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Hsa_circ_0079929 in lung adenocarcinoma and its biological implications in lung adenocarcinoma progression

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Abstract

Background This report investigated the expression, prognostic and biological implications of hsa_circ_0079929 in lung adenocarcinoma, which was based on clinical and experimental data.

Methods Patients with lung adenocarcinoma were screened and their clinical data and tissues (including cancerous tissues and adjacent normal tissues) were collected. The total RNA in tissues and cell lines was analyzed to obtained hsa_circ_0079929 level. The clinical significance was examined using the Chi-square test, Multi-variate Cox proportional hazards regression, and Kaplan-Meier curve. Cell malignant features were evaluated from three aspects (proliferation, migration, and invasion), detected by CCK-8 and Transwell methods.

Results Hsa_circ_0079929 raised in expression level in lung adenocarcinoma. This upregulation of hsa_circ_0079929 was correlated with adverse clinical parameters and poor outcome in terms of overall survival, resulting in an independent prognostic purpose molecular for overall survival. Overexpression of hsa_circ_0079929 could contribute to cell proliferation/migration/invasion, whereas its knockdown could inhibit these malignant features. Hsa_circ_0079929 was a molecular decoy for miR-1184 in lung adenocarcinoma cells.

Conclusions Hsa_circ_0079929 could promote the malignant features of lung adenocarcinoma cells and may aid the follow up and therapeutic target discovery of lung adenocarcinoma.

Keywords Lung adenocarcinoma, Hsa_circ_0079929, Prognosis, Progression

Background

Lung adenocarcinoma remains the common fatal subtype of non-small cell lung cancer, with a share of 40–50% in all lung cancers [1, 2]. Most lung adenocarcinomas originate from the bronchial epithelium, and a few originate from the mucous glands of the large bronchi [3]. The disease progresses slowly, and initial symptoms are generally less obvious [4]. The incidence and mortality of lung adenocarcinoma are increasing year by year [5]. Percent of cases and 5-year relative survival by stage at diagnosis ranged from 57.4 to 5.2% [6, 7]. Small-molecule tyrosine kinase inhibitors and immunotherapies are already in use, bringing

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unprecedented survival benefits to patients with specific genetic mutations [8]. But there are currently no targeted agents for lung cancer patients without specific genetic mutations, who are often treated with chemotherapy and radiation therapy with relatively low efficacy [9]. Due to serious side effects, they can seriously affect the quality of life for lung cancer patients, even leading to premature death and shorter survival. Consequently, overall curable and survival rates for lung adenocarcinoma patients remain low, especially in metastatic disease. Tissue biomarkers may be of use in determining prognosis [10]. To be able to predict the natural history of disease in individual patients, the availability of prognostic biomarkers for lung adenocarcinoma patients become an important goal for clinicians.

Circular RNAs (circRNAs) range in size (100 nt-over 4 kb) and contain single or even multiple exons [11]. Unlike known one-dimensional ncRNAs, circRNAs are RNA molecules with covalent link between 3' and 5' ends, which form a single-stranded continuous loop structure [12]. CircRNAs have a high abundance in eukaryotes, is evolutionarily conserved, and is specifically expressed in certain cell types or specific tissue, pointing toward a potential biomarker role [13]. They have extraordinary stability, due to their lack of exposed ends and certain RNA folds based on their structure. CircRNAs have shown to be potent tumor marker aiding the diagnosis and follow up of cancer [14]. Hsa_circ_0079929 (hsa_circCDK13_005) locates in chr7: 40,027,197–40,027,857 with a length of 660 nt. CircCDK13 has been reported to be elevated in colorectal cancer and prostate carcinogenesis [15]. Hsa_circ_0079299 can suppress hepatocellular carcinoma cell growth [16]. More recently, hsa_circ_0079929 was a differentially expressed circRNA in lung adenocarcinoma [17]. However, further investigation on the assessment of hsa_circ_0079929 in lung adenocarcinoma prognosis and progression hasn't been reported.

In this report, detection of the expression of hsa_circ_0079929 by quantitative real-time PCR was compared in a retrospective study of 147 lung adenocarcinoma cancerous samples and adjacent healthy lung tissues, with a follow-up study of these samples using the Multi-variate Cox proportional hazards regression and Kaplan-Meier analyses. Finally, cell function experiments were performed, in order to discover the correlation of hsa_circ_0079929 overexpression or knockdown with tumor growth and metastasis in human lung adenocarcinoma cell lines.

Methods

Subjects

Clinical records of patients with lung cancer were retrospectively evaluated, to extract the visit and hospitalization information of patients who underwent curative resection by reason of primary lung adenocarcinoma, between 2011 and 2015 ($n=147$). Patients who received any tumor-specific therapy, incomplete resection of other metastatic disease, or the presence of any nodules at the time of surgery were excluded from the study. Medical records of these patients were collected, recorded, and classified for age, sex, smoking status, TNM stage (the 7th edition of IASLC proposals), *N* status, distant metastasis, pleural invasion, vascular invasion, differentiation, and carcinoembryonic antigen (CEA) values. The follow-up information was collected, and the mean duration of clinical follow-up was 28 months (minimum/maximum: 3–60 months). This single-center retrospective study was with the approval of the ethical committee of Yuhuan Second People's Hospital. All data and information derived from the tissues and clinical data was followed with written informed consent.

Cell lines and culture conditions

For investigation of human normal lung cell lines, MRC-5 and HFL1 were obtained from the Cell Bank of Chinese Academy of Sciences (Beijing, China), along with their specific culture mediums. For experiments of human lung adenocarcinoma cells, Calu-3, PC-9, NCI-H1975, and NCI-H1395, from the same institution as above, were cultured in RPMI 1640 (C11875500BT, GIBCO, Gaithersburg, USA), or MEM (41500034, GIBCO, Grand Island, USA), all supplanted with 10% FBS (GIBCO, Carlsbad, USA). The culture conditions for all cells were 37 °C with 5% CO_2.

Overexpression and knockdown of hsa_circ_0079929

Small interfering RNA (siRNA) against hsa_circ_0079929 (siCIRC and siCIRC-2), the negative control scrambled siRNA (siCON), pCD25-ciR vector containing hsa_circ_0079929 (ovCIRC), and the negative pCD25-ciR vector (ovCON) were purchased from Geneseed Biotech (Guangzhou, China). Transfection of cells used Lipofectamine® 3000 (Life Technologies, Carlsbad, USA). Hsa_circ_0079929 overexpression and knockdown were confirmed using quantitative real-time PCR.

