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Inhibition of hsa_circ_0001313 (circCCDC66) induction enhances the radio-sensitivity of colon cancer cells via tumor suppressor miR-338-3p Effects of cicr_0001313 on colon cancer radio-sensitivity

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ABSTRACT

Circular RNA_0001313 (circ_0001313), also known as circCCDC66, is a novel circRNA that recently found to be upregulated in colon cancer tissues and promote colon cancer progression. However, the role of circ_0001313 in regulating radio-sensitivity of colon cancer and its molecular mechanism remain undetermined. Here we found circ_0001313 was significantly upregulated and miR-338-3p was downregulated in radio-resistant colon cancer tissues compared to radio-sensitive tissues. Radiation treatment in colon cells triggered a remarkable upregulation of circ_0001313 and a downregulation of miR-338-3p. Knockdown of circ_0001313 reduced cell viability, colony formation rate and increased caspase-3 activity in colon cancer cells under irradiation. Moreover, circ_0001313 act as a sponge for miR-338-3p in colon cancer cells. Furthermore, miR-338-3p could reverse the effects of circ_0001313 knockdown on cell viability, colony formation, and caspase-3 activity. These findings revealed that knockdown of circ_0001313 could induce radio-sensitivity of colon cancer cells by negatively regulating miR-338-3p.

1. Background

Colon cancer, characterized by uncontrolled cell growth in the large intestine, is one of the most common human malignancies in both males and females [1]. There are no obvious symptoms in the early stages of colon cancer, and the lack of effective diagnostic techniques, patients with colon cancer are frequently diagnosed at the advanced stages [2]. Although the conventional therapeutic strategies for colon cancer, mainly include chemotherapy, radiotherapy, and surgical resection have been improved [3], the effects of these therapeutic treatment are limited by the acquired or inherent drug- or radio-resistance [4]. Therefore, understanding the mechanism of chemo-therapy and radio-therapy resistance of colon cancer is important for the treatment of colon cancer patients.

Circular RNAs (circRNAs), a subfamily of RNA molecules produced by lariat-typed and spliceosome-mediated splicing, was recently reported to be widely expressed in eukaryotes [5,6]. According to its source, circRNAs could be divided into two major groups: exonic circRNAs (exclusively containing the exon sequences) mainly located in the cytoplasm; and exon intronic circRNAs (retaining intron sequences) largely remain in the nuclei [5]. Increasing evidences have suggested that circRNAs expression profiles in various cancerous samples were different from that in normal tissues and cell lines [7,8]. Moreover, circRNAs were reported to be involved in multiple cellular tumor-associated physiological processes, including tumor initiation, progression, and metastasis [9]. For instance, circ_BCRC-3 was found to be lowly expressed in the tissues and cell lines of bladder cancer, and it was demonstrated to inhibit the progression of bladder cancer through miR-182-5p/p27 pathway in the functional assays [10]. Circ_0052112 was reported to be significantly upregulated in breast cancer cells, and in the functional assays, it was revealed to promoting cancer cell invasion and migration by sponging miR-125a-5p [11]. Circ_0001313, also known as circ_CCDCC6, is a novel circRNA that identified to be significantly upregulated in colon cancer tissues by RNA sequencing, and it was demonstrated to promote colon cancer progression by Shaw-Jenq Tsai et al [12]. However, whether circ_0001313 plays a role in the

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radio-sensitivity of colon cancer cells remains undetermined.

In the present study, we found that circ_0001313 was highly expressed and miR-338-3p was lowly expressed in the tissue and cell samples of colon cancer than normal samples. Moreover, we focus on that circ_0001313 could modulate the radio-resistance of colon cancer by negatively regulating miR-338-3p expression, which may supply a potential therapeutic target for radio-resistant colon cancer patients.

2. Materials and methods

2.1. Colon cancer tissue samples and cell lines

Radio-sensitive colon cancer tissue samples (n = 42) were collected from radiosensitive patients who were diagnosed at the Department of General Surgery, Nanhua Hospital Affiliated to Nanhua University between 2016–2018, and never received chemo- or radiotherapy before. Radio-sensitivity was evaluated 6 months after radiotherapy through histological examination. Radio-resistant colon cancer tissue samples (n = 42) were recruited from the patients exposed to 40 Gy irradiation. All protocols were approved by the ethics committee of the Department of General Surgery, Nanhua Hospital Affiliated to Nanhua University. Written informed consents were obtained from every colon patient.

The colon cancer cell lines SW480 and SW620 were purchased from the American Type Culture Collection (ATCC, Manassas, USA). SW480 and SW620 were cultured in Eagles MEM (Sigma-Aldrich, St. Louis, MO) containing 10% fetal bovine serum (Invitrogen, UK) and 1% penicillin/streptomycin under the conditions of 95% air and 5% CO_2 at 37°C.

2.2. Irradiation

Treated SW480 and SW620 cells were seeded into 25-T culture flasks at a concentration of 2×10^4 cell/ml and then subjected to different doses of 6-MV X-ray (0, 2, 4, and 6 Gy) treatment by using a linear accelerator (Varian 2300EX, USA). Cells under different doses of irradiation were collected every 6 h within 24 h after irradiation.

2.3. RNA interference and transfection assay

To knockdown circ_0001313, two siRNA against circ_0001313 (si-Circ#1, and si-Circ#2) were synthesized, and qRT-PCR was utilized to examine their knockdown efficiency. The cDNA of circ_0001313 was amplified and inserted into pcDNA vector(contain the cyclization sequence) to establish pcDNA-circ_0001313, which was used to overexpress circ_0001313 expression. The empty pcDNA vector was used as controls. To block miR-338-3p expression in colon cells, miR-338-3p inhibitor was used. SW480 and SW620 cells cultured in 6-well plates and allowed to growth to 80% confluence. Subsequently, SW480 and SW620 cells were transfected with 50 nM si-Circ#1 + 2, 100 nM miR-338-3p inhibitor, or 50 ng pcDNA-circ_0001313 by Lipofectamine 2000 (Invitrogen) according to the guidance of manufacturers.

