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miR-4270 Modulates the Irradiation-Sensitivity of Nasopharyngeal Carcinoma Cells through Modulation of p53 *in Vivo*

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The lowered sensitivity to irradiation considerably impacted on the prognosis of nasopharyngeal carcinoma treatments. This study aimed to explore the functions of *miR-4270* in nasopharyngeal carcinoma. Bioinformatic analysis was performed online accessing GSE139164 dataset to screen the top 30 differential microRNAs in nasopharyngeal carcinoma patients with radio-sensitivity. Cancer cell lines, 6-10B and 5-8F, were cultured and measured for expression of *miR-4270* and *TP53* (the gene of the tumor suppressor protein p53) with the normal nasopharyngeal epithelial cells as a control. The *miR-4270* expression was regulated in cells via the introduction of *miR-4270* inhibitor or mimic in different concentrations (25, 50, 100 nmol/L). Targetscan predicted the target of *miR-4270* and the bindings while luciferase was used to confirm this. CCK8 methods were used to evaluate the irradiation sensitivity of the cells after exposure to increasing X-Ray irradiation. RT-PCR detected the RNA expression and Western blot examined the protein expression of p53. Flow cytometry detected the cell apoptosis rates respectively. *miR-4270* is among the top differential microRNAs between the radio-sensitive and -resistant patients. *In vivo*, *miR-4270* expression was lower in cancer cell lines. The inhibition of *miR-4270* raised the cell sensitivity to irradiation. *miR-4270* negatively mediated *TP53* and targeted *TP53*. Additionally, p53 increased cell sensitivity to irradiation and modulated by *miR-4270* in nasopharyngeal carcinoma cells. In conclusion, this study first reports that *miR-4270* is lower in the radio-sensitive patients and modulated the irradiation-sensitivity of nasopharyngeal carcinoma cells through modulation of p53 *in vivo*.

Keywords: *miR-4270*; nasopharyngeal carcinoma; p53; radio-sensitivity
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Introduction

Nasopharyngeal carcinoma (NPC) presents higher occurrences in the population of Southeast Asia, which might be greatly due to the environment and lifestyles (Chen et al. 2019). Currently intensity-regulated radiation plus concurrent chemotherapy has been a good option for most NPC patients (Perri et al. 2019). However, the efficacy of radiotherapy was not optimistic in NPC patients at advanced stage especially in those with distant metastasis after radiotherapy (Sun et al. 2019b).

Recent years, small molecules have emerged as endogenous regulators in cell functions related to various diseases and tumors (Zhang et al. 2020). microRNAs were identified to be abnormally expressed in NPC tissues and cells (Zou et al. 2020). *miR-9* was downregulated in NPC cells and upregulation of *miR-9* could suppress the NPC tumorigenesis via PDK/AKT signaling pathway (Lu et al. 2018). *miR-506-3p* was down-regulated in NPC cells and increase of *miR-506-3p* expression led to the suppression in cell proliferation, migration, invasion and induced apoptosis (Liang et al. 2019). *miR-23* was reported to be upregulated in NPC tissues and related to tumor metastasis in patients and it might regulate angiogenesis through targeting *TSGA10* (Bao et al. 2018). Recently increasing studies focused on unveiling the radio-sensitivity from molecular perspectives. Annexin VII (*ANXA7*) inhibition increased radio-sensitivity of NPC cells through modulation of cell cycles, apoptosis and *in vivo*, tumor growth (Gui et al. 2020). *miR-483-5p* upregulation inhibited the cellular sensitivity to irradiation

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by lowering the apoptosis rates and provoking cell proliferation through targeting *DAPK1*, a cell death-related gene (Tian et al. 2019). Earlier, *miR-24* expression was discovered to be low in NPC cells and patients and inhibition of *miR-24* in NPC cells led to a decrease in the cellular radiosensitivity via targeting SP1 (Kang et al. 2016). Similarly, *miR-372* upregulation promoted apoptosis, p53 and Bax, yet inhibited cell proliferation and Bcl-2 in NPC cells, adding to cellular sensitivity to irradiation (Wang et al. 2019). However, a lot more microRNAs have been identified to display different expression in NPC cells and tissues compared to their normal counterparts (Bruce and Liu 2014; Spence et al. 2016; Zou et al. 2020).

NCBI database provided the GSE139164 dataset openly (https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE139164) and we analyzed and screened out a new microRNA, *miR-4270*, which presented differential expression in the NPC patient samples with sensitivity or resistance to radiotherapy. Furthermore, it was predicted that *miR-4270* might target the apoptosis-associated gene *TP53* in homo sapiens. *TP53*, the gene of the tumor suppressor protein p53, was widely reported as a tumor-related gene, presenting abnormal expression in tumor tissues or cells and participating in the regulation of cancer progression (Ventura et al. 2007). In NPC cases, previous clinical studies showed that p53 was correlated with low 5-year survival rate (Yang et al. 2019). In NPC cells, p53 and Caspase 3 could regulate the cell apoptosis (Weng et al. 2019). In addition, it was revealed that p53 might modulate the ionizing radiation reactivity in NPC cells or animal models (Sun et al. 2020b).

