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Inhibition of miR-146b expression increases radioiodine-sensitivity in poorly differential thyroid carcinoma via positively regulating NIS expression

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ABSTRACT

Dedifferentiated thyroid carcinoma (DTC) with the loss of radioiodine uptake (RAIU) is often observed in clinical practice under radioiodine therapy, indicating the challenge for poor prognosis. MicroRNA (miRNA) has emerged as a promising therapeutic target in many diseases; yet, the role of miRNAs in RAIU has not been generally investigated. Based on recent studies about miRNA expression in papillary or follicular thyroid carcinomas, the expression profiles of several thyroid relative miRNAs were investigated in one DTC cell line, derived from normal DTC cells by radioiodine treatment. The top candidate miR-146b, with the most significant overexpression profiles in dedifferentiated cells, was picked up. Further research found that miR-146b could be negatively regulated by histone deacetylase 3 (HDAC3) in normal cells, indicating the correlation between miR-146b and Na^+/I^- symporter (NIS)-mediated RAIU. Fortunately, it was confirmed that miR-146b could regulate NIS expression/activity; what is more important, miR-146b interference would contribute to the recovery of radioiodine-sensitivity in dedifferentiated cells via positively regulating NIS. In the present study, it was concluded that NIS-mediated RAIU could be modulated by miR-146b; accordingly, miR-146b might serve as one of targets to enhance efficacy of radioactive therapy against poorly differential thyroid carcinoma (PDTC).

1. Introduction

Thyroid cancer is the most common endocrine malignant tumor, the majority of which are differentiated thyroid carcinomas (DTCs), including papillary or follicular types [1,2]. Radioactive iodine therapy is the primary mean of treating metastatic DTC, based on the iodine-uptake of the residual lesion or the metastases [3]. However, about 30% thyroid cancer metastases can lose their ability to concentrate iodine, a phenomenon called dedifferentiation, indicating a poor prognosis [4,5]. Reestablishing the ability to concentrate iodine becomes the principal problem faced by the radioactive iodine therapy for poorly differential thyroid carcinoma (PDTC). MicroRNAs (miRNAs), a class of small noncoding messenger RNA species, predominantly bind to 3′UTR of target genes resulting in either degradation of target mRNA or inhibition of translation [6], and have emerged as a promising therapeutic target in many diseases [7]. Moreover, it has been reviewed the tremendous potential of miRNAs for use in the diagnosis and prognosis of thyroid tumors, including miR-196, miR-146b, miR-155 and so on [8–10].

Differentiated thyroid cells express a number of differentiation markers such as thyroglobulin (Tg), thyroperoxidase (TPO) and the Na^+/I^- symporter (NIS), that are responsible for iodine uptake, synthesis of thyroid hormones and the differentiated thyroid phenotype [11]. Studies have revealed that decreased TSH receptor (TSHR) expression correlate with FTC-133 ongoing dedifferentiation after ^{131}I radiation, while *TSHR* transfection can also improve the expression of thyroid-specific molecules, including TSHR, NIS and TPO, and radioiodine uptake (RAIU) [12,13]. The sodium-iodide symporter (NIS), located on the basolateral membrane of thyroid follicular cells, is the key molecule responsible for the iodide

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concentration. Down-regulated expression of NIS has been observed in DTC resistance to ^{131}I therapy [14], on the other hand, increased NIS expression is also reported in follicular cell lines after treated with Trichostatin A (TSA), one of histone deacetylase (HDAC) inhibitors [15]. Currently, NIS-mediated RAIU, the basis for targeted ablation of thyroid cancer remnants, has been proven to be modulated by miR-339-5p through *in silico* analysis, showing the important role of miR-339-5p in the development or maintenance of thyroid malignancy [16].

In the present study, dedifferentiated FTC-133 (dFTC-133) cells, obtained by monoclonal culture of FTC-133 cell line after ^{131}I radiation, then identified as PDTC, were investigated for the reasons about decreased radioiodine-sensitivity, which might be related to functional roles of thyroid relative microRNAs, especially for significantly upregulated miR-146b. NIS-mediated RAIU, with HDAC-related regulation, was also studied correlatively.

2. Materials and methods

2.1. Cell cultures

The human DTC cell line FTC-133 was were purchased from ATCC (Bethesda, MD), and were grown according to ATCC guidelines. The cells were cultured in Dulbecco's modified Eagle's medium and Ham's F-12 nutrient mixture (DMEM/F12; Invitrogen) supplemented with 10% fetal bovine serum containing 1% penicillin/streptomycin (Invitrogen), and grown at 37 °C and 5% CO_2.

2.2. dFTC-133 cell line establishment

Referring to early studies, dedifferentiation of FTC-133 cells can be enhanced by ^{131}I radiation [17]. And, dedifferentiated FTC-133 (dFTC-133) cell line has been established by monoclonal culture of FTC-133 cell line after ^{131}I radiation [18]. Accordingly, FTC-133 cells were seeded in 6-well plates incubated with 15 μCi Na^{131}I for three days and then cultured in activity free medium for few months. Through subsequent incubation by graded dilutions, wells with one cell clone formation were selected and cultured. Following ^{131}I treatment for different time points, cells were also subjected to radioiodine (^{125}I) uptake analysis as following described. Cells with stable lowest radioiodine (^{125}I) uptake were picked up as dFTC-133. The expression of thyroid differentiated markers and thyroid relative microRNAs were detected by western blot and real-time PCR respectively, as following described.

