

Circular RNA CSPP1 motivates renal cell carcinoma carcinogenesis and the Warburg effect by targeting RAC1 through microRNA-493-5p

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Circular RNAs (circRNAs) take on regulatory roles in renal cell carcinoma (RCC). The research's goal was to figure out circ-CSPP1's role and molecular mechanism in RCC. The results clarified that circ-CSPP1 expression was enhanced in RCC. Down-regulating circ-CSPP1 refrained the proliferation, migration, invasion, and Warburg effect (aerobic glycolysis), but accelerated apoptosis of RCC cells. The luciferase activity assay exhibited that circ-CSPP1 could perform as an endogenous sponge for miR-493-5p. Elevating miR-493-5p repressed RCC progression. The bioinformatics website starBase confirmed that ras-related C3 botulinum toxin substrate 1 (RAC1) was a target gene of miR-493-5p. Circ-CSPP1 up-regulated RAC1 by sponging miR-493-5p, and elevating RAC1 could turn around the effect of down-regulating circ-CSPP1 on RCC cells. Taken together, circ-CSPP1 is identified as a novel RCC-promoting RNA that could serve as a latent therapeutic target for RCC therapy.

Keywords: Circ-CSPP1, MiR-493-5p, RAC1; renal cell carcinoma, Warburg effect

Received: 31 March, 2022; revised: 23 May, 2023; accepted: 28 June, 2023; available on-line: 18 September, 2023

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Acknowledgements of Financial Support: The National Natural Science Foundation of China (NSFC No.81101937).

Abbreviations: circRNAs, Circular RNAs; HCC, hepatocellular carcinoma; miRNAs, MicroRNAs; RCC, Renal cell carcinoma; RAC1, Ras-related C3 botulinum toxin substrate 1

INTRODUCTION

Renal cell carcinoma (RCC) is a class of diversified malignant neoplasms of the renal cortex with diverse clinical, morphological, and genetic features (Rysz *et al.*, 2021). Though clinical interventions like chemotherapy, surgery, and radiotherapy have made great progress, RCC patients' prognosis is still unpleasing. Therefore, it is imperative to develop new therapeutic strategies considering the biological characteristics of RCC cells. It is well known that metabolic reprogramming, a recognized hallmark of cancer, achieves tumor cell proliferation and survival by changing bioenergetic and biosynthetic pathways. Tumor cells having glycolysis high rates (Warburg effect) is a phenomenon that has been extensively studied in RCC (Emberley *et al.*, 2021). Therefore, figuring out the regulatory mechanism of the Warburg effect is likely to be beneficial for the development of effective RCC treatments.

Circular RNAs (circRNAs), a class of covalently closed endogenous RNAs without a 5' cap or 3' poly(A) tail, are cell-specific, tissue-specific, and developmental stage-specific (Huang *et al.*, 2020). For a long time, circRNAs have been regarded as waste products of splicing errors. Recent studies have discovered circRNAs can perform as gene regulators in diversified physiological and pathological processes and can regulate tumorigenesis and cancer progression (Li *et al.*, 2020). Meanwhile, circRNAs have also been clarified to exert biological functions by performing as miRNA sponges or protein sponges (van Zonneveld *et al.*, 2021). Therefore, circRNAs have been identified as latent biomarkers for tumor diagnosis (Zhu *et al.*, 2021). Circ-CSPP1 is a newly identified circRNA that has been confirmed to take on a momentous role in tumorigenesis (Xue, 2020; Sun *et al.*, 2020). However, its role in RCC and its latent mechanisms remain uncertain.

MicroRNAs (miRNAs) are a class of single-stranded non-coding RNAs that post-transcriptionally control gene expression (Bushati *et al.*, 2007). miRNA dysregulation is the key to the pathogenesis of diversified diseases, including cancer (Ali Syeda *et al.*, 2020) and can function as latent oncogenes or tumor suppressor genes (Iorio *et al.*, 2012). For instance, miR-133b impacts cell proliferation, chemosensitivity, and invasion of RCC cells by refraining the ERK signaling pathway (Xu *et al.*, 2020). A former study clarified that miR-493-5p expression is reduced in hepatocellular carcinoma (HCC) (Yang *et al.*, 2021). However, miR-493-5p's functions in RCC and its interaction with circ-CSPP1 remain ambiguous.

Ras-related C3 botulinum toxin substrate 1 (RAC1) is also commonly present in mammalian tissues and cells as a multiplex regulator of epithelial differentiation and is involved in apoptosis-related pathways through the production of reactive oxygen species (Lee *et al.*, 2012). RAC1 has been implicated in multiple cellular processes, such as cell proliferation, tumor metastasis, and epithelial-mesenchymal transition (Yoon *et al.*, 2017). The same pathways shared by circRNAs, miRNAs, and RAC1 suggest that they might function coordinately to orchestrate tumorigenesis. RAC1 has been reported to contribute to the tumorigenesis of RCC (Zhang, 2022; Shan *et al.*, 2017). Thus, RAC1 could be the target gene of miRNAs to regulate the tumorigenesis of RCC.

