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miR-625-3p Regulates Transcription Factor 21 (TCF21) Expression to Enhance Radio-resistance of Hepatoma Cells

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ABSTRACT miR-625-3p regulates cell functions in various cancers. However, its role in radio-resistance in hepatoma remains to be unveiled. This study aims at investigating the functions of miR-625-3p in hepatoma. RT-PCR detected miR-625-3p/TCF21 expression in hepatoma tissues and cell line. MTT assays were conducted to analyze the miR-625-3p role in regulations of radio-resistance of hepatoma cells. Overall survival was evaluated in correlation to miR-625-3p among patients. Luciferase reporter experiments validated the predicted TCF21 as miR-625-3p target. Higher miR-625-3p expression was discovered in hepatoma patients. In tissues of radio resistant patients, miR-625-3p was higher. The overexpression of miR-625-3p increased the radio-resistance of hepatoma cells to irradiation. miR-625-3p adversely moderated TCF21 via binding to the 3'UTR of TCF21. Downregulating miR-625-3p promoted the cell sensitivity to irradiation, which was reversed by TCF21 inhibition.

INTRODUCTION

Hepatoma is a prevalent aggressive cancer, which accounts for 90 percent of liver digestive cancers and has poor survival rate because of its relapse in spite of remarkable advances in therapy for the past ten years (Wang et al. 2019). Hepatocellular Carcinoma (HC) can be treated with focal high-dose irradiation employing a diversity of cutting-edge and specialized treatment strategies including conformal irradiation planning, radiofrequency ablation, and image-guided irradiation (Craig et al. 2020; Dawson and Guha 2008; Maruyama et al. 2008; Yu et al. 2018). Radio-resistance poses challenges in ineffective clinical result and resistance is reported to be promoted by several factors including aberrant patterns of microRNA (miRNAs) expression (Lin et al. 2018; Ma et al. 2018; Wu et al. 2020; Zhang et al. 2015). miRNAs were discovered to regulate cancer cell apoptosis after irradiation (Ebrahimi and Hashemy 2019; Niemoeller et al. 2011). miR-20a enhanced cell radio-resistance through activation of the PTEN/PI3K/Akt in HC in vitro (Zhang et al. 2015). Furthermore, miR-193a-3p modified the radio-resistance of HC cells via regulation of PSEN1 (Ma et al. 2018). Screening miRNAs and exploring possible downstream targets may also facilitate the development of diagnosis and prognosis of cancers. miRNAs regulated canonical pathways in lung cancer, colorectal cancer, osteosarcoma through inhibiting the target genes (Gmerek et al. 2019; Wang et al. 2019; Wu et al. 2019). miRNAs significantly dysregulated in cancer cells or tissues are of interest. miR-210 was discovered to be upregulated in HC tissues and its inhibition induced cell apoptosis and reduced cell proliferation (Li et al. 2020). miR-625-3p was abnormally increased in thyroid cancer and its overexpression promoted cancer cell invasion and migration (Fang et al. 2018; Fang et al. 2019). miR-625-3p was reported as a biomarker in colorectal cancer and induced drug resistance and inhibitory strategy could be used to attenuate the resistance situation in vitro (Rasmussen et al. 2016). However, miR-625-3p functional roles in hepatoma and responses to irradiation remain unknown.

TCF21 is an important transcriptional factor that functions in cell cycle, proliferation, apoptosis, differentiation and so on (Ao et al. 2020). TCF21 can

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be regulated by noncoding RNAs like miRNAs. In ovarian cancer, TCF21 was targeted and suppressed by miR-205, which induced the cancer progression (Wei et al. 2017). Similarly, miR-3648 could target and negatively regulate TCF21, promoting the bladder cancer progression (Sun et al. 2019). However, the role of TCF21 in hepatoma and responses to irradiation remain unknown. Based on the online tool TargetScan, miR-625-3p might target TCF21. Therefore, the researchers hypothesized miR-625-3p/TCF21 axis may be engaged in the regulation of HC radio resistance in vitro.

Objectives

This study aims at investigating the functions of miR-625-3p/TCF21 in hepatoma and the responses to irradiation in vitro.

MATERIAL AND METHODS

Time of Data Collection and Experiments

Tissues were collected from March 2019 to June 2020 in Shengzhou People's Hospital. Experiments were performed from July to October of 2020.

Tissue Collection

Researchers collected 60 fresh human hepatoma tissue samples and respective adjacent non-tumor tissue samples from patients without undergoing preoperative chemotherapy or irradiation before biopsy sampling at Shengzhou People's Hospital. The specimen were frozen and stored at minus 80^0C after sampling during surgery. Then, the researchers conducted irradiation and effectiveness was analyzed toward the end of irradiation. Subgroups, Resistant (n=38) and Sensitive (n=22) groups were formed.

Ethical Declaration

The Committee of Ethics in Shengzhou People's Hospital authorized and approved these experiments and patients signed informed consent voluntarily. The declaration of Helsinki during the experiments was adhered to.

