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LINC02323 facilitates development of lung squamous cell carcinoma by miRNA sponge and RBP dysregulation and links to poor prognosis

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Abstract

Background: The poor outcome of patients with lung squamous cell carcinoma (LUSC) highlights the importance of the identification of novel effective prognostic markers and therapeutic targets. Long noncoding RNAs (lncRNAs) have generally been considered to serve important roles in tumorigenesis and the development of various types of cancer, including LUSC.

Methods: Here, we aimed to investigate the role of *LINC02323* in LUSC and its potential mechanisms by performing comprehensive bioinformatic analyses.

Results: *LINC02323* was elevated and positively associated with unfavorable prognosis of LUSC patients. *LINC02323* exerted oncogenic function by competitively binding to miR-1343-3p and miR-6783-3p, thereby upregulating *L1CAM* expression. Indeed, we also determined that *LINC02323* could interact with the RNA-binding protein DDX3X, which regulates various stages of RNA expression and processing.

Conclusion: Taken together, we identified that *LINC02323* and its indirect target *L1CAM* can act as novel biomarkers for determining the prognosis of patients with LUSC and thus deserves further study.

KEYWORDS
DDX3X, L1CAM, *LINC02323*, miR-1343-3p, miR-6783-3p, lung squamous cell carcinoma

INTRODUCTION

Lung cancer is the leading cause of tumor-related mortality worldwide.[1] Non-small cell lung cancer (NSCLC), which accounts for more than 85% of all lung cancers, consists of two predominant subtypes: lung adenocarcinoma (LUAD) and lung squamous cell carcinoma (LUSC). The 5-year overall survival (OS) rate of advanced NSCLC has greatly improved due to the targeted therapies and immune checkpoint inhibitors.[2,3] However, these agents preferentially benefit patients with LUAD,[5] and the current OS of LUSC remains 20%–30% less than that of LUAD.[6–8] These differences in available therapeutic strategies and clinical outcomes result from different molecular patterns according to

tumor genomic aberration or transcript alterations.[4] Hence, elucidating the molecular mechanisms underlying tumorigenesis and progression might be conducive to developing more effective therapeutic targets for LUSC.

Noncoding RNAs (ncRNAs), especially microRNAs (miRNAs) and long noncoding RNAs (lncRNAs), contribute to various biological processes in cancer tumorigenesis and progression.[5,6] LncRNA with a length >200 nucleotides may be involved in more diverse and complex mechanisms for regulating the biogenetic process because of the longer sequences and more complex spatial structures compared with small RNAs.[7,8] LncRNAs interact with DNAs, RNAs, and proteins to participate in numerous physiological and pathological processes at the transcriptional, translational, and epigenetic levels and other mechanisms such as chromatin remodeling, cell cycle regulation, splicing regulation, mRNA degradation, and translational regulation.[9,10] Several lncRNAs are expressed abnormally in lung cancer, particularly in LUAD, where they may act as oncogenes or tumor suppressor genes, suggesting lncRNA as novel tumorigenic drivers in lung cancer. LncRNA H19 interacts with mutant-type p53 (R175H), leading to LUAD progression.[11] LncRNA-MALAT1 promotes the spread and invasion of lung cancer by regulating the expressions of miR-124/STAT3 and miR-206/AKT.[12] LINC00261 decreases hypoxia-induced tumor angiogenesis and its loss was associated with a poor prognosis for patients with LUAD.[13] However, the relationship between lncRNAs and the progression of LUSC remained unclear.

Systemic biology and bioinformatic approaches can facilitate the understanding of the pathogenesis of LUSC and the identification of potential novel biomarkers and druggable targets.[14–16] Many transcriptomic analyses and datasets of LUSC samples have been established. Nevertheless, compared with coding genes and microRNAs, the specific lncRNAs involved in the onset and development of LUSC remain unknown. The current study was designed to investigate *LINC02323* expression (located between 545 253 and 102 592 425 on chromosome 14) and its prognostic value in LUSC. Our results showed that *LINC02323* functioned as an oncogene in LUSC by sponging miR-1343, miR-6783, and DDX3X-interacted axis, then boosting cell growth and maintaining CSC features. Our findings may be used to further investigate the characteristics of LUSC and are useful in determining the prognosis and therapy of patients with LUSC.

METHODS

Data collection

The gene expression quantification datasets of LUSC were extracted from samples of The Cancer Genome Atlas (TCGA) (available online: https://portal.gdc.cancer.gov, accessed on August 24, 2022). The criterion in the analysis was a *p*-value <0.05 which was calculated using the University of Alabama at Birmingham CANcer (UALCAN) data analysis portal (available online: http://ualcan.path.uab.edu,

accessed on August 24, 2022), the Encyclopedia of RNA Interactomes (ENCORI) (available online: https://starbase.sysu.edu.cn/index.php, accessed on October 9, 2022), or TNM plotter (available online: https://tnmplot.com/analysis/, accessed on August 24, 2022).

Survival analysis of *LINC02323*, L1CAN, and DDX3X in LUSC

The survival analyses of the various candidate genes in LUSC were assessed via the Kaplan Meier (KM) plotter (available online: http://kmplot.com/analysis/, accessed on October 7, 2022). The hazard ratios (95% confidence intervals) were calculated using the Cox proportional model.

Survival analysis of miR-1343-3p and miR-6783-3p in LUSC

The Pan-Cancer Analysis Platform of ENCORI was used to analyze the association of the miR-1343-3p and miR-6783-3p expression with overall survival (OS). Patients were divided into two groups by median value, which was computed with median survival.

Screening for targets of miR-1343-3p and miR-6783-3p

The target gene of miR-1343-3p and miR-6783-3p was predicted via miRDB (available online: http://mirdb.org/, accessed on October 7, 2022), which searched the target genes based on the conserved sites 8 mer and 7 mer sites matching the seed regions of miRNAs. Target score >90% was set as the cutoff criteria.

Interaction of RNA-binding protein (RBP) and miRNAs with *LINC02323*

The miRNA or RBP interaction analyses with lncRNA (miRNA-lncRNA, and RBP-lncRNA) were performed using the ENCORI online database. The potential interactive RBPs with *LINC02323* were searched using RBP-lncRNA module, with a selection criterion of the stringency equal to, or more than 1, in the crosslinking immunoprecipitation (CLIP) database.

