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Circular RNA circLRCH3 promotes oxaliplatin resistance in gastric cancer through the modulation of the miR-383-5p/FGF7 axis

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Summary. Background. Gastric cancer (GC) is a common malignant tumor of the digestive system. Circular RNAs (circRNAs) play a vital role in tumorigenesis and chemoresistance. The current study aimed to explore the possible role and mechanism of circRNA leucine rich repeats and calponin homology domain containing 3 (circLRCH3) in GC chemoresistance.

Methods. The levels of circLRCH3, microRNA-383-5p (miR-383-5p) and fibroblast growth factor 7 (FGF7) were determined by quantitative real-time PCR or Western blot. Cell Counting Kit-8 (CCK-8) assay was utilized to evaluate cell survival rate and proliferation ability. Colony formation, transwell and flow cytometry assays were used to assess cell proliferation, migration, invasion and apoptosis. The expression of multidrug resistance proteins was detected by Western blot. The binding relationship between miR-383-5p and circLRCH3/FGF7 was verified by dual-luciferase reporter assay or RNA immunoprecipitation assay. Xenograft assay was conducted to analyze the role of circLRCH3 in OXA resistance *in vivo*.

Results. CircLRCH3 and FGF7 levels were up-regulated, while miR-383-5p level was reduced in OXA-resistant GC tissues and cells. Depletion of circLRCH3 attenuated the resistance of OXA-resistant cells to OXA. CircLRCH3 silence reduced OXA resistance by regulating miR-383-5p. Besides, miR-383-5p elevated OXA sensitivity of GC cells by repressing FGF7. Moreover, the deletion of circLRCH3 increased OXA sensitivity *in vivo*.

Conclusions. Knockdown of circLRCH3 alleviated OXA resistance of GC by modulating the miR-383-5p/FGF7 axis, which provided a promising therapeutic target for GC chemoresistance.

Key words: Gastric cancer, circLRCH3, miR-383-5p, FGF7, Oxaliplatin

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Introduction

Gastric cancer (GC) is a frequent malignancy of the digestive system, ranking third in tumor-related mortality (Bray et al., 2018). Since most GC patients are diagnosed at an advanced stage, their prognosis is unsatisfactory (Lott and Carvajal-Carmona 2018). Surgical resection and chemotherapy are effective treatment strategies for GC, but the existence of drug resistance has become a stumbling block for GC therapy (Biagioni et al., 2019). Oxaliplatin (OXA) is a third-generation platinum anti-cancer drug and exerts a certain effect on GC (Wang et al., 2019). Hence, in-depth exploration of the potential mechanism of OXA resistance in gastric carcinoma is essential for prolonging the survival of GC patients.

Circular RNAs (circRNAs) are single-stranded molecules with covalent closed-loop structures formed by connecting 5' and 3' terminals (Li et al., 2020a). In recent years, many studies have identified that circRNAs participate in regulating a range of biological functions of gastrointestinal tumors, including proliferation, metastasis and inflammation (Nie et al., 2020). Besides, many circRNAs have been verified to be implicated in cancer chemoresistance (Cui et al., 2020). For instance, circ_0007841 contributed to multiple myeloma progression and bortezomib resistance by binding to microRNA-129-5p (miR-129-5p) and elevating JAG1 expression (Wang et al., 2020b). In addition, silencing of circ_0000260 increased cisplatin sensitivity of gastric adenocarcinoma by regulating the microRNA-129-

5p/MMP11 pathway (Liu et al., 2020). CircLRCH3 (hsa_circ_0005873) derived from leucine rich repeats and calponin homology domain containing 3 (LRCH3) gene was substantially elevated in GC tissues, as revealed through the GEO database (GSE78092). However, the potential function of circLRCH3 in GC remains unknown.

Moreover, increasing evidence has validated that circRNAs weaken the suppressive effect of miRNAs on target genes by targeting miRNAs (Zhong et al., 2018). Also, extensive investigations have confirmed the critical role of miRNAs in tumorigenesis, development and drug resistance of GC (Azarbarzin et al., 2020). For example, miR-424-3p accelerated the deterioration of GC by modulating ABCC2-mediated chemoresistance (Li et al., 2020b). Exhaustion of miR-195 enhanced 5-fluorouracil resistance by up-regulating HMGA1 (Wang 2019). Additionally, Wei and Gao revealed that miR-383-5p level was prominently dropped in GC, and up-regulation of miR-383-5p impeded GC cell growth and migration by participating in various signaling pathways (Wei and Gao, 2019). Nevertheless, the role of miR-383-5p in GC chemoresistance has not been elucidated.

Herein, we first studied the expression pattern of circLRCH3 in GC. Furthermore, we explored the possible function of circLRCH3 in OXA resistance of GC and the relationship between circLRCH3 and miR-383-5p/fibroblast growth factor 7 (FGF7) axis in OXA resistance of GC.

Materials and methods

Clinical samples

Paired tumor tissues (n=31) and adjacent normal tissues (n=31) were collected from GC patients undergoing surgery at Soochow University Affiliated Taicang Hospital. OXA clinical resistance was defined as the appearance of new lesions or an increase in tumor growth of more than 30% after 2 months of chemotherapy. All patients were classified into OXA-sensitive (n=13) group and OXA-resistant (n=18) group. All participants signed the written informed consent, and this research was endorsed by the Ethics Committee of Soochow University Affiliated Taicang Hospital.

Cell culture and transfection

Human normal gastric epithelial cells (GES-1), GC cells (N87, AGS, MKN-45 and HGC-27), and OXA-resistant cell lines (MKN-45/OXA and HGC-27/OXA) were commercially obtained from TongPai (Shanghai, China) and incubated in RPMI-1640 medium (Youkang Biotech, Beijing, China) containing 10% fetal bovine serum (FBS; Youkang Biotech) with 5% CO_2 at 37°C.

CircLRCH3 small interference RNA (si-circLRCH3), circLRCH3 overexpression plasmid (circLRCH3), miR-383-5p mimics (miR-383-5p), miR-383-5p inhibitor (in-miR-383-5p), FGF7 overexpression vectors (FGF7) and corresponding controls (si-NC, Vector, miR-NC, in-miR-NC and pcDNA) were purchased from Genechem (Shanghai, China). Lipofectamine 3000 (Invitrogen, Carlsbad, CA, USA) was utilized for cell transfection when cell confluence reached ~80%.

Quantitative real-time PCR (qRT-PCR)

Total RNA was isolated using TRIzol reagent (Leagene, Beijing, China). Next, cDNA was synthesized using the specific cDNA synthesis kit (Vazyme, Nanjing, China). Then, qRT-PCR reaction was carried out using SYBR Green Master Mix (Vazyme). GAPDH or U6 (for miR-383-5p) was regarded as an endogenous control. All primers are shown: circLRCH3-F: 5'-TTAACAATGGCGAAATGCAG-3', circLRCH3-R: 5'-AGATGGAGGAAGGGGTGAAG-3'; miR-383-5p-F: 5'-GGGAGATCAGAAGGTGATT-3', miR-383-5p-R: 5'-CAGTGCGTGTCGTGGAGT-3'; FGF7-F: 5'-TCCTGCCAACTTTGCTCTACA-3', FGF7-R: 5'-CAGGGCTGGAACAGTTCACAT-3'; GAPDH-F: 5'-GCACCGTCAAGGCTGAGAAC-3', GAPDH-R: 5'-ATGGTGGTGAAGACGCCAGT-3'; U6-F: 5'-CTCGCTTCGGCAGCACATA-3', U6-R: 5'-AACGCTTCACGAATTTGCGT-3'.

