Original Article

circRACGAP1 Promotes Proliferation of Non-Small Cell Lung Cancer Cells through the miR-1296/CDK2 Pathway

(NSCLC / circRACGAP1 / miR-1296 / CDK2 / proliferation)

YANG ZHAO, LIYONG DENG, YI XIE, WEIMING WANG, QIN CHAI, GUIHUA WANG

The Affiliated Changsha Central Hospital, Department of Oncology, Hengyang Medical School, University of South China, Changsha, Hunan, China

Abstract. Circular RNAs (circRNAs) have played an essential role in cancer development. This study aimed to illustrate the impact and potential mechanism of circRACGAP1 action in NSCLC development. The expression patterns of circRACGAP1, miR-1296, and CDK2 in NSCLC tissues and cell lines were analysed by RT-qPCR. The function of circRACGAP1 in NSCLC cell proliferation and apoptosis was investigated using the CCK-8 assay, flow cytometry, TUNEL staining, and Western blot. The interaction among circRACGAP1, miR-1296, and CDK2 was clarified by dual-luciferase reporter assay while the correlation was confirmed by the Pearson correlation coefficient. The expression of circRACGAP1 and CDK2 was up-regulated in NSCLC tissues, while the expression of miR-1296 was down-regulated. Cell function studies further revealed that circRACGAP1 could promote NSCLC cell proliferation, accelerate the cell cycle process, up-regulate B-cell lymphoma 2 (Bcl2) expression, and down-regulate Bcl2-associated X (Bax) expression. miR-1296 was identified as a downstream target to reverse circRACGAP1-mediated cell proliferation. miR-1296 directly targeted the 3'-UTR of CDK2 to regulate proliferation and apoptosis of NSCLC cells. Additionally, the dual-luciferase reporter assay and Pearson correlation coefficient analysis proved that circRACGAP1 acted in NSCLC cells by negatively regulating miR-1296 expression and positively regulating CDK2 expression. In summary, our study revealed that circRACGAP1 promoted NSCLC cell proliferation by regulating the miR-1296/CDK2 pathway, providing potential diagnostic and therapeutic targets for NSCLC.

Introduction

Non-small cell lung cancer (NSCLC), the most common type of lung cancer, accounted for over 85 % of lung cancer cases in 2014 (Reck et al., 2014). NSCLC is usually diagnosed at an advanced stage. In recent years, immunotherapy and molecular-targeted therapy have improved the prognosis of NSCLC (Shepherd et al., 2011). Despite advances in treatment research, NSCLC patients still suffer from a poor 5-year survival rate (Ko et al., 2018). Thus, there is an urgent need to explore early diagnostic targets for NSCLC. In recent years, researchers at home and abroad have discovered the role of non-coding RNAs (ncRNAs) in the biology of NSCLC and their clinical potential (Kiełbowski et al., 2023).

The research on ncRNAs mainly focuses on microRNAs (miRNAs), long non-coding RNAs (lncRNAs), as well as circular RNAs (circRNAs). Among them, circRNAs are popular for researchers due to their unique structure and important functions (Zhang et al., 2020a). circRNAs can affect gene regulation by acting as miRNA and protein baits or competitors, thereby influencing various physiological processes, including early development, immune response, neurogenesis, and tumorigenesis (Yang et al., 2022). circRNAs have been proven to be key regulatory factors in cancer pathogenesis (Kristensen et al., 2018). The circRNA-miRNA-mRNA

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Corresponding authors: Yang Zhao, The Affiliated Changsha Central Hospital, Department of Oncology, Hengyang Medical School, University of South China, No. 161, Shaoshan South Road, Yuhua District, 410004 Changsha, Hunan, China; e-mail: zhaoyang19810411@gmail.com; Guihua Wang; e-mail: wangguihua8890@163.com

Abbreviations: Bax – Bcl2-associated X, Bcl2 – B-cell lymphoma 2, CCK-8 – cell counting kit-8, CDKL1 (2) – cyclin-dependent kinase-like 1 (2), circRNA(s) – circular RNA(s), E2F7 – E2F transcription factor 7, FSCN1 – fascin actin-bundling protein, 1, HCC – hepatocellular carcinoma, lncRNA(s) – long non-coding RNA(s), MAPK1 – mitogen-activated protein kinase 1, miRNA(s) – microRNA(s), ncRNA(s) – non-coding RNA(s), NSCLC – non-small cell lung cancer, PTBP1 – polypyrimidine tract-binding protein 1, RIF1 – regulatory factor 1, RT-qPCR – quantitative real-time polymerase chain reaction, SFPQ – splicing factor proline/glutamine-rich, SIRT3 – sirtuin 3.

regulatory network plays an important role in the progression of NSCLC (Balasundaram and Doss C, 2023). For example, circSATB2 can regulate fascin actin-bundling protein 1 (FSCN1) expression by directly binding to miR-326 to promote NSCLC development (Zhang et al., 2020a). Additionally, circRNA_101237 accelerates NSCLC progression through the miRNA-490-3p/mitogen-activated protein kinase 1 (MAPK1) axis (Zhang et al., 2020b). Moreover, the expression of circRACGAP1 may drive the development of NSCLC. In a further mechanism study, high expression of circRACGAP1 promoted NSCLC development through regulation of the miR-144-5p/cyclin-dependent kinase-like 1 (CDKL1) pathway (Lu et al., 2021). Furthermore, circRACGAP1 enhances NSCLC cell migration by recruiting polypyrimidine tract-binding protein 1 (PTBP1) to facilitate sirtuin 3 (SIRT3)-mediated replication timing regulatory factor 1 (RIF1) deacetylation (Xiong et al., 2023). Therefore, circRACGAP1 is considered a potential target for NSCLC treatment.