Therefore, we hypothesize that *miR-4270*/p53 might be involved in the modulation of radio-sensitivity of NPC cells. This study aimed to examine the potential role of *miR-4270* and p53 in NPC *in vivo* in association to radiosensitivity.

Materials and Methods

Online database analysis

The GSE139164 dataset was downloaded from NCBI (https://www.ncbi.nlm.nih.gov/). The dataset included 7 samples of NPC radio-sensitive patients and 5 samples with radio-resistance. The most differential microRNAs in the two groups were identified by using the online GEO2R analyzer, from which we listed the top 30 genes and selected one microRNA, *miR-4270*, which has not been researched yet in NPC.

Cell culture

The human NPC cell lines, 6-10B and 5-8F, and normal human NPC cell line, NP69, were purchased from Procell (Wuhan, China). 6-10B is a non-metastatic cell line with high tumorigenicity while 5-8F is derived from metastatic squamous cell cancer. Dulbecco's modified eagle medium (DMEM) and fetal bovine serum (FBS) were bought from Thermo Fisher Scientific (Shanghai, China). The cells were cultured in DMEM with 10% FBS and incubated at 37ºC with 95% O_2 and 5% CO_2. When the cell density reached 80%-90%, cells were passaged. Cells at log growth stage were collected for this study.

Cell transfection and treatment

The cells from 6-10B and 5-8F cell lines were collected and planted onto 6-well plates. The 6-10B cells were transfected with the *miR-4270* inhibitor (25, 50, 100 nmol/L), which was used to target and inhibit the *miR-4270* expression in cells using Lipofectamine 3000 (Thermo Fisher Scientific). 6-10B cells were divided into the control group (without transfection), and the *miR-4270* inhibitor groups, including miR in (25), miR in (50) and miR in (100) groups, which were respectively transfected with the *miR-4270* inhibitor 25, 50 and 100 nmol/L. The 5-8F cells were transfected with *miR-4270* mimic (25, 50, 100 nmol/L) to endogenously upregulate *miR-4270* expression in cells using Lipofectamine 3000. The 5-8F cells were grouped into the control (without transfection), miR m (25), miR m (50) and miR m (100), which were respectively transfected with the *miR-4270* mimic 25, 50 and 100 nmol/L. After 48 h incubation, cells were collected for reverse transcription-polymerase chain reaction (RT-PCR) detection. Cells from all groups were collected after gene regulation and exposed to irradiation of 6-MV accelerator (LINAC, CA, USA) at a dosage of 2, 4, 6 and 8 Gy for 30 min. After cells underwent indicated transfection and irradiation, cells were seeded into 96-well plates for survival analysis.

The *miR-4270* in (100) and control group from 6-10B cell line and the *miR-4270* m (100) and its control group from 5-8F cell line were selected for further study in association with the interaction between *miR-4270* and p53, including RT-PCR and Western blot analysis for p53 in both cell lines. The 6-10B cells were transfected with *sh-TP53* alone or collectively with *miR-4270* inhibitor and the parental 6-10B cells served as a control group. The 5-8F cells were transfected with overexpressed-*TP53* (*oe-TP53*) alone or co-transfected with *oe-TP53* and *miR-4270* mimic with the parental 5-8F cells as a control group. After transfection, cells in these groups were exposed to 6 Gy irradiation for 30 min and thereafter underwent cell viability and apoptosis analysis. The *miR-4270* inhibitor and mimic, and their control plasmids, short hairpin *TP53* (*sh-TP53*, used to knock down *TP53* expression in cells), *oe-TP53* and their control plasmids were all synthesized in GenePharma (Shanghai, China).

Reverse transcription-polymerase chain reaction (RT-PCR)

Total RNA was extracted from each sub-group indicated above, using Beyozol Reagent (Beyotime, Shanghai, China), strictly according to the protocols. RNAs were reversely transcribed into cDNAs with the assistance of RNase H-Kit from Beyotime. Thereafter, BeyoFast™ SYBR Green qPCR Mix Kit was used (Beyotime). The PCR reaction procedure was as follows, 95℃ for 2 min,

pre-denaturation; 95°C 15 sec, denaturation; annealing/extension, 60°C 20-30 sec. The cycle from denaturation to annealing/extension was repeated for 40 times. The ABI 7900HT PCR machine and its software was used to quantify the expression. *U6* and *ACTB* were used as internal control for *miR-4270* and *TP53*. The primer sequences used in this study was listed below. *miR-4270*, Forward: 5′-TCAGGGAGTCAGGG-3′; Reverse: 5′-TGTCGTGGAGTCGGC-3′. *U6*, Forward: 5′-CTCGCTTCGGCAGCACA-3′; Reverse: 5′-AACGCTTCACGAATTTGCGT-3′. *TP53*, Forward: 5′-CAGCACATGACGGAGGTTGT-3′; Reverse: 5′-TCATCCAAATACTCCACACGC-3′. *ACTB*, Forward: 5′-TCGTCCACCGCAAATGCT-3′; Reverse: 5′-AACCGACTGCTGTCACCT-3′.