Cell Counting Kit-8 (CCK-8) assay

CCK-8 assay was utilized to detect cell survival and proliferation. In short, cells were grown in 96-well plates after different transfections or treatments. After cells were cultured for appropriate times, 10 μL of CCK-8 (Boster, Wuhan, China) was injected into each plate and maintained for an additional 4h. Finally, the absorbance was examined using a microplate reader (Bio-Rad, Hercules, CA, USA).

Colony formation assay

Following transfection, MKN-45/OXA and HGC-27/OXA cells were plated into 6-well plates. Afterwards, the cells were cultivated for 14 days and treated with 4% paraformaldehyde (Solarbio) and 0.1% crystal violet (Solarbio). Finally, the colonies were counted under a microscope (Nikon, Tokyo, Japan).

Transwell assay

Cell migration and invasion capacity was determined using transwell chambers (Corning, Corning, NY, USA) without or with Matrigel (Corning). Briefly, MKN-45/OXA and HGC-27/OXA cells were injected into the upper chamber together with FBS-free RPMI-1640 medium after transfection. Meanwhile, the bottom chamber was filled with 10% FBS. Following cultivationfor 24h, the transferred cells were reacted with 4% paraformaldehyde (Solarbio) and 0.1% crystal violet (Solarbio), and counted under a microscope (Nikon; magnification: 100×).

Flow cytometry

MKN-45/OXA and HGC-27/OXA cells were collected and rinsed with phosphate buffer saline (PBS; Solarbio). Subsequently, the cells were incubated with Annexin V-FITC and Propidium Iodide (PI; Vazyme). Finally, the apoptotic cells were determined by a flow cytometer (Beckman Coulter, Miami, FL, USA).

Western blot assay

After extracting protein with RIPA buffer (Keygen, Nanjing, China), the protein concentration was determined using BCA Protein Assay Kit (Beyotime, Shanghai, China). Afterwards, protein samples were separated by 10% SDS-PAGE and transferred onto PVDF membranes (Beyotime). Then, the membranes were incubated with anti-multidrug resistance-associated protein 1 (MRP1) (1:1000, ab233383, Abcam, Cambridge, UK), anti-multidrug resistance 1 (MDR1) (1:2000, ab129450, Abcam), anti-FGF7 (1:5000, ab131162, Abcam) or anti-β-actin (1:2500, ab8227, Abcam) after being blocked with 5% non-fat milk. Subsequently, after incubating goat anti-rabbit IgG secondary antibody with the membranes (1:20000, ab205718, Abcam), the protein bands were developed by ECL system (Beyotime).

Dual-luciferase reporter assay

The binding sequence between miR-383-5p and circLRCH3 or FGF7 3'UTR was predicted using the

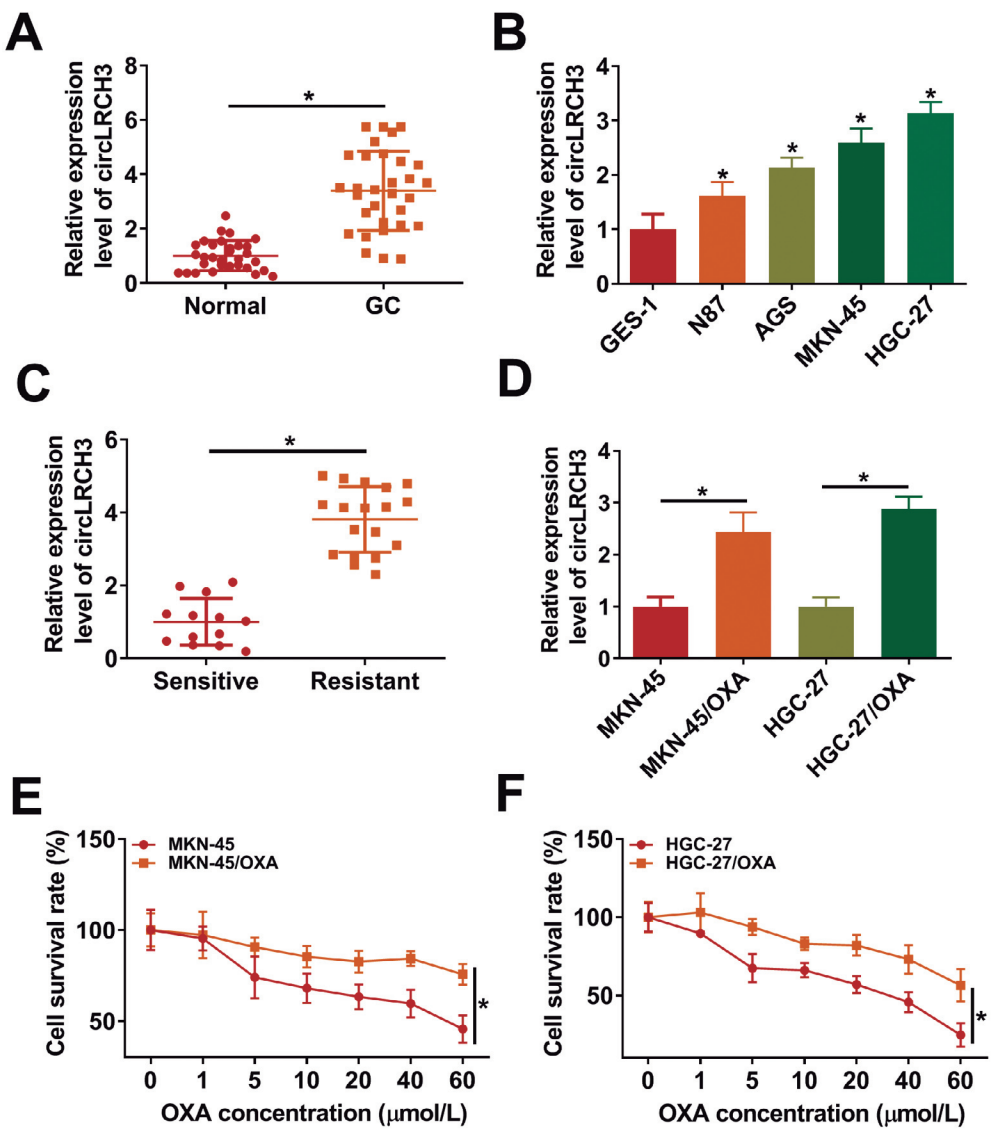

Fig. 1. Expression of circLRCH3 in OXA-resistant GC tissues and cells. **A.** The level of circLRCH3 in GC tissues (n=31) and adjacent normal tissues (n=31) was measured by qRT-PCR. **B.** CircLRCH3 level in GES-1 cells and GC cells (N87, AGS, MKN-45 and HGC-27) was detected using qRT-PCR. **C.** CircLRCH3 expression in OXA-sensitive (n=13) and OXA-resistant (n=18) GC patients was examined via qRT-PCR. **D.** CircLRCH3 level in parental GC cells (MKN-45 and HGC-27) and OXA-resistant cells (MKN-45/OXA and HGC-27/OXA) was measured using qRT-PCR. **E, F.** Cell survival rate was evaluated by CCK-8 method. **P*<0.05.

circinteractome (https://circinteractome.irp.nia.nih.gov/) or TargetScan (http://www.targetscan.org/vert_72/) database. Subsequently, the wild-type reporter plasmid (circLRCH3 WT or FGF7 3'UTR WT) and the mutant reporter plasmid (circLRCH3 MUT or FGF7 3'UTR MUT) were constructed by Genechem. Then, the corresponding plasmids together with miR-NC or miR-383-5p were introduced into MKN-45/OXA and HGC-27/OXA cells, and the luciferase activity was measured with Dual-Luciferase Reporter Assay Kit (Vazyme).