This study was conducted to figure out the biological characteristics and latent mechanisms of circ-CSPP1 in RCC progression and Warburg effect. Circ-CSPP1 motivated RCC progression and the Warburg effect by targeting miR-493-5p to control RAC1, suggesting that circ-CSPP1 may offer a novel biomarker and latent therapeutic target for RCC.

MATERIALS AND METHODS

Clinical sample collection

The research was approved by the Ethics Committee of The Second Affiliated Hospital of Xi'an Jiaotong University (Approval number.201803XAHT), and informed consent was obtained from each subject before sample collection. Forty pairs of RCC tumor tissues and adjacent normal tissues were harvested from patients in the urology department at The Second Affiliated Hospital of Xi'an Jiaotong University, and two pathologists independently made a definite pathological diagnosis. Adjacent normal tissue was harvested at least 3 cm from the tumor site. Tissues were kept in liquid nitrogen. Clinical data including clinical stage, age, gender, tumor stage, lymph node metastasis, and distant metastasis were recorded.

Cell culture

RCC cell lines (786-O, ACHN, CAKI-1, and OSRC2) and human normal kidney cell line (HK-2) were bought from the Chinese Academy of Sciences (Shanghai, China). Cells were cultured in Roswell Park Memorial Institute (RPMI) 1640 medium plus 10% fetal bovine serum (Gibco, Carlsbad, CA, USA), 100 units (U)/ml penicillin, and 100 U/ml streptomycin (Gibco).

Cell transfection

Transfection of miRNA mimics, inhibitors, small interfering RNAs (siRNAs), and plasmids was done with Lipofectamine 3000 (Invitrogen, Carlsbad, CA) in the light of the manufacturer's instructions. MiR-493-5p mimic, miR-493-5p inhibitor, circ-CSPP1 siRNA (si-circ-CSPP1), RAC1 siRNA (si-RAC1) and their negative controls (NCs) were bought from GenePharma (Shanghai, China). pcDNA 3.1 circRNA mini vector was utilized to elevate circ-CSPP1 expression in RCC cells.

Cell counting kit (CCK)-8 assay

Transfected cells were seeded in 96-well plates (Gibco BRL, Gaithersburg, MD, USA). Measurement of optical density at 450 nm was done with a microplate reader (Millipore, Billerica, MA, USA) (Chen *et al.*, 2020).

Colony formation assay

Transfected cells were seeded in 6-well plates and cultivated for colony formation assay. The colonies were fixed with 4% formaldehyde (Macklin, Shanghai, China), and then staining with 0.3% crystal violet was implemented. Counting and analyzing visible colonies were done by Image J software 1.8.0 (Wang *et al.*, 2020).

Flow cytometry

Apoptosis was tested by Annexin V- fluorescein isothiocyanate/propidium iodide (PI) apoptosis detection kit (Invitrogen). Briefly, double-staining was done with Annexin V and PI, and then the apoptosis rate was examined via FACSCalibur (Becton Dickinson) and FlowJo 10.0 software (Lin *et al.*, 2020).

Transwell assays

For cell invasion assay, transfected cells were seeded into 24-well transwell chambers with Matrigel. After incubation, non-invasive cells were wiped off, invasive

cells were fixed with paraformaldehyde, and then staining with crystal violet was implemented. Counting and photographing stained cells in each well were conducted under an inverted microscope (Olympus, Japan). As for the migration experiment, cells were seeded into the upper chamber without Matrigel, and other steps were similar to the cell invasion experiments.

Lactate production, glucose uptake, and ATP

Quantification of glucose levels was done using a glucose assay kit (Sigma-Aldrich, St. Louis, MO, USA). Measurement of lactate levels was implemented using a lactate assay kit (BioVision, Mountain View, CA, USA). ATP levels were tested by the CellTiter-Glo fluorescent cell viability assay (Promega, Madison, MI, USA).

Reverse transcription quantitative polymerase chain reaction (RT-qPCR)

Total RNA was extracted from tissues or cells using the RNeasy Mini Kit (QIAGEN, Hilden, Germany) according to the manufacturer's protocol. cDNA was generated using the Promega reverse transcription kit. RT-qPCR was carried out in the ABI7500 system using the SYBR Green method. Relative gene expression was determined by the $2^{-\Delta\Delta Ct}$ method. The primers were clarified in Table 1 (Cao *et al.*, 2020).

Western blot

RIPA lysis buffer (Beyotime) was used to extract total protein. Proteins were separated by 12% Sodium dodecyl sulfate-polyacrylamide gel electrophoresis and electro-blotted onto polyvinylidene fluoride membranes. The membranes were incubated with primary antibodies RAC1 (05-389, 1:1000, MilliporeSigma) and GAPDH (2118, 1:1000, Cell Signaling Technology). Bands were visualized via enhanced chemiluminescence (Millipore) after incubation with the HRP-conjugated secondary antibody. Relative band intensities were quantified by the ChemiDoc XRS imaging system (Liu *et al.*, 2020).

