used for patients with different molecular subtypes of hepatocellular carcinoma, providing several insights into the development of precision oncology.

As we all know, immunotherapy lacks efficacy predictors due to its complex mechanism of action. Recently, TMB has been widely explored as a predictive biomarker in some cancers on immunotherapy efficacy (Hellmann et al., 2018; Tang et al., 2021). In the present study, HCC patients with high-risk scores presented a considerably higher TMB than those with low-risk scores. Published research studies reported that patients with high TMB have a worse OS in HCC (Cao et al., 2019). The results were consistent with the present study.

Within our perception, this is the first study to investigate the correlation between m7G-related lncRNAs and HCC pathogenesis in the immune microenvironment and to establish a prognostic model based on public databases. There are, however, several innate limitations in our research. First, the conclusions we had drawn above were based on public databases. Despite abundant biological information being used to elucidate the m7G-related lncRNA, further studies are needed to support these conclusions in a larger HCC patient cohort. More importantly, despite our growing understanding of m7G and lncRNAs, more experiments are required to illustrate how these m7G-related lncRNAs affect HCC.

Conclusion

The m7G-LPS model had promising prediction performance for OS in HCC. Our results provided comprehensive evidence for further studies indicating the function of m7G-related lncRNAs in HCC, which could shed new perspectives on the tumorigenesis and guidance of tumor immunotherapy in HCC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

Ethics statement

Written informed consent was obtained from the individual(s) for the publication of any potentially identifiable images or data included in this article.

Author contributions

Study concept and design: TW, ZZ, and XW. Acquisition of data: TW and LY. Analysis of data: TW. Drafting of the manuscript: TW, ZZ, XW, and WL. Revision of the manuscript: all authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.929035/full#supplementary-material

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Expression of T cell-related lncRNAs in multiple sclerosis

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Long non-coding RNAs (lncRNAs) have been demonstrated to in the pathophysiology of multiple sclerosis (MS). In order to appraise the role of T cell-related lncRNAs in this disorder, we assessed expressions of NEST, RMRP, TH2-LCR, MAFTRR and FLICR in MS patients and healthy individuals. We detected significant difference in the expression of RMRP and FLICR between cases and controls. There were substantial correlations between expressions of NEST, RMRP, TH2-LCR, MAFTRR and FLICR lncRNAs among patients, but not controls. The strongest correlations were found between RMRP and TH2-LCR, and between MAFTRR and RMRP with correlation coefficients of 0.69 and 0.59, respectively. ROC curve analysis revealed appropriate power of FLICR in differentiating between MS patients and healthy controls (AUC value = 0.84). Expression of NEST lncRNA was positively correlated with disease duration in MS patients, but negatively correlated with age at onset. In brief, we reported dysregulation of two T cell-related lncRNAs in MS patients and proposed FLICR as a putative marker for this disorder.

KEYWORDS

FLICR, NEST, RMRP, TH2-LCR, lncRNA, multiple sclerosis, T cell

Introduction

Multiple sclerosis (MS) is an autoimmune condition in which the underlying cause is not completely understood. Autoimmune reactions may occur as a result of inappropriate function of regulatory T cells (Tregs). This population of T cells expresses Foxp3 and low level of CD127 (Seddiki et al., 2006). Several genes associated with MS such as CD25, CTLA4, CD127, IL-10 have been shown to be related to Treg function or differentiation (ANZgene, 2009; Rioux et al., 2009). Numerous studies have reported abnormal function or quantities of Tregs in blood (Viglietta et al., 2004; Venken et al., 2008; Dalla Libera et al., 2011; Rodi et al., 2016), central nervous system (CNS) lesions and cerebrospinal fluid (Fritzsching et al., 2011) of MS patients. Notably, expression levels of Foxp3 have been

TABLE 1 General parameters of study participants.

Study groups	Parameters	Values	
Patients	Sex (number)	Male	12
		Female	38
	Age (Years, mean ± SD)	Male	41.08 ± 9.5
		Female	39.13 ± 8.99
	Disease duration (Years, mean ± SD)	Male	3.75 ± 2.3
		Female	10.34 ± 6.34
	Age of onset (Years, mean ± SD)	Male	37.33 ± 10.49
		Female	28.76 ± 8.84
	EDSS Score	Male	2.62 ± 0.77
		Female	2.3 ± 1.24
Controls	Sex (number)	Male	12
		Female	38

revealed to be diminished in Tregs of MS patients (Venken et al., 2008; Frisullo et al., 2009). Moreover, their ability in inhibition of T cell response to myelin basic protein has been diminished (Haas et al., 2005; Venken et al., 2007). A recent study has reported decline in the number of resting and increase in activated $CD4^{+}CD25+FOXP3+$Tregs in MS patients (Verma et al., 2021). Cumulatively, abnormal function of Tregs is a predominant finding in MS patients.

Recent studies have indicated the importance of non-coding RNAs in the regulation of Treg function and their plasticity as well as commitment of Treg lineage (Ghafouri-Fard and Taheri, 2020; Luo and Wang, 2020; Jalaiei et al., 2021; Taheri et al., 2021). A number of newly identified long non-coding RNAs (lncRNAs), namely FLICR (FOXP3 Regulating Long Intergenic Non-Coding RNA), MAFTRR (MAF Transcriptional Regulator RNA), NEST (IFNG-AS1), RMRP (RNA Component Of Mitochondrial RNA Processing Endoribonuclease) and TH2-LCR (Th2 Cytokine Locus Control Region) are supposed to affect function of Tregs. However, experimental data for confirmation of their effect in the pathogenesis of MS is missing. We conducted this study to evaluate expression of these lncRNAs in the peripheral blood of MS patients in comparison with healthy persons.

Materials and methods

Study participants

The study included 12 male MS patients and 38 female MS patients. Additionally, 50 age and sex matched healthy individuals were recruited as controls. The latter group of individuals had no history of neuropsychiatric or immune-mediated diseases. MS patients were diagnosed based on the revised McDonald criteria (Polman et al., 2011). Relapsing-remitting MS was diagnosed based on the presence of at least two separate areas of damage in the CNS that have happened at dissimilar points in time. MRI results, history of symptoms and findings on the neurological examination were used for diagnosis. Informed consent was obtained from all MS patients and healthy controls. The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1400.562). General data of MS patients and controls is summarized in Table 1.

Expression studies

Four milliliters of whole peripheral blood were gathered from cases and controls. Blood specimens were used for RNA extraction using the Hybrid-RTM blood RNA extraction kit (GeneAll Biotechnology Co Ltd., South Koera). Then, cDNA was produced using the extracted RNA and a commercial Reverse Transcription kit (Applied Biosystems). Expressions of NEST, RMRP, TH2-LCR, MAFTRR and FLICR were quantified using the primers listed in Table 2. B2M was used as the reference gene for qPCR.

Statistical analysis

GraphPad Prism version 9.0 (La Jolla, CA, United States) was used for data assessment. Expression levels of five lncRNAs, namely NEST, RMRP, TH2-LCR, MAFTRR, and FLICR were compared between MS patients and healthy controls using the comparative–delta Ct method. The normal/gaussian distribution of the values was assessed using the Shapiro-Wilk test. Mann-Whitney U test was applied to recognize differentially expressed genes between MS patients and controls. Two-way ANOVA test was applied to examine the effects of disease and gender on expressions of lncRNAs.

TABLE 2 Primer sequences.

Gene	Sequence 5→3	Primer length (bp)
B2M	F- AGATGAGTATGCCTGCCGTG	20
	R- GCGGCATCTTCAAACCTCCA	20
FLICR	F- GGG CTT TTC CAG AAG GGT CT	20
	R- AGC CCA GGG TTC TAG TCG	18
MAFTRR	F- CTG AAG GGA CAG GAC GGA CAA C	22
	R- GGG GAA AAC CTG GAA AGA GGG A	22
NEST	F- AGC TGA TGA TGG TGG CAA TCT	21
	R- TGA CTT CTC CTC CAG CGT TTT	21
RMRP	F- GTA GAC ATT CCC CGC TTC CCA	21
	R- GAG AAT GAG CCC CGT GTG GTT	21
TH2-LCR	F- GCT CCC CAG GCT TTT GAG ATA	21
	R- TGG TGA TGC TGA AGG GAG AC	20

TABLE 3 Information about selected lncRNAs.

Name/Gene ID	Accession number	Location	Official full name
IFNG-AS1 (NEST)	NR_104124.1	12q15	IFNG antisense RNA 1
	NR_104125.1		
RMRP	NR_003051.3	9p13.3	RNA component of mitochondrial RNA processing endoribonuclease
TH2LCRR (TH2-LCR)	NR_132124.1	5q31.1	T helper type 2 locus control region associated RNA
	NR_132125.1		
	NR_132126.1		
MAFTRR	NR_104663.1	16q23.2	MAF transcriptional regulator RNA
FLICR	NR_147988.1	Xp11.23	FOXP3 regulating long intergenic non-coding RNA

Correlations between expression levels NEST, RMRP, TH2-LCR, MAFTRR and FLICR were measured using Spearman's rank correlation coefficient since expression data was not normally distributed. Correlations between expressions of lncRNAs and demographic/clinical data such as age, disease duration, age at onset and EDSS were assessed using Spearman's rank correlation coefficients.

Receiver operating characteristic (ROC) curve was illustrated to value the diagnostic power of expression levels of differentially expressed genes. p value < 0.05 was considered as significant.

Results

Expression data

In the current study, we assessed expression of five lncRNAs, namely NEST, RMRP, TH2-LCR, MAFTRR and FLICR. Table 3 shows the information about selected lncRNAs.

Expression levels of RMRP and FLICR were different between MS patients and controls (Figure 1).

We detected significant effect of group (disease) factor on expression levels of RMRP and FLICR. However, gender factor had no significant effect on expressions of these lncRNAs, which means that there is no significant difference in the expression of these lncRNAs between males and females. Moreover, the interaction of gender and group had no effect on expressions of any of the studied genes (Table 4). Therefore, we did not perform post hoc tests for multiple comparisons.

Expression levels of RMRP and FLICR were significantly elevated in MS patients compared with controls (Figure 2).

Our data revealed significant correlations between expression levels of NEST, RMRP, TH2-LCR, MAFTRR and FLICR lncRNAs among patients, but not controls. The strongest correlations were found between RMRP and TH2-LCR, and between MAFTRR and RMRP with correlation coefficients of 0.69 and 0.59, respectively (Tables 5, 6).

ROC curve analysis revealed appropriate power of FLICR in differentiating between MS patients and healthy controls (AUC

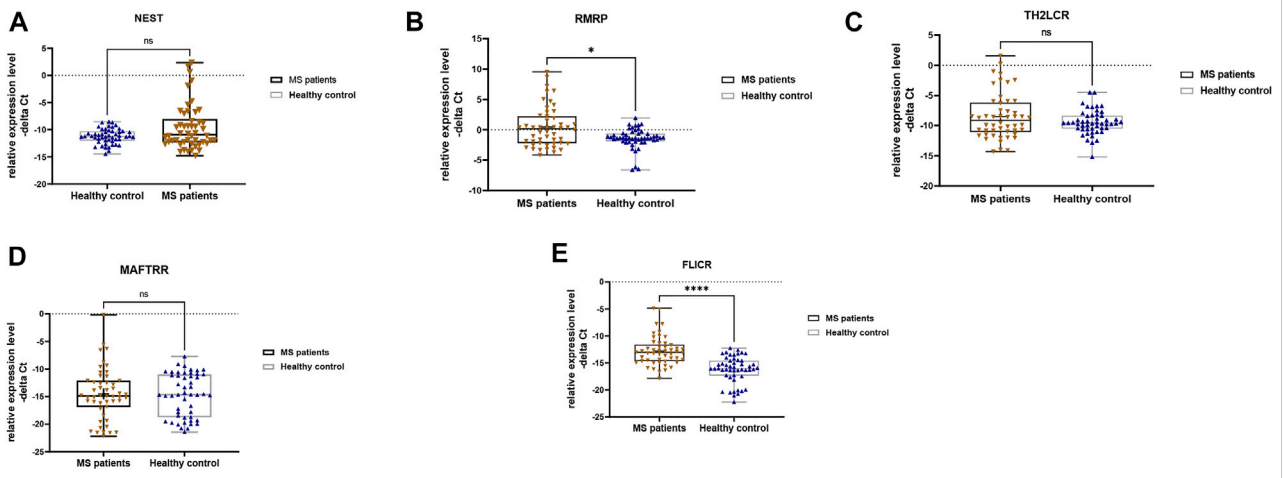

FIGURE 1
Relative expression levels of five lncRNAs in total multiple sclerosis (MS) patients and controls as measured by−delta Ct values **(A–E)**. Mann-Whitney U test was applied to find differentially expressed genes between cases and controls (**p value <0.01 and ****p value <0.0001).

TABLE 4 Graphpad prism output from analysis of effect of Group and Gender (Tests of Between-Subjects Effects) on expression of studied genes in cases compared to healthy controls.

Source of variation	Group effect			Gender effect			Interactions		
	SS[a]	F[b]	p Value	SS	F	p Value	SS	F	p Value
NEST	31.28	3.03	0.072	22.9	2.42	0.12	7.18	0.75	0.38
RMRP	58.05	8.21	0.005*	3.74	0.53	0.46	0.48	0.068	0.79
TH2-LCR	34.13	3.67	0.058	0.22	0.023	0.87	9.85	1.059	0.3
MAFTRR	0.13	0.007	0.93	3.33	0.17	0.67	0.61	0.03	0.85
FLICR	233.1	34.11	<0.0001*	6.92	1.01	0.31	0.50	0.07	0.78

[a]Sum of Squares.
[b]F of Variance.

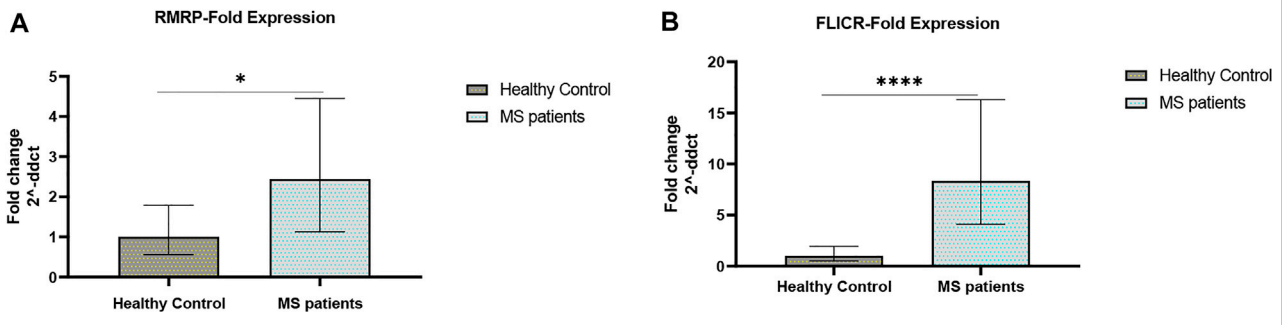

FIGURE 2
Fold changes of RMRP and FLICR expression **(A,B)** in MS patients versus healthy controls (*p value <0.05, and ****p value <0.0001).

TABLE 5 Spearman's correlation between expression levels of lncRNAs among MS patients and healthy subjects (* p-Value at a significance level of $p < 0.05$.** p-Value at a significance level of $p < 0.001$).

	RMRP		TH2-LCR		MAFTRR		FLICR	
	Patients	Controls	Patients	Controls	Patients	Controls	Patients	Controls
NEST	0.4*	0.25	0.37*	0.21	0.38*	0.04	0.49**	0.046
RMRP			0.69**	0.016	0.59**	0.15	0.49**	-0.24
TH2-LCR					0.51**	0.08	0.44*	0.23
MAFTRR							0.42*	-0.13

TABLE 6 The results of ROC curve analyses for two differentially expressed lncRNAs in patients with MS disease.

FLICR				RMRP			
AUC±SD	Sensitivity	Specificity	p value	AUC±SD	Sensitivity	Specificity	p value
0.84 ± 0.03	0.86	0.72	<0.0001	0.63 ± 0.05	0.5	0.88	0.02

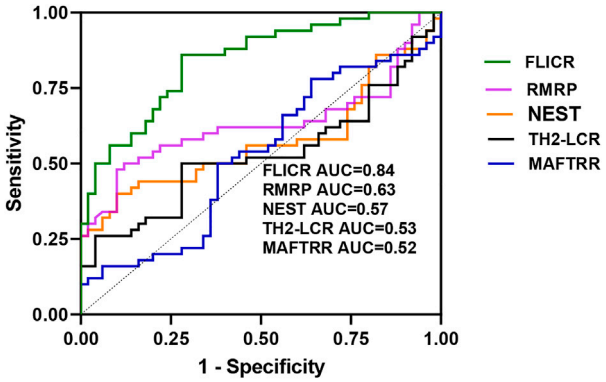

FIGURE 3
ROC curves of five lncRNAs levels in patients with multiple sclerosis.

value=0.84). RMRP, NEST, TH2-LCR and MAFTRR had AUC values of 0.63, 0.57, 0.53 and 0.52, respectively (Figure 3).

Sensitivity and specificity values for two differentially expressed genes between cases and controls, i.e., FLICR and RMRP are shown in Tables 5, 6.

Expression of NEST lncRNA was positively correlated with disease duration in MS patients, but negatively correlated with age at onset (Table 7).

TABLE 7 The results of Spearman's rank correlation between expressions of five lncRNAs and clinical data.

	Age	Disease duration	Age at onset	EDSS
NEST	−0.207	**0.289***	**−0.32***	0.09
RMRP	−0.076	−0.03	−0.04	−0.03
TH2-LCR	−0.183	−0.121	−0.1	0.047
MAFTRR	−0.058	0.014	−0.08	−0.04
FLICR	−0.157	0.194	−0.26	0.094

*Significance level of $p < 0.05$.
**Significance level of $p < 0.001$.
Disease duration was classified into 3 ranges (1–5, 6–10 and more than 4 years).
EDSS, scores was classified into 2 ranges (1-2 and 3–5).

Discussion

Tregs have important functions in the pathoetiology of MS. Alterations in the frequency and suppressive effects of Tregs in MS patients is implicated with the evolution and exacerbation of MS (Kimura, 2020). Therefore, genes that regulate function of Tregs are potentially involved in the pathoetiology of this disorder. We compared expression of five Treg-associated lncRNAs between MS patients and healthy persons. We noticed higher expression levels of RMRP and FLICR in MS patients compared with controls. Consistent with our findings, a recent investigation has reported significant up-regulation of RMRP in drug-naïve relapsing remitting MS patients compared to healthy persons (Rahmani et al., 2021). However, expression of this lncRNA has been lower in IFNβ-1α-treated patients compared with drug-naïve patients (Rahmani et al., 2021). FLICR has a role as negative regulator of FOXP3 *in cis*. Notably, it is expressed in Tregs (Zemmour et al., 2017). Its role in the regulation of FOXP3 expression is exerted through cooperation with TGFβ and IL-2 signaling (Zemmour et al., 2017). Up-regulation of this lncRNA in MS patients might reflect abnormal activity of at least a subgroup of Tregs in these patients.

Besides, our data supported significant correlations between expression levels of NEST, RMRP, TH2-LCR, MAFTRR and FLICR lncRNAs among MS patients, but not controls. This finding indicates the presence of a disease-related regulatory or interactive mechanism among these lncRNAs. Therefore, interruption of this network might influence pathogenesis of MS.

Consistent with substantial up-regulation of FLICR in MS patients, ROC curve analysis revealed appropriate power of FLICR in differentiating between MS patients and healthy controls, suggesting this lncRNA as a possible peripheral marker of MS.

Finally, we detected positive correlation between expression of NEST lncRNA and disease duration in MS patients, but its expression was inversely correlated with age at onset of MS. NEST has been previously reported to participate in the epigenetic activation of the *IFNG* locus (Gomez et al., 2013). IFN-γ is a key cytokine detected in MS lesions. Moreover, IFN-γ levels are impressively up-regulated during MS activity. Besides, IFN-γ–producing Th1 responses are associated with inflammation both in MS patients and animal models of this disorder (Zamvil and Steinman, 1990; Liblau et al., 1995).

Taken together, we reported up-regulation of two Treg-related lncRNAs in MS patients and suggested one of these lncRNAs as a putative marker for MS. We recommend conduction of additional studies for verification of these results.

Data availability statement

The original contributions presented in the study are included in the article/supplementary materials, further inquiries can be directed to the corresponding authors.

Ethics statement

The studies involving human participants were reviewed and approved by The study protocol was approved by the ethical committee of Shahid Beheshti University of Medical Sciences (IR.SBMU.MSP.REC.1400.562). The patients/participants provided their written informed consent to participate in this study.

Author contributions

SG-F wrote the manuscript and revised it. MT and AS designed and supervised the study. SE designed and supervised the study. BH, MD and FE collected the data and performed the experiment. SE analyzed the data. All authors read and approved the submitted manuscript.

Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Identification of a apoptosis-related LncRNA signature to improve prognosis prediction and immunotherapy response in lung adenocarcinoma patients

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Apoptosis is closely associated with the development of various cancers, including lung adenocarcinoma (LUAD). However, the prognostic value of apoptosis-related lncRNAs (ApoRLs) in LUAD has not been fully elucidated. In the present study, we screened 2, 960 ApoRLs by constructing a co-expression network of mRNAs-lncRNAs associated with apoptosis, and identified 421 ApoRLs that were differentially expressed between LUAD samples and normal lung samples. Sixteen differentially expressed apoptosis-related lncRNAs (DE-ApoRLs) with prognostic relevance to LUAD patients were screened using univariate Cox regression analysis. An apoptosis-related lncRNA signature (ApoRLSig) containing 10 ApoRLs was constructed by applying the Least Absolute Shrinkage and Selection Operator (LASSO) Cox regression method, and all LUAD patients in the TCGA cohort were divided into high or low risk groups. Moreover, patients in the high-risk group had a worse prognosis ($p < 0.05$). When analyzed in conjunction with clinical features, we found ApoRLSig to be an independent predictor of LUAD patients and established a prognostic nomogram combining ApoRLSig and clinical features. Gene set enrichment analysis (GSEA) revealed that ApoRLSig is involved in many malignancy-associated immunomodulatory pathways. In addition, there were significant differences in the immune microenvironment and immune cells between the high-risk and low-risk groups. Further analysis revealed that the expression levels of most immune checkpoint genes (ICGs) were higher in the high-risk group, which suggested that the immunotherapy effect was better in the high-risk group than in the low-risk group. And we

Abbreviations: ApoRLs, apoptosis-related lncRNAs; ApoRLSig, apoptosis-related lncRNA signature; AUC, the area under the curve; DE-ApoRLs, Differentially expressed ApoRLs; CGs, Immune checkpoint genes; KEGG, Kyoto Encyclopedia of Genes and Genomes; LASSO, the least absolute shrinkage and selection operator; lncRNAs, Long non-coding RNAs; LUAD, Lung adenocarcinoma; OS, overall survival; PCA, Principal component analysis; ROC, receiver operating characteristic; ssGSEA, the single sample gene set enrichment analysis; TCGA, The Cancer Genome Atlas

found that the high-risk group was also better than the low-risk group in terms of chemotherapy effect. In conclusion, we successfully constructed an ApoRLSig which could predict the prognosis of LUAD patients and provide a novel strategy for the antitumor treatment of LUAD patients.

KEYWORDS

lung adenocarcinoma, apoptosis, long non-coding RNA, prognostic, signature, immunotherapy

Introduction

Lung cancer still has the highest mortality rate in the cancer spectrum worldwide, with a 5-years survival rate of only 10%–20% (Sung et al., 2021). Lung adenocarcinoma (LUAD) accounts for approximately 40%–50% of all lung cancer cases (Bray et al., 2018). Although molecular targeted therapies and immunotherapies have been developed for LUAD, long-term survival remains suboptimal for most patients (Saito et al., 2018). Therefore, it remains urgent to identify new and effective prognostic biomarkers to improve the low survival rate of patients with LUAD.

Apoptosis is one of the most common and well-studied forms of programmed cell death (Fuchs and Steller, 2015), the initiation of which depends on the activation of a series of Caspase proteases that subsequently induce extensive cleavage of hundreds of substrates and rapid cell death (D'Arcy, 2019). Apoptosis has a dual role in cancer, on the one hand, it can inhibit tumor development by deleting malignant or pre-malignant cells; on the other hand, it can promote tumor development by stimulating reparative and regenerative responses in the tumor microenvironment (Morana et al., 2022). Moreover, apoptosis plays an important role in the development and progression of non-small cell lung cancer, and targeting apoptosis may be a new and effective treatment for lung cancer (Liu et al., 2017).

Long non-coding RNAs (lncRNAs), which typically exceed 200 nucleotides in size and are transcribed by RNA polymerase II, have an important regulatory role in the induction of apoptosis (Ghafouri-Fard et al., 2021). Many studies have demonstrated that lncRNAs are key regulators involved in the progression of human cancer including lung cancer. Different lncRNAs can modulate the sensitivity of chemotherapy, radiotherapy and egfr-targeted therapy through distinct mechanisms (Chen Y. et al., 2021b). In recent years, several studies have constructed a series of prognostic lncRNA signatures in LUAD to improve patient prognosis by exploring lncRNAs associated with ferroptosis (Lu et al., 2021), pyroptosis (Song et al., 2021), autophagy (Chen et al., 2021), necroptosis (Lu et al., 2022), and immunity (Wu G. et al., 2021). Whereas, the apoptosis-related lncRNA signature (ApoRLSig) and its relationship with prognosis have not been systematically evaluated in LUAD.

In this study, an ApoRLSig was constructed based on The Cancer Genome Atlas (TCGA) database, and the relevance of apoptosis-related lncRNAs (ApoRLs) to the prognosis of patients with LUAD was systematically assessed. Then, we analyzed the relationship between ApoRLs and clinicopathological characteristics of LUAD patients, and established a nomogram to individually predict patient's survival. In addition, the relationship between risk score and tumor immune microenvironment, immune checkpoint genes (ICGs), and chemotherapy sensitivity was further evaluated. The results of this study may help to improve individualized treatment effectiveness and prognostic assessment of patients with LUAD.

Materials and methods

Data acquisition and processing

RNA sequencing (RNA-seq) data and corresponding clinical survival information for LUAD samples from TCGA database were downloaded via the UCSC xena website (https://xenabrowser.net/datapages/). There were 510 tumor samples and 58 normal samples in the TCGA-LUAD dataset. To reduce statistical bias in the analysis, patients with missing overall survival (OS) or short survival (<30 days) were excluded, and 487 patients were finally included in the study (Supplementary Table S1). A total of 326 patients with complete clinicopathological data were included in the subsequent analysis (Supplementary Table S2).

Apoptosis-related gene detection

A total of 136 apoptosis-related genes were collected by searching the Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway database (https://www.kegg.jp/kegg/pathway.html) with the keyword "Apoptosis." Eventually, 134 apoptosis-related genes were retrieved from the mRNA expression profile of TCGA-LUAD (Supplementary Table S3).

Screening of apoptosis-related lncRNAs

Pearson correlation analysis was performed to identify potential lncRNAs associated with apoptosis-related genes. The apoptosis-related mRNA-lncRNA co-expression network was constructed using |Pearson correlation coefficient|>0.4 and $p < 0.001$ as thresholds. A total of 2, 960 ApoRLs were identified. The co-

expression network was visualized using Cytoscape 3.8.2. Using the R package "ggalluvial" to draw sankey diagrams. Differentially expressed ApoRLs (DE-ApoRLs) between tumor and normal samples were identified by the "DESeq2" package (Love et al., 2014). |log2FC|>2 and FDR<0.05 were considered to be significant.

Construction of apoptosis-related lncRNA prognostic model in lung adenocarcinoma

Univariate Cox analysis of OS was performed to identify DE-ApoRLs with prognostic value ($p < 0.001$). Then, using the R package "glmnet," the least absolute shrinkage and selection operator (LASSO) Cox regression was performed to screen for key DE-ApoRLs. Risk scores of patients were calculated based on the expression levels of lncRNAs and the corresponding lasso coefficients. The risk score is calculated by the formula: risk score = $\sum$exp(i)×coef(i). Using the median risk score as the cut-off point, patients were divided into a low-risk group and a high-risk group. Survival analysis was performed to compare the OS of the high-risk and low-risk groups by using the R packages "survivor" and "survminer." Using the R package "timeROC," time-dependent receiver operating characteristic analysis and the area under the curve (AUC) were performed to assess the predictive power of the model. Principal component analysis (PCA) was performed to evaluate the distribution of patients with different risk scores, and PCA plots were generated by the "scatterplot3D" package of R. In addition, the distribution of patient survival status was evaluated based on risk score levels.

Predictive nomogram construction

The Wilcoxon test was used to explore the potential relationship between the risk score and multiple clinical characteristics (age, sex, stage, TNM stage). $p < 0.05$ was considered to be significant. Then, univariate and multivariate Cox regression analyses were performed on the independent prognostic factors, and the results were visualized using the R package "forestplot." Subsequently, independent risk factors with clinical prognostic significance were integrated, and a nomogram was constructed to predict 1-, 3-, and 5-years survival in LUAD patients by using the R package "rms." Finally, the predictive accuracy of the model was further evaluated by the consistency index, calibration curve, and receiver operating characteristic (ROC) curve.

Gene set enrichment analysis

Gene set enrichment analysis was performed for genes in the high-risk and low-risk group using the R package "clusterProfiler" and "org.Hs.eg.db." The c5.go.v7.5.1.entrez.gmt and c2.cp.kegg.v7.5.1.entrez.gmt were selected as predefined gene sets from the Molecular Signature Database (MSigDB; https://www.gsea-msigdb.org/gsea/msigdb/index.jsp). Biological processes and pathways that were significantly enriched were screened according to the criteria of NOM $p < 0.05$ and FDR<0.25.

Immune infiltration and chemotherapeutic drug sensitivity analysis

The immune, stromal and estimete score for each patient were calculated by the R "estimate" package. The level of immune cell infiltration was quantified for each patient using CIBERSORT (https://cibersort.stanford.edu/). A heat map of the correlation between lncRNAs and immune cell infiltration was drawn by the R package "corrplot." The proportions of 22 immune cells in the high- and low-risk groups were compared and the results were visualized using the R package "vioplot." In addition, the single-sample gene set enrichment analysis (ssGSEA) in the "GSVA" package was used to quantify the relative infiltration of 28 immune cell types in the tumor microenvironment (Barbie et al., 2009). The set of characteristic genes for each immune cell type was obtained from a publication (Jia et al., 2018). In the ssGSEA analysis, the relative abundance of each immune cell type was represented by an enrichment score. Seventy-nine ICGs were obtained from the literature (Hu et al., 2021), 78 of which were expressed in the TCGA-LUAD dataset, and the relationship between the risk score and expression levels of ICGs was assessed. The IC50 values of common antitumor drugs used in the treatment of LUAD, such as cisplatin, etoposide, docetaxel, gefitinib, erlotinib, gemcitabine, and paclitaxel, were compared between two groups using the R packages "pRRophetic" and "ggplot2".

Statistical analysis

All calculations and statistical analyses for this study were performed in R (version 4.1.3) (https://www.r-project.org/). Survival analysis was performed using the Kaplan-Meier method. The Wilcoxon signed-rank test was used to compare the differences between groups. Spearman or Pearson correlation coefficients were used to evaluate the relationships among lncRNA expression, estimate scores, and immune infiltration. $p < 0.05$ was considered a significant difference.

Results

Identification of apoptosis-related lncRNAs with prognostic value in lung adenocarcinoma

We first screened 136 apoptosis-related genes (mRNAs), of which 134 genes had expression data in the TCGA-LUAD

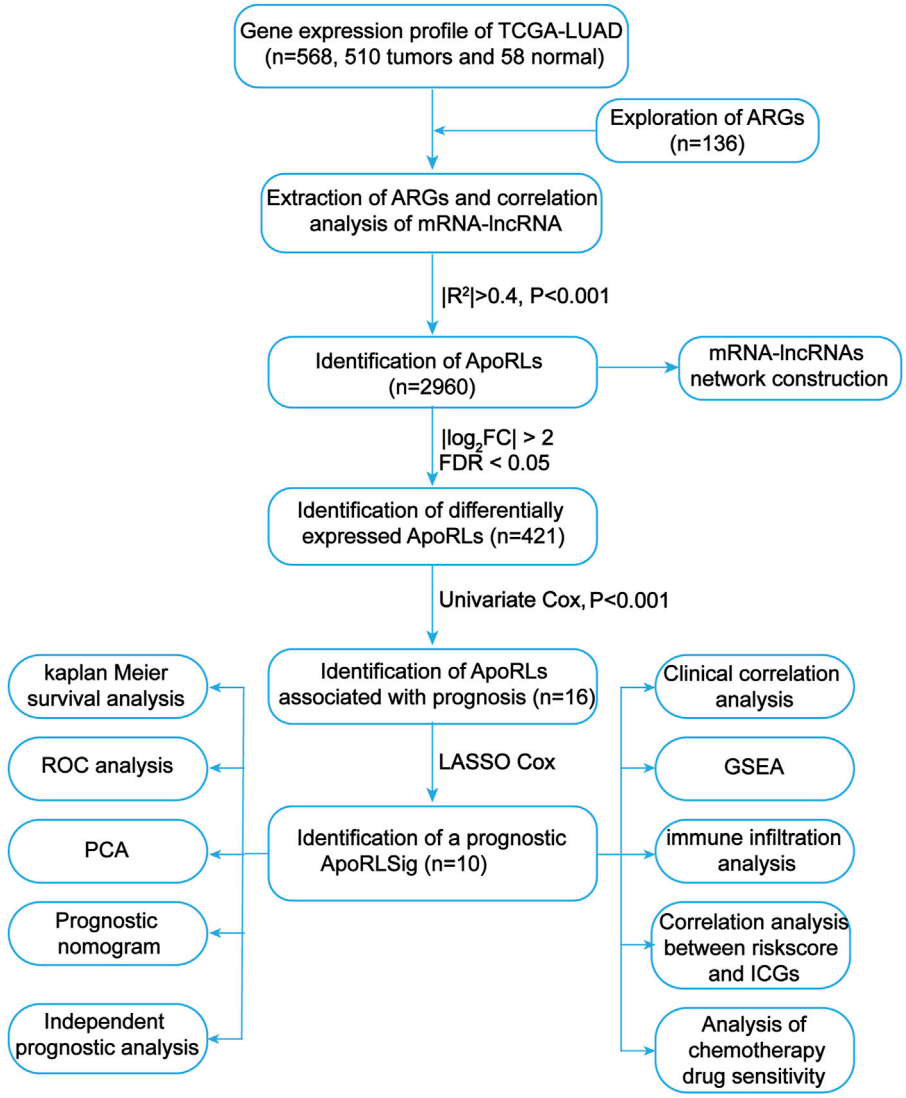

FIGURE 1
Flowchart of the present study. TCGA, The Cancer Genome Atlas; LUAD, lung adenocarcinoma; ARGs, apoptosis-related genes; ApoRLs, apoptosis-related lncRNAs; ApoRLSig, apoptosis-related lncRNA signature; ICGs, immune checkpoint genes.

dataset (Supplementary Table S3). The workflow of this study is shown in Figure 1. Peasron correlation analysis identified 2, 960 ApoRLs ($|R^2|>0.4$, $p < 0.001$). Then, differential analysis of tumor and normal samples identified 421 DE-ApoRLs ($|\log_2 FC|>2$, $p < 0.05$, Figure 2A; Supplementary Figure S1A). Next, 16 lncRNAs whose expression levels correlated with patient prognosis were screened by univariate Cox regression, suggesting their prognostic value for LUAD ($p < 0.001$, Figure 2B; Supplementary Table S4). Eleven lncRNAs were poor prognostic factors (HR > 1, Figure 2C) and five lncRNAs were favorable prognostic factors (HR < 1, Figure 2D).

Construction of a prognostic apoptosis-related lncRNA signature

LASSO Cox regression analysis identified 10 ApoRLs (RP11.1105O14.1, CTD.2510F5.4, AC018647.3, CTD.3179P9.1, CTD.2555C10.3, LINC01312, LINC00968, RP11.462L8.1, LINC00857, FAM83A.AS1) , and established a prognostic ApoRLSig (Figures 3A,B). The correlations of these 10 lncRNAs with apoptosis genes are shown in Figure 4A. Among them, seven lncRNAs (RP11.1105O14.1, CTD.2510F5.4, CTD.2555C10.3, LINC01312, RP11.462L8.1, LINC00857, FAM83A.AS1) were significant adverse prognostic factors, while the remaining

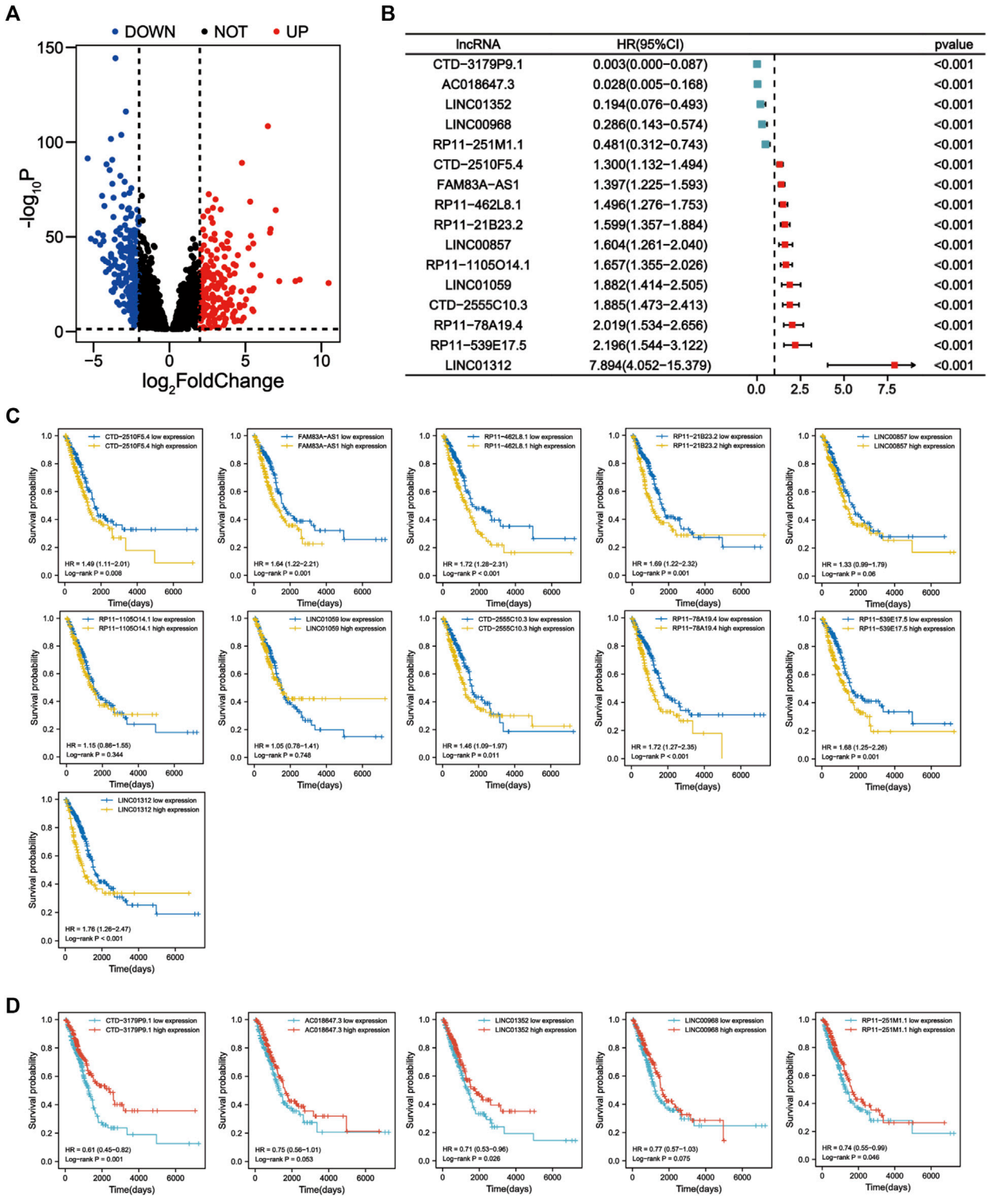

FIGURE 2
Identification of prognostic apoptosis-related lncRNAs in LUAD patients. **(A)** The differentially expressed apoptosis-related lncRNAs were shown in the volcano plot. **(B)** Forest plot showing the HR (95% CI) and *p* values of lncRNAs screened by univariate Cox regression analysis (all $p < 0.001$). **(C)** Kaplan–Meier survival curves for eleven unfavorable prognostic apoptosis-related lncRNAs. **(D)** Kaplan-Meier survival curves for five apoptosis-related lncRNAs with a good prognosis.

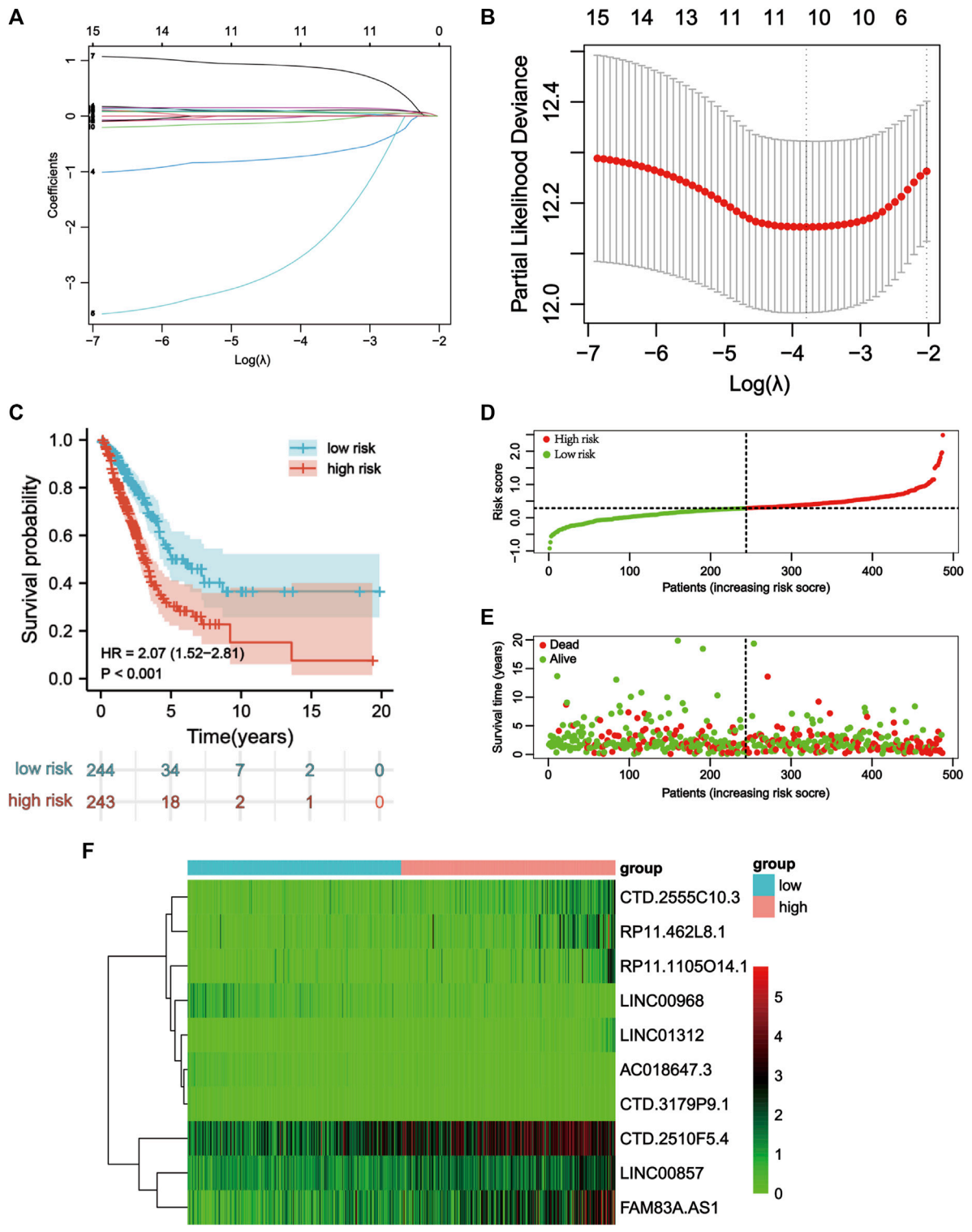

FIGURE 3
Construction of the apoptosis-related lncRNA signature. **(A)** Lasso coefficients profiles of the 16 apoptosis-related lncRNAs. **(B)** Lasso regression analysis obtained 10 prognostic apoptosis-related lncRNAs. **(C)** Kaplan–Meier curves for OS in the high-risk and low-risk groups stratifiied by ApoRLSig ($p < 0.001$). **(D)** Risk curve based on the risk score for each sample, where red indicates a high risk and green indicates a low risk. **(E)** Scatterplot based on the survival status of each sample. Red and green dots indicate death and survival, respectively. **(F)** The heatmap shows the distribution of 10 apoptosis-related lncRNAs in the high-risk and low-risk groups.

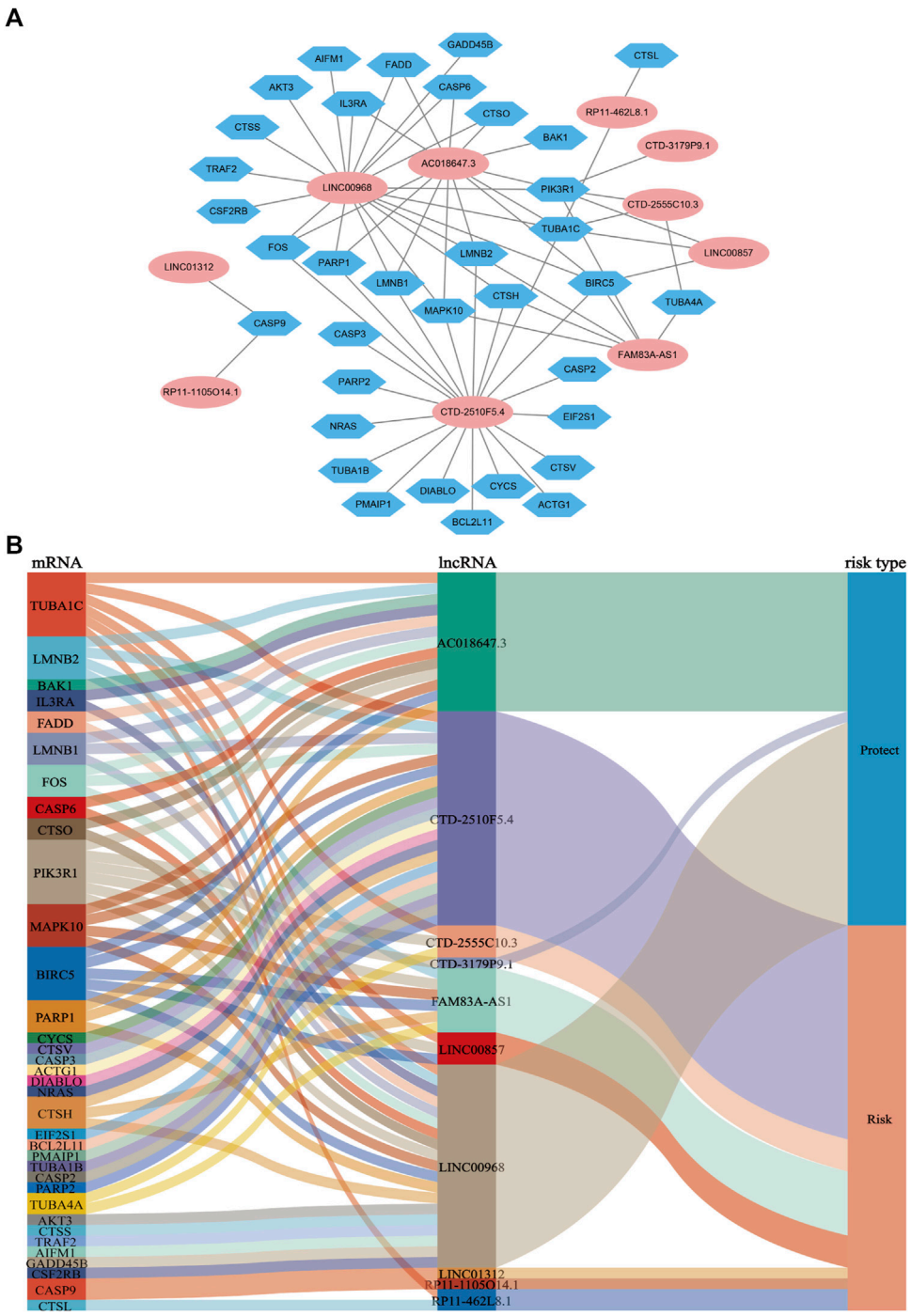

FIGURE 4
Coexpression network and Sankey diagram of prognostic apoptosis-related lncRNAs. **(A)** A co-expression network of apoptosis-related lncRNAs and mRNAs was constructed. Pink ellipses indicate prognostic AR-lncRNAs, and blue hexagons indicate apoptosis-related mRNAs. The levels of the 10 apoptosis-related lncRNAs were associated with the levels of 35 apoptosis-related mRNAs. **(B)** Sankey diagram showing the associations between prognostic apoptosis-related lncRNAs, mRNAs, and risk type.

lncRNAs (AC018647.3, CTD.3179P9.1, LINC00968) were favorable prognostic factors for OS (Figure 4B). The risk score was calculated as follows: risk score = (0.1072 × RP11.1105O14.1 expression level) + (0.0555 × CTD.2510F5.4 expression level) + (−0.6456 × AC018647.3 expression level) + (−2.0068 × CTD.3179P9.1 expression level) + (0.1503 ×

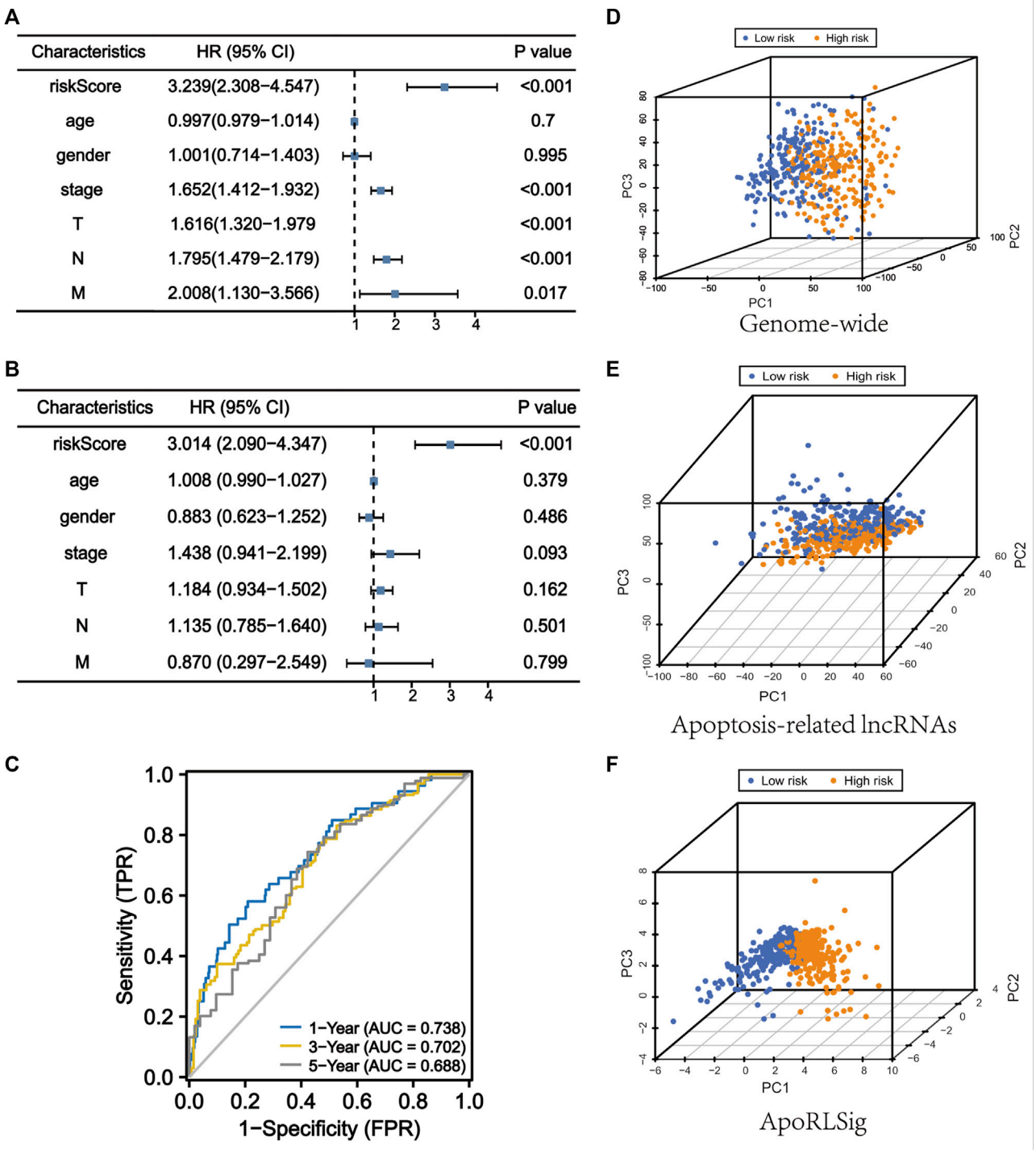

FIGURE 5
ApoRLSig is an independent prognostic factor for overall survival. Univariate **(A)** and multivariate **(B)** Cox regression analysis of the relationship between clinical characteristics (including FerRLSig) and OS. **(C)** Time-dependent ROC curves of OS at 1, 3, and 5 years. Principal component analysis (PCA) of low-risk and high-risk groups based on the **(D)** genome-wide, **(E)** apoptosis-related lncRNAs, and **(F)** the ApoRLSig including 10 apoptosis-related lncRNAs. Patients with high risk scores are indicated in orange, and those with low risk scores areindicated in blue. T, tumor stage; N, lymph node metastasis stage; M, distant metastasis stage.

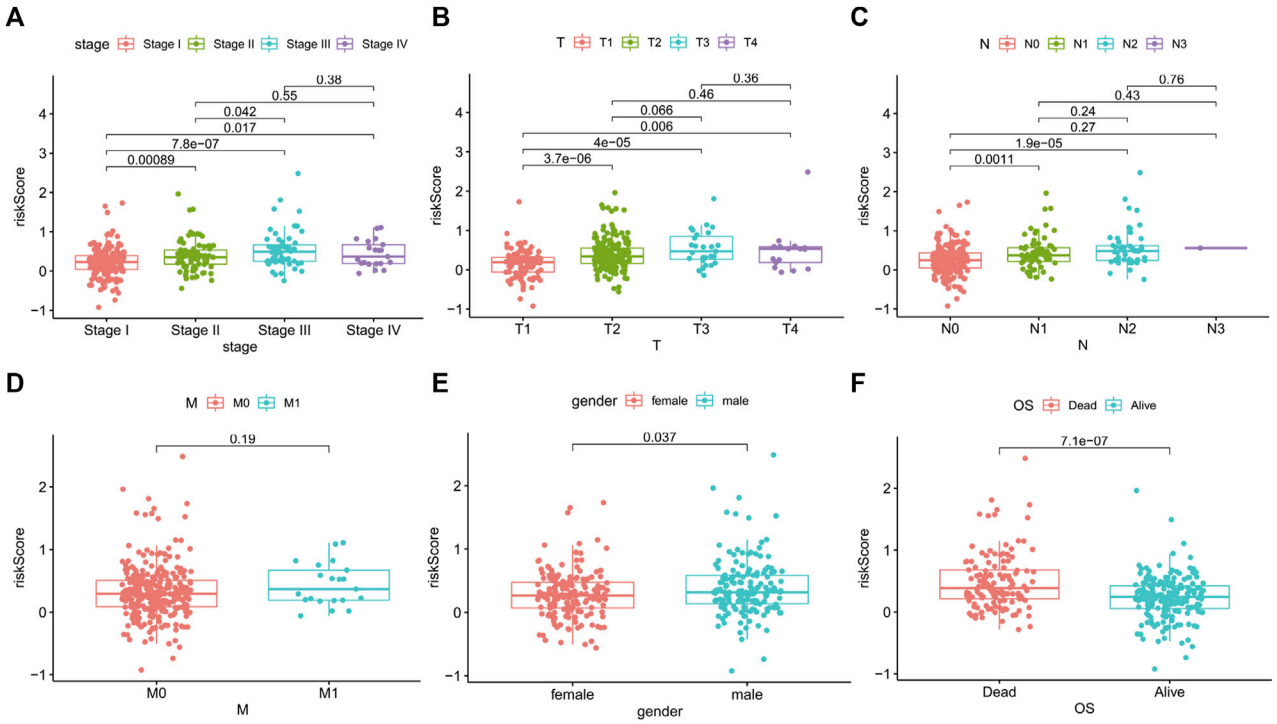

FIGURE 6
ApoRLSig was associated with the clinicopathological factors of patients with LUAD. Correlation analysis between risk score and Stage **(A)**, T **(B)**, N **(C)**, M **(D)**, Gender **(E)**, survival outcome **(F)**. T, tumor stage; N, lymph node metastasis stage; M, distant metastasis stage.

CTD.2555C10.3 expression level) + (0.8414 × LINC01312 expression level) + (−0.0628 × LINC00968 expression level) + (0.0605 × RP11.462L8.1 expression level) + (0.0702 × LINC00857 expression level) + (0.0708 × FAM83A.AS1 expression level). We calculated the risk score for each patient according to the formula, and divided patients into a high-risk group (n = 243) and a low-risk group (n = 244) using the median risk score as the threshold. Kaplan-Meier curves showed a significant difference in OS between the high-risk and low-risk groups of LUAD patients (p < 0.001, Figure 3C), indicating that the newly developed signature is effective in predicting survival. Meanwhile, the risk curve, scatter plot based on survival status and heat maps of expression distribution for these 10 lncRNAs are shown in Figures 3D–F.

Evaluation of ApoRLSig as an independent prognostic factor for lung adenocarcinoma

We performed univariate and multivariate Cox regression analyses to determine whether ApoRLSig is an independent prognostic model for OS in LUAD patients. The HRs (95% CI) for the risk score in univariate and multivariate Cox regression analyses were 3.239 (2.308–4.547) (p < 0.001, Figure 5A) and 3.014 (2.090–4.347) (p < 0.001, Figure 5B), indicating that ApoRLSig is an independent prognostic indicator. In addition, the predictive accuracy of the model was assessed by time-dependent receiver operating characteristic analysis at 1, 3, and 5 years, with AUC values of 0.738, 0.702, and 0.688, respectively (Figure 5C). Then, we compared the low-risk and high-risk groups based on genome-wide, ApoRLs, and the risk model using PCA. As shown in Figures 5D,E, genome-wide or ApoRLs could not effectively distinguish between high-risk and low-risk groups, while ApoRLSig could clearly distinguish between high-risk and low-risk patients, further supporting the accuracy of the model (Figure 5F). The above results illustrated that ApoRLSig is an important independent prognostic risk factor for patients with LUAD.

Correlations between the risk score and clinicopathological factors

To further assess the role of ApoRLSig in the development of LUAD, we evaluated the correlations between the risk score and clinicopathological factors. As shown in Figure 6 and Supplementary Table S5, there was a significant correlation between the risk score and pathological stage (p < 0.01),

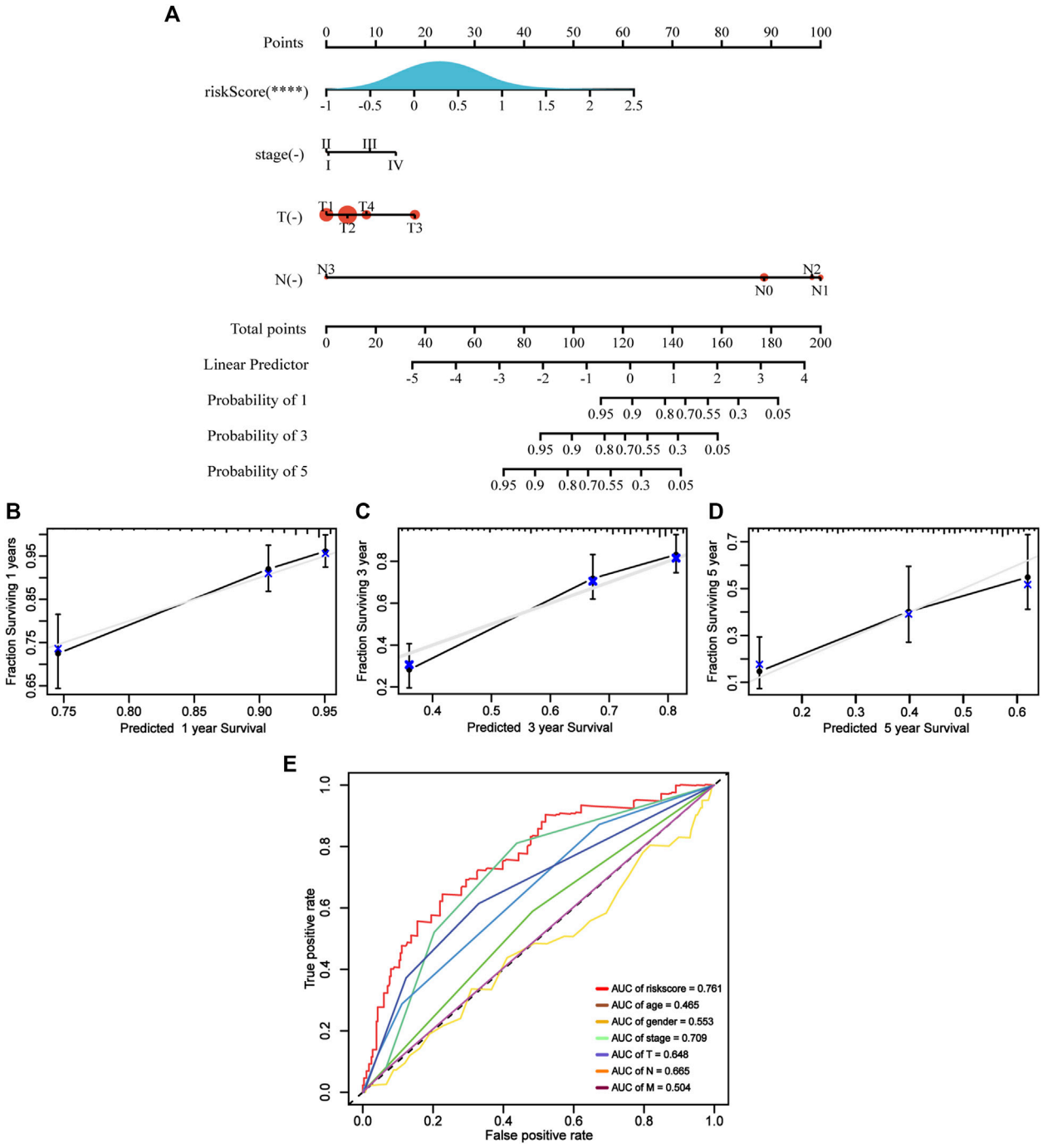

FIGURE 7
Clinical predictive nomogram construction and evaluation. **(A)** A clinical predictive nomogram based on the clinicopathological factors and risk score.The calibration curves of the nomogram in predicting 1-year **(B)**, 3-years **(C)**, and 5-years **(D)** survival of LUAD patients. **(E)** Time-dependent ROC curve analyses for predicting OS at 1 year by risk score age, sex, stage, T (tumor size), N (lymph node metastasis and M (distant metastasis).

especially for stages II-IV, which were significantly higher than stage I (Figure 6A, $p < 0.05$). The signature correlated with tumor stage (Figure 6B, $p < 0.05$), and patients with lymph node metastases had significantly higher risk scores than those without lymph node metastases (Figure 6C, $p < 0.01$). In addition, there was a correlation between the signature and gender (Figure 6E, $p < 0.05$). Figure 6F illustrated that patients with high risk scores had a

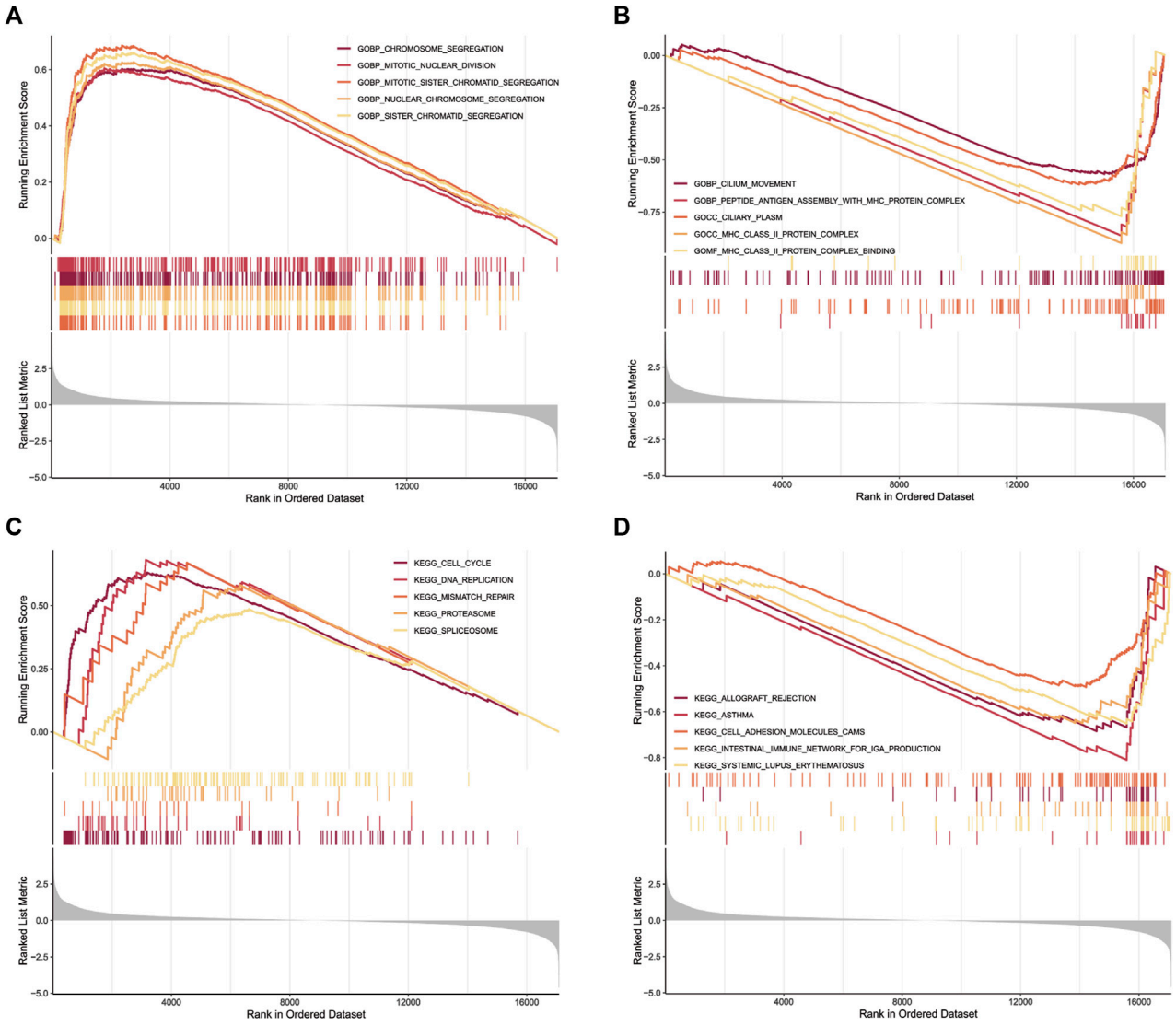

FIGURE 8
GSEA for samples with high risk scores and low risk scores. **(A)** Enriched gene sets in C5 collection, the Go gene sets, by patients with high risk scores. Only gene sets both with NOM $p < 0.05$ and FDR q < 0.25 were considered signifificant. Only five top gene sets are shown in the plot. **(B)** The enriched gene sets in C5 collection by patients with low risk scores. **(C)** Enriched gene sets in C2 collection, the KEGG gene sets, by patients with high risk scores. **(D)** The enriched gene sets in C2 collection by patients with low risk scores.

significantly poorer prognosis in terms of survival status than patients with low-risk scores. These results suggested that ApoRLSig is closely associated with the progression and prognosis of LUAD.

Construction of a predictive nomogram

Using ApoRLSig in combination with other clinicopathological factors (stage, T, and N), we constructed a clinically applicable nomogram to estimate the probability of survival at 1, 3 and 5 years for patients with LUAD (Figure 7A). The consistency index of the model was: 0.73 (95% CI: 0.68–0.78, $p < 0.001$) and its 1-, 3-, and 5-years calibration curves indicated that the mortality rates estimated by the nomogram were close to the actual mortality rates (Figures 7B–D). In the time-dependent ROC curve for 1-year OS, the AUC value of ApoRLSig was 0.761, which was significantly higher than other clinical features, further supporting the predictive ability of ApoRLSig for survival in patients with LUAD (Figure 7E).

Identification of ApoRLSig-related biological pathways

GO functional annotation and KEGG pathway enrichment were performed using Gene set enrichment analysis. GO functional annotation results (Supplementary Table S6) showed that chromosome segregation (NES = 2.50, p = 0.000), mitotic nuclear division (NES = 2.48, p = 0.000), mitotic sister chromatid segregation (NES = 2.63, p = 0.000), nuclear chromosome segregation (NES = 2.54, p = 0.000) and sister chromatid segregation (NES = 2.58, p = 0.000) were enriched in LUAD patients with high risk scores (Figure 8A). In contrast, cilium movement (NES = −2.19, p = 0.000), peptide antigen assembly with MHC protein complex (NES = −2.27,p = 0.000), ciliary plasm (NES = −2.26, p = 0.000), MHC class II protein complex (NES = −2.23, p = 0.000) and MHC class II protein complex binding (NES = −2.18, p = 0.0002) were enriched in patients with low risk scores (Figure 8B). In addition, 19 KEGG pathways were enriched (Supplementary Table S7). Cell cycle (NES = 2.35, p = 0.000), DNA replication (NES = 2.07, p = 0.001), mismatch repair (NES = 1.84, p = 0.031), proteasome (NES = 1.82, p = 0.014) and splicesome (NES = 1.81, p = 0.001) signaling pathways were enriched in the high-risk group (Figure 8C). Meanwhile, Allograft rejection (NES = −2.05, p = 0.001), asthma (NES = −2.26, p = 0.000), cell adhesion molecules CAMs (NES = -1.83, p = 0.001), intestinal immune network for IgA production (NES = −2.06, p = 0.001) and systemic lupus erythematosus (NES = -2.12, p = 0.000) signaling pathways were enriched in the low-risk group (Figure 8D). We found that multiple of these pathways are immune response-related pathways. The results indicated that the lncRNAs signature may be related to the tumor immune microenvironment.

Correlation of the risk score with tumor immune microenvironment

To further assess the correlations between the risk score and tumor microenvironment, we quantified the level of tumor immune cells infiltration in both groups of patients using ESTIMATE, CIBERSORT and ssGSEA algorithms. The results showed that three ApoRLs were positively correlated with stromal, immune and estimate score, including AC018647.3, CTD-3179P9.1 and LINC00968, while LINC01312, RP11-1105O14.1 and LINC00857 were negatively correlated with them (p < 0.001, Figure 9A; Supplementary Figure S1B). Differences in infiltration of 22 immune cell types in patients with LUAD in TCGA are shown in Figure 9B, reflecting the intrinsic characteristics of individual differences. The high-risk group of LUAD patients had a higher proportion of T cells CD4 memory activated (p < 0.001), Macrophages M0 (p < 0.001), Mast cells activated (p < 0.001) and Neutrophils (p = 0.031). In contrast, B cells memory (p < 0.001), T cells CD4 memory resting (p < 0.001), Monocytes (p < 0.001), Macrophages M1 (p = 0.005), Dendritic cells resting (p < 0.001) and Mast cells resting (p < 0.001) were negatively associated with risk score (Figure 9C). Furthermore, we analyzed the correlations between 10 ApoRLs and 22 immune cells (Figure 9D). Correlation analysis of immune cell subsets based on ssGSEA showed more immune cell infiltration in the low-risk group, including B cells, central memory $CD4^+$ T cells, dendritic cells, natural killer cells, Eosinophi, Macrophage , Mast cells, MDSC , Monocyte , $CD8^+$ T cells, T follicular helper cells, Regulatory T cells and Type 1 T helper cell (p < 0.001, Supplementary Table S8; Figure 9E). In contrast, only memory B cells, Activated $CD4^+$ T cells, CD56dim natural killer cells, neutrophils and Type 2 T helper cells infiltrated in the high-risk group. The results suggested that our signature is not only a prognostic marker but also reflects the level of immune cell infiltration.

Differences in response to immunotherapy and chemotherapy between the high-risk and low-risk groups

The expression levels of ICGs may be predictive biomarkers for immune checkpoint blockade therapy. We investigated the relationship between the expression of 78 ICGs and two groups. The results showed that 19 ICGs were expressed at higher levels in the low-risk group, including BTNL9, HLA-DRB5, HLA-DPB1, HLA-DOA, HLA-DQB1, CD40LG, HLA-DRB1, HLA-DRA, HLA-DPA1, HLA-DMA, HLA-DQA1, HLA-DMB, CD96,BTLA, HLA-DOB, CD48, TNFSF15, CD200R1, CD28, while the other 41 ICGs were highly expressed in the high-risk group (Supplementary Table S9). The first 10 ICGs were shown in Figures 10A–E and Supplemenatry Figures S1C–G. These results demonstated that ApoRLSig could be a candidate biomarker for immunotherapy in patients with LUAD. In addition, the results of the correlation analysis between risk score and the sensitivity of chemotherapeutic agents to LUAD were shown in Figures 10F–L. Patients with high risk scores were highly sensitive to cisplatin (p = 0.032), docetaxel (p < 0.001), gemcitabine (p = 0.026) and paclitaxel (p < 0.001), while patients with low risk scores were only sensitive to erlotinib (p = 0.006). There was no significant difference in the sensitivity of etoposide and gefitinib between the two groups (p > 0.05). The results indicate that ApoRLSig is a potential predictor of chemotherapy sensitivity.

Discussion

Lung cancer is the leading cause of cancer deaths worldwide. LUAD is one of the most common histological types of lung cancer (Carrillo-Perez et al., 2021). In recent years,

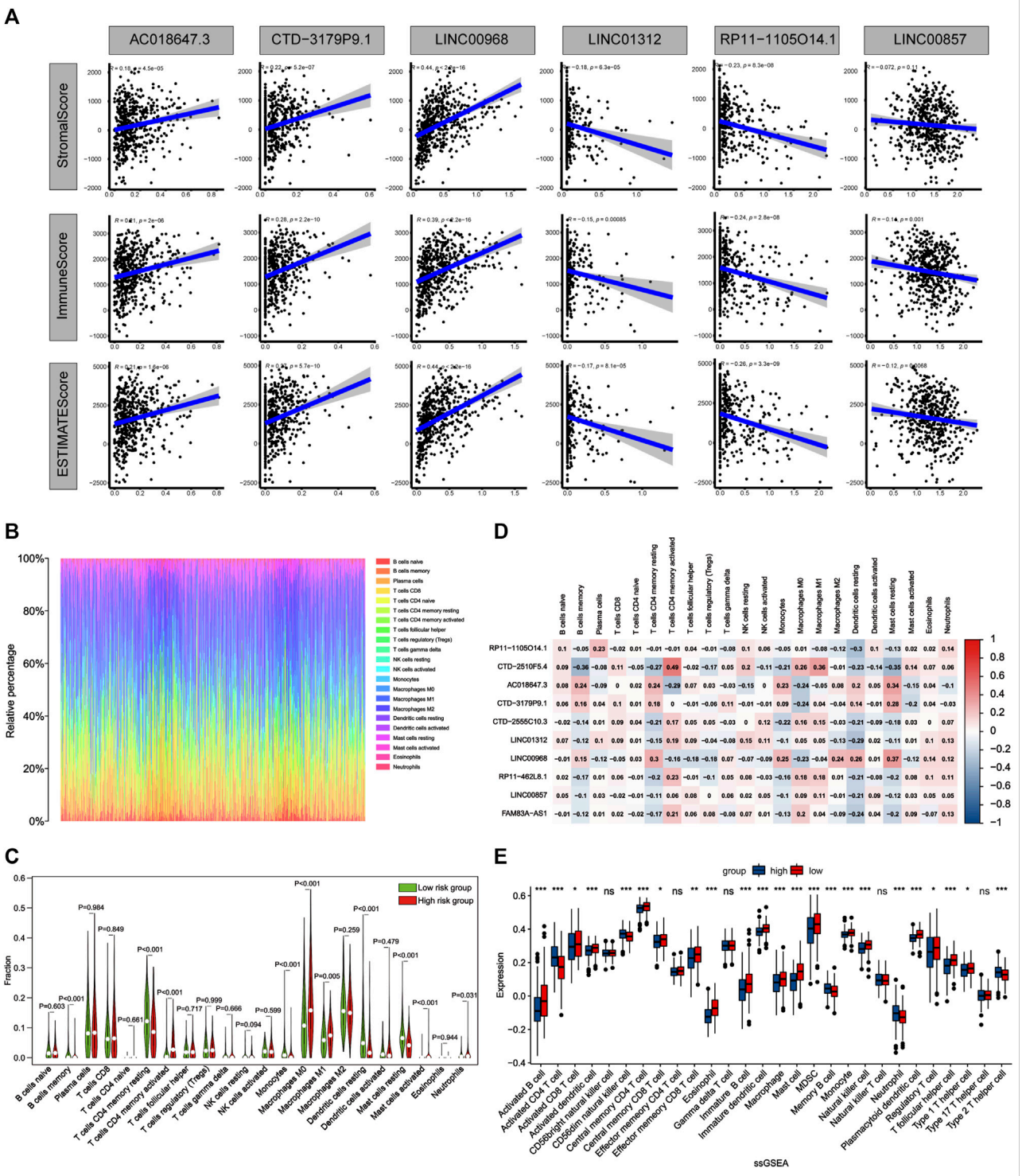

FIGURE 9
Comparison of the immune microenvironment of LUAD patients between the high- and low-risk groups. **(A)** Correlation matrices between six lncRNAs expression and stromal score, immune score, and estimate score. **(B)** Barplot shows the proportion of 22 types of TICs in LUAD samples. The column names of the plot were sample ID. **(C)** Violin plot showed the ratio of 22 immune cell types between the low-risk and high-risk groups, and Wilcoxon signed-rank test was used for significance test. Red indicates the high-risk group and green indicates the low-risk group. **(D)** Heatmap showing the correlation between 22 TICs and 10 lncRNAs. **(E)** The single sample gene set enrichment analysis (ssGSEA) algorithm compares the expression of 28 immune cells between patients in high and low risk groups. *$p < 0.05$; **$p < 0.01$; ***$p < 0.001$.

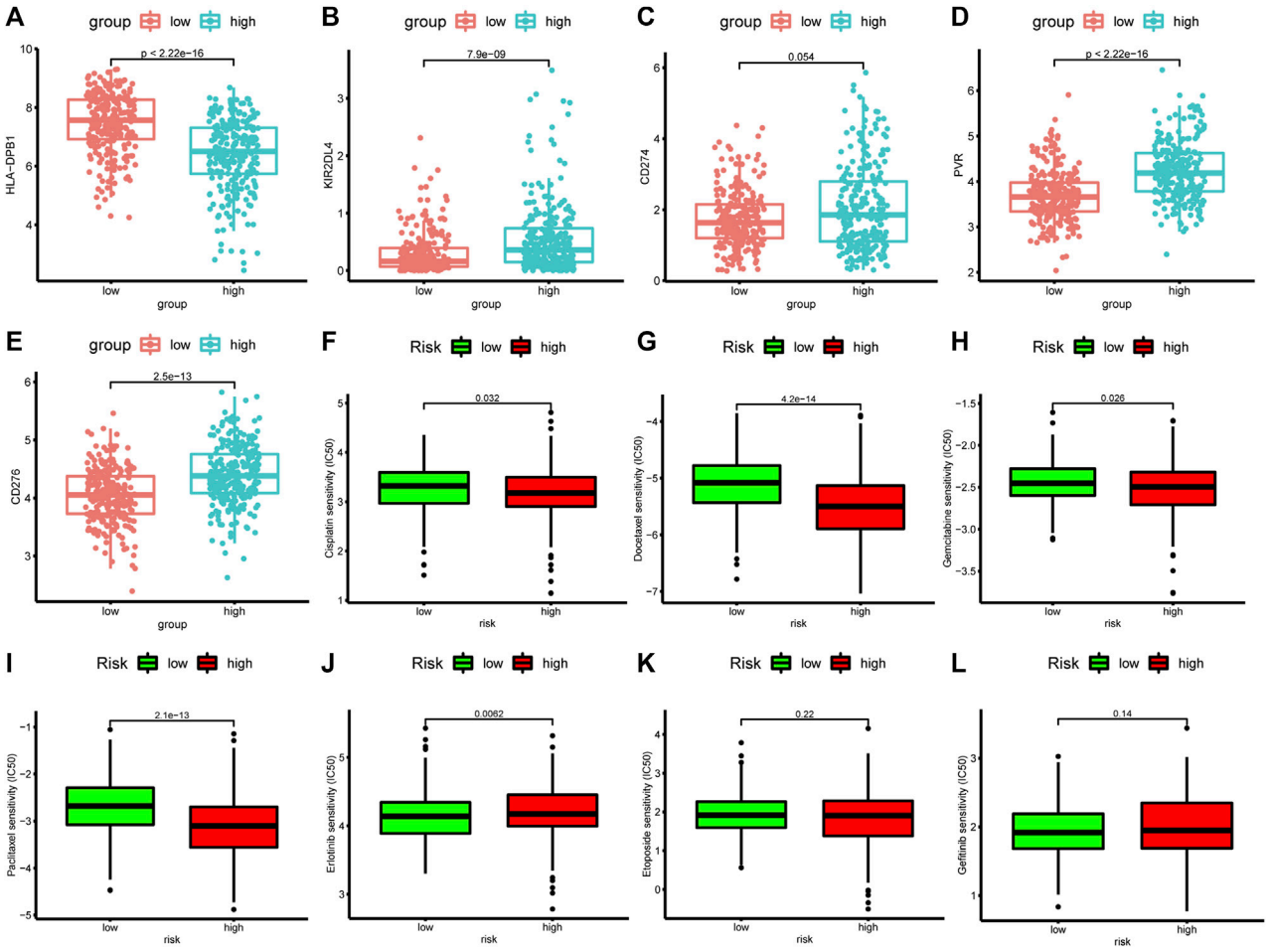

FIGURE 10
Correlation analysis between risk score, ICGs, and chemotherapeutics sensitivity. The differential expression of five immune checkpoint genes, **(A)** HLA-DPB1, **(B)** KIR2DL4, **(C)** CD27, **(D)** PVR, and **(E)** CD276, between the high-risk group and the low-risk group. Half-maximal inhibitory concentration (IC50) values for seven common antineoplastic drugs, **(F)** cisplatin, **(G)** docetaxel, **(H)** gemcitabine, **(I)** paclitaxel, **(J)** erlotinib, **(K)** etoposide, and **(L)** gefitinib, between the high-risk and low-risk groups.

chemotherapy and molecular targeted therapy can prolong the overall survival of patients with LUAD, and the emergence of immunotherapy also brings a promising future to LUAD treatment (Lou et al., 2020; Deshpand et al., 2022). However, the prognosis for patients with LUAD remains poor due to late diagnosis and the emergence of drug resistance (Blandin et al., 2017; Li et al., 2020). Hence, there is an urgent need to develop safe and feasible predictive biomarkers that will facilitate accurate and timely personalized treatment of LUAD patients and greatly improve their prognosis.

Apoptosis is a specific programmed cell death process regulated by molecules, and regulating apoptosis can treat a variety of diseases, including cancer (Ketelut-Carneiro and Fitzgerald, 2022). Moreover, the cytotoxic effects of most oncological chemotherapeutic agents are mediated through activation of apoptotic pathways, and apoptosis targeting holds promise as a key strategy for cancer treatment (Johnstone et al., 2002; Singh and Lim, 2022). Increasing evidence showed that lncRNAs can regulate apoptosis through different mechanisms, and their regulatory effects on apoptosis in lung cancer cells have been investigated (Wang et al., 2020; Xiang et al., 2020; Ghafouri-Fard et al., 2021; Ouyang et al., 2021). Whereas, the role of ApoRLs in the prognosis, chemotherapy and immunotherapy of LUAD is not well understood.

In this study, we constructed a prognostic signature using 10 ApoRLs, and the ROC curve demonstrated that this lncRNA signature had moderate predictive performance for OS in LUAD patients. We then evaluated the relationship between the risk score and clinical features of LUAD and constructed a nomogram diagnostic model. Next, we linked the lncRNA signature to the tumor immune microenvironment and found that these ApoRLs play a key role in the regulation of tumor

immune infiltration, suggesting that they may be potential targets for tumor immunotherapy. Finally, the correlation between ICGs, chemotherapeutic sensitivity and risk score was analyzed to assess the role of this signature in immune response and chemotherapy effect in LUAD. These results strongly suggested that the lncRNA signature may play an important role in LUAD.

Among the identified lncRNAs, five were closely associated with tumor development, namely CTD.2510F5.4, LINC01312, LINC00857, FAM83A.AS1, and LINC00968. CTD.2510F5.4 was found to be significantly upregulated in cancerous tissues and was strongly associated with poor prognosis in LUAD (Wang et al., 2018). We found that LINC01312 could be used as a prognostic marker to predict survival in LUAD (Li et al., 2018). However, the biological functions of LINC01312 in apoptosis and LUAD have not been systematically analyzed and need to be further investigated. LINC00857 is considered to be an oncogenic lncRNA that promotes proliferation and metastasis of cancer cells in pancreatic (Chen et al., 2022), colorectal (Chang et al., 2021) and breast (Zheng et al., 2020) cancers, and it regulates apoptosis and autophagy (Su et al., 2020). FAM83A.AS1 regulates the proliferation, migration, invasion and epithelial-mesenchymal transition process of LUAD cells by targeting microRNA-141-3p (Huang et al., 2022). Notably, LINC00857 and FAM83A.AS1 are components of the immune-associated lncRNA signature (Mu et al., 2021; You et al., 2021), suggesting a possible strong link between apoptosis and immune regulation in LUAD. LINC00968 is significantly downregulated in LUAD and inhibits tumor proliferation, migration and invasion, and may serve as a prognostic marker and potential therapeutic target for LUAD (Wu C. et al., 2021). In addition, LINC00968 was found to be closely associated with ferroptosis (Lu et al., 2021) and N-6 methylation (m6A) (Zheng et al., 2021), and could attenuate drug resistance in cancer cells (Xiu et al., 2019). However, the prognostic value of five lncRNAs (RP11.1105O14.1, CTD.2555C10.3, RP11.462L8.1, AC018647.3, and CTD.3179P9.1) for cancer and their contribution to apoptosis have been lacking studies. Therefore, further studies are needed to explore the role of these lncRNAs in LUAD and apoptosis.