RNA immunoprecipitation (RIP) assay

Magna RIP kit (Millipore, Billerica, MA, USA) was applied for RIP analysis following the manufacturer's requirements. MKN-45/OXA and HGC-27/OXA cells were lysed and reacted with magnetic beads conjugated with anti-AGO2 or anti-lgG. Finally, the contents of circLRCH3 and miR-383-5p were examined using qRT-PCR analysis.

In vivo assay

This experiment was endorsed by the Animal Ethics Committee of Soochow University Affiliated Taicang Hospital. Lentivirus plasmids containing circLRCH3 shRNA (sh-circLRCH3) or the control (sh-NC) synthesized by Genechem were transfected into HGC-27/OXA cells. Afterwards, stably transfected cells (2×10^6) were subcutaneously inoculated into the right back of BALB/c nude mice (five-week-old, n=20). After 7 days of injection, mice were intraperitoneally injected with 3 mg/kg OXA or equivalent PBS every 7 days

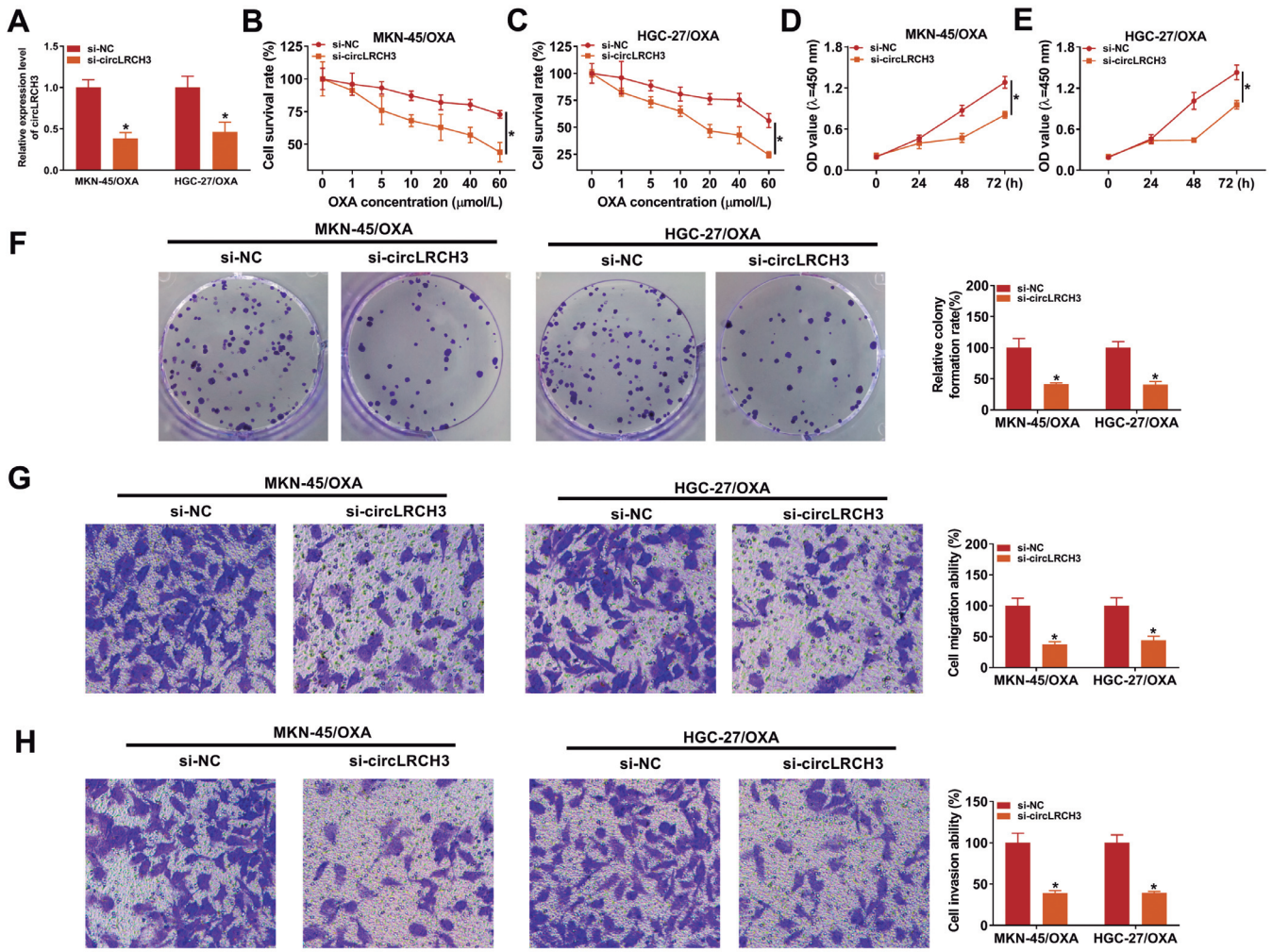

Fig. 2. Depletion of circLRCH3 enhanced OXA sensitivity in OXA-resistant GC cells. MKN-45/OXA and HGC-27/OXA cells were introduced with si-NC or si-circLRCH3. **A.** CircLRCH3 level was determined via qRT-PCR. **B, C.** Cell survival rate was detected by CCK-8 assay after stimulation with various concentrations of OXA. Cell proliferation was assessed with the CCK-8 method **(D, E)** and colony formation assay **(F)**. **G, H.** Cell migration and invasion abilities were determined by transwell assay. **P*<0.05.

according to the following groups: sh-NC+PBS, sh-circLRCH3+PBS, sh-NC+OXA and sh-circLRCH3+OXA (n=5 per group). Additionally, tumor volume was monitored every 7 days. Following 28 days, the mice were sacrificed, and the tumors were excised for weight measurement and RNA analysis.

Statistical analysis

Data are expressed as mean ± standard deviation in three independent replicates using GraphPad Prism 7 software (GraphPad, San Diego, CA, USA). The differences were evaluated by Student's *t*-test or one-way analysis of variance. Statistical significance was considered when P value <0.05.

Results

CircLRCH3 was up-regulated in OXA-resistant GC tissues and cells

To clarify the expression profile of circLRCH3 in GC, we first examined circLRCH3 level in GC tissues and cells. As displayed in Fig. 1A, circLRCH3 expression was remarkably elevated in GC tissues (n=31) compared to normal tissues (n=31). Simultaneously, qRT-PCR analysis showed that circLRCH3 level was strikingly increased in GC cells (N87, AGS, MKN-45 and HGC-27) relative to normal gastric epithelial cells (GES-1) (Fig. 1B). In addition, all GC patients were divided into OXA-sensitive (n=13) and OXA-resistant (n=18) groups according to the sensitivity of GC patients to OXA. As shown in Fig. 1C, circLRCH3 level was markedly elevated in OXA-resistant GC tissues compared to OXA-sensitive GC tissues. As expected, circLRCH3 expression in OXA-resistant cells (MKN-45/OXA and HGC-27/OXA) was remarkably higher than that in parental GC cells (MKN-45 and HGC-27) (Fig. 1D). In addition, CCK-8 analysis showed that MKN-45/OXA and HGC-27/OXA cells were less sensitive to OXA than parental GC cells (Fig. 1E,F).