Functional analysis and the gene set variation analysis (GSVA) of gene sets

To investigate the role of L1 cell adhesion molecule (*L1CAM*) and *DDX3X*, the LUSC patients of TCGA were divided into *L1CAM* or *DDX3X* high- and low-expression groups according to the highest and lowest quartiles, and gene set enrichment analysis (GSEA) was conducted to

assess the enrichment of datasets between high- and low-target gene groups. False discovery rate (FDR) < 0.05 and nominal *p*-value <0.05 were set as the cutoff criteria. The gene set "c2.cp.kegg.v6.2.symbols.gmt" was chosen as the reference gene set. The correlation of the gene sets positively correlated with *LINC02323*, *L1CAM*, or *DDX3X* was also extracted from the UALCAN. The criteria in gene extraction were Pearsons correlation coefficient (CC) > 0.3 and *p*-value <0.05, which was calculated using the UALCAN. The GSVA score of signaling pathway on survival rate was calculated using gene set cancer analysis (GSCA) (available online: http://bioinfo.life.hust.edu.cn/GSCA/#/, accessed on October 10, 2022). Metascape (available online: http://metascape.org/gp/index.html#/main/step1) and NetworkAnalyst (available online: https://www.networkanalyst.ca/, accessed on October 7, 2022) were used to visualize for KEGG of positively-correlated genes of *L1CAM*.

Signaling pathway and network analysis

The significant enrichment analysis of the gene sets positively-correlated with *L1CAM* was also assessed based on the Kyoto Encyclopedia of Genes and Genomes (KEGG) using the Database for Annotation, Visualization and Integrated Discovery (DAVID) (Available online: https://david.ncifcrf.gov/, accessed on October 10, 2022).

## Statistical analysis

Spearman's correlation was applied for the analysis of the correlation. Most analyses were conducted using GraphPad Prism 9.0. Logistic regression, univariate, and multivariate analyses were used to assess the influence of clinical variables on patient survival. A *p*-value less than 0.05 was considered statistically significant.

RESULTS

Analysis of *LINC02323* expression in patients with lung cancer

We first analyzed *LINC02323* expression in LUSC. As shown in Figure 1a, *LINC02323* expressions were higher (5.5-fold, *p*-value = 2.6×10^{-39}) in LUSC in the cancer genome atlas (TCGA) database collected from UALCAN. However, the expression did not depend on the progress of cancer stages and smoking/reformed smoking status in LUSC (Figure 1b, c). In addition, *LINC02323* expression was significantly increased in an additional 13 cancer types other than lung cancer (acute myelogenous leukemia, bladder, colon, liver, ovary, pancreas, rectum, renal papillary cell carcinoma, stomach, testis, thyroid, uterine, and uterine endometrial carcinoma) on the TMN plotter website and seven types in TCGA cohort (bladder, urothelial carcinoma,
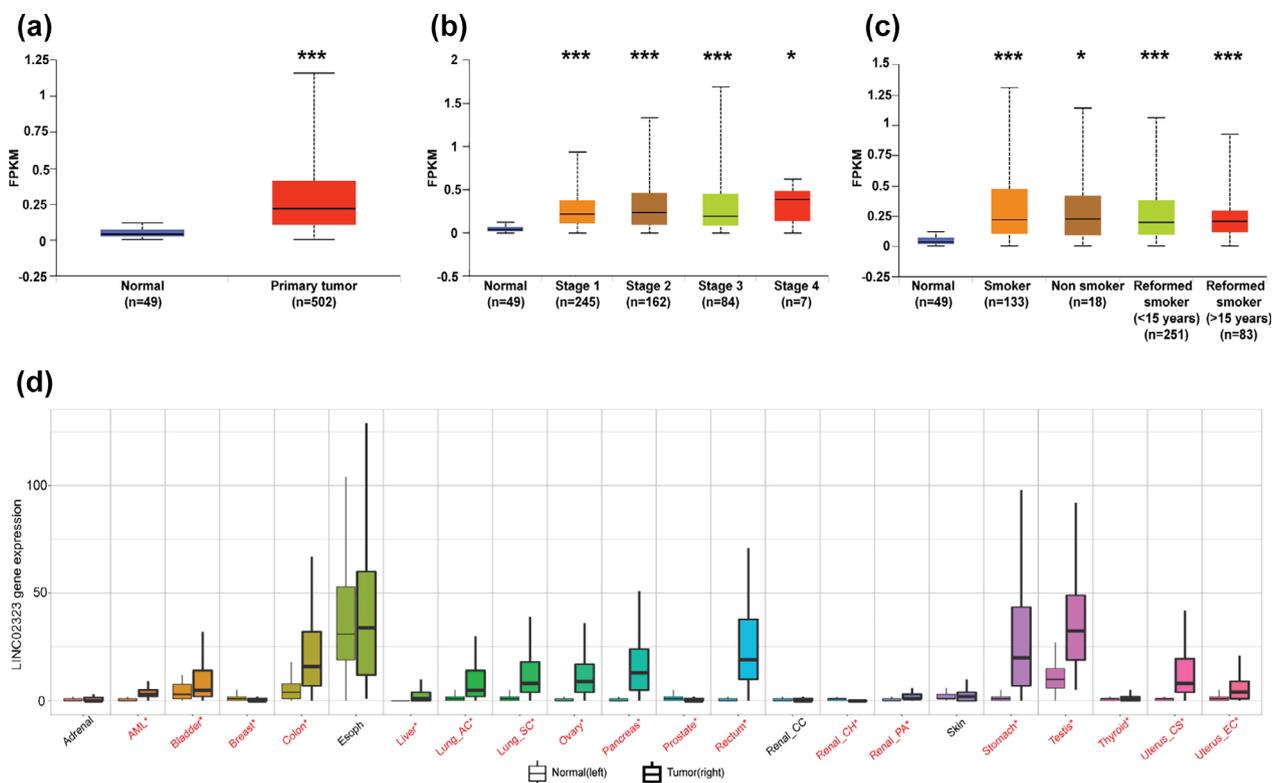
FIGURE 1 The expression of *LINC02323* in cancers. (a) The expression of *LINC02323* in TCGA lung squamous cell carcinoma (LUSC) dataset. The levels of *LINC02323* in (b) the different stages and (c) smoking status. (d) The levels of *LINC02323* in various cancers extracted from TNM plotter. ***p* < 0.01; ****p* < 0.005; NS, not significant

