

RESEARCH ARTICLE

circCORO1C promotes the proliferation and metastasis of hepatocellular carcinoma by enhancing the expression of PD-L1 through NF- κ B pathway

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Abstract

Background: Circular RNA (circRNA) affects the occurrence and development of human cancers, but the specific mechanism of hepatocellular carcinoma (HCC) has not yet been fully understood.

Methods: CircRNAs were determined by human circRNA array analysis and quantitative reverse transcription polymerase chain reaction (qRT-PCR). Cell viability, migration, invasion, and other indicators were used for cell function analysis. Knockdown and overexpression techniques were used to explore the mechanism of circCORO1C in the occurrence and development of HCC by RNA sequencing, qRT-PCR, western blot, and other methods.

Results: Among the thousands of circRNAs, 1238 circRNAs were significantly changed. As for the top 10 upregulated circRNAs, the expression of circRNAs, hsa_circ_0036412, hsa_circ_0036411, hsa_circ_0028071, hsa_circ_0036409, hsa_circ_0000437, hsa_circ_0021427, hsa_circ_0097182, hsa_circ_0028067, hsa_circ_0006852, and hsa_circ_0003620 were significantly increased. In regard to the top 10 downregulated circRNAs, the expression of hsa_circ_0123629, hsa_circ_0096121, hsa_circ_0038932, hsa-circRNA3310-44, hsa_circ_0045746, hsa_circ_0016836, hsa-circRNA10899-9, hsa_circ_0050116, hsa_circ_0035543, and hsa_circ_0092118 decreased significantly. About these circRNAs, the downregulation of hsa_circ_0006852 (circCORO1C) can inhibit the tumorigenesis of HCC cells in vivo and in vitro, and the overexpression of circCORO1C can enhance the proliferation and metastasis ability of HCC cells. Mechanistically, circCORO1C activated the NF- κ B signaling pathway, increased P65 phosphorylation and upregulation of c-Myc and COX-2, leading to increased PD-L1 expression.

Conclusion: CircCORO1C upregulates c-Myc and COX-2 through NF- κ B signaling pathway, leading to the upregulation of PD-L1, which jointly promotes the development of HCC, suggesting that circCORO1C is a promising biomarker and therapeutic target for HCC.

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KEYWORDS

circRNA, hepatocellular carcinoma, NF- κ B, PD-L1

1 | INTRODUCTION

Hepatocellular carcinoma (HCC) is currently the fifth most common cancer in the world, as well as the second leading cause of cancer-related deaths.¹ It is estimated that there are more than 2 million deaths died of liver disease and liver cancer every year, accounting for about 4% of all deaths worldwide.² Most of deaths from liver diseases including liver cirrhosis and viral hepatitis, as well as liver cancer, can be attributed to hepatitis B virus (HBV) and hepatitis C virus (HCV).³ Due to the high rate of HBV infection, the regions with the highest incidence rate are Asia and sub-Saharan Africa.⁴ Unlike other cancers, we have clearly defined the main risk factors associated with HCC, which include metabolic syndrome, viral hepatitis (B and/or C) in patients with diabetes, alcoholism, and non-alcoholic fatty liver disease. Other co-factors for the occurrence and development of HCC include aflatoxin B1, tobacco, etc.⁵ However, due to its insidious onset, high malignancy, and rapid development, the treatment of HCC is still difficult, with poor prognosis and short survival. Therefore, studying the mechanism of the occurrence and development of HCC is of great significance to prolong the survival of patients with HCC.

For a long time in the past, non-coding RNA has been a star in the RNA field.⁶ With the continuous development of sequencing technology, circular RNAs (circRNAs), as a new type of RNA including non-coding circRNAs and coding circRNAs,⁷ stepped onto the stage and became well-known. circRNA has higher stability due to its special closed-loop structure and is not easily degraded.⁸ CircRNA is involved in a variety of physiological and pathological processes,⁹ and the abnormal expression of circRNA is closely related to a variety of diseases, such as immune diseases, cell proliferation, and tumor formation.¹⁰ In addition, circRNA can be detected in body fluids, which indicates that it is expected to become a new promising biomarker.¹¹

This study found that in the tumor tissues of HCC cases, the expression of a circRNA called circCORO1C was increased compared with normal tissues. Down-expression of circCORO1C could inhibit the tumorigenesis of HCC cells *in vivo* and *in vitro*, whereas overexpression of circCORO1C enhanced the proliferation and metastasis ability of HCC cells. In addition, we proved that circCORO1C could regulate the expression of PD-L1 on HCC cells through NF- κ B pathway, and further accelerate the development of HCC cells. These results will help to explain the occurrence and development mechanism of HCC and bring new hope for the diagnosis and treatment of HCC in future.

2 | MATERIALS AND METHODS

2.1 | CircRNA microarray

CapitalBio Technology Human CircRNA Array v2 was established by using four identical arrays on the respective slides. A total of three pairs of matched cancerous tissue and adjacent normal tissue were tested.

2.2 | Cases and tissue samples were collected

This work was approved by Nanjing First Hospital and the First Affiliated Hospital of Nanjing Medical University. We communicated about the study in accordance with the Declaration of Helsinki. Prior to the study, the cases provided written informed consent. Human HCC and surrounding normal tissue were collected from 38 patients who underwent surgery with informed consent during 2015–2019 from Nanjing First Hospital and the First Affiliated Hospital of Nanjing Medical University.

2.3 | Cell culture

Shanghai Institute of Biological Sciences in China provided human HCC cell lines (HCCLM3, Hep3b). All cell lines were cultured in DMEM medium (Life Technologies, USA) containing 10% fetal bovine serum (Gibco, Australia), 100 IU/ml penicillin and 100 mg/ml streptomycin, and covered with 5% CO₂ at 37°C in a moist incubator.

2.4 | Sanger sequencing

The amplified product was inserted into a T vector for Sanger sequencing. Primers were designed to confirm the splicing connection of circCORO1C. Sanger sequencing was performed by RealGene (China).

2.5 | Quantitative reverse transcription polymerase reaction (qRT-PCR)

Total RNA in both tissues and cells was isolated using Trizol reagent (Invitrogen, USA) according to the manufacturer's operating procedures. Based on a reverse transcription kit (Takara, Japan), cDNA synthesized circRNA and mRNA. We quantified mRNA and circRNAs under the SYBR Green PCR Kit (RiboBio, China). circCORO1C primer pair included: 5'-CAAATGTGAGATTGCCAGGTAT-3' (Forward) and 5'-TCACAGCTGCACTAAGAAG-3' (Reverse). Before calculation, GAPDH was used to standardize the expression level of mRNA and circRNA.