Depletion of circLRCH3 enhanced OXA sensitivity in OXA-resistant GC cells

To investigate the potential role of circLRCH3 in OXA resistance, circLRCH3 siRNA was introduced into OXA-resistant cells to knock down circLRCH3. As illustrated in Fig. 2A, si-circLRCH3 transfection strikingly reduced the expression of circLRCH3 in MKN-45/OXA and HGC-27/OXA cells compared with the si-NC group. Additionally, interference of circLRCH3 enhanced the sensitivity of OXA-resistant cells to OXA (Fig. 2B,C). Meanwhile, CCK-8 and colony formation assays suggested that circLRCH3 silencing markedly reduced the proliferatory ability of MKN-45/OXA and HGC-27/OXA cells (Fig. 2D-F). Besides, transwell analysis showed that depletion of circLRCH3 significantly decreased the migration and invasion capabilities of MKN-45/OXA and HGC-27/OXA cells (Fig. 2G,H). Flow cytometry revealed that down-regulation of circLRCH3 accelerated the apoptosis of OXA-resistant cells (Fig. 3A). Moreover, the introduction of si-circLRCH3 remarkably reduced the expression of multidrug resistance proteins (MRP1 and

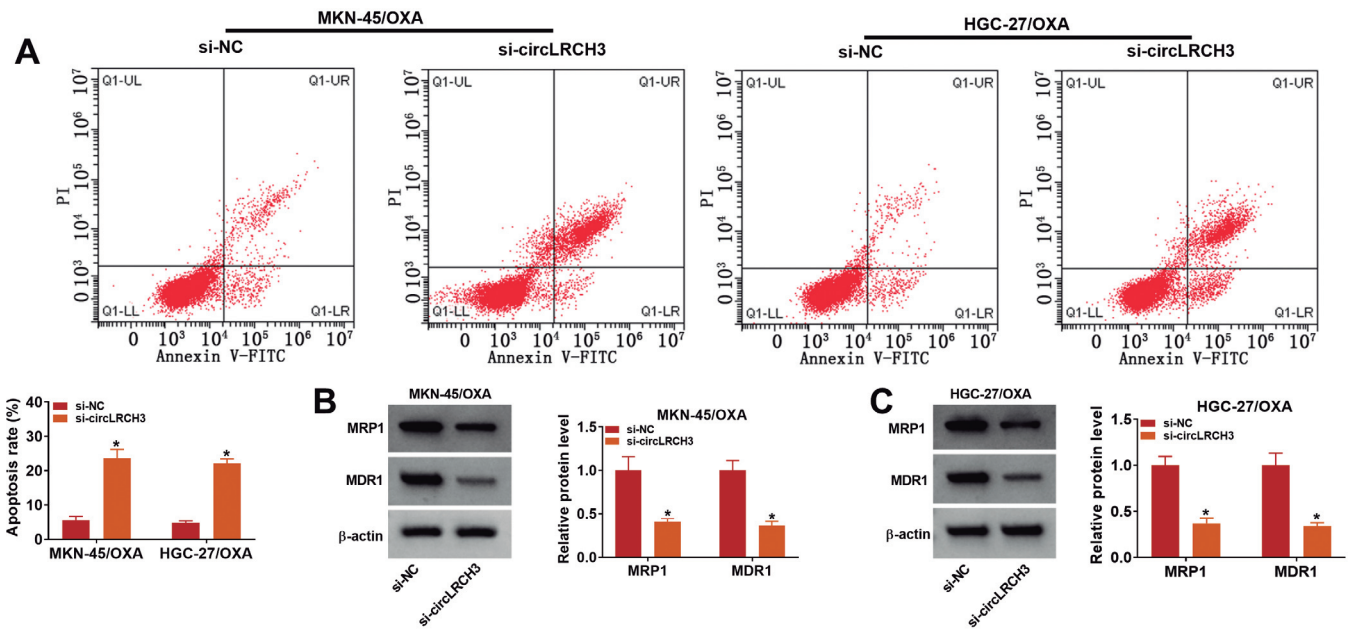

Fig. 3. A. Cell apoptosis was analyzed using flow cytometry. **B and C.** The expression of MRP1 and MDR1 was measured by Western blot. **P*<0.05.

MDR1) relative to the control group (Fig. 3B,C). These data evidenced that silence of circLRCH3 alleviated the resistance of OXA-resistant cells to OXA.

CircLRCH3 directly interacted with miR-383-5p

We screened six candidate miRNAs that might combine with circLRCH3 through the circinteractome and starbase online databases (Fig. 4A). Simultaneously, qRT-PCR assay showed that circLRCH3 overexpression efficiency was significant after circLRCH3 transfection (Fig. 4B). In addition, up-regulation of circLRCH3 led to the most significant decrease in miR-383-5p expression, so miR-383-5p was chosen for the follow-up research (Fig. 4C,D). As shown in Figure 4E, circLRCH3 shared complementary binding sites with miR-383-5p. Besides, qRT-PCR analysis showed that the overexpression and knockdown efficiency of miR-383-5p was significant (Fig. 4F). To verify the predicted binding relationship, dual-luciferase reporter and RIP assays were performed in MKN-45/OXA and HGC-27/OXA cells. The results showed that miR-383-5p