miRNA plays a crucial role in cancer development, mainly through its interaction with the 3'UTR of target mRNA to participate in transcriptional regulation (Hwang and Mendell, 2006). Research on colorectal cancer has shown that miRNA-1296 promotes the proliferation and metastasis of cancer cells by regulating splicing factor proline/glutamine-rich (SFPQ) (Tao et al., 2018). In hepatocellular carcinoma (HCC), miRNA-1296 suppresses HCC cell proliferation and angiogenesis by negatively regulating E2F transcription factor 7 (E2F7) expression (Liu et al., 2023). In NSCLC, miR-1296 expression inhibits proliferation and invasion by regulating the Wnt signalling pathway (Deng et al., 2020). Cyclin-dependent kinase 2 (CDK2) is crucial in cell cycle regulation and is closely associated with the progress of various cancers (Tadesse et al., 2020). Small-molecule CDK2 inhibitors have shown therapeutic benefits for certain cancers (Tadesse et al., 2019). In cervical cancer research, miR-1296 blocked the cell cycle and promoted apoptosis by inhibiting CDK2 expression (Zheng et al., 2021). Furthermore, miR-1296 can serve as a potential target for inhibiting CDK2 expression in prostate cancer (Majid et al., 2010). However, there is currently no research on the role of the circRACGAP1/miR-1296/CDK2 axis in NSCLC.

Based on the above findings, this study utilized clinical samples and cell experiments to explore the role and specific mechanisms of circRACGAP1 in NSCLC. In general, this study found that circRACGAP1 increased CDK2 expression by sponging miR-1296 to promote NSCLC, providing new insights for the discovery of therapeutic targets and development of novel strategies for NSCLC.

Material and Methods

Human tissue samples

Experiments involving clinical tissues were approved by the Affiliated Changsha Central Hospital, University of South China Ethics Committee. Fifteen pairs of NSCLC tissues and adjacent normal tissues obtained from patients in the Affiliated Changsha Central Hospital were analysed. Patients who accepted chemotherapy or radiation therapy before surgery were not included.

Cell culture and treatment

16HBE, A549, Calu-3, H1299, and H1975 cells were obtained from Abiowell and incubated in RPMI-1640 medium (AW-MC002, Abiowell, Changsha, China) including 10 % of foetal bovine serum (FBS, 10099141, Gibco, Grand Island, NY) and 1 % of penicillin/streptomycin (AWI0070a, Abiowell). They were cultured in a saturated humidity incubator (DH-160I, SANTN, Shanghai, China) with 5 % CO_2 at 37 °C. In addition, cell slides were prepared by respective inoculation of appropriate densities of H1299 cells in culture dishes for TUNEL staining. H1299 cells were transfected with sh-NC, sh-circRACGAP1, inhibitor-NC, miR-1296 inhibitor, sh-NC, sh-CDK2, oe-NC, and oe-CDK2 using Lipofectamine 2000 (11668019, Invitrogen, Waltham, MA). All plasmids and miRNA mimics were obtained from HonorGene (Changsha, China).

Quantitative real-time polymerase chain reaction (RT-qPCR)

16HBE, A549, Calu-3, H1299, and H1975 cells, NSCLC tissues and adjacent normal tissues were treated with Trizol reagent (15596026, Thermo Fisher Scientific, Waltham, MA) to extract total RNA. Reverse transcription was performed by using an mRNA reverse transcription kit (CW2569, CWBIO, Taizhou, China), followed by RT-qPCR with UltraSYBR Mixture (CW2601, CWBIO). All primers used are displayed in Table 1. The gene expression was quantified by the $2^{-\Delta\Delta Ct}$ method using β-actin or *U6* as the internal reference.

Table 1. Sequences of primers

Gene name	Forward (5'-3')	Reverse (5'-3')
β-actin	ACCCTGAAGTACCCCATCGAG	AGCACAGCCTGGATAGCAAC
circRACGAP1	GACGTTGAATAGGGAGAGCCAA	ATCCATCTTTCCTAAGTGCTACAGG
CDK2	GACACGCTGCTGGATGTCA	CAGAAAGCTAGGCCCTGGAG
U6	CTCGCTTCGGCAGCACA	AACGCTTCACGAATTTGCGT
miR-1296	TTAGGGCCCTGGCTCCAT	CACCTGGGTTAGGGTCGAAG

Cell counting kit-8 (CCK-8) assay

H1299 cells were digested and cultured at 37 °C (5 × 10^3 cells/well). After adhesion, they were treated accordingly for 48 h. Next, 100 µl of medium containing 10 % CCK-8 reagent (NU679, DOJINDO, Kumamoto, Japan) replaced the original medium. Finally, H1299 cells were cultured for 4 h, and then optical density (OD) at 450 nm was read by a multifunctional microplate reader (MB-530, Shenzhen Heales Technology Development, Guangdong, China).

Flow cytometry

H1299 cells were supplemented with pre-cooled 100 % ethanol (1.2 ml) at a final concentration of 75 % and placed at 4 °C overnight for fixation. H1299 cells were washed and suspended with PBS to remove ethanol. Then, they were supplemented with 150 µl of propidium iodide (PI, MB2920, MeilunBio, Dalian, China) and stained for 30 min. Finally, the H1299 cell cycle was measured using a flow cytometer (A00-1-1102, Beckman, Brea, CA).

TUNEL staining

After the fixation with 4 % paraformaldehyde, cell slides were reacted with 100 µl of Proteinase K solution prepared using the TUNEL cell apoptosis detection kit (40306ES50, Yeasen, Shanghai, China) at 37 °C for 20 min. Next, cell slides were incubated with 100 µl of equilibration buffer and 50 µl of TDT solution in that order. After staining with 4′,6-diamidino-2-phenylindole (DAPI), cell slides were sealed with glycerol before observation by a fluorescence microscope (BA410T, Motic, Xiamen, China).