cervical squamous cell carcinoma and endocervical adeno-carcinoma, colon adenocarcinoma, esophageal carcinoma, head and neck squamous cell carcinoma, pancreatic adeno-carcinoma, rectum adenocarcinoma, and stomach adenocarcinoma) (Figure 1e, f) compared with these in normal tissues. These data showed that *LINC02323* may exert an oncogenic role in various cancers, including LUSC.
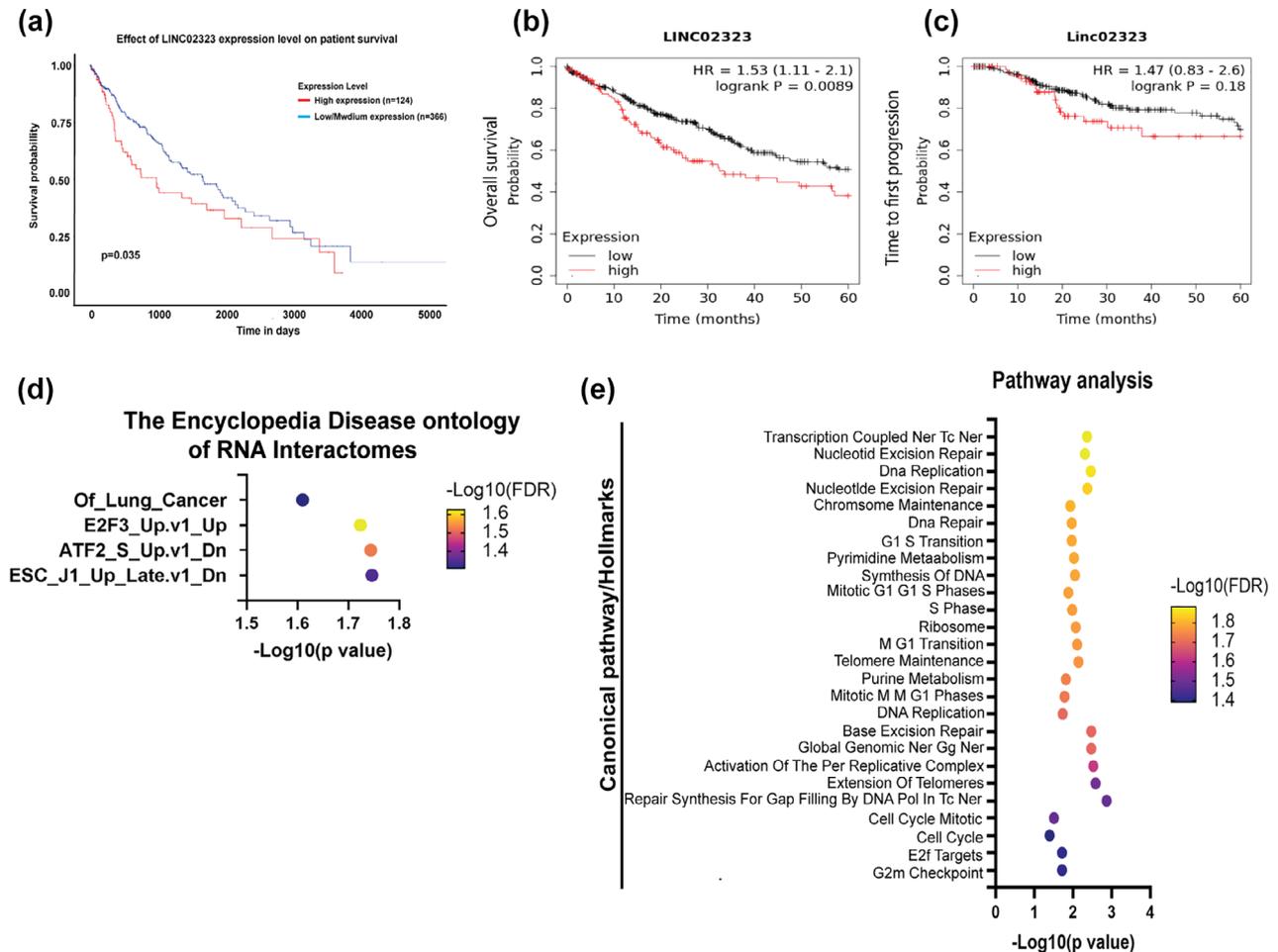
Correlation between *LINC02323* expression and the prognosis of patients with LUSC

Next, we investigated the correlation between *LINC02323* expression and overall survival (OS) in the TCGA database. The results indicated that high *LINC02323* expression was related to poor survival in LUSC in TCGA database (Figure 2a). These results were validated by data from the KM plotter website (Figure 2b). However, *LINC02323* was not correlated with relapse-free survival (RFS) in LUSC (Figure 2c). Disease ontology analysis by ENCORI website

also showed that *LINC02323* was associated with the occurrence of lung cancer, and also correlated with cell cycle-related oncogenic genes, including *E2F* (Figure 2d). Pathway analysis also indicated that the targets of *LINC02323* were involved in cell cycle regulation, DNA replication and repair, and chromosome maintenance (Figure 2e).

Interactions between *LINC02323* with miR-1343-3p/miR-6783-3p-L1CAM axis in LUSC

LncRNAs are known to act as a sponge of miRNAs, thereby inhibiting the degradation of mRNAs targeted by miRNAs. We thus predicted the interactions of *LINC02323* using the ENCORI website. The results showed that miR-1343-3p and miR-6783-3p were complementary to LINC02323 (Figure 3a). The levels of miR-1343-3p and miR-6783-3p were higher in LUSC by 3.5- (p-value = 0.031) and 3.84-fold (p-value = 0.0011) compared with these in normal tissues,