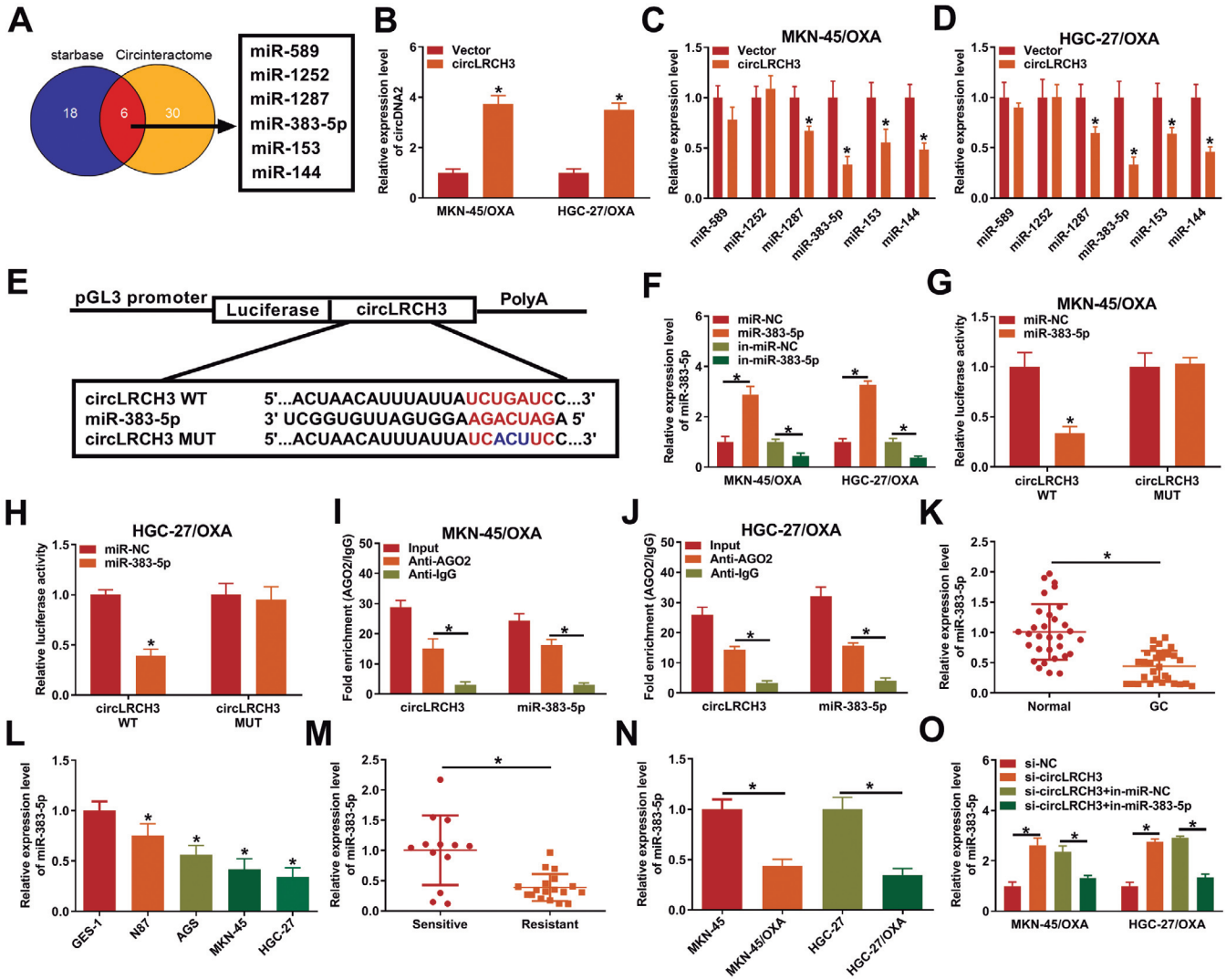

Fig. 4 CircLRCH3 directly interacted with miR-383-5p. **A.** Venn diagram showing the possible target miRNAs of circLRCH3. **B.** The overexpression efficiency of circLRCH3 was confirmed by qRT-PCR. **C and D.** The levels of six possible targets (miR-589, miR-1252, miR-1287, miR-383-5p, miR-153 and miR-144) were examined by qRT-PCR in MKN-45/OXA and HGC-27/OXA cells after circLRCH3 overexpression. **E.** The putative binding site between circLRCH3 and miR-383-5p was displayed. **F.** After the introduction of miR-383-5p or in-miR-383-5p, miR-383-5p level was measured via qRT-PCR. Dual-luciferase reporter assay **(G and H)** and RIP assay **(I and J)** were utilized to validate the association between circLRCH3 and miR-383-5p. **K and L.** The expression of miR-383-5p in GC tissues and cells was examined by qRT-PCR. **M and N.** The level of miR-383-5p in OXA-resistant GC tissues and cells was detected using qRT-PCR. **O.** The level of miR-383-5p was tested using qRT-PCR in MKN-45/OXA and HGC-27/OXA cells transfected with si-circLRCH3 or/and in-miR-383-5p. **P*<0.05.

mimics strikingly decreased the luciferase activity of circLRCH3 WT but not that of circLRCH3 MUT (Fig. 4G,H). RIP analysis showed that circLRCH3 directly bound to miR-383-5p (Fig. 4I,J). Compared with the control group, miR-383-5p was significantly down-regulated in GC tissues (n=31) and cells (N87, AGS, MKN-45 and HGC-27) (Fig. 4K,L). Furthermore, miR-383-5p level was remarkably reduced in OXA-resistant GC tissues and cells relative to OXA-sensitive GC tissues and parental GC cells (Fig. 4M,N). Additionally, co-transfection of si-circLRCH3 and in-miR-383-5p abolished circLRCH3 knockdown-mediated increase in miR-383-5p level (Fig. 4O). These data indicated that circLRCH3 negatively regulated miR-383-5p expression.

Down-regulation of miR-383-5p overturned the effect of circLRCH3 on OXA resistance

To further explore whether circLRCH3 sponged miR-383-5p to play a role in OXA resistance, rescue experiments were implemented in MKN-45/OXA and HGC-27/OXA cells transfected with si-circLRCH3 or/and in-miR-383-5p. CCK-8 assay showed that deletion of circLRCH3 increased OXA sensitivity of MKN-45/OXA and HGC-27/OXA cells, while this impact was abrogated by repressing miR-383-5p (Fig. 5A,B). In addition, silencing of circLRCH3 hindered cell proliferation, migration and invasion and triggered cell apoptosis in MKN-45/OXA and HGC-27/OXA cells, whereas these effects were reversed by down-regulating miR-383-5p (Fig. 5C-H). Furthermore, the introduction of in-miR-383-5p restored the decrease in MRP1 and MDR1 levels caused by circLRCH3 knockdown (Fig. 5I,J). Overall, these data evidenced that depletion of circLRCH3 elevated OXA sensitivity of OXA-resistant GC cells by up-regulating miR-383-5p.

FGF7 served as a target for miR-383-5p

Next, TargetScan online database predicted that FGF7 was a possible target mRNA for miR-383-5p (Fig. 6A). Dual-luciferase reporter assay showed that miR-383-5p overexpression markedly reduced the luciferase activity of FGF7 3'UTR WT in MKN-45/OXA and HGC-27/OXA cells, but did not affect FGF7 3'UTR MUT (Fig. 6B,C). In addition, FGF7 mRNA and protein levels were prominently increased in GC tissues compared with normal tissues (Fig. 6D,E). Meanwhile,

