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Knockdown of long non-coding RNA TINCR decreases radioresistance in colorectal cancer cells

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ABSTRACT

An increasing number of studies have revealed the role of long non-coding RNAs in cancer. However, the mechanisms of action and functional utility in colorectal cancer (CRC) have not been fully elucidated. Here we describe the functional role and potential mechanism of TINCR (terminal differentiation-induced non-coding RNA) in CRC. Firstly, TINCR was selected using sequencing analyses and the starBase database. Cell Counting Kit-8, scratch wound healing, and transwell assays revealed that TINCR inhibited proliferation and migration in SW620 and HTC116 cells. Intriguingly, TINCR expression was up-regulated in a radioresistant CRC cell line (SW620R). Although TINCR had no significant effects on SW620R cell proliferation or migration, knockdown of TINCR reduced the radioresistance, and its overexpression had opposite effects. We then focused on transcription factor 4 (TCF4) as it is downregulated in CRC and associated with increased stemness in tumors. We found that TINCR and TCF4 levels were positively related in SW620R cells. TINCR knockdown reduced sphere formation ability in SW620R cells. TINCR also suppressed the OCT4 and SOX2 stemness genes, despite having no effect on NANOG. The expression levels of these genes were substantially higher in SW620R than in SW620 cells. To further explore the mechanism of TINCR and radioresistance, miR-137 was analyzed as it targets TCF4. We firstly confirmed that TCF4 is a target of miR-137. We then identified that TINCR knockdown enhanced miR-137 expression in SW620R cells. Collectively, these findings suggest that TINCR knockdown inhibits TCF4 by regulating miR-137 expression.

1. Introduction

Colorectal cancer (CRC) is a leading cause of death from human malignant tumors [1]. Approximately 10–15% of patients with CRC have synchronous lung metastases [2]. Several chemotherapeutic agents such as 5-fluorouracil, oxaliplatin, and methotrexate have been used to treat CRC, but the outcome of most patients with CRC has not markedly improved [3]. Radiation therapy is an effective option to manage cancers including CRC [4,5]. However, radioresistance is often induced and is a major factor leading to the failure of radiotherapy and poor prognosis in CRC patients [6]. Intriguingly, CRC cell stemness has been hypothesized as being at least partially responsible for radioresistance in CRC [7]. Therefore, increasing radiotherapy efficacy by targeting factors involved in radioresistance appears to be an attractive strategy for CRC treatment.

Recently, researchers found that a group of long noncoding RNAs (lncRNAs) more than 200 nucleotides long regulate a host of physiologic functions such as proliferation, migration, invasion, and apoptosis in various cancers such as breast cancer [8], nasopharyngeal carcinoma [9], bladder cancer [10], gastric cancer [11] and non-small cell lung cancer [12]. Many studies have also shown that lncRNAs such as HOTAIR [13], UCA1 [14], HULC [15], ROR [16] and DNM3OS [17] have an impact on radioresistance in cancer cells.

Several lncRNAs have been associated with CRC and radiation therapy through literature reading, and we aimed to explore their differential expression. We found that TINCR was differentially expressed in radioresistant CRC cell lines. According to the UCSC database (http://genome.ucsc.edu/), TINCR is located at chr19:5,560,933-5,561,388. One study has suggested that its genetic variation contributes to CRC susceptibility and progression [18]. Another study

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showed that loss of TINCR expression promotes proliferation and metastasis [19], but little is known about the association between TINCR and radioresistance.

To explore the mechanism of TINCR in CRC, we searched for related literature and found that TINCR binds to miRNA-137 [20]. This small noncoding RNA frequently regulates gene expression by binding to 3′-UTRs [21,22]. miR-137 has been found to inhibit certain functions of colon cancer stem cells [23] and is closely related to chemoresistance [24]. There is growing evidence that a regulatory network composed of lncRNAs and miRNAs has significant roles in cancer [25], but the association between TINCR and miR-137 in CRC cells is unclear. More importantly, miR-137 has been identified to suppress CRC cell proliferation, migration, and invasion by targeting TCF4 [26]. Interestingly, researchers found that TCF4 played different functions in a wide range of CRC cases [27], and a genome-wide RNA-mediated interference (RNAi) screen identified TCF4 as a transcriptional repressor that is partly responsible for decreased Wnt pathway signaling and restricted CRC cell growth [28]. Based on this evidence, we investigated the association between TINCR and TCF4. It is tempting to speculate that TINCR could influence stemness features together with TCF4 to induce radioresistance in CRC cells; the potential relationship between TINCR and miR-137 may also influence other biological processes.

In the present study, TINCR was investigated to determine its functional roles in proliferation and migration, and particularly its effects on radioresistance in CRC cells. The links among TINCR, TCF4, and miR-137 were analyzed to identify their potential mechanisms of inducing radioresistance in CRC cells.