Table 1. RT-qPCR primers

Genes	Primer sequence (5'-3')
Circ-CSPP1	F: CCATCCCATCAGTTCATCCT
	R: TTCACCTCCAAGAGCATCC
miR-493-5p	F: TTGTACATGGTAGGCTTTCATT
	R: AACCATTTATTCTCCCGACC
RAC1	F: AACCAATGCATTTCTGGAG
	R: TGTTTGCGGATAGGATAGGG
U6	F: CTCGCTTCGGCAGCAC
	R: AACGCTTCACGAATTTGCGT
GAPDH	F: CACCCACTCCTCCACCTTTG
	R: CCACCACCCTGTTGCTGTAG

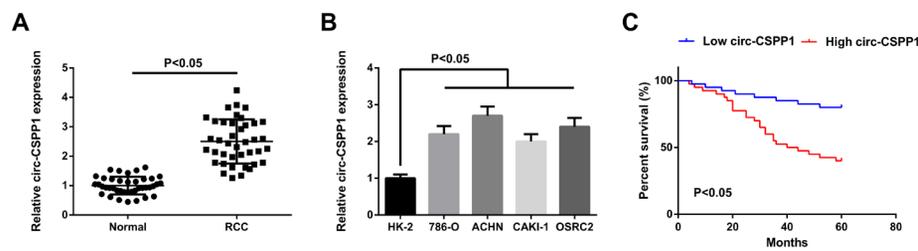


Figure 1. Circ-CSPP1 is elevated in RCC and is linked with poor prognosis (A, B) RT-qPCR to test circ-CSPP1 in RCC tissues and adjacent tissues (n=40), and RCC cell lines; (C) Kaplan-Meier survival analysis. Measurement data were shown as mean \pm S.D.

Luciferase activity assay

After predicting the binding sites of circ-CSPP1 and RAC1 3'UTR in miR-493-5p, pmirGLO reporter vector (Promega) was utilized to construct circ-CSPP1 and RAC1 3'UTR wild-type (WT) and Mutant (MUT) reporter vectors. A549 cells were incubated with miR-545-5p mimic or mimic NC with the reporter vectors. Then cell luciferase activity was tested by the dual luciferase reporter gene detection kit (Promega) (Yuan *et al.*, 2021).

Statistics

All data were processed by SPSS 21.0 statistical software (SPSS, Inc, Chicago, IL, USA). Measurement data were shown as mean \pm standard deviation (S.D.). For the measurement data obeyed a normal distribution, a t-test was used for comparison between the two groups, and a one-way analysis of variance and the Bonferroni post-hoc test were used for comparison among multiple groups. To evaluate the relationship between circ-CSPP1 and the survival prognosis of RCC patients, the Kaplan-Meier method was used for survival analysis, and a log-rank test was used to determine that the difference between high and low expression curves was statistically

significant. Pearson correlation analysis in clinical samples was conducted. $P < 0.05$ emphasized obvious statistical meaning.

RESULTS

Circ-CSPP1 is elevated in RCC and is associated with poor prognosis

Circ-CSPP1 was up-regulated in RCC tissues (Fig. 1A). Endogenous circ-CSPP1 was elevated in RCC cell lines, and highest in ACHN cells (Fig. 1B). Therefore, ACHN cells were chosen for subsequent experiments. The 40 patients were divided into two groups according to circ-CSPP1 expression, and circ-CSPP1 was associated with distant metastasis, clinical stage, and tumor stage (Table 2). Meanwhile, it came out that clinical stage, tumor stage, distant metastasis, and circ-CSPP1 were independent predictors of overall survival (OS) of RCC patients (Table 3). Kaplan-Meier survival analysis exhibited that RCC patients with higher circ-CSPP1 expression had poor OS (Fig. 1C). These results suggested

Table 2. The link between circ-CSPP1 and clinicopathological features of RCC

Characteristics	Number	circ-CSPP1		P
		Low (n = 20)	High (n = 20)	
Age				0.527
<60	21	12	9	
≥ 60	19	8	11	
Gender				1.000
Male	23	11	12	
Female	17	9	8	
Clinical stage				0.022
I-II	24	16	8	
III-IV	16	4	12	
Tumor stage				0.025
T1+T2	22	15	7	
T3+T4	18	5	13	
Distant metastasis				0.001
M0	25	18	7	
M1	15	2	13	

Table 3. Univariate and multivariate analysis of clinicopathological indexes for overall survival within RCC patients

Risk factors	Univariate analysis		Multivariate analysis	
	HR (95%CI)	<i>P</i>	HR (95%CI)	<i>P</i>
Age (years)	1.023 (0.348-2.316)	0.874		
Gender	0.912 (0.556-2.875)	0.456		
Clinical stage	1.686 (1.004-4.731)	0.005	1.712 (1.013-4.178)	0.016
Tumor stage	2.616 (1.063-4.129)	0.006	2.175 (1.238-3.662)	0.018
Distant metastasis	3.174 (1.319-7.627)	0.004	2.747 (1.312-6.533)	0.002
circ-CSPP1 expression	1.389 (1.086-5.643)	0.001	1.266 (1.064-5.242)	0.003

that circ-CSPP1 is elevated in RCC and is associated with poor prognosis.

