LINC00511 aggravates the malignancy of lung adenocarcinoma through sponging microRNA miR-4739 to regulate pyrroline-5-carboxylate reductase 1 expression

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Abstract

Background: Long non-coding RNA LINC00511 is known to exacerbate lung adenocarcinoma (LUAD) progression. However, the specific mechanism by which LINC00511 affects LUAD progression has not been investigated as yet, and we aimed to elucidate the same in the present study.

Methods: The expression levels of LINC00511, microRNA miR-4739, and pyrroline-5-carboxylate reductase 1 (PYCR1) were determined by quantitative reverse transcription PCR and Western blotting. The Cell Counting Kit-8 and bromodeoxyuridine assays were used to evaluate cell proliferation. Apoptosis was evaluated by flow cytometry, and Bax and Bcl-2 protein levels were determined by western blotting. Cell migration was assessed using transwell assay. The interaction between LINC00511, miR-4739, and PYCR1 was analyzed using luciferase, RNA immunoprecipitation, and RNA pull-down assays.

Results: The expression levels of LINC00511 and PYCR1 in LUAD were downregulated, whereas that of miR-4739 was upregulated. Functional studies showed that knockdown of LINC00511 or PYCR1 suppressed the proliferation and migration of LUAD cells, and promoted apoptosis. On the contrary, knockdown of miR-4739 had tumor-promoting effects. Mechanistically, LINC00511 prevented the miR-4739 led inhibition of PYCR1, resulting in PYCR1 overexpression.

Conclusion: This study demonstrates for the first time that LINC00511 aggravates the malignancy of LUAD by sponging miR-4739 to upregulate PYCR1 expression.

KEYWORDS
apoptosis, LINC00511, lung adenocarcinoma, miR-4739, PYCR1

1 INTRODUCTION

Lung cancer, one of the most common cancers in the world, is the main cause of cancer-related deaths, and lung adenocarcinoma (LUAD) is its most frequent subtype.¹⁻⁴ With an increasing understanding of molecular cancer biology, as well as identification of potential target genes and molecular aberrations that promote tumor growth, LUAD can be subjected to effective diagnostic and
therapeutic approaches. However, despite significant advances in inchoate diagnosis and innovative treatment strategies (such as the anticancer applications of fungus-derived substances), survival rates of patients with LUAD remain low. Therefore, understanding the factors determining the gene or protein expression levels in LUAD will provide a new avenue for its treatment at multiple functional levels.

Long non-coding RNAs (lncRNAs, >200nt long) regulate the expression of various genes, and are thus involved in various functions, including cell growth, differentiation, and development. Several studies have demonstrated a regulatory role of lncRNAs in LUAD. For instance, lncRNA DLG2-AS1 expression has been highly correlated with the malignancy of LUAD. Another lncRNA, MACC1-AS1, was upregulated in LUAD, resulting in poor survival rates and increases malignant cell behavior. Notably, LINC00511 has been shown to promote progression of a variety of cancers, including breast, stomach, and cervical. In addition, Wei et al. found that LINC00511 was upregulated in LUAD and its downregulation inhibited cancer cell proliferation. Similarly, Xue et al. reported increased expression of LINC00511 in LUAD, suggesting a positive correlation between LINC00511 levels and poor prognosis in patients with LUAD. Furthermore, they found that LINC00511 overexpression promotes LUAD progression. Thus, LINC00511 plays an important role in LUAD, and the mechanism of LINC00511 in LUAD warrants further investigation. LncRNAs can regulate mRNA expression through competitive interactions with shared miRNAs and our bioinformatics analysis showed that miR-4926 and PYCR1 were the downstream regulating axis of LINC00511 in LUAD.