2. Materials and methods

2.1. Cell culture

The SW620 and HTC116 human CRC cell lines were obtained from ATCC (Manassas, VA, USA) and maintained in Dulbecco's modified Eagle's medium (DMEM; Gibco, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum (FBS; Invitrogen, Carlsbad, CA, USA), 100 U/mL penicillin (Sigma, St. Louis, MO, USA), and 100 μg/mL streptomycin (Sigma) in a humidified atmosphere of 5% (v/v) CO_2 and 95% air at 37 °C.

2.2. Establishment of a radioresistant cell line

The cells were first grown to approximately 50% confluence in 25-cm^2 culture flasks. Cells were treated with 2 Gy of X-ray irradiation (120 kV and 3.5 mA for 4 min with a 0.5-mm A1 filter) using an X-ray generator (M-150 WE: SOFTEX Co., Tokyo, Japan) and then returned to the incubator. When reached approximately 90% confluence, the cells were digested using trypsin and cultured into new flasks. When they reached approximately 50% confluence, the cells were again irradiated (second fraction). We irradiated cells with fractionated X-ray until the total doses reached 60 Gy. For all assays on irradiated cells, there was at least a 2-week interval between the last 2 Gy of X-ray irradiation and the experiment.

2.3. Cell transfection

A fragment of TINCR cDNA was cloned into a pcDNA3.1 vector at the BamHI-EcoRI sites (named as pcDNA TINCR, pcDNA). Then pcDNA TINCR or pcDNA was separately transfected into cells using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer's instructions. Small interfering RNAs (siRNAs) for TINCR and negative control (NC) siRNAs were purchased from Ribobio (Guangzhou, China). The siRNA target sequences were as follows: TINCR-si1: 5′-GCAGAGT CATCACTACCT

T-3′ (sense) and TINCR-si2: 5′−CCACCCATTTCTTAAGCCT-3′ (sense). Cells were cultured for approximately 18 h before transfection. siRNA transfection was performed using the X-tremeGENE transfection reagent (Roche, Basel, Switzerland) in accordance with the manufacturer's instructions. At 48 h after transfection, cells were harvested and then prepared for the following assays. The transfection efficacy of cells was shown in Figure S1, S2, S3.

2.4. RNA extraction and real-time polymerase chain reaction (qPCR)

Total RNA were extracted from CRC cells using the TRIzol Total RNA Reagent (Invitrogen) following the manufacturer's protocol. cDNA synthesis was performed with 2 μg of total RNA using the RevertAidTM H Minus First Strand cDNA Synthesis Kit (Takara, Ohtsu, Japan). The following sequences were used: TINCR forward: 5′-TGTGGCCCAAACT CAGGGATACAT-3′, TINCR reverse: 5′-AGATGACAGTGGC

TGGAGTTGTCA-3′; miR-137 forward: 5′−CCATTCATTCGTTATTG CTTAAGA-3′, miR-137 reverse: 5′-TATGCTTGTTCTCGTCTCTGT GTC-3′; TCF4 forward: 5′-CGGCGGTGGAGGGGATGAC-3′, TCF4 reverse: 5′-GGCCGCTTCTTCCAAACTT

TCC-3′; glyceraldehyde 3-phosphate dehydrogenase (GAPDH) forward: 5′-ACGGATTTGGTCGTATTGGGCG-3′, GAPDH reverse: 5′-GCTC CTGGAAGAT

GGTGATGGG-3′. GAPDH was used for normalization.

qPCR assays were performed using the SYBR PrimeScript Real-Time Quantitative PCR kit (Takara, Ohtsu, Japan) with an Applied Biosystems 7500 Fluorescent Quantitative PCR System (Applied Biosystems, Foster City, CA, USA). The reaction mixtures started at 95 °C for 30 s, followed by 40 amplification cycles of 95 °C for 5 s, and 60 °C for 34 s. Gene expression quantification was performed using the ΔΔCT calculation with CT as the threshold cycle.

2.5. Cell proliferation assay

The proliferative ability of transfected cells was assessed using a Cell Counting Kit-8 (CCK-8) assay (Dojindo Molecular Technologies, Inc., Kumamoto, Japan), following the manufacturer's protocol. Cells were seeded at a density of 3000 cells/well in 96-well culture plates and cultured for 18 h prior to transfection. Subsequently, cells were transfected with pcDNA or pcDNA TINCR. Lipofectamine 2000 was used at a density of 0.3 μL/well, and the corresponding RNA was used at 3 pmoL/well. The culture medium was replaced with DMEM containing 10% CCK-8 solution at 48 h post-transfection. Each group was repeated three times independently. At 0–4 days post-transfection, optical density was measured at a wavelength of 450 nm using a Thermo Scientific Multiskan FC (Thermo Fisher Scientific, Waltham, MA, USA). Growth curves were produced based on the calculated number of viable cells.