Circ-CSPP1 motivates RCC progression and glycolysis

For studying the biological function of circ-CSPP1 in RCC, si-circ-CSPP1, pcDNA3.1-circ-CSPP1, and corresponding NCs were introduced into ACHN cells, and the transfection efficiency was verified (Fig. 2A). The results clarified that repression of circ-CSPP1 restrained cell proliferation, invasion, and migration, and induced

apoptosis, but elevation of circ-CSPP1 had the opposite effect (Fig. 2B–F). Meanwhile, Warburg effect correlation analysis clarified that suppressing circ-CSPP1 reduced lactate production, glucose uptake, and ATP, while up-regulation of circ-CSPP1 resulted in the opposite results (Fig. 2G–I). In addition, similar results were found in KAKI-1 cells (Supplementary Fig. 1A–I at <https://ojs.ptbioch.edu.pl/index.php/abp>). These data suggested that circ-CSPP1 motivated RCC progression and glycolysis.

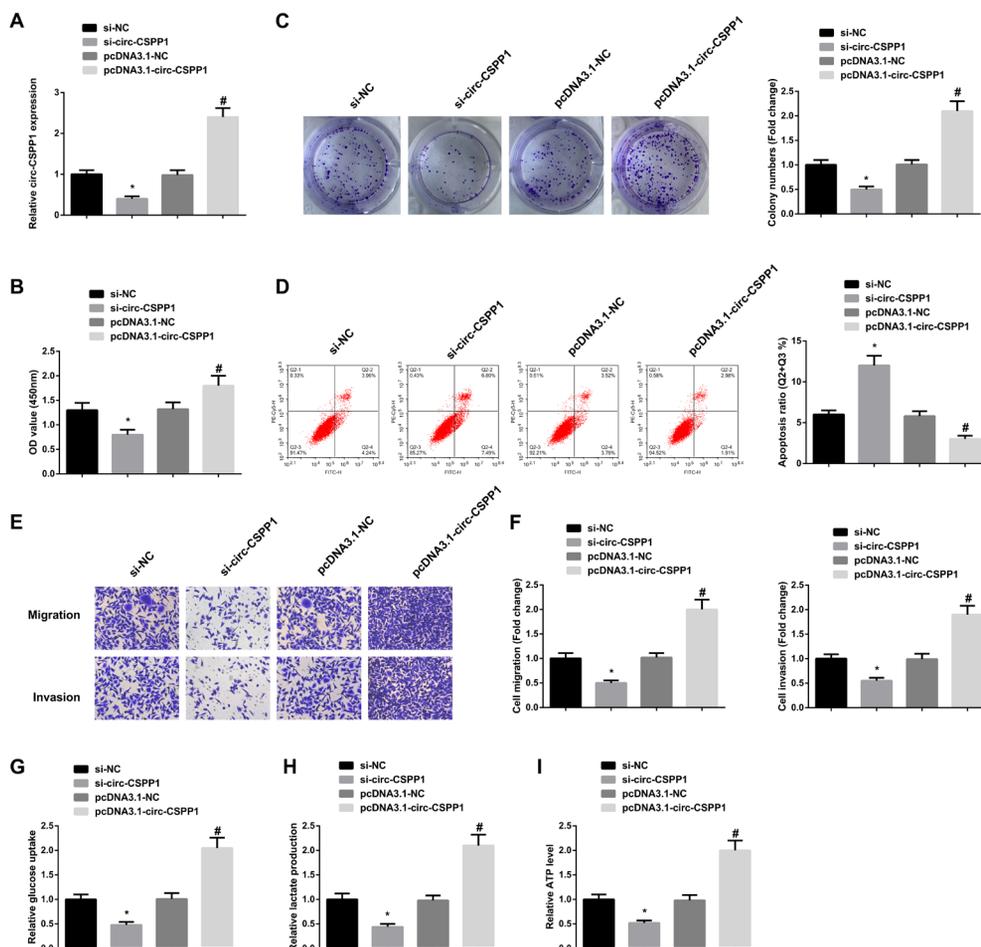


Figure 2. Circ-CSPP1 motivates RCC progression and glycolysis.

(A) RT-qPCR to test circ-CSPP1; (B/C) CCK-8 and clone formation assay to examine cell proliferation; (D) Flow cytometry to detect cell apoptosis; (E–F) Transwell to detect cell migration and invasion; (G–I) Glucose uptake, lactate production and ATP. Measurement data were shown as mean \pm S.D. **vs the si-NC, $P < 0.05$; #vs the pcDNA3.1-NC, $P < 0.05$.