There are complex interactions between tumor cells and the tumor microenvironment that significantly influence tumor progression (Arneth, 2020). Therefore, this study demonstrates the relationship between ApoRLSig and tumor immune microenvironment. Significant differences in immune cell infiltration were found between high- and low-risk groups, confirming the role of ApoRLs in the regulation of tumor immune infiltration. Tumor immunity depends on the balance between immune cells that promote tumor or inhibit tumor progression (Wang et al., 2019). Type 1 T helper cells, which release TNF-a, IL-2, and interferon-g (IFN-g), exert antitumor effects, while Type 2 T helper cells mainly produce IL-4 to suppress the host immune system and promote tumor growth (Becker, 2006). M1 macrophages and natural killer cells have been shown to exert antitumor effects during tumorigenesis, and natural killer cells can drive tumor immunotherapeutic responses (Biswas et al., 2008; Huntington et al., 2020). It has been shown that the presence of $CD8^+$ T cells is a hallmark of the anti-tumor immune response (Chen Y. et al., 2021a). Dendritic cells are specialized antigen-presenting cells that play a key role in the initiation, programming and regulation of tumor-specific immune responses (Melief, 2008; Li and He, 2018). $CD4^+$ T regulatory cells, MDSC and mast cells may promote tumor progression (Ostrand-Rosenberg, 2008). In addition, an increase in neutrophil count is often strongly associated with poor cancer prognosis (Mollinedo, 2019). Consistent with previous studies, our study found more infiltration of immune cells performing anti-tumor responses (e.g., Activated $CD8^+$ T cells, Type 1 T helper cells, Activated dendritic cells, M1 macrophages, and Natural killer cells) in the tumor microenvironment of patients with low risk scores, reflecting a reduction in malignancy and the effects of various treatments. In contrast, more immune cells that promote tumor progression (e.g., $CD4^+$ T regulatory cells, mast cells, and neutrophils) were found in high-risk scoring patients. An exception emerged, with higher levels of MDSC infiltration in the low-risk population. Lung cancer has high levels of MDSCs, which are associated with resistance to chemotherapy, targeted therapy and immunotherapy and can predict poor prognosis (Liu et al., 2010; Feng et al., 2012; Heuvers et al., 2013; Huang et al., 2013; Zhou et al., 2018). This also explains the fact that patients with low risk scores are less sensitive to multiple chemotherapeutic agents than patients with high risk scores in our study.

In addition, apoptosis not only plays an important role in tumor development, but also has an impact on the effectiveness of immunotherapy and molecular targeted therapy for tumors (Carneiro and El-Deiry, 2020; Michie et al., 2020). Since immunotherapy with checkpoint inhibitors plays a key role in LUAD, we further investigated the differences in the expression of ICGs between high- and low-risk groups. The expression levels of HLA-DPB1, KIR2DL4, CD274, PVR, CD276, HLA-DRA, HLA-DOA, HLA-DRB5, HLA-DPA1, and HLA-DRB1 were found to be significantly different in the two groups of patients. Meanwhile, we found higher expression levels of most immune checkpoint genes in patients with high risk scores, prompting a superior immunotherapy effect in the high-risk group than in the low-risk group. Of note, patients with high risk scores were found to be highly sensitive to the chemotherapeutic agents cisplatin, docetaxel, gemcitabine and paclitaxel, indicating that the high-risk group was also outperformed by the low-risk group in terms of chemotherapy efficacy. These results suggest that lncRNAs in this signature may influence the development of LUAD by regulating immune responses in tumors and play a crucial role in chemotherapy drug resistance in LUAD.

The strength of this study is that we have constructed the first prognostic model of ApoRLs in LUAD and analyzed the

relationship of the risk score with immunotherapy response and chemotherapy drug sensitivity. Most importantly, the lncRNA signature constructed in this study has higher predictive accuracy and is more comprehensively studied than another existing apoptosis-related signature that is used to predict the prognosis of lung adenocarcinoma (Zou et al., 2022). However, there are limitations in our study. First, we used only one dataset to construct the model. Second, this is a retrospective study. Third, this study lacks functional experimental validation. Hence, prospective cohort studies and molecular biology experiments are needed in this study to further validate the prognostic value of ApoRLSig and to explore the molecular mechanisms of ApoRLs.

In summary, we constructed a novel ApoRLSig to predict the prognosis of LUAD patients, and established an effective nomogram model including ApoRLSig. Furthermore, the most important contribution of this study is that we demonstrated the relationship between ApoRLSig and tumor immune microenvironment and further evaluated the relationship between ICGs, chemotherapy drug sensitivity, and risk score.These findings are of great importance in guiding the treatment and prognostic evaluation of patients with LUAD.

Data-availability statement

RNA sequencing (RNA-seq) data and corresponding clinical survival information for TCGA-LUAD samples were downloaded through the UCSC xena website (https://xenabrowser.net/datapages/). Apoptosis-related genes can be found at hsa04210 in the KEGG pathway database (https://www.kegg.jp/kegg/pathway.html). c5.go.v7.5.1.entrez.gmt and c2.cp.kegg.v7.5.1.entrez.gmt were downloaded from the Molecular Signature Database (MSigDB; https://www.gsea-msigdb.org/gsea/msigdb/index.jsp).

Author contributions

TL and XW conceived and designed the study. SY and FZ conducted the literature search and drafted the initial manuscript. JO and LG analyzed the data. SH confirmed the authenticity of all the raw data. All authors have read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

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N^6-methyladenosine-modified lncRNA and mRNA modification profiles in cerebral ischemia-reperfusion injury

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Cerebral ischemia-reperfusion injury (CIRI) is common in ischemic stroke and seriously affects the prognosis of patients. At present, N^6-methyladenosine (m^{6}A) modification of lncRNAs and mRNAs has been reported in other diseases, such as cancer, but its role in CIRI has not been clarified. In this study, we aimed to investigate the m^{6}A lncRNA and m^{6}A mRNA modification profiles in CIRI. First, we detected the total level of m^{6}A and the changes in related m^{6}A methyltransferases and demethylases in the brain tissue of rats with CIRI and then identified differentially modified lncRNAs and mRNAs in CIRI by lncRNA and mRNA epigenetic transcriptomic microarray. In addition, bioinformatics analysis was used to predict the underlying functions and related pathways of related lncRNAs and mRNAs. We found that the total m^{6}A methylation level was significantly increased, and the expression of fat mass and obesity-associated protein (*FTO*) was downregulated after CIRI. In addition, a large number of m^{6}A-modified lncRNAs and mRNAs appeared after CIRI, and these genes were mainly enriched for the Toll-like receptor signaling pathway, peroxisome proliferator-activated receptor (PPAR) signaling pathway, and mitogen-activated protein kinase (MAPK) signaling pathway. Our findings provide the basis and insights for further studies on m^{6}A modification in CIRI.

KEYWORDS

cerebral ischemia-reperfusion injury, N^6-methyladenosine, lncRNA, mRNA, FTO

Introduction

The primary treatment principle for ischemic stroke is to recanalize the blood flow in the ischemic area and restore the blood oxygen supply to the infarcted brain tissue as soon as possible, but it may cause cerebral ischemia-reperfusion injury (CIRI) and secondary injury to the brain tissue (van der Steen et al., 2022). The mechanism underlying CIRI is very complex and may be related to oxidative stress, calcium

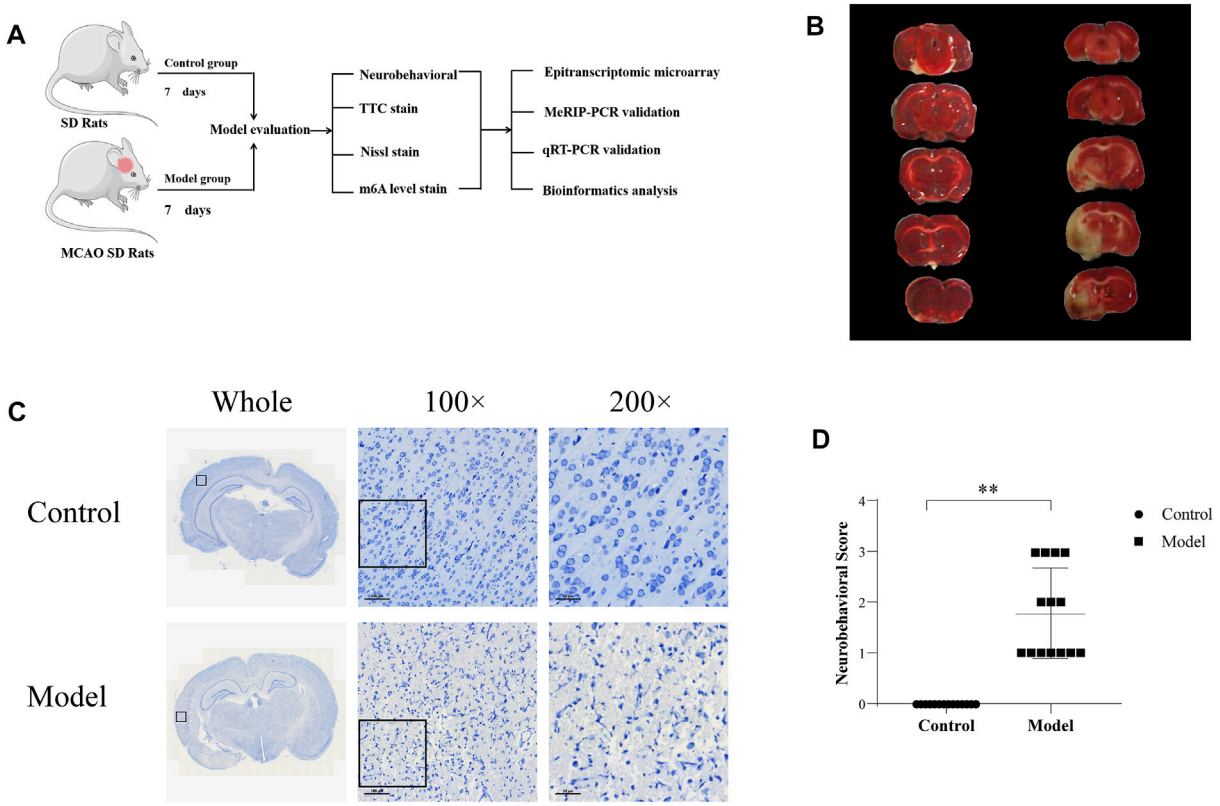

FIGURE 1
Establishment of the CIRI model **(A)** Experimental design **(B)** TTC detection (TTC staining displayed the infarcted areas in white and the normal areas in red) **(C)** Nissl staining **(D)** Neurobehavioral score.

overload, inflammation, *etc.* (Stegner et al., 2019), which eventually lead to nerve cell damage, apoptosis, or necrosis. Further exploration of the pathogenesis and prognostic biomarkers of CIRI or identification of therapeutic targets may be of great significance.

Long noncoding RNAs (lncRNAs) are a class of RNAs that cannot encode translational proteins but can actively participate in many important biological processes by regulating gene expression at the transcriptional and posttranscriptional levels. In recent years, studies have shown that lncRNAs are involved in pathophysiological responses such as inflammation, oxidative stress, angiogenesis, and nerve regeneration after cerebral ischemia (Akella et al., 2019). Currently, the number of studies involving related lncRNAs involved in CIRI is increasing, and a recent study reported lncRNA expression profiles after CIRI (Yang et al., 2022). However, the roles of N^6-methyladenosine (m^6A)-related lncRNA posttranscriptional modifications in CIRI remain unknown.

M^6A is the most extensive modification of mRNA and ncRNA observed in eukaryotes, accounting for 80% of RNA methylation modifications, which can functionally regulate the transcriptome of eukaryotes, thereby affecting RNA splicing, nucleation, localization, translation, and stabilization (Meyer et al., 2012; Frye et al., 2018). M^6A RNA methylation occurs in a variety of important cellular life processes, such as stem cell differentiation and the production of biological rhythms, and is also involved in the occurrence of various diseases (Dominissini et al., 2012). The process of methylation is reversible, and the level of RNA m^6A methylation modification is regulated by methyltransferases and demethylases (Roundtree et al., 2017). Several current studies suggest that lncRNAs may regulate tumor growth through m^6A modification in cancer (Steponaitis et al., 2022). Unfortunately, the role of m^6A-related lncRNAs and mRNAs in CIRI has not been elucidated.

In the present study, we identified the m^6A lncRNA and mRNA modification profiles for the first time in CIRI. Bioinformatics analysis was used to predict the potential functions and related pathways of lncRNAs and mRNAs dysregulated by m^6A modification in CIRI. Moreover, further analysis indicated that m^6A modification of lncRNAs may exert biological functions through the lncRNA-miRNA-mRNA transcriptional network, as shown in Figure 1A.

Materials and methods

Animals

Specific pathogen-free (SPF) male Sprague Dawley (SD) rats that were 8 weeks old and weighed 250–260 g were purchased from Hunan Silaike Jingda Co., LTD (Changsha, China) using production license number SCXK (Xiang) 2019-0004. The animals were housed in the SPF animal room of the First Affiliated Hospital of Hunan University of Chinese Medicine at a temperature of 24–26°C, a relative humidity of 40%–60%, a day-night cycle of 12 h, and free access to food and water, and adaptive rearing was performed for 1 week before the experiment. This animal experiment was approved by the Experimental Animal Ethics Committee of the First Affiliated Hospital of Hunan University of Chinese Medicine (ZYFY20201215-1).

Drugs and reagents

2,3,5-Triphenyl tetrazolium chloride (TTC) dye (Chengdu clone Chemicals Co., Ltd., CAS 298-96-4); arterial embolus (Beijing Cinontech CO., Ltd, 2636A2); RNA wait tissue preservation solution (Dalian Meilunbio Co., Ltd, MA0208); m^6A RNA methylation assay kit (Abcam, ab185912); TRIzol reagent (Life Technologies, T9424); affinity-purified anti-m^6A rabbit polyclonal antibody (Synaptic Systems, 202003); sheep anti-Rabbit IgG (Invitrogen, 11203D); and a lncRNA&mRNA epigenetic transcriptomic microarray (8 × 60 k) customized by Arraystar were used.

Instruments

A centrifuge 5418 (Eppendorf, Germany); ultramicro spectrophotometer (Nanodrop, United States); bioanalyzer 2100 (Agilent, United States); G2505C microarray scanner (Agilent, United States); Vectra3 intelligent tissue slice imaging system (Perkin Elmer, United States); and Gene Amp PCR System 9700 (Applied Biosystems, United States) were used.

Model preparation and grouping

Consistent with the method described previously, rats were randomly selected to establish the CIRI model (She et al., 2019): after the rats were anesthetized, an incision was made in the middle of the neck, the left internal and external carotid arteries and the common carotid arteries were exposed; the common carotid arteries were clipped to one small orifice, a wire plug was inserted until reaching the internal carotid artery at a depth of 2 mm from the bifurcation of the artery, and the internal carotid artery was ligated. Reperfusion was performed after the insertion of wire plugs for 2 h, and blood could re-enter the middle cerebral artery by the circle of Willis to achieve cerebral vascular reperfusion. The rats in the control group were operated on in the same way as the model group but were not given a plug line to occlude the middle cerebral artery. Finally, the wound was sutured, and the state of the rats was observed. The degree of neurological deficit in the rats was assessed by reference to the Zea-Longa scoring method (Longa et al., 1989), and a score of one–three points indicated successful modeling.

Neurobehavioral score

Recent studies have indicated that the seventh day after cerebral ischemia is the optimal time for brain tissue recovery (Dos Santos et al., 2021). Therefore, in this study, the Longa 5-point scale was used to score neurobehavioral scores on the seventh day after CIRI in rats. The scoring criteria were as follows: 0 points, normal, no neurological signs; one point, the animal could not fully extend the left forelimb; two points, the animal's left limb was paralyzed, the animal turned around to the left when walking, and tail-collision occurred; three points, the animal walked to the left and fell sideways, or the animal was unable to stand or roll; four points, no spontaneous movement, impaired consciousness.

TTC detection

Consistent with the method described previously (Ni et al., 2022), after neurological evaluation, the rats were anesthetized; the whole brain was quickly removed; and the olfactory bulb, cerebellum, and lower brainstem were removed. The brains in each group were removed after being frozen in a freezer at -20°C for 15 min; placed on ice disks from the frontal pole to the occipital pole; centered at the level of the optic chiasm; and subjected to coronal, equal thickness sectioning with a slice thickness of 2 mm. The brain slices were immersed in 2% TTC staining solution and incubated at 37°C at constant temperature in the dark for 15 min. The stained hindbrain slices were placed in 10% formalin after fixation, removed, and blotted with filter paper to dry the surface moisture. The brain slices were arranged neatly in the anterior-posterior order of the brain, and a digital camera was used to obtain images.

Nissl staining

After anesthetizing the rats, the whole brains were removed, fixed with 4% paraformaldehyde, and embedded in paraffin. The processed brain tissue was sectioned coronally and

morphologically evaluated by Nissl staining. The sections were observed and photographed under a microscope.

Quantification of total m^{6}A levels in brain tissues

Total m^{6}A levels were detected using a commercial m^{6}A RNA methylation Quantification Kit. In brief, total RNA was first extracted using the TRIzol method, 200 ng of total RNA was added to each well, and reagents were added in steps according to the manufacturer's instructions. Then, m^{6}A levels were measured colorimetrically by reading the absorbance of each well at a wavelength of 450 nm.

RT–qPCR validation

The relative gene expression of m^{6}A methylase was verified by RT–qPCR. These genes included methyltransferases (writers), including methyltransferase like 3 (*METTL3*), methyltransferase like 14 (*METTL14*) and Wilms tumor one associated protein (*WTAP*), and demethylases (erasers), including fat mass and obesity-associated protein (*FTO*) and alkylation repair homolog 5 (*ALKBH5*). The experimental process strictly followed the steps of the kit. The internal reference gene was β-actin, and the $2^{-\Delta\Delta Ct}$ method was used to calculate the relative expression of the gene. The sequences of each primer are shown in Supplementary Table S1.

Epigenetic transcriptomic microarray assays

The brains were decapitated directly after the rats were anesthetized, and the cortical tissue on the ischemic side was rapidly separated on ice, placed into a cryovial prefilled with RNA wait solution, stored strictly according to the operation procedure, and sent to Aksomics Biotechnology (Shanghai, China) for microarray assays. In brief, total RNA was first extracted using the TRIzol method, then the total RNA was immunoprecipitated with anti-m^{6}A antibodies. The "IP" grade fraction of the immunoprecipitation was highly enriched for m^{6}A methylated RNA, and the supernatant "Sup" grade contained unmodified RNA. The above two RNA types were amplified as cRNAs and mixed after labeling with Cy5 and Cy3. The samples were hybridized to the microarray for 17 h at 65°C in an Agilent Hybridization Oven. Slides were scanned with an Agilent G2505C microarray scanner. Data were extracted using Agilent feature extraction software. The generated raw data obtained from the files were normalized by GeneSpring software for subsequent data analysis.

Gene ontology (GO) functional analysis and kyoto encyclopedia of genes and genomes (KEGG) pathway enrichment analysis

GO function and KEGG pathway enrichment analyses were performed on the associated mRNAs (Chen et al., 2022).

MeRIP-PCR validation

Three lncRNAs (*LOC100912312*, *uc.440-* and *uc.77-*) and three mRNAs (protein phosphatase 1F (*PPM1F*), o-linked N-acetylglucosamine transferase (*OGT*) and schlafen family member 13 (*SLFN13*)) were randomly selected for validation. Total RNA was first immunoprecipitated with anti-m^{6}A antibodies. The immunoprecipitated "IP" fraction contained enriched m^{6}A methylated RNA, and the supernatant "Supernatant" fraction contained unmodified RNA. The above two kinds of RNA were converted into cDNA, amplified, and subsequently subjected to RT–qPCR using gene-specific primers. In addition, MeRIP-PCR assays were performed in three replicates in each group (n = 3). The primer sequences are shown in Supplementary Table S2, and the proportion of m^{6}A methylation modification of each gene was calculated according to the following formula.

$$\%\text{Input} = \frac{2^{-\text{Ct MeRIP}}}{2^{-\text{Ct MeRIP}} + 2^{-\text{Ct Supernatant}}} \times 100\% \quad (1)$$

Competing endogenous RNA (ceRNA) network establishment

We selected the above three validated lncRNAs for ceRNA network establishment. Similar to the results of a previous study (Chen et al., 2022), differentially methylated mRNAs with levels that were significantly positively correlated with lncRNA levels were first screened based on a Pearson coefficient >0.8. Then, miRBase and TargetScan were used to predict lncRNA–miRNA relationship pairs. mRNA–miRNA relationship pairs were predicted by the miRDB and miRWalk. Finally, a lncRNA–miRNA-mRNA transcription network was established with miRNA as a bridge.

Statistical analysis

Differentially expressed m^{6}A methylation genes were screened by fold changes (FCs) of ≥1.5 and p values of <0.05. Measurement data are expressed as the mean ± standard deviation. If the data in each group conformed to a normal distribution, a one-way ANOVA was performed, and results with

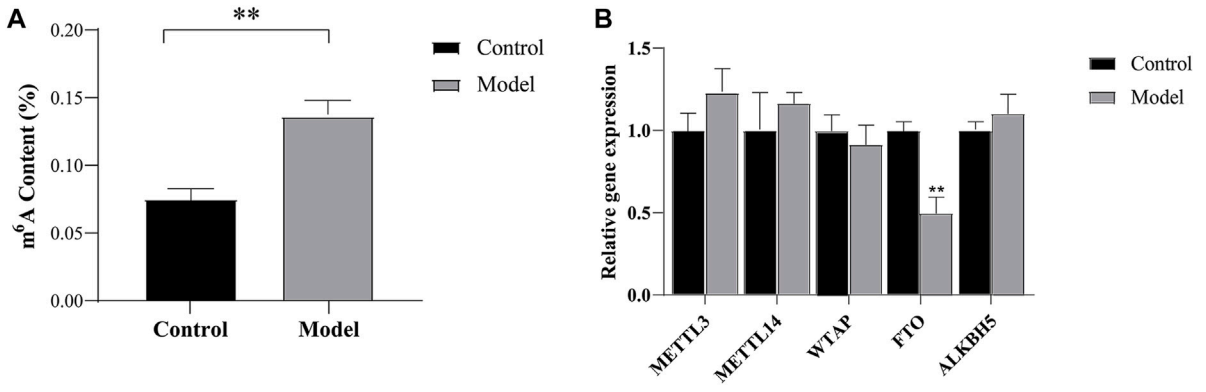

FIGURE 2
M^{6}A modification and methyltransferases and demethylases validation **(A)** The total level of m^{6}A modification measured in cortical tissue on the ischemic **(B)** Expression analysis of m^{6}A methyltransferases and demethylases by RT-qPCR. $^{**}p < 0.01$ vs Control group.

p values of <0.05 were considered statistically significant. Analysis was performed using GraphPad Prism 8 graphing software.

Results

Establishment of the CIRI model

TTC staining showed that the brain tissue in the control group was dark red, and no obvious pale cerebral infarction was found, while the model group showed obvious pale infarction, as shown in Figure 1B. Nissl staining showed a clear and complete neuronal cytoarchitecture in the cortical region of control rats, with uniform cytoplasmic and nuclear staining showing a pale blue color, normal intercellular spaces, and abundant numbers of Nissl bodies showing a dark blue color. Unlike in the control group, in the ischemic side of the cortex in the model group, neuronal cell body shrinkage was severe, vacuolar degeneration in the cytoplasm was obvious, cell arrangement lost regularity, and the number of Nissl bodies was reduced, as shown in Figure 1C. The neurobehavioral score of the rats in the model group was significantly higher than that observed in the control group ($p < 0.01$), as shown in Figure 1D. In conclusion, it was suggested that the rat model of CIRI was successfully established, and pathological damage was observed on the ischemic side of the rat model of cerebral ischemia.

Detection of total m^{6}A levels after CIRI

The results showed that the total m^{6}A methylation level in the cortex tissue of the ischemic side was significantly higher in the model group than in the control group ($p < 0.01$), as shown in Figure 2A. This result suggested that CIRI resulted in the abnormal methylation of cortical tissue on the ischemic side.

Verification of m^{6}A methyltransferases and demethylases

We studied the expression levels of five m^{6}A methyltransferases and demethylases and found that the expression of *FTO* was lower ($p < 0.01$) in the model group than in the control group, while no significant changes were observed in the expression of methyltransferases, including *METTL3, WTAP,* and *METTL14*. In addition, we also found no significant changes in the expression levels of *ALKBH5*, as shown in Figure 2B. These results suggest that the increase in total m^{6}A levels in CIRI may be caused by the imbalance in the expression of *FTO*.

M^{6}A modification profiles of lncRNAs and mRNAs

Samples from the model and control groups were analyzed using m^{6}A lncRNA and mRNA epigenetic transcriptomic microarrays. The results showed that a total of 108 lncRNAs exhibited differences in m^{6}A modification between the model group and the control group, of which 54 were hypermethylated and 54 were hypomethylated, as shown in Figure 3A. In addition, 590 mRNAs harbored differential m^{6}A modifications, of which 375 were hypermethylated and 215 were hypomethylated, as shown in Figure 3B. The raw data supporting this result have been uploaded to the GEO database (GSE201258).

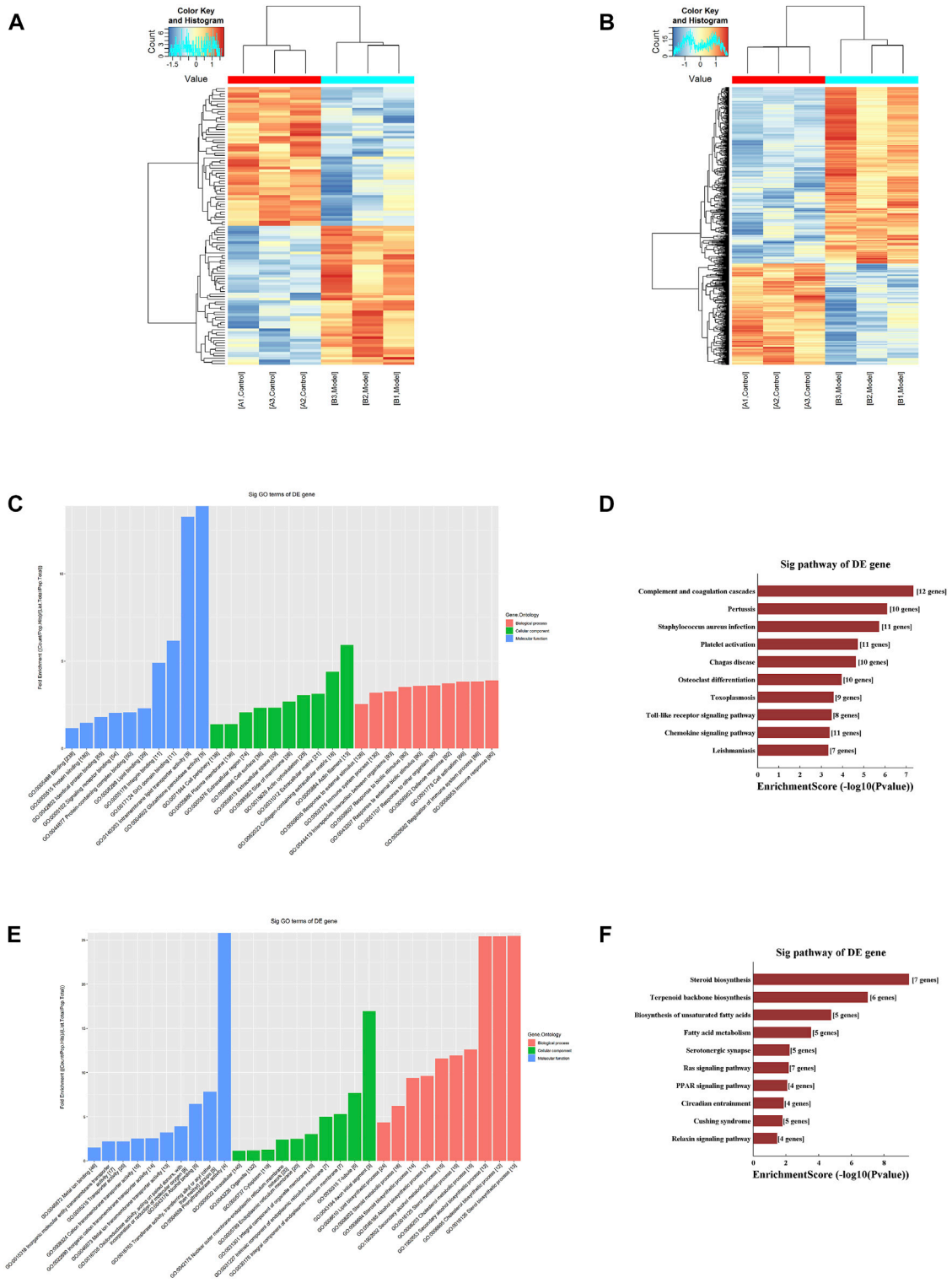

FIGURE 3
M^{6}A modification profiles **(A)** Hierarchical clustering analysis of the differentially methylated lncRNAs **(B)** Hierarchical clustering analysis of the differentially methylated mRNAs **(C)** GO functional analysis of the hypermethylated mRNAs **(D)** KEGG pathway enrichment analysis of the hypermethylated mRNAs **(E)** GO functional analysis of the hypomethylated mRNAs **(F)** KEGG pathway enrichment analysis of the hypomethylated mRNAs.

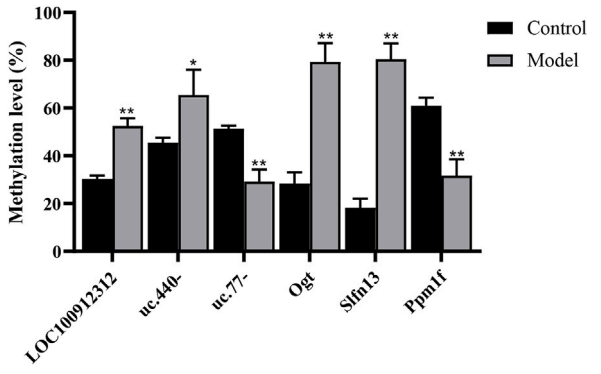

FIGURE 4
M^{6}A analysis of differentially methylated genes by MeRIP-PCR. *p < 0.05, **p < 0.01 vs Control group.

GO function and KEGG pathway enrichment analysis of differentially methylated mRNAs

First, we performed an enrichment analysis of the hypermethylated mRNAs and found that the biological processes involved were mainly protein binding, lipid binding, and glutathione peroxidase activity, and the cellular functions involved were mainly cell activation and immune response. The results of the KEGG pathway enrichment analysis mainly identified the chemokine signaling pathway and toll-like receptor signaling pathway, as shown in Figures 3C, D.

Moreover, we performed an enrichment analysis of the hypomethylated mRNAs and found that the biological processes involved were mainly ion binding and oxidoreductase activity, and the cellular components involved were mainly cell junctions, organelles, and synapses. The molecular functions involved mainly included various types of metabolism and biosynthesis. The results of the KEGG pathway enrichment analysis were mainly for steroid biosynthesis and the peroxisome proliferator-activated receptor (PPAR) signaling pathway, as shown in Figures 3E, F.

Validation of differentially methylated genes

Validation of the microarray results was performed by MeRIP-PCR. Three differentially methylated lncRNAs and three mRNAs were randomly picked. The results showed that in the model group, the m^{6}A methylation ratios of *LOC100912312*, *uc.440-*, *OGT* and *SLFN13* were significantly increased (p < 0.05 or 0.01), and the m^{6}A methylation ratios of *uc.77-* and *PPM1F* were significantly decreased (p < 0.01), consistent with the trends of change observed in the microarray results, as shown in Figure 4.

CeRNA analysis of lncRNAs

To clarify the biological function of related lncRNAs, we performed ceRNA network analysis on the validated *LOC100912312*, *uc.440-* and *uc.77-* lncRNAs based on the ceRNA hypothesis. A ceRNA network consisting of three lncRNAs, 17 miRNAs, and 16 mRNAs was established, as shown in Figure 5A. The enrichment analysis of mRNAs in this network showed that the biological processes were mainly involved in the regulation of voltage-gated calcium channel activity and neuromuscular process controlling posture, the cellular components were mainly involved in the costamere and sarcolemma, and the molecular functions were mainly involved in transforming growth factor beta-activated receptor activity (Figure 5B). The results of the KEGG pathway enrichment analysis mainly identified the mitogen-activated protein kinase (MAPK) signaling pathway, as shown in Figure 5C.

Discussion

M^{6}A is one of the most prevalent modifications present in the RNA of higher eukaryotes, and increasing amounts of evidence suggest that m^{6}A modification of RNA plays important biological roles in physiological and pathological processes in the central nervous system (CNS) (Zhang et al., 2022). This finding was confirmed in cerebral ischemic disease, in which lncRNAs were identified as important biomarkers (Chokkalla et al., 2019; Xu et al., 2020a). Unfortunately, to date, reports on the dysregulation of lncRNA m^{6}A modification in cerebral ischemia-reperfusion injury and studies on the biological functions of related lncRNAs have not been reported.

In this study, we first detected significantly elevated m^{6}A levels in ischemic lateral brain tissue, which is consistent with the results of previous studies (Xu et al., 2020b). M^{6}A modifications are known to be dynamic and reversible, installed by "writers" and removed by "erasers" (Shi et al., 2019). Therefore, we further investigated the expression levels of five common m^{6}A methyltransferases and demethylases and found that *FTO* expression was downregulated after CIRI, while no significant changes were observed in the expression of methyltransferases, including *METTL3, WTAP,* and *METTL14*. The high expression of *METTL3*, *WTAP,* and *METTL14* may lead to an increase in the m^{6}A methylation level (Zhang et al., 2022), but at present, there are few reports related to cerebral ischemia, which need to be further studied. *FTO* was initially considered related to obesity (Jia et al., 2011). With further research, *FTO* has been confirmed to be an important regulator of m^{6}A methylation (Wei et al.,

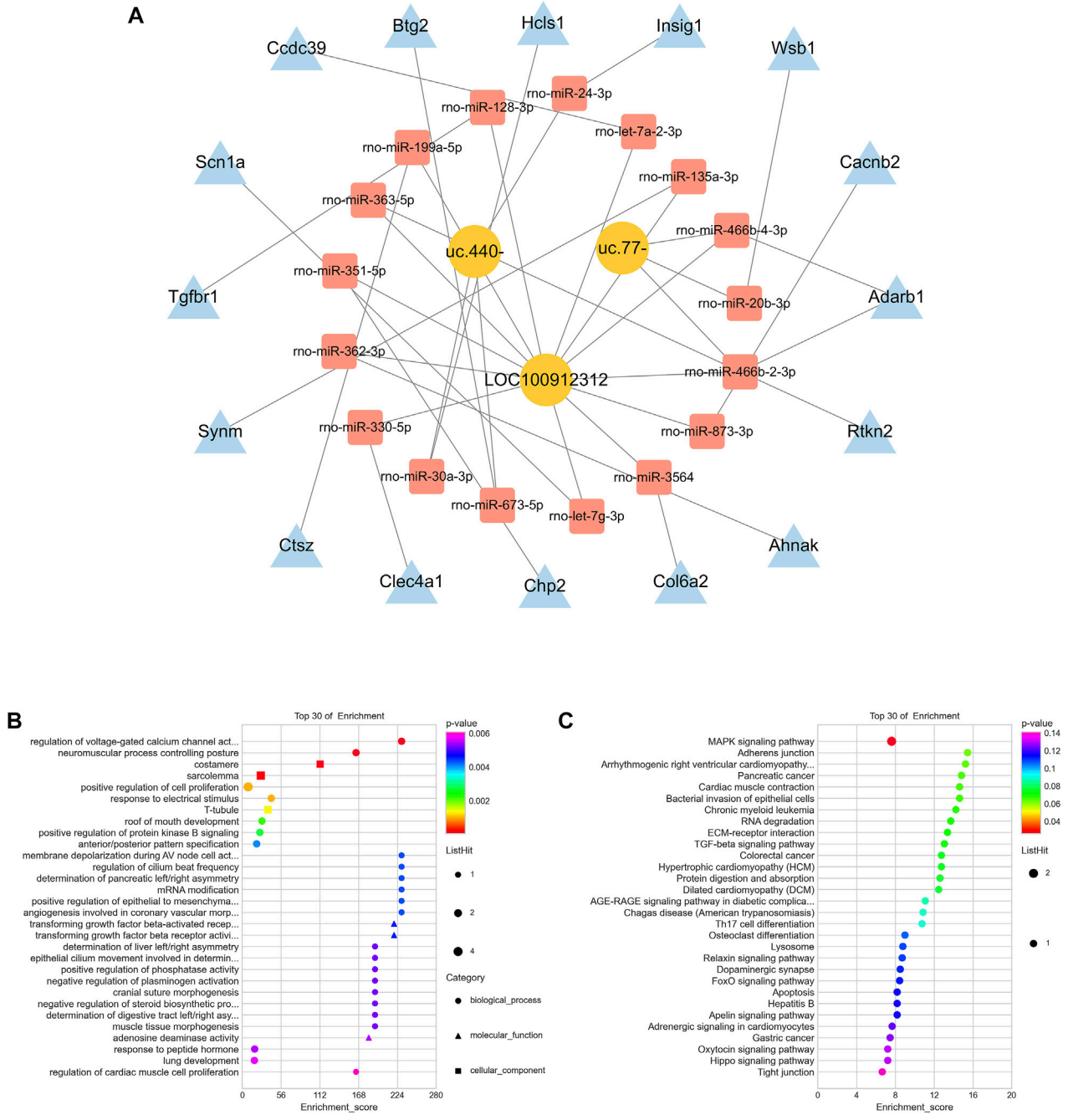

FIGURE 5
CeRNA analysis of lncRNAs **(A)** CeRNA network for *LOC100912312*, *uc.440-* and *uc.77-* **(B)** GO functional analysis involved in the ceRNA network **(C)** KEGG pathway enrichment analysis involved in the ceRNA network.

2018; Wei et al., 2022). *FTO* is an m^{6}A demethylase with abundant expression in the brain (Li et al., 2018). Studies have shown that *FTO* expression is specifically downregulated in cerebral ischemic cortical neurons (Yi et al., 2021). In addition, the expression of *FTO* is downregulated in myocardial infarction (Mathiyalagan et al., 2019), suggesting that the expression of FTO is generally low in ischemic injury. Conversely, overexpression of *FTO* was shown to reverse m^{6}A methylation and reduce the levels of neuronal apoptosis caused by cerebral ischemia (Xu et al., 2020b). Moreover, *FTO* has also been found to affect neurogenesis, memory formation, regulation of neuropsychiatric disorders, *etc.* (Li et al., 2017; Walters et al.,

2017; Wang et al., 2022), suggesting that it may be closely related to the CNS. Moreover, we also found no significant changes in the expression levels of *ALKBH5*. Although *ALKBH5* has been reported to selectively demethylate *BCL2* transcripts after cerebral ischemia, which prevents degradation of B-cell lymphoma-2 (*BCL2*) mRNA, enhances expression of anti-apoptotic *BCL2* protein, and inhibits neuronal apoptosis, the role of *ALKBH5* is still less understood (Xu et al., 2020b).

Microarray analysis showed that 590 mRNAs exhibited differences in m^{6}A modification in CIRI, of which 375 were hypermethylated and 215 were hypomethylated. Several studies have reported on the roles of related mRNAs. For instance, apolipoprotein E (*ApoE*) was recognized as a hypomethylated RNA in this study. In the CNS, *ApoE* is synthesized and secreted by astrocytes and is involved in maintaining the homeostasis of cholesterol and phospholipids, regulating the mobilization and redistribution of cholesterol and phospholipids during neural membrane remodeling and thus regulating the maintenance of synaptic plasticity as well as repair when neuronal cells are damaged (Cantuti-Castelvetri et al., 2018). In addition, vascular endothelial growth factor A (*VEGFA*) is recognized as a hypermethylated RNA; *VEGFA* is a factor that is closely related to angiogenesis after cerebral ischemia, and hypermethylated *VEGFA* can promote angiogenesis through multiple pathways (Xin et al., 2022). GO function and KEGG pathway enrichment analyses showed that these m^{6}A-modified mRNAs were mainly involved in lipid binding, immune reactions, and oxidoreductase activity, and the signaling pathways involved were the Toll-like receptor signaling pathway and PPAR signaling pathway. The Toll-like receptor signaling pathway is a bridge between innate immunity and acquired immunity and has been confirmed to be closely related to the inflammatory cascade observed after cerebral ischemia (Eltzschig and Eckle, 2011). Proliferator-activated receptors (PARs) are ligand-activated receptors in the nuclear hormone receptor family that control metabolic processes in many cells. Studies have shown that ligand-activated PPAR can inhibit the expression of a variety of related inflammatory factors, thus inhibiting the inflammatory response and playing a protective role in CIRI (Wu et al., 2018). In addition, studies have supported the idea that PPAR activation has a direct transcriptional regulatory effect on the expression of several key endogenous antioxidants. For example, the activation of PPAR can activate the expression of antioxidant enzymes in female rats and protect them from CIRI (Mohagheghi et al., 2013).

In recent years, m^{6}A-modified lncRNAs have received extensive attention. For example, m^{6}A modification can drive the interaction between related lncRNAs and downstream genes, which indicates that the m^{6}A modification status in lncRNAs may control the biological functions of lncRNAs (Lan et al., 2021). In this study, we identified 108 lncRNAs with differential m^{6}A modification, of which 54 were hypermethylated and 54 were hypomethylated. Studies have found that although lncRNA cannot directly encode translational proteins, it can act as a "miRNA sponge", indirectly reducing the binding between miRNA and downstream mRNA targets by absorbing the miRNA into this sponge, thus affecting the expression of target genes. This is essentially the mechanism underlying ceRNA regulation (Salmena et al., 2011). Subsequently, we established a potential ceRNA network of validated lncRNAs *LOC100912312*, *uc.440-*, and *uc.77-* to clarify the biological functions of relevant lncRNAs. Enrichment analysis indicated that this network was mainly closely related to the MAPK signaling pathway. MAPK signaling is an important regulatory pathway after cerebral ischemia and hypoxia and is closely related to pathological processes such as oxidative stress, the inflammatory response, apoptosis, and autophagy (Zhen et al., 2016). Experiments have confirmed that the activated MAPK pathway after cerebral ischemia can activate the NF-E2–related factor 2(Nrf2) pathway, and inhibiting the phosphorylation of the MAPK pathway can reverse the nuclear translocation of Nrf2 and reduce oxidative stress (Meng et al., 2018); other studies have indicated that the MAPK signaling pathway is associated with inflammatory responses. It can activate the downstream nuclear factor kappa-B (NF-κB) pathway after cerebral ischemia and promote the inflammatory response (Wang et al., 2014) while inhibiting the activation of the MAPK pathway can reduce the activation of the inflammatory response and have neuroprotective effects (Guo et al., 2012).

Undeniably, this study has several limitations. First, the sample size of this study was relatively small, and the above results may need to be validated in a large sample study in the future. In addition, targeting *FTO* in CIRI requires further exploration. Despite these limitations, we present the first systematic analysis of m^{6}A lncRNA and m^{6}A mRNA modification profiles in CIRI and constructed a related lncRNA–miRNA–mRNA network, laying a foundation for further revealing the pathogenesis of CIRI.

Conclusion

In summary, we identified for the first time that m^{6}A lncRNA and m^{6}A mRNA were differentially modified in CIRI, and total m^{6}A levels were increased in CIRI, which might be caused by the downregulation of *FTO* expression. In addition, bioinformatics analysis was used to predict the potential functions of differentially m^{6}A-modified lncRNAs and m^{6}A mRNAs, which could provide a reference for further revealing the mechanism of CIRI.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and

accession number(s) can be found below: https://www.ncbi.nlm.nih.gov; GSE201258.

Ethics statement

The animal study was reviewed and approved by This animal experiment was approved by the Experimental Animal Ethics Committee of the First Affiliated Hospital of Hunan University of Chinese Medicine (ZYFY20201215-1).

Author contributions

BL designed the experiments, analyzed the data, and prepared the manuscript. LS and BC performed the experiments, analyzed the data, and prepared the manuscript. QW optimized the language of the manuscript. YX and JY performed the experiments. The manuscript was revised by QW and ZG. All authors confirmed the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2022.973979/full#supplementary-material.

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Non-coding RNAs in ossification of the posterior longitudinal ligament

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Ossification of the posterior longitudinal ligament (OPLL) is a kind of disease that involves a variety of factors leading to ectopic bone deposition of the spinal ligament. Although the detailed mechanism is not clear, genetic factors play important roles in the development of this disease. Noncoding RNA (ncRNA) refers to an RNA molecule that is not translated into a protein but participates in the regulation of gene expression. Functionally important types of ncRNA associated with OPLL include long noncoding RNA, microRNA, and circular RNA. We listed the differentially expressed ncRNAs in OPLL patients and normal controls to find the ncRNAs most relevant to the pathogenesis of the disease. The potential regulatory networks of ncRNA in OPLL cells were analyzed based on their most abundant signal transduction pathway data. The analysis of the highly connected ncRNAs in the regulatory network suggests that they play an important role in OPLL. These findings provide new directions for the study of OPLL pathogenesis and therapeutic targets. In this paper, we reviewed and analyzed the literature on ncRNAs in OPLL published in recent years, aiming to help doctors better understand and treat this disease.

KEYWORDS

lncRNA, circRNA, ossification of the posterior ligament, ncRNA, microRNA

Introduction

Ossification of the posterior longitudinal ligament (OPLL) is a disease in which fibroblasts appear in the ligament tissue and form osteoblasts due to a variety of factors. OPLL is more common in East Asia than in North America. OPLL often occurs in the cervical spine, most commonly at the C5 level (Kawaguchi et al., 2016). Cervical OPLL is also related to sex and age. The risk of cervical OPLL is higher in males than in females and higher in the elderly than in adolescents. In a report from Taiwan, the estimated

Abbreviations: BMP-2, Bone morphogenetic protein-2; BMSC, Bone mesenchymal stem cells; BMSC-sEVs, Bone marrow mesenchymal stem cell-derived small extracellular vesicles; Col 1, Collagen 1; ERK, Extracellular regulated protein kinases; IFNGR1, Interferon gamma receptor; MAPK, Mitogen-activated protein kinase; miR, MicroRNA; OCN, Osteocalcin; OSX, Osterix; RT-qPCR, Reverse transcription quantitative polymerase chain reaction; RUNX2, Runt-related transcription factor 2; SNHG1, Small nucleolar RNA host gene 1; STAT, Signal transducer and activator of transcription.

prevalence of cervical OPLL was 7.7 per 100,000 person-years (Wu et al., 2011; Wu et al., 2018). CT images of 1,500 patients in Japan showed that men were 4.9% more likely than women to develop OPLL in the cervical spine, while men were 0.6% less likely than women to develop OPLL in the thoracic spine (Fujimori et al., 2016). Although the underlying mechanism of OPLL has not been thoroughly studied, recent theories suggest that OPLL is associated with genetic factors (Nishida et al., 2015).

Noncoding RNA (ncRNA) refers to a class of RNA transcribed from the genome that does not encode proteins, and there are tens of times more of them than protein-coding RNAs. In the past decade, ncRNAs have been regarded as useless "junk" RNAs, but in recent years, research has shown that ncRNAs play a crucial role in regulating gene expression as information carriers, potential biomarkers, and therapeutic targets (Han et al., 2013; Kopp and Mendell, 2018; Tsuru et al., 2018). There are many kinds of functionally important ncRNAs that form ncRNA action networks through the permutations and combinations of secondary and/or tertiary structures (non-coding RNA interactor networks/NINs) and play important roles in the development of OPLL (Fabbri et al., 2019).

At present, some reports have found that ncRNAs regulate ossification and further influence OPLL progression. For example, miR-146a may inhibit osteogenic differentiation by blocking tumor necrosis factor (TNF)-α activation of nuclear factor κB (NF-κB) in bone mesenchymal stem cells (BMSCs) derived from human adipose tissue (Cho et al., 2010). TNF-α-induced NF-KB activation stimulates the growth of microRNA-150-3p and inhibits the osteogenic differentiation of mesenchymal stem cells by targeting β-catenin (Wang et al., 2016). Currently, we have very little knowledge of ncRNAs in patients with OPLL, both in terms of quantity and mechanism of action. The purpose of our study was to identify some ncRNAs and mRNAs that are most correlated with OPLL, to study their roles in the progression of OPLL according to their targets and to obtain the potential therapeutic value of these ncRNAs and mRNAs for OPLL.

LncRNAS involved in ossification of the posterior longitudinal ligament

Aberrantly expressed lncRNAs of osteogenically differentiated mesenchymal stem cells in ossification of the posterior longitudinal ligament

Cai et al. (2020) investigated the interaction between abnormally expressed lncRNAs and mRNAs in OPLL mesenchymal stem cells and predicted lncRNA targets and transcription factors. They found 651 upregulated lncRNAs and 74 downregulated lncRNAs in the MSCs of the OPLL patients (Table 1). They also used the top 10 differentially expressed lncRNAs and mRNAs for reverse transcription quantitative polymerase chain reaction (RT-qPCR) assays, and the results confirmed the microarray data. Interestingly, the authors found that in patients with OPLL, the most important upstream potential transcription factor of lncRNA was Octamer transcription factor 1 (Oct-1), which had the most interactions. Oct1 is widely distributed in tissues. It is a complex and versatile transcription factor that plays an essential role in maintaining cellular homeostasis and signaling pathways. It regulates the underlying expression of genes by recruiting and activating RNA polymerase III. Moreover, Oct1 is directly linked to the core transcription mechanism. However, it can also induce pauses to balance genes for rapid transcription (Pance, 2016). Oct1 may be an important target for studying the regulation of OPLL transcription in the future, and many mechanisms have not been thoroughly studied.

Not only upstream transcription factors but also some LncRNAs can affect the relationship between the bone morphogenetic protein-9-guided signal transduction pathway and the receptor and ligand of Notch, thus affecting the osteogenic differentiation process of MSCs. Zhang et al. (2019) found that lncRmst knockout reduces the expression of Notch receptors and ligands to inhibit Bmp9-induced osteogenic, chondrogenic and adipogenic differentiation and weakens BMP9-induced ALP activity and ectopic formation of bone matrix. BMP9 can directly induce osteogenic differentiation through Notch or other mediators. However, the ligand and receptor of Notch are inhibited by some microRNAs, such as miR-107, miR-125a, miR-27b, miR-34a, miR-449a and miR-449b. LncRmst can sponge microRNAs to promote osteogenic differentiation, such as miR-106, miR-125a, miR-449a and miR-449b. Not coincidentally, other researchers have found that lncRNA H19 and HOTAIR are closely associated with BMP9-induced osteogenic differentiation in MSCs. LncRNA H19 affects the early stages of BMP9-induced osteogenic differentiation by regulating Notch ligand expression, and either silencing or overexpression of lncRNA H19 inhibits BMP9-triggered terminal osteogenic differentiation of MSCs. However, activation of Notch signaling in turn rescues the inhibited osteogenic differentiation (Liao et al., 2017).

LncRNA expression profiles in peripheral blood mononuclear cells

Jiang et al. (2021) profiled the differential lncRNA expression from peripheral blood mononuclear cells (PBMSCs) and identified 751 upregulated and 650 downregulated lncRNAs by using RNA-sequencing. The lncRNA with the most upregulated expression was LNC-Cryba4-1:21, which was upregulated 1790-fold. The lncRNA with the most downregulated expression was lncILF3-AS1, which was

TABLE 1 LncRNAs expression profiles in OPLL.

No.	Evaluation method	Sample	Dysregulation (up)	Dysregulation (down)	Signal pathway	References
1	Microarray	Human MSCs	651 lncRNAs	74 lncRNAs	—	Cai et al. (2020)
	RT-qPCR			LncRNA-p26842		
2	RNA-sequencing	PBMCs	751 lncRNAs	650 lncRNAs	—	Jiang et al. (2021)
3	qRT-PCR	Ligament fibroblasts	LncRNA XIST	—	Targets miR-17-5p to promote the expression of AHNAK and BMP2	Liao et al. (2019)
4	lncRNA microarray	Ligament fibroblasts	73 lncRNAs	69 lncRNAs	—	Wang et al. (2020)

downregulated 1185-fold. To prove the authenticity of the RNA sequencing results, the authors randomly chose two upregulated lncRNA groups and two downregulated lncRNA groups to perform RT-qPCR. The results of RT-qPCR reconfirmed the results of RNA sequencing. LncRNA ILF3-AS1 (ILF3-AS1) has been reported to be abnormally expressed in some tumors. Hu et al. (2019) found that lncIlF3-AS1 levels were significantly upregulated by the nuclear transcription factor SP1 in osteosarcoma tissues and cells. Moreover, ILF3-AS1 plays a tumor-promoting role in the progression of osteosarcoma through the miR-212/SOX5 axis. LncILF3-AS1 is a ceRNA (competing endogenous RNA) of miR-212, and overexpression of ILF3-AS1 can significantly reduce the expression of miR-212 and increase the expression of SOX5. However, downregulation of ILF3-AS1 inhibited the proliferation, migration and invasion of osteosarcoma cells and ultimately promoted their apoptosis. Some mechanisms of lncRNA ILF3-AS1 have been explored in bone tumors, but its specific role in OPLL is still unclear, which may be a research direction for further development in the future.

LncRNA expression profiles in primary human ligament fibroblasts

Liao et al. (2019) found that lncRNA X inactive specific transcript (XIST) was significantly increased in primary human ligament fibroblastic cells from OPLL tissues. Some researchers have found that lncRNA XIST regulates miR-17-5p/AHNAK/BMP2 through a signaling pathway to affect posterior longitudinal ligament ossification. Significantly higher levels of lncRNA XIST in response to mechanical stress led to lower levels of miR-17-5p. This further led to increased levels of AHNAK, BMP2 and runt-related transcription factor 2 (RUNX2), which promoted osteogenic differentiation of posterior longitudinal ligament cells. METTL3 affects the osteogenic differentiation of primary ligament fibroblasts *via* the lncRNA XIST/miR-302a-3p/USP8 axis. METTL3 not only increases the expression level of the lncRNA XIST but also promotes the m6A methylation modification of lncRNA (Yuan et al., 2021). For example, Song et al. (2021) found that METTL3 can activate the mitogen-activated protein kinase (MAPK) signaling pathway by regulating the m6A modification and expression of a lncRNA. This enhances the osteogenic differentiation of human adipose-derived stem cells. However, miR-302a-3p can in turn affect METTL3 levels through a negative feedback mechanism. Wang et al. (2020) reported that 73 lncRNAs were upregulated and 69 lncRNAs were downregulated in ligament fibroblasts from OPLL tissues. In particular, the greatest upregulated small nucleolar RNA host gene 1 (SNHG1) expression was confirmed *via* RT-qPCR; even more surprising was the authors' finding that SNHG1 acts as a sponge for miR-320b. This led to increased expression of interferon gamma receptor (IFNGR1) and activation of the JAK/STAT signaling pathway, ultimately leading to increased ligament fibroblast mineralization and osteogenic differentiation.

MiRNA involved in ossification of the posterior longitudinal ligament

MiRNA expression profiles in primary ligament cells

By comparing the primary posterior longitudinal ligament cells of OPLL and normal PLL, Xu et al. (2016) demonstrated that 144 miRNAs were upregulated and 74 miRNAs were downregulated (Table 2). After verification, it was found that the expression of Runx2 and IBSP in the miR-10a-5p overexpression group was the most upregulated, and had a strong role in promoting bone matrix mineralization and calcium deposition. Jiang et al. (2020) found that miR-497 and miR-195 were downregulated in PLL tissues of OPLL patients, while the expression levels of ADORA2A were significantly increased. Mechanical stress can promote the process of osteogenic differentiation. The interesting finding is that the expression of miR-195 and miR-497 is also reduced in PLL cells treated with cyclic mechanical stress (CMS). This is

TABLE 2 MiRNAs expression profiles in OPLL.

No.	Evaluation method	Sample	Dysregulation (up)	Dysregulation (down)	Signal pathway	References
1	RNA-sequencing	primary ligament cell	144	74	—	Xu et al. (2016)
2	RT-qPCR Western blot	OPLL cells	—	miR-195	Targets ADORA2A to inhibit the cAMP/PKA pathway	Jiang et al. (2020)
3	RT-qPCRWestern blot	OPLL cells	—	miR-497	Targets ADORA2A to inhibit the cAMP/PKA pathway	Jiang et al. (2020)
4	RT-qPCRWestern blot	OPLL cells	miR-181a-5p	—	Targets PBX1 and ACAN	Liu et al. (2020)
5	RT-qPCRWestern blot	OPLL cells	miR-181a-3p	—	—	Liu et al. (2020)
6	High through-put microrna sequencing	OPLL blood samples	miR-563	—	Targets SMURF1to stabilize SMAD protein and promote the activity of BMP2	Zhang et al. (2017)
14	High through-put microrna sequencing	OPLL blood samples	miR-10a-3p	—		Xu et al. (2019)
15	High through-put microrna sequencing	OPLL blood samples	miR-10a-5p	—	—	Xu et al. (2019)
7	High through-put microrna sequencing	OPLL blood samples	miR-210-3p	—	Targets MAPK to inhibit osteogenic differentiation	Xu et al. (2019)
8	High through-put microrna sequencing	OPLL blood samples	miR-885-5p	—	Targets β-catenin	Xu et al. (2019)
9	High through-put microrna sequencing	OPLL blood samples	—	miR-129-2-5p	—	Xu et al. (2019)
10	High through-put microrna sequencing	OPLL blood samples	—	miR-199b-5p	Targets JAG1 and modulates the Notch signaling pathway to inhibits osteogenic differentiation	Xu et al. (2019)
11	High through-put microrna sequencing	OPLL blood samples	—	miR-212-3p	Targets GDF5 and further activates Smad1/5/8 phosphorylation to inhibits osteogenic differentiation	Xu et al. (2019)
12	High through-put microrna sequencing	OPLL blood samples	—	miR-196b-5p	Targets FGF2	Xu et al. (2019)
13	High through-put microrna sequencing	OPLL blood samples	—	miR-218-1-3p	—	Xu et al. (2019)
16	Edger package of R software	OPLL cell specimens	80	264	—	Xu et al. (2020)
17	High through-put microrna sequencing	OPLL cells	miR-320e	—	Targets TAK1 to promote osteogenic differentiation	Xu et al. (2022)
18	High through-put microrna sequencing	OPLL cells	miR-6720-3p	—	—	Xu et al. (2022)
19	High through-put microrna sequencing	OPLL cells	miR-3185	—	—	Xu et al. (2022)
20	RT-qPCRWestern blot	OPLL tissue	miR-497-5p	—	Targets RSPO2 to inhibit the Wnt3α/β-catenin pathway	Chen et al. (2021)
21	microRNA array	OPLL cells	—	12	—	Yayama et al. (2018)

because CMS significantly enhances CpG island methylation of the miR-195 and miR-497 promoters in PLL cells. miR-497-5p has been reported to be a modulator of cartilage-related genes (Hou et al., 2019). Moreover, BMSCS-EVs can upregulate miR-497-5p levels to reduce RSPO2 levels and thus inhibit the Wnt/β-catenin pathway to promote osteogenic differentiation. Ma et al. (2020) found that serum levels of miR-497-5p are significantly decreased in postmenopausal women, and overexpression of miR-497-5p promotes osteogenic differentiation and bone mineralization. Liu et al. (2020) found that miR-181a-5p and miR-181a-3p were highly expressed in OPLL ligament cells, and the level of miR-181a-5p in OPLL was significantly higher than that of miR-181a-3p compared with normal PLL primary ligament cells. MiR-181a-5p and miR-10a-5p have a similar mechanism of action, both of which have a strong role in promoting osteogenic differentiation. Moreover, miR-181a-5p significantly promoted ALP activity and calcium deposition after overexpression of the microRNA mimic, but miR-181A-3p had little overexpression effect. The use of modified miRNA antisense inhibitors (antagomirs) also confirmed that miR-181a-5p plays a major role in regulating cell ossification *in vitro*. *In vivo*, the authors further demonstrated that miR-181a-5p significantly

increased the osteogenic capacity of posterior ligament cells and led to increased cervical osteophyte formation in Tip-Toe-walking OPLL mice. By means of RT–PCR, Zhang et al. (2017) found that the expression of miR-563 in OPLL cultured ligament cells was significantly higher than that in PLL ligament cells. After the miR-563 overexpression treatment, the expression levels of the osteogenic-related genes RUNX2, IBSP and ALP were higher than those in the control group. After miR-563 inhibition treatment, the levels of osteoblast-related genes decreased. Furthermore, the mechanism of action of miR-563 was further studied. The high expression of miR-563 can reduce the expression of SMURFl, thus stabilizing SMAD protein, promoting the activity of BMP2 and other pathways, and ultimately promoting osteogenic differentiation.

The miRNA expression profiles in blood

Based on the high-throughput miRNA sequencing data of OPLL and PLL cells, Xu et al. (2019) selected 10 miRNAs with the largest expression differences. Among the 10 selected miRNAs, miR-10a-3p, miR-10a-5p, miR-563, miR-210-3p and miR-885-5p were highly upregulated in OPLL tissues. However, miR-129-2-5p, miR-199b-5p, miR-196b-5p, miR-212-3p and miR-218-1-3p were highly downregulated. In addition, miR-10a-5p, miR-563 and miR-210-3p showed high accuracy and significance in patients who had OPLL in the normal group and IDD (intervertebral disc degenerated) group. However, the authors found no significant difference in serum bone metabolites in differentiating OPLL from IDD and non-IDD patients, so the determination of these miRNAs may play an important role in the early detection of OPLL. Moreover, a combination of three miRNAs is more diagnostic than a single miRNA. Significant increases in miR-10a-5p and miR-10a-3p were also found in other studies involving OPLL. This suggests that these two miRNAs are closely associated with the progression of OPLL and may have the potential to function as hub RNAs in a complex mechanism of action.

The miRNA expression profiles in mesenchymal stem cells

Xu et al. (2020) analyzed a total of 344 differentially expressed miRNAs from the database by using the edgeR Package of R Software, among which 80 miRNAs were upregulated and 264 were downregulated. More interestingly, they identified miR-520d-3p, miR-4782-3p, miR-6766-3p and miR199b-5p as hub miRNAs by FunRich prediction. These miRNAs have been shown to be associated with the development of hepatocellular carcinoma, lung cancer and peripheral vascular disease in reported studies, but their specific mechanisms in OPLL are still poorly understood. It is only known that SP1 can act as an upstream regulator and participate in the process of posterior longitudinal ligament ossification *via* the Wnt signaling pathway through LEF1 and Wnt2.

An interesting phenomenon was found by Xu et al. (2022) Although the level of miR-320e was upregulated in OPLL cells, it was far less than that in OPLL mesenchymal stem cell-derived exosomes. This indicates that miRNAs strongly associated with certain diseases can spontaneously accumulate in sEVs and be transmitted to other cells to exert their effects. The authors also found that miR-6720-3p and miR-3185 were also significantly upregulated, similar to miR-320e. However, the exact mechanism of their action is not yet understood, and may be a direction for future research. Chen et al. (2021) reported that mesenchymal stem cells from fat and muscle tissue have high osteogenic differentiation ability. Immunofluorescence showed that mesenchymal stem cells released extracellular vesicles and were entoxified by ligament fibroblasts to upregulate miR-497-5p. Yayama et al. (2018) detected a total of 177 microRNA factors through the microRNA array, and the expression of 12 factors in OPLL cells was significantly downregulated compared with non-OPLL cells in the control group (CSM patients). Hsa-miR-487b-3p is downregulated in OPLL patient cells, and the expression of genes involved in Wnt signal transduction may be related to this RNA. Wnt signaling regulates the osteogenic differentiation of chondrocytes and osteoblasts and regulates cartilage osteogenesis in the pathogenesis of OLF and posterior longitudinal ligament ossification.

In OLF cells, Han et al. (2018) revealed that miRNA-342-3p was significantly elevated during osteogenic differentiation and significantly decreased during bone loss by miRNA sequencing analysis. MiRNA-342-3p promotes osteogenic differentiation by directly targeting ATF3 and inhibiting ATF3 inhibition. After miRNA-342-3p was knocked out in human mesenchymal stem cells (MSCs), the ALP, osteocalcin (OCN), COL I and RUNX2 genes related to osteogenesis were downregulated. Although there are no research results of miR-342-3p in OPLL, its role in promoting osteogenic differentiation in OLF and MSCs is sufficient for us to conduct correlation studies. In addition, miR-342-3p may be a vital potential target for the treatment of OPLL in the future.

CircRNA involved in ossification of the posterior longitudinal ligament

CircRNA expression profiles in peripheral blood mononuclear cells

Jiang et al. (2021b) reported that 177 circRNAs were upregulated and 154 circRNAs were downregulated in peripheral blood mononuclear cells (PBMCs) (Table 3). The

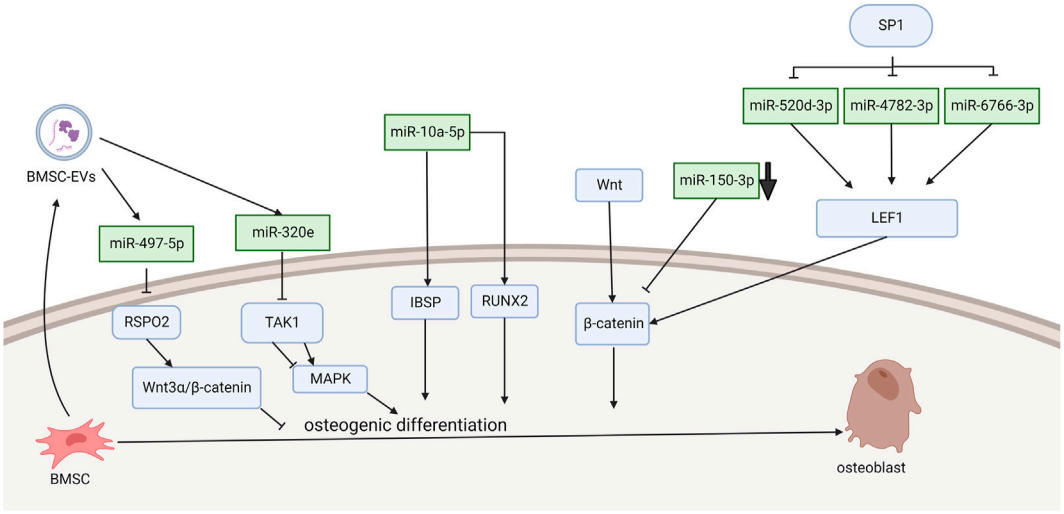

FIGURE 1
Functions of specific ncRNAs in bone marrow mesenchymal stem cells. ("Created with BioRender.com").

circRNA with the most upregulated expression was hsa-circ-0092429, which was upregulated 38.78-fold. The circRNA with the most downregulated amount was hsa-circ-0137606, which was downregulated 292-fold. To prove the authenticity of the RNA sequencing results, the authors randomly choose two upregulated circRNA groups and two downregulated circRNA groups to perform RT-qPCR. The results of RT-qPCR reconfirmed the results of RNA sequencing.

CircRNA expression profiles in ossification of the posterior longitudinal ligament cells

By comparing OPLL tissue samples and non-OPLL tissue samples, Jiang et al. (2021a) demonstrated that 72 circRNAs were upregulated and 74 circRNAs were downregulated. Moreover, their follow-up study showed that hsa-circ-0007292 was the most elevated in OPLL cells. MiR-508-3p can inhibit the expression of osteogenesis-related genes by inhibiting SATB2 mRNA expression. Hsa-circ-0007292 is most commonly found in the cytoplasm and acts as a sponge for miR-508-3p to promote the progression of OPLL.

CircRNA expression profiles in primary posterior longitudinal ligament cells

Sun et al. (2021) found that circSKIL expression levels were significantly elevated in primary cervical posterior longitudinal ligament cells from patients with OPLL and promoted osteogenic differentiation through the JNK/STAT3 pathway. In addition, the overexpression of circSIKL can also improve the expression levels of RUNX2, ALP, COL I, OCN and OPN, thus proving the role of circSIKL in the ossification of the posterior longitudinal ligament.

The classic pathways in patients with ossification of the posterior longitudinal ligament

The classical pathways of bone mesenchymal stem cells

Xu et al. (2016) demonstrated that miRNA-10a-5p expression activated Runx2 and Ibsp signaling, which promoted osteogenic differentiation (Figure 1). They found that PLL cells can transdifferentiate into an osteoline by Alizarin Red staining. The SP1 transcription factor leads to OPLL through the WNT signaling pathway, and this process is achieved by downregulating miR-520d-3p, miR-4782-3p, and miR-6766-3p to stimulate LEF1 overexpression (Xu et al., 2020). Wang et al. (2016) demonstrated that TNF-α can upregulate the level of miR-150-3p. Moreover, there is a complementary sequence region on the 3′-UTR of β-catenin, which is predicted to bind specifically to miR-150-3p and reduce the level of β-catenin. The following literature provides the new research idea that microRNA delivery by extracellular vesicles plays a crucial role in the formation of OPLL. Chen et al. (2021) found that immunofluorescence showed that mesenchymal stem cells released extracellular vesicles and were entoxified by ligament fibroblasts. Bone marrow mesenchymal stem cell-

TABLE 3 CircRNAs expression profiles in OPLL.

No.	Evaluation method	Sample	Dysregulation (up)	Dysregulation (down)	Signal pathway	References
1	RNA-sequencing RT-qPCR	PBMCs	177 circRNAs	154 circRNAs	—	Jiang et al. (2021a)
2	OPLL tissue	72 circRNAs		74circRNAs	—	Han et al. (2018)
3	qRT-PCR	OPLL tissue	circSKIL	—	Promotes the expression levels of RUNX2, ALP, COL I, OCN and OPN *via* JNK/STAT3 pathway	Jiang et al. (2021b)

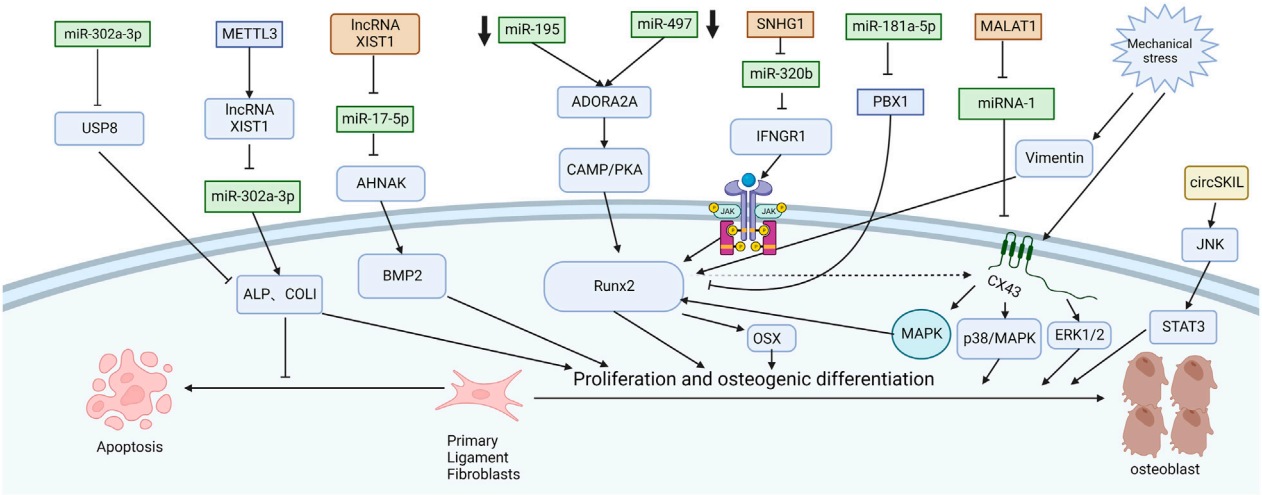

FIGURE 2
Functions of specific ncRNAs in ligament fibroblasts. ("Created with BioRender.com").

derived extracellular vesicles (BMSC-EVs) upregulate miR-497-5p and target the reverse regulation of RSPO2. However, RSPO2 can activate the Wnt3α/β-catenin pathway to inhibit osteogenic differentiation. Therefore, BMSCS-EVS promotes osteogenic differentiation through upregulation of miR-497-5p and then targets downregulation of RSPO2 to reduce the Wnt3α/β-catenin pathway. Moreover, Western blot results showed that the expression of osteogenic genes (BMP2, ALP, Collagen I, Osteocalcin, and Osteopontin) in BMSC-EV-treated ligament fibroblasts was upregulated. This conclusion is different from the well-known function of the WNT signaling pathway. Moreover, Xu et al. demonstrated in more detail one of the mechanisms by which exosomes promote ossification of the posterior longitudinal ligament. They discovered that miR-320e was more abundant in both OPLL cells and exosomes than in PLL. In their study, miR-320e targeted TAK1 and significantly downregulated it, thereby promoting osteogenic differentiation and inhibiting osteoclast formation. Different studies suggest that TAK1 can both promote and inhibit osteogenic differentiation. Therefore, TAK1 is considered to be a bidirectional regulator of early and late osteogenic differentiation.

Classical pathways and functions of mechanical stress in primary ligament fibroblasts

Chen et al. (2016) explored the mechanism of promoting osteogenic differentiation from the perspective of mechanical stress. They established an *in vitro* stress loading model. Connexin 43 (Cx43) was highly expressed in the posterior longitudinal ligament fibroblasts of OPLL patients. The extracellular regulated protein kinase (ERK) 1/2 protein, p38 MAPK protein and JNK protein were significantly increased in both OPLL and non-OPLL cells after mechanical loading. Compared with siRNA interference of Cx43, blocking the ERK1/2 or p38 MAPK pathway alone only partially weakened the osteogenic effect of mechanical stress, while blocking the JNK pathway alone had no significant difference. These results suggest that the ERK1/2 and p38 MAPK pathways mediate Cx43 osteogenesis in ligamentous fibroblasts to some extent, while the JNK pathway has little correlation with Cx43 osteogenesis (Figure 2). Moreover, Yang et al. (2016) found that downregulation of connexin 43 could inhibit the

osteogenic differentiation induced by dexamethasone. This is achieved by inhibiting the activity of RUNX2 and ERK and decreasing the expression of bone genes. Zhang et al. (2014) examined the effects of mechanical stress on vimentin and osteogenic genes by Western blotting and semiquantitative reverse transcription-polymerase chain reaction. They found that mechanical stress-induced vimentin downregulation in ligament fibroblasts further increased OCN, ALP, and COL I expression. Ultimately, it promoted fibroblast ossification, leading to OPLL.

CircSKIL promoted the proliferation and differentiation of primary posterior longitudinal ligament fibroblasts through the JNK/STAT3 pathway. Liao et al. showed that lncXIST was significantly increased in primary human ligament fibroblastic cells from OPLL tissues. This reduced the expression level of miR-17-5p and increased the levels of AHNAK and BMP2, which promoted the proliferation and differentiation of primary posterior longitudinal ligament fibroblasts.

Wang et al. reported that the lncRNA SNHG1 could inhibit the expression of miR-320b to upregulate IFNGR1 by serving as a sponge for miR-320b. Moreover, it activates the JAK/STAT pathway. When the authors silenced SNHG1, the osteogenic differentiation and mineralization of ligament fibroblasts were reduced.

METTL3 inhibited the expression of miR-302a-3p by upregulating lncRNA XIST and ultimately induced the expression of ALP and COL I to promote osteogenic differentiation of primary ligament fibroblasts (Yuan et al., 2021). MALAT1 promoted osteogenic differentiation of primary ligament fibroblasts by inhibiting the inhibitory effect of miR-1 on Connexin-43 (Yuan et al., 2019). After PLL fibroblasts were successfully isolated, Jiang et al. (2020) used siRNA to specifically silence ADORA2A and administered CMS treatment. The expression of osteogenic factors (ALP, OCN, Runx2, COL I) and calcium content were significantly decreased in the si-Adora2A group. Moreover, the cAMP/PKA pathway inhibitor H89 confirmed that ADORA2A could activate the cAMP/PKA pathway. Therefore, the downregulation of miR-497 and miR-195 leads to the elevation of ADORA2A, which then activates the cAMP/PKA pathway to increase the expression of osteogenic factors and promote the formation of OPLL. Liu et al. (2020) first demonstrated that miR-181a-5p, rather than miR-181a-3p, directly targeted PBX1 and ACAN in ligament cells and reduced their levels by a luciferase reporter assay. After miR-181a-5p was inhibited, ALP activity was restored only after PBX1 was knocked out. PBX1 overexpression also downregulated the level of miR-181a-5p. After ACAN was knocked out, the activity of ALP could not be restored after miR-181a-5p was inhibited. These results further demonstrate the indispensable role of PBX1 in the OPLL process and the unique relationship between miR-181a-5p and PBX1. In patients with ankylosing spondylitis, the Wnt/β-catenin pathway is also found to play an important role. Tang et al. (2018) found that miR-124 increased and GSK-3βdecreased in AS ligament tissue. Upregulation of miR-124 significantly reduced the expression of GSK-3β in osteoblast differentiation, weakened the inhibition of Wnt/β-catenin pathway activity, enhanced the expression of Osterix and RUNX2, and promoted osteoblast differentiation of ligament fibroblasts. Some studies have also shown that the Wnt/β-catenin signaling pathway can immediately promote osteoblast differentiation by upregulating the expression of osteoblast-specific genes such as RUNX2 (Kugimiya et al., 2007), Osterix and ALP and downregulating the expression of CCAAT/enhancer-binding protein alpha (CEBPα) and peroxisome proliferator-activated receptor gamma (PPARγ) (Qiu et al., 2007; Cawthorn et al., 2012). It inhibited the differentiation of mesenchymal fibroblasts into adipocytes and indirectly promoted the differentiation of mesenchymal fibroblasts into osteoblasts.

Challenges and perspectives

OPLL is a common spinal surgical condition in which the posterior longitudinal ligament at the posterior border of the vertebral body ossifies and causes a series of clinical symptoms due to compression of the spinal cord or nerve roots under the influence of various related factors. The patient's condition will worsen, especially when subjected to external forces, which seriously affects the patient's daily life. However, OPLL is highly genetically heterogeneous and has a complex formation mechanism, which is still not understood. Currently, there is no effective treatment except surgery. With the advancement of sequencing technology and the rapid development of bioinformatics, the etiology of OPLL and the establishment of therapeutic targets are also flourishing.

The current understanding of the regulatory roles of different lncRNAs, miRNAs and circRNAs in the osteogenic differentiation of OPLL cells can be used to prevent or treat OPLL. For example, some circRNAs act as sponges of miRNAs to inhibit the osteogenic differentiation effect of miRNAs. Alternatively, some ncRNAs that promote osteogenic differentiation are used to treat osteoporosis. However, there is no relevant report on the clinical application of ncRNA, and the safety and efficacy of clinical research should be the focus of future studies. If scholars in the future create large-scale deep phenotypic biobanks and map the characterization of the relationship between genome and disease, this can provide a strong theoretical basis for the study of disease etiology. When the genotype of the relevant potential drug target is presented, the biological rationale of clinical research on the drug will also be determined.

Conclusion

In general, great progress has been made in the study of the regulatory mechanism of ncRNA in the development of OPLL. The deep mining of ncRNA provides a good basis for OPLL research both in predicting the pathogenesis and in treating it.

Although lncRNA and miRNA have been studied the most among ncRNAs, there are still many underlying mechanisms of osteogenic differentiation of OPLL cells that have not been clarified, especially circRNA and siRNA. In summary, ncRNAs mainly regulate the mechanism of OPLL in the following aspects. First, ncRNA can regulate gene expression at the epigenetic level. For example, lncRNAs can promote DNA methylation to promote bone formation. Second, ncRNA regulates OPLL at the transcriptional level, and miRNA-10a-5p expression activates osteogenic transcription factor Runx2 signaling, thereby promoting osteogenic differentiation. Finally, ncRNA can regulate OPLL progression through endogenous competition. For example, lncRNA, as a ceRNA of miRNA, is adsorbed on miRNA as a sponge, thus inhibiting miRNA expression.

In this paper, we reviewed the recent literature on OPLL-related RNA research at home and abroad, analyzed the impact of OPLL on its occurrence and development, and briefly introduced the shortcomings of current research and the latest research trends on OPLL susceptibility genes. We also present a preliminary investigation of the potential ossification mechanism of OPLL and discuss the effects of mechanical stress and exosomes on OPLL.

Author contributions

JD, ZY, and QZ conceived the project and revised the manuscript; HZ and QZ wrote the first draft and drew the figures.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Research advances in roles of microRNAs in nasal polyp

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MicroRNAs (miRNAs), a subset of endogenous RNAs highly conservative with short chains, play key regulatory role in the biological relevant events of the cells. Exosomes are extracellular vesicles like the plasma membrane components being able to deliver information molecules such as miRNA between cells and to regulate the fate of the target cells. The progression of chronic rhinosinusitis with nasal polyps (CRSwNP) is closely associated with significant alterations of miRNA levels in both cells and exosomes. RNA-binding proteins (RBPs) have been acknowledged to play important roles in intracellular miRNA transport to exosomes, and specific membrane proteins such as caveolin-1 critically involved in HNRNPA1 -mediated transport of miRNA to exosomes. Aberrant alteration in endogenous miRNA levels significantly contributes to the process of airway remodeling in the nasal tissue and to the occurrence and progression of inflammatory responses in CRSwNP. Exogenous miRNAs delivered *via* exosomes has also been shown to play an important role in activating macrophages or in regulating vascular permeability in the CRSwNP.This paper highlights the mechanism of RBP-mediated delivery of miRNAs to exosomes and the important contribution of endogenous miRNAs to the development of CRSwNP in response to inflammation and airway remodeling. Finally, we discuss the future research directions for regulation of the miRNAs to CRSwNP.Delivery of exogenous miRNAs by exosomes alters the endogenous miRNAs content in nasal mucosal epithelial cells or in associated inflammatory cells in the CRSwNP, and altered endogenous miRNAs affects the inflammatory response and airway remodeling, which then regulates the occurrence and progression of CRSwNP.RBPs and associated membrane proteins such as caveolin-1 may play a crucial role in the entry of exogenous miRNA into exosomes.

KEYWORDS

microRNA, chronic rhinosinusitis with nasal polyps, RNA binding protein, inflammatory response, airway remodeling, exosomes

1 Introduction

MiRNAs are a set of endogenous single-stranded small molecule RNAs with length of 18–22 nt, non-translatable, and generated *via* two successive steps of processing from primary-miRNAs (pri-miRNAs) to precursors of miRNAs (pre-miRNA) and from pre-miRNAs to mature miRNAs (Lee et al., 2004) (Figure 1). Molecularly, miRNAs regulate gene expression at the transcriptional and post-transcriptional levels, whereas biologically, they are critically involved in almost all the known biological events such as cell proliferation and differentiation, development, reproduction, and aging (Chen and Wang, 2012; Gerasymchuk et al., 2020; Wang et al., 2021). Aberrant alterations of the miRNA abundance and the species often initiate pathogenesis of many diseases, initially making it plausible to speculate that the miRNAs could become the potential biomarkers translatable to clinic therapy for many kind of diseases. Indeed, so far many miRNAs have been acknowledged as the key regulators implicated in various disease such as cancers and neurological disorders, some of which have been developed as therapeutic targets for some stubborn diseases (Sun et al., 2018; Peng et al., 2020).

The abundance of miRNAs in cells is dynamically regulated in accordance with physiological conditions and development stage. Aberrant alteration of miRNAs may significantly contribute to initiation and/or progression of diseases. Several mechanisms account for the dynamic alteration of miRNAs abundance. The common one is up-regulation or downregulation of miRNA genes, while the enhanced or blocked miRNA package into exosomes and transportation to outside cells also become factors.

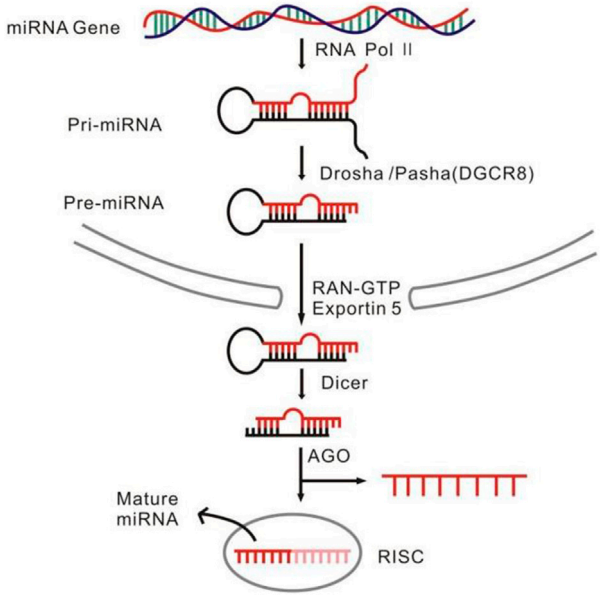

FIGURE 1
The miRNA formation and function process.

The occurrence and progression of CRSwNP is associated with alteration of intracellular miRNA levels, although the differential expression of miRNA was also detected in the nasal lavage fluid (NLF)-EV (extracellular vesicles) of CRSwNP patients (Cha et al., 2021). Some miRNAs can regulate the epithelial-mesenchymal transition (EMT) to affect the airway remodeling of CRSwNP (Liu et al., 2021b), of which is miR-155 that could be regulated by glucocorticoids, commonly serving as clinical therapy (Zheng et al., 2012). Both studies demonstrate that miRNA is involved in the development and progression of nasal polyps at both the phenotypic and molecular levels, suggesting the importance of miRNAs in CRSwNP in exploration of the occurrence mechanism of CRSwNP and clinic therapy.

The CRSwNP patients with the prevalence of 0.5%–4.3% developed clinical manifestations such as unconsciousness, dizziness, memory loss and hyposmia, and is often accompanied by asthma comorbidity and postoperative recurrence (Table 1). The CRSwNP mainly showed Th2 cell inflammation, mainly involving the effects of IL-4, IL-5, and IL-13, accompanied by changes in IL-25, IL-33, TSLP and IgE levels, with eosinophil infiltration and nasal polyps phenomenon (Fossiez et al., 1996). Besides, CRSwNP is associated with elevated levels of eosinophils, innate lymphoid cells (ILC2), macrophages and mast cells (Lee et al., 2021). NP is characterized by epithelial cell alterations, epithelial mesenchymal transformation, goblet cell hyperplasia, extracellular matrix degradation, fibrin deposition, and tissue edema.

2 The function of inflammatory cells in the CRSwNP

One of the important pathological features exhibited by CRSwNP is inflammatory cell infiltration. Infiltrating inflammatory cells include nasal epithelial cells, ILC2, eosinophils, mast cell, macrophage and Th1/Th2/Th17/Treg cell, mostly Th2 cells. The inflammatory cell infiltration is accompanied by changes of cytokines and chemokine expression. Nasal epithelial cells are capable of regulating the development of Type II inflammatory response by releasing TSLP, IL-33 and IL-25. Activated ILC2 leads to eosinophilia mainly by releasing IL-5, whereas release of IL-13 promotes airway hyperresponsiveness, goblet cell hyperplasia, mucus production, and DC activation. Th2 cells releases IL-4, IL-5, and IL-13 participates in the inflammatory response, and eosinophils releases the chemokine CCL23 to recruit M2 macrophages to release chemical mediators, such as histamine, to induce mucosal oedema and local inflammation. Activated macrophages release proinflammatory factors and

TABLE 1 Introduction of the CRSwNP.