RNA isolation and quantitative real-time PCR

Samples for total RNA extraction were subjected to TRIzol Reagent (Introvigen, Carlsbad, USA) and RNase R (Epicentre, Madison, USA), following the manufacturer's instructions. For miRNA, complementary

DNAs were pre-amplified using the miScript II RT Kit (Qiagen, Hilden, Germany). Quantitative real-time PCR were performed, with primers for miR-1184 and the internal control U6, under the usage of miScript SYBR Green PCR Kit (Qiagen, Hilden, Germany). A SYBR Green qPCR system was used for circRNA expression analysis: an M-MLV First Strand Kit (Life Technologies, Grand Island, USA) and a Platinum SYBR Green qPCR Super Mix UDG Kit (Invitrogen, Carlsbad, USA). Transcript levels were quantified at the ABI 7500 FAST system (Life Technologies, Grand Island, USA). Relative levels were normalized to GAPDH or U6, using the $2^{-\Delta\Delta Ct}$ formula.

Cell proliferation assay

Cell counting kit-8 (DOJINDO, Kumamoto, Japan) was taken for cell proliferation. After transfection, the cells were seeded at specific densities (Calu-3: 5000 cells/well; NCI-H1395: 8000 cells/well). 2-hour incubation post indicated points (0, 12, 24, 48, and 72 h), kit reagent was added and absorbance at 450 nm was measured on a plate reader (Bio-Rad, Hercules, USA).

Cell invasion and migration assays

Transfected and trypsinized Calu-3 and NCI-H1395 cells were resuspended and subjected to 24-hour starvation in their respective FBS-free media. Cell migration/invasion were determined using CytoSelect 24-well Cell Migration/Invasion Assays (8 μm, Cell Biolabs, San Diego, USA), with cell in serum-free medium in the top chamber and mediums containing 10% FBS in the bottom wells. After a 24-hour incubation, the migratory or invasive cells were dissociated and lyzed, following a measurement at 480 nm/520 nm (Bio-Rad Laboratories, Hercules, USA). The coefficient of variation was < 15%. The fluorescence was in ratio to that of the non-transfected cells.

Bioinformatics analysis

The downstream miRNAs of hsa_circ_0079929 were retrieved from Circular RNA Interactome, using the "miRNA Target Sites" module. MiR-1184 has been identified to inhibit lung adenocarcinoma previously [18].

Dual-luciferase reporter assay

The sequences of hsa_circ_0079929 including wild-type (WT-CIRC) or mutant miR-1184-binding sites (MUT-CIRC), were synthesized and inserted into luciferase reporter vectors by Genechem (Shanghai, China). Calu-3 cells were plated (10,000 cells per well) for a 24-hour incubation on 24-well plates. Then, cells were co-transfected with 100 ng of vector coding for either wildtype or mutant hsa_circ_0079929, 100 ng of either miR-1184 mimic or inhibitor (Geneseed Biotech, Guangzhou, China) using FuGENE HD Transfection Reagent (Promega, Madison, USA), for 50 h. After washing and lysing, dual luciferase assays were completed in the presence of Dual-Luciferase Reporter Assay Kit (Promega, Madison, USA). The values were displayed as Firefly luciferase values/Renilla luciferase values.

Statistical analysis

A $P < 0.05$, at two-tailed level, was considered significant. Associations between tumor expression of hsa_circ_0079929 and clinicopathologic characteristics were assessed using either the Chi-square tests, or Fisher's exact test. Correlations between hsa_circ_0079929 and miR-1184 levels were analyzed using Pearson's correlations. Overall survival time was estimated using the Kaplan-Meier method (the log-rank test). Multivariate Cox regression was utilized to examine the associations between relevant clinicopathologic features, along with hsa_circ_0079929 expression, with overall survival. Two-tailed Student's t-test (or two-way analysis of variance test) was introduced to compare quantitative data.

Results

Expression of hsa_circ_0079929 in lung adenocarcinoma and its prognostic value

The assay detected the hsa_circ_0079929 in 147 cases precluded a significant upregulation in lung adenocarcinoma tissues (2.022) vs. adjacent normal lung tissues (1.000) ($P<0.001$) (Fig. 1A). The median value of hsa_circ_0079929 relative expression level was 2.031, a value to stratify the patients as high or low hsa_circ_0079929 groups. After grouping, patients with high hsa_circ_0079929 tend to be correlated with advanced TNM stage ($P=0.036$), pleural ($P=0.037$), and vascular invasion ($P=0.025$) (Table 1). Patients whose tumors expressed high hsa_circ_0079929 levels had poorer overall survival ($P=0.010$) (Fig. 1B). Multivariate regression analyses screened hsa_circ_0079929 ($P=0.014$) as one of the independent factors that affected overall survival (Table 2). The increased expression of hsa_circ_0079929 was also observed in lung adenocarcinoma cell lines ($P<0.001$) (Fig. 1C).

Hsa_circ_0079929 contributed to proliferation and migration/invasion in Calu-3 cells

We then examined whether hsa_circ_0079929 is necessary for the promotion of lung adenocarcinoma progression. We overexpressed hsa_circ_0079929 in Calu-3 cells by transfecting with a plasmid containing hsa_circ_0079929 (Fig. 2A) and checked cell proliferative, migratory, and invasive capacity. CCK-8 assays