Western blot analysis

Cells were collected as indicated above in the cell transfection and treatment section. Total protein was extracted using radio immunoprecipitation (RIPA) lysis buffer from Beyotime. Then proteins were separated on sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) and thereafter transferred onto the polyvinylidene fluoride (PVDF) membranes. The membranes were blocked for 1 h using Tris buffered saline Tween (TBST) with 5% skimmed milk. After washing the membranes using TBST, the PVDF membranes were prepared for antibody incubation. First, the primary antibodies after dilution were added and incubated with the membranes overnight at 4°C. The next day, TBST washed the PVDF membranes for at least 5 times and then the secondary antibody was added after dilution and the membranes were incubated for 1 h. The p53 antibody (ab32389, 1:1,000) and the beta-actin antibody (ab115777, 1:200) were bought from Abcam (Shanghai, China). The secondary antibody was goat anti-rabbit IgG H&L (HRP) preadsorbed (H&L HRP) (ab97080, 1:20,000) from Abcam. The BeyoECL Plus (Beyotime) was used and the protein bands were shown after developing and fixation. The grey values of the protein bands were analyzed using ImageJ (NCBI).

Cell counting kit 8 (CCK-8) for cell viability

Cells after transfection underwent respectively different dose rate of irradiation (0, 4, 6 and 8 Gy) from each group from the 6-10B and 5-8F cell lines were collected and seeded onto 96-well plates with 2,000 cells/μl in each

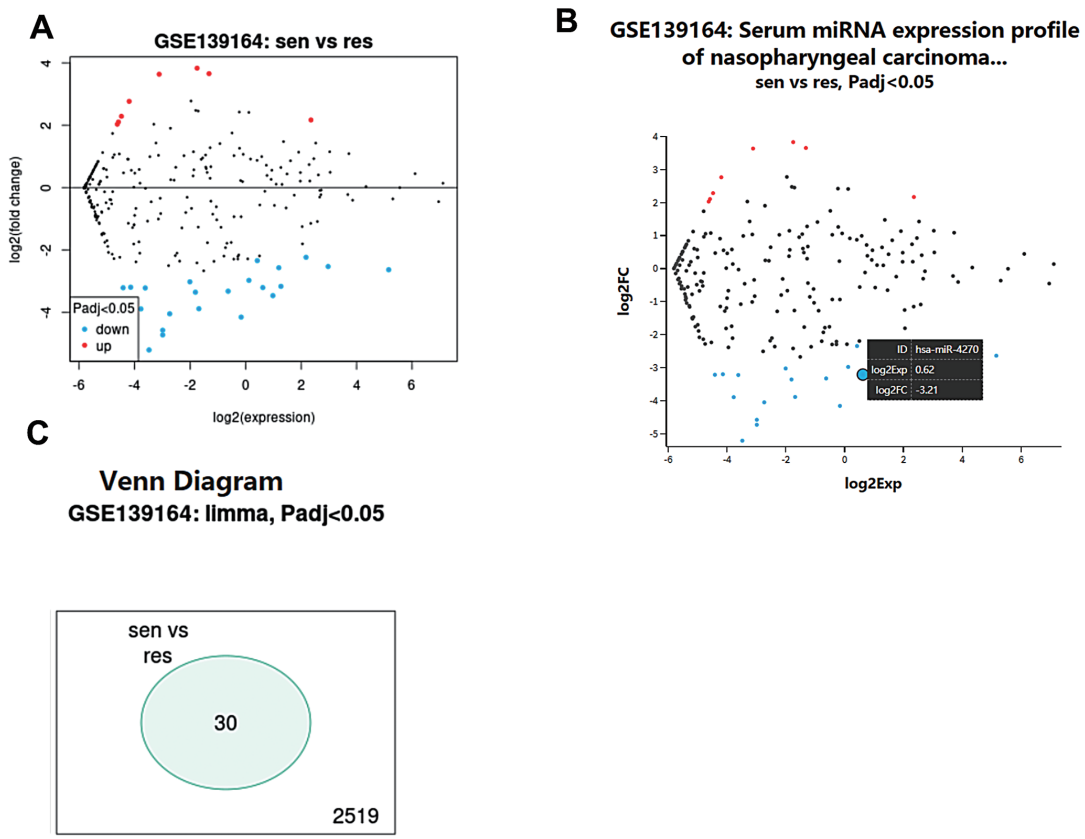

Fig. 1. *miR-4270* is downregulated in the radio-sensitive nasopharyngeal carcinoma (NPC) patients.
GSE139164 dataset was assessed (https://www.ncbi.nlm.nih.gov/) and analyzed for differential microRNAs in NPC patients with radio-sensitivity or -resistance. A) The top differential miRNAs were plotted. The red parts are the upregulated genes in the radio-sensitive group versus the radio-resistant, and the green parts are the most downregulated in the radio-sensitive group. B) *miR-4270* was the most downregulated. C) Venn Diagram demonstrated that there were 30 miRNAs in common in all the NPC samples.