FIGURE 2 The correlation of *LINC02323* with survival of lung squamous cell carcinoma (LUSC) patients. *LINC02323* is associated with a poor prognosis in LUSC. The association of *LINC02323* expression and survival time through data extracted from the Kaplan-Meier plotter website on overall survival disadvantage when (a) expression is high with divergent trends ($p < 0.05$), (b) time to first progression, and (c) post-progression survival. Red and black lines: High- and low-expressed *LINC02323*, respectively. HR, hazard ratio. (d) Disease ontology (DO) annotated *LINC02323* was associated with lung cancer occurrence. (e) Pathway analysis of *LINC02323*, as assessed by ENCORI website
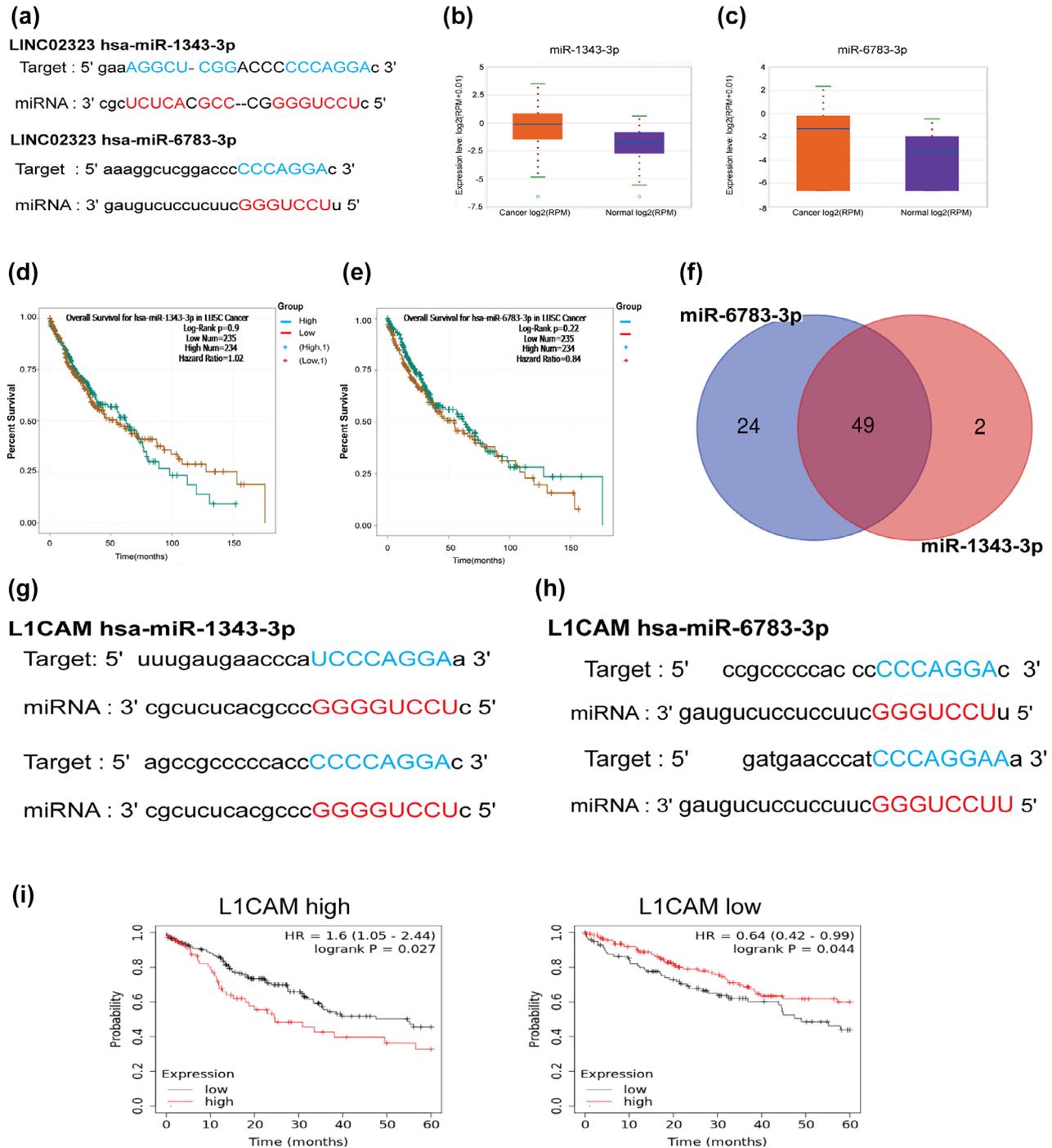
FIGURE 3 The interaction of *LINC02323* with miR-1343-3p or miR-6783-3p. (a) The binding of miR-1343 and miR-6783 on the sequence of *LINC02323*. (b) The expression of miR-1343-3p and (c) miR-6783-3p in lung squamous cell carcinoma (LUSC). (d) The association of miR-1343-3p and (e) miR-6783-3p on OS of LUSC patients. (f) The common targets of miR-1343 and miR-6783. (g) The binding sites of mi-1343-3p and (h) miR-6783-3p in the 3'UTR of L1CAM. (i) The cross-analysis of *LINC02323* with *L1CAM*-high and *L1CAM*-low LUSC patients. *$p < 0.05$; **$p < 0.01$; ***$p < 0.005$; NS, not significant