MicroRNAs (miRNAs) are noncoding RNAs that regulate cellular biological processes by binding to target miRNAs. Previous studies have revealed that miR-4739 is associated with pleural fibrosis, osteogenic differentiation in mice, and critical limb ischemia in patients with diabetes. Additionally, miR-4739 has recently been reported to act as a tumor suppressor in some cancers. For example, the expression of miR-4739, which inhibits the proliferation, migration, and invasion of cancer cells, was significantly downregulated in prostate cancer. miR-4739 expression was also downregulated in gastric cancer, and it regulated the malignant behavior of cancer cells by targeting ZNF703. However, the effects of miR-4739 in LUAD have rarely been reported.

Pyrroline-5-carboxylate reductase 1 (PYCR1), located in the mitochondria, is one of the most overexpressed metabolic enzymes in cancer. Functional gene screening has showed that PYCR1 overexpression in LUAD is associated with poor prognosis, and its knockout inhibits LUAD cell proliferation, migration, and invasion. However, the specific mechanism of action of the LINC00511/miR-4739/PYCR1 axis in LUAD requires further investigation.

In the present study, we investigated the functional role of the LINC00511/miR-4739/PYCR1 axis in LUAD. We hypothesized that LINC00511 might be acting as an oncogenic factor in LUAD progression by regulating miR-4739/PYCR1 axis. The findings from this study might provide a valuable target for the clinical diagnosis and treatment of LUAD, as well as improve the prognosis of patients with LUAD through LINC00511-based molecular targeting.

2 MATERIALS AND METHODS

2.1 Tissue samples

Each patient signed an informed consent form for this study, which was approved by the Ethics Committee of our hospital. LUAD tissues and paired adjacent normal tissues from 50 patients were collected for this study. The tissues were immediately placed in liquid nitrogen for storage at −80°C.

2.2 Cell culture

Human normal lung epithelial cells (BEAS-2B) and LUAD cells (HCC827, NCI-H1975, A549, and PC9) obtained from ATCC (USA) were cultured in Dulbecco’s modified Eagle’s medium (DMEM; Gibco, USA) supplemented with 10% fetal bovine serum (FBS; Gibco). 1% P/S (Invitrogen, USA) was placed into the culture medium and maintained in an incubator (37°C, 5% CO2).

2.3 Cell transfection

The short hairpin RNAs (shRNAs) targeting LINC00511 or PYCR1 (sh-LINC00511 or sh-PYCR1) and its negative control shRNA (sh-NC) were constructed by Genepharm (Shanghai, China). MiR-4739 inhibitor, mimic, inhibitor-NC, and mimic-NC, were synthesized by Switchgear Genomics (USA). Mimics (100 pmol), inhibitor (50 nM) or shRNA (50 nM) were transfected into HCC827 and A549 cells using Lipofectamine2000 (Thermo Fisher Scientific, USA) and collected after 48h.

2.4 Quantitative reverse transcription PCR (qRT-PCR) assay

Total RNA and miRNAs were extracted according to the protocols of the TRIzol kit (Invitrogen) and mirNeasy EFPE kit (BioTeke, Beijing, China), respectively. First-strand cDNA synthesis for total mRNA was accomplished using the PrimeScript First Strand cDNA Synthesis kit (Takara, Dalian, China), and targeted reverse transcription of miRNA was performed using the Hairpin-it miRNA qPCR Quantitation Kit (GenePharma, China). Subsequently, mRNA and miRNA levels were assessed by qRT-PCR assay with glyceraldehyde-3-phosphate dehydrogenase (GAPDH) or Uracil6 (U6) as reference, using the 2−ΔΔCt method. The primers of the genes involved are listed in Table 1.
TABLE 1 Primers used in the presence work

| Gene     | Primer sequence (5’-3’) |
|----------|------------------------|
| LINC00511 | Forward:CTAAACAAGAGGTAAAGTGTTCAG  
Reverse:AAGTCGCAACACCCCTCATTTAC |
| miR-4739  | Forward:GCTGGGACATTGAAAGTCTCA  
Reverse:GATGTTCCATCGGGTGTTC |
| PYCR1     | Forward:ACACCCCACAACAGGAGAC  
Reverse:GATGTTCCATCGGGTGTTC |
| GAPDH     | Forward:CTAAGGCTAAACCATGAAAAG  
Reverse:ACCAGGACATACAGGGACA |
| U6        | Forward:CACCAAGAGTCTTCTCTCTATTAGT  
Reverse:GCTGAGGCTTCCACAGCT |