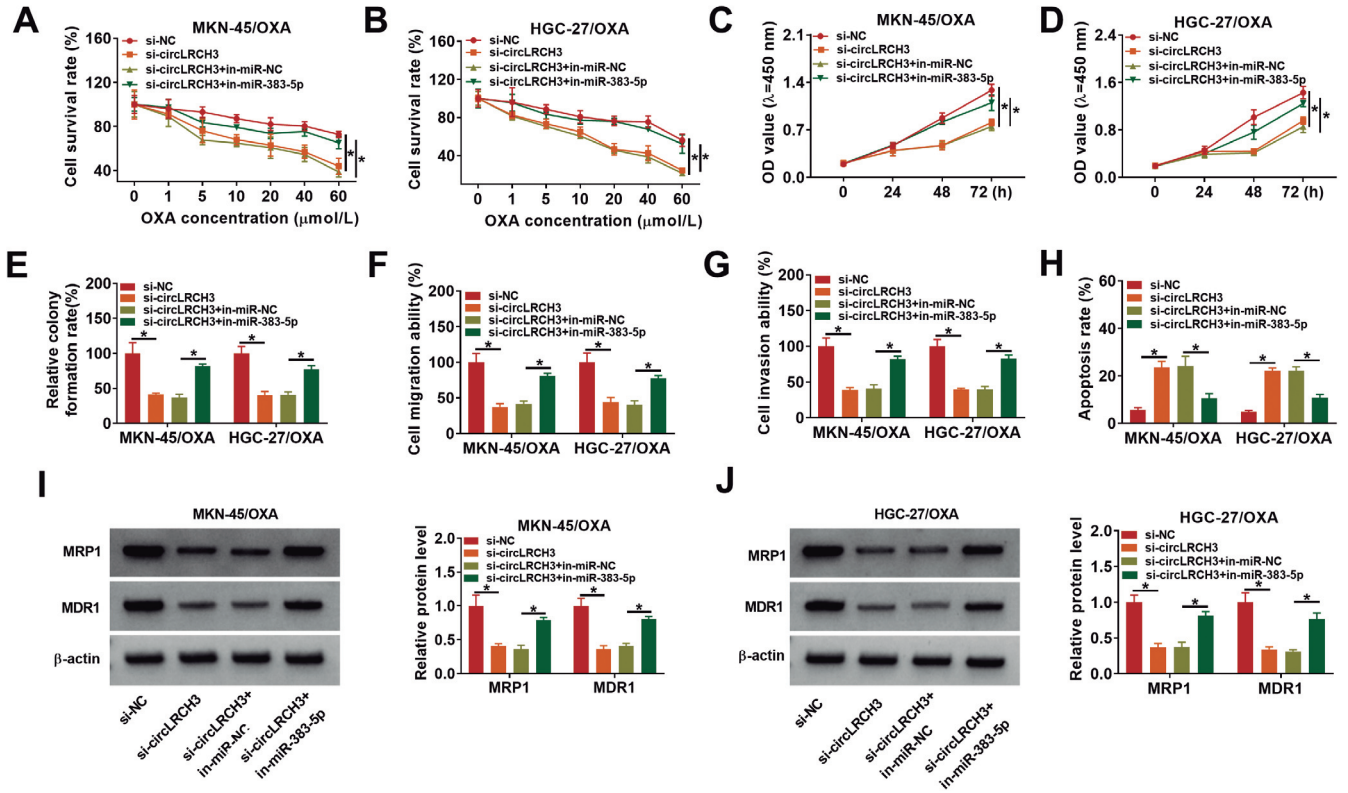

Fig. 5. Down-regulation of miR-383-5p overturned the effect of circLRCH3 on OXA resistance. MKN-45/OXA and HGC-27/OXA cells were introduced with si-NC, si-circLRCH3, si-circLRCH3+in-miR-NC or si-circLRCH3+in-miR-383-5p. Cell survival rate **(A and B)**, cell proliferation ability **(C-E)**, cell migration and invasion capabilities **(F and G)**, cell apoptosis **(H)**, and multidrug resistance protein (MRP1 and MDR1) levels **(I and J)** were examined using CCK-8, colony formation, transwell, flow cytometry and Western blot assays. **P*<0.05.

FGF7 protein level was markedly elevated in GC cells relative to GES-1 cells (Fig. 6F). Also, FGF7 mRNA and protein levels were remarkably higher in OXA-resistant GC tissues than in OXA-sensitive GC tissues (Fig. 6G,H). As expected, FGF7 protein expression was strikingly increased in MKN-45/OXA and HGC-27/OXA cells compared to parental GC cells (Fig. 6I). Furthermore, co-transfection of miR-383-5p and FGF7 restored the reduction in FGF7 protein level caused by miR-383-5p overexpression (Fig. 6J). Additionally, in-miR-383-5p transfection recovered the decrease in FGF7 protein level caused by circLRCH3 depletion (Fig. 6K). Collectively, these data indicated that circLRCH3 elevated FGF7 expression by absorbing miR-383-5p.

FGF7 reversed the repressive effect of miR-383-5p on OXA resistance

To further investigate whether miR-383-5p affected OXA resistance by modulating FGF7, rescue

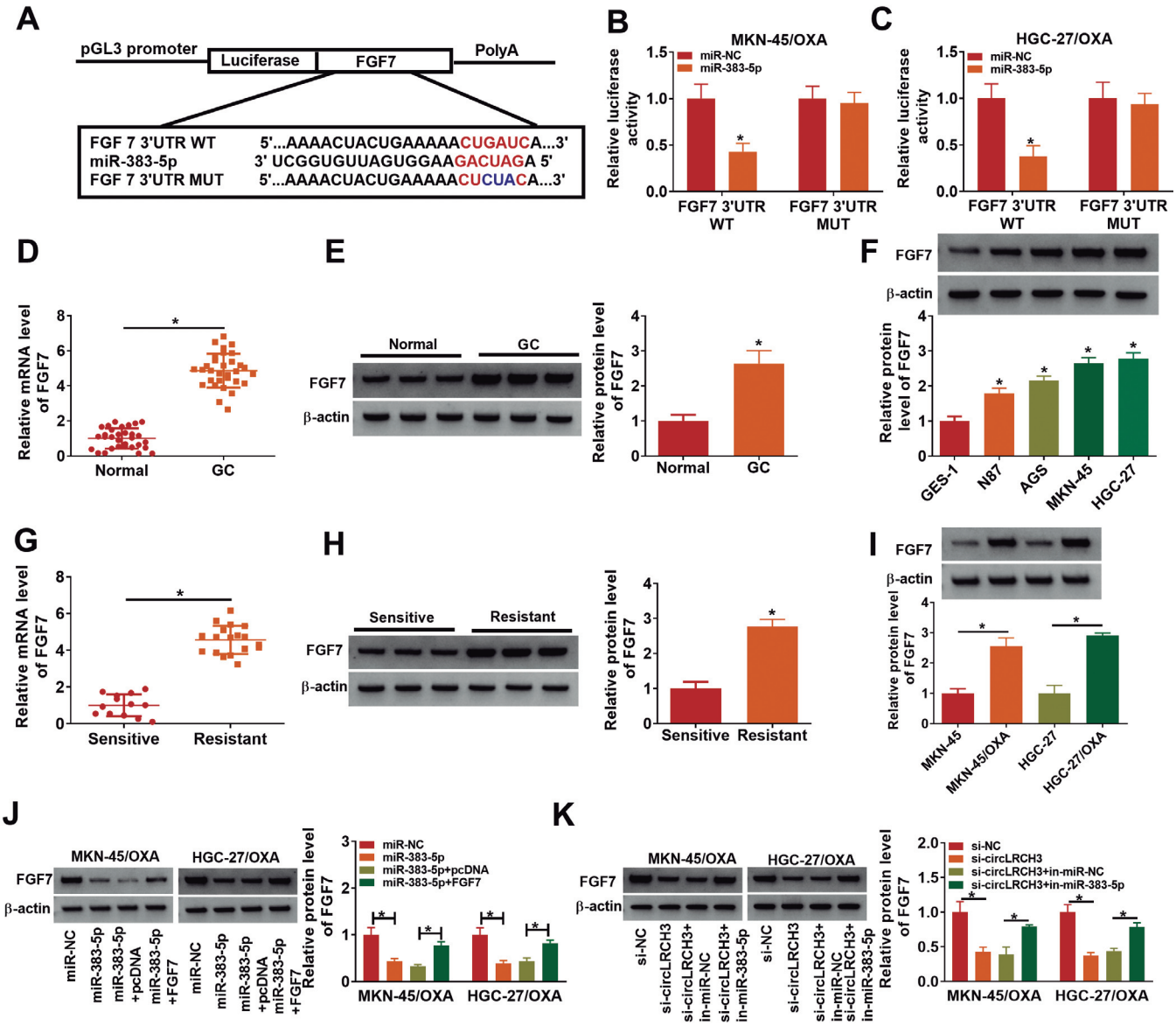

Fig. 6. FGF7 served as a target for miR-383-5p. **A.** The complementary sequence between miR-383-5p and FGF7 3'UTR was predicted by TargetScan. **B abd C.** Dual-luciferase reporter assay was used to confirm the targeting relationship of FGF7 and miR-383-5p. **D and E.** FGF7 mRNA and protein levels in GC (n=31) and normal tissues (n=31) were examined by qRT-PCR and Western blot. **F.** FGF7 protein level in GES-1 cells and GC cells was detected using Western blot. **G and H.** FGF7 mRNA and protein levels in OXA-sensitive (n=13) and OXA-resistant (n=18) GC patients were tested by qRT-PCR and Western blot. **I.** FGF7 expression in parental GC cells and OXA-resistant cells was examined using Western blot. **J.** After transfection with miR-NC, miR-383-5p, miR-383-5p+pcDNA or miR-383-5p+FGF7 in GC cells, FGF7 protein level was measured by Western blot. **K.** FGF7 protein level was detected by Western blot in OXA-resistant cells transfected with si-circLRCH3 or/and in-miR-383-5p. **P*<0.05.