Western blot

H1299 cells were treated with RIPA lysate (AWB0136, Abiowell) to extract total proteins. The proteins were transferred to nitrocellulose (NC) membranes after SDS-PAGE. After blocking, NC membranes were cultured with primary antibodies overnight at 4 °C, including CDK2 (1 : 1000, 60312-1-Ig, Proteintech, Rosemont, IL), Bax (1 : 5000, ab32503, Abcam, Cambridge, UK), B-cell lymphoma 2 (Bcl2, 1 : 5000, 60178-1-Ig, Proteintech), and β-actin (1 : 5000, 66009-1-Ig, Proteintech). Then, the NC membranes were incubated with the secondary antibodies, respectively. NC membranes were incubated with Super ECL Plus detection reagent (AWB0005, Abiowell) for chemiluminescence imaging. The grey values of bands were read by Quantity One 4.6.6 (Bio-Rad Inc., Hercules, CA). Finally, protein expression was calculated with β-actin as the internal reference.

Dual-luciferase reporter assay

The binding sites of circRACGAP1 and miR-1296, miR-1296 and CDK2 were analysed using the Circinteractome database. psiCHECK-2-Hum-circRACGAP1-WT, psiCHECK-2-Hum-circRACGAP1-MUT, psiCHECK-2-Hum-CDK2-WT, and psiCHECK-2-Hum-CDK2-MUT were obtained from HonorGene. HEK-293T cells (AW-CNH086, Abiowell) were cultured for 24 h and then transfected with the above vectors and miRNA mimics using Lipofectamine 2000. After culture, HEK-293T cells were lysed and then treated according to the dual-luciferase detection kit (E1910, Promega, Madison, WI). Relative luciferase activity was analysed by a chemiluminescence detector (GloMax 20/20, Promega).

Statistical analysis

Experimental data were analysed by GraphPad Prism 8.0 and expressed as mean ± standard deviation (SD). The paired Student's *t*-test was utilized to compare two groups in clinical samples. One-way analysis of variance (ANOVA) and Tukey's post-hoc test were employed to compare multiple groups. The correlation among circRACGAP1, miR-1296, and CDK2 expression was confirmed by the Pearson correlation coefficient. $P < 0.05$ was considered statistically significant.

Results

Up-regulation of circRACGAP1 expression in NSCLC tissues and cell lines

First, we performed RT-qPCR analysis of circRACGAP1 expression in 15 pairs of clinical tissues. Significant up-regulation of circRACGAP1 expression was exhibited in NSCLC tissues in comparison with adjacent normal tissues (Fig. 1A). Next, circRACGAP1 expression in 16HBE cells and NSCLC cells (A549, Calu-3, H1299, and H1975) was analysed. Consistently, circRACGAP1 was highly expressed in NSCLC cell lines. Among these, H1299 cells exhibited the highest

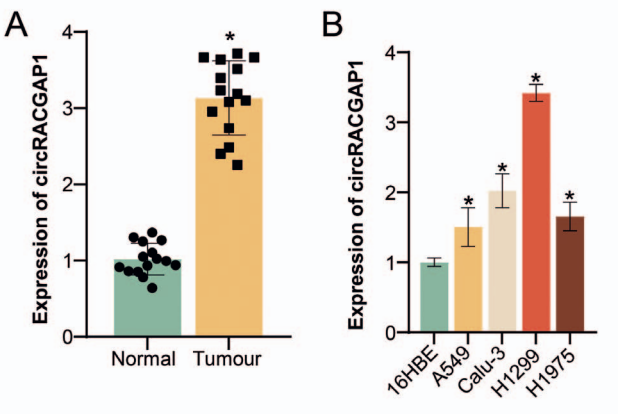

Fig. 1. The expression of circRACGAP1 was up-regulated in NSCLC tissues and cell lines. **(A)** The expression of circRACGAP1 in NSCLC and adjacent normal tissues was analysed by RT-qPCR. $^*P < 0.05$ vs Normal. **(B)** The expression of circRACGAP1 in the normal cell line (16HBE) and NSCLC cell lines (A549, Calu-3, H1299, and H1975) was analysed by RT-qPCR. $^*P < 0.05$ vs 16HBE.

circRACGAP1 expression. Therefore, H1299 cells were selected as the experimental subjects (Fig. 1B). These results suggested up-regulation of circRACGAP1 expression in NSCLC tissues and cell lines.

Silencing of circRACGAP1 suppressed proliferation and induced apoptosis of NSCLC cells

To illustrate the impact of circRACGAP1 on NSCLC, H1299 cells were transfected with sh-circRACGAP1. RT-qPCR validation demonstrated a significant reduction in circRACGAP1 expression in H1299 cells of the sh-circRACGAP1 group, indicating successful transfection (Fig. 2A). After transfection with sh-circRACGAP1, the proliferation of H1299 cells significantly decreased (Fig. 2B). Further investigations revealed that sh-circRACGAP1 transfection prolonged the G1 phase but shortened the S phase of H1299 cells (Fig. 2C). Moreover, H1299 cell apoptosis markedly increased upon transfection with sh-circRACGAP1 (Fig. 2D). sh-circRACGAP1 transfection down-regulated Bcl2 expression while up-regulating Bax expression. These findings proved that silencing of circRACGAP1 could suppress proliferation and induce apoptosis of NSCLC cells.

circRACGAP1 targeted miR-1296 in NSCLC cells

The binding sites between circRACGAP1 and miR-1296 were identified using the Circinteractome database (Fig. 3A). miR-1296 mimic transfection significantly weakened luciferase activity in circRACGAP1-WT-transfected HEK-293T cells, indicating that miR-1296 has a significant regulatory effect on circRACGAP1 expression (Fig. 3B). RT-qPCR further confirmed that sh-circRACGAP1 transfection promoted miR-1296 expression in H1299 cells (Fig. 3C). Moreover, miR-1296 expression was suppressed in NSCLC tissues compared to adjacent normal tissues (Fig. 3D). Pearson correlation coefficient analysis revealed a significant negative correlation between circRACGAP1 and miR-1296 (Fig. 3E). The above suggested that circRACGAP1 targeted miR-1296 and negatively regulated its expression.