respectively (Figure 3b, c). However, these two elevated miRNAs had no significant effect on the OS of LUSC patients (Figure 3d, e). We further assessed the potential targets of miR-1343-3p and miR-6783-3p predicted by miRDB (Supporting Information, Table S1, Figure 3f). According to the screening by bioinformatics, *L1CAM* was the target for both miR-1343-3p and miR-6783-3p, which have high affinity on the binding of 3'UTR of *L1CAM* (Figure 3g, h). In addition, cross-analysis revealed that the HR of *LINC02323* for survival was decreased from 1.6 (with higher level of *L1CAM*) to 0.64 (with lower level of *L1CAM*) in LUSC (Figure 3i).
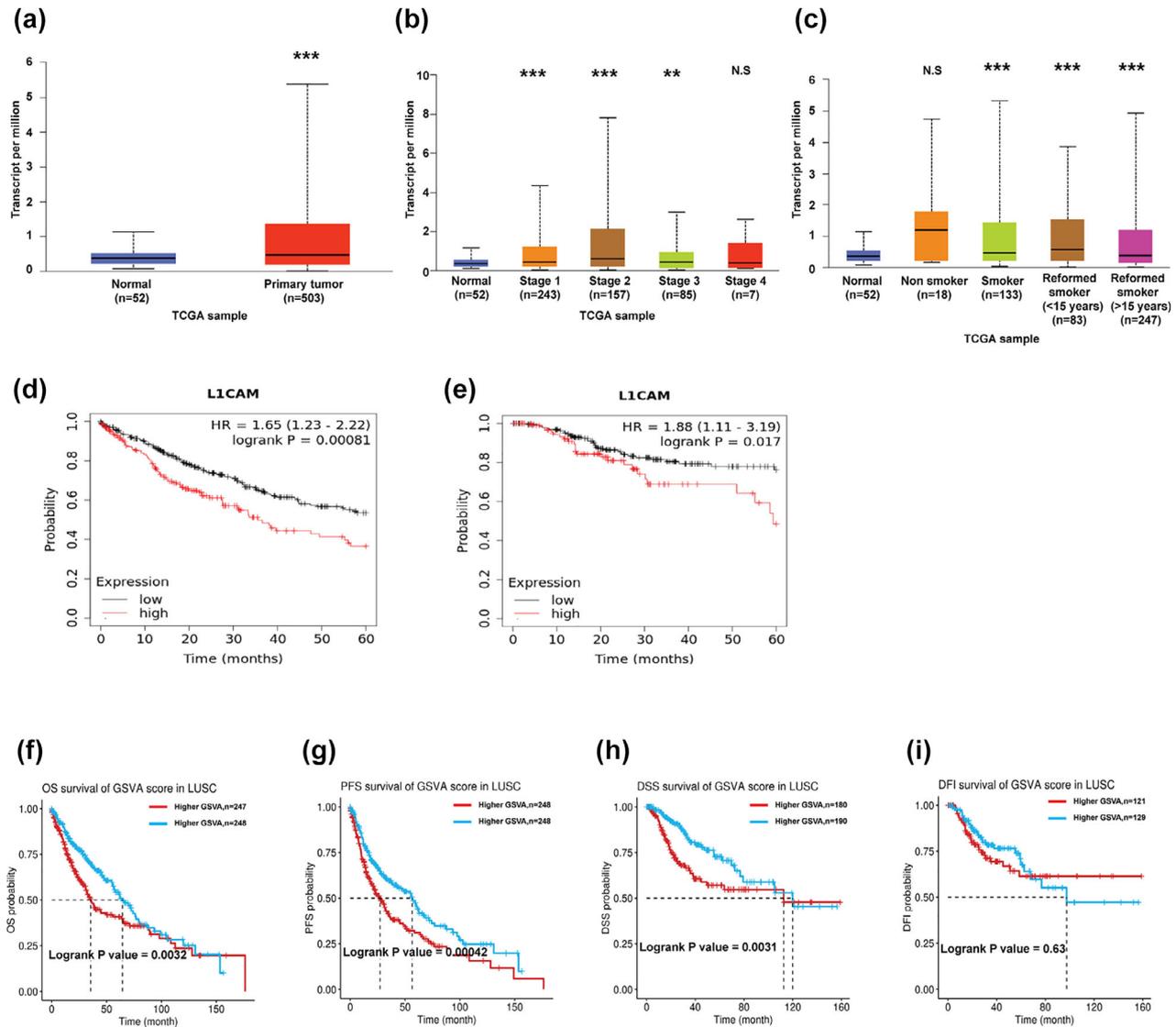
FIGURE 4 The correlation of *L1CAM* in the survival rate of lung squamous cell carcinoma (LUSC) patients. (a) *L1CAM* expression in LUSC. The levels of *L1CAM* in (b) different stages and (c) smoking status. (d) Elevated *L1CAM* was also correlated with poor OS and (e) PRF in the patients with LUSC. (f) The influence of gene set positively correlated with *L1CAM* levels on the OS, (g) PFS and (h) disease-specific survival (DSS) and (i) disease-free interval (DFI). $*p < 0.05$; $**p < 0.01$; $***p < 0.005$; NS, not significant

Upregulation of L1CAM conferred poor survival in LUSC

To identify whether *L1CAM* expression was correlated with LUSC development, we assessed the *L1CAM* expression in normal and LUSC tumor tissue using a TCGA cohort. The results showed that *L1CAM* expression was increased (5.01-fold; p-value = 0.0037; FDR = 0.0087) in TCGA cohort (Figure 4a), although expression did not depend on the progress of cancer stages and smoking status in LUSC (Figure 4b, c). The elevated *L1CAM* was also correlated with poor OS and RFS in patients with LUSC (Figure 4d, e). To elucidate the prognostic value of coexpression gene sets of *L1CAM* in LUSC development, we extracted the gene sets with a positive correlation of *L1CAM* in LUSC and assessed by GCSC website. We found that 54 genes ($r > 0.3$, $p < 0.05$)

were strongly associated with *L1CAM* expression (Supporting Information, Table S2). The higher GSVA score of this gene set was linked to poor prognosis, including shorter OS (HR = 1.52, p-value = 0.002), PFS (HR = 1.42, p-value = 0.005), and disease-specific survival (DSS) (HR = 1.62, p-value = 0.02), but not disease-free interval (DFI) (HR = 0.71, p-value = 0.19) (Figure 4f–i).

Biological functions of *LINC02323*

For biological analysis, the gene set which was positively correlated with *L1CAM* expression had a positive correlation with the epithelial to mesenchymal transition (EMT) and RAS-dependent mitogen-activated protein kinase (RAS/MAPK) pathways (Figure 5a, b). To validate the
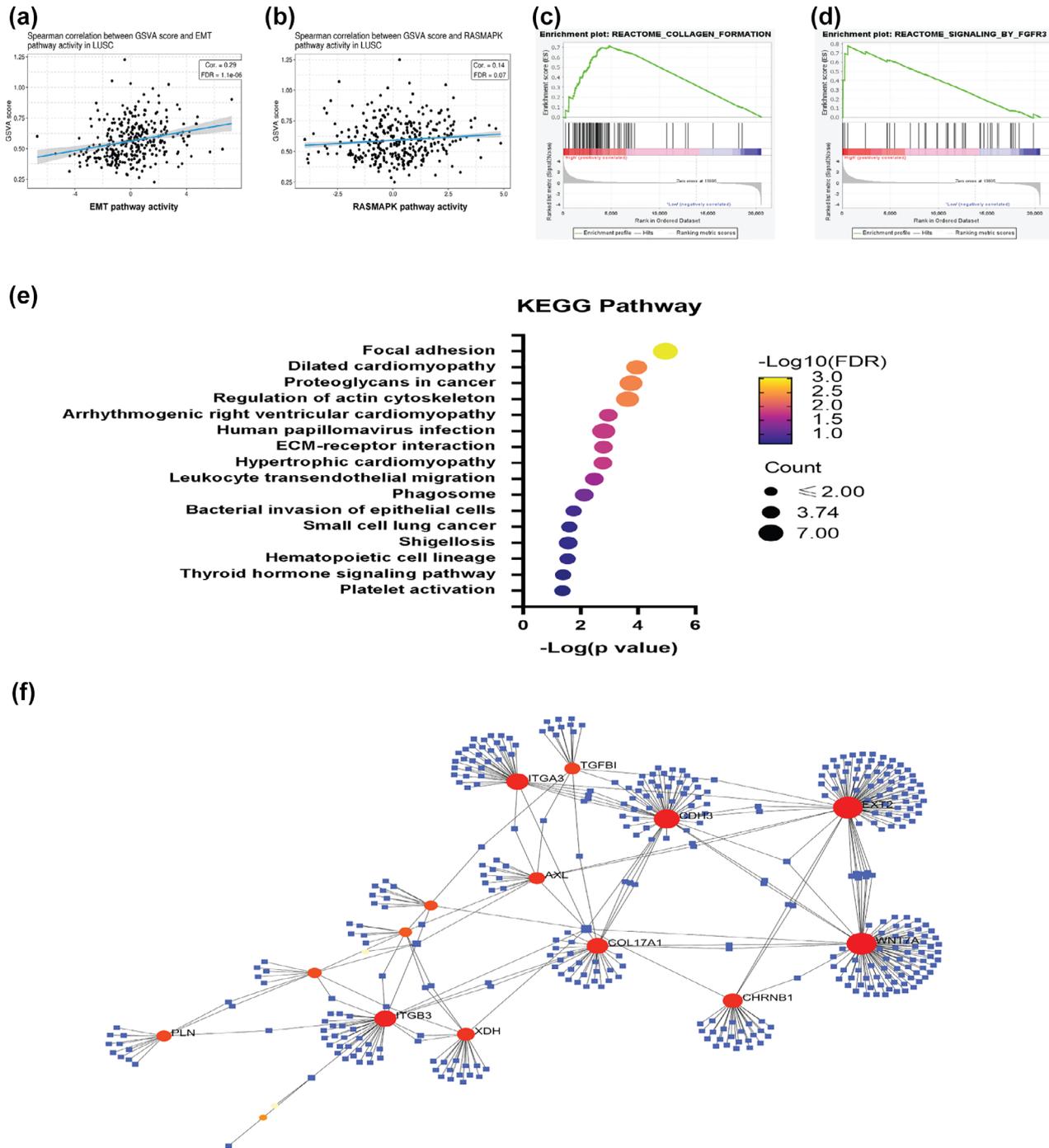
FIGURE 5 Elevated *L1CAM* expression is associated with extracellular matrix (ECM) remodeling and RAS activation. The gene set positively correlated with *L1CAM* levels associated with (a) EMT and (b) RAS/MAPK pathway. The transcriptomes of LUSC patients with high *L1CAM* expression are associated with (c) collagen formation and (d) *FGFR3* activation. (e) Pathway and (f) hub gene analysis of the transcriptomes of LUSC patients with high *L1CAM* expression. ***$p < 0.005$

