LncRNA KCNQ1OT1 inhibits the radiosensitivity and promotes the tumorigenesis of hepatocellular carcinoma via the miR-146a-5p/ACER3 axis

Ganghua Yang*, Lijing Zhou*, Qinhong Xu, Fandi Meng, Yong Wan, Xiankui Meng, Lin Wang, and Lei Zhang

Department of Geriatric Surgery, The First Affiliated Hospital of Xi’an Jiaotong University, Xi’an, China

ABSTRACT

Hepatocellular carcinoma (HCC) is the third leading cause of cancer-related death, and radiotherapy is currently one of the main treatments. Long non-coding RNAs (lncRNAs) are associated with the radiosensitivity and tumorigenesis of HCC. However, the role and molecular mechanism of potassium voltage-gated channel subfamily Q member 1 overlapping transcript 1 (KCNQ1OT1) in HCC are still unclear. The relative expression of KCNQ1OT1, microRNA-146a-5p (miR-146a-5p) and alkaline ceramidase 3 (ACER3) was quantified by quantitative real-time polymerase chain reaction (qRT-PCR). Cell proliferation was measured by 3-(4, 5-dimethylthiazol-2-yl)-2, 5-diphenyl tetrazolium bromide (MTT) assay. Clonogenic assay was used to assess the radiosensitivity of cells. Cell apoptosis and metastasis were evaluated by flow cytometry and transwell assays, respectively. The protein levels of apoptosis markers, metastasis markers and ACER3 were detected by western blot (WB) analysis. The relationship between miR-146a-5p and KCNQ1OT1 or ACER3 was determined by dual-luciferase reporter assay. Additionally, animal experiments were carried out to explore the effect of KCNQ1OT1 silencing on HCC tumor growth in vivo. KCNQ1OT1 was highly expressed in HCC, and its knockdown hindered the proliferation and metastasis, while increased the radiosensitivity and apoptosis of HCC cells. MiR-146a-5p could interact with KCNQ1OT1, and its inhibition reversed the effects of silenced-KCNQ1OT1 on the radiosensitivity and tumorigenesis of HCC cells. Besides, ACER3 was a target of miR-146a-5p, and its overexpression inversed the effects of miR-146a-5p mimic on the radiosensitivity and tumorigenesis of HCC cells. The expression of ACER3 was regulated by KCNQ1OT1 and miR-146a-5p. Furthermore, KCNQ1OT1 also could reduce the growth of HCC by regulating the miR-146a-5p/ACER3 axis in vivo. Our study suggested that KCNQ1OT1 improved ACER3 expression to regulate the radiosensitivity and tumorigenesis of HCC through sponging miR-146a-5p, indicating that KCNQ1OT1 might be a new therapeutic target for HCC.

ARTICLE HISTORY
Received 30 March 2020
Revised 6 August 2020
Accepted 8 August 2020

KEYWORDS
Hepatocellular carcinoma; KCNQ1OT1; miR-146a-5p; ACER3; radiosensitivity; tumorigenesis

INTRODUCTION

Hepatocellular carcinoma (HCC) is the sixth most common malignancy in the world and is most common in men aged 40–50 years old [1,2]. Patients are usually accompanied by unexplained liver pain, wasting and progressive liver large phenomenon, seriously affect the quality of life of patients [3]. Radiotherapy is one of the most common methods for the treatment of HCC [4,5], but the development of radioresistance severely hinders the progress of HCC treatment [6,7]. Furthermore, HCC metastasis and recurrence are also crucial factors affecting HCC treatment [8,9]. Therefore, it is very necessary to explore the mechanisms that affect the radiosensitivity and tumorigenesis of HCC.

Long non-coding RNAs (lncRNAs) are non-protein coding transcripts with a length of > 200 nucleotides [10,11]. LncRNAs have multiple functions, the most important of which is to participate in the regulation of gene expression as the natural microRNAs (miRNAs) sponges [12,13]. In recent years, researchers have found that lncRNAs play vital roles in tumor development or regulation [14,15]. They were commonly present as oncogenes in many types of human cancers, including HCC. For instance, lncRNA HAGLROS could regulate the expression of ATG12 to increase cell proliferation and autophagy, while decrease the

CONTACT Lin Wang  essdba@163.com; Lei Zhang  gzzxxo@163.com
*These authors contributed equally to this work.

An Expression of Concern has been issued for this article. Please see Expression of Concern: (http://dx.doi.org/10.1080/15384101.2021.1973200)

Supplemental data for this article can be accessed here.