2.4. RNA extraction and quantitative real-time PCR (RT-PCR) assay

TRIzol reagent (Invitrogen) was utilized to extract total RNAs of colon tissues and cell lines, and RNA quality was evaluated by NanoDro2000c (Thermo Scientific, Waltham, USA). The synthesis of first-strand cDNA was carried out through a PrimerScript RT reagent Kit (Takara, Japan). RT-PCR analyses for circ_0001313 and miR-338-3p were performed using BestarTM qPCR MasterMix (#2043, DBI Bioscience, China) on an ABI7500 system (Applied Biosystems). The primers used in this study were listed in Table 1.

2.5. MTT assay

Cell viability of treated SW480 and SW620 cells was measured by MTT dye reduction method. Treated SW480 and SW620 cells were seeded into 96 well plates and maintained at 37°C for 24 h. subsequently, dye solution (30 μl) was added into each well and incubated for another 4 h. Reaction was abolished by adding 200 μl stop solution into each well and incubated for 1 h. The Infinite® 200 PRO (FPRO-T; Tecan, Seestrasse, Switzerland) was utilized to assess the absorbance at 570 nm.

Table 1
Primer sequences for qRT-PCR analysis.

Gene	Primer sequences
GAPDH	Forward: 5′-TATGATGATATCAAGAGGGTAGT-3′ Reverse: 5′-TGTATCCAAACTCATTGTCATAC-3′
Hsa_circ_0001313	Forward: 5′-GTATCTTGCCAGCTTTCTCCG-3' Reverse: 5′-TGCAGTTCTTGTTTCACAGC-3'
miR-338-3p	Forward: 5′-TGCGGTCCAGCATCAGTGAT-3' Reverse: 5′-CCAGTGCAGGGTCCGAGGT-3’
U6	Forward: 5'-CGCTTCACGAATTTGCGTGTCAT-3' Reverse: 5′-GCTTCGGCAGCACATATACTAAAAT-3'

2.6. Caspase-3 activity assay

The caspase-3 activities in treated SW480 and SW620 cells were detected through the caspase activity assay kit (Beyotime, Haimen, China) under the instructions of manufacturer. The absorbance value of samples was determined at 405 nm under a microplate reader (Infinite M200, Tecan, Switzerland).

2.7. Colony formation assay

Treated SW480 and SW620 cells (2×10^4 cells/well) were seeded into 6-well plates and cultured at 37 °C in an incubator under 5% CO_2 and 95% O_2 for two weeks. Subsequently, colonies were firstly fixed in methanol for 30 min and then stained with Giemsa for 15 min, and finally number of colonies was calculated under a microscope.

2.8. Plasmid construction and dual luciferase activity assay

The putative miR-338-3p binding sequence in wild-type (WT) and mutant (MUT) circ_0001313 were amplified using PCR and then inserted into the luciferase vector pmirGLO (Promega, USA). The recombinant vectors were named as circ_0001313 WT and circ_0001313 MUT, respectively. SW480 and SW620 cells (1×10^4 cells/well) were plated into 96-well plates and then co-transfected with circ_0001313 WT or circ_0001313 MUT and miR-338-3p mimics or miR-338-3p negative control (miR-control) by Lipofectamine 2000 (Invitrogen). The Dual-Luciferase Assay System (Promega) was utilized to examine the firefly and renilla luciferase activities. Renilla luciferase activity was normalized to Firefly luciferase activity.

2.9. RNA immunoprecipitation (RIP)

The EZ-Magna RIP kit (Millipore, Billerica, MA, USA) was utilized to carried out RIP assay. In brief, SW480 and SW620 cells were allowed to growth at 80% confluency, and then cells were incubated with NP-40 RIP lysis buffer containing PMSF (1 mM), DTT (1 mM), 1% protease inhibitor, and RNase inhibitor (200 U/ml). Subsequently, the RIP buffer, which supplemented with magnetic beads conjugated with Ago2 antibody, was added into 200 μl of whole cell lysates. Beads were rinsed with pre-cold NT2 buffer and incubated with Proteinase K (10 mg/ml) for 30 min to block the non-specific binding. Immunoprecipitated RNAs correlated with Ago2 were isolated by TRIzol reagent and then analyzed by qRT-PCR assay.