2.6. Scratch wound healing assay

Cells were seeded in 24-well culture plates at a density of 1×10^5 cells/well and cultured for 18 h prior to transfection. Uniform wounds were created in transfected cells, and then cells were cultured in serum-free medium. Images of wound closure were viewed at 40× magnification using a microscope and photographed 0, 24, and 48 h after scratching.

2.7. Transwell assay

To determine cell metastasis, transfected cells were plated in serum-free medium in the top chamber with a non-coated membrane (Corning, Corning, NY, USA), while medium containing 20% FBS was placed in the lower well. After a 24-h incubation, the cells were fixed using 4% formaldehyde for 15 min, stained with crystal violet dye for 20 min, and photographed under 100× magnification using a microscope. Experiments were carried out at least three times.

2.8. Bioinformatics analysis

The BiBiserv2 database (https://bibiserv.cebitec.uni-bielefeld.de/) was used to assess the relationship between TINCR and miR-137. The starBase database (http://starbase.sysu.edu.cn/) was used to assess the expression of TINCR and TCF4 in CRC and the correlation between the expression of TINCR, miR-137 and TCF4 in CRC.

2.9. Dual-luciferase assay

A pmirGLO luciferase vector was purchased from Promega (Madison, WI, USA). TINCR DNA was obtained through PCR using cDNA, Pfu DNA polymerase, and synthetic oligonucleotide primers incorporating restriction sites. PCR products were ligated into the pmirGLO luciferase vector in accordance with the manufacturer's instructions and then sequenced for confirmation. We constructed wild-type TCF4 (TCF4-WT) reporter plasmids, or mutant reporter plasmids (TCF4-MUT) with pmirGLO luciferase vectors (Promega). SW620R cells were transfected with the luciferase reporter plasmid including wild-type or mutant plasmids. The cells were then transfected with an miR-137 mimic or miRNA negative control (miR-NC) using Lipofectamine 2000 (Invitrogen) in accordance with the manufacturer's instructions. Luciferase activity was then measured consecutively using the dual luciferase assay system (Promega).

2.10. Tumor sphere formation assay

Cells were digested using 0.25% trypsin (Sigma, MA, USA); washed twice using calcium/magnesium-free phosphate-buffered saline (PBS); suspended in serum-free DMEM-F12 medium supplemented with 1% penicillin-streptomycin solution, 20 ng/mL epidermal growth factor, 10 ng/mL basic fibroblast growth factor, and 2% B27; and seeded in a low-attachment T25 flask (Corning, NY, USA). Cells were collected after 5 days, and then digested using Accutase enzyme and seeded into 96-well plates (100 cells/well). Cells were cultivated for 7–14 days, and the spheres were then assessed using a microscope.

2.11. Clonogenic survival assay

SW620 or SW620R cells were plated in 100-mm dishes and irradiated using 0, 2, 4, 6, and 8 Gy. After irradiation, cells were cultured for 2 weeks, fixed in 4% formaldehyde for 15 min, and then in absolute ethanol containing 1% methyl violet for 20 min. Finally, the number of surviving colonies was counted (defined as a colony with > 50 cells). Plating efficiency (PE) was measured for each cell line. Survival fractions (SFs) were calculated using the equation SF = colonies counted/(cells seeded × PE) × 100%. All assays were independently performed in triplicate.

2.12. Western blot assay

Cells were collected, washed in 3 mL pre-cooled PBS, placed, on ice and then boiled in sodium dodecyl sulfate sample buffer. Proteins samples were resolved through electrophoresis on a 10% polyacrylamide gel and then transferred to polyvinylidene fluoride (PVDF) membranes. The membranes were incubated for 80 min with 5% skimmed milk powder at room temperature and then incubated with primary antibodies against TINCR, TCF4, or GAPDH (ABclonal Biotechnology, Wuhan, China) at 4 °C overnight. Membranes were then incubated with corresponding secondary goat anti-rabbit and goat anti-mouse antibodies (ABclonal Biotechnology) at 37 °C for 1 h. PVDF membranes were developed using an ECL chemiluminescent reagent. Finally, the protein bands were measured using the Bio-Rad Gel Doc XR + system (Bio-Rad, Hercules, CA, USA).

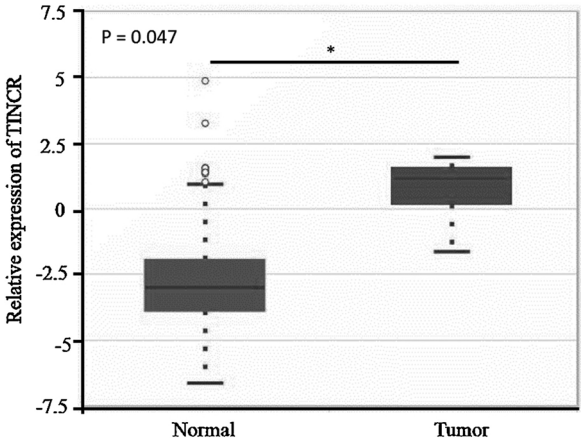

Fig. 1. Abnormal expression of TINCR in CRC. The starBase database (http://starbase.sysu.edu.cn/) suggested that TINCR was differentially expressed in CRC.