This article has been retracted. Please see Retraction (http://dx.doi.org/10.1080/15384101.2023.2171200)

© 2020 Informa UK Limited, trading as Taylor & Francis Group
apoptosis of HCC cells through sponging miR-5095 [16]. LncRNA HOXA-AS3 promoted cell proliferation, metastasis, and EMT process in HCC via regulating miR-29c expression [17]. Also, lncRNA H19 increased PSEN1 expression to improve the radiosensitivity and chemotherapeutic tolerance of HCC cells by sponging miR-193a-3p [18]. LncRNA potassium voltage-gated channel subfamily Q member 1 overlapping transcript 1 (KCNQ1OT1) is a chromatin regulatory RNA [19], which has been shown to participate in the regulation of many cancers as an oncogene [20–22]. However, studies on the role and mechanism of KCNQ1OT1 in HCC are still limited.

The purpose of this study was to investigate the effect of KCNQ1OT1 on the radiosensitivity and tumorigenesis of HCC. The molecular mechanism of KCNQ1OT1 in HCC was further elucidated through bioinformatics prediction and experimental verification. The presentation of KCNQ1OT1/miR-146a-5p/alkaline ceramidase 3 (ACER3) axis might provide a new method for the effective treatment of HCC patients.

Materials and methods

Patient tissues collection

32 cases of HCC patients were recruited from The First Affiliated Hospital of Xi’an Jiaotong University for this study. 32 paired HCC tissues (Tumor) and adjacent normal tissues (Normal) were collected from HCC patients and stored at −80°C. All patients had not received any other treatment and signed written informed consent. This study was authorized by the Ethics Committee of The First Affiliated Hospital of Xi’an Jiaotong University.

Cell culture and transfection

HCC cells (Huh7, Hep3B, SK-Hep-1 and SNU-182) were obtained from Procell (Wuhan, China). Human liver epithelial cells (THLE-2) were bought from American Type Culture Collection (ATCC, Manassas, VA, USA). All cells were cultured in Dulbecco’s Modified Eagle’s Medium (DMEM; HyClone, Logan, Utah, USA) containing 10% fetal bovine serum (FBS; HyClone), 1% Penicillin-Streptomycin solution (10 kU/mL-10 mg/mL; Procell) at 37°C with 5% CO₂. KCNQ1OT1 small interfering RNA (siRNA), lentivirus short hairpin RNA (shRNA) and overexpression vector (si-KCNQ1OT1#1/#2, sh-KCNQ1OT1 and KCNQ1OT1) or the negative controls (si-NC, sh-NC and vector), miR-146a-5p mimic and inhibitor (miR-146a-5p and anti-miR-146a-5p) or their negative controls (miR-NC and anti-miR-NC), ACER3 overexpression plasmid (ACER3) and its negative control (pcDNA) were bought from Geneseed (Guangzhou, China). The above plasmid vectors were transfected into Huh7 and Hep3B cells using Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was extracted using TRIzol reagent (Invitrogen). One-step cDNA First Strand Reverse Transcription Kit (HaiGene, Haerbin, China) was used to transcribe RNA into cDNA. SYBR Green real-time PCR (Takara, Dalian, China) was performed to execute qRT-PCR assay using IQ5 Real-Time PCR instrument (Bio-Rad, Hercules, CA, USA). Glyceraldehyde 3-phosphate dehydrogenase (GAPDH) or U6 was considered as internal control. The relative expression was calculated using 2−ΔΔCt methods. The primer sequences were as follows: KCNQ1OT1: F 5ʹ-GCACTCTGGGTCTGTCTTC-3ʹ, R 5ʹ-CACCT TCCCTGCCTCCTACAC-3ʹ; ACER3: F 5ʹ-ATGCTCATAGGTCTGTTCTC-3ʹ, R 5ʹ-AGTGGTTATAGTTACCAGGC-3ʹ; GAPD H F 5ʹ-GACTCTGGGTCTGTCTTC-3ʹ, R 5ʹ-CACCT TCCCTGCCTCCTACAC-3ʹ; ACER3: F 5ʹ-ATGCTCATAGGTCTGTTCTC-3ʹ, R 5ʹ-AGTGGTTATAGGTACCCAGGC-3ʹ; GAPD H F 5ʹ-GACTCATGACCACTCATCAGGC-3ʹ, R 5ʹ-AGAGGCAGGGATGATGTTC-3ʹ; miR-146a-5p: F 5ʹ-AGCTGTGGTGTTCTTACGGAT-3ʹ, R 5ʹ-CCATGCTGTCAGGCAAGAAAC-3ʹ; U6: F 5ʹ-GAGGGCGTATTTCCTCCATGATT-3ʹ, R 5ʹ-TAATTAGATTAATTTGACT-3ʹ.
**Cell proliferation assay**

3-(4, 5-dimethyl-2 thiazolyl)-2, 5-diphenyl-2-H-tetrazolium bromide (MTT) Assay Kit (Beyotime, Shanghai, China) was used in this study. Huh7 and Hep3B cells were incubated with MTT solution for 4 h. Then, dimethylsulfoxide (DMSO) was added into cells to dissolve for 15 min. The absorbance was measured using a microplate reader (Baiiu, Shanghai, China) at 490 nm.