Inhibition of miR-1296 restored the function of circRACGAP1 in the proliferation and apoptosis of NSCLC cells

To illustrate the impact of miR-1296 on the function of circRACGAP1, H1299 cells were transfected with sh-circRACGAP1 and miR-1296 inhibitor. A significant decrease in miR-1296 expression in miR-1296 inhibitor-transfected H1299 cells indicated successful transfection (Fig. 4A). The miR-1296 inhibitor promoted proliferation in H1299 cells following sh-circRACGAP1 transfection (Fig. 4B). Further research revealed that miR-1296 inhibitor shortened the G1 phase and prolonged the S phase in sh-circRACGAP1 transfected H1299 cells (Fig. 4C). Additionally, the apoptosis rate of H1299 cells transfected with sh-circRACGAP1 and miR-1296 inhibitor was reduced (Fig. 4D). The miR-1296 inhibitor up-regulated Bcl2 expression and down-regulated Bax expression in H1299 cells transfected with

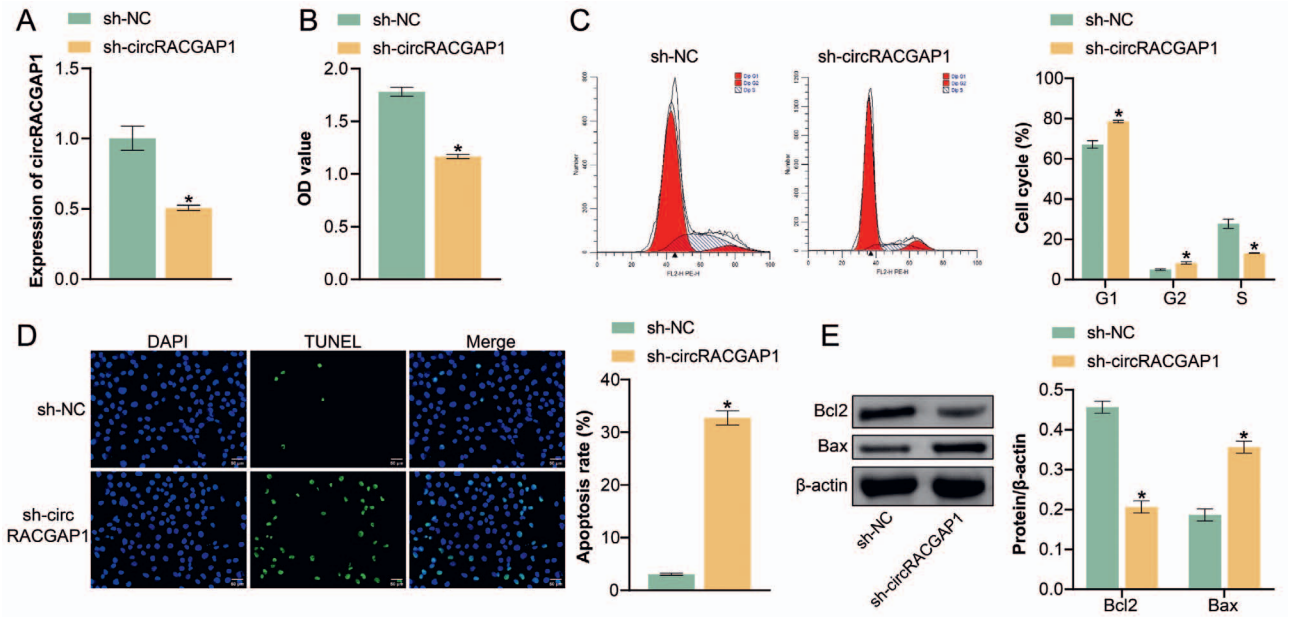

Fig. 2. Silencing of circRACGAP1 suppressed proliferation and induced apoptosis of NSCLC cells. **(A)** The expression of circRACGAP1 in H1299 cells was analysed by RT-qPCR. **(B)** The cell viability of H1299 cells was measured by CCK-8 assay. **(C)** The cell cycle of H1299 cells was analysed by flow cytometry. **(D)** The apoptosis of H1299 cells was detected by TUNEL staining. **(E)** The expression of Bcl2 and Bax in H1299 cells was evaluated by Western blot. $^{*}P < 0.05$ vs sh-NC.