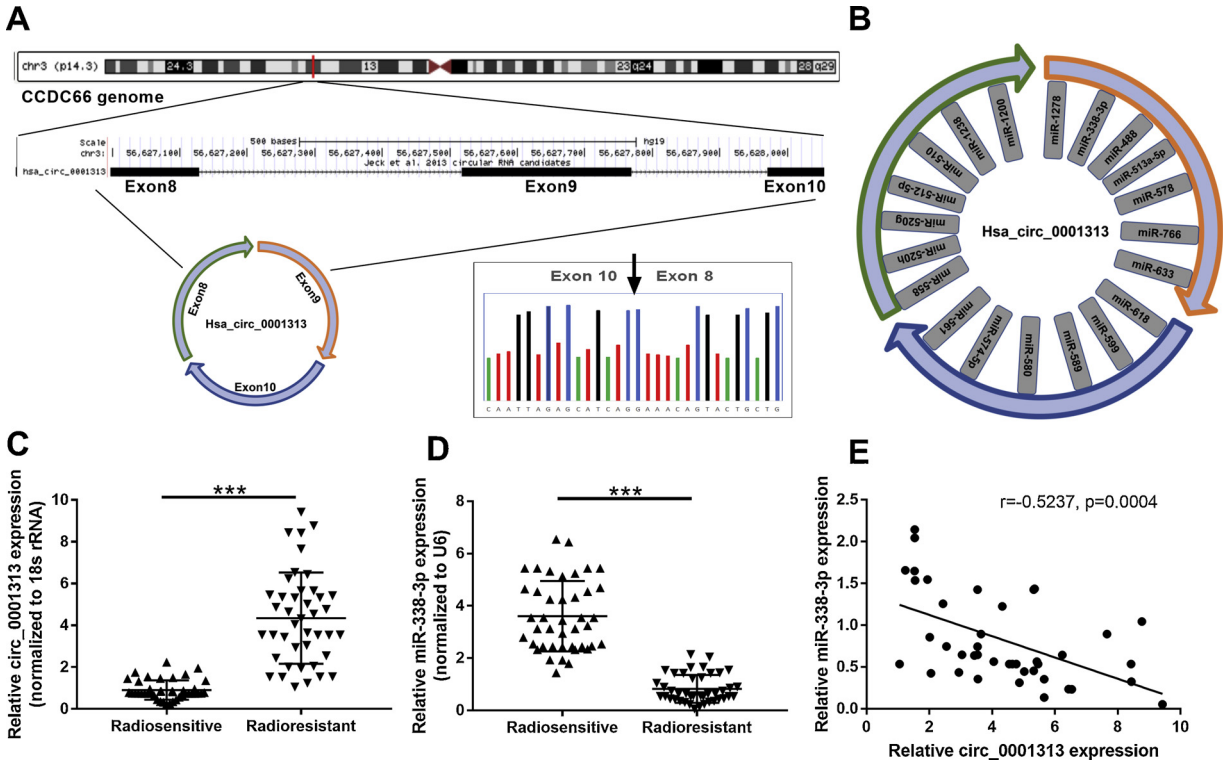

Fig. 1. circ_0001313 was increased and miR-338-3p was decreased in radio-resistant colon cancer tissues. (A) The schematic diagram of genomic location and formation of circ_0001313. **(B)** Predictive miRNAs of circ_0001313. **(C)** Circ_0001313 and **(D)** miR-338-3p expression in radio-sensitive and –resistant colon cancer tissues were measured by qRT-PCR (***$P <$ 0.001, vs radio-sensitive group). **(E)** Correlation between circ_0001313 and miR-338-3p expression.

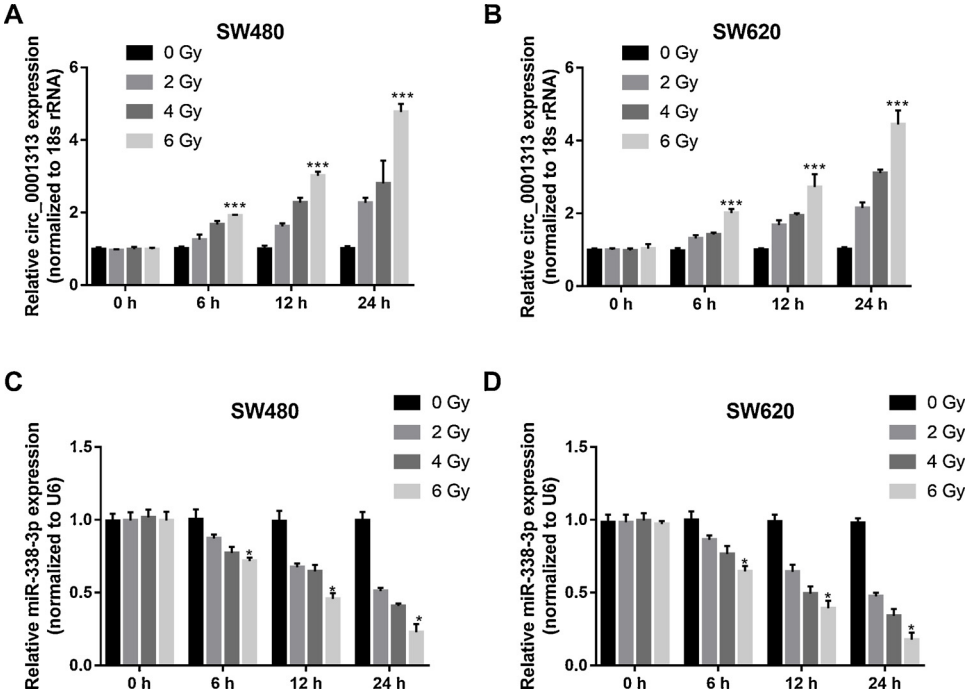

Fig. 2. The expression of circ_0001313 and miR-338-3p in response to different dosage of Gy irradiation in colon cancer cells. (A and B) The expression of circ_0001313 was detected in SW480 and SW620 cells under 0, 2, 4, and 6 Gy irradiation at 0, 6, 12 and 24 h (***$P <$ 0.001, vs 0 h group). **(C and D)** The expression of miR-338-3p was examined in SW480 and SW620 cells under 0, 2, 4, and 6 Gy irradiation at 0, 6, 12 and 24 h (***$P <$ 0.001, vs 0 h group).

2.10. Statistical analysis

Data were presented as mean ± SEM in the present study, and were analyzed by the Graphpad (Ver. Prism 7, GraphPad Prism Software, La Jolla, CA, USA). One-way analysis of variance was utilized to analyze the difference between groups, and P value less than 0.05 was considered significant.