CRSwNP	
prevalence	0.5%–4.3%
clinical manifestation	unconsciousness,dizziness,memory loss and hyposmia
Inflammation type	Th2 cell inflammation
Associated inflammatory substances	IL-4, IL-5 and IL-13, accompanied by changes in IL-25, IL-33, TSLP and IgE levels
Related cells	Nasal epithelial cells, ILC2, eosinophils, mast cell,macrophage,Th1/Th2/Th17/Treg cell
Tissue feature	EMT, airway remodeling, fibrin accumulation,Increased mucus,polyp
Disease feature	Asthma comorbidity and postoperative recurrence

recruit inflammatory cells to participate in the inflammatory response. The Th1/Th2/Th17/Treg cell balance directed towards Th2 mediates the progression of the type 2 inflammatory response.

2.1 Nasal epithelial cells

Nasal epithelium cells in CRSwNP are able to release IL-25, IL-33 and TSLP by which are able to activate type 2 innate lymphoid cells (ILC2) and to increase the production of type 2 cytokines (Fujieda et al., 2019). At the same time, TLSP can activate dendritic cells (DCs) to present antigen and costimulatory signals, and differentiate naive T cells into effector Th2 cells, causing the release of type 2 cytokine to increase (Patel et al., 2019). Type II inflammatory response can damage the epithelial cells of the nasal mucosa, improve the nasal mucosal epithelial permeability, and decrease mucosal barrier function, basement membrane thickening, stromal fibrosis, epithelium to mesenchymal transformation (EMT), airway remodeling and other phenomena.

2.2 ILC2

Epithelial-derived cytokines as well as other biological mediators such as lipid mediators enable to activate ILC2. The activated ILC2 can release IL-4, IL-5, IL-8, IL-9, IL-13, GM-CSF, and Th2 cells to enhance type 2 immune responses. Releases of IL-5 and IL-13, but only the IL-5 by the activated ILC2 could raise eosinophil levels in contrast to IL-13 that leads to airway hyper-response (AH), increasing the number of goblet cells and enhancing mucus secretion. Furthermore, IL-4, IL-5 and IL-9 released by the activated ILC2 could induce B cell proliferation and IgE production. By contrast, IL-8 and GM-CSF released by ILC2 activate neutrophils and macrophages (Poposki et al., 2017; van der Ploeg et al., 2020). IL-9 released by IL2 could maintain ILC2 survival in polyp tissue and increase the levels of mast cells. ICL2 releases cytokines, such as IL-2, IL-4, and IL-13, and induces Th2 cell proliferation and promote the occurrence of dimorphic inflammatory response (Li et al., 2021).

2.3 Th2 cells, together with eosinophils

DCs stimulate the differentiation of naive T cells into effector Th2 cells, increasing the secretion of type-2 cytokines such as IL-4, IL-5, and IL-13 (Fossiez et al., 1996). While IL-4 promotes IgE production (Punnonen et al., 1997), IL-5 is able to induce the production of eosinophilic extracellular traps (Hamilos et al., 1995). The chemokine eosinophils (Cho-C motif) ligand (CCL) 23 recruits macrophages and differentiate into M2 macrophages. CCL23 released by eosinophils recruits macrophages and differentiates into M2 macrophages (Poposki et al., 2011).

2.4 Mast cell

Activated mast cells release chemical mediators, such as histamine, to induce mucosal oedema and local inflammation. Upon stimulation with TSLP, IL-33, and IL-1, mast cells secrete IL-3 and IL-13 (Saluja et al., 2016). Both IgE and mast cells numbers increase in eosinophilic CRSwNP, and IgE is involved in regulating the regulation and activation of mast cell degranulation. Allergens were found to be able to bind to specific IgE on CRSwNP mast cells, activate mast cells and release a variety of pro-inflammatory mediators and cytokines such as prostaglandin D2, prompting the development of an inflammatory response (Cao et al., 2014).

2.5 Macrophage

Activated macrophages not only release the proinflammatory cytokines TNF and IL-1, but also could differentiate into M2 macrophages thereby releasing paclitaxel and chemokine CCL18 and recruiting eosinophils, Th2 cells and myeloid dendritic cells involved in inflammatory cell infiltration and inflammatory response of CRSwNP (Peterson et al., 2012).

2.6 Th1/Th2/Th17/treg cell

The traditional view is that Th0 cells from CRSwNP patients tend to differentiate into Th2 cells stimulated by inflammatory factors, disrupting the original Th1/Th2 cell balance and mediating the occurrence of type 2 inflammatory response (Omori and Ziegler, 2007). Recent studies have found that the Th17/Treg cell balance has an important role in the occurrence and progression of the inflammatory response. CD4 + T cells differentiate into Th17 cells in the presence of inflammatory factors, and the released IL-17 is able to trigger an inflammatory response (Fossiez et al., 1996), both of which can differentiate into Treg cells and play an immunosuppressive function in the absence of inflammatory factors and TGF-β (Fontenot et al., 2017). The development of Th17/Treg balance towards Th17 is considered an important feature of the inflammatory response as well. More importantly, the extended Th1/Th2/Th17/Treg cell pattern could be essential to understand inflammatory mechanisms at the molecular level.

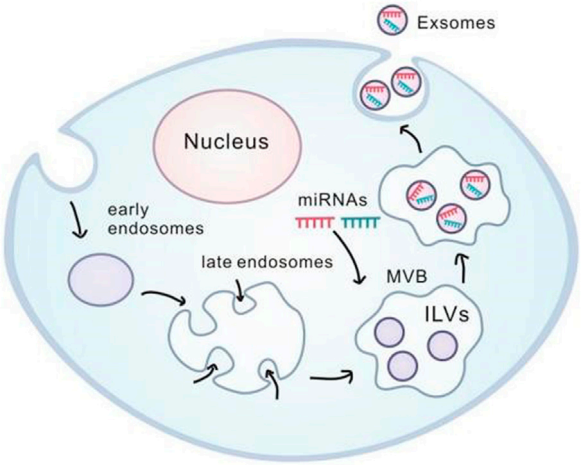

FIGURE 2
Generation and internalization of exosome.

3 Exosomes

3.1 Structure and components of the exosomes

Exosomes are membrane-bound vesicles with a similar structure to the plasma membrane, containing miRNA, mRNA, and proteins and being capable of transferring from the original cells to the corresponding target cells *via* body fluids (Jia et al., 2017). It has been confirmed that the nasal lavage fluid contained exosomes and the components have been identified to be surface markers CD9, CD63 and CD81 using flow cytometry (Lässer et al., 2011). These findings are to compare the difference in exosome components in nasal lavage fluid between normal persons and CRSwNP patients.

3.2 Generation and exportation of exosome

Exosomes are microvesicles with diameter in 30–100 nm and morphologically with a "dish" morphology consisting of a lipid bilayer. Their precursors are luminal vesicles (ILVs) in cellular polyvesicles (MVBs), and after MVB fusion with the cell membrane the precursors are released into the extracellular form of exosomes.

Exosomes contain receptors and transmembrane proteins on the cell membrane, and their formation starts as early endosomes formed by cell membrane depressions. Following that, they gradually become a late endosome, budding into multiple vesicles (MVBs), and selectively transporting intracellular proteins and specific miRNA to the luminal vesicles (ILVs). Subsequently, the multi-vesicle bodies fuse with the cell membrane to release luminal vesicles outside the cell and thereby become exosomes. Exosomes secreted by cells can be transported through extracellular fluid such as serum, lymph and taken up by target cells, accordingly causing biological consequences (Jia et al., 2017). However, the involvement of exosomes in signaling between inflammatory cells, as well as the migration of nasal polyps and inflammatory responses has been less explored. It is plausible to speculate that the understanding exosomes-mediated intercellular signaling may provide essential information for pathogenesis and clinical therapy for the nasal polyps.(Figure 2)

3.3 Differential expression of miRNA in CRSwNP

Significant difference in exosomes miRNA levels were detected between normal persons and CRSwNP patients. In the study of Xuan and Xia, the levels of miR-155, miR-375, miR-671-3p, and miR-92a-3p were associated with CRSwNP development *via* the same controls (Xia et al., 2015; Xuan et al., 2019). Analysis on GO enrichment and KEGG pathways showed that 192 of miRNA expression was significantly downregulated, but no substantial upregulation was observed in CRSwNP relative to normal control (Yu et al., 2021). Therefore, it could be concluded that the occurrence of CRSwNP is associated with the downregulation of miRNAs, suggesting that miRNAs with significant alteration in the expression levels could contribute to physiolpathology and be potentially developed as therapeutic targets for clinical therapy of CRSwNP patients.

4 Alteration of miRNAs levels in the inflammatory cells in response to inflammation stimuli

CRSwNP mainly showed an inflammatory response in Th2 cells, along with the phenomenon of eosinophil infiltration. The level of the inhibitory cytokine IL-10 was able to affect CRSwNP progression, and the TNF-α expression is also important for CRSwNP progression. Some miRNA were found to influence the occurrence and progression of nasal polyps by regulating the levels of IL-10 or TNF-α.

4.1 The miRNA-21 was able to increase the IL-10 levels in the nasal epithelial cells to inhibit the inflammatory response

The miR-21 functions as negative feedback regulator for the CRSwNP *via* reducing the levels of proinflammatory cytokines. By targeting to PDCD4 mRNA, the miR-21 inhibited PDCD4 translation, thereby reducing the P65 phosphorylation of NF-kB and consequently repressing the inhibitory effect of nuclear IL-10 gene expression and elevating IL-10 levels. IL-10 could inhibit the expression of proinflammatory cytokines such as IL-25, IL-33, TSLP, IL-1, IL-6 and IL-8, thus inhibiting the progression of inflammatory response (Liu et al., 2021a). Therefore, the miR-21 confers anti-inflammatory effect *via* directly elevating the level of IL-10 targeting to the miR-21/PDCD4/NF-kB pathway thereby downregulating the expression of inflammatory factors.

4.2 Elevation of miR-142-3p level enhances TNF-α expression in nasal epithelial cells and exaggerates the inflammatory response

Co-elevation of both miR-142-3p and TNF-α levels were observed in human nasal epithelial cells upon stimulation with LPS in a way that the abundance of both miR-142-3p and TNF-α were positively correlated. It has been acknowledged that co-upregulation of both miR-142-3p and TNF-α in nasal epithelial cells could enhance the inflammatory response. The related molecular mechanism is the induced expression of TNF-α by the elevation of miR-142-3p promotes the release of IL-6, IL-10, and IFN-γ from monocyte macrophages and eosinophils, whereas induced release of NF-kB, C-fos and C-Jun subsequently regulates the inflammatory response, and *vice versa* (Yang et al., 2018). Therefore, it could be speculated that miR-142-3p might be involved in the inflammatory response through the LPS-TLR-TNF-α signaling pathway (Qing et al., 2021).

4.3 The miRNA-19a down-regulates IL-10 levels in peripheral dendritic cells and promoted the inflammatory response

In response to TLSP, peripheral dendritic cells (DCs) activate and present antigen and costimulatory signals to differentiate naive T cells into effector Th2 cells, while secrete IL-10 to reduce the inflammatory response. Significantly upregulated level of the IL-4 due to elevated miR-19a levels in CRSwNP patients could suppress IL-10 expression in dendritic cells. Furthermore, IL-4 suppresses the transcription of the IL-10 gene in response to HDAC11, the gene transcriptional repressor (Luo et al., 2017), thus significantly increasing the levels of miR-19a and IL-10 in DC cells of CRSwNP patients (Simpson et al., 2014).

5 Alteration of miRNAs in CRSwNP effects airway remodeling

5.1 Airway remodeling in the CRSwNP

In CRSwNP, airway remodeling is mainly characterized by fibrin accumulation, increasing mucin-containing mucus and epithelial mesenchymal transition. The increase of coagulation factor expression and decreased fibrinolytic activity in nasal tissue induced the accumulation of fibrin in nasal polyps. Th2 cytokines in CRSwNP can inhibit t-PA expression in nasal epithelial cells and inhibit fibrin degradation, resulting in edema eosinophilic nasal polyps in type 2 inflammation, and the increase of coagulation factor XIIIa in type 2 nasal polyps contribute to fibrin accumulation as well (Takabayashi et al., 2013a; Takabayashi et al., 2013b). Type 2 inflammatory cytokines such as IL-4, IL-13, IL-8, and IL-33 in CRSwNP patients enhanced expression of MUC5AC in nasal polyp-derived epithelial cells (Zhang et al., 2019). In response to IL-13, the expression of anion transporter pendrin protein was increased in the epithelial cells of CRSwNP, leading to the increased mucus production (Seshadri et al., 2015). EMT causes loss of cell connectivity and polarity in CRSwNP tissues, conversion of nasal epithelial cells to mesenchymal cell phenotypes, downregulation of epithelial markers expression such as e-cadherin and SIRT1 and upregulation of mesenchymal markers expression such as-SMA and MMP (Thiery et al., 2009). Of the markers, MMP can degrade specific extracellular matrix (ECM) components. Indeed, significant elevation of MMP2 and MMP9 levels observed, while the TIMP1 and TIMP4 levels are significantly attenuated in CRSwNPs, which is closely related to the occurrence of polyps in CRSwNP (Li et al., 2010).

TABLE 2 Effect of some miRNA on the immune response and airway remodeling.

Effect of the inflammatory cell miRNA levels	
Inflammatory response	The miR-21/PDCD4/NF-kB pathway regulates IL-10 together with pro-inflammatory factor levels
	miR-142-3p enhances TNF-α levels through the LPS-TLR-TNF-α signaling pathway to promotes the inflammatory response
	The miRNA-19a decreased IL-10 levels in peripheral dendritic cell promoted the inflammatory response
Airway remodeling	Increasing miR1555p levels promote EMT
	Regulation of miR-21 levels or affecting a portion of the TGF-β1-miR-21 -PTEN-Akt axis is important for the treatment of CRSwNP
	miR29b3p promotes a-tubulin deacetylation-mediated tissue remodeling

5.2 Airway remodeling-related miRNA in CRSwNP

5.2.1 Upregulation of miR1555p promotes EMT

Elevation of miR155-5p levels in nasal epithelial cells caused by enhanced TGF-β1 levels in CRSwNP patients could more efficiently target to SIRT1, participate in EMT progression in epithelial cells and affect the airway remodeling process in nasal tissue (Yang et al., 2020). This is further evidenced by the factor that a miR155-5p inhibitor can attenuate the effect on SIRT1 and subsequently inhibit EMT, thus surpressing the polyposogenesis of CRSwNP.

5.2.2 TGF-β1 induces the nasal epithelial cell-mesenchymal transformation *via* miR-21

TGF-β1, a representative cytokine in tissue remodeling, could activate EMT signaling to initiate tissue remodeling of the airway epithelium and nasal tissue, leading to the altered epithelial cell morphology and upregulation of mesenchymal protein and miR-21 expression. Previous studies have observed significant upregulation of both TGF-β1mRNA and miR-21 in CRSwNP patients relative to normal control group. Consistently, the miR-21 inhibitor and an Akt-specific inhibitor could inhibit TGF-β1-induced EMT, whereas the reduction in miR-21 abundance was accompanied by elevation of the PTEN levels and decrease in Akt phosphorylation (Li et al., 2019). Therefore, it is reasonable to suggest that miR-21 or the TGF-β1-miR-21-PTEN-Akt axis may potentially developed as targets for clinical therapy of CRSwNP.

5.2.3 miR29b3p promotes α-tubulin deacetylation-mediated tissue remodeling

MMP9 is able to bind to integrin proteins and regulate α-tubulin acetylation and deacetylation. Acetyl-α-tubulin could affect cell morphology and got involved in the formation of nasal polyps in CRSwNP. MiR29b-3p and TMP1 play counterpart roles in terms of MM9 expression regulation in ways that miR-29b-3p stimulates expression of MMP9, while TIMP1 reduces MMP9 abundance. miR29b3p affects acetyl-α-tubulin levels by enhancing the interaction between MMP9 and integrin 1, the lack of which is associated with epithelial mesenchymal transformation induced by TGF-β (Liu et al., 2021b). Thus, regulation of α-tubulin acetylation levels by miR-293p could be another promising strategy for the treatment of nasal polyps (Table 2).

6 The miRNA is secreted to the exosome machinery

6.1 The role of RNA-binding protein in miRNA secretion

Cells selectively transport miRNA into exosomes, and the proteins involved in this process mainly include RNA-binding proteins such as hnRNPA2B1 with Argonaute-2, as well as exosome assembly-synthesized membrane proteins such as caveolin-1 and sphingophospholipase 2. RNA-binding protein (RBP) is a class of proteins that can specifically bind RNA molecules to mediate their entry into exosomes. Studies have found significant differences in concentrations between the intracellular miRNA and the secreted miRNA, indicating the essential role of RBP in miRNA entry into exosome.

6.2 The RNA-binding protein involved in the regulation of miRNA secretion to the exosomes

A number of RBPs have been characterized to be associated with the secretion of miRNA into exosomes, including hnRNPs, Ago2, YBX-1, MEX3C, MVP, and La proteins. hnRNPs play diversified roles in regulating transcriptional and post-transcriptional gene expression, Including RNA splicing, polyadenylation, capping, modification, export, localization and translation. hnRNPs bind to short RNA single strands through specific domains. For example, hnRNP-q contains a N-terminal domain that recognizes the GGCU sequence highly affinitive to the miRNAs. The miRNA binding domain in hnRNP-q could initiate and regulate transportation of miR-3470a and miR-194-2-3p into

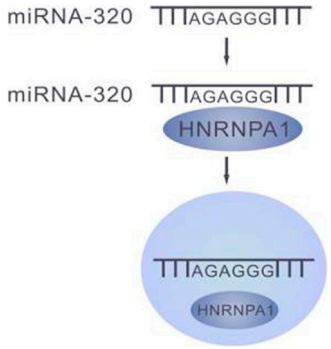

FIGURE 3
HNRNPA1 mediates transportation of the miRNAs with AGAGGG motif into the exosomes.

exosomes (Santangelo et al., 2016). In consideration of the essential roles of hnRNP-q, hnRNP-q downregulation could repress miRNA transportation into exosomes, leading to accumulation of miRNA in the cytoplasm.

Like hnRNP-q, ARGONAUNT2 (AGO2) is highly affinitive to miRNAs and can serve as carrier for transportation of miRNAs into exosomes. The AGO2 mediated miRNAs transportation is regulated by the KRAS-MEK-ERK pathway *via* alteration of the AGO2 phosphorylation levels. This was confirmed by the elevated miR-100 content of hyperphosphorylated KRAS mutations *in vitro* (Cha et al., 2015). The RNA-binding domain of YBX-1 enables it to interact with miR-133 and miR-223 for transportation of both miRNAs into exosomes. To provide evidence, reducing YBX-1 expression levels in H/R EPC reduced the abundance of miR-133 in exosomes, while the miR-133 abundance in the cytoplasm remains largely unaltered (Lin et al., 2019). Unlike hnRNP-q and AGO2 with miRNA binding domains for miRNA transportation into exosomes, it was found that MEX3C is not a direct target of miR-451a during miR-451a transportation to exosomes. The RBP MEX3C was involved in forming the MEX3C-Ago2 complex, mediating miR-451a entry into exosomes (Lu et al., 2017). This finding suggests the two RBP involved participation in the process of miRNA transport into exosomes, providing new perspectives on miRNA transport. Another RBP, Dome principal protein (MVP) is involved in the transport of RNA from nucleus to cytoplasm and from cytoplasm to exosomes. MVP overexpression in CT26 colon cancer cells leads to decreased abundance of miR-193a in the cells and increased levels in exosomes, suggesting the important role the MVP plays in the miR-193a transportation to exosomes. The La protein, a transcription factor of the RNA polymerase III and a RBP, was found to play important role in transportation of some miR-122 into exosomes. Downregulation of the La content in the cytoplasm would cause a decrease in miR-122 abundance in exosomes and *vice versa* (Temoche-Diaz et al., 2019).

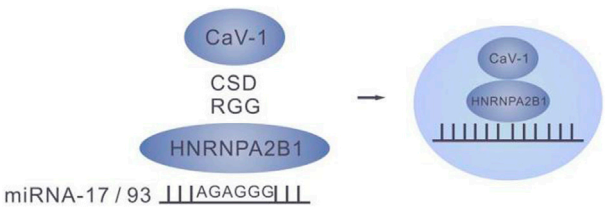

FIGURE 4
The cav-1/HNRNPA2B1 complex.

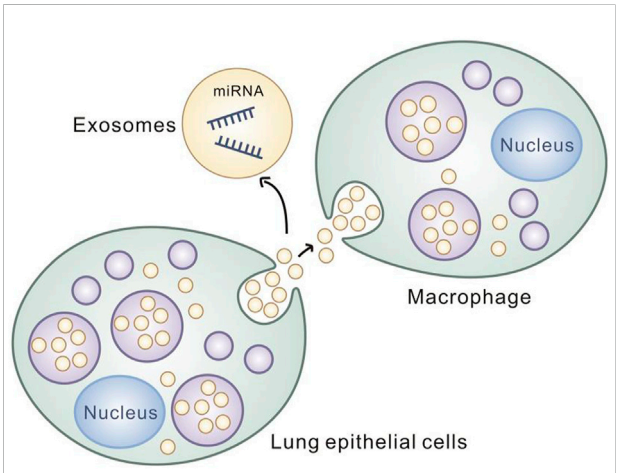

FIGURE 5
Lung epithelial cells transmit inflammatory signals to macrophages through exosomes.

6.3 The HNRNP-A1 recognizes the miRNA containing the AGAGGG motif and allows for delivery to the exosomes

HNRNP-A1 was found to specifically recognize miRNAs that contain AGAGGG motifs such as miR-320 to mediate miRNA secretion to exosomes. Upon knockdown of HNRNPA1, miR-320 level increased significantly in the cytosol, and accordingly dramatic decrease in exosomes, suggesting the essential role of HNRNPA1 in maintenance of some miRNAs accumulation in exosomes (Gao et al., 2019). This further suggests that the synchronously dynamic fluctuation of miRNAs in cells and exosomes is mediated by RNPs. (Figure 3)

6.4 Membrane protein caveolin-1 is involved in HNRNPA2B1 regulation of miRNA transport to exosomes

RBPs, such as hnRNPs, Ago2, YBX-1, YBX-1, MEX3C, MVP and La proteins all bind miRNA and facilitate their

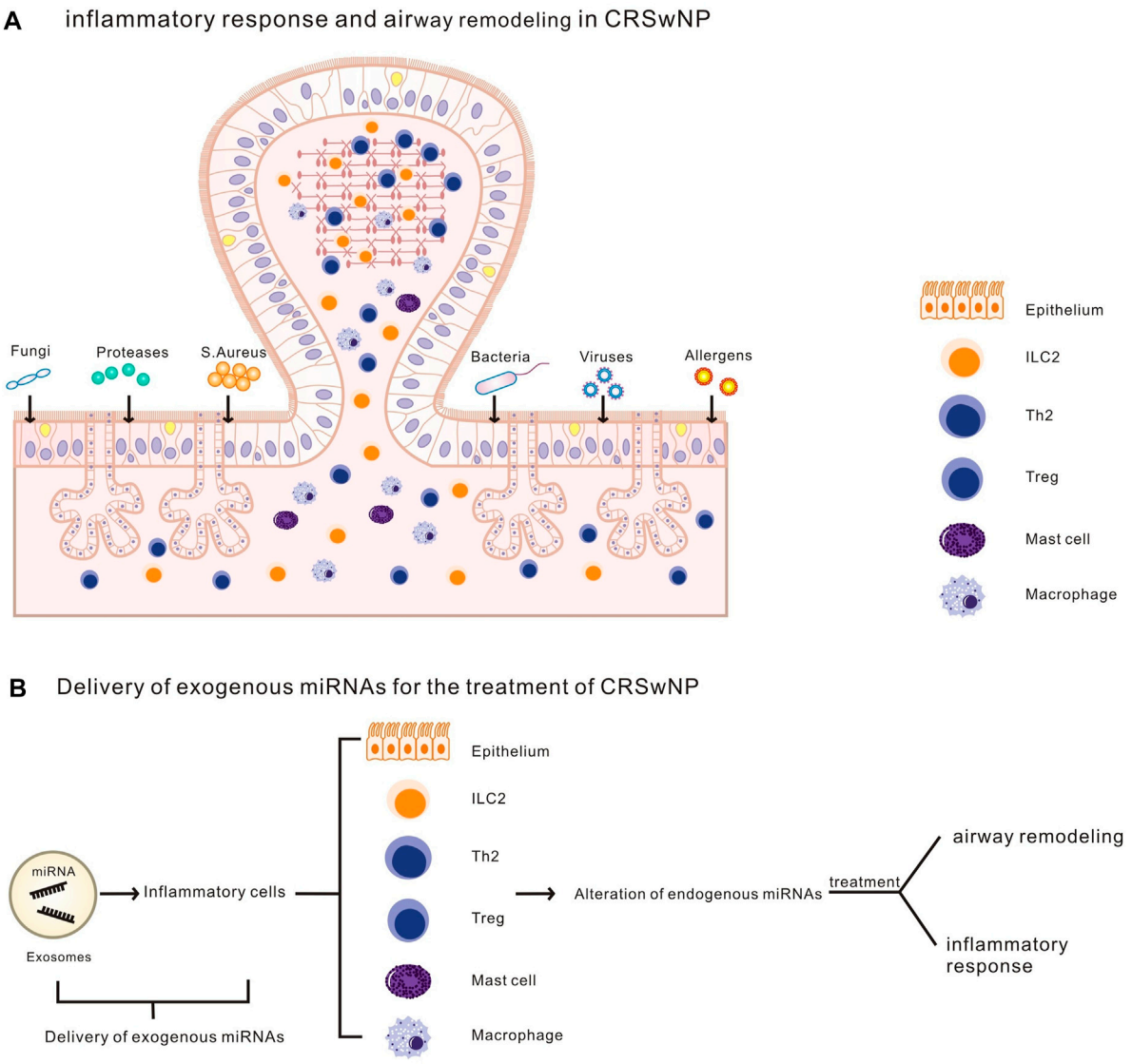

FIGURE 6
(A). Airway remodeling and an inflammatory response in CRSwNP.**(B)**.Exosomes deliver exogenous miRNA to inflammatory cells (including nasal epithelial cells and other CRSwNP-related inflammatory cells), altering cellular endogenous miRNA levels, regulating airway remodeling and inflammatory response in CRSwNP.

transfer to exosomes. The membrane protein caveolin-1 was involved in the RBP- mediated miRNA entry into exosomes. Upon induction of oxidative stress, Y-14 of caveolin-1was phosphorylated in the lung epithelial cells to enable the CSD of caveolin-1 to interact with the RGG of HNRNPA2B1 to form a cav-1/HNRNPA2B1 complex (Lee et al., 2019). Importantly, previous study demonstrated the involvement of membrane proteins in the RBP-mediated entry of miRNA into exosomes.(Figure 4)

7 Effect of exosome-delivered miRNA on target cells

7.1 The miR-22-3p in the CRSwNP exosomes regulates vascular permeability by targeting the VE-calcium adhesion proteins

MiR-22-3p targets mRNA 3′-UTR, coding for VE-calcium adhesion protein. Significant upregulation of miR-22-3p expression

in nasal lavage fluid of CRSwNP patients could downregulate expression of VE-cadherin, leading to abnormal vascular permeability and consequently causing the aberrant tissue edema and nasal polyp growth (Zhang et al., 2020). Different study further supports the conclusion that miR-22-3p in exosomes downregulates expression of the VE-calcium adhesion proteins to regulate CRSwNP occurrence and progression (Gu et al., 2017). Altogether suggests that exosome-delivered miRNA is capable of regulating CRSwNP progression *via* targeting adhesion protein molecules。

7.2 Exosome-delivered miR-17/93 is able to downregulate Irf2bp2 in macrophages to activate them

It was found that miR17/93-containing exosomes released by lung epithelial cells under oxidative stress were able to be ingested by macrophages, and thedelivered miR17/93 was able to reduce Irf2bp2 levels in macrophages. The Irf2bp2 suppresses macrophage activation, and the reduced level of Irf2bp2 in macrophages can activate macrophages and promote macrophage migration and the synthesis and release of cytokines such as TNF and IL-1 (Chen et al., 2015). It is confirmed that exogenously delivered miRNA can regulate the expression level of intracellular related proteins followed by causing the corresponding biological consequences. It is of great significance for miRNA transmission in CRSwNP to explore the occurrence mechanism and to develop clinical therapy for CRSwNP. (Figure 5)

8 Future

CRSwNP is an inflammatory respiratory disease with high global incidence and high recurrence, and limited information for its pathogenesis is available. Nasal epithelial cells, myeloid dendritic cells, ICL2 cells, eosinophils, mast cells, macrophages and Th2 cells are all involved in the inflammatory process of CRSwNP. The new Th1/Th2/Th17/Treg cell pattern opened a novel angle towards the mechanism of inflammatory response and progression.

Changes in cellular endogenous miRNA content have important effects on both inflammatory cytokine production and airway remodeling in CRSwNP. Regulation of intracellular miRNA levels has significant potential in treating CRSwNP and studying the pathogenesis of CRSwNP.

Given that the exosomes could be isolated from the Nasal lavage fluid in CRSwNP patients and as an easy way miRNAs could be profiled to identify and characterize, the exosomes and the contained miRNAs have become an efficient tool convenient for investigating the miRNA-mediated pathology for CRSwNP.

Exosome-delivered miR-22-3p in CRSwNP regulates vascular permeability by targeting ve-calcium adhesion proteins, and the exosome-delivered miR-17/93 in lung epithelial cells is able to downregulate Irf2bp2 in macrophages to activate macrophages. Cell-secreted exosomes have been shown to regulate the inflammatory response in the target cells, of which miRNA plays an important role. Extracellular stimulation signals transmitted by miRNA in exosomes can activate or inhibit inflammatory cells, cause intracellular-related biological events such as generation and release of the related inflammatory cytokines, and regulate the occurrence and progression of CRS. The study of miRNA in exosomes provides new angle for the development of novel drugs for therapy of CRSwNP.

Studies on the process of RBP in miRNA transport to exosomes revealed the causes of synchronized changes between endogenous miRNA and foreign miRNA in exosomes. Moreover, RBP is important for the entry of endogenous miRNA into exosomes. Several specific membrane proteins such as caveolin-1 have also been shown to be involved in RBP-mediated miRNA entry into exosomes, miRNA entry processes may involve more intracellular components, and miRNA transport into exosomes with greater research potential.

Altered miRNAs levels have important effects on inflammatory progression and airway remodeling in nasal polyps. Exogenous exosome-delivered miRNAs targets nasal mucosal epithelial cells as well as associated inflammatory cells in CRSwNP, changes their cellular endogenous miRNA content, and regulates the occurrence and progression of CRSwNP by regulating inflammatory responses and airway remodeling. This provides new ideas and methods to study the pathogenesis, treatment and prevention of nasal polyps, as well as to realize the precise treatment of nasal polyps and reduce the recurrence rate of nasal polyps after surgery (Figure 6).

Author contributions

SJ and MC conceived the idea of the study; HZ interpreted the results; NZ wrote the paper; all authors discussed the results and revised the manuscript. All authors agree to be accountable for the content of the work.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Molecular mechanisms of long noncoding RNAs associated with cervical cancer radiosensitivity

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Despite advances in cervical cancer screening and human papilloma virus (HPV) vaccines, cervical cancer remains a global health burden. The standard treatment of cervical cancer includes surgery, radiation therapy, and chemotherapy. Radiotherapy (RT) is the primary treatment for advanced-stage disease. However, due to radioresistance, most patients in the advanced stage have an adverse outcome. Recent studies have shown that long noncoding RNAs (lncRNAs) participate in the regulation of cancer radiosensitivity by regulating DNA damage repair, apoptosis, cancer stem cells (CSCs), and epithelial–mesenchymal transition (EMT). In this review, we summarize the molecular mechanisms of long noncoding RNAs in cervical cancer and radiosensitivity, hoping to provide a theoretical basis and a new molecular target for the cervical cancer RT in the clinic.

KEYWORDS

cervical cancer, long noncoding RNA (lncRNA), radiosensitive, DNA damage repair, apoptosis

Introduction

Cervical cancer is the fourth most common female lower genital tract malignancy, and over 570000 women are diagnosed with cervical cancer each year (Ferlay et al., 2019). The staging system of the International Federation of Gynecology and Obstetrics (FIGO) determines the clinical decision of cervical cancer. Patients with cervical cancer in its early stages (stages I, II) usually undergo surgical treatment, and those who have risk factors will receive adjuvant radiotherapy (RT). Patients with advanced cervical cancer (stage III, IV) or patients with severe complications or surgical contraindications will receive RT as the main treatment (Meng et al., 2018). Approximately three-fifths of all cervical cancer patients undergo RT (Chung et al., 2005). RT has greatly improved therapeutic effectiveness in cervical cancer. However, in the clinic, some patients experience

Abbreviations: RT, radiotherapy; lncRNAs, long noncoding RNAs; CSCs, cancer stem cells; EMT, epithelial–mesenchymal transition; ATM, ataxia telangiectasia-mutated; ATR, ATM and Rad-3 related; NHEJ, nonhomologous end joining; PKM2, pyruvate kinase isozyme type M2; HK2, hexokinase 2; hTERT human telomerase reverse transcriptase.

radioresistance, and those patients often suffer an unfavorable treatment outcome. Hence, enhancing the radiosensitivity of cervical cancer cells is important for patients. Researchers have looked for various strategies to solve this urgent problem. In recent years, researchers have found that long noncoding RNAs (lncRNAs) have a crucial function in regulating cervical cancer radioresistance.

LncRNAs are a class of noncoding RNAs that are longer than 200 nucleotides and have a complex function in regulating biological processes, including cell proliferation, migration, invasion, the cell cycle and apoptosis (Esteller, 2011). Recent studies suggest that lncRNAs interact with DNA, RNA, and protein, thus participating in a variety of cellular processes, including transcription, translation, and posttranscriptional regulation (Huarte et al., 2010). LncRNAs can affect gene expression epigenetically *via* diverse mechanisms: 1) lncRNAs can regulate histone modification and chromatin status to affect gene transcription. 2) lncRNAs can attract transcription factors or repressors to the promoter of a particular gene (Long et al., 2017), 3) lncRNAs can prevent transcription-related proteins from binding to their DNA targets by acting as decoys. LncRNAs can compete with endogenous RNAs to silence target genes by binding to miRNAs and blocking their function (Gutschner and Diederichs, 2012). Many studies have demonstrated that there is an association between lncRNAs and radiosensitivity of malignant tumors (Yang Q. et al., 2019; Tang T. et al., 2019; Yang Z. et al., 2019; Liu R. et al., 2020; Wang et al., 2020). However, there is no summary of the systematic and comprehensive molecular mechanisms of long noncoding RNAs associated with cervical cancer radiosensitivity. Hence, we performed a systematic literature review on lncRNAs and the detailed mechanisms by which they enhance or weaken the radiosensitivity of cervical cancer, and we discuss these topics in seven sections.

Radiation causes DNA damage, inhibits cell proliferation, and induces cellular apoptosis to treat cancer (Yao et al., 2019). The conventional radiobiological principles proposed by Withers are known as the "4Rs", repair of sublethal cellular damage, reoxygenation of cells within the cell cycle, redistribution of the surviving cells and repopulation of cells after radiation (Withers, 1975). Radiotherapy induces tumour cell death by causing cell cycle arrest and leading to DNA damage. Repair of sublethal cellular damage and repopulation of cells after radiation make tumor cells resistant to radiation and decrease radiosensitivity; on the other hand, reoxygenation of cells within the cell cycle and redistribution of the surviving cells decrease the radiosensitivity of tumor cells (Brown et al., 2014).

Numerous studies have shown that lncRNAs are functionally involved in various critical cellular processes that regulate RT in cervical cancer. LncRNAs can modulate DNA damage repair, the cell cycle, and apoptosis and can also influence the redistribution of surviving cells by regulating EMT, cancer stem cells (CSCs) and the reoxygenation of cells by regulating aerobic glycolysis after RT in cervical cancer (Figure 1).

LncRNAs modulate DNA damage repair and the cell cycle

Radiation-induced DNA damage includes base damage, single- and double-strand breaks, and DNA crosslinks caused by direct ionization or free oxygen radical effects (Olive, 1998), DNA double-strand breaks (DSBs) are the most critical. When DNA damage occurs, the cell cycle checkpoint is active, causing a delay in the cell cycle, and providing time to repair DNA damage. Finally, damage that cannot be repaired will induce cancer cell apoptosis. Different DNA repair pathways initiate and repair different types of DNA damage. However, there is also functional overlap between different DNA repair pathways (Mao and Wyrick, 2016). Among these repairs are nonhomologous end joining (NHEJ) repair, homologous recombination (HR) nucleotide excision repair, mismatch repair, and base excision repair (BER) (Sancar, 1995; Hoeijmakers, 2001; Schärer, 2003; Chapman et al., 2012; Krokan and Bjørås, 2013; Mao and Wyrick, 2016). In mammalian cells, ataxia telangiectasia-mutated (ATM) and ATM and Rad-3 related (ATR) are two phosphatidylinositol kinases that are involved in these pathways (Bakkenist and Kastan, 2004). DNA damage and DNA repair pathways are crucial determinants of radiosensitivity.

In mammalian cells, ATM and ATR are two phosphatidylinositol kinases that are involved in DNA damage repair (Bakkenist and Kastan, 2004). When DNA damage occurs, the MRE11-RAD50-NBS1 (MRN) complex first detects damaged DNA and recruits ATM to damage sites (Lee and Paull, 2004). Then ATM is activated by autophosphorylation at Ser 1981 (Bakkenist and Kastan, 2003) and acetylation at K3106 (Sun et al., 2005). Activated ATM can also activate p53-dependent and independent pathways to initiate the downstream protein kinase phosphorylation cascade (Shiloh and Ziv, 2013). In p53-independent pathways, activated ATM phosphorylates downstream targets Chk2 at Thr68 and leading to phosphorylation of Cdc25 at Ser 216. Phosphorylated Cdc25 bind to 14-3-3 proteins and sequestrate Cdc25 in cytoplasm inhibiting activation of cyclin B/Cdk1 by Cdc25 and resulting in G2/M arrest. In p53 dependent pathways, the p53 protein activates downstream genes such as p21 which can inhibit CDK2 resulting in G1 arrest, and 14-3-3, which can inhibit cyclin B/Cdk1, resulting in G2/M arrest. Teng et al. (Teng et al., 2015) revealed that after IR-induced DNA damage, ATM activation activates ATR, and then Chk1 is phosphorylated at Ser345. ATR-Chk1 restricts CDK2 activity and finally cause G1 arrest. Through the above steps, Cell cycle arrest provide more time for DNA damage repair. This will cause radioresistance. Therefore, the use of checkpoint inhibitors to

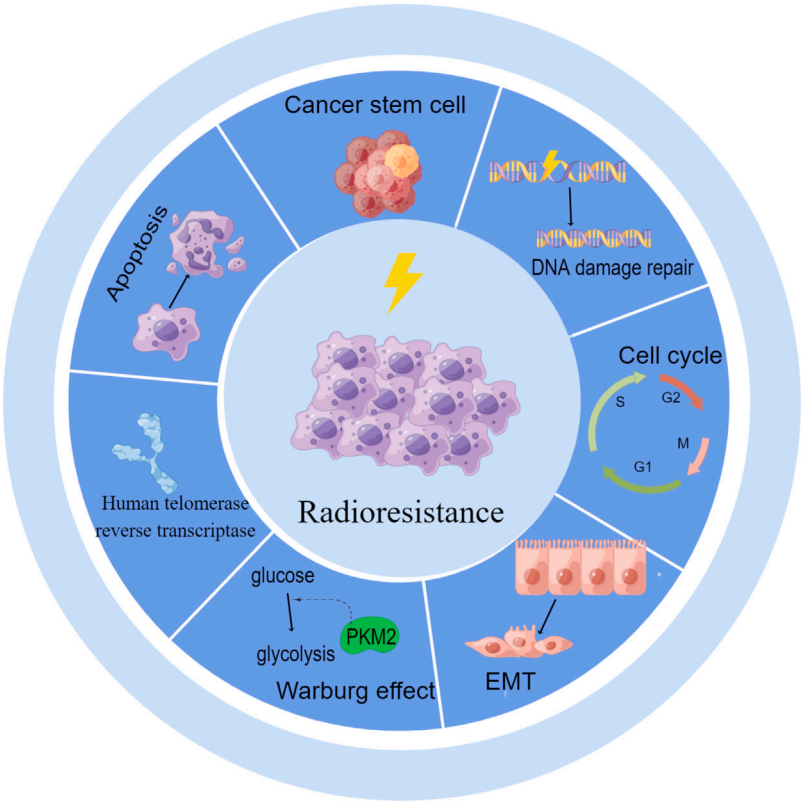

FIGURE 1
LncRNAs regulate radioresistance by DNA damage repair, cell cycle, apoptosis, EMT, CSCs, aerobic glycolysis and human telomerase reverse transcriptase.

improve the therapeutic effects of radiotherapy is a clinical treatment strategy in the future.

ATM has been proved to be connected with therapeutic effects of radiotherapy in cervical cancer. Roossink et al. (2012) recruited 349 advanced stage cervical cancer patients to detect the expression of p-ATM in their pretreatment tissue using immunohistochemical analysis and results revealed that 344 patients (98.6%) had positive nuclear staining and 183 patients (52.4%) had high expression. By analyzing clinical data, they found that high p-ATM expression was related to poor survival. To determine the relationship between p-ATM and the response of cervical cancer to irradiation, they carried out *in vitro* studies and found that the expression of activated ATM was high in Caski cells even before irradiation while in HeLa and SiHa cells was extremely low. And in comparison to HeLa and SiHa cells, Caski cells were significantly more resistant to irradiation. This result reveals us there is a positive correlation between baseline level of p-ATM and radioresistance. Then they proved ATM inhibition led to the destroy of G2/M arrest and finally sensitized tumour cells to radiation. The same result was also reported by. Teng et al. (2015) They use inhibitors of ATR (ETP-46464) (Hickson et al., 2004; Toledo et al., 2011) and ATM (KU55933) to treat Hela and SiHa cervical cancer cells for 15 min before IR exposure and then assessed clonogenic survival. Both ETP-46464 and KU55933 could enhance the response to IR by affecting the expression levels of p-ATM/P-Chk1 and p-ATM/P-Chk2. A compound named Ro 90-7501 can enhance the adiosensitivity of cervical cancer by inhibiting ATM (Tamari et al., 2019), A Chinese herbal medicine Osthole can also inhibited phosphorylation of ATM and enhances irradiation sensitivity of cervical cancer cells (Che et al., 2018).

Many studies showing that lncRNAs can directly act on DNA damage repair-related protein to regulate DNA damage response. We retrieved related literature and found that lncRNAs can regulate the expression of ATM in various tumors, including cervical cancer (Sharma et al., 2020), esophageal squamous cell cancer (Chen et al., 2018; Zhang et al., 2019), pancreatic cancer (Chen et al., 2017), and thyroid cancer (Yuan et al., 2020). Zhao et al. (2020) identified an lncRNA named HITT that maps to 14q32, and contains three exons. LncRNA HITT inhibits ATM activity by affecting MRN. ATM inhibitors may serve as an attractive target to overcome radioresistance in cervical cancer (Figure 2).

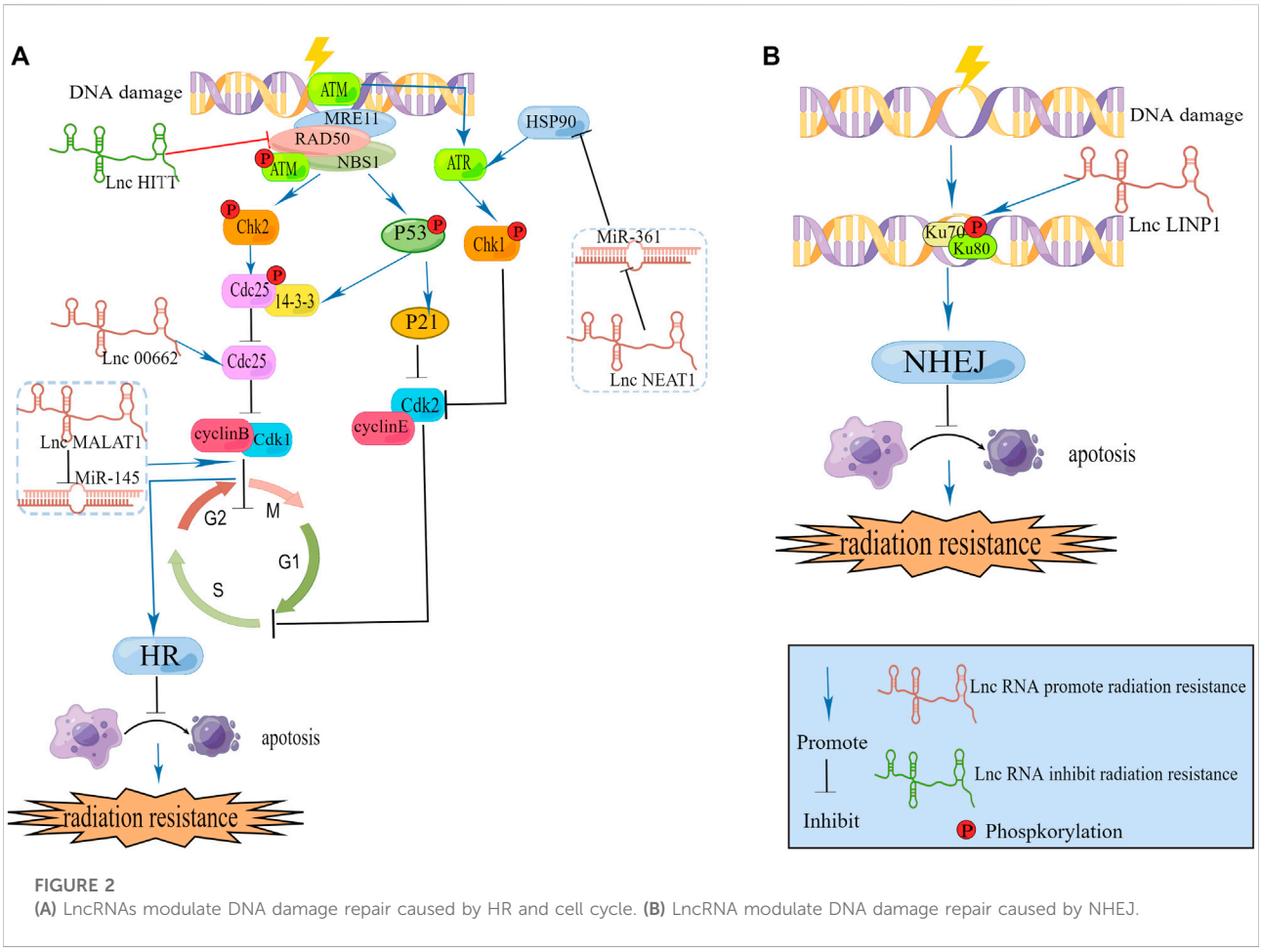

FIGURE 2
(A) LncRNAs modulate DNA damage repair caused by HR and cell cycle. **(B)** LncRNA modulate DNA damage repair caused by NHEJ.

Kyungsoo Ha c (Ha et al., 2011) found in cervical cancer that ATR, which is a *bona fide* heat shock protein (hsp) 90 client protein, is downregulated when hsp90 is inhibited. Additionally, they found that treatment with hsp90 inhibitors decreased both ATR and CHK1 levels, and the hsp90 inhibitor increased radiation-induced DNA damage as well as apoptosis of transformed cells by blocking ATR-CHK1-mediated DNA damage repair signaling. Xu et al. (2020) carried out a luciferase reporter assay and demonstrated that in cervical cancer, miR-361 reduces the expression of hsp90 directly, and lncRNA NEAT1. Directly downregulates the expression of miR-361. From the two above studies, we can establish a link between the lncRNAs NEAT1, hsp90, ATR and radiation-mediated DNA damage repair. We infer that lncRNA NEAT1 could increase the expression of hsp90 and ATR, DNA damage repair, causing radioresistance. Han et al. (2018) have already revealed that NEAT1 can facilitate radioresistance in cervical cancer. They use the luciferase reporter analysis and RNA immunoprecipitation (RIP) assay to prove lncRNA NEAT1 can spong miR-193b-3p as a ceRNA to regulate cyclin D1. More experiments are needed to investigate this hypothesis.

As we mentioned above CDC25 is required for DNA damage repair. LINC00662 is reported to be connected with CDC25A in cervical cancer. Wei et al. (2020) collected 39 samples from patients (cancer samples and adjacent cervical samples) with cervical cancer and found that the expression of LINC00662 was markedly elevated in cervical cancer tissues in comparison with adjacent tissues. They subsequently used two CC cell lines (C33A and Caski) to investigate the function of LINC00662. According to their findings, LINC00662 facilitated cervical cancer progression and radioresistance *via* miR-497-5p adsorption and upregulation of CDC25A. However they did not compare LINC00662 expression between different patients' tumor tissues and collected clinical information to assess the therapeutic effects of radiotherapy in different expression level. Follow-up researchers can supplement the above points if they carry out experiments in this area, which can significantly improve the quality of the article.

LncRNA in nonhomologous end joining pathway 1 (LINP1) is located on chromosome 10p14 and is associated with the NHEJ repair pathway (Thapar et al., 2020). The NHEJ repair pathway is a major DNA damage repair pathway involved in the repair of

DSBs in DNA that are damaged after radiotherapy (Lord and Ashworth, 2012). Wu et al. (2020) collected twenty paired cervical cancer tissues and adjacent normal tissues from Guizhou Provincial People's Hospital (Guizhou, China) to examine each tissue's LINP1 expression level and revealed that CC tissues exhibit higher expression of LINP1 than adjacent noncarcinoma tissues and that high expression of LINP1 can suppress KLF2 and PRSS8 to aggravatecervical cancer development. Similarly, Wang et al. (2018) isolated total RNA from five tissues of multiple patients and then carried out qRT–PCR experiments. In comparison to adjacent tissues, tumor tissues from cervical cancer patients contained higher levels of LINP1. They found that within 30 min of RT, LINP1 translocates into the nucleus, and the protein was upregulated later. We know that RT can cause DNA DSBs and then Ku70–Ku80 heterodimers are recruited and activated by NHEJ. Additionally, by promoting NHEJ protein recruitment to DSBs, Ku80 can bind breaks in DNA. One of the Ku-interacting proteins called DNA-PKCs is a serine/threonine-protein kinase required for NHEJ in human cells; Wang et al. (2018) revealed that LINP1 associates with Ku80 and DNA-PKCs in cervical cancer cell lines. Moreover, LINP1-deficient cells were more prone to radiation-induced cell death and DNA DSBs after RT than control cells. Together, these data indicated that in cervical cancer, the lncRNA LINP1 contributes to radiation resistance by increasing the efficiency of DNA damage repair through the NHEJ pathway (Figure 2).

DNA damage induced by radiation induces DNA repair pathways and alters the expression of cell cycle checkpoint molecules. Affected cells are able to arrest cell cycle progression and repair damaged DNA or undergo apoptosis if the damage cannot be repaired. Radiosensitivity is closely associated with cell cycle arrest, since different phases of the cell cycle exhibit different levels of radiosensitivity, and the G2/M phase is the most radiosensitive phase of the cell cycle (Wilson, 2004). LncRNAs regulate radiosensitivity in cervical cancer by arresting cell cycle growth to interfere with DNA damage repair. G0/G1 phase Han et al. (2018) found that in cervical cancer, the absence of NEAT1 leads to cell cycle arrest in G0/G1, and this change allows cells to undergo apoptosis. This is because NEAT1 binds to miR-193b in a competitive manner to regulate the expression of CCND1, enhancing radioresistance in cervical cancer. In another study, Jiang et al. (2014) investigated lncRNA MALAT1 in cervical cancer and found that MALAT1 downregulation causes cells to enter the G1 phase and that cyclin D1, cyclin E, and CDK6 levels are significantly altered. Other researchers Lu et al. (2016) reported that lncRNA MALAT1 can regulate the cell cycle to influence radiosensitivity. They revealed that lncRNA MALAT1 was negatively correlated with miR-145, which can enhance G2/M phase block. These two studies provide evidence that the function of MALAT1 in cervical cancer is related to the cell cycle. In the future, we can investigate more lncRNAs that are connected with the cell cycle to learn their roles in regulating radiosensitivity in cervical cancer.

LncRNAs modulate apoptosis

Among the mechanisms of irradiation-induced toxicity, apoptosis plays a significant role. Tumor cells that undergo apoptosis display a number of morphological features, including condensed chromatin, shrinkage and fragmentation, blebbing of the plasma membrane, and the formation of apoptotic bodies (Wyllie, 2010). Extrinsic death receptor signals and intrinsic mitochondrial signals are the two main inducers of apoptosis.

To determine the changes in signaling pathways in cervical cancer cells after radiation, (Khalilia et al., 2018) exposed cervical cancer cells to different doses of radiation and revealed that apoptosis, RAS, TGF-β, WNT, the oxidative stress response, and p53 were significant downstream signals. Among these pathways, the p53 pathway is considered to be the most crucial regulator. Accumulating evidence indicates that the p53 pathway participates in proliferation and apoptosis to regulate radiosensitivity in various cancers (Concin et al., 2000; Oei et al., 2015; Granados-López et al., 2021). In p53 dependent apoptosis, p53 promotes apoptosis by activating the transcription of apoptotic factors including Bax, PUMA, Noxa at the same time it can inhibit the transcription of anti-apoptotic factors such as Bcl-2, Bcl-xL, Survivin. On the other hand, p53 can accumulate in the cytoplasm and directly connect to the mitochondria, leading to changes in the permeability of the mitochondrial membrane.

By interacting with p53 pathway factors, LncRNAs regulate radiosensitivity. Under hypoxic conditions, p53 participates in the degradation of HIF-1α (Ravi et al., 2000). Fu et al. (2015) found that HIF-1α increased radiation resistance in cervical cancer cells by inhibiting the expression of p53 and increasing the expression of VEGF. Li N. et al. (2018) found that radiation reduced lncRNA levels and HIF-1α levels in mice bearing HeLa cells as well as in HeLa cells and C33A cells, and they carried out a series of experiments and finally revealed that overexpression of lncRNA HOTAIR induces radiation resistance by increasing the expression of HIF-1α in cervical cancer cells. The lncRNA DINO is a p53 transcriptional target and functional modulator (Sharma et al., 2020). LncRNA DINO can bind to TP53 to stabilize and upregulate transcriptional target genes of TP53. The lncRNA DINO has been found to be a potent tumor suppressor in specific subsets of human and mouse tissues (Marney et al., 2022). However, the relationship between DINO and radiation sensitivity has not been revealed. From previous research, we infer that DINO may have a positive effect on radiosensitivity.

LncRNA GAS5 is reported to connected with cancer cell apoptotic to affect radiosensitivity. Gao et al. (2019) collected twenty cervical cancer patient biopsy tumor samples before RT. These patients had never received any chemotherapy before

radiation therapy. The twenty patients were divided into two groups: 1) radioresistant (9 cases) and 2) radiosensitive (11 cases). Researchers compared the expression of related molecules between the two groups. And found lncRNA GAS5 and miR-106b expression was significantly different between radioresistant tissues and radiosensitive tissues. Then, they used SiHa and ME180 cervical cancer cells to investigate the biological role of GAS5 in the radiosensitivity of cervical cancer. They found that upregulation of GAS5 enhanced radiosensitivity in SiHa cells, while downregulation of GAS5 decreased radiosensitivity in ME180 cells. GAS5 acted as a miR-106b sponge, inhibiting miR-106b and promote IER3 expression increasing cervical cancer cell radiosensitivity both *in vitro* and *in vivo*. IER3 is necessary for cervical cancer cell apoptotic activity by inhibiting the expression of BCL-2 and BCL-xL (Yoon et al., 2009). Fang et al. (2020) illustrated the relationship between GAS5 expression and the overall survival (OS) of cervical cancer patients using Kaplan–Meier analysis. They confirmed that poor OS was associated with low GAS5 expression. Univariate analysis indicated that GAS5 could be an independent prognostic factor for patients with CC. These above studies indicate that lncRNA GAS5 may become an important topic for cervical cancer clinical research, and researchers could detect these lncRNAs to infer the effect of RT in cervical cancer patients.

A majority of miRNAs play key roles in regulating radiation-induced apoptosis. In various cancers, lncRNAs function as miRNA sponges to regulate target gene expression. In recent studies, lncRNAs were reported to regulate radiosensitivity in cervical cancer cells by working as miRNA sponges. For example, in cervical cancer tissues and cells, the lncRNA SNHG6, which is a small nucleolar RNA host gene (SNHG family), was upregulated, knockdown of SNHG6 can improve the radiosensitivity of cervical cancer cells by promoting radiation-induced apoptosis. SNHG6 can directly sponge miR-485-3p.

Researchers have mentioned that in cervical cancer, miR-21 could facilitate cell proliferation by downregulating the expression of programmed cell death 4 (PDCD4) (Yao et al., 2009) as well as in breast cancer. MiR-21 inhibits the expression of p53-regulated genes. In various tumors, including non-small cell lung cancer (Song et al., 2017), and esophageal squamous cell carcinoma (ESCC) (Li F. et al., 2018; Lin et al., 2020), miR-21 has been shown to be related to radiation sensitivity. In cervical cancer, Zhang L et al. (2020) found that lncRNA MEG3 was negatively related to miR-21 to affect cell proliferation and apoptosis. These studies revealed that lncRNA MEG3 may also sensitize cervical cancer cells to radiation treatment by affecting the expression of miR-21, and this hypothesis needs to be proven. There are also many miRNAs that can interact with lncRNAs to affect the radiation effect in cervical cancer.

Above all, lncRNAs are believed to affect cervical cancer cell radiosensitivity through their ability to induce apoptosis. Several mechanisms can be employed to achieve this effect, including affecting signaling pathways such as p53 or miRNA sponges that manipulate the expression of target genes (Figure 3). Hence, we can identify the difference between apoptotic and nonapoptotic cervical cancer cells by detecting noncoding RNAs in the future. Their relationship with RT sensitivity was studied, and a corresponding database was created for further exploration of the relationship between these noncoding RNAs and apoptotic signaling and to identify genes that can regulate the expression of these noncoding RNAs and therefore achieve RT sensitivity regulation.

LncRNAs modulate EMT and CSCs

EMT plays an important role during tumor development. Tumor cells acquire an invasive phenotype when EMT programs aberrant activation. When tumor cells accept radiation, they may acquire stemness and oncogenic metabolism by inducing EMT (Lee et al., 2017). In tumors, there are cells that can self-renewing called CSCs. CSCs are similar to multipotent embryonic stem cells and possess self-renew capabilities (Reya et al., 2001). . Under specific conditions, CSCs and non-CSCs can convert to each other. CSCs play a crucial role in tumorigenesis and metastasis (Ponti et al., 2005). Researchers have shown that CSCs are generated by EMT (Mani et al., 2008). CSCs existence in cervical cancer with CD44+CK17+/sphere-forming and express stemness-related genes (Oct-4, Sox2 and so on) (Feng et al., 2009). In cervical cancer CSCs, EMT marker vimentin is high expression and perform a higher level of radioresistance than normal cervical cancers (López et al., 2012). And in HeLa, high expression of EMT marker Twist can induce EMT and elevate the expression of CD44 which is cancer stem marker of CSCs (Li and Zhou, 2011). These data indicate that there is a tight link between EMT and CSCs.

In cervical cancer, lncRNAs can regulate EMT-related gene and transcription factors (Figure 4). For example, lncRNA NEAT1 can downregulate miR-361, which can inhibit EMT process in cervical cancer cells by targeting on EMT activator HSP90 (Xu et al., 2020). LncRNA ZEB1-AS1, SPRY4-IT1, MALAT1 and TUG1 are high-expression in cervical cancer cells and associated with poor prognosis. Overexpression of ZEB1-AS1 can increase the level of N-cadherin and vimentin and increase EMT promoter ZEB1 to facilitate EMT process (Cheng et al., 2018). Lnc RNA SPRY4-IT1 and HOTAIR can also target on ZEB1 to promote EMT process. SPRY4-IT1 can bind to miR-101-3p to down regulate its target gene ZEB1 (Fan et al., 2019). HOTAIR can inhibit the expression of miR-203 which is negatively correlated with ZEB1. LncRNA MALAT1 has a positive influence on EMT transcription factor snail and promote the EMT process (Sun et al., 2016). Knockdown the expression of lncRNA TUG1 can down regulate EMT-related

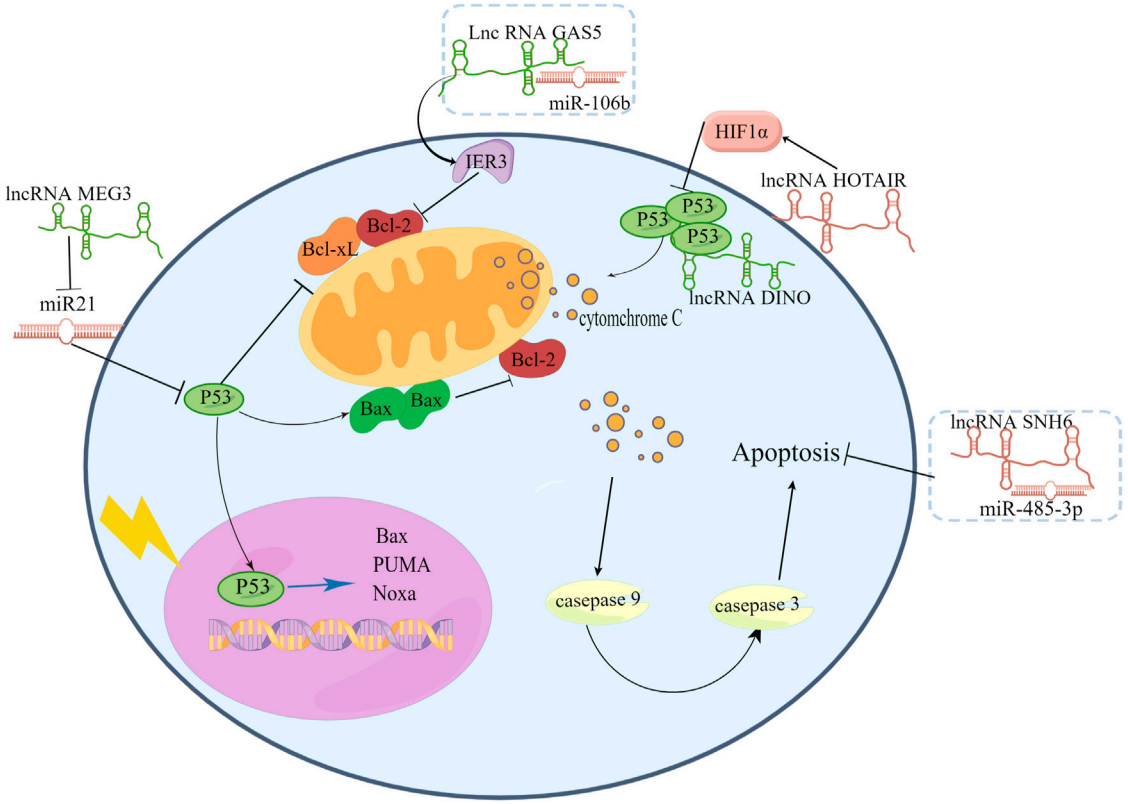

FIGURE 3
LncRNAs affect signaling pathways such as p53 or miRNA sponges to affect cervical cancer cell radiosensitivity.

markers (fibronectin, vimentin, and cytokeratin) (Hu et al., 2017).

LncRNAs are involved in the EMT process of cervical cancer cells by different signaling pathways (Figure 4). LncRNA tyrosine protein kinase transmembrane receptor one antisense RNA 1 (ROR1-AS1) is high-expression in cervical cancer cells and tissues. Downregulate ROR1-AS1 in cervical cancer can inhibit EMT-related markers N-cadherin and vimentin by influencing Wnt/β-catenin signaling pathway (Zhang J et al., 2020). LncRNA-CTS is high-expression in cervical cancer tissues and can bind to miR-505 to enhance EMT process by activating the TGF/SMAD pathway (Feng S. et al., 2019). TNF-α can upregulated lncRNA LOC105374902 by promoting it binding to STAT3. LncRNA LOC105374902 promote EMT process in cervical cancer by sponging miR-1285-3p (Feng Y. et al., 2019).

Increasing evidence indicates that lncRNAs may regulate CSCs in various cancers, including liver cancer (Wang et al., 2015; Ding et al., 2016), colorectal cancer (Tang D. et al., 2019), non-small cell lung cancer (Wu and Wang, 2017). In cervical cancer lncRNA HOXA11-AS has an association of EMT/CSCs. Compared with normal tissues, LncRNA HOXA11-AS is highly expressed in cervical cancer cells and has a negative effect on overall survival. Overexpression of LncRNA HOXA11-AS promotes sphere formation, CSC markers CD133+/CD44+ and self-renewal of cervical cancer cells. Knockdown of HOXA11-AS can downregulate the EMT-related genes (β-catenin, vimentin, snail) and stemness genes (SOX2, Oct-4, Nanog). At the same time, sphere-forming capacity of cervical cancer cells is also decreased. *In vivo*, knockdown of HOXA11-AS can decrease tumor growth. From this study, we predict that LncRNA HOXA11-AS plays an important role in the establishment of EMT/CSCs in cervical cancer cells (Kim et al., 2016). And in gastric cancer cells overexpression of LncRNA HOXA11 can also promote the expression of cancer stem markers including CD44, CD90, CD133, and Bmi1 as well as pluripotency markers Nanog and Sox2 (Wang C et al., 2019). These data indicate LncRNA HOXA11 may be a promising therapeutic target for the treatment of cancers.

According to the cancer stem cell theory, the resistance of cancer to chemotherapy and RT is due to resident CSCs (Wang et al., 2013). Radiation treatment can directly kill the majority of tumor cells by inducing apoptosis. However, a small portion of tumor cells can endure treatment, exhibit radioresistant properties and dedifferentiate. These tumor cells transform into CSCs *via* EMT (Chi et al., 2017). This shift leads to radioresistance in tumor cells, and CSCs can lead to cancer

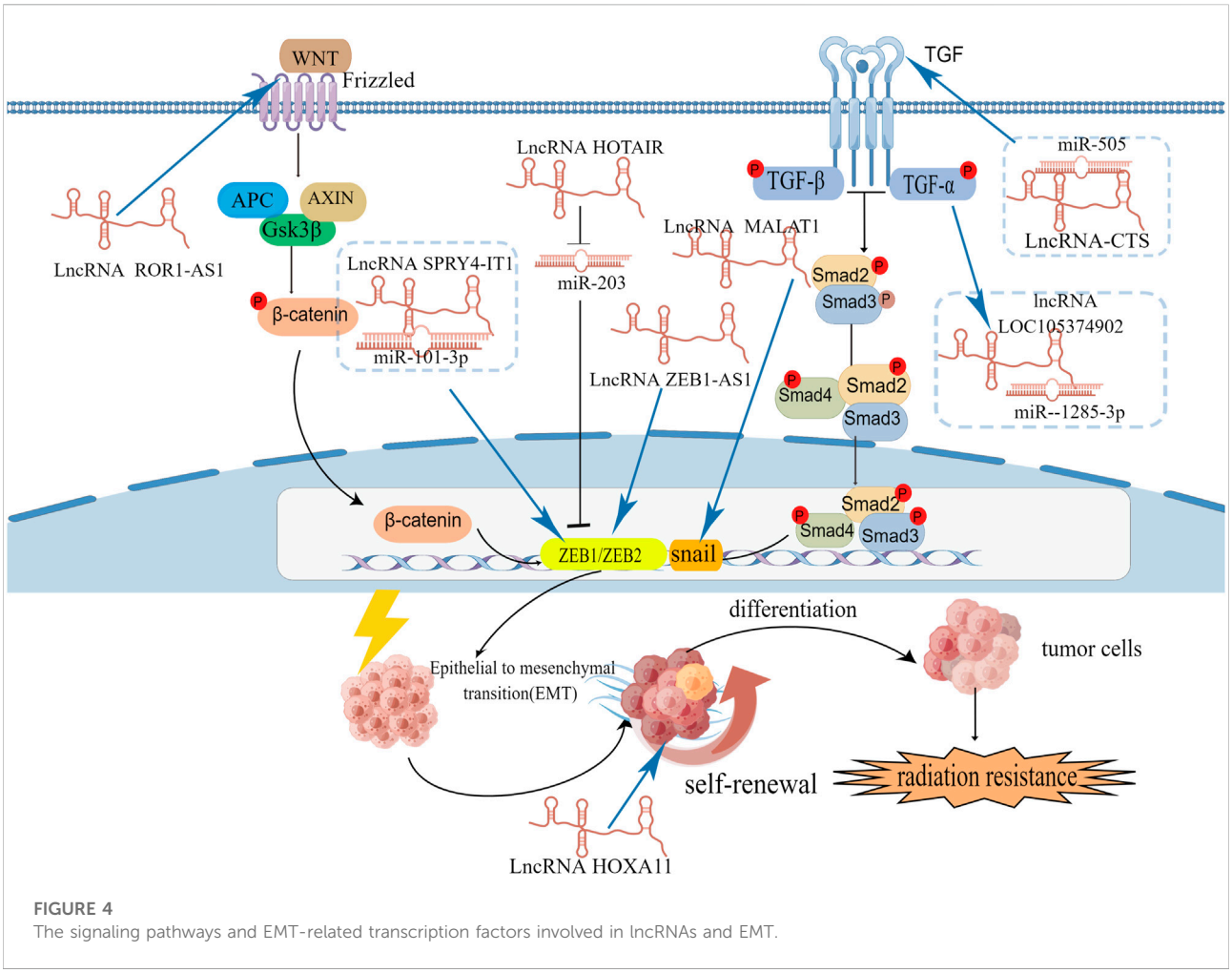

FIGURE 4
The signaling pathways and EMT-related transcription factors involved in lncRNAs and EMT.

recurrence and metastasis. In cervical cancer, high levels of CSCs predict poor outcomes after radiotherapy (Fu et al., 2018). Blocking irradiation-induced CSCs activation may increase radiosensitivity in cervical cancer (Prabakaran et al., 2019). LncRNA HOTAIR has been proved to be necessary for EMT and stemness maintenance of cancer cell lines (Pádua Alves et al., 2013; Łuczak et al., 2016). In breast cancer, overexpression of HOTAIR can downregulate miR-34a expression and at the same time, can promote p53 binding to the p21 promoter and enhance CSC-MCF7(enrich the CSCs subpopulation from MCF7) proliferation, colony formation and migration. Overexpression of HOTAIR can upregulate Sox2, which is the key transcription factor regulate that regulates self-renewal capacity, and epigenetic expression of miR-34a can disturb this regulation (Deng et al., 2017). In cervical cancer, the lncRNA HOTAIR is also connected with stemness acquisition. Knockdown of the expression of HOTAIR can downregulate the expression of six stem cell markers, including NANOG, Oct4, CD44, ALDH1, CD133, and *vice versa* (Zhang et al., 2021). Compared with normal cervical epithelial cells (NCECs), the expression level of HOTAIR was significantly upregulated in cervical cancer cells such as HeLa and C33A cells (Li N. et al., 2018). High HOTAIR expression was correlated with shorter overall survival (Kim et al., 2015). The same outcome has been reported by other researchers. Initially, they used serum samples to detect the different levels of HOTAIR between cervical cancer patients and normal women. They found that circulating HOTAIR levels were significantly higher in cervical cancer patients and connected with a poor prognosis. Then, they performed further research to investigate the biological functions of HOTAIR in cervical cancer. They used four cervical cancer cell lines to detect HOTAIR expression and carried out a series of experiments, such as MTT assays and migration and invasion assays. They finally concluded that HOTAIR acts as an oncogene in cervical cancer and then cultured primary cervical cancer cells from 25 fresh cervical cancer tissues to investigate the relationship between HOTAIR and the response to RT. They demonstrated that the expression level of HOTAIR was negatively correlated with that of P21. By hindering p21 expression in HeLa cells, HOTAIR can induce radioresistance (Jing et al., 2015). This result is consistent

with the results of the above breast cancer studies, HOTAIR is also reported to be connected with radiosensitivity in colorectal cancer (Yang et al., 2016; Liu Y. et al., 2020), pancreatic cancer (Wu et al., 2018), and liver cancer (Zhai et al., 2020). Therefore, HOTAIR may be a potential target against cervical cancer.

However, very few studies have investigated the link between lncRNAs and CSCs in cancer RT, and more *in vitro* experimental data are needed. With suitable experimental methods, *in vivo* experimental data would be more convincing. It may be possible to develop new methods to improve therapeutic effects of radiotherapy in cervical cancer by gaining a deeper understanding of lncRNAs, CSCs and EMT.

LncRNAs regulate aerobic glycolysis

Malignant tumors have a high aerobic glycolysis rate, leading to high lactic acid production, and this phenomenon is called the Warburg effect. Aerobic glycolysis is necessary for cancer cell proliferation (Vander Heiden et al., 2009). Lactate is the product of aerobic glycolysis and is necessary for cancer cell migration and metastasis (San-Millán and Brooks, 2017). In previous studies, it has been found that glycolysis increases radiosensitivity (Quennet et al., 2006; Shimura et al., 2014). The enzyme pyruvate kinase isozyme type M2 (PKM2), which produces ATP, is a rate-limiting enzyme at the end of glycolysis (Wang H et al., 2019). To investigate the role of PKM2 in the RT of cervical cancer, Lin et al. (2019) collected human tissue samples from 94 patients who accepted radiation therapy. According to the response after the completion of RT, 94 patients were divided into two groups: a complete response (CR) and a noncomplete response (nCR) group. They carried out IHC staining to detect the expression of PKM2 in these two groups and found that the expression of PKM2 in the nCR group was higher than that in the CR group, and PKM2 expression was enhanced in cervical cancer cells after radiation. Then, they used HeLa and SiHa cell lines with stable low PKM2 expression to demonstrate that knocking down PKM2 can enhance the radiosensitivity of cervical cancer cells. Luan and Wang (2018) found that in cervical cancer cells, SiHa and Caski, lncRNA XLOC_006390 were related to PKM2. Puckett et al. (2021) summarized the relationship between noncoding RNAs and PKM2. All these studies revealed that lncRNAs may affect the efficiency of RT by influencing the expression of PKM2.

Like PKM2, hexokinase 2 (HK2) is another rate-limiting enzyme during aerobic glycolysis. LncRNA urothelial cancer associated 1 (UCA1) was reported to be connected with HK2 in cervical cancer. LncRNA UCA1 first discovered and researched in bladder cancer as an oncogene (Wang et al., 2008). Fan et al. (2018) explored the role of the lncRNA UCA1 in cervical cancer radioresistance. They establish irradiation-resistant (IRR) cervical cancer cell lines, SiHa–IRR and HeLa–IRR. The expression of UCA1 was significantly increased in SiHa–IRR and HeLa–IRR compared with SiHa and HeLa. To explore the function of lncRNA UCA1 in regulating the therapeutic effects of radiotherapy in cervical cancer, they carried out siRNA knockdown and plasmid overexpression assays. And they revealed overexpression of LncRNA UCA1 lead to radioresistance in cervical cancer. And in SiHa–IRR and HeLa–IRR glycolysis related proteins (HK2 and PKM, HIF-1α) were increased. Then the glycolysis inhibitor 2-dG which compete with glucose for HK2 is used in SiHa–IRR and HeLa–RR to detect the radiosensitivity. 2-dG improves the radiosensitivity of SiHa–IRR and HeLa–IRR. These findings suggest that LncRNA UCA1 affect the effects of radiotherapy by the HK2/glycolytic pathway in cervical cancer cells. The abnormal activation of glycolysis may have a role in cervical cancer radioresistance. Inhibition of glycolysis restored the radiosensitivity of irradiation-resistant cervical cancer cells. Although further research regarding the mechanism underlying the regulatory effects of lncRNAs regulating aerobic glycolysis to affect radiation is needed, existing studies have expanded the understanding of the role of aerobic glycolysis in radioresistance and provided potential novel targets to enhance radiosensitivity in cervical cancer. Targeting glycolysis may provide an improved radiotherapy for cervical cancer.

Other factors regulating RT sensitivity

Human telomerase reverse transcriptase (hTERT) is a subunit of telomerase capable of preserving telomere integrity. Cancer cells contain a highly active promoter of hTERT, whereas most normal cells contain a weak promoter (Shay and Wright, 2011). Barczak et al. (2018) noted that in head and neck cancer cell lines, inhibiting hTERT expression can induce apoptosis and result in G1/S/G2 cell cycle arrest, thus enhancing the response to RT. This study reminds researchers that hTERT may be an important target to improve the efficacy of RT in malignancy. In 2020, Li et al. (2020) reported that in cervical cancer, binding of HMGB3 to the hTERT promoter promotes the transcriptional upregulation of hTERT and promotes cell growth and radioresistance. HMGB3 is the most abundant nonhistone protein in eukaryotes (Nemeth et al., 2006). Researchers have used bioinformatics and experimental analyses of several cell lines and identified an antisense transcript upstream in the human telomerase reverse transcriptase (hTERT) promoter region named hTERT antisense promoter-associated (hTAPAS) RNA. By analyzing The Cancer Genome Atlas (TCGA), they found that the expression of hTERT was negatively correlated with that of hTAPAS (Malhotra et al., 2018). Hu et al. (2015) reported that in esophageal squamous cell carcinoma, lncRNA CDKN2B-AS1 has a positive correlation with hTERT. Therefore, we suggest that in tumor cells, special lncRNAs may affect the efficacy of RT by regulating hTERT.

TABLE 1 LncRNAs and radiotherapy in cervical cancer.

LncRNA	Expression in cervical cancer	Radiosensitivity	Mechanism	References
NEAT1	Increased	Decreased	NEAT1 competitively binds miR-193b-3p to upregulate the expression of cyclin D1	Han et al. (2018)
LINC00662	Increased	Decreased	LINC00662 adsorpt miR-497-5p and upregulation of CDC25A	Wei et al. (2020)
LINP1	Increased	Decreased	LINP1 knockdown enhanced cell apoptosis and delayed repairs of DNA DSBs after radiation	Wang et al. (2018)
MALAT1	—	Decreased	Sponges miR-145 and enhance G2/M phase block	Lu et al. (2016)
GAS5	Decreased	Increased	miR-106b/IER3	Gao et al. (2019)
SNHG6	Increased	Decreased	LncRNA SNHG6 sponges miR-485-3p to release STYX.	Liu et al. (2020a)
HOTAIR	Increased	Decreased	HOTAIR inhibiting p21 in HeLa cells	Jing et al. (2015)
UCA1	—	Decreased	UCA1 regulates radioresistance through the glycolytic pathway by modulating HK2 in cervical cancer	Fan et al. (2018)
PCAT1	Increased	Decreased	Sponges miR-128	Ge et al. (2020)

Conclusion and future perspectives

Radiotherapy is a key treatment modality for cervical cancer, but the efficacy is limited by radioresistance. Emerging research has demonstrated that lncRNAs are associated with radioresistance, and the relationship between them has gradually become a research hotspot. Studies have shown that lncRNAs have potential for use as biomarkers of the response to RT and can modulate the RT response of cervical cancer.

The radiation response of cervical cancer differs greatly among different patients; thus, it is important to predict the response to radiation treatment for each patient. In addition, the overall RT treatment time for cervical cancer patients is usually several weeks. Thus, early identification of patients who are not responding to therapy would provide an opportunity to consider alternative treatment options. This would benefit individualized treatment. However, it is difficult to distinguish different lncRNAs that correlate with only the RT response between responders and nonresponders because in clinical studies, patients usually undergo combined chemoradiotherapy. Fortunately, a limited number of recent cervical cancer studies have shown that lncRNAs indeed exhibit differences in the setting of RT alone. At present, there are few studies on the ability to predict the RT effect of cervical cancer based on lncRNAs, but this will be a hot topic that requires large-scale clinical research.

Studies have focused on the effect of the expression level of lncRNAs on the radiosensitivity of cervical cancer *via* basic experiments. However, the molecular mechanisms underlying radioresistance have not yet been completely elucidated. In this review, we summarized the potential mechanisms of lncRNAs that are associated with radiosensitivity in cervical cancer (Table 1). The role of different lncRNAs in the RT sensitivity of different malignant tumors could be twofold. Some can enhance radiosensitivity, while others may enhance radioresistance. Therefore, it is of great significance to identify abnormally expressed lncRNAs in cervical cancer and to investigate their function and potential related signaling pathways in the response to RT. In this way, researchers can develop more effective radiotherapies and institute individualized treatment.

The study of lncRNAs in RT in cervical cancer remains in the initial stage, and most existing studies have only been carried out *in vitro*; thus, clinical trials are lacking. Moreover, the particular molecular biological mechanisms by which lncRNAs regulate radiosensitivity have not yet been completely described.

Understanding the molecular pathways regulated by lncRNAs should greatly improve the effectiveness of RT in cervical cancer and reveal better treatment strategies. Through summarizing the existing research articles, it was found that lncRNAs mainly affect RT sensitivity by affecting the DNA damage repair, cell cycle, apoptosis, EMT, CSCs, and glycolysis in cervical cancer RT. Recent studies have reported that human telomerase reverse transcriptase may also be related to the effect of RT, which is a very important finding and needs to be verified by more experiments. After recognizing the role of lncRNAs in RT of cervical cancer, we need to conduct *in vivo* experiments to verify these conjectures. We believe that research on lncRNAs in the response to RT will provide new RT sensitization strategies for cervical cancer. Studies on lncRNAs that affect the cervical cancer RT response will provide new

sensitization strategies for cervical cancer RT and lay the foundation for the clinical application of lncRNAs in the future.

Author contributions

SW wrote the manuscript. YW and CW drew the figures by Figdraw. XD and HZ participated in discussions and commented on the manuscript. TX conceived the idea, and directed and critically reviewed the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Development of a novel pyroptosis-related LncRNA signature with multiple significance in acute myeloid leukemia

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Background: Pyroptosis, a programmed cell death (PCD) with highly inflammatory form, has been recently found to be associated with the origin of hematopoietic malignancies. Long noncoding RNA (lncRNA) had emerged as an essential mediator to regulate gene expression and been involved in oncogenesis. However, the roles of pyroptosis-related lncRNA (PRlncRNA) in acute myeloid leukemia (AML) have not yet been completely clarified.

Methods: We collected AML datasets from public databases to obtain PRlncRNA associated with survival and constructed a PRlncRNA signature using Lasso-Cox regression analysis. Subsequently, we employed RT-PCR to confirm its expression difference and internal training to further verify its reliability. Next, AML patients were classified into two subgroups by the median risk score. Finally, the differences between two groups in immune infiltration, enrichment analysis and drug sensitivity were further explored.

Results: A PRlncRNA signature and an effective nomogram combined with clinicopathological variables to predict the prognosis of AML were constructed. The internal validations showed that the PRlncRNA risk score model was an accurate and productive indicator to predict the outcome of AML. Furthermore, this study indicated that higher inflammatory cell and immunosuppressive cells, and less sensitive to conventional chemotherapy drugs were highlighted in the high-risk group.

Abbreviations: LncRNA, Long noncoding RNA; PRlncRNA, pyroptosis-related lncRNA; AML, Acute myeloid leukemia; allo-HSCT, allogeneic hematopoietic stem cell transplantation; HSCs, hematopoietic cells; TCGA, The Cancer Genome Atlas; GTEx, Genotype-Tissue Expression; DEGs, Differentially Expressed Genes; FDR, false discovery rate; OS, overall survival; LASSO, least absolute shrinkage and selection operator; ROC, receiver operating characteristic; ssGSEA, single-sample gene-set enrichment analysis; GSVA, gene set variation analysis; IC50, the half inhibitory centration; RT-qPCR, Real-Time Quantitative PCR; IDA, iron-deficiency anemia; cDNA, complementary DNA; AUC, the area under curves; PCA, principal component analysis; CCR, chemokine receptor; TMB, tumor mutation burden; TME, tumor microenvironment; PCD, programmed cell death.

Conclusion: Through comprehensive analysis of PRlncRNA model, our study may offer a valuable basis for future researches in targeting pyroptosis and tumor microenvironment (TME) and provide new measures for prevention and treatment in AML.

KEYWORDS

acute myeloid leukemia, pyroptosis, lncRNA, prognosis, lasso-cox regression, tumor microenvironment

1 Introduction

Acute myeloid leukemia (AML) is one of the severe and life threatening hematological malignant tumor arising from hematopoietic stem cells (HSCs) (Thomas and Majeti, 2017) with a high recurrence and mortality rate. About 10–40% of younger AML patients are primarily refractory to induction therapy, while a higher recurrence rate was observed in elderly patients (Döhner et al., 2015). After receiving allogeneic hematopoietic stem cell transplantation (allo-HSCT), up 50% of AML patients finally relapse (de Lima et al., 2014), and the 2-year survival rates are below 20% (Schmid et al., 2012; Schmid et al., 2018). Currently, guideline for the risk stratification of AML had included cytogenetic abnormalities, as well as gene mutations in *NPM1, CEBPA, FLT3,* and *KIT* (Dohner et al., 2017), improving the diagnosis and prognosis of AML patients. Although most AML patients could reach initial remission after induction chemotherapy, the long-term survival was still dismal. Accordingly, it is still urgently needed to find novel treatment strategies for improving the outcome of AML patients.

Pyroptosis, a highly inflammatory form of programmed cell death (PCD) (Miao et al., 2010), has garnered increasing attention as it relates to innate immunity and various diseases. Unlike apoptosis, pyroptosis was induced by the activation of gasderminsis through classical and non-classical pathways (Kayagaki et al., 2015; Ding et al., 2016; Liu et al., 2016; Rogers et al., 2017; Broz et al., 2020; Zhou et al., 2020), leading to cell swelling and plasma membrane rupture, and then triggering a strong inflammatory response (Chen et al., 2016). This process, however, could be either beneficial or detrimental to clearance of malignancies. Pyroptosis can inhibit the occurrence and development of tumors by mediating cell death and favourable immune response, while its hyper-inflammatory state could form a microenvironment conducive to tumor development and metastasis. During the inflammatory response, inflammasome activation exerts a critical role in maintaining multiple stages of hematopoietic homeostasis (Yang et al., 2021). Irreversible pyroptotic cell death may be caused by the activation of inflammasome in HSCs and thus leading to the origin of hematopoietic malignancies (Wei et al., 2022). An immunity and pyroptosis gene-pair signature performed stronger survival predictive efficacy than 10 existing signatures (Kong et al., 2022). The researches for the relationship between pyroptosis and tumor may provide some inspirations for clinical treatments. Recent several researches showed pyroptosis-related protein-coding genes could predict prognosis in AML (He et al., 2022; Liu et al., 2022; Pan et al., 2022; Shao et al., 2022; Zhou et al., 2022).

With a complex secondary and tertiary structure, long noncoding RNA (lncRNA) is RNA with over 200 nucleotides in length. Now, lncRNA has emerged as an essential mediator to participate in the regulation of gene expression such as chromatin modification, transcriptional regulation, and post-transcriptional regulation (Mercer et al., 2009) in almost all aspects of biology, particularly in tumorigenesis (Yang et al., 2014; Fang and Fullwood, 2016). Currently, accumulating researches have reported the effect of lncRNA as prognostic and diagnostic markers in leukemia (Garzon et al., 2014; Lei et al., 2018; Mer et al., 2018; Zhu et al., 2021). Moreover, several lncRNA recently were found to play roles in the pyroptosis pathway (He et al., 2020; Xu et al., 2020; Zhang et al., 2022). However, the relationship between pyroptosis-related lncRNA (PRlncRNA) and clinical prognosis in AML is still ambiguous.