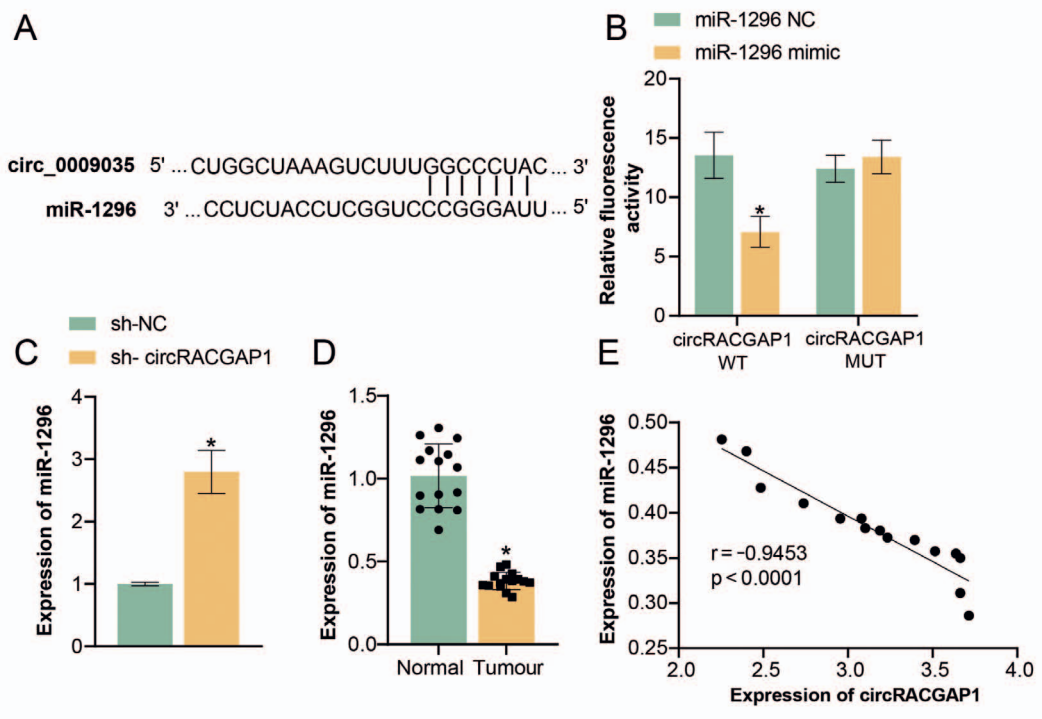

Fig. 3. circRACGAP1 acted as a sponge of miR-1296 in NSCLC. **(A)** The binding sites between circRACGAP1 and miR-1296 were predicted using the Circinteractome database. **(B)** The targeted binding between circRACGAP1 and miR-1296 was validated by dual-luciferase reporter assay. $^{*}P < 0.05$ vs miR-1296 NC. **(C)** The expression of miR-1296 in H1299 cells was analysed by RT-qPCR. $^{*}P < 0.05$ vs sh-NC. **(D)** The expression of miR-1296 in NSCLC and adjacent normal tissues was analysed by RT-qPCR. $^{*}P < 0.05$ vs Normal. **(E)** The correlation between circRACGAP1 and miR-1296 was demonstrated by Pearson correlation coefficient analysis.

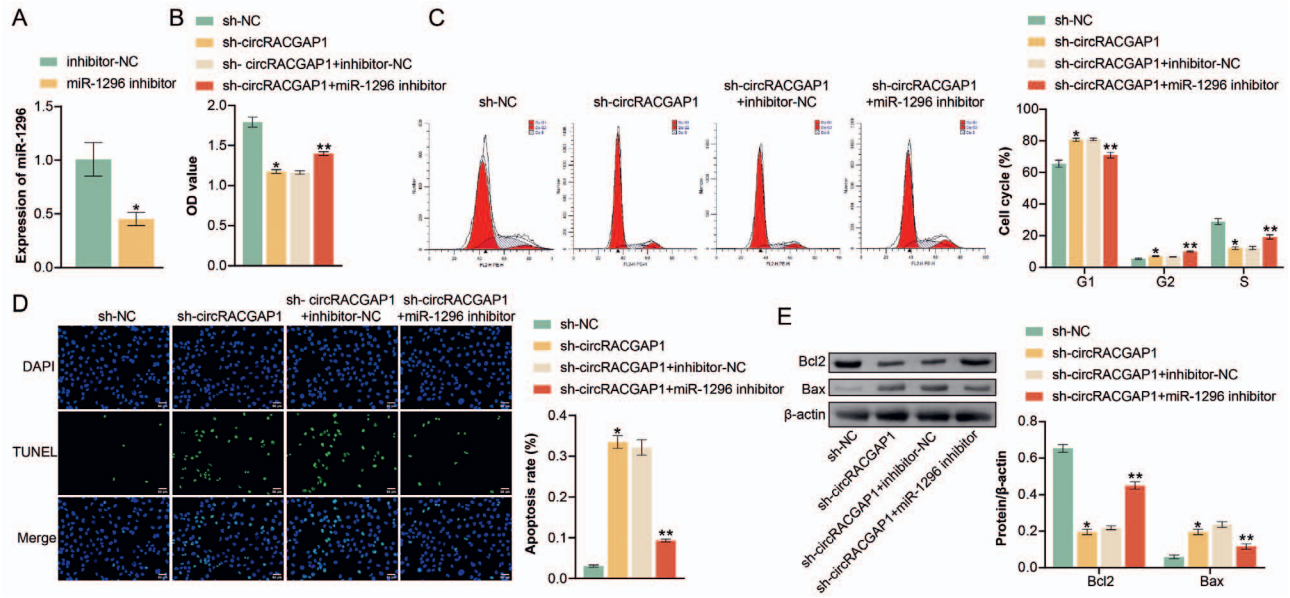

Fig. 4. Inhibition of miR-1296 restored the ability of circRACGAP1 to promote proliferation and inhibit apoptosis of H1299 cells. **(A)** The expression of miR-1296 in H1299 cells was analysed by RT-qPCR. $^{*}P < 0.05$ vs inhibitor-NC. **(B)** The cell viability of H1299 cells was measured by CCK-8 assay. **(C)** The cell cycle of H1299 cells was analysed by flow cytometry. **(D)** The apoptosis of H1299 cells was detected by TUNEL staining. **(E)** The expression of Bcl2 and Bax in H1299 cells was evaluated by Western blot. $^{*}P < 0.05$ vs sh-NC, $^{**}P < 0.05$ vs sh-circRACGAP1+inhibitor NC.