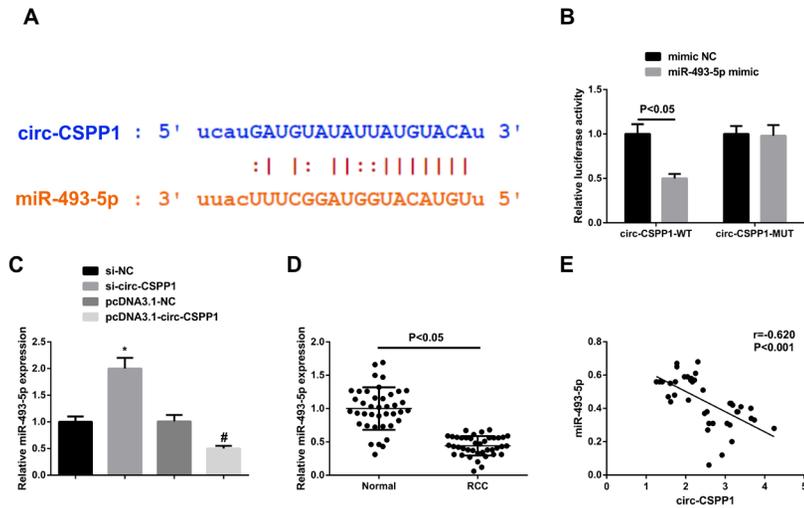


Figure 3. Circ-CSPP1 adsorbs miR-493-5p in RCC.

(A) StarBase to forecast binding sites for circ-CSPP1 and miR-493-5p; (B) The luciferase activity assay to testify the interaction between circ-CSPP1 and miR-493-5p; (C) RT-qPCR detection of miR-493-5p after the intervention of circ-CSPP1; (D) RT-qPCR to test miR-493-5p in RCC tissues; (E) Pearson correlation analysis of circ-CSPP1 and miR-493-5p in clinical samples. Measurement data were shown as mean \pm S.D. **vs the si-NC, $P < 0.05$; #vs the pcDNA3.1-NC, $P < 0.05$.

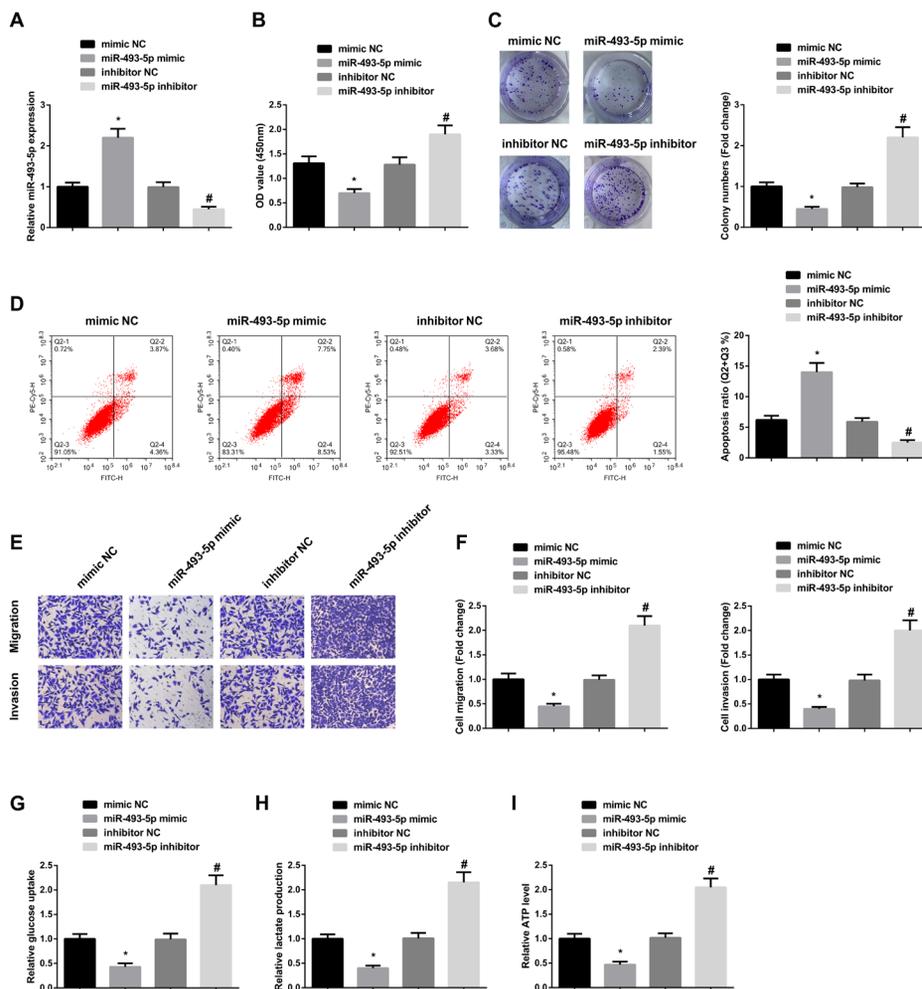


Figure 4. MiR-493-5p represses RCC progression and glycolysis.

