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ORIGINAL ARTICLE

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CDR1as is overexpressed in laryngeal squamous cell carcinoma to promote the tumour's progression via miR-7 signals

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Abstract

Objectives: To investigate the roles played by the circular RNA (circRNA) molecule ciRS-7 (CDR1as) and tumour suppressor miRNA-7 (miR-7) in laryngeal squamous cell carcinoma (LSCC).

Methods: Specimens of LSCC tissue (n = 30) and corresponding relative normal tissue (n = 30) were collected to determine their levels and clinical significance of CDR1as/mir-7 expression. The CDR1as and miR-7 were overexpressed in LSCC cells to investigate its function and mechanism in vitro and in vivo.

Results: Patients with high TNM stages, poorly differentiated tumours, lymph node metastases and poor prognosis had high CDR1as levels but low miR-7 levels. CDR1 expression was negatively associated with miR-7 expression in LSCC. Overexpression of CDR1as in vitro enhanced cell vitality, and promoted the proliferation, migration, and invasion of two LSCC cell lines (Hep2 and AMC-HN-8.) However, these effects could be abrogated by knockdown of CDR1as or the forced expression of miR-7. Mechanistically, overexpressed CDR1 molecules functioned as miR-7 sponges and upregulated the key targets of miR-7, CCNE1, and PIK3CD in Hep2 and AMC-HN-8 cells. In vivo studies demonstrated the tumourigenic role of CDR1as. Overexpression of CDR1as alone promoted tumour growth and increased expression of the proliferation indices ki-67, CCNE1, and PIK3CD. Although the tumour suppressor miR-7 effectively inhibited the tumour growth, this effect could be counteracted by co-treatment with CDR1as in vivo.

Conclusion: CDR1as is an oncogene that promotes LSCC progression by regulating miR-7 signals.

1 | INTRODUCTION

Laryngeal cancer is the second most common respiratory tract malignancy, and the majority of laryngeal cancer (85%-95%) is laryngeal squamous cell carcinoma (LSCC). The incidence of LSCC has been increasing each year. In 2016, an estimated 13 430 new cases of laryngeal cancer were diagnosed, and ~3620 patients died from the disease.[1] Despite therapeutic advances in the last two decades, the clinical outcomes of patients with advanced LSCC have not improved, and the 5-year survival rate has decreased from 66% to 63%. This decrease in survival can be possibly attributed to a lack of definitive diagnosis, high local and regional recurrent rates after surgery, the increased use of chemoradiation therapy, and related therapy resistance.[2,3] These factors highlight the need for further research and innovation in identifying the underlying molecular mechanisms of LSCC, developing more effective diagnostic methods, and finding new therapeutic targets.

Non-coding RNAs (ncRNAs) account for 95% of the total RNA transcribed in eukaryotic cells, and include short microRNAs (miRNAs), long non-coding RNAs (lncRNAs), and circular RNAs (circRNAs).[4] Circular RNAs (circRNAs) are newly discovered endogenous non-coding RNA (ncRNA) molecules with covalently closed continuous loops, and act as gene regulators in mammals. Mounting evidence suggests that circRNAs may function as competing

endogenous RNAs or microRNA sponges in regulating alternative splicing or transcription mechanisms, and are thought to be involved in gene regulation during various biological processes and diseases.[5,6]

Cerebellar degeneration-related protein 1 antisense RNA (CDR1as), also known as circular RNA sponge for miR-7 (ciRS-7), was reported to antagonize miR-7 availability.[7] In islet cells, overexpression of CDR1as significantly increased both insulin content and secretion via miR-7/*Myrip/Pax6* signals.[7] *CDR1as*-deficient brains of animals displayed impaired sensorimotor gating, which manifests in psychiatric disorders as a deficiency in the ability to filter out unnecessary information, and is associated with miR-7 and miR-671.[8] Moreover, CDR1as was found to be upregulated in gastric cancer,[9] colorectal cancer,[10] and hepatocellular carcinoma[11] tissues, and be predictive of a poor clinical outcome for cancer patients. However, the clinical role played by CDR1as in LSCC is unclear.

The availability of miR-7, a tumour suppressor, is reduced by CDR1as during carcinogenesis by its targeting of EGFR/RAF1/MAPK signals or the PTEN/PI3K/AKT signalling pathway.[6,12] Interestingly, a detailed genomic analysis from the Cancer Genome Atlas Research Program (TCGA) indicated that these pathways have multiple potential therapeutic targets for laryngeal cancer, and these targets include EGFR, ERBB2, CCND1, TP53, and PIK3CD.[13] Thus, we speculated that CDR1as/miR-7 signalling might be a promising therapy target for LSCC. In this study, we estimated the levels of CDR1as and miR-7 expression in LSCC samples, and determined its clinical significance. The functions of CDR1as and miR-7 were also investigated in vitro and in vivo.

2 | MATERIAL AND METHODS

2.1 | Tumour samples, cell lines, and reagents

Thirty specimens of LSCC tissue and corresponding relative normal tissue were collected from the Fifth Hospital Affiliated with the Medical University of Guangzhou. All diagnoses of laryngeal cancer were confirmed by experienced pathologists after haematoxylin and eosin staining. Each patient who participated in the study provided their written informed consent. All these retrospective specimens were processed according to ethical and legal standards of the Fifth Hospital Affiliated with the Medical University of Guangzhou. The 30 patients were followed-up until 4 March 2018. Hep2 and AMC-HN-8 LSCC cells were purchased from the cell bank of the Chinese Academy of Sciences (Shanghai, China) and cultured in Dulbecco's Modified Eagle's medium (DMEM) supplemented with 10% FBS (Life Technologies, Carlsbad, CA), ampicillin, and streptomycin at 37°C, in a 5% CO_2 atmosphere. Vector-CDR1as and its negative control (which inserted a ciRS-7 sequence only, but no invert repeat flanking introns into the Vector-empty plasmid, resulting in no circular CDR1as), shRNA-CDR1as were synthesized by GenePharma (Shanghai, China). MiR-7 mimics and negative controls were purchased from RiboBio (Guangzhou, China). Anti-GAPDH, E-cadherin, N-cadherin, vimentin, CCNE1, and PIK3CD antibodies were obtained from Cell Signalling Tech (Danvers, MA) and Abcam (Cambridge, MA).