Cell Culture and Cell Transfection

Researchers purchased the following human hepatoma cell lines human HC cell lines (Huh-7, BEL-7405, HB611, HepG2 and SMMC7721) and the human normal hepatocytes (L02) from ATCC (CA, USA). The cells were incubated using RPMI-1640 supplemented with 10 percent FBS (Gibco, Shanghai). The HB611s were incubated with 5 percent CO_2 at 37^0C and were trypsinized and collected following 80–90 percent convergence. Lipofectamine 2000 (Beyotime, Shanghai, China) was used for transfection of cells. The cells were transfected using Lipofectamine 2000 (Thermo Fisher, Beijing) with miR-625-3p mimics, miR-625-3p inhibitor, and respective negative control mimics (NC mimics), negative control inhibitor (NC inhibitor), sh-TCF21, and sh-NC (Guangzhou Fulengen Co., Ltd, Guangzhou, China).

Irradiation Treatment

Researchers performed cell treatment with irradiation for the transfected or non-transfected HB611 cells. The cells were subjected to 0, 2, 4, 6, 8 and 10 Gy 6-MV x-ray produced by a linear accelerator (Varian 2300EX at a prescription rate of 5 Gy/minute; CA, USA).

RT-PCR

Total RNA was separated using Beyozol reagent (#R0011, Beyotime, China), followed by reverse transcription of 1 μg RNA to cDNA employing BeyoRT™ cDNA First Chain Synthesis Kit (#D7166, Beyotime). RT-PCR assays were performed using Beyofast™ SYBR Green QPCR Mix (Beyotime) to examine levels of miR-625-3p and TCF21 expression (Xiao et al. 2020). GAPDH and U6 were used as the internal control and the $2^{-\Delta\Delta CT}$ calculation was followed. The primers were designed and then sent for synthesis to Genelily Bio-Tech (Shanghai, China) and summarized in Table 1. All experiments were conducted in triplicates.

Table 1: Primer sequences

Name	*Sequences*
miR-625-3p (forward)	5'- GGGGAGGGGGAAAGTTCTA-3'
miR-625-3p (reverse)	5'- TATCCAGTGCGTGTCGTG-3'
TCF21 (forward)	5'- GCCCACTTGAGGCAGATCCT-3'
TCF21 (reverse)	5'- CCACCATAAAGGGCCACGTC-3'
GAPDH (forward)	5'- AGAAGGCTGGGGCTCATTTG-3'
GAPDH (reverse)	5'- AGGGGCCATCCACAGTCTTC-3'

MTT Assay

Researchers performed MTT assays to determine cell survival rates in different groups. The cells were inoculated onto 96-well plates (5×10^3 cells each well). 20 µl of Methyl thiazolyl tetrazolium (MTT) solution at 5mg/ml was supplemented and the cells were further cultured for 5 hours in a saturated incubator (Sigma-Aldrich) (Liu et al. 2019). 150 µl of DMSO was added (Sigma, NJ, USA) after discarding the supernatant to dissolve the formazan. Absorbance was then observed at the wavelength of 450 nm on the lab microplate reader (Thermo Fisher, USA).

Western Blotting

Researchers performed western blot assays to measure the protein changes of TCF21. The HB611 cells were lysed using RIPA (Beyotime). The proteins were thereafter separated using SDS-PAGE method and then were transferred onto PVDF membranes. The membranes were cultured with primary and then secondary antibodies: TCF21 Polyclonal Antibody (1:1000, #PA5-53031, Invitrogen, USA) and anti-GAPDH antibody (1: 1000, #AF1186, Beyotime) at 25^0C for one night, subsequently incubated with IgG HRP (1:5000, G-21234, Invitrogen). Then, enhanced chemiluminescence reagent kit (Beyotime) was used. The blots were developed using an ECL imaging system (Tanon, Tanon-5200) and protein expression was analyzed on Image J software (NCBI).

Bioinformatics Analysis

Researchers used TargetScan (targetscan.org/vert_72/, a Bioinformatics analyzer to predict the putative binding sites between TCF21 and miR-625-3p. Furthermore, StarBase tool (http://starbase. sysu.edu.cn/) was used to analyze the overall survival rates in relation to TCF21 expression in HC patients.

Luciferase Reporter Assay

The target sequence TCF21 comprising of the wild type (WT) or mutant type (MT) miR-625-3p binding sites were inserted into a pGL3 Dual luciferase Vector (Promega, WI, USA). These WT or MT-TCF21 plasmids were co transfected into HB611 cells with miR-625-3p mimic, miR-625-3p inhibitor or their controls using Lipofectamine 2000 (Liu et al. 2019). After 48 hours, the luciferase activity was analyzed using the Dual Luciferase reporter Kit (Promega, WI, USA).

Statistical Analysis

All the experiments were done in triplicate. One-way ANOVA method and student's t test, were used for analysis of the statistical differences in groups. Kaplan Meir revealed the connections between overall survival and miR-625-3p or TCF21 expression levels in patients. Results were regarded statistically significant when $P<0.05$. The statistical analysis was conducted on GraphPad Prism 5 (GraphPad Software, CA, USA) and SPSS 18.0 version (SPSS Inc., IL, USA).