sh-circRACGAP1 (Fig. 4E). The above findings showed that the inhibition of miR-1296 restored the function of circRACGAP1 to enhance proliferation and suppress apoptosis of H1299 cells.

miR-1296 targeted CDK2 in NSCLC cells

The binding sites between miR-1296 and the 3'-UTR of CDK2 were identified using the Circinteractome database (Fig. 5A). miR-1296 mimic transfection markedly reduced luciferase activity in CDK2-WT-transfected HEK-293T cells, indicating a significant regulatory effect of miR-1296 on CDK2 expression (Fig. 5B). Further verification demonstrated that transfection of the miR-1296 inhibitor promoted CDK2 expression in H1299 cells (Fig. 5C–5D). Additionally, clinical results indicated high CDK2 expression in NSCLC tissues compared to adjacent normal tissues (Fig. 5E). Pearson correlation coefficient analysis revealed a significant positive correlation between circRACGAP1 expression and CDK2 expression, yet a significant negative correlation was observed between miR-1296 and CDK2 (Fig. 5F). These results indicated that miR-1296 targeted CDK2 and negatively regulated its expression in NSCLC cells.

Silencing of circRACGAP1 down-regulated CDK2 expression via miR-1296 to affect proliferation and apoptosis of NSCLC cells

To clarify the specific mechanism of circRACGAP1 action in NSCLC, H1299 cells were transfected with sh-circRACGAP1, miR-1296 inhibitor, sh-CDK2, and oe-CDK2. Significant changes in CDK2 at the transcriptional and translational levels in H1299 cells suggested the transfection was effective and successful (Fig. 6A–B). sh-CDK2 significantly inhibited proliferation of miR-1296 inhibitor-transfected H1299 cells, leading to the lengthening of the G1 phase while shortening the S phase. oe-CDK2 promoted proliferation of H1299 cells transfected with sh-circRACGAP1, leading to the shortening of the G1 phase while lengthening the S phase (Fig. 6C–D). Furthermore, sh-CDK2 significantly induced apoptosis in miR-1296 inhibitor-transfected H1299 cells, accompanied by inhibition of Bcl2 expression and promotion of Bax expression. oe-CDK2 inhibited apoptosis of H1299 cells transfected with sh-circRACGAP1, characterized by raised Bcl2 expression and reduced Bax expression (Fig. 6E–F). These results suggested that silencing of circRACGAP1 down-regulated CDK2 expression via miR-1296 to suppress proliferation and promote apoptosis of NSCLC cells.

Discussion

With the increasing incidence and mortality rates of NSCLC, research on its pathogenesis and treatment strategies is constantly advancing (Herbst et al., 2018). As a novel type of RNA, circRACGAP1 has shown potential as a diagnostic and therapeutic target for NSCLC (Lu et al., 2021). Here, we identified high circRACGAP1 expression in NSCLC tissues and cell lines. circRAC-

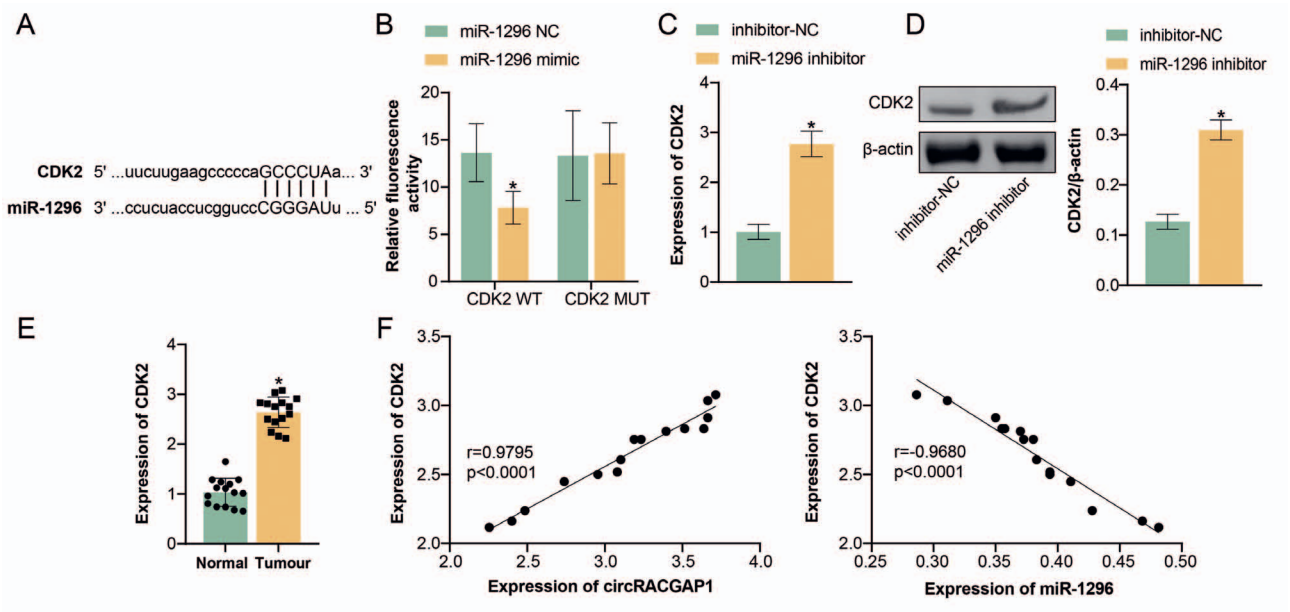

Fig. 5. miR-1296 targeted CDK2 in NSCLC. **(A)** The binding sites between CDK2 and miR-1296 were predicted using the Circinteractome database. **(B)** The targeted binding between miR-1296 and CDK2 was confirmed by dual-luciferase reporter assay. $^{*}P < 0.05$ vs miR-1296 NC. **(C–D)** The expression of CDK2 in H1299 cells was analysed by RT-qPCR and Western blot. $^{*}P < 0.05$ vs inhibitor-NC. **(E)** The expression of CDK2 in NSCLC and adjacent normal tissues was analysed by RT-qPCR. $^{*}P < 0.05$ vs Normal. **(F)** The correlation between CDK2 and circRACGAP1, as well as CDK2 and miR-1296, was demonstrated by Pearson correlation coefficient analysis.