2.3. RNA extraction and quantitative real-time PCR

Total RNA was extracted from two subpopulations using Trizol (Invitrogen) following the manufacturer's instructions. For miRNA expression analysis, RNA was reverse transcribed using a TaqMan miRNA reverse transcription kit (Applied Bio-systems) and RT primers for miR-146b, for example, were provided with the miR-146b-5p TaqMan miRNA assay (PN4373178; Applied Bio-systems) according to the manufacturer's instructions. Experimental details were described in a previous study [19]. For HDAC3 expression, moderate RNA sample was reverse transcribed with SuperScript III First-Strand Synthesis System (Invitrogen) in the presence of random hexamers to synthesis cDNA. Primers used for mRNA were: HDAC3-F: 5′-ACTCGTGCTGGGTGGTGGTG-3′, HDAC3-R: 5′-CTGCA GGCACGTCAGAATCT-3′; β-actin-F: 5′-AAAGACCTGTACGGGAACAC-3′, β-actin-R: 5′-GTCATACTCCTGCTTGCTGAT-3′. Quantitative real-time PCR was performed using the Sybgreen I real-time PCR kit (TaKaRa) in an ABI PRISM 7500 Real-time PCR System (Applied Bio-systems). All amplification reactions were done in triplicate. The results were analyzed using the $2^{-\Delta\Delta Ct}$ method [20], with β-actin used as internal control for HDAC3.

2.4. Western blotting

As for western blotting analysis, cells were washed with phosphate buffered solution (PBS) and harvested by scraping with RIPA lysis buffer. After quantified spectrophotometrically using the bicinchoninic-acid-based (BCA) method (Pierce Chemical), whole-cell-lysates was separated through 12% SDS-PAGE and electrophoretically transferred to PVDF (polyvinylidene difluoride) membranes (Millipore). Membranes were incubated with one of the primary anti-mouse antibodies for detection of NIS, TSHR, TPO, HDAC1, HDAC3, and β-actin overnight at 4 °C in TBST buffer. Then, these membranes were incubated with a horseradish peroxidase conjugated anti-mouse antibody (Santa Cruz Biotechnology). Immunoblot images were acquired and analyzed using ImageQuant LAS 4000 System (GE Healthcare).

2.5. Transfection of microRNA mimics and small interfering RNA (siRNA)

The cells were seeded in antibiotic-free medium for 24 h prior to transfection. DharmaFECT transfection reagent (Dharmacon) was used to transfect cells with miR-146b mimic or inhibitor (Dharmacon) for 24 h at 50% confluence following the manufacturer's instructions. The miRIDIAN miRNA mimic or inhibitor negative control (Dharmacon) was used as control. For siRNA experiments, the following sequences were designed as targets for human HDAC3 and NIS (3′-UTR): HDAC3, 5′-GCCGGUUAUCAACCAGGUATT-3′; NIS, 5′-GCAAAUGAGUUCAGGACUAUU-3′. Pools of siRNAs directed against human NIS or HDAC3 and nontargeting siRNAs (ON-TARGETplus SMARTpools) were purchased from Dharmacon. RNA interference was carried out with an siRNA-Lipofectamine 2000 (Invitrogen) mixture at 37 °C for 4 h, followed by addition of fresh complete medium. Cell samples were collected 24 h after transfection for quantification of miRNA or mRNA. The subsequent experiments were performed 48 h after the transfection.

2.6. Plasmids and transient transfection

Recombinant eukaryotic expression plasmid pcDNA3.1-NIS and pcDNA3.1-HDAC3 was constructed by cloning corresponding amplified products into the eukaryotic expression plasmid pcDNA3.1 (Invitrogen) as described before [12]. The pcDNA3.1-NIS, pcDNA3.1-HDAC3 and empty pcDNA3.1 (+) were transfected into cells by using Lipofectamine 2000 reagent (Invitrogen) according to the manufacturer's protocol. Briefly, cells were seeded for one day to achieve 90%–95% confluency per well at the time of transfection. Purified pcDNA3.1-NIS, pcDNA3.1-HDAC3 or pcDNA3.1 (+) was mixed with Opti-MEM respectively. And, moderate Lipofectamine in Opti-MEM was added and incubated for 20 min at room temperature. The DNA and Lipofectamine mixture was added in the cells overlaid with serum free DMEM for 5 h, then the complexes were removed and the cells were cultured in normal medium for at least 24 h. Blank group of cells were set without transfection. Cell samples were also collected for quantification of miRNA or mRNA, with subsequent experiments performed 48 h after the transfection.

2.7. Radioiodine (^{125}I) uptake assay

Radioiodine uptake (RAIU) was measured as described before [18,21]. In brief, cells were washed with 1 ml of HBSS and then incubated at 37 °C with 500 μl buffered HBSS (Hanks Balanced Salt

