

HNF1 α Contributes to Radio-Resistance in Cervical Cancer Via Regulating the CircHNF1 α /miR-204-3p/RAD51D Regulatory Axis

Hui Du

Second Hospital of Hebei Medical University

Nai-Yi Zou

Fourth Hospital of Hebei Medical University

Hong-Ling Zuo

Second Hospital of Hebei Medical University

Xue-Yuan Zhang

Fourth Hospital of Hebei Medical University

Shu-Chai Zhu (✉ sczhu_1965@163.com)

Hebei Medical University First Affiliated Hospital <https://orcid.org/0000-0003-0199-7431>

Primary research

Keywords: cervical cancer, radio-resistance, HFN1 α , circRNA, RAD51D

Posted Date: December 21st, 2020

DOI: <https://doi.org/10.21203/rs.3.rs-131012/v1>

License:   This work is licensed under a Creative Commons Attribution 4.0 International License.

[Read Full License](#)

Abstract

Background: Radiotherapy as an important primary treatment has effectively improved the survival of patients with cervical cancer (CC). However, some patients do not show optimal benefits of radiotherapy due to radio-resistance. Therefore, identification of biomarkers of radio-resistance and unraveling the underlying mechanisms of radio-resistance is a key imperative for these patients.

Methods: The expression levels of circHNF1 α , miRNAs, and mRNA in tissues and cell lines were detected by qRT-PCR analysis. The levels of proteins were analyzed by western blot. Cell proliferation ability was measured by colony formation assay. RNA pull-down and luciferase reporter assay analysis were performed to identify the sponging microRNAs of circHNF1 α . The target gene of miR-204-3p was determined by luciferase reporter assay. Chromatin immunoprecipitation (ChIP) analysis and luciferase reporter assay were performed to identify the transcription factor of circHNF1 α .

Results: We found significant upregulation of HNF1 α expression in radio-resistant cervical cancer tissues and cell lines. Depletion of HNF1 α reduced whereas overexpression of HNF1 α promoted the resistance of CC cells to irradiation in vitro and in vivo. HNF1 α positively regulated RAD51D at the protein level but not at the mRNA level, thus attenuating radio-resistance of CC cells. Mechanistically, upregulation of HNF1 α enhanced circHNF1 α transcription and promoted circHNF1 α biogenesis, which in turn sponged miR-204-3p and thus relieved their repression of the RAD51D expression. The HNF1 α /circHNF1 α /miR-204-3p/RAD51D regulatory axis was found to play a critical role in conferring radio-resistance of CC cells.

Conclusions: Dysregulation of the HNF1 α /circHNF1 α /miR-204-3p/RAD51D axis may promote the radio-resistance of CC cells. Blocking this pathway may provide therapeutic benefits against CC radio-resistance.

Background

Globally, cervical cancer (CC) is the 4th most frequently cancer and the second lethal malignancy in females in females. In the United States, there were an estimated 570,000 new cases of CC and 311,000 deaths in 2018 [1, 2]. Recent advances in diagnostic technology, surgical methods, and radiotherapy have helped improve the 5-year OS and PFS of patients with CC. However, more than 25% patients develop local or distant relapse due to radio-resistance, leading to poor 5-year PFS rates (< 20%) [3, 4]. Therefore, characterization of the underlying molecular mechanisms of CC radio-resistance and development of alternative therapeutic targets is a key imperative.

Circular RNAs (circRNAs) is a kind of new endogenous non-coding RNA which has a covalently closed loops structure with neither poly-adenylated tail in 3'ends nor the cap structure at 5'end [5, 6]. Accumulate evidence has confirmed that circRNAs may regulate gene expression and pathways by sponging miRNAs, interacting with RNPs and moderating transcription [7–9]. Recent studies have unraveled a wide range of functions of circRNAs; this has led to increasing recognition of the role of circRNAs in diseases, especially human cancers [10]. Some studies have confirmed the effects of circRNAs on cervical cell tumorigenesis

and tumor progression. For example, circSLC26A4 was upregulation in CC tissues and cell lines. Overexpression of circSLC26A4 had a relationship with poor survival [11]. Similarly, CircCLK3 was shown to be upregulated in CC tissues and cell lines. Knockdown of circCLK3 inhibited CC cell growth, invasion and migration. Additionally, studies have documented the involvement of circAMOTL1, circMOT1, and circ_0023404 in cell viability, chemo-resistance, and autophagy of CC[12–14]. However, the potential role of circRNAs in CC radio-resistance has not been explored.

HNF1 α is a key member of the hepatocyte nuclear factor 1 (HNF-1) transcription factor family; it has been identified as one of the critical transcription factors in the early development of liver, pancreas, and kidney[15, 16]. An increasing body of evidence has implicated mutant of HNF1 α in the causation of diabetes, including maturity onset diabetes of the young and type 2 diabetes [17, 18]. Recent studies have shown that HNF1 α may affect survival of cells by moderating cell growth and metastasis in different human cancers [19, 20]. For example, HNF1 α as an oncogene, was upregulated in pancreatic ductal adenocarcinoma (PDA) cells. Overexpression of HNF1 α in PDA cells was shown to induce cell proliferation *in vivo* and *in vitro* [21]. Knockdown of HNF1 α was found to promote epithelial-mesenchymal transition (EMT) in liver cancer cell lines via decreasing the protein level of E-cadherin and increasing the level of TGF β 1[22]. Of note, HNF1 α functions as an oncogene in CC and has involvements in CC cell survival and EMT, all of which enhance cell motility and metastasis [23]. However, the effect of HNF1 α in radio-resistance of CC cells is not clear.

In this study, we revealed that the expression of HNF1 α was increased in radio-resistant CC tissues and cell lines. Overexpression of HNF1 in CC cells increased cell resistance to irradiation, *in vitro* and *in vivo*. Furthermore, HNF1 α positively regulated RAD51D protein level at the post-transcriptional level. HNF1 α , as a transcription factor, was found to regulate circHNF1 level in CC cells. circHNF1 α induced RAD51D protein level by sponging miR-204-3p, which directly targeted RAD51D in CC cells. The HNF1 α /circHNF1 α /miR-204-3p/RAD51D axis was performed to play an important role in the radio-resistance of CC and represents a novel therapeutic target to overcome the resistance of CC to radiotherapy.

Methods

The detailed procedures cell proliferation assays, chromatin immunoprecipitation-qPCR, luciferase reporter assay, analyses of apoptosis, RNA synthesis and biotin pull-down as well as key reagents are described in Supplementary Experimental Procedures.

Patients And Tumor Samples

Ten pairs of cervical carcinoma radio-sensitive tissues and cervical carcinoma radio-resistance tissues were obtained from the fourth Hospital of the Hebei Medical University between July 2012 to June 2017. The effectiveness of radiotherapy was evaluated as previously described [24]. All patients had a

confirmed histopathological diagnosis of CC (stage IIIB) and had not been treated with chemotherapy or radiotherapy.

Cell Culture And Transfection

Human CC cell lines (Hela, ME180, SiHa, and MS751) were obtained from our laboratory. All cell lines were cultured in high-glucose DMEM medium containing 10% FBS and 100 units/mL of penicillin and streptomycin at 37 °C in an atmosphere of 5% CO₂.

Lipofectamine 2000 (Invitrogen) was used for cell transfection according to the manufacturer's protocols. The siRNA, miRNA mimic and anti-miRNA, and negative controls were designed by GenePharma Co. Ltd. (Shanghai, China). The overexpression plasmids and luciferase assay plasmids were purchased from the Genewiz Company (Suzhou, China).

Statistical analysis

All data are presented as mean \pm standard error of the mean. Student's *t* test was used to assess between-group differences; for multiple comparisons or repeated measurements, ANOVA or repeated ANOVA followed by Tukey's post hoc test was used. *P* values < 0.05 were considered indicative of statistical significance. All statistical analyses were performed using the Graphpad Prism 7 software (GraphPad Software, San Diego, CA, USA).