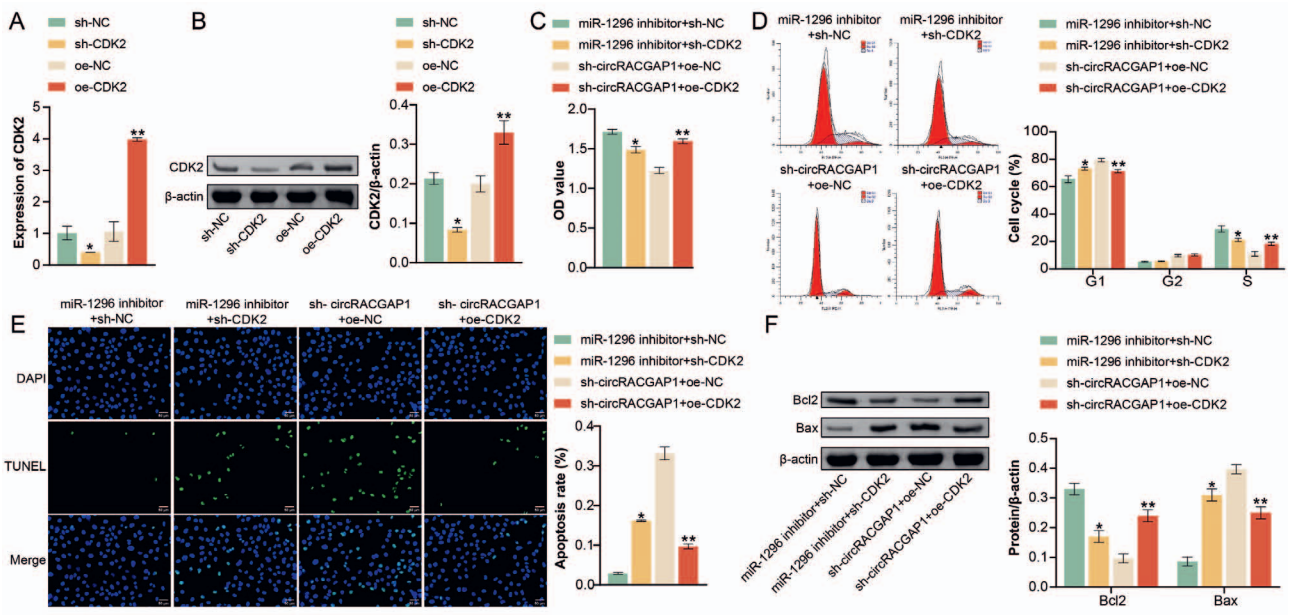

Fig. 6. Silencing of circRACGAP1 down-regulated CDK2 expression via miR-1296 to suppress proliferation and induce apoptosis of NSCLC cells. **(A–B)** The expression of CDK2 in H1299 cells was detected by RT-qPCR and Western blot. $^{*}P < 0.05$ vs sh-NC and $P < 0.05$ vs oe-NC. **(C)** The cell viability of H1299 cells was measured by CCK-8 assay. **(D)** The cell cycle of H1299 cells was analysed by flow cytometry. **(E)** The apoptosis of H1299 cells was detected by TUNEL staining. **(F)** The expression of Bcl2 and Bax in H1299 cells was evaluated by Western blot. $^{*}P < 0.05$ vs miR-1296 inhibitor+sh-NC, $^{**}P < 0.05$ vs sh-circRACGAP1+oe-NC.

GAP1 contributed to NSCLC progression by down-regulating Bax expression and up-regulating Bcl2 expression to inhibit cell apoptosis. Besides, we revealed the potential mechanism by which circRACGAP1 acted as a miR-1296 sponge to up-regulate CDK2 expression to promote NSCLC (Fig. 7).

Unlike linear RNAs, circRNA owns high conservation and stability, making it an ideal biomarker for disease diagnosis (Kristensen et al., 2019). Recently, abnormal circRNA expression has been exhibited in various human cancers (Li et al., 2018b; Zhong et al., 2018). In lung cancer, circRNA participates as a competitive endogenous RNA in proliferation, migration, and invasion. Differentially expressed circRNA can serve as a diagnostic biomarker for lung cancer (Chen et al., 2018). Studies have revealed that many circRNAs are up-regulated in NSCLC tissues and cell lines, such as circFARSA (Hang et al., 2018), circRNA_100876 (Yao et al., 2017), Hsa_circ_004325 (Tian et al., 2017), circ_0067934 (Wang and Li, 2018), F-circEA (Tan et al., 2018), and Hsa_circ_0079530 (Li et al., 2018a). In this study, circRACGAP1 was highly expressed in NSCLC tissues and cell lines, which is similar to the trend reported in the literature (Lu et al., 2021). These findings proved that circRACGAP1 might also promote NSCLC development and be a potential target for prediction and diagnosis.