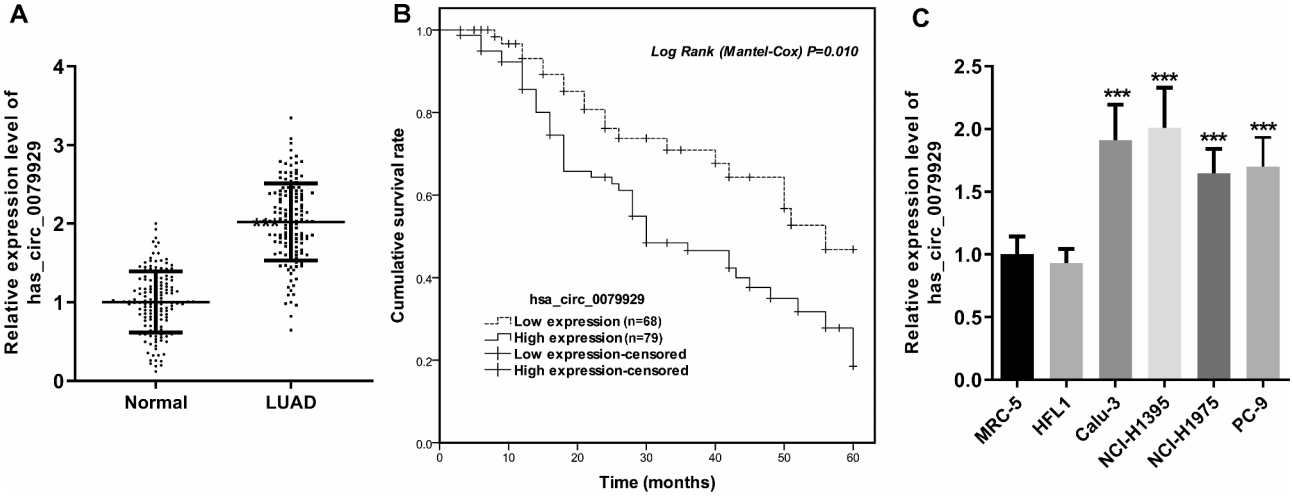

Fig. 1 Hsa_circ_0079929 expression in lung adenocarcinoma and the Kaplan-Meier curve based on hsa_circ_0079929 expression. (**A**) Expression of hsa_circ_0079929 was increased in lung adenocarcinoma compared to that in adjacent normal tissue. The comparison was achieved using Paired t-test. ***$P<0.001$. (**B**) Kaplan-Meier curve was constructed followed a Log Rank test ($P=0.010$). (**C**) Expression of hsa_circ_0079929 in lung adenocarcinoma cell lines (Calu-3, NCI-H1395, NCI-H1975, PC-9). The comparison was achieved using Unpaired t-test. ***$P<0.001$

demonstrated that overexpression of hsa_circ_0079929 increased cell proliferation (Fig. 2B and C). Moreover, cell migration and invasion were markedly heightened in Calu-3 cells overexpressing hsa_circ_0079929 (Fig. 2D and E). Taken together, these data demonstrated that hsa_circ_0079929 could function as a positive regulator in lung adenocarcinoma progression.

Knockdown of hsa_circ_0079929 contributes to the decreases in cell proliferation and migration/invasion in NCI-H1395 cells

Then, we examined whether a deficiency of hsa_circ_0079929 could inhibit lung adenocarcinoma progression. We selected NCI-H1395 cells because of the weak expression of hsa_circ_0079929. The siRNA showed significant knock-down efficiency in NCI-H1395 cell line (Fig. 3A). CCK-8 assay demonstrated that reduction in hsa_circ_0079929 levels significantly suppressed cell proliferation in NCI-H1395 cells (Fig. 3B and C). The migratory property of cells was markedly hindered by the knock-down of hsa_circ_0079929 (Fig. 3D) and that cell invasion was also decreased in the hsa_circ_0079929-knockdown NCI-H1395 cells (Fig. 3E).

Hsa_circ_0079929 acted as a miR-1184 decoy

CircRNA with competitive endogenous RNA (ceRNA) potential can compete with mRNA for miRNA binding sites, thereby affecting cell function and disease progression (19). Among the bioinformatics-predicting miRNAs, miR-1184 expression in lung adenocarcinoma cell lines has been verified to down-regulation (Fig. 4A). Additionally, miR-1184 was negatively and significantly hsa_circ_0079929 in lung adenocarcinoma tissues (Fig. 4B). The pairing bases between miR-1184 and hsa_circ_0079929 provided the sequence used to transfect Calu-3 cells (Fig. 4C). The dual-luciferase reporter assay data indicated the inhibition of miR-1184 mimic on the activity of luciferase in the cells wild-type hsa_circ_0079929-co-transfected cells (Fig. 4D). Therefore, hsa_circ_0079929 could act as a molecular decoy for miR-1184.

Inhibition of miR-1184 rescued the antiproliferative, anti-migratory, and anti-invasive effects of silencing hsa_circ_0079929

To further study whether the suppression of lung adenocarcinoma by hsa_circ_0079929 knockdown was linked to the expression of miR-1184, a rescue experiment was designed. Hsa_circ_0079929 was silenced in NCI-H1395, which increased miR-1184 levels, while at the same miR-1184 was antagonized. As shown in Fig. 5A, miR-1184 inhibition partially restored the increased level of miR-1184 after hsa_circ_0079929 was silenced. Cell proliferation was measured, and results showed that the decreased levels of cell proliferation were almost fully rescued by miR-1184 inhibition (Fig. 5B). Similar effects were observed by monitoring cell migration and invasion (Fig. 5C and D). Collectively, these results support the notion that miR-1184 is a target that is responsible for Hsa_circ_0079929 oncogenic activities in lung adenocarcinoma, including proliferation, migration, and invasion.

Discussion

In the present study, we found the expression level of hsa_circ_0079929 in lung adenocarcinoma cells and tissues was upregulated. Then the potential of

Table 1 Comparison of patient characteristics according to the expression level of has_circ_0079929 in patients with lung adenocarcinoma

Variables	*N*	Low has_circ_0079929 (*n* = 68)	High has_circ_0079929 (*n* = 79)	*P*-value
Age (years)				0.761[a]
≤60	69	31	38	
>60	78	37	41	
Sex				0.439[a]
Female	59	25	34	
Male	88	43	45	
Smoking				0.475[a]
Yes	91	40	51	
No	56	28	28	
TNM stage				0.044*[a]
I-II	112	57	55	
III-IV	35	11	24	
N status				0.036*[a]
N0	95	50	45	
N+	52	18	34	
Distant metastasis				0.123[b]
M0	140	67	73	
M1	7	1	6	
Pleural invasion				0.037*[a]
Negative	119	60	59	
Positive	28	8	20	
Vascular invasion				0.025*[b]
Negative	128	64	64	
Positive	19	4	15	
Differentiation				0.091[a]
Well	82	43	39	
Moderately, poorly	65	25	40	
CEA				0.285[a]
<45 ng/mL	113	55	58	
≥45 ng/mL	34	13	21	

[a] indicates the analysis was conducted using the Chi-square tests; b indicates the analysis was conducted using Fisher's exact test. *$P < 0.05$. CEA, carcinoembryonic antigen

Table 2 Multivariable analysis for the risk factors of overall survival

Variables	HR	95%CI	*P*
has_circ_0079929	2.056	1.159–3.646	0.014
Age	1.203	0.711–2.035	0.492
Sex	1.250	0.739–2.113	0.405
Smoking	1.154	0.682–1.952	0.593
TNM stage	1.647	0.959–2.828	0.071
Differentiation	1.167	0.691–1.971	0.565
CEA	2.001	1.323–6.402	0.048

CEA, carcinoembryonic antigen

hsa_circ_0079929 as a prognostic purpose biomarker in view of overall survival was assessed. The correlation of hsa_circ_0079929 with tumor stage and pleural/vascular invasion suggests a possible involvement of hsa_circ_0079929 in lung adenocarcinoma progression. Hsa_circ_0079929 expression can predict a poor survival rate, and multivariate analysis revealed hsa_circ_0079929 as a prognostic purpose sign for lung adenocarcinoma overall survival. Collectively, our findings indicate that hsa_circ_0079929 expression may aid to lung adenocarcinoma prognosis and follow-up.