2.6 | RNase R resistance analysis

Total RNA was extracted using the Trizol method and then 2 mg of total RNA was incubated at 37°C for 30 min with or without 3 U/mg RNase R (Epicentre Technologies, USA). To determine the amount of mRNA and circRNA, we reverse transcribed RNA to obtain cDNA

after incubation. Specific primers were then used for analysis by qRT-PCR.

2.7 | HCC cell transfection

The plasmid pcDNA3.1vector-circCORO1C, si-circCORO1C, and sh-circCORO1C relevant lentiviruses were obtained from RiboBio (Guangzhou, China). HCC cells were transduced with individual types of lentivirus at a multiplicity of infection (MOI) of 10 in the presence of 5 µg/ml puromycin or transfected with the specific plasmid by using Lipofectamine 2000 (Invitrogen, USA). circCORO1C-siRNA1#: 5'-GTGAGATTGCCAGGTATGGCA -3'; siRNA2#: 5'-AGAT TGCCAGGTATGGCAGAT -3'; siRNA3#: 5'-TGCCAGGTATGGCAGATCCCA -3'.

2.8 | Cell Counting Kit 8 for proliferation assay and 5-acetyl-20-deoxyuridine (EDU) assay

HCC cells were inoculated in 96 wells at a density of 4000 cells per well. When cultured at 0 h, 24 h, 48 h, 72 h, and 96 h, seed cells were treated with 10 µl CCK-8 solution (RiboBio, China). Then follow the manufacturer's instructions (Synergy4, USA), and the cell absorbance was analyzed at 450 nm at the corresponding time using a microplate analyzer. The EDU assay was performed to evaluate Cell proliferation under the Cell-Light EDU DNA Cell Proliferation Kit (RiboBio, China). HCC cells were subjected to the plating process in 24 wells and cultured at 24h. After incubation with 50mM EDU solution for 2 h, the cell lines were treated with 4% paraformaldehyde immobilization. Next, the cell lines were treated with Apollo Dye Solution and Hoechst seal treatment, according to the manufacturer's agreement. EDU cell lines were captured and counted under the Olympus FSX100 microscope (Olympus, Japan).

2.9 | Scratch wound experiment

When the degree of cell fusion reached about 90% 24 h after transfection, the wound was aspirated with 200 µl pipetting head, and the cells were washed with culture medium to remove the free cells and debris. The culture medium was added and the plates were incubated at 37°C. All aspects of wound healing were investigated. In addition, the representative scratch line is captured.

2.10 | Cell invasion and migration analysis

The difference between the invasion and migration experiments was the presence or absence of Matrigel on the Transwell suprapentriangular membrane. According to the study design, 10,000 HCC cells were uniformly dispersed in the upper compartment of Transwell (Corning, USA) and serum-free medium was added. Complete

medium containing 10% FBS was added to the lower compartment. After 24 h, the cells in the upper layer of the membrane were removed, fixed with methanol, and then stained with crystal violet. The results were observed with an inverted microscope.

2.11 | Western blot

In the RIPA lysis buffer, the cells undergo the lysis process. The protein was prepared and quantified by BCA assay (Beyotime, China). The same number of proteins were extracted by 10% SDS-PAGE and PVDF membrane transfer (Millipore, Germany). Proteins sealed with 5% skim milk powder were incubated at 4°C with primary antibodies, namely anti-GAPDH (ab9485), anti-PD-L1 (ab213524), anti-COX-2(ab62331), anti-NF-κB p65(ab76311), anti-c-Myc(ab32072), and anti-Phospho-NF-κB(q04206) for 12 h. The resultant membrane was then incubated with a secondary antibody for 2 h. Finally, using an enhanced chemiluminescence kit (Pierce, USA), the imprints were detected and the relevant information was studied based on the Image Lab Software.

2.12 | Xenografts in mice

The Animal Control Committee of Nanjing Medical University has approved the animal experiments, and all the procedures and care for animals related to the experiments comply with the institutional ethics of the experimental procedures related to animals. To establish a xenograft tumor system, sh-NC, sh-circCORO1C ($n = 5$, each group) were randomly isolated from 20 5-week-old male BALB/c nude mice in a series of HCC cells. HCC cells were injected subcutaneously into the axilla of nude mice. The study determined the volume of all nude mice injected every 4 days based on a digital caliper.

2.13 | Statistical analysis

This study was analyzed primarily based on SPSS 19.0 (IBM, USA), with a p -value <0.05 reported to be statistically significant. In this study, a comparison process of continuous information was obtained based on a single t experiment in the two groups, while information related to classification was analyzed by a chi-square test procedure.

3 | RESULTS

3.1 | Profiling of abnormally expressed circRNAs in HCC tissues and characterization of circCORO1C in HCC

In order to identify the circRNA associated with HCC tumorigenesis, we selected the cancerous tissues and adjacent normal tissues of 3 HCC cases and performed a human circRNA microarray

analysis with significant throughput. A total of 54311 differentially expressed circRNAs were identified. From these different circRNAs, based on the multiple of difference folder greater than 5, the parental genes related to cancer development, and the primer sequence design, we finally screened a total of 3 target circRNA (Figure 1A). The top 10 upregulated and top 10 downregulated genes are shown in Table 1. Among them, the most upregulated circRNA comes from a parental gene (CORO1C), which is related to tumor susceptibility. According to the circbase database (<http://www.circbase.org/>), the splicing mature sequence of circCORO1C at chr12:109046047-109055934 is 683 bp in length, and circCORO1C is derived from exons 8-12 (Figure 1B). In order to confirm the circular form of circCORO1C, we analyzed by qRT-PCR that compared with linear CORO1C, circCORO1C is resistant to degradation induced by RNase R exonuclease (Figure 1C). These results suggest that circCORO1C has a basic circRNA structure.

3.2 | CircCORO1C is significantly upregulated in HCC

Using qRT-PCR technology to compare the expression levels of circCORO1C in 38 pairs of HCC tissues and adjacent normal tissues, we found that the expression level of circCORO1C in HCC tissues was significantly higher than that in adjacent tissues (Figure 1D).