RESULTS

miR-625-3p is Upregulated in HC Tissues, Cells and it is Associated with Low Overall Survival Rate

RT-PCR assays were performed in 60 matched hepatoma and non-cancer tissue specimens. Significant upregulation of miR-625-3p in HC tissues in contrast to normal ones (Fig. 1A, $P<0.05$). miR-625-3p was upregulated in the irradiation-resistant group rather than the irradiation-sensitive tissues (Fig.1B, $P<0.05$). Furthermore, to analyze overall survival associated with miR-625-3p expression, 60 hepatoma patients were classified into high miR-625-3p group and low miR-625-3p group. Generally, low survival rate was noticed in hepatoma patients with higher miR-625-3p as opposed to high survival rate in hepatoma patients with lower miR-625-3p (Fig. 1C, $P<0.05$).

miR-625-3p Inhibition in HC Cells Decreases Cellular Radio-resistance

miR-625-3p expression was higher in 42 cases that were resistant to irradiation (Fig. 1A, $P<0.05$). miR-625-3p was found to be higher in the HB611 cells with irradiation ranging from 0, 2, 4, 6, 8 and 10Gy (Fig. 2B, $P<0.05$). miR-625-3p was overexpressed in the miR-625-3p mimic group and downregulated in the miR-625-3po inhibitor group in HB611 cells after transfection (Fig. 2C-D, $P<0.05$). Cells were exposed to irradiation with doses rang-

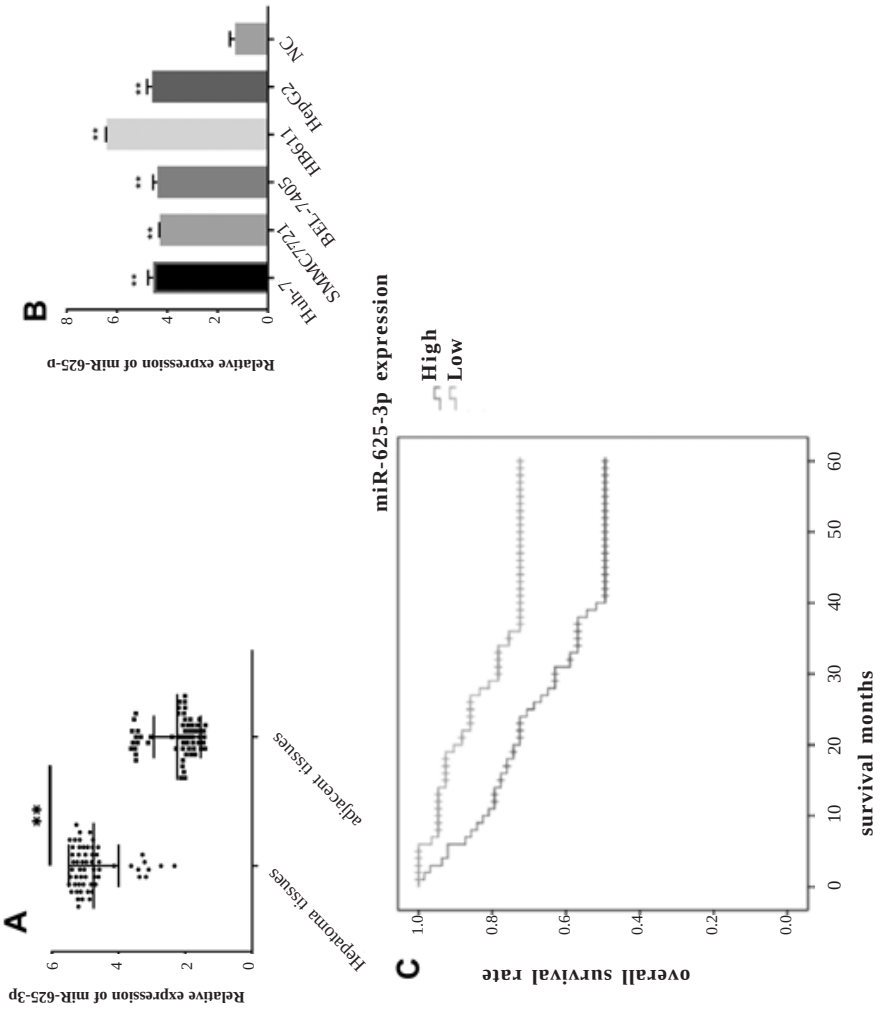

Fig. 1. miR-625-3p is upregulated in HC tissues, cell lines and it is associated with low overall survival rate
A) miR-625-3p expression in HC tissues(n=60) and paired normal ones(n=60) by RT-PCR assays
B) miR-625-3p expression in HC subgroups, radioresistant(n=38) and radiosensitive (n=22) groups
C) Kaplan-Meier overall survival analysis for HC patients(n=60). VS NC, $^{**}P < 0.05$.