2.5 | Cell counting kit-8 (CCK-8) assay

This assay was used to evaluate cell proliferation after transfection. Cells (1 x 10^5 cells/well) were seeded in a 96-well microtiter plate, and CCK-8 solution (Dojindo, Japan) was added at the specified time point. Absorbance was measured at 450 nm using a microplate reader (BioTek Instruments, USA) after 1.5 h of incubation.26

2.6 | Bromodeoxyuridine (BrdU) assay

Cell proliferation was determined using a BrdU Cell Proliferation Assay Kit (CST, USA). After 24 h of cultivation in FBS-free cell medium, the cells were labeled with 1 x BrdU solution for 6 h to induce proliferation. Subsequently, the cells were fixed and denatured, and BrdU was added for further culture for 2 h. Horseradish peroxidase (HRP) antibody was used to detect the binding antibody, and TMB was added to develop color. Finally, absorbance was measured at 450 nm using a microplate reader.

2.7 | Apoptosis assay

The Annexin V/fluorescein isothiocyanate (FITC) apoptosis assay kit (BD Biosciences, USA) was used to evaluate cell apoptosis. Briefly, cells were suspended in 1 x binding buffer, and cell number was adjusted to 1 x 10^5 cells/mL. Cells were incubated with annexin V-FITC and propidium iodide (PI) for 15 min in the dark. The apoptosis rate was then analyzed using a FACSCalibur flow cytometer (BD Biosciences).27

2.8 | Transwell migration assay

The transfected cells were collected to prepare a single-cell suspension, and cell migration potential was determined using a 24-well plate transwell assay with a polycarbonate transwell filter (Corning, USA). Serum-free medium (100 µl) was implanted with the cells in the upper compartment, and the medium containing 20% FBS (500 µl) was injected into the lower compartment. Next, the collected cells were stained with 0.5% crystal violet (Sigma-Aldrich, USA) at 25°C for approximately 20 min after incubation for 24 h. Migratory cells were photographed and counted using an inverted microscope (Olympus, Japan).28

2.9 | Luciferase assay

The LINC00511 fragment containing the potential miR-4739 binding site and the 3′-UTR of PYCR1 were inserted into the pmirGLO vector (Promega, USA) to obtain the wild-type LINC00511 and PYCR1 plasmids (LINC00511-WT and PYCR1-WT). Additionally, the LINC00511 and PYCR1 mutants (LINC00511-MUT and PYCR1-MUT) were obtained using the Q5 Site-Directed Mutagenesis Kit (BioLabs, USA). The cells were then transfected with the mimic or mimic-NC, and LINC00511-WT/MUT or PYCR1-WT/MUT luciferase constructs. Luciferase activity was assessed using a dual-luciferase reporter system (Promega).29

2.10 | RNA immunoprecipitation (RIP) assay

RIP analysis was performed strictly following the protocol of the Magna RIP™ RNA kit (Millipore, USA), to ascertain the coexistence of LINC00511 and miR-4739 in the RNA-induced silencing complex (RISC). After the A549 and HCC827 cells were lysed with RIP lysis buffer, the cell extracts were treated with antibody (anti-AGO2)-binding magnetic beads for 6 h at 4°C. The magnetic beads were then removed, and the enrichment levels of purified LINC00511 were analyzed by qRT-PCR.