Results

1. HNF1 α is increased in radio-resistant human CC tissues and CC cells.

To identify the role of HNF1 α in the radio-resistance of CC, we explored the level of HNF1 α in radio-sensitive and radio-resistant CC tissues. As shown in Fig. 1A and 1B, the protein level of HNF1 α was significant up-regulated in radio-resistant CC tissues compared with in radio-sensitive CC tissues by using IHC and western blot analysis. Additionally, the radio-resistant CC tissues had a higher mRNA level of HNF1 α than radio-sensitive CC tissues (Fig. 1C). To demonstrate the expression level HNF1 α in different CC cell lines, we performed the colony formation assay to observe to evaluate the sensitivity to radiotherapy in different CC cell lines. As shown in Fig. 1D, CC cells were treated with 0, 2, 4, and 8 Gy of irradiation. Cell proliferation was markedly decreased after 4 Gy of IR in 4 CC cell lines. Importantly, the survival percentage of SiHa cell line was much higher than other cell lines after treated with different different doses of irradiation considering that the SiHa cell line as the radio-resistant cell line. While the ME180 cells has a lower survival percentage after IR considering the ME180 cell line as the radio-sensitive cell line. Then, we detected the mRNA and protein levels of HNF1 α in CC cell lines by using qRT-PCR and western blot analysis. The results revealed that the the mRNA and protein levels of HNF1 α cell line were lowest in ME180 than in other CC cell lines; therefore, this cell line was selected for gain-of-function experiments. While the SiHa cell line which had a higher level of HNF1 α was chose for loss-of-

function experiments (Fig. 1E and 1F). Together, these findings suggest that the higher expression level of HNF1 α may contribute to radio-resistance in CC cells.

2. HNF1 α plays an essential role in CC cell radio-resistance

In order to clarify the effect of HNF1 α on CC radio-resistance, some loss-and-gain experiments were performed. First, to knock down of HNF1 α level in CC cell, SiHa cells were transfected with specific shRNA. Western blot and qRT-PCR were used to detect the level of HNF1 α . As the results showed in Fig. 2A and 2B, the mRNA and protein levels of HNF1 α were significantly suppressed in shRNA-transfected SiHa cells. Colony formation analysis showed that depletion of HNF1 α in SiHa cells suppressed the cell proliferation after IR compared with the control group, suggesting that downregulated HNF1 α level promoted cell radio-sensitivity in CC cells (Fig. 2C). On the contrast, transfection of overexpression plasmid of HNF1 α significantly enhanced the mRNA and protein levels in Me180 cells (Fig. 2D and 2E). Colony formation analysis result revealed that enforced HNF1 α expression in ME180 cells significantly increased cell radio-resistance in ME180 cells (Fig. 2F). These findings suggest that knockdown of HNF1 α reduces while overexpression of HNF1 α induces the radio-resistance in CC cells.

3. HNF1 α positively regulates RAD51D protein expression in CC cells

HNF1 α is known to be involved in chemotherapeutic and radiotherapeutic resistance of cancer cells by directly or indirectly regulating the expression of multiple proteins[25]. To determine the mechanism by which HNF1 α regulated radio-resistance of CC cells, we detected the protein levels of the genes that have previously been shown to be regulated by HNF1 α and that had shown a correlation with radiotherapeutic resistance. As shown in Fig. 3A and 3B, the radio-resistant CC cell line-SiHa showed dramatic upregulation of the protein expression of DNA repair protein homolog 4 (RAD51D); however, there was no significant difference at the mRNA level. Western blot result showed that increase of HNF1 α level dramatically reduced the protein expression in ME180 cells (Fig. 3C). However, the mRNA level of RAD51D was not influenced by the increase in HNF1 α (Fig. 3D). On the contrary, knockdown of HNF1 α level in SiHa cells suppressed the expression of RAD51D at the protein level, but not at the mRNA level; these findings indicated that HNF1 α regulated the RAD51D level at the post-transcriptional level (Fig. 3E and 3F). Subsequently, we detected the expression level of RAD51D in different kinds of CC tissues. Western blot results showed higher protein expressions of RAD51D in radio-resistant CC tissues compared to that in radio-sensitive CC tissues (Fig. 3G). However, the mRNA expressions of RAD51D were not significantly different between the radio-resistant and radio-sensitive CC tissues (Fig. 3H); these findings suggested that the higher protein level of RAD51D may be involved in radio-resistance of CC cells. To further identify the role of RAD51D in conferring CC cell radio-resistance, we transfected the ME180 cells with overexpression plasmid of RAD51D. The colony formation assay result showed that overexpression of RAD51D could increase the resistance to radiotherapy. Co-transfection of overexpression plasmid of RAD51D with shHNF1 α in ME180 cells partly abrogated the induced-effect of RAD51D on cell proliferation (Fig. 3I). Collectively, these findings suggested a positive correlation between HNF1 α and RAD51D protein level, which is involved in radio-resistance of CC cell.

4. Upregulation of HNF1 α increases the expression of circHNF1 α in radio-resistant CC cells

HNF1 α regulates the gene and non-coding RNA expressions at the transcriptional level. In CC cells, we found upregulation of hsa_circ_0028940 (named circHNF1 α), which is formed from the HNF1 α exon 2–7 (1392 bp length), in radio-resistant CC cell line (Fig. 4A and B). Interestingly, upregulation of HNF1 α in ME180 cells increased the expression level of circHNF1 α , while knockdown of HNF1 α in SiHa cell decreased the expression level of circHNF1 α (Fig. 4C and D); these findings indicated that HNF1 α may positively regulate the expression of circHNF1 α . Furthermore, colony formation assay result performed that overexpression of circHNF1 α increased the radio-resistance of CC cells compared with the control group. However, this increase was reversed by concomitant depletion of HNF1 α (Fig. 4E). These findings suggest that HNF1 α positively regulates circHNF1 α expression, which participates in radio-resistance of CC cells.

Next, we determined whether HNF1 α affected the expression of circHNF1 α at the transcriptional level. First, PROMO prediction software was used to identify the putative transcriptional factor of HNF1 α and circHNF1 α ; The results showed that promoter region of HNF1 α and circHNF1 α has 2 putative binding sites of HNF1 α . CHIP-PCR analysis confirmed that HNF1 α could directly bind to the region of the HNF1 α and circHNF1 α promoter (Fig. 4F). Luciferase activity assay further revealed that overexpression of HNF1 α could significantly increase the luciferase activity of the plasmid which containing the promoter region of HNF1 α and circHNF1 α (Fig. 4G). These findings indicated that HNF1 α regulates the expression level of circHNF1 α at the transcriptional level.

5. circHNF1 α elevated the expression of RAD51D by sponging miR-204-3p

As described above, we sought to investigate the involvement of circHNF1 α in mediating the correlation between HNF1 α and RAD51D. Previous studies have shown that circRNAs function as ceRNAs by sponging microRNAs (miRNAs) to regulate the downstream gene expression in the post-transcriptional level. We first analyzed the potential binding microRNAs of circHNF1 α different target prediction programs, miRanda, RNA22, and Rnahdrid. The Venn diagram showed that 11 miRNAs contained the binding-sequences circHNF1 α , including miR-33b-3p, miR-184-3p, miR-193a-3p, miR-204-3p, miR-323-3p, miR-363-5p, miR-367-5p, miR-373, miR-483-3p, miR-650, miR-1285-3p (Fig. 5A). Subsequently, we used the biotin-labeled circHNF1 α probe pull-down assay to explore the expressions of miRNAs in the circHNF1 α -overexpressed ME180 cells. qRT-PCR and agarose gel electrophoresis of PCR products showed significant enhancement of circHNF1 α in circHNF1 α -overexpressed ME180 cells by using biotin-labeled circHNF1 α probes (Fig. 5B and 5C). Subsequently, RT-qPCR was performed to detect the expressions of the candidate miRNAs in the precipitates. The results showed enrichment of miR-184-3p, miR-650, and miR-204-3p in the circHNF1 α -overexpressed precipitates (Fig. 5D). Furthermore, luciferase assays showed that co-transfection with circHNF1 α -luciferase-reporter vector and miR-204-3p mimics, but not miR-650 mimics or miR-184-3p mimics, significantly decreased the luciferase activity (Fig. 5E). These findings suggested that circHNF1 α can sponge miR-204-3p in CC cells.