(A) RT-qPCR to test miR-493-5p; (B–C) CCK-8 and clone formation assay to examine cell proliferation; (D) Flow cytometry to detect cell apoptosis; E/F: Transwell to detect cell migration and invasion; (G–I) ATP, glucose uptake and lactate production. Measurement data were shown as mean \pm S.D. ** $P < 0.05$, vs the mimic NC; # $P < 0.05$, vs the inhibitor-NC.

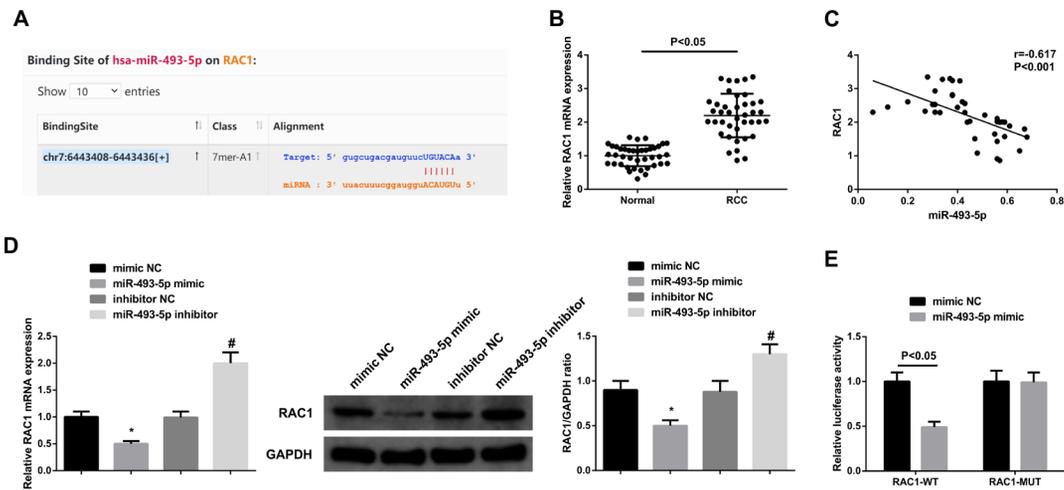


Figure 5. RAC1 is miR-493-5p's downstream target in RCC.

(A) StarBase to predict that miR-493-5p and RAC1 had targeted binding sites; (B) RT-qPCR examination of RAC1 in RCC tissues; (C) Pearson correlation analysis of miR-493-5p and RAC1 in clinical samples; (D) RT-qPCR and Western blot test of RAC1 after intervening miR-493-5p; (E) The luciferase activity detection to testify the targeting relationship between miR-493-5p and RAC1. Measurement data were shown as mean \pm S.D. ** $P < 0.05$, vs the mimic NC; # $P < 0.05$, vs the inhibitor-NC.

Circ-CSPP1 adsorbs miR-493-5p in RCC

The targeting sites for circ-CSPP1 and miR-493-5p were predicted by starBase (Fig. 3A). Luciferase activity assay was performed to testify the binding relationship between circ-CSPP1 and miR-493-5p, and it came out that circ-CSPP1-WT with miR-493-5p mimic reduced luciferase activity, indicating that circ-CSPP1 could combine with miR-493-5p (Fig. 3B). As expected, depression of circ-CSPP1 elevated miR-493-5p expression in ACHN cells, whereas upregulating circ-CSPP1 suppressed miR-493-5p expression (Fig. 3C). Moreover, it was discovered that circ-CSPP1 was inversely correlated with miR-493-5p expression in RCC tissues (Fig. 3D–E). Taken together, these findings suggested that circ-CSPP1 adsorbed miR-493-5p in RCC.

MiR-493-5p represses RCC progression and glycolysis

For studying miR-493-5p's biological function in RCC, miR-493-5p mimic, miR-493-5p inhibitor and corresponding NCs were introduced into ACHN cells, and the transfection efficiency was testified (Fig. 4A). It turned out that elevating miR-493-5p refrained cell progression while repressing miR-493-5p had the opposite effect (Fig. 4B–F). The Warburg effect correlation analysis clarified that elevation of miR-493-5p reduced ATP, glucose uptake and lactate production, while depression of miR-493-5p caused the opposite results (Fig. 4G–I). These results suggested that miR-493-5p depressed RCC progression and glycolysis.

RAC1 is miR-493-5p's downstream target in RCC

starBase was applied for predicting that miR-493-5p had a targeted binding site with RAC1 (Fig. 5A). RAC1 expression was elevated in RCC tissues (Fig. 5B), and miR-493-5p was inversely linked with RAC1 expression in RCC tissues (Fig. 5C). Furthermore, it was found that miR-493-5p mimic could reduce RAC1 expression, whereas miR-493-5p inhibitor could elevate RAC1 expression (Fig. 5D). In the luciferase activity assay, it was found that the luciferase activity was suppressed after introduction of RAC1-WT and miR-493-5p mim-

ic (Fig. 5E). All in all, RAC1 was miR-493-5p's downstream target in RCC.

