Silencing of lncRNA H19 Enhances the Sensitivity to X-rays and Carbon-Ions Through the miR-130a-3p /WNK3 Signal Axis in Non-Small-Cell Lung Cancer Cells

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Abstract

Background: LncRNA H19 was believed to act as an oncogene in various types of tumors and was considered to be a therapeutic target and diagnosis marker. However, the role of lncRNA H19 in regulating the radiosensitivity of non-small cell lung cancer (NSCLC) cells was unknown. However, the effects of lncRNA H19 on radiosensitivity of NSCLC were not clear.

Methods: The expression profiles of lncRNAs were explored via transcriptome sequencing in NSCLC. The CCK-8, EDU, and clonogenicity survival assay were conducted to explore the proliferation and radiosensitivity in NSCLC cells.

Results: Expression patterns of lncRNAs revealed that compared with A549 cells, lncRNA H19 was upregulated in radioresistant NSCLC(A549-R11) cells. Knockdown experiments revealed that lncRNA H19 enhanced the *radiation sensitivity* of both A549 and H460 cancer cell lines to X-rays and carbon ion irradiation. Mechanistically, lncRNA H19 upregulated With-No-Lysine Kinase 3 (WNK3) expression via serving as a sponge of miR-130a-3p and promoted the resistance of NSCLC cells to both X-rays and carbon ion irradiation.

Conclusion: Knockdown of lncRNA H19 promoted the *radiation sensitivity* of NSCLC cells to X-rays and carbon ion irradiation. Hence, lncRNA H19 might function as a potential therapeutic target which enhance the anti-tumor effects of radiotherapy in NSCLC.

Introduction

The incidence of lung cancer ranked second diagnosed malignancy, just behind breast cancer. However, lung cancer is the most common cause of cancer deaths, a fatality of 1.8 million, in 2020.[1]. The treatment of lung cancer include surgery, radiotherapy, chemotherapy, targeted therapy, immunotherapy and so on. Despite the application of targeted therapy, immunotherapy and other treatment methods, the prognosis of lung cancer is very poor, where 5-year survival rate varies from 4%-17%[2]. Radiotherapy could be applying in all stages of disease. Our previous results study showed that inspiring result were obtained in the modality of radiotherapy combined with immunotherapy [3]. However, radiation resistance is acknowledged as one of cancer therapy-failure factors. It is crucial for us to identify key factors which lead to radio-resistance and to improve anti-tumor effect. It is a strategy that overcome radio-resistance and improve tumor response to radiation via combining radiation therapy with other approaches in NSCLC. Hence, suitable therapeutic targets should be screen to help aiding radiation sensitivity.

Compared with photon radiotherapy, carbon ion radiotherapy (CIRT) has its unique advantages, such as Bragg peak, higher relative biological effect, higher linear energy transfer (LET), sharper dose distribution, better target conformity [4]. Especially, it was proven to be safety for patients who undergo interstitial lung disease in older lung cancer patients [5, 6]. One recent prospective phase II study showed that the 2-year local control (LC) rates and overall survival (OS) rates were 91.2% and 91.9%, and 5-year LC rates and 5-year OS rates were 88.1% and 74.9%, respectively in early-stage peripheral NSCLC patients [7]. The

latest clinical outcome showed that CIRT group resulted in a higher 5-year LC rates (92.3% vs 42.4%) and 5-year OS rates (71.8% vs 34.4%) than the stereotactic body radiation therapy(SBRT)group in early-stage NSCLC[8]. Although CIRT can achieve certain results, it remains a large amount of room for improvement.

According to recent studies, the dysregulation of the expression microRNAs (miRNAs) and long non-coding RNAs (lncRNAs) could alter cellular radiosensitivity [9, 10]. LncRNAs, > 200 nucleotides, and miRNAs about 22 nucleotides belong to non-coding RNAs and mediate biological behaviors of various cancers [11]. It is lncRNAs and miRNAs that exert a significant role in modulating biological behaviors of tumor, such as invasion and metastasis. For instance, lncRNA CASC9.5 was involved in the proliferation and metastasis of lung adenocarcinoma (LUAD) [12]. The H19 gene, 2.3kb RNA molecule, locate on chromosome 11p15.5[13]. LncRNA H19 was believed to act as an oncogene in NSCLC and was considered to be a therapeutic target and diagnosis marker [14]. For example, a study revealed that lncRNA H19 was high level in NSCLC patients and may be a diagnosis marker for diagnostic sensitivity and the specificity were 67.74% and 63.08%, respectively [15]. In addition, single nucleotide polymorphisms (SNPs) in lncRNA H19 were associated with susceptibility to lung cancer especially in NSCLC [16]. Numerous previous studies had confirmed that lncRNA H19 was highly expressed in samples of lung cancer [17, 18]. To date, it remains unknown whether lncRNA H19 regulates the radiosensitivity of NSCLC. This work was devoted to the mechanism where lncRNA H19 regulates radiosensitivity in NSCLC.

Materials And Methods

Cell culture

Human NSCLC lines A549 and H460 were grown in RPMI 1640 medium supplemented with 10% fetal bovine serum (FBS). Human embryonic kidney cells (HEK-293T) were cultured in 10% FBS in DMEM medium. These cell lines were all purchased from the Cell Resource Centre of the Chinese Academy of Sciences and cultured at 37 °C in 5% CO_2.

Irradiation

X-rays were generated by a X-RAD generator (Faxitron, USA). The dose rate was 2.0 Gy/min (225 kV, 0.2 mm Al filter). Irradiations were also performed using the carbon ion beam (80.55 MeV/u) which were generated by the Deep Therapy Terminal, Institute of Modern Physics, Chinese Academy of Sciences (HIRFL-CSR). Ray parameters are as follow: dose rate of 2Gy/min and broadened Bragg peak of 5mm.