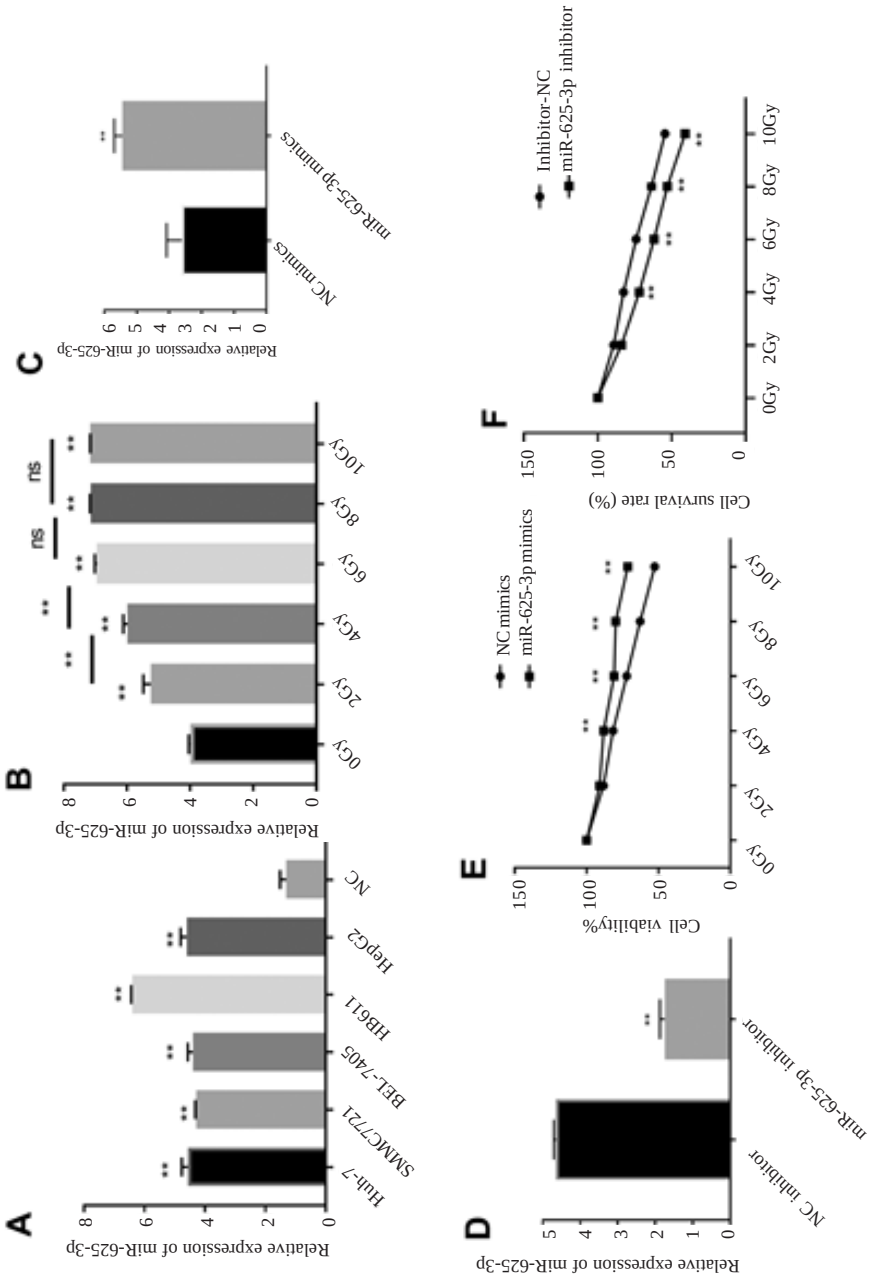

Fig. 2. Downregulated miR-625-3p in HC cells decreases cellular radio-resistance
A) RT-PCR assays for miR-625-3p expression in HC tissues of radio-resistant group and radio-sensitive group
B) HB611 cells were exposed to irradiation (0, 2, 4, 6, 8, 10Gy) and RT-PCR detected the expression of miR-625-3p
C-D) RT-PCR detected the expression of miR-625-3p in HB611 cells that were transfected with miR-625-3p mimics and miR-625-3p inhibitor and their negative controls
E-F) Cells were subjected to irradiation and cell survival was examined using MTT assays. $^{**}P < 0.05$

ing from 0, 2, 4, 6, 8 and 10Gy for 24 hours and the MTT assays examined the cell survival. miR-625-3p overexpression significantly alleviated the inhibiting effects by irradiation, suggesting miR-625-3p could add to the radio resistance of HC cells while the miR-625-3p inhibition could add to the efficacy of irradiation in HC cells (Fig. 2E and F, $P<0.05$).

miR-625-3p targets TCF21 and High TCF21 Expression is Correlated with High Survival Rates in HC Patients

TCF21 was predicted as a target for miR-625-3p on TargetScan, which revealed putative bindings between miR-625-3p and TCF21 (Fig. 3A). miR-625-3p mimic, NC mimic or miR-625-3p inhibitor, NC inhibitor, were co-transfected with TCF21-WT and or TCF21-MUT to cells. miR-625-3p mimic decreased luciferase activity of TCF21-WT whereas increased luciferase activity was observed in cells transfected with miR-625-3p inhibitor and TCF21-WT in HB611 cells, suggesting that TCF21 was a direct target gene of miR-625-3p and could be adversely moderated by miR-625-3p in hepatoma cells (Fig. 3B-C, $P<0.05$). TCF21 mRNA expression was reduced in the miR-625-3p upregulation group and was enhanced in miR-625-3p inhibition group compared to the negative controls (Fig. 3D, $P<0.05$). Using StarBase, the researchers found that among HC patients with high expression of TCF21 tended to have higher survival rates (Fig. 3E, $P<0.05$). Furthermore, RT-PCR results discovered that TCF21 mRNA expression was lower in the radio-resistant patients (Fig. 3F, $P<0.05$).

miR-625-3p Influences the Radio-Resistance of HC Cells via Regulation of TCF21

Western blot results supported that upregulation of miR-625-3p could decrease the protein expression of TCF21 in HC cells and the downregulation could increase the TCF21 protein (Fig. 4A-B, $P<0.05$). miR-625-3p downregulation could inhibit TCF21 in mRNA and protein levels and knockdown of TCF21 further decreased the TCF21 mRNA and protein levels (Fig. 4C-E, $P<0.05$). Furthermore, the knockdown of TCF21 decreased the cell survival rate, which was enhanced by the downregulation of miR-625-3p in HC cells (Fig. 4F, $P<0.05$).