**Clonogenic assay**

Huh7 and Hep3B cells were exposed with graded doses (0, 2, 4, 6 and 8 Gy) of X-ray at 37°C with 5% CO₂. After continued culture for 2 weeks, the colonies were fixed with methanol and stained with crystal violet to count the number of colonies (> 50 cells/colony). The survival curve after ionizing radiation was calculated as follows: survival fraction = the colony formation rate in the treatment group/control group.

**Flow cytometry**

After transfection for 24 h, Huh7 and Hep3B cells were exposed with 0 Gy or 6 Gy X-ray for 24 h. After that, the cells were collected and washed with PBS. Then, the cells were stained using Annexin V-FITC/PI Apoptosis Detection Kit (Vazyme Biotech, Nanjing, China). Fluorescence signals were collected by Flow Cytometer (BD Biosciences, San Jose, CA, USA) to measure the apoptotic rate of Huh7 and Hep3B cells.

**Western blot (WB) analysis**

Huh7 and Hep3B cells were lysed with RIPA lysis buffer (Beyotime). After centrifugation, the supernatant was collected and boiled with the 1× loading buffer for 10 min. Protein samples were resolved by sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) gel and then transferred onto polyvinylidene fluoride (PVDF) membranes (Millipore, Billerica, MA, USA). After blockage with 5% nonfat milk, membranes were incubated with primary antibodies against Bcl-2 (1:1,000, Beyotime), Bax (1:5,000, Beyotime), Cleaved PARP (1:3,000, Beyotime), E-cadherin (1:2,000, Beyotime), N-cadherin (1:1,000, Beyotime), Vimentin (1:5,000, Beyotime), ACER3 (1:500, Saierbio, Tianjin, China) or GAPDH (1:500, Saierbio) overnight at 4°C and then incubated with horseradish peroxidase-coupled secondary antibody (1:1,000, Saierbio) for 1 h. Following the incubation, protein signals were visualized with an enhanced chemiluminescence (ECL) solution (Pierce, Waltham, MA, USA).

**Transwell assay**

Chambers with 8 μm polycarbonate membrane (Corning Inc., Corning, NY, USA) were used to detect cell migration and invasion ability. Additionally, the chambers were pre-coated with Matrigel (BD Biosciences) for invasion detection, while not coated with Matrigel for migration detection. Huh7 and Hep3B cells (1 × 10^5) were seeded into the upper chambers with serum-free medium, and the lower chambers were filled with DMEM containing 10% FBS. After incubation for 24 h, cells were fixed with paraformaldehyde and stained with crystal violet. The cells on the submembrane side were photographed and counted by microscope (Novel, Ningbo, China).

**Dual-luciferase reporter assay**

The sequence of KCNQ1OT1 containing miR-146a-5p binding sites or mutant binding sites was cloned into pmirGLO vectors (Promega, Madison, WI, USA) to obtain KCNQ1OT1-WT or KCNQ1OT1-MUT reporter vector. Similarly, ACER3-WT or ACER3-MUT reporter vector was also built in the same way. The above reporter vectors were co-transfected with miR-146a-5p mimic or miR-NC into Huh7 and Hep3B cells. To confirm KCNQ1OT1 could regulate ACER3 expression by sponging miR-146a-5p, ACER3-WT or ACER3-MUT reporter vector were co-transfected with KCNQ1OT1 overexpression vector or vector into Huh7 and Hep3B cells. Following transfection for 48 h, the activities of Firefly luciferase and Renilla luciferase
were detected using the Dual-luciferase Reporter Gene Assay (Beyotime). The relative luciferase activity was calculated using the formula: Firefly luciferase/Renilla luciferase.

**Animal experiments**

Huh7 cells transfected with sh-NC or sh-KCNQ1OT1 were subcutaneously injected into BALB/c nude mice (n = 3 per group, Kay Biological, Shanghai, China), respectively. After 7 days, the length and width of the tumors were detected every 4 days until 27 days. After that, the mice were euthanized, and the tumors were removed for further analysis. The tumor volume was calculated according to the formula: weight = length × width²/2. Animal experiments were approved by the Animal Ethics Committee of The First Affiliated Hospital of Xi’an Jiaotong University and performed according to the Guide for the Care and Use of Laboratory Animals.