2.2 | Cell transfection

Hep2 and AMC-HN-8 cells were seeded into 12 plates. Next, Lipofectamine 2000 (Invitrogen, Waltham, MA) was used to transfect the cells with Vector-CDR1as, shRNA-CDR1as, miR-7 mimics or a negative control for the indicated time period at a concentration of 1 ng/mL as described in the manufacturer's instructions.

2.3 | CCK-8 assay

Hep2 and AMC-HN-8 cells that had been transfected with Vector-CDR1as, shRNA-CDR1as, miR-7 mimics or a negative control were harvested and washed with PBS. Regents contained in a Cell Counting Kit-8 (Kumamoto, Japan) were mixed with DMEM and used for the cell viability assays. Absorbance was measured at 450 nm with a microplate reader.

2.4 | Colony formation assay

Hep2 and AMC-HN-8 cells overexpressing CDR1as and/or miR-7 were harvested and re-suspended in complete medium containing 10% FBS; after which, they were seeded into 12-well plates and cultured for 10 days. The cells were then stained with 0.1% crystal violet, fixed with methanol for 15 minutes, and visualized under a dissection microscope (Olympus, Tokyo, Japan). Colonies containing ≥50 cells or were counted.

2.5 | Flow cytometry assays

Hep2 and AMC-HN-8 cells used for cell cycle analysis were stained with PI staining solution (10 μg/mL RNase A and 50 μg/mL PL) at 37°C for 30 minutes in the dark. The cell cycle distribution was analysed by using a flow cytometer equipped with Cell-Quest software (BD Biosciences, Franklin Lakes, NJ, USA). Cells used for apoptosis analysis were fixed in cold 70% ethanol at −20°C for 2 hours. After fixation, RNase (10 mg/mL) was added and the cells were stained with 2 μL of annexin V mixed with 2 μL of propidium iodide (PI; eBioscience, San Diego, CA) according to the manufacturer's instructions. The cells were then analysed by flow cytometry.

2.6 | Transwell assay

Aliquots containing 2×10^4 Hep2 or AMC-HN-8 cells transfected with CDR1as and/or miR-7 were added to the upper chamber of a non-coated Transwell insert. For the invasion assay, tumour cells were added to the upper chambers of Transwell inserts that had been coated with Matrigel. Cells that did not migrate or invade were removed with a cotton swab, stained with crystal violet, and counted under an inverted microscope. Five randomly selected microscopic fields were used for cell counting.

2.7 | Western blot

Total cellular protein extracts were separated by sodium dodecyl sulphate-polyacrylamide gel electrophoresis (SDS-PAGE), and the protein bands were transferred onto a nitrocellulose membrane (Bio-Rad, Hercules, CA), which was then blocked with 5% non-fat milk in TBST buffer (Tris Buffer Saline containing 0.1% Tween-20) for 1 hour at room temperature. After blocking, the membrane was incubated with primary antibodies overnight at 4°C. The membrane was then washed with TBST buffer, and the immunostained protein bands were incubated with a HRP-conjugated secondary antibody for 1 hour at room temperature. The resulting protein blots were washed with TBST buffer and visualized with \ECL-Plus reagent (Millipore, Billerica, MA). GAPDH was used as a loading control in the studies.

2.8 | Real-time PCR

Quantitative real-time RT-PCR (qRT-PCR) was performed according to a standard protocol. Total RNA was extracted from cells and tumour samples using TRIzol reagent (Invitrogen) according to the manufacturer's instructions. cDNA was synthesized using a PrimeScript RT Reagent Kit (Takara, Dalian, China). PCR amplification was performed using conditions of 95°C for 10 seconds, 40 cycles of 94°C for 30 seconds, 60°C for 30 seconds, and 72°C for 30 seconds on an ABI 7900 PCR system (Applied Biosystems, Foster City, CA) with SYBR Green Real-time PCR Master Mix (Takara). The expression levels of genes for CDR1as, miR-7, CCNE1, PIK3CD, E-cadherin, N-cadherin, and vimentin were normalized to that for GAPDH. The relative levels of gene expression were calculated by the $2^{-\Delta\Delta Ct}$ method. Each experiment was performed in triplicate. The primers used for studies are listed below:

Gene	Primer 5′-3′
CDR1as	F: TAGTACGTCGTGCCCTGA
	R: CACTTGACGTGCAGCATC
miR-7	F: CCACGTTGGAAGACTAGTGATTT
	R: TATGGTTGTTCTGCTCTCTGTCTC
U6	F: CTCGCTTCGGCAGCACA
	R: AACGCTTCACGAATTTGCGT
CCNE1	F: ATGAAGAAGTTGAACCATGCCA
	R: CCTCCAGAACAGTATTCCATTGC
E-Cadherin	F: ATGGCTTCCCTCTTTCATCTC
	R: ATAGTTCCGCTCTGTCTTTGG
N-Cadherin	F: TCAGGCGTCTGTAGAGGCTT
	R: ATGCACATCCTTCGATAAGACTG
PIK3CD	F: AAGGAGGAGAATCAGAGCGTT
	R: GAAGAGCGGCTCATACTGGG
GAPDH	F: ACACCCACTCCTCCACCTTT
	R: TTACTCCTTGGAGGCCATGT

2.9 | Immunohistochemistry and immunofluorescence assays

Immunohistochemistry and immunofluorescence assays were performed as previously described.[14] Immunohistochemistry (IHC) was performed to determine the Ki-67 scores of the tumour tissues in the different groups.