RNA extraction and PCR

RNA extraction, using TRIzol reagent (Sangon, China), was done at 24 h after transfection for RNA sequence. Reverse transcription of mature miRNA130a-3p was conducted with specific miRNA reverse transcriptase primers (Ribobio, China) and the internal reference was U6. For qRT-PCR of mRNA and lncRNA H19, cDNA was synthesized with Prime Script RT Mix (Takara, China). The

level of each mRNA and lncRNA H19 was normalized to that of a GAPDH control. Changes relative to endogenous controls were calculated using the $2^{-\Delta CT}$ method.

The primers of H19 were as follows:

Fw: 5'TCCTGAACACCTTAGGCTGG3'

Rev: 5'TGATGTTGGGCTGATGAGGT3'

The primers of WNK3were as follows:

Fw: 5'TGTTGAAATGACGGAAGATGACA3'

Rev: 5'TCTGCCACTAGGAGAAGTAGC3'

siRNA knockdown and miRNA mimics experiments

NSCLC cells were plated at 50% confluency in 35-mm petri dishes. The riboFECTTM (Ribobio, China) was used as a transfection reagent. The concentration of both MiR-130a-3p mimic and si-H19 (Ribobio, China) was 50 nM and si-WNK3 (Ribobio, China) was 100 nM. After a further 24 h, cells were harvested. Knockdown or mininc efficiency was measured relative to housekeeping gene by RT-PCR.

lncRNAH19: siRNA: CCTCTAGCTTGGAAATGAA

WNK3: siRNA: GACCGACAGTTGTTTCACA

Dual-luciferase reporter assay

HEK-293T cells were seeded at 1.0×10^6 cells/well in 35-mm petri dishes 1 day before transfection. Cells were transfected with miR-130-3p mininc and WNK3 wild type plasmids/ mutation type plasmids(2.5mg) or lncH19 wild type plasmids/ mutation type plasmids(2.5mg). Transfection reagents were jetPRIME® and Polyplus-transfection (USA). Cells were incubated for 24 hours and, when indicated, were treated by the Dual-Luciferase Reporter Gene Assay Kit (Beyotime, China) following the manufacturer's instructions. The vector of dual-luciferase reporter was pmiR-RB-Report (Ribobio, China), which included Renilla luciferase (Rluc) reporter gene and Firefly luciferase (Fluc) reporter gene. Rluc was used as an internal control.

CCK-8 assay

Cell viability was evaluated using a Cell Counting Kit-8 (CCK8, APExBIO, USA). Cells were seeded at 3×10^3 cells/plate in 96-well plates. Cells are irradiated 24 hours after transfection. After a 24h,48h,72h treatment, every plate was added with 10 mL CCK-8 reagent for two hours. Then, the optical density (OD) was read at 450 nm.

5-Ethynyl-20-deoxyuridine (EDU) assay

The EDU assay was performed using the Cell-Light EDU DNA Cell Proliferation Kit (RiboBio, China). Images were obtained from a fluorescence microscope (Olympus, Japan).

Colorogenic survival assay

After transfection and irradiation, the appropriate amounts of cells were seeded in triplicate in 60-mm petri dishes. After 2 weeks of culture, with a reagent of 4% polyformaldehyde and 1% crystal violet, the cells were fixed and stained. Clones contained at least 50 cells were counted.

Western blot analysis

Cells were lysed in RIPA buffer with protease and phosphorylase inhibitors. The protein concentration was measured by the BCA assay kit (Thermo Scientific, USA). Lysates were denatured at 100 °C for 10 minutes, and separated on a 10% or 15% SDS polyacrylamide gel. Protein was transferred to a PVDF membranes (Millipore, USA), then blocked with BSA (Solarbio, China) for 1.5 h. Antibodies used were phosphorylated p38 (Immunoway, YP0338 1:1000), Bcl-2 (Gene Tex, GTX50413 1:500), Bax (Gene Tex, GTX56246 1:500), GAPDH (Gene Tex, GTX100118 1:5000). Western blot gels for Fig4D, 4E.

Flow cytometry

Both A549 and H460 Cells were seeded in 35-mm petri dishes and then irradiated with 6 Gy, 24 hours after transfection. After a further 24h, 48h, 72h incubation, the cells were collected and stained with Annexin V and PI. In summary, cells were stained by apoptosis kit (Roche, USA) following the manufacturer's instructions. Data was analyzed with FlowJo v10.1.

Statistics

Statistical analyses were performed using GraphPad Prism software v7.0. Changes in paired samples were assessed using paired t-tests. Comparisons between treatment groups were experimentally hypothesized or not were made by Student's t-test or ANOVA. All the experiment was repeated three times. Significant difference was defined as $p < 0.05$.