Though the clinical medical methods and medical conditions are improving, there still have small improvements in 5-year survival among patients with lung cancer. Five-year survival of 23% was found for non-small cell lung cancer [19]. Good prognostic markers are demanded for the proper selection of therapy and follow-up management of patients. As a heterogeneous disease that reflects gene changes, biomarkers for lung adenocarcinoma prognosis can consist of various biomolecules, including RNAs [20]. For example, circ-PDCD11 is specifically upregulated in lung large-cell carcinoma, relating to poor survival [21]. The prognostic value of hsa_circ_0079929 has been confirmed in several aggressive malignancies, and tested to be promising targets for several cancer therapy [15, 16]. Here, the prognostic value of hsa_circ_0079929 in lung adenocarcinoma was identified. It shows a great promise to become part of routine clinical follow-up practice. Larger studies in future underway will be needed to confirm whether hsa_circ_0079929 has a high predictive power.

Previous studies have shown that the change in circRNA expression level is related to involvement in important pathological processes [22]. Cancerous cells escape antigrowth signals via suppressing the tumor suppressor genes and embark on the path of infinite proliferation. The overexpression or knockdown of specific circRNA can inhibit or promote cell proliferation, migration, invasion, and metastasis of cancerous cells [23]. For instance, knockdown of circ_0002483 can inhibited growth of lung cancer cells [24]. Another upregulated type of circRNA known as Circ-HMGA2 can promote lung adenocarcinoma cell metastasis [25]. In this report, the overexpression of hsa_circ_0079929 resulted in an increase in cell malignant behavior, whereas knockdown of hsa_circ_0079929 inhibited cell malignant behavior. A similar effect of hsa_circ_0079929 has been found in prostate cancer, which showed as knockdown of hsa_circ_0079929 inhibiting the growth of prostate cancer cells in vivo and in vitro [15]. Therefore, hsa_circ_0079929 can promote the progression of lung adenocarcinoma, and

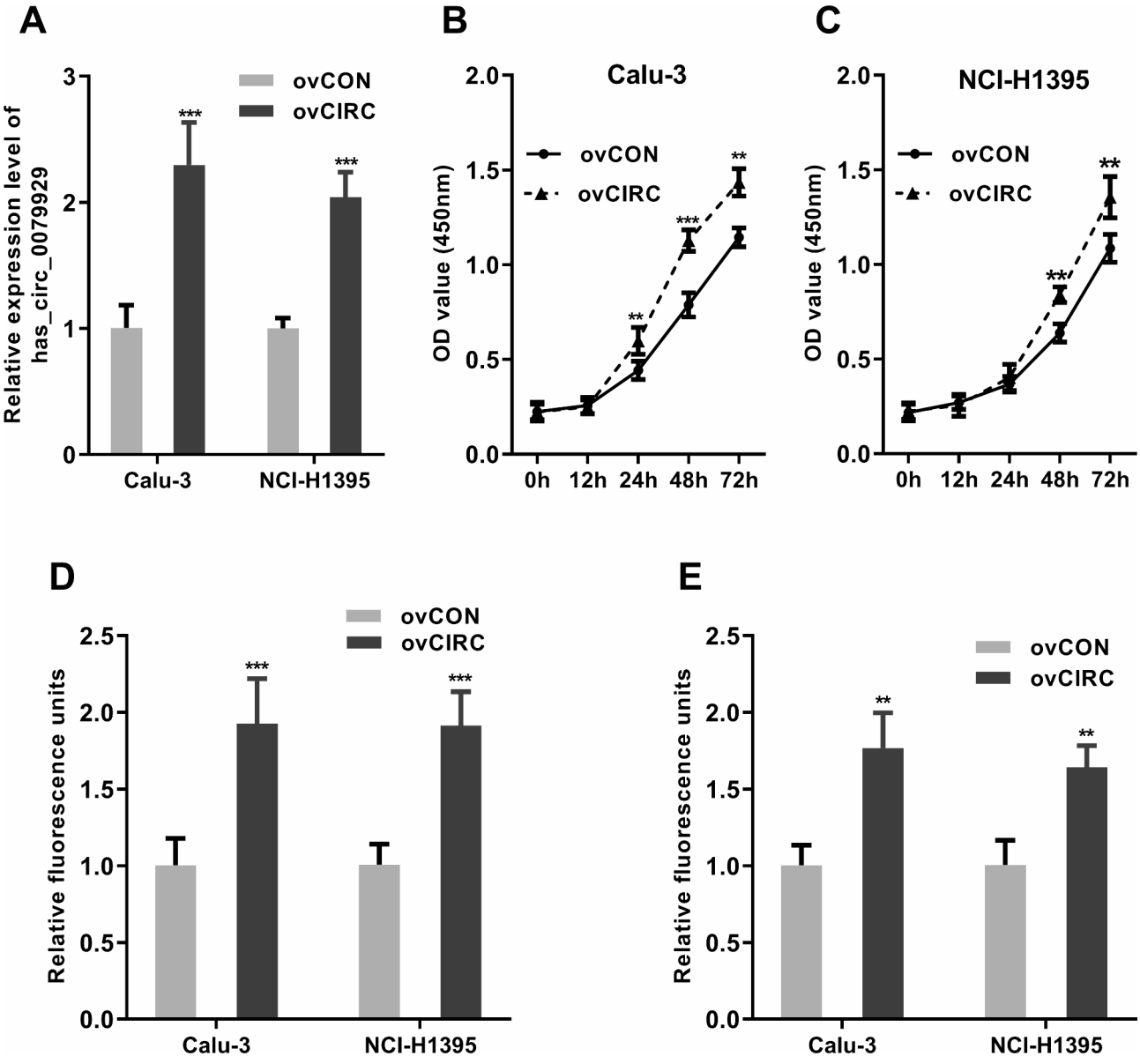

Fig. 2 Overexpression of hsa_circ_0079929 promoted the proliferative, migratory, and invasive capacity of lung adenocarcinoma cells. (**A**) Quantitative real-time PCR confirmed the transfection efficiency. (**B**) (**C**) Overexpression of hsa_circ_0079929 increased cell growth, significantly different from ovCON. The comparison was achieved using two-way analysis of variance test. (**D**) Overexpression of hsa_circ_0079929 increased cell migration, significantly different from ovCON. (**E**) Overexpression of hsa_circ_0079929 increased cell invasion, significantly different from ovCON. The comparison was achieved using two-way analysis of variance test. $^{**}P<0.01$. $^{***}P<0.001$

have the potential to be a therapeutic target for lung adenocarcinoma.