To clarify the role of miR-204-3p in mediating the relationship between HNF1 α and RAD51D, we overexpressed miR-204-3p or suppressed miR-204-3p in CC cells. The results showed that overexpression of miR-204-3p in CC cells reduced the protein level of RAD51D while silencing of miR-204-3p increased the protein level of RAD51D (Fig. 5F). Next, we found that the RAD51D 3'-UTR has a putative miR-204-3p binding site by using TargetScan. Subsequently, we co-transfected ME180 cells with wild-type (WT) or mut RAD51A 3'-UTR-luciferase reporter and miR-204-3p mimic. Results of luciferase assay result revealed that miR-204-3p mimic markedly reduced luciferase activity of WT RAD51D 3'-UTR but not in the mutation type (Fig. 5H). Additionally, we transfected miR-204-3p mimics or circHNF1 α overexpression plasmid in ME180 cells, respectively, or co-transfected them together. Western blot analysis showed that the increased protein level of RAD51D induced by miR-204-mimic was reversed by enforced circHNF1 α expression (Fig. 5I). Collectively, these findings indicated that circHNF1 α regulates RAD51D protein level by sponging miR-204-3p.

6. Hnf1 α /circHnf1 α /mir-204-3p/rad51d Axis Regulates The Radio-resistance Of Cc Cells

In order to explore the role of the HNF1 α /circHNF1 α /miR-204-3p/RAD51D axis in CC cell radio-resistance, some rescue experiments were performed. Firstly, we transfect the shHNF1 α or miR-204-3p inhibitor in ME180 cells, respectively, or transfected them together. The protein level of RAD51D was detected by western blot. As shown in Fig. 1A, suppression of miR-204-3p increased the protein level of RAD51D. However, their co-transfection reversed miR-204-3p-induced RAD51D increase alone. Then, colony formation assay showed that downregulated of miR-204-3p in ME180 cells markedly decreased cell sensitivity to irradiation and this inhibitory effect was reversed by concomitant knockdown of HNF1 α (Fig. 6B). Similarly, we circHNF1 α overexpression plasmid or shRAD51D in Hep2 cells, respectively, or transfected them together. Western blot result showed that overexpression of circHNF1 α increased the protein level of RAD51D (Fig. 6C). However, co-transfection of shRAD51D almost reversed the circHNF1 α -induced increase in RAD51D. Colony formation assay also showed that overexpression of circHNF1 α in CC cells significantly increased their resistance to irradiation and this effect was reversed by depletion of RAD51D (Fig. 6D). These findings further confirmed that the HNF1 α /circHNF1 α /miR-204-3p/RAD51D axis regulates CC cell radio-resistance.

7. Hnf1 α Is Involved In Cc Radio-resistance In Vivo

To determine the pathophysiological effect of HNF1 α on CC radio-resistance, we established a CC xenograft model of nude mouse. First, the stable-knockdown-HNF1 α SiHa cells or normal SiHa cells were implanted into nude mice. After 14 days, the mice were administered 16 Gy of irradiation. As shown in Fig. 7A and Fig. 7B, the suppression of HNF1 α group had a smaller tumor volume compared with that in the control group. Consistently, knockdown of HNF1 α resulted in decreased level of RAD51D protein. The results of qRT-PCR showed decreased expression of circHNF1 α in the HNF1 α -suppression group

compared with the control group (Fig. 7D). These results suggested that knockdown of HNF1 α increases the radio-sensitivity of CC in vivo.

Discussion

The main findings of this study were: 1) HNF1 α was up-regulated in radio-resistant CC tissues compared with radio-sensitive CC tissues; 2) Overexpression of HNF1 α promoted CC cell radio-resistance, while depletion of HNF1 α reduced CC cell radio-resistance in vivo and in vitro; 3) HNF1 α was found to regulate circHNF1 α expression at the transcriptional level which could sponge of miR-204-3p in CC cells; 4) HNF1 α was found to positively regulate RAD51D protein expression in CC cells via the circHNF1 α /miR-204-3p axis. These findings suggest a critical role of the HNF1 α /circHNF1 α /miR-204-3p/RAD51D axis in CC cell radio-resistance.

Radiotherapy is the primary treatment for CC that confers survival benefit. However, failure of radiotherapy due to radio-resistance contributes to poor clinical outcomes and high mortality in these patients. Previous studies have found that abnormal expression of gene may be associated with radio-resistance. For example, Yao reported that higher level of SOX2 was responsible for CC cell radio-resistance. Overexpression of SOX2 was observed to be closely related to cell growth, survival, and cell cycle changes by regulating the Hedgehog pathway [26]. Suzuki also revealed that GRP94 may serve as one of the molecular targets for combating radio-resistance in CC cells. Silencing of GRP94 in CC cells induced increased sensitivity to radiotherapy [27]. Besides, Zou found that depletion of HMGB3 significantly enhanced the radio-sensitivity of CC cells, while overexpression of HMGB3 had the opposite effect; this effect was mediated via transcriptional upregulation of hTERT [28]. The present study found a correlation between high level of HNF1 α and radio-resistance of CC tissues. Importantly, knockdown of HNF1 α markedly induced radio-sensitivity of CC cells while overexpression of HNF1 α had the opposite effect. These findings suggest that HNF1 α may serve as a biomarker for predicting the sensitivity of CC cells to radiotherapy.

An increasing body of evidence has implicated the abnormal expression of non-coding RNAs (including miRNA, lncRNA, and circRNA) in the development and radio-resistance of CC cells. For example, studies have demonstrated reduced expression of lncRNA GAS5 in the radio-resistant human CC tissues and cell lines. Overexpression of GAS5 was found to enhance the radio-sensitivity of CC cells by inhibiting the function of miR-106 [29]. Additionally, high level of lncRNA HOTAIR showed an association with radio-resistance. Depletion of HOTAIR in CC cells led to upregulation of apoptosis and increased the sensitivity to radiotherapy [30]. Moreover, lncRNA UCA1, and lncRNA DANCR have been indicated to play an important role in radio-resistance in CC cells [31, 32]. Furthermore, miR-181a, miR-4429, and miR-16-5p were found to be involved in radio-resistance of CC cells [24, 33, 34]. Despite the recent advances regarding lncRNAs and miRNA, the biogenesis of circRNAs and the molecular mechanisms underlying CC cell radio-resistance are not well characterized. In the present study, we identified for the first time the upregulation of hsa_circ_0028940 (which we named as circHNF1 α), formed from the exon 2–7 of HNF1 α , in radio-resistant CC cell line and tissues. Overexpression of circHNF1 α significantly decreased the radio-

sensitivity of CC cells. According to a previous study, circular RNAs may be generated along with the transcription of its parental gene [35]. Similarly, in the present study, we demonstrated that HNF1 α as transcription factor can bind to the promoter region of circHNF1 α and positively regulate the expression of circHNF1 α . However, further research is required to determine whether the circHNF1 α in turn regulates the expression of its parental gene.