3.3 | CircCORO1C promotes proliferation, invasion and metastasis in HCC cells in vitro

In order to test the role of circCORO1C in HCC cells, we used a plasmid transfection system to establish HCCLM3 and Hep3b that stably expressing circCORO1C (Figure 2A), thereby achieving ectopic expression of circCORO1C. In addition, this experiment also developed three siRNAs targeting circCORO1C (si-circCORO1C). Comparing the silencing efficiency, we finally chose the si-circCORO1C 2# with the most significant suppression efficiency to perform the following experimental process (Figure 3A). Based on the expression level of circCORO1C in the cell line, we selected the corresponding overexpression and knockdown cell lines. CCK-8 analysis showed that overexpression of circCORO1C increased the proliferation rate of HCC, while knocking down circCORO1C inhibited the proliferation of HCC (Figures 2B and 3B). The EdU experiment also verified this result (Figures 2C and 3C). Furthermore, we used Transwell assay to detect whether circCORO1C affects the invasion and migration ability of HCC cells. The results showed that si-circCORO1C significantly inhibited the invasion and migration of HCC, while overexpression was the opposite (Figures 2D and 3D). A scratch-wound assay also verify this phenomenon (Figures 2E and 3E). The above results suggest that circCORO1C greatly promotes the occurrence and development of HCC.

3.4 | RNA sequencing results of si-circCORO1C in HCC cells

In order to explore the mechanism by which circCORO1C promotes the development of HCC and evaluate the changes in downstream gene expression of circCORO1C, we knocked down circCORO1C in HCC cells and used RNA transcriptome sequencing to evaluate the overall effect. Comparing si-NC group and si-circCORO1C group, we can identify a total of 12815 differentially expressed genes in HCC cells (Figure 4A). We found that 513 genes were upregulated and 725 genes were downregulated that were statistically different (Figure 4B). The pathway analysis results showed that these differentially expressed genes were mainly enriched in tumors, metabolism, and immune pathways (mainly affecting the immune process of T cells) (Figure 4C,D). The results of GO analysis showed that the most obvious molecular functions involved catalytic activity, binding, and signal transduction activities, while the most obvious biological processes involved cellular polymer metabolism and biogenesis (Figure 4E,F).

3.5 | CircCORO1C facilitates the proliferating and metastasis processes pertaining to HCC by regulating PD-L1 through NF- κ B pathway

Based on relevant literature and our previous research, we selected a candidate gene related to tumor immunity-PD-L1, to further identify the mechanism by which circCORO1C affects the occurrence and development of HCC. We used qRT-PCR to compare the changes in PD-L1 mRNA expression after knockdown and overexpression of circCORO1C. The results showed that when the expression of circCORO1C decreased, the expression level of PD-L1 mRNA also decreased to varying degrees (Figure 5A). On the contrary, overexpression of the former and the latter also showed a significant upregulation (Figure 5B). Existing studies have shown that the high expression of PD-L1 is often related to the induction of c-Myc, and c-Myc is the key downstream molecule of the NF- κ B classical cellular pathway. Further, we studied whether circCORO1C can activate the NF- κ B pathway, affect the expression of PD-L1 and promote the occurrence and development of HCC. p65 is a key molecule in NF- κ B signaling. We investigated whether circCORO1C regulates the activity of the NF- κ B signaling pathway by regulating the phosphorylation level of p65. At the protein level, we found that the overexpression of circCORO1C caused the level of phosphorylated p65 (p-p65) to rise, and the expression of c-Myc, COX-2, and PD-L1 was also upregulated (Figure 5C). Knock down of circCORO1C gave the opposite result (Figure 5D).

3.6 | circCORO1C facilitates the growth of HCC in vivo

In order to explore the related process of circCORO1C and HCC development in vivo, HCCLM3 and Hep3b cells transfected with