Result

lncRNA H19 regulates the radiosensitivity of A549 and H460 cells to X-rays and carbon ion irradiation

In A549 cells and compared radioresistant cells, the differentially expressed lncRNAs were investigated by high-throughput sequencing. Compared with A549 cells, lncRNA H19 was upregulated in radioresistant NSCLC cells (Fig.1A). The radioresistant cells were obtained from our previous study [19]. According to the TCGA database (https://tcga-data.nci.nih.gov/tcga/), the high level of lncRNA H19 was related to poor survival in lung cancer patients who received radiotherapy (Fig.1B). LncRNA H19 knockdown assays were conducted to elucidate the function of lncRNAH19 on the radiation sensitivity of NSCLC cells. Firstly, the expression of lncRNA H19 was downregulated after siRNA transfection in both A549 cells and H460

cancer cells. The inhibitory efficiency was confirmed by qRT-PCR(Fig.1C). Furthermore, lncRNA H19 knockdown co-treated with X-rays irradiation inhibited colony formation (Fig. 1D). Similarly, lncRNA H19 knockdown together with carbon irradiation inhibited colony formation in A549 cells (Fig.S1A). Compared with the NC treated cells, the proliferation of A549 and H460 cells was suppressed when they were treated with lncRNA H19 downregulation combined with 6 Gy irradiation (Fig. 1E). The EDU-incorporation experiment indicated that DNA synthesis was decreased after inhibition of lncRNA H19 and irradiation (Fig.S1B). When both A549 and H460 cells were treated with 6 Gy of X-rays irradiation, compared with those cells with lncRNA H19 knockdown, the rate of apoptosis was significantly restrained (Fig. 1F). Taken together, these results revealed that lncRNA H19 inhibition sensitizes NSCLC cells to both X-rays irradiation and carbon irradiation.

lncRNA H19 acts as a competing endogenous RNA via sponging miR-130a-3p

According to an online software(http://www.mirbase.org/), a great many miRNAs, including miR-130a-3p, could bind to lncRNA H19(Fig. 2A). As an online lncRNA prediction software (http://starbase.sysu.edu.cn/) predicted, there was a potential combination of H19 and miR-130a-3p. Dual luciferase reporter gene assays was performed to confirm that H19 was the direct target gene of miR-130a-3p (Fig. 2B). The luciferase activity of miR-130a-3p+H19 Wt in the miR-130a-3p mimic group was lower compared with NC group. The two mutant plasmid groups were similar luciferase activities. After knockdown of lncRNA H19, compared with in the NC-treated, the expression of miR-130a-3p was upregulated (Fig.S2A). All of above data suggest that H19 can combined with miR-130a-3p. What is more, after 24 hours transfection of the miR-130a-3p mimic, the level of miR-130a-3p is significant upregulated in both A549 and H460 cells (Fig.S2B). Colony formation assays was used to explore whether miR-130-3p regulates the *radiation sensitivity* of NSCLC cells. Compared to negative control cells, overexpression of miR-130a-3p increased the radiosensitivity of NSCLC cells (Fig. 2C). The result of CCK-8 assay revealed that miR-130a-3p mimic significantly inhibitor cell viability compared with the control groups after irradiation (Fig. 2D). The EDU-incorporation experiment showed that DNA synthesis in NSCLC cells was decreased after miR-130a-3p mimic and irradiation (Fig.S2C). Furthermore, the flow cytometry assay was conducted to explore whether miR-130a-3p regulates apoptosis of A549 and H460 cells (Fig. 2E). The miR-130a-3p mimic promoted the apoptosis of both A549 and H460 cancer cells after 6 Gy irradiation. These results revealed that indicated that lncRNA H19 acted as a competing endogenous RNA via sponging miR-130a-3p which sensitizes NSCLC cells to irradiation.

WNK3 functions as the downstream target gene of miR-130a-3p

The online database (starBase v2.0) was used to predict miR-130a-3p target genes.WNK3 was a candidate. First of all, the inhibitory efficiency of WNK3 was conducted by qRT-PCR in both A549 cells and H460 cells (Fig.3A). Due to suitable target genes should negatively correlate with miRNA expression. In NSCLC cells, when miR-130a-3p was overexpressed, WNK3 was downregulated, compared with NC (Fig. 3B). According to this result, WNK3 was suitable target gene of miR-130a-3p. Hence, WNK3 was chosen for further validation. A dual luciferase reporter assay was used to validate the direct binding

between miR-130a-3p and WNK3 (Fig. 3C). The dual luciferase reporter systems were constructed, including miR-130a-3p recognition elements of the 3'-UTR -WNK3 which were both wild type and mutated type. When the mixture which contain the miR-130a-3p mimic and WNK3 wild-type reporter plasmids was co-transfected into 293T cells, the luciferase activity was attenuated, compared with NC group and both mutation groups. These findings validate that WNK3 was a target of miR-130a-3p. The online database (http://ualcan.path.uab.edu) was used to analysis WNK3 expression. WNK3 was highly expressed in NSCLC samples (Fig. 3D). Additionally, the online website analysis (http://kmplot.com) revealed that high level of WNK3 was related to poor OS in NSCLC samples (Fig. 3E).

WNK3 modulated the *radiation sensitivity* of NSCLC cells and affected the P38 signaling pathway

The function of WNK3 on the *radiation sensitivity* of NSCLC cells was explored. Colony formation assays showed that WNK3 knockdown co-treated with X-rays irradiation inhibited colony formation (Fig.4A). After 6 Gy X-rays irradiation, compared with NC-treated cells, WNK3 inhibitor dramatically restrained the proliferation of NSCLC cells (Fig. 4B). The EDU-incorporation experiment indicated that proliferation in both A549 and H460 cells was decreased after inhibition of WNK3 and irradiation (Fig.S3). Additionally, WNK3 inhibition together with irradiation promoted the apoptosis of NSCLC cells (Fig. 4C). Taken together, these results revealed that WNK3, the downstream target gene of miR-130a-3p, modulates the *radiation sensitivity* of both A549 and H460 cancer cells. Previous studies had proven that WNK3 and p38 modulated apoptotic response in Hela cells[20, 21]. Hence, there had been speculation of whether such function could occur in NSCLC cells. Consequently, the expression of phosphorylated p38 (p-p38) was detected (Fig. 4D). WNK3 inhibition together with irradiation decreased the expression of p-p38. In addition, we ascertain the famous protein of the apoptotic signaling pathway by western blotting. After WNK3 inhibition together with irradiation, the ratio of Bax / Bcl-2 was increased (Fig. 4E). Additionally, a rescue experiment was conducted to analysis whether lncRNA H19 regulates the radiosensitivity through the miR-130a-3p in NSCLC cells. Both A549 and H460 cancer cells were transfected with NC, over-expressing lncRNA H19 plasmid and over-expressing lncRNA H19 plasmid together with miR-130a-3p minic, and then exposed to 6 Gy X-rays. The overexpression of lncRNA H19 enhanced the *radiation sensitivity* of NSCLC cells, which was rescued by the miR-130a-3p minic(Fig. 4F).