Circ-CSPP1 accelerates RCC progression and glycolysis through the miR-493-5p/RAC1 axis

After verifying that RAC1 is the target of miR-493-5p, we investigated whether circ-CSPP1 accelerates the development of RCC by controlling the miR-493-5p/RAC1 axis. It was found that upregulation of circ-CSPP1 elevated RAC1 expression, and repression of RAC1 restored RAC1 expression following upregulation of circ-CSPP1 (Fig. 6A). It turned out that repression of RAC1 abolished the motivation of cell proliferation by elevation of circ-CSPP1 (Fig. 6B, C). Likewise, the repressive effect of up-regulated circ-CSPP1 on apoptosis was abolished by down-regulated RAC1 (Fig. 6D). Moreover, RAC1 repression attenuated the promoting effect of circ-CSPP1 upregulation on cell invasion and migration (Fig. 6E, F). Also, repression of RAC1 could turn around the motivation of cellular glycolysis by up-regulation of circ-CSPP1 (Fig. 6G–I). Taken together, these data suggested that circ-CSPP1 motivated RCC progression and glycolysis through the miR-493-5p/RAC1 axis.

DISCUSSION

CircRNAs are extensively studied cancer markers recently (Yan *et al.*, 2021). More studies have shown that circRNA takes on a crucial role in the genesis and progression of diversified tumors. For example, hsa_circ_0065217 is elevated in RCC and motivates RCC growth and metastasis *via* controlling the miR-214-3p-ALPK2 axis (Zhang *et al.*, 2021). Circ_001842 is elevated in RCC and exerts an oncogenic role in RCC *via* a miR-502-5p-dependent SLC39A14 mechanism (Zeng *et al.*, 2020). Our research revealed that circ-CSPP1 was upregulated in RCC tissues, and RCC patients with high circ-CSPP1 expression had a poor prognosis, suggesting that circ-CSPP1 may be a prognostic biomarker for RCC patients. Meanwhile, circ-CSPP1 silencing restrained RCC cell progression *in vitro*, while elevation of circ-CSPP1

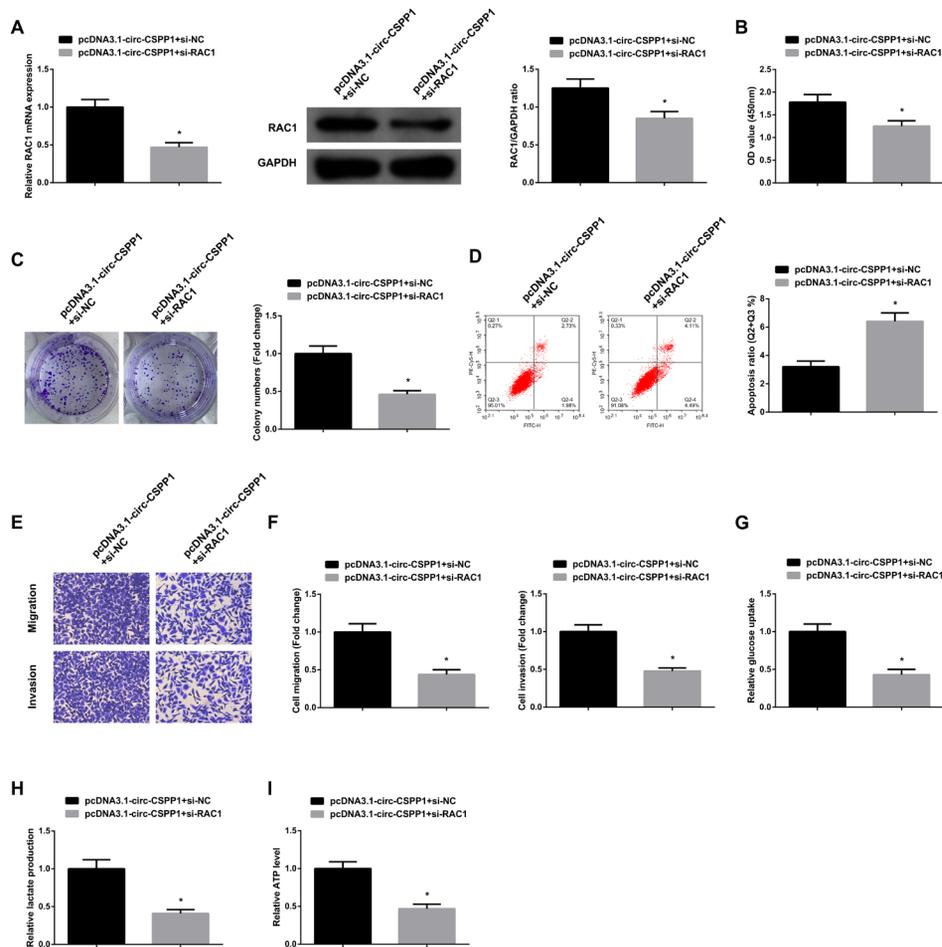


Figure 6. Circ-CSPP1 accelerates RCC progression and glycolysis through the miR-493-5p/RAC1 axis.