2.11 | RNA pull-down assay

A biotinylated miR-4739 probe (Bio-miR-4739) synthesized by GenePharma (USA) was transfected into A549 and HCC827 cells. The cells were then lysed, and the lysate was incubated with M-280 streptavidin magnetic beads (Invitrogen) for 10 min, 48 h after transfection. Finally, the amount of PYCR1 in the magnetic bead eluent was determined using qRT-PCR.30

2.12 | Western blot analysis

Proteins extracted from the radioimmunoprecipitation assay (RIPA) lysis buffer were electrophoresed and separated by 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (SDS-PAGE) and transferred to a polyvinylidene fluoride (PVDF) membrane. Membranes were blocked with 5% non-fat milk for 1 h and then blotted using primary antibodies against Bax (ab32503,
Abcam, UK), Bcl-2 (ab32124, Abcam), PYCR1 (ab279385, Abcam), and GAPDH (ab181602, Abcam) at 4°C overnight. After washing, the membranes were probed with antibody against horseradish peroxidase (HRP) (ab6728, Abcam). The antigen–antibody complex was observed using an enhanced chemiluminescence detection system (Bio-Rad).28

2.13 | Statistical analysis

Data analysis was performed using SPSS 21 (SPSS, USA), and data were expressed as the mean ± standard deviation (SD). Student’s t test was used to compare the two treatment groups, and analysis of variance (ANOVA) was used to compare differences between multiple groups. Statistical significance was set at p < 0.05. All experiments were performed at least three times.

3 | RESULTS

In this study, we investigated the functional role and mechanism of LINC00511 in LUAD via bioinformatic analysis and functional loss experiments. The results showed that downregulation of LINC00511 and PYCR1 levels in LUAD led to antitumor effect. In addition, miR-4739 was downregulated in LUAD, while downregulation of miR-4739 resulted in tumor-promoting behavior. Mechanistically, LINC00511 regulated PYCR1 via a miR-4739 sponge, promoted the proliferation and migration of LUAD cells, and inhibited apoptosis.
3.1 PYCR1 and miR-4936 were shown to be the downstream regulating axis of LINC00511 in LUAD

The significantly upregulated genes by the criteria of adjusted 
\( p < 0.05 \) and \( \log FC > 2 \) in LUAD and lung squamous cell carcinoma (LUSC) were obtained from GEPIA database (http://gepia2.cancer-pku.cn/#analysis). A total of 245 genes in LUAD and 545 genes in LUSC were obtained. By comparing these with the 14 significantly upregulated genes in GSE130779 using the same criteria, two genes were shortlisted: secreted phosphoprotein 1 (SPP1) and PYCR1 (Figure 1A). The role of SPP1 in lung cancer has been thoroughly elucidated; however, studies on PYCR1 in lung cancer are very limited. Thus, we studied how PYCR1 affects lung cancer progression through its involvement in a competitive endogenous RNA (ceRNA) network. First, the expression of LINC00511 in the tissues was studied, and it was found that LINC00511 levels in the cancer tissues were upregulated by approximately 3 times compared with those in the normal tissues (Figure 1B). Similarly, the LINC00511 expression in LUAD cells was upregulated compared with that in BEAS-2B cells (Figure 1C). Then, to establish a link between LINC00511, a significantly upregulated IncRNA in both LUAD and LUSC in the GEPIA database (Figure 1D), and PYCR1, we predicted the potential miRNAs that target PYCR1 using the TargetScan 7.2 algorithm and those that could be targeted by LINC00511 using the starBase algorithm. Five candidate miRNAs were identified using both algorithms: hsa-miR-129-1-3p, hsa-miR-129-2-3p, hsa-miR-1321, hsa-miR-4739, and hsa-miR-4756-5p (Figure 1E). Literature survey indicated that miR-4739 has been reported to act as a tumor suppressor in prostate cancer via a lncRNA-ceRNA network, \(^{21} \) but has not been studied in context of lung cancer so far. We also detected the expression of the five miRNAs in our collected samples and found that miR-4739 was the most significantly downregulated among them (Figure 1F).
3.2 LINC00511 knockdown inhibited the proliferation of LUAD cells