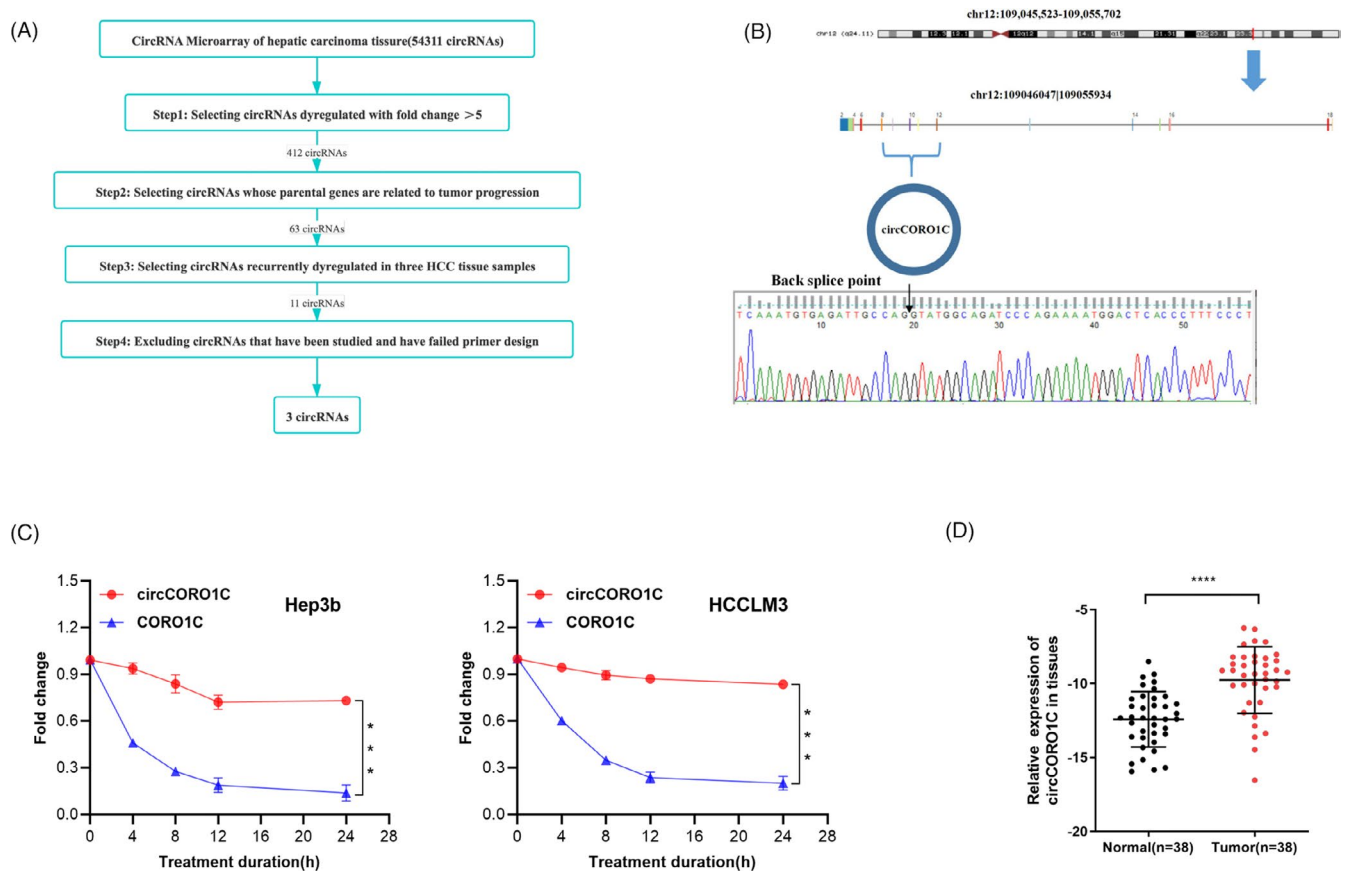


FIGURE 1 Analysis of abnormally expressed circRNA in HCC tissue and its characteristics. (A) The flow chart illustrates the screening criteria for potential regulatory circRNAs that are highly expressed in HCC. (B) The genomic locus of circCORO1C. (C) RNase R exonuclease treatment was used to evaluate the stability of circCORO1C and CORO1C mRNA in Hep3b and HCCLM3 cells. (D) Measure the expression of circCORO1C in 38 pairs of HCC (tumor) and matched non-cancerous tissues using qRT-PCR. *** $p < 0.001$, **** $p < 0.0001$

sh-circCORO1C and sh-NC were injected into nude mice to establish a xenograft tumor system (Figure 6A). From the results, it can be found that the low expression state of circCORO1C can effectively reduce the size and weight of nude mice (Figure 6B–D). Overall, we clarified that circCORO1C upregulates the expression status of PD-L1 through the NF- κ B pathway, and further promotes the tumorigenesis of HCC cells.

4 | DISCUSSION

With the continuous deepening of the research, we have found that circRNA is closely related to the occurrence and development of a variety of cancers, which further suggests that it is expected to become a new biomarker and therapeutic target.^{12,13} In this study, we identified that the expression of circCORO1C in HCC tissues and cells was abnormally increased. In addition, *in vivo* and *in vitro* functional experiments have proved that high expression of circCORO1C can promote the malignant phenotype of HCC. These results suggest that circCORO1C is a promising therapeutic target for HCC (Figure 7).

In order to find the downstream target genes of circCORO1C, we performed RNA sequencing and successfully proved that

circCORO1C can promote the occurrence of HCC through NF- κ B/PD-L1 signaling. Previous related studies have found that circRNA can exert its biological functions by regulating signal transduction pathways. For example, ciRS-7 can trigger the migration of esophageal squamous cell carcinoma (ESCC) cells through NF- κ B signal.¹⁴ Zhang Xiu-Li et al. also found that hsa_circ_0020397 can target the expression of TERT and PD-L1 by promoting miR-138 to regulate the vitality, apoptosis and invasion of colorectal cancer cells.¹⁵ Whether circCORO1C can also affect other signal pathways requires further research.

NF- κ B is a wide-spread nuclear transcription factor with multiple regulatory functions. It can exist in the form of dimers or heterodimers, the most common being in the form of P50/P65 heterodimers in the cytoplasm.¹⁶ We have proved that circCORO1C can activate the NF- κ B pathway, causing the increase of phosphorylation level of P65. At this time, p-P65 can be translocated into the nucleus and combined with the downstream target gene promoter to rapidly induce the target gene. The mRNA synthesis is involved in the regulation of multiple biological effects.¹⁷ Existing studies have shown that COX-2 and c-Myc are classic downstream molecules of NF- κ B.¹⁸ COX is a rate-limiting enzyme that catalyzes the production of arachidonic acid to produce prostaglandins. Among them, COX-2 is inductively expressed and is almost undetectable

Probe name	Gene symbol	p	FC (abs)	Regulation
hsa_circ_0036412	ETFA	8.90706E-06	55.03696462	Up
hsa_circ_0036411	ETFA	3.3006E-05	37.68342002	Up
hsa_circ_0028071	CORO1C	2.94807E-07	32.46962911	Up
hsa_circ_0036409	ETFA	4.63354E-05	32.03530897	Up
hsa_circ_0000437	CORO1C	0.00060834	30.63929082	Up
hsa_circ_0021427	HPS5	0.2345992	29.9899456	Up
hsa_circ_0097182	CORO1C	0.000910002	28.26077256	Up
hsa_circ_0028067	CORO1C	0.001445578	27.81472622	Up
hsa_circ_0006852	CORO1C	0.00063447	23.62487842	Up
hsa_circ_0003620	ETFA	4.40611E-06	22.29468638	Up
hsa_circ_0123629	ARPP21	0.000773124	14.51632141	Down
hsa_circ_0096121	TIMM10B	0.002628483	14.43738951	Down
hsa_circ_0038932	KIF22	0.01422177	11.71387095	Down
hsa-circRNA3310-44	NF1	0.000322456	10.89922009	Down
hsa_circ_0045746	ACOX1	0.01524767	10.56932985	Down
hsa_circ_0016836	URB2	0.009926082	9.256077859	Down
hsa-circRNA10899-9	ADAMTS17	0.01563557	9.200000885	Down
hsa_circ_0050116	ELL	0.1080323	8.615795457	Down
hsa_circ_0035543	ANXA2	0.02181139	8.321271554	Down
hsa_circ_0092118	G6PD	0.00012548	8.283772885	Down