In this study, we identified that PRlncRNA could effectively predict the outcome of AML and were associated with immunity and pro-inflammatory signaling. We first constructed a novel PRlncRNA prognostic model. Next, two risk groups were identified by the median risk score. The relationship between risk groups in immune infiltration, functional analysis and response to chemotherapy were further explored. Our study may facilitate an understanding of the mechanism underlying PRlncRNA in AML and provide precision therapies.

2 Materials and methods

2.1 Data collection

We downloaded the expression profiles of RNA-seq (n = 150) and clinicopathological information of AML tumorous tissue from the Cancer Genome Atlas (TCGA) website (https://portal.gdc.cancer.gov/), while the RNA-seq profiles of normal blood (n = 337) were obtained from UCSC XENA of Genotype-Tissue Expression (GTEx) (https://xenabrowser.net/datapages/) (Li et al., 2021). Then we normalized and processed the RNA-seq profiles of counts value from TCGA and GTEx with "limma" package (Mounir et al., 2019). From Genecards (https://www.genecards.org/), 176 pyroptosis-related genes (PRGs) (Supplementary Table S1) were finally retrieved.

2.2 Functional pathway enrichment of pyroptosis-related differentially expressed genes (DEGs)

According to the screening criteria of a false discovery rate (FDR) < 0.05 and |log2 fold change (FC)| > 0.25, 57 pyroptosis-related DEGs were obtained with the "limma" package in R (Ritchie et al., 2015). For better understanding of the functions of DEGs, functional enrichment analysis, including the GO terms and KEGG pathways were carried out with "clusterProfiler" and "ggplot2″ packages (Yu et al., 2012).

2.3 Construction and validation of the prognostic model

We evaluated the association between 57 pyroptosis-related DEGs and lncRNA by the pearson correlation coefficient. In total, 877 PRlncRNA were screened out following the criteria of absolute correlation coefficient >0.3 and a *p*-value < 0.001. To minimize the possible statistical bias, the samples were sifted when they lack overall survival (OS) value, of which 125 AML patients with complete clinical information were obtained. First, univariate Cox regression was applied to determine the lncRNA associated with prognosis. Subsequently, to prevent overfitting, least absolute shrinkage and selection operator (LASSO) regression was performed using the "glmnet" package. Finally, multivariate Cox regression was used to establish a four-lncRNA risk model of AML. We computed a risk score of each patient *via* the expression level of lncRNA and its regression coefficient. The formula was as follows: Risk score = $\sum_{i}^{n}$Coefi × A_i. AML patients were randomly assigned into high- and low-risk groups by median value of the risk score and a Kaplan–Meier survival curve was further employed with the "survival" package. Then we applied "survivalROC" package to plot the receiver operating characteristic (ROC) curve of the 1-, 3-, and 5-year for OS rates of AML. Patients of entire set were randomized into two internal test sets with "caret" package (Mahmoudian et al., 2021). Finally, the same above formula was used to further validate the reliability of the signature.

2.4 Construction of the nomogram and analysis of potential clinical relevance

We constructed a nomogram combining risk score and clinical factors (age and molecular risk) with the 'rms' package to predict the 1-, 2-, and 4-year survival of AML patients (Iasonos et al., 2008). Then calibration curves were plotted to assess the clinical utility of the nomogram. At first, we took 1-,3- and 5-year as the criteria for nomogram, however the calibration curve showed no score in 5-year. Thus, a 1-,3-, and 4-year setting was drawn back. For further exploration of the predictive signature, we compared the survival difference of risk groups and expression differences of predictive genes in clinicopathological subgroups.

2.5 Evaluation of immune status

First, Immune checkpoints activation was compared between risk groups using "ggpubr" package. In order to explore the association between risk scores and immune cells and functions, we employed the single-sample gene-set enrichment analysis (ssGSEA) score to quantify the enrichment levels of immune cells, related functions or pathways in different subgroups, using the "gene set variation analysis (GSVA)" package (Dalangood et al., 2020). Moreover, the proportions of 22 immune cells between the risk groups were explored by the CIBERSORT algorithm (Newman et al., 2015).

2.6 Gene set enrichment analysis (GSEA)

GSEA was carried out to determine the predominant genes enriched pathways with GSEA 4.1.0 (http://www.broad.mit.edu/gsea/). We applied "c2. cp.kegg.v7.5. symbols", "c5. go.v7.5. symbols" and "h.all.v7.5. Symbols" to complete functional enrichment analysis. The threshold for statistical significance was considered as nominal $p < 0.05$ and FDR <0.25.

2.7 Drug sensitivity prediction

To evaluate the therapeutic value of signature in AML, the "pRRophetic" package (Geeleher et al., 2014) was applied to analyse the half inhibitory concentration (IC50) of typical chemotherapy drugs. Thereafter, we compare the IC50 values between two risk groups to search for potential drugs.

2.8 Human clinical specimens preparation

Samples of newly diagnosed AML and iron-deficiency anemia (IDA) were acquired from patients who were treated in the Qilu Hospital of Shandong University in Jinan, China. Ten pairs of bone marrow samples were included and IDA samples served as control.

2.9 RNA extraction and real-time quantitative PCR (RT-qPCR)

Total RNA was extracted using the Trizol method as previously reported (Invitrogen, Carlsbad, CA, United States). Complementary DNA (cDNA) was synthesized using Evo M-MLV RT Mix Kit with gDNA Clean for qPCR (Accurate Biology, Human, China). Four lncRNA gene expressions were

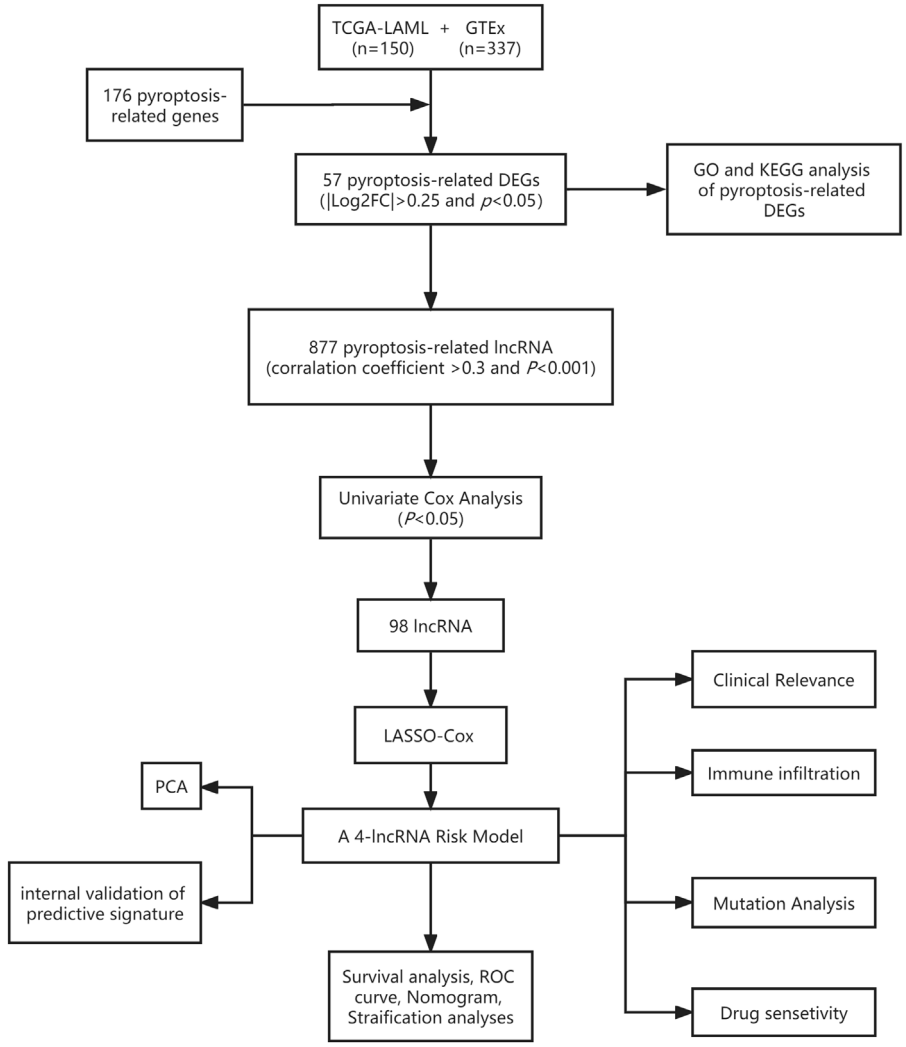


FIGURE 1
Detailed flow chart of our research.

verified by PCR using SYBR® Green Premix Pro Taq HS qPCR Kit (Accurate Biology, Human, China) with GAPDH as a control. The primer sequences of four lncRNA were shown in Supplementary Table S2.

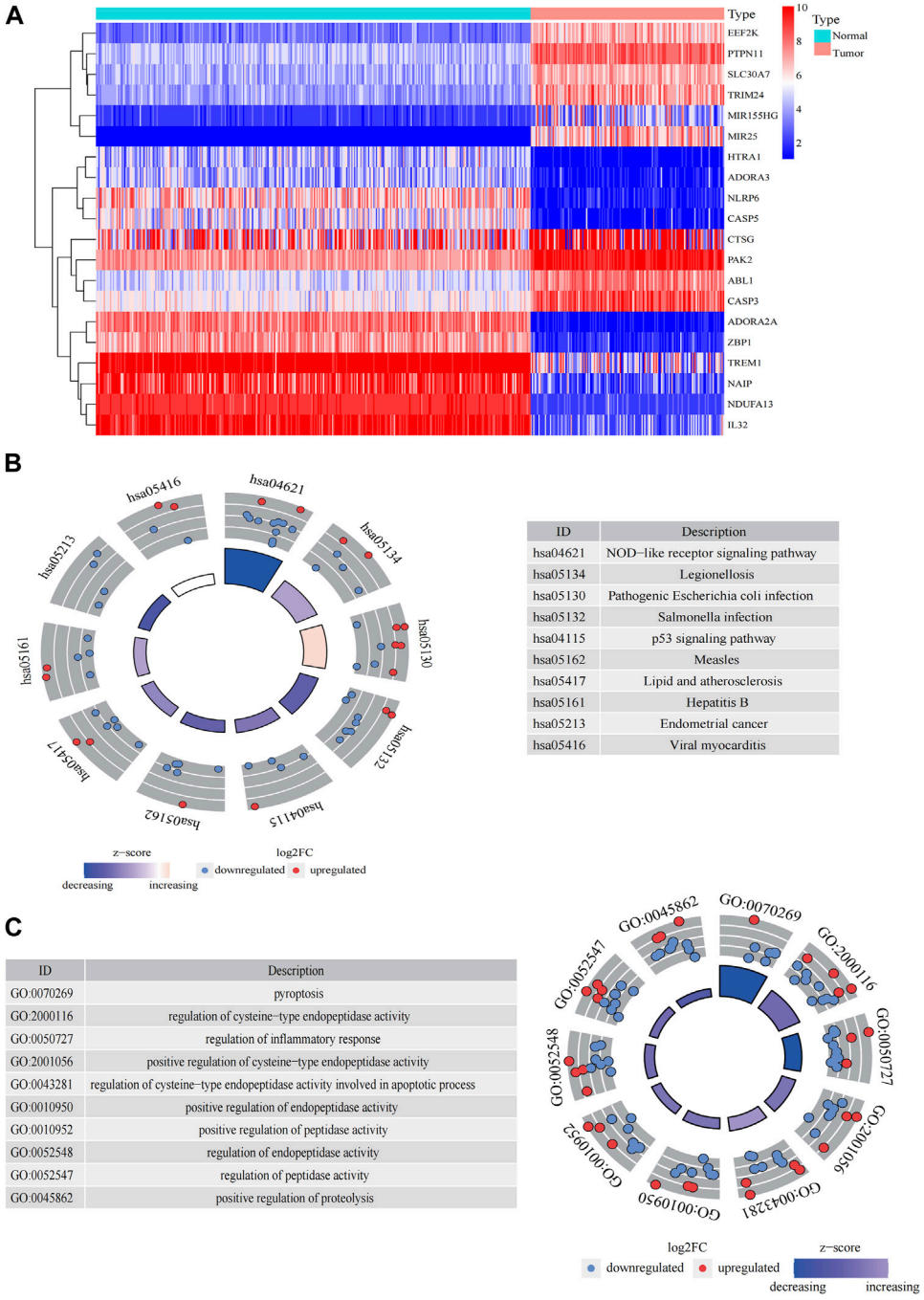
2.10 Statistical analysis

Results of the following analysis were performed with the R software (Version 4.1.0). Comparison of OS between risk groups was conducted by Kaplan-Meier analysis as well as log-rank tests. The time-dependent ROC curve and the area under curve (AUC) were carried out with the "timeROC" package. For continuous variables, Student's t-test and Wilcox test were performed to test the differences between two groups while One-way ANOVA or Kruskal–Wallis test was used in three or more groups. Chi-square tests were employed in categorical variables. The optimal cut-off value was 0.876 between two risk groups determined by "survminer" package. P-values are two-sided in all statistical, and $p < 0.05$ was considered as statistically significant results.

3 Results

3.1 Identification and enrichment analysis of pyroptosis-related DEGs

The general workflow (Figure 1) describes the whole screening and analysis processes. From TCGA and GTEx matrix, we obtained 150 tumor samples and 337 normal samples. According to the expressions of PRGs and DEGs ($|Log2FC| > 0.25$ and $p < 0.05$), 57 pyroptosis-related DEGs



ID	Description
hsa04621	NOD−like receptor signaling pathway
hsa05134	Legionellosis
hsa05130	Pathogenic Escherichia coli infection
hsa05132	Salmonella infection
hsa04115	p53 signaling pathway
hsa05162	Measles
hsa05417	Lipid and atherosclerosis
hsa05161	Hepatitis B
hsa05213	Endometrial cancer
hsa05416	Viral myocarditis

ID	Description
GO:0070269	pyroptosis
GO:2000116	regulation of cysteine−type endopeptidase activity
GO:0050727	regulation of inflammatory response
GO:2001056	positive regulation of cysteine−type endopeptidase activity
GO:0043281	regulation of cysteine−type endopeptidase activity involved in apoptotic process
GO:0010950	positive regulation of endopeptidase activity
GO:0010952	positive regulation of peptidase activity
GO:0052548	regulation of endopeptidase activity
GO:0052547	regulation of peptidase activity
GO:0045862	positive regulation of proteolysis

FIGURE 2
Significantly enriched GO terms and KEGG pathways of pyroptosis-related DEGs between AML and the healthy control. **(A)** Heatmap of top 10 pyroptosis-related DEGs of upregulated and downregulated genes. Enrichment analysis of pyroptosis-related DEGs of KEGG pathway **(B)** and GO terms **(C)**.

including 35 downregulated genes and 22 upregulated genes were obtained (Supplementary Figure S1A). The expression level of the top 10 upregulated and downregulated pyroptosis-related DEGs were characterized (Figure 2A). Then KEGG and GO analysis were performed. Pyroptosis-related DEGs were mainly enriched in the NOD−like receptor signalling pathway, Legionellosis, Pathogenic Escherichia coli infection, *Salmonella* infection, p53 signalling pathway, Measles, Lipid and atherosclerosis, Hepatitis B, Endometrial cancer and Viral myocarditis according to KEGG pathway analysis (Figure 2B;

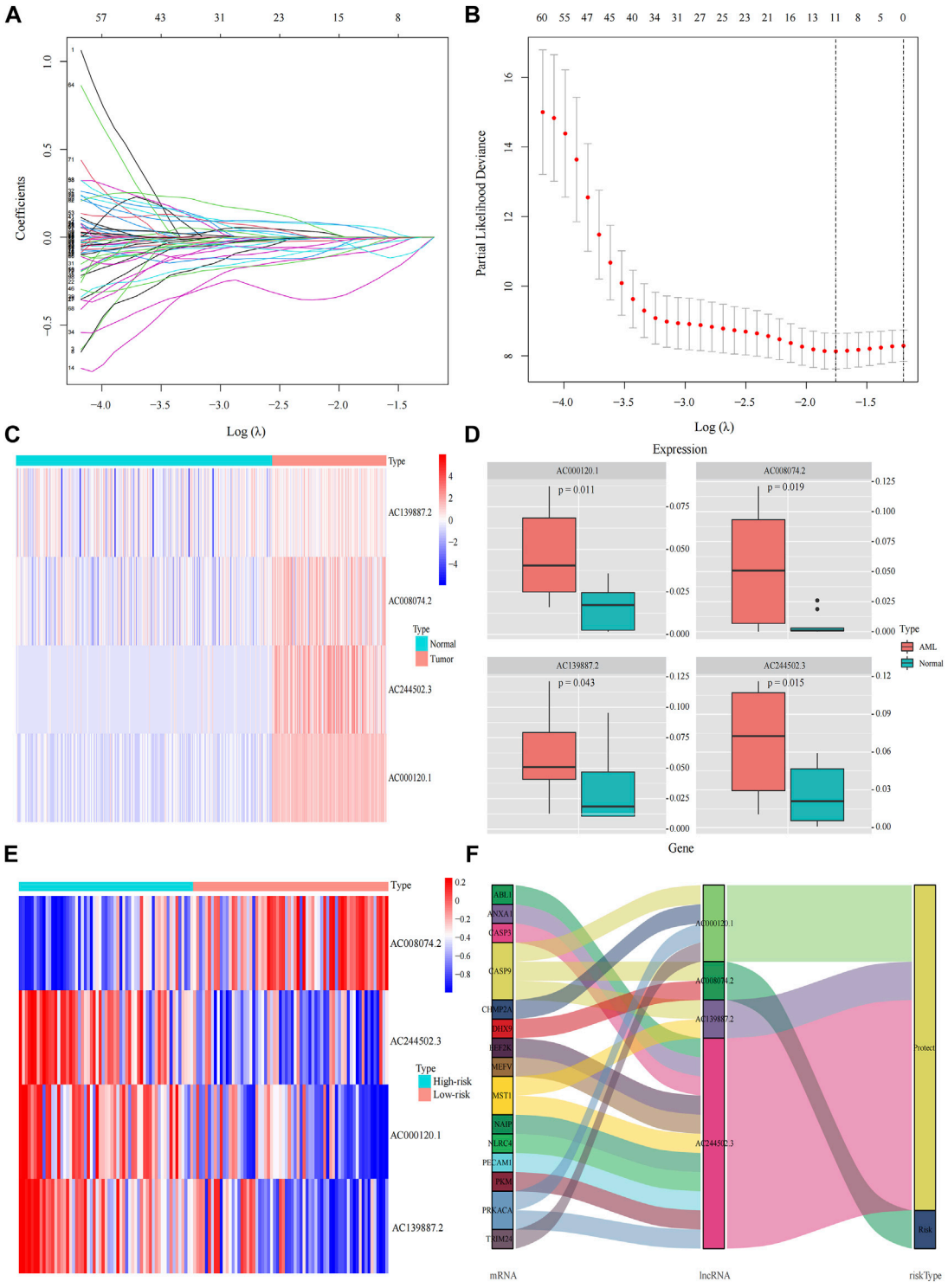


FIGURE 3
Construction the pyroptosis-related prognostic model by DEGs. **(A,B)** Least absolute shrinkage and selection operator (LASSO) regression analysis with ten-fold cross validation to determine the lambda number. **(C)** The expression levels of four pyroptosis-related lncRNA in AML and healthy control (Wilcoxon tests). **(D)** RT-PCR-detected RNA expression of four genes in our own samples, 10 AML *de novo*, and 10 IDA as normal control samples. **(E)** The expression levels of four pyroptosis-related lncRNA in different risk groups. **(F)** Sankey diagram of prognostic pyroptosis-based lncRNA.

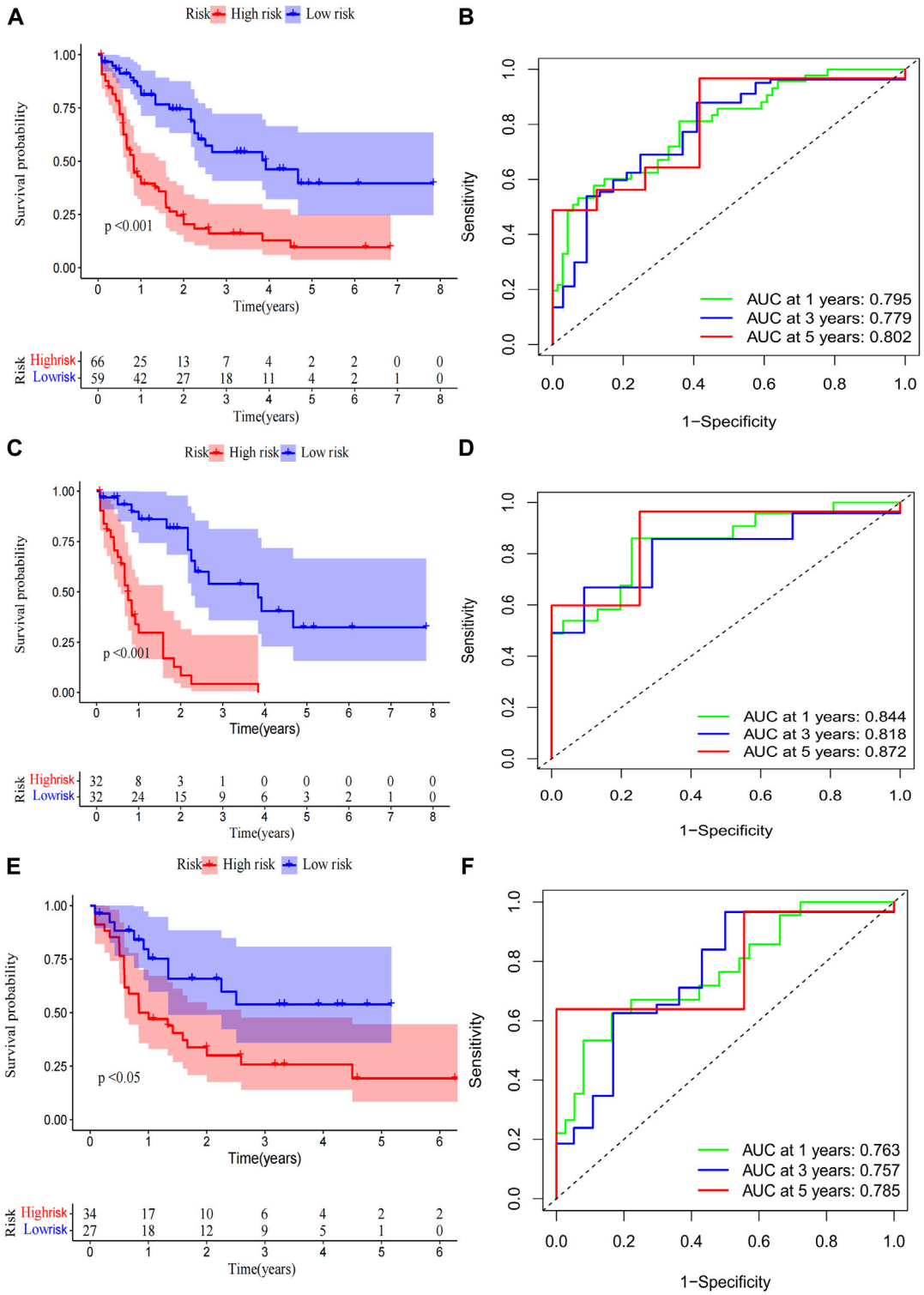


FIGURE 4
Risk score of the pyroptosis-related prognostic signature for overall survival (OS). Kaplan-Meier survival curve of high- and low-risk patients and ROC curve and AUCs at 1-year, 3-year and 5-year survival in the entire cohort **(A,B)**, the first internal cohort **(C,D)** and the second internal cohort **(E,F)**.

Supplementary Table S3). Furthermore, pyroptosis, regulation of cysteine–type endopeptidase activity, regulation of inflammatory response and positive regulation of proteolysis were highlighted in GO enrichment (Figure 2C; Supplementary Table S4).

3.2 Establishment and validation the pyroptosis-related prognostic model

A total of 98 PRlncRNA correlated with OS were identified by univariate Cox regression (Supplementary Figure 1B). Then, we used the LASSO-Cox regression to reduce overfitting (Figures 3A,B). Finally, a pyroptosis-related prognostic risk model composed of four lncRNA (*AC244502.3*, *AC000120.1*, *AC139887.2*, *AC008074.2*) was established by multivariate Cox regression. Higher expression level of four PRlncRNA were observed in tumor tissues (Figure 3C). Their higher expressions in AML patients were validated according to RT-qPCR (Figure 3D). *AC244502.3*, *AC000120.1* and *AC139887.2* were up-regulated in low-risk group as protective factors in this prognostic model, while *AC008074.2* was a risk factor (Figures 3E,F). The risk score was as follows: risk score = *AC008074.2* expression * 0.751252629082265 + *AC139887.2* expression * (-1.43187457646711) + *AC000120.1* expression * (-1.49861878505506) + *AC244502.3* expression * (-0.278256123715056). We further visualized the correlation between lncRNA and mRNA. The co-expression network including 19 pairs mRNA-lncRNA was characterized in Supplementary Figure 2A ($|R2| > 0.3$ and $p < 0.001$). Two risk groups were randomised by the median value of the risk score. The patients of high-risk group were observed significantly shorter OS than those in low-risk group (Figure 4A). ROC curve was applied to assess the reliability of our prognostic signature. The AUC value was 0.795 at 1 year, 0.779 at 3 years, and 0.802 at 5 years which indicated excellent stability (Figure 4B). To determine the prediction ability of the model, we stratified entire dataset into two groups. Similar results were observed in two internal cohorts. The high-risk group showed lower OS rate and good evaluated prognostic power was also observed in validation cohorts according to the ROC curves (Figures 4C–F). The 1-,3-, and 4-year ROC curves for entire cohort and two internal cohorts were shown in Supplementary Figure S2B–D. Patients' baseline characteristics of the entire and two validation cohorts are outlined in Table 1.

3.3 Construction of nomogram and analysis of the potential clinical relevance of the prognostic signature

The clinical relevance of our signature was further explored. We performed univariate Cox regression analysis incorporating eleven variables that were easily accessible (Supplementary Figure 2E). Besides risk score, molecular risk and age were shown to be independent risk factors for OS and then were enrolled to multivariate Cox regression (Figure 5A). The risk score demonstrated a higher predictive ability than that of clinicopathological variables in AML (Figure 5B) and other previously reported prognostic signatures [Leu 2022 (Kong et al., 2022), AJH 2021 (Chen et al., 2021), Int Imp 2022 (Shao et al., 2022)] (iAUC-Our signature: 0.806, iAUC-Leu2022: 0.691, iAUC-AJH2021: 0.570, and iAUC-IntImp2022: 0.658; all the *p*-values in comparisons between Our signature and the above three models were <0.001). Based on multivariate Cox regression, we developed a novel nomogram, aiming to optimize the predictive accuracy of the risk model (Figure 5D). The ROC curve exhibited a reliable predictive efficacy (Figure 5E). The 1-,2-, and 4-year calibration curves of our constructed nomogram yielded good agreement between prediction and observation (Figures Figure5F–H). The clinical characteristics of two risk groups were further compared. The heatmap showed that the distribution of FAB type varied significantly in risk groups while age, gender, molecular risk, cytogenetic risk and blood cell counts were no statistically differences (Supplementary Figure S3). To assess the predictive ability of the predictive signature in different clinicopathological subgroups, we separated AML patients into groups by age, sex, FAB, molecular risk and cytogenetic risk. For each clinical subgroups, the patients of high-risk group had worse OS than those of low-risk group (Supplementary Figure S4). We further explored association between the four predictive genes and clinical variables (Supplementary Figure S5).

3.4 Enrichment analysis of the prognostic model

Principal component analysis (PCA) indicated that AML patients could not be well separated into high- and low-risk groups by all genes (Figure 6A) but the shortfalls could be compensated by our signature (Figure 6B). The genes of immune checkpoint such as CD70, LAIR1, CD276, HAVCR2, CD200R1, CD86 and LGALS9, were higher expressed in high-risk group, demonstrated that high-risk group may profit from immune checkpoint inhibitors (Figure 6C). Subsequent analysis of the tumor microenvironment was carried out. Inflammatory and immunosuppressive cells were abundant in high-risk group, including the presence of Monocytes, Neutrophils along with M2 macrophages, thus conferring a significant survival disadvantage (Figure 6D). Then, we investigated the differences in immune signatures between low- and high-risk groups. Neutrophils, tregs, chemokine receptor (CCR), check–point, type-I and type-II IFN response and parainflammation were observed with higher expression level in high-risk group, indicating that high-risk group was correlated with the inflammatory response and tumor immune escape (Figures 6E,F). To assess the potential biological processes between two groups, enrichment analysis of GO, KEGG and hallmarker were performed (Figures 7G,H;

TABLE 1 The clinical characteristics of patients in different cohorts.

	Entire TCGA dataset (n = 125)	Validation cohorts		
		First cohort (n = 61)	second cohort (n = 64)	*p*-value
Age (Mean ± SD)	54.1 ± 1.4	53.4 ± 2.0	54.7 ± 2.1	0.514
Gender, n (%)				0.063
Female	55 (44.0%)	29 (47.5%)	41 (64.1%)	
Male	70 (56.0%)	32 (52.5%)	23 (35.9%)	
FAB, n (%)				0.930
Non-M3	113 (90.4%)	55 (90.2%)	55 (90.2%)	
M3	12 (9.6%)	6 (9.8%)	6 (9.4%)	
WBC, ×109/L (Mean ± SD)	30.5 ± 3.2	33.6 ± 5.1	27.7 ± 4.0	0.516
HB, g/L (Mean ± SD)	9.6 ± 0.1	9.7 ± 0.2	9.5 ± 0.2	0.700
PLT, ×109/L (Mean ± SD)	66.6 ± 5.0	68.8 ± 6.5	64.5 ± 7.7	0.261
PB_blast_cell (Mean ± SD)	66.2 ± 2.0	65.9 ± 3.1	66.5 ± 2.5	0.906
BM_blast_cell (Mean ± SD)	36.9 ± 2.7	36.4 ± 4.0	37.4 ± 3.8	0.943
[a]RISKCyto, n (%)				0.805
Favorable	28 (22.4%)	14 (23.0%)	14 (21.9%)	
Intermediate	71 (56.8%)	33 (54.1%)	38 (59.4%)	
Poor	26 (20.8%)	14 (23.0%)	12 (18.8%)	
[b]RISKMole, n (%)				0.906
Favorable	29 (23.2%)	15 (24.6%)	14 (21.9%)	
Intermediate	68 (54.4%)	32 (52.5%)	36 (56.3%)	
Poor	28 (22.4%)	14 (23%)	14 (21.9%)	
Riskscore (Mean ± SD)	1.9 ± 0.2	2.1 ± 3.5	1.6 ± 0.2	0.374

[a]Cytogenetic risk.
[b]Molecular risk.

Supplementary Figure S6A; Supplementary Tables S5–7). High-risk patients were closely related to myeloid leukocyte activation, inflammatory response such as Chemokine, Cytokine-cytokine receptor interaction, Interleukin-6 production, Leukocyte transendothelial migration, Toll like receptor, Apoptosis and Interferon gamma, and tumor-related signaling pathways such as IL6-JAK-STAT3, MAPK, VEGF, Wnt, PI3K-AKT-mTOR, KRAS, NF-κB and P53. The inflammasome-related genes (Liu et al., 2016) exhibited a high expression level in the high-risk group (Supplementary Figure S6B).

3.5 Mutation profile and chemotherapeutics of the prognostic model

Gene mutations in AML could modify the disease process and subsequently influence the outcomes (Daver et al., 2019). Therefore, gene mutation status was analysed, among which *FLT3* had the highest mutation rate (Supplementary Figure S7A). There were no statistically differences in tumor mutation burden (TMB) between risk groups and survival rates between the TMB groups (Supplementary Figure S7B, C). The high-TMB group was observed poor prognosis in subgroup analysis stratified by risk groups (Supplementary Figure S7D). Then the 20 highest point mutations were further compared (Supplementary Figure S7E). Higher frequencies of *TP53* mutations (low vs. high: 0–11%) was found in the high-risk group (Supplementary Figure S7F). Chemotherapy is still the mainstream treatment for AML. We analysed the association between risk groups and the sensitivity to typical chemotherapeutic agents in AML (Figure 7). The low-risk group showed more sensitivity to the conventional chemotherapeutics including Doxorubicin, Cytarabine, Methotrexate, Etoposide, Midostaurin, Lenalidomide, ATRA and HDAC inhibitor Vorinostat, while the high-risk group was less resistant to protease inhibitor such as bortezomib and MG.132. Our risk signature may become a potential indicator of drug sensitivity.

4 Discussion

AML is an aggressive hematologic malignancy with complex and dysregulated microenvironment that, in part, promotes

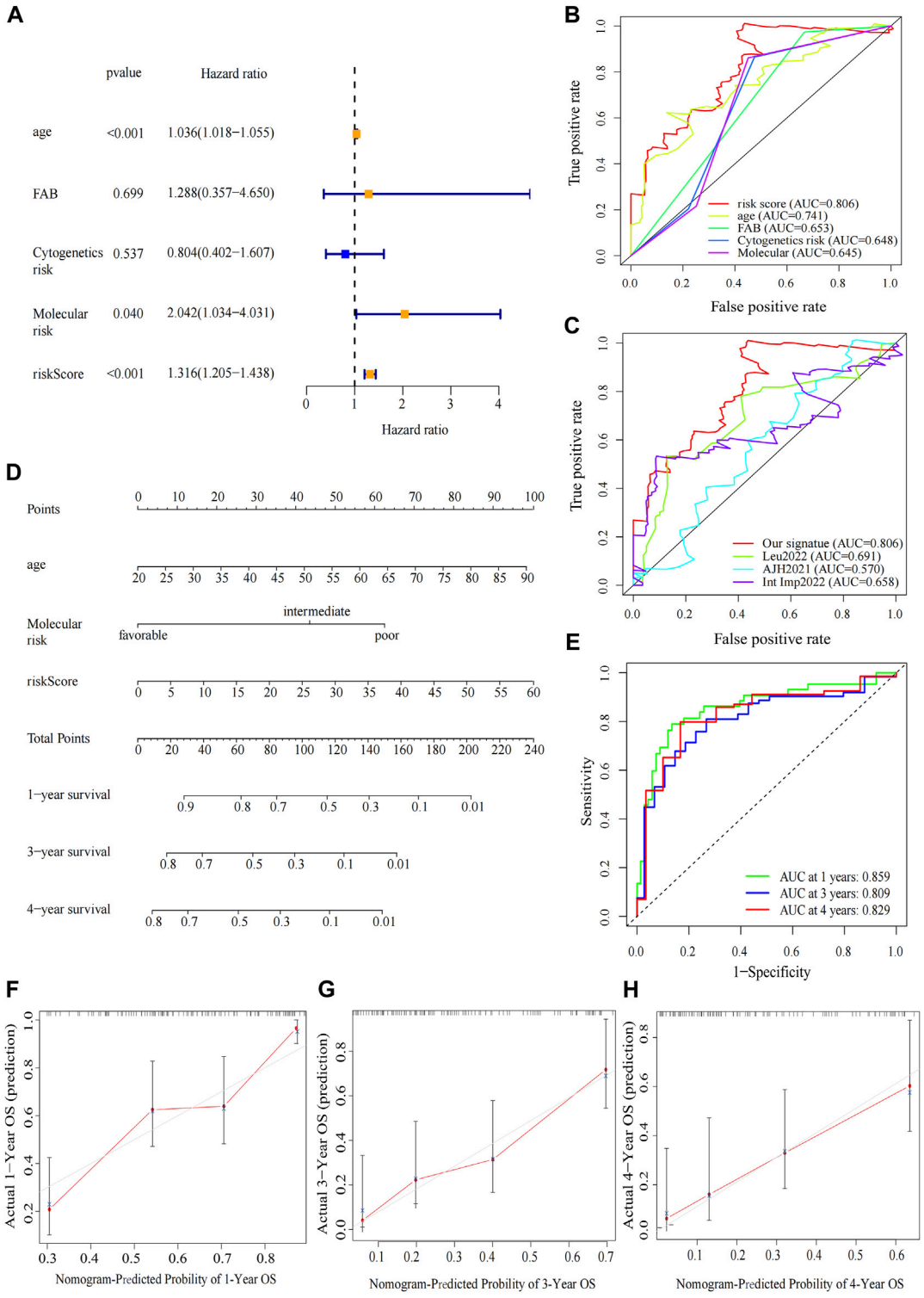


FIGURE 5
Construction and verification of the nomogram. **(A)** Forest map of multivariate Cox regression analysis of the risk scores and clinical parameters. **(B)** The ROC curve of multivariate Cox regression variables. **(C)** Prognostic ROC AUC comparison of Our signature and other three signatures. **(D)** The nomogram of 1-year, 3-year or 4-year OS of AML patients based on risk score, age and molecular risk. **(E)** ROC curves and AUCs for the nomogram. **(F–H)** The calibration curves test consistency between the actual OS rates and the predicted survival rates at 1, 3 and 4 years.

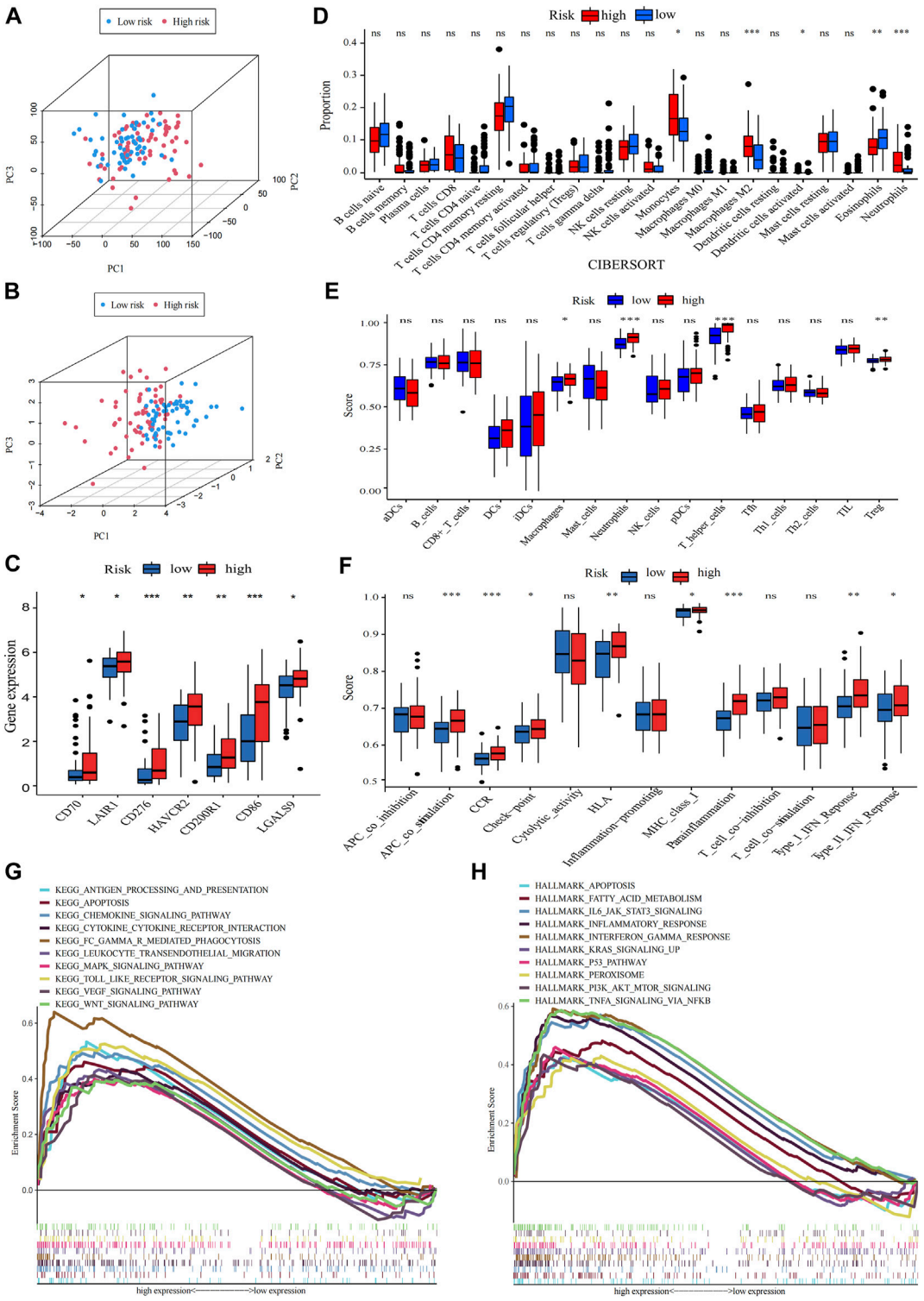


FIGURE 6
Analysis of differences in immunological characteristics and functional enrichment of AML in different risk groups. **(A,B)** PCA analysis of the expression patterns of grouped samples using all genes and prognostic signature. **(C)** Differential expression of immune checkpoint genes in low- and high-risk group (Wilcox test). **(D)** The proportion of every type of TME infiltrating cells between the two risk groups analyzed, respectively, by CIBERSORT. **(E,F)** Differences between immune cell infiltration and immune-related functions in two subtypes, with ssGSEA algorithm (Wilcoxon test, $*p < 0.05$; $**p < 0.01$; $***p < 0.001$; ns, non-significant). **(G,H)** The high-risk group enriched gene sets of GSEA-based KEGG and Hallmark analysis.

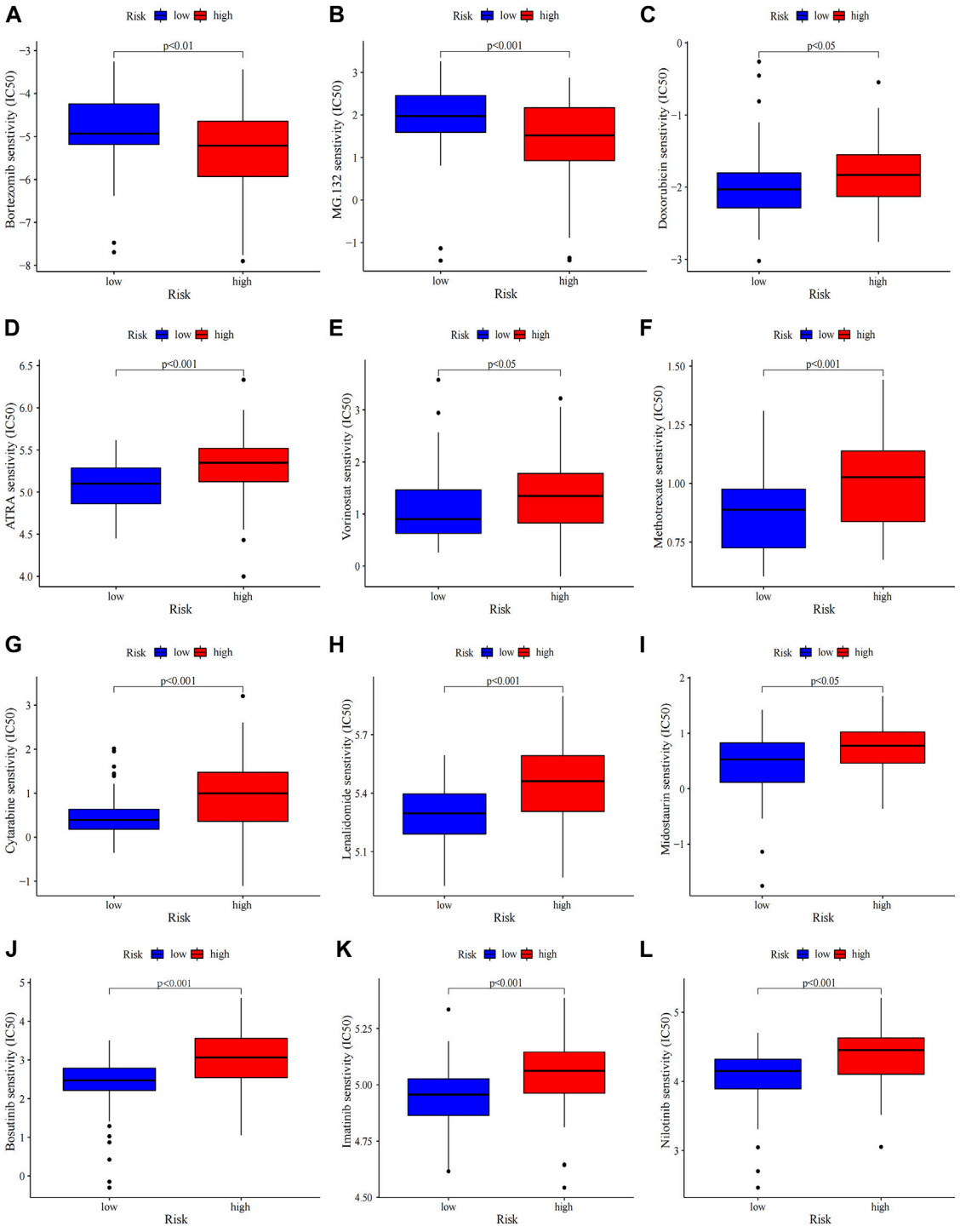


FIGURE 7
Chemosensitivity prediction. Higher drug sensitivity in high-risk group **(A–C)** and low-risk group **(D–L)** (Wilcoxon tests). Lower half inhibitory centration (IC50) means better drug sensitivity.

leukemogenesis (Teague and Kline, 2013). During the leukemic transition, AML blasts modified the immune microenvironment to evade immune surveillance. Accumulating evidences indicated that chronic inflammation in tumor microenvironment (TME) played a major role in immune invasion, thus contributing to tumorigenesis (Grivennikov et al., 2010; Shin and Brusselle, 2014;

Lu et al., 2021). In some studies of pyroptosis in AML, TME was found remarkably different between risk groups (He et al., 2022; Shao et al., 2022; Zhou et al., 2022). Pyroptosis, an inflammation-dependent form of PCD, was proved to participate in tumor growth and chemosensitivity (Xia et al., 2019), while its mechanisms are complicated. For instance, pyroptosis serves as a tumor inhibitor in hepatocellular and nasopharyngeal carcinoma (Zaki et al., 2010; Chen et al., 2012) and a two-edged sword of promotor and inhibitor in cervical cancer (Xia et al., 2019). A growing body of evidences indicated that lncRNA participated in multiple biological processes of various tumors *via* pyroptosis-related pathways. ADAMTS9-AS2 inhibited gastric cancer progression and promoted cisplatin chemosensitivity by regulating the pyroptosis process (Ren et al., 2020). Moreover, the activation of MEG3 enhanced Cisplatin-induced pyroptosis in triple-negative breast cancer (Yan et al., 2021). In liver cancer, SNHG7 suppressed the NLRP3-related pyroptosis pathway (Chen et al., 2020). The effect of PRlncRNA in AML has not been yet clarified. Therefore, a PRlncRNA signature was established to explore its possible clinical relevance with AML.

In this study, 57 pyroptosis-related DEGs were obtained which were highly related to pyroptosis, inflammatory, p53 signaling pathway and positive regulation of endopeptidase activity based on KEGG and GO analysis. The genes of positive regulation of endopeptidase activity were related in numerous cellular processes, including apoptosis, DNA damage repair, or cell cycle progression (Jorgensen et al., 2006) and mediated PCD(Yamada et al., 2020). Next, we used Lasso-Cox regression analysis to identify a risk model of four-PRlncRNA (*AC244502.3, AC000120.1, AC139887.2 and AC008074.2*). Then, the signature was validated in two internal cohorts. The ROC curves of whole dataset and two internal training sets demonstrated high accuracy of the clinical prognostic model. Our signature exhibited greater predictive ability than clinicopathological variables such as molecular risk and cytogenetic risk. In our study, *AC244502.3*, *AC000120.1* and *AC139887.2* were observed as potentially protective lncRNA with higher expression in low-risk group, while *AC008074.2* was potentially dangerous. *AC000120.1* had been found to associated with the prognosis of Bladder Cancer (Cui et al., 2021). The functions of the three remaining lncRNA have not been studied and reported specifically yet. Thus, more researches of lncRNA are needed to be conducted.

The recruitment of inflammatory cells, cytokines and chemokines results in the inflammatory TME which contributes to metastasis and invasion of tumor cells, modulates the anti-tumor immune response and influences the sensitivity to chemotherapeutic drugs. Previous studies suggested the high-risk group of pyroptosis-related signature was enriched in immunosuppressive cells and had lower drug sensitivity to classical chemotherapy (Pan et al., 2022; Shao et al., 2022; Zhou et al., 2022). In pediatric patients with AML, PRGs were found to predict recurrence (He et al., 2022). To probe the underlying mechanisms further of our signature, the TME and enrichment analysis between risk subgroups were investigated. In this study, the high-risk group demonstrated higher inflammatory response mediated by IL-6-related signaling pathways and lower immune response regulated by immunosuppressive cells such as M2 macrophages, Treg cells and so on. Recent study indicated that M2 macrophages could induce immune suppression (Ruffell and Coussens, 2015) and were associated with the worse survival status (Italiani and Boraschi, 2014). Monocytes preferentially differentiated into immunosuppressive tumor-associated macrophages (TAMs) in solid tumor (Richards et al., 2013), which would suppress immune response. Higher level of IL-6 was considered to be related to bone marrow failure (Zhang et al., 2020) and the high risk of relapse for AML (Stevens et al., 2017). In TME, IL-6 could drive tumor cells proliferation, invasiveness, and metastasis through activating JAK/STAT3 signaling pathway which could in return promote IL-6 transcription (Chang et al., 2014). Meanwhile, NF-κB was identified as a key transcription factor that drove the IL-6 signaling (Yoon et al., 2012; McFarland et al., 2013). Receptor activator of nuclear NF-κB was associated with dismal disease course and chemoresistance in AML (Clar et al., 2021). The signaling pathway of JAK/STAT3 and NF-κB have been universally recognized as the bridge linking tumor and inflammation. As is well known, the inflammation induced by bacterial and viral infections could increase the cancer risk. Intrinsic inflammatory response could be triggered by tumors which may build up a protumor microenvironment (Tye et al., 2012). Additionally, upregulated procancer pathways (Wnt, p53, PI3K-AKT-mTOR, VEGF, and KRAS) were seen more frequently in high-risk group. This complicated cancer-immune crosstalk in the TME finally facilitate tumor cell growth and survival, with decreased antitumor immunity. Our research also showed that the low-risk group was less resistant to the conventional chemotherapy drugs, while high-risk patients may profit from proteasome inhibitor. Bortezomib may exert anti-inflammatory effects by inhibiting the expression of NF-κB, IL-6 and TNF-α. (Teague and Kline, 2013). Summarizing all findings, we constructed two groups, of which high-risk group had lower immune response, more inflammatory cell infiltration, and lower sensitivity to classical chemotherapy; together these characters resulted in a significant reduction in the overall survival.

In this study, based on four PRlncRNA, we constructed a prognostic risk score model for AML patients which demonstrated a reliable predictive validity. Moreover, the risk score model increased the knowledge of the mechanisms of PRlncRNA in AML and had the potential to provide more precisely targeted interventions. There are still some shortcomings that must be noted. First, external validation was not conducted due to the lack of expression profiles of lncRNA in other databases. Second, although with the validation

of differential expression between normal and tumor samples, our study mainly based on public databases. Further experiments *in vivo* and *in vitro* should be needed to explore the underlying mechanisms of PRlncRNA in AML.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found in the article/Supplementary Material.

Ethics Statement

The studies involving human participants were reviewed and approved by the Ethics Committee of Shandong University School of Medicine. The patients/participants provided their written informed consent to participate in this study.

Author contributions

All authors read and approved the final manuscript. CC, YF, MZ, and GZ designed the study. GZ, CG, YS, MS, LZ, and HF collected and analysed the data. ZC performed the expression verification in clinical samples. GZ and CG wrote the manuscript. CC supervised the study. All the authors have read and approved the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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Supplementary material

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LncRNAs could play a vital role in osteosarcoma treatment: Inhibiting osteosarcoma progression and improving chemotherapy resistance

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Osteosarcoma (OS) is one of the most common primary solid malignant tumors in orthopedics, and its main clinical treatments are surgery and chemotherapy. However, a wide surgical resection range, functional reconstruction of postoperative limbs, and chemotherapy resistance remain as challenges for patients and orthopedists. To address these problems, the discovery of new effective conservative treatments is important. Long non-coding RNAs (lncRNAs) are RNAs longer than 200 nucleotides in length that do not encode proteins. Researchers have recently found that long non-coding RNAs are closely associated with the development of OS, indicating their potentially vital role in new treatment methods for OS. This review presents new findings regarding the association of lncRNAs with OS and summarizes potential clinical applications of OS with lncRNAs, including the downregulation of oncogenic lncRNAs, upregulation of tumor suppressive lncRNAs, and lncRNAs-based treatment to improve chemotherapy resistance. We hope these potential methods will be translated into clinical applications and greatly reduce patient suffering.

KEYWORDS

long non-coding RNA (LncRNA), osteosarcoma-pathology, progression, chemotherapy resistance, regulatory mechanism

1 Introduction

Osteosarcoma (OS) is the most common primary solid malignant tumor in orthopedics and is derived from primitive mesenchymal cells (Ritter and Bielack, 2010). Long diaphyses such as the distal femur, proximal tibia, and proximal humerus are most often affected by OS. Data show that 50% of OS occurs around the knee, which can seriously impair patient motor ability (Arndt and Crist, 1999; Bielack et al., 2002; Bielack et al., 2009). The annual incidence of OS is 2–3 million people, with adolescents most at risk (Corre et al., 2020). Although most patients initially present local pain and swelling, pathological fracture of the affected limbs and metastasis-related symptoms can occur early in the disease as OS is highly aggressive and metastasizes early (Bielack and CarrleCasali, 2009). In addition, the 5-year survival rate of patients with early metastasis is usually <20%, with lung metastasis the most likely to occur (Wang et al., 2020a). At present, due to rapid tumor development and the high invasion level of local tissue, surgery combined with chemotherapy is the main treatment for patients with OS (Schwarz et al., 2009; Ta et al., 2009). However, early metastasis, a wide surgical resection range,

a high risk of postoperative recurrence, and difficult postoperative function reconstruction challenge OS treatment methods (Grimer, 2005; Gosheger et al., 2006; Bielack et al., 2009). Therefore, new clinical methods are needed, including accurate monitoring methods for early diagnosis and postoperative surveillance, less invasive treatment methods to avoid massive limb function loss, and more effective methods to lower postoperative recurrence.

LncRNAs are RNAs >200 nucleotides in length that do not encode any protein and can modulate the development of OS in different biological processes (Chen et al., 2021). Some lncRNAs are highly expressed in OS cells and promote tumor proliferation and migration. Recent studies demonstrated that the knockdown of oncogenic lncRNAs not only suppressed the proliferation and promoted the apoptosis of OS cells but also reduced the invasion and migration of OS cells (Zhang et al., 2019; Fei et al., 2019; Li et al., 2019; Lian et al., 2019). LncRNAs act in different ways to reduce OS cell proliferation, including regulating the cell cycle, suppressing cell metabolism, and reducing angiogenesis. However, some lncRNAs are expressed at low levels in OS cells and their overexpression can inhibit tumor development *via* different mechanisms (Wan et al., 2020; Zhou et al., 2020). Most overexpressed lncRNAs suppress tumor progression by targeting miRNAs, although other pathways have also been described. In clinical treatment, chemotherapy resistance is common, resulting in recrudescence and metastases. LncRNAs also play a vital role in chemotherapy resistance through different pathways (Cheng Y et al., 2019; Zhang Q-Q et al., 2021). Various kinds of chemotherapy resistance in OS are related to lncRNAs, and a single lncRNA may be related to multiple kinds of chemotherapy resistance in OS.

This review describes recent studies related to lncRNAs and OS and summarizes three main aspects of the potential applications of lncRNAs in the treatment of OS. These aspects are the downregulation of oncogenic lncRNA promoting tumor progression, the upregulation of tumor-suppressive lncRNA, and lncRNA treatments to improve chemotherapy resistance (Figure 1).

2 Oncogenic lncRNA silencing inhibits the progression of osteosarcoma

2.1 Oncogenic lncRNA silencing inhibits osteosarcoma proliferation and promotes apoptosis

Recent studies have demonstrated that the knockdown of oncogenic lncRNAs can directly inhibit the proliferation of OS cells and promote apoptosis *in vitro*. These data are summarized in Table 1. Li et al. (2019) reported that the upregulation of lncRNA NNT-AS1 promoted OS progression by inhibiting the tumor suppressor miR-320a. They confirmed that lncRNA NNT-AS1 knockdown repressed the proliferation and promoted the apoptosis of OS-732 cells by CCK-8 assays and western blot. Fei et al. (2019) reported that lncRNA ROR functioned as an oncogene in OS by sponging miR-206. They performed CCK-8 and colony formation assays to demonstrate the inhibition of proliferation and colony formation ability of U2OS cells after lncRNA ROR knockdown. Zheng et al. (2020) proposed that the LINC00266- 1/miR-548c-3p/SMAD2 feedback loop stimulated OS development and showed that lncRNA LINC00266- 1 downregulation partially inhibited MG63 and U2OS cell proliferation by CCK-8 and colony formation assays. In the protein imprinting assay, LINC00266-1 downregulation led to the downregulation of Ki67 and PCNA proteins in OS cells; flow cytometry and TUNEL assays showed

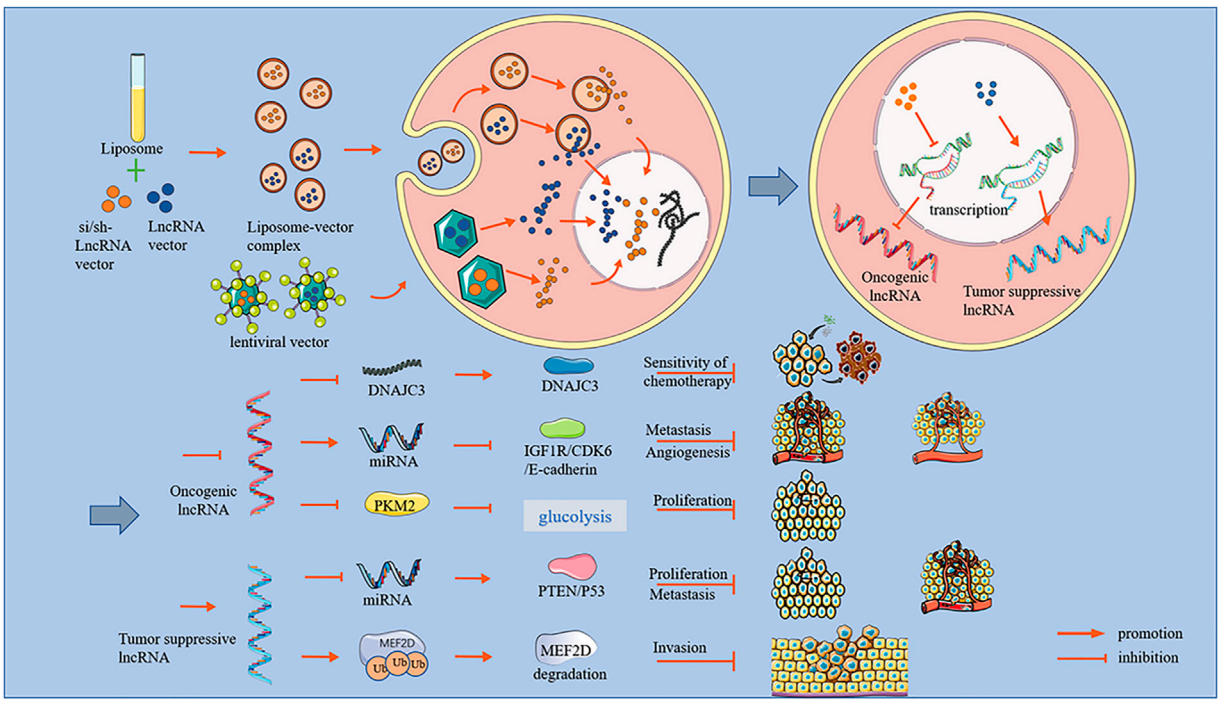

FIGURE 1
An overview of lncRNA effects and mechanisms.

TABLE 1 Oncogenic lncRNAs associated with the inhibition of osteosarcoma proliferation and the promotion of apoptosis.

LncRNA	Expression in OS	Regulatory pathway	Key function *in vitro* after knocked-down	Function *in vivo* after knocked-down	Refs
NNT-AS1	↑	MiR-320a/beta-catenin and RUNX2 axis	Proliferation ↓	Tumor formation↓	Li et al. (2019)
			Apoptosis ↑		
			Cell cycle arrested		
ROR	↑	Targeting miR-206	Proliferation ↓	Tumor growth↓	Fei et al. (2019)
			Apoptosis ↑		
00266-1	↑	Linc00266-1/miR-548c-3p/SMAD2 feedback loop	Proliferation↓	Tumor growth↓	Zheng et al. (2020)
			Apoptosis ↑	PCNA and Ki-67 ↓	
			Cell cycle arrested		
00963	↑	miR-204-3p/FN1 axis	Proliferation↓	—	Zhou et al. (2019)
			Apoptosis ↑		
ADPGK-AS1	↑	Targeting miR-542-3p	Proliferation ↓	—	Luo et al. (2019)
			Apoptosis ↑		
			Cell cycle arrested		
AFAP1-AS1	↑	miR-497/IGF1R axis	Proliferation ↓	Tumor growth↓	Fei et al. (2020)
			Apoptosis ↑	Ki-67 expression↓	
BLACAT1	↑	miR-608/SOX12 axis	Proliferation ↓	—	Chen X et al. (2020)
			Apoptosis ↑		
CCDC144NL-AS1	↑	miR-490-3p/HMGA2 axis	Proliferation ↓	Tumor growth↓	He et al. (2021)
			Apoptosis ↑	Tumor volume↓	
				Tumor weight↓	
H19	↑	MiR-29a3p/LASP1 axis	Proliferation↓	Tumor growth↓	Jin et al. (2021)
			Apoptosis ↑	Tumor volume↓	
				Tumor weight↓	
KCNQ1OT1	↑	MiR-3666/KLF7 axis	Proliferation ↓	Tumor volume↓	Huang et al. (2021)
			Apoptosis ↑	Tumor weight↓	
SND1-IT1	↑	miR-665/POU2F1	Apoptosis ↑	—	Jin et al. (2019)
LINC01116	↑	silencing p53 and EZH2	Proliferation ↓	—	Zhang Z. F et al. (2019)
			Apoptosis ↑		
			Cell cycle arrested		

that LINC00266-1 knockdown induced OS apoptosis cells. In *in vivo* experiments, LINC00266-1 knockdown inhibited OS growth in nude mice. Zhou et al. (2019) reported that the lncRNA LINC00963 inhibited miR-204-3p by directly binding to it. CCK-8 assay to assess cell growth and viability demonstrated that lncRNA LINC00963 knockdown inhibited OS proliferation by promoting the miR-204-3p/FN1 axis. Luo et al. (2019) found that the lncRNA ADPGK-AS1 affected cell proliferation, invasion, migration, and apoptosis by targeting miR-542-3p in OS. LncRNA ADPGK-AS1 knockout resulted in a significantly decreased cell proliferation rate and protein expression of CDK4 and CyclinD1. TUNEL experiments showed apoptosis induction. Meanwhile, Fei et al. (2020) reported lncRNA AFAP-AS1 as a novel oncogene that promoted OS tumorigenesis and progression by competitively binding miR-497 and regulating IGF1R expression. AFAP1-AS1 knockout significantly inhibited U2OS cell proliferation and colony formation in the CCK-8, plate clone formation, and flow cytometry assays. Chen X et al. (2020) demonstrated that lncRNA BLACAT1 facilitated OS cell growth and motility by upregulating SOX12 through sponging miR-608. They observed reduced 143B and MG63 cell proliferation and increased apoptosis by TUNEL, EdU, and colony formation assays. He et al. (2021) reported that lncRNA CCDC144NL-AS1 promoted OS tumorigenicity by acting as a molecular sponge for microRNA-490-3p, thereby increasing HMGA2 expression. They confirmed through CCK-8 assay and flow cytometry that OS cell proliferation was significantly inhibited

and the apoptosis rate was significantly increased after the knockdown of lncRNA CCDC144NL-AS1. *In vivo*, compared to the control group, tumors grew significantly slower and weighed less when lncRNA CCDC144NL-AS1 was silenced. These results indicated that CCDC144NL-AS1 knockdown significantly upregulated the expression of miR-490-3p in HOS and SAOS-2 cells and that CCDC144NL-AS1 expression was positively correlated with HMGA2 mRNA expression in OS tissues. Jin et al. (2021) reported that lncRNA H19 regulated LASP1 expression in OS by competitively binding to miR29a3p. CCK-8 assays showed that RNAH19 knockdown increased miR-29a-3p expression and decreased LASP1 expression, which inhibited Saos2 and MG63 cell proliferation. Huang et al. (2021) confirmed significantly inhibited proliferation and colony formation in U2OS and SAOS-2 cells after lncRNA KCNQ1OT1knockdown, as assessed by CCK-8 assay, colony formation assay, and western blot analysis. The authors suggested that lncRNA KCNQ1OT1 promoted tumor growth and activated the Wnt/β catenin signaling pathway in OS by targeting the miR3666/KLF7 axis. When KCNQ1OT1 was silenced, miR-3666 expression increased significantly. KCNQ1OT1 was negatively correlated with miR-3666 expression. KLF7 expression was negatively correlated with miR-3666 expression and positively correlated with KCNQ1OT1 expression. Jin et al. (2019) reported that lncRNA SND1-IT1 accelerated OS proliferation and migration by upregulating POU2F1 through the sponging of miRNA-665. After knocking down lncRNA SND1-IT1, CCK-8 and EdU assays showed decreased OS cell viability at 48, 72, and 96 h. Compared to the non-knockdown group, the number of colonies formed was significantly reduced and the proportion of EdU-positive cells was lower in the lncRNA SND1-IT1 knockdown group. Zhang Z. F et al. (2019) suggested that lncRNA LINC01116 promoted the proliferation of OS cells by silencing p53 and EZH2. Knockdown of lncRNA LINC01116 significantly reduced OS cell viability and promoted apoptosis, as assessed by CCK8 assay and cell apoptosis determination.

2.2 Oncogenic lncRNA silencing could arrest the cell cycle of OS cells

In addition to directly inhibiting OS cell proliferation and apoptosis, lncRNA knockdown could also affect OS cells by regulating the cell cycle. Duan et al. (2019) found that lncRNA MALAT1 exerted an oncogenic function in OS by regulating the miR-34a/CCND1 axis. When lncRNA MALAT1 was silenced, the expression of CCND1, a regulator of the cell cycle, was significantly reduced in Western blot assays. Deng et al. (2019) reported that the lncRNA SNHG1 promoted OS cell proliferation, migration, and invasion by downregulating miRNA-101-3p expression, which enhanced ROCK1 expression. The downregulation of lncRNA SNHG1 induced cell apoptosis and maintained the cell cycle at the G0/G1 phase, thus reducing the overall cell viability. Deng et al. observed that lncRNA LINC00514 promoted OS progression by sponging microRNA-708, thereby increasing the expression of cell proliferation upregulated factor (URGCP). LINC00514 knockdown significantly increased the apoptosis rate of HOS and MG-63 cells; moreover, the proportion of cells in the G0/G1 phase increased according to the proportion of cells in the S phase, as shown by flow cytometry analysis (Yu et al., 2020). Zhang G. F et al. (2021) demonstrated that lncRNA LINC01278 was highly expressed in OS and participated in tumor development by mediating the miR-134-5p/KRAS axis. After LINC01278knockdown, cell cycle analysis showed a significantly increased number of cells in the G1/G0 phase and a significantly decreased number of cells in the S and G2/M phases. These results suggested that LINC01278 knockdown could induce cell apoptosis, arrest the cell cycle at G1/G0, and further inhibit cell proliferation. The mechanism was that miR-134-5p expression increased significantly after LINC01278 knockdown. MiR-134-5p was negatively correlated with LINC01278, and LINC01278 may specifically bind miR-134-5p to participate in OS progression. Therefore, lncRNA LINC01278 could be involved in the regulation of KRAS expression as a sponge of miR-134-5p. Wei et al. (2020) reported that the Usf1-mediated upregulation of lncRNA gAS6-AS2 promoted OS progression through the miR-934/BCAT1 axis. In their study, after AS6-AS2 knockdown, western blot assay showed increased levels of the apoptosis-related molecules caspase 3 and 9, which indicated cell cycle inhibition. Wang et al. (2021) observed that lncRNA HCG9 promoted OS progression through RAD51 by acting as a ceRNA of miR-34b-3p. RAD51 is a key enzyme that regulates the cell cycle and preserves the G2/M phase, whereas H2A preserves cell cycle arrest. After RNAHCG9 knockdown, cell cycle analysis showed that cell arrest was induced, the G0/G1 phase was significantly enhanced, and the G2/M phase was significantly inhibited, thus inhibiting OS progression. After lncRNA SPRY4IT1 knockdown, Yao et al. (2020) demonstrated that cells with cell cycle progression significantly accumulated in the G1 phase, indicating that SPRY4IT1 downregulation could lead to G1 phase arrest. They also found that miR-101 mimic transfection led to S-phase cell cycle arrest. One possible mechanism for these results was that lncRNA SPRY4- IT1 promoted OS progression by regulating the expression of ZEB1 and ZEB2 through miR-101. Shi et al. (2020) proposed that lncRNA AFAP1-AS1 played an oncogenic role in OS through the RhoC/ROCK1/p38MAPK/Twist 1 signaling pathway. Flow cytometry and cell cycle analysis after lncRNA AFAP1-AS1 knockdown showed that compared to the control group, the cell distribution of the knockdown group showed a significant increase in the G0/G1 phase and a significant decrease in the S phase; however, the two groups did not differ significantly in the G2/M phases. In summary, the cell cycle was arrested in the G0/G1 phase, and S cell cycle progression was inhibited after AFAP1-AS1 knockdown. Wang and Zhang (2021) observed that lncRNA CASC15 promotes IS proliferation and metastasis by regulating the epithelial-mesenchymal transition *via* the Wnt/β-catenin signaling pathway. CASC15 affected the cell cycle by activating the Wnt/β-catenin pathway, thereby promoting cell proliferation. After CASC15 knockdown, CCK-8 and EdU assays showed decreased OS cell proliferation. The number of cells in the G0/G1, S, and G2/M phases detected by flow cytometry showed a decreased number of cells in the S phase. The effect of lncRNA knockdown on cell metabolism may also have an inhibitory effect on OS progression. Pan et al. (2022) reported that lncRNA HCG18 promoted OS growth by enhancing aerobic glycolysis *via* the miR-365a-3p/PGK1 axis. HCG18 knockdown partly reduced the extracellular acidification rate (ECAR) in MNNG-HOS and 143B cells but enhanced the oxygen consumption rate (OCR) of these cells. The results of the cell metabolism experiment showed decreased glucose consumption, increased ATP levels, and decreased lactate production in OS cells when lncRNA HCG18 was silenced. These results indicated that

TABLE 2 Oncogenic lncRNAs associated with the cell cycle arrest of osteosarcoma cells.

LncRNA	Expression in OS	Regulatory pathway	Key function *in vitro* after knocked-down	Function *in vivo* after knocked-down	Refs
MALAT1	↑	miR-34a/CCND1 axis	Proliferation ↓	—	Duan et al. (2019)
			Apoptosis↑		
			Cell cycle arrested		
SNHG1	↑	miR-1013p/ROCK1 axis	Proliferation ↓	—	Deng et al. (2019)
			Apoptosis ↑		
			Cell cycle arrested		
00514	↑	LINC00514/miR-708/URGCP pathway	Proliferation ↓	Tumor volume↓	Yu et al. (2020)
			Apoptosis ↑	Tumor weight↓	
			Cell cycle arrested		
LINC01278	↑	miR-134-5p/KRAS axis	Proliferation↓	Tumor volume↓	Zhang G. F et al. (2021)
			Apoptosis ↑	Tumor weight↓	
			Cell cycle arrested		
GAS6-AS2	↑	miR-934/BCAT1 axis	Proliferation ↓	Tumor volume↓	Wei et al. (2020)
			Apoptosis ↑	Tumor weight↓	
			Cell cycle arrested		
HCG9	↑	miR-34b-3p/RAD51 axis	Proliferation ↓	Tumor volume↓	Wang et al. (2021)
			Apoptosis↑	Tumor weight↓	
			Cell cycle arrested	Cell cycle arrested	
SPRY4-IT1	↑	miR-101/ZEB1 and ZEB2 axis	Proliferation ↓	Tumor volume↓	Yao et al. (2020)
			Apoptosis ↑	Tumor weight↓	
			Cell cycle arrested	E-cadherin ↑	
			EMT ↓		
AFAP1-AS1	↑	RhoC/ROCK1/p38MAPK/ Twist1 pathway	Proliferation↓	Tumor volume↓	Shi et al. (2020)
			Apoptosis ↑	Tumor weight↓	
			Cell cycle arrested		
			Angiogenesis↓		
			EMT ↓		
CASC15	↑	Wnt/β-catenin pathway	Proliferation ↓	Tumor volume↓	Wang and Zhang (2021)
			Apoptosis ↑		
			Cell cycle arrested		
			EMT ↓		
HCG18	↑	miR-365a-3p/PGK1 axis	Proliferation ↓	Tumor growth↓	Pan et al. (2022)
			Apoptosis↑	Tumor volume↓	
			Cell metabolism ↓	Tumor weight↓	
				Ki-67 expression↓	
OR3A4	↑	miR-1207-5p/G6PD axis	Proliferation ↓	—	Wang et al. (2020b)
			Apoptosis ↑		
			Cell metabolism↓		

(Continued on following page)

TABLE 2 (*Continued*) Oncogenic lncRNAs associated with the cell cycle arrest of osteosarcoma cells.

LncRNA	Expression in OS	Regulatory pathway	Key function *in vitro* after knocked-down	Function *in vivo* after knocked-down	Refs
PCAT-1	↑	miR-508-3p/ZEB1 axis	Proliferation ↓	—	Chang et al. (2021)
			Apoptosis ↑		

HCG18 knockdown inhibited aerobic glycolysis in OS cells, thereby inhibiting tumor cell growth. Additionally, Wang et al. (2020b) found that lncRNA OR3A4 regulated OS cell growth by modulating miR-1207-5p/G6PD signaling. Glucose consumption assays using MG-63 and SaoS-2 cells with lncRNA OR3A4 knockdown showed significantly reduced NADPH levels and inhibited glucose consumption and lactate production in OS cells. These results suggested that OR3A4 knockdown suppressed glycolysis in OS cells. Chang et al. (2021) reported inhibited OS cell metabolism following the downregulation of lncRNA PCAT-1. Because lncRNA PCAT-1 promoted OS progression through the mir-508-3p/ZEB1 axis, miR-508-3p levels increased significantly after lncRNA PCAT-1 knockdown. miR-508-3p expression was negatively correlated with pCAT-1. LncRNA PCAT-1 knockdown reduced ZEB1 expression, thereby inhibiting OS progression. These data are summarized in Table 2.

2.3 Oncogenic lncRNA silencing reduces osteosarcoma angiogenesis

Inhibition of OS cell proliferation is also related to the regulation of angiogenesis by lncRNAs. Xiao et al. (2020) reported that LINC00265 targets miR-382-5p and regulates SAT1, VAV3, and angiogenesis in OS. They demonstrated inhibited cell tube production capacity *via* LINC00265 silencing. Therefore, LINC00265 knockdown caused a decreased ability to form blood vessels. Vimalraj et al. (2021) hypothesized that lncRNA MALAT1 promotes tumor angiogenesis by regulating microRNA-150-5p/VEGFA signaling in OS. They analyzed the levels of angiogenin-related genes and VEGFA by Western blot and ELISA and found that lncRNA MALAT1 knockdown significantly reduced the expression and secretion levels of VEGFA in MG63 and SaOS2 cells. Using chicken embryos, they established a new model to examine the regulation of angiogenesis in tumors. Compared to the control group, the MALAT1 knockdown group showed reduced blood vessel length and size and connections between blood vessels, as well as decreased mRNA expression levels of the angiogenic markers VEGFA, ANG1, Tie2, CXCR4, and FGF2. LncRNA AFAP1-AS1 is overexpressed in OS and plays an oncogenic role through the RhoC/ROCK1/p38MAPK/Twist-1 signaling pathway. Shi et al. (2019) reported that AFAP1-AS1 knockdown reduced vasculogenic mimicry in OS cells. Vasculogenic mimicry (VM) is the formation of microvascular structures in malignant tumors and is believed to be closely related to cancer cell growth, invasion, and metastasis. To investigate whether AFAP1-AS1 affects VM formation in OS cells, the authors performed a tube formation assay. Compared to the control group, the AFAP1-AS1 knockdown group showed a significantly reduced number of tubular structures in OS cells, indicating that AFAP1-AS1 knockdown could inhibit VM formation and that AFAP1-AS1 may play an important role in VM formation in OS cells. Chen et al. (2021b) demonstrated that the LOC100129620/miR-335-3p/CDK6 signaling pathway promotes OS metastasis. The results of cell scratch experiments showed that the knockdown of lncRNA LOC100129620 reduced the stimulating effect of OS cells on endothelial cell migration. Similarly, LOC100129620 knockdown reduced the number of endothelial cells within the xenograft model. These results indicated that LOC100129620 knockdown can inhibit OS angiogenesis. Thus, lncRNAs play an important regulatory role in various pathophysiological processes of OS, including cell proliferation, cell cycle progression, apoptosis, and carcinogenesis. These data are summarized in Table 3.

2.4 Oncogenic lncRNA silencing suppresses invasion and migration in OS

An increasing number of studies have revealed that the knockdown of oncogenic lncRNAs can suppress OS development, especially cell invasion and cell migration. These data are summarized in Table 4. Zhang C et al. (2019) reported that lncRNA MIAT might function as a sponge of miR-128-3p in OS and that the invasion and migration of MG63 cells after downregulation of lncRNA CMIAT were inhibited by transwell and wound healing assays, respectively. Meanwhile, Lian et al. (2019) confirmed that lncRNA LINC00460 knockdown remarkably repressed OS cell invasion and migration by transwell and wound healing assays; they also reported that Linc00460 functioned as a competitively endogenous RNA (ceRNA) by sponging miR-1224-5p in OS, thereby promoting OS progression. LncRNA LINC00665 facilitated OS progression by reducing miR-3619 expression. Zhang D. W et al. (2020) verified that LINC00665 knockdown suppressed OS cell invasion and migration in transwell assays. Xing et al. (2020) confirmed that upregulated LINC00689 competitively bound to miR-655, which prevented SOX18 from miRNA-mediated degradation, thus facilitating OS progression. As evidenced by wound healing and transwell assays, when LINC00689 was silenced, the migratory and invasive abilities of MG63 and 143B cells were notably impaired. Moreover, transwell and wound healing assays to investigate the role of lncRNA LINC01614 in the regulation of cell migration and invasion in OS cells demonstrated reduced cell migration and invasion ability in the LINC016141 knockdown group compared to the control group. Additionally, Cai et al. (2021) demonstrated that lncRNA LINC01614 could function as a competing endogenous RNA and promote OS cell proliferation and invasion through the miR-520a-3p/SNX3 axis. Zhang W et al. (2020) reported that the downregulation of lncRNA DANCR inhibited OS cell migration and metastasis and proposed a mechanism in which lncRNA DANCR regulates OS migration and invasion by targeting the miR-49/MSI2 axis. Dai et al. (2021) reported that the lncRNA EBLN3P promoted OS *via*

TABLE 3 Oncogenic lncRNAs associated with reduced osteosarcoma angiogenesis.

LncRNA	Expression in OS	Regulatory pathway	Key function *in vitro* after knocked-down	Function *in vivo* after knocked-down	Refs
00265	↑	targeting miR-382-5p	Proliferation ↓	Tumor growth↓	Xiao et al. (2020)
			Apoptosis ↑	Tumor volume↓	
			Angiogenesis↓	SAT1, VAV3↓	
LncRNA MALAT1	↑	miRNA-150-5p/VEGFA axis	Proliferation↓	Angiogenesis ↓	Vimalraj et al. (2021)
			Apoptosis ↑		
			Angiogenesis↓		
AFAP1-AS1	↑	RhoC/ROCK1/p38MAPK/ Twist1	Proliferation ↓	Tumor growth↓	Shi et al. (2019)
			Angiogenesis↓	Tumor volume↓	
LOC100129620	↑	CDK6 expression	Proliferation ↓	Tumor growth↓	Chen et al. (2021b)
			Apoptosis ↑	Tumor volume↓	
			Cell cycle arrested	Angiogenesis ↓	
			Angiogenesis↓		

TABLE 4 Oncogenic lncRNAs associated with osteosarcoma invasion and migration.

LncRNA	Expression in OS	Regulatory pathway	Key function *in vitro* after knockdown	Function *in vivo* after knockdown	Refs
MIAT	↑	MIAT/miR-128-3p/VEGFC axis	Invasion ↓	—	Zhang C et al. (2019)
			Migration ↓		
			VEGFC ↑		
00460	↑	00460/miR-1224-p/ FADS1 axis	Invasion ↓	—	Lian et al. (2019)
			Migration ↓		
00665	↑	sponging miR-3619	Invasion ↓	—	Zhang D. W et al. (2020)
			Migration ↓		
00689	↑	miR-655/SOX18 axis	Invasion ↓	—	Xing et al. (2020)
			Migration ↓ caspase3/9 ↑		
01614	↑	miR-520a-3p/SNX3 axis	Invasion ↓	—	Cai et al. (2021)
			Migration ↓		
DANCR	↑	miR-149/MSI2 axis	Invasion ↓	—	Zhang W et al. (2020)
			Migration ↓		
BLN3P	↑	miR-224-5p/Rab10 axis	Invasion ↓	—	Dai et al. (2021)
			Migration ↓		
HCG18	↑	miR-148b/ETV5	Invasion ↓	—	Zheng and Lin (2021)
		axis	Migration ↓		
HIF1A-AS2	↑	Sponging miR-129-5p	Invasion ↓	—	Wang et al. (2019)
			Migration ↓		

the miR-224-5p/Rab10 regulatory loop. When lncRNA EBLN3P was knocked down, transwell and wound healing experiments with OS cells showed inhibited invasion and migration by OS cells. Zheng and Lin (2021) found that lncRNA HCG18 promotes OS progression through miR-148b/ETV5 regulation; a transwell assay in OS cells after LncRNAHCG18 knockdown showed significantly suppressed migration and invasion. Wang et al. (2019) proposed that lncRNA HIF1A-AS2 overexpression promoted OS progression through the

modulation of miR-129-5p. The authors used wound healing and invasion assays of OS cells measure determine cell proliferation and invasion ability and concluded that silencing lncRNA HIF1A-AS2 significantly decreased OS cell invasion and metastasis. In summary, many experiments have shown that the knockout of specific lncRNAs can inhibit OS cell invasion and migration.

2.5 Oncogenic lncRNA regulates the expression of EMT-related proteins in OS cells

The epithelial-interstitial transformation (EMT) is a basic process that controls the morphogenesis of organisms. EMT manifests as a loss of E-cadherin and an increase in vimentin expression and is a process in which epithelial cells lose their polarity and adhesion to gain migratory ability and adopt a mesenchymal phenotype. Knockdown of lncRNAs can also regulate the expression of EMT-related proteins in OS cells. LINC00324 accelerated OS proliferation and migration by regulating WDR66. Wu et al. (2020) used western blot analysis to evaluate the effect of LINC00324 on EMT progression. After the knockdown of LINC00324 in OS cells, the expression level of E-cadherin increased whereas the expression levels of N-cadherin and Vimentin decreased. Yan et al. (2021) reported that LINC00467 serves as a molecular sponge for miR-217, while karyopherin subunit α4 (KPNA4) is a downstream target gene of miR-217; LINC00467 facilitates OS progression by sponging miR-217 to regulate KPNA4 expression. The authors reported decreased expression levels of EMT-associated proteins after LINC00467 silencing, as assessed by western blot analysis. In contrast, for the same lncRNA LINC00467, Ma et al. (2020) concluded that lncRNA LINC00467 contributes to OS growth and metastasis by regulating HMGA1 by directly targeting miR-217. In their western blot analysis, lncRNA LINC00467 downregulation increased E-cadherin expression and decreased N-cadherin and vimentin expression. Zhou and Mu (2021) also reported that the results of Western blot analysis showed that LINC00958 knockdown downregulated N-cadherin and vimentin expression while upregulating E-cadherin expression. The authors concluded that LINC0095 promotes tumorigenesis and metastasis in OS by competitively inhibiting miR-4306 expression, leading to elevated CEMIP expression. Gu Z. Q et al. (2020) reported that lncRNA LINC01419 mediated malignant phenotypes in OS by targeting the miR-519a-3p/PDRG1 axis and that LINC01419 downregulation increased the expression of E-cadherin protein while lowering the expression levels of N-cadherin, vimentin, and β-catenin. Zhang H et al. (2020) demonstrated that DDX11-AS1 could sponge miR-873-5p to upregulate DDX11 expression and that DDX11-AS1 contributed to OS progression by stabilizing DDX11. Meanwhile, western blot analysis indicated that DDX11-AS1 deficiency markedly reduced MMP2, MMP9, N-cadherin, Slug, and Twist expression but increased E-cadherin expression, suggesting that silencing DDX11-AS1 could suppress cell metastasis and the EMT process. Additionally, Wei et al. (2020) proposed that USF1-mediated upregulation of lncRNA GAS6-AS2 facilitated OS progression through the miR-934/BCAT axis. They measured the expression levels of EMT-relevant molecules, N-cadherin, E-cadherin, and vimentin in OS cells after GAS6-AS2 knockdown. The data demonstrated that GAS6-AS2 depletion dramatically reduced N-cadherin and vimentin levels, while GAS6-AS2 silencing significantly increased E-cadherin levels. Liu et al. (2021) reported that lncRNA X-inactive (XIST) promoted OS metastasis by modulating microRNA-758/Rab 16. The results of Western blot analysis showed that transfection with si-XIST upregulated E-cadherin at the protein level while downregulating the protein levels of N-cadherin and vimentin in OS cells. In brief, EMT is an important way to promote OS invasion and migration and enhance its malignant behaviors. Knockdown of specific lncRNAs could inhibit EMT progression and delay tumor invasion and metastasis by reducing N-cadherin and vimentin levels while enhancing E-cadherin expression. These data are summarized in Table 5.