CeRNA theory is one of the mechanisms explaining the role of circRNAs in disease. CeRNA molecules such as circRNA, contain the shared microRNA response elements, then act as miRNA sponges and allow competition for miRNA [26]. For instance, circ_0020123 can interact with miR-1283, then regulating PDZD8 expression, thus promoting lung cancer [27]. Bioinformatics analysis by Circular RNA Interactome based on the Targetscan prediction tool can predict the miRNAs potentially targeting the circRNAs and map the binding sites for miRNAs on selected circRNA. MiR-1184 has been reported as a downregulated miRNA in non-small cell lung cancer and lung adenocarcinoma, inhibiting cell migration and invasion [28]. Hsa_circ_0001666 and circNEIL3 both act as sponges of miR-1184. In this report, we verified that hsa_circ_0079929 also was a sponge of miR-1184. In lung adenocarcinoma, miR-1184 can target PIF1 to influence lung adenocarcinoma radiotherapy, and moderate CCL22 to hinder resistance to

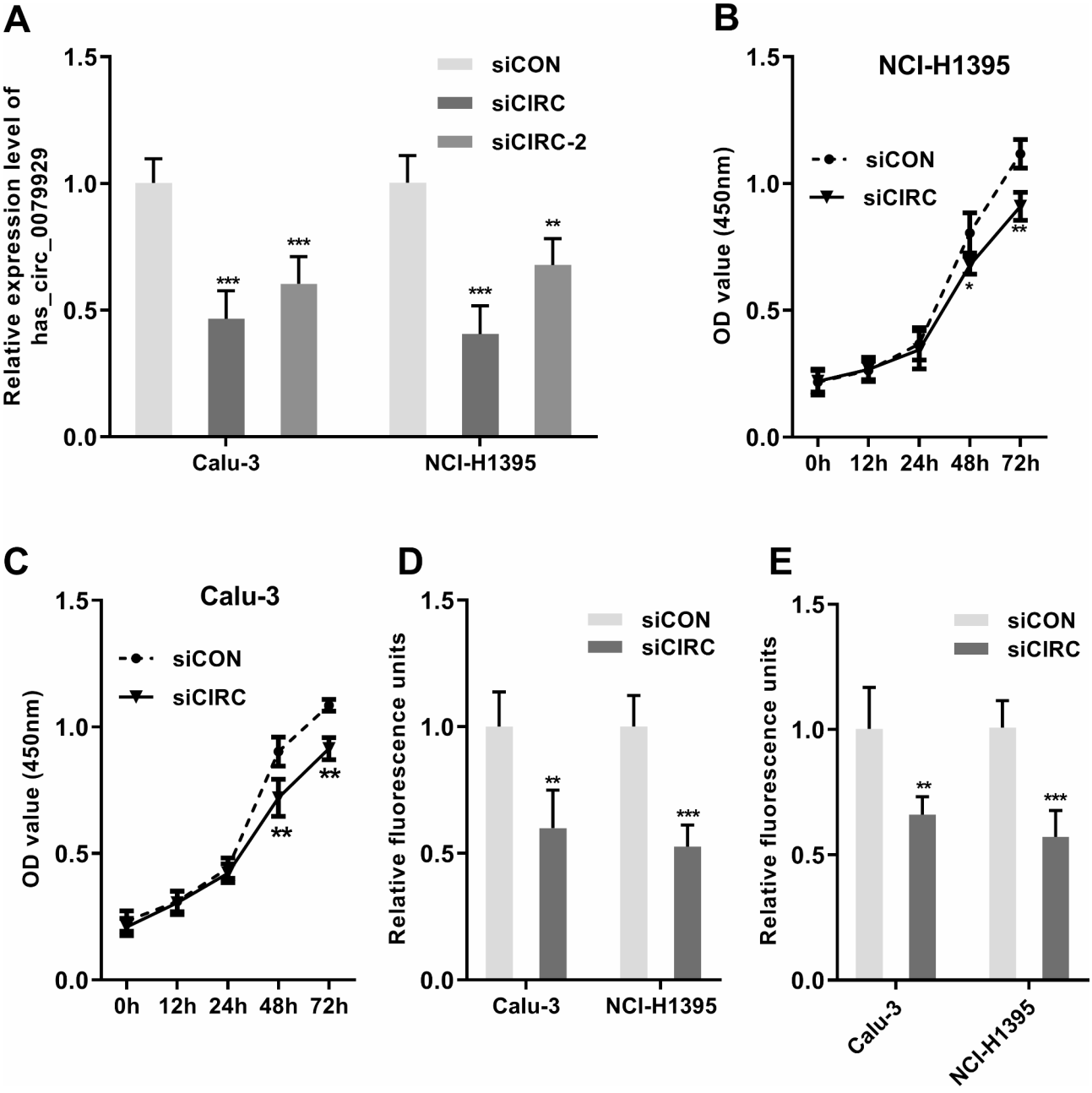

Fig. 3 Knockdown of hsa_circ_0079929 inhibited cell growth, migration, invasion in lung adenocarcinoma cells. (**A**) Hsa_circ_0079929 expression in NCI-H1395 cells significantly decreased. (**B**) (**C**) Suppression of hsa_circ_0079929 inhibited cell growth. (**D**) Suppression of hsa_circ_0079929 hindered cell migration. (**E**) Suppression of hsa_circ_0079929 hindered cell invasion. The comparison was achieved using two-way analysis of variance test. (**P*<0.05. ***P*<0.01. ****P*<0.001, significantly different from siCON)

anti-PD-1 immunotherapy [18, 29]. Moreover, miR-1184/SLC7A11 axis mediates glutathione synthesis, thus inducing lung adenocarcinoma cell ferroptosis [30]. Therefore, hsa_circ_0079929 may exert its effect via miR-1184.

This study revealed the prognostic and therapeutic potential of hsa_circ_0079929 in lung adenocarcinoma. The main limitation of the study is that, instead of in vivo experiments, only cellular experiments were conducted. Of course, a large-scale assessment of has_circ_0079929 expression in clinical practice is needed to implement.