To validate the promoting effect of circRACGAP1 on NSCLC development, we silenced circRACGAP1 in

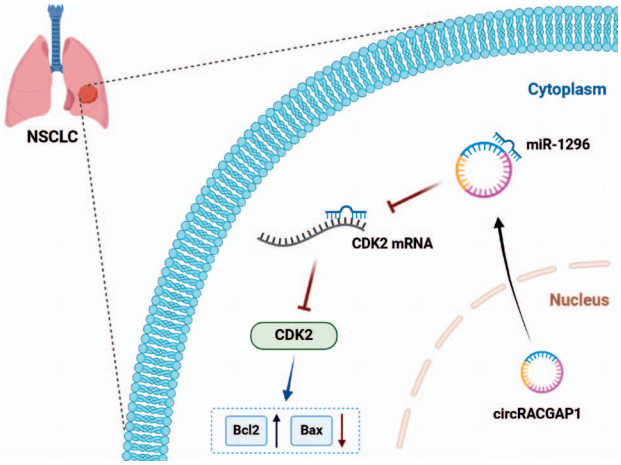

Fig. 7. Schematic diagram of the relationship among circRACGAP1, miR-1296, CDK2, Bcl2, Bax, and apoptosis in NSCLC.

H1299 cells. The silencing of circRACGAP1 inhibited the proliferation of H1299 cells and arrested them in the G1 phase. Research has shown that RACGAP1 is essential for cytoplasmic division, and knockdown of RACGAP1 can arrest lung cancer cells in the G1 phase and ultimately induce cell death (Liang et al., 2013). Selective inhibition of Bcl2 expression has been found to exhibit anti-tumour effects in lung cancer (American

Association for Cancer Research, 2015). Additionally, over-expression of Bax in human lung cancer cells can induce cell apoptosis (Kaliberov et al., 2002). In NSCLC research, regulation of the Bax/Bcl2 cascade has been reported to enhance cancer cell apoptosis (Alam et al., 2022). In this study, silencing of circRACGAP1 up-regulated Bax expression and down-regulated Bcl2 expression in H1299 cells to induce cell apoptosis. The above findings proved that high expression of circRACGAP1 promoted NSCLC development by enhancing cell proliferation.

miRNA, as a small single-stranded ncRNA, plays a prominent role in tumour development through the regulation of the cell cycle, angiogenesis, metabolism, and apoptosis. Moreover, miRNA usually inhibits target gene expression through post-transcriptional regulation (Iqbal et al., 2019). Research has shown the role of the circRNA-miRNA-mRNA regulatory axis in cancer development (Liang et al., 2020). Hsa_circRNA_101237 raises MAPK1 expression through miRNA-490-3p, thereby promoting NSCLC progression (Zhang et al., 2020b). circRACGAP1 promotes CDKL1 expression through miR-144-5p, thereby promoting the proliferation of NSCLC cells (Lu et al., 2021). Hsa_circ_0000520 increases CDK2 expression through miR-1296 to promote the proliferation of cervical cancer cells (Zheng et al., 2021). However, there are currently no reports on the role of the circRACGAP1-miR-1296-CDK2 regulatory axis in NSCLC. Here, we found that miR-1296 was down-regulated, while CDK2 was up-regulated in NSCLC tissues. Bioinformatic analysis confirmed miR-1296 as the target of circRACGAP1, and CDK2 as the target of miR-1296. The dual-luciferase reporter assay confirmed these predictions. Furthermore, Pearson correlation coefficient analysis revealed negative regulation of miR-1296 by circRACGAP1 and of CDK2 by miR-1296. To elucidate the role of the circRACGAP1-miR-1296-CDK2 axis in NSCLC, sh-circRACGAP1, miR-1296 inhibitor, sh-CDK2, and oe-CDK2 were transfected into H1299 cells. oe-CDK2 reversed the inhibitory effect of sh-circRACGAP1 on the proliferation in H1299 cells, while sh-CDK2 reversed the inhibitory effect of miR-1296 inhibitor on the cell apoptosis in H1299 cells. These results suggest that circRACGAP1 promotes NSCLC development by promoting CDK2 expression through miR-1296.

This study revealed one of the mechanisms by which circRACGAP1 promoted the development of NSCLC through the miR-1296/CDK2 axis. In future studies, we will develop strategies targeting circRACGAP1 for NSCLC treatment and validate its potential applications as a biomarker for diagnosis and prognosis assessment in NSCLC. Furthermore, we will unravel the complex regulatory network of circRACGAP1 with other non-coding RNAs, laying the theoretical and experimental foundations for improving the efficacy of NSCLC treatment and developing new targeted therapeutic strategies.

Conclusion

In summary, circRACGAP1 promoted the proliferation of NSCLC cells by regulating the miR-1296/CDK2 axis. This study will advance the study of NSCLC pathogenesis and the development of novel therapeutic strategies.

Data availability

The data used to support the findings of this study are available from the corresponding author upon request.

Conflict of interest

The authors declare no conflict of interest.

Ethical approval

The study was approved by the Ethics Committee of The Affiliated Changsha Central Hospital, University of South China (No. 2020-S0009). The research was conducted according to the World Medical Association Declaration of Helsinki. All the information about the study will be fully explained to the subjects by the researchers. All the participants provided informed consent before sampling.

Authors' contributions

Y.Z. and L.D. contributed to conceptualization, data curation, validation, supervision and writing of the original draft. Y.Z. and G.W. contributed to funding acquisition. W.W., Q.C. and Y.X. contributed to investigation, methodology. G.W. conceptualization, project administration, supervision and review. Y.Z. and L.D. contributed equally to this study. These are the co-first authors. All authors read and approved the final manuscript.

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