experiments were carried out in MKN-45/OXA and HGC-27/OXA cells transfected with miR-383-5p or/and FGF7. As expected, CCK-8 analysis showed that FGF7 up-regulation partially abrogated the promotion of miR-383-5p overexpression on OXA sensitivity of MKN-45/OXA and HGC-27/OXA cells (Fig. 7A,B). Moreover, up-regulation of miR-383-5p suppressed cell proliferation, migration and invasion and induced cell apoptosis in MKN-45/OXA and HGC-27/OXA cells, while co-transfection of miR-383-5p and FGF7 overturned these effects (Fig. 7C-H). Besides, introduction of FGF7 overexpression vector restored the reduction of MRP1 and MDR1 levels caused by miR-383-5p up-regulation (Fig. 7I,J). Altogether, these data demonstrated that miR-383-5p enhanced OXA sensitivity of OXA-resistant GC cells by targeting FGF7.

CircLRCH3 silencing increased OXA sensitivity in vivo

To validate the effect of circLRCH3 on OXA resistance *in vivo*, HGC-27/OXA cells transfected with sh-NC or sh-circLRCH3 were inoculated into nude mice, followed by administration with 3 mg/kg OXA or PBS. As illustrated in Fig. 8A,B, circLRCH3 depletion or OXA treatment remarkably decreased tumor volume and weight, and simultaneous circLRCH3 knockdown and OXA administration enhanced these effects. Furthermore, qRT-PCR showed that circLRCH3 and FGF7 mRNA levels were significantly reduced, while miR-383-5p level was markedly increased in xenograft tumors after circLRCH3 silencing (Fig. 8C-E). Meanwhile, Western blot analysis suggested that circLRCH3 knockdown strikingly decreased FGF7 protein level (Fig. 8F). These data evidenced that silence of circLRCH3 enhanced OXA sensitivity *in vivo*.

Discussion

Chemoresistance leads to tumor recurrence and increased mortality by severely impairing the efficacy of chemotherapy (Sarmento-Ribeiro et al., 2019). In view of this, studying the underlying mechanism of chemoresistance is essential to develop new targets for chemotherapy. Besides, oxaliplatin is an effective drug for the treatment of advanced GC due to its lower toxicity and better tolerability (Montagnani et al., 2011). Several investigations have shown that circRNAs exert crucial effects on OXA resistance of GC. For example,

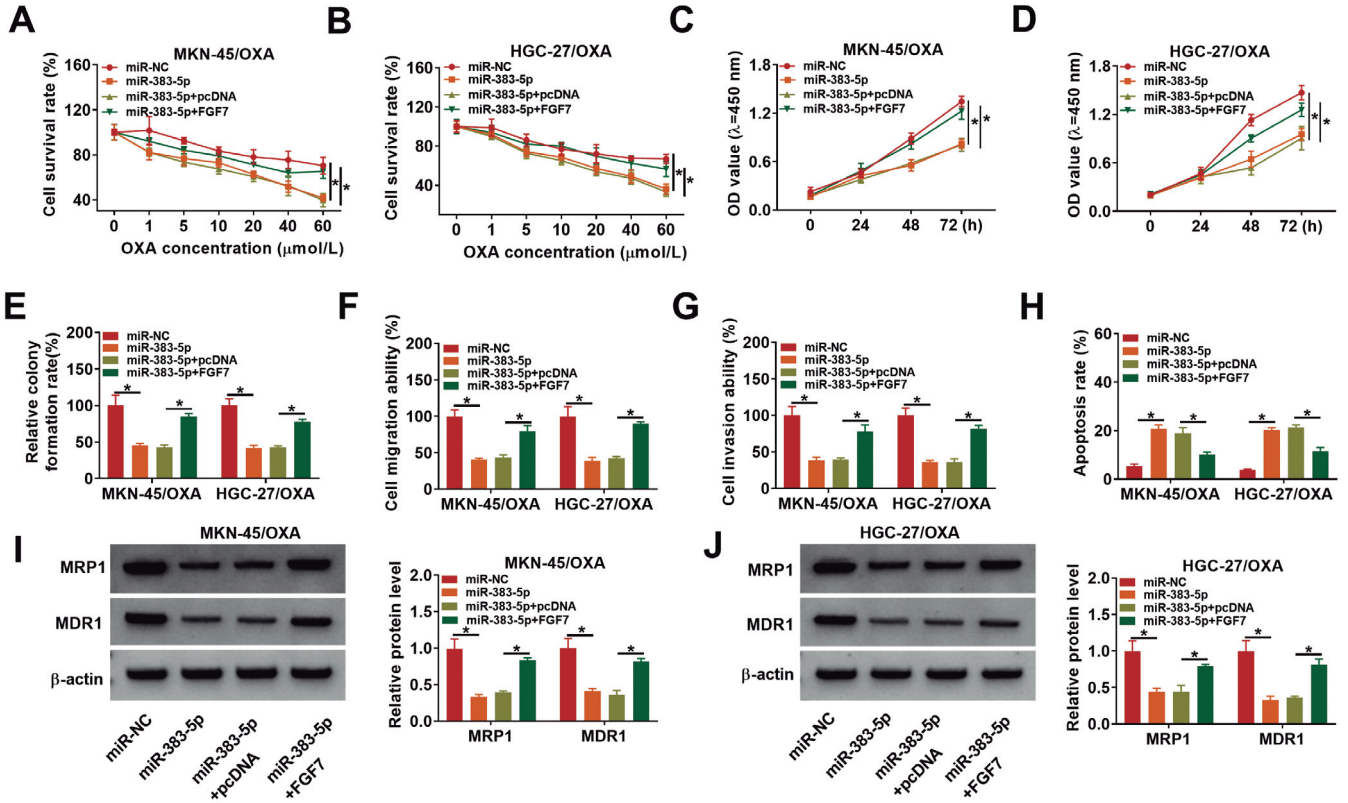

Fig. 7. FGF7 reversed the repressive effect of miR-383-5p on OXA resistance. MKN-45/OXA and HGC-27/OXA cells were introduced with miR-NC, miR-383-5p, miR-383-5p+pcDNA or miR-383-5p+FGF7. Cell survival rate **(A and B)**, cell proliferation ability **(C-E)**, cell migration and invasion abilities **(F and G)**, cell apoptosis **(H)**, and the levels of MRP1 and MDR1 **(I and J)** were assessed using CCK-8, colony formation, transwell, flow cytometry and Western blot assays. **P*<0.05.

up-regulation of circ_0001546 restrained the resistance of GC cells to oxaliplatin by absorbing miR-421 and activating ATM (Wu et al., 2020). Zhong et al., suggested that exosomal circ_0032821 triggered oxaliplatin resistance of GC cells by competitively binding to miR-515-5p and up-regulating SOX9 (Zhong et al., 2020). In the present research, we confirmed that circLRCH3 level was conspicuously boosted in OXA-resistant GC tissues and cells. In addition, the deletion of circLRCH3 improved the sensitivity of OXA-resistant GC cells to OXA.