**Statistical analysis**

All experiments were performed in triplicate, and all independent experiments were set for 3 times to take the average value. Results were expressed as mean ± standard deviation (SD). All data were analyzed using GraphPad Prism 5.0 software (GraphPad, La Jolla, CA, USA). Student’s t-test or one-way analysis of variance (ANOVA) was used to analyze the differences between the paired groups or among the multiple groups, respectively. *P* < 0.05 was regarded as statistically significant.

**Results**

**KCNQ1OT1 expression was upregulated in HCC tissues and cells**

Firstly, we detected the expression of KCNQ1OT1 in HCC tissues and adjacent normal tissues. The results showed that KCNQ1OT1 was highly expressed in HCC tissues (Figure 1(a)). Similarly, compared with human liver epithelial cells (THLE-2), KCNQ1OT1 expression also was markedly increased in HCC cells, especially in Huh7 and Hep3B cells (Figure 1(b)). These data revealed that KCNQ1OT1 was abnormally expressed in HCC tissues and cells.

**Knockdown of KCNQ1OT1 improved the radiosensitivity and inhibited the tumorigenesis of HCC cells**

To investigate the role of KCNQ1OT1 in the radiosensitivity and tumorigenesis of HCC cells, we transfected si-KCNQ1OT1#1/#2 or si-NC into Huh7 and Hep3B cells. Through detecting the expression of KCNQ1OT1, we found that the transfection efficiency of si-KCNQ1OT1#1/#2 was excellent, especially si-KCNQ1OT1#1 (Figure 2(a)). Then, MTT assay results indicated that compared with si-NC, silencing of KCNQ1OT1 decreased the proliferation of Huh7 and Hep3B cells (Figure 2(b-c)). Also, we studied the effect of KCNQ1OT1 on the radiosensitivity of HCC cells using clonogenic assay. The results showed that the survival fraction of Huh7 and Hep3B cells in the KCNQ1OT1 knockdown group significantly decreased with the increase of ionizing radiation, suggesting that silenced-KCNQ1OT1 led to a dose-dependent inhibitory effect on the survival fraction of HCC cells (Figure 2(d-e)). Flow cytometry results showed that knockdown of KCNQ1OT1 promoted the apoptosis of Huh7 and Hep3B cells. Besides, compared with that in the absence of ionizing radiation (0 Gy), the apoptosis of the si-NC group and si-KCNQ1OT1#1 group was markedly increased...
in the presence of ionizing radiation (6 Gy) (Figure 3(a-b)). Additionally, KCNQ1OT1 knockdown also inhibited the protein expression of anti-apoptosis marker (Bcl-2) and promoted the protein expression of apoptosis markers (Bax and Cleaved PARP) in Huh7 and Hep3B cells, and this effect was also more pronounced in the presence of ionizing radiation (6 Gy) (Figure 3(c-d)). These indicated that KCNQ1OT1 knockdown could enhance the radiosensitivity of HCC cells. Furthermore, as shown in Figure 3(e-f), crystal violet staining pictures and statistical results indicated that the numbers of migrating and invading Huh7 and Hep3B cells were significantly reduced after silencing of KCNQ1OT1. In addition, silenced KCNQ1OT1 also remarkably promoted the protein expression of E-cadherin and suppressed the protein expression of N-cadherin and Vimentin (Figure 3(g-h)). These suggested that KCNQ1OT1 knockdown blocked the metastasis of HCC cells. Hence, these results revealed that KCNQ1OT1 played a crucial role in HCC progression.

**KCNQ1OT1 targeted miR-146a-5p in HCC cells**

To investigate the mechanism of KCNQ1OT1 on HCC cells, we used the Starbase2.0 tools to predict the target of KCNQ1OT1. According to bioinformatics predictions, there was a direct interaction between KCNQ1OT1 and miR-146a-5p (Figure 4(a)). Dual-luciferase reporter assay demonstrated that miR-146a-5p mimic could significantly repress the luciferase activity of KCNQ1OT1-WT.
in Huh7 and Hep3B cells, while did not affect KCNQ1OT1-MUT (Figure 4(b-c)). Besides, we found that knockdown of KCNQ1OT1 increased the expression of miR-146a-5p in Huh7 and Hep3B cells (Figure 4(d)). Also, we discovered that miR-146a-5p was significantly downregulated in HCC tissues compared to adjacent normal tissues (Figure 4(e)). In addition, we also detected a significant low expression of miR-146a-5p in four HCC cell lines (Figure 4(f) and Supplement Figure 1a). Correlation analysis revealed that the expression of miR-146a-5p was negatively correlated with KCNQ1OT1 in HCC tissues (Figure 4(g)). Therefore, we confirmed that miR-146a-5p could interact with KCNQ1OT1 in HCC.