2.13. Statistical analyses

All *in vitro* experiments were performed in triplicate. Data are presented as mean ± SD. Comparisons between two groups were analyzed using Student's t-test. One-way or two-way analysis of variance were used for the comparison of more than two groups. All statistical calculations and analyses were performed using OriginPro software (OriginLab Corporation, Northampton, MA, USA). $P < 0.05$ was considered statistically significant.

3. Results

3.1. Abnormal TINCR expression in CRC

Based on the sequencing analysis findings, we assessed TINCR expression. Furthermore, we collected samples and assessed its differential expression **(Figure S4)**. The results suggested that TINCR may play a pivotal role in CRC. The starBase database (http://starbase.sysu.edu.cn/) suggested that TINCR is differentially expressed in CRC (Fig. 1).

3.2. TINCR inhibited CRC cell proliferation and migration

To assess the impact of TINCR on the proliferation and migration of CRC cells, CCK-8, scratch wound healing, and transwell assays were conducted in SW620 and HTC116 cells. The results suggested that the proliferation and migration of both cell lines were decreased in the pcDNA TINCR group compared with the control group (Fig. 2A–F). The data indicate that TINCR inhibits proliferation and migration ability in CRC cell lines.

3.3. Knockdown of TINCR decreases the radioresistance of SW620 cells

We confirmed establishment of a stable radioresistant SW620 cell line (named SW620R). Afterwards, we examined the TINCR expression in SW620R. Interestingly, it was higher in SW620R cells (Fig. 3A). To examine the functional role of TINCR in radioresistant CRC cells, we performed CCK-8, scratch wound healing, and transwell assays. TINCR overexpression or knockdown had no significant effect on proliferation or migration in SW620R cells compared with SW620 cells (Fig. 3B–E). Subsequently, overexpression and knockdown experiments were conducted to determine the role of TINCR in radioresistance. TINCR knockdown reduced the resistance to ionizing radiation compared with control cells, while its overexpression enhanced the resistance (Fig. 3F, G). These findings suggest that knockdown of TINCR decreases the