2.6 Oncogenic lncRNA silencing demonstrates antitumor effects *in vivo*

As described above, the knockdown of oncogenic lncRNAs significantly inhibited the progression of OS cells *in vitro*. Furthermore, several studies have shown similar results *in vivo*. Researchers typically transferred OS cells with silenced oncogenic lncRNAs into mice and observed tumor cell growth to determine the role of lncRNAs *in vivo*. Ning and Bai (2021) injected MG63 cells after DSCAM-AS1 silencing subcutaneously into the groin of each mouse. The results showed decreased tumor size and weight in the DSCAM-AS1-silenced group after 4 weeks. They proposed that DSCAM-AS1 accelerated OS cell proliferation and migration through miR-186-5p/GPRC5A signaling. Li et al. (2021) reported that FGD5-AS1 increased OS cell proliferation and migration by sponging miR-506-3p to upregulate RAB3D. They injected OS cells transfected with pcDNA3.1-FGD5-AS1 or FGD5-AS1 siRNA into nude mice *via* the caudal vein, in which FGD5-AS1 overexpression promoted the lung metastasis of OS cells, while FGD5-AS1 depletion repressed the formation of metastatic nodules in the lung. Li et al. (2020) established an OS mouse model to study the effects of lncRNA HULC on OS cell invasion and metastasis, and observed smaller tumor tissues and fewer metastatic tumors in the HULC knockdown group compared to those in the control group. Finally, lncRNA HULC induces OS progression by regulating the miR-372-3p/HMGB1 signaling axis. Chen et al. (2021a) confirmed that lncRNA NEAT1 promoted the epithelial-mesenchymal transition and metastasis of OS cells by sponging miR-483 to upregulate STAT3 expression *in vivo*. They observed significantly smaller tumor volumes, lighter tumor weights, slower tumor growth rates, and fewer metastatic tumors in the lncRNA NEAT1-silenced group compared to those in the control group. Liu W et al. (2020) found that lncRNA PGM5-AS1 silencing inhibited OS cell tumorigenesis and reduced the number of lung metastases in a mouse tumor model. They suggested that lncRNA PGM5-AS1 promoted the epithelial-mesenchymal transition, invasion, and metastasis of OS cells by impairing miR-140-5p-mediated FBN1 inhibition. Jiang et al. (2021) injected OS cells into mice to construct an OS model *in vivo*. Six weeks later, compared to the control group, the lncRNA RUSC1-AS1 knockdown group showed significantly reduced tumor volume; attenuated tumor node weight; increased E-cadherin expression; and decreased N-cadherin, vimentin, and Snail expression. They demonstrated that lncRNA RUSC1-AS1

TABLE 5 Oncogenic lncRNA associated with the regulation of EMT-related protein expression in osteosarcoma cells.

LncRNA	Expression in OS	Regulatory pathway	Key function *in vitro* after knockdown	Function *in vivo* after knockdown	Refs
00324	↑	Regulating WDR66	Invasion ↓	—	Wu et al. (2020)
			Migration ↓		
			E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓		
00467	↑	miR-217/KPNA4 axis	Invasion ↓	—	Yan et al. (2021)
			Migration ↓		
			E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓		
00467	↑	miR-217/HMGA1 axis	Invasion ↓	—	Ma et al. (2020)
			Migration ↓		
			E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓		
00958	↑	miR-4306/CEMIP axis	Invasion ↓	—	Zhou and Mu (2021)
			Migration ↓ caspase-3 ↑ E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓		
01419	↑	miR-519a-3p/ PDRG1 axis	Invasion ↓	Tumor volume↓	Gu Z. Q et al. (2020)
			Migration ↓	PCNA/Ki-67 ↓	
			E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓		
			β-catenin ↓		
DDX11-AS1	↑	Stabilizing DDX11	Invasion ↓	Tumor volume↓	Zhang H et al. (2020)
			Migration ↓	Tumor weight↓	
			E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓		
GAS6-AS2	↑	miR-934/BCAT1 axis	Invasion ↓	—	Wei et al. (2020)
			Migration ↓		
			E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓ caspase-3 ↑		
			caspase-9 ↑		

(Continued on following page)

TABLE 5 (*Continued*) Oncogenic lncRNA associated with the regulation of EMT-related protein expression in osteosarcoma cells.

LncRNA	Expression in OS	Regulatory pathway	Key function *in vitro* after knockdown	Function *in vivo* after knockdown	Refs
X-inactive	↑	microRNA-758/ Rab16 axis	Invasion ↓	—	Liu et al. (2021)
			Migration ↓		
			E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓		

functioned as a competing endogenous RNA (ceRNA) to competitively bind miR-101-3p, thus upregulating Notch 1 expression and mediating the malignant behaviors of OS cells. Yu et al. (2019) proposed that the lncRNA taurine promoted OS cell metastasis by mediating HIF-1α *via* miR-143-5p. In their tumor mouse model, compared to the control group, the lncRNA TUG1 knockdown group showed significantly less visible peritoneal and pulmonary nodules and smaller tumor volumes and weights. Ding et al. (2021) reported the results of a tail vein injection lung metastasis model, in which the number of pulmonary metastasis nodules formed by OS cells in the MELTF-AS1-silenced group (shRNA MELTF-AS1) was significantly reduced compared to that in the control group. Additionally, the survival time of mice in the MELTF-AS1-silenced group was longer than that in the control group. Immunofluorescence assays revealed that after silencing MELTF-AS1 in OS cells, the protein level of Vimentin decreased, and the protein level of E-cadherin increased. These results suggested a weakened metastasis ability of OS cells *in vivo* after MELTF-AS1 silencing. Indeed, MELTF-AS1 adsorbed miR-485-5p in the cytoplasm and acted as a ceRNA to promote MMP14 expression. In general, lncRNA plays an important role in OS progression *in vivo*; the downregulation of specific lncRNA significantly inhibited tumor growth, invasion, and metastasis in transfection and tail vein injection experiments in mice. These data are summarized in Table 6.

3 Tumor-suppressive lncRNA overexpression inhibits OS progression

According to different regulatory mechanisms of lncRNAs in OS progression, not only the knockdown of oncogenic lncRNAs but also the overexpression of tumor-suppressive lncRNAs could inhibit OS progression. These data are summarized in Table 7.

3.1 Tumor-suppressive lncRNAs can suppress OS by targeting miRNAs

Tumor-suppressive lncRNAs always inhibit OS progression by affecting miRNAs. The expression level of lncRNA LINC00588 is low in HOS and U2OS cell lines. Zhou et al. (2020) transfected these cell lines with the lentiviral vector pLVX-LINC00588. The results of CCK-8, wound healing, and transwell assays showed inhibition of cell viability, migration, and invasion, respectively, due to LINC00588 overexpression. Cell cycle analysis with a cell cycle analysis kit showed arrested cell cycles in the G2 phase in OS. Furthermore, to assess tumor cell metastasis ability, the expression of EMT in transfected cells showed upregulated expression of E-cadherin and downregulated expression of ZEB1, Snail, and Fibronectin. Finally, the authors demonstrated that lncRNA LINC00588 was a ceRNA for miRNA-1972 and inhibited TP53 expression. Wan et al. (2020) overexpressed lncRNA LINC00691 *via* pLVX-IRES-Puro vectors in U2OS and Saos-2 cell lines. They observed reduced proliferation and invasion of OS cells overexpressing lncRNA LINC00691 in CCK-8 analysis and transwell assay, respectively. They also demonstrated increased E-cadherin expression and decreased ZEB1, snail, and fibronectin expression with lncRNA LINC00691 overexpression in EMT analysis. Finally, they demonstrated that lncRNA LINC00691 acts as a ceRNA for miRNA-1256 and promotes ST5 expression. LncRNA GAS5 has been identified as a tumor suppressor in several human cancers involving OS. Liu J et al. (2020) transfected U2OS and Saos-2 cell lines with the pcDNA-GAS5 vector, in which GAS5 overexpression not only inhibited OS cell proliferation and invasion in CCK-8 and transwell assays but also promoted apoptosis, as shown by flow cytometry. LncRNA GAS5 acted as a ceRNA for miR-23a-3p and inhibited the activation of the PI3K/AKT pathway. Finally, in their tumor xenograft BALB/c nude mouse model, compared to the pcDNA3.1 empty vector group, the pcDNA-GAS5 vector group showed a smaller tumor size and a lower miR-23a-3p expression level. HCG11 is another lncRNA with decreased expression in OS cells. Gu et al. (2021) transfected MG63 and 143B cell lines with the lncRNA HCG11 vector and reported that HCG11 overexpression decreased OS cell proliferation in CCK-8 assays; moreover, the cell cycle was arrested in cell cycle analysis, and DNA replication activity was inhibited in EdU assays. *In vivo*, a BALB/c nude mouse tumor model was established. Compared to the control group, tumor volumes were smaller in the HCG11 overexpression group. Finally, the authors demonstrated that lncRNA HCG11 works by binding to miR-942-5p and IGF2BP2 and increasing p27Kip1 expression. Chen J et al. (2020) reported that lncRNA NR_027471 suppresses OS both *in vitro* and *in vivo*. After transfecting the NR_027471 vector into U2OS and Saos-2 cell lines, inhibition of proliferation, migration, and invasion were observed in the CCK-8 assay, scratch test, and transwell assay respectively. A nude mouse tumor xenograft model was established, which showed a significantly lower tumor weight in the NR_027471 group compared to that in the control group. The authors also confirmed the regulatory mechanism by which lncRNA NR_027471 upregulates TP53INP1 by sponging miR-8055. After observing significantly downregulated lncRNA TUSC7 expression in OS tissues, Zhao et al. (2021) transfected U2OS and MG63 cells

TABLE 6 Oncogenic lncRNAs associated with antitumor effects *in vivo*.

LncRNA	Expression in OS	Regulatory pathway	Key function *in vitro* after knockdown	Function *in vivo* after knockdown	Refs
DSCAM-AS1	↑	miR-186-5p/GPRC5A axis	Invasion ↓	Tumor volume↓	Ning and Bai (2021)
			Migration ↓	Tumor weight↓	
			E-cadherin ↑	E-cadherin ↑	
			N-cadherin ↓	N-cadherin ↓	
			Vimentin ↓	Vimentin ↓	
FGD5-AS1	↑	miR-506-3p/RAB3D axis	Invasion ↓	lung metastasis ↓	Li et al. (2021)
			Migration ↓		
HULC	↑	miR-372-3p/HMGB1 axis	Invasion ↓	Tumor volume↓	Li et al. (2020)
			Migration ↓	Tumor weight↓ lung metastasis ↓	
NEAT1	↑	miR-483 to upregulate STAT3 expression	Invasion ↓	Tumor volume↓	Chen et al. (2021a)
			Migration ↓	Tumor weight↓ lung metastasis ↓	
			E-cadherin ↑	Liver metastasis ↓	
			N-cadherin ↓	E-cadherin ↑	
				N-cadherin ↓	
				Vimentin ↓	
			Vimentin ↓		
PGM5-AS1	↑	miR-140-5p/FBN1 axis	Invasion ↓	Tumor volume↓	Liu W et al. (2020)
			Migration ↓	Tumor weight↓ lung metastasis ↓	
			E-cadherin ↑	E-cadherin ↑	
			N-cadherin ↓	N-cadherin ↓	
			Vimentin↓	Vimentin ↓	
RUSC1-AS1	↑	Notch1 pathway	Invasion ↓	Tumor volume↓	Jiang et al. (2021)
			Migration ↓	Tumor weight↓ lung metastasis ↓	
			E-cadherin ↑	E-cadherin ↑	
			N-cadherin ↓	N-cadherin ↓	
			Vimentin ↓	Vimentin ↓	
Taurine	↑	miR-143-5p/HIF-1α axis	Invasion ↓	Tumor volume↓	Yu et al. (2019)
			Migration ↓	peritoneal spread ↓	
			Angiogenesis ↓		
			E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓		
MELTF-AS1	↑	miR-485-5p/MMP14 axis	Invasion ↓	Tumor volume↓	Ding et al. (2021)
			Migration ↓	Tumor weight↓ lung metastasis ↓	
			E-cadherin ↑		
			N-cadherin ↓		
			Vimentin ↓		

TABLE 7 Tumor suppressive lncRNAs associated with the inhibited progression of osteosarcoma targeting miRNA.

LncRNA	Expression in OS	Regulatory pathway	Key function after overexpression	Refs
LINC00588	↓	miR-1972/TP53 axis	Proliferation↓	Zhou et al. (2020)
			Invasion ↓	
			Migration ↓	
			Cell cycle arrested	
			E-cadherin ↑	
			N-cadherin ↓	
			Vimentin ↓	
LINC00691	↓	miR-1256/ST5 axis	Proliferation↓	Wan et al. (2020)
			Invasion ↓	
			E-cadherin ↑	
GAS5	↓	miR-23a-3p/PI3K/AKT pathway	Proliferation ↓	Liu J et al. (2020)
			Invasion ↓	
			Apoptosis ↑	
HCG11	↓	miR-942-5p/p27Kip1 axis	Proliferation ↓	Gu et al. (2021)
			Cell cycle arrested	
NR_027471	↓	miR-8055/TP53INP1 axis	Proliferation↓	Chen J et al. (2020)
			Invasion ↓	
			Migration ↓	
TUSC7	↓	miR-181a/RASSF6 axis	Proliferation ↓	Zhao et al. (2021)
			Invasion ↓	
			Apoptosis ↑	

with pcdNA3. 1-TUSc7 (a TUSc7 overexpression vector). CCK-8 assay, colony formation assay, transwell assay, and flow cytometry analysis, showed lncRNA TUSC7 overexpression inhibited OS cell proliferation and invasion and promoted their apoptosis *in vitro*. *In vivo*, a BALB/c nude mouse tumor xenograft model was established, which showed significantly smaller tumors in the TUSc7-overexpressing group. Finally, they demonstrated that lncRNA TUSC7 acted as a ceRNA for miR-181a and upregulated RASSF6 expression. These data are summarized in Table 7.

3.2 Tumor-suppressive lncRNAs inhibit OS by mechanisms other than miRNAs

In addition to targeting miRNAs, overexpressed lncRNAs can suppress OS *via* other methods. Zhao et al. (2019) transfected U2OS and MG63 cells with pcDNA-EPIC1, in which the lncRNA EPIC1 level was significantly higher than that of the control group. The results of the MTT assay and BALB/c nude mouse xenograft model showed inhibited cell viability and suppressed tumor growth, which indicated that OS progression could be inhibited by lncRNA EPIC1 overexpression. Their research also indicated that the effect of lncRNA EPIC1 was mediated by promoting the ubiquitylation of MEF2D. The authors observed decreased lncRNA HIF2PUT expression in U2OS and MG-63 stem cell lines and transfected these cells with pcDNA-HIF2PUT. The results of the wound-healing and transwell assays suggested that a high lncRNA HIF2PUT level inhibited OS cell migration and invasion. These results were reversed in the HIF2PUT knockdown groups. Finally, the inhibitory effect of lncRNA HIF2PUT was attributed to a positive correlation between lncRNA HIF2PUT and HIF-2a (Ding et al., 2019). Xin et al. (2022) transfected OS cells isolated from frozen OS tissue with the lncRNA LINC00619 vector. In addition to the inhibition of OS cell proliferation, migration, and invasion by LINC00619 overexpression in the MTT assay, scratch test, and transwell assay, respectively, LINC00619 overexpression also promoted OS apoptosis in flow cytometry analysis. Furthermore, the PI3K/Akt pathway and HGF expression were repressed in OS cells overexpressing LINC00619. The tumor-suppressive effect of lncRNA H19 was verified *in vitro* and *in vivo*. Xu et al. (2020) infected U2OS cells with a virus containing H19, and H19-bound protein complexes were identified by mass spectrometry. Wiki pathway and GO_BP analyses showed that these putative H19-associated protein complexes mainly functioned in DNA damage response, DNA repair, and the cell cycle. These findings indicated that lncRNA H19 suppresses OS genesis through DNA repair protein complexes. To determine the effects of lncRNA MEG3 on OS progression, Chen L et al. (2020) transfected MG-63 cells with lncRNA MEG3 vectors. As a result, the cell proliferation of the MEG3 group was significantly inhibited in the CCK-8 assay; the cell apoptosis of the MEG3 group was remarkably promoted in Hoechst 33258 staining, and the expression of the apoptosis-related protein

TABLE 8 Tumor suppressive lncRNAs associated with osteosarcoma inhibition by mechanisms other than miRNAs.

LncRNA	Expression in OS	Regulatory pathway	Key Function after overexpression	Refs
EPIC1	↓	MEF2D ubiquitylation	Proliferation ↓	Zhao et al. (2019)
HIF2PUT	↓	Promoting HIF-2a	Invasion ↓	Ding et al. (2019)
			Migration ↓	
LINC00619	↓	PI3K/Akt and HGF inhibition	Proliferation↓	Xin et al. (2022)
			Invasion ↓	
			Migration ↓	
H19	↓	DNA repair protein complexes	Cell cycle arrested	Xu et al. (2020)
MEG3	↓	Notch pathway inhibition	Proliferation↓	Chen L et al. (2020)
			Apoptosis ↑	
MEG3	↓	—	Proliferation↓	Huang et al. (2022)
			Invasion ↓	
			Apoptosis ↑	

TABLE 9 LncRNAs associated with improved chemotherapy resistance.

LncRNA	Expression in OS	Regulatory pathway	Affected chemotherapy-resistance types	Refs
FOXD2-AS1	↑	Targeting miR-143	Cisplatin	Zhang Q-Q et al. (2021)
NCK-AS1	↑	Targeting miR-137	Cisplatin	Cheng Y et al. (2019)
ROR	↑	Sponging miR-153-3p	Cisplatin	Cheng F-H et al. (2019)
SARCC	↓	upregulating miR-143	Cisplatin	Wen et al. (2020)
LINC00922	↑	TFAP2C/LINC00922/miR-424-5p	Doxorubicin	Gu Z et al. (2020)
lncARSR	↑	AKT/MRP1 axis	Doxorubicin	Shen and Cheng (2020)
Sox2OT-V7	↑	Targeting miR-142 and miR-22	Doxorubicin	Zhu et al. (2020)
ANCR	↑	—	Doxorubicin	Hu et al. (2021)
LAMTOR5-AS1	↑	Regulating NRF2	Etoposide	Pu et al. (2021)
			Carboplatin	
			Cisplatin	
OIP5-AS1	↑	sponging miR-137-3p	Doxorubicin	Sun et al. (2020)
OIP5-AS1	↑	miR-377-3p/FOSL2 axis	Cisplatin	Liu and Wang (2020)

Caspase3 in the MEG3 group was markedly increased in western blotting. Furthermore, they reported that lncRNA MEG3 overexpression inhibited the Notch signaling pathway in OS cells. Huang et al. (2022) prepared c(RGDyK)-modified and MEG3-loaded exosomes (cRGD-Exo-MEG3) and proved that they could be delivered more efficiently to OS cells both *in vitro* and *in vivo*. In OS cells targeted by cRGD-Exo-MEG3, cell proliferation was inhibited in CCK-8 and colony formation assays; cell invasion ability was suppressed in transwell assays, and cell apoptosis was promoted in flow cytometry analysis. *In vivo*, a xenograft tumor model was applied, in which cRGD-Exo-MEG3 treatment markedly reduced the tumor volume compared to that of the control groups. These results indicated that lncRNA MEG3-loaded exosomes may be an effective therapy to suppress OS. These data are summarized in Table 8.

4 LncRNA treatment improves chemotherapy resistance

Although obvious improvements have been made, chemotherapy resistance remains a major obstacle causing recrudescence and metastases. Recent studies have also demonstrated the vital role of lncRNAs in OS chemotherapy resistance, as shown in Table 9.

4.1 LncRNAs could be treatment targets to suppress cisplatin resistance in OS

Various lncRNAs can sensitize OS cells to cisplatin *via* knockdown or overexpression. Zhang Q-Q et al. (2021) reported that lncRNA FOXD2-AS1 knockdown inhibited miR-143 expression to inhibit OS resistance to cisplatin. They transfected siRNA targeting FOXD2-AS1 into cisplatin-resistant OS cells. The CCK-8 assay showed a significantly lower IC50 in the si-FOXD2-AS1 group compared to that in the control group. Transwell and wound-healing assays also showed suppressed invasion and migration by cisplatin-resistant OS cells when FOXD2-AS1 was knocked down. OS resistance to cisplatin was also inhibited by knocking down lncRNA NCK-AS1. Cheng Y et al. (2019) transfected shRNA-NCK1-AS1 into DDP-resistant MG63 cells to knock down lncRNA NCK-AS1. Cell growth, cell migration, and cell invasion were extremely inhibited in DDP-resistant MG63 cells after NCK-AS1 knockdown in the CCK-8, wound healing, and transwell assays, respectively. Cheng F-H et al. (2019) reported increased lncRNA ROR expression in cisplatin-resistant OS cells and transfected shRNA targeting ROR into cisplatin-resistant OS cells (MG63/DDP and U2OS/DDP). The MTT and transwell assays showed reduced cell proliferation and invasion in MG63/DDP and U2OS/DDP cells following ROR knockdown. This indicated that ROR knockdown suppressed cisplatin resistance in OS cells. Wen et al. (2020) observed decreased lncRNA SARCC expression in OS cells and transfected lncRNA SARCC into SaoS-2 and U2OS cells. When SaoS-2 and U2OS cells were exposed to cisplatin, cells overexpressing SARCC showed more impaired cell viability compared to cells without SARCC overexpression. They also demonstrated that OS cells sensitized to cisplatin with miR-143 overexpression and lncRNA SARCC upregulated miR-143.

4.2 LncRNAs as potential treatment targets to suppress doxorubicin resistance in OS

LncRNAs may also be treatment targets for doxorubicin resistance in OS. Gu Z et al. (2020) reported that lncRNA LINC00922 accelerated OS doxorubicin (DXR) resistance *via* the TFAP2C/LINC00922/miR-424-5p feedback loop. When they transfected short hairpin RNA (shRNA) specifically targeting LINC00922 into DXR-resistant OS cells (MG63/DXR), the CCK-8 assay showed inhibition of the IC 50 (50% inhibitory concentration). *In vivo*, a mouse xenograft model showed that tumor volume and weight were also inhibited by LINC00922 knockdown in MG63/DXR cells. Shen and Cheng (2020) reported that lncRNA ARSR enhanced OS adriamycin resistance by upregulating multidrug resistance-associated protein-1 (MRP1) through AKT activation. After transfected U2OS and MG63 cells with siRNA targeting the lncRNA ARSR, the cells were exposed to adriamycin at stepwise increasing concentrations. Cells expressing siRNA targeting lncRNA ARSR showed inhibited cell proliferation, an inhibitory effect that was enhanced with increased adriamycin concentrations. In their ADM-resistant OS mouse model, the suppression of tumor growth by ADM was recovered by si-lncARSR, and tumor growth was significantly suppressed in the si-lncARSR group compared to the siRNA-negative control group. Zhu et al. (2020) focused on the relationship between lncRNA Sox2OT-V7 and doxorubicin (DOX) resistance in OS and transfected a Sox2OT-V7-silenced lentivirus recombinant plasmid into U2OS cells. They reported an IC50 value of 1.533 μM in DOX-resistant U2OS (U2OS/DOX) cells after Sox2OT-V7 silenced plasmid transfection, compared to 4.380 μM in non-transfected DOX-resistant U2OS cells. Finally, they suggested that Sox2OT-V7 silencing sensitized U2OS/DOX cells *via* upregulated expression of miR-142/miR-22 and regulated autophagy in U2OS cells. Hu et al. (2021) reported that lncRNA ANCR exosomes from doxorubicin (DOX)-resistant U2OS cells induced drug resistance among Dox-sensitive cells. When the authors transfected siRNA targeting lncRNA ANCR into DOX-resistant KHOS and U2OS cells, MTT assays showed a significantly lower IC50 value in the ANCR-silenced group compared to that in the control group. This indicated that ANCR silencing could sensitize OS to DOX.

4.3 A single lncRNA can reduce multiple chemotherapy resistances

Some lncRNAs play a vital role in multidrug resistance. Pu et al. (2021) reported that the lncRNA LAMTOR5-AS1 is upregulated in chemotherapy-sensitive cells and transfected LAMTOR5-AS1 into OS cells. After exposure to IC 50 doses of VP-16 (etoposide), CBP (carboplatin), and DDP (cisplatin), the CCK-8 assay showed increased cell death in the LAMTOR5-AS1 group compared to the negative control. An annexin V-FITC staining assay showed that LAMTOR5-AS1 overexpression promoted DDP-induced apoptosis of SJSA-1 cells. *In vivo*, a tumor xenograft mouse model was constructed, in which LAMTOR5-AS1 overexpression promoted the inhibitory effects of DDP on tumor formation, weight, and volume. Sun et al. (2020) discovered that OIP5-AS1 served as a sponge of miR-137-3p and that OIP5-AS1 suppression reduced OS resistance to doxorubicin. They transfected siRNAs targeting OIP5-AS1 into DOX-resistant MG63 cells. The CCK-8 assay and flow cytometry analysis showed that OIP5-AS1 inhibition not only sensitized DOX-resistant MG63 (MG63/DOX) cells to doxorubicin-mediated cytotoxicity but also promoted the apoptosis of MG63/DOX cells. *In vivo*, compared to the control group, MG63/DOX cells transfected with lenti-sh-OIP5-AS1 grew slower and had smaller tumor volumes. LncRNA OIP5-AS1 also exhibited the ability to reduce cisplatin resistance in OS cells. Liu and Wang (2020) transfected siRNA against OIP5-AS1 into MG63 and Saos-2 cells and proved that OIP5-AS1 knockdown could enhance cisplatin sensitivity in OS cells *in vitro* and *in vivo*.

5 Discussion

OS is one of the most common malignant tumors in orthopedics, is highly aggressive, and is always transferred early. However, existing treatments for OS have many shortcomings, such as wide surgical resection ranges, a high risk of postoperative recurrence, and chemotherapy resistance. Recent research has revealed the potentially vital role of lncRNAs in OS progression. This review broadly divided lncRNAs into two categories: oncogenic lncRNAs and tumor-suppressive lncRNAs. Oncogenic lncRNAs are highly expressed in OS cells, while the expression levels of tumor-suppressive lncRNAs are low (Fei et al., 2019; Li et al., 2019; Wan et al., 2020; Zhou et al., 2020). After oncogenic

lncRNA knockdown, several aspects of OS progression can be inhibited, including inhibited proliferation, arrested cell cycle at G0/G1 phase, reduced angiogenesis, suppressed invasion, and weakened migration ability. These anti-osteosarcoma effects of lncRNA were not only confirmed *in vitro* but have also been demonstrated *in vivo*. However, inhibiting OS progression required enhanced expression of tumor-suppressive lncRNAs (Chen L. et al., 2020; Huang et al., 2022). Moreover, regardless of oncogenic or tumor-suppressive lncRNAs, most of their mechanisms of action are to act as ceRNAs to miRNAs and then affect the expression of downstream proteins (Figure 1).

Although chemotherapy is an important tool in the treatment of OS today, especially in combination with surgery, which can narrow the scope of surgery and reduce the recurrence rate, chemotherapy resistance is still an inevitable topic. To find a new method to reduce the chemotherapy resistance of OS, researchers have turned to lncRNAs, with rich results. LncRNA FOXD2-AS1, NCK-AS1, and ROR were highly expressed in cisplatin-resistant OS cells, and cisplatin resistance in OS cells improved after their silencing (Cheng Y et al., 2019; Cheng F-H et al., 2019; Zhang Q-Q et al., 2021). Similarly, silencing LINC00922, lncARSR, Sox2OT-V7, and ANCR could improve doxorubicin resistance in OS. Moreover, silencing lncRNA LAMTOR5-AS1 and lncRNA OIP5-AS1 may also promote multiple kinds of chemotherapy resistance.

Although studies on osteosarcoma-related lncRNAs have achieved fruitful results, there remain many deficiencies in lncRNAs as targets for the treatment of osteosarcoma. First, the current research on osteosarcoma-related lncRNAs is still in the experimental stage, and clinical evidence for the treatment of osteosarcoma by lncRNAs is lacking. Second, few studies have assessed targeted delivery methods of lncRNAs or their silencers in osteosarcoma. Huang et al. (2022) purified and collected exosomes from osteosarcoma cells transfected with lncRNA MEG3. These exosomes were rich in lncRNA MEG3 and were modified with cRGD peptides to enhance their targeting capability. After the xenograft osteosarcoma model was established, fluorescently labeled exosomes were intravenously injected. The results showed that compared to the MEG3 group, the cRGD-modified exosomes emitted stronger fluorescence signals in the tumor site, confirming that the cRGD-modified exosomes had a stronger ability to target lncRNA delivery to osteosarcoma. However, most studies established animal models of osteosarcoma suppression using osteosarcoma cells transfected with lncRNA or lncRNA silencer plasmids rather than the targeted delivery of lncRNAs and their silencer. Third, while many lncRNAs are potential therapeutic targets for osteosarcoma, there remains a lack of standardized evaluation of efficacy and transverse comparison of efficacy.

The future research directions in osteosarcoma-related lncRNAs include more effective methods of targeted delivery of lncRNAs to osteosarcoma. Second, evaluation criteria for the effectiveness of targeted lncRNAs in the treatment of osteosarcoma are needed. Third, whether for diagnosis, treatment, or prognosis, we hope to see the clinical application of lncRNAs in osteosarcoma.

Author contributions

Writing and table design: FC and SX; review and editing: SX, LX, LS, and BZ; conceptualization, review, and critical revision: FC and LJ. All authors have read and approved the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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In silico analysis to identify novel ceRNA regulatory axes associated with gallbladder cancer

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Competitive endogenous RNA (ceRNA) networks are reported to play a crucial role in regulating cancer-associated genes. Identification of novel ceRNA networks in gallbladder cancer (GBC) may improve the understanding of its pathogenesis and might yield useful leads on potential therapeutic targets for GBC. For this, a literature survey was done to identify differentially expressed lncRNAs (DELs), miRNAs (DEMs), mRNAs (DEGs) and proteins (DEPs) in GBC. Ingenuity pathway analysis (IPA) using DEMs, DEGs and DEPs in GBC identified 242 experimentally observed miRNA-mRNA interactions with 183 miRNA targets, of these 9 (CDX2, MTDH, TAGLN, TOP2A, TSPAN8, EZH2, TAGLN2, LMNB1, and PTMA) were reported at both mRNA and protein levels. Pathway analysis of 183 targets revealed p53 signaling among the top pathway. Protein-protein interaction (PPI) analysis of 183 targets using the STRING database and cytoHubba plug-in of Cytoscape software revealed 5 hub molecules, of which 3 of them (TP53, CCND1 and CTNNB1) were associated with the p53 signaling pathway. Further, using Diana tools and Cytoscape software, novel lncRNA-miRNA-mRNA networks regulating the expression of TP53, CCND1, CTNNB1, CDX2, MTDH, TOP2A, TSPAN8, EZH2, TAGLN2, LMNB1, and PTMA were constructed. These regulatory networks may be experimentally validated in GBC and explored for therapeutic applications.

KEYWORDS

gallbladder cancer, ceRNA regulatory network, lncRNA-miRNA-mRNA regulatory axes, P53 signaling pathway, therapeutic applications

1 Introduction

Gallbladder Cancer (GBC) is among the most common malignant tumor of the gastrointestinal system. The only effective treatment is complete surgical resection for this cancer which can be given to only about 10% of patients because this cancer is usually diagnosed at advanced stages, however, recurrence rates after surgical resections are high (Zhu et al., 2010). GBC patients have an overall median survival of 19 months and a 5-year survival rate of 28.8% (Zhu et al., 2020) despite standard treatment, including chemotherapy, radiotherapy and targeted therapy. Therefore, it is necessary to understand the molecular mechanism associated with GBC and identify novel targets for therapeutic applications in GBC.

MicroRNAs (miRNAs) are a class of small non-coding RNAs (ncRNAs) of approximately ~22 nucleotides long. Their mRNA targets are based on limited sequence complementarity between the miRNAs seed region (first 2–7 nucleotides from the 5′ end) and regions in the 3′ untranslated region (3' -UTR) of the mRNA. miRNAs play a key role in the negative regulation of target genes and thus change the cellular environment. Nearly 60% of the total known mRNAs are regulated by miRNAs (Friedman et al., 2008). Another group of ncRNA—long non-coding RNAs (lncRNAs), which are >200 nucleotides in length, also affect mRNA stability (Sebastian-delaCruz et al., 2021). LncRNAs containing a 'miRNA response element' can compete with other RNAs and are believed to act as competing endogenous RNAs (ceRNAs). LncRNA-mediated ceRNA regulatory network, namely, lncRNA/miRNA/mRNA axis, is important in promoting tumorigenesis and can potentially serve as a handle to identify key therapeutic targets (Xu et al., 2022).

There are several high-throughput studies available in GBC to identify differentially expressed mRNAs (DEGs) and proteins (DEPs) (Kim et al., 2008; Miller et al., 2009; Huang et al., 2014; Wang et al., 2020a). Knowledge of the non-coding RNAs regulating the expression of these cancer-associated genes/proteins and the corresponding regulatory networks would be important to understand the pathogenesis of GBC and for therapeutic applications. Various groups have analyzed the differential expression of miRNAs (DEMs) in tissue (Letelier et al., 2014; Zhou et al., 2014; Goeppert et al., 2019), serum/plasma extracellular vesicles (Ueta et al., 2021; Yang et al., 2022) using high-throughput studies in GBC. Similarly, other researchers have analyzed differentially expressed lncRNA (DELs) using high-throughput studies (Ma et al., 2016; Wang et al., 2017a; Wu et al., 2017), however, the majority of the studies on lncRNA in GBC are targeted studies. Few of these studies revealed the "lncRNA-miRNA-mRNA network" in GBC in a targeted manner (Wang et al., 2016a; Hu et al., 2019; Yang et al., 2020; Zhang et al., 2020). Another study has constructed a ceRNA network using DELs, DEGs data and predicted miRNAs based on the DEGs from a single GBC dataset (GSE76633) (Kong et al., 2019a). In view of the availability of various targeted and high-throughput studies at lncRNA, miRNA, mRNA, and protein levels in GBC, the construction of a "ceRNA regulatory network" (lncRNA/miRNA/mRNA axis) based on multiple experimental datasets would be highly relevant.

We aimed to uncover the potential ceRNA regulatory networks regulating cancer-associated processes involved in the development of GBC. The present study applied multi-omics datasets available in GBC, including high-throughput GBC-proteomics data from our lab (Akhtar et al., 2023), and RNA interaction databases (predicted/experimentally verified) to identify novel lncRNA-miRNA-mRNA regulatory networks in GBC which may be explored for their therapeutic applications in GBC.

2 Methodology

2.1 Data collection

The literature search was performed and studies with high-throughput expression data in GBC were included for miRNA and mRNA. As the detection of proteins is low in comparison to mRNAs or miRNAs, due to technical limitations, here, both high-throughput studies as well as targeted studies in GBC were used to achieve a comprehensive DEP dataset. The high-throughput proteomic studies include the data from our lab (Akhtar et al., 2023). For lncRNA, only targeted studies were used as their interactions with miRNA are well-annotated.

2.2 miRNA-mRNA regulatory axis of gallbladder cancer

Non-redundant lists of DEMs and DEGs were imported into QIAGEN IPA (QIAGEN Inc., https://digitalinsights.qiagen.com/IPA) (Krämer et al., 2014) and identified the miRNA-mRNA interactions. Similarly, non-redundant lists of DEMs and DEPs were imported into IPA and identified the miRNA-mRNA interactions. miR IDs (for DEMs) and gene symbols (for DEGs and DEPs) were used for IPA analysis.

The expression of lncRNA/miRNA/mRNA/protein was considered "Up" or "Down" based on the expression trend in >50% of the studies. The ones showing an opposite expression in equal no. of studies (50%) were not considered for any further analysis. Similarly, in the case of multiple transcripts of a gene the expression was considered "Up" or "Down" based on the expression trend in >50% of the transcripts.

For IPA analysis, an expression pairing filter was applied to include miRNA-mRNA pairs which are showing an opposite correlation in expressions (miRNA-Up/mRNA-Down; miRNA-Down/mRNA-Up). The confidence level filter was used to include only those interactions which were 'experimentally observed' or 'predicted with high confidence' [the cumulative weighted context score (or "CWCS") as defined by TargetScan is −0.4 or lower]. The datasets from miRNA-mRNA interactions from DEGs and DEPs were integrated and obtained a non-redundant list of miRNA-mRNA interactions. "Experimentally observed" miRNA-mRNA interactions (from the non-redundant list) were selected and miRNA regulatory network was constructed using Cytoscape software v3.9.1 (https://cytoscape.org/) (Shannon et al., 2003).

2.3 Functional enrichment analysis

The Search Tool for Retrieval of Interacting Genes database (STRING version 11.5; https://string-db.org) (Szklarczyk et al., 2019) is an online database and tool that can build protein-protein interaction (PPI) network based on known and predicted interactions. Gene ontology analysis for "experimentally observed" miRNA targets was performed through STRING (cellular components and biological processes) and IPA (molecular and cellular functions and canonical pathways).

2.4 Protein-protein interaction analysis

The PPI of the "experimentally observed" miRNA targets were analyzed using STRING 11.5, [Organism: *Homo sapiens* and PPI

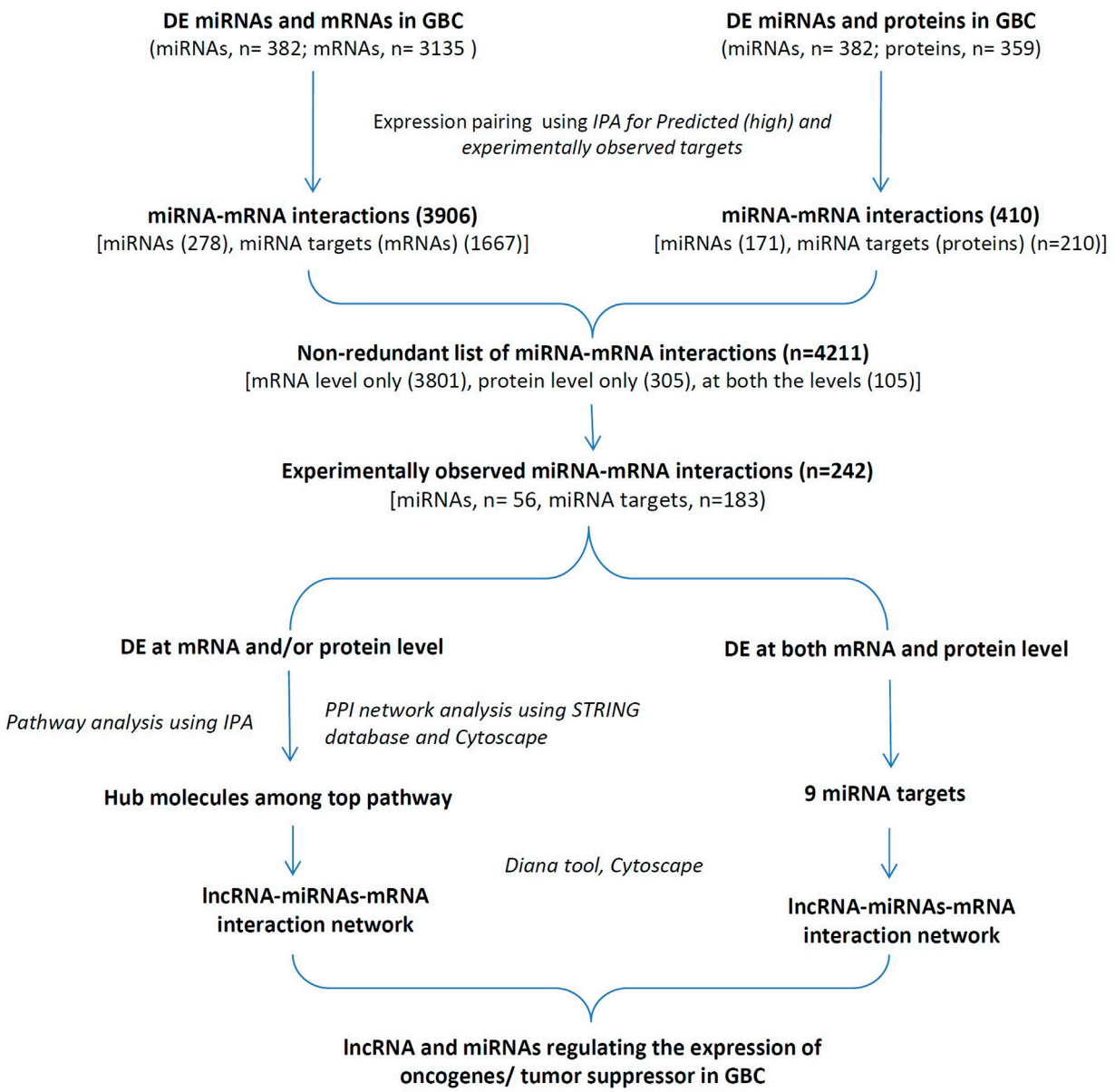

FIGURE 1
The overall workflow of the study. DE miRNAs, mRNAs, proteins and lncRNAs in GBC based on a literature survey were used for the study. DE, Differentially expressed; GBC, Gallbladder Cancer; IPA, Ingenuity pathway Analysis; PPI, protein-protein interaction.

score was set as 0.9 (highest confidence)]. The network was visualized by cytoscape v3.9.1. Cytohubba, a plugin of cytoscape software, was used to identify the hub genes of the PPI network (Chin et al., 2014). The intersection of the top 10 nodes ranked by degree, closeness, betweenness and bottleneck centrality were considered hub genes.

2.5 ceRNA regulatory network construction

DELs (targeted studies) and DEMs (miRNAs associated with hub molecules among the top pathway and the targets reported at both mRNA and protein levels) were used to screen the experimentally validated interaction between them by DIANA-LncBase v3 (https://diana.e-ce.uth.gr/lncbasev3) (Karagkouni et al., 2020). Both the subunits of miRNA, i.e., "-3p" and "-5p" were considered for finding associated lncRNAs in those cases where the subunits were not specified. Then, lncRNA-miRNA and miRNA-mRNA co-expression pairs (positive relation) were then used to construct ceRNA interaction networks (lncRNA-miRNA-mRNA). The networks were visualized using Cytoscape. Another database, mirTarBase (mirtarbase.cuhk.edu.cn), containing more than three hundred and sixty thousand miRNA-mRNA interactions was then used to categorize the miRNA-mRNA interactions with strong evidence (Reporter assay/Western blot/qPCR) or less strong evidence (Microarray, NGS, pSILAC, CLIP-Seq and others).

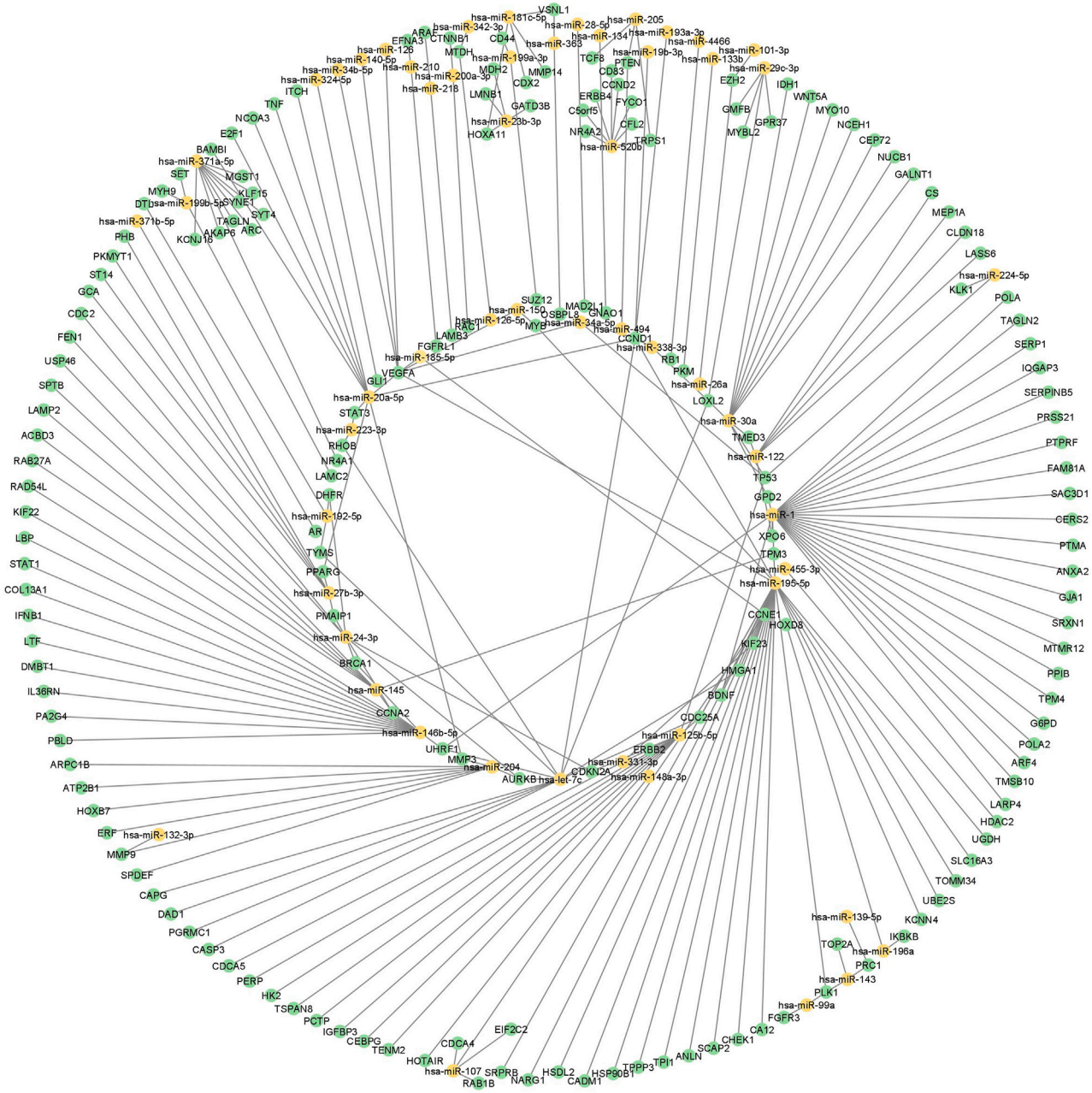

FIGURE 2
miRNA regulatory network in GBC. miRNA-mRNA regulatory network using DE miRNAs, mRNAs and proteins in GBC. Yellow color represents miRNAs and green represents mRNA or protein. GBC, Gallbladder Cancer; DE, Differentially expressed.

3 Results

The study design and workflow of the study is shown in Figure 1. 'Tissue-based' datasets (DELs, DEMs, DEGs, and DEPs) were used from high-throughput and/or targeted studies and performed IPA analysis for miRNA-mRNA interactions (predicted and experimentally observed) using DEMs and DEGs or DEPs. The "experimentally observed targets" were further used for associated canonical pathways and PPI analysis to identify hub molecules. In addition, miRNA targets DE at both mRNA and protein levels with a positive correlation in expression were also analyzed. Finally, lncRNA-miRNA-mRNA regulatory networks were constructed for the selected targets possibly functional in GBC.

3.1 DEM, DEG, DEP, and DEL dataset

Literature search revealed three high-throughput studies on tissue miRNA analysis in GBC (Letelier et al., 2014; Zhou et al., 2014; Goeppert et al., 2019). A total of 382 DEMs (non-redundant

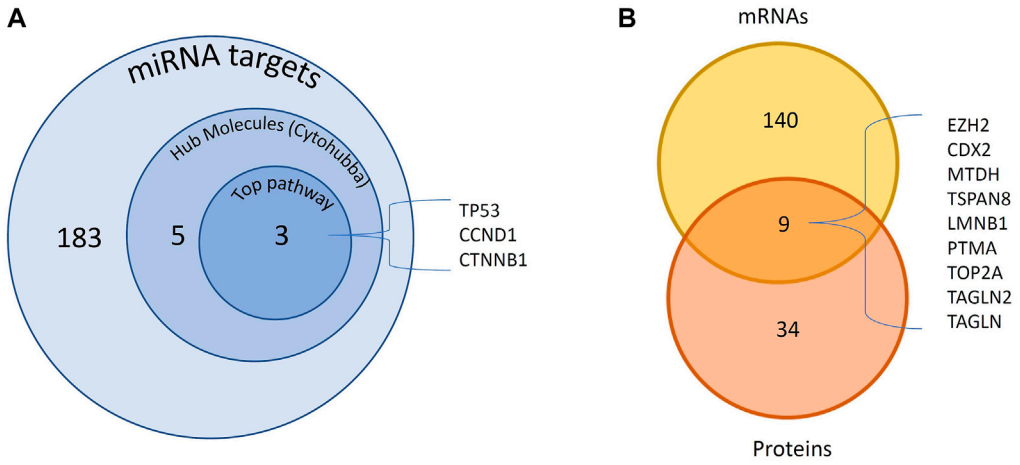

FIGURE 3
DE miRNAs and their targets DE in GBC. A total of 183 experimentally observed targets were identified. **(A)** PPI network analysis of 183 proteins showed 5 hub molecules. Pathway analysis showed p53 signaling among the top pathway which includes 3 of the hub molecules. **(B)** We found 9 miRNA targets reported to be DE at both mRNA and protein levels. DE- Differentially expressed; GBC, Gallbladder Cancer; PPI- protein-protein interaction.

list) were obtained and shown in Supplementary Table S1. A total of seven high-throughput studies were found analyzing the expression of mRNAs in tissues. A total of 3,135 DEGs (non-redundant list) were obtained and are shown in Supplementary Table S2. Both high-throughput and targeted studies on the differential expression of proteins in GBC tissues were used for the analysis. Data on DEPs from our lab (Akhtar et al., 2023) was also included. A total of 359 DEPs (non-redundant list) were obtained and are shown in Supplementary Table S3. For lncRNAs, 45 targeted studies representing expressions of 43 different lncRNAs (non-redundant list) were found and is shown in Supplementary Table S4.

3.2 miRNA-mRNA regulatory axis of GBC

First, non-redundant lists of DE miRNAs (n = 382) (miRNA IDs) and DE mRNAs (n = 3,135) (gene symbol) were imported into IPA, out of which 372 miRNAs and 2,996 mRNAs were mapped. Inverse expression pairing resulted in a total of 3,906 miRNA and mRNA interactions "Dataset 1" (with high prediction and/or experimentally observed) that includes 278 miRNAs and 1,667 miRNA targets (mRNAs).

Similarly, non-redundant lists of DE miRNAs (n = 382) (miRNA IDs) and DE proteins (n = 359) (gene symbol) were imported into IPA, out of which 372 miRNAs and 356 mRNAs were mapped. Expression pairing resulted in a total of 410 miRNA and mRNA interactions "Dataset 2" (with high prediction and/or experimentally observed) that includes 171 miRNAs and 210 miRNA targets (proteins).

The above two datasets were integrated and obtained a non-redundant list of 4,211 miRNA-mRNA interactions (Supplementary Table S5). This includes 242 interactions with "experimentally observed targets" (Figure 2) and 3,969 interactions with targets "predicted with high confidence". These 242 interactions include 183 targets (Figure 3, Supplementary Table S6) and were used for gene ontology, pathway, and protein-protein interaction (PPI) network analysis. Out of 242, a total of 11 interactions include 9 targets (CDX2, MTDH, TOP2A, TSPAN8, EZH2, TAGLN2, LMNB1, PTMA, and TAGLN) that are reported to be differentially expressed at both mRNA and protein level in GBC (Figure 3B; Supplementary Table S7).

3.3 Functional enrichment analysis

Gene ontology analysis of 183 proteins through STRING showed that these are localized in intracellular organelle lumen, cytoplasm, membrane-bound organelle, nucleoplasm and chromosome (Figure 4A). The top biological processes include positive regulation of cellular process, biological process, metabolic process and developmental process (Figure 4B). Molecular and cellular function and canonical pathways were analyzed through IPA and the threshold criteria considered for the analysis are -log p-value >1.3 or p-value <0.05. The top molecular and cellular functions include cell death and survival, cancer, organismal injury and abnormalities, organismal survival, and cell cycle (Figure 4C). The top canonical pathways include p53 signaling pathway, pancreatic adenocarcinoma signaling, ovarian carcinoma signaling, Aryl hydrocarbon receptor signaling, cyclins and cell cycle regulation (Figure 4D; Supplementary Table S8).

3.3 Protein-protein interaction analysis

PPI analysis of 183 miRNA targets using STRING was visualized by cytoscape (Figure 5A). A total of 5 hub molecules (TP53, STAT3, CTNNB1, CDK1, CCND1) were identified based on the intersection of the top 10 nodes ranked by degree, closeness, betweenness and bottleneck centrality (Figure 5B). Three of the hub molecules (TP53, CCND1, CTNNB1) belong to "p53 signaling pathway" (Figure 3A).

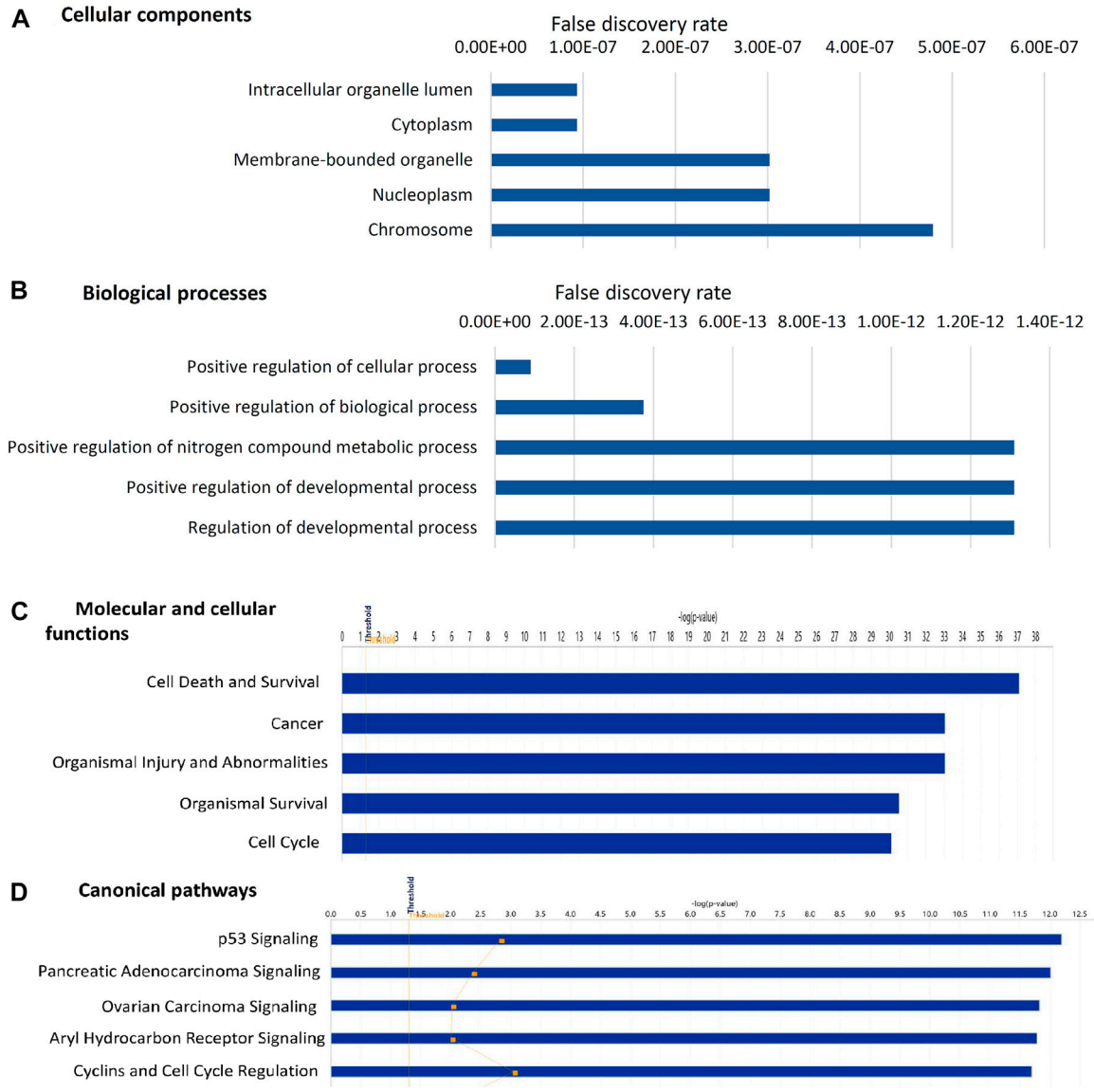

FIGURE 4
Gene ontology analysis of 183 experimentally observed miRNA targets. The top five cellular components **(A)** biological processes **(B)** molecular and cellular functions **(C)** canonical pathways **(D)** associated with these targets are shown in the figure. The threshold criteria considered for the analysis are -log *p*-value >1.3 or false discovery rate <0.05. The genes associated with canonical pathways are provided in Supplementary Table S5.

3.4 ceRNA regulatory networks

ceRNA regulatory networks were constructed for miRNAs associated with 3 hub molecules (TP53, CTNNB1, CCND1) associated with "p53 signaling pathway". Since lncRNAs can bind to miRNA and indirectly regulate the translation of targeted mRNAs, the expression of lncRNAs and mRNAs should be positively correlated (López-Urrutia et al., 2019). The lncRNA-miRNAs-mRNA networks for p53, CCND1, and CTNNB1 is shown in Figure 6. The ceRNA regulatory networks for 8 out of 9 miRNA targets (reported to be DE at both mRNA and protein levels) were also constructed. No lncRNA-miRNA interaction was found for one of the targets, TAGLN. The lncRNA-miRNAs-mRNA networks for CDX2, MTDH, TOP2A, TSPAN8, EZH2, TAGLN2, LMNB1, and PTMA is shown in Figure 7. All the lncRNA-miRNA interactions were reported to be strong interactions except for hsa-miR-23b-3p and associated lncRNAs. The miRNA-mRNA interactions with "strong evidence" as per miRTarBase are shown with thick lines and the ones that are with "less strong evidence" is shown with a dashed line (Figures 6, 7).

4 Discussion

The molecular mechanism associated with the development and progression of GBC is not clear. An understanding of the ceRNA regulatory networks targeting the "tumor-associated proteins" in GBC would be highly important. In the present study, we integrated

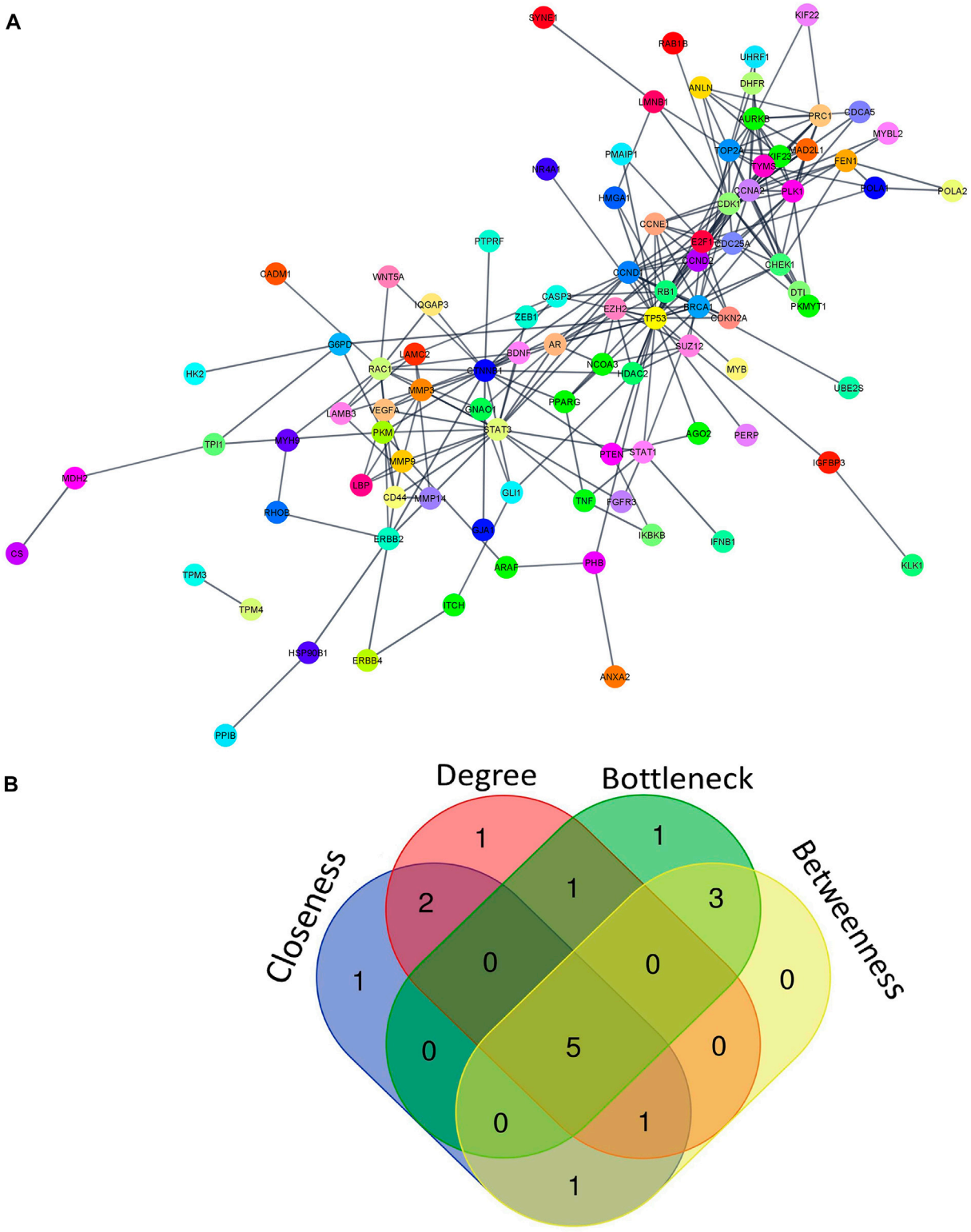

FIGURE 5
miRNA-regulated protein-protein interaction network. **(A)** PPI network of 183 miRNA targets **(B)** The intersection of the top 10 nodes ranked by degree, betweenness, closeness and bottleneck. We found 5 hub genes including TP53, STAT3, CTNNB1, CDK1, and CCND1. PPI- protein-protein interaction.

the "tissue-based" datasets (lncRNAs, miRNAs, mRNAs and proteins) from high-throughput and/or targeted studies to identify novel ceRNA regulatory networks in GBC. IPA analysis was performed for miRNA-mRNA interactions (predicted and experimentally observed) using DEMs and DEGs or DEPs. We focused on "experimentally observed targets" for associated

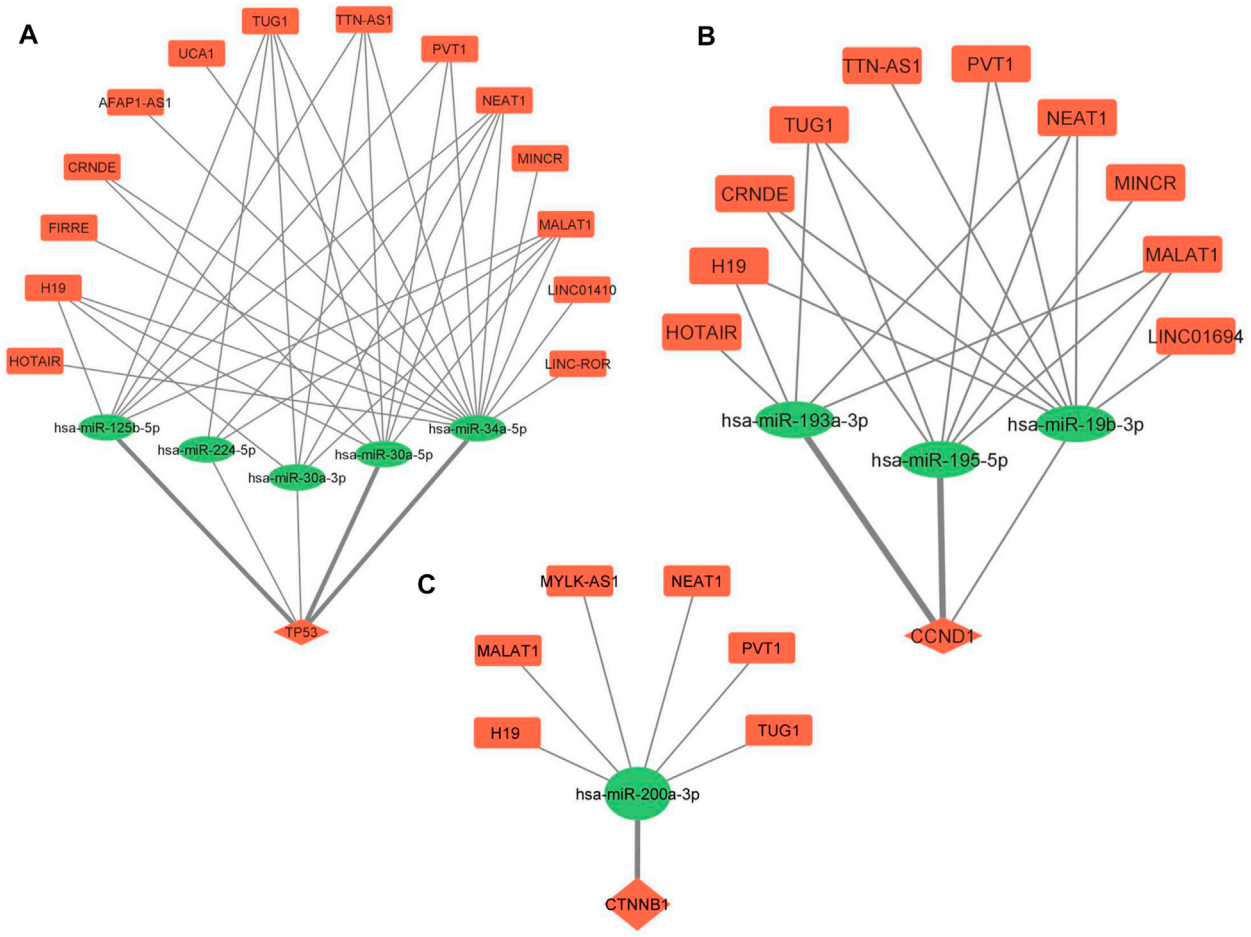

FIGURE 6
lncRNA-miRNA-mRNA regulatory network of 3 hub molecules among p53 signaling pathway in GBC. **(A)** TP53 **(B)** CCND1 and **(C)** CTNNB1. Different shapes indicate different RNA molecules (Round rectangle–lncRNA, Ellipse-miRNAs, Diamond-mRNA). The red color indicates "upregulation" and the green color indicates "downregulation" of RNA molecules. mRNA-miRNA interactions with strong evidence, as per the miRTarBase database, are marked with thick lines. GBC, Gallbladder Cancer.

canonical pathways and PPI analysis to identify hub molecules. In this study, we included the protein dataset to explore the interactions in which the miRNA target is DE at both mRNA and protein levels with a positive correlation in expression in GBC. Then, lncRNA-miRNA-mRNA regulatory networks were constructed for the selected targets, and the following strategy was used for screening the potential ceRNA regulatory network. First, the miRNA-mRNA interactions with strong evidence were screened as per miRTarBase and a literature survey was done for any report on these interactions in cancer conditions. Then, lncRNA-miRNA pairs were also screened for any report of cancer. Further, using the miRNA-mRNA interactions and lncRNA-miRNA interactions, we propose ceRNA networks (lncRNA-miRNA-mRNA) possibly functional in GBC and may have a potential for therapeutic applications in GBC.

We found "p53 signaling pathway" to be among the top pathway associated with 183 miRNA targets. PPI network analysis using these miRNA targets showed 5 hub molecules, 3 of them (p53, CCND1 and CTNNB1) were associated with p53 signaling pathway. TP53, a tumor suppressor gene, affects the cell cycle mechanism and programmed cell death (apoptosis) (Lowe and Lin, 2000; Yildirim et al., 2015). It is reported to be overexpressed in ~56% of GBC cases (Ghosh et al., 2013) and 70% of GBC cases (Misra et al., 2000). Cyclin D1 (CCND1) participates in the cell cycle phase transition (G1/S phase) (Luo et al., 2017). Accumulation of β-catenin promotes the transcription of many oncogenes such as c-Myc and CyclinD-1 (Shang et al., 2017). Both CCND1 and CTNNB1 are negatively regulated by the p53 genes (Liu et al., 2001; Swaminathan et al., 2012).

Further, lncRNA-miRNA-mRNA regulatory networks for p53, CCND1 and CTNNB1 revealed novel ceRNAs possibly regulating the expression of p53 in GBC. We found three p53-miRNAs interactions (miR-125b-5p, miR-34a-5p, and miR-30a-5p), with strong evidence (miRTarBase), regulating the expression of p53 (Figure 6A). Le et al. (2009) highlighted the importance of miR-125b, a brain-enriched miRNA, in the negative regulation of p53 and p53-induced apoptosis during development and stress response. miR-125b has been reported as an oncogene that inhibits cell apoptosis by negatively regulating p53 expression (Wu et al., 2013). There is no report on the regulation of p53 through miR-34a-5p and miR-30a-5p in cancer. In one of our networks (Figure 6B), miR-193a-3p and miR-195-5p are regulating the expression of CCND1, and they are also reported to participate

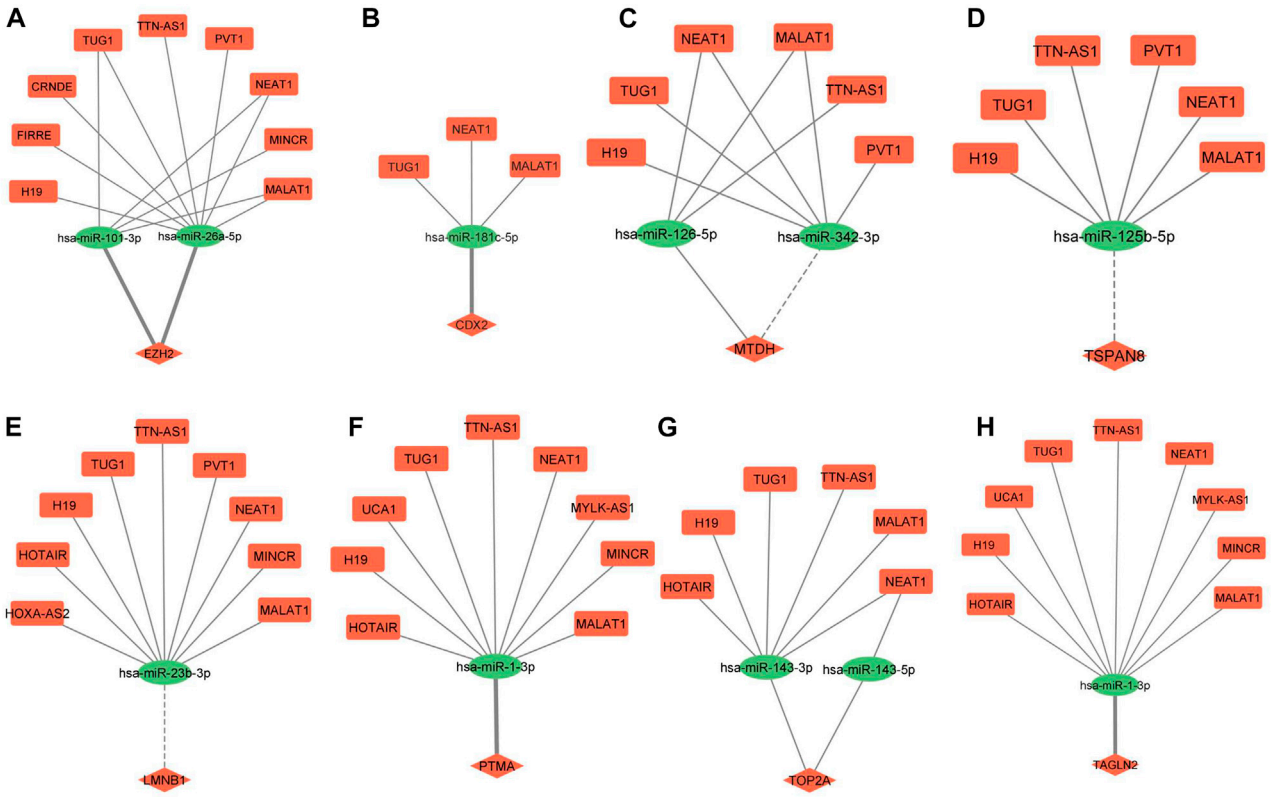

FIGURE 7
lncRNA-miRNA-mRNA regulatory network of eight miRNA targets DE at both mRNA and protein levels in GBC. **(A)** EZH2 **(B)** CDX2 **(C)** MTDH **(D)** TSPAN8 **(E)** LMNB1 **(F)** PTMA **(G)** TOP2A **(H)** TAGLN2. Different shapes indicate different RNA molecules (Round rectangle–lncRNA, Ellipse-miRNAs, Diamond-mRNA). The red color indicates "upregulation" and the green color indicates "downregulation" of RNA molecules. mRNA-miRNA interactions with strong evidence are marked with thick lines, and the ones with less strong evidence are marked with dotted lines as per the miRTarBase database. DE, Differentially expressed; GBC, Gallbladder Cancer.

in the pathogenesis of hepatocellular carcinoma and pancreatic ductal adenocarcinoma, respectively, by targeting CCND1 (Chen et al., 2019; Wang et al., 2020b). miRNA-195 inhibits cell proliferation, migration and invasion in epithelial ovarian carcinoma (EOC). In addition, a negative correlation of miR 195 expression with that of CDC42 and CCND1 expression levels was also observed in EOC (Hao et al., 2020). We found miR-200a to be regulating the expression of CTNNB1 in our network (Figure 6C). miR-200a is demonstrated to target CTNNB1 in nasopharyngeal carcinoma (Xia et al., 2010).

Literature search on "lncRNA-miRNA pairs" in cancer revealed one lncRNA (MALAT1) -miR125b interaction in laryngocarcinoma (Zong et al., 2021), three lncRNA (TUG1, NEAT1, MALAT1) -miR-34a interactions reported in endometrial cancer, Nasopharyngeal Cancer, Melanoma (Liu et al., 2017; Ji et al., 2019; Li et al., 2019) and three lncRNA (PVT1, NEAT1, and MALAT1) -miR-30a-5p interactions in papillary thyroid carcinoma, gastric cancer, Hepatocellular Carcinoma (Feng et al., 2018; Pan et al., 2018; Rao et al., 2021). Previous studies revealed that the lncRNAs H19 and NEAT1 were found to directly target miR-193a-3p in Hepatocellular Carcinoma and Lung Adenocarcinoma, respectively (Ma et al., 2018; Xiong et al., 2018). PVT1 is reported to target miR-195 in osteosarcoma (Zhou et al., 2016). H19 has been reported to competitively bind to miR-200a and indirectly regulate β-catenin in colorectal cancer (Yang et al., 2017). MALAT1 is found to competitively bind to miR-200a-3p in non-small cell lung cancer (Wei et al., 2019).

Here, six ceRNA regulatory networks (MALAT1-miR125b-p53; PVT1/MALAT1-miR-195-CCND1, H19/NEAT1-miR-193a-3p-CCND1, H19/MALAT1-miR-200a-CTNNB1) were found for which lncRNA-miRNA and/or miRNA-mRNA interactions have been reported in other cancers. In view of the correlation in expression of these lncRNA, miRNA and mRNA, the ceRNA networks appear to play key regulatory roles and may be explored in GBC.

Out of 242, a total of 11 interactions include 9 targets reported to be DE at both mRNA and protein levels in GBC. Out of 9, we found lncRNA-miRNA-mRNA regulatory networks for 8 of them (CDX2, MTDH, TOP2A, TSPAN8, EZH2, TAGLN2, LMNB1, and PTMA) (Figure 7). The same strategy as explained earlier was used for screening the potential ceRNA regulatory networks possibly functional in GBC. We found miRNA-mRNA interaction for a total of 4 genes (EZH2, CDX2, TAGLN2, and PTMA). EZH2 has different roles in cancer, such as oncogenic, tumor suppressor, cancer cell metastasis, cancer immunity, and metabolism. Previous studies have shown its overexpression in different cancers, including prostate cancer, breast cancer, etc. (Duan et al., 2020). PTMA is upregulated and associated with the

development of various cancers, including esophageal squamous cell carcinoma, colorectal, bladder, lung, and liver cancer (Zhu et al., 2019). The Caudal-type homeobox transcription factor 2 (CDX2) gene is a specific intestinal transcription factor that is involved in the embryonic development and differentiation of the intestine. Its overexpression in gastric carcinoma cells significantly inhibits cell growth and proliferation (Xie et al., 2010). Transgelin 2 (TAGLN2) is known to bind to actin to facilitate the formation of cytoskeletal structures. Downregulation of transgelin 2 is reported to promote breast cancer metastasis (Yang et al., 2019).

We found miRNA-mRNA interactions (miR-26a-5p/miR-101-3p-EZH2; miR-181c-5p-CDX2; miR1-3p-TAGLN2/PTMA) involving four genes. Interestingly, miR-26a-5p-EZH2 interaction was found to be involved in cell proliferation, cell invasion and apoptosis in GBC cells (Wang et al., 2016b). EZH2 is reported to be a direct target of miR-26a in Uveal Melanoma (UM) cells. Further, the knockout of EZH2 mimicked the tumor inhibition of miR-26a in UM cells (Li et al., 2021). A recent study demonstrated that miR-101-3p prevented retinoblastoma cell proliferation by targeting EZH2 and HDAC9 (Jin et al., 2018), prevented autophagy in endometrial cancer cells by targeting EZH2 (Wang and Liu, 2018) and inhibits invasion and metastasis in renal cell carcinoma by Targeting EZH2 (Dong et al., 2021). PTMA was identified as a target gene regulated by the miR-1 in bladder cancer (Yamasaki et al., 2012).

A literature search on "lncRNA-miRNA pairs" in cancer revealed two lncRNA (NEAT1, MALAT1) -miR-101-3p interactions in lung cancer (Wang et al., 2017b; Kong et al., 2019b), two lncRNA (TUG1, MALAT1) -miR-26a-5p interactions observed in colon cancer and colorectal cancer (Tian et al., 2019; Zhou et al., 2021) and two lncRNA (TUG1, MALAT1) -miR-1-3p interactions in hepatic carcinoma, esophagus cancer (Li et al., 2020; Tang et al., 2022). We found six ceRNA regulatory networks (TUG1/MALAT1-miR-26a-5p-EZH2; NEAT1/MALAT1-miR-101-3p- EZH2; TUG1/MALAT1-miR-1-3p-PTMA) targeting two genes, EZH2 and PTMA. In view of the functional role of EZH2 and PTMA in cancer, as discussed earlier, the ceRNA regulatory network targeting these two genes may be investigated in GBC.

Overall, we identified twelve ceRNA regulatory networks which might be functional in GBC, however, the experimental validation of these networks (expression analysis) in the clinical samples is the limitation of the present study. In future, *in vitro* and *in vivo* studies would be planned that might establish the functional role of these networks in GBC.

5 Conclusion

The present study used the experimental data from high-throughput/targeted studies on miRNAs, mRNAs and proteins in GBC and identified 183 miRNA targets. IPA analysis showed "p53 signaling pathway" to be the top pathway associated with them. Three of the targets were among the top 5 hub molecules in PPI network analysis. A total of 9 targets were reported to be DE at both mRNA and protein levels. Further, lncRNA-miRNA-mRNA regulatory networks were constructed for 3 targets (TP53, CCND1, and CTNNB1) associated with p53 signaling pathway and 9 targets (CDX2, MTDH, TOP2A, TSPAN8, EZH2, TAGLN2, LMNB1, and PTMA) DE at both mRNA and protein level. Overall, twelve ceRNA regulatory networks (MALAT1-miR125b-p53; PVT1/MALAT1-miR-195-CCND1, H19/NEAT1-miR-193a-3p-CCND1, H19/MALAT1-miR-200a-CTNNB1, TUG1/MALAT1-miR-26a-5p-EZH2; NEAT1/MALAT1-miR-101-3p- EZH2; TUG1/MALAT1-miR-1-3p-PTMA) were identified for which lncRNA-miRNA and/or miRNA-mRNA interactions have been reported in other cancers. These and other lncRNA-miRNA-mRNA regulatory networks may be experimentally validated and explored for their therapeutic applications in GBC.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material, further inquiries can be directed to the corresponding author.

Author contributions

PG, RS were involved in study design; NS, JA were involved in literature search; NS, VC were involved in bioinformatics analysis; PG, SKU, NS, JA, and VC were involved in data analysis, and manuscript writing; RS, SKU had critically reviewed the manuscript; All authors read and approved the final manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Development and validation of an endoplasmic reticulum stress long non-coding RNA signature for the prognosis and immune landscape prediction of patients with lung adenocarcinoma

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Background: Lung adenocarcinoma (LUAD), the most common histotype of lung cancer, may have variable prognosis due to molecular variations. This work investigated long non-coding RNA (lncRNA) related to endoplasmic reticulum stress (ERS) to predict the prognosis and immune landscape for LUAD patients.

Methods: RNA data and clinical data from 497 LUAD patients were collected in the Cancer Genome Atlas database. Pearson correlation analysis, univariate Cox regression, least absolute shrinkage and selection operator regression analyses, as well as the Kaplan-Meier method, were used to screen for ERS-related lncRNAs associated with prognosis. The risk score model was developed using multivariate Cox analysis to separate patients into high- and low-risk groups and a nomogram was constructed and evaluated. Finally, we explore the potential functions and compared the immune landscapes of two groups. Quantitative real-time PCR was used to verify the expression of these lncRNAs.

Results: Five ERS-related lncRNAs were shown to be strongly linked to patients' prognosis. A risk score model was built by using these lncRNAs to categorize patients based on their median risk scores. For LUAD patients, the model was found to be an independent prognostic predictor ($p < 0.001$). The signature and clinical variables were then used to construct a nomogram. With 3-year and 5-year OS' AUC of 0.725 and 0.740, respectively, the nomogram's prediction performance is excellent. The 5-lncRNA signature was associated with DNA replication, epithelial-mesenchymal transition, and the pathway of cell cycle,

Abbreviations: LUAD, Lung adenocarcinoma; lncRNA, Long non-coding RNA; OS, Overall survival; ERS, Endoplasmic reticulum stress; TCGA, the Cancer Genome Atlas; LASSO, Least absolute shrinkage and selection operator; ROC, Receiver operating characteristic; GSEA, Gene set enrichment analysis; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; qRT-PCR, Quantitative real-time PCR; EMT, Epithelial-mesenchymal transition; ICIs, Immune checkpoint inhibitors; TIME, Tumor immunological microenvironment.

P53 signaling. Between the two risk groups, immune responses, immune cells, and immunological checkpoints were found to be considerably different.

Conclusion: Overall, our findings indicate that the 5 ERS-related lncRNA signature was an excellent prognostic indicator and helped to predict the immunotherapy response for patients with LUAD.

KEYWORDS

lung adenocarcinoma, long non-coding RNA, endoplasmic reticulum stress, prognostic model, immune landscape

1 Introduction

Lung cancer is the cancer with the highest fatality rate in the world, with around 25% of cancer patients dying of it (Sung et al., 2021). Lung adenocarcinoma (LUAD) is the most common histotype of lung cancer, accounting for around 40% of all lung malignancies with an increasing prevalence (Zappa and Mousa, 2016). Despite the employment of a variety of treatments, including surgery, chemoradiotherapy, targeted therapy, and immunotherapy, lung cancer patients had a dismal prognosis, with 5-year survival rates ranging from 4% to 17% (Hirsch et al., 2017). Due to molecular variations, patients with histologically identical malignancies may have a different prognosis. Therefore, compared to a single biomarker, integrating multiple biomarkers into a signature to predict the prognosis and high-risk group of patients with LUAD is of great significance. As a result, combining molecular biomarkers into a signature for predicting the prognosis of LUAD patients is of great importance.

Long non-coding RNAs (lncRNAs) have a role in a variety of physiological and pathological processes, including tumor progression and metastasis (Hu et al., 2018; Chi et al., 2019). LncRNAs are important in multi-gene regulatory networks, and they can be utilized to diagnose and predict survival in a variety of cancers (Van Leeuwen and Mikkers, 2010; Jiang et al., 2016). For example, upregulated lncRNA LINC00691 was a poor prognostic predictor for patients with lung cancer, and through modulation of SATB2, the downregulated lncRNA SATB2-AS1 inhibits tumor metastasis in colorectal cancer (Xu et al., 2019). Increasing researches suggested that lncRNA-based signatures can accurately predict overall survival (OS) in LUAD patients (Lin et al., 2018; Ren et al., 2021).

Endoplasmic reticulum stress (ERS) is defined as an imbalance in endoplasmic reticulum homeostasis, which includes the unfolded protein response and perturbation in calcium (Ca^{2+}) (Hetz, 2012; Leprivier et al., 2015). Relevant studies have shown that ERS has a major part in the genesis and progression of a variety of human malignancies (Obacz et al., 2017; Da Silva et al., 2020). ERS have a good predictive value as prognostic indicators in diffuse gliomas and hepatocellular carcinoma, according to previous researches (Liu et al., 2021a; Huang et al., 2021). According to previous research achievements, ERS has been linked to drug-induced apoptosis in lung carcinoma (Joo et al., 2007). However, few sequence-based studies have examined the characteristics of lncRNA related with ERS and their relationship to OS in patients with LUAD.

By analyzing lncRNA expression data from the Cancer Genome Atlas (TCGA), we were able to identified ERS-related lncRNAs. Then, to appropriately assess patients' prognosis, we developed a predictive multi-lncRNA signature of differentially expressed ERS-related lncRNAs.

2 Materials and methods

2.1 Identification of ERS-related lncRNAs

We access the TCGA database to obtain RNA-seq (Fragments Per Kilobase Million format) and clinical data for 497 patients with LUAD (https://cancergenome.nih.gov/). RNA-seq data was normalized by limma R package, and the mean value was used if the genes were duplicated. Two hundred and fifty-two ERS-related genes were retrieved by investigating previous studies (Supplementary Table S1) (Huang et al., 2021).

To identify the differentially expressed lncRNAs as well as the genes related to ERS between LUAD and adjacent normal tissues, we then used the criterion $|\log2FC| > 0.5$ and False Discovery Rate (FDR) < 0.05. Pearson correlation analysis was utilized to screened out ERS-related lncRNAs, and then we employed univariate Cox regression to explore lncRNAs related prognosis. In order to narrow the range of prognosis-related lncRNAs, the least absolute shrinkage and selection operator (LASSO) regression and Kaplan-Meier analysis were used.

2.2 Establishment of the risk score model

Following that, to establish the optimal risk model with ERS-related lncRNAs, we conducted a multivariate Cox regression analysis and made use of its regression coefficient. The risk score was calculated using linear integration of the regression coefficient and the expression value of 5 ERS-related lncRNAs. As a result, we computed the risk score as follow:

$$\text{Risk score} = \sum_{i=1}^{5} (\beta_i \times Exp_i).$$

The coefficient (βi) was the corresponding lncRNAs' regression coefficient of the above Cox regression model. The expression level of the identified ERS-related lncRNAs is referred to as *Expi*. This formula was used for each patient to calculate his corresponding risk score. Furthermore, we considered median risk score as the cutoff value to divide all the patients into high- and low-risk groups. The Kaplan-Meier survival curve and log-rank test were applied to demonstrate the prognostic difference between the two groups.