**Inhibition of miR-146a-5p recovered the effects of KCNQ1OT1 silencing on the radiosensitivity and tumorigenesis of HCC cells**

To verify the role of KCNQ1OT1 in HCC cells through sponging miR-146a-5p, we co-transfected si-KCNQ1OT1#1 or si-NC with anti-miR-146a-5p or anti-miR-NC into Huh7 and Hep3B cells. The detection of miR-146a-5p expression was used to reflect the suppression efficiency of anti-miR-146a-5p and the promoting effect of KCNQ1OT1 knockdown on miR-146a-5p expression (Figure 5(a)). Then, MTT assay results revealed that miR-146a-5p inhibitor could reverse the inhibition effect of KCNQ1OT1 silencing on the proliferation of Huh7 and Hep3B cells (Figure 5(b-c)). As detected by clonogenic assay, the inhibitory effects of si-KCNQ1OT1#1 on the survival fraction of Huh7 and Hep3B cells were blocked by anti-miR-146a-5p (Figure 5(d-e)). Besides, flow cytometry results indicated that regardless of the
presence of ionizing radiation, inhibition of miR-146a-5p also could reverse the promotion effect of downregulated KCNQ1OT1 on the apoptosis of Huh7 and Hep3B cells (Figure 5(f-g)), as well as the effect on the protein expression levels of Bcl-2, Bax and Cleaved PARP (Figure 5(h-i)). Furthermore, miR-146a-5p inhibitor also abolished the inhibition effect of silenced KCNQ1OT1 on the migration and invasion of Huh7 and Hep3B cells (Figure 5(j-k)), and reversed the effect of KCNQ1OT1 knockdown on the protein expression levels of E-cadherin, N-cadherin and Vimentin (Figure 5(l-m)). These results suggested that KCNQ1OT1 regulated the radiosensitivity and tumorigenesis of HCC cells through sponging miR-146a-5p.

**ACER3 was a target of miR-146a-5p in HCC cells**

Targetscan analysis predicted that the 3′UTR of ACER3 harbored putative binding sites of miR-146a-5p (Figure 6(a)). To confirm whether ACER3 was a direct target of miR-146a-5p, we co-transfected ACER3-WT or ACER3-MUT reporter vector into Huh7 and Hep3B cells with miR-146a-5p or miR-NC. After 48 h post-transfection, the luciferase activity of ACER3-WT was strikingly reduced by miR-146a-5p overexpression. However, the luciferase activity of ACER3-MUT was unaffected (Figure 6(b-c)). We also found that miR-146a-5p mimic could decrease the mRNA and protein levels of ACER3 in Huh7 and Hep3B cells (Figure 6(d-e)). Furthermore, through qRT-PCR and WB analysis, we detected the expression of ACER3 and concluded that ACER3 was highly expressed in HCC tissues compared with that in adjacent normal tissues (Figure 6(f-g)). And the mRNA and protein expression levels of ACER3 also were upregulated in four HCC cell lines compared to THLE-2 cells (Figure 6(h-i) and Supplement Figure 1b-c). Besides, correlation analysis indicated that ACER3 expression was negatively correlated with miR-146a-5p in HCC tissues (Figure 6(j)). Hence, these results revealed that miR-146a-5p targeted ACER3 in HCC.

**Overexpressed ACER3 restored the effects of miR-146a-5p on the radiosensitivity and tumorigenesis of HCC cells**

To confirm that miR-146a-5p regulated the progress of HCC through targeting ACER3, we transfected ACER3 overexpression plasmid with miR-146a-5p mimic into Huh7 and Hep3B cells. The detection results of the mRNA and protein expression showed that ACER3 overexpression plasmid could restore the inhibitory effect of miR-146a-5p on ACER3 expression, indicating its high overexpression efficiency (Figure 7(a-b)). Further experimental verification showed that overexpression of miR-146a-5p blocked the proliferation of Huh7 and Hep3B cells, while ACER3 overexpression had the opposite effect (Figure 7(c-d)). Clonogenic assay results revealed that
miR-146a-5p decreased the survival fraction of Huh7 and Hep3B cells, but the entry of ACER3 could increase the survival fraction, suggesting that ACER3 could reverse the promotion effect of miR-146a-5p overexpression on the radiosensitivity of HCC cells (Figure 7(e-f)). Also, ACER3 overexpression recovered the acceleration effect of miR-146a-5p overexpression on the apoptosis of Huh7 and Hep3B cells (Figure 7(g-h)). As shown in Figure 7(i-j), the suppressive effect of miR-146a-5p on the protein expression of Bcl-2 and the promotion effect on the protein expression of Bax and Cleaved PARP also could be reversed by ACER3 overexpression. Furthermore, miR-146a-5p overexpression also repressed the numbers of migrating and invading cells, enhanced the protein expression of E-cadherin, and suppressed the protein expression of N-cadherin and Vimentin in Huh7 and Hep3B cells, while ACER3 overexpression also could reverse this effect (Figure 7(k-n)). These results further confirmed that miR-146a-5p regulated the radiosensitivity and tumorigenesis of HCC cells through targeting ACER3.