TABLE 1 Top 10 upregulated and top 10 downregulated circRNAs

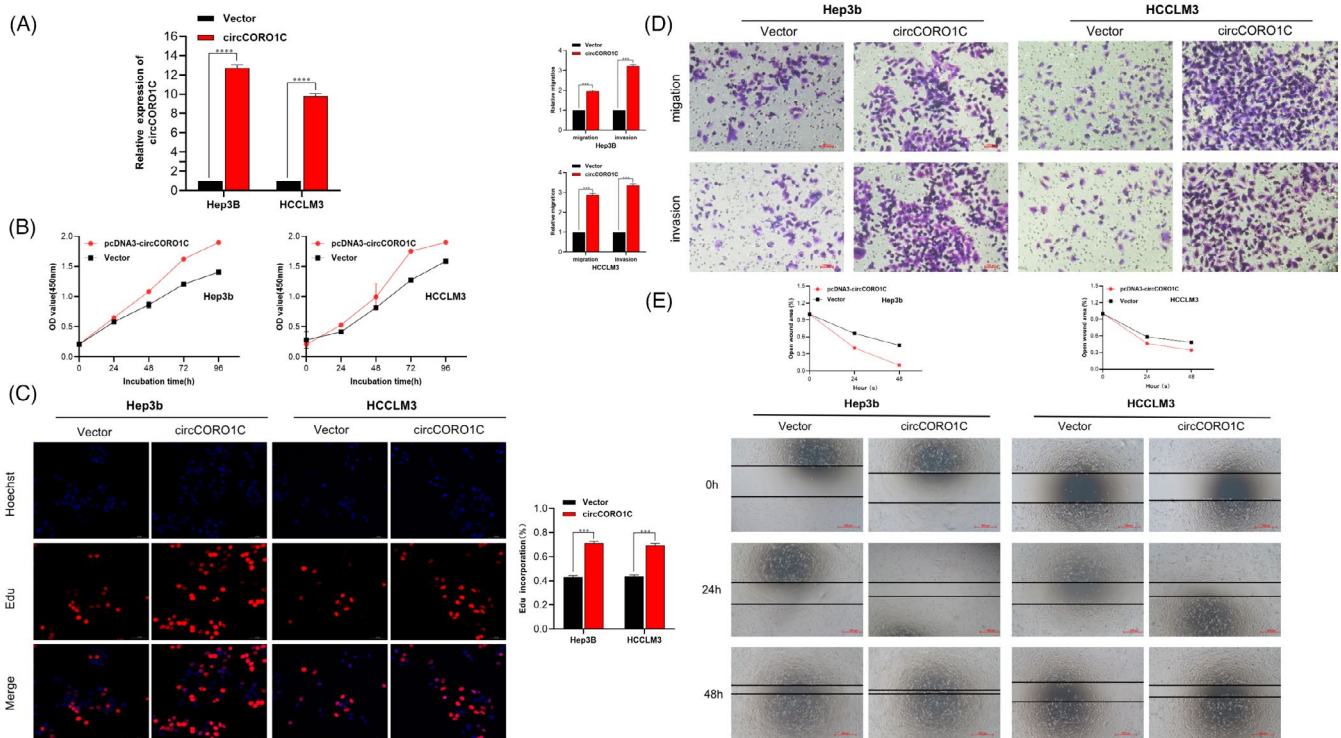


FIGURE 2 Overexpression of circCORO1C promotes the proliferation, migration, and invasion of HCC cells in vitro. (A) After the cells were transfected with plasmids overexpressing circCORO1C, the expression of circCORO1C was evaluated by qRT-PCR. (B) After transfection with Vector or pcDNA3-circCORO1C, the growth curve of the cells was measured by the CCK-8 assay. (C) HCC cells transfected with Vector or pcDNA3-circCORO1C were subjected to EdU analysis to assess cell proliferation. (D) Transwell analysis is used to detect the influence of circCORO1C on the invasion and migration ability of HCC cells. (E) Cell migration was assessed using a wound healing assay. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$