The expression of LINC00511 in HCC827 and A549 cells was silenced by approximately 70% in sh-LINC00511 group compared with sh-NC group (Figure 2A). The functional effects of LINC00511 on proliferation, apoptosis, and migration of LUAD cells were further investigated. CCK-8 assay indicated that silencing of LINC00511 reduced the viability of HCC827 and A549 cells by 40% and 55%, respectively (Figure 2B). BrdU assay indicated that the extent of proliferation of cells with low LINC00511 expression was approximately 50% of that of cells in the sh-NC group (Figure 2C). Moreover, an approximately 1.5-fold increase in apoptosis was observed in the sh-LINC00511 group compared with that in the sh-NC group via flow cytometry (Figure 2D). Western blotting showed an increase in Bax levels and a decrease in Bcl-2 levels after LINC00511 knockdown (Figure 2E). In addition, the transwell assay showed that compared with the sh-NC group, the migration of HCC827 and A549 cells in the sh-LINC00511 group was reduced by 70% and 75%, respectively (Figure 2F).

3.3 LINC00511 sponges miR-4739

Next, we studied the relationship between LINC00511 and miR-4739. Analysis of the starBase database showed that LINC00511 contains sequences that bind to miR-4739 (Figure 3A). Pearson’s correlation analysis showed that miR-4739 expression was negatively correlated to LINC00511 expression in LUAD tissues (Figure 3B). In addition, analysis at the cellular level showed that miR-4739 was downregulated in cancer cells compared with its expression in BEAS-2B cells (Figure 3C). These results indicate that miR-4739 is abnormally expressed in LUAD, which may be related to LINC00511 expression. Therefore, we investigated the relationship between LINC00511 and miR-4739. The luciferase activity of miR-4739 mimic-treated cells containing LINC00511-WT decreased (Figure 3D). RIP analysis showed that the enrichment level of LINC00511 on Ago2 was approximately 50 times higher than that on IgG. These results indicated that LINC00511 binds to miR-4739 (Figure 3E). Additionally, qRT-PCR showed more than 3-fold increase in miR-4739 expression after LINC00511 silencing.
**Figure 4** LINC00511 acted on the biological functions of LUAD cells through miR-4739. A. The miR-4739 level in A549 and HCC827 cells transfected with sh-LINC00511 or miR-4739 inhibitor. B. The viability of A549 and HCC827 cells transfected with sh-LINC00511 or miR-4739 inhibitor was assessed using the CCK-8 assay. C. The proliferation of A549 and HCC827 cells transfected with sh-LINC00511 or miR-4739 inhibitor was assessed using the BrdU assay. D. The viability of A549 and HCC827 cells transfected with sh-LINC00511 or miR-4739 inhibitor was assessed using the flow cytometry assay. E. The relative RNA and protein levels of Bax and Bcl-2 of A549 and HCC827 cells transfected with sh-LINC00511 or miR-4739 inhibitor were measured using western blotting. F. Migration of A549 and HCC827 cells transfected with sh-LINC00511 or miR-4739 inhibitor were evaluated using a transwell assay. **p < 0.001 vs. inhibitor-NC. #p < 0.05, ##p < 0.001 vs. sh-NC. $p < 0.05, $$p < 0.001$ vs. inhibitor+sh-LINC00511.

(Figure 3F). Hence, LINC00511 binds to miR-4739 and thus negatively regulates its expression.

### 3.4 LINC00511 performs its functions in LUAD cells through miR-4739

Next, we investigated the mechanism of action of LINC00511. qRT-PCR showed that miR-4739 levels were downregulated by more than 70% after miR-4739 knockdown and were upregulated by more than 2.5-fold after LINC00511 knockdown (Figure 4A). LINC00511 knockdown also reversed the inhibitory effect of miR-4739 knockdown on miR-4739 levels. CCK-8 and BrdU assays showed an increase in cell viability and proliferation in the inhibitor group compared with those in the inhibitor-NC group. Moreover, the inhibitory effect of LINC00511 silencing on cell viability and proliferation was partially eliminated by miR-4739 knockdown (Figure 4B,C). Flow cytometry showed a decrease in apoptosis...
after miR-4739 knockdown, whereas LINC00511 silencing reversed this effect (Figure 4D). In addition, Western blotting results confirmed that miR-4739 knockdown enhanced the Bcl-2 protein expression, inhibited Bax, and reversed the pro-apoptotic effect of sh-LINC00511 (Figure 4E). Furthermore, miR-4739 knockdown upregulated cell migration by approximately 2 times and reversed the inhibitory effect of low LINC00511 expression on cell migration (Figure 4F).