Discussion

Radiotherapy is an important treatment modality with indications in all stages of NSCLC. However, it is underutilized. According to an evidence-based indication, it was estimated that seventy-seven percent of patients who suffered from lung cancer should receive radiotherapy [22, 23]. Actually, to achieve a better outcome of radiotherapy, tumors should be delivered higher doses which may lead to unsatisfactory side effects. Therefore, potential targets should be identified to help to enhance radio-sensitivity. NSCLC is characterized by alterations in multiple cellular pathways. Unsatisfactorily, most of targeted treatments only act on single pathway, which may lead to drug resistance. A single miRNA can affect the expressions of multiple mRNAs and each mRNA is, in turn, regulated by lots of miRNAs, which form a complex miRNA-mRNA network. Hence, it is hopeful that the drug resistance will be conquered by miRNA-

targeted drugs in the near future. LncRNAs and miRNAs could regulate a great many varieties of processes through competing endogenous RNAs in numerous cancer types, which suggested that they may be used as therapeutic targets, biomarkers, prognostic indicators.

Radio-sensitivity is modulated by lncRNAs in tumor. For example, lncRNA CRNDE/PRC2 targeting p21 enhance radio-resistance of NSCLC [24]. A recent study revealed that linc-SPRY3-2/3/4, Y chromosome non-coding RNA, regulated radiation sensitivity and affected apoptosis and cell viability in NSCLC [25]. LncRNA PVT1 defined as a poor prognosis factor, enhances radio-resistance by regulating cell apoptosis in nasopharyngeal carcinoma [26]. The interaction between lncRNA H19 and miR-193a-3p regulates radio-sensitivity of hepatocellular carcinoma cells [27]. Hence, identification of radio-sensitive relevant molecular mechanisms may help to improve the efficacy of radiotherapy and increase the anti-tumor effect. In this study, lncRNA H19 was proven highly expressed in radioresistant NSCLC cells. LncRNA H19 inhibition sensitizes NSCLC cells to both X-rays irradiation and CIRT. The result seemed, at least in part, to confirm our hypothesis that lncRNA H19 could modulate the radiosensitivity of NSCLC. Recent years, several advances arose from the H19-related drug development. BC-819, a DNA plasmid, is a potential therapeutic approach for cancers that overexpress the H19 gene. BC-819 was safety and well tolerated for unresectable pancreatic cancer, recurrent ovarian cancer and bladder cancer. Inspiringly, BC-819 together with chemotherapy may enhance anti-tumor therapeutic efficacy [28–30]. Therefore, inspiring results that BC-819 combined with radiotherapy can be expected in the near future.

It is miRNAs that indirectly contribute to coding for proteins. However, once miRNAs bind to 3’ untranslated region (UTR) of messenger RNA (mRNA), mRNA was degradation or could not be translated [31]. MiRNAs have been demonstrated to influence treatment outcome and to predict prognosis in cancer. In our study, lncRNA H19, serving as an endogenous sponge, can directly target miR-130a-3p. A dual luciferase reporter assay seemed to confirm this notion. Moreover, it was miR-130a-3p that inhibited cell proliferation, induced cell apoptosis and determined the radiosensitivity in NSCLC. Similarly, miR130a served as a tumor suppressor in NSCLC. Low expression of miR130a was related to the poor 5-year OS of NSCLC patients. Mechanically, miR-130a downregulated the level of KLF3 to inhibit the growth of NSCLC cells [32]. MiR130a, in vivo and ex-vivo experiments, played an anti-tumor role in cutaneous squamous cell carcinoma [33]. MiR-130a level was low in primary NK cells of NSCLC patients. The killing ability of NK cells was enhanced by the biological function of MiR-130a [34].

Radiotherapy is applied in the treatment of various types of tumors. Many miRNAs, associating with response to radiation treatment, can be used to predict efficacy of radiotherapy. While some render tumors radioresistant, others promote tumor radiosensitivity. For example, the radio sensitivity of cervical cancer in vitro was promoted by miR-22, which were regulated by promoting apoptosis [35]. MiR-27 promoted the sensitivity of NSCLC cells to radiotherapy via homologous recombination-mediated DNA repair and was recognized as a therapeutic target of NSCLC [10]. In recent years, several preclinical trials targeting miRNAs had been initiated. Miravirsen, first miRNA-targeted drug, an anti-miRNA-122, was investigated to compromise HCV replication [36]. TargomiR, miR-15/107 miRNA mimics, was shown well tolerated and safety in patients with recurrent thoracic cancer in Phase I study. Interim data indicated that

six patients receive the eight weeks of protocol treatment and five of them achieved disease control [37]. Similarly, miR-155 regulates multiple pathways, including JAK/STAT, MAPK/ERK and PI3K/AKT. Cobomarsen, antagomiR of miR-155, underwent phase 1 clinical trial in mycosis fungoides, the most common type of cutaneous T-cell lymphoma (CTCL) [38]. Taken together, targeting miRNAs drugs in combination with radiotherapy may be a hopeful anti-tumor approach.