(A) RT-qPCR and Western blot to test RAC1; (B–C) CCK-8 and clone formation assay to examine cell proliferation; (D) Flow cytometry to detect cell apoptosis; (E–F) Transwell to detect cell migration and invasion; (G–I) ATP, glucose uptake and lactate production. Measurement data were shown as mean \pm S.D. *vs. the pcDNA3.1-circ-CSPP1+si-NC, $P < 0.05$.

showed opposite results. These data suggested that circ-CSPP1 motivated the progression of RCC.

It is well known that mammalian cells generate energy through oxidative phosphorylation under aerobic conditions, and during tumorigenesis, cancer cells must rewire their metabolism to maintain survival and reproduction (Urbano *et al.*, 2021). Most cancer cells utilize the Warburg effect to support anabolic growth and tumorigenesis, namely aerobic glycolysis, a metabolic mode that is favored even in the presence of oxygen (Lundø *et al.*, 2020). Warburg effect was found to be beneficial for cancer cell progression and was involved in immune responses and drug resistance (Samec, 2020; Icard *et al.*, 2018). Recent studies have shown that circRNAs can modulate tumor phenotypes, such as metastasis and the Warburg effect. For example, circFOXP1 motivates RCC progression and Warburg effect by sponging miR-423-5p to upregulate U2AF2 (Fang *et al.*, 2021). This study identified a novel circRNA as a regulator of the Warburg effect. The results clarified that downregulation of circ-CSPP1 suppressed the Warburg effect in RCC cells.

Several studies have shown that circ-CSPP1 performs as an oncogene in diversified human cancers and exerts its biological functions by adsorbing miRNAs. For example, circ-CSPP1 is elevated in cervical cancer (CC), and circ-CSPP1 knockdown suppresses CC tumor

growth and migration via downregulating ITGB1, PI3K and Akt expression by sponging miR-361-5p (Yang *et al.*, 2020). Circ-CSPP1 is upregulated in ovarian cancer (OC), and silencing circ-CSPP1 represses OC cell biological functions via upregulating miR-1236-3p (Li *et al.*, 2019). Here, it was confirmed that circ-CSPP1 was a decoy of miR-493-5p. Furthermore, repressing miR-493-5p turned around the effect of circ-CSPP1 silencing on RCC cell progression and Warburg effect, while elevating miR-493-5p turned around the effect of upregulating circ-CSPP1 on RCC progression and Warburg effect in HCC. These results suggested that circ-CSPP1 controlled RCC progression and the Warburg effect via performing as a sponge for miR-493-5p.

MiRNAs control gene expression by combining with mRNAs' 3'-UTR, thereby regulating diversified aspects of cellular activity (Huang *et al.*, 2011). Here, RAC1 was a target of miR-493-5p. RAC1 is a highly conserved rho family small GTPase that is essential for diversified cellular and developmental processes (Banka *et al.*, 2022). RAC1 is elevated in different types of tumors, including RCC, and motivates tumorigenesis by participating in tumor cell biological behaviors (Liang *et al.*, 2021; Goka *et al.*, 2020). This study also confirmed that RAC1 was elevated in RCC tissues, and its expression was up-regulated by elevation of circ-CSPP1 but refrained by upreg-

ulation of miR-493-5p. Moreover, repression of RAC1 clearly turned around the effect of circ-CSPP1 elevation on RCC cell progression and the Warburg effect. These results suggested that circ-CSPP1 motivated RCC progression and glycolysis through miR-493-5p/RAC1 axis.

However, the research still takes on several limitations. Although the role of circ-CSPP1 in RCC progression has been partially elucidated, the study had a small sample size, and more tissue samples are still needed to further examine circ-CSPP1 expression trend in RCC. Also, further animal experiments are required to validate the effect of the circ-CSPP1/miR-493-5p/RAC1 axis on RCC tumor growth *in vivo*. Moreover, the Crabtree effect (inhibitory effect of high glucose on mitochondrial oxidative phosphorylation) may also exist in cancer cells. In other words, under the condition of high glucose concentration, glycolytic metabolism is dominated in cells while aerobic respiration is inhibited, thus generating lactic acid (Diaz *et al.*, 2011; Redman *et al.*, 2013). The Crabtree effect is similar to the Warburg effect, the only difference being that the Crabtree effect is caused by high concentrations of glucose (Hammad *et al.*, 2016), therefore, in future studies, exogenous glucose concentration will be a parameter that needs to be designed more precisely to exclude the interference of Crabtree effect as much as possible.

CONCLUSION

All in all, circ-CSPP1 motivates RCC progression and the Warburg effect by acting as a miR-493-5p sponge to upregulate RAC1. The study findings suggest that circ-CSPP1 may serve as a hopeful diagnostic and therapeutic target for RCC.

Declarations

Acknowledgments. Not applicable.

Competing interests. The authors have no conflicts of interest to declare.

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