3. Results

3.1. Circ_0001313 expression was increased and miR-338-3p was decreased in radio-resistant colon cancer tissues

Circ_0001313 arose from the CCDC66 gene and consisted of the head-to-tail splicing of exon 8–10 (Fig. 1A). Bioinformatics analysis

tools predicted 20 miRNAs that targeted by circ_0001313 (Fig. 1B). In order to explore the functions of circ_0001313 and miR-338-3p in regulating radio-sensitivity of colon cancer, expression of circ_0001313 and miR-338-3p were detected by qRT-PCR in radio-resistant and radio-sensitive colon cancer tissues. Results showed that circ_0001313 expression was significantly increased, whereas miR-338-3p expression was substantially decreased in the radio-resistant tissues than that in the radio-sensitive tissues (***$P < 0.001$, Fig. 1**C and D**). Moreover, we found that there was a negative correlation between circ_0001313 and miR-338-3p expression (Fig. 1E). These results suggested that circ_0001313 and miR-338-3p may be involved in the radio-sensitivity of colon cancer.

3.2. Circ_0001313 and miR-338-3p showed the opposed expression trend in response to irradiation in colon cancer

Since there was a negative correlation between circ_0001313 and miR-338-3p expression in colon cancer, we then examined their expression trends under different dosages of irradiation (0, 2, 4, and 6 Gy) in colon cancer cells. In both SW480 and SW620 cells, circ_0001313 expression was remarkably upregulated in response to 6 Gy irradiation in a time-dependent manner (***$P < 0.001$, Fig. 2**A and B**). Conversely, miR-338-3p expression showed a remarkable downregulation under 6 Gy exposure in both SW480 and SW620 cells in a time-dependent manner (*$P < 0.05$, Fig. 2**C and D**).

3.3. Circ_0001313 knockdown improved radio-sensitivity of colon cancer cells

To further investigate the biological roles of circ_0001313 in the radio-sensitivity of colon cancer, we blocked its expression by using specific siRNAs (si-Circ#1-2) in colon cancer cells. Results from qRT-PCR showed that circ_0001313 expression was significantly downregulated in SW480 and SW620 cells transfected with si-Circ#1 or si-Circ#2 compared with those cells transfected with si-control (*$P < 0.05$, Fig. 3**A and B**). Subsequently, si-Circ#1 + 2 transfected colon cancer cells were subjected to irradiation treatment under 0, 2, 4, and 6 Gy. MTT assay indicated that circ_0001313 knockdown dramatically reduced cell viability of both SW460 and SW620 cells compared with si-control treated colon cancer cells (*$P < 0.05$, Fig. 3**C and D**). Results from caspase-3 activity assay suggested that circ_0001313 knockdown significantly enhanced caspase-3 activity of both SW460 and SW620 cells compared with si-control trated colon cancer cells at the presence of 6 Gy irradiation of not (*$P < 0.05$, Fig. 3**E and F**). Meanwhile, colony formation assay revealed that circ_0001313 knockdown remarkably reduced the colony formation rate of SW460 and SW620 cells than that in si-control transfected colon cancer cell at the presence of 6 Gy irradiation of not (*$P < 0.05$, Fig. 3**G and H**).

Table 2
TargetScan miRNA prediction binding sites with hsa_circ_0001313.

Mirbase ID	Site Type	CircRNA Start	CircRNA End
hsa-miR-1200	7mer-m8	257	263
hsa-miR-1238	7mer-1a	109	115
hsa-miR-1278	7mer-m8	5	11
hsa-miR-338-3p	**7mer-1a**	**11**	**17**
hsa-miR-488	7mer-m8	22	28
hsa-miR-510	8mer-1a	229	236
hsa-miR-512-5p	7mer-1a	230	236
hsa-miR-513a-5p	7mer-1a	32	38
hsa-miR-520g	8mer-1a	160	167
hsa-miR-520h	8mer-1a	160	167
hsa-miR-558	7mer-1a	416	422
hsa-miR-561	7mer-m8	381	387
hsa-miR-574-5p	7mer-1a	254	260
hsa-miR-578	7mer-m8	39	45
hsa-miR-580	7mer-1a	189	195
hsa-miR-589	8mer-1a	187	194
hsa-miR-599	7mer-1a	219	225
hsa-miR-618	7mer-1a	176	182
hsa-miR-633	7mer-1a	344	350
hsa-miR-766	7mer-m8	12	18

3.4. Circ_0001313 negatively regulated miR-338-3p expression by directly binding

TargetScan miRNA prediction binding sites with hsa_circ_0001313 were shown in Table 2, and our results from bioinformatics prediction