2.10 | Xenograft animal studies

To examine the role played by CDR1as in vivo, Hep2 cells were transfected with lentivirus vector for miR-7, Vector-CDR1as, both miR-7 and vector-CDR1, or a negative control. Hep2 cells (2×10^6) were subcutaneously injected into the rear flank of nude mice (6 mice per group). The tumour sizes were measured at 3-day intervals, and the tumour volumes were calculated using the formula: V (cm^3) = width^2 (cm^2) × length (cm)/2. The protocol used for these studies was approved by the Institutional Animal Care and Use Committee of the Fifth Hospital Affiliated with the Medical University of Guangzhou.

2.11 | Statistical analyses

All data were analysed using SPSS for Windows, Version 16.0 (SPSS 16.0, SPSS Inc, Chicago, IL) and the Prism statistical software package (Version 5.0; Graphpad Software Inc, La Jolla, CA, USA). Unpaired *t*-tests or Mann-Whitney *U* tests were used for comparisons between two groups, and multiple group comparisons were analysed by one-way ANOVA. A *P*-value <0.05 was considered statistically significant. All experiments were performed at least three times.

3 | RESULTS

3.1 | CDR1as predicted a poor clinical outcome in LSCC

To investigate the clinical significance of CDR1as in LSCC, the levels of CDR1as expression were assessed in the LSCC tissues (n = 30) and corresponding relatively normal tissues (n = 30). Q-PCR results indicated that CDR1as expression was significantly upregulated in the LSCC tissues (tumour) when compared with its expression in the corresponding non-neoplastic tissues (Normal; Figure 1A). Moreover, we also analysed the correlations between CDR1as overexpression and various clinicopathological characteristics of the LSCC patients, including tumour–node–metastasis, cellular differentiation, and lymph node metastasis etc The results showed that LSCC patients with a higher TNM stage (Figure 1B), poor cellular differentiation (Figure 1C), and extensive lymph node metastasis (Figure 1D) had higher levels of CDR1as in their tumour samples. But there were no relationships between CDR1as and gender or age of LSCC patients (Figure 1E,F). The correlation between survival time and the CDR1as expression were analysed in LSCC patients. The results showed that the LSCC patients with higher expression of CDR1as have shorter survival time (HR ratio: 0.2484; 95% CI: 0.07053-0.8745; Figure 1G). Furthermore, we found that tumour suppressor miR-7 was