3.5 | PYCR1 is the target of miR-4739

qRT-PCR analysis was conducted to study the expression of PYCR1 in tissues. As shown in Figure 5A, PYCR1 levels in LUAD tissues were approximately 4-fold those in normal tissues. In addition, Pearson’s correlation analysis revealed a negative correlation between PYCR1 levels and miR-4739 expression in LUAD tissues (Figure 5B). Furthermore, PYCR1 levels were higher in LUAD cells than in BEAS-2B cells (Figure 5C). Additionally, analysis of the starbase database revealed a miR-4739 binding site on PYCR1 (Figure 5D). Moreover, dual-luciferase and RNA pull-down assays revealed that co-treatment of miR-4739 mimic with PYCR1-WT decreased luciferase activity, and biotin-labeled miR-4739 increased PYCR1 enrichment by more than 60 times (Figure 5E,F). These results revealed that PYCR1 was targeted by miR-4739.

3.6 | PYCR1 silencing reversed the effect of miR-4739 knockdown on LUAD cell function

As shown in Figure 6A,B, miR-4739 knockdown increased both the mRNA and protein levels of PYCR1, whereas PYCR1 knockdown reduced them. In addition, the cell viability and proliferation of HCC827 and A549 cells in the sh-PYCR1 group decreased compared with those in the sh-NC group. Moreover, the positive effect of the miR-4739 inhibitor on cell survival was eliminated after transfection with sh-PYCR1 (Figure 6C,D). Flow cytometry showed that knockdown of PYCR1 upregulated the apoptosis of LUAD cells and reversed the effect of the miR-4739 inhibitor on apoptosis (Figure 6E). Furthermore, Western blotting showed that low PYCR1 expression increased Bax levels, decreased Bcl-2 levels, and reversed the inhibitory effect of the miR-4739 inhibitor on apoptosis (Figure 6F). Additionally, transwell assay revealed that PYCR1 silencing reduced cell migration by more than 70% and reversed the enhancing effect of miR-4739 knockdown on cell migration (Figure 6G).

4 | DISCUSSION

In this study, we predicted using bioinformatic analysis that miR-4739 was downregulated in LUAD and confirmed it through
large-scale screening of differentially expressed miRNAs in tumor tissues. Furthermore, we showed that LINC00511 and PYCR1 were upregulated in LUAD and silencing of LINC00511 and PYCR1 resulted in a decrease in cell viability, proliferation, and migration, and an increase in apoptosis. In addition, we showed that miR-4739 could bind to both LINC00511 and PYCR1, and the expression levels of LINC00511 and PYCR1 were negatively correlated with miR-4739 expression in A549 and HCC827 cells. Altogether, our results demonstrate for the first time that LINC00511 exacerbates LUAD by upregulating PYCR1 expression via miR-4739 suppression.

LINC00511 is known to be an oncogene that influences tumor size, metastasis, and prognosis by binding to histone methyltransferase.
Overexpression of LINC00511 was observed in LUAD tissues compared with its levels in normal tissues. CCK-8 assay, flow cytometry, and Western blotting revealed that silencing LINC00511 inhibited proliferation and promoted the apoptosis of A549 cells. Invasion is an important manifestation of the malignancy of tumors. Transwell invasion assay revealed that invasion was significantly reduced when LINC00511 was silenced in A549 cells. Overall, these results indicate that LINC00511 increases the proliferation and invasion of malignant cells. This observation is in accordance with a previous report on the function of LINC00511 in pancreatic ductal adenocarcinoma and squamous cell carcinoma.\(^{31}\)