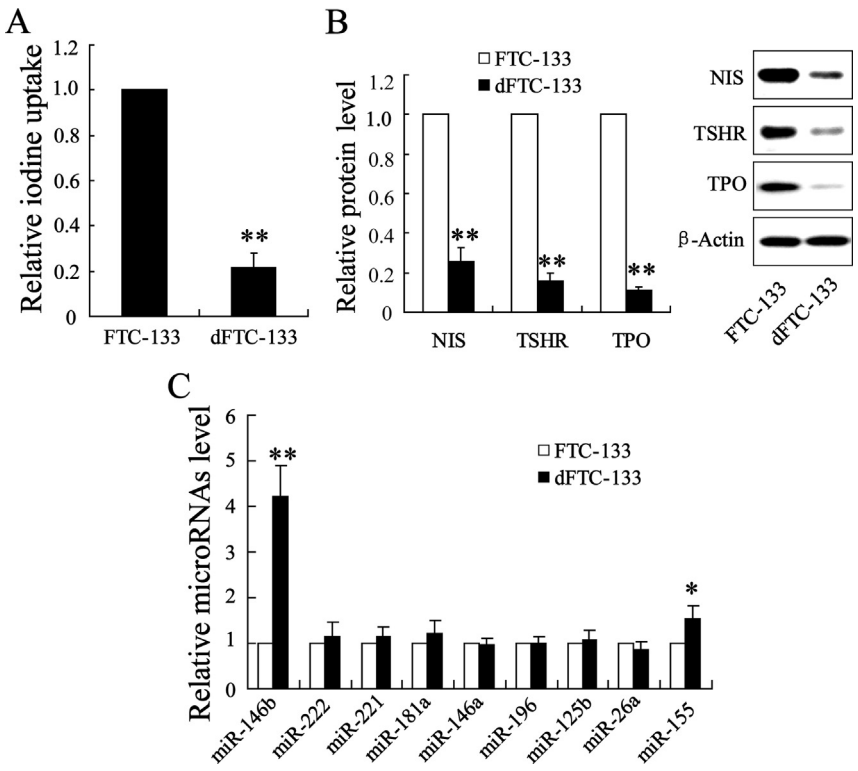

Fig. 1. Establishment of poorly differential thyroid carcinoma (PDTC) cell line (dFTC-133) from FTC-133 cells after co-incubation with 15 μCi $Na^{131}I$ for 3 days. A. RAIU ability of dFTC-133 cells decreased to a statistically significant degree compared to parental cells. B. Expression of NIS, TSHR, and TPO was down-regulated significantly in dFTC-133 cells, compared to that of parental cells. C. Relative expression levels of some thyroid relative microRNAs were detected in dFTC-133 cells. MiR-146b was upregulated most significantly among these thyroid relative microRNAs. $^{*}P < 0.05$; $^{**}P < 0.01$.

Solution, Sigma–Aldrich) containing 0.1 μCi carrier-free $Na^{125}I$ and 10 μM sodium iodide for 5, 10, 15, 30 and 60 min respectively. Then, radioactive medium was aspirated and cells were washed with 1 ml of ice-cold HBSS for 1 min. Cells were harvested using trypsin and counted with a hemacytometer. One milliliter of 95% ethanol was added to each well for 20 min and then transferred into vials for counting with a gamma counter. The radioactivity was normalized to the number of cells present at the time of the assay as cpm every 10^6 cells. Three independent experiments were repeated for each point.

2.8. Luciferase reporter assay

In silico prediction of miR-146b potential binding sites within the 3′-UTR of NIS mRNA was performed using TargetScan Human 6.2 (http://www.targetscan.org/). Effect of miR-146b dysregulation on NIS activity was verified by luciferase-hNIS-3′UTR reporter assay as described before [16]. After seeding for 24 h, FTC-133 cells were transfected with plasmid containing firefly luciferase-hNIS-3′UTR (GeneCopoeia), with a Renilla luciferase gene as an internal control, for 14 h using Fugene HD (Promega). Subsequently, cells were transfected with miR-146b mimics or inhibitors before being subjected to luciferase assay. Luciferase activity was measured in a Glomax-Multi Detection System (Promega) using Dual-Luciferase Reporter 1000 Assay System (Promega). Data are represented as relative fold change of the ratios of firefly luciferase activity normalized to Renilla luciferase activity.

2.9. Cell viability assay

MTT assay was used here to observe cellular viability as previously described [22]. After incubation with MTT (5 mg/ml) for 4 h at 37 °C, 100 μl dimethyl sulfoxide (Sigma–Aldrich) was added and mixed thoroughly. The amount of formazan crystals was determined quantitatively by absorbance measurements in spectrophotometric assays at 570 nm. The cell apoptosis was also analyzed by the detection of DNA fragments using TUNEL assay, for further evaluating the cell viability. Cytospin preparations of both floating and attached cells were collected and fixed with 4% paraformaldehyde for 15 min at room temperature. Then, cells were washed in PBS and permeabilized with 0.1% Triton X-100 in 0.1% sodium citrate for 2 min on ice. TUNEL analysis was performed using the “In situ cell death detection kit, fluorescein” (Roche), following the manufacturer's instructions. In each sample, a total of 300 cells including normal and apoptotic cells were counted under a microscope. Three independent experiments were repeated for each point.

2.10. Statistical analysis

The results of the quantitative data in this study are expressed as the mean ± standard deviation (S.D). Two-tailed Student's t test was used for comparisons of two independent groups, with Welch's corrected t test for unequal variances in SPSS 13.0 software. P-value < 0.05 was considered statistically significant.