functional analysis of *L1CAM*, we also divided LUSC patients in the TCGA cohort into *L1CAM* higher and lower expression groups, and then applied the gene expression profiles onto GSEA analysis. The results showed that *L1CAM* and its regulated gene sets in LUSC were mainly involved in collagen formation and *FGFR3* activation (Figure 5c, d). KEGG

pathway analysis also supported that the gene set was associated with the process of ECM, including focal adhesion, proteoglycans in cancer and ECM-receptor interaction (Figure 5e). The hub genes of the *L1CAM*-positive gene set were *ITGA1, ITGB3, TGFBI, AXL, XDH, PLNCDH3, WNT7A, CHRNB1,* and *COL17A1* (Figure 5f).

TABLE 1 The potential RBPs of *LINC02323*

| Gene	Fold change (tumor/normal)		HR of OS		Gene	Fold change (tumor/normal)		HR of OS	
	FC	*p*-value	HR	*p*-value		FC	*p*-value	HR	*p*-value
AUH	0.80	<0.001	1.62	0.01	ILF3	1.71	<0.001	0.78	0.10
BCCIP	1.63	<0.001	0.62	0.04	KHDRBS1	1.36	<0.001	0.78	0.11
BUD13	1.11	<0.001	0.73	0.04	LIN28B	n.a		1.20	0.23
CSTF2T	1.03	0.004	0.69	0.04	LSM11	1.12	<0.001	1.28	0.11
DDX3X	0.87	0.001	0.85	0.27	NONO	1.74	<0.001	0.74	0.04
DDX42	1.18	<0.001	0.86	0.34	NUMA1	0.77	0.06	0.74	0.04
DGCR8	1.49	<0.001	0.76	0.13	PCBP2	1.27	<0.001	0.67	0.01
DKC1	3.59	<0.001	0.67	0.02	PRPF8	0.77	<0.001	1.23	0.18
EIF4A3	2.21	<0.001	0.81	0.17	PTBP1	1.72	<0.001	0.84	0.26
EIF4G2	1.05	<0.001	1.49	0.01	RBFOX2	n.a.		0.75	0.05
FAM120A	1.18	<0.001	1.27	0.15	RBM27	1.09	0.006	0.74	0.05
FBL	2.85	<0.001	0.72	0.03	SAFB2	1.01	0.02	0.74	0.08
FKBP4	4.09	<0.001	0.70	0.02	SF3A3	1.25	<0.001	0.77	0.11
FMR1	1.06	<0.001	0.69	0.02	SF3B4	1.84	<0.001	0.70	0.02
FUS	1.36	<0.001	0.68	0.03	SLTM	1.06	0.01	1.23	0.17
FXR1	2.61	<0.001	0.71	0.02	SMNDC1	1.50	<0.001	0.78	0.09
FXR2	0.76	<0.001	1.31	0.08	SND1	1.33	<0.001	1.26	0.19
GTF2F1	1.12	<0.001	0.72	0.03	SRSF1	n.a.		0.72	0.04
HNRNPA1	1.34	<0.001	0.75	0.05	SRSF7	n.a.		0.79	0.12
HNRNPC	1.69	<0.001	0.72	0.06	SRSF9	n.a.		0.83	0.26
HNRNPK	1.50	<0.001	0.78	0.10	TAF15	1.27	<0.001	0.88	0.42
HNRNPM	1.34	<0.001	0.73	0.03	TARBP2	1.91	<0.001	0.81	0.19
HNRNPU	1.31	<0.001	0.64	0.01	TNRC6A	1.08	0.001	0.70	0.02
HNRNPUL1	1.25	<0.001	0.70	0.02	TRA2A	1.02	0.02	0.70	0.03
IGF2BP2	4.91	<0.001	0.75	0.05	U2AF2	1.68	<0.005	0.69	0.05
IGF2BP3	29.88	<0.001	0.69	0.02	XRN2	1.31	<0.005	1.17	0.36

Abbreviations: FC, fold change; HR, hazard ratio; n.a., not available; OS, overall survival; RBPs, RNA-binding proteins.