More specifically, WNK3, an important factor in many pathways, functioned as an accelerator of cancer. In this study, starBase v2.0 (http://starbase.sysu.edu.cn/) was used to ascertain miR-130a-3p-targeted mRNA. A dual luciferase reporter assay was used to confirm that miR-130a-3p had specific binding sites with WNK3. In addition, WNK3 was highly expressed in NSCLC, which was correlated with poor prognosis. Functionally, WNK3 inhibition promoted apoptosis and increased radiosensitivity of NSCLC cells to X-rays irradiation. The mechanism of action of WNK3 involved in procaspase-3 and heat shock protein 70. Due to the suppression of WNK3, the apoptotic response was promoted and the activation of caspase-3 was accelerated in HeLa cells [20]. Similarly, WNK3 function as a “bad boy” for the reason that it promoted invasion in glioma [39]. However, the function of WNK3 is completely unknown in NSCLC. Our results revealed that downregulation of WNK3 combined with radiation, the radiosensitivity of tumor cells was increased. Anti-tumor effect, inducing tumor cell apoptosis, is the main mechanism of radiotherapy. Bax and bcl-2 were famous markers of the apoptotic signaling pathway. Our study revealed that WNK3 inhibition co-treated with X-rays irradiation increased the ratio of Bax /Bcl-2. In addition, p38 pathways known as stress activated protein kinases are activated by various environmental and genotoxic stress agents and regulates various cellular functions, including apoptosis, cell proliferation, cell migration and survival and so on [40, 41]. Similar biological function was found in the present study. After receiving a 6 Gy irradiation and WNK3 knockdown, the level of phosphorylated p38 was downregulated. With the expanding development of targeted agents, ralimetinib (LY2228820), an inhibitor of p38, had been developed and been applied in clinical treatment. A randomized controlled trial involving 118 patients revealed that ralimetinib in combination with gemcitabine and carboplatin could improve the PFS of recurrent ovarian cancer patients [42]. What is more, encouraging results were obtained, including ralimetinib together with radiotherapy plus temozolomide in the treatment of glioblastoma [43],as well as with tamoxifen to treat advanced breast cancer[44]. Combining our results, radiotherapy combined with ralimetinib could be considered in future clinical trials.

Conceivably, more mechanisms of lncRNAs which promote radiosensitivity or radio-resistance, especially function as biomarkers, prognostic indicators, or therapeutic targets, are needed to identify in different cancer types. LncRNAs could bind to miRNA, serve as ceRNA to modulate the radio-sensitivity and we focused on the lncRNA H19-miRNA130a-3p-WNK3 of NSCLC in this study. Powered by advanced RNA-based therapeutics, in no distant future, radio-RNA agents can emerge to enhance the anti-cancer effect of radiotherapy in NSCLC.

Abbreviations

NSCLC: non-small cell lung cancer; WNK3: With-No-Lysine Kinase 3; CIRT: carbon ion radiotherapy; LET: higher linear energy transfer; LC: local control rates; OS: overall survival; lncRNAs: long non-coding RNAs; LUAD: lung adenocarcinoma; SNPs: single nucleotide polymorphisms; CCK8: cell counting kit-8; EDU :5-Ethynyl-20-deoxyuridine; UTR: untranslated region; mRNA: messenger RNA

Declarations

Authors' contributions

Xiaohu Wang, Qiang Li and Xiaodong Jin contributed to choose research directions. Xueshan Zhao finished the manuscript. Ruifeng Liu and Hongtao Luo conducted the data analyses. Zhen Yang, Yichao Geng, Shuangwu Feng, Chengcheng Li and Lina Wang performed experiments. All authors read and approved the final manuscript.

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Availability of data and materials

The datasets generated and analyzed during the current study are publicly available from the following online databases: Mirbase database (http://www.mirbase.org/); TCGA(https://tcga-data.nci.nih.gov/tcga/); starBase v2.0 (http://starbase.sysu.edu.cn/) ; UALCAN(http://ualcan.path.uab.edu); Kaplan-Meier Plotter(http://kmplot.com)

Ethics approval and consent to participate

Not applicable.

Consent for publication

Not applicable.

Competing interests

The authors have no conflict of interest.