Table 1. The most downregulated 30 miRNAs in the radio-sensitive group.

	log2 (fold change) (sen vs. res)	-log10 (P value) (sen vs. res)
hsa-miR-3610	−5.212	6.294
hsa-miR-6840-3p	−4.729	3.689
hsa-miR-4634	−4.58	3.398
hsa-miR-4763-3p	−4.158	3.1
hsa-miR-3195	−4.051	2.467
hsa-miR-197-5p	−3.894	2.684
hsa-miR-4532	−3.888	2.426
hsa-miR-6749-5p	−3.469	2.792
hsa-miR-4507	−3.358	1.85
hsa-miR-1915-3p	−3.327	1.99
hsa-miR-7108-5p	−3.223	1.662
hsa-miR-3620-5p	−3.216	2.181
hsa-miR-4270	−3.21	2.293
hsa-miR-4530	−3.199	1.901
hsa-miR-1225-5p	−3.17	2.539
hsa-miR-6724-5p	−3.025	1.516
hsa-miR-1202	−2.979	1.859
hsa-miR-6090	−2.639	4.144
hsa-miR-6821-5p	−2.573	1.774
hsa-miR-6869-5p	−2.536	2.398
hsa-miR-4516	−2.345	1.339
hsa-miR-3665	−2.239	1.681
hsa-let-7f-1-3p	2.029	1.375
hsa-miR-7111-3p	2.104	1.45
hsa-miR-574-5p	2.166	1.452
hsa-miR-6732-3p	2.283	1.648
hsa-miR-6865-3p	2.764	2.267
hsa-miR-425-3p	3.634	2.441
hsa-miR-1825	3.652	1.991
hsa-miR-1908-3p	3.829	2.134

The 30 miRNAs are all listed, and *miR-4270* is among the most down-regulated ones.
sen, patients sensitive to radiotherapy; res, patients resistant to radiotherapy.

well. The parental cell lines without transfection underwent respectively different dose rate of irradiation (0, 4, 6 and 8 Gy) were regarded as control groups. After 48-h culture, 10 μl CCK8 solution (Beyotime) was added in each well and the plates were incubated in an incubator for 1.5 h at 37°C. The lab microplate reader was used to read the optical density (OD) values at the wavelength of 450 nm.

Further, the *sh-TP53* and *sh-TP53+miR-4270* in and the control groups derived from 6-10B cell line and the *oe-TP53*, *oe-TP53+miR-4270* m and their control groups from 5-8F cell line were selected after 6 Gy irradiation for cell viability analysis. Cells from each group was planted into 96-well plates and after 0, 24, 48 and 72 h, 10 μl CCK8 solution was added respectively. After 1.5 h, the OD values were read.

Luciferase reporter assay

Targetscan Human (http://www.targetscan.org/vert_72/) was utilized to predict targets for *miR-4270* in homo sapiens, from which, *TP53* was selected for further analysis. The targeted sites were predicted and verified using Luciferase reporter gene methods. The 6-10B cells were selected and co-transfected with *TP53*-WT (wild type) or *TP53*-MU (mutant) and *miR-4270* inhibitor or inhibitor control(in) while the 5-8F cells were co-transfected with *TP53*-WT (wild type) or *TP53*-MU (mutant) and *miR-4270* mimic or mimic control(m) using Lipofectamine 3000. Briefly, the luciferase activity in all groups were measured using Dual-Luciferase Reporter Assay System from Promega (Shanghai, China) after 48-h transfection.

Flow cytometer for cell apoptosis analysis

Cells were collected for apoptosis analysis and seeded onto 96-well plates. Annexin V-FITC apoptosis kit was purchased from Beyotime. Phosphate buffered saline (PBS) washed the cells for three times and then 10 μl prodium iodide (PI) and 5 μl Annexin V-FITC were added and blended gently. The plates were incubated at room temperature for 20 min in dark room and then were placed in ice bath. The flow cytometer, CytoFLEX was used and the apoptosis rates were analyzed on the CytExpert Software (Beckman Coulter, Beijing, China).

Statistical analysis

All the results displayed in mean and standard deviation (SD) values were derived from three independent experiments for each group. Graphpad7 (Graphpad, CA, USA) was used to analyze the results and evaluated the significance of the differences between two groups (t-test) or among multi-groups (one-way ANOVA for single index by Tukey's correction method; two-way ANOVA for multiple indexes by Sidak's correction method). Alpha is 0.05 in this study.