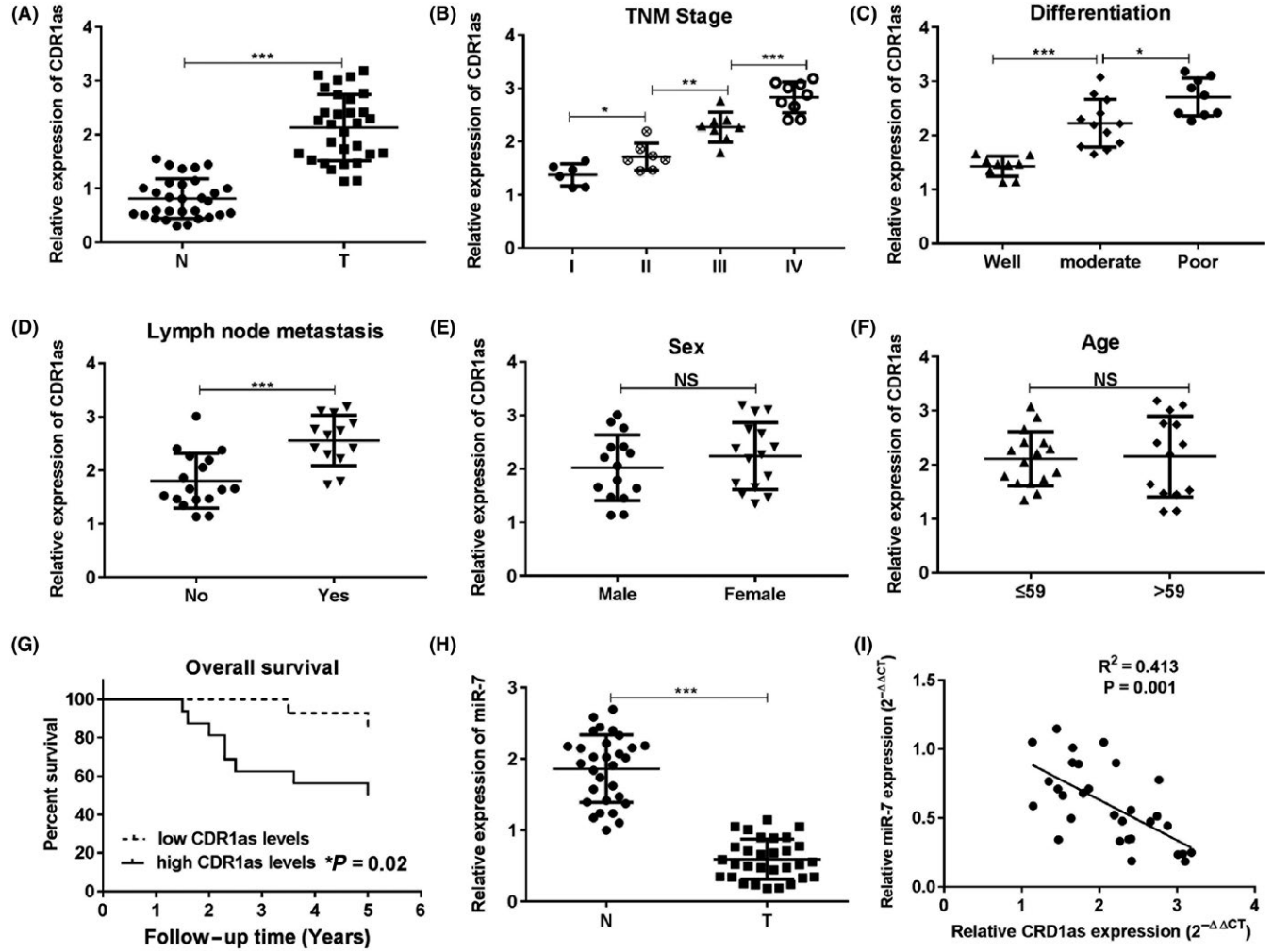

FIGURE 1 Expression of CDR1as and miR-7 in LSCC. A, The mRNA levels for CDR1as in samples of LSCC and relatively normal were determined by Q-PCR. B-F, The clinicopathological characteristics tumour–node–metastasis (TNM) stage (B), tumour differentiation (C), lymph node metastasis (D) gender (E) and age (F) of LSCC patients were analysed based on the expression of CDR1as. G, The correlation between survival time and the CDR1as expression were analysed in LSCC patients. H, The levels of mRNA for miR-7 in samples of LSCC tissue and relatively normal tissue were determined by Q-PCR. I, The correlation between CDR1as and miR-7 in LSCC was analysed. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$. Data represent the mean ± SD

downregulated in the tumour tissues (Figure 1H). An analysis of the correlation between CDR1as and tumour suppressor miR-7 showed that CDR1as levels were negatively associated with miR-7 levels in the LSCC samples (Figure 1I). These data indicated that upregulated CDR1as levels were associated with the progression of LSCC.

3.2 | CDR1as promoted the growth and metastasis of tumour cells in vitro

Because CDR1as predicts a poor clinical outcome for LSCC patients, we next determined the function of CDR1as in vitro by forcing its overexpression or knockdown. The Q-PCR results demonstrated that we had successfully overexpressed or knocked down the CDR1as levels in the two LSCC cell lines, Hep2 and AMC-HN-8 (Figure 2A). We then determined the vitality of the two cell lines at different time points and found that overexpression of CDR1as enhanced the vitality of the tumour cells, while knockdown of CDR1 abrogated that effect (Figure 2B). Results of the EdU proliferation assay indicated that the proliferation of Hep2 and AMC-HN-8 cells was remarkably upregulated by CDR1as overexpression (Figure 2C). The colony forming ability of cells that overexpressed of CDR1as was elevated, but was inhibited in cells in which CDR1as had been knocked down (Figure 2D). The cycle assays showed that knockdown of CDR1as induced G1/S phase arrest and apoptosis in both cell lines (Figure 2E,F, respectively).

We also examined the migration and invasion abilities of the Hep2 and AMC-HN-8 cells. Overexpression of CDR1as enhanced the migration and invasion abilities of the cells when compared to cells transfected with a negative control vector. However, inhibition of CDR1as produced the opposite effects (Figure 2G). The EMT phenotype was also induced by CDR1as, and this induction was accompanied by a decreased level of E-cadherin and increased levels of N-cadherin and vimentin (Figure 2H,I).