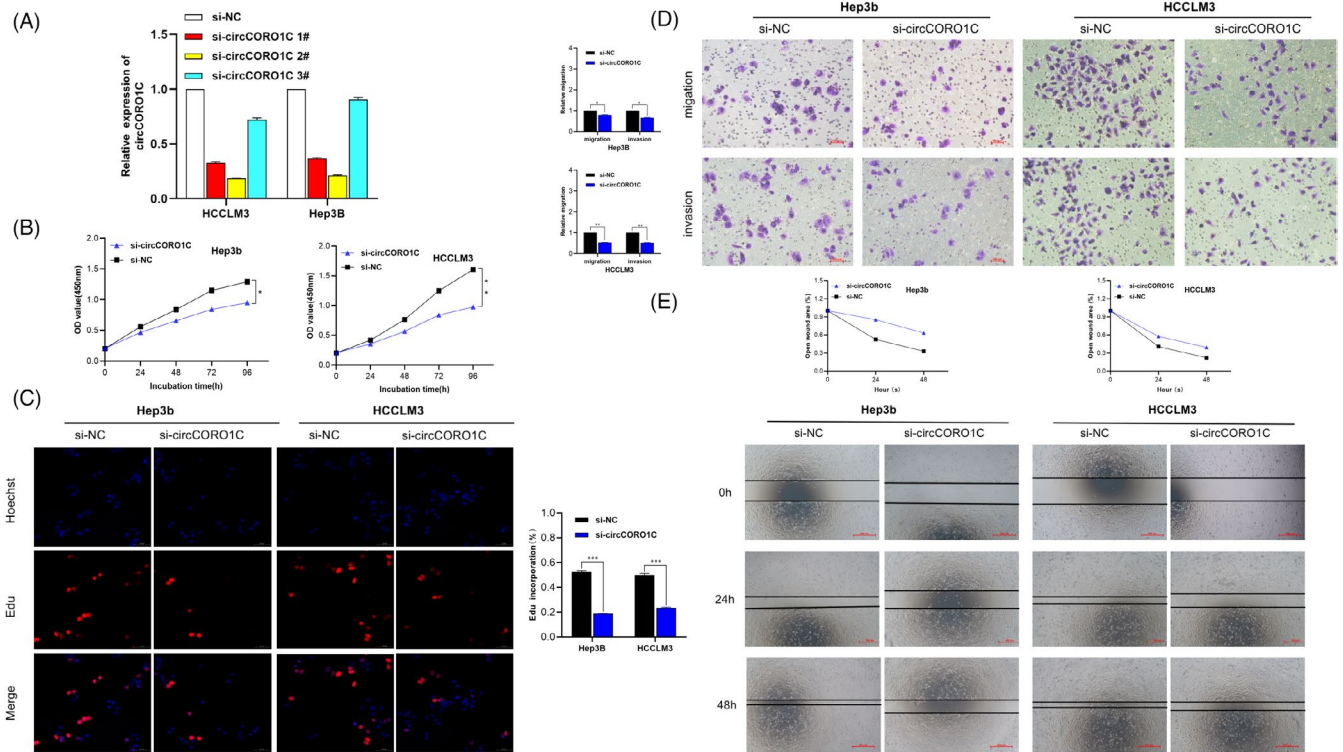


FIGURE 3 Knockdown of circCORO1C inhibits the proliferation, migration, and invasion of HCC cells in vitro. (A) siRNA for circCORO1C was designed, and qRT-PCR was used to evaluate the expression of circCORO1C in each group after transfection with si-circCORO1C. (B) After transfection with si-circCORO1C or si-NC, the growth curve of the cells was measured by the CCK-8 assay. (C) HCC cells transfected with si-circCORO1C or si-NC are subjected to EdU analysis to assess cell proliferation. (D) Transwell analysis is used to detect the influence of circCORO1C on the invasion and migration ability of HCC cells. (E) Cell migration was assessed using a wound healing assay. $^{*}p < 0.05$, $^{**}p < 0.01$, $^{***}p < 0.001$

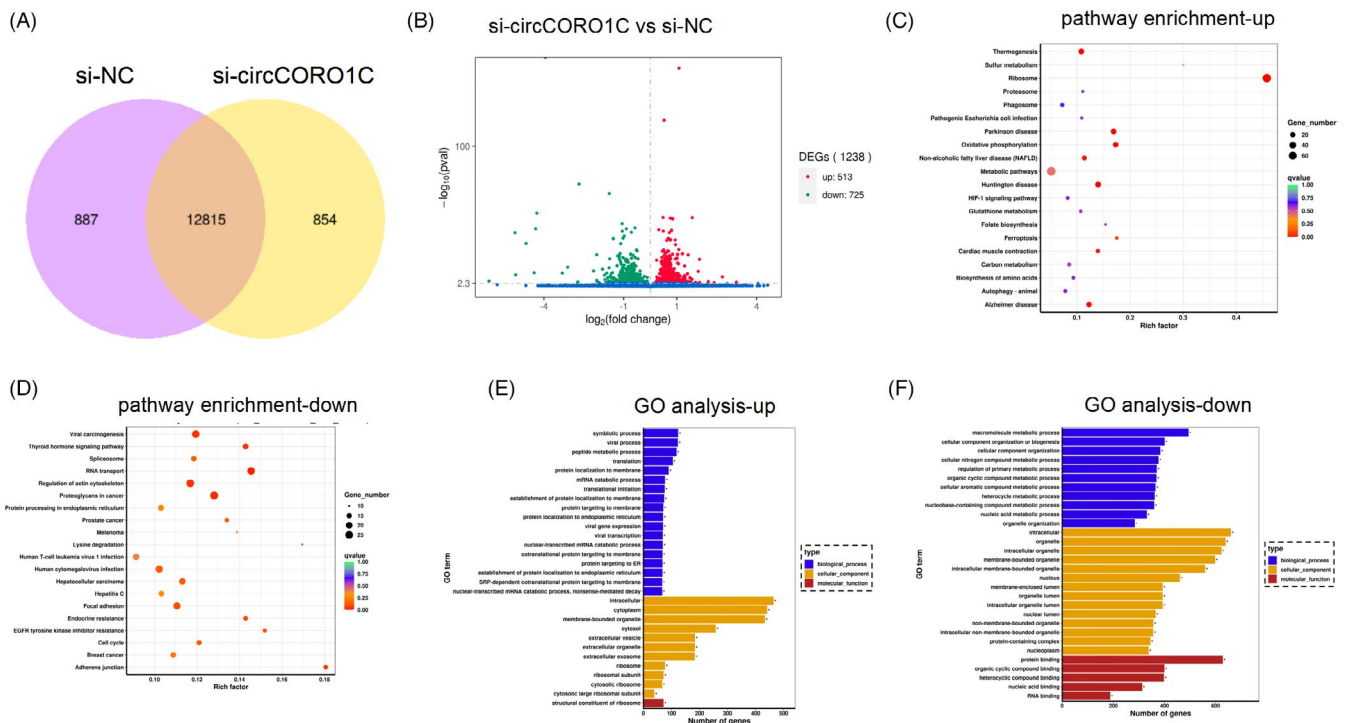


FIGURE 4 RNA sequencing results of si-circCORO1C in HCC cells. (A) Venn diagram of differential genes. (B) Volcano diagram showing the overall distribution of differential genes. (C–F) GO and pathway analysis for downstream genes in the si-circCORO1C and si-NC groups analysis