3. Results

3.1. MiR-146b was up-regulated most significantly among the thyroid relative miRNAs in dFTC-133, with decreased RAIU ability and inhibited thyroid differentiated markers

RAIU of FTC-133 cells decreased after co-incubation with ^{131}I gradually and did not recover even if ^{131}I was removed [18]. In this

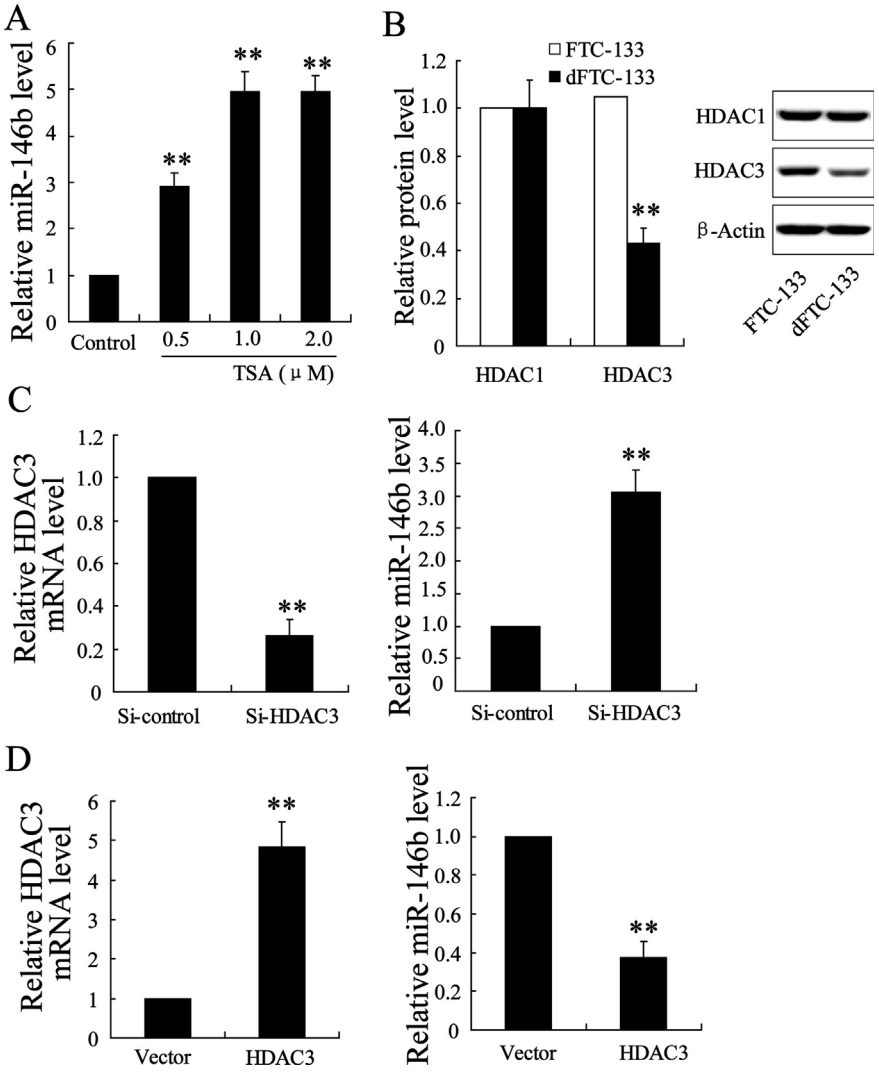

Fig. 2. Investigation for the relevance between miR-146b overexpression and HDAC inhibition. A. Up-regulation of miR-146b in FTC-133 cells after treated with different concentrations of TSA, one of HDAC inhibitors, for 72 h. B. Expression of HDAC1 and HDAC3 in dFTC-133 cells, compared to FTC-133. C. Induced expression of miR-146b in si-HDAC3 transfected FTC-133 cells. D. Down-regulation of miR-146b in HDAC3 overexpressing dFTC-133 cells. $^{**}P < 0.01$.

study, similar results were concluded with the lowest RAIU observed after 48 h co-incubation. In order to avoid any possibility for RAIU recovery, dedifferentiated FTC-133 (dFTC-133) cells were obtained by monoclonal culture of FTC-133 cell line after ^{131}I radiation for three days. The relative RAIU of obtained dFTC-133 decreased significantly compared to primary FTC-133 (Fig. 1A), as described before. Expression of thyroid-specific proteins, such as NIS, TSHR and TPO, also decreased in dFTC-133 cells to a statistically significant degree compared to FTC-133 (Fig. 1B). So, the poorly differentiated thyroid cancer (PDTC) cell line (dFTC-133) was successfully established from FTC-133.

Several studies have demonstrated the utility of microRNAs as class identifiers especially in the context of follicular thyroid carcinoma (FTC), papillary thyroid carcinoma (PTC) and anaplastic thyroid carcinoma [9,10,23]. Here, the expression of several thyroid relative microRNAs was compared in this two cell lines, to find the possible key microRNA upon radiation. By calculating the data of real-time PCR, it was found that most of microRNAs were up-regulated in dFTC-133 compared with FTC-133. Especially, miR-146b increased most significantly than other microRNAs (Fig. 1C).

3.2. Expression of miR-146b was negatively regulated by HDAC3

TSA, one common used HDAC inhibitor, has been reported to alter microRNA expression profiles in several human carcinomas [24]. Thus, the effect of TSA treatment in FTC-133 cells was investigated on the miR-146b dysregulation, with results showing that miR-146b could be up-regulated significantly by moderate amount of TSA treatment (Fig. 2A). Moreover, expression profiles of HDACs indicated that HDAC3 might play more important role in dedifferentiation or miR-146b expression, as significantly decreased HDAC3 expression was observed in dFTC-133 compared with FTC-133 (Fig. 2B). Further study was performed to study the effect of HDAC3 dysregulation on the expression of miR-146b. Results showed that the expression level of miR-146b increased in FTC-133 cells with interfered HDAC3 (Fig. 2C), and decreased in dFTC-133 cells with HDAC3 overexpression (Fig. 2D). It was concluded that the expression of miR-146b could be negatively regulated by HDAC3 distinctively, while normal HDAC inhibition under micromolar TSA treatment is also proven to increase the effectiveness of radioactive iodine therapy [15]. What's more important, HDAC3-regulated dysexpression of miR-146b showed us direction into investigating