DISCUSSION

This study has investigated the influence of miR-625-3p in radio-resistance of hepatoma cells. It has been shown that miR-625-3p was upregulated in hepatoma tissues particularly in radio-resistant ones. It has also been shown that increased miR-625-3p expression promoted radio-resistance of hepatoma cells. In addition, this study has shown a direct binding between miR-625-3p and TCF21 from which miR-625-3p adversely modulated TCF21. Moreover, TCF21 was significantly lower in radio-resistant HC patients than in radio-sensitive ones and high TCF21 was correlated with high overall survival rate among HC patients.

MiRNAs act as diagnostic biomarkers for therapeutic intervention strategies due to their roles as suppressor of tumors or oncogenes in hepatoma progression (Grimaldi et al. 2019; Guan et al. 2019; Qiu et al. 2019; Rupaimoole et al. 2016; Sartorius et al. 2018). In this study, researchers have also shown the aberrant expression of miR-625-3p as an oncogene which might be related to the promotion of tumor progression and radio-resistance, agreeing with existing knowledge. Previous studies also further explored the effect of miRNAs on cellular resistance to irradiation (Chen et al. 2016; Gandellini et al. 2014; Ni et al. 2017; Rogers et al. 2020) through the interactions with target genes (Kong et al. 2019; Li et al. 2019; Xu et al. 2019). Previously, miR-625-3p was high expressed in lung cancer cells and miR-625-3p upregulation contributed to the gefitinib resistance in vitro through inhibiting AXL (Du et al. 2020), which was similar to the discoveries of the miR-625-3p/TCF21 in radio resistance in HC cells.

TCF21 is reported to be a tumor suppressor with growth-inhibitory, anti-angiogenic and anti-chemoresistance features and increased expression of TCF21 might exert therapeutic benefits (Zhao et al. 2019). In this study, miR-625-3p was shown to promote radio-resistance of hepatoma cells. Furthermore, downregulation of miR-625-3p could decrease the radio-resistance and further knockdown of TCF21 could revert this partly, suggesting that miR-625-3p may exert its role in HC via regulation of TCF21. Therefore, miR-625-3p might influence radio-resistance by affecting TCF21 in HC cells.

The expression of TCF21 was downregulated significantly in irradiation-resistant human hepato-