In terms of mechanism, many studies have corroborated that circRNAs indirectly regulate gene expression by functioning as miRNA sponges (Liu et al., 2017). For instance, circMCTP2 decelerated GC progression and improved the sensitivity of GC to cisplatin by modulating the miR-99a-5p/MTMR3 pathway (Sun et al., 2020). Wang et al., discovered that circTMEM87A expedited the malignant growth of GC by decoying miR-142-5p to up-regulate ULK1 (Wang et al., 2020a). In this research, we predicted some miRNAs that might bind to circLRCH3 using bioinformatics software. In view of the differences in miRNA expression after overexpression of circLRCH3, we chose the most significantly down-regulated miR-383-5p as the follow-up research object. Besides, miR-383-5p has been identified as a suppressor of diverse cancers, including lung adenocarcinoma (Zhao et al., 2017), cervical cancer (Hu et al., 2019) and breast cancer (Zhang et al., 2020). Xu et al., revealed that miR-383-5p was beneficial to the overall survival of GC patients by suppressing HDAC9 (Xu et al., 2019). In the current research, we disclosed that miR-383-5p level was remarkably reduced in OXA-resistant GC tissues and cells. Furthermore, depletion of circLRCH3 attenuated OXA resistance by repressing miR-383-5p.

In addition, bioinformatics analysis and experimental verification showed that FGF7 directly interacted with miR-383-5p. Accumulating evidence has corroborated that miRNAs regulate translation repression or mRNA degradation by base-paring with mRNA 3'UTR, thereby affecting gene expression (Mo 2012). Fibroblast growth factors (FGFs) drive tumor progression by regulating tumor growth, angiogenesis

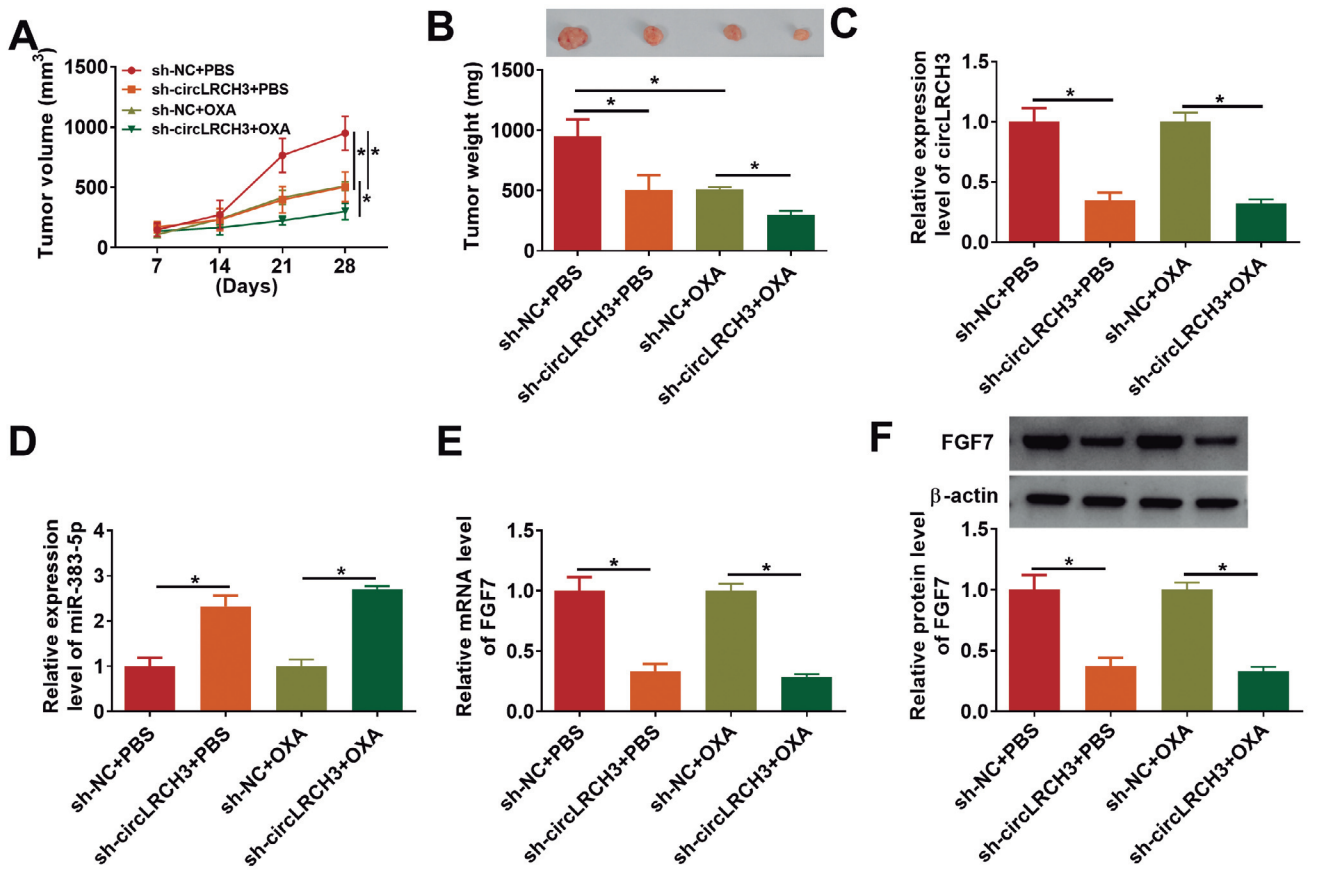

Fig. 7. CircLRCH3 silencing increased OXA sensitivity *in vivo*. HGC-27/OXA cells transfected with sh-NC or sh-circLRCH3 were inoculated into nude mice. **A.** After 7 days, the mice were injected with 3 mg/kg OXA or PBS every 7 days, and tumor volume was measured. **B.** After 28 days, the mice were killed and the xenografts were weighed. **C-F.** The levels of circLRCH3, miR-383-5p and FGF7 in tumor tissues were measured by qRT-PCR and Western blot. *P < 0.05.

and drug resistance (Presta et al., 2017). FGF7 (also called KGF) belongs to the FGFs family and regulates the differentiation and migration of different types of epithelial cells (Yen et al., 2014). In gastric carcinoma, lncRNA AFAP1-AS1 indirectly up-regulated FGF7 by combining with miR-155-5p to accelerate tumor progression (Ma et al., 2020). In this report, we revealed that FGF7 expression was conspicuously increased in GC. More importantly, miR-383-5p contributed to the OXA sensitivity of GC cells by targeting FGF7.

In summary, our research unveiled that circLRCH3 boosted oxaliplatin resistance in GC by modulating the miR-383-5p/FGF7 axis. These findings might provide an effective therapeutic target for GC chemotherapy.

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