LncRNAs are known to regulate mRNA expression at transcriptional and post-transcriptional levels through miRNA response elements.\(^{32}\) This mechanism of action is enabled through interactions between IncRNAs and miRNAs to establish the ceRNA axis.\(^{33}\) In addition, there is growing evidence that the ceRNA axis plays a key role in the formation and progression of various cancers.\(^{8}\) For example, IncRNA DGC5 promotes the malignant proliferation of cancer cells in vitro and growth in vivo in LUAD, by regulating miR-22-3p.\(^{34}\) LncRNA TTN-AS1 leads to poor LUAD prognosis and malignant behavior of cancer cells and modulates cyclin-dependent kinase 5 (CDK5) expression by sponging miR-142-5p.\(^{35}\) LncRNA PGM5P4-AS1 upregulates the expression of leucine zipper tumor suppressor (LZTS3) by sponging miR-1275, to inhibit the tumorigenicity of LUAD cells.\(^{28}\) Furthermore, in LUAD, LINC00511 has been found to modulate pyruvate kinase M1/2 (PKM)/baculoviral IAP repeat containing 5 (BIRC5) expression by sponging miR-625-5p/miR-182-3p.\(^{33,36}\) However, in this study, bioinformatic analysis predicted that miR-4739 might be a potential target of LINC00511, which was further confirmed by targeting analysis. In addition, studies have shown that miR-4739 is expressed at low levels in gastric cancer,\(^{20}\) prostate cancer,\(^{21}\) and pancreatic ductal adenocarcinoma,\(^{37}\) and inhibits the proliferation, migration, and invasion of these cancer cells. However, the role of miR-4739 in LUAD remains to be elucidated. In this study, miR-4739 was found to be downregulated in LUAD, and the interference of miR-4739 promoted the malignant behavior of LUAD cells. These results are consistent with those of studies on miR-4739 in other cancers. This study therefore demonstrated that miR-4739 can play an inhibitory role in LUAD but is sponged by LINC00511.

PYCR1 controls proline biosynthesis and converts pyrroline-5-carboxylate to proline.\(^{34}\) Several studies have reported the role of PYCR1 in cancer. For example, Wand et al. found that PYCR1 promotes the malignant phenotype of non-small cell lung carcinoma.\(^{38}\) Wang et al. also reported that PYCR1 was overexpressed and associated with poor prognosis in papillary renal cell carcinoma.\(^{39}\) Furthermore, Gao et al. reported the carcinogenic effect of PYCR1 in LUAD.\(^{24}\) We reached a similar conclusion about LUAD, where PYCR1 was found to be upregulated in cancer tissues and cell lines, and its knockdown inhibited the malignancy of cancer cells. Furthermore, for the first time, we demonstrated that PYCR1 was targeted by miR-4739.

The limitation of this study is that it was conducted only at the cellular level, as its results were not verified in animals with LUAD tumor xenografts. In addition, carcinogenesis is a very complex process, and we did not study the signaling pathway in detail, only confirming the effect of the interaction between LINC00511, miR-4739, and PYCR1 on prostate cancer. We will conduct animal studies and explore the underlying mechanisms of LUAD in the future.

5 | CONCLUSION

This study indicated that LINC00511 acts as an oncogenic RNA and is upregulated in LUAD, functioning as a miR-4739 “sponge,” and thus upregulating PYCR1. Overall, characterization of the LINC00511/miR-4789/PYCR1 axis in this study provides a novel avenue for LUAD treatment.

AUTHOR CONTRIBUTIONS

LY performed the experiments and data analysis. LW conceived and designed the study. YZ made the acquisition of data. LY and LW did the analysis and interpretation of data. All authors read and approved the article.

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CONFLICTS OF INTEREST

There is no conflict of interest declared by the authors.

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All data generated or analyzed during this study are included in this article.

CODE AVAILABILITY

Not available.

CONSENT FOR PUBLICATION (INCLUDE APPROPRIATE STATEMENTS)

Consent for publication was obtained from the participants.

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