Conclusions

Hsa_circ_0079929 is highly expressed in lung adenocarcinoma. Upregulation of hsa_circ_0079929 was linked to unfavorable clinical parameters, associated

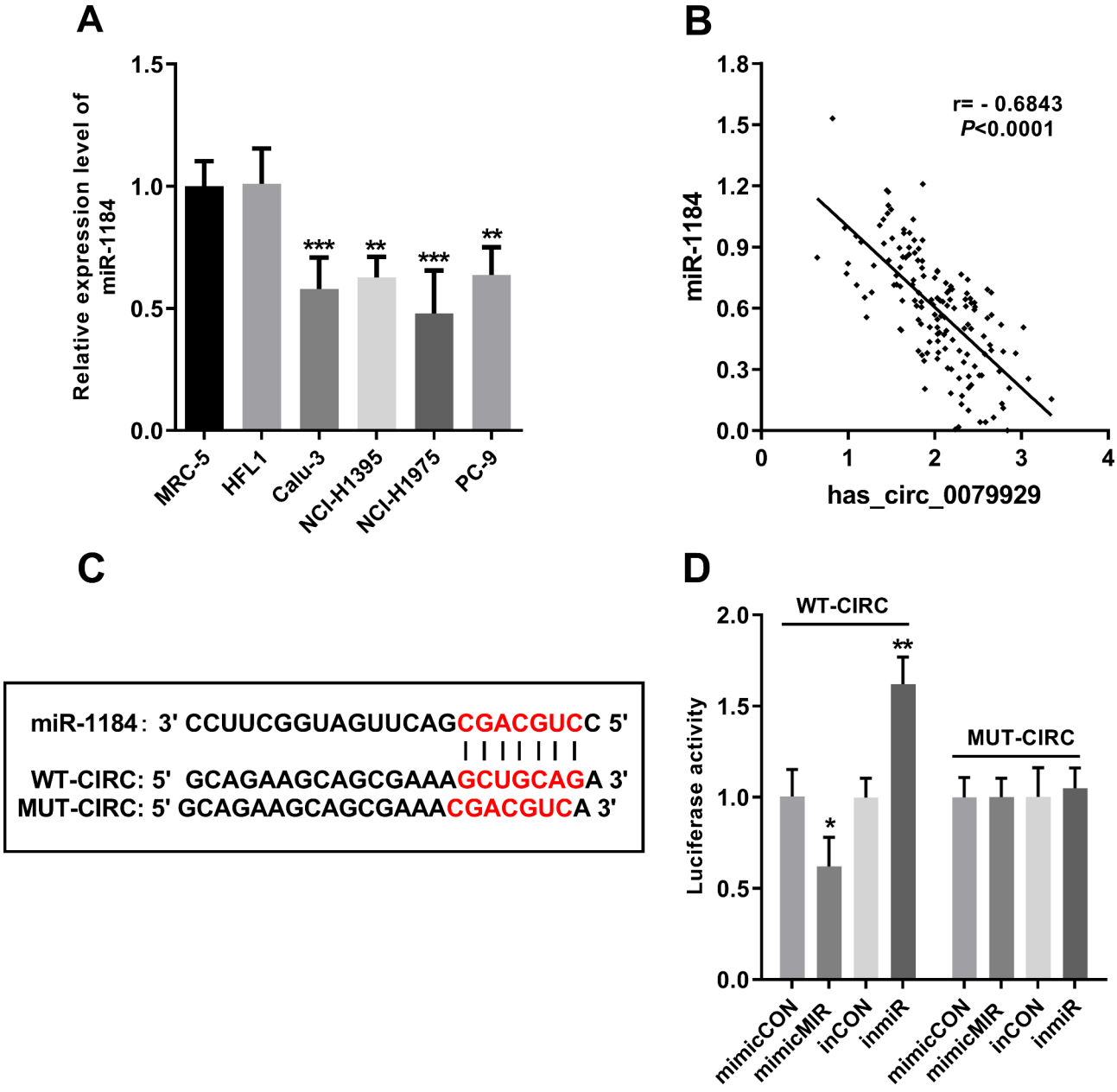

Fig. 4 Hsa_circ_0079929 acted as a molecular decoy for miR-1184. (**A**) Expression of miR-1184 in lung adenocarcinoma cell lines. The comparison was achieved using Unpaired t-test. ***P*<0.01. ****P*<0.001. (**B**) The correlation between miR-1184 and hsa_circ_0079929 in lung adenocarcinoma. The correlation coefficient was computed by Pearson analysis (*P*<0.0001). (**C**) The binding sites between hsa_circ_0079929 and miR-1184 (Circular RNA Interactome). (**D**) The luciferase activity of wild- and mutant-type hsa_circ_0079929. The comparison was achieved using two-way analysis of variance test. **P*<0.05. ***P*<0.01

with a poor prognosis. Hsa_circ_0079929 could sponge miR-1184, acting as promoter in lung adenocarcinoma.