2.3 Construction and evaluation of the nomogram

Using both univariate and multivariate Cox regression analysis, the prognostic relationship between the risk score and clinical

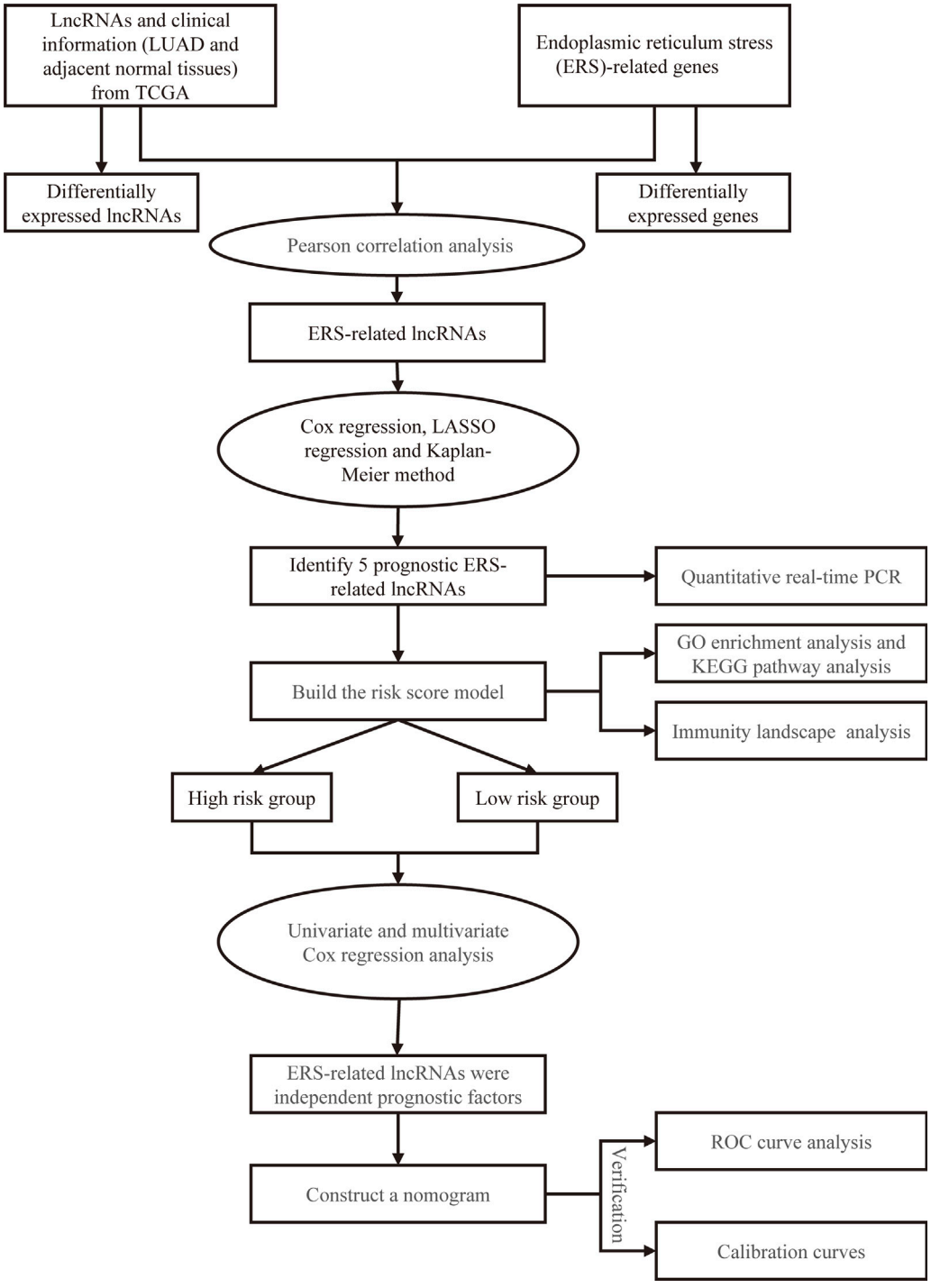

FIGURE 1
Flowchart of our design for the study.

features (including age, gender, and TMN stage) was investigated. Following that, in order to predict the prognosis of each patient with LUAD more accurately, we created a nomogram for 3- and 5-year OS by using the results of multivariate Cox regression model. To evaluate the prediction performance of the nomogram, we then employed the receiver operating characteristic (ROC) curve analysis and the calibration curves.

2.4 Gene set enrichment and immunity landscape analysis

Gene set enrichment analysis (GSEA) was performed for the low- and high-risk groups to investigate the potential pathways and functions of these ERS-related lncRNAs. Not only Gene Ontology (GO) enrichment analysis but also Kyoto Encyclopedia of Genes and

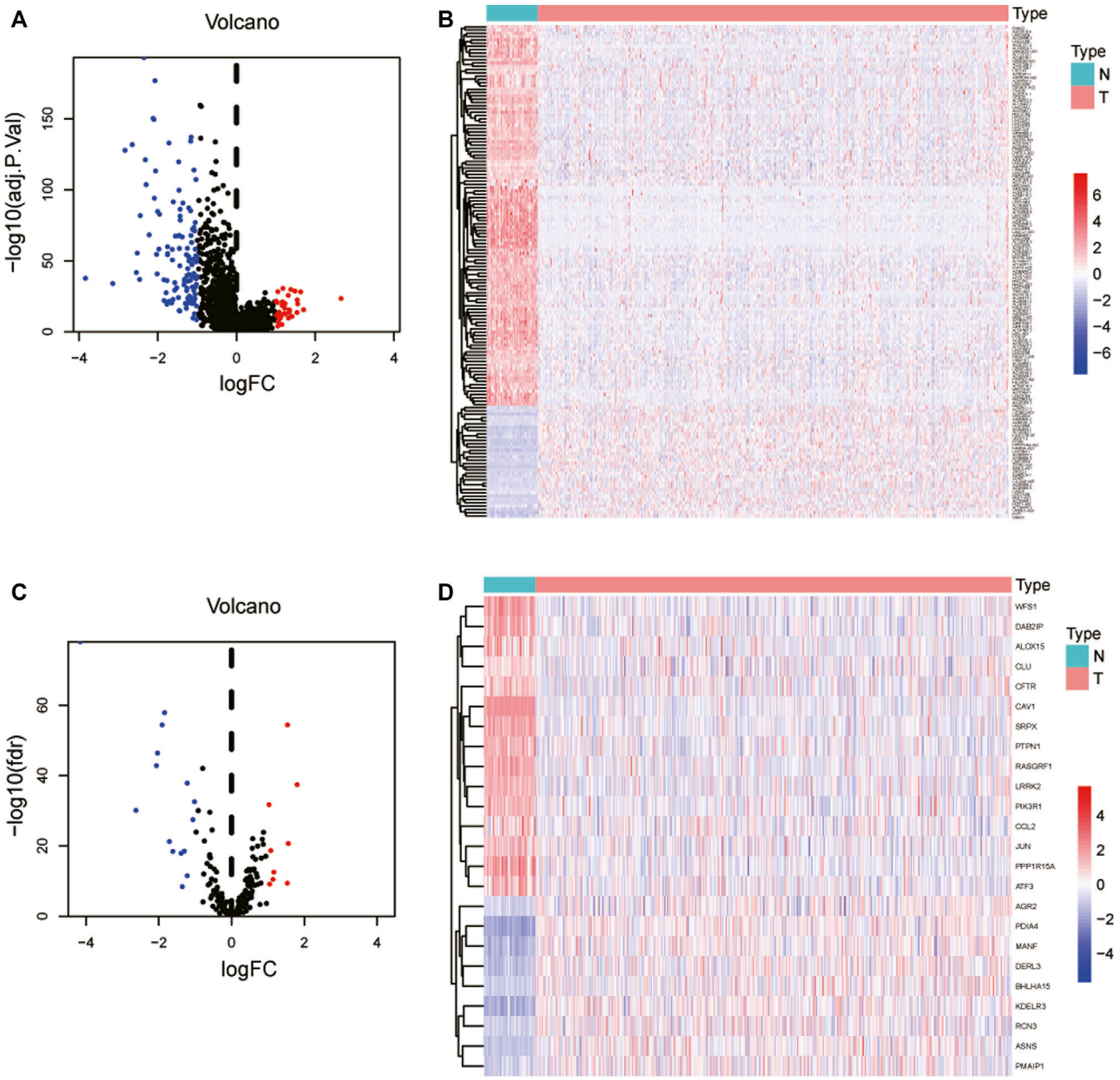

FIGURE 2
Identification of ERS-related lncRNAs in LUAD. **(A)** As shown in volcano plot, the 34 lncRNAs were upregulated in tumors, while 116 were downregulated. **(B)** Differential expression lncRNA heatmaps in tumors and normal adjacent tissues. **(C)** Nine ERS-related genes were found to be upregulated in tumors, whereas 15 were found to be downregulated. **(D)** Heatmaps of differential expression ERS-related genes in tumors and normal adjacent tissues. LUAD: Lung adenocarcinoma; ERS: Endoplasmic reticulum stress.

Genomes (KEGG) pathway analysis were conducted. Furthermore, we compared the differences immune cell abundance, immune function, and immune checkpoints between high- and low risk groups.

2.5 RNA extraction and quantitative real-time PCR

Human LUAD cells (A549, PC-9 and SKLU1) and normal bronchial epithelial cells (16HBE) were obtained from Procell Life Science and Technology (Wuhan, China). Using TRIzol reagent (BioTeke, Beijing, China), we isolated the whole RNA of the LUAD cells. Subsequently, qRT-PCR was performed utilizing the HiScript II Q RT SuperMix for qPCR (Bioer Technology, Hangzhou, China), according the instructions provided by the manufacture for all program steps. The $2^{-\triangle\triangle Ct}$ method was used to calculate the relative expression of each lnRNA. We utilized the unpaired *t*-test to compare the LUAD cells and normal cells in the expression level differences. Supplementary Table S2 shows the primer sequences.

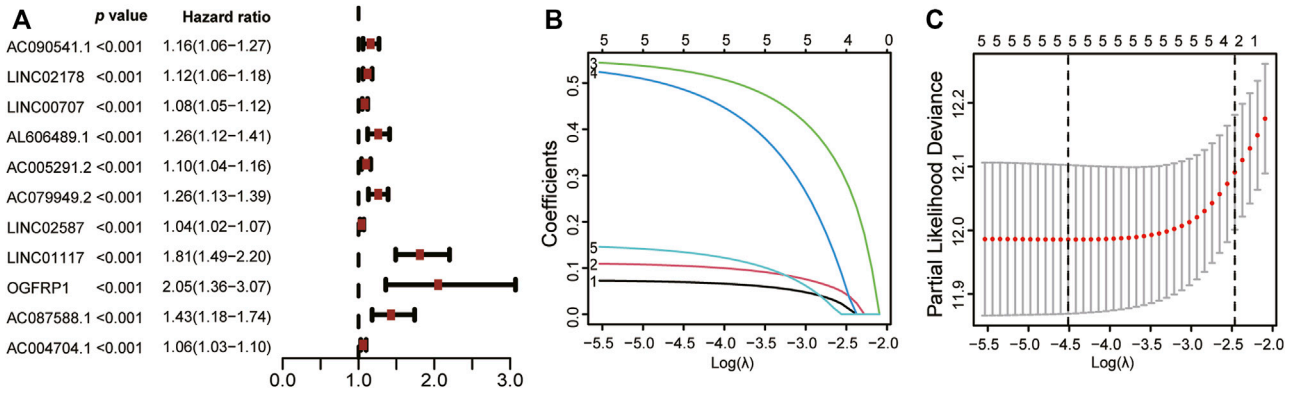

FIGURE 3
Screening ERS-related lncRNAs related to prognosis. **(A)** Based on univariate Cox analysis, 11 ERS-related lncRNAs were significantly correlated with LUAD patients' OS, as shown in the forest plot. **(B)** LASSO analysis was utilized to filter out ERS-related lncRNAs in LUAD patients. **(C)** The LASSO coefficient spectrum for lncRNAs linked to ERS is shown. OS: Overall survival; LUAD: Lung adenocarcinoma; ERS: Endoplasmic reticulum stress; LASSO: Least absolute shrinkage and selection operator.

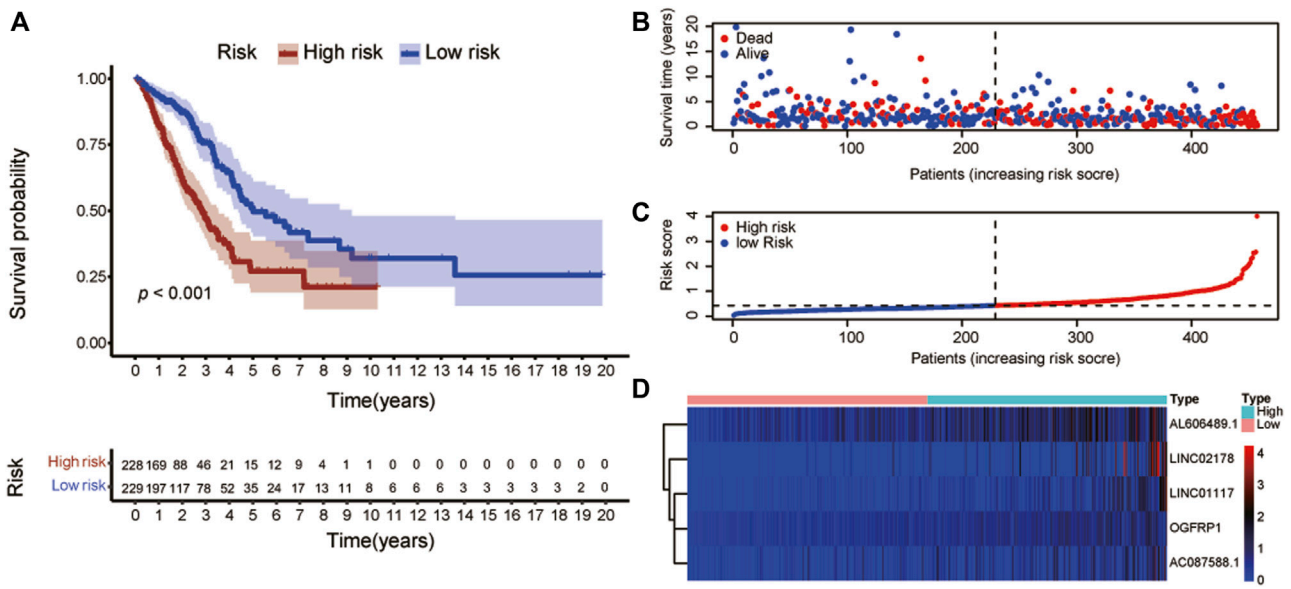

FIGURE 4
Establishment of the risk score model utilizing ERS-related lncRNAs. **(A)** Kaplan-Meier analysis showed that the patients in the high-risk group had a significantly lower overall survival than that in the low-risk group. **(B)** The survival status for each patient. **(C)** Distributions of patient risk ratings. **(D)** Heatmaps of lncRNAs associated to ERS in low- and high-risk groups. Warm colors denoted a high level of expression, whereas cold colors denoted a low level of emotion. ERS: Endoplasmic reticulum stress.

2.6 Statistical analysis

We conducted all statistical analyses in this research using R software (Institute for Statistics and Mathematics, Vienna, Austria; https://www.r-project.org, version 4.0.2) and GraphPad Prism 8. 0 software. The unpaired Wilcoxon Rank Sum test was used to assess the differences between continuous variables. The chi-square test was used to examine the relationship between categorical factors. The statistically significant setting for each analysis was two-tailed $p < 0.05$.

3 Results

3.1 Identification of ERS-related lncRNAs

Figure 1 depicts the flowchart for this research. We examined 497 lung cancer patients using the TCGA database to find differentially expressed lncRNAs and their associated activities of ERS-related genes in LUAD. Subsequently, 497 tumors and 54 normal adjacent tissues were retrieved from these patients. We retrieved previous studies to extract 252 ERS-related genes in total (Huang et al., 2021). We identified

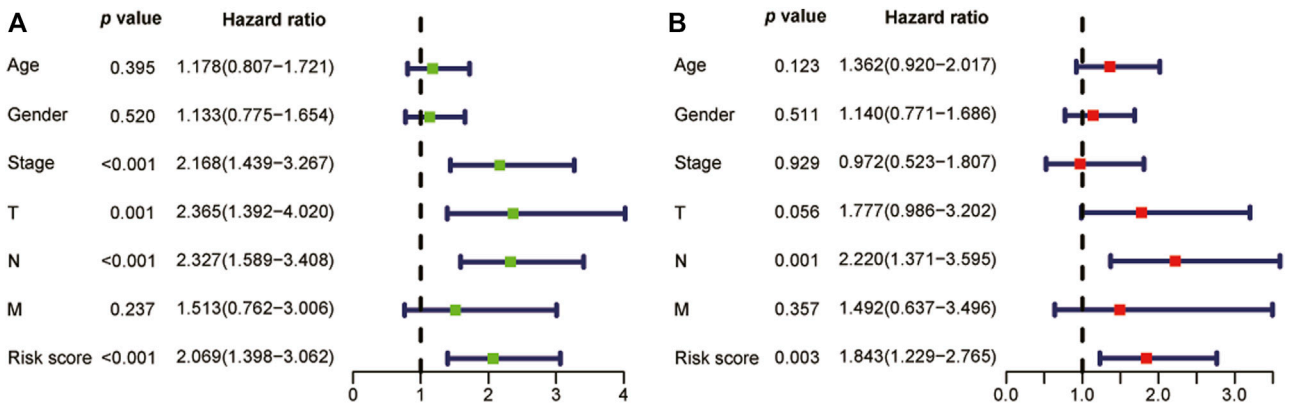

FIGURE 5
The univariate and multivariate Cox analysis analyses utilizing the risk score and clinical variables. **(A)** The univariate Cox analysis results showed that the risk score was highly associated with overall survival (n = 453). **(B)** In LUAD patients, the lncRNA signature, as shown in the multivariate Cox analysis, was determined to be an independent prognostic factor (n = 453). LUAD: Lung adenocarcinoma.

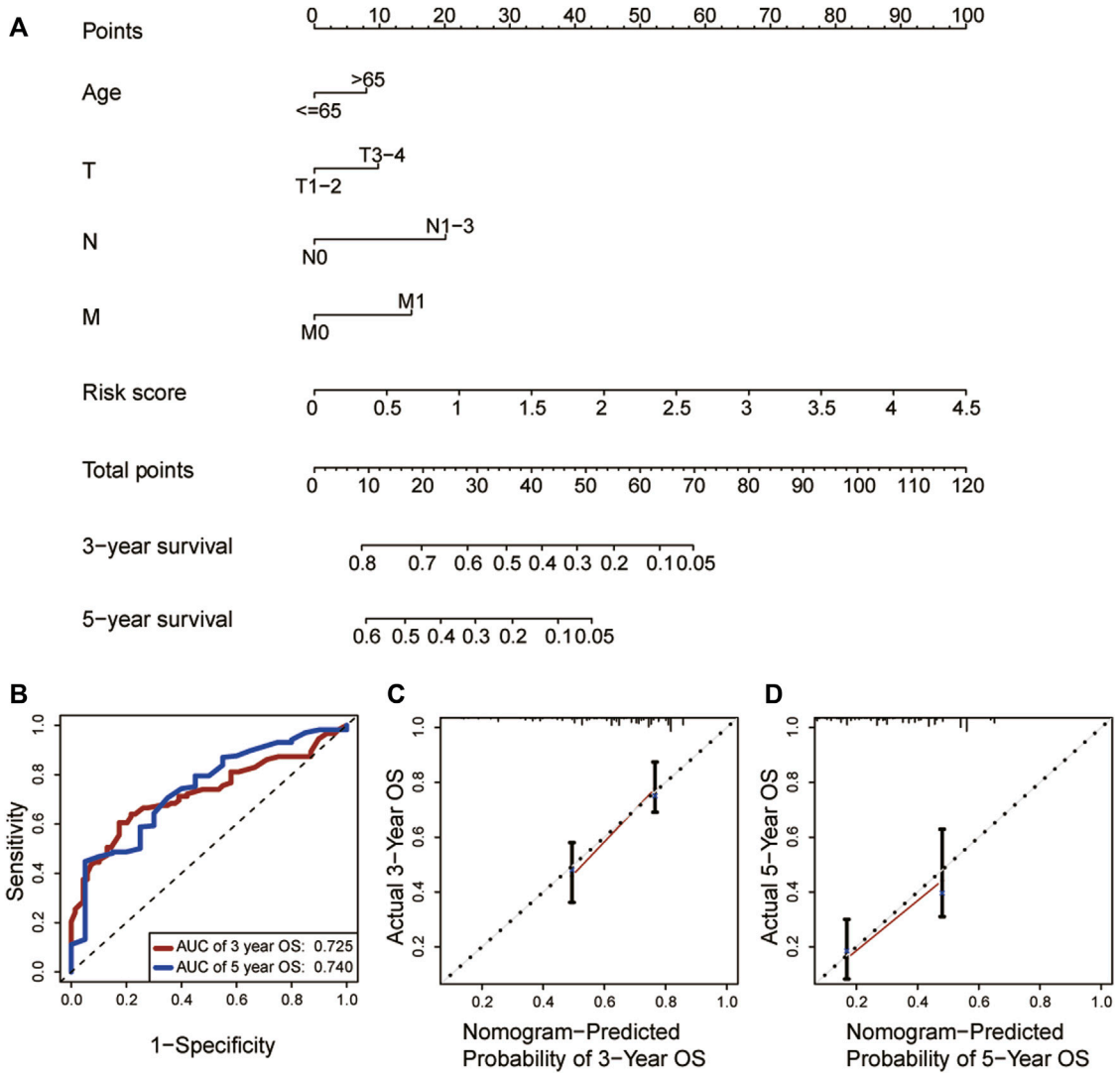

FIGURE 6
Construction and assessment of the nomogram (n = 453). **(A)** Establishment of the nomogram. **(B)**According to ROC curve analysis for predicting OS by the nomogram, the AUCs for 3-year OS was 0.725 and that for 3-year OS 0.740. **(C, D)** The calibration curve for nomogram's 3-year and 5-year OS, respectively. ROC: Receiver operating characteristic; OS: Overall survival; AUC: Area under curve.

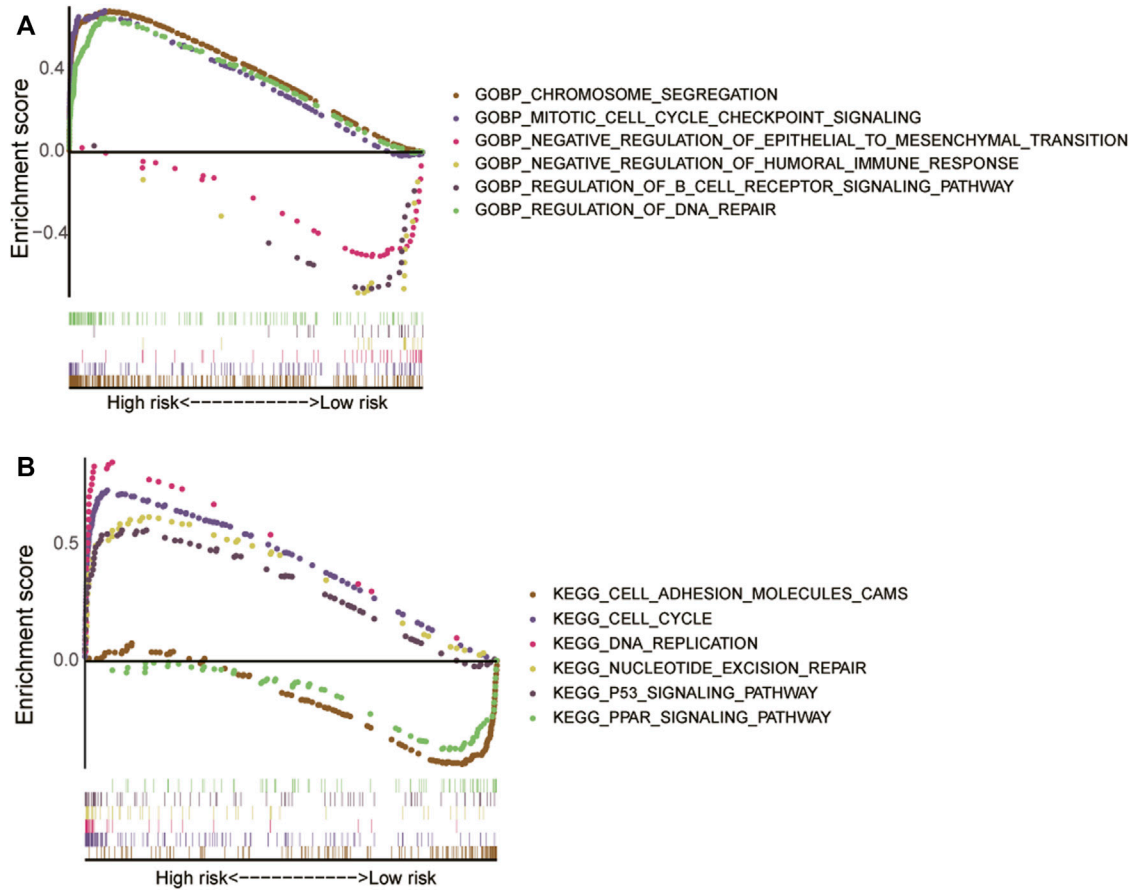

FIGURE 7
The results of Gene Set Enrichment Analysis for high- and low-risk groups. **(A)** Gene Ontology enrichment analysis. **(B)** Kyoto Encyclopedia of Genes and Genomes pathway analysis.

that a total of 150 lncRNAs were deferentially expressed in tumors and normal adjacent tissues, with 34 being upregulated and 116 being downregulated in tumors (Figures 2A,B). A total of 24 genes were evaluated for differential expression among 252 ERS-related genes acquired from previous publications, including 9 upregulated and 15 downregulated in tumors (Figures 2C, D).

Furthermore, we were successful in screening out 2643 ERS-related lncRNAs utilizing Pearson correlation analysis (Supplementary Table S4). Following that, univariate Cox regression analysis showed that 11 lncRNAs were tightly associated with prognosis of LUAD patients (Figure 3A). Finally, the LASSO regression analysis and the Kaplan-Meier method incorporated 5 lncRNAs: AL606489.1, LINC02178, LINC01117, OGFRP1, and AC087588.1 (Figures 3B,C), which were found to be strongly associated to OS in lung adenocarcinoma patients (Supplementary Figure S1).

3.2 Establishment of the risk score model

To construct risk score model, these well-characterized lncRNAs were filtered applying multivariate Cox regression. The formula was "Risk score = (0.1048) × AL606489.1 expression + (0.0697) × LINC02178 expression + (0.5262) × LINC01117 expression + (0.4863) × OGFRP1 expression + (0.1346) × AC087588.1 expression". Based on the median risk score, the patients were separated into two parts: high-risk and low-risk. Compared with the patients in low-risk group, the patients in the high-risk group presented significantly shorter OS ($p < 0.001$, Figure 4A). The risk score distribution and survival status of these 5 lncRNAs were showed in Figures 4B,C, and the risk coefficient and patient mortality were greater in the high-risk group. The expression patterns of the 5-lncRNA signature in the two groups were revealed in the heatmap (Figure 4D).

3.3 Construction and evaluation of the nomogram

As shown in the univariate and multivariate Cox regression, the risk score was strongly related to OS (Figure 5A) and was also demonstrated to be an independent prognostic predictor of OS (Hazard ratio: 1.843; 95% Confidence interval:1.229–2.765; $p = 0.003$, Figure 5B). Next, we constructed a nomogram utilizing the results of the multivariate Cox regression (Figure 6A). The nomogram's AUC of 3-year OS was 0.725 and that of 5-year OS was 0.740 (Figure 6B). The calibration curve also demonstrated that the nomogram with the risk score was

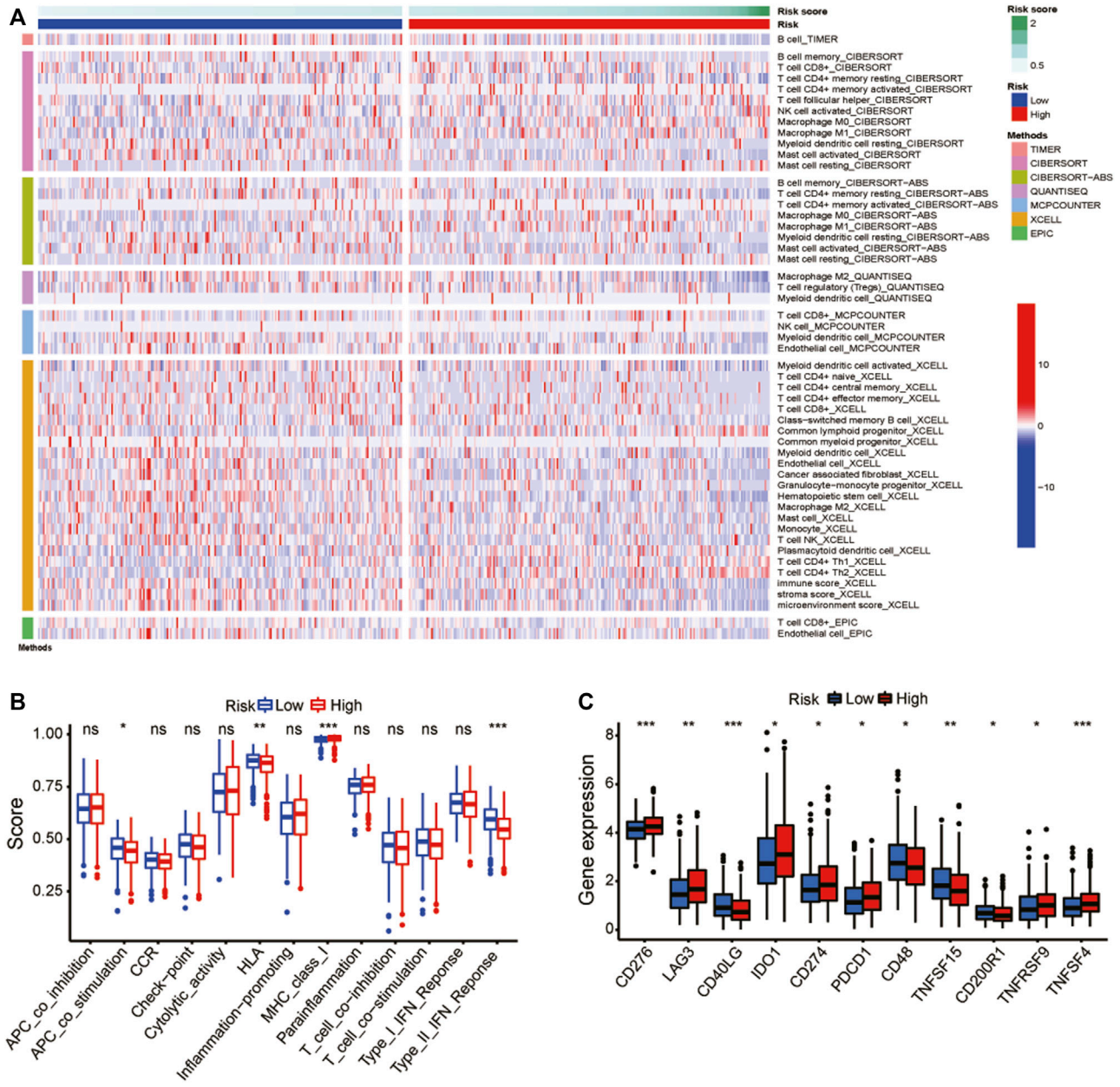

FIGURE 8
Gene expression and immune function. **(A)** A heat map for immunological responses between high- and low-risk groups. **(B)** Immune cell subpopulations and associated functions. **(C)** Expression of immune checkpoints in high-risk and low-risk groups. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$; ns: no significance.

effective in the prediction of the prognosis for patients with LUAD (Figures 6C, D).

3.4 Gene set enrichment analysis

We found that the ERS-related lncRNAs were abundant in chromosomal segregation, mitotic cell cycle checkpoint signaling, humoral immune response, epithelial-mesenchymal transition (EMT), B cell receptor signaling pathway, and DNA repair, according to GO enrichment analysis (Figure 7A). The pathway of cell cycle, DNA replication, cell adhesion, PPAR signaling, nucleotide excision repair, and P53 signaling were shown to be related to these lncRNAs (Figure 7B). These factors may inspire people to further investigate the role of ERS-related lncRNAs in the pathogenesis further.

3.5 Immunity expression based on the risk model

Figure 8A showed a heatmap of immune cell infiltration using several different algorithms. The immune functions were shown to

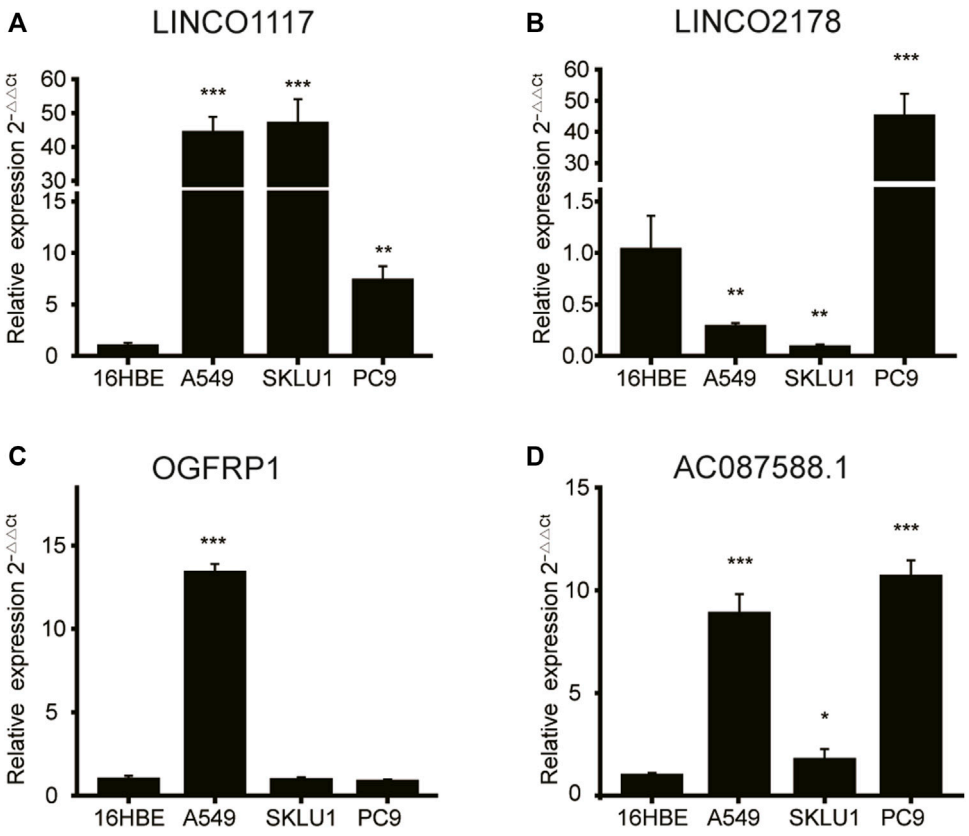

FIGURE 9
The qRT-PCR results of 4 lncRNAs' expression in lung adenocarcinoma and normal bronchial epithelial cell lines. **(A)** LINC01117, **(B)** LINC02178, **(C)** OGFRP1, **(D)** AC087588.1. lncRNA: Long non-coding RNA. *$p < 0.05$, **$p < 0.01$, and ***$p < 0.001$.

be significantly different between the two groups (Figure 8B). Correlation analysis between immune cell subpopulations and related functions revealed that co-stimulation antigen-presenting cells, chemokine receptors, type II IFN response, and human leukocyte antigen showed higher expression in high-risk group than that in low-risk group, but the MHC class I was the opposite. Our research was expanded to explore the expression levels of immune checkpoint in two groups (Figure 8C). The expression of most immune checkpoints, such as CD276, LAG3 and CD48, have a lot of variation between the two groups. The results suggested that risk scores may be useful for patients with LUAD in predicting the response to immune checkpoint inhibitors (ICIs).

3.6 Expression level of selected lncRNAs in lung adenocarcinoma cells

Furthermore, we confirm the expression levels of 4 lncRNAs using a qRT-PCR test in the LUAD cells (A549, PC-9 and SKLU1). The expression of the lncRNA LINC01117, as shown by the results, was significantly upregulated in all the LUAD cell lines (Figure 9A). The expression of LINC02178 was significantly elevated in the PC9 cell line, but was downregulated in other cell lines (Figure 9B). OGFRP1 were obviously upregulated in the A549 cell line (Figure 9C). In all LUAD cell lines, AC087588.1 was considerably upregulated as well (Figure 9D).

4 Discussion

The objective of this study was to find out a better predictive marker related to ERS in order to provide approaches to develop innovative treatments for LUAD. As a result, we developed a new lncRNA signature based on ERS for the prediction of the prognosis of LUAD patients. Patients with LUAD were divided into high-risk and low-risk groups based on the signature. Patients in the low-risk group had significantly favorable OS, according to the results of the prognostic analysis. In addition, a nomogram was constructed and shown to have an excellent predictive effect. To our knowledge, this is the first study to identify and analyze prognostic lncRNAs related to ERS in patients with LUAD.

In our study, all five lncRNAs, AL606489.1, LINC02178, LINC01117, OGFRP1 and AC087588.1, were shown to be independent unfavorable prognostic indicators for LUAD. Previous research has linked AL606489.1 to ferroptosis (Guo et al., 2021), pyroptosis (Song et al., 2021) and autophagy (Wu et al., 2021), as well as a poor prognosis in LUAD patients, which is consistent with our findings. In LUAD, ERS may be associated to ferroptosis, pyroptosis, and autophagy. LINC02178 was also

identified a risk factor for bladder urothelial cancer (Sun et al., 2020). Ding and Liu., 2019 discovered that OGFRP1 was a risky lncRNA in LUAD, and that OGFRP1 increased the proliferation, migration, and invasion of non-small cell lung cancer through the miR-4640–5p/eIF5A axis (Liu et al., 2021b). Furthermore, OGFRP1 acts as an oncogene in prostate cancer (Wang et al., 2021), endometrial cancer (Lv et al., 2019) and gastric cancer (Zhang et al., 2021a). However, further work is needed to investigate the biological and molecular mechanisms of these lncRNAs.

According to GSEA results, chromosome segregation, mitotic cell cycle checkpoint signaling, DNA replication, and the pathway of cell cycle, P53 signaling were abundant in the high-risk group, whereas EMT, DNA repair, and the pathway of cell adhesion, PPAR signaling were enriched in the low-risk group. Wang et al. (2016) discovered that Rac3 in lung cancer might enhance cell proliferation *via* the cell cycle pathway. A previous study found that the lncRNA PIK3CD-AS2 promotes progression in patients with LUAD by suppressing p53 signaling (Zheng et al., 2020), which implies that ERS-related lncRNA may have a similar effect. Some lncRNAs in LUAD, such as LINC0047 (Deng et al., 2020), LINC01006 (Zhang et al., 2021b) and SGMS1-AS1 (Liu et al., 2021c), have been shown to influence EMT processes.

Immune responses, immune cells, and immunological checkpoints were shown to be significantly different between the two risk groups in this study, suggesting that ERS-related lncRNAs are likely to affect the immune system in patients with LUAD. Low-risk patients with LUAD may have a stronger immunological response. ICIs, as we all know, have greatly improved the prognosis of lung cancer patients in recent years (Aldarouish and Wang, 2016). Accordingly, the efficacy of ICIs is thought to be highly linked to the immune systems of the hosts and the tumor immunological microenvironment (TIME) (Taube et al., 2018). TIME might be modulated by ERS, affecting tumor growth and response to immunotherapy (Chen and Cubillos-Ruiz, 2021). The ERS-related five-lncRNA signature in LUAD may reflect the effect of ICIs and immune response rate. Immunotherapy may be more advantageous for people with LUAD who are in the low-risk group.

There are a few shortcomings in our current study. To begin with, we were unable to gather information on more specific clinic pathological parameters, such as treatment information and tumor markers. In the future, external validation of the signature in other datasets and prospective large-scale multicenter cohorts will be required for reliability and precision. Second, due to lack of funds, the expression levels of lncRNA AL606489.1 were missing from our qPCR data. Finally, more functional experiments are needed to understand the specific mechanisms by which these ERS-related lncRNAs interact with LUAD.

5 Conclusion

In conclusion, the 5 ERS-related lncRNAs and a well-validated nomogram based on those lncRNAs were found to be beneficial in predicting the prognosis of LUAD patients. Our findings also point to promising avenues for LUAD immunotherapy and provide a significant foundation for future research.

Data availability statement

The datasets presented in this study can be found in online repositories. The names of the repository/repositories and accession number(s) can be found below: https://portal.gdc.cancer.gov, The Cancer Genome Atlas.

Author contributions

The research was planned by JX and MC. The data was analyzed and the original article was written by ZW and JZ. ML and WB gathered references and reviewed the paper. The data was gathered by XX and GX. The final manuscript was reviewed and approved by all authors.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2023.1024444/full#supplementary-material

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The Tet2–Upf1 complex modulates mRNA stability under stress conditions

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Introduction: Environmental stress promotes epigenetic alterations that impact gene expression and subsequently participate in the pathological processes of the disorder. Among epigenetic regulations, ten–eleven Translocation (Tet) enzymes oxidize 5-methylcytosine (5mC) to 5-hydroxymethylcytosine (5hmC) in DNA and RNA and function as critical players in the pathogenesis of diseases. Our previous results showed that chronic stress increases the expression of cytoplasmic Tet2 in the hippocampus of mice exposed to chronic mild stress (CMS). Whether the cytoplasmic Tet2 alters RNA 5hmC modification in chronic stress-related processes remains largely unknown.

Methods: To explore the role of cytoplasmic Tet2 under CMS conditions, we established CMS mice model and detected the expression of RNA 5hmC by dot blot. We verified the interaction of Tet2 and its interacting protein by co-immunoprecipitation combined with mass spectrometry and screened downstream target genes by cluster analysis of Tet2 and upstream frameshift 1 (Upf1) interacting RNA. The expression of protein was detected by Western blot and the expression of the screened target genes was detected by qRT-PCR.

Results: In this study, we found that increased cytoplasmic Tet2 expression under CMS conditions leads to increase in total RNA 5hmC modification. Tet2 interacted with the key non-sense-mediated mRNA decay (NMD) factor Upf1, regulated the stability of stress-related genes such as Unc5b mRNA, and might thereby affect neurodevelopment.

Discussion: In summary, this study revealed that Tet2-mediated RNA 5hmC modification is involved in stress-related mRNA stability regulation and may serve as a potential therapeutic target for chronic stress-related diseases such as depression.

KEYWORDS

Tet2, Upf1, RNA, 5hmC, mRNA stability

Introduction

Epidemiological studies have revealed that chronic stress caused by environmental factors has a significant impact on diseases (Jirtle and Skinner, 2007; Burrage et al., 2018; Wen-Yue and Xiao-Dong, 2021). Adverse experiences, such as emotional abuse (LeMoult et al., 2020), isolation (Slavich and Sacher, 2019), smoking (Fluharty et al., 2017), alcoholism (Schuckit, 2009), and obesity (Milaneschi et al., 2019), are highly sensitive to environmental stress, especially during early life, a critical period of brain developmental plasticity. These environmental stress factors influence an

individual's genome through inducing changes in epigenetic modifications and are high-risk factors for psychiatric disorders such as depression (Green et al., 2010; Barbu et al., 2021).

Chronic environmental stress induced several types of epigenetic modifications to regulate gene expression in the pathological process of depression (Dalton et al., 2014; Mayer et al., 2021). For instance, people with major depressive disorder (MDD) showed a decreased global DNA 5hmC in peripheral leukocytes (Tseng et al., 2014; Reszka et al., 2021). Some antidepressants also showed the effect of increasing the level of DNA 5hmC (Wei et al., 2014; Wang et al., 2019). Therefore, as the dioxygenase of DNA or RNA 5-methylcytosine (Ito et al., 2010; Fu et al., 2014; Lan et al., 2020; Ma et al., 2022), the Tet family may be one of the genetic modifiers responsible for MDD. The Tet family includes three members: Tet1, Tet2, and Tet3 (Rasmussen and Helin, 2016). Our previous study found that the expression of Tet2 increased in the hippocampus of mice after chronic stress and Tet2 mainly accumulated in the cytoplasm (Zhang et al., 2021). However, the role of Tet2 accumulation in the cytoplasm under stress conditions remains unclear.

Recent studies have shown that methylation and demethylation modifications were also found in RNA cytosine, namely, RNA 5mC and RNA 5hmC (Motorin et al., 2010). These modifications are involved in RNA processing (Yang et al., 2017), mRNA stability (Chen et al., 2019; Yang et al., 2019), neural system development (Flores et al., 2017), and tumorigenesis (Liang et al., 2020). Tet enzymes, such as the dioxygenase of DNA 5mC (Tsiouplis et al., 2020), have also been found to serve as the hydroxymethylase of RNA 5 mC (Fu et al., 2014; Guallar et al., 2018). The ATP-dependent RNA helicase upstream frameshift 1 (Upf1) has potential interactions with Tet2 protein according to the mass spectrometry result of Tet2 protein. Upf1 impacts mRNA stability (Staszewski et al., 2023) and is the most enriched RNA-binding protein of the 5mC site in mRNA (Amort et al., 2017). Whether Upf1 participates in the Tet2-mediated hydroxymethylation of RNA 5mC is still unknown.

In this study, we found that the expression of RNA 5hmC modification increased under CMS conditions accompanied with increased cytoplasmic Tet2. The ATP-dependent RNA helicase Upf1 was identified as a Tet2-interacting protein to modify RNA 5hmC and regulate RNA stability such as Unc5b mRNA, which is involved in axonal guidance and stress responses. Our findings, therefore, provide novel mechanistic insights into the role of epigenetic modification induced by Tet2 in chronic stress, suggesting the pathological importance of cytoplasmic Tet2 and RNA 5hmC modification in psychiatric disorders.

Materials and methods

Reagents, antibodies, cell lines, and oligos

The lists of the reagents, antibodies, cell lines, and oligos used in this study are given in Supplementary Table S1.

Animals and establishment of chronic mild mice model

All procedures and protocols in this study were approved by the Institutional Animal Care and Use Committee of Soochow University. C57BL/6 mice (25.0 ± 3.0 g, 8–12 weeks old) were purchased from the Shanghai Research Center for Model Organisms. The mice were randomly divided into two groups: the control group and the model group and exposed to various, randomly scheduled, low-intensity social and environmental stressors two–three times a day for 5 weeks. The stressors included the following: (Jirtle and Skinner, 2007) water deprivation for 24 h, (Wen-Yue and Xiao-Dong, 2021) food deprivation for 24 h, (Burrage et al., 2018) wet cage for 24 h, (LeMoult et al., 2020) empty cage for 24 h, (Slavich and Sacher, 2019) cage tilt for 24 h (45°), (Fluharty et al., 2017) restraint stress for 2 h, (Schuckit, 2009) forced swimming at 4°C for 6 min, (Milaneschi et al., 2019) cage exchange for 6 min, (Green et al., 2010) shaking the cage for 6 min, (Barbu et al., 2021) and giving noise for 6 min. The control mice were housed under normal conditions (four to five per cage) and only switched to individual housing during testing for the depressive-like behavior phase.

Tail suspension test (TST)

Depression in mice was assessed using the TST (Sidransky et al., 2009). First, the mice were suspended 55 cm above the floor with a medical tape placed about 2 cm from the tip of their tails. The mice were made to turn to the camera to prevent them from being affected by the camera. The immobility time of the mice was recorded using a video camera for 6 min. The criterion for mouse immobility was immobility for at least 2 s.

Forced swimming test (FST)

The FST was used to assess mice for depressive behavior. The FST apparatus consisted of a transparent glass cylinder (18 cm diameter and 40 cm height) filled with water up to 15 cm at about 25°C. The cylinder was wrapped in tin foil paper to prevent interference while the mice swam. Pre-testing is required before a formal experiment. Mice were placed in a cylinder and allowed to swim and then removed from the water and returned to the cage after been dried in a heated environment for several minutes. In the formal test, the camera was placed directly above the cylinder, and the immobility time of the mice was measured during a 6-min swim after being placed in the water. At the end of each test, the warm water should be refilled so as not to affect the subsequent measurement tests. The criterion for immobility in mice is to stay afloat without struggling.

Sucrose preference test (SPT)

In this test, mice with anhedonia were evaluated with reference to the method of Liu et al., (2018) mentioned in published articles. Before the test, the mice were acclimated to the cage with two randomly placed tubes containing 1% (w/v) sucrose solution and water for 3 days. During the adaptation period, new tubes will be replaced daily, and the tube is placed at random positions each day. In the SPT, mice could prefer to

drink 2% (w/v) sucrose solution or water freely, and the volume of sucrose solution or water was recorded at the second hour and the 24th hour, respectively. The sucrose preference is calculated using the following equation: ratio of sucrose preference = consumption of sucrose/[consumption of water + consumption of sucrose] × 100%.

Cell culture and transfection

Neuro 2A (N2a) cells and HEK293T cells were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum (FBS) and were placed in a humidified incubator at 37°C with 5% CO_2. For transfection, HEK293T cells were transfected at 70% confluency using polyethylenimine (PEI), and N2a cells were transfected at 70% confluency using PL transfection reagent according to the manufacturer's instructions.

Immunoprecipitation (IP), protein extraction, and Western blot

For endogenous IP, three 90-mm dishes containing N2a cells at 70% confluency were lysed in 1 mL RIPA buffer [50 mM Tris (pH 8.0), 0.1% SDS, 150 mM NaCl, 1 mM EDTA (pH 8.0), 1 mM EGTA (pH 8.0), 0.5% deoxycholate, and 1% Triton X-100] containing 100 × cocktail and 200 × DTT at 4°C under rotary agitation for 30 min. About 5% input was taken after centrifuging at 13,200 rpm for 30 min at 4°C. Total protein was divided into two groups, and each group contained not less than 2 mg. The protein supernatant was added after agarose beads were activated and was pre-cleared by rotating for 2 h at 4°C. The supernatant was transferred to another tube, and the same amount of Tet2 and IgG antibody was added into the two tubes and then rotated at 4°C overnight. The supernatant was transferred to the agarose beads and rotated at 1,000 rpm at 4°C overnight. The supernatants were removed after centrifuging at 1,000 rpm at 4°C for 3 min, 50 μL 2 × loading buffer was added to the precipitate, and the samples were boiled at 95°C for 10 min.

For exogenous IP, HEK293T cells were transiently transfected with target plasmids using PEI. Three 90-mm dishes of HEK293T cells were used as one group. HA-Upf1 (30 μg) + Vector (30 μg) and HA-Upf1 (30 μg) + Flag-Tet2 (30 μg) plasmids were transfected for 48 h. Cell pellets were lysed using lysis buffer [150 mM NaCl, 25 mM tris-HCl (pH 7.4), 10% glycerol, 0.5% Triton X-100, 2 mM $MgCl_2$] containing 100 × cocktail and 200 × DTT at 4°C for 30 min; centrifuged at 4°C for 30 min at 13,200 rpm; and 60 μL of the supernatant was collected as the input sample. The remaining supernatant was put into Flag beads and rotated at 4°C overnight. The supernatant was removed by a magnetic shelf, and the beads were cleaned twice with lysis buffer. About 50 μL 1 × loading buffer was added to the precipitate, and the samples were boiled at 95°C for 10 min.

For total protein extraction, cell pellets were lysed using RIPA buffer containing 50 mM Tris (pH 8.0), 0.1% SDS, 150 mM NaCl, 1 mM EDTA (pH 8.0), 1 mM EGTA (pH 8.0), 0.5% deoxycholate, and 1% Triton X-100 on ice for 30 min and then centrifuged at 4°C at 12,000 rpm for 15 min. The supernatant was collected and boiled for 10 min at 95°C after adding 1/4 volume of 5 × loading buffer. About 30-μg protein aliquot of each sample was separated using standard SDS-PAGE and transferred onto a PVDF membrane. After transferring the protein, the PVDF membrane was blocked with 10% nonfat milk for 1 h at room temperature (RT) and incubated overnight at 4°C with target primary antibody. Immunoreactive bands were detected by ECL chemiluminescence reagent after incubating with secondary antibody for 1 h. The antibody and reagents used in this experiment are given in the Supplementary Material.

Immunofluorescence

The samples were fixed overnight at 4°C in 4% PFA in PBS for 30 min, treated with 0.3% Triton X-100 in PBS (PBST) for 10 min, and then blocked with 5% BSA in 0.3% PBST for 1 h at RT. Subsequently, the samples were incubated with sample-specific primary antibodies overnight at 4°C, followed by three washes with PBS, and incubated with florescent-labeled secondary antibody containing DAPI for 1 h at RT. The samples were imaged using a fluorescence microscope (Axio Scope A1, Zeiss) after patching on the microslide.

Construction and transformation of the expression plasmids

RNA was extracted from the hippocampus of wild-type C57BL/6 mice and reverse-transcribed onto cDNA. Target fragments were amplified from this cDNA as templates. The gel extraction products of target fragments were linked with linearized pCMV-HA or pCMV-flag plasmids by homologous recombination. DH5α *E.coli* was used to transform the connection products obtained in the aforementioned experiments. After transformation, monoclonal colony was selected for plasmid amplification. The plasmids were extracted and verified by sequencing.

Overlapping genes and functional enrichment analysis

The overlapping genes list was obtained from the jvenn database (http://jvenn.toulouse.inra.fr/app/example.html). Metascape (http://metascape.org/gp/index.html#/main/step1) is a gene-list analysis tool for gene functional enrichment analysis (Zhou et al., 2019). The identified overlapping genes were inputted into the Metascape database for Gene Ontology (GO), Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, and disease enrichment analysis.

RNA isolation and quantitative RT–PCR

Total RNA was extracted using RNA TRIzol and was reverse-transcribed using the Reverse Transcription Kit as per the manufacturer's protocol. Quantitative real-time PCR was performed using 2 × SYBR Green PCR Master Mix with a 7500 real-time PCR system (Applied Biosystems, Foster City, CA, United States). GAPDH was used as an endogenous control for real-

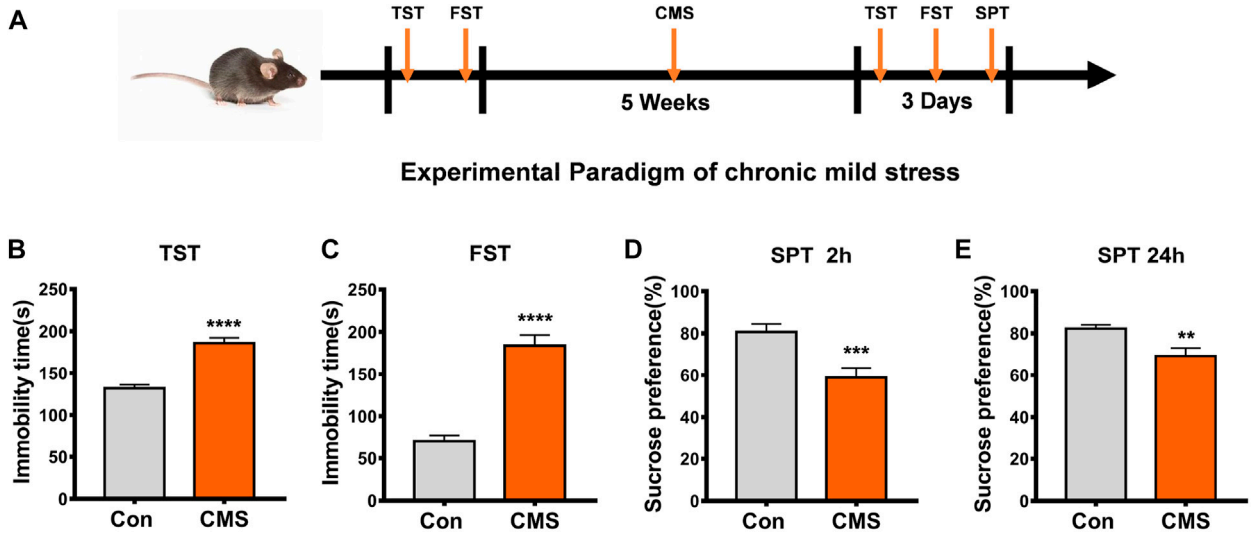

FIGURE 1
Establishment and identification of CMS model mice. **(A)** Experimental paradigm of the CMS model. **(B–E)** Behavioral tests were performed after the experimental procedure. **(B)** TST, $n = 29, 23$, $^{****}p < 0.0001$. **(C)** FST, $n = 40, 33$, $^{****}p < 0.0001$. **(D)** SPT at the second h, $n = 38, 37$, $^{***}p < 0.001$. **(E)** SPT at the 24th h, $n = 31, 33$, $^{**}p < 0.01$. All data are presented as the mean ± S.E.M. Unpaired two-tailed Student's t-test was used.

time PCR amplification. For data analysis, fold change was calculated using the ΔΔCt method according to the threshold cycle (Ct) value obtained from RT-PCR. The sequences of the primers used in this study are given in Supplementary Material.

RNA isolation and dot blot

Total RNA was extracted using RNA TRIzol and was reverse-transcribed using the Reverse Transcription Kit as per the manufacturer's protocol. During RNA extraction, DNase I, RNase-free pipettor tips, RNase-free centrifugal tubes, and other RNase-free plastic products were used, and RNA was dissolved in RNase-free water treated by DEPC. After the extraction, the RNA purity was tested using a NanoDrop 2000 spectrophotometer. For dot blot, a 20-μL system containing 2,000 ng RNA, 10 μL 2M NaOH (denature solution), and double distilled water was prepared. Then, the mix was allowed to stand at 4°C for 20 min. We spotted 2 μL of samples onto the nitrocellulose (NC) membrane and allowed the membrane to dry at RT. RNA was fixed to the membrane by incubating at 80°C for 30 min. Then, the NC membrane was blocked with 10% nonfat milk for 1 h at RT and incubated with 5hmC antibody overnight at 4°C. The immunoreactive signal was detected by ECL chemiluminescence reagent after 2-h incubation with secondary antibody at RT. Also, the dot signal was quantified by using ImageJ software.

Statistical analysis

All statistical analyses were performed using Prism 7.0 (GraphPad Software) and are expressed as means ± SEM. The differences with different treatments were determined by *t*-test or one-way ANOVA, followed by the Tukey's *post hoc* test, and were considered statistically significant at $p < 0.05$.

Results

RNA 5hmC modification and Tet2 expression increased in the hippocampus of CMS mice

Our previous results showed high levels of RNA 5hmC modification in the brainstem, hippocampus, and cerebellum of mice, and RNA 5hmC modification was downregulated in the MPTP-induced mouse model (Miao et al., 2016), but the changes of RNA 5hmC in depressed mice are still unclear. Therefore, by establishing a CMS mouse model (Figure 1), we detected the RNA 5hmC level in the hippocampus and prefrontal cortex, which are most sensitive to the neurotoxic effects of stress (McEwen et al., 2016). Dot blot results showed that the level of RNA 5hmC was increased in the hippocampus ($p < 0.01$, Figures 2A, B) and prefrontal cortex ($p < 0.05$, Figures 2C, D) of CMS mice compared with the control group. Because RNA 5hmC is relatively less distributed in the cortex (Miao et al., 2016), the hippocampal region was selected for further research.

Studies have shown that RNA 5hmC is derived from RNA 5mC and is mainly catalyzed by Tet proteins (Fu et al., 2014). Thus, we detected the expression of Tet proteins including Tet1, Tet2, and Tet3 in the hippocampus of CMS mice. Compared with the control group, the mRNA level ($p < 0.01$, Figure 2F) and protein level ($p < 0.01$, Figures 2J, K) of Tet2 were increased in the hippocampus of

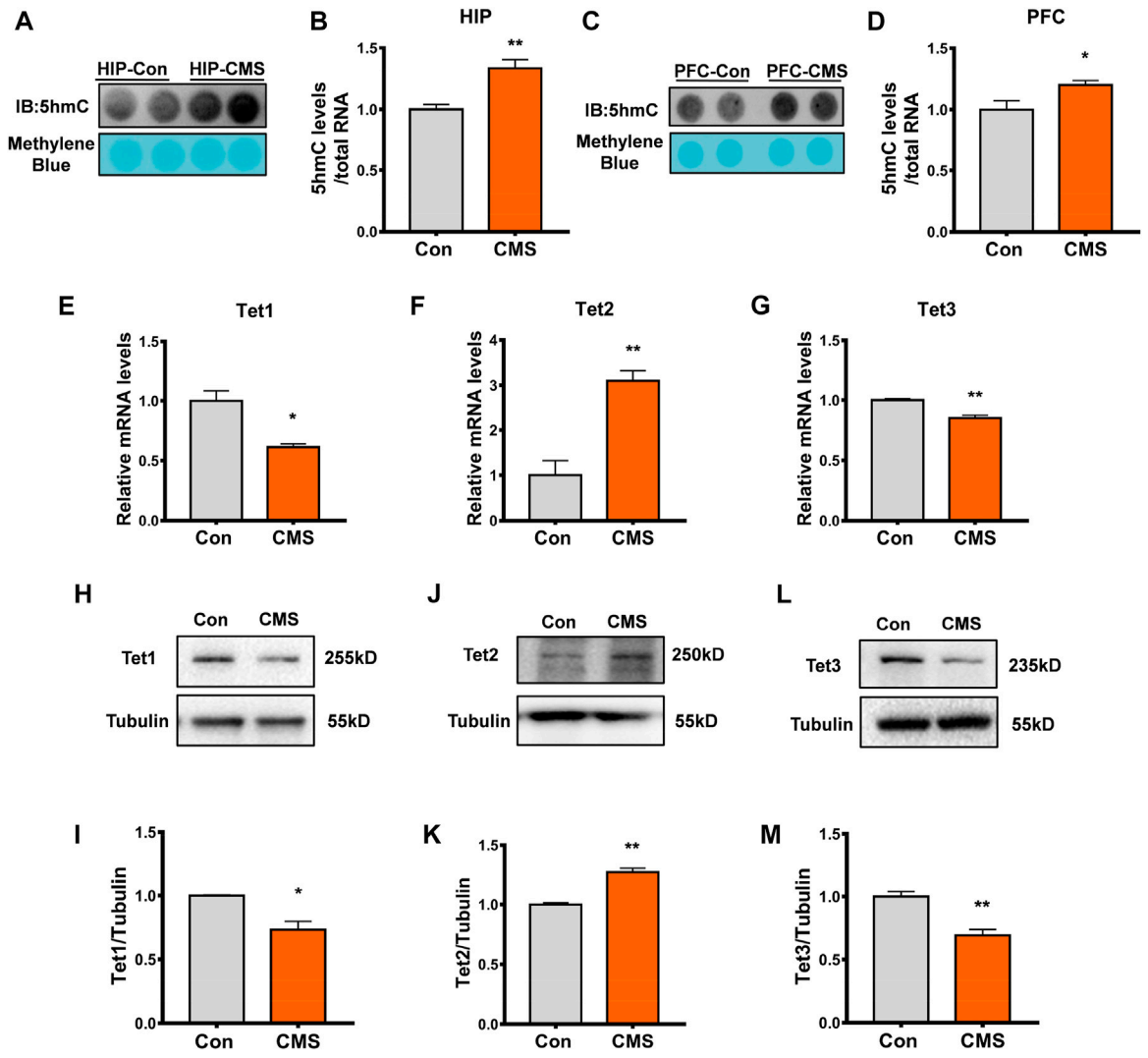

FIGURE 2
RNA 5hmC modification and Tet2 expression increased in the hippocampus of CMS mice. **(A,B)** Representative dot blot **(A)** and quantitative analysis **(B)** of RNA 5hmC abundance in the hippocampus (HIP) brain regions of control and CMS mice. $n = 4$, $^{**}p < 0.01$. **(C,D)** Representative dot blot **(C)** and quantitative analysis **(D)** of RNA 5hmC abundance in the brain regions of the prefrontal cortex (PFC) of control and CMS mice. $n = 4$, $^{*}p < 0.05$. **(E–G)** Quantitative RT-PCR of Tet1 **(E)**, Tet2 **(F)**, and Tet3 **(G)** mRNA levels in the hippocampus of control and CMS mice. $n = 3$, $^{*}p < 0.05$, $^{**}p < 0.01$. **(H, I)** Representative Western blot **(H)** and quantitative analysis **(I)** of Tet1 protein levels in the mouse hippocampus. $n = 3$, $^{*}p < 0.05$. **(J, K)** Representative Western blot **(J)** and quantitative analysis **(K)** of Tet2 protein levels in the mouse hippocampus. $n = 3$, $^{**}p < 0.01$. **(L,M)** Representative Western blot **(L)** and quantitative analysis **(M)** of Tet3 protein levels in the mouse hippocampus. $n = 3$, $^{**}p < 0.01$. Quantified data are normalized to the control group, whose value is equal to 1. All data are presented as the mean ± S.E.M. Unpaired two-tailed Student's t-test was used.

CMS mice, while the level of Tet1/Tet3 mRNA ($p < 0.05$, Figures 2E, G), Tet1 protein ($p < 0.05$, Figures 2H, I), and Tet3 protein ($p < 0.01$, Figures 2L, M) significantly decreased. Therefore, this indicated that the increase in RNA 5hmC modification in the hippocampus of CMS mice results from the increase in Tet2, rather than Tet1 and Tet3.

Tet2 regulated RNA 5hmC levels

To further validate the role of Tet2 in the oxidation of RNA 5mC to 5hmC, we examined RNA 5hmC levels in N2a cells by either Tet2 knockdown or overexpression. Tet2 knockdown markedly decreased Tet2 mRNA and protein expression in N2a cells ($p < 0.0001$, 0.01, Figures 3A–C). At the same time, RNA 5hmC level was significantly reduced in Tet2 knockdown N2a cells ($p < 0.01$, Figures 3D, E). Due to high molecular weight of full-length Tet2, and previous studies having confirmed that the catalytic domain of Tet2 (Tet2 CD) also has the 5mC hydroxymethylation modification function as the full-length Tet2 (Fu et al., 2014), we overexpressed Tet2 CD in N2a cells ($p < 0.0001$, Figures 3F, G). The RNA 5hmC level significantly increased after Tet2 CD overexpression ($p < 0.05$, Figures 3H, I).

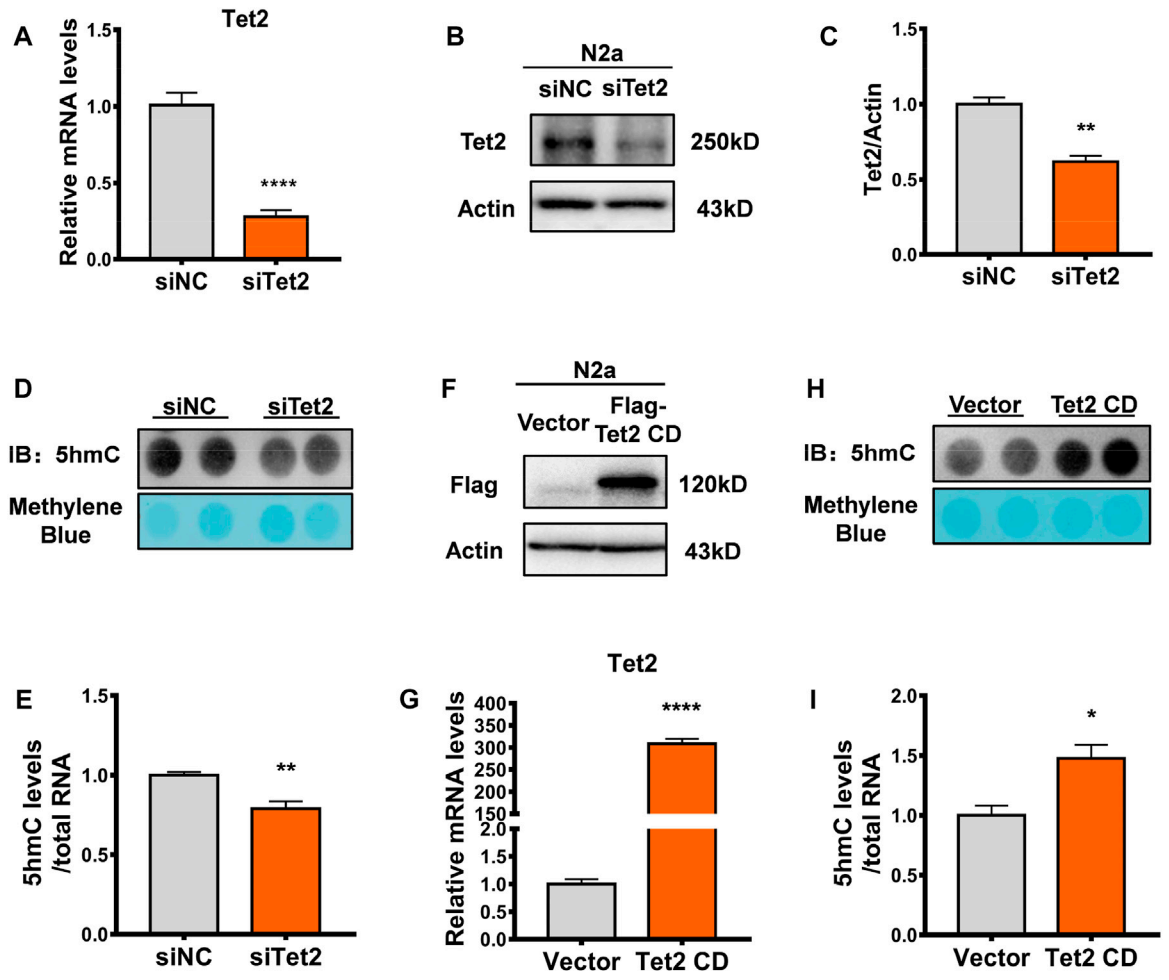

FIGURE 3
Tet2 regulated RNA 5hmC levels. **(A)** Quantitative RT-PCR of Tet2 mRNA levels in Tet2 knockdown N2a cells. $n = 5$, $^{****}p < 0.0001$. **(B,C)** Representative Western blot **(B)** and quantitative analysis **(C)** in Tet2 knockdown N2a cells. $n = 3$, $^{**}p < 0.01$. **(D,E)** Dot blot quantification of RNA 5hmC abundance before and after Tet2 knockdown. $n = 4$, $^{**}p < 0.01$. **(F)** Representative images of immunoblots of the transfection efficiency of Tet2 CD in the N2a cells. **(G)** Quantitative analyses of Tet2 mRNA levels before and after overexpression of Tet2 CD. $n = 3$, $^{****}p < 0.0001$. **(H,I)** Dot blot quantification of RNA 5hmC abundance before and after Tet2 CD overexpression. $n = 3$, $^{*}p < 0.05$. Quantified data are normalized to the control group, whose value is equal to 1. All data are presented as the mean ± S.E.M. Unpaired two-tailed Student's t-test was used.

The expression of Upf1, a Tet2-interacting protein, increased in the hippocampus of depressed mice

To further investigate the function of Tet2-mediated RNA 5hmC modification, we analyzed the mass spectrometry data of the potential interacting proteins of Tet2 (Guallar et al., 2018) and found that a large portion of the proteins interacting with Tet2 were involved in the mRNA processing pathway (Figure 4A). Among the Tet2-interacting protein candidates, Upf1 was found to potentially interact with Tet2. A previous study showed the Upf1 protein has binding sites that significantly overlap with 5mC sites in mouse embryonic stem cells and brain (Amort et al., 2017). This suggests that 5mC may contribute to the binding and functionality of Upf1. To explore the role of Upf1 in Tet2-mediated RNA hydroxymethylation modification, we co-transfected Flag-Tet2 plasmids with HA-Upf1 plasmids in HEK293T cells and found that HA-Upf1 could be precipitated by Flag-Tet2 (Figure 4B). The interaction was also confirmed with endogenous Tet2 and Upf1 in N2a cells (Figure 4C). In addition, we also observed the colocalization of Tet2 and Upf1 in the cytoplasm of HEK293T cells with Flag-Tet2 and HA-Upf1 overexpression (Figure 4D). Subsequently, we detected the expression of Upf1 in the hippocampus of CMS mice. Our results showed that the expression of Upf1 significantly increased in the hippocampus of CMS mice ($p < 0.05$, 0.001, Figures 4E–G).

Downstream genes of the Tet2–Upf1 complex reduced in the hippocampus of stressed mice

In order to investigate the influence of Tet2 and Upf1 on RNA 5hmC modification, we screened candidate downstream target

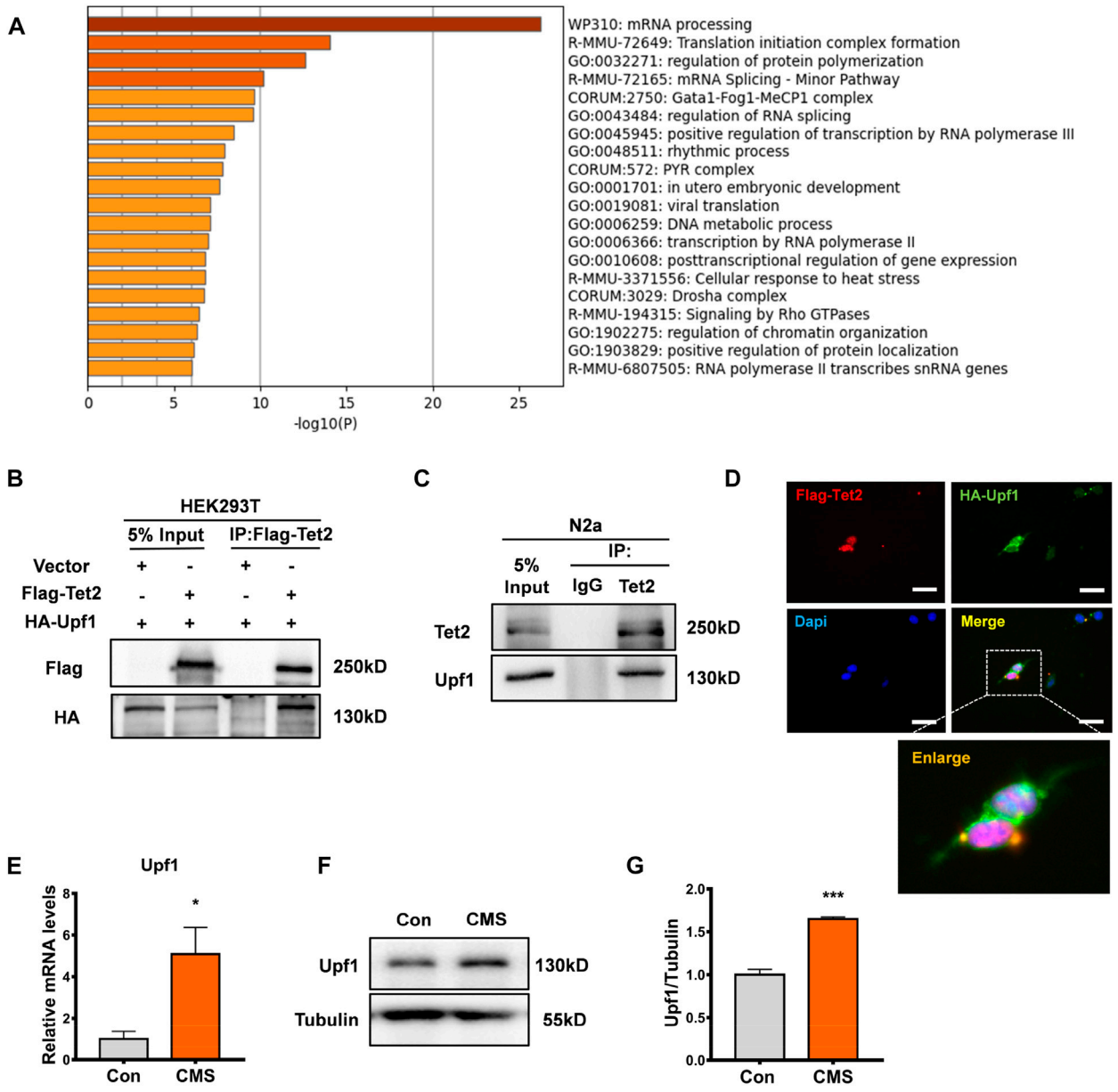

FIGURE 4
Tet2-interacting protein Upf1 increased in the hippocampus of stressed mice. **(A)** Pathway map of Tet2-interacting proteins constructed by GO gene function analysis. **(B)** Exogenous Co-IP experiment was performed using HEK293T cells. Flag-Tet2 and HA-Upf1 were transfected into HEK293T cells as indicated, and the cell lysates were subsequently immunoprecipitated using anti-Flag. The immunoprecipitates were examined by Western blotting with anti-HA antibody. Input represented 10% of cell lysates used in the Co-IP experiment. **(C)** Endogenous Co-IP experiment was performed using N2a cells. Cell lysates from cells were incubated with anti-Tet2 antibody. For Western blotting of immunoprecipitates, anti-Upf1 antibody was used. **(D)** HEK293T cells were transfected with Flag-Tet2 plasmids and HA-Upf1 plasmids and stained for Flag (red), HA (green), and DAPI (blue). Representative immunofluorescence images revealed the colocalization of Flag-Tet2 and HA-Upf1 in the cytoplasm of HEK293T cells. Scale bar, 25 μm. **(E)** RT-PCR detection of Upf1 mRNA levels in the hippocampus of control and CMS mice. $n = 3$, $^{*}p < 0.05$. **(F,G)** Western blot detection of Upf1 protein levels in the hippocampus of control and CMS mice. $n = 3$, $^{***}p < 0.001$. Quantified data are normalized to the control group, whose value is equal to 1. All data are presented as the mean ± S.E.M. Unpaired two-tailed Student's t-test was used.

genes of the interaction of Tet2 and Upf1 from the data of Tet2 RNA co-immunoprecipitation (Tet2-RIP) (Lan et al., 2020) and Upf1 RNA co-immunoprecipitation (Upf1-CLIP) (Hurt et al., 2013). GO analysis and cluster analysis of the overlapped genes (Supplementary Table S2) revealed the existence of stress-related pathways such as axonal guidance and neural development (Figure 5A). The potential target genes Ntn1, Unc5b, Ptpn11, and Src are included in this pathway (Figure 5B) and participate in the process of psychiatric disorders such as depression (Zeng et al., 2017; Perrino et al., 2018; Meng et al., 2022). The changes of these target genes under CMS were detected by RT-PCR, and the results showed that the expression of these target genes at the mRNA level was reduced after stress compared with the control group ($p < 0.05$, 0.01,

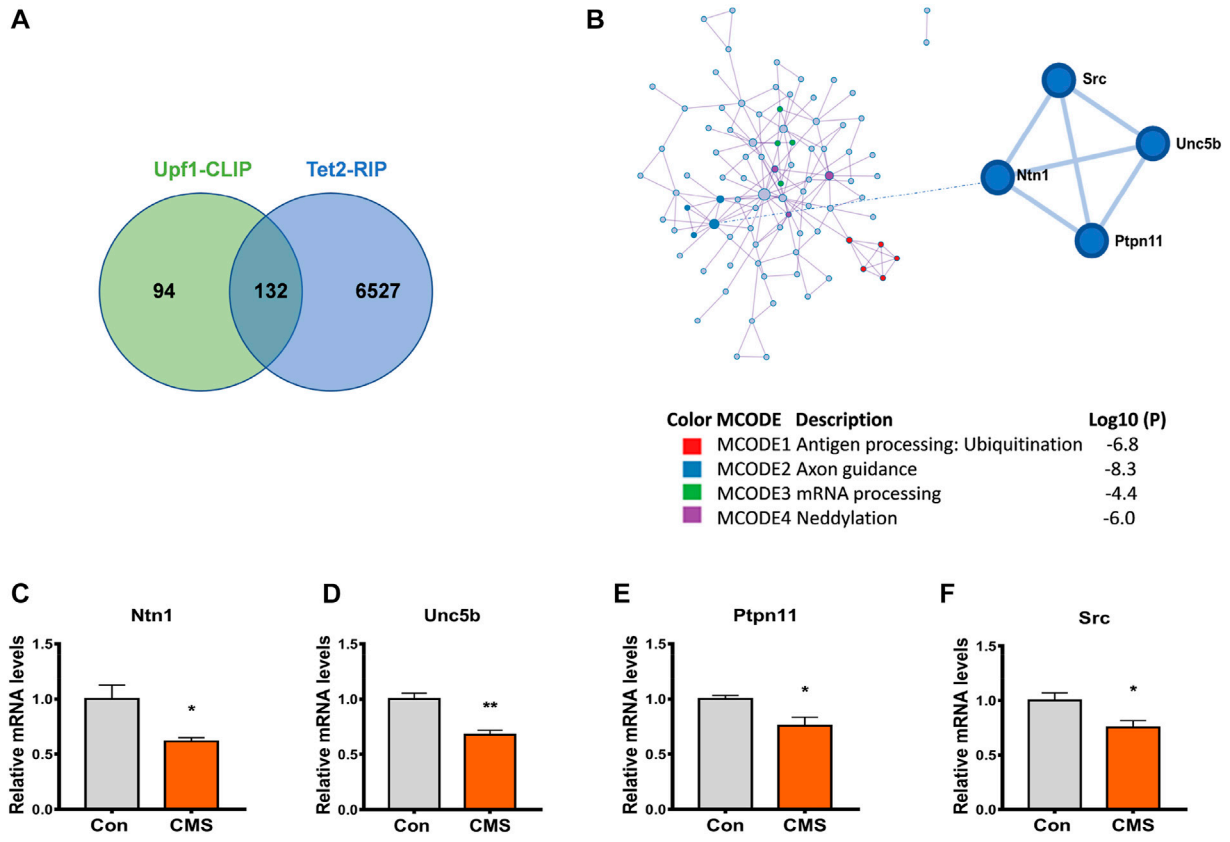

FIGURE 5
Downstream genes of the Tet2–Upf1 complex reduced in the hippocampus of stressed mice. **(A)** Screening of intersection target genes using Tet2-RIP and Upf1-CLIP data. **(B)** Screening of stress-related intersection target genes. **(C–F)** RT-PCR detection of the mRNA levels of target genes Ntn1 (*$p < 0.05$) **(C)**, Unc5b (**$p < 0.01$) **(D)**, Ptpn11 (*$p < 0.05$) **(E)**, and Src(*$p < 0.05$) **(F)** in the hippocampus of control mice and CMS mice. $n = 5$. Quantified data are normalized to the control group, whose value is equal to 1. All data are presented as the mean ± S.E.M. Unpaired two-tailed Student's t-test was used.

Figures 5C–F). Therefore, we selected Ntn1, Unc5b, Ptpn11, and Src as the candidate genes in this study to explore the effect of the Tet2–Upf1 complex to mRNA stability due to the RNA helicase property of Upf1.

The target gene Unc5b was significantly altered after Tet2 or Upf1 knockdown

To investigate the mechanism by which the Tet2–Upf1 complex affects downstream target genes, we knocked down Tet2 and Upf1 in N2a cells, respectively ($p < 0.05$, 0.001, Figures 6A–D), and analyzed the expression of target genes Ntn1, Unc5b, Ptpn11, and Src. The results showed that Unc5b had the most significant change in mRNA level after knockdown of Tet2 ($p < 0.01$, Figures 6E–H) and Upf1 ($p < 0.05$, 0.01, Figures 6I–L). Unc5b is involved in many biological processes, including neural development (Boyé et al., 2022), angiogenesis (Potente et al., 2011), tumor processes (Kong et al., 2016), and the occurrence of post-stress depression (Zeng et al., 2017), so we next examined Unc5b RNA 5hmC modification as a representative target gene of the Tet2–Upf1 complex.

Tet2–Upf1 complex affects the stability of Unc5b mRNA

Previous studies have demonstrated that RNA 5hmC modification regulates mRNA stability at the post-transcriptional level (Lan et al., 2020). In order to investigate whether Tet2 regulates the stability of Unc5b mRNA through RNA 5hmC modification, we treated the Tet2 knockdown N2a cells with actinomycin D (ActD) to inhibit transcription and examined the Unc5b mRNA stability. The results showed that Tet2 knockdown increased the stability of Unc5b mRNA ($p < 0.05$, Figure 7A). At the same time, we examined the Unc5b mRNA level after overexpression of Tet2 CD, and the results showed that the expression level of Unc5b mRNA decreased after overexpression of Tet2 CD ($p < 0.01$, Figure 7B), and the stability of Unc5b mRNA decreased after Tet2 CD overexpression ($p < 0.05$, Figure 7C). A previous study showed that Upf1 is a major protein involved in regulating mRNA stability [38]. To verify whether Upf1 is also involved in the regulation of Unc5b mRNA stability, we knocked down Upf1 in N2a cells. Upf1 knockdown increased the stability of Unc5b mRNA ($p < 0.05$, Figure 7D). Similarly, the mRNA level of Unc5b decreased after

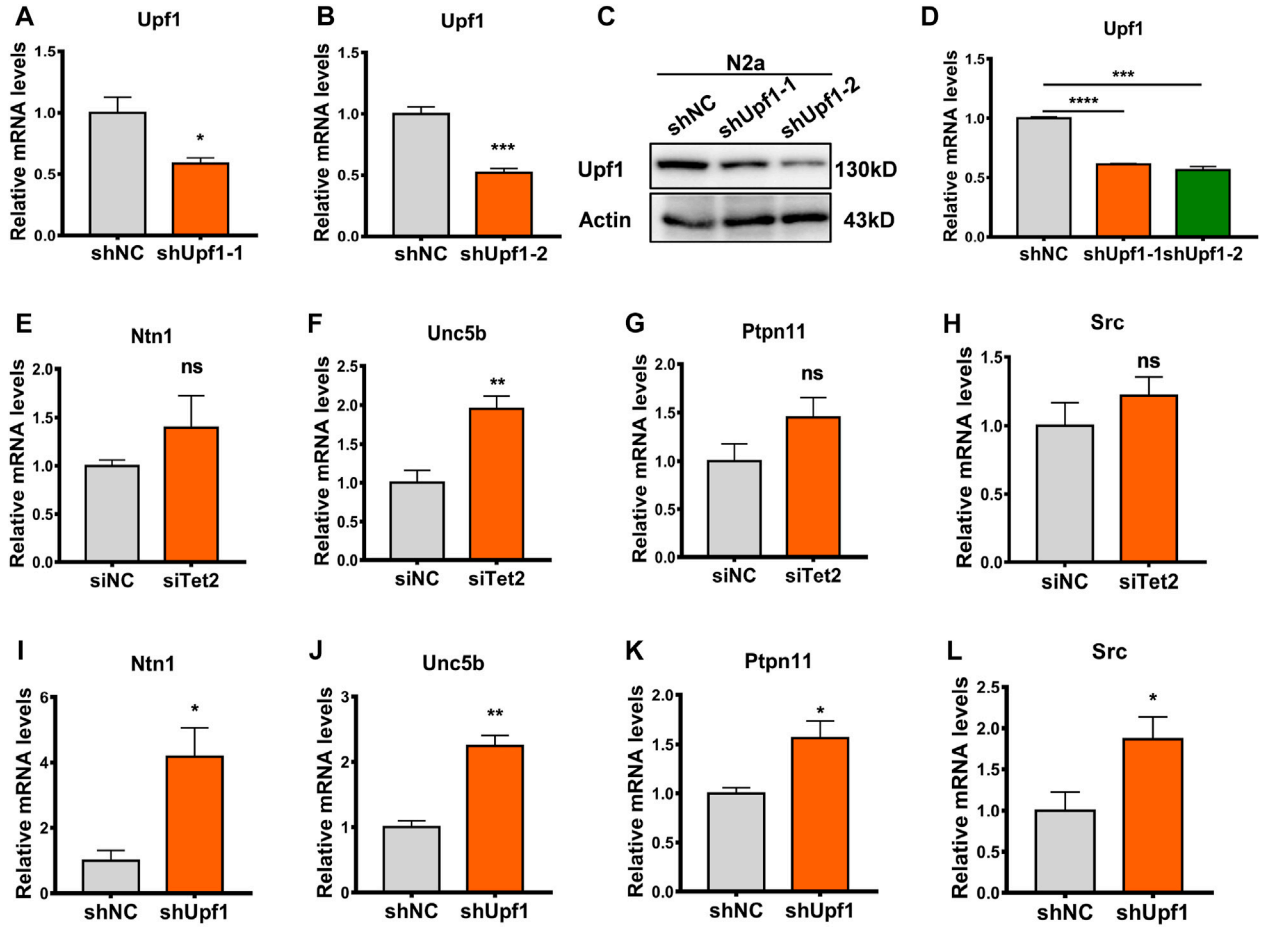

FIGURE 6
Unc5b reduced after knockdown of Tet2 or Upf1. **(A)** RT-PCR detection of the mRNA level of knockdown Upf1 in the constructed shUpf1-1 plasmid. $n = 3$, $^{*}p < 0.05$. **(B)** RT-PCR detection of the mRNA level of knockdown Upf1 in the constructed shUpf1-2 plasmid. $n = 4$, $^{***}p < 0.001$. **(C,D)** Western blot detection of the protein level of knockdown Upf1 in the constructed shUpf1-2 plasmid. $n = 3$, $^{***}p < 0.001$, $^{****}p < 0.0001$. **(E–H)** RT-PCR detection of mRNA levels of Ntn1 **(E)**, Unc5b **(F)**, Ptpn11 **(G)**, and Src **(H)** before and after knockdown of Tet2. $n = 4–6$. ns: no significant, $^{**}p < 0.01$. **(I–L)** RT-PCR detection of mRNA levels of Ntn1 **(I)**, Unc5b **(J)**, Ptpn11 **(K)**, and Src **(L)** before and after knockdown of Upf1. $n = 3–6$. $^{*}p < 0.05$, $^{**}p < 0.01$. Quantified data are normalized to the control group, whose value is equal to 1. All data are presented as the mean ± S.E.M. Unpaired two-tailed Student's t-test was used.

overexpression of Upf1 ($p < 0.05$, Figures 7E, F) and the stability of Unc5b mRNA was decreased after overexpression of Upf1 ($p < 0.05$, Figure 7G). To further verify that Tet2 and Upf1 participate in the regulation of Unc5b mRNA stability through a complex, we co-transfected HA-Upf1 plasmids with siTet2 in N2a cells and detected the Unc5b mRNA level. Our results showed that Upf1 overexpression decreased the Unc5b mRNA expression and stability, while Tet2 knockdown partially rescued the reduction of Unc5b mRNA expression and stability ($p < 0.01$, Figures 7H, I). Taken together, our results suggested that Tet2 and Upf1 have a synergistic effect on Unc5b mRNA stability.