Interaction of *LINC02323* with RNA-binding proteins (RBPs)

RBPs are known to be abnormally expressed in cancer and regulate the function of large noncoding RNAs. To better understand the potential regulatory mechanisms of *LINC02323* in LUSC, we examined the expression of RBPs, which interacts with *LINC02323* in LUSC. We used the ENCORI database to explore possible RBPs for *LINC02323*. The results showed that 52 RBPs might modulate *LINC02323* function. A total of 43 RBPs were upregulated and four RBPs were downregulated in LUSC. Among them, *DDX3X* expression was reduced and associated with better OS in LUSC. In contrast, the levels of *FAM120A*, *LSM11*, and *XRN2* were increased and correlated with shorter OS in LUSC (Table 1). To identify the RBP which most probably regulates the *LINC02323*, we conducted a cross-analysis of *LINC02323* with these four RBPs. Cross-analysis of *DDX3X* with *LINC02323* on OS showed that the hazard ratio (HR) of *LINC02323* declined in the high *DDX3X* expression

group (HR = 0.74, *p* = 0.16), compared with that in low *DDX3X* expression LUSC patients (HR = 1.9, *p* = 0.0034) (Figure 6a). In contrast, the effect of *LINC02323* only slightly, or insignificantly, decreased the OS of LUSC patients with lower expressions of *FAM120A*, *LSM11*, and *XRN2* in LUSC (Figure 6b–d). These results showed that *DDX3X* could be a RBP of *LINC02323*, contributing to the development of LUSC.

For biological analysis, we extracted the gene sets which were positively correlated with *DDX3X* expression and assessed it using Metascape. The results showed the gene sets were grouped into functional mcodes, which were involved in RNA splicing, mRNA destabilization and metabolism, histone modification, and stress granule assembly (Figure 6e). In addition, GSEA analysis also supported that *DDX3X* was associated with *E2F* targets, as the pathway analysis of *LINC02323* (Figure 6f). In addition, the NRAS pathway was also involved in the gene enrichment analysis of *DDX3X*, as it was in the analysis of *L1CAM*, a downstream factor of *LINC02323* (Figure 6g).
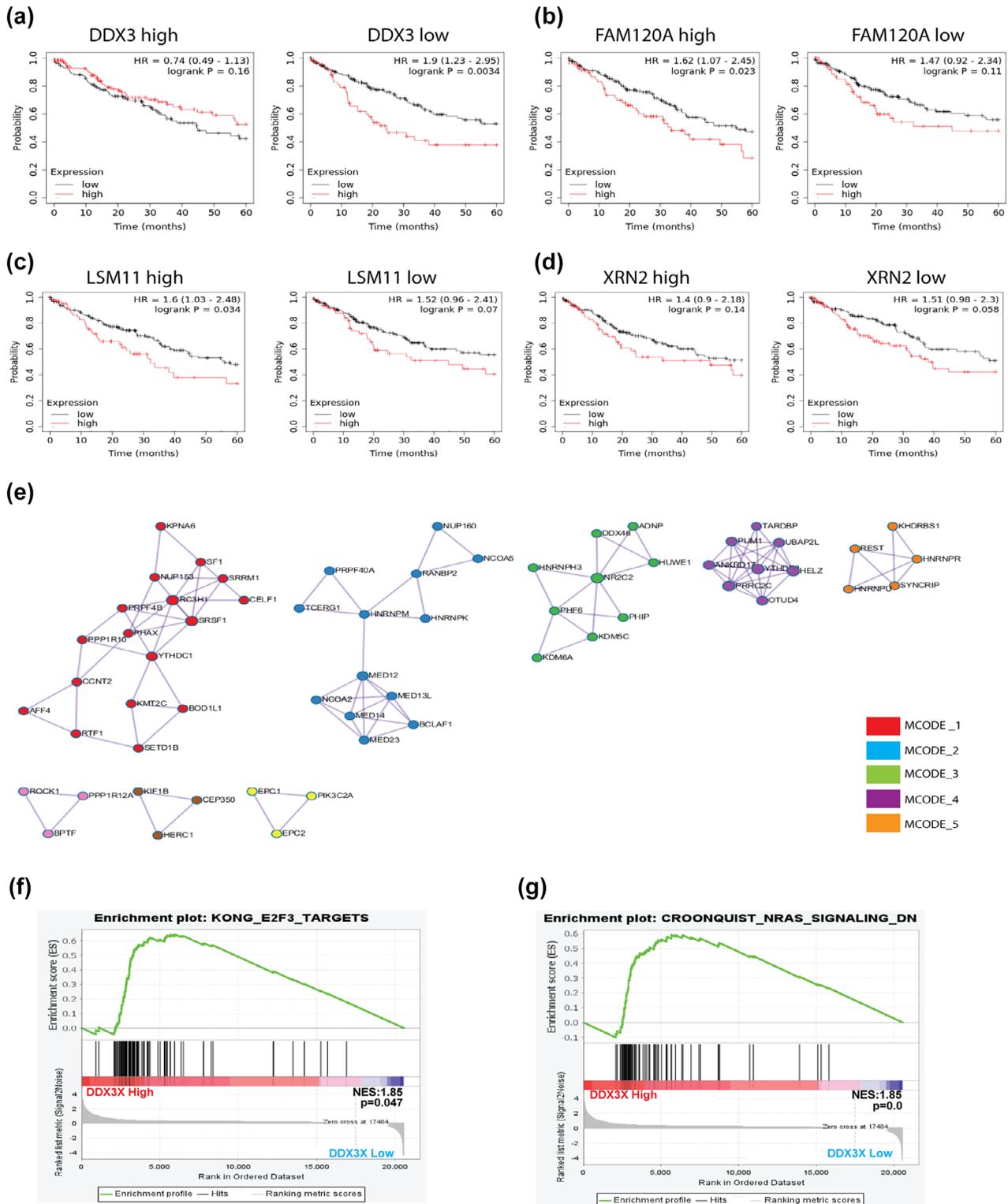
FIGURE 6 DDX3X is the RNA-binding protein (RBP) of *LINC02323* in LUSC. The cross-analysis of *LINC02323* in (a) *DDX3X*, (b) *FAM120A*, (c) *LSM11*, and (d) *XRN* on the OS of LUSC patients. (e) Pathway analysis of the transcriptomes of LUSC patients with high *DDX3X* expression. (f) The gene set positively correlated with *DDX3X* levels associated with (g) *E2F3* and (h) N-RAS pathway in LUSC patients. *p < 0.05; **p < 0.01; ***p < 0.005; NS, not significant

DISCUSSION

Based on the poor outcome, improving the prognosis of LUSC patients by finding novel biomarkers and targets for drug development is urgently required. The association between the dysregulated lncRNAs and the prognosis of patients has been reported in various malignancies.[17] Zhang et al. has reported that *LINC02323* increased metastasis of

LUAD by promoting EMT.[18] The correlations of *LINC02323* expression have not yet been described in LUSC. In our results, *LINC02323* expression was elevated in LUSC tissues. Survival analysis showed that high expression of *LINC02323* was positively correlated with the poor prognosis of patients with LUSC. This is the first comprehensive evaluation of *LINC02323* that implicates its potential oncogenic role in LUSC.