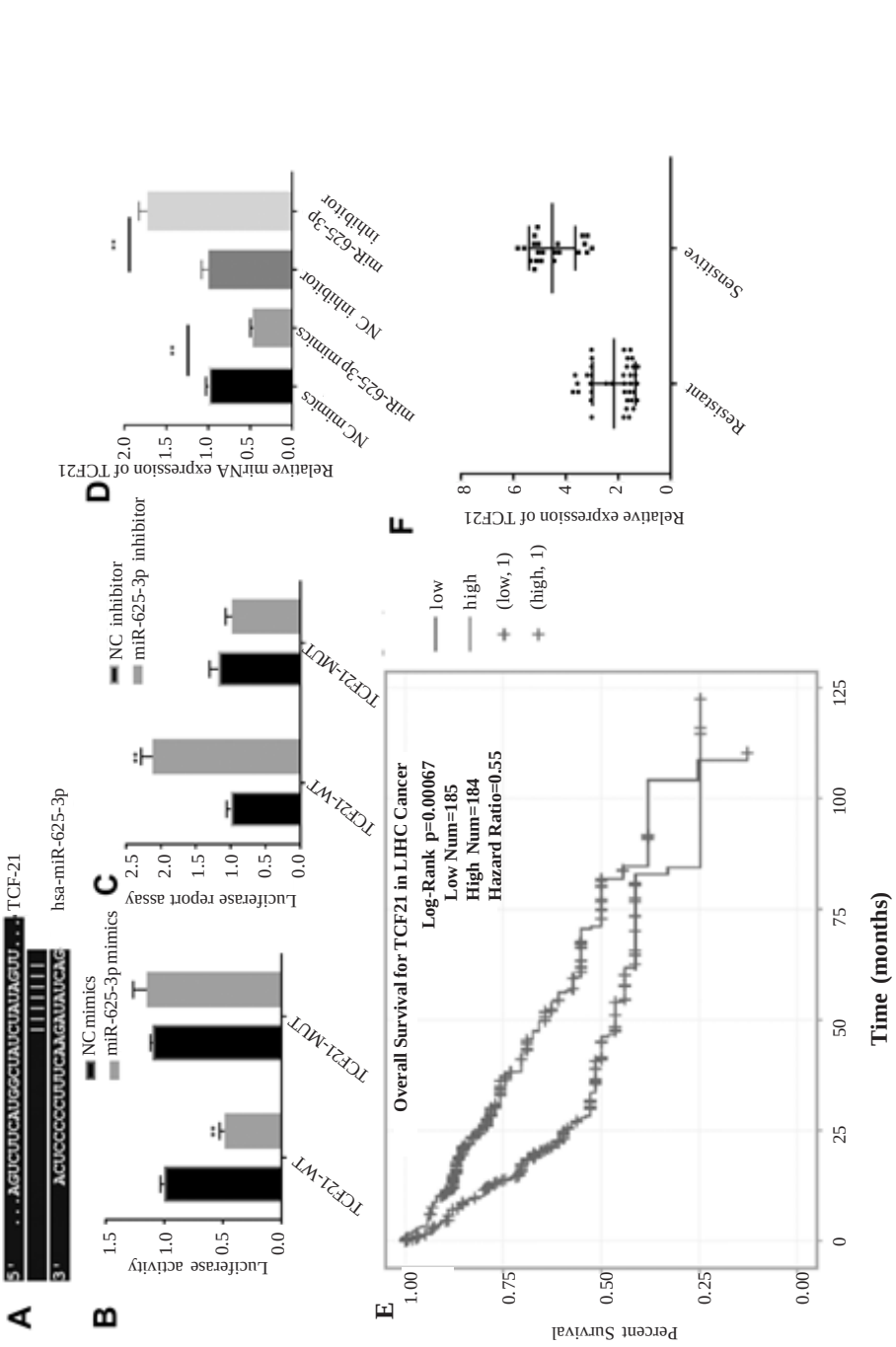

Fig. 3. miR-625-3p directly targets TCF21 and high TCF21 expression is associated with high overall survival rates in HC patients
A) TCF21 was predicted to be targeted by miR-625-3p in homo sapiens on Targetscan tool. B-C) The direct bindings between miR-625-3p and TCF21 were validated by luciferase reporter gene assays
D) RT-PCR measured the expression of TCF21 in cells after miR-625-3p regulation
E) starBase online tool was used to analyze the overall survival rates in correlation with TCF21 expression among HC patients(n=369)
F) RT-PCR was used to further measure the TCF21 mRNA expression in patients (radio-resistant, n=38; radio-sensitive, n=22)
****P < 0.05**

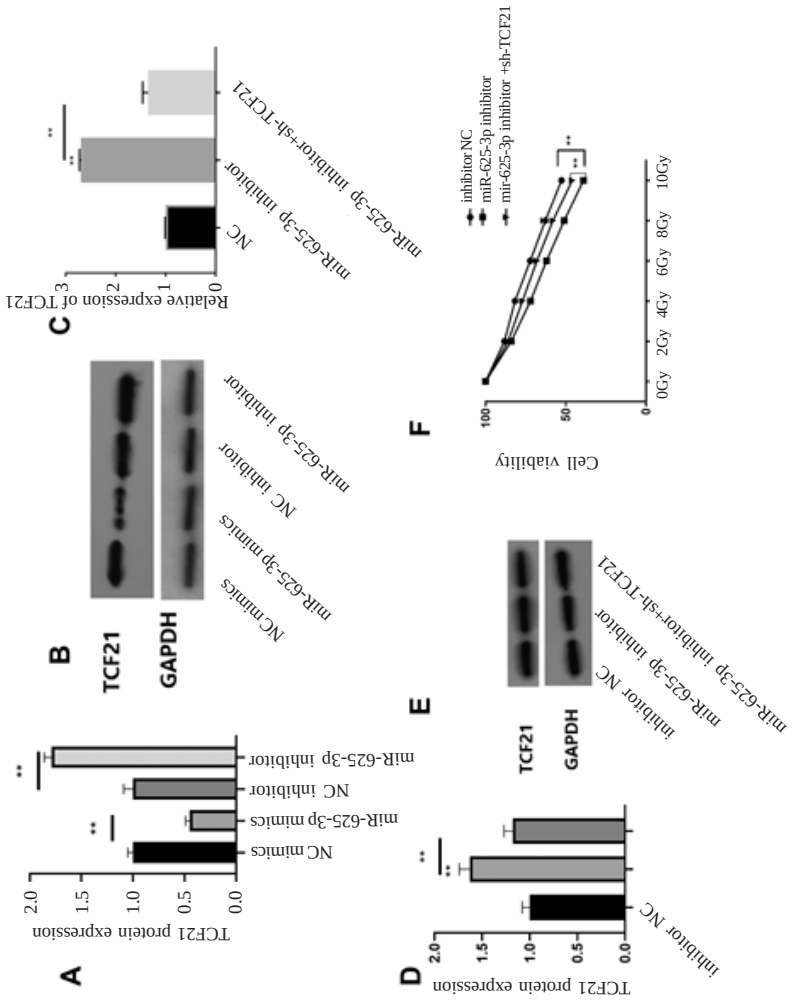

Fig. 4. miR-625-3p influences the radio-resistance of HC cells via regulation of TCF21
A-B) Western blot and ImageJ were used to analyze the TCF21 protein expression in HC cells after miR-625-3p regulation
C) RT-PCR detected the mRNA expression of TCF21 in cells transfected with inhibitor NC, miR-625-3p inhibitor and co-transfected with miR-625-3p and sh-TCF-21
D-E) Western blot and Image J
F) MTT methods were used to analyze the cell survival after cells were exposed to irradiation (0, 2, 4, 6, 8, 10Gy) in each group
****P < 0.05**

ma tissue specimens in contrast to irradiation-sensitive tissues. The results were consistent with the prognostic value of TCF21,which was examined in HC , showing that patients with higher TCF21 tended to have better prognosis for overall survival than those with lower expression (Lu et al. 2019). Additionally, this study has shown a negative correlation among TCF21 and miR-625-3p expressions which is important in understanding the underlying interaction between TCF21 and miR-625-3p. Thus, this study indicated that miR-625-3p enhanced cell viability by regulating TCF21 expression to enhance radio-resistance in HC cells.