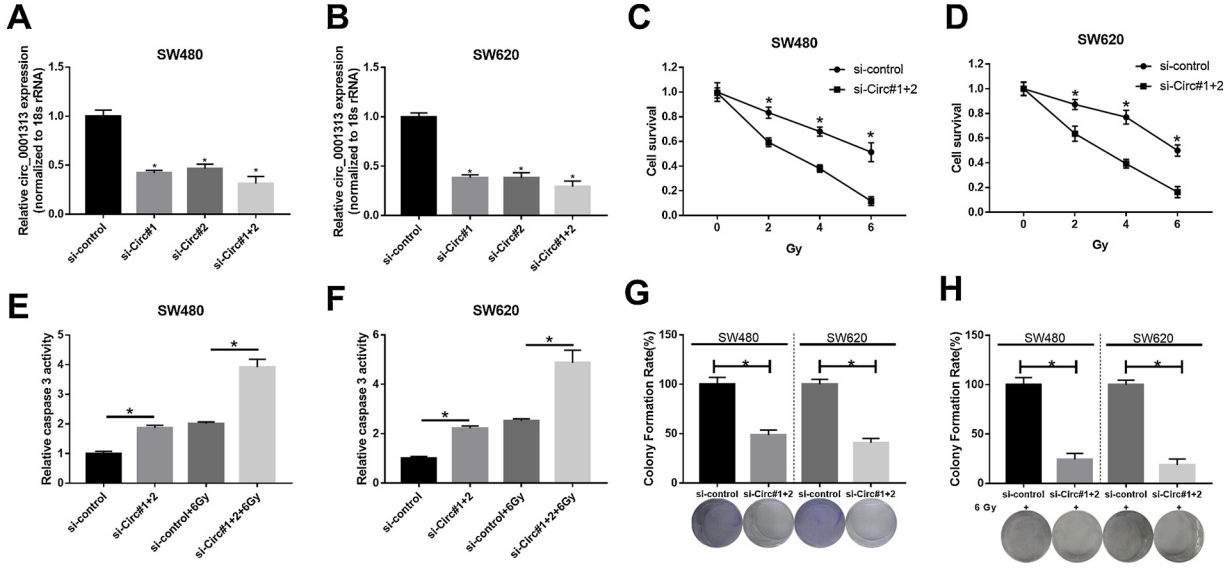

Fig. 3. Effects of circ_0001313 knockdown on proliferation and apoptosis of colon cancer cells under irradiation. (A and B) Knockdown efficiency of circ_0001313 siRNAs (si-Circ#1-3) in SW480 and SW620 cells were evaluated by qRT-PCR (*$P < 0.05$, vs si-control group). **(C and D)** Cell viability of SW480 and SW620 cells under different dosage of irradiation (0, 2, 4, and 6 Gy) were assessed by MTT assay at 48 h after circ_0001313 siRNAs transfection (*$P < 0.05$, vs si-control group). **(E and F)** Caspase-3 activity in circ_0001313 blocked SE480 and SW620 cells were measured by a caspase activity assay kit at the presence of 6 Gy irradiation or not (*$P < 0.05$, vs si-control group or si-control + 6Gy group). **(G and H)** Colony formation assay was performed to analyze the proliferation of SW460 and SW620 cells transfected with si-control or circ_0001313 siRNAs under the presence of 6 Gy irradiation or not (*$P < 0.05$, vs si-control group).