Results

miR-4270 is downregulated in the radio-sensitive NPC patients

GSE139164 dataset was assessed (https://www.ncbi.nlm.nih.gov/) and analyzed for differential microRNAs in NPC patients with radio-sensitivity or -resistance. The top differential miRNAs were plotted and the red parts are the upregulated genes in the radio-sensitive group versus the radio-resistant and the green parts are the most downregulated in the radio-sensitive group (Fig. 1A) and *miR-4270* was among the downregulated as explained in the Fig. 1B. Venn Diagram demonstrated there were 30 miRNAs in common in all the NPC samples. The 30 miRNAs were all listed and *miR-4270* was among the most down-regulated ones (Table 1).

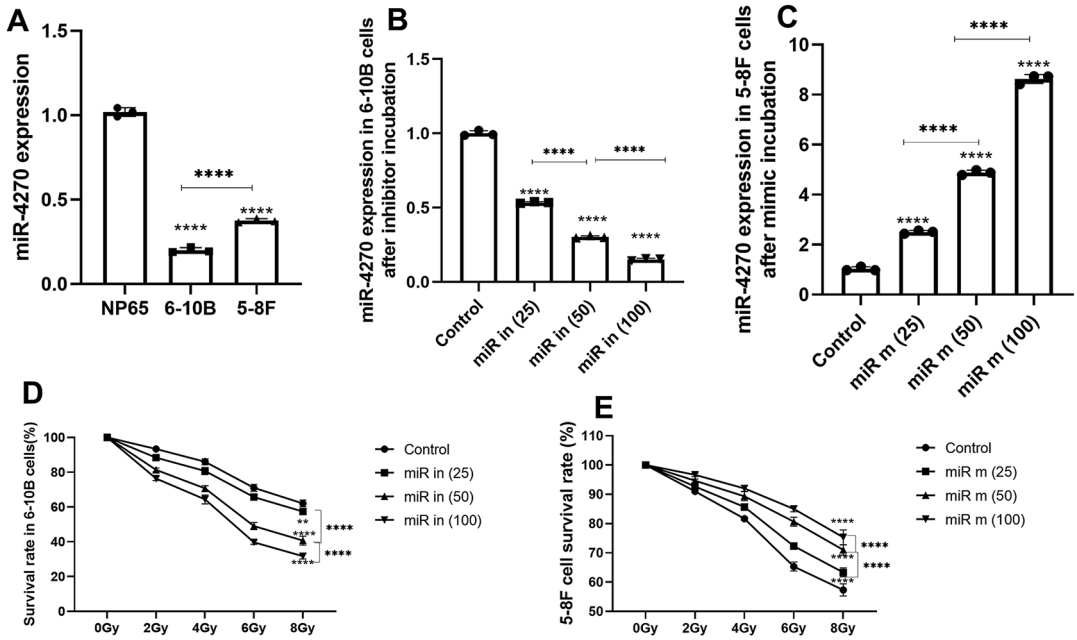

Fig. 2. *miR-4270* inhibition raised the cell sensitivity to irradiation in nasopharyngeal carcinoma (NPC) cells. A) RT-PCR measured the *miR-4270* expression in NPC cell lines, 5-8F and 6-10B with the normal immortal nasopharyngeal epithelial cell line NP69 as a control. B, C) *miR-4270* expression was downregulated in 6-10B cells (B) and upregulated in 5-8F cells (C). RT-PCR measured the RNA expression of *miR-4270* in cells after treatment. The control group here was the untreated cell lines. D, E) CCK8 assessed the consequent survival rates after cells underwent the X-Ray irradiation. The control group here was the untreated cell lines. ****P < 0.0001, ***P < 0.0002, **P < 0.002, compared to the control group or compared between two groups that are shown by the lines.

miR-4270 inhibition raised the cell sensitivity to irradiation in NPC cells

miR-4270 expression was measured in the NPC 5-8F and 6-10B cell lines with the normal immortal nasopharyngeal epithelial cell line NP69 as a control, and RT-PCR results showed that the expression of *miR-4270* was downregulated in the NPC cell lines and in detail, lower in the 6-10B cell line than 5-8F cell line (Fig. 2A). Then we inhibited the *miR-4270* expression in 6-10B cell line and RT-PCR further verified the inhibition of *miR-4270* in cells (Fig. 2B). Also, 5-8F cells were selected to increase the *miR-4270* expression and RT-PCR validated the upregulation of *miR-4270* in 5-8F cells (Fig. 2C). Cells after regulation of *miR-4270* expression underwent X-Ray radiation (0, 2, 4, 6 and 8 Gy) for 30 min. CCK8 kit was used to measure the cell survival rates in each group. It was discovered that as *miR-4270* expression decreased, the cell survival rate was inhibited, meaning that *miR-4270* inhibition enhanced the radio-sensitivity of 6-10B cells (Fig. 2D). On the other hand, increase of *miR-4270* in 5-8F cells resulted in the inhibition of radio-sensitivity (Fig. 2E).