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Figures

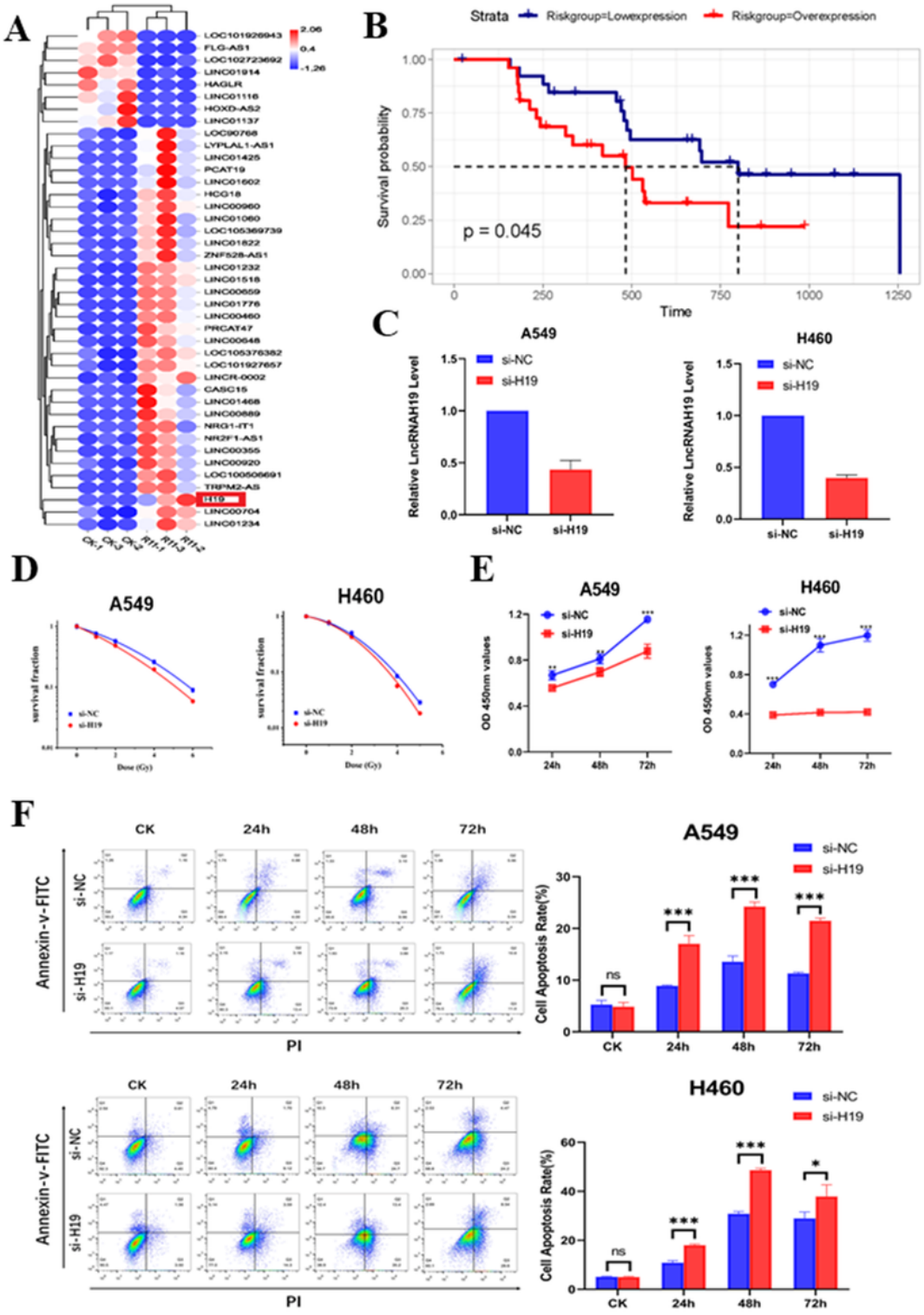

Figure 1

lncRNA H19 regulates the radiosensitivity of NSCLC cells to X-rays and carbon ion irradiation. (A) Differential lncRNAs expression was found between A549 and compared radioresistant cells (A549-R11) cells. (B) The OS of NSCLC patients with different expression of lncRNA H19 after radiotherapy. (C) Transfection efficiency of H19 was measured by qRT-PCR. (D) Colony formation ability after H19 knockdown and irradiation. (E) Cell viability after H19 knockdown and irradiation measured by CCK-8. (F)

Flow cytometric on cells apoptosis after H19 knockdown and irradiation. Data are represented as the mean ± SD. * p < 0.05, ** p < 0.01, *** p < 0.001 compared with the control group.