Increasing evidence has suggested that the most important function and mechanism of action of circRNAs is their role as miRNA sponge molecules. In the present study, we found that circHNF1 α may directly sponge miR-204-3p, which targeted and repressed the expression of RAD51D. According to a previous study, the correlation between HNF1 α and RAD51D is likely mediated by circHNF1 α . These results suggests that the knockdown of HNF1B may play a role in drug resistance in ovarian cancer by directly relating with these drug resistance-related proteins including RAD51 (as determined by bioinformatics analyses). However, the underlying mechanism of the relationship between HNF1 α and RAD51 was not elaborated. In the present study, we found that HNF1 α regulates circHNF1 α at the transcriptional level. Upregulation of HNF1 α enhances circHNF1 α transcription and promotes circHNF1 α biogenesis, which in turn sponges miR-204-3p and thus relieves their repression of the RAD51D expression. In other words, on one hand, HNF1 α may function as a transcriptional factor and directly regulate the downstream gene expression. On the other hand, the TF may also regulate the protein level indirectly by affecting the expression of non-coding RNAs.

Conclusion

In summary, our findings indicate that HNF1 α upregulation in CC cells led to the formation of the HNF1 α /circHNF1 α /miR-204-3p/RAD51D regulatory axis. These results highlight the importance of the HNF1 α /circHNF1 α /miR-204-3p/RAD51D axis in conferring radio-resistance in CC. The identified regulatory axis may serve as a potential therapeutic target against radio-resistance of CC.

Abbreviations

CC

cervical cancer

PFS

progression-free survival

circRNAs

circular RNAs

HNF-1

hepatocyte nuclear factor 1

RAD51D

DNA repair protein homolog 4

miRNAs

microRNAs

UTR

untranslated region

Declarations

Ethics approval and consent to participate

The present study and was authorized Ethics Committee of the Fouth Hospital of Hebei Medical University. All patients and volunteers were anonymous and provided written informed consent. All animal studies were approved by the Institutional Animal Care Committee of Hebei Medical University

Consent for publication

Written consent was obtained from all participants.

Availability of data and materials

Not applicable.

Competing interests

The authors declare that they have no competing interests.

Funding

Not applicable

Authors' contributions

Conception and design: S.Z. and H.D.; Tissues collection: H.Z. and N.Z.; Development of methodology: X.Z., H.Z. and H.D.; Acquisition of the data: H.D. and H.Z.; Analysis of data: H.D.and X.Z.; Writing the manuscript: H.D.

Acknowledgements

Not applicable

Publisher's Note

Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Siegel RL, Miller KD, Jemal A. Cancer statistics, 2018. CA Cancer J Clin. 2018;68(1):7–30.

2. Cohen PA, Jhingran A, Oaknin A, Denny L. Cervical cancer. *Lancet*. 2019;393(10167):169–82.
3. Liontos M, Kyriazoglou A, Dimitriadis I, Dimopoulos MA, Bamias A. Systemic therapy in cervical cancer: 30 years in review. *Crit Rev Oncol Hematol*. 2019;137:9–17.
4. Wakatsuki M, Kato S, Kiyohara H, Ohno T, Karasawa K, Tamaki T, Ando K, Shiba S, Kamada T, Nakano T. The prognostic value of rectal invasion for stage IVA uterine cervical cancer treated with radiation therapy. *BMC Cancer*. 2016;16:244.
5. Chen LL, Yang L. Regulation of circRNA biogenesis. *Rna Biology*. 2015;12(4):381–8.
6. Jeck WR, Sharpless NE. Detecting and characterizing circular RNAs. *NATURE BIOTECHNOLOGY*. 2014;32(5):453–61.
7. And ROB, Mikkelsen JG. **miRNA sponges: soaking up miRNAs for regulation of gene expression.** *Wiley Interdisciplinary Reviews Rna* 2014, 5(3).
8. Kristensen LS, Andersen MS, Stagsted LVW, Ebbesen KK, Hansen TB, Kjems J. The biogenesis, biology and characterization of circular RNAs. *Nat Rev Genet*. 2019;20(11):675–91.
9. Huang S, Yang B, Chen BJ, Bliim N, Ueberham U, Arendt T, Janitz M. The emerging role of circular RNAs in transcriptome regulation. *Genomics*. 2017;109(5–6):401–7.
10. Chen Y, Li C, Tan C, Liu X. Circular RNAs: a new frontier in the study of human diseases. *J Med Genet*. 2016;53(6):359–65.
11. Ji F, Du R, Chen T, Zhang M, Zhu Y, Luo X, Ding Y: **Circular RNA circSLC26A4 Accelerates Cervical Cancer Progression via miR-1287-5p/HOXA7 Axis.** *Mol Ther Nucleic Acids* 2020, **19**:413–420.
12. Guo J, Chen M, Ai G, Mao W, Li H, Zhou J. Hsa_circ_0023404 enhances cervical cancer metastasis and chemoresistance through VEGFA and autophagy signaling by sponging miR-5047. *Biomed Pharmacother*. 2019;115:108957.
13. Ou R, Lv J, Zhang Q, Lin F, Zhu L, Huang F, Li X, Li T, Zhao L, Ren Y, et al. circAMOTL1 Motivates AMOTL1 Expression to Facilitate Cervical Cancer Growth. *Mol Ther Nucleic Acids*. 2020;19:50–60.
14. Chen M, Ai G, Zhou J, Mao W, Li H, Guo J. circMTO1 promotes tumorigenesis and chemoresistance of cervical cancer via regulating miR-6893. *Biomed Pharmacother*. 2019;117:109064.
15. Shih DQ, Bussen M, Sehayek E, Ananthanarayanan M, Shneider BL, Suchy FJ, Shefer S, Bollileni JS, Gonzalez FJ, Breslow JL, et al. Hepatocyte nuclear factor-1alpha is an essential regulator of bile acid and plasma cholesterol metabolism. *Nat Genet*. 2001;27(4):375–82.
16. Odom DT, Zizlsperger N, Gordon DB, Bell GW, Rinaldi NJ, Murray HL, Volkert TL, Schreiber J, Rolfe PA, Gifford DK, et al. Control of pancreas and liver gene expression by HNF transcription factors. *Science*. 2004;303(5662):1378–81.
17. Hu M, Cherkaoui I, Misra S, Rutter GA. Functional Genomics in Pancreatic β Cells: Recent Advances in Gene Deletion and Genome Editing Technologies for Diabetes Research. *Front Endocrinol (Lausanne)*. 2020;11:576632.
18. Lau HH, Ng NHJ, Loo LSW, Jasmen JB, Teo AKK. The molecular functions of hepatocyte nuclear factors - In and beyond the liver. *J Hepatol*. 2018;68(5):1033–48.