miR-4270 negatively mediated TP53 in NPC cells by targeting it

In 6-10B cells, *TP53* mRNA level and p53 protein level were enhanced when *miR-4270* was inhibited (Fig. 3A, C). When *miR-4270* was upregulated in 5-8F cells, the expression of *TP53* mRNA and p53 protein was inhibited (Fig. 3B, D). Thereafter, Targetscan predicted the potential bindings between *TP53* and *miR-4270* in homo sapiens (Fig. 3E). Luciferase gene reporter method was introduced to validate this and results confirmed the prediction (Fig. 3F, G).

TP53 increased cell sensitivity to irradiation and modulated by miR-4270 in NPC cells

6-10B cells were transfected with *sh-TP53* alone or additionally with *miR-4270* inhibitor while 5-8F cells were transfected with *oe-TP53* plasmid or modulated with *miR-4270* m collectively. RT-PCR and Western blot revealed that the expression of *TP53* mRNA and p53 protein was both inhibited in the *sh-TP53* group but restored in the co-transfected group in 6-10B cells (Fig. 4A, B). Otherwise, the same methods discovered that the expression of *TP53* mRNA and p53 protein was enhanced, but was inhibited with the introduction of *miR-4270* mimic into the 5-8F cells (Fig. 4C, D). Further, cell viability was attenuated in the *sh-TP53* group of 6-10B cells but was restored after the involvement of *miR-4270* inhibitor (Fig. 4E). On the other hand, flow cytometer experiments showed that the apoptosis rate was inhibited as the inhibition of *TP53* in the *sh-TP53* group of 6-10B cells and was recovered due to the co-transfection of *miR-4270* inhibitor (Fig. 4G). Additionally, in the 5-8F cells, cell viability was enhanced as the upregulation of *TP53* and was significantly reduced by the additional transfection of *miR-4270* mimic (Fig. 4F).