**KCNQ1OT1/miR-146a-5p regulated the expression of ACER3 in HCC cells**

At the same time, we detected the expression of ACER3 under the condition of knocking down KCNQ1OT1 and miR-146a-5p. As shown in Figure 8(a), qRT-PCR results indicated that silenced-KCNQ1OT1 inhibited the expression of ACER3 in Huh7 and Hep3B cells, while miR-146a-5p inhibitor improved its expression. Also, WB results confirmed that the inhibition of si-KCNQ1OT1 on the protein level of ACER3 could be alleviated by interfering of miR-146a-5p in Huh7 and Hep3B cells (Figure 8(b)). In addition, we also performed the dual-luciferase reporter assay to confirm the regulation of KCNQ1OT1 on ACER3. After verifying the transfection efficiency of KCNQ1OT1 overexpression vector (Supplement Figure 7.)
Figure 2a), we co-transfected with KCNQ1OT1 overexpression vector and ACER3-WT or ACER3-MUT into Huh7 and Hep3B cells. The results suggested that KCNQ1OT1 overexpression could enhance the luciferase activity of ACER3-WT, while no effect on the luciferase activity of ACER3-MUT (Supplement Figure 2B-C). These revealed that KCNQ1OT1 acted as a sponge of miR-146a-5p to regulate ACER3 level in HCC cells.

**KCNQ1OT1 silencing reduced HCC tumor growth in vivo**

To further confirm the role of KCNQ1OT1 in HCC, we constructed mice xenograft model for HCC to perform the in vivo experiments. By detecting the volume and weight of the tumor, we found that the knockdown of KCNQ1OT1 could significantly inhibit the growth rate of the transplanted tumor and reduce the volume and weight of the tumors (Figure 9(a-b)). In addition, the detection results of KCNQ1OT1 expression in the transplanted tumor suggested that the expression of KCNQ1OT1 in the sh-KCNQ1OT1 group was successfully suppressed (Figure 9(c)). Furthermore, we also measured the expression of miR-146a-5p and ACER3 in the tumors. The results showed that the expression of miR-146a-5p was markedly increased (Figure 9(d)), while the mRNA and protein expression levels of ACER3 was notably decreased in the sh-KCNQ1OT1 group (Figure 9(e-f)). All data suggested that KCNQ1OT1 silencing could repressed HCC tumor growth by regulating the miR-146a-5p/ACER3 axis.

**Discussion**

Many lncRNAs have been shown to be involved in the regulation of HCC radiosensitivity. For example, knockdown of lncRNA NEAT1_2 reinforced the radiosensitivity of HCC cells via miR-101-3p/WEE1 axis [23]. LncRNA ROR regulated RAD18 expression to improve the radioresistance of HCC cells by sponging miR-145 [24]. LncRNA KCNQ1OT1 had an abnormal expression in a variety of cancers. In HCC, Li et al. reported that KCNQ1OT1 could regulate the growth of HCC through inhibiting miR-504 expression [25]. Also, results of Wan et al. demonstrated that KCNQ1OT1 expression was related to the development of HCC [26]. However, there had no report on the radiosensitivity to HCC. Here, we found that KCNQ1OT1 was abnormally expressed in HCC tissues and cells. Moreover, silencing of KCNQ1OT1 inhibited the proliferation, improved the radiosensitivity, promoted the apoptosis and hindered the metastasis of HCC cells. Most importantly, in vivo experiments further confirmed that KCNQ1OT1 knockdown could reduce the growth of HCC tumor. These indicated that KCNQ1OT1 was involved in the progression of HCC as an oncogene.

A large number of studies have confirmed that lncRNA can act as a sponge for miRNA, thus relieving the inhibition of miRNA on downstream genes [12,13]. As a class of small non-coding protein RNAs, miRNAs are related to the regulation of many cancers. For example, miR-548b inhibited proliferation and invasion of HCC cells through targeting SP1 [27], and miR-2053 downregulated the PI3K and Wnt/β-catenin signaling pathways to hinder HCC progression [28]. In addition, in terms of radiosensitivity, miR-621 was considered to be a tumor radiosensitizer in HCC [29], and miR-203 could improve the radiosensitivity of HCC via targeting Bmi-1 [30]. In our research, through prediction and verification, we found that KCNQ1OT1
could directly sponge miR-146a-5p. In addition, we also confirmed that KCNQ1OT1 could negatively regulate miR-146a-5p in vitro and in vivo. Luo et al. research confirmed that miR-146a-5p enhanced the radiosensitivity and apoptosis of HCC [31]. Consistent with previous research results, we discovered that miR-146a-5p inhibitor could reduce the increased radiosensitivity after KCNQ1OT1 knockdown, and promote the blocked tumorigenesis after KCNQ1OT1 silencing. All data revealed that miR-146a-5p might act as a tumor suppressor in HCC.