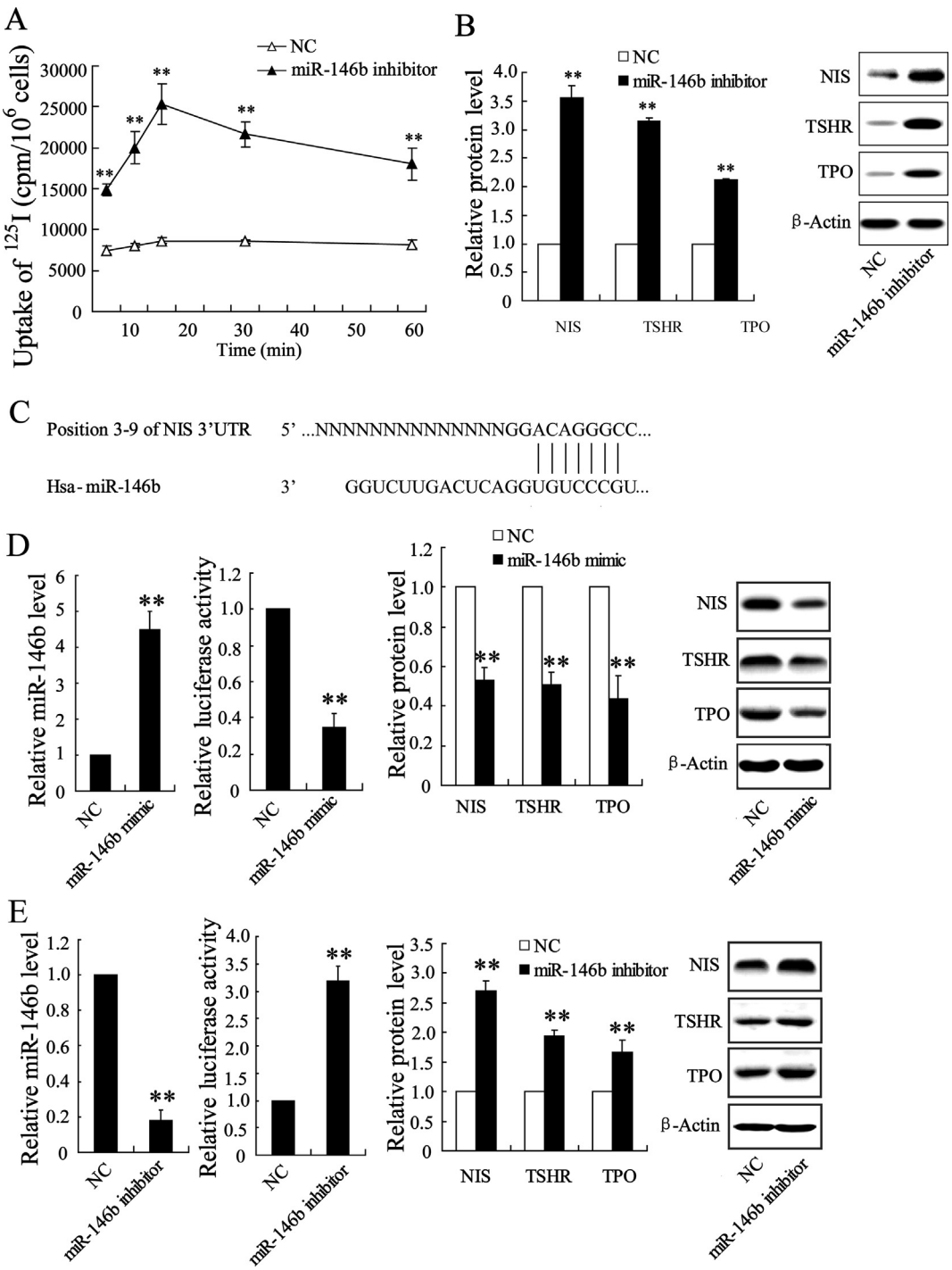

Fig. 3. Effect of miR-146b dysregulation on the RAIU ability of dFTC-133 cells and expression of thyroid differentiated markers, especially for NIS and its 3′-UTR activity. A. RAIU assay with 0.1 μCi $Na^{125}I$ for different time in dFTC-133 cells under miR-146b inhibitor transfection. B. Expression of NIS, TSHR, and TPO in dFTC-133 cells after transfected with miR-146b inhibitor. C. The potential miR-146b binding sites of NIS-3′UTR through bioinformatics analysis. D. Down-regulated effect of miR-146b overexpression by mimics transfection in FTC-133 cells. E. Upregulated effect in miR-146b inhibitors transfected dFTC-133 cells. $^{**}P < 0.01$.

the potential role of miR-146b in the improvement of radioiodine-sensitivity in dFTC-133, related to NIS-mediated RAUI pathways.

3.3. RAIU ability was increased under miR-146b interference in dFTC-133, with induced thyroid differentiated markers

Since RAIU ability decreased in miR-146b upregulated dFTC-133 cells, miR-146b silence was carried out to examine some feedback effect on RAIU ability, and also the expression of thyroid differentiated markers. Results of RAIU assay indicated that RAIU ability could be recovered to some extent in dFTC-133 cells with miR-146b inhibitor transfection (Fig. 3A). Additionally, increased expression of thyroid differentiated markers was also detected correspondingly (Fig. 3B). Taken together, miR-146b might regulate important pathways in the dedifferentiation of thyroid carcinoma.