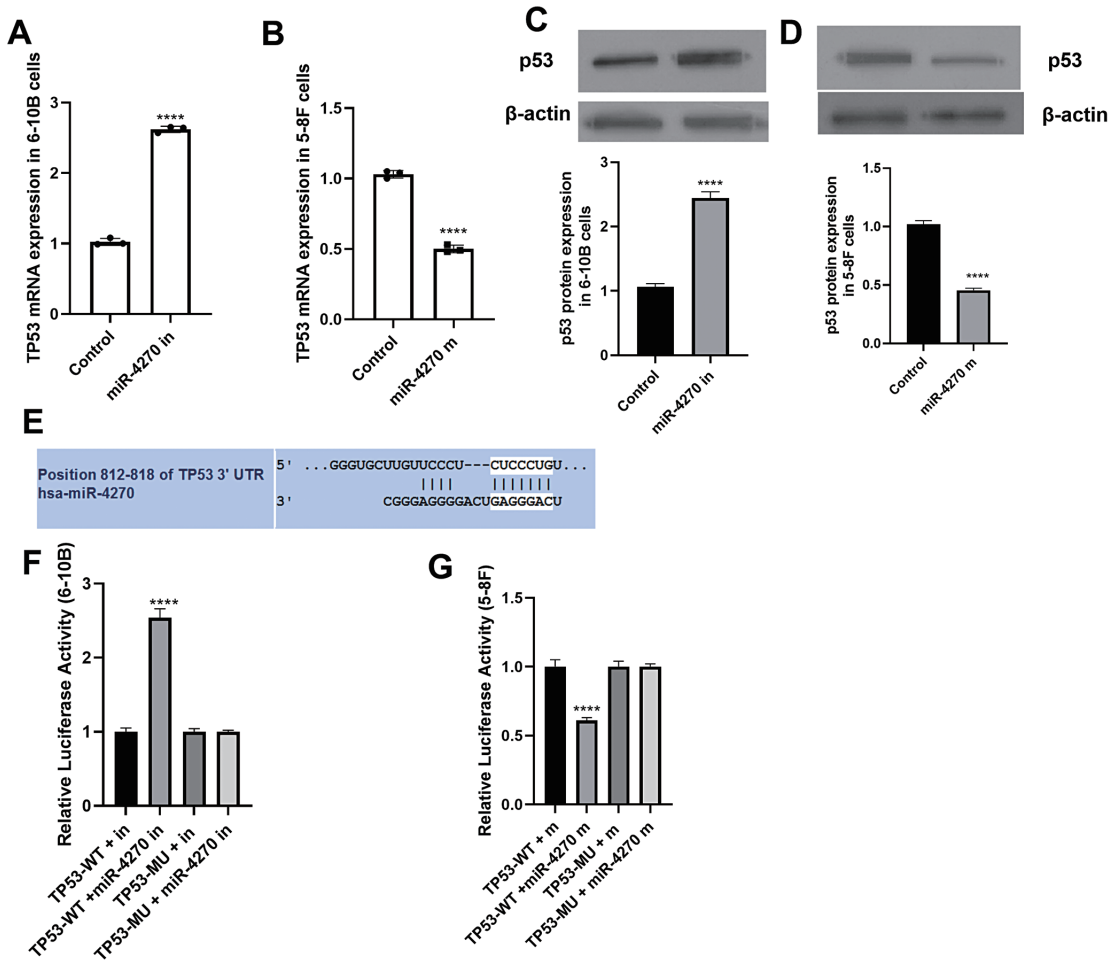

Fig. 3. *miR-4270* negatively mediated *TP53* in nasopharyngeal carcinoma (NPC) cells by targeting it.
A, B) RT-PCR measured the mRNA expression of *TP53* in 6-10B cells (A) and 5-8F cells (B) after regulation of *miR-4270*. ****P < 0.0001 compared to the control group without *miR-4270* regulation. C, D) Western blot methods analyzed the protein expression of p53 in 6-10B cells (C) and 5-8F cells (D), and the bands were evaluated using ImageJ. ****P < 0.0001 compared to the control group without *miR-4270* regulation. E) Taregetscan was used to predict the potential targets of *miR-4270* in homo sapiens. F, G) Luciferase reporter gene assays were used to evaluate the luciferase activity in 6-10B cells (F) and 5-8F cells (G). ****P < 0.0001 compared to the other groups transfected with *TP53-WT*.

Apoptosis rates were respectively increased in the *oe-TP53* group and suppressed by the introduction of *miR-4270* mimic in 5-8F cells (Fig. 4H).

Discussion

In this study, we first screened a new microRNA, *miR-4270*, which showed significantly different expression between the NPC patients with radio-sensitivity or radio-resistance using the open dataset GSE139164 (NCBI), which made us hypothesize that *miR-4270* might be involved in the NPC progression *in vivo*. Previously, *miR-4270* was discovered to be downregulated in retinoblastoma tissues and cells, and worked as a tumor suppressor by inhibiting the cell proliferation, invasiveness and mobility (Feng et al. 2021). Interestingly, in breast cancer tissues, *miR-4270* was among the top upregulated genes and closely associated with the stage of cancer (Hamam et al. 2016). Microarray method also discovered that *miR-4270* expression was considerably lower in the hepatocellular carcinoma tissues and its diagnostic value was also indicated previously (Zhu et al. 2018). In gastric cancer, *miR-4270* was downregulated and inhibition of *miR-4270* in cells resulted in the decrease in proliferation, invasion and migration and the functions of *miR-4270* could be modulated by the *Hsa_circ_0005556* and *MMP19* (Shen et al. 2020). In addition, *miR-4270* was revealed previously to be associated with brain metastasis in lung adenocarcinoma (Sun et al. 2019a; Sun et al. 2020a,). In non-small cell lung cancer, *miR-4270* was upregulated and targeted *Linc00472* and *KLLN*, and regulated the cancer progression through p53 pathway (Zou et al. 2019). We examined the role of *miR-4270* in NPC cells in this study and found that down-regulation of *miR-4270* could increase the cell radio-sensitivity *in vivo*.

Furthermore, we validated that *TP53* was a target of *miR-4270* in NPC cells and *miR-4270* could negatively reg-