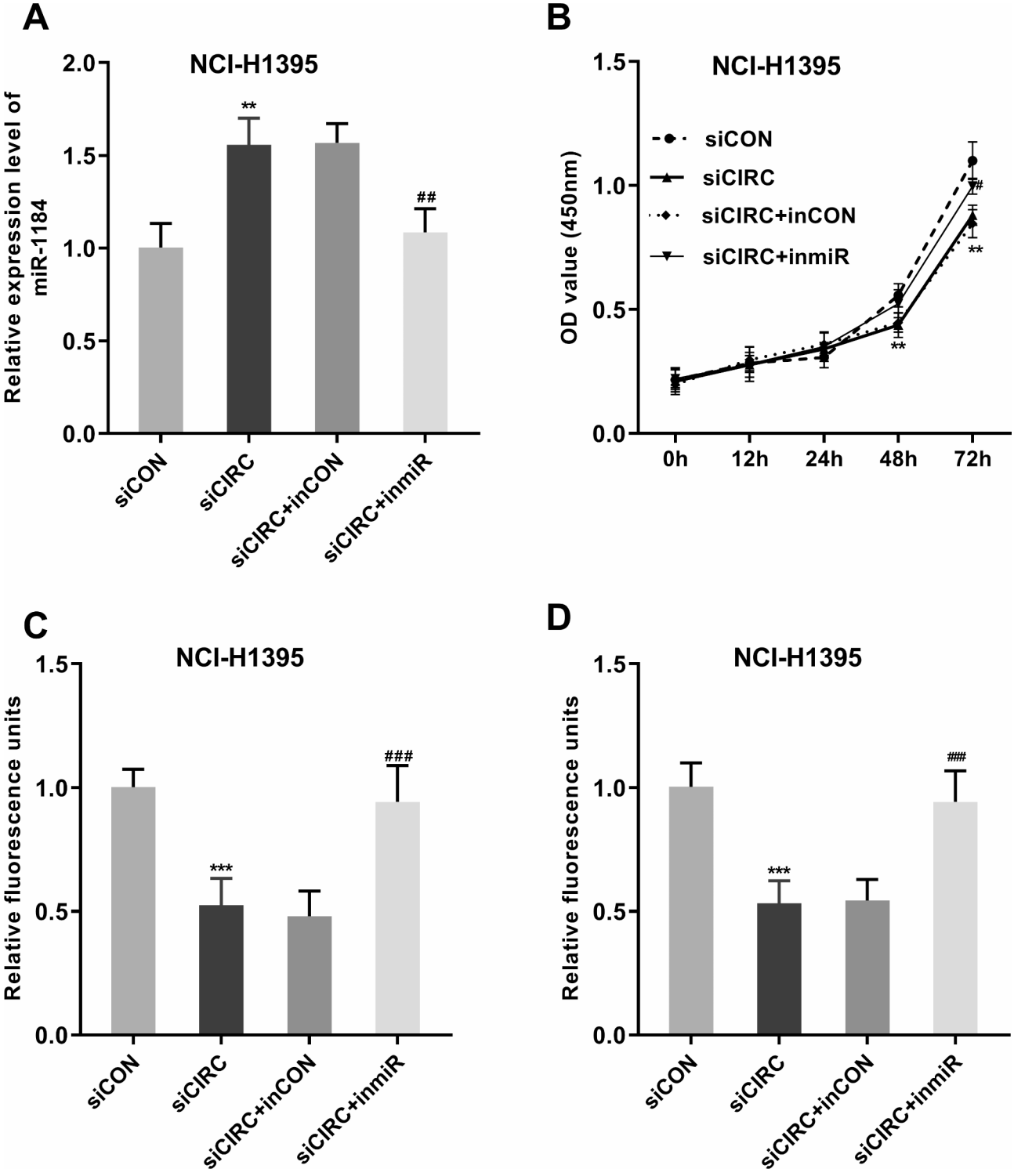

Fig. 5 Inhibition of miR-1184 rescues the antiproliferative, anti-migratory, and anti-invasive effects of silencing hsa_circ_0079929. (**A**) NCI-H1395 cells were transfected with siRNA negative control (siCON) or hsa_circ_0079929 siRNA together with miR-1184 inhibitor (inmiR) or negative control (inCON). (**B**) Cell proliferation was assayed using cell counting kit-8. Cell migration (**C**) and invasion (**D**) were determined transwell assays. $^{**}P < 0.01$; $^{***}P < 0.001$, vs. siCON. $^{\#}P < 0.05$, $^{\#\#}P < 0.01$, $^{\#\#\#}P < 0.001$, vs. siCIRC + inCON

Abbreviations

CEA	Carcinoembryonic antigen
ceRNA	Competitive endogenous RNA
circRNAs	Circular RNAs
siRNA	Small interfering RNA

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Author contributions
Y S, RX H, X Y and J M designed the research study, performed the research. Y S and J M analyzed the data. Y S and RX H wrote the manuscript. All authors contributed to editorial changes in the manuscript. All authors read and approved the final manuscript.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
All procedures performed in studies involving human participants were in accordance with the ethical standards of the institutional and/or national research committee and with the 1964 Helsinki Declaration and its later amendments or comparable ethical standards. The study was approved by the Bioethics Committee of Yuhuan Second People's Hospital.

Consent for publication
Not applicable.

Conflict of interest
The authors declare that they have no conflicts of interest to report regarding the present study.