19. Xu J, Filetti S, Hershman JM. Expression of hepatocyte nuclear factor-1alpha mRNA in human anaplastic thyroid cancer cell lines and tumors. *Thyroid*. 2008;18(5):533–9.
20. Yu Y, Liang S, Zhou Y, Li S, Li Y, Liao W. HNF1A/CASC2 regulates pancreatic cancer cell proliferation through PTEN/Akt signaling. *J Cell Biochem*. 2019;120(3):2816–27.
21. Abel EV, Goto M, Magnuson B, Abraham S, Ramanathan N, Hotaling E, Alaniz AA, Kumar-Sinha C, Dziubinski ML, Urs S, et al. **HNF1A is a novel oncogene that regulates human pancreatic cancer stem cell properties.** *Elife* 2018, 7.
22. Pelletier L, Rebouissou S, Vignjevic D, Bioulac-Sage P, Zucman-Rossi J. HNF1 α inhibition triggers epithelial-mesenchymal transition in human liver cancer cell lines. *BMC Cancer*. 2011;11:427.
23. Hu Y, Wu F, Liu Y, Zhao Q, Tang H. DNMT1 recruited by EZH2-mediated silencing of miR-484 contributes to the malignancy of cervical cancer cells through MMP14 and HNF1A. *Clin Epigenetics*. 2019;11(1):186.
24. Ke G, Liang L, Yang JM, Huang X, Han D, Huang S, Zhao Y, Zha R, He X, Wu X. MiR-181a confers resistance of cervical cancer to radiation therapy through targeting the pro-apoptotic PRKCD gene. *Oncogene*. 2013;32(25):3019–27.
25. Li J, Zhang Y, Gao Y, Cui Y, Liu H, Li M, Tian Y. Downregulation of HNF1 homeobox B is associated with drug resistance in ovarian cancer. *Oncol Rep*. 2014;32(3):979–88.
26. Huang C, Lu H, Li J, Xie X, Fan L, Wang D, Tan W, Wang Y, Lin Z, Yao T. SOX2 regulates radioresistance in cervical cancer via the hedgehog signaling pathway. *Gynecol Oncol*. 2018;151(3):533–41.
27. Kubota H, Suzuki T, Lu J, Takahashi S, Sugita K, Sekiya S, Suzuki N. Increased expression of GRP94 protein is associated with decreased sensitivity to X-rays in cervical cancer cell lines. *Int J Radiat Biol*. 2005;81(9):701–9.
28. Li Z, Zhang Y, Sui S, Hua Y, Zhao A, Tian X, Wang R, Guo W, Yu W, Zou K, et al. Targeting HMGB3/hTERT axis for radioresistance in cervical cancer. *J Exp Clin Cancer Res*. 2020;39(1):243.
29. Gao J, Liu L, Li G, Cai M, Tan C, Han X, Han L. LncRNA GAS5 confers the radio sensitivity of cervical cancer cells via regulating miR-106b/IER3 axis. *Int J Biol Macromol*. 2019;126:994–1001.
30. Jing L, Yuan W, Ruofan D, Jinjin Y, Haifeng Q. HOTAIR enhanced aggressive biological behaviors and induced radio-resistance via inhibiting p21 in cervical cancer. *Tumour Biol*. 2015;36(5):3611–9.
31. Fan L, Huang C, Li J, Gao T, Lin Z, Yao T. Long non-coding RNA urothelial cancer associated 1 regulates radioresistance via the hexokinase 2/glycolytic pathway in cervical cancer. *Int J Mol Med*. 2018;42(4):2247–59.
32. Cao L, Jin H, Zheng Y, Mao Y, Fu Z, Li X, Dong L. DANCR-mediated microRNA-665 regulates proliferation and metastasis of cervical cancer through the ERK/SMAD pathway. *Cancer Sci*. 2019;110(3):913–25.
33. Sun H, Fan G, Deng C, Wu L. miR-4429 sensitized cervical cancer cells to irradiation by targeting RAD51. *J Cell Physiol*. 2020;235(1):185–93.

34. Zhang S, Wang W, Wu X, Liu W, Ding F. miR-16-5p modulates the radiosensitivity of cervical cancer cells via regulating coactivator-associated arginine methyltransferase 1. *Pathol Int.* 2020;70(1):12–20.
35. Li Z, Huang C, Bao C, Chen L, Lin M, Wang X, Zhong G, Yu B, Hu W, Dai L, et al. Exon-intron circular RNAs regulate transcription in the nucleus. *Nat Struct Mol Biol.* 2015;22(3):256–64.

Figures

Image not available with this version

Figure 1

HNF1 α was increased in radio-resistant human CC tissues and CC cells. (A and B) The protein level of HNF1 α in radio-resistant CC tissues (n = 10) and radio-sensitive CC tissues (n = 10) was detected by western blot Immunohistochemistry staining. (C) The mRNA expression of HNF1 α in radio-resistant CC tissues (n = 10) and radio-sensitive CC tissues (n = 10) was detected by qRT-PCR. mRNA expressions are normalized by β -actin. **P < 0.01 vs. radio-sensitive CC tissues. (D) Four different CC cell lines were treated with different dose of irradiation (0, 2, 4, and 8 Gy). Then the colony formation assay was used to detect the cell viability. (E and F) The mRNA and protein levels of HNF1 α in the 4 different CC cell lines (Hela, ME180, MS751, and SiHa) were detected by qRT-PCR and western blot. *P < 0.05, **P < 0.01 vs. Hela cell.

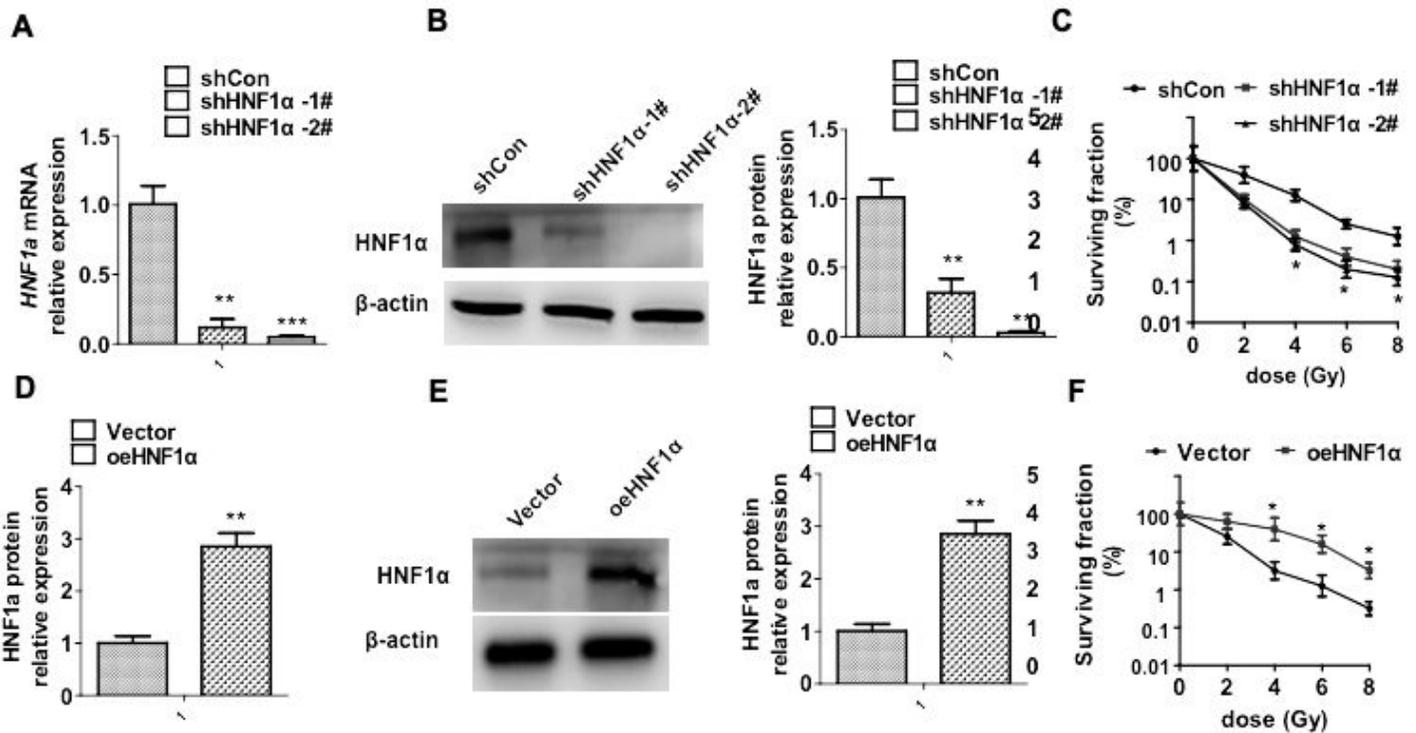


Figure 2

HNF1α plays an essential role in CC cell radio-resistance. (A) SiHa cells were transfected with sh-HNF1α-1, sh-HNF1α-2, or sh-control. qRT-PCR was performed to determine the mRNA level of HNF1α. **P < 0.01, ***P < 0.001 vs. sh-control. (B) SiHa cells were prepared as in (A). Western blot was used to detect HNF1α protein level. (C) SiHa cells were prepared as in (A) and cell survival was assessed using colony formation assay. *P < 0.05 vs. sh-control. (D) ME180 cells were transfected with HNF1α-overexpression vector or empty vector. qRT-PCR was used to detect HNF1α mRNA level. **P < 0.01 vs. empty vector. (E) ME180 cells were prepared as in (D) and Western blot was used to detect HNF1α protein level. (F) ME180 cells were prepared as in (D) and cell survival was assessed using colony formation assay. *P < 0.05 vs. empty vector.