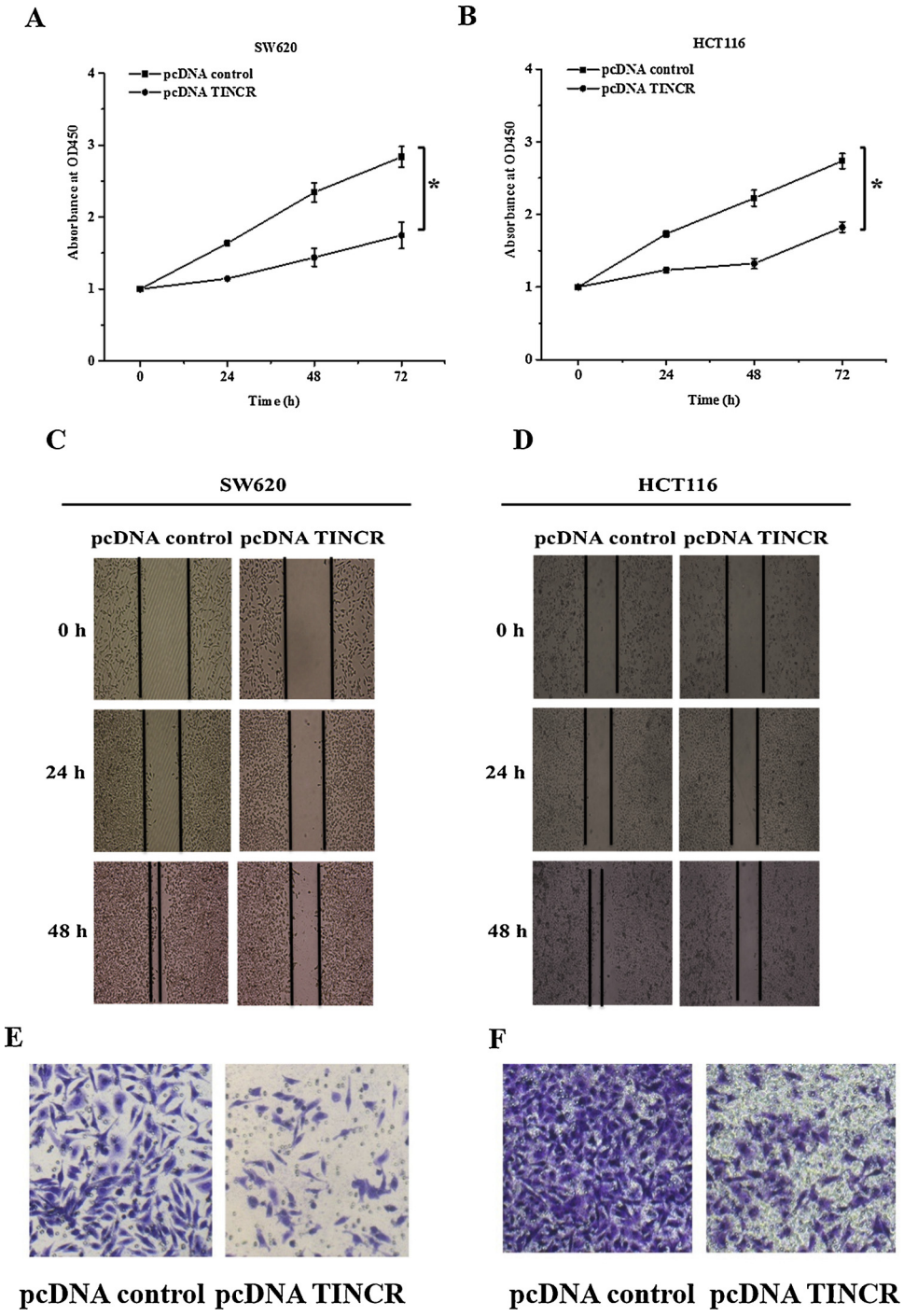

Fig. 2. TINCR inhibited CRC cell proliferation and migration *in vitro*. (**A**)–(**F**) CCK-8, scratch wound healing, and transwell assays showed that TINCR inhibited proliferation and migration in SW620 and HCT116 cells. Error bars represent the SDs obtained from three independent experiments, and the data are shown as mean ± SD. CCK-8, Cell Counting Kit-8; NC, negative control; si, small interfering RNA; * $P < 0.05$.

radioresistance of SW620 cells.

3.4. Knockdown of TINCR suppresses the stemness of radioresistant SW620 cells

To determine the cause of the induced radioresistance in SW620 cells, we focused on TCF4 as it has been reported to influence stem features [29,30]. Using the starBase database (http://starbase.sysu.edu.cn/), we found that TCF4 was downregulated in CRC cells (Fig. 4A), and a positive relationship between TINCR and TCF4 has been identified (Fig. 4B). To further verify the relationship between TINCR and TCF4 in radioresistant CRC cells, we conducted a knockdown experiment in SW620R cells. The results suggested that TCF4 expression decreased after TINCR knockdown (Fig. 4C). TINCR-si1 was administered to cells prior to western blot assays. The results showed reduced TCF4 protein levels in the TINCR-si1 group compared with the control group (Fig. 4D). Knockdown of TINCR inhibited sphere formation ability in SW620R cells (Fig. 4E). The expression levels of stemness-related genes were measured with qPCR. Of the two effective siRNAs, TINCR-si1 was selected for the knockdown experiment. The expression levels of OCT4 and SOX2 were lower in the TINCR-si1 group than in control cells, but no significant differences in NANOG expression were observed