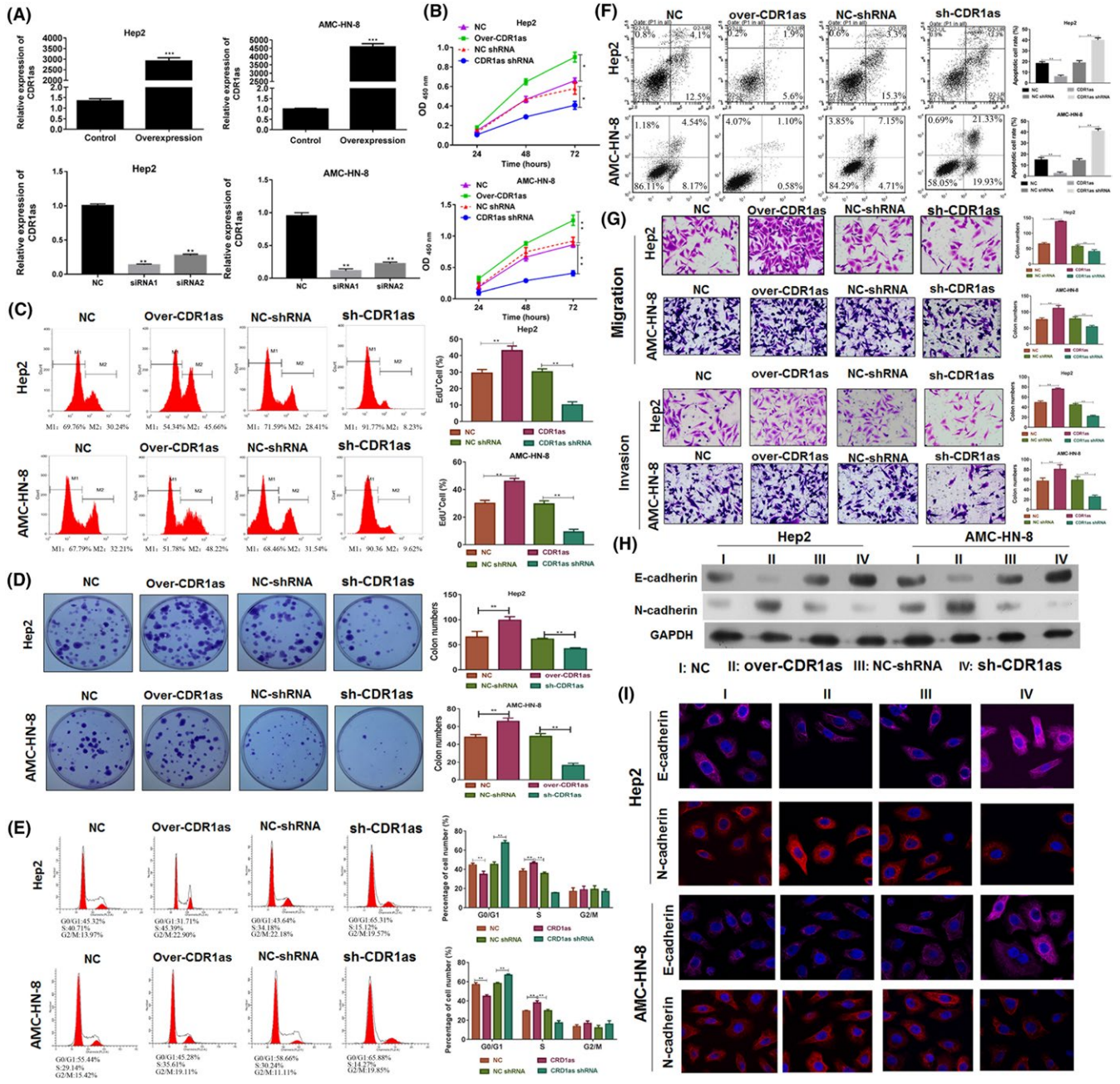

FIGURE 2 CDR1as promoted the tumour characteristics of LSCC in vitro. A, The efficiency of transfection with Vector-CDR1as, shRNA-CDR1as or a negative control for 48 h was determined in Hep2 and AMC-HN-8 cells by Q-PCR. B, The effects of CDR1as on cell vitality were estimated by the CCK-8 assay. C and D, The EdU proliferation assay and colony formation assay were used to examine the proliferation of Hep2 and AMC-HN-8 cells with overexpressed or decreased CDR1as. E and F, The effects of CDR1as on the cell cycle and apoptosis were analysed in Hep2 and AMC-HN-8 cells by flow cytometry. G, The migration and invasion abilities of two cell lines with overexpressed or decreased CDR1as were determined by the Transwell assay. H and I, The EMT phenotype (N-cadherin and E-cadherin) of the two cells was determined by WB and IF. $^{**}P < 0.01$, $^{***}P < 0.001$, data represent the mean ± SD

3.3 | Mir-7 signals impaired the tumourigenic function of CDR1as in vitro

Our studies showed that CDR1as upregulates the miR-7 targets for LSCC growth and migration in vitro. To confirm the role of miR-7 in CDR1as-induced carcinogenesis, we forced overexpression of miR-7 in LSCC cells. When compared with cells that overexpressed CDR1as alone, cells that co-expressed CDR1as and miR-7 displayed decreased proliferation, impaired vitality (Figure 3A,B), reduced rates of colony formation (Figure 3C), G1/S arrest, increased rates of apoptosis (Figure 3D,E), and reduced migration and invasion capabilities (Figure 3F). In addition, the CDR1as-induced EMT phenotype of the LSCC cells was reversed by miR-7 (Figure 3G,H). These data indicated that restoration of miR-7 expression in LSCC cells could inhibit the tumourigenic function of CDR1as.