LncRNAs can act as sponges to regulate gene expression by competitively binding miRNAs.[19] *LINC02323* as an oncogenic gene has been found in some studies, and it is linked to numerous malignancies, such as ovarian cancer, liver cancer, and LUAD.[20] Recent evidence showed that *LINC02323* upregulates *E2F1* expression by competitively binding to miR-29c-3p, thereby changing the immune microenvironment and chemoresistance in hepatocellular carcinoma (HCC).[21] miR-129-5p has also been found to inhibit the proliferation, invasion, and migration of gastric cancer cells by selectively decreasing *COL1A1* expression; miR-1343-3p have been indicated to involve *LINC02323*-mediated cancer growth and progression by suppression of TGF-β receptor in LUAD and ovarian cancer.[18,20] Chen et al. reported that LINC00173.v1 binds to miR-511-5p to regulate VEGFA expression, thereby promoting angiogenesis and development in LUSC. Animal experiments have demonstrated that LINC00173.v1 increases the therapeutic sensitivity of LUSC cells to cisplatin and that targeting LINC00173.v1 could be a potential treatment for combating LUSC. The current study identified two miRNAs, miR-1343-3p and miR-6783-3p, associated with various cancers, including colorectal cancer, glioma, HCC, and LUAD.[22–24] Regardless of high expression of miR-1343-3p and miR-6783-3p in LUSC, they were not significantly associated with an OS in LUSC, suggesting the functions of miRNAs were probably via a regulatory network. Indeed, the target gene of miR-1343-3p and miR-6783-3p, of L1 cell adhesion molecule (*L1CAM*), was upregulated in LUSC and also positively correlated with *LINC02323* expression in LUSC ($R = 0.18$, $p < 0.05$), supporting the findings that *LINC02323* inhibited the function of miR-1343-3p and miR-6783-3p. However, the network of *LINC02323*-miR-1343-3p and *LINC02323*-miR-6783-3p interactions warrants further experimental studies in LUSC.

L1CAM is a transmembrane glycoprotein that plays a critical role in cell adhesion, cell migration and cancer development, and is associated with poor prognosis in patients with cancer.[25] *L1CAM* is also regarded as a potential important gene as a predictor of platinum response in high-risk endometrial carcinoma.[26] LUAD patients with high *L1CAM* expression in surgically-resected brain metastases have been reported to have an unfavorable overall survival time.[27] Antagonistic mimetic compounds targeting *L1CAM* decreases glioblastoma cell migration.[28] Specifically, increased *L1CAM* expression has been observed in LUSC patients and has a close correlation with an adverse prognosis in LUSC. Functional enrichment analysis showed that the upregulated genes correlated with *L1CAM* were primarily implicated in various tumor microenvironment remodeling and growth factor-related pathway, including the EMT process, collagen formation and NRAS pathway. Particularly, the upregulated gene set also predicted an adverse prognosis in LUSC patients, including OS, PRF, and DSS. Additional studies and clinical trials are needed to evaluate whether L1CAM blockade may have significant therapeutic efficacy to improve the treatment of LUSC.

LncRNAs can specifically bind to RBPs and influence the biological functions of RBPs. Conversely, certain specific RBPs can bind to lncRNAs to influence the function of lncRNAs on the regulation of downstream gene expression. On the other hand, RBPs can also regulate the expression of lncRNAs at the transcriptional level. DDX3X, one of the members of the DEAD-box helicase family, is able to control most stages of RNA metabolism from transcription, splicing, RNA export to translation.[29,30] DDX3X can bind to lncRNA *TUG1* and increases the stabilization of Bcl-2-associated athanogene 5.[31] LncRNA Rncr4 (retinal noncoding RNA 4) regulates the inhibitory function of DDX3X to mouse retina architecture.[32] The role of DDX3X in lung cancer is controversial. DDX3X is the RBP of LINC00673-v4, which promotes LUAD progression by activating the Wnt pathway.[33] Inhibition of DDX3X by chemical inhibitor RK-33 has been reported to induce apoptosis and increased radiation sensitivity in LUAD A549 cell and NSCLC H1299 cells.[34] In contrast, DDX3X has been reported to be a target of tumor suppressor p53, which enhances the cyclin-dependent kinase inhibitor p21, resulting in cell cycle arrest in lung cancer. Indeed, loss of p53 decreased the expression of DDX3X which in turn contributes to cancer progression and poor patient outcome in NSCLC. Loss of DDX3X decreases E-cadherin expression by MDM2-Slug pathway expression and promotes tumor metastasis.[35] The present study revealed that DDX3X could be a RBP of *LINC02323* due to the loss of *LINC02323* impact on OS in the patient with lower level of *DDX3X*. Reduced expression of DDX3X predicts adverse outcomes and is related to a poor prognosis in patients with LUSC. The consistency between upregulated *LINC02323* and downregulated *DDX3X* was associated with E2F targets and cell cycle regulation, indicating a negative feedback existed between *LINC02323* and *DDX3X*. However, research on the impact of *LINC02323* on the biological function of *DDX3X* is still limited, and further exploration of the associated regulatory mechanism is required.

The limitations of our study are as follows: Biological experiments are required to confirm our results because the study was based on a bioinformatic analysis, and the characteristic details (age, race, tumor grade, size, and therapy) were not taken into account due to our study mainly concentrating on the genes and those commonly identified as significantly changed in TCGA datasets. Therefore, biological information may be needed for further validation.

This study found that the *LINC002323*-miR-1343/6783-3p-*L1CAM* and *LINC02323*-*DDX3X* axis might be the critical network for determining important molecular mechanisms in the progression of LUSC and they might be

valuable prognostic markers and potential therapeutic targets in the progression of LUSC.

AUTHORS' CONTRIBUTIONS

KLW and JYH conceptualized the entire study. YCH, YYW, and CYC provided the technical support, performed the experiments and acquired the data. CYC provided the software management and analyzed the data. YWL and YCH validated the results. YMT and YCH executed the formal analysis. JYH pursued the investigation and provided the resources. YMT, KLW, and JYH performed data curation and interpreted the data. YMT and KLW wrote original draft. JYH and YLH wrote, reviewed and edited the final manuscript. JYH supervised the study, was the project administrator and acquired the funding. All authors read and confirmed the final version of the manuscript.

CONFLICT OF INTEREST
The authors declare that they have no competing interests.

DATA AVAILABILITY STATEMENT
Datasets of the present study are not available in public based on some ongoing studies in our laboratory. However, it is available from the corresponding author on reasonable request.

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