Overexpression of NDRG2 Increases Iodine Uptake and Inhibits Thyroid Carcinoma Cell Growth In Situ and In Vivo

Anqi Yin,*†[1] Chengguo Wang,‡[1] Jiachen Sun,*[1] Jianjun Gao,§ Liang Tao,† Xilin Du,‡ Huadong Zhao,‡ Jiandong Yang,§ and Yan Li*†

*Department of Biochemistry and Molecular Biology, The Fourth Military Medical University, Xi'an, China
†Department of Anesthesiology, Xijing Hospital, The Fourth Military Medical University, Xi'an, China
‡Department of General Surgery, Tangdu Hospital, The Fourth Military Medical University, Xi'an, China
§Department of General Surgery, 210 Hospital of Chinese People's Liberation Army, Dalian, China

Medullary thyroid carcinoma (MTC) is an uncommon and highly aggressive tumor of the neuroendocrine system, which derives from the neuroendocrine C cells of the thyroid gland. Except for surgical resection, there are not very many effective systemic treatment options for MTC. N-Myc downstream-regulated gene 2 (*NDRG2*) had a significantly lower expression in MTC compared with normal thyroid tissue. However, the function of *NDRG2* in MTC oncogenesis is largely unknown. In this study, we found that overexpression of *NDRG2* inhibited the proliferation of TT cells (human medullary thyroid carcinoma cells) in vitro and suppressed the development of MTC in a nude mouse xenograft model. Further analysis revealed that NDRG2 arrested the cell cycle G_0/G_1 phase progression and induced TT cell apoptosis. Moreover, NDRG2 overexpression may mediate the antiproliferative effect by reducing cyclin D1 and cyclin E protein levels. We also found aberrant *NDRG2*-mitigated TT cell migration and invasion in vitro. Sodium/iodide symporter (NIS) mediates active I^- transport into the thyroid follicular cells, and radionuclide treatment is a promising therapy for MTC. Our current data revealed that NDRG2 overexpression enhanced NIS level in TT cells and increased their iodine uptake in vitro. Furthermore, $^{99m}TcO_4^-$ radionuclide imaging of the xenograft tumors indicated that NDRG2 could promote NIS-mediated radionuclide transport. In conclusion, the present study suggested that *NDRG2* is a critical molecule in the regulation of MTC biological behavior and a potential promoter in radioactive iodine therapy.

Key words: N-Myc downstream-regulated gene 2 (*NDRG2*); Medullary thyroid carcinoma (MTC); Sodium/iodide symporter (NIS); Tumor-suppressor gene

INTRODUCTION

Medullary thyroid carcinoma (MTC) derives from the neuroendocrine C cells of the thyroid gland and constitutes 5–10% of thyroid carcinomas. The majority of MTC cases are sporadic, and approximately 20% are hereditary (1–3). Its metastases to distant sites usually occur at the early stage: including lung, liver, and bone (4). MTC responds poorly to either chemotherapy or radiotherapy. Previous studies showed that the survival rates of MTC patients have not been improved much in the past few decades (5). In the pathogenesis of thyroid carcinoma, evidence indicates that there are many genetic alterations and unique chromosomal rearrangements that occur, including the RET–Ras–BRAF signaling cascade and PI3K–AKT–mTOR pathway (6–9). However, the development and pathogenesis of MTC are largely unknown.

As one member of the human N-myc downstream-regulated gene (*NDRG*) family, *NDRG2* (GenBank Accession No. AF159092) was first identified by polymerase chain reaction-based subtractive hybridization in our laboratory. *NDRG2* encodes a 41-kDa protein containing an acyl-carrier protein (ACP)-like domain and is involved in cell growth and proliferation (10). Some studies reported that NDRG2 was shown to be upregulated in Alzheimer's brains (11) and could induce the differentiation of dendritic cells (12). We have demonstrated that NDRG2 was a candidate tumor-suppressor gene (13): the expression of NDRG2 was significantly reduced in various cancer tissues, and the overexpression of NDRG2 inhibits the proliferation of some cancer cells (14–17). Importantly, we have found that the levels of NDRG2 mRNA and protein were significantly low in all histotypes of thyroid tumors (18).

These results are consistent with previous observations by Mordalska et al. (19) that the levels of NDRG2 mRNA expression were statistically significantly decreased in papillary thyroid carcinoma (PTC). However, whether NDRG2 is involved in oncogenesis and is associated with the development of MTC cells is still unknown.