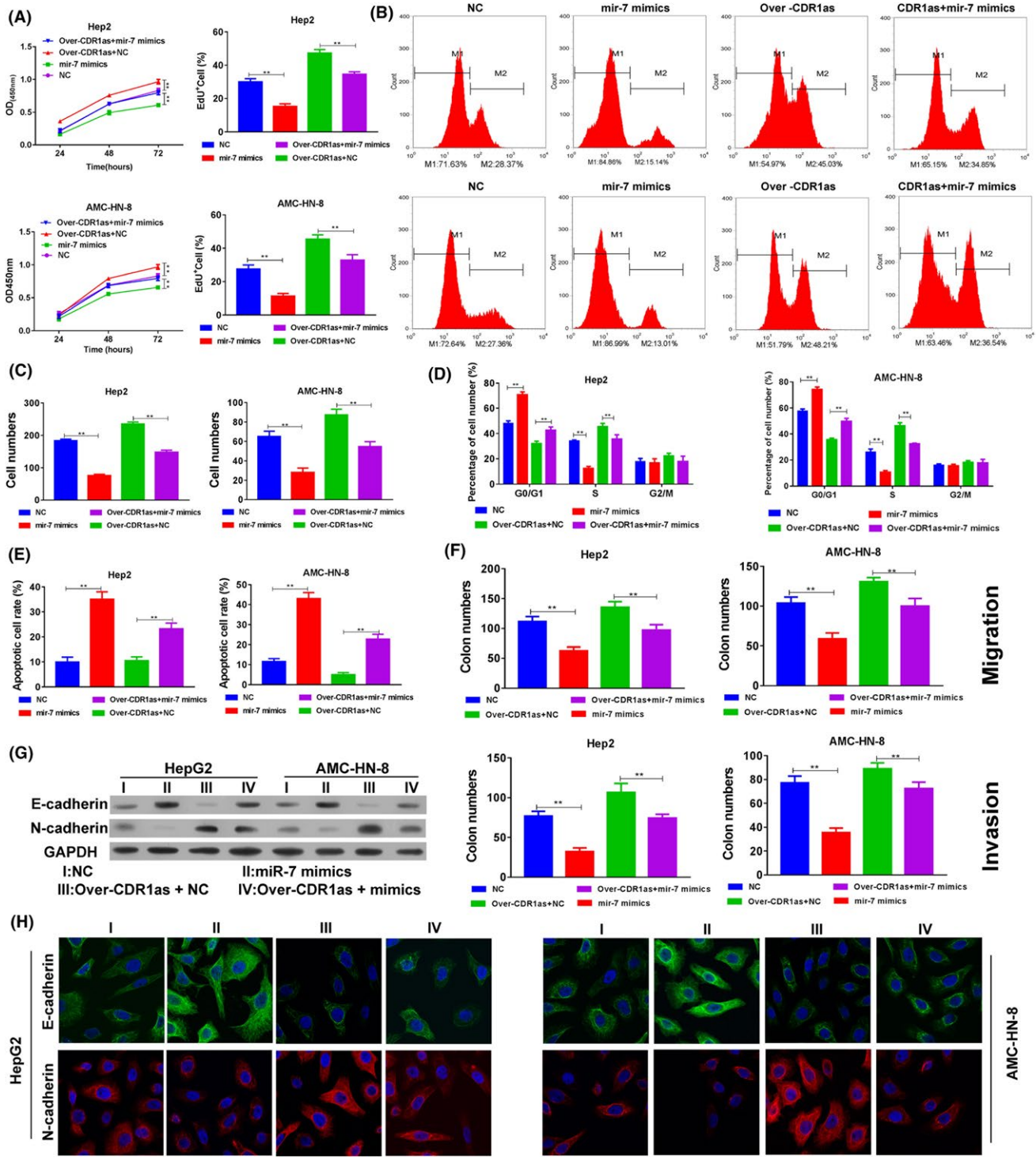

FIGURE 3 MiR-7 inhibited the CDR1as-induced progression of LSCC in vitro. A, Hep2 and AMC-HN-8 cells were transfected with vector-CDR1as, miR-7 mimics or a negative control for 48 h and cell vitality was estimated by the CCK-8 assay. B and C, The EdU proliferation assay and colony formation assay were used to indicate the proliferation of Hep2 and AMC-HN-8 cells transfected with CDR1as or miR-7. D and E, The effects of CDR1as on the cell cycle and apoptosis were analysed by flow cytometry. F, The migration and invasion abilities of two cell lines transfected with CDR1as or miR-7 were determined by the Transwell assay. G and H, The EMT phenotype (N-cadherin and E-cadherin) of two cells lines was determined by WB and IF. $^{**}P < 0.01$, $^{***}P < 0.001$, data represent the mean ± SD

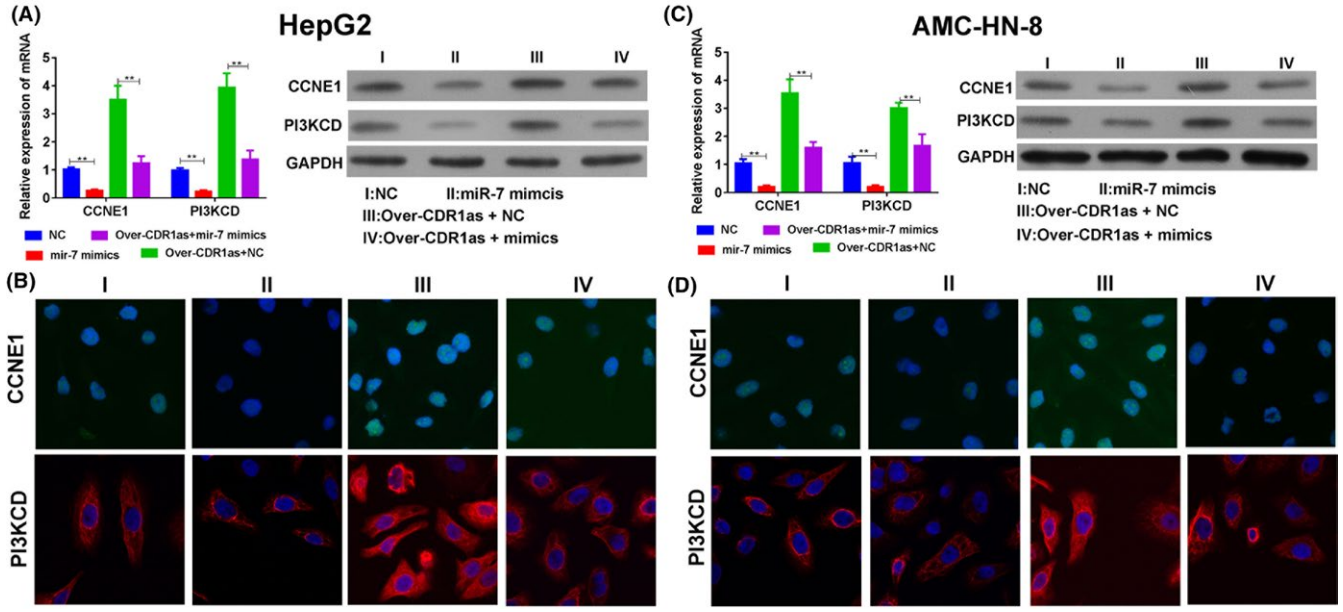

FIGURE 4 CDR1as upregulated the miR-7 target genes. Hep2 and AMC-HN-8 cells were transfected with vector-CDR1as, miR-7 mimics or a negative control for 48 h and the mRNA and protein levels of CCNE1 and PIK3CD in (A and B) Hep2 and (C and D) AMC-HN-8 cells were determined by Q-PCR, WB and IF. $^{**}P < 0.01$, data represent the mean ± SD