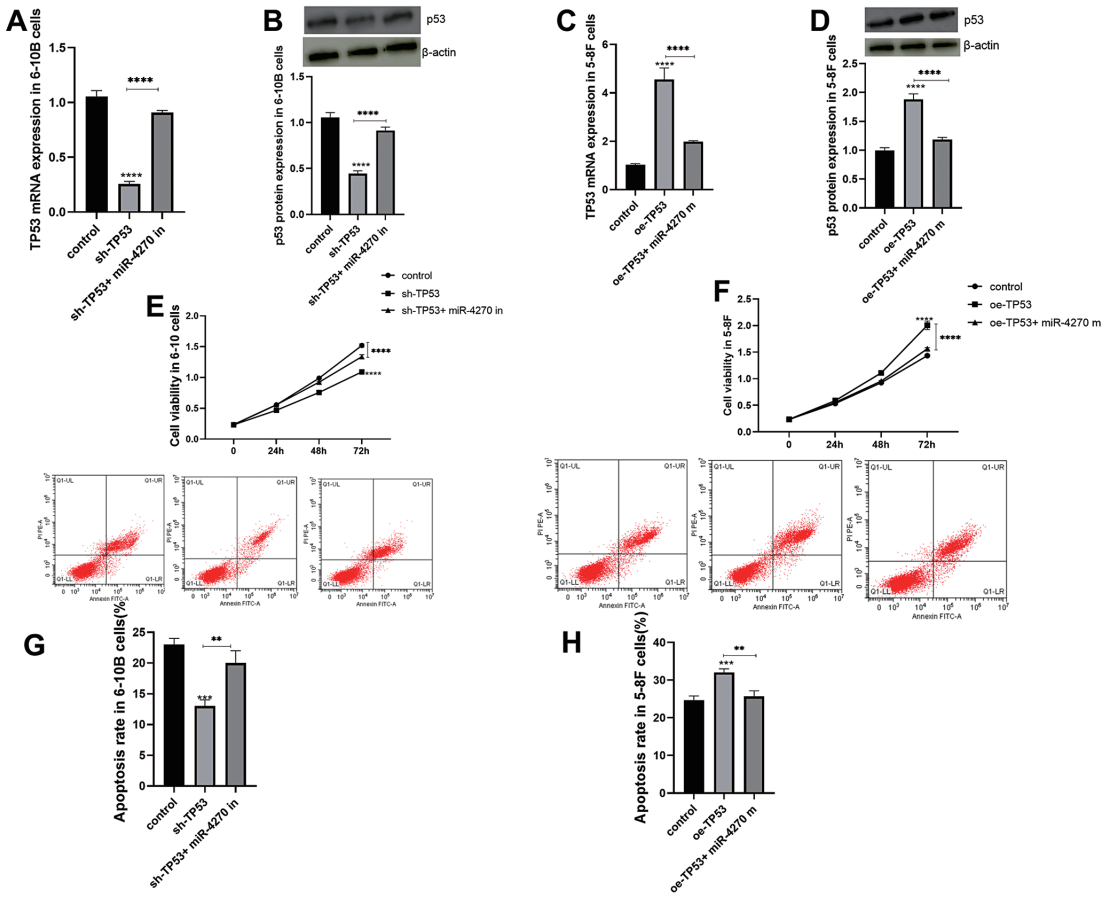

Fig. 4. *TP53* increased cell sensitivity to irradiation and modulated by *miR-4270* in nasopharyngeal carcinoma (NPC) cells. 6-10B cells (A, C, E, G) were transfected with *sh-TP53* alone or additionally with *miR-4270* inhibitor while 5-8F cells (B, D, F, H) were transfected with *oe-TP53* plasmid or modulated with *miR-4270* m collectively. A-D) RT-PCR and Western blot methods measured the p53 mRNA and protein expression levels. Image J was used to evaluate the bands accordingly. E, F) CCK8 method evaluated the cell viability. G, H) Flow cytometry assays were used to detect the apoptosis rates in each group. ****P < 0.0001, ***P < 0.0002, **P < 0.002, compared to the control group, or compared between two groups that are shown by the lines.

ulate *TP53* mRNA and protein levels. *TP53* could encode the tumor suppressor protein p53, and p53 is normally inhibited in cancers and the restoration of p53 function was revealed to curb the tumor progression (Ventura et al. 2007, Wasylishen and Lozano 2016). Due to its significant role in modulating cancer cell apoptosis, p53 has been revealed to be associated with the radio-sensitivity in the context of drug treatment *in vivo* (Fernández-Aroca et al. 2019). The depletion or mutation of *TP53* could induce radio-resistance or chemoresistance in cancer cells and *in vivo* (El-Deiry 2003). Furthermore, the phosphorylated p53 was an indispensable part of DNA repair pathway, whose activation could improve the DNA repair and decrease the cellular apoptosiss (Zhang et al. 2019). In neuroblastoma mice model, loss of p53 induced radio-resistance *in vivo* (Yogev et al. 2016). In melanoma, the restoration of p53 benefited the radio-sensitivity *in vivo* (Krayem et al. 2019). In NPC cells and mice, an inhibitor of *MDM2* was revealed to inhibit *MDM2* and enhance *TP53* in mRNA and protein levels, and promoted the cell apoptosis and tumor growth (Fan et al. 2019). This study correlated the *TP53* and *miR-4270* with the cell apoptosis and viability in NPC cells and it was shown that inhibition of p53 in cells could decrease the cell apoptosis and increase cell viability while the restoration of p53 could induce higher cell apoptosis rate, and the upregulation of *miR-4270* could curb the p53 effect in cells through negative modulation of mRNA and protein levels of *TP53*.

The limitation of this study exists in the absence of potential canonical pathways associated with p53 pathway. Further studies could feature on a bigger picture of the regulatory mechanisms involved.

The current study demonstrated an *in-vitro* analysis and posed a new microRNA, *miR-4270* in NPC. *miR-4270* expression was lower in the NPC patients with higher radio-sensitivity. Furthermore, the downregulation of *miR-4270* increased the sensitivity of NPC cells against irradiation via p53 regulation. Therefore, this research might help to identify the *miR-4270* as a new biomarker in radio-sensitivity among NPC patients.

Conflict of Interest

The authors declare no conflict of interest.

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