Discussion

Our previous findings showed that Tet2 protein expression was increased in the mouse hippocampus under chronic mild stress conditions and accumulated in the cytoplasm (Zhang et al., 2021). Some studies have reported that RNA 5hmC modification is highly expressed in the mouse hippocampus and other brain tissues (Miao et al., 2016), but the changes in RNA 5hmC after stress are not clear. In this study, we detected the RNA 5hmC level in the hippocampus of CMS model mice, and the results showed that the level of RNA 5hmC increased after chronic mild stress. Among the Tet family, only Tet2 showed an increase in mRNA and protein levels, while Tet1 and Tet3 decreased at both mRNA and protein levels, suggesting that the increase of cytoplasmic Tet2 may be an important contributor for the increased level of RNA 5hmC. Therefore, in this study, we illustrated that cytoplasmic Tet2 participates in the regulation of mRNA expression through RNA 5hmC modification under stress conditions.

Among the epigenetic modifications, the most widely studied are DNA methylation modification and hydroxymethylation modification (Moore et al., 2013; Zhang et al., 2014). With the advancement of RNA modification identification methods, researchers found that RNA, including tRNA, rRNA, and mRNA,

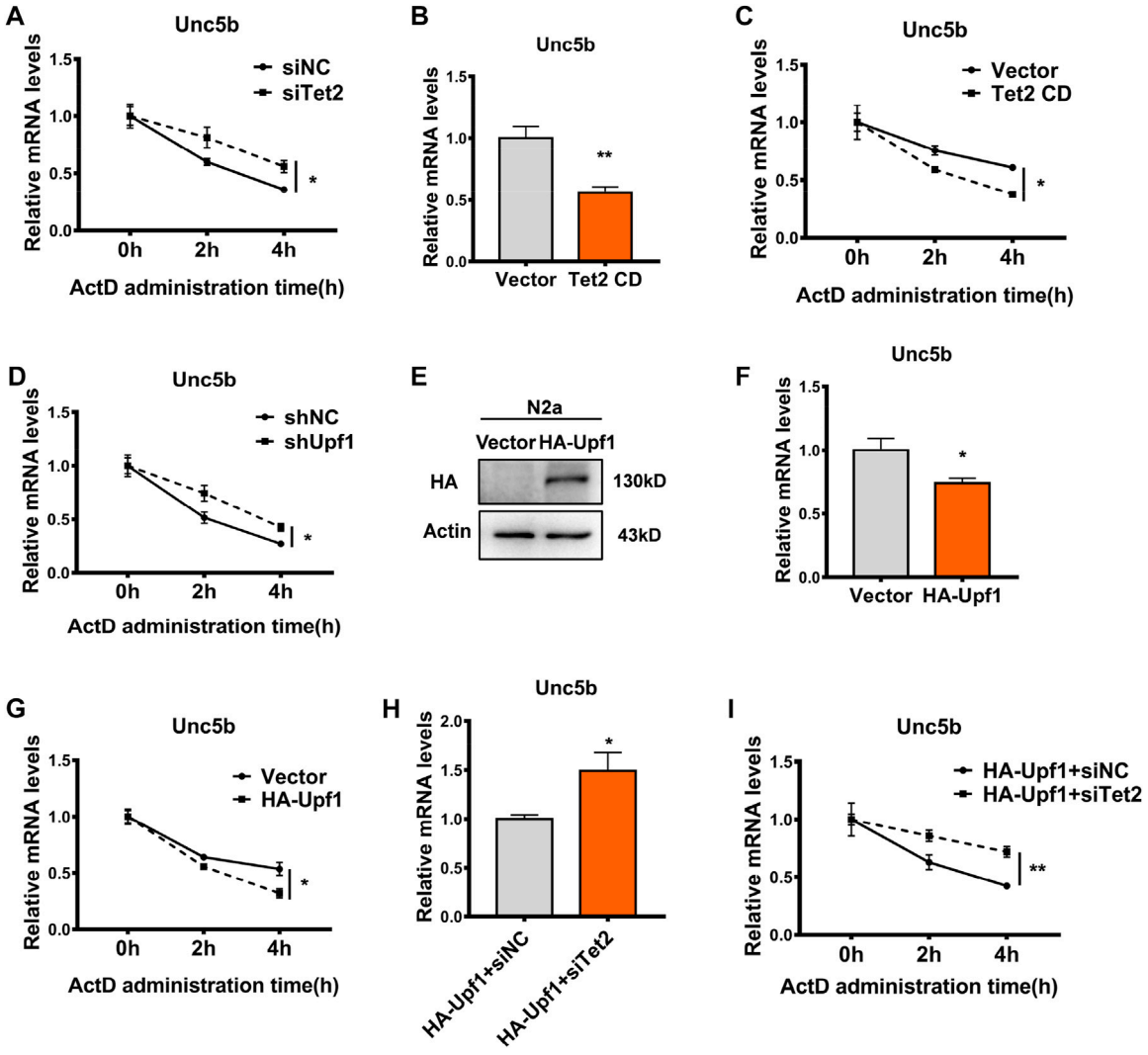

FIGURE 7
Tet2–Upf1 complex regulated Unc5b mRNA stability. **(A)** Stability of Unc5b mRNA before and after knockdown of Tet2 was detected by RT-PCR. n = 6, *p < 0.05. **(B)** mRNA levels of Unc5b before and after overexpression of Tet2 CD were detected by RT-PCR. n = 4, **p < 0.01. **(C)** Stability of Unc5b mRNA before and after overexpression of Tet2 CD was detected by RT-PCR. n = 6, *p < 0.05. **(D)** RT-PCR to detect the stability of Unc5b mRNA before and after knockdown of Upf1. n = 3, *p < 0.05. **(E)** Western blot to detect the transfection efficiency of HA-Upf1 in N2a cells. **(F)** RT-PCR detection of Unc5b mRNA levels before and after Upf1 overexpression. n = 4, *p < 0.05. **(G)** RT-PCR detection of Unc5b mRNA stability before and after Upf1 overexpression. n = 6, *p < 0.05. **(H)** Unc5b mRNA levels before and after Tet2 knockdown after Upf1 overexpression were detected by RT-PCR. n = 4, *p < 0.05. **(I)** mRNA stability of Unc5b before and after Tet2 knockdown after Upf1 overexpression was detected by RT-PCR. n = 6, **p < 0.01.3.2. Quantified data are normalized to the control group, whose value is equal to 1. All data are presented as the mean ± S.E.M. Unpaired two-tailed Student's t-test was used.

all have RNA methylation modification and hydroxymethylation modification, namely, RNA 5mC and RNA 5hmC (Machnicka et al., 2013; Boccaletto et al., 2018). Studies have reported that RNA 5hmC modifications mainly exist in mRNA (Xu et al., 2016) and are involved in regulating mRNA stability (Lan et al., 2020). Tet enzymes, as the dioxygenase of DNA 5 mC (Tsiouplis et al., 2020), have also been found to act as the hydroxymethylase of RNA 5mC in recent years (Fu et al., 2014; Guallar et al., 2018).

To further confirm the modification of RNA 5hmC by Tet2, we performed Tet2 knockdown in N2a cells using siTet2. The results were consistent with expectations, and the expression of RNA 5hmC was reduced after knockdown of Tet2 and increased by overexpression of Tet2 CD. Taken together, we confirmed that Tet2 is the dominant protein responsible for the increased modification of RNA 5hmC under chronic stress. Studies reported that RNA-modifying enzymes and RNA-binding proteins cooperate in the regulation of transcripts (Boo and Kim, 2020). Here, we found a potential interaction between Tet2 and the RNA-binding protein Upf1 by performing GO analysis on the mass spectrometry data of Tet2 and confirmed their interaction by CO-IP. NMD, which is arguably the best-characterized translation-dependent regulatory pathway in

mammals, selectively degrades mRNAs as a means of post-transcriptional gene control (Hug et al., 2016). This control can be used for ensuring the quality of gene expression. Alternatively, such genetic control can facilitate the adaptation of cells to changes in their environment. The key to NMD, irrespective of its purpose, is the ATP-dependent RNA helicase Upf1, without which NMD fails to occur (Brogna et al., 2016; Hug et al., 2016). Subsequently, we found that Tet2 and Upf1 are involved in the regulation of mRNA stability in axonal guidance and neurodevelopmental pathways through complex-mediated RNA 5hmC modification.

In conclusion, we found that increased expression of Tet2 after chronic stress in the cytoplasm led to an increase in RNA 5hmC modification. We, therefore, focused on the role and mechanism of 5hmC modification of RNA under chronic stress. In addition, Tet2 and its new interacting protein Upf1 can participate in the regulation of stress-related mRNA stability in the form of a complex, and the common target genes of Tet2 and Upf1 are mainly involved in axon guidance-related genes, also indicating that neuronal development plays a critical role in chronic stress-related diseases such as depression. However, there are still some gaps in our study, such as where the increase of Tet2 and RNA 5hmC in nerve cells mainly occurs after chronic stress, and other roles of Tet2 on RNA 5hmC modification in chronic stress, which are also the focus of our future research.

Data availability statement

The original contributions presented in the study are included in the article/Supplementary Material; further inquiries can be directed to the corresponding authors.

Ethics statement

The animal study was reviewed and approved by the Institutional Animal Care and Use Committee of Soochow University.

Author contributions

XX, BW, and WG designed this study; RY and MX performed the experiments and analyzed the data. WW, AK, MZ, and ZM assisted in the experiments. RY and MX wrote the first draft; XX and BW revised the paper. All authors have read and agreed to the published version of the manuscript.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

Supplementary material

The Supplementary Material for this article can be found online at: https://www.frontiersin.org/articles/10.3389/fgene.2023.1158954/full#supplementary-material

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A review on the role of LINC00511 in cancer

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Long Intergenic Non-Protein Coding RNA 511 (LINC00511) is an RNA gene being mostly associated with lung cancer. Further assessments have shown dysregulation of this lncRNA in a variety of cancers. LINC00511 has interactions with hsa-miR-29b-3p, hsa-miR-765, hsa-mir-150, miR-1231, TFAP2A-AS2, hsa-miR-185-3p, hsa-miR-29b-1-5p, hsa-miR-29c-3p, RAD51-AS1 and EZH2. A number of transcription factors have been identified that regulate expression of LINC00511. The current narrative review summarizes the role of LINC00511 in different cancers with an especial focus on its prognostic impact in human cancers.

KEYWORDS

LINC00511, cancer, biomarker, expression, diagnostic

Introduction

Long non-coding RNAs (lncRNAs) are widely expressed transcripts with essential roles in gene regulation. Based on the results of the Human GENCODE project, the number of lncRNA genes in the human genome is estimated to surpass 16,000 (Fang et al., 2018). These transcripts embrace lncRNAs transcribed by RNA polymerase II, gene-overlapping antisense transcripts as well as lncRNAs from intergenic regions (lincRNAs) (Ghafouri-Fard et al., 2020; Ghafouri-Fard et al., 2021; Statello et al., 2021). Typically, lncRNAs have a 7-methyl guanosine cap at the 5′end and a polyadenylated tail at the 3′ends. Moreover, lncRNAs are spliced in a similar manner to mRNAs (Statello et al., 2021). However, several RNA polymerase II-transcribed lncRNAs are incompetently processed and are held in the nuclear compartment (Statello et al., 2021). LncRNAs interact with DNA, RNA and proteins. Through these interactions, lncRNAs influence chromatin structure and function, affect the assembly of nuclear bodies, change the stability and expression of mRNAs within the cytoplasm and regulate signaling pathways (Statello et al., 2021; Hussen et al., 2022). In comparison with mRNA promoters, lincRNA promoters have been found to be devoid of transcription factor binding sites, but having binding sites for a number of specific factors such as GATA and FOS (Melé et al., 2017).

Long Intergenic Non-Protein Coding RNA 511 (LINC00511) is an RNA gene being mostly associated with lung cancer. This lncRNA is encoded on chr17:72,290,091-72,640,472 (GRCh38/hg38), minus strand. Notably, more than 100 alternatively splice variants have been recognized for LINC00511. Except for two variants with retained introns (LINC00511-279 with 3766 bp and LINC00511-278 with 508 bp), other are affiliated with lncRNA group of transcripts (https://asia.ensembl.org/Homo_sapiens/Gene/Summary?db=core;g=ENSG00000227036; r=17:72290091-

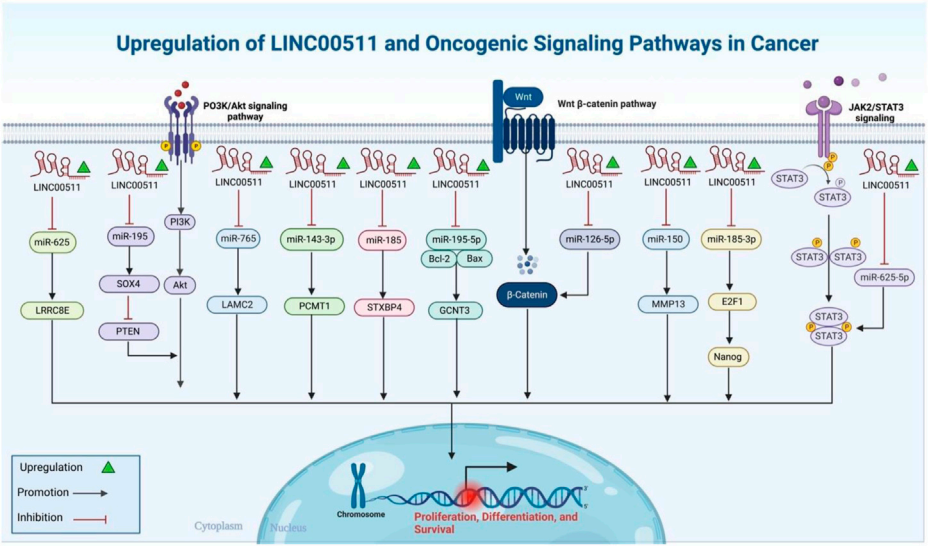

FIGURE 1
A graphical illustration of the oncogenic role that LINC00511 performs in the environment of the many types of cancer.

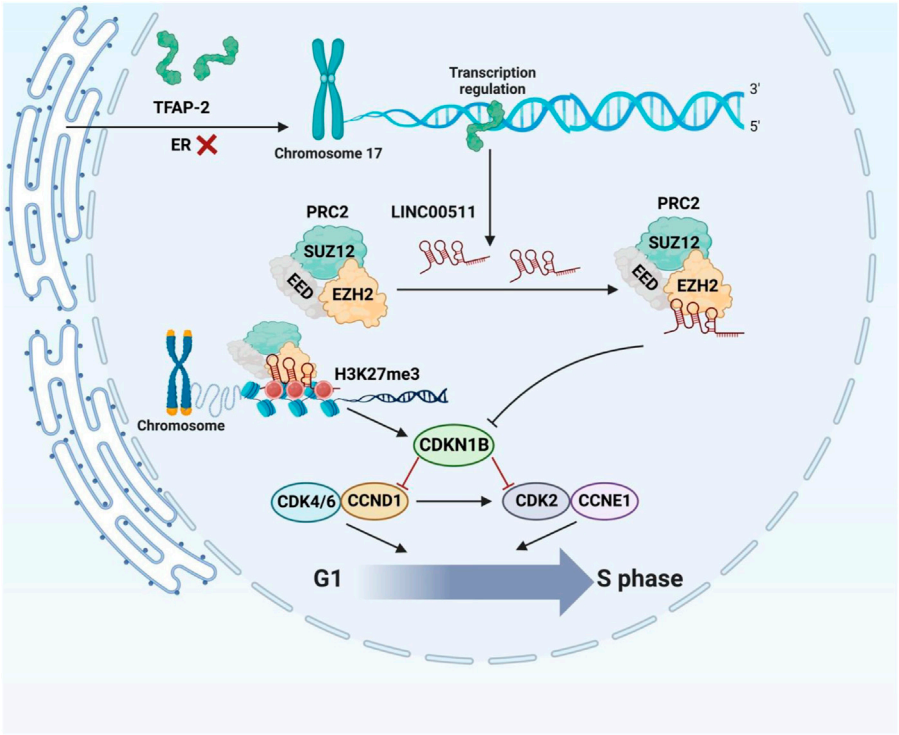

FIGURE 2
The carcinogenic role of LINC00511 in ER-negative tumorigenesis. ER insufficiency enhanced TFAP-2 activity at particular promoter regions, promoting LINC00511 expression. By interacting with EZH2 to attract PRC2 to regulate histone methylation, the ER-negative-associated LINC00511 repressed the expression of CDKN1B, assisting in the G1/S transition to maintain cellular growth.

72640472). This lncRNA has important roles in the development of cancers and can be used as a possible diagnostic and prognostic marker in cancer. Abnormal expression of LINC00511 in a wide array of malignancies potentiates it as a target for therapeutic interventions. Based on DIANA-LncBase database (https://diana.e-ce.uth.gr/lncbasev3) (Karagkouni et al., 2020), expression of LINC00511 in various tissues and cell types have been investigated. With medium and high TPM levels and in *homosapiens*, the highest level of expression

TABLE 1 Role of LINC00511 in breast cancer.

Cancer type	Expression/ Role	Samples/Assessed cell lines	Pathways	Targets/ Regulators	Function	Ref
Breast Cancer (BC)	Upregulated/ Oncogene	39 cases of breast cancer/ MCF-10A, MDA-MB-468, MDA-MB-231, MDA-MB-453, MCF-7	miR-185-3p/E2F1/ Nanog axis	miR-185-3p/E2F1	LINC00511/miR-185-3p/E2F1/ Nanog axis has a role in breast cancer stemness and malignancy	Lu et al. (2018)
	Upregulated/ Oncogene	10 TNTPs/MDA-MB-231, MCF-7	LINC00511/miR-150/MMP13 axis	miR-150/MMP13	The LINC00511/miR-150/ MMP13 axis could represent a novel treatment strategy for sufferers with breast cancer, because it is a breast cancer promoter	Shi et al. (2021)
	Upregulated/ Oncogene	MDA-MB-231, MCF-7, T47D, MDA-MB-468, MCF-10a	-	MET, E2F2, TGFA, and WNT10A	Patients with breast cancer who express LINC00511 more than usual have a bad outcome. Breast cancer progression is facilitated by LINC00511	Liu et al. (2021a)
	Upregulated/ Oncogene	21 TNTPs/MDA-MB-231, MCF-7, Hs-578T, T47D, MCF-10A	LINC00511/miR-29c/CDK6 axis	miR-29c/CDK6	Through controlling the miR-29c/ CDK6 axis, LINC00511 lowering increased paclitaxel cytotoxicity in BC cells. A viable BC therapeutic option might be LINC005111	Zhu et al. (2019)
	Upregulated/ Oncogene	25 patients and 25 control samples	LINC00511/miR-185-3p axis	miR-301a-3p	A more accurate diagnosis of BC may be made using serum LINC00511 and miR-301a-3p as prospective molecular indicators	Mahmoud et al. (2021)
	Upregulated/ Oncogene	MDA-MB-231, MDA-MB-436	LINC00511/miR-185/STXBP4 axis	miR-185/STXBP4	Inhibition of LINC00511 reduces It has ability to bind competitively to miR-185, which boosts STXBP4 production and enhances radiation responsiveness in BC. A prospective treatment approach for boosting the prognosis of BC is the LINC00511/miR-185/STXBP4 axis	Liu et al. (2019)
	Upregulated/ Oncogene	-	Apoptosis pathway	-	Through the suppression of antiapoptotic genes, LINC00511 deletion procedures using CRISPR/Cas9 improved the apoptosis of breast cancer cells	Azadbakht et al. (2022)
	Upregulated/ Oncogene	MCF7, UACC-812, MDA-MB-231	-	EZH2 and CDKN1B	In ER-negative breast cancer, lncRNAs have a role in controlling the network of cell cycle regulation, and this has led to speculation that LINC00511 may be used as an anticancer treatment	Zhang et al. (2019)
	Upregulated/ Oncogene	15 TNTPs	LINC00511/hsa-miR-573/GSDMC axis	miR-573/GSDMC	The most probable ncRNA-related mechanisms that drive GSDMC in BRCA are thought to be the LINC00511/hsa-miR-573 axis	Sun et al. (2022)
	Upregulated/ Oncogene	7 TNTPs (HER-2-enriched)	-	-	A putative chemical indicator and viable treatment option for breast cancer of the HER-2-enriched subgroup is LINC00511	Yang et al. (2016)

of LINC00511 is in prostate tissue and PC3 cell type along with cancer/ malignant category.

The current narrative review summarizes the role of LINC00511 in different cancers with an especial focus on its prognostic impact in human cancers.

LINC00511 in cancers

Several studies have reported dysregulation (mainly upregulation) of LINC00511 in different cancers. These studies have also identified miRNAs that are sponged by LINC00511.

TABLE 2 Role of LINC00511 in gastric cancer.

Cancer type	Expression/ Role	Samples/Assessed cell lines	Pathways	Targets/ Regulators	Function	Ref
Gastric Cancer (GC)	Upregulated/ Oncogene	AGS, HGC-27, ACP01, SNU-1, Het-1A	PI3K/AKT pathway	miR-195-5p/SOX4	After epigenetically suppressing PTEN to activate the PI3K/AKT pathway by engaging EZH2, SOX4-induced LINC00511 stimulated SOX4 via ceRNA pattern, promoting GC cell proliferation, migration and stemness while preventing GC cell death	Wang et al. (2021)
	Upregulated/ Oncogene	MKN-45, BGC-823, HGC-27, MGC-803, GES-1	LINC00511/miR-124-3p/PDK4 axis	miR-124-3p/ PDK4	By functioning as a ceRNA to control the miR-124-3p/PDK4 axis, which could be a viable therapeutic option for GC, LINC00511 encourages the tumor cell growth	Sun et al. (2020a)
	Upregulated/ Oncogene	25 patients with gastric cancer/ GES-1, AGS, SGC7901, BGC823, MKN45, MGC803	MAPK signaling pathway	miR-515-5p	By influencing miR-515-5p, LINC00511 can stimulate the expansion of tumor cells, suggesting that it might be a viable option for the creation of anti-cancer medications	Wang et al. (2020a)
	Upregulated/ Oncogene	35 TNTPs/GES1, GC27, BGC823, MGC803, SGC7901	LINC00511/miR-625-5p/NFIX axis	miR-625-5p/NFIX	By targeting NFIX in GC cells, LINC00511 is a tumor activator to sponge miR-625-5p. Elimination of this lncRNA might be viewed as a treatment option for GC therapy	Chen et al. (2019a)
	Upregulated/ Oncogene	80 patients with GC/GES-1, MGC, HGC, MKN25, MKN28	LINC00511/miR-124-3p/EZH2 pathway	miR-124-3p/ EZH2 pathway	In sufferers with GC, LINC00511 is linked to a poor overall survival. It was predicted that LINC00511 would be a suitable target for treating human GC since it has a function in encouraging the cancerous cells growth	Huang et al. (2020)
	Upregulated/ Oncogene	50 TNTPs/MKN28, BGC-823, MKN-45, MGC-803, SGC-7901, GES-1	STAT3 signal pathway	miR-625-5p/ STAT3	By controlling miR-625-5p and STAT3, LINC00511 encourages GC cell growth, implying that LINC00511 has oncogenic capabilities that influence the formation of GC.	Cui et al. (2021)
	Upregulated/ Oncogene	AGS, SGC7901	LINC00511/miR-29b/ KDM2A axis	miR-29b/KDM2A axis	In GC, LINC00511 depletion boosted the apoptosis and hindered cell growth. It is possible to exploit the LINC00511/ miR-29b/KDM2A axis as a viable treatment option for GC.	Zhao et al. (2020)

Breast cancer

In breast cancer, LINC00511 has been found to be highly expressed in the clinical samples and its over-expression has been correlated with poor prognosis (Lu et al., 2018). Functional studies have shown that LINC00511 promotes proliferation, sphere-formation capacity, expression of stem factors and growth of breast tumors (Lu et al., 2018). From a mechanical point of view, LINC00511 acts as a molecular sponge for miR-185-3p to enhance expression of E2F1 protein. Besides, E2F1 binds with the promoter of Nanog gene and increases its expression (Figure 1). Therefore, LINC00511/miR-185-3p/E2F1/Nanog axis has been identified as an important route for induction of stemness and tumorigenesis in breast cancer (Lu et al., 2018). Another study in breast cancer has revealed more than 180 potential targets for LINC00511 through siRNA and RNA-seq assays. Bioinformatics analyses have shown relation between differently expressed genes and signaling pathways mediated by p38-α and p38-β. LINC00511 has been found to be mainly located in the cytoplasm regulating expression of MMP13 through sponging miR-150 (Shi et al., 2021). Expression of LINC00511 in breast cancer samples has been closely correlated with the presence of lymph node metastasis, greater tumor size and molecular subtypes of breast cancer. This lncRNA has been found to increase migratory potential and invasive ability of MDA-MB-231 and MCF-7 cells. Moreover, expression of LINC00511 has been shown to be increased by DNA hypomethylation. In turn, LINC00511 could promote expressions of Wnt10A, E2F2, TGFA, and MET and reduce sensitivity of breast cancer cells to Panobinostat (Liu et al., 2021a). LINC00511 can also influence the cytotoxic effects of paclitaxel on breast cancer cells through regulating miR-29c/CDK6 axis (Zhu et al., 2019).

Another study in patients with breast cancer has reported upregulation of LINC00511 and miR-301a-3p in patients' blood parallel with downregulation of miR-185-3p (Mahmoud et al., 2021). Notably, LINC00511 expression has been increased in

TABLE 3 Role of LINC00511 in lung cancer.

Cancer type	Expression/ Role	Samples/Assessed cell lines	Pathways	Targets/ Regulators	Function	Ref
Lung Squamous Cell Carcinoma (LUSC)	Upregulated/ Oncogene	SK-MES-1, H226, 16-HBE	LINC00511/miR-150-5p/ TADA1 axis	miR-150-5p/ TADA1	A plan for the therapeutic targeting of LINC00511 in LUSC should be developed, because it stimulates the advancement of LUSC.	Wu et al. (2020)
Lung Adenocarcinoma (LUAD)	Upregulated/ Oncogene	45 LAC tissues and corresponding adjacent normal lung tissues/BEAS-2B, H1299, A549	PKM2 signaling pathway	miR-625-5p/ PKM2	By influencing miR-625-5p/PKM2, LINC00511 was found to be engaged in the evolution of LAC, demonstrating that LINC00511/ miR-625-5p/PKM2 may represent interesting treatment targets for LUAC.	Xue and Zhang (2020)
	Upregulated/ Oncogene	40 TNTPs/A549, Calu-3, BEAS-2B, DV-90, PC-9	LINC00511/miR-195-5p/ GCNT3 axis	miR-195-5p/ GCNT3	By suppressing miR-195-5p, LINC00511 reduction encourages GCNT3 production, and as a result, supports the malignant growth of LUAD.	Zhang et al. (2022)
	Upregulated/ Oncogene	A549/DDP, 16HBE	LINC00511/miR-182/ BIRC5 axis	miR-182-3p/ BIRC5	After injection of cisplatin (DDP), LINC00511 silencing prevents the growth of A549/DDP cells into tumors. In order to understand the mechanism of gained DDP tolerance, this research offers a unique LINC00511/miR-182/ BIRC5 model	Zhu et al. (2022)
	Upregulated/ Oncogene	35 TNTPs/A549, PC9, BEAS-2B	Linc00511/miR-126-5p/ miR-218-5p/ COL1A1 axis, PIK3/AKT pathway	miR-126-5p, miR-218-5p/ COL1A1	By targeting miR-126-5p and miR-218-5p, LINC00511 influences COL1A1 production, encouraging cancerous cell growth and motility. LINC00511 could be a viable treatment approach for LUAD.	Wang et al. (2022)

early stages of breast cancer. Area under the reciever operating characteristic curves of LINC00511, miR-185-3p, and miR-301a-3p has been superior to classical tumor markers indicating the diagnostic values of these transcripts as molecular biomarkers in liquid biopsy. Moreover, expression of LINC00511 has been correlated with lymph node metastasis and advanced tumor grades (Mahmoud et al., 2021). Additionally, the sponging effect of LINC00511 on miR-185 has been shown to be involved in breast cancer recurrence and radioresistance via regulation of STXBP4 expression (Liu et al., 2019). In breast cancer cells, LINC00511 expression induced by TFAP-2 expression and directly affected by ER deficiency at the transcriptional level. Through its interaction with EZH2, LINC00511 has been shown to encourage tumor development and suppress apoptosis (Figure 2) (Zhang et al., 2019). Table 1 summarizes the results of studies regarding the role of this lncRNA in breast cancer.

Gastric cancer

LINC00511 has been shown to promote progression of gstaric acnecr through regulating SOX4 expression and epigenetically suppressing PTEN to induce activity of PI3K/AKT pathway (Wang et al., 2021). Moreover, LINC00511 can promote growth of gastric tumors through acting as a molecular sponge for miR-124-3p and regulating expression of PDK4 (Sun et al., 2020a). miR-515-5p is another miRNA that is sponged by LINC00511 in gastric cancer cells leading to enhancemnet of proliferation and invasion of these cells (Wang et al., 2020a). Finally, miR-625-5p/NFIX (Chen et al., 2019a), miR-124-3p/EZH2 (Huang et al., 2020), miR-625-5p/STAT3 (Cui et al., 2021) and miR-29b/KDM2A (Zhao et al., 2020) are other molecular axes regulated by LINC00511 in gastric cancer. Table 2 shows the role of LINC00511 in gastric cancer.

Lung cancer

LINC00511 has ben shown to promote proliferation, invasive capacities, and migration of non-small cell lung cancer cells through regulating miR-625-5p/GSPT1 axis (Cheng et al., 2021). Moreover, LINC00511 can promote progression of this type of cancer through binding to EZH2 and LSD1 and decreasing expression levels of LATS2 and KLF2 (Zhu et al., 2019). Besides, this lncRNA has a role in induction of tumor recurrence in this type of cancer through sponging miR-98-5p and increasing expression of TGFBR1 (Li et al., 2022). LINC00511 can also induce resistance of lung cancer cells to

TABLE 4 Dysregulation of LINC00511 in different cancers (TNTP: tumor and non-tumor pairs of tissues).

Cancer type	Expression/ Role	Samples/Assessed cell lines	Pathways	Targets/ Regulators	Function	Ref
Hepatocellular Carcinoma (HCC)	Upregulated/ Oncogene	Huh7, Hep3B	-	RAB27B	There seems to be a link between the creation of invadopodia and the generation of exosomes and LINC00511 dysregulation. In HCC, LINC00511 could be a treatment option	Peng et al. (2021)
	Upregulated/ Oncogene	SMCC7721, HepG2, Huh7, Hep3B	LINC00511/miR195/ EYA1 axis	miR195/EYA1	A prospective therapeutic option for the detection of HCC has been provided by LINC00511 that interacted with EYA1 to accelerate HCC formation through miR-195	Hu et al. (2020a)
	Upregulated/ Oncogene	127 TNTPs/LO2, Hep3B, HepG2, SMMC-7721, MHCC97H, Huh7, HCCLM3	-	miR-424	LINC00511 may have a significant impact on how HCC develops, and that it will also act as a viable prognostic and therapeutic target.	Wang et al. (2019a)
Liver Hepatocellular Carcinoma (LIHC)	Upregulated/ Oncogene	LO2, MHCC-97H, Huh7, HCC-LM3, Hep3B, MHCC-97L, Huh6	-	miRNA-29c	Through boosting tumor cell proliferative ability, LINC00511 worsens LIHC's development. A predictive marker for LIHC could be LINC00511	Liu et al. (2019)
Osteosarcoma (OS)	Upregulated/ Oncogene	24 TNTPs/SW1353, U2OS	LINC00511/miR-185-3p/E2F1 axis	miR-185-3p/ E2F1	As an oncogenic RNA, LINC00511 leads to the formation and spread of tumor cells. The LINC00511/miR-185-3p/E2F1 axis may be extremely important for the onset of osteosarcoma	Xu et al. (2020)
	Upregulated/ Oncogene	30 TNTPs s/(MG-63, Saos-2, U2OS, HOS, NHOst	-	miR-765	Via substantially controlling the production of miR-765, aberrant transcription of LINC00511 boosted osteosarcoma cell tumorigenesis and motility	Yan et al. (2018)
	Downregulated/ Tumor suppressor gene	45 patients with osteosarcoma/hFOB1.19, MG-63, U-2OS, Saos-2, HOS	-	-	Elevated amounts of LINC00511 slow the growth of tumors. LINC00511 could be a new indicator and prospective osteosarcoma treatment approach	Qiao et al. (2020)
	Upregulated/ Oncogene	10 TNTPs/hFOB 1.19, MG-63, HOS, Saos-2, 143B	LINC00511/miRNA-618/MAEL axis	miRNA- 618/ MAEL	OS cell growth and malignancy were both hindered by lower LINC00511 production. It could be a viable biomarker for more exploration on the treatment of OS.	Guo et al. (2019)
T-cell Acute Lymphoblastic Leukemia (T-ALL)	Upregulated/ Oncogene	Blood samples of 35 T-ALL patients and 30 normal controls/HPB-ALL, TALL-1, ALL-SIL, CUTLL1, PBMC	LINC00511/miR-195-5p/LRRK1 axis	miR-195-5p/ LRRK1	Through the miR-195-5p/ LRRK1 axis, LINC00511 accelerated the evolution of T-ALL, pointing a possible therapeutic hint for the T-ALL sufferers	Li et al. (2020)
Glioma	Upregulated/ Oncogene	HEB, NHA, T98 G cells (CRL-1690), A172 cells (CRL-1620), LN229 cells (CRL-2611), U-87MG cells (HTB-14IG)	LINC00511/miR-15a-5p/AEBP1 axis	miR-15a-5p/ AEBP1	Glioma formation can be slowed down by LINC00511 knockdown. Additionally, via the miR-15a-5p/ AEBP1 axis, the process of LINC00511 influences the onset of glioma	Liu et al. (2021b)
	Upregulated/ Oncogene	U87, U251, SHG44, A172, NHA	SP1/LINC00511/miR-124-3p/CCND2 axis	miR-124-3p/ CCND2	The transcription factor SP1 served as the inspiration for the overexpression of LINC00511 in glioma cells. Additionally, the upregulation of LINC00511 competitively sponges the miR-124-3p, driving the production of CCND2 and the cyclin D2 protein produced by this gene, which may hold significant potential for glioma therapies	Li et al. (2019)

(Continued on following page)

TABLE 4 (*Continued*) Dysregulation of LINC00511 in different cancers (TNTP: tumor and non-tumor pairs of tissues).

Cancer type	Expression/ Role	Samples/Assessed cell lines	Pathways	Targets/ Regulators	Function	Ref
Papillary Thyroid Carcinoma (PTC)	Upregulated/ Oncogene	41 TNTPs/B-CPAP, KTC-1, KTC-1	-	CDKs and EZH2	As an oncogene in PTC, LINC00511 promotes proliferation through CDKs. It will serve as a fundamental therapeutic target for PTC.	Xiang et al. (2020)
Colorectal Cancer (CRC)	Upregulated/ Oncogene	85 CRC tissues and adjacent normal tissues/ HCT116, HT-29, LoVo, SW480, SW620, NCM460	HNF4α/LINC00511/ IL24 axis	HNF4α and IL24	The proliferative, spreading and aggressive features of CRC cells are lowered by LINC00511 reduction, that eventually reduces tumorigenicity of CRC.	Lu et al. (2021)
	Upregulated/ Oncogene	120 TNTPs/SW480, SW620, HCT16, HT29, NCM460	LINC00511-mediated microRNA (miR)-625-5p/WEE1 axis	miRNA-625-5p/ WEE1	By suppressing WEE1 and restoring miR-625-5p, downregulated LINC00511 prevents the carcinogenesis of CC, establishing a fundamental standard for CC-targeted treatment	Qian et al. (2022)
	Upregulated/ Oncogene	12 TNTPs/HT-29, HCT8, HCE8693, SW620, NCM460	LINC00511/miR-29c-3p/NFIA axis	miR-29c-3p/ NFIA	By inhibiting the LINC00511/miR-29c-3p/NFIA axis, LINC00511 assisted in the onset of CRC, proposing that LINC00511 could be a viable therapeutic target.	Hu et al. (2020b)
Glioblastoma (GBM)	Upregulated/ Oncogene	160 TNTPs/U87, A172, U138, U251, U373, LN-18, T98G, Human HEK293T	Wnt/β-catenin signaling	miR-126-5p	In GBM cells, LINC00511 controlled Wnt/Catenin stimulation by functioning as a molecular sponge for miR-126-5p. It stated that LINC00511 could operate as a marker for the treatment of GBM.	Wang et al. (2021)
	Upregulated/ Oncogene	36 GBM tissues and 8 non-tumour brain tissues (NBT)/U87, LN229, U251, A172, 293T	LINC00511/miR-524-5p/YB1/ZEB1 axis	miR-524-5p/YB1	By boosting EMT, the LINC00511/ miR524-5p/YB1/ZEB1 positive feedback loop might encourage GBM cell motility and infiltration. LINC00511 could be a viable therapeutic target for GBM sufferers	Du et al. (2020)
Esophageal Cancer (ECa)	Upregulated/ Oncogene	-	-	miR-150-5p	By attaching to miR-150-5p and sponging this miRNA, LINC00511 controls the creation of cancerous cells and, could be a treatment option for ECa	Sun et al. (2020a)
Cervical Cancer (CC)	Upregulated/ Oncogene	-	LINC00511/miR-497-5p/MAPK1 axis	miR-497-5p/ MAPK1	Overexpression of LINC00511 boosted CC cell growth, motility and infiltration, whereas LINC00511 reduction had the opposite effects	Lu et al. (2022)
	Upregulated/ Oncogene	92 cervical cancer tissues and 40 adjacent normal tissues/SiHa, HeLa, C33A, Caski, Ect1/E6E7	-	-	In cervical cancer sufferers, high LINC00511 transcription is linked to clinical deterioration. The growth, motility and infiltration of tumor cells are restricted by LINC00511 suppression	Yu et al. (2019)
	Upregulated/ Oncogene	40 TNTPs/HT29, LOVO, SW620, SW480	LINC00511/miR-153-5p/HIF-1α axis	miR-153-5p/ HIF-1α	A crucial part of the CRC carcinogenesis is played by LINC00511. HIF-1α/LINC00511/ miR-153-5p might be used as a therapeutic target in CRC.	Sun et al. (2020b)
	Upregulated/ Oncogene	19 TNTPs/SiHa, CaSki, C33A, HUCEC	LINC00511/miR-324-5p/DRAM1 axis	miR-324-5p/ DRAM1	The miR-324-5p/DRAM1 axis is regulated by LINC00511, acting as a ceRNA which, promotes the progression of both HPV-negative and HPV-positive cervical cancer	Zhang et al. (2020)

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TABLE 4 (*Continued*) Dysregulation of LINC00511 in different cancers (TNTP: tumor and non-tumor pairs of tissues).

Cancer type	Expression/ Role	Samples/Assessed cell lines	Pathways	Targets/ Regulators	Function	Ref
	Upregulated/ Oncogene	84 CC patients/PTX-resistant Hela/PTX	-	-	Suppression of LINC00511 may lessen CC cell motility and infiltration as well as paclitaxel tolerance, and may boost cell death in CC cells. LINC00511 suppression offers CC a brand-new treatment option	Mao et al. (2019)
	Upregulated/ Oncogene	47 TNTPs/SiHa, CaSki, C33A, ME180, HeLa, NCECs	-	RXRA or PLD1	In CC, LINC00511 promotes the production of PLD1, which is controlled by RXRA. It could be a viable indicator for the therapy of CC.	Shi et al. (2020)
Cervical Squamous Carcinoma (CESC)	Upregulated/ Oncogene	115 cases of CESC, 79 cases of cervical intraepithelial neoplasia and 101 healthy controls	-	-	A diagnostic model consisting of CCAT2 and LINC01133 has exhibited significant clinical utility for the identification of cervical intraepithelial neoplasia in both healthy individuals and patients. The serum concentrations of CCAT2, LINC01133 and LINC00511 could be used as effective non-invasive indicators for the diagnosis of CESC.	Wang et al. (2020b)
Clear Cell Renal Cell Carcinoma (ccRCC)	Upregulated/ Oncogene	49 TNTPs/HK-2, A498, 786-O, ACHN, Caki-2	LINC00511/miR-625/ CCND1 pathway	miRNA-625/ cyclin D1	As a ceRNA, LINC00511 controls the transcription of CCND1 in ccRCC via sponging miR-625. As a result, the LINC00511/miR-625/ CCND1 pathway could offer ccrCC patients a prospective treatment approach	Deng et al. (2019)
Bladder Cancer (BcA)	Upregulated/ Oncogene	47 TNTPs/TCCSUP, SW780	LINC00511/miR-143-3p/PCMT1 axis	miR-143-3p/ PCMT1	By suppressing the production of miR-143-3p and promoting the production of PCMT1, LINC00511s molecular mechanism may prevent bladder cancer cells from proliferating and invading. A novel target for bladder cancer treatment may be offered by LINC00511	Dong et al. (2021)
	Upregulated/ Oncogene	45 TNTPs/SV-HUC-1, BIU87, T24, 5637	Wnt/β-catenin signaling pathway	miR-15a-3p	By inhibiting the Wnt/β-catenin signaling pathway activity, LINC00511 suppression decreases bladder cancer cells ability to proliferate and increases their likelihood of dying. LINC00511 could also be a putative bladder cancer indicator and possible therapeutic target.	Li et al. (2018)
Tongue Squamous Cell Carcinoma (TSCC)	Upregulated/ Oncogene	Tca-8113	LINC00511/miR-765/ LAMC2 axis	miR-765/LAMC2	By sponging miR-765 with ceRNA, LINC00511 increases the production of LAMC2. the ceRNA regulation network contributes to new knowledge about the pathophysiology of TSCC and given information on how to take advantage of the emerging area of lncRNA-directed treatment for TSCC.	Ding et al. (2018)
Non-small Cell Lung Cancer (NSCLC)	Upregulated/ Oncogene	67 patients/16HBE, A549, NCIH1299, NCIH1650, NCIH 1975, NCIH460	LINC00511/miR-625-5p/GSPT1 axis	miR-625-5p/ GSPT1	By targeting miR-625-5p/GSPT1, LINC00511 boosts NSCLC cell growth, infiltration and motility. LINC00511 is a possible diagnostic indicator and treatment option for NSCLC.	Cheng et al. (2021)

(Continued on following page)

TABLE 4 (*Continued*) Dysregulation of LINC00511 in different cancers (TNTP: tumor and non-tumor pairs of tissues).

Cancer type	Expression/ Role	Samples/Assessed cell lines	Pathways	Targets/ Regulators	Function	Ref
	Upregulated/ Oncogene	57 TNTPs	-	EZH2 and LSD1/ LATS2 and KLF2	Apoptosis is triggered in NSCLC cells when LINC00511 is knocked down, although this reduces the capability of the cells to spread and infiltrate	Zhu et al. (2019)
	Upregulated/ Oncogene	62 stage I NSCLC patients and the age- and gender-matched healthy controls	LINC00511/miR-98-5p/ TGFBR1 axis	miR-98-5p/ TGFBR1	In NSCLC, LINC00511 quantities were raised, which may contribute to distant postoperative recurrence of NSCLC and enhance NSCLC cell growth, motility and penetration by targeting and controlling the miR-98-5p/TGFBR1 axis	Li et al. (2022)
	Upregulated/ Oncogene	40 TNTPs/A549, H522, A549/DDP, H522/DDP, BEAS-2B	LINC00511/miR-625/ LRRC8E pathway	miR-625/ LRRC8E	LINC00511 enhanced LRRC8E production by downregulating miR-625 to boost DDP tolerance in NSCLC. A viable therapeutic target to reduce DDP tolerance in NSCLC is LINC00511	Liu et al. (2022)
	Upregulated/ Oncogene	124 TNTPs/A549, SK-MES-1, H1299, 95D, H460, H520, H1975, H157, SK-LU-1, SPC-A-1, 16HBE	-	EZH2 and p57	In NSCLC, LINC00511 is clinically, physiologically and molecularly oncogenic	Sun et al. (2016)
Pancreatic Cancer (PC)	Upregulated/ Oncogene	91 TNTPs/BxPC-3, CFPAC-1, PANC-1, SW 1990, MIAPaCa-2, HPDE6-C7	LINC00511/miR-370-5p/p21 Axis	miR-370-5p/p21, Snail, and ZEB1	The LINC00511/miR-370-5p/ p21 promoter region axis was responsible for the suppressive impact of DET (deoxyelephantopin) on the growth and spread of PC cells	Ji et al. (2022)
Pancreatic Ductal Adenocarcinoma (PDAC)	Upregulated/ Oncogene	140 TNTPs, PANC-1, MIA PaCa-2, Capan-2, SW 1990, ASPC-1, BxPC-3, HPDE6	LINC005/hsa-miR29b-3p/VEGFA axis	miR-29b-3p/ VEGFA	The etiology of PDAC is profoundly influenced by the new lncRNA LINC00511. LINC00511 is a unique predictive indicator that can forecast the clinical outcomes of PDAC sufferers following surgery and could be used as a treatment option for PDAC.	Zhao et al. (2018)
Thyroid Carcinoma (TC)	Upregulated/ Oncogene	TPC-1, BCPAP, IHH-4, Nthy-ori 3–1	JAK2/STAT3 signaling pathway and LINC00511/TAF1/ JAK2 axis	TAF1 and JAK2	Through TAF1-mediated JAK2/ STAT3 signaling, enhanced expression of LINC00511 increased the radiosenitivity of TC cells. The potential biomarker function of LINC00511 in the management of TC was shown by the recent research	Chen et al. (2019b)
Ovarian Cancer (OC)	Upregulated/ Oncogene	CAOV3, OVCAR3, SKOV3, UWB1.289	-	miR-424-5p and miR-370-5p/ ESR1	With the suppression of cell death, upregulated LINC00511 boosted the vitality, motility and penetration of CAOV3 cells. The disruption of miR-424-5p and miR-370-5p is likely responsible for these actions that promote malignancy	Wang et al. (2019b)
	Upregulated/ Oncogene	SKOV3, SNU840	-	EZH2 and P21	The growth of OC cells is slowed by LINC00511 silencing. This information may offer a valuable lncRNA as a predictive indicator and possible treatment option	Deng et al. (2019)

TABLE 5 LINC00511 interactions with miRNAs.

Target gene	LncRNA experiment	Tissue	Cell line	Disease state	Reference
MIR29B1	Luciferase reporter assays, RNA immunoprecipitation	Pancreatic cancer	PANC-1, MIA PaCa-2, Capan-2, SW 1990, ASPC-1, BxPC-3	Pancreatic cancer	Zhao et al. (2018)
MIR765	Dual luciferase reporter assays	NA	Tca-8113	Tongue squamous cell carcinoma	Ding et al. (2018)
MIR524	Dual-luciferase gene reporter assay, RNA immunoprecipitation (RIP)	Brain	U87, LN229, U251, A172, 293T	Glioblastoma Multiforme	Du et al. (2020)
miR-124-3p	Luciferase assay	GC tissue	GES-1, MGC, HGC, MKN25, MKN28	Gastric cancer	Huang et al. (2020)
MIR150	Luciferase reporter assay	Breast	MDA-MB-231, MCF-7	Breast Cancer	Shi et al. (2021)

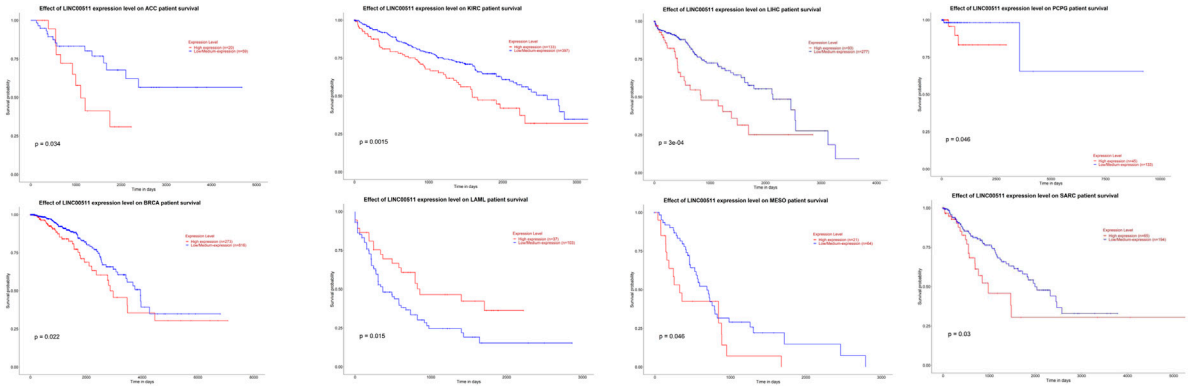

FIGURE 3
The impact of LINC00511 on survival of patients with different cancers.

cisplatin through sponging miR-625 and influencing expression of LRRC8E (Liu et al., 2022). Finally, this lncRNA exerts its oncogenic effects in lung cancer through binding to EZH2 and decaresing expression of p57 (Sun et al., 2016). Table 3 shows the role of LINC00511 in lung cancer.

Other types of cancers

Over-expression of LINC00511 has been reported in a variety of cancers including colorectal, pancreatic, liver, thyroid and other types of cancers (Table 4).

Transcriptional regulation of LINC00511

Investigations in the Hormonizome database (Rouillard et al., 2016) indicated that 18 transcription factors (CTCF, EP300, ESR1, EZH2, FOXA1, GATA3, H2AFZ, MAX, MYC, NFIC, NR2F2, NR3C1, POLR2A, RAD21, TCF12, TEAD4, YY1, and ZBTB7A) possibly bind to the promoter of LINC00511 gene based on ChIP-seq data from the ENCODE Transcription Factor Target dataset.

LINC00511 related pathways and functions.

Based on lncHUB database (https://maayanlab.cloud/lnchub/), 10 KEGG pathways with the highest Z-score in which LINC00511 is predicted to be involved include glycosphingolipid biosynthesis, bacterial invasion of epithelial cells, basal cell carcinoma, central carbon metabolism in cancer, notch signaling pathway, RNA polymerase, DNA replication, cell cycle, bladder cancer and mismatch repair. Additionally, 10 gene ontology (GO) terms with the highest Z-score that are associated with LINC00511 include regulation of hydrogen peroxide-induced cell death (GO:1903205), negative regulation of response to reactive oxygen species (GO:1901032), protein heterotetramerization (GO:0051290), viral release from host cell (GO:0019076), exit from host cell (GO:0035891), signal complex assembly (GO:0007172), regulation of striated muscle tissue development (GO:0016202), regulation of myelination (GO:0031641), positive regulation of kidney development (GO:0090184) and regulation of proteolysis (GO:0030162). Also, IGSF11, PHLPP1, CPOX, SOX2, PACC1, SOX21, TMPRSS5, HEY1, MARCKS, KCTD5, OLIG2, ATAT1, CDK5R1, BCAN and BAALC are 15 genes with the highest Z-score predicted to be co-expressed with LINC00511.

LINC00511 interactions with miRNAs and other molecules

Based on RNAInter (RNA Interactome Database) (Kang et al., 2022), LINC00511 has interactions with hsa-miR-29b-3p, hsa-miR-765, hsa-mir-150, miR-1231, TFAP2A-AS2, hsa-miR-185-3p, hsa-miR-29b-1-5p, hsa-miR-29c-3p, RAD51-AS1 and EZH2 with score ≥0.1. Also, based on LncRNA2Target v3.0 (Cheng et al., 2019), LINC00511 interactions with miRNAs has been showed in Table 5.

Impact of LINC00511 dysregulation on clinical outcome of patients with cancers

We investigated the survival rate caused by LINC00511 in different cancers using ualcan database (Chandrashekar et al., 2017). This database performs survival analysis using TCGA data. The difference was statistically significant with a log-rank *p*-value less than 0.05. As a result, LINC00511 has an effect on the survival rate of patients with adrenocortical carcinoma (ACC), breast invasive carcinoma (BRCA), kidney renal clear cell carcinoma (KIRC), acute myeloid leukemia (LAML), liver hepatocellular carcinoma (LIHC), Mesothelioma (MESO), pheochromocytoma and paraganglioma (PCPG) and sarcoma (SARC) (Figure 3). While in patients with LAML, overexpression of this lncRNA is associated with better clinical outcome, in other types of cancers, its upregulation is associated with lower survival.

Discussion

LINC00511 is a lincRNA being over-expressed in a variety of human tumors and cancer cell lines. Except for a single study in osteosarcoma (Qiao et al., 2020), other studies in this type of cancer and other cancers have reported upregulation of LINC00511 in tumoral tissues compared with their non-tumoral counterparts. Dysregulation of LINC00511 affects cancer pathogenesis through increasing cell proliferation and inhibiting cell apoptosis. It can also increase activity of several cancer-promoting signaling pathways.

A previous meta-analysis has reported association between over-expression of LINC00511 and poor prognosis in different cancers in terms of overall, progression-free or relapse-free survival times (Agbana et al., 2020). Moreover, upregulation of this lncRNA has been associated with larger tumor size, recurrent disorder and metastasis to lymph nodes or distant organs (Agbana et al., 2020).

Mechanistically, LINC00511 can act as a molecular sponge for a variety of miRNAs regulating their targets. miR-185-3p/E2F1, miR-150/MMP13, miR-29c/CDK6, miR-185/STXBP4, miR-573/GSDMC, miR-195-5p/SOX4, miR-124-3p/PDK4, miR-625-5p/NFIX, miR-124-3p/EZH2, miR-625-5p/STAT3, miR-29b/KDM2A, miR-195/EYA1, miR-185-3p/E2F1, miRNA-618/MAEL, miR-195-5p/LRRK1, miR-15a-5p/AEBP1, miR-124-3p/CCND2, miRNA-625-5p/WEE1, miR-29c-3p/NFIA, miR-150-5p/TADA1, miR-524-5p/YB1, miR-625-5p/PKM2, miR-195-5p/GCNT3, miR-182-3p/BIRC5, miR-218-5p/COL1A1, miR-497-5p/MAPK1, miR-153-5p/HIF-1α, miR-324-5p/DRAM1, miR-625/cyclin D1, miR-143-3p/PCMT1, miR-765/LAMC2, miR-625-5p/GSPT1, miR-98-5p/TGFBR1, miR-625/LRRC8E, miR-370-5p/p21, miR-29b-3p/VEGFA and miR-370-5p/ESR1 are examples of miRNA/mRNA axes that are regulated by LINC00511. Molecular axes being regulated by LINC00511 in more than one type of cancer represent better targets for design of anti-cancer therapies since they can be applied in a wider range of malignancies. Therefore, identification of the impact of above-mentioned molecular axes in the progression of different types of cancer is an important step in design of novel therapeutics.

LINC00511 can induce stemness in cancers and facilitate tumor progression and metastasis (Lu et al., 2018). Therefore, LINC00511-modifying modalities can be used as possible strategies for defeating cancer metastasis.

The prognostic role of over-expression of LINC00511 in different cancers has been evaluated thoroughly by various research groups indicating its important effects on survival of affected individuals. Future studies should assess its expression in biofluids to provide a non-invasive route for cancer diagnosis and patients' follow-up.

Author contributions

MT designed and supervised the study. SG-F wrote the draft and revised it. AS, BH, and SA collected the data and designed the figures and tables. All the authors read the submitted version and approved it.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Interaction between SIRT1 and non-coding RNAs in different disorders

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SIRT1 is a member of the sirtuin family functioning in the process of removal of acetyl groups from different proteins. This protein has several biological functions and is involved in the pathogenesis of metabolic diseases, malignancy, aging, neurodegenerative disorders and inflammation. Several long non-coding RNAs (lncRNAs), microRNAs (miRNAs) and circular RNAs (circRNAs) have been found to interact with SIRT1. These interactions have been assessed in the contexts of sepsis, cardiomyopathy, heart failure, non-alcoholic fatty liver disease, chronic hepatitis, cardiac fibrosis, myocardial ischemia/reperfusion injury, diabetes, ischemic stroke, immune-related disorders and cancers. Notably, SIRT1-interacting non-coding RNAs have been found to interact with each other. Several circRNA/miRNA and lncRNA/miRNA pairs that interact with SIRT1 have been identified. These axes are potential targets for design of novel therapies for different disorders. In the current review, we summarize the interactions between three classes of non-coding RNAs and SIRT1.

KEYWORDS

SIRT1, lncRNA, miRNA, circRNA, biomar

Introduction

As a member of the sirtuin family, Sirt1 has a function in removal of acetyl groups from different proteins. This nicotinamide adenosine dinucleotide (NAD)-dependent deacetylase has several biological functions and is involved in the pathogenesis of metabolic diseases, malignancy, aging, neurodegenerative disorders and inflammation (Rahman and Islam, 2011). SIRT1 has a lot of substrates including a number of transcription factors. These transcription factors include p53, FoxO family, HES1, HEY2, PPARγ, CTIP2, p300, PGC-1α, and NF-κB (Haigis and Guarente, 2006; Michan and Sinclair, 2007; Yamamoto et al., 2007; Pillarisetti, 2008). The enzymatic reaction catalyzed by SIRT1 leads to generation of nicotinamide and transfer of the acetyl group of the substrate to cleaved NAD, producing a distinctive metabolite, namely, O-acetyl-ADP ribose (Pillarisetti, 2008).

SIRT1 has an important role in the regulation of energy homeostasis in response to accessibility to nutrients. In the liver tissue, SIRT1 enhances expression of the nuclear

TABLE 1 SIRT1-interacting miRNAs.

Type of diseases	miRNA	Sample	Cell line	SIRT1 expression	Targets and pathways	Discussion	Ref
Sepsis	miR-181a (Up)	-	RAW264.7	(Down)	Nrf2, p-65, NF-κβ, TNF-α, IL-1β, IL-6, Bcl-2, Bax	Inhibition of miR-181a via targeting SIRT1 by activating Nrf2 and inhibiting NF-κB could attenuate sepsis-induced inflammation and apoptosis	Wu et al. (2021a)
Sepsis	miR-133a (Up)	Serum samples: sepsis (n = 60), normal group (n = 30), C57BL/6J mice	RAW264.7	(Down)	ALT, AST, IL-1β, IL-6, TNF-α	miR-133a by targeting SIRT1 could aggravate inflammatory responses in sepsis	Chen et al. (2020a)
Sepsis	miR-195 (Up)	-	NCM460	(Down)	Bcl-2, Bax, elF2α, ATF4, CHOP, GRP78	miR-195 via targeting the SIRT1/eIF2α axis could enhance intestinal epithelial cell apoptosis	Yuan et al. (2020)
Sepsis	miR-197		H9c2	(Down)	Bcl-2, Bax, IL-6, IL-1β, Caspase-3, p53	miR-197 by modulating SIRT1 could participate cardiomyocyte injury	Liu et al. (2022a)
Septic cardiomyopathy	miR-22 (-)	miR-22-flox mice, αMHC-Cre mice, littermates wild-type (WT) mice	Cardiomyocyte	(Down)	TNF-α, IL-6, IL-1β, LC3-I/II, p62, Atg7, Caspase-3/9, Bax, Bcl-2	Downregulation of miR-22 by targeting SIRT1 could alleviate septic cardiomyopathy	Wang et al. (2021a)
Non-alcoholic steatohepatitis (NAFLD)	miR-29a (-)	C57BL/6 mice	HepG2	(-)	GSK-3β, CD36, PERK, IRE1α, XBP1s, CHOP	miR-29a via modulating the GSK-3β/SIRT1 could ameliorate mouse non-alcoholic steatohepatitis	Yang et al. (2020)
NAFLD	miR-34a (Up)	Wistar rats	-	(Down)	FXR, p53, ALT, AST, γ-GGT, TNF-α, IL-6,	Alteration of miR-34a/SIRT1/FXR/p53 axis could induce NAFLD in rats	Alshehri et al. (2021)
Chronic hepatitis C (CHC)	miR-34a (Up)	CHC (n = 41), healthy control samples (n = 18)	-	(Down)	p53, TBA, AST, ALT	miR-34a via mediating the SIRT1/p53 axis could enhance liver fibrosis in patients with chronic hepatitis	Li et al. (2020a)
Hepatic I/R Injury	miR-182 (-)	Black/Swiss mice, C57BL/6J WT mice	Hepatocyte	(Down)	XBP1, NLRP3, ALT, IL-1β, TNF-α, IL-18, Caspase-1	SIRT1 via modulating the miR-182-mediated XBP1/NLRP3 axis could alleviate hepatic IR injury	Li et al. (2021a)
Cardiac Fibrosis	miR-128 (Up)	C57BL/6 J mice	H9c2	(Down)	PIK3R1, p53, p62, Bcl-2, Bax, Beclin-1, LC3-I/II, AKT, mTOR	Downregulation of miR-128 via targeting the SIRT1/PIK3R1 axis could ameliorate cardiac dysfunction	Zhan et al. (2021)
Congestive heart failure (CHF)	miR-22 (-)	C57BL/6 mice	Cardiomyocyte	(Down)	PGC-1α, TFAM, p62, LC3-I/II	Downregulation of miR-22 by targeting SIRT1/PGC-1α could alleviate CHF.	Wang et al. (2021b)
HF	miR-199a (Up)	C57Bl/6J mice	CMs, CFs, CECs	(Down)	P300, Yy1, sST2	miR199/SIRT1/P300 axis via upregulating the circulation of soluble sST2 isoform could modulate heart failure	Asensio-Lopez et al. (2021)
Myocardial I/R Injury	miR-29a (Up)	C57BL/J6	H9c2	(Down)	NLRP3, IL-1/6, IL-1β, TNF-α, eNOS, iNOS, Caspase-1	Downregulation of miR-29a by targeting SIRT1 and inhibiting NLRP3-mediated pyroptosis could ameliorate myocardial I/R Injury	Ding et al. (2020)

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TABLE 1 (*Continued*) SIRT1-interacting miRNAs.

Type of diseases	miRNA	Sample	Cell line	SIRT1 expression	Targets and pathways	Discussion	Ref
Cardiotoxicity	miR-200a-3p (Up)	Wistar rats	H9c2, 293T	(-)	PEG3, NF-κβ, Bax, Bcl-2, IKK, p65, IκBα	miR-200a-3p via modulating SIRT1/NF-κB axis and by targeting PEG3 could aggravate cardiotoxicity	Fu et al. (2021)
Acute myocardial infarction (AMI)	miR-181a-5p (-)	-	H9C2	(-)	Bcl-2, Bax, Caspase-3	miR-181a-5p via regulating SIRT1 could involve cardiomyocyte apoptosis induced by hypoxia–reoxygenation	Qi et al. (2020)
AMI	miR-124-3p (Up)	SD rats	H9C2	(Down)	FGF21, CREB, PGC1-α, g IL-1α, IL-1β, IL-2/6, IFN-γ, TNF-α, Bax, Bcl-2, Caspase-3	miR-124-3p via targeting SIRT1 by modulation FGF21/CREB/PGC1α axis could regulate cell apoptosis and oxidative stress of acute myocardial infarction	Wei et al. (2021)
Osteoarthritis (OA)	miR-30b-5p (Up)	OA tissue samples (n = 40) and adjacent (n = 15) normal tissue samples, SD rats	HC-A,	(Down)	FoxO3a, NLRP3, NF-κβ, IL-1β, IL-6/18, TNF-α, Bax, Caspase-1/3, MMP-3/13, ASC	NF-κB-inducible miR-30b-5p via modulating SIRT1-FoxO3a-mediated NLRP3 inflammasome could aggravate joint pain	Xu et al. (2021a)
OA	miR-122 (Up)	OA tissue samples (n = 29), normal cartilage tissue samples (n = 29)	-	(Down)	Collagen-II, Aggrecan, MMP-13, ADAMTS4	miR-122 via targeting SIRT1 could regulate chondrocyte extracellular matrix degradation in osteoarthritis	Bai et al. (2020)
Kidney Injury	miR-34a (Up)	Kunming mice	-	(Down)	p53, TNF-α, IL-6, IL-1β, Caspase-9, Bax, Bcl-2	miR-34a/SIRT1/p53 axis could modulate kidney injury	Hao et al. (2021)
Acute kidney injury (AKI)	miR-183-3p (Up)	SD rats	NRK-52E	(Down)	PUMA, FOXO3a, TGF-β1, *a*-SMA, Vimentin,E-Cadherin	Depletion of miR-183-3p via the SIRT1/PUMA/FOXO3a axis could improve renal tubulointerstitial fibrosis after AKI.	Li et al. (2021b)
Diabetic nephropathy (DN)	miR-150-5p (Up)	(n = 60) diabetes mellitus patients, C57BL/6J mice	Podocyte	(Down)	p53, p62, AMPK, p-cadherin, ZO-1, LC3-I/II	Downregulation of miR-150-5p by targeting the SIRT1/p53/AMPK axis could ameliorate diabetic nephropathy	Dong et al. (2021)
DN	miR-34a (Up)	C57BL/6J mice	Podocyte	(Down)	p53, LC3A/B-I, LC3A/B-II	The p53/miR-34a/SIRT1 axis inhibition could ameliorate podocyte injury in DN.	Liang et al. (2021)
Cerebral I/R Injury	miR-19a/b-3p (Up)	SD rats	-	(Down)	FoxO3, SPHK1, NF-κβ p65, TNF-α, IL-6, IL-1β	miR-19a/b-3p via targeting the SIRT1/FoxO3/SPHK1 axis could promote inflammation during cerebral I/R injury	Zhou et al. (2021)
SCI	miR-324-5p (Up)	SD rats	PC12	(Down)	Bcl-2, Caspase-3, Bax, TNF-α, IL-1β	Silencing miR-324-5p by modulating SIRT1 could alleviate rats SCI.	Wang et al. (2021c)
CCIS	miR-34c-5p (Up)	SD rats	-	(Down)	TNF-α, IL-6, IL-1β, STAT3	Downregulation of miR-34c-5p via targeting the SIRT1/STAT3 axis could alleviate neuropathic pain	Mo et al. (2020)
Epilepsy	miR-135a-5p (Up)	-	BV2	(-)	Caspase-3/9	Downregulation of miR-135a-5p via targeting SIRT1 could protect glial cells against apoptosis in epilepsy	Wang et al. (2021d)

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TABLE 1 (*Continued*) SIRT1-interacting miRNAs.

Type of diseases	miRNA	Sample	Cell line	SIRT1 expression	Targets and pathways	Discussion	Ref
MDD	miR-138 (Up)	C57BL/6J mice	-	(Down)	PGC-1α, FNDC5, BDNF	miR-138 by targeting SIRT1 could enhance depressive-like behaviors in the hippocampus	Li et al. (2020b)
Migraine	miR-34a-5p (-)	SD rats	trigeminal ganglionic cells	(-)	COX2, PGE2, p65, NF-κβ, IL-1β, IL-13	miR-34a-5p via inhibiting SIRT1 could enhance the IL-1β/COX2/PGE2 axis and stimulate the release of CGRP in trigeminal ganglion neurons in rats	Zhang et al. (2021a)
DFUs	miR-489-3p (-)	SD rats	HUVECs	(-)	VEGF, Bcl-2, Bax, Caspases-3/9, PI3K, AKT, eNOS, iNOS	Alteration in miR-489-3p/SIRT1 axis could enhance wound healing in DFU.	Huang et al. (2021a)
DR	miR-221 (Up)	-	hRMEC	(Down)	Nrf2, Caspase-3 Bax, Bcl-2, Keap-1	Overexpression of miR-221 via inhibiting SIRT1 could enhance apoptosis of hRMEC.	Chen et al. (2020b)
ALI	miR-146a-3p (Up)	SD rats	BEAS-2B	(Down)	NF-κβ, TNF-α, IL-1β, IL-4, IL-6, IL-10	Depletion of miR-146a-3p via upregulating SIRT1 and mediating NF-κB could attenuate ALI.	Yang and Li (2021)
UUO	miR-155-5p (Up)	-	NRK-49F	(Down)	α-SMA, Collage-I, Fibronectin	miR-155-5p via modulating SIRT1 promotes renal interstitial fibrosis	Wang et al. (2021e)
-	miR-217 (Up)	-	HUVECs	(-)	p53, SA-β-gal	miR-217 via modulating the SIRT1/p53 axis could enhance endothelial cell senescence	Wang et al. (2021f)
-	miR-204-5p (-)	C57BL/6J	HC11	(-)	PPARγ	miR-204-5p by targeting SIRT1 could enhance lipid synthesis in mammary epithelial cells	Zhang et al. (2020a)
-	miR-128-3p (Up)	-	BMSCs	(-)	IL-6, IL-1β, MMP-9, MCP-1	MiR-128-3p by regulating SIRT1 expression could mediate inflammatory responses in BMSCs	Wu et al. (2020)
-	miR-34a-5p, miR-34a-3p (-)	Human submandibular gland tissue samples (n = 114), human parotid gland tissue samples (n = 114), serum samples (n = 114), SD rats	SMG-C6,	(-)	CTRP6, AMPK, TNF-α, Bcl-2, Bax Caspase-3/8/9/12, Cytochrome-C,	CTRP6 via targeting the AMPK/SIRT1 axis by modulating miR-34a-5p expression could attenuate TNF-α-induced apoptosis	Qu et al. (2021)
-	miR-146a-5p (Up)	(n = 45) bone tissue samples, KO mice	MC3T3-E1	(-)	Collagen-I	miR-146a-5p via targeting SIRT1 could regulate bone mass	Zheng et al. (2021)
PCa	miR-373 (-)	-	AsPC-1, PANC-1	(-)	PGC-1α, NRF2, Bax, Bcl-2, Caspase-3/8/9, PARP, eNOS, iNOS	miR-373 via modulating the SIRT1/PGC-1α/NRF2 axis could suppress cell proliferation in pancreatic cancer cells	Yin et al. (2021)
CRC	miR-34a (Up)	CRC tissue and ANT samples, DAB1/J mice, NOD-SCID mice	HCT-8, HCT-116, CHO, PBMCs	(-)	NF-κβ, p65, B7-H3, TNF-α	miR-34a via modulating the SIRT1/NF-κB and B7-H3/TNF-α axis could induce immunosuppression in colorectal cancer	Meng et al. (2021)

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TABLE 1 (*Continued*) **SIRT1-interacting miRNAs.**

Type of diseases	miRNA	Sample	Cell line	SIRT1 expression	Targets and pathways	Discussion	Ref
cSCC	miR-199a-5p (Down)	BALB/c nude mice	A431, NHSF	(Up)	CD44ICD, OCT4, SOX2, Nanog	miR-199a-5p by targeting SIRT1 and CD44ICD cleavage signaling could repress stemness of cSCC stem cells	Lu et al. (2020)

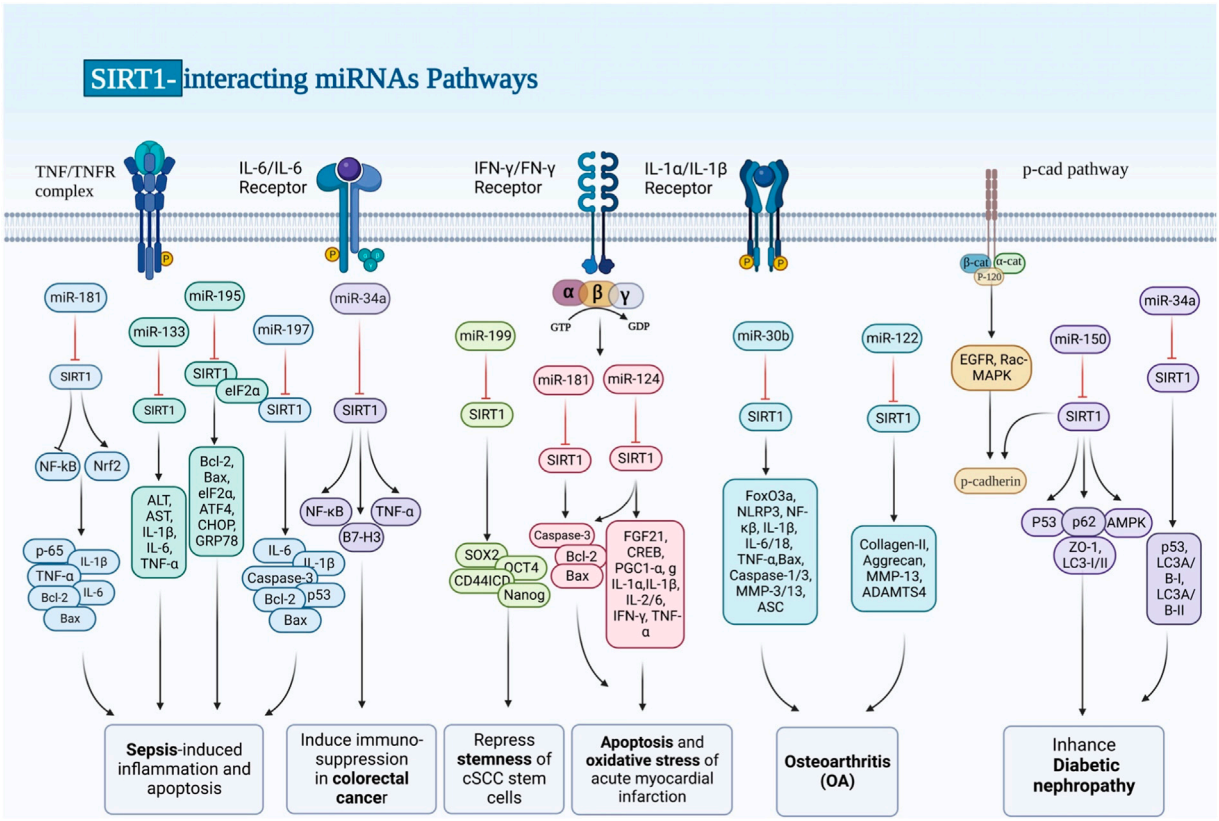

FIGURE 1
SIRT1 works with a lot of molecules, some of which are transcription factors. p53, the FoxO family, HES1, HEY2, PPAR, CTIP2, p300, PGC-1, and NF-B are all transcription factors. Dysregulation of SIRT1-targeting miRNAs plays a role in the pathogenesis of sepsis and its complications, chronic hepatitis, ischemia/reperfusion (I/R) injury to the liver and heart, cardiac fibrosis, myocardial infarction, osteoarthritis, diabetic nephropathy, and a number of malignant diseases like colorectal cancer.

receptor PPARα, thus regulating lipid homeostasis. Deletion of Sirt1 in this tissue has been shown to impair PPARα signaling and decrease ß-oxidation of fatty acids, resulting in the development of hepatic steatosis, induction of inflammatory responses in liver, and endoplasmic reticulum stress (Purushotham et al., 2009).

In addition to the regulation of metabolic pathways, SIRT1 is involved in the carcinogenic processes. Its expression has been found to be increased in both hematological malignancies (Bradbury et al., 2005) and solid tumors (Huffman et al., 2007; Stünkel et al., 2007). Possibly acting as an oncogene, SIRT1 interacts with p53 and induces its deacetylation at its C-terminal Lys382 residue (Vaziri et al., 2001), thus inactivating this tumor suppressor.

In fact, SIRT1 is involved in a variety of human disorders including malignant and nonmalignant conditions. Recently, researchers have focused on identification of the interaction between non-coding RNAs and SIRT1 in these disorders. These investigations have led to identification of a number of long non-coding RNAs (lncRNAs), microRNAs (miRNAs) and circular RNAs (circRNAs) that regulate expression of SIRT1. In the current review, we provide an overview of these non-coding RNAs.

SIRT1-interacting miRNAs

A class of non-coding RNAs known as miRNAs regulate gene expression by binding to specific target genes in distinct pathways, thereby modulating the expression of various genes (Ghafouri-Fard et al., 2021a; Hussen et al., 2021; Hussen et al., 2022a). Mature

TABLE 2 SIRT1-interacting circRNAs.

Type of diseases	Circular-RNAs	Sample	Cell line	SIRT1 expression	Targets and pathways	Discussion	Ref
T2DM	hsa_circ_0115355 (Down)	Serum samples of T2DM patients (n = 20)	INS-1	(Down)	miR-145	hsa_circ_0115355 via targeting the miR-145/SIRT1 axis could enhance pancreatic β-cell function	Dai et al. (2022)
DN	HIPK3 (Down)	-	HK-2	(Down)	miR-326, miR-487a-3p, Caspase-3, Bax, Bcl-2	Circ-HIPK3 via modulating the miR-326/miR-487a-3p/SIRT1 axis could alleviate high glucose toxicity to HK-2 Cells	Zhuang et al. (2021)
RA	hsa_circ_0044235 (Down)	Serum samples of RA (n = 48), healthy control group (n = 36), DBA/1 J mice	FLSs	(Down)	miR-135b-5p, Caspase-1, TNF-α, IL-6, IL-1β, NLRP3	hsa_circ_0044235 could regulate pyroptosis via modulating miR-135b-5p-SIRT1 axis	Chen et al. (2021)
CCI	circ_0000296 (Down)	C57BL/6J mice	HT22	(Down)	miR-194-5p, Runx3	Upregulation of circ_0000296 via miR-194-5p/Runx3 axis could increase transcription of SIRT1 and inhibit apoptosis of hippocampal neurons	Huang et al. (2021b)
OA	Circ_0001103 (Down)	OA samples (n = 30), normal tissues (n = 10), OA serum (n = 10) and normal (n = 10) samples	Chondrocyte	(Down)	miR-375, IL-1β, COL2A1, ADAMTS4	Circ_0001103 via targeting miR-375 by upregulating SIRT1 could alleviate IL-1β-induced chondrocyte cell injuries	Zhang et al. (2021b)
IDD	CIDN (Down)	IDD tissue samples (n = 30) and healthy control tissues (n = 50), SD rats	NP	(Down)	miR-34a-5p, MMP-3/13, Bax, caspase-3, Bcl-2, Collagen-II	Circ-CIDN via the miR-34a-5p/SIRT1 axis could mitigate compression loading-induced damage	Xiang et al. (2020)
GC	NOP10 (Up)	10 pairs of GC and ANT samples	GES-1, AGS, MNK-45, HGC-27, BGC-823	(Up)	miR-204, NF-κβ, E-cadherin, p65, Vimentin, Bcl-2, Caspase-3, Bax	Circ-NOP10 by regulating the miR-204/SIRT1 axis could mediate gastric cancer progression	Xu et al. (2021b)
Glioma	Circ-0082374 (Up)	glioma samples (n = 42), non-cancer tissue samples (n = 28), BALB/c nude mice	A172, BT325, LN229, U251, SHG44, HA1800	(-)	miR-326, MMP-9, E-cadherin, Vimentin	Knockdown of Circ-0082374 by modulating the miR-326/SIRT1 axis could inhibit viability, migration, invasion, and glycolysis of glioma cells	Wang et al. (2020)

miRNAs are formed by further processing of pre-miRNAs, which are formed from the transcribed nucleic acids that make up primary miRNAs. Several miRNAs have been shown to target SIRT1, thus regulating its expression. Dysregulation of SIRT1-targeting miRNAs is involved in the pathogenesis of sepsis and its complications, non-alcoholic fatty liver disease (NAFLD), chronic hepatitis, hepatic and myocardial ischemia/reperfusion (I/R) injury, cardiac fibrosis, heart failure, myocardial infarction, osteoarthritis, kidney injury, diabetic nephropathy, cerebral I/R Injury, spinal cord injury, epilepsy and a number of malignant conditions (Table 1; Figure 1). In sepsis, upregulation of miR-181a (Wu Z. et al., 2021), miR-133a (Chen L. et al., 2020) and miR-195 (Yuan et al., 2020) has been shown to lead to downregulation of SIRT1 and aggravation of inflammatory responses. miR-29a, miR-34a and miR-182 are among SIRT1-interacting miRNAs being involved in the pathogenesis of hepatic disorders. For instance, miR-29a via modulating the GSK-3β/SIRT1 could ameliorate mouse non-alcoholic steatohepatitis (Yang et al., 2020). Alterations in the miR-34a/SIRT1/FXR/p53 axis have been found to induce NAFLD in rats (Alshehri et al., 2021). Moreover, miR-34a via mediating the SIRT1/

p53 axis could enhance liver fibrosis in patients with chronic hepatitis (Li X. et al., 2020).

miR-128 has been shown to be involved in the pathogenesis of chronic angiotensin II infusion-induced cardiac remodeling through modulation of SIRT1. Silencing this miRNA in the heart tissues of mice could ameliorate angiotensin II-induced cardiac dysfunction, hypertrophy, fibrosis and oxidative stress damage. Angiotensin II could induce upregulation of miR-128 in cell culture. Treatment of cells with miR-128 antagomir could attenuate angiotensin II -induced apoptosis and oxidative damage possibly through targeting the SIRT1/p53 pathway. Suppression of this miRNA could also activate PIK3R1/Akt/mTOR pathway, restrain angiotensin II-induced autophagy in cardiomyocytes, and mitigate oxidative stress and apoptosis (Zhan et al., 2021).

SIRT1-interacting miRNAs are also involved in the pathogenic processes in the acute myocardial infarction. Suppression of miR-29a has been shown to protect against myocardial I/R injury through influencing expression of SIRT1 and subduing oxidative stress and NLRP3-associated pyroptosis (Ding et al., 2020). In addition, miR-200a-3p has been found to aggravate doxorubicin-induced cardiotoxic effects through targeting PEG3 via SIRT1/NF-κB signaling pathway (Fu et al., 2021). miR-181a-5p is another miRNA which participates in the cardiomyocyte apoptosis induced by hypoxia–reoxygenation via regulation of SIRT1 (Qi et al., 2020). Moreover, an experiment in an animal model of acute myocardial infarction has shown that miR-124-3p targets SIRT1 to influence cell apoptosis, inflammatory responses, and oxidative stress through regulation of the FGF21/CREB/PGC1α axis (Wei et al., 2021). Besides, miRNAs that modulate expression of SIRT1 can affect pathogenesis of heart failure. For instance, downregulation of miR-22 by targeting SIRT1/PGC-1α could alleviate this disorder (Wang et al., 2021b). Finally, miR199/SIRT1/P300 axis has apotential function in the patheticlogy of this disorder (Asensio-Lopez et al., 2021).

Lastly, three SIRT1-interacting miRNAs have been revealed to participate in the carcinogenesis. miR-373 is a tumor suppressor miRNA that inhibits proliferation of pancreatic cancer cells through influencing activity of SIRT1/PGC-1α/NRF2 axis (Yin et al., 2021). On the other hand, miR-34a acts as an immunosuppressive miRNA in colorectal cancer via regulation of SIRT1/NF-κB/B7-H3/TNF-α axis (Meng et al., 2021). Lastly, miR-199a-5p has a role in repression of stemness of squamous cell carcinoma cells through influencing activity of SIRT1 and CD44ICD cleavage signaling (Lu et al., 2020).

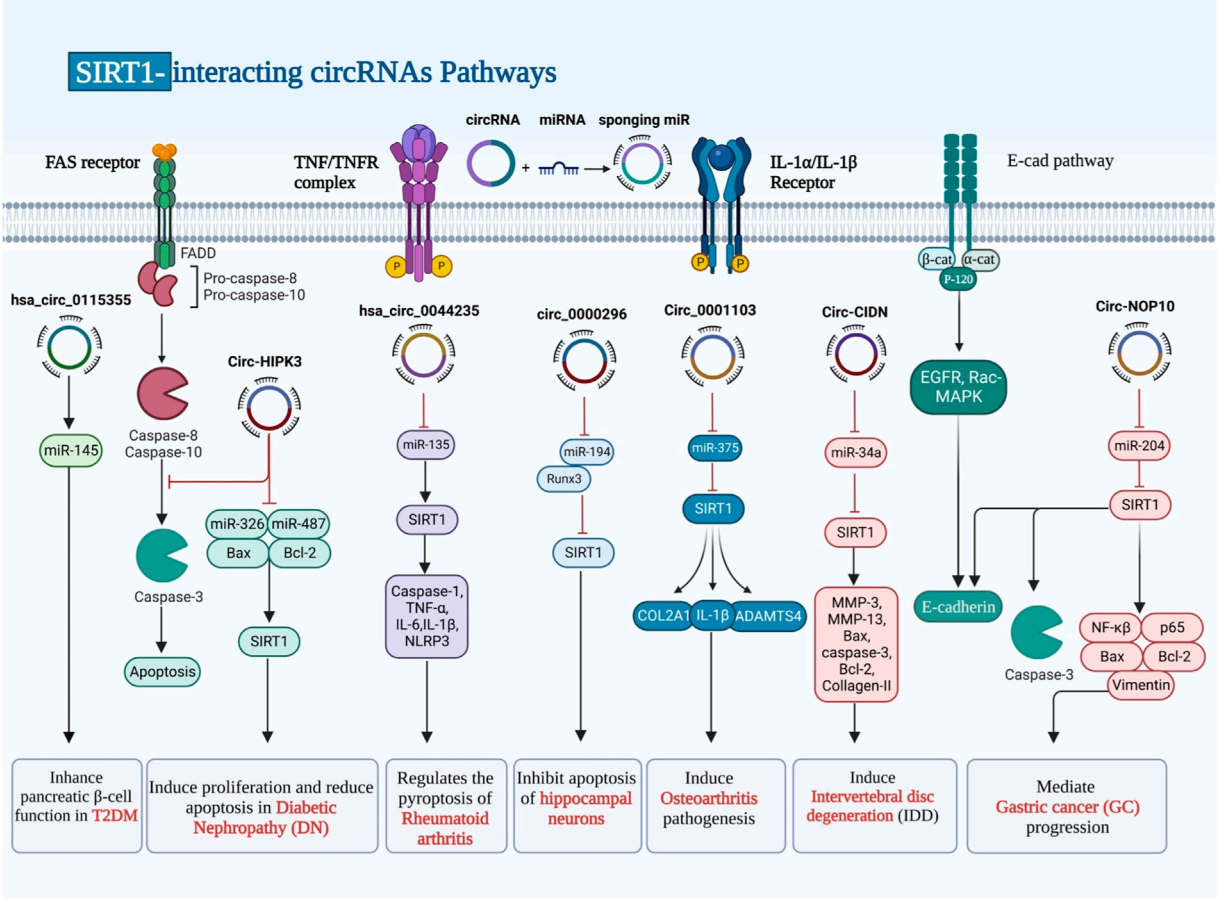

FIGURE 2
CircRNAs have been proven to serve as molecular sponges for miRNAs, thereby influencing the expression of miRNA targets. SIRT1 has been found to be the target of miRNA genes, which were already being sponged by different types of circRNAs that prevented or enhanced gene expression.