Through prediction and analysis of target genes, we discovered that ACER3 was the target gene of miR-146a-5p. Studies had shown that the expression of ACER3 was associated with the development of colitis-related cancers [32], and promoted the acute myeloid leukemia progression [33]. Yin et al. results discovered that ACER3 could promote cell growth and decrease the apoptosis of HCC [34]. Similarly, in our research, ACER3 was upregulated in HCC, and its overexpression could increase the proliferation and metastasis of HCC cells blocked by overexpressed miR-146a-5p. Besides, overexpressed ACER3 also decreased the apoptosis and radiosensitivity of HCC cells stimulated by miR-146a-5p. Furthermore, our data also revealed that the expression of ACER3 also was positively regulated by KCNQ1OT1 in vitro and in vivo. Hence, our results proposed that KCNQ1OT1 regulated the HCC progression through the miR-146a-5p/ACER3 axis.

To sum up, we indicated that IncRNA KCNQ1OT1 was overexpressed in HCC and could regulate the radiosensitivity and tumorigenesis of HCC. Functional analysis revealed that KCNQ1OT1 restrained the radiosensitivity and facilitated the tumorigenesis of HCC cells through regulating the miR-146a-5p/ACER3 axis. The perfected molecular mechanism of KCNQ1OT1 might provide a new strategy for HCC treatment.

### Highlights

1. The interaction between KCNQ1OT1 and miR-146a-5p was confirmed for the first time;
2. ACER3 was first identified as a target gene of miR-146a-5p;
3. The existence of KCNQ1OT1/miR-146a-5p/ACER3 axis was first verified.

### Disclosure statement

No potential conflict of interest was reported by the authors.

### References

[1] Siegel RL, Miller KD, Jemal A. Cancer statistics, 2017. CA Cancer J Clin. 2017;67:7–30.
[2] Armengol C, Sarrias MR, Sala M. Carcinoma hepatocelular: presente y futuro. Med Clin (Barc). 2018;150:390–397.
[3] Usmani A, Mishra A, Ahmad M. Nanomedicines: a theranostic approach for hepatocellular carcinoma. Artif Cells Nanomed Biotechnol. 2018;46(4):680–690.
[4] Park J, Jung J, Kim D, et al. Long-term outcomes of the 2-week schedule of hypofractionated radiotherapy for recurrent hepatocellular carcinoma. BMC Cancer. 2018;18:1040.
[5] Shui Y, Yu W, Ren X, et al. Stereotactic body radiotherapy based treatment for hepatocellular carcinoma with extensive portal vein tumor thrombosis. Radiat Oncol. 2018;13:188.
[6] Cai J, Xiong Q, Jiang X, et al. RNF6 facilitates metastasis and radioresistance in hepatocellular carcinoma through ubiquitination of FoxA1. Exp Cell Res. 2019;374:152–161.
[7] Wu J, Li Y, Dang YZ, et al. HAb18G/CD147 promotes radioresistance in hepatocellular carcinoma cells: a potential role for integrin 1 signaling. Mol Cancer Ther. 2015;14:553–563.
[8] Hong SK, Lee KW, Yoon KC, et al. Different prognostic factors and strategies for early and late recurrence after adult living donor liver transplantation for hepatocellular carcinoma. Clin Transplant. 2019;33(10):e13703.
[9] Wang L, Ye G, Zhan C, et al. Clinical factors predictive of a better prognosis of pulmonary metastasectomy for hepatocellular carcinoma. Ann Thorac Surg. 2019;108:168–1691.
[10] Bergmann JH, Spector DL. Long non-coding RNAs: modulators of nuclear structure and function. Curr Opin Cell Biol. 2014;26:10–18.
[11] Mercer TR, Dinger ME, Mattick JS. Long non-coding RNAs: insights into functions. Nat Rev Genet. 2009;10:155–159.
[12] Tay Y, Rinn J, Pandolfi PP. The multilayered complexity of ceRNA crosstalk and competition. Nature. 2014;505:344–352.
[13] Wilusz JE, Sunwoo H, Spector DL. Long noncoding RNAs: functional surprises from the RNA world. Genes Dev. 2009;23:1494–1504.

[14] Peng WX, Koirala P, Mo YY. LncRNA-mediated regulation of cell signaling in cancer. Oncogene. 2017;36:5661–5667.

[15] Yang G, Lu X, Yuan L. LncRNA: a link between RNA and cancer. Biochim Biophys Acta. 2014;1839:1097–1109.