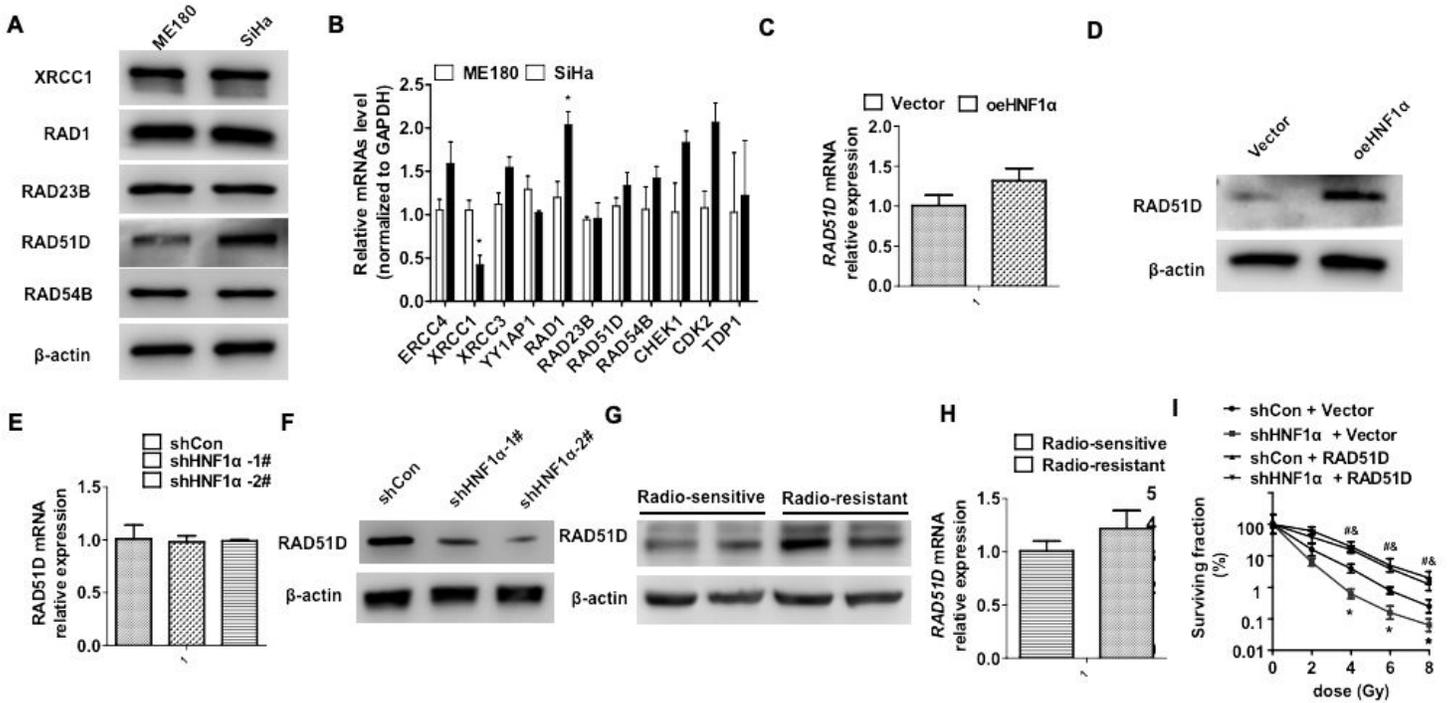


Figure 3

HNF1α positively regulates RAD51D protein expression in CC cells. (A and B) Western blot and qRT-PCR were performed to detect the protein and mRNA levels of XRCC1, RAD1, RAD23B, RAD51D, and RAR54B in ME180 cells and SiHa cells, respectively. (C and D) ME180 cells were transfected with HFN1α overexpression vector or empty vector. qRT-PCR and western blot were used to detect the mRNA and protein expressions of RAD51D. (E and F) SiHa cells were transfected with sh-HFN1α-1#, sh- sh-HFN1α-2#, or sh-control. qRT-PCR and western blot were used to detect the mRNA and protein expressions of RAD51D. (G and H) Western blot and qRT-PCR were used to detect the protein and mRNA expressions of RAD51D resistant CC tissues (n = 10) compared with that in the radio-sensitive CC tissues (n = 10). (I) ME180 cells were transfected with overexpression plasmid of RAD51D or shHNF1α, respectively, or co-transfected with both and then treated with 0, 2, 4, and 8 Gy of irradiation. Cell survival was analyzed using colony formation assay. *P< 0.05, # P<0.05 vs corresponding control.