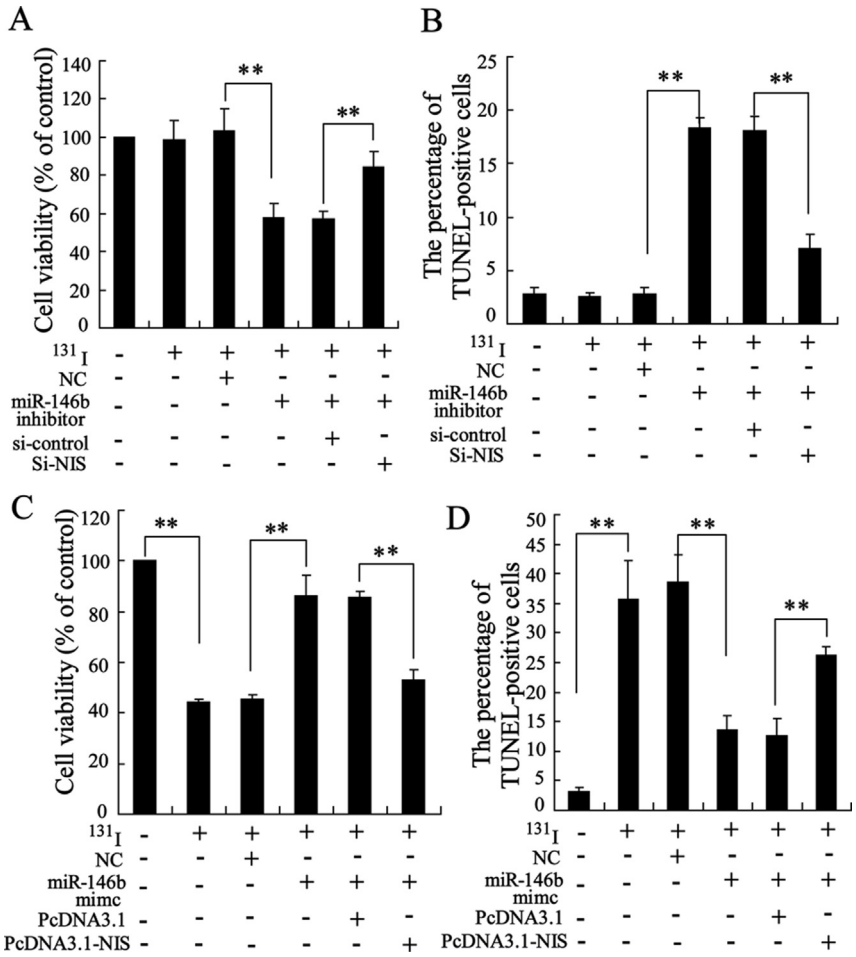

Fig. 4. Detection of cell viability and apoptosis in miR-146b interfered dFTC-133 cells and miR-146b overexpressing FTC-133 cells. After treated with 60 μCi $Na^{131}I$ for 20 min and normal incubation for 24 h, diminished cell viability (A) and increased apoptosis (B) were observed in miR-146b interfered dFTC-133 cells, while this change could be recovered by additional NIS inhibition. For miR-146b overexpressing FTC-133 cells, increased cell viability (C) and decreased apoptosis (D) were detected, and this change could also be recovered by additional NIS overexpression. Here, NIS inhibition or overexpression was carried out after at least 72 h transfection with miR-146b mimic or inhibitor. $^{**}P < 0.01$.

3.4. MiR-146b could negatively regulate NIS-3′UTR activity and also the expression of NIS, TSHR and TPO in FTC-133

In order to gain more insights of the functional role of miR-146b in radioiodine-induced dedifferentiation of thyroid carcinoma, the relationship between miR-146b and thyroid differentiated markers, especially for NIS, was deeply investigated. Bioinformatics analysis showed that miR-146b could directly target the position 3–9 of NIS-3′UTR (Fig. 3C), while this binding ability was further demonstrated by luciferase analysis. As shown in Fig. 3D, miR-146b overexpression resulted in significant decrease of luciferase activity in FTC-133 cells using luciferase-hNIS-3′UTR reporter, when the expression of NIS, TSHR and TPO was also detected to be down-regulated. On the other side, improved NIS-3′UTR activity was observed in miR-146b silencing FTC-133 cells, along with increased expression of NIS, and also TSHR and TPO (Fig. 3E). Therefore, it was speculated that miR-146b might influence the radioiodine uptake by targeting NIS-3′UTR, resulting in decreased NIS-mediated RAIU activity.

3.5. NIS could recover the effect of miR-146b dysregulation on the radioiodine-sensitivity in dFTC-133 and FTC-133

The relationship of miR-146b expression and NIS-3′UTR activity indicated that the effect of miR-146b on the RAIU activity change might involve NIS-mediated pathways. So, the effect of miR-146b dysregulation on the radioiodine-sensitivity of dFTC-133 and FTC-133 was observed subsequently, including the detection of cell viability and apoptosis. For dFTC-133 cells with low radioiodine-sensitivity, significant decrease of cell viability and increase of apoptosis were observed under miR-146b inhibitor transfection (Fig. 4A, B). Interestingly, the change in cell viability and apoptosis could be both reversed by succedent NIS interference. On the other hand, significant increase of cell viability and decrease of apoptosis were observed in radioiodine-sensitive FTC-133 cells under the treatment of miR-146b mimic transfection (Fig. 4C, D). Similarly, the change in cell viability and apoptosis could also be recovered by following NIS overexpression. Results indicated that inhibition of miR-146b expression could increase the radioiodine-sensitivity of dFTC-133 involving regulated expression and function of NIS. What's more important, miR-146b can be used as one potential candidate for improving the radioactive therapy against poorly differential thyroid carcinoma.