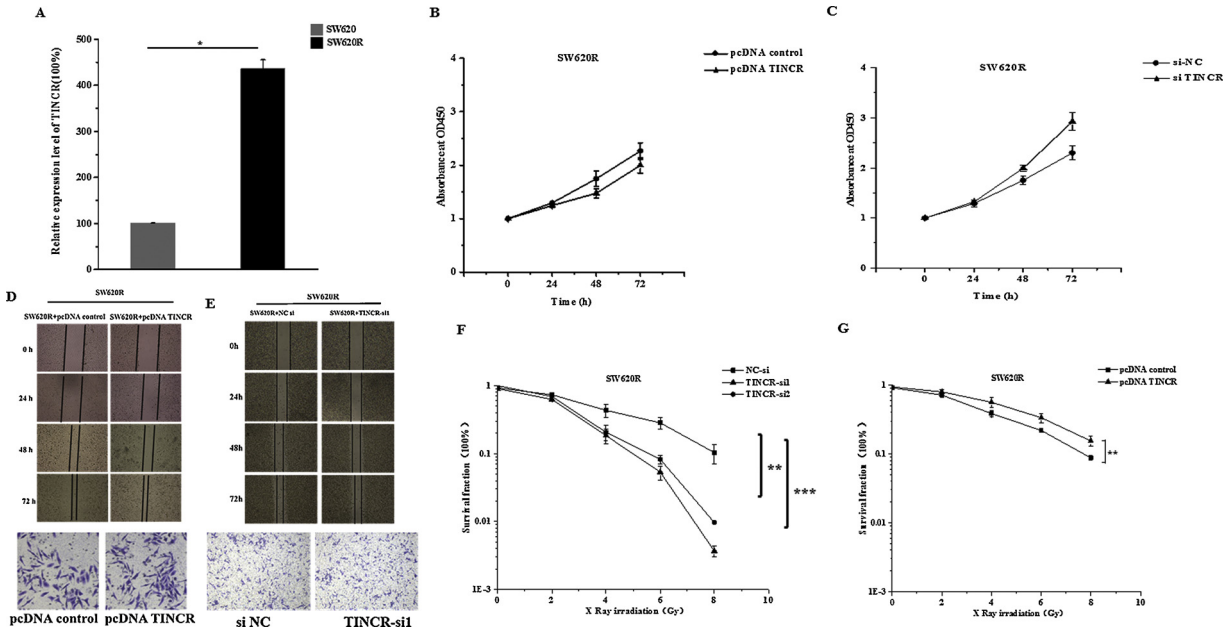

Fig. 3. Knockdown of TINCR decreases the radioresistance of SW620 cells. (**A**) TINCR expression was higher in SW620R cells than SW620 cells. (**B**–(E) CCK-8, scratch wound healing, and transwell assays showed that TINCR overexpression and knockdown had no effects on SW620R cell proliferation or migration. (**F**) (**G**) Clonogenic survival assays showed that reduced TINCR expression diminished resistance to ionizing radiation compared with control cells, its overexpression showed opposite effects. Error bars represent the SDs obtained from three independent experiments, and the data are shown as mean ± SD. CCK-8, Cell Counting Kit-8; NC, negative control; si, small interfering RNA; *, $P < 0.05$; **, $P < 0.01$; ***, $P < 0.001$.

(Fig. 4F). In addition, OCT4, SOX2, and NANOG RNA levels were lower in SW620 cells compared to SW620R cells (Fig. 4G). Our findings suggest that knockdown of TINCR suppresses stemness in radioresistant SW620 cells.

3.5. Knockdown of TINCR inhibits TCF4 by regulating miR-137 expression

To further explore the potential link between radioresistance and stemness in radioresistant CRC cells, we investigated the link between TINCR and miR-137 in SW620 cells. A bioinformatics search of the BiBiserv2 database (https://bibiserv.cebitec.uni-bielefeld.de/) revealed