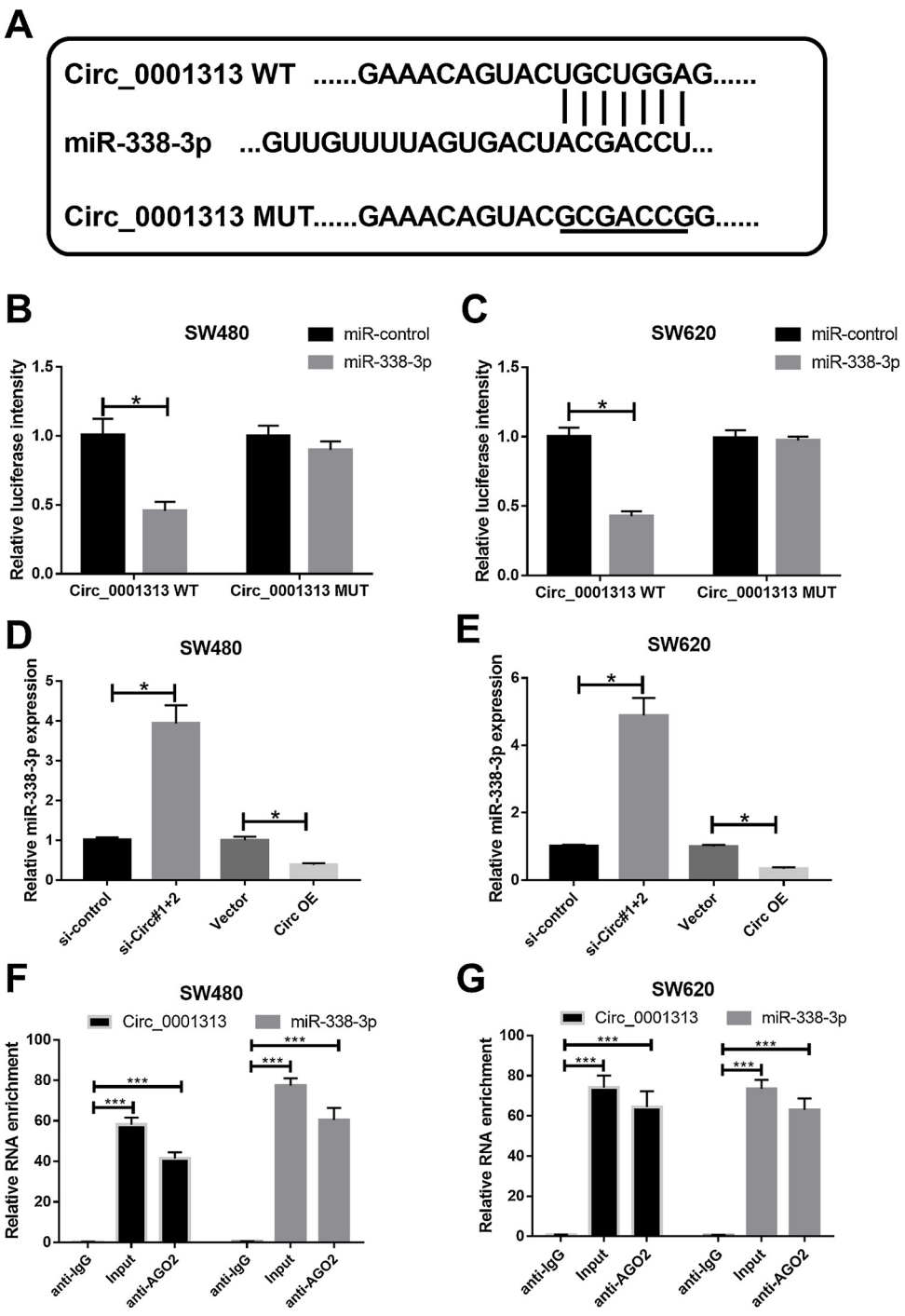

Fig. 4. Circ_0001313 act as a sponge for miR-338-3p and negatively regulated its expression. (A) Predictive binding sites between circ_0001313 and miR-338-3p. **(B and C)** Dual luciferase reporter assay was carried out to validate the interaction between circ_0001313 and miR-338-3p in SW460 and SW620 cells (*$P <$ 0.05, vs miR-control group). **(D and E)** Expression of miR-338-3p in SW460 and SW620 cells transfected with si-Circ_0001313, pcDNA-circ_0001313 or matched controls were measured by qRT-PCR (*$P <$ 0.05, vs si-control or vector). **(F and G)** RIP assay was carried out using SW460 and SW620 cell extracts with Ago2 antibody, qRT-PCR was used to detect the RNA levels in immunoprecipitates (***$P <$ 0.001, vs anti-IgG group).

showed that miR-338-3p may contain the putative binding sites with circ_0001331 (Fig. 4A). In the dual-luciferase reporter assay, compared with miR-control group, we found that miR-338-3p mimics could significantly attenuate the luciferase activity of both SW460 and SW620 cells driven by circ_0001313 WT, but not that driven by circ_0001313 MUT (*$P <$ 0.05, Fig. 4**B and C**). In order to further verify the interaction between circ_0001313 and miR-338-3p, expression of miR-338-3p was evaluated in SW480 and SW620 cells transfected with si-Circ#1 + 2, pcDNA-circ_0001313 or matched controls by qRT-PCR. Results showed that miR-338-3p expression was significantly upregulated in si-Circ#1 + 2 treated SW480 and SW620 cells, whereas its expression was remarkably downregulated in pcDNA-circ_0001313 treated SW480 and SW620 cells compared to matched control cells (*$P <$ 0.05, Fig. 4**D and E**). To explore whether circ_0001313 interacted with miR-338-3p through Ago2, RIP assay with Ago2 antibody was carried out using colon cancer cell extracts. After examined with qRT-PCR, we revealed that circ_0001313 and miR-338-3p were both markedly enriched in the Ago2 immunoprecipitates relative to input control in SW480 and SW620 cell extracts (***$P <$ 0.001, Fig. 4**F and G**).

3.5. MiR-338-3p inhibitor reversed the improved effects of circ_0001313 knockdown on radio-sensitivity of colon cancer cells

Based on the regulatory effects of circ_0001313 on miR-338-3p expression in colon cancer cells, we further evaluated the effects of miR-338-3p silencing on circ_0001313 function in colon cancer cells. Results from qRT-PCR showed that the si-Circ#1 + 2 induced