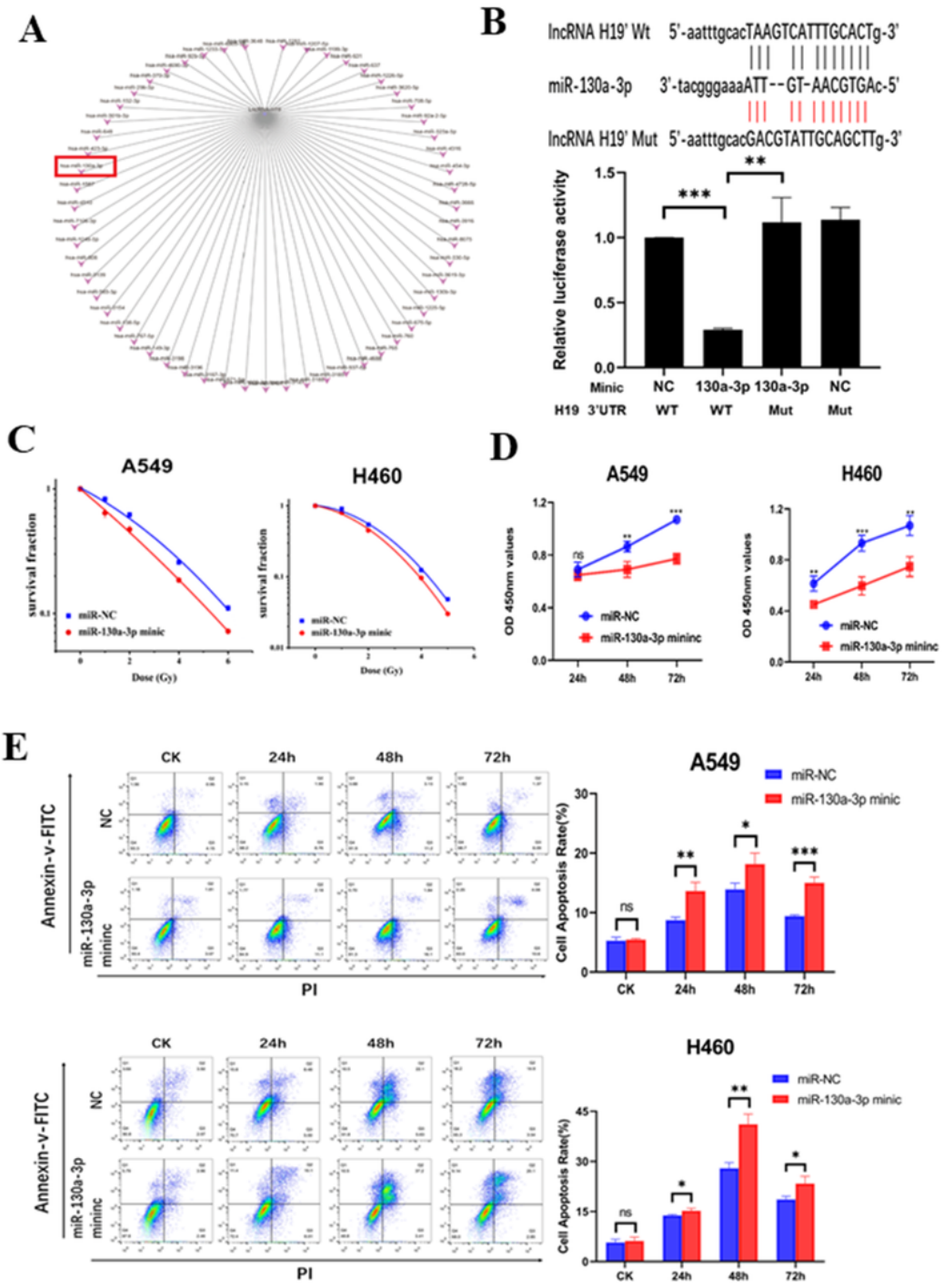

Figure 2

lncRNA H19 acts as a competing endogenous RNA via sponging miR-130a-3p. (A) Predicted miRNAs interact with lncRNA H19. (B)Above: H19 3'-UTR binding site for miR-130a-3p. Below: HEK-293T cells were transfected with a miR-130a-3p mimic/ NC and luciferase reporter containing H19 3'-UTR (WT) or its

mutant construct (Mut). WT-NC was set to “1.0”. (C) Colony formation ability after miR-130a-3p mimic and irradiation. (D) Cell viability after miR-130a-3p mimic and irradiation measure by CCK-8. (E) Flow cytometric on cells apoptosis after miR-130a-3p mimic and irradiation. Data are represented as the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group.