TABLE 3 SIRT1-interacting lncRNAs.

Type of diseases	LncRNA	Sample	Cell line	SIRT1 expression	Target and pathways	Discussion	Ref
RA	GAS5 (Down)	Serum samples of RA patients (n = 35) and Serum samples of healthy control (n = 35)	RA-FLSs	(Down)	miR-222-3p, TNF-α,IL-1β, IL-6, Bcl-2, Bax, Caspase-3/9	GAS5 via regulating the miR-222-3p/Sirt1 axis could alleviate RA.	Yang et al. (2021)
Atherosclerosis	LincRNA-p21 (Down)	Serum samples of AS patients (n = 25), C57BL/6 mice	HAECs, 293T	(Down)	miR-221, Pcsk9, Caspase-3, Bcl-2, Bax	LncRNA-p21 via modulating the miR-221/SIRT1/Pcsk9 axis could alleviate atherosclerosis progression	Wang et al. (2021g)
SARI	GAS5 (Down)	C57BL/6 mice	HK-2	(Down)	miR-579-3p, Nrf2, IL-1β, IL-18, NLRP3, PGC-1α, Caspase-1,	GAS5 via inhibiting the miR-579-3p by activating the SIRT1/PGC-1α/Nrf2 axis could reduce cell pyroptosis in SARI.	Ling et al. (2021)
OA	MCM3AP-AS1 (Down)	30 pairs of OA and ANTs	CHON-001, ATDC5	(Down)	miR-138-5p, IL-6, IL-8, TNF-α	MCM3AP-AS1 via modulating the miR-138-5p/SIRT1 axis could protect chondrocytes from IL-1β-induced inflammation	Shi et al. (2021)
Sepsis	TUG1 (Down)	C57BL/6 mice	RAW264.7	(Down)	miR-9-5p, TNF-α, MCP-1, IL-6, IL-10, iNOS, Arg-1,	TUG1 via impairing miR-9-5p targeted SIRT1 inhibition could confer anti-inflammatory macrophage polarization in sepsis effects	Ma et al. (2021)
DN	TUG1 (Down)	-	HK-2	SIRT1 (Down)	miR-29c-3p, ERS, Bax, Bcl-2, caspase-3/12, GRP78, CHOP, PERK, eIF-2α	The TUG1/miR-29c-3p/SIRT1 axis could regulate endoplasmic reticulum stress-mediated cell injury in DN.	Wang et al. (2021h)
Ischemic Stroke	SNHG8 (-)	C57BL/6 mice	BMEC	(Down)	miR-425-5p, NF-κβ, caspase-3, ZO-1, Occludin, TNF-α, IL-1β, IL-6	SNHG8 via regulating miR-425-5p mediated SIRT1/NF-κβ axis could attenuate blood-brain barrier damage	Tian et al. (2021)
Ischemic Stroke	SNHG7 (Down)	C57BL/6 mice	PC12,	(Down)	miR-9	SNHG7 by targeting the miR-9/SIRT1 axis could alleviate damage in PC12 Cells	Zhou et al. (2020)
Cerebral I/R Injury	SNHG15 (Up)	-	SH-SY5Y	(-)	miR-141, TNF-α, IL-1β, IL-6, iNOS, p65	SNHG15 by targeting the miR-141/SIRT1 axis could enhance oxidative stress damage	Kang et al. (2021)
Myocardial I/R Injury	Oip5-as1 (Down)	SD rats	NRVMs, H9c2	(Down)	miR-29a, AMPK, PGC1α, LDH, ROS, Bax, Bcl-2, Cyt-c, caspase-3, 15-F2t-isoprostane, SOD, GPx	Oip5-as1 via activating the SIRT1/AMPK/PGC1α axis by sponging miR-29a could attenuate myocardial I/R injury	Niu et al. (2020)
MI	ILF3-AS1 (Down)	-	H9c2, 293T	(-)	miR-212-3p, PI3K, AKT, Bcl-2, Bax, caspase-3/9	ILF3-AS1 via targeting the miR-212-3p/SIRT1 axis and the PI3K/Akt pathway could regulate MI.	Zhang et al. (2020c)
AMI	ANRIL (Up)	-	H9c2	(-)	miR-7-5p, Bcl-2, Bax, Caspase-3/9, HIF-1α	ANRIL via targeting the miR-7-5p/SIRT1 axis could protect H9c2 cells against hypoxia-induced injury	Shu et al. (2020)

(Continued on following page)

TABLE 3 (*Continued*) SIRT1-interacting lncRNAs.

Type of diseases	LncRNA	Sample	Cell line	SIRT1 expression	Target and pathways	Discussion	Ref
Diabetes	TUG1 (Down)	C57BL/6J mice	3T3-L1	(Down)	miR-204, AMPK, ACC, ATGL, PGC-1α, PPARα, UCP-1	TUG1 via targeting SIRT1 by regulating miR-204 could enhance brown remodeling of white adipose tissue in diabetic mice	Zhang et al. (2020d)
NSCLC	SNHG10 (Down)	60 pairs of NSCLC and ANT samples	H1581, H1703	(-)	miR-543	SNHG10 via sponging miR-543 could upregulate tumor suppressive SIRT1 in NSCLC.	Zhang et al. (2020b)
HCC	SNHG7 (Up)	25 pairs of HCC and ANT samples	THLE-3, 293T, HepG2, SK-hep-1	(Up)	miR-34a, NLRP3, Caspase-1, IL-1β	SNHG7 via targeting the miR-34a/SIRT1 axis could inhibit NLRP3-dependent pyroptosis	Chen et al. (2020c)
CRC	GAS5 (Down)	75 pairs of CRC and ANT samples, Wistar rats	HT29, HCT116, SW480, SW620	(Up)	miR-34a, mTOR, LC3 I/II. Beclin-1, Bcl-2, Bax	GAS5 via targeting the miR-34a/mTOR/SIRT1 axis could inhibit malignant progression in CRC.	Zhang et al. (2021c)
AML	UCA1 (Up)	Serum samples: AML (n = 27), normal (n = 9)	KG-1a, THP-1, HS-5	(-)	miR-204, Caspase-3, iNOS, COX-2	Silencing UCA1 via targeting miR-204 by repressing SIRT1 could accelerate apoptosis in pediatric AML.	Liang et al. (2020)
RB	KCNQ1OT1 (UP)	3 pairs of RB and ANTs, nude mice	hTERT RPE-1, Y79, WERI-Rb-1	(Down)	miR-124, SP1, Cyclin-D1, Caspase-3, Vimentin, E/N-cadherin,	KCNQ1OT1 by targeting the miR-124/SP1 axis could modulate RB cell proliferation and invasion	Zhang et al. (2021d)

SIRT1-interacting circRNAs

Circular RNAs (CircRNAs) are common in all animals, from viruses to mammals. They are single-stranded, endogenous covalently closed RNA molecules with highly stability. The biosynthesis, regulation, localization, destruction, and modification of circRNAs have all seen great progress (Sayad et al., 2022). CircRNAs play a role in a wide range of human disorders, particularly malignancies (Ghafouri-Fard et al., 2021b; Ghafouri-Fard et al., 2022). The impact of SIRT1-interacting circRNAs in the regulation of SIRT1 has been assessed in diabetes and its complications, rheumatoid arthritis, chronic cerebral ischemia, osteoarthritis, intervertebral disc degeneration as well as malignant disorders, particularly glioma (Table 2). All of these circRNAs have been shown to act as molecular sponges for miRNAs to subsequently affect expression of miRNAs targets (Figure 2). For instance, hsa_circ_0115355 has been found to regulate activity of miR-145/SIRT1 axis, thus enhancing function of pancreatic *β* cells in patients with type 2 diabetes mellitus (Dai et al., 2022). CircHIPK3 is another circRNA which participates in the pathogenesis of diabetic complications. Expression of this circRNA has been significantly reduced in HK-2 cells following exposure with high glucose. Forced upregulation of circHIPK3 could reverse high glucose-induced pathologic events in HK-2 cells. SIRT1 has been found to be the target of miR-326 and miR-487a-3p, two downstream genes of circHIPK3. Silencing of these two miRNAs could induce proliferation and decrease apoptosis in high glucose-induced HK-2 cells. Taken together, upregulation of circHIPK3 can reduce the effects of high glucose in HK-2 cells via sponging miR-326 or miR-487a-3p and influencing expression of SIRT1 (Zhuang et al., 2021).

Hsa_circ_0044235 is another circRNA which has been shown to be downregulated in patients with rheumatoid arthritis (RA). Downregulation of this circRNA has been correlated with low levels of SIRT1 expression in these patients. Overexpression of hsa_circ_0044235 could attenuate joint inflammation, cell apoptosis, and joint injury, and reduce NLRP3-mediated pyroptosis but increasing SIRT1 expression. Upregulation of this circRNA could also inhibit caspase-1 content. Mechanistically, hsa_circ_0044235 increases expression of SIRT1 through sponging miR-135b-5p (Chen et al., 2021).

CircularNOP10 and circ0082374 are two putative oncogenic circRNAs that regulate expression of SIRT1. CircularNOP10 has a role in induction of progression of gastric cancer through

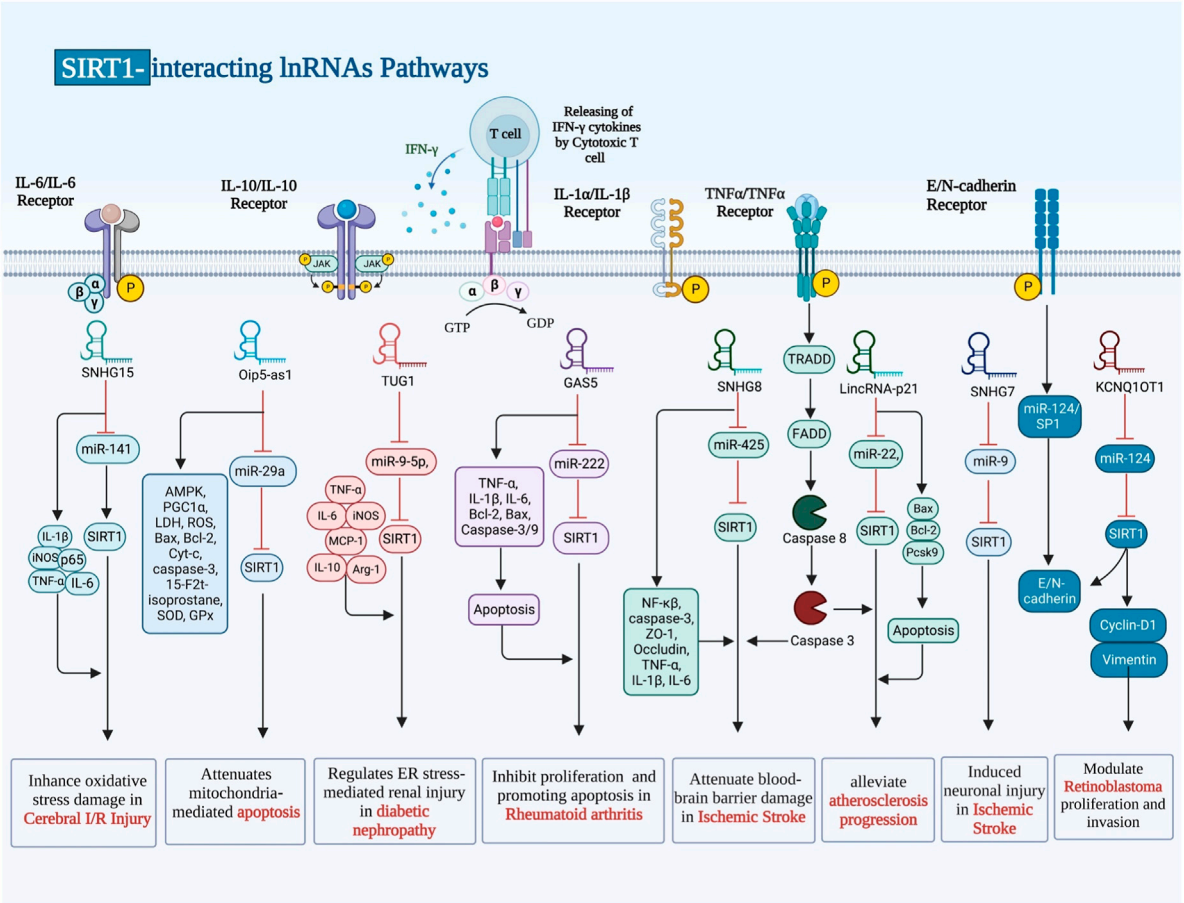

FIGURE 3
There are numerous ways in which SIRT1 and the other lnRNAs interact, and it has been demonstrated that these interactions have an impact on pathogenesis conditions.

regulation of miR-204/SIRT1 pathway (Xu J. et al., 2021). In glioma cells, circ0082374 has a role in induction of cell viability, migration, invasion and glycolysis through regulation of miR-326/SIRT1 axis (Wang et al., 2020).

SIRT1-interacting lncRNAs

Transcripts larger than 200 nt are known as long non-coding RNAs (lnRNAs), which cannot code for proteins and may possess small open reading frames (ORFs). Because they interact with various proteins, mRNAs and DNA sequences, lncRNAs play significant roles in a number of disorders (Sabaie et al., 2021; Hussen et al., 2022b). GAS5, LincRNA-p21, MCM3AP-AS1, TUG1, SNHG7, SNHG8, SNHG10, SNHG15, Oip5-as1, ILF3-AS1, ANRIL, UCA1 and KCNQ1OT1 are examples of lncRNAs that regulate expression of SIRT1 through sponging miRNAs. These lncRNAs can affect pathogenesis of RA, atherosclerosis, sepsis-associated renal injury (SARI), diabetic nephropathy, ischemic stroke and a number of malignant conditions (Table 3). For instance, GAS5 via regulating the miR-222-3p/Sirt1 axis could alleviate RA (Yang et al., 2021). Moreover, GAS5 via inhibiting the miR-579-3p and activating the SIRT1/PGC-1α/Nrf2 axis could reduce cell pyroptosis in SARI (Ling et al., 2021). In the context of osteoarthritis, MCM3AP-AS1 via modulating the miR-138-5p/SIRT1 axis could protect chondrocytes from IL-1β-induced inflammation (Shi et al., 2021).

SIRT1-interacting lncRNAs have also been shown to affect pathogenesis of malignant conditions. For instance, SNHG10 has been found to sponge miR-543 in non small cell lung cancer (Zhang Z. et al., 2020). Moreover, SNHG7 has been demonstrated to inhibit NLRP3-associated pyroptosis through regulating miR-34a/SIRT1 axis in liver cancer (Chen Z. et al., 2020). GAS5 can inhibit malignant progression of colorecatl cancer cells through regulating macroautophagy and forming a negative feedback loop with the miR-34a/mTOR/SIRT1 axis (Zhang HG. et al., 2021). On the other hand, UCA1 has a role in induction of cell proliferation and suppression of apoptosis through affecting expression of SIRT1 and miR-204 in pediatric AML (Liang et al., 2020). The known interactions that SIRT1 has with a variety of lnRNAs are illustrated in Figure 3.

A number of therapeutic agents such as anthocyanins, ginsenoside-R3, dexmedetomidine hydrochloride, berberine, sorafenib, 17β-Estradiol, phenylpyridinium, tetrahydroxy stilbene glycoside, cisplatin, resveratrol, sulforaphane and liraglutide have been found to affect expression of non-coding RNAs/SIRT1 axes (Table 4). For instance, experiments in animal model of asthma

TABLE 4 Effects of drugs on SIRT1-interacting ncRNAs.

Type of diseases	Drug	Non-coding RNAs	Sample	Cell line	SIRT1 expression	Target	Discussion	Ref
Asthma	Anthocyanins (Anth)	miR-138-5p (Up)	Balb/c mice; treated with 250 mg/kg Anth before each atomization for 1 h	HBE; treated with 10 μg/mL Anth for 1 h	(-)	NF-κβ p65, IL-4/5/13, IFN-γ	Anth via targeting the miR-138-5p/SIRT1 axis by downregulating NF-κβ could inhibit airway inflammation in asthmatic mice	Liu et al. (2022b)
Sepsis	Ginsenoside-R3 (Rg3)	TUG1 (-), miR-200c-3p	C57BL/6 mice; treated with 20 mg/kg Rg3, I.P, for 1 h	Hepatocyte; pretreated with 25 μM Rg3 for 6 h	(Down)	LC3-I/II, p62, Beclin-1, PGC1-α, AMPK	Rg3 by modulating the TUG1/miR-200c-3p/SIRT1 axis could alleviate septic liver injury	Wu et al. (2021b)
LI	Dexmedetomidine hydrochloride (DEX)	TUG1 (-), miR-194	-	WRL-68; pretreated with 0.01, 0.1, and 1, 5 nM DEX for 1 h	(Down)	Bax, Bcl-2, TNF-α, IL-1β, IL-6	DEX by activating the TUG1/miR-194/SIRT1 axis could inhibit hepatocyte inflammation and apoptosis	Gu et al. (2021)
Insulin resistance	Berberine (BBR)	miR-146b (-)	C57BL/6J mice; treated with 5, 10 mg/kg/day, I.P, for 4 weeks,	HepG2; treated with 5–30 μM BBR for 24h and 48 h	(Down)	FOXO1	BBR by regulating the miR-146b/SIRT1 axis could ameliorate hepatic insulin resistance	Sui et al. (2021)
Liver cancer	Sorafenib	miR-425 (-)	TCGA and GEO databases	HepG2, PLC, Hep3B, Huh7, MIHA; treated with 10 μM for 48 h	(-)	LC3-I/II, ATGL	miR-425 via SIRT1 to promote sorafenib resistance could regulate lipophagy in liver cancer	Sun et al. (2021)
PMOP	17β-Estradiol (E2)	H19 (Down), miR-532-3p	Bone tissue (n = 10), serum samples (n = 10), control group (n = 10), Wistar rats; treated with 0.5 mg/kg/day E2 subcutaneously	BMSCs; treated with 10^{-7} M E2 for 14 days	(Down)	ALP, RUNX2,	E2 via targeting the miR-532-3p/SIRT1 axis could enhance the expression of H19 to regulate osteogenic differentiation	Li et al. (2021c)
PD	Phenylpyridinium (MPP)	miR-132 (-)	FVB littermate wild-type mice	SH-SY5Y; treated with 1.25 and 2.5 mM MPP, for 12, 24, 48 h	(Down)	p53, NF-κB	Upregulation of miR-132 via activating the SIRT1/p53 axis could induce PD.	Qazi et al. (2021)
-	Tetrahydroxy Stilbene Glycoside (TSG)	miR-34a (Up)	-	HUVECs; pretreated with 20, 40 μg/ml TSG for 24 h	(Down)	PAI-1, p21	TSG via targeting the miR-34a/SIRT1 axis could attenuate endothelial cell premature senescence	Zhang et al. (2022)
AKI	Cisplatin (DDP)	miR-132-3p (-)	C57BL/6J mice; treated with 20 mg/kg DDP for 24, 48 h	HK-2; treated with 5 μg/ml DDP for 24, 48 h	(Down)	NF-κβ,	miR-132-3p via targeting NF-κβ by modulating SIRT1 could promote DDP-induced apoptosis in renal tubular epithelial cells	Han et al. (2021)
BLC	DDP	MST1P2 (-), miR-133b	-	SW 780/DDP, RT4/DDP	(-)	p53, Caspase-3	LncRNA MST1P2/miR-133b axis via the SIRT1/p53 axis can influence chemoresistance to DDP-based therapy	Chen et al. (2020d)

(Continued on following page)

TABLE 4 (*Continued*) Effects of drugs on SIRT1-interacting ncRNAs.

Type of diseases	Drug	Non-coding RNAs	Sample	Cell line	SIRT1 expression	Target	Discussion	Ref
-	Resveratrol (RSV)	miR-155 (-)	-	N9; treated with 10 μM RSV for 1 h	(-)	AMPK, NLRP3, NF-κβ, IL-1β, IL-18	RSV via targeting the SIRT1/AMPK axis could inhibit NLRP3 inflammasome-induced pyroptosis and miR-155 expression in microglia	Tufekci et al. (2021)
-	Sulforaphane (SFN)	miR-34a (Up)		HUVECs; pretreated with 1.0 μmol/l SFN for 4, 8, 12 h	(-)	Nrf2, ARE	SFN via modulating the miR-34a/SIRT1 axis by upregulating Nrf2 could protect endothelial cells from oxidative stress	Li et al. (2021d)
DN	Liraglutide (LRG)	miR-34a (-)	SD rats; treated with 6 mg LRG subcutaneously for 12 weeks	-	(-)	AST, ALT, HIF-1α, Egr-1, TGF-β1	LRG via targeting the miR-34a/SIRT1 axis could regulate kidney and liver in DN rats	Xiao et al. (2021)

have shown that anthocyanins suppresses inflammatory responses in airways through decreasing activity of NF-κB pathway via the miR-138-5p/SIRT1 axis (Liu Y. et al., 2022). Moreover, ginsenoside Rg3 can alleviate sepsis-related hepatic injury through modulation of TUG1/miR-200c-3p/SIRT1 axis (Wu P. et al., 2021). TUG1/miR-194/SIRT1 axis has been found to be targeted by dexmedetomidine hydrochloride to inhibit hepatocytes apoptosis and inflammatory responses (Gu et al., 2021). Additionally, the effects of berberine in amelioration of hepatic insulin resistance have been revealed to be mediated through regulation of miR-146b/SIRT1 axis (Sui et al., 2021).

Discussion

SIRT1 has a role as a deacetylase and is able to deacetylate a range of substrates. Thus, it participates in the regulation of a wide array of physiological processes such as gene expression, metabolic pathways and aging (Haigis and Guarente, 2006; Michan and Sinclair, 2007). This protein has functional interactions with lncRNAs, miRNAs and circRNAs. In fact, a complicated network exists between these non-coding RNAs and SIRT1. Hsa_circ_0115355/miR-326, hsa_circ_0115355/miR-487a-3p, HIPK3/miR-145, hsa_circ_0044235/miR-135b-5p, circ_0000296/miR-194-5p, circ_0001103/miR-375, CIDN/miR-34a-5p, NOP10/miR-204, circ-0082374/miR-324 are examples of circRNA/miRNA pairs that interact with SIRT1. Similarly, GAS5/miR-222-3p, GAS5/miR-579-3p, GAS5/miR-34a, MCM3AP-AS1/miR-138-5p, TUG1/miR-9-5p, TUG1/miR-29c-3p, TUG1/miR-204, SNHG8/miR-425-5p, SNHG7/miR-9, SNHG7/miR-34a, SNHG15/miR-141, SNHG10/miR-543, Oip5-as1/miR-29a, ILF3-AS1/miR-212-3p, ANRIL/miR-7-5p, UCA1/miR-204 and KCNQ1OT1/miR-124 are lncRNA/miRNA pairs that regulate expression of SIRT1 in different contexts. These interactions are possibly involved in the pathoetiology of a number of human disorders such as sepsis, cardiomyopathy, heart failure, non-alcoholic fatty liver disease, chronic hepatitis, cardiac fibrosis, myocardial ischemia/reperfusion injury, diabetes, ischemic stroke, immune-related disorders and cancers. In cancers, SIRT1-interacting non-coding RNAs not only affect cell proliferation but also regulate stemness and immunosuppressive responses in the tumor niche.

SIRT1 is a potential target for design of novel therapies. Most importantly, a number of drugs used for treatment of diverse asthma, sepsis, liver injury, insulin resistance, postmenopausal osteoporosis, Parkinson's disease, diabetic nephropathy and cancers exert their effects through modulation of non-coding RNAs/SIRT1 axis. Thus, identification of the interactions between non-coding RNAs and SIRT1 has practical significance in design of novel therapeutic strategies for diverse disorders. Remarkably, non-coding RNAs that modulate expression of SIRT1 are putative modulators of the response of patients to different drugs.

Author contributions

SG-F wrote the manuscript and revised it. MT and GS supervised and designed the study. HS, YP, and BH collected the data and designed the figures and tables. All authors read and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Publisher's note

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Glossary

ANT	Adjacent normal tissue
PPARα	peroxisome proliferators-activated receptor *a*
CTRP6	C1q/Tumor Necrosis Factor-Related Protein-6
HMGCoAR	β-Hydroxy *ß*-Methylglutaryl-CoA Reductase
hUC-MSCs	Human Umbilical Cord-Derived Mesenchymal Stem Cell
HBE	Human Bronchial Epithelial
hTERT RPE-1	Human Retinal Pigment Epithelial Cell Line
FLSs	Fibroblast-Like Synoviocytes
PBMCs	Human Peripheral Blood Mononuclear Cells
BMSCs	Human Bone Marrow Mesenchymal Stem Cells
ALP	Alkaline Phosphatase
MPO	Myeloperoxidase
CECs	Cardiac Endothelial Cells
CFs	Cardiac Fibroblasts
CMs	Cardiomyocytes
CGRP	Calcitonin Generated Peptide
PGE2	Prostaglandin E2
BMEC	Microvascular Endothelial Cell
HASMCs	Human Aortic Smooth Muscle Cells
HIF-1α	Hypoxia-Inducible Factor-1 *a*
Egr-1	Early Growth Response-1
UA	Uric Acid
UREA	Urea
Nrf2	Nuclear Factor Erythroid-2-Related Factor 2
ARE	Antioxidant Response Element
NRVMs	Neonatal Rats Ventricular Myocytes
BMSCs	Bone Marrow Mesenchymal Stem Cells
hRMEC	Human Retinal Microvascular Endothelial Cells
CUMS	Chronic Unpredictable Mild Stress
HBDH	Hydroxybutyratse Dehydrogenase
CK-MB	Creatine Kinase MB Activity
NHSF	Normal Human Skin Fibroblast
RA	Rheumatoid Arthritis
AS	Atherosclerosis
SARI	Sepsis-associated renal injury
AML	Pediatric acute myeloid leukemia
HCC	Hepatocellular carcinoma
NSCLC	Non-small cell lung cancer
I/R	Ischemia-reperfusion
NAFLD	Non-Alcoholic Fatty Liver Disease
CHC	Chronic Hepatitis C
SCMP	Septic Cardiomyopathy
CHF	Congestive Heart Failure
HF	Heart Failure
MI	Myocardial Injury
AMI	Acute Myocardial Infarction
AKI	Osteoarthritis (OA), Acute Kidney Injury
DN	Diabetic Nephropathy
DR	Diabetic Retinopathy
SCI	Spinal Cord Injury
CCIS	Chronic Constriction Injury of Sciatic Nerve
MDD	Major Depressive Disorder
DFUs	Diabetic Foot Ulcers
ALI	Acute Lung Injury
UUO	Unilateral Ureteral Obstruction
PCa	Pancreatic Cancer
CRC	Colorectal Cancer
cSCC	Cutaneous Squamous Cell Carcinoma
RB	retinoblastoma
T2DM	Type 2 Diabetes Mellitus
CCI	Chronic Cerebral Ischemia
IDD	Intervertebral Disc Degeneration
GC	Gastric Cancer
LI	Liver Injury
HIR	Hepatic Insulin Resistance
PMOP	Postmenopausal osteoporosis
PD	Parkinson's Disease
BLC	Bladder Cancer

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MIR31HG, a potential lncRNA in human cancers and non-cancers

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Long non-coding RNAs have recently attracted considerable attention due to their aberrant expression in human diseases. LncMIR31HG is a novel lncRNA that is abnormally expressed in multiple diseases and implicated in various stages of disease progression. A large proportion of recent studies have indicated that MIR31HG has biological functions by triggering various signalling pathways in the pathogenesis of human diseases, especially cancers. More importantly, the abnormal expression of MIR31HG makes it a potential biomarker in diagnosis and prognosis, as well as a promising target for treatments. This review aims to systematically summarize the gene polymorphism, expression profiles, biological roles, underlying mechanisms, and clinical applications of MIR31HG in human diseases.

KEYWORDS

MIR31HG, long non-coding RNA, expression, biological function, signalling pathways

1 Introduction

Cancer is one of the leading causes of death worldwide with increasing incidence and mortality. Based on recent cancer statistics in 185 countries, 19.3 million new cases were diagnosed in 2021, and 10.0 million cancer patients died (Sung et al., 2021). In various types of cancers, abnormal expression of long non-coding RNAs (lncRNAs) can be detected, which is supposed to be related to proliferation, invasion, metastasis, and other biological aspects of cancer (Kopp and Mendell, 2018; Gyamfi et al., 2022; Vervoort et al., 2022). LncRNAs, more than 200 nucleotides in length, are non-coding RNA molecules that lack an open reading frame (Birney et al., 2007; Derrien et al., 2012). Unlike microRNAs(miRNAs), they cannot encode any protein but regulate chromatin dynamics, gene expression, growth, differentiation, and development due to their special length (Wang and Chang, 2011; Roberts et al., 2014). Several recent studies have indicated that lncRNAs play numerous roles in human malignant tumours (Cao et al., 2019; Zhou et al., 2021; Yang et al., 2022a).

LncRNA MIR31HG, also known as long non-coding HIF-1α coactivating RNA (LncHIFCAR) or LOC554202, is located in 9p21.3 with 2,166 bp in length and acts as a host gene for miR-31 in intron 2 (Augoff et al., 2012; Yan et al., 2018). We shed light on this recently-discovered lncRNA because several reports have shown that the lncRNA MIR31HG is aberrantly expressed in different cancers and affects numerous biological processes, including proliferation, metastasis, epithelial-mesenchymal transition (EMT), cellular senescence, and apoptosis in tumour development (Gupta et al., 2020; Tu et al., 2020). An increasing number of studies have also reported it can also participate in some signalling pathways (Zhang et al., 2021; Feng et al., 2022). Furthermore, the different functions performed by lncRNA MIR31HG depend on the tumour types and pathways involved. The complexity of these pathways presents great challenges but provides opportunities for the discovery of original cancer therapeutic targets and potential diagnostic biomarkers.

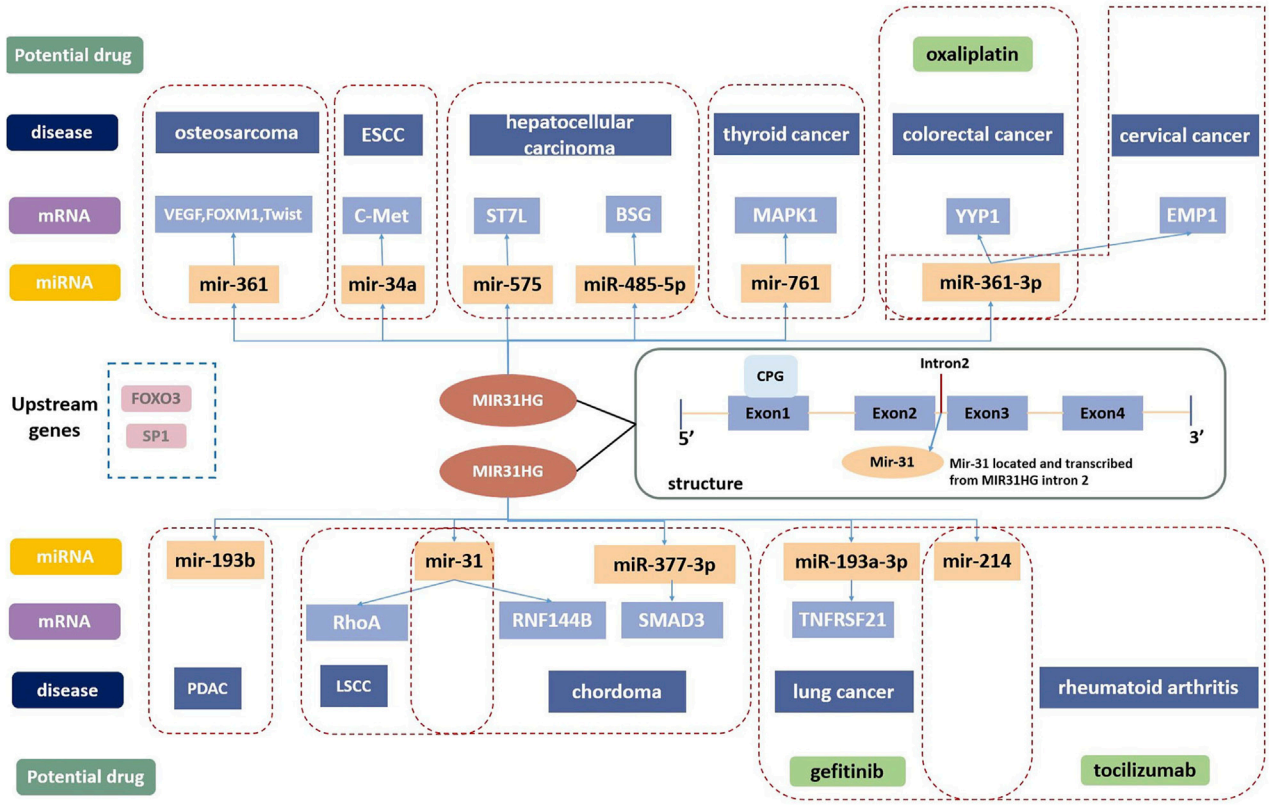

FIGURE 1
The structure of MIR31HG contains four exons and three junctions. Mir-31 is located in intron2. SP1 and FOXO3 are the upstream genes of MIR31HG. MIR31HG plays an important role in the lncRNA-miRNA-mRNA ceRNA network in disease development. MIR31HG can serve as a therapeutic target, and oxaliplatin, gefitinib and tocilizumab are related potential drugs for human diseases.

Recently, more evidence has also shown that MIR31HG also participates in other diseases in addition to cancer, such as psoriasis, IgA nephropathy (IgAN), hirschsprung's disease, rheumatoid arthritis (RA), and osteonecrosis of the femoral head (ONFH) (Cai et al., 2018; Gao et al., 2018; Yuan et al., 2020; Cao et al., 2021; Liu et al., 2022a).

This review aims to elaborate on the gene polymorphism, aberrant expression levels, biological roles, related mechanisms, and potential clinical applications of lncRNA MIR31HG in human diseases and concludes its function as a biomarker for the diagnosis, prediction, and treatment of human diseases.

2 MIR31HG gene polymorphisms

MIR31HG was first mentioned to participate in the translation process of mir31 and proved to be the host gene of mir31 in 2009 (Corcoran et al., 2009). MIR31HG contains four exons and three junctions, and mir31 is possibly located in intron2 (Augoff et al., 2012; Shih et al., 2017). A study in 2012 also assumed that there could be a large CPG island in its promoter, and showed the role of MIR31HG in promoting hypermethylation in human cancer (Augoff et al., 2012) (Figure 1).

Single nucleotide polymorphisms (SNPs) are involved in developing the role of specific genes in disease occurrence, and several recent analyses have revealed the supportive role of MIR31HG gene variations in the susceptibility of human diseases. Rs10965059, rs72703442, rs55683539, rs1332184, rs2181559, rs10965064, and rs2025327 were common SNPs used for MIR31HG polymorphism analyses (Daly et al., 2001). As an intronic variant of MIR31HG, rs10965059 is a crucial SNP because it was associated with susceptibility to various diseases including LDH, IgA nephropathy, steroid-induced osteonecrosis, and alcohol-induced osteonecrosis (Yuan et al., 2020; Liu et al., 2022a; Wang et al., 2022a; Hu et al., 2022). Aside from these non-cancers, MIR31HG polymorphisms also affect breast cancer (Wei et al., 2023). In Chinese women, three SNPS, rs72703442-AA, rs55683539-TT, and rs2181559-AA, were related to a lower risk of breast cancer (BC), while rs55683539 was considered the best risk-predictive single-locus model. According to estrogen receptor (ER) and progesterone receptor (PR) status analysis, rs79988146 was a relative gene variant for ER-positive and PR-positive breast cancer patients.

3 The expression level of MIR31HG in human diseases

Considerable research in recent years has continually investigated MIR31HG expression levels in cancer, including

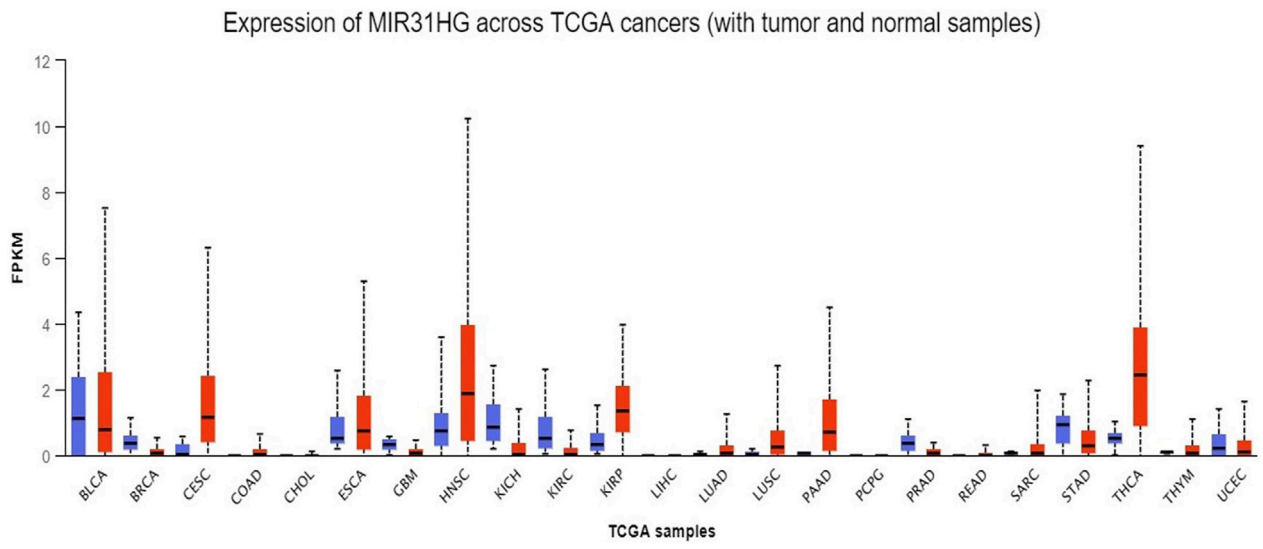

FIGURE 2
A pan-cancer analysis of MIR31HG expression in various cancers shows the overexpression of MIR31HG in BRCA, CESC, COAD, HNSC, LUAD, PAAD and THCA. Lower expression is found in KIRC and UCEC.

gastric cancer (Nie et al., 2016; Lin et al., 2018), breast cancer (Augoff et al., 2012; Shi et al., 2014; Xin et al., 2021), lung cancer (Qin et al., 2018; Dandan et al., 2019; Zheng et al., 2019), colorectal cancer (Ding et al., 2015; Yang et al., 2016a; Li et al., 2018; Eide et al., 2019), bladder cancer (He et al., 2016; Sveen et al., 2020), head and neck squamous cell carcinoma (Ren et al., 2017; Wang et al., 2018a; Chu et al., 2020), osteosarcoma (Sun et al., 2019), melanoma (Xu and Tian, 2020) and other cancer types (Yang et al., 2016b; Shih et al., 2017; Feng et al., 2020; Li, 2020; Chen et al., 2022; Ko et al., 2022; Tu et al., 2022). A pan-cancer analysis of gene expression was performed by the UALCAN database using The Cancer Genome Atlas (TCGA) data to determine the expression of MIR31HG in human cancers (Figure 2). Furthermore, MIR31HG expression seemed to share a strong relationship with clinical characteristics in a number of cancers, such as tumour node metastasis (TNM), differentiation, distant metastasis, disease-free survival (DFS), and overall survival (OS) (Table 1). In addition to human cancer, in specific human non-cancer MIR31HG overexpression was able to be found in psoriasis (Gao et al., 2018), IgAN (Yuan et al., 2020), RA, and ONFH (Cao et al., 2021). However, it is downregulated in hirschsprung's disease (Cai et al., 2018). MIR31HG significantly influenced this human non-cancer with pathological progression and clinical traits (Table 2).

3.1 The expression of MIR31HG in cancers

3.1.1 Pan-cancer analysis of MIR31HG expression

First, to determine the expression of MIR31HG in human cancers, the UALCAN database was used to investigate the pancancer expression of MIR31HG (Chandrashekar et al., 2017). Based on the data from TCGA, overexpression of the lncRNA MIR31HG was discovered in breast invasive carcinoma (BRCA), cervical squamous cell carcinoma and endocervical adenocarcinoma (CESC), colon adenocarcinoma (COAD), head and neck squamous cell carcinoma (HNSC), lung adenocarcinoma (LUAD), pancreatic adenocarcinoma (PAAD) and thyroid carcinoma (THCA). On the contrary, lower MIR31HG expression was found in kidney renal clear cell carcinoma (KIRC) and uterine corpus endometrial carcinoma (UCEC). As this is a simple analysis only dependent on the database, we further examined the MIR31HG expression in various types of cancers by searching related experiments and clinical research.

3.1.2 MIR31HG dysregulation in gastric cancer

Gastric cancer is the third most prevalent cancer all over the world, and its incidence and mortality have remained high in recent years (Thrift and El-Serag, 2020; Ajani et al., 2022; Yeoh and Tan, 2022). According to Nie's research, a low expression level of MIR31HG was discovered in gastric cancer tissues compared with adjacent normal tissues. Its downregulated expression level was associated with larger tumour size, advanced pathological stage, and relatively poor prognosis (Nie et al., 2016). In contrast, MIR31HG was found to be overexpressed in gastric cancer in Lin's study, especially in HGC27 and MGC-803 cell lines (Lin et al., 2018). On the one hand, MIR31HG played an important role in promoting cell proliferation and migration in MGC-803. On the other hand, in HGC27 cells, MIR31HG inhibited cell proliferation and migration, demonstrating the diverse functions of MIR31HG in different gastric cancer cell lines.

3.1.3 MIR31HG dysregulation in breast cancer

Breast cancer accounts for nearly 25% of cancers in women and is currently the most lethal cancer in females worldwide (Bray et al., 2018; Wang et al., 2021a). The expression of lncRNA MIR31HG was upregulated in various types of breast cancers (Shi et al., 2014; Xin et al., 2021), while MIR31HG was downregulated in triple-negative breast cancer (Augoff et al., 2012). For the first time, Shi et al. (2014) showed that MIR31HG knockdown could result in diminished cell proliferation by modulating the G1–S checkpoint and apoptosis in breast cancer. Experimental evidence from another study also indicated that silencing MIR31HG can suppress breast cancer proliferation migration, and invasion by targeting polymerase

TABLE 1 The expression, related functions, clinical features and related genes of MIR31HG in cancers.

NO	Cancer types	Regulation	Related functions	Clinical features	Related genes	Source of evidence	Ref
1	Gastric cancer	Up Down	Proliferation, migration	Poor prognosis	—	Cell line studies	Nie et al. (2016), Lin et al. (2018)
						Cell line, animal, and clinical studies	
2	Breast cancer	Up Down (TNBC)	Proliferation, migration	Tumour size, clinical stage	POLDIP2	Cell line, animal, and clinical studies	Augoff et al. (2012), Shi et al. (2014), Xin et al. (2021)
3	Lung cancer	Up	Proliferation, migration, invasion, metastasis, apoptosis, EMT	Clinical stage, TNM and OS	Mir-31 Mir-214	Cell line, animal, and clinical studies	Qin et al. (2018), Dandan et al. (2019), Zheng et al. (2019)
4	Colorectal cancer	Up Down	Proliferation, apoptosis, cell cycle, angiogenesis, glycolysis	OS and DSS Tumour size and pathologic stage	Mir-361-3p	Cell line, animal, and clinical studies	Ding et al. (2015), Yang et al. (2016a), Li et al. (2018), Eide et al. (2019)
5	Bladder cancer	Up Down	Proliferation, migration	TNM stage, OS and DFS	-	Cell line and clinical studies	He et al. (2016), Sveen et al. (2020)
6	ESCC	Up Down	Proliferation, migration, invasion	TNM, distant metastasis; differentiation, OS	Mir-34a/c-met	Cell line, animal, and clinical studies	Ren et al. (2017), Chu et al. (2020)
7	LSCC	Up	Proliferation, tumourigenesis, apoptosis	Advanced T category, OS, and RFS	Mir-31, RhoA HIF1A and P21	Cell line, animal, and clinical studies	Wang et al. (2018a)
8	Osteosarcoma	Up	Proliferation, invasion, migration, apoptosis, EMT	Metastasis	—	Cell line, animal, and clinical studies	Sun et al. (2019)
9	Melanoma	Up	Proliferation, invasion, migration	Metastasis, TNM stage	—	Cell line and clinical studies	Xu and Tian (2020)
10	Thyroid cancer	Up	Proliferation, invasion, migration, and apoptosis	Tumour size, lymph node metastasis	Mir-761	Cell line and clinical studies	Chen et al. (2022)
11	Cervical cancer	Up	Cell growth and invasion	Tumour size, lymph node metastasis and OS	Mir-361-3p,EMP1	Cell line, animal, and clinical studies	Li (2020)
12	Oral cancer	Up	Proliferation, invasion, and wound healing	Poor survival in stage IV, node metastasis in stage I-III	Mir-31, HIF-1α	Cell line, animal, and clinical studies	Shih et al. (2017), Tu et al. (2022)
13	PDAC	Up	Proliferation, invasion, apoptosis and EMT	DFS	Mir-193b	Cell line, animal, and clinical studies	Yang et al. (2016b), Ko et al. (2022)
14	NPC	Up	Proliferation, migration, and invasion	-	Mir-31/hroA	Cell line study	Feng et al. (2020)

TABLE 2 The expression, related functions, clinical features of MIR31HG in non-cancers.

NO	1	2	3	4	5
Non-cancer types	Psoriasis	IgA nephropathy	Hirschsprung's disease	Rheumatoid arthritis	Osteonecrosis of femoral head
Regulation	Up	Up	Down	Up	Up
Related functions	Proliferation and cell cycle	—	Proliferation and migration	Proliferation, migration, inflammation	—
Clinical features	Diagnosis	Susceptibility	—	Treatment	Susceptibility
Source of evidence	Cell line study	Silico analysis	Cell line study	Cell line study	Silico analyses
Ref	Gao et al. (2018)	Yuan et al. (2020)	Cai et al. (2018)	Cao et al. (2021)	Liu et al. (2022a), Wang et al. (2022a)

(DNA-directed), delta interacting protein 2(POLDIP2) (Xin et al., 2021). MIR31HG expression was notably upregulated in 20 breast cancer tissues in two cell lines (MDA-MB-231 and MDA-MB435S) collected from BC patients who received surgical resection, contrasting with the former result in 2012 (Augoff et al., 2012). Functionally, executed as an oncogene, MIR31HG influences the

apoptotic, proliferative, and invasive capabilities, as well as tumour size and clinical stage in breast cancer (Shi et al., 2014).

3.1.4 MIR31HG dysregulation in lung cancer (LC)

It was estimated that nearly 2 million new lung cancer cases occurred and caused 1.76 million deaths in the past year, proposing the severity of LC worldwide (Brody, 2020; Thai et al., 2021). A great number of studies have demonstrated that MIR31HG overexpression in lung cancer tissues and related cell lines (A549, H2228, H1975, H1229) (Qin et al., 2018; Tu et al., 2020). A study on 152 tissue samples consisting of both lung adenocarcinoma and adjacent normal samples revealed that MIR31HG was related to advanced clinical staging and TNM stage (Qin et al., 2018). Zheng et al. (2019) performed research with 88 patients and demonstrated that the expression of MIR31HG also exhibited a close relationship with histological differentiation grade and lymph node metastasis in non-small cell lung cancer (NSCLC). In agreement with Zheng's result, patients with MIR31HG overexpression are likely to have unfavourable OS (Dandan et al., 2019).

3.1.5 MIR31HG dysregulation in colorectal cancer (CRC)

Colorectal cancer is the third most prevalent malignant tumour and the second most common cause of cancer deaths worldwide (Ciardiello et al., 2022; Sinicrope, 2022). For the first time, a study revealed that MIR31HG expression was lower than that in non-cancerous colorectal tissues, and another study from Yang et al. involving 178 samples also supported this finding (Ding et al., 2015; Yang et al., 2016a). Patients with lower MIR31HG appeared to have a worse outcome of OS and DFS in colorectal cancer (Yang et al., 2016a). Contrary to the former study, emerging evidence has shown an elevated level of MIR31HG in CRC tissues compared with adjacent tissues (Li et al., 2018; Wang et al., 2022b). Moreover, in association with miR-31-5p, patients with MIR31HG outlier status had shorter relapse-free survival (RFS), highlighting the prognostic role of MIR31HG in colorectal cancer (Eide et al., 2019). Furthermore, in an experiment on 221 patients treated with oxaliplatin, the high performance of MIR31HG was also associated with high DFS and OS rates (Li et al., 2018).

3.1.6 MIR31HG dysregulation in bladder cancer

As the fourth most common malignancy in men and the most common malignancy in women, bladder cancer has been associated with high mortality and morbidity (Lenis et al., 2020). Growing evidence shows that MIR31HG expression is suppressed in bladder cancer and was consistent with the pan-cancer analysis (He et al., 2016). This study on 55 samples revealed that a lower level of MIR31HG was linked with advanced TNM stage, while another study indicated that in patients with the basal subtype, MIR31HG overexpression was discovered and correlated with poor OS and DFS (Sveen et al., 2020). The differentiation may be caused by different subtypes of bladder cancer in those studies.

3.1.7 MIR31HG dysregulation in head and neck squamous cell carcinoma (HNSCC)

As one of the most lethal cancers in the world, esophageal squamous cell carcinoma (ESCC) accounts for nearly 90% of new esophageal cancer cases per year (Abnet et al., 2018; Yang et al., 2020). Based on research including 185 samples, lower MIR31HG expression was detected in ESCC tissues from patients with ESCC compared with the control. A lower MIR31HG level was associated with worse differentiation, advanced lymph node metastasis, positive distant metastasis, and poorer OS (Ren et al., 2017). In the other two studies, MIR31HG expression was found to be upregulated in ESCC (Sun et al., 2018; Chu et al., 2020). Fifty-three blood samples from ESCC patients and 39 blood samples from healthy people were collected in Sun's study which proposed that higher expression of MIR31HG was positively related to advanced TNM stage and lymphatic metastasis. Moreover, they discovered that MIR31HG could distinguish ESCC patients from healthy individuals through ROC curve analysis in plasma, indicating the role of MIR31HG in diagnosis (Sun et al., 2018).

Laryngeal squamous cell carcinoma (LSCC) remains one of the most common HNSCCs and leads to approximately 20% of all cases (Mody et al., 2021). Indeed, MIR31HG expression was reported to be upregulated in LSCC. One study *in vitro* and *in vivo* revealed that combined with HIF1α (hypoxia-inducible factor 1α) and P21, MIR31HG significantly accelerated cell growth and impaired apoptosis. Furthermore, in this experiment, sixty LSCC patients were divided into two groups according to diverse expression levels, and patients with lower MIR31HG expression had significantly better OS and RFS. Therefore, more research with a larger sample size is demanded to clarify the function of MIR31HG in HNSCC (Wang et al., 2018a).

3.1.8 MIR31HG dysregulation in osteosarcoma

Osteosarcoma, the most frequent type of primary malignant bone tumour, occurs mostly in adolescents and young adults (Kansara et al., 2014). With the development of curative therapy, wide resection surgery combined with chemo radiotherapy was applied and significantly improved the 5-year survival rate, but remains insufficient (Gill and Gorlick, 2021; Meltzer and Helman, 2021). An elevated level of MIR31HG was discovered in osteosarcoma samples. In detail, high expression of MIR31HG was associated with poor tumour stages and distant metastasis. Loss of MIR31HG further inhibited miR-361, which is a tumour suppressor that inhibits cell proliferation and migration. Except for miR-361, its downstream genes, such as vascular endothelial growth factor (VEGF), forkhead box protein 1(FOXM1), and Twist, were also suppressed in osteosarcoma cells, resulting in EMT and tumour growth (Sun et al., 2019). In summary, MIR31HG exerted oncogenic function by directly regulating miR-361 for tumour growth.

3.1.9 MIR31HG dysregulation in melanoma

Malignant melanoma (MM) is known as a fatal malignant tumour caused by mutant melanocyte proliferation (Nassar and Tan, 2020). One study demonstrated that the expression of MIR31HG was significantly upregulated in melanoma. Abundant expression of MIR31HG was significantly correlated with lymph node metastasis, distal metastasis, and TNM stage, and served as a prognostic biomarker for MM (Xu and Tian, 2020).

3.1.10 MIR31HG and other cancers

Based on research containing 57 papillary thyroid cancer (PTC) with a reference sample and four relative adjacent normal thyroid tissues, MIR31HG expression was correlated with M stage, N stage,

and lymph nodes. For the first time, the research presented that higher expression of MIR31HG was connected with a higher level of immune infiltration in thyroid cancer (Chen et al., 2022). In cervical cancer, Li (2020) verified that MIR31HG silencing inhibited cervical cancer cell proliferation and invasion, whereas anti-miR-361-3p or overexpression of epithelial membrane protein 1 (EMP1) led to the opposite effect. Higher expression of MIR31HG can also be found in oral carcinoma, pancreatic ductal adenocarcinoma (PDAC), and nasopharyngeal carcinoma (NPC). The upregulated MIR31HG level in oral carcinoma was related to poor clinical outcomes and contributed to cancer progression (Shih et al., 2017). MIR31HG functions as an oncogene in PDAC, and the overexpression of MIR31HG is closely associated with poorer DFS in PDAC patients (Yang et al., 2016b; Ko et al., 2022). Increasing levels of MIR31HG enhanced NPC cell growth and metastasis, and could be inhibited by the regulation of mir-31 (Feng et al., 2020).

3.2 The expression of MIR31HG in non-cancers

3.2.1 Psoriasis

Psoriasis is commonly regarded as a chronic immune-related papulosquamous skin disease and negatively affects the life quality of patients (Griffiths and Barker, 2007; Walter, 2022). Previous research indicated that interleukin-17A (IL-17A), interleukin-22(IL-22), tumor necrosis factor-α (TNF-α), and interleukin-1α (IL-1α) were pro-inflammatory cytokines in psoriasis and linked with NF-κB signalling activation (Guilloteau et al., 2010; De Simone et al., 2015). In psoriasis, MIR31HG was found to be elevated in psoriasis lesions and the upregulation of MIR31HG required IL-17A, IL-22, TNF-α, and IL-1α stimulation, demonstrating that nuclear factor kappa B inhibitor (NF-κB) signalling could be crucial crosstalk in psoriasis. Moreover, p65 targeted by specific small interfering RMA (siRNA) suppressed MIR31HG overexpression in human immortalized keratinocytes (HaCaT), consistent with the effect of BAY11-7082 (BAY, NF-κB) on cytokine-induced MIR31HG expression. Additionally, the inhibition of MIR31HG also dampened HaCaT cell proliferation (Gao et al., 2018).

3.2.2 IgA nephropathy

IgA nephropathy (IgAN) is a chronic disease characterized by the deposition of IgA in the glomerular mesangium and is the most common glomerulonephritis worldwide (Saha et al., 2018; Pattrapornpisut et al., 2021). Expressed in human kidneys and participating in autoimmune diseases, more attention has been given to MIR31HG in recent years (Gupta et al., 2020). Through a study including 413 Chinese IgAN patients and 423 healthy people, several single nucleotide polymorphisms (SNPs) in MIR31HG, such as rs1332184 and rs55683539, were significantly associated with an increased risk of IgA nephropathy, creatively indicating that individuals with MIR31HG overexpression were likely to be more susceptible to IgAN (Yuan et al., 2020).

3.2.3 Hirschsprung's disease (HSCR)

Known as a congenital disease with a disorder of the enteric nervous system, HSCR commonly occurs among children (Langer, 2013; Das and Mohanty, 2017). Cai et al. (2018) made a hypothesis that miR-31, miR-31*, and their host gene MIR31HG may participate in the pathogenesis of hirschsprung's disease. Then, they found that the downregulation of MIR31HG could suppress cell migration and proliferation through the MIR31HG-miR31/31*-ITIH5/PIK3CG pathway. While the downregulation of MIR31HG, miR-31, and miR-31* is not associated with the cell cycle and apoptosis.

3.2.4 Rheumatoid arthritis

Rheumatoid arthritis (RA) is a chronic autoimmune disorder affected by multiple factors and mostly damages the joints (Smolen et al., 2016; Deane and Holers, 2021). Fibroblast-like synoviocytes (FLSs) the most abundant cell type in the joint synovium become inflamed and even invade bones in RA (Tu et al., 2018). Using specific siRNA to downregulate MIR31HG expression promoted cell proliferation, migration, and inflammation in RA-FLS. For clinical applications, tocilizumab could suppress RA-FLSs inflammation by targeting MIR31HG, indicating the protective role of MIR31HG in RA (Cao et al., 2021)

3.2.5 Osteonecrosis of the femoral head (ONFH)

Osteonecrosis of the femoral head is a complicated hip disability characterized by bone necrosis and usually occurs in young adults with an average age of 30–50 years old (Petek et al., 2019). Steroid use, alcohol abuse, and smoking are defined as common non-traumatic leading causes of ONFH (Wang et al., 2018b). Since previous studies have discovered that abnormal lncMIR31HG expression can affect osteogenic differentiation and that its polymorphism is correlated with radius bone mineral content in boys (Chesi et al., 2015). Two studies further investigated the MIR31HG gene variant in steroid-induced and alcohol-induced osteonecrosis. In one study including 708 Chinese Han volunteers, both age and sex were related to MIR31HG polymorphisms, and MIR31HG-rs10965059 was associated with a lower risk of bilateral steroid-induced osteonecrosis (Wang et al., 2022a). MIR31HG-rs10965059 and MIR31HG-rs10965064 were strongly associated with a lower risk of disease occurrence especially in patients over 40 years old and rs10965059 served as a protective gene in alcohol-induced osteonecrosis (Liu et al., 2022a). However, the precise mechanism by which MIR31HG participates in pathogenesis remains unclear.

4 The biological role of MIR31HG in human diseases

4.1 MIR31HG and the cell cycle

LncRNA MIR31HG has been revealed to control the cell cycle during human disease development. For instance, Ding et al. (2015) indicated that overexpression of MIR31HG may promote colorectal cancer cell arrest in the G0/G1 phase and induce apoptosis through the activation of specific caspase cleavage cascades. A study performed by Yang et al. (2016b) demonstrated that the knockdown of MIR31HG significantly induced G1/S arrest in pancreatic ductal adenocarcinoma (PDAC), whereas enhanced expression of MIR31HG had the opposite effects. Qin et al. (2018) also showed that downregulation of MIR31HG inhibited

the proliferation of lung adenocarcinoma cells and blocked the G0/G1 to S-phase transition in cell cycle progression, but had no effect on cell apoptosis. In psoriasis, the silencing of lncRNA MIR31HG induced cell cycle arrest in the G2/M phase potentially via the mediation of siRNA (Gao et al., 2018).

4.2 MIR31HG and the EMT

Epithelial-to-mesenchymal transition (EMT) is a biological process by which polarized epithelial cells are transformed into highly motile mesenchymal cells (Thiery and Sleeman, 2006). Numerous types of research have proven that EMT plays an essential role in tumour progression and metastasis (Thiery, 2002; Hugo et al., 2007). Moreover, lncRNAs including HOTAIR, H19, ATB, and MIR100HG are commonly known as regulators that are involved in the EMT process (Dhamija and Diederichs, 2016). In particular, the lncRNA MIR100HG also acts as a host gene for miRNAs like MIR31HG, and a recent study emphasized its function as a positive regulator in EMT to advance colorectal cancer cell evasion and metastasis (Liu et al., 2022b).

Recent advances in sequencing technology have revealed that the lncRNA MIR31HG may serve as an oncogenic regulator in PDAC by promoting EMT. Transforming growth factor β (TGFβ) signalling plays dual roles in cancer progression, especially in the later stages of tumourigenesis. The high amounts of TGFβ secreted by cancer cells promote tumour progression by inducing EMT (Zhang et al., 2017a). Thus, Ko et al. (2022) found that the enrichment of TGFβ signalling in PDAC and the presence of MIR31HG enhanced TGFβ-induced EMT, suggesting that MIR31HG could serve as an oncogene in PDAC. Furthermore, as an epithelial marker, E-cadherin levels were found to be elevated, while mesenchymal signs such as Vimentin and transcription factors like Twist1 were remarkably downregulated in NSCLC cells through the silencing of MIR31HG (35). Patients with MIR31HG overexpression tend to share higher TGFβ and EMT gene expression in colorectal cancer, implying a potential relationship between MIR31HG and the EMT gene signature in other cancer types (Eide et al., 2019).

4.3 MIR31HG and senescence

Cellular senescence is an irreversible arrest of cell proliferation that occurs when cells experience potentially oncogenic stress. Because of its limitation on cellular fission, it is regarded as an anticancer mechanism (Kuilman et al., 2010; Storer et al., 2013). Cellular senescence mainly relies on two tumour suppressor pathways: p14ARF/p53 and p16INK4A/pRB. Several lncRNAs are involved in cellular senescence. By modulating SAFA-PANDA-PRC communication, lncRNA PANDA restricted the cell proliferative state and dampened cellular senescence. Conversely, the loss of PANDA promoted senescence, serving as an opportunity for dropping out of senescence (Kotake et al., 2011; Puvvula et al., 2014). Recent research has shown that the lncRNA MIR31HG is associated with oncogene-induced senescence (OIS) through various targets.

From Marta Montes's study in 2015, MIR31HG was upregulated in OIS, and the knockdown of MIR31HG could induce a senescence phenotype. Moreover, this research has also demonstrated that the role of nuclear MIR31HG in senescence relies on p16INK4A since depleting p16INK4A can eliminate these effects. On the contrary, no evidence suggests that MIR31HG has a relationship with the p14ARF/p53 pathway in modulating senescence. The senescence-associated secretory phenotype (SASP) has been shown to either restrain or enhance tumour progression. Therefore, it is promising to find certain factors that could affect SASP without influencing the tumour-suppressive effects related to senescence at the same time for advanced therapies (Montes et al., 2015). In 2021, Marta Montes further discovered that cytoplasmic MIR31HG modulated the expression and secretion of SASP-related components by interacting with YBX1 to induce IL1A translation. In conclusion, the lncRNA MIR31HG both promotes and suppresses senescence, and these different effects mainly rely on MIR31HG localization, further indicating that inhibition of MIR31HG could potentially be used in senescence-related pathology therapies (Montes et al., 2021).

4.4 MIR31HG and cell differentiation

Numerous studies have shown the relevance of lncRNAs in cell differentiation (Richart et al., 2022; Wu et al., 2022). For example, the overexpression of lncRNA Snhg6 was found in tumour-derived myeloid-derived suppressor cells (MDSCs) and contributed to MDSC differentiation by inhibiting the ubiquitination of EZH2 (Lu et al., 2021). Recent studies have explored whether lncRNA MIR31HG participates in cell differentiation, especially in adipocyte and osteogenic differentiation.

According to Huang's research, inhibition of MIR31HG suppressed adipocyte differentiation of human adipose-derived stem cells (hASCs) via histone modification of fatty acid binding protein 4 (FABP4) (Huang et al., 2017). FABP4 is a kind of protein that is highly expressed in adipose tissue and can be targeted for metabolic disease treatment (Haunerland and Spener, 2004). This research suggests potential determinants of the applications of MIR31HG in obesity and other disorders. Apart from participating in adipocyte differentiation, MIR31HG may play an important role in osteogenic differentiation. HASCs are a type of mesenchymal stem cell (MSC) capable of bone regeneration and repair, making them attractive in bone tissue engineering (Tapp et al., 2009). Upregulated by inflammatory cytokines such as TNF-α and IL-17 by the NF-κB Signalling pathway, MIR31HG inhibited osteoblast differentiation of hASCs. In contrast, the knockdown of MIR31HG could promote bone formation, demonstrating that the inhibition of MIR31HG benefits bone regeneration and relieves inflammation (Jin et al., 2016).

5 Mechanism of MIR31HG-mediated biological function in human diseases

5.1 The Wnt/β-catenin signalling pathway

Wnt/β-catenin is a family of proteins that play an important role in controlling embryonic and organ development, as well as cancer

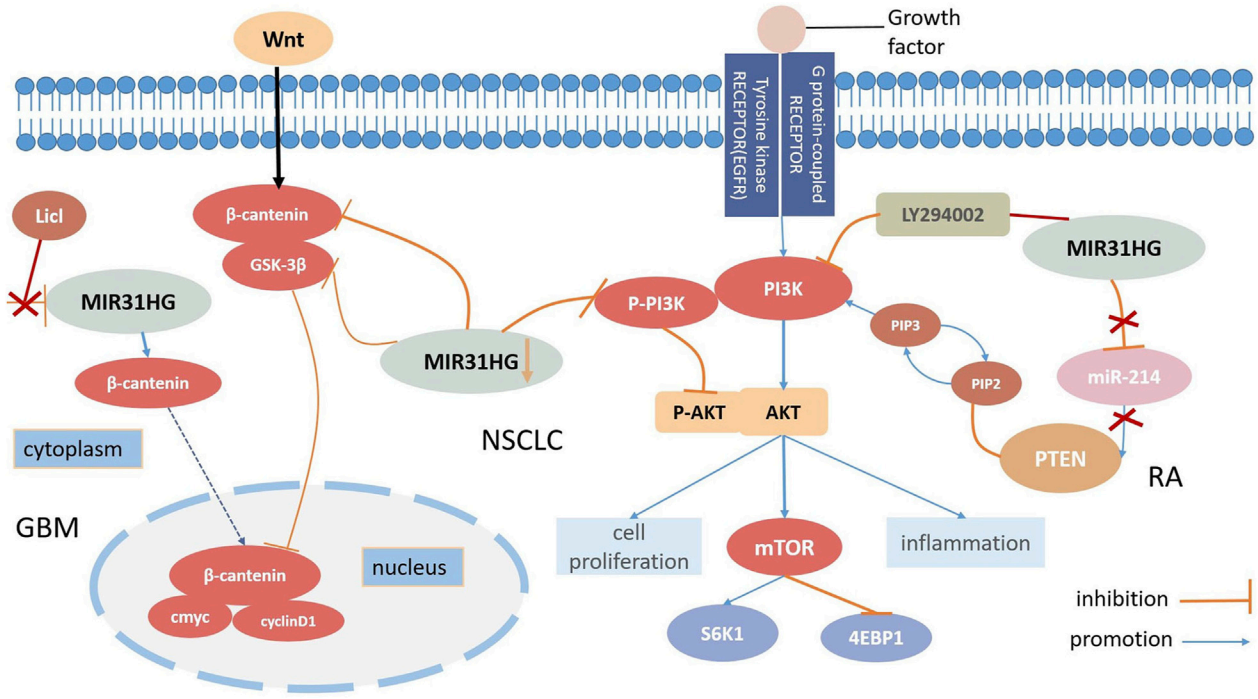

FIGURE 3
MIR31HG mediates biological function by participating the Wnt-β-catenin pathway and the AKT pathway in human diseases.

progression (Clevers and Nusse, 2012). The Wnt/β-catenin pathway is able to confine the transcription of downstream genes by activating the expression of β-catenin (Chatterjee et al., 2022). It was reported that a high level of MIR31HG contributes to the progression of a variety of cancers by activating canonical Wnt signaling, which is also recognized as the Wnt/β-catenin signalling pathway.

In contrast to some lncRNAs, MIR31HG is located mainly in the cytoplasm (Montes et al., 2015). Zheng et al. elucidated that the downregulation of MIR31HG depressed the Wnt/β-catenin signalling pathway via the inhibition of GSK3β and β-catenin expression levels but induced p-GSK3β overexpression in NSCLC cells. On the contrary, MIR31HG was demonstrated to enhance cell proliferation and invasion by activating this pathway in NSCLC (Zheng et al., 2019). The activation of the Wnt/β-catenin pathway was also found in glioblastoma (GBM) progression (Zhang et al., 2021). Triggered by STAT1, MIR31HG could transcribe β-catenin from the cytoplasm into the nucleus, and the Wnt/β-catenin pathway activator LiCl was utilized to invert both the ability to inhibit proliferation and impress apoptosis caused by MIR31HG knockdown in glioblastoma (Zhang et al., 2021). Therefore, we can safely conclude that the activation of the Wnt/β-catenin signaling pathway regulated by MIR31HG enhances cell growth in glioblastoma (Figure 3).

5.2 The AKT pathway

AKT kinases which are also called protein kinase B(PKB), are signalling molecules of cell growth and differentiation and the AKT pathway is commonly involved in inhibiting cell apoptosis and stimulating cell proliferation (Mundi et al., 2016; Stratikopoulos and Parsons, 2016). Among those AKT pathways, the PI3K (phosphatidylinositol 3-kinase)/AKT signaling pathway is one of the most prevalent and distinctive pathways related to growth, which modifies biological mechanisms in human diseases. For instance, the PI3K/AKT/mTOR (mammalian target of rapamycin) signalling pathway exerts an essential role in the tumourigenesis of malignant cancers (Barrett et al., 2012; Alzahrani, 2019). Moreover, phosphatase and tensin homolog (PTEN) is an important tumour suppressor that can dephosphorylate phosphatidylinositol (Birney et al., 2007; Gyamfi et al., 2022; Vervoort et al., 2022)-triphosphate (PIP3) to phosphatidylinositol 4,5-biphosphate (PIP2) and suppress the PI3K/AKT signalling pathway in various cancers (Bonneau and Longy, 2000). Many lncRNAs employ a cooperative effect as fine-tuners on both tumour inhibition and oncogenesis (Ghafouri-Fard et al., 2021). LncRNA FER1L4 evokes cell cycle arrest and AB073614 stimulates proliferation and hampers apoptosis; both of these processes are regulated by the AKT signalling pathway (Wang et al., 2019). Recently, several types of research have shown that the lncRNA MIR31HG may have a relationship with the AKT signalling pathway in various diseases (Figure 3).

In the case of NSCLC, Wang discovered that the overexpression of MIR31HG could not directly modify all epidermal growth factor receptor (EGFR), PI3K, or AKT levels but could affect the expression levels of P-EGFR, P-PIP3 and P-AKT. Research has elucidated that MIR31HG influences NSCLC cell proliferation, apoptosis, and the cell cycle by driving the EGFR/PI3K/AKT pathway, and even contributing to gefitinib resistance (Wang et al., 2017). The

downregulation of P-PIP3 and P-AKT can also be seen in nasopharyngeal carcinoma (NPC) through MIR31HG silencing. In this study, silencing of MIR31HG decreased cell proliferation but promoted apoptosis; however, 740Y-P, a PI3K agonist successfully reversed this process. A positive correlation between MIR31HG and AKT expression levels was further demonstrated, suggesting that MIR31HG enhanced cell proliferation and induced apoptosis in NPC at the same time through the PI3K/AKT signalling pathway (Feng et al., 2022). RA-FLSs share several tumour cell-like characteristics with cancers, and a number of studies have demonstrated that PTEN may participate in RA-FLS formation. According to Cao's study, MIR31HG and PTEN played roles as suppressive targets in RA-FLS inflammation regulated by mir214 and further motivated the AKT signalling pathway. Furthermore, the attachment of the PI3K inhibitor LY294002 remedied RA-FLS hyperinflammation induced by the loss of MIR31HG, suggesting that MIR31HG may serve as an upstream target for the AKT signalling pathway (Cao et al., 2021). In conclusion, MIR31HG inhibited proliferation, migration, and inflammation via regulation of the downstream miR-214-PTEN-AKT pathway. According to the gene set enrichment analysis (GSEA), MIR31HG may also contribute to colorectal cancer invasion and metastasis by modulating the PI3K-AKT-mTOR-signalling pathway (Wang et al., 2022b).

5.3 The lncRNA-miRNA-mRNA ceRNA network

Apart from the two pathways described above, MIR31HG also acts on a series of targets and participates in other pathways. The competing endogenous RNA (ceRNA) regulatory network is the main mechanism of MIR31HG in cancer development (Li and Zhan, 2019). Through ceRNA mechanisms, MIR31HG could act as a miRNA sponge to regulate the expression of downstream messenger RNAs (mRNAs), and affect tumour progression (Figure 1).

MIR31HG serves as an oncogene in cervical carcinoma by acting as a sponge for miR-361-3p to modulate the miRNA target EMP1 (Li, 2020). In colorectal cancer, miR-361-3p was inhibited by MIR31HG which thereby increased the YY1 level, contributing to tumour progression (Guo et al., 2021). By sponging miR-34a, MIR31HG enhances the expression of c-Met and promotes the development of ESCC (Chu et al., 2020). In hepatocellular carcinoma, FOX3-induced lncRNA LOC554202 upregulated BSG by competitively binding to miR-485-5p and promoted tumour progression (Yang et al., 2022b). In addition, MIR31HG inhibited hepatocellular carcinoma (HCC) proliferation and metastasis by directly binding miR-575 to positively modulate the expression of ST7L (Yan et al., 2018). MiR-31 is suppressed by the lncRNA MIR31HG in various cancers, including chordoma and LSCC. MIR31HG plays a crucial role in the progression of chordoma by indirectly promoting RNF144B via miR-31 (Ma et al., 2017). In LSCC, MIR31HG acted as an oncogene directly by inhibiting miR-31 expression and promoting its target gene RhoA expression (Yang et al., 2018). The modulation function of MIR31HG in regulating MAPK1 expression was completed by competitively sponging miR-761 in thyroid cancer (Peng et al., 2022). Furthermore, overexpression of MIR31HG promoted tumour growth in osteosarcoma cells by downregulating miR-361 expression and elevating the expression of VEGF, FOXM1 and Twist, which are target genes of miR-361 (Sun et al., 2019). Sp1-activated-MIR31HG advanced cell migration and invasion by directly binding to miR-214 in NSCLC (Dandan et al., 2019). According to the latest research from Mo's team, MIR31HG serves as an oncogene by targeting mir-193a-3p and positively enhances recombinant tumor necrosis factor receptor superfamily, member 21 (TNFRSF21) expression in lung adenocarcinoma (Mo et al., 2022). In pancreatic ductal adenocarcinoma, lncMIR31HG exhibited oncogenic properties through the downregulation of miR-193b (Yang et al., 2016b).

5.4 Interaction with hypoxia-inducible factor 1α (HIF-1α)

Hypoxia-inducible factor 1α (HIF-1α) is the HIF-1 transcription factor and can modulate the expression of hypothetical genes (LaGory and Giaccia, 2016). As hypoxia induction can cause tumour metastasis and lead to worse prognosis, a number of studies have demonstrated the role of the HIF-1α pathway in cancer development in recent years (Zhou et al., 2015; Wu et al., 2017). In a recent review, upregulated lncRNAs including HOTAIR, H19, and MALAT1 were found and concluded to be hypoxia-responsive lncRNAs in cancers (Wang et al., 2021b). Similar to LncH19, lncMIR31HG was also induced by hypoxia and was subsequently named lncHIFCAR (long non-coding HIF-1α coactivating RNA) (Shih et al., 2017).

Through a previous integrated analysis of the expression of lncRNA-mRNA in advanced LSCC, lncMIR31HG was positively related to HIF-1α (Wang et al., 2018a). Western blotting further proved that the knockdown of MIR31HG inhibited HIF-1α expression and increased P21 expression. Moreover, in oral cancer, MIR31HG directly binds with HIF-1α and forms a special complex (Shih et al., 2017). This complex recruits HIF-1α and its coactivator p300, contributing to the overexpression of MIR31HG and activation of the HIF-1α pathway. Surprisingly, even under normoxic conditions, MIR31HG still enhanced the target genes of HIF-1α, indicating that MIR31HG could serve as a HIF-1α coactivator. Unfortunately, studies on lncMIR31HG and HIF-1α were only conducted before 2020 and the specific mechanisms were unclear.

6 MIR31HG as a potential biomarker in human diseases

6.1 MIR31HG as a diagnostic biomarker

Multiple kinds of research have elucidated that long non-coding RNAs participate in human diseases (Yang et al., 2022a; Hao et al., 2023). In view of the aberrant expression level, wide functions and gradually displayed underlying mechanisms, we will further discuss that lncRNA MIR31HG acts as a potential diagnostic biomarker in human cancer and non-cancers.

Differential expression of MIR31HG in specific tissues in cancers helps to distinguish diseased tissues from normal tissues,

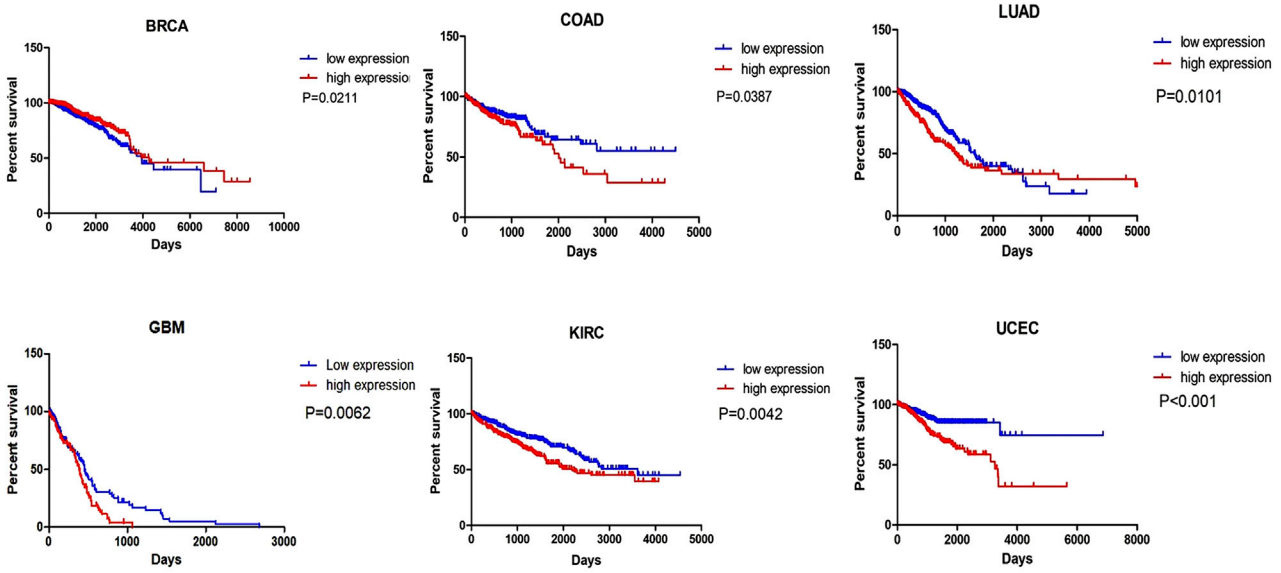

FIGURE 4
The survival analysis of MIR31HG indicates that MIR31HG is related to the OS of patients in six types of cancers. And in BRCA, different from other cancers, MIR31HG overexpression is associated with a better outcome.

indicating that MIR31HG could be a potential biomarker for early cancer diagnosis (Ren et al., 2017; Yan et al., 2018). Apart from cancer diagnosis, the latest research focuses on the diagnostic role of MIR31HG in human non-cancers. Genetic factors play a crucial role in the development of lumbar disc herniation (LDH), which is a common spinal disease that poses a great threat to human health both worldwide and in China (Deyo and Mirza, 2016; Zhang et al., 2017b). By performing a multifactor dimensionality reduction (MDR) analysis, individuals with the MIR31HG polymorphism rs10965059 were found to be at great risk of LDH providing the possibility for early screening, prevention and diagnosis of Chinese Han LDH high-risk populations (Hu et al., 2022).

6.2 MIR31HG as a prognostic biomarker

Numerous studies have revealed that MIR31HG is positively related to clinicopathological features, including tumour size, clinical stage, TNM stage, advanced T category, lymph node metastasis, distant metastasis, overall survival, and progression-free survival (Table1). These clinical features elucidate the possibility that MIR31HG could serve as a prognostic biomarker in human cancer. For example, downregulation of MIR31HG in colorectal cancer was significantly associated with TNM stage, histologic grade, and lymph node metastasis, indicating that MIR31HG expression was linked with poor prognosis in CRC (Yang et al., 2016a). In oral squamous cell carcinoma, MIR31HG overexpression is related to a worse survival tendency in stage IV diseases (Tu et al., 2022). Furthermore, we conducted a survival analysis of MIR31HG in 21 kinds of human cancers using the Oncolnc database (http://www.oncolnc.org) (Figure 4). All data used for analysis in this database came from TCGA, and a log-rank *p*-value less than 0.05 was viewed as a great difference. LncRNA MIR31HG has a relationship with the OS of patients with breast invasive carcinoma (BRCA), lung cancer, colorectal cancer, glioblastoma, kidney renal clear cell carcinoma (KIRC) and uterine corpus endometrial carcinoma (UCEC). In breast cancer, a higher level of MIR31HG was associated with longer OS, while in other cancers, its overexpression was related to a worse outcome. This analysis suggested the potential role of MIR31HG in predicting cancer prognosis. Nevertheless, research has yet to systematically investigate the association between MIR31HG and human non-cancer.

6.3 MIR31HG as a therapeutic reagent

Drug resistance is a leading obstacle to human disease treatment (Vasan et al., 2019). With further research on the mechanisms involved in lncRNA-related disease pathogenesis, lncMIR31HG could be a promising biomarker for specific disease treatments. In NSCLC patients with EGFR mutations, LOC554202 reduced the sensitivity of NSCLC cells to gefitinib and promoted gefitinib resistance by regulating mir31 expression (He et al., 2019). For colorectal patients who have been treated with oxaliplatin-based chemotherapy, higher expression levels of LOC554202 were associated with DFS and OS rates. This result suggested that LOC554202 may be a potential marker for evaluating the outcome of colorectal cancer therapy (Li et al., 2018).

Tissue inflammation is one of the classic symptoms of RA (Buch et al., 2021). For treating moderate to severe RA in adults, either utilizing tocilizumab alone or in combination with other doses was viewed as a common therapy. MIR31HG, targeted by tocilizumab, was indicated to suppress RA-FLS inflammation and become a potential therapeutic target for RA in Cao's study (Cao et al., 2021).

7 Conclusion and perspective

Plenty of existing evidence shows that lncRNA play an important role in the pathogenesis of human diseases. With further study of lncRNAs in human cancer, it is worth exploring and concluding the role of lncRNAs in tumour development. In this review, we summarize the current research on the role of MIR31HG in human cancer and non-cancer. MIR31HG gene polymorphism is associated with susceptibility to several diseases and plays an oncogenic role or acts as a tumour suppressor by regulating tumour cell proliferation, apoptosis, the cell cycle, EMT, and senescence. MIR31HG also participated in the differentiation of hASCs in non-cancer. These effects are realized by diverse mechanisims, such as the Wnt/β pathway, the AKT pathway, the lncRNA-miRNA-mRNA ceRNA network and interaction with HIF-1α (Figure 3).

MIR31HG, upregulation or downregulation, may act as a biomarker for the prognosis and diagnosis of cancer. Moreover, the dysregulation of MIR31HG in various cancers is significantly related to important clinical features including tumour size, TNM staging, histological grade, OS, and DFS (Table1). For human disease treatment, MIR31HG also serves as a therapeutic target for different diseases including NSCLC, colorectal cancer, and RA.

In conclusion, the lncRNA MIR31HG participates in the pathogenesis of human diseases and has great potential for clinical application by functioning as a diagnostic or prognostic biomarker and therapeutic target in human diseases.

However, there are some limitations in those studies. First, controversy still exists regarding the specific MIR31HG expression level in certain cancers, such as breast cancer, colorectal cancer, bladder cancer and ESCC. These expression differences may be the consequence of the diverse cell lines used and the specific patients selected. Second, in cancers such as PDAC and NPC, only one or two studies have depicted the role of MIR31HG in their development. The lack of multiple experiments can mislead our cognitions and obtain controversial results.

Although it has been shown that MIR31HG is also dysregulated in non-cancers, those studies are merely limited to cell line studies and *in silico* analysis. More animal experiments and clinical research are needed in the future. To date, MIR31HG has been shown to participate in only five human non-cancers, far less than its participation in cancers. Whether lncMIR31HG plays an important role in human non-cancer pathogenesis needs more solid support in wider types of diseases.

Functional experiments show that lncMIR31HG affects human diseases through four main mechanisms. However, the related mechanistic pathways remain in their primary stage, especially in the WNT pathway. Moreover, current studies mostly focus on the investigation of MIR31HG downstream regulators, and other studies on its upstream genes or regulators should be performed in the future. In addition, the lncRNA MIR31HG is correlated with drug resistance and treatment outcomes in cancers. However, its clinical value in non-cancers is not clear. Consequently, further high-equality experiments and credible clinical research are required to explore the latent value underlying MIR31HG in disease pathogenesis and treatment.

Author contributions

QZ and JW provided ideas for this review. LR wrote the manuscript. YY and JL drew the tables and figures. HL and HY revised this manuscript. All authors contributed to the article and approved the submitted version.

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Conflict of interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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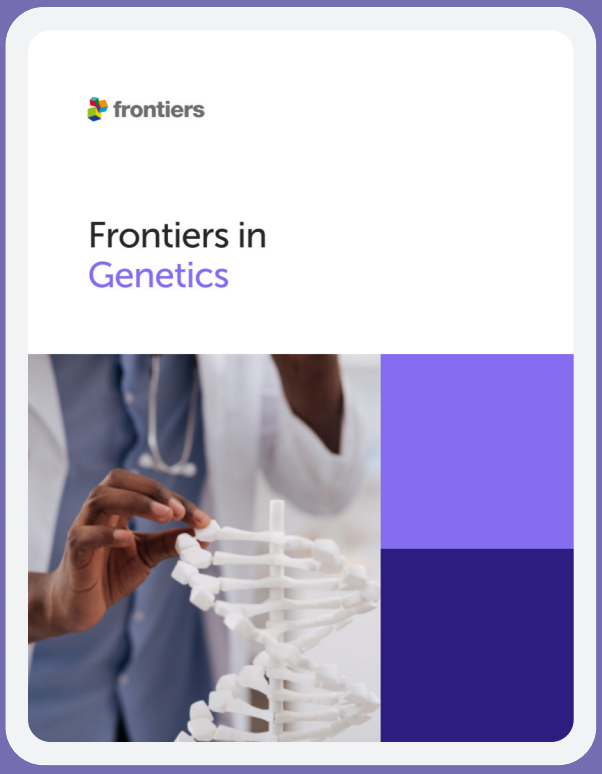

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