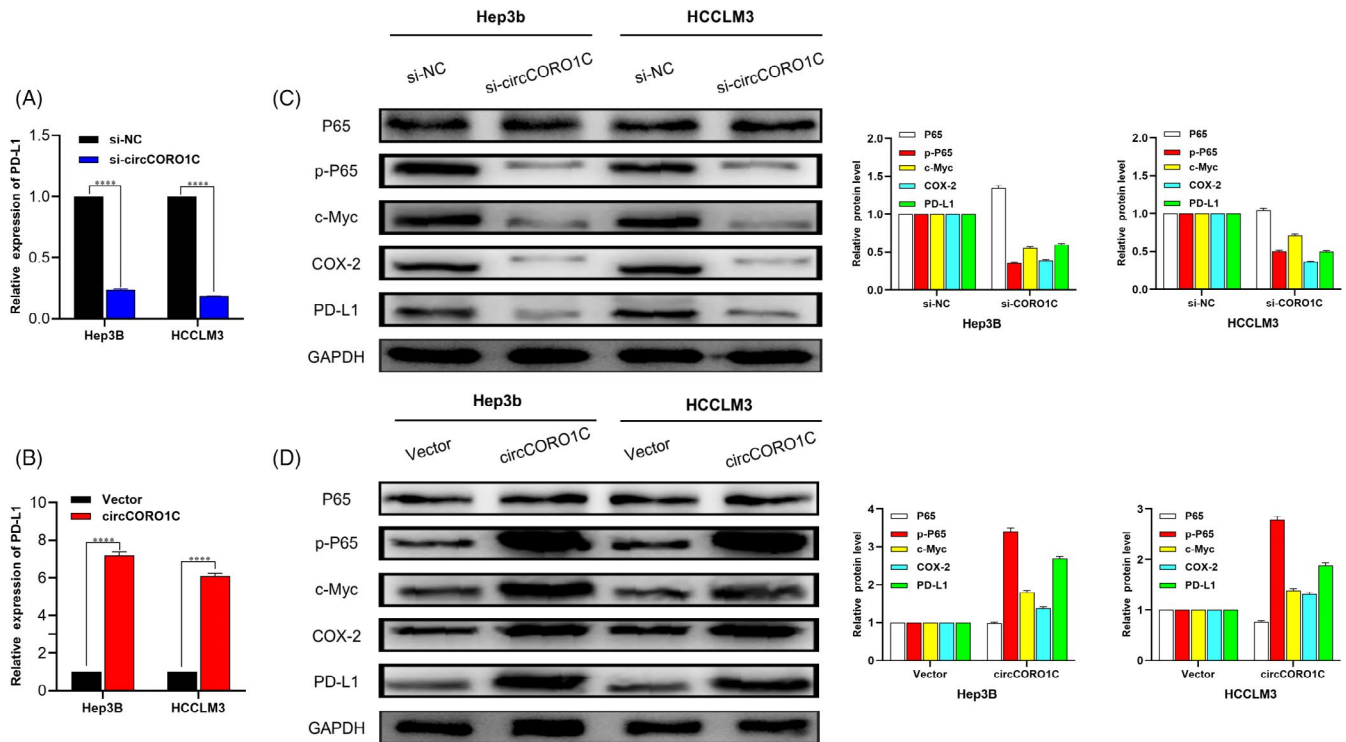


FIGURE 5 CircCORO1C regulates PD-L1 through the NF- κ B pathway and promotes the occurrence and development of HCC. (A) After knocking down circCORO1C, qRT-PCR was used to analyze the expression level of downstream PD-L1 mRNA. (B) After overexpression of circCORO1C, qRT-PCR was used to analyze the expression level of downstream PD-L1 mRNA. (C) Detect the expression of NF- κ B protein marker and PD-L1 protein in designated cells by Western blot analysis. **** $p < 0.0001$

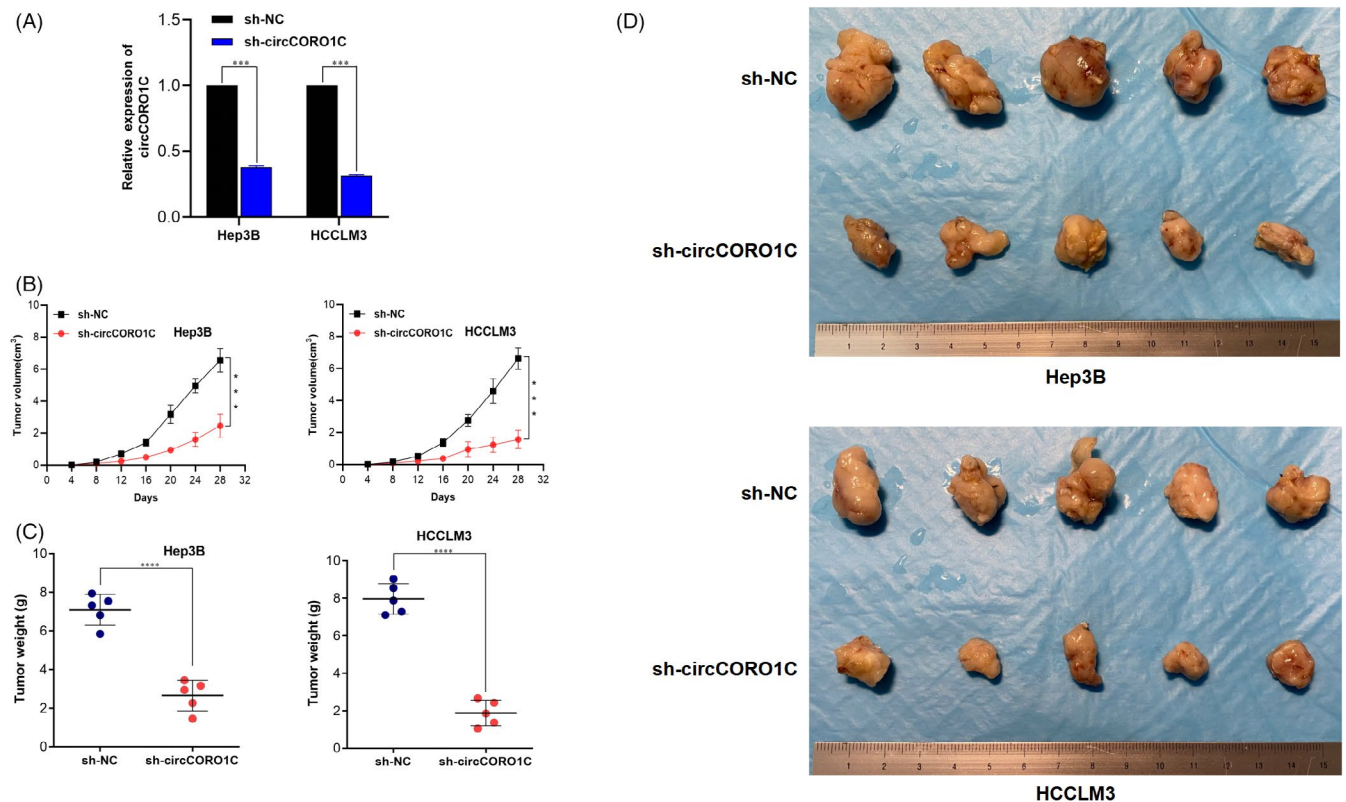


FIGURE 6 CircCORO1C facilitates the growth of HCC in vivo. (A) qRT-PCR was used to analyze the expression level of circCORO1C in HCC cells transfected with sh-circCORO1C and sh-NC. (B) The growth curves of xenograft tumors. The tumor volumes were measured every 4 days. (C) The relative weights of tumors were evaluated. (D) Representative images of the HCC tumor bearing BALB/c nude mice and xenograft HCC tumors. *** $p < 0.001$, **** $p < 0.0001$