The sodium/iodide symporter (NIS) is an integral plasma membrane lycoprotein that mediates active I⁻ transport into the thyroid follicular cells, the first step in thyroid hormone biosynthesis (20). The correlation between NIS expression in thyroid tumors and their ability to uptake the radioiodine has been confirmed (21,22). Evidence indicates that the accumulation of I⁻ in the thyroid via NIS is in close correlation with Na^+/K^+-ATPase (23). Na^+/K^+-ATPase locates on the basolateral side of thyroid cells and can pump Na^+ outside by expending energy, and this can form the Na^+ concentration gradient, which serves as the driving force for NIS cotransporting two Na^+ along with one I⁻ into the thyrocytes (24). It is worth mentioning that Na^+/K^+-ATPase β1 subunit was identified as a protein that interacts with NDRG2. The activity of Na^+/K^+-ATPase β1 is directly proportional to NDRG2 protein level. NDRG2 colocalized with Na^+/K^+-ATPase β1 in the perinuclear region of the cytoplasm, increased the stability, and extended the half-life of Na^+/K^+-ATPase β1(25). We speculate that NDRG2 may interact with Na^+/K^+-ATPase β1 maintaining the Na^+ concentration gradient to facilitate the function of NIS.

To further identify whether and how NDRG2 is involved in the pathogenesis of MTC, we upregulated the expression of NDRG2 in MTC TT cells by lentivirus and revealed its role in the regulation of MTC both in vitro and in vivo. We found that NDRG2 appeared to be a candidate tumor suppressor, suppressing the proliferation and migration of MTC cells and tumor development. We discuss the implication of our findings in the pathogenesis of MTC and provide a potential strategy for increasing the sensitivity of MTC to radionuclide therapy.

MATERIALS AND METHODS

Cell Culture

Human medullary thyroid carcinoma TT cells were obtained from the American Type Culture Collection (ATCC) and maintained in Kaighn's modification of Ham's F-12 medium (Sigma-Aldrich) supplemented with 10% fetal bovine serum and 2.5 g/L sodium bicarbonate. 293T cells for producing lentiviral particles were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 10% fetal bovine serum. All the cells were maintained in a humidified 5% CO_2 atmosphere at 37°C.

Amplification of NDRG2 Overexpression Lentivirus

The plasmids of pLenti6.3/V5-DEST-NDRG2 and the control plasmids of pLenti6.3/V5-DEST-CHERRY

were generously provided by Ph.D. Jian Zhang (Fourth Military Medical University, Shaanxi Province, China). The plasmid, together with plasmids psPAX2 (Addgene, USA) and pMD2.G (Addgene, USA), was cotransfected into 293T cells with Lipofectamine 2000 (Invitrogen, USA), according to the manufacturer's instructions for the generation of lentivirus-overexpression-NDRG2 or lentivirus-overexpression-CHERRY, respectively. The culture supernatants containing lentivirus were harvested and ultracentrifuged. The virus titers of each viral preparation were determined.

Cell Infection

TT cells were seeded in a six-well plate and grown at 70–80% confluence and infected with lentivirus-NDRG2 or lentivirus-CHERRY (at MOI 30), respectively. The blasticidin (Invitrogen, USA) was added into the medium 48 h after infection with a concentration of 9 μg/ml for selection of stably transduced cells. Approximately 4 weeks later, the stably transduced cells were established with the overexpression of NDRG2 or CHERRY gene and named as TT-NDRG2 or TT-CHERRY, respectively.

RT-PCR

The total RNA was extracted from cells using the TRIzol reagent (Takara, Japan). After quantification, total RNA (2 μg) was reverse transcribed into cDNA using the PrimeScript® RT reagent Kit (Takara, Japan), according to the manufacturer's instructions. Subsequently, the cDNA was used as the template in a Takaka Taq™ (Takara, Japan) and in triplicate subjected to denaturation at 95°C for 5 min and 30 cycles of 94°C for 30 s and 58°C for 30 s, followed by extension at 72°C for 7 min using the specific primers. The sequences of primers were as follows: human NDRG2: 5′-AACCACCCGGACACTGTTGAA-3′ (forward) and 5′-AAGGATCATCTCCGGAATGGAAG-3′ (reverse); NIS: 5′-CTGTCCACCGGAATTATCTGCAC-3′ (forward) and 5′-TTAGCATCACCACGACCTGGAA-3′ (reverse); GAPDH as a control: 5′-GCACCGTCAAGG CTGAGAAC-3′ (forward) and 5′-TGGTGAAGACGCCA GTGGA-3′ (reverse). Mean values from three independent experiments were taken as results.