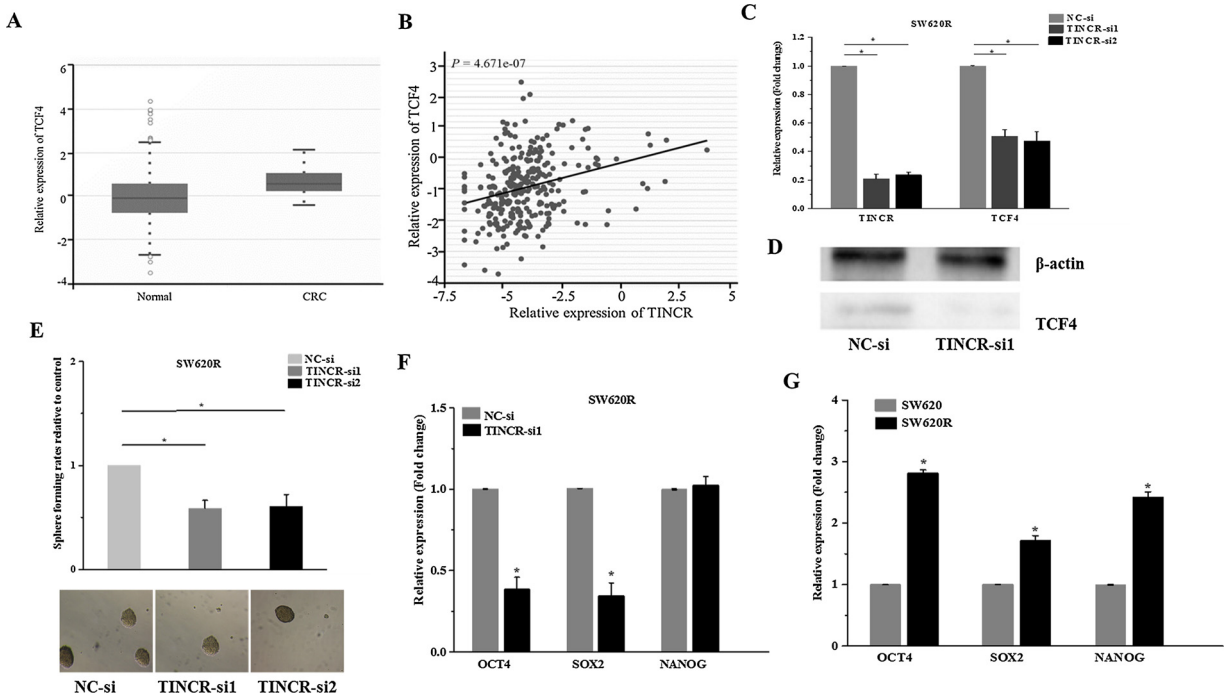

Fig. 4. Knockdown of TINCR suppresses stemness in radioresistant SW620 cells. **(A)** (**B**) The starBase database showed that TCF4 was downregulated in CRC cells, and a positive relationship between TINCR and TCF4 was identified. (**C**) (**D**) Knockdown of TINCR suppressed TCF4 expression. (E) Knockdown of TINCR inhibited sphere formation ability in SW620R cells. (**F**) OCT4 and SOX2 expression levels were lower in the TINCR-si1 group than in control cells, but there was no significant difference in NANOG expression. (**G**) OCT4, SOX2, and NANOG expression levels were lower at the RNA level in SW620 cells compared to SW620R cells. Error bars represent the SDs obtained from three independent experiments, and the data are shown as mean ± SD. NC, negative control; si, small interfering RNA; *, $P < 0.05$.

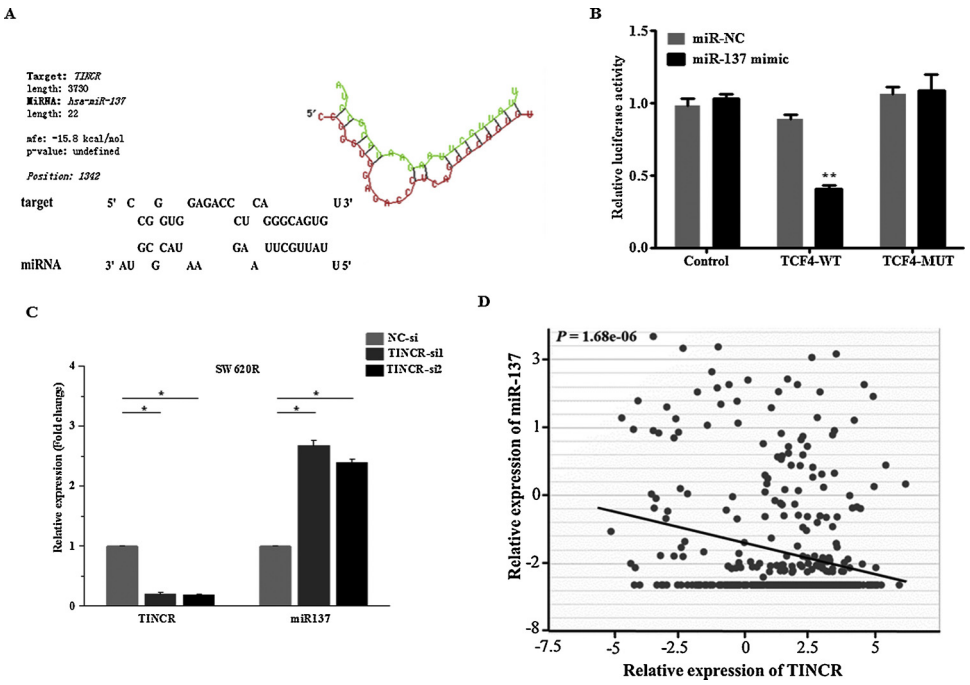

Fig. 5. Knockdown of TINCR inhibits TCF4 by regulating miR-137 expression. (**A**) (**B**) TCF4 is a target of miR-137. (**C**) (**D**) TINCR expression was negatively related to miR-137 expression. Error bars represent the SD obtained from three independent experiments, and the data are shown as mean ± SD. NC, negative control; si, small interfering RNA; *, $P < 0.05$; **, $P < 0.01$.

that TINCR and miR-137 interact with each other (Fig. 5A).

To determine whether TCF4 is a direct target of miR-137, a dual-luciferase reporter assay was conducted. Co-transfection of a miR-137 mimic and TCF4-WT into cells significantly decreased luciferase activity compared with miR-NC, whereas the transfection of a mimic with the mutated TCF4 (TCF4-MUT) did not affect luciferase activity (Fig. 5B).

To explore whether TINCR restrained miR-137 expression, SW620 cells were transfected with TINCR-si1 or TINCR-si2. qPCR assays were conducted, and we found that miR-137 expression increased after transfection with TINCR-si1 or TINCR-si2, compared with the corresponding control group (Fig. 5C). We subsequently obtained CRC clinic sample data from the starBase database (http://starbase.sysu.edu.cn/). Correlation analyses showed that TINCR levels are linked with miR-137 expression (Fig. 5D; $P < 0.001$). Taken together, TINCR knockdown inhibits TCF4 by downregulating miR-137 expression.