3.4 | The targets of mir-7 were upregulated by CDR1as

Because miR-7 and its targets appeared to be regulated by CDR1as for tumour progression, we estimated the impacts of CDR1as on the miR-7 targets in LSCC cells. We found that CDR1as increased the levels of CCNE1 and PIK3CD mRNA and proteins in Hep2 (Figure 4A,B) and AMC-HN-8 (Figure 4C,D) cells, which was conducive to tumour proliferation and metastasis. Moreover, we also confirmed that although the miR-7 target genes CCNE1 and PIK3CD could be induced by CDR1as, restoration of tumour suppressor miR-7 expression abrogated this process and inhibited the expression of CCNE1 and PIK3CD in the two cell lines. This indicated the involvement of miR-7/CCNE1/PIK3CD in CDR1as-induced LSCC tumour growth.

3.5 | The tumourigenic role of CDR1as in vivo

We next examined the tumourigenic role of CDR1as in vivo. Nude mice were implanted with human Hep2 cells that overexpressed miR-7, CDR1as or both. The results indicated that overexpression of CDR1as alone significantly promoted tumour growth and increased tumour volume, whereas the mice implanted with Hep2 cells that highly expressed both miR-7 and CDR1as had smaller tumours (Figure 5A,B). Tumour proliferation was confirmed by the index Ki-67 level in the tumour (Figure 5C). The EMT phenotype and levels of CCNE1 and PIK3CD expression were determined and showed that CDR1as could promote the EMT and increase cellular CCNE1 and PIK3CD levels in vivo; both of these processes were inhibited by miR-7 (Figure 5D-F). These results demonstrated that CDR1as participated into the progression of LSCC via miR-7 signals.

4 | DISCUSSION

As the second most common cancer in the head and neck region, the overall survival time of LSCC patients, and especially advanced-stage patients, has not significantly improved during the past several decades.[1] In this study, we sought to discover new therapeutic targets for LSCC. CircRNAs play important roles in the initiation and development of cancers, and mainly serve as microRNA sponges that regulate gene expression.[15] CDR1as is one of the few circRNAs that has been proposed to inhibit tumour suppressor miR-7. We found that CDR1as functions as an oncogene and is upregulated in LSCC tissue to promote tumour progression via miR-7 both in vitro and in vivo.

Unlike linear RNA molecules, the 3′ and 5′ ends of circRNAs are joined together by covalent bonds, leading to circularization. This prevents the degradation of circRNA by RNA exonucleases.[16] It also increases the structural stability of circRNAs and makes them very abundant in the cytoplasm. Given their conservation, abundance, and tissue specificity, circRNAs may play roles as special molecular markers in cancer.[17] In this study, we found that the levels of CDR1as expression in samples of LSCC tissue were significantly increased when compared to those in normal tissues. High CDR1as levels were predictive of a high TNM stage, poor tumour differentiation, and lymph node metastasis in LSCC patients, indicating that CDR1as could serve as a diagnostic marker for LSCC. Xuan et al[18] reported that hsa_circRNA_100855 was the most upregulated circRNA and hsa_circRNA_104912 was the most downregulated circRNA in LSCC. Patients with a T3-4 stage tumour, neck nodal metastasis, a poorly differentiated tumour, or who were at an advanced clinical stage of their disease had relatively lower levels of