Western Blot Analysis

The cells were washed with ice-cold PBS and lysed in RIPA buffer (50 mM Tris, 150 mM NaCl, 1% NP-40, 0.1 mM PMSF, and protease inhibitor cocktail) on ice for 30 min. Fifty micrograms of total protein (quantified by BCA protein assay; Pierce, Rockford, IL, USA) was subjected to SDS-PAGE and transferred to Hybond ECL nitrocellulose membranes (Amersham Biosciences, Piscataway, NJ, USA). After being blocked with TBST (20 mM Tris, 150 mM NaCl, 0.05% Tween-20) containing

5% fat-free milk for 1 h at room temperature, the blots were probed with the following primary antibodies: NDRG2 (Abnova, Taiwan), NIS (Abcam, UK), cyclin D1, cyclin E, Cdk2 (Cell Signaling Technology, USA), and β-actin (Abcam, UK). Subsequently, species-matched horseradish peroxidase-conjugated secondary antibodies (Abcam, UK) were detected by an Odyssey infrared imaging system (LI-COR Inc., Lincoln, NE, USA).

Cell Cycle and Apoptosis Analysis

The cell cycle was detected by flow cytometry analysis, as described previously (6). TT, TT-CHERRY, and TT-NDRG2 cells were washed with ice-cold PBS twice and fixed with 70% ethanol for 30 min at 4°C, and then filtered through a 50-mm nylon mesh, respectively. The DNA content of stained nuclei was analyzed by a flow cytometer. Cell apoptosis was measured using Annexin V-FITC Apoptosis Detection Kit (Sigma-Aldrich) and subsequently analyzed by flow cytometry. Each experiment was performed in triplicate.

MTT Assay

TT, TT-CHERRY, and TT-NDRG2 cells were seeded, respectively, in an initial density of 1×10^4/well in 96-well plates in triplicate with 10% FBS F12K at 37°C 5% CO_2 for varying periods and exposed to fresh media every other day. During the constant time of each day, MTT [3-(4, 5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] was added into each well at a final concentration of 0.5 mg/ml for one plate. The insoluble formazan was dissolved in dimethyl sulfoxide and measured at OD (490 nm) for determining the cell viability.

Colony Formation Assay

TT, TT-CHERRY, and TT-NDRG2 cells at 100 cells per well in 6-cm plates were cultured in 10% FBS F12K at 37°C 5% CO_2 for 3 weeks. The cell colonies were washed twice with PBS, fixed by 4% paraform for 15 min, and stained with Giemsa for 30 min. Individual clones with more than 50 cells were counted. Clone-forming efficiency for individual type of cells was calculated, according to the number of colonies/number of inoculated cells × 100%.

Monolayer Wound Healing Assay

TT, TT-CHERRY, and TT-NDRG2 cells were seeded in 24-well plates with 10% FBS F12K, respectively. After the cells reached subconfluence, the monolayer cells were wounded by scraping off the cells with 200-μl pipette tips and then grown in medium for 48 h. The migrated distance of cells was monitored and imaged under a microscope. The distances of cell migration were calculated by subtracting the distance between the lesion edges at 48 h from the distance measured at 0 h. The total wound areas were analyzed using the Nikon Image software.

Transwell Invasion Assay

The impact of upregulating NDRG2 expression on the invasiveness of MTC TT cells was measured using the Matrigel invasion assay. Briefly, Transwell inserts (Corning Incorporated, USA) with 8.0-μm pores were coated with Matrigel (0.77 μg/μl; Corning Incorporated, USA). Cells at 10^4/well were seeded in the upper chambers in 200 μl FBS-free F12K, and the lower wells were filled with 500 μl of 10% FBS F12K for inducing cell migration. Following incubation for 24 h, the cells on the filter surface were fixed with 4% formaldehyde, stained with hematoxylin and eosin, and examined under a microscope. A total of six random high-power microscopic fields (200×) per filter were photographed, and the numbers of cells were directly counted.

Iodine Uptake Assay

TT, TT-NDRG2, and TT-CHERRY cells were seeded in six-well plates. Once they reached 80% confluence, the medium was replaced with F-12 medium supplemented with 0.5 μCi ^{125}I and incubated for 1 h. After 5 min, 10 min, 20 min, 30 min, 60 min, and 90 min, the medium containing ^{125}I was discarded. Cells were washed with PBS twice and collected following trypsin digestion. These cells were then transferred into tubes, and their cpm values were measured.

Tumorigenesis Assay

The influence of NDRG2 overexpression on the tumor development of