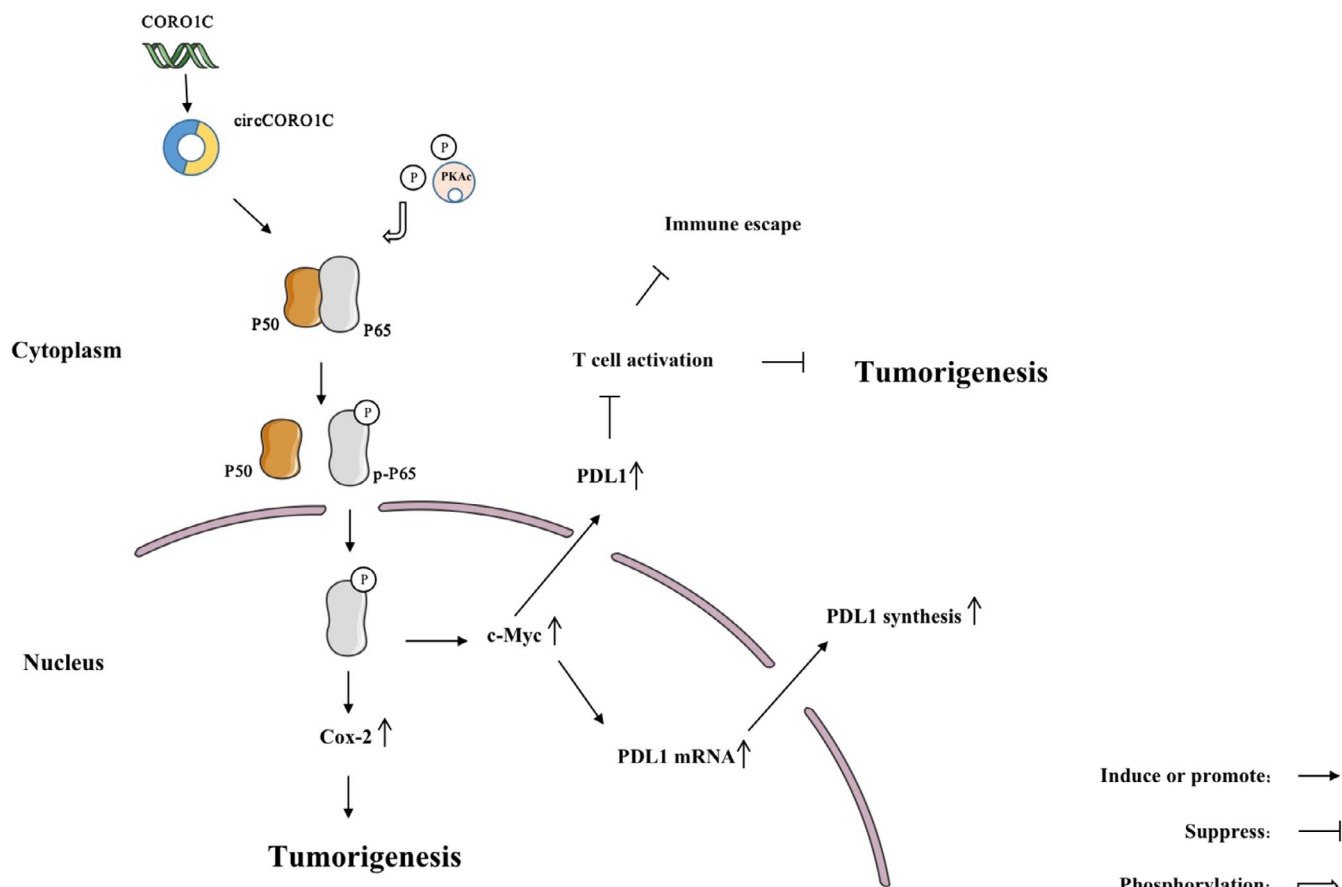


FIGURE 7 Schematic diagram of the mechanism of circCORO1C in HCC

in most tissues under normal physiological conditions.¹⁹ Buskens et al. found that overexpression of COX-2 can cause excessive proliferation of intestinal mucosal cells, which becomes an early event in the formation of esophageal adenocarcinoma and plays an important role in the invasion and metastasis of esophageal adenocarcinoma.²⁰ Shen S et al. found that c-Myc can promote the occurrence and development of osteosarcoma by affecting cell glucose metabolism.²¹

We also found that the NF- κ B pathway can increase the expression of c-Myc to increase the expression of PD-L1.²² Programmed death-ligand 1 (PD-L1, B7-H1) is a member of the B7 family of cell surface ligands.²³ It is a membrane ligand that is found significant upregulating on the surface of many cells in inflammatory and/or carcinogenic lesions.²⁴ PD-L1 binds to the receptor PD-1 on T cells, causing the Sh2p-driven T-cell receptor and its core receptor CD28 to dephosphorylate, inhibiting antigen-driven T-cell activation,²⁵ and anti-PD-1/PD-L1 treatment can reactivate the antibody immune response.²⁶ This treatment has produced significant clinical benefits in patients with non-small cell lung cancer (NSCLC).²⁷ In addition, anti-PD-1/PD-L1 blockade immunotherapy has been used to treat advanced hepatocellular carcinoma associated with hepatitis B virus infection and has achieved certain effects.²⁸

In summary, we clarified a new circRNA, named circCORO1C, whose expression is increased significantly in HCC cases and cell lines. CircCORO1C promotes the proliferation and metastasis of

hepatocellular carcinoma by enhancing the expression of PD-L1 through NF- κ B pathway, suggesting that circCORO1C is a promising biomarker and therapeutic target for HCC.

ACKNOWLEDGMENTS

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CONFLICT OF INTEREST

We declare that they have no competing interests.

AUTHORS CONTRIBUTIONS

Fan Wu, Shijie Ma, Hongyong Cao designed the study. Fan Wu, Guoqiang Sun, Wubin Zheng, LiangLiang Wu, Xiao Li, Jing Tao done the experiments. Weiwei Tang, Ye Cheng analyzed the data. Fan Wu prepared the manuscript. Shijie Ma, Hongyong Cao reviewed the manuscript. The manuscript was approved by the authors.

CONSENT FOR PUBLICATION

Written informed consent for publication was obtained from all participants.

DATA AVAILABILITY STATEMENT

Data can be required from the corresponding author.

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