[16] Wei H, Hu J, Pu J, et al. Long noncoding RNA HAGLROS promotes cell proliferation, inhibits apoptosis and enhances autophagy via regulating miR-5095/ATG12 axis in hepatocellular carcinoma cells. Int Immunopharmacol. 2019;73:72–80.

[17] Tong Y, Wang M, Dai Y, et al. LncRNA HOXA-AS3 sponges miR-29c to facilitate cell proliferation, metastasis, and EMT process and activate the MEK/ERK signaling pathway in hepatocellular carcinoma. Hum Gene Ther Clin Dev. 2019;30:129–141.

[18] Ma H, Yuan L, Li W, et al. The LncRNA H19/miR-193a-3p axis modifies the radio-resistance and chemotherapeutic tolerance of hepatocellular carcinoma cells by targeting PSEN1. J Cell Biochem. 2018;119:8325–8335.

[19] Pandey RR, Mondal T, Mohammad F, et al. Kcnq1ot1 antisense noncoding RNA mediates lineage-specific transcriptional silencing through chromatin-level regulation. Mol Cell. 2008;32:232–246.

[20] Bian Y, Gao G, Zhang Q, et al. KCNQ1OT1/miR-217/ZEB1 feedback loop facilitates cell migration and epithelial-mesenchymal transition in colorectal cancer. Cancer Biol Ther. 2019;20:886–896.

[21] Shao J, Pan X, Yin X, et al. KCNQ1OT1 affects the progression of diabetic retinopathy by regulating miR-1470 and epidermal growth factor receptor. J Cell Physiol. 2019;234:17269–17279.

[22] Dong Z, Yang P, Qiu X, et al. KCNQ1OT1 facilitates progression of non-small-cell lung carcinoma via modulating miRNA-27b-3p/HSP90A1 axis. J Cell Physiol. 2019;234:11304–11314.

[23] Chen X, Zhang N. Downregulation of LncRNA NEAT1_2 radiosensitizes hepatocellular carcinoma cells through regulation of miR-101-3p/WEE1 axis. Cell Biol Int. 2019;43:44–55.

[24] Chen Y, Shen Z, Zhi Y, et al. Long non-coding RNA ROR promotes radioresistance in hepatocellular carcinoma cells by acting as a ceRNA for microRNA-145 to regulate RAD18 expression. Arch Biochem Biophys. 2018;645:117–125.

[25] Li C, Miao R, Zhang J, et al. Long non-coding RNA KCNQ1OT1 mediates the growth of hepatocellular carcinoma by functioning as a competing endogenous RNA of miR-504. Int J Oncol. 2018;52:1603–1612.

[26] Wan J, Huang M, Zhao H, et al. A novel tetranucleotide repeat polymorphism within KCNQ1OT1 confers risk for hepatocellular carcinoma. DNA Cell Biol. 2013;32(11):628–634.

[27] Qiu H, Zhang G, Song B, et al. MicroRNA-548b inhibits proliferation and invasion of hepatocellular carcinoma cells by directly targeting specificity protein 1. Exp Ther Med. 2019;18(3):2332–2340.

[28] Song T, Ma K, Zhao C, et al. MicroRNA-2053 overexpression inhibits the development and progression of hepatocellular carcinoma. Oncol Lett. 2019;18(2):2043–2049.

[29] Shao Y, Song X, Jiang W, et al. MicroRNA-621 Acts as a Tumor Radiosensitizer by Directly Targeting SETDB1 in Hepatocellular Carcinoma. Mol Ther. 2019;27(2):355–364.

[30] Shao Y, Zhang D, Li X, et al. MicroRNA-203 increases cell radiosensitivity via directly targeting Bmi-1 in hepatocellular carcinoma. Mol Pharm. 2018;15(8):3205–3215.

[31] Luo J, Si ZZ, Li T, et al. MicroRNA-146a-5p enhances radiosensitivity in hepatocellular carcinoma through replication protein A3-induced activation of the DNA repair pathway. Am J Physiol Cell Physiol. 2019;316:C299–C311.

[32] Wang K, Xu R, Snider AJ, et al. Alkaline ceramidase 3 deficiency aggravates colitis and colitis-associated tumorigenesis in mice by hyperactivating the innate immune system. Cell Death Dis. 2016;7:e2124.

[33] Chen C, Yin Y, Li C, et al. ACER3 supports development of acute myeloid leukemia. Biochem Biophys Res Commun. 2016;478:33–38.

[34] Yin Y, Xu M, Gao J, et al. Alkaline ceramidase 3 promotes growth of hepatocellular carcinoma cells via regulating S1P/S1PR2/P13K/AKT signaling. Pathol Res Pract. 2018;214:1381–1387.