4. Discussion

CRC is one of the most common malignancies worldwide with a high morbidity rate [1]. Moreover, a high proportion of patients with early onset CRC are diagnosed with advanced-stage tumors [31,32], which have a poor prognosis. The factors involved in poor prognosis was also related to proliferation [33], migration [34], tumor progression [35]. Particularly, radioresistance is a major cause of treatment failure in cancer treatment [36–38]. It is therefore important to explore new diagnostic and therapeutic biological targets for CRC.

Recently, the differential expression profiles of lncRNAs have been found to influence the radioresistance. For example, DNM3OS served as an attractive target to reverse tumor radioresistance in Esophageal Squamous Cell Carcinoma [39]; AHIF promotes glioblastoma progression and radioresistance via exosomes [40]; FAM83H-AS1 contributes to the radioresistance, proliferation, and metastasis in ovarian cancer [41]. Intriguingly, Zhang et al. previously demonstrated that loss of TINCR expression promotes CRC proliferation and metastasis [19], and another study suggested that genetic variation of TINCR contributes to CRC susceptibility and progression [18]. Radioresistance, however, is the most commonly reported phenomenon, and often results in radiotherapy failure [4,5]. It is therefore worth investigating if TINCR affects radioresistance in CRC.

The *in vitro* studies suggested differentially expressed TINCR in CRC cell lines. A series of functional experiments were conducted. The results suggested that TINCR inhibited CRC cell proliferation and migration. We also established a radioresistant cell line (SW620R) and found increased TINCR expression. Notably, TINCR is related to radioresistance in SW620 cells. Moreover, researches suggested that stemness also takes responsible for the effect of radioresistance [42,43]. Thereafter, we focused on TCF4 as it was shown to be downregulated in CRC through bioinformatics analyses. A previous study also reported that it was related to enhanced stem cell features in tumors [30]. Another showed that TCF4 has various effects in different tumor subtypes [26], and it has been reported that TCF4 is partly responsible for restricted CRC cell growth [28], We hypothesized that TINCR might play a similar role in CRC. In addition, previously reported evidence suggests that cancer cells with healthy stemness are highly resistant to common anticancer treatments, and this characteristic promotes cancer development [44,45]. In our study, radioresistance downregulated the TCF4 and TINCR expression. Furthermore, we found that knockdown of TINCR inhibited sphere formation ability in SW620R cells. We therefore analyzed stemness-related genes (OCT4, SOX2, and NANOG). Our results suggested that OCT4 and SOX2 expression levels were positively related with that of TINCR. Stemness-related gene expression levels were higher in SW620R cells compared with SW620 cells. The role of TINCR in cancers could vary, as it has been found to promote gastric cancer [46] and breast cancer [47], but it suppresses proliferation and invasion in lung cancer [48]. While, computational prediction suggests that TINCR could regulate multiple mRNAs; therefore, its functions could vary in carcinogenesis and tumor progression [49]. In addition, it has recently become apparent that cross-regulation exists between lncRNAs and miRNAs in cancers [25]. In CRC, for example, CCAT2 regulates miR-145 expresssion by suppressing its maturation process in colon cancer cells [50]; CHRF-induced miR-489 loss promotes metastasis in CRC [51]; SNHG1 regulates CRC cell growth through interactions with EZH2 and miR-154-5p [52]. Interestingly, TCF4 is a reported target of miR-137 [26], and this was confirmed in our dual-luciferase assay results. Consequently, we used the BiBiserv2 database (https://bibiserv.cebitec.uni-bielefeld.de/) bioinformatics tool and conducted a series of experiments and found that TINCR knockdown inhibits TCF4 to reduce stemness. It is well known that lncRNAs play roles in cancer stem cells [53,54]. For example, H19 regulates cancer stem cells and is

associated with poor prognosis in breast cancer patients [55]; HOTAIR suppresses cancer stemness and metastasis in oral carcinomas [56]; and TALNEC2 is associated with increased glioma stem cells and their resistance to radiation [57]. Thus, the associations among TINCR, TCF4, and miR-137 were tentatively identified in radioresistant CRC cells.

5. Conclusion

In the current study, the role of TINCR in CRC was investigated using sequencing analyses and the starBase database. We found that TINCR inhibited proliferation and migration in CRC cells, but knockdown of TINCR decreased radioresistance in radioresistant CRC cells. Our results provide new insights into the functions of TINCR in radioresistant CRC cells. Moreover, knockdown of TINCR may inhibit TCF4 to decrease cell radioresistance through regulating miR-137 expression in CRC. However, there are some limitations of the current study. For instance, the causes of TINCR influenced stemness in radioresistant cells remains to be fully understood; stemness-related signaling pathways are expected to be clarified. It will be of great interest to link more potential events to the TINCR in future studies.

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Declaration of Competing Interest

The authors declare no conflict of interest.

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None.

Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.prp.2019.152622.

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