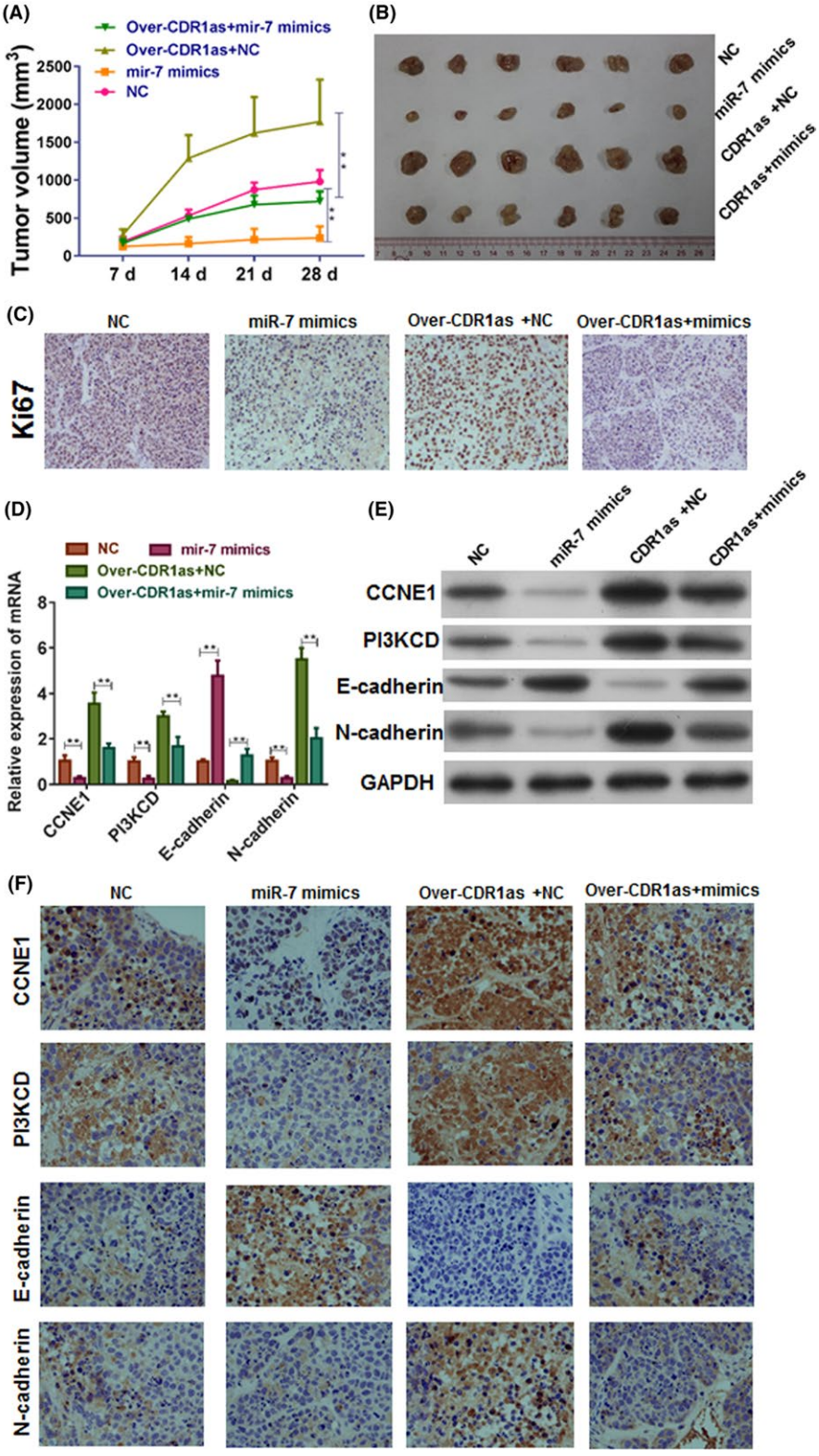

FIGURE 5 CDR1as regulated miR-7 activity to promote tumour growth in vivo. Hep-2 cells containing the lentivirus vector for vector-CDR1as or miR-7 alone, or both were subcutaneously injected into nude mice. A-B, The growth rates and average weights of the tumours in the different treatment groups were estimated at the time the animals were sacrificed. C, The levels of Ki67 expression in the tumour tissues in the different groups were estimated by IHC. D-F, The EMT phenotype (N-cadherin, E-cadherin, CCNE1, and PIK3CD levels) was determined by Q-PCR, WB and IHC assays. $^{**}P < 0.01$, $^{***}P < 0.001$, data represent the mean ± SD

hsa_circRNA_104912 expression.[18] Similarly, other circRNAs have also been proposed as cancer biomarkers. Qin et al[19] reported that hsa_circ_0001649 expression was significantly downregulated in hepatocellular carcinoma tissues when compared with its expression in adjacent liver tissues, and that hsa_circ_0001649 expression was correlated with the size of an HCC tumour. In gastric cancer, downregulation of hsa_circ_002059 expression was associated with metastasis and the TNM stage. Furthermore, the levels of hsa_circ_002059 in plasma could serve as stable biomarker for the diagnosis of gastric carcinoma.[20]

Although CDR1as and hsa_circRNA_104912 are thought to be involved in the progression of LSCC, the mechanisms of their functions are unclear. CircRNAs are relatively stable, ubiquitous, conserved, and highly expressed in animal cells, and can regulate the expression of their target genes in several ways.[16,20] The functions of circRNAs as miRNA sponges have been intensively investigated, because their circular loop consists of various miRNA binding sites known as miRNA response elements (MREs); these MREs enable circRNAs to act as miRNA sponges.[21] CDR1as is the circRNA that has been most often studied as a miR-7 sponge. CDR1as contains >70 binding sites for miR-7, and is extensively bound by Argonaute (AGO) proteins.[7] Similarly, another cir-ITCH, derived from the ITCH gene, possesses a sequence enriched with three miRNA binding sites (miR-7, miR-17, and miR-214).[22] In this study, we found that CDR1as overexpression promoted LCSS tumour growth and the EMT phenotype; however, these effects could be inhibited by miR-7 via its ability to regulate CCNE1 and PIK3CD levels in LSCC. This finding was consistent with that in hepatocellular carcinoma (HCC), where knockdown of Cdr1as suppressed the proliferation and invasion of HCC cells and promoted the expression of miR-7 to inhibit CCNE1 and PIK3CD expression.[23] In colorectal cancer (CRC), inhibition of CDR1as suppressed CRC cell proliferation and invasion, and a miR-7 inhibitor was able to rescue the function of CDR1as knockdown. This rescue might attributed to tumour growth and the invasion-related miR-7 targets, EGFR and IGF-1R.[9] Another study showed that EGFR-RAF1 activity was regulated by CDR1as/miR-7 signals in CRC patients. Furthermore, the results of in vivo studies showed that CDR1as impaired the tumour-induced suppression of miR-7 and thereby promoted tumour growth in CRC.[19] In this study, we confirmed the tumourigenic role of CDR1as in vivo. Interestingly, we found that overexpressed CDR1as alone could accelerate the growth of LSCC tumours, and this effect helped to counteract the function of miR-7 by promoting tumour growth and upregulating the invasion-related miR-7 targets, CCNE1 and PIK3CD, in tumour cells.

5 | CONCLUSION

In conclusion, we reported the presence of oncogene CDR1as in LSCC cells. High levels of CDR1as promoted LSCC growth and metastasis via regulation of the miR-7/CCNE1/PIK3CD signalling pathway. High levels of CDR1as were predictive of a poor clinical outcome and might serve as a novel biomarker for LSCC patients.

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CONFLICT OF INTEREST

No competing financial interests exist.

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