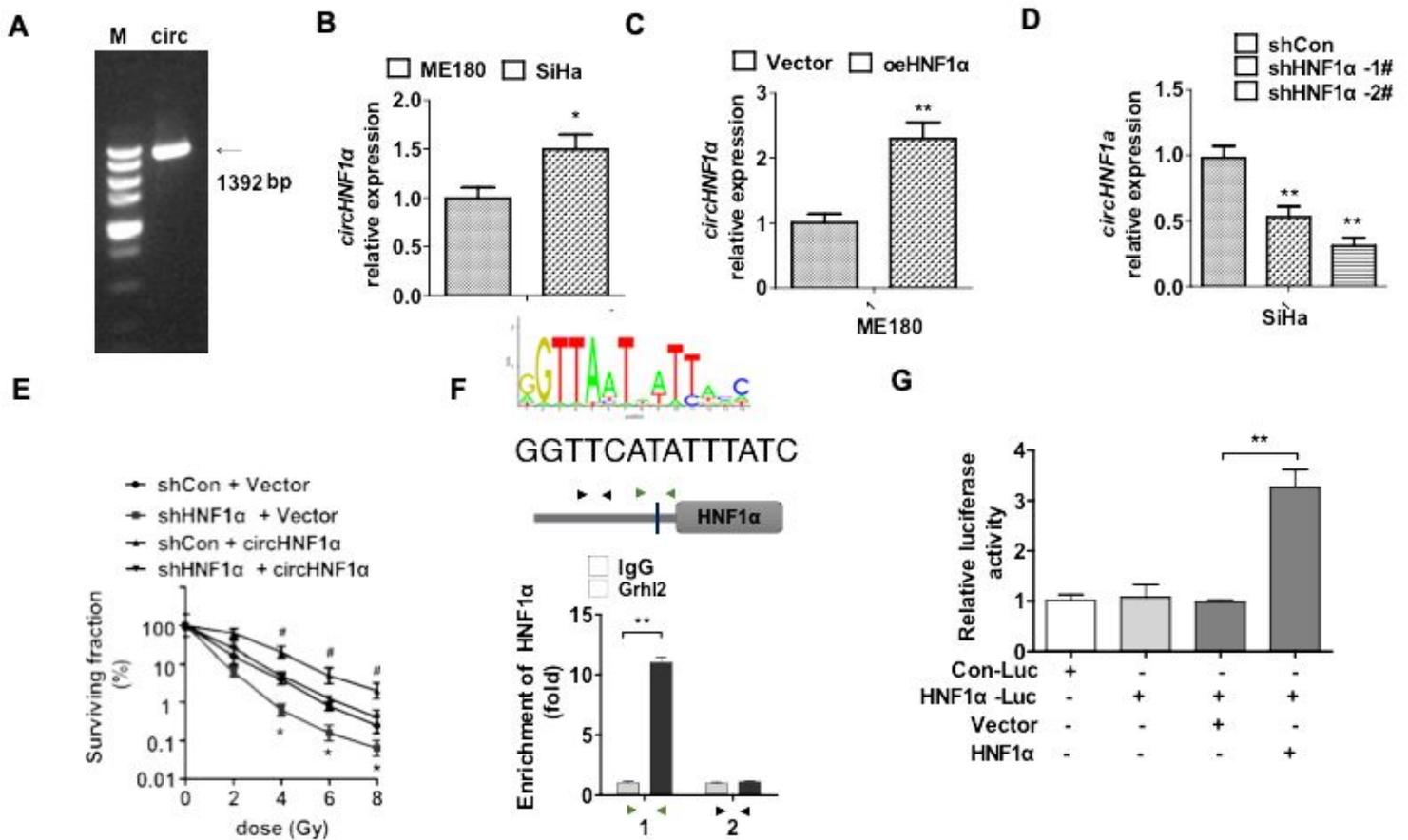


Figure 4

Upregulation of HNF1α increases the expression of circHNF1α in radio-resistant CC cells. (A) Agarose gel electrophoresis of PCR products was used to detect the existence of circ-HFN1α. (B) qRT-PCR was used to detect the circHNF1α level in ME180 cells and SiHa cells. *P < 0.05 vs ME180 cells. (C) ME180 cells were transfected with HFN1α overexpression vector or empty vector. qRT-PCR was performed to detect circHNF1α level. **P < 0.01 vs. empty vector. (D) SiHa cells were transfected with sh-HFN1α-1, sh-HFN1α-2, or sh-control, respectively. qRT-PCR was used to detect circHNF1α level. **P < 0.01 vs. sh-control. (E) ME180 cells were transfected with overexpression plasmid of circHNF1α and shHNF1α, respectively, or co-transfected with both and then treated with 0, 2, 4, and 8 Gy of irradiation. Cell survival was analyzed using colony formation assay. *P < 0.05, #P < 0.05 vs corresponding control. (F) ChIP-qPCR was used to detect HNF1 binding to the HFN1α and circ HFN1α promoter regions. **P < 0.01, vs. IgG. (G) HFN1α-promoter-luciferase reporter were co-transfected with HFN1α overexpression vector or empty vector, and then luciferase reporter assays were performed. **P < 0.05 vs. empty vector

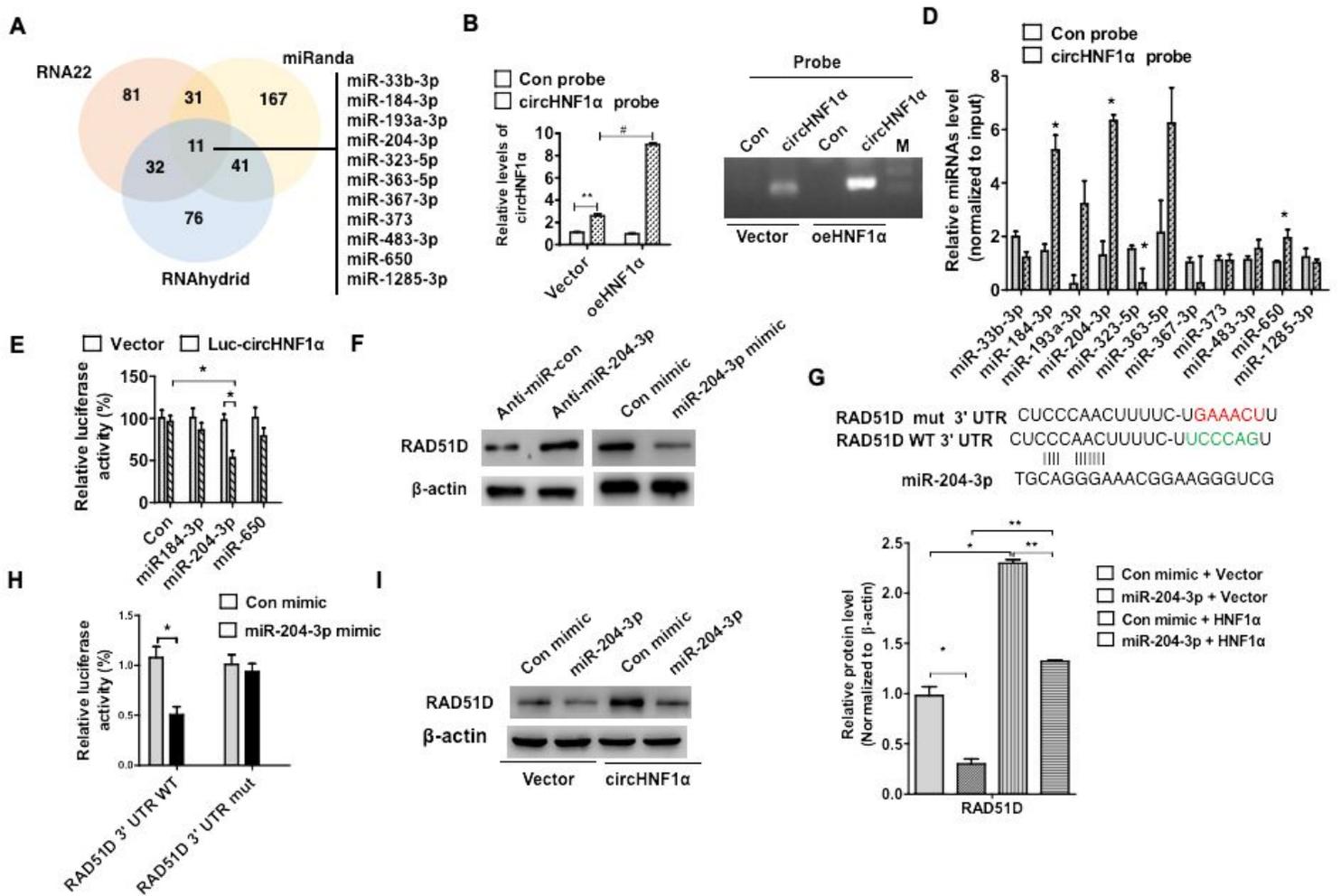


Figure 5

circHNF1α elevates the expression of RAD51D by sponging miR-204-3p. (A) The potential binding miRNAs of circHNF1α were identified from three different target prediction programs using Venn diagram. (B) ME180 cells were transfected with circHNF1α or empty vector, pulled down from ME180 cell lysates with biotin-labeled circHNF1α or control probe; qRT-PCR was performed to determine the pull-down efficiency. * $P < 0.05$ vs. corresponding control. (C) Agarose gel electrophoresis was used to detect the PCR products. (D) The miRNAs were pulled down from ME180 cell lysates with biotin-labeled circHNF1α or control probe. qRT-PCR was used to detect the relative expression of the indicated miRNAs. Expressions are normalized to that of U6. * $P < 0.05$ vs. control probe. (E) ME180 cells were co-transfected with wild-type (WT) or mutant (mut) circHNF1α-luciferase reporter and miRNA mimics. Luciferase reporter assays were performed to detect the luciferase activity. * $P < 0.05$ vs. empty vector. (F) ME180 cells were transfected with miR-204-3p mimic, mimic-con, anti-miR-202-5p, or anti-miR-con. Western blot analysis was performed to determine RAD51D protein level. (G) Potential binding site of miR-204-3p at the 3'UTR of RAD51D. (H) ME180 cells co-transfected with miR-204-3p mimic and WT or mutant (mut) RAD51D 3'-UTR-luciferase reporter. Luciferase reporter assays were used to detect luciferase activity. * $P < 0.05$ vs. control mimic. (I) ME180 cells were transfected with overexpression plasmid of circHNF1α and miR-204-3p mimic, respectively, or co-transfected with both. Western blot was used to detect RAD51D protein level.