CONCLUSION

Taken together, suppressing miR-625-3p reduced hepatoma cell resistance to irradiation which was reversed through knocking down TCF21. Therefore, miR-625-3p regulated the radio-resistance of hepatoma cells via TCF21.

RECOMMENDATIONS

Higher miR-625-3 in hepatoma patients and cell lines was observed. miR-625-3p was higher in the radioresistant group than those in the radiosensitive. Furthermore, high miR-625-3p was associated with low survival rate in patients. The upregulation of miR-625-3p enhanced the radio-resistance of hepatoma cells. miR-625-3p adversely moderated the protein levels of TCF21 via direct binding to the 3'-UTR of TCF21.

REFERENCES

Ao X, Ding W, Zhang Y, Ding D, Liu Y 2020. Tcf21: A critical transcription factor in health and cancer. *J Mol Med (Berl)*, 98(8): 1055-1068.

Chen X, Xu Y, Liao X, Liao R, Zhang L, Niu K, Li T, Li D, Chen Z, Duan Y, Sun J 2016. Plasma mirnas in predicting radiosensitivity in non-small cell lung cancer. *Tumour Biol*, 37(9): 11927-11936.

Craig AJ, Von Felden J, Garcia-Lezana T, Sarcognato S, Villanueva A 2020. Tumour evolution in hepatocellular carcinoma. *Nat Rev Gastroenterol Hepatol*, 17(3): 139-152.

Dawson LA, Guha C 2008. Hepatocellular carcinoma: Radiation therapy. *Cancer J*, 14(2): 111-116.

Du W, Sun L, Liu T, Zhu J, Zeng Y, Zhang Y, Wang X, Liu Z, Huang JA 2020. The mir 625 3p/axl axis induces non t790m acquired resistance to egfr tki via activation of the tgf â/smad pathway and emt in egfr mutant non small cell lung cancer. *Oncol Rep*, 44(1): 185-195.

Ebrahimi S, Hashemy SI 2019. Microrna-mediated redox regulation modulates therapy resistance in cancer cells: Clinical perspectives. *Cell Oncol (Dordr)*, 42(2): 131-141.

Fang L, Kong D, Xu W 2018. Microrna-625-3p promotes the proliferation, migration and invasion of thyroid cancer cells by up-regulating astrocyte elevated gene 1. *Biomed Pharmacother*, 102: 203-211.

Fang L, Xu W, Kong D 2019. Icariin inhibits cell proliferation, migration and invasion by down-regulation of microrna-625-3p in thyroid cancer cells. *Biomed Pharmacother*, 109: 2456-2463.

Gandellini P, Rancati T, Valdagni R, Zaffaroni N 2014. Mirnas in tumor radiation response: Bystanders or participants? *Trends Mol Med*, 20(9): 529-539.

Gmerek L, Martyniak K, Horbacka K, Krokowicz P, Scierski W, Golusinski P, Golusinski W, Schneider A, Masternak MM 2019. Microrna regulation in colorectal cancer tissue and serum. *PLoS One*, 14(8): e0222013.

Grimaldi AM, Incoronato M 2019. Clinical translatability of "identified" circulating mirnas for diagnosing breast cancer: Overview and update. *Cancers (Basel)*, 11(7): 1-16.

Guan Y, Guan X, An H, Baihetiya A, Wang W, Shao W, Yang H, Wang Y 2019. Epigenetic silencing of mir-137 induces resistance to bicalutamide by targeting trim24 in prostate cancer cells. *Am J Transl Res*, 11(5): 3226-3237.

Kong L, Wei Q, Hu X, Chen L, Li J 2019. Mir-193a-3p promotes radio-resistance of nasopharyngeal cancer cells by targeting srsf2 gene and hypoxia signaling pathway. *Med Sci Monit Basic Res*, 25: 53-62.

Li X, Yuan M, Song L, Wang Y 2020. Silencing of microrna-210 inhibits the progression of liver cancer and hepatitis b virus-associated liver cancer via targeting egr3. *BMC Med Genet*, 21(1): 48.

Li Y, Song X, Liu Z, Li Q, Huang M, Su B, Mao Y, Wang Y, Mo W, Chen H 2019. Upregulation of mir-214 induced radioresistance of osteosarcoma by targeting phlda2 via pi3k/akt signaling. *Front Oncol*, 9(298): 1-10.

Lin LC, Chen CF, Ho CT, Liu JJ, Liu TZ, Chern CL 2018. Gamma-glutamylcysteine synthetase (gamma-gcs) as a target for overcoming chemo- and radio-resistance of human hepatocellular carcinoma cells. *Life Sci*, 198: 25-31.

Liu Z, Yu Y, Huang Z, Kong Y, Hu X, Xiao W, Quan J, Fan X 2019. Circrna-5692 inhibits the progression of hepatocellular carcinoma by sponging mir-328-5p to enhance dab2ip expression. *Cell Death Dis*, 10(12): 900.

Lu W, Yang C, Du P, Zhang JL, Zhang JC 2019. Expression tendency and prognostic value of tcf21 in hepatocellular carcinoma. *Artif Cells Nanomed Biotechnol*, 47(1): 1466-1470.

Ma H, Yuan L, Li W, Xu K, Yang L 2018. The lncrna h19/mir-193a-3p axis modifies the radio-resistance and chemotherapeutic tolerance of hepatocellular carcinoma cells by targeting psen1. *J Cell Biochem*, 119(10): 8325-8335.