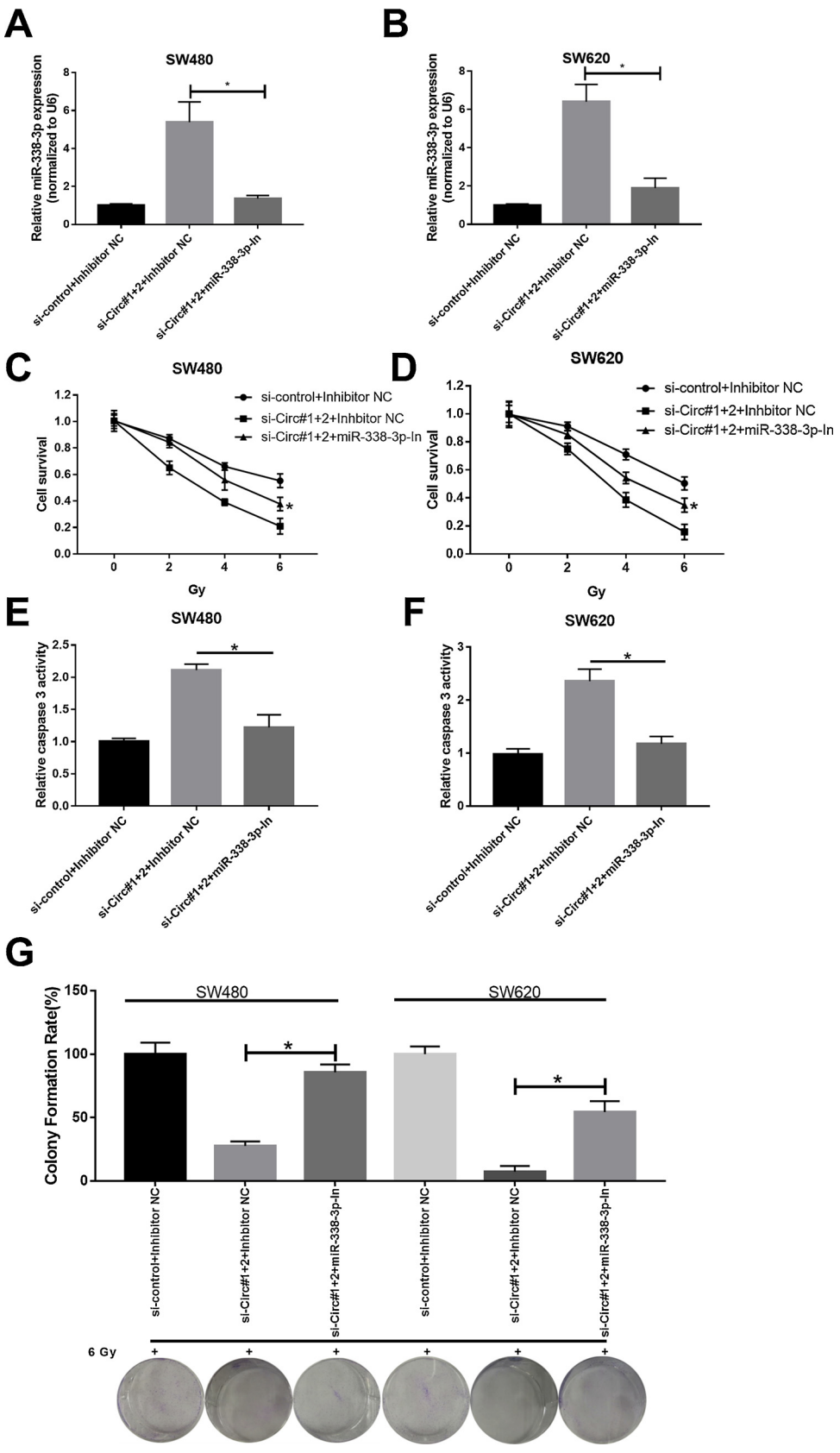

Fig. 5. Effects of miR-338-3p inhibitor on cell proliferation and apoptosis of circ_0001313 knockdown SW460 and SW620 cells. (A and B) Expression of miR-338-3p was detected by qRT-PCR in SW460 and SW620 cells treated with si-Circ_0001313 and miR-338-3p inhibitor (*P < 0.05, vs si-Circ_0001313 + inhibitor NC). **(C and D)** Cell viability of SW460 and SW620 cells treated with si-Circ_0001313 and miR-338-3p inhibitor were evaluated by MTT assay under different dosage of irradiation (*P < 0.05, vs si-Circ_0001313 + inhibitor NC). **(E and F)** Caspase-3 activity of SW460 and SW620 cells treated with si-Circ_0001313 and miR-338-3p inhibitor were measured (*P < 0.05, vs si-Circ_0001313 + inhibitor NC). **(G)** Colony formation assay was used to assess proliferation of SW460 and SW620 cells treated with si-Circ_0001313 and miR-338-3p inhibitor (*P < 0.05, vs si-Circ_0001313 + inhibitor NC).

upregulation of miR-338-3p was abolished by miR-338-3p inhibitor in SW480 and SW620 cells (*P < 0.05, Fig. 5**A and B**). In MTT assay, we found that si-Circ#1 + 2 induced reduction of cell viability under irradiation was reversed by the treatment of miR-338-3p inhibitor in SW480 and SW620 cells (*P < 0.05, Fig. 5**C and 5D**). The si-Circ#1 + 2 induced enhancement of caspase-3 in SW480 and SW620 cells abolished by the application of miR-338-3p inhibitor (*P < 0.05, Fig. 5**E and 5 F**). Meanwhile, results from colony formation showed