4. Discussions

DTCs are often curable with surgical resection and ^{131}I ablation, because ^{131}I can effectively treat tumor foci exhibiting high ^{131}I uptake [3]. By contrast, the loss or absence of radioiodine uptake is often observed in less differentiated tumors with an unfavorable

prognosis. So, the prediction of progression of differentiated carcinoma to more aggressive forms like poorly differentiated and anaplastic types is of considerable interest for determining prognosis or therapeutic decisions [25]. Studies on restoring iodide uptake of DTC had been done targeting NIS with agents such as retinoic acids or gene transfection in a model of a nonsmall cell lung cancer cell line, with recent results showing that radioiodine uptake and retention can be improved under *TSHR* gene transfection with enhanced thyroid specific molecular expression [12]. However, studies about miRNAs regulated radioiodine uptake and retention have not been enriched yet, even though miRNAs regulation on thyroid specific molecules have been revealed gradually [26]. Some thyroid relative miRNAs have been confirmed to contribute to a state of oncogenesis and dedifferentiation [27]. Hence, their expression profiles were examined in dFTC-133 derived from FTC-133, and then significantly overexpressed miR-146b were picked up and speculated to play an important role in the decrease of radioiodine uptake.

Recent studies have suggested that miR-146b, deregulated in PTC, is associated with advanced tumor characteristics, while overexpression of miR-146b could significantly increase cell migration and invasiveness, and enhance resistance to chemotherapy-induced apoptosis in PTC cell line [28]. However, the miR-146b deregulation in FTC cells hasn't been explored yet, let alone the mechanistic explanation for its function in poorly differentiated FTC. Here, our research firstly reported the miR-146b deregulation in FTC and also revealed the important role of miR-146b in the decreased radioiodine sensitivity of FTC under ^{131}I radiation. NIS-mediated RAIU, with HDAC-related regulation, were investigated as a try to find the relevance between miR-146b deregulation and decreased radioiodine sensitivity. TSA, one HDAC inhibitor, was used to establish down-regulated profiles of HDACs which resulted in the up-regulation of miR-146b in normal FTC-133, while significantly down-regulated HDAC3 was also detected in dFTC-133 cells compared to FTC-133, and more data confirmed that miR-146b expression cold be negatively regulated by HDAC3. Due to the correlation between HDAC and NIS-mediated RAIU pathways [15], it was speculated that miR-146b might play functional role in improving the radioiodine sensitivity in poorly differential dFTC-133 cells.

Improved RAIU activity and also thyroid specific molecular expression was observed in miR-146b silencing cells. And, the effect of miR-146b dysregulation on NIS, especially for NIS-3′UTR activity, was deeply discussed. Findings from both bioinformatics analysis and luciferase assays showed that miR-146b had direct effect on the activity and expression of NIS, indicating its roles in NIS-mediated RAIU pathways. Moreover, increased radioiodine sensitivity of dFTC-133 cells under miR-146b inhibition was observed to go down again by NIS silence, while depressed radioiodine sensitivity of FTC-133 cells under miR-146b overexpression could be also recovered by NIS up-regulation. We could conclude that the effect of miR-146b dysregulation on the radioiodine-sensitivity change was mediated by NIS activity.

In summary, the expression of miR-146b was significantly upregulated in dFTC-133 cells that derived from FTC-133 after ^{131}I radiation. Further research revealed that miR-146b expression was negatively regulated by HDAC3 in FTC-133 cells. Interestingly, it was confirmed that the inhibition of miR-146b expression could increase the radioiodine sensitivity of dFTC-133 cells via positively regulating NIS expression. On the other side, induced miR-146b also decreased the radioiodine sensitivity of FTC-133 cells involving similar NIS-mediated RAIU pathways. Our study revealed the potential of miR-146b to be one candidate for improving the radioactive therapy against poorly differential thyroid carcinoma. Further study can be established to clarify the mechanisms of miR-146b regulation in dedifferentiated thyroid carcinoma for improving radioactive therapy.

Conflict of interest

None.

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Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.04.134.