Maruyama H, Yoshikawa M, Yokosuka O 2008. Current role of ultrasound for the management of hepatocellular carcinoma. *World J Gastroenterol*, 14(11): 1710-1719.

Ni J, Bucci J, Chang L, Malouf D, Graham P, Li Y 2017. Targeting micrornas in prostate cancer radiotherapy. *Theranostics*, 7(13): 3243-3259.

Niemoeller OM, Niyazi M, Corradini S, Zehentmayr F, Li M, Lauber K, Belka C 2011. Microrna expression pro-

files in human cancer cells after ionizing radiation. *Radiat Oncol*, 6(29): 1-5.
Qiu L, Wang T, Ge Q, Xu H, Wu Y, Tang Q, Chen K 2019. Circular rna signature in hepatocellular carcinoma. *J Cancer*, 10(15): 3361-3372.
Rasmussen MH, Lyskjær I, Jersie-Christensen RR, Tarpgaard LS, Primdal-Bengtson B, Nielsen MM, Pedersen JS, Hansen TP, Hansen F, Olsen JV, Pfeiffer P, Ørntoft TF, Andersen CL 2016. Mir-625-3p regulates oxaliplatin resistance by targeting map2k6-p38 signalling in human colorectal adenocarcinoma cells. *Nat Commun*, 7(12436): 1-15.
Rogers CJ, Lukaszewicz AI, Yamada-Hanff J, Micewicz ED, Ratikan JA, Starbird MA, Miller TA, Nguyen C, Lee JT, Olafsen T, Iwamoto KS, Mcbride WH, Schaue D, Menon N 2020. Identification of mirna signatures associated with radiation-induced late lung injury in mice. *PLoS One*, 15(5): e0232411.
Rupaimoole R, Calin GA, Lopez-Berestein G, Sood AK 2016. Mirna deregulation in cancer cells and the tumor microenvironment. *Cancer Discov*, 6(3): 235-246.
Sartorius K, Sartorius B, Winkler C, Chuturgoon A, Makarova J 2018. The biological and diagnostic role of mirna's in hepatocellular carcinoma. *Front Biosci (Landmark Ed)*, 23: 1701-1720.
Sun W, Li S, Yu Y, Jin H, Xie Q, Hua X, Wang S, Tian Z, Zhang H, Jiang G, Huang C, Huang H 2019. Microrna-3648 is upregulated to suppress tcf21, resulting in promotion of invasion and metastasis of human bladder cancer. *Mol Ther Nucleic Acids*, 16: 519-530.
Wang J, Liu S, Shi J, Li J, Wang S, Liu H, Zhao S, Duan K, Pan X, Yi Z 2019a. The role of mirna in the diagnosis, prognosis, and treatment of osteosarcoma. *Cancer Biother Radiopharm*, 34(10): 605-613.
Wang J, Zhao H, Yu J, Xu X, Liu W, Jing H, Li N, Tang Y, Li Y, Cai J, Jin J 2019b. Mir-92b targets p57kip2 to modulate the resistance of hepatocellular carcinoma (hcc) to ionizing radiation (ir) -based radiotherapy. *Biomed Pharmacother*, 110: 646-655.
Wei J, Zhang L, Li J, Zhu S, Tai M, Mason CW, Chapman JA, Reynolds EA, Weiner CP, Zhou HH 2017. Microrna-205 promotes cell invasion by repressing tcf21 in human ovarian cancer. *J Ovarian Res*, 10(1): 33.
Wu CH, Chen CY, Yeh CT, Lin KH 2020. Radiosensitization of hepatocellular carcinoma through targeting radio-associated microrna. *Int J Mol Sci*, 21(5): 1-14.
Wu KL, Tsai YM, Lien CT, Kuo PL, Hung AJ 2019. The roles of microrna in lung cancer. *Int J Mol Sci*, 20(7): 1611.
Xiao J, Liu Y, Wu F, Liu R, Xie Y, Yang Q, Li Y, Liu M, Li S, Tang H 2020. Mir-639 expression is silenced by dnmt3a-mediated hypermethylation and functions as a tumor suppressor in liver cancer cells. *Mol Ther*, 28(2): 587-598.
Xu R, Li H, Wu S, Qu J, Yuan H, Zhou Y, Lu Q 2019. Microrna-1246 regulates the radio-sensitizing effect of curcumin in bladder cancer cells via activating p53. *Int Urol Nephrol*, 51(10): 1771-1779.
Yu Y, Feng M 2018. Radiotherapy for hepatocellular carcinoma. *Semin Radiat Oncol*, 28(4): 277-287.
Zhang Y, Zheng L, Ding Y, Li Q, Wang R, Liu T, Sun Q, Yang H, Peng S, Wang W, Chen L 2015. Mir-20a induces cell radioresistance by activating the pten/pi3k/akt signaling pathway in hepatocellular carcinoma. *Int J Radiat Oncol Biol Phys*, 92(5): 1132-1140.
Zhao Q, Wirka R, Nguyen T, Nagao M, Cheng P, Miller CL, Kim JB, Pjanic M, Quertermous T 2019. Tcf21 and ap-1 interact through epigenetic modifications to regulate coronary artery disease gene expression. *Genome Med*, 11(1): 23.

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