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References

1. Duma N, Santana-Davila R, Molina JR. Non-small Cell Lung Cancer: Epidemiology, Screening, diagnosis, and treatment. Mayo Clin Proc. 2019;94:1623–40. https://doi.org/10.1016/j.mayocp.2019.01.013
2. Siegel RL, Miller KD, Wagle NS, Jemal A. Cancer statistics, 2023. Cancer J Clin. 2023;73:17–48. https://doi.org/10.3322/caac.21763
3. Lambe G, Durand M, Buckley A, Nicholson S, McDermott R. Adenocarcinoma of the lung: from BAC to the future. Insights into Imaging. 2020;11:69. https://doi.org/10.1186/s13244-020-00875-6
4. Tamiya Y, Nakai T, Suzuki A, Mimaki S, Tsuchihara K, Sato K, Yoh K, Matsumoto S, Zenke Y, Nosaki K, Izumi H, Shibata Y, Sakai T, Taki T, Miyazaki S, Watanabe R, Sakamoto N, Sakashita S, Kojima M, Hashimoto N, Tsuboi M, Goto K, Ishii G. The impact of tertiary lymphoid structures on clinicopathological, genetic and gene expression characteristics in lung adenocarcinoma. Lung cancer (Amsterdam Netherlands). 2022;174:125–32. https://doi.org/10.1016/j.lungcan.2022.11.001
5. Skřičková J, Kadlec B, Venclíček O, Merta Z. Lung cancer. Casopis Lekaru Ceskych. 2018;157:226–36.
6. Oliver AL. Lung Cancer: Epidemiology and Screening. Surg Clin North Am. 2022;102:335–44. https://doi.org/10.1016/j.suc.2021.12.001
7. Bade BC, Dela Cruz CS. Lung Cancer 2020: epidemiology, etiology, and Prevention. Clin Chest Med. 2020;41:1–24. https://doi.org/10.1016/j.ccm.2019.10.001
8. Saito M, Shiraishi K, Kunitoh H, Takenoshita S, Yokota J, Kohno T. Gene aberrations for precision medicine against lung adenocarcinoma. Cancer Sci. 2016;107:713–20. https://doi.org/10.1111/cas.12941
9. Abu Rous F, Singhi EK, Sridhar A, Faisal MS, Desai A. Lung Cancer treatment advances in 2022. Cancer Invest. 2023;41:12–24. https://doi.org/10.1080/07357907.2022.2119479
10. Guo H, Zhang J, Qin C, Yan H, Liu T, Hu H, Tang S, Tang S, Zhou H. Biomarker-targeted therapies in Non-small Cell Lung Cancer: current status and perspectives. Cells. 2022;11. https://doi.org/10.3390/cells11203200
11. Ghafouri-Fard S, Shoorei H, Sabernia T, Hussen BM, Taheri M, Pourmoshtagh H. Circular RNAs and inflammation: epigenetic regulators with diagnostic role. Pathol Res Pract. 2023;251:154912. https://doi.org/10.1016/j.prp.2023.154912
12. He Z, Zhu Q. Circular RNAs: emerging roles and new insights in human cancers. Biomed Pharmacotherapy = Biomedecine Pharmacotherapie. 2023;165:115217. https://doi.org/10.1016/j.biopha.2023.115217
13. Sufianov A, Begliarzade S, Beilerli A, Liang Y, Ilyasova T, Beylerli O. Circular RNAs as biomarkers for lung cancer. Non-coding RNA Res. 2023;8:83–8. https://doi.org/10.1016/j.ncrna.2022.11.002
14. Ghaedrahmati F, Nasrolahi A, Najafi S, Mighani M, Anbiyaee O, Haybar H, Assareh AR, Kempisty B, Dzięgiel P, Azizidoost S, Farzaneh M. Circular RNAs-mediated angiogenesis in human cancers. Clin Translational Oncology: Official Publication Federation Span Oncol Soc Natl Cancer Inst Mexico. 2023;25:3101–21. https://doi.org/10.1007/s12094-023-03178-6
15. Qi JC, Yang Z, Lin T, Ma L, Wang YX, Zhang Y, Gao CC, Liu KL, Li W, Zhao AN, Shi B, Zhang H, Wang DD, Wang XL, Wen JK, Qu CB. CDK13 upregulation-induced formation of the positive feedback loop among circCDK13, miR-212-5p/miR-449a and E2F5 contributes to prostate carcinogenesis. J Experimental Clin cancer Research: CR. 2021;40:2. https://doi.org/10.1186/s13046-020-01814-5
16. Zheng H, Chen T, Li C, Xu C, Ding C, Chen J, Ju S, Zhang Z, Liang Z, Cui Z, Zhao J. A circular RNA hsa_circ_0079929 inhibits tumor growth in hepatocellular carcinoma. Cancer Manage Res. 2019;11:443–54. https://doi.org/10.2147/cmar.S189338
17. Li Y, Sun R, Li R, Chen Y, Du H. Prognostic Nomogram based on circular RNA-Associated competing endogenous RNA network for patients with Lung Adenocarcinoma. Oxidative Med Cell Longev. 2021;2021(9978206). https://doi.org/10.1155/2021/9978206
18. Zhang T, Wu DM, Luo PW, Liu T, Han R, Deng SH, He M, Zhao YY, Xu Y. CircNEIL3 mediates pyroptosis to influence lung adenocarcinoma radiotherapy by upregulating PIF1 through miR-1184 inhibition. Cell Death Dis. 2022;13:167. https://doi.org/10.1038/s41419-022-04561-x
19. Xia C, Dong X, Li H, Cao M, Sun D, He S, Yang F, Yan X, Zhang S, Li N, Chen W. Cancer statistics in China and United States, 2022: profiles, trends, and determinants. Chin Med J. 2022;135:584–90. https://doi.org/10.1097/cm9.0000000000002108
20. Sears CR, Mazzone PJ. Biomarkers in Lung Cancer. Clin Chest Med. 2020;41:115–27. https://doi.org/10.1016/j.ccm.2019.10.004
21. Ma T, Miao H, Xiong Y, Ma Y, Dong Z. CircRNA circ-PDCD11 is highly expressed in lung large-cell carcinoma and predicts poor survival. Immunopharmacol Immunotoxicol. 2023;45:89–93. https://doi.org/10.1080/08923973.2022.2117628
22. Yu Y, Zheng W, Ji C, Wang X, Chen M, Hua K, Deng X, Fang L. Tumor-derived circRNAs as circulating biomarkers for breast Cancer. Front Pharmacol. 2022;13:811856. https://doi.org/10.3389/fphar.2022.811856
23. Ning J, Luo Y, Chen L, Xiao G, Tanzhu G, Zhou R. CircRNAs and lung cancer: insight into their roles in metastasis. Biomed Pharmacotherapy = Biomedecine Pharmacotherapie. 2023;166:115260. https://doi.org/10.1016/j.biopha.2023.115260
24. Wan J, Ding G, Zhou M, Ling X, Rao Z. Circular RNA hsa_circ_0002483 promotes growth and invasion of lung adenocarcinoma by sponging miR-125a-3p. Cancer Cell Int. 2021;21:533. https://doi.org/10.1186/s12935-021-02241-y
25. Yu Z, Zhu X, Li Y, Liang M, Liu M, Liu Z, Qin L, Wu X, Du K, Liu L, Wang Y, Zheng Y. Circ-HMGA2 (hsa_circ_0027446) promotes the metastasis and epithelial-mesenchymal transition of lung adenocarcinoma cells through the miR-1236-3p/ZEB1 axis. Cell Death Dis. 2021;12:313. https://doi.org/10.1038/s41419-021-03601-2
26. Singh S, Sinha T, Panda AC. (2023) Regulation of microRNA by circular RNA. Wiley interdisciplinary reviews RNA:e1820. https://doi.org/10.1002/wrna.1820
27. Wei W, Wang C, Wang L, Zhang J. circ_0020123 promotes cell proliferation and migration in lung adenocarcinoma via PDZD8. Open Med (Warsaw Poland). 2022;17:536–49. https://doi.org/10.1515/med-2022-0434
28. Wang X, Li R, Feng L, Wang J, Qi Q, Wei W, Yu Z. Hsa_circ_0001666 promotes non-small cell lung cancer migration and invasion through miR-1184/miR-548l/AGO1 axis. Mol Therapy Oncolytics. 2022;24:597–611. https://doi.org/10.1016/j.omto.2022.02.011
29. Liu Y, Zhang H, Zhang W, Xiang L, Yin Z, Xu H, Lu P, Ma Y, Xiong L, Zhang X, Liang X, Luo J, Liang X. circ_0004140 promotes LUAD tumor progression and

immune resistance through circ_0004140/miR-1184/CCL22 axis. Cell Death Discovery. 2022;8:181. https://doi.org/10.1038/s41420-022-00983-w

30. Pan CF, Wei K, Ma ZJ, He YZ, Huang JJ, Guo ZZ, Chen ZP, Barr MP, Shackelford RE, Xia Y, Wang J. CircP4HB regulates ferroptosis via SLC7A11-mediated glutathione synthesis in lung adenocarcinoma. Translational lung cancer Res. 2022;11:366–80. https://doi.org/10.21037/tlcr-22-138

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