References

[1] L.A. Akslen, A.O. Myking, Differentiated thyroid carcinomas: the relevance of various pathological features for tumour classification and prediction of tumour progress, Virchows Arch. A Pathol. Anat. Histopathol. 421 (1992) 17–23.
[2] J.C. Desbois, J. Bienayme, J.P. Bouveret, et al., [Multiple endocrine adenomas type II b. (Syndrome "Marfanoid aspect, multiple mucosal neuromatosis, medullary carcinoma of the thyroid, pheochromocytoma"). Family study and review of the literature], Ann. Pediatr. Paris 24 (1977) 587–597.
[3] W. Sacks, C.H. Fung, J.T. Chang, et al., The effectiveness of radioactive iodine for treatment of low-risk thyroid cancer: a systematic analysis of the peer-reviewed literature from 1966 to April 2008, Thyroid 20 (2010) 1235–1245.
[4] D.T. Woodrum, P.G. Gauger, Role of ^{131}I in the treatment of well differentiated thyroid cancer, J. Surg. Oncol. 89 (2005) 114–121.
[5] M.A. Walter, D. Wild, H. Rasch, et al., Coregistered iodine-131 single photon emission computed tomography/computed tomography reveals dedifferentiation in a metastatic follicular thyroid carcinoma, Thyroid 16 (2006) 1063–1064.
[6] J. Krol, I. Loedige, W. Filipowicz, The widespread regulation of microRNA biogenesis, function and decay, Nat. Rev. Genet. 11 (2010) 597–610.
[7] R. Maqbool, M. Ul Hussain, MicroRNAs and human diseases: diagnostic and therapeutic potential, Cell Tissue Res. 358 (2014) 1–15.
[8] H. He, K. Jazdzewski, W. Li, et al., The role of microRNA genes in papillary thyroid carcinoma, Proc. Natl. Acad. Sci. U. S. A. 102 (2005) 19075–19080.
[9] M. Swierniak, A. Wojcicka, M. Czetwertynska, et al., In-depth characterization of the microRNA transcriptome in normal thyroid and papillary thyroid carcinoma, J. Clin. Endocrinol. Metab. 98 (2013) E1401–E1409.
[10] B. Wojtas, C. Ferraz, T. Stokowy, et al., Differential miRNA expression defines migration and reduced apoptosis in follicular thyroid carcinomas, Mol. Cell Endocrinol. 388 (2014) 1–9.
[11] G. Brabant, C. Maenhaut, J. Kohrle, et al., Human thyrotropin receptor gene: expression in thyroid tumors and correlation to markers of thyroid differentiation and dedifferentiation, Mol. Cell Endocrinol. 82 (1991) R7–R12.
[12] F. Feng, H. Wang, S. Hou, et al., Re-induction of cell differentiation and ^{131}I uptake in dedifferentiated FTC-133 cell line by TSHR gene transfection, Nucl. Med. Biol. 39 (2012) 1261–1265.
[13] W. Cheng, H. Fu, F. Feng, et al., Efficacy of lentiviral-mediated transfection of hTSHR in poorly differentiated thyroid carcinoma cell line, Nucl. Med. Biol. 40 (2013) 576–580.
[14] S. Trouttet-Masson, S. Selmi-Ruby, F. Bernier-Valentin, et al., Evidence for transcriptional and posttranscriptional alterations of the sodium/iodide symporter expression in hypofunctioning benign and malignant thyroid tumors, Am. J. Pathol. 165 (2004) 25–34.
[15] R. Zarnegar, L. Brunaud, H. Kanauchi, et al., Increasing the effectiveness of radioactive iodine therapy in the treatment of thyroid cancer using trichostatin A, a histone deacetylase inhibitor, Surgery 132 (2002) 984–990 (discussion 990).
[16] A. Lakshmanan, A. Wojcicka, M. Kotlarek, et al., microRNA-339-5p modulates Na+/I- symporter-mediated radioiodide uptake, Endocr. Relat. Cancer 22 (2015) 11–21.
[17] S. Tepmongkol, S. Keelawat, S. Honsawek, et al., Rosiglitazone effect on radioiodine uptake in thyroid carcinoma patients with high thyroglobulin but negative total body scan: a correlation with the expression of peroxisome proliferator-activated receptor-gamma, Thyroid 18 (2008) 697–704.
[18] F. Feng, H. Wang, H. Fu, et al., Dedifferentiation of differentiated thyroid carcinoma cell line FTC-133 is enhanced by ^{131}I pretreatment, Nucl. Med. Biol. 38 (2011) 1053–1058.
[19] C.K. Chou, R.F. Chen, F.F. Chou, et al., miR-146b is highly expressed in adult papillary thyroid carcinomas with high risk features including extrathyroidal invasion and the BRAF(V600E) mutation, Thyroid 20 (2010) 489–494.

[20] T.D. Schmittgen, K.J. Livak, Analyzing real-time PCR data by the comparative C(T) method, Nat. Protoc. 3 (2008) 1101–1108.
[21] S.J. Weiss, N.J. Philp, E.F. Grollman, Iodide transport in a continuous line of cultured cells from rat thyroid, Endocrinology 114 (1984) 1090–1098.
[22] M. Tuncel, D. Aydin, E. Yaman, et al., The comparative effects of gene modulators on thyroid-specific genes and radioiodine uptake, Cancer Biother Radiopharm. 22 (2007) 443–449.
[23] R. Visone, P. Pallante, A. Vecchione, et al., Specific microRNAs are down-regulated in human thyroid anaplastic carcinomas, Oncogene 26 (2007) 7590–7595.
[24] L.V. Rhodes, A.M. Nitschke, H.C. Segar, et al., The histone deacetylase inhibitor trichostatin A alters microRNA expression profiles in apoptosis-resistant breast cancer cells, Oncol. Rep. 27 (2012) 10–16.
[25] T. Higashi, R. Nishii, S. Yamada, et al., Delayed initial radioactive iodine therapy resulted in poor survival in patients with metastatic differentiated thyroid carcinoma: a retrospective statistical analysis of 198 cases, J. Nucl. Med. 52 (2011) 683–689.
[26] H.-J. Schulten, R. Alotibi, A. Al-Ahmadi, et al., Effect of BRAF mutational status on expression profiles in conventional papillary thyroid carcinomas, BMC Genomics 16 (2015) S6.
[27] M.P. Menon, A. Khan, Micro-RNAs in thyroid neoplasms: molecular, diagnostic and therapeutic implications, J. Clin. Pathol. 62 (2009) 978–985.
[28] C.K. Chou, K.D. Yang, F.F. Chou, et al., Prognostic implications of miR-146b expression and its functional role in papillary thyroid carcinoma, J. Clin. Endocrinol. Metab. 98 (2013) E196–E205.