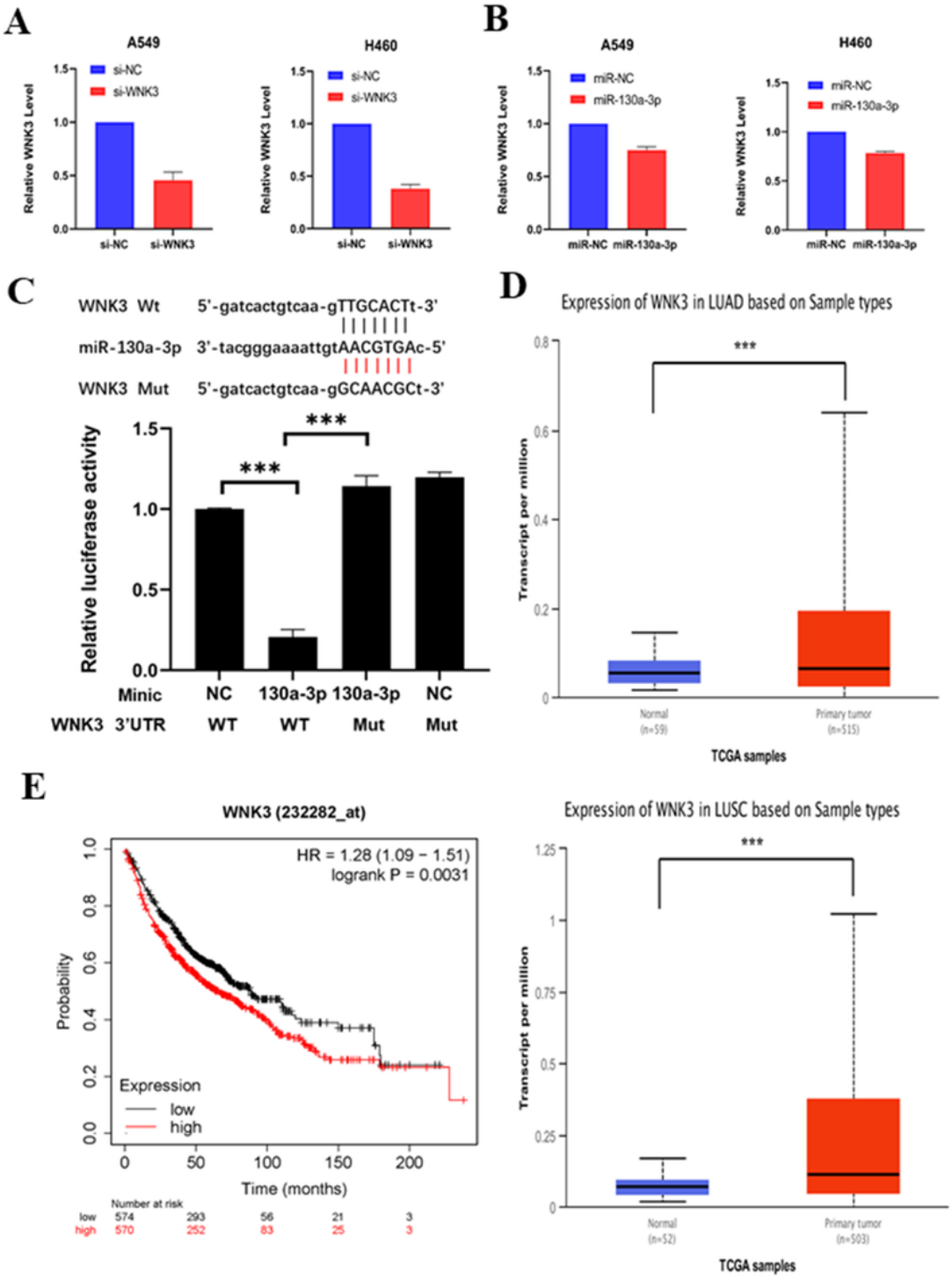

Figure 3

WNK3 functions as the downstream target gene of miR-130a-3p. (A) Transfection efficiency measured by qRT-PCR. (B) Relative expression of WNK3 after transfection with miR-130a-3p mimic. (C) Above: The WNK3 3'-UTR binding site for miR-130a-3p. Below: HEK-293T cells were transfected with a miR-130a-3p mimic/ NC and luciferase reporter containing WNK3 3'-UTR (WT) or its mutant construct (Mut). WT-NC was set to “1.0”. (D) Above: Relative expression of WNK3 between normal tissue and tumor in LUAD. Below: Relative expression of WNK3 between normal tissue and tumor in lung squamous cell carcinoma (LUSC). (E) High level of WNK3 was related to poor OS in NSCLC samples.

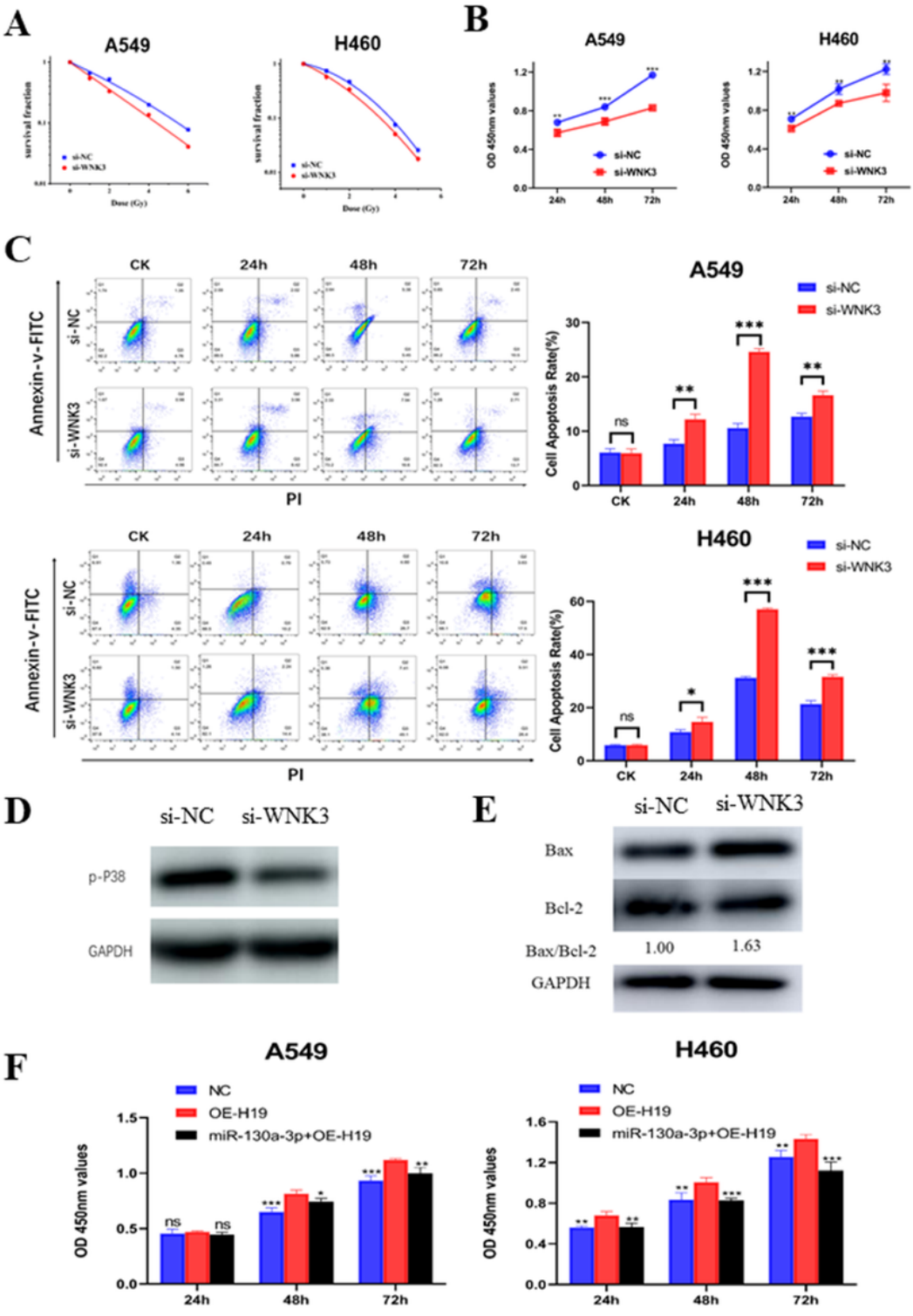

Figure 4

WNK3 modulated the radiation sensitivity of NSCLC cells and affected the P38 signaling pathway. (A) Colony formation ability after WNK3 knockdown and irradiation. (B) Cell viability after WNK3 knockdown and irradiation measured by CCK-8. (C) Flow cytometric on cells apoptosis after WNK3 knockdown and irradiation. (D) The expression of P-P38 in H460 cells after WNK3 knockdown and irradiation. GAPDH was used as the control. (E) The expression of Bax, Bcl-2 after WNK3 knockdown and irradiation. GAPDH was used as the control. (F) Cell viability after transfection with NC or H19 overexpression, or co-transfection with H19 overexpression and miR-130a-3p minic and irradiation tested using CCK-8. Data are represented as the mean ± SD. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$ compared with the control group.

Supplementary Files

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