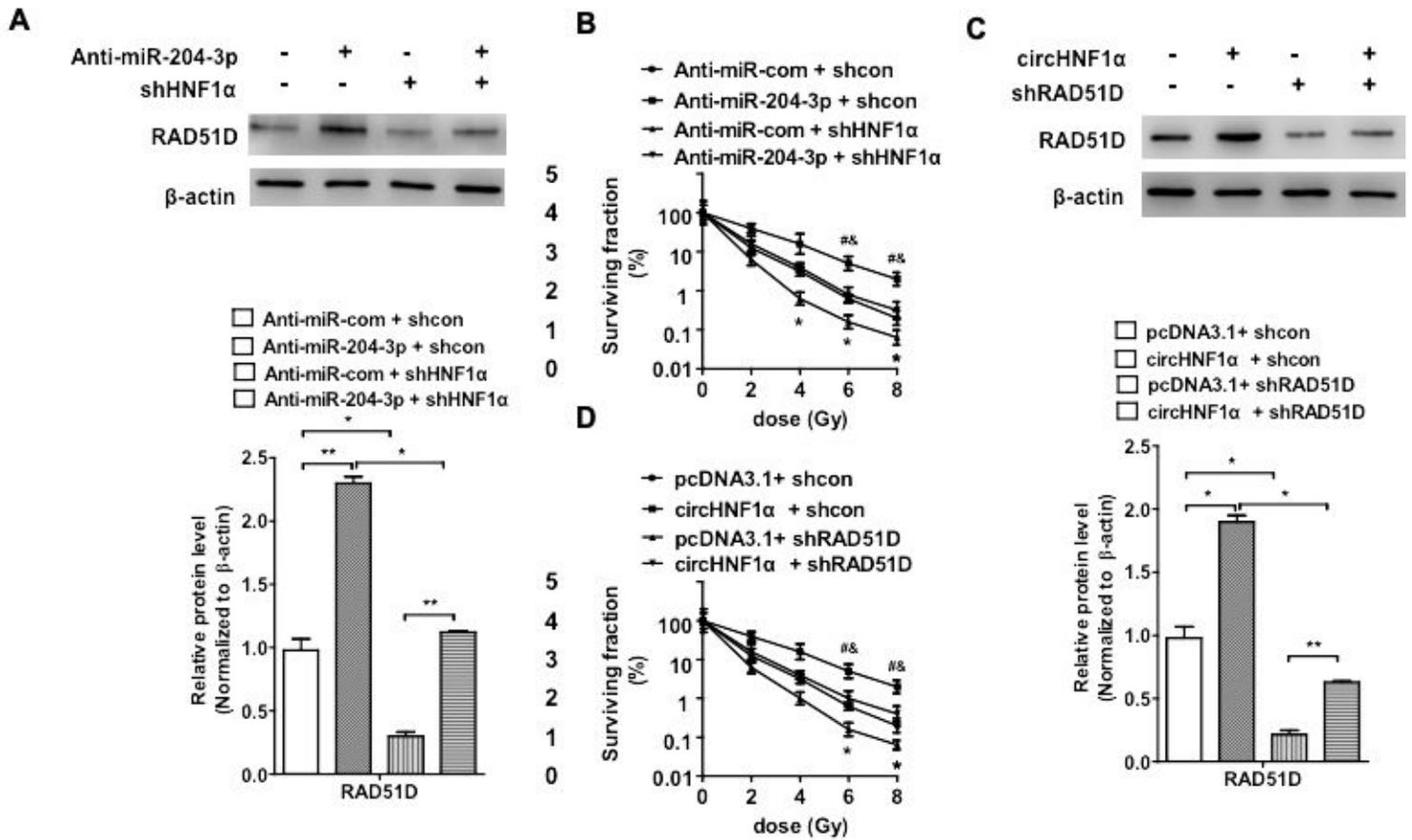


Figure 6

HNF1 α /circHNF1 α /miR-204-3p/RAD51D axis regulates the radio-resistance of CC cells. (A) ME180 cells were transfected with shHNF1 α and anti-miR-204-3p, respectively, or co-transfected with both of them. Western blot was used to detect RAD51D protein level. (B) Cells were prepared as in (A) and then treated with 0, 2, 4, and 8 Gy of irradiation. Cell survival was analyzed using colony formation assay. * $P < 0.05$, ** $P < 0.05$ vs corresponding control. (C) ME180 cells were transfected with overexpression of circHNF1 α and anti-miR-204-3p, respectively, or co-transfected with both of them. Western blot was used to detect RAD51D protein level. (D) Cells were prepared as in (A) and then treated with 0, 2, 4, and 8 Gy of irradiation. Cell survival was analyzed using colony formation assay. * $P < 0.05$, ** $P < 0.01$ vs corresponding control.

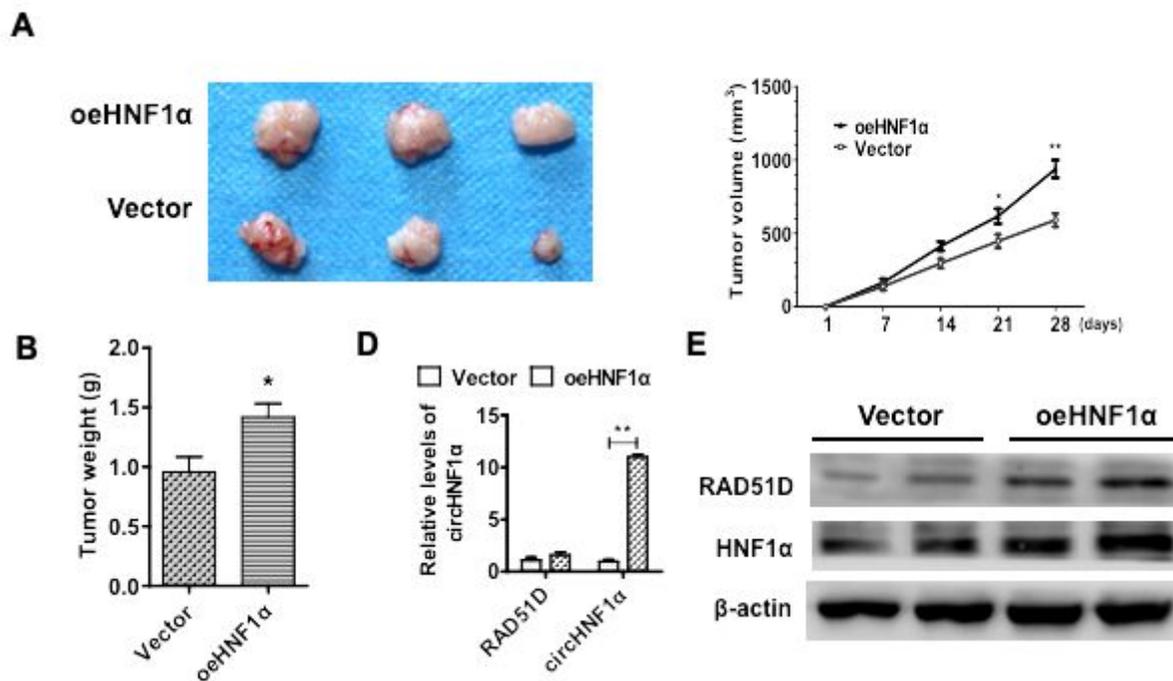


Figure 7

HNF1α is involved in CC radio-resistance in vivo. (A) ME180 cells with stable expression of HNF1α or negative control ME180 cells were injected into nude mice to establish xenograft tumors. Tumor volume was monitored by direct measurement with calipers and calculated using the formula: $(\text{length} \times \text{width}^2)/2$. * $P < 0.05$, ** $P < 0.01$ vs. control group. (B) Xenograft tumor wet weight in each group of mice. * $P < 0.05$ vs. control group. (C) qRT-PCR was used to detect the circHNF1α and RAD51D mRNA level in xenograft tumor tissues. ** $P < 0.01$ vs. control group. (D) Western blot analysis was performed to measure the protein expressions of HNF1α and RAD51D in xenograft tumor tissues.

Supplementary Files

This is a list of supplementary files associated with this preprint. Click to download.

- [Supplementaryinformation.docx](#)