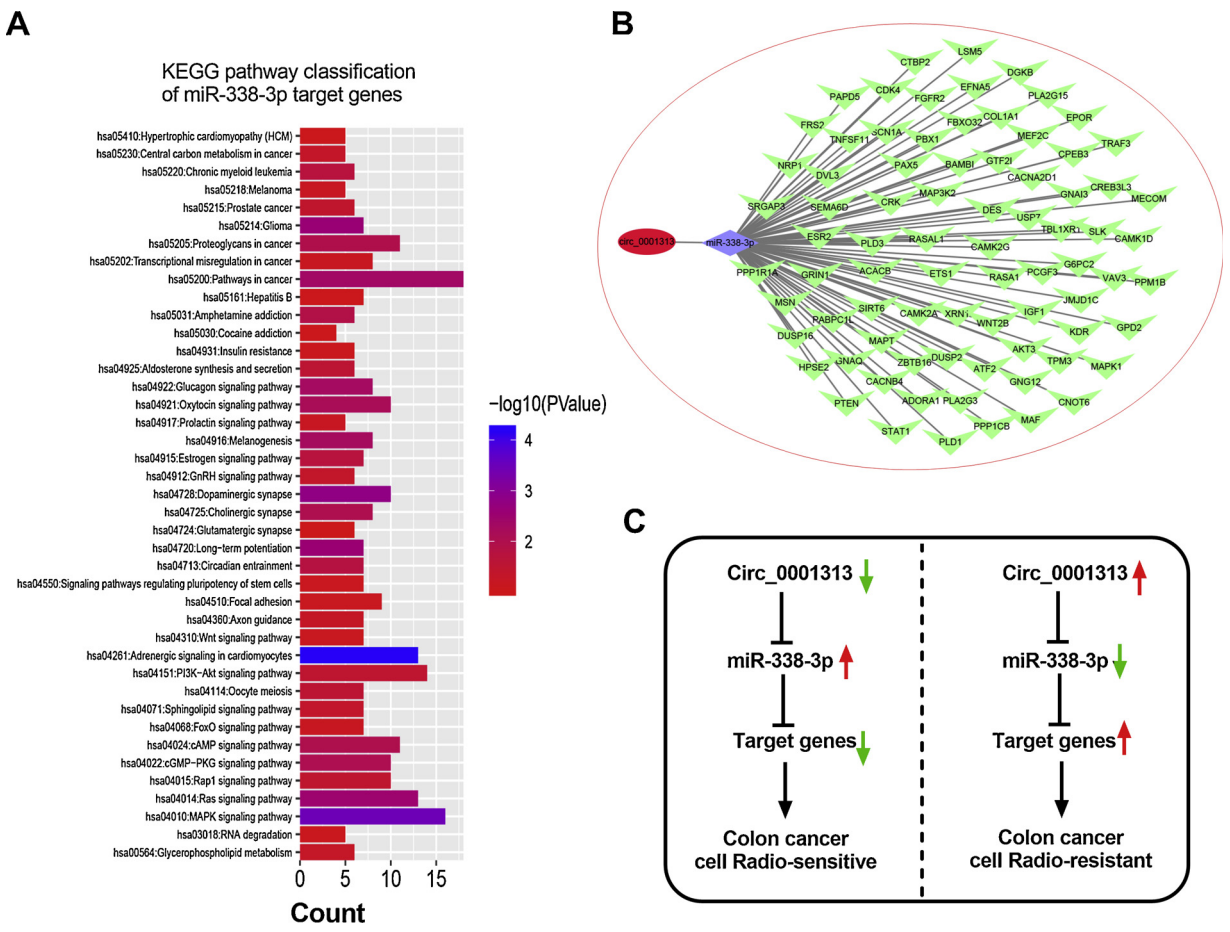

Fig. 6. Construction of miR-338-3p target gene network by KEGG analysis. (A) KEGG pathway enrichments of predicted target genes of miR-338-5p. **(B)** The ceRNA network of circ_0001313 within miR-338-3p and its targeted genes. **(C)** The schematic diagram of circ_0001313 effects on colon cancer radio-resistant.

that miR-338-3p inhibitor could reverse the si-Circ#1 + 2 induced reduction of colony formation rate in SW480 and SW620 cells (*P < 0.05, Fig. 5G).

3.6. MiR-338-5p-gene network and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment

KEGG pathway enrichments of miR-338-3p were performed to predict target genes, and "Hypertrophic cardiomyopathy (HCM, hsa05410)", "Central carbon metabolism in cancer (hsa05230)", "Chronic myeloid leukemia (hs05220)", "Melanoma (hsa05218)", "Prostate cancer (05,215)", and "Pathways in cancer (hsa05200)" were commonly top pathways by function prediction of miR-388-3p (Fig. 6A). Moreover, the target genes of miR-338-3p were mapped by using Cytoscape software (Fig. 6B). Taken together, our findings suggested that decreased circ_0001313 expression could upregulate miR-338-3p, which cause the downregulation of miR-338-3p target genes, resulting in the sensitivity of colon cancer cell (Fig. 6C). Conversely, increased circ_0001313 expression could reduce miR-338-3p, which cause the upregulation of miR-338-3p target genes, resulting in the resistance of colon cancer cell (Fig. 6C).

4. Discussion

Currently, radiotherapy is used as a standard pre-operative therapy strategy for multiple human cancers [13]. Evidences suggested that colon cancer patients with radiotherapy before standardized total mesorectal excision (TME) exhibited a higher survival rate than those with TME alone [14]. However, the radio-resistance of tumor cells remains to be the most fundamental barrier in the irradiation treatment of various human tumors, including colon cancer, resulting in an upregulation of tumor recurrence and reducing the therapeutic effects [15]. Previously studies have indicated that long non-coding RNAs (lncRNAs) and microRNAs (miRNAs) involved in the radiotherapy and chemotherapy resistance of various cancers, such as breast cancer, prostate cancer, and colon cancer [3,16,17]. However, to our best knowledge, few of literatures specifically evaluated the roles of circRNAs in the radio-resistance of cancers. Congying Xie et al. performed a circRNAs microarray in human radio-resistant esophageal cancer cell line and its parental cell line KYSE-150 in 2016, they identified 57 upregulated and 17 downregulated circRNAs, however, they have not evaluated the exact functions of corresponding circRNAs in radio-resistant esophageal cancer [18]. Circ_0001313 (circ_CCDC66) was recently identified to be significantly increased in colon cancer tissues by RNA sequencing, and it exhibited promotive effects on colon cancer progression [12]. In the present study, we further demonstrated that circ_0001313 expression was remarkably increased in the radio-resistant colon cancer tissues than that in the radio-sensitive tissues, and it was highly expressed in the colon cancer cells under irradiation exposure, indicating a role in radiotherapy resistance.

Recently, increasing studies have indicated that miRNAs play a critical role in multiple cancer cell radio-sensitivity, such as breast cancer, cervical cancer, and hepatocellular carcinoma [19–21]. MiR-338-3p was proposed as a tumor suppressor and it was frequently found to be decreased in various cancer types [22,23]. Previously studies have reported a significant role of miR-338-3p in the chemo-resistance of cancers. For instance, miR-338-3p expression was lower in the hepatocarcinoma tissues and cells, and overexpression of miR-338-3p

significantly reduced cell viability, promoted cell apoptosis, and enhanced the sensitivity of hepatocarcinoma cells to sorafenib [24]. However, whether miR-338-3p involved in the radio-resistance of cancer cells remains largely unknown. In the present study, we found that miR-338-3p was lowly expressed in the radio-resistant colon tissues and cells under irradiation.

Moreover, circRNAs was demonstrated to exhibit their functions by acting as miRNAs sponges in various biological processes [25,26]. Circ_0001313 was identified to sponge more than 99 miRNAs by Shaw-Jenq Tsai in 2018 [12]. In the present study, we revealed that circ_0001313 could also sponge miR-338-3p, and miR-338-3p mimics could reduce cell viability, suppress colony formation rate, and increase caspase-3 activity under irradiation in colon cancer cells, which were exacerbated by circ_0001313 knockdown, indicating that miR-338-3p might be a downstream regulator of circ_0001313.

5. Conclusion

In conclusion, circ_0001313 was highly expressed and miR-338-3p was lowly expressed in colon cancer tissues and cell lines under irradiation. Functional assays indicated that circ_0001313 knockdown improved radio-sensitivity of colon cells by upregulating miR-338-3p expression, providing a potential therapeutic target for radio-resistant colon cancer patients.

Conflict of interest

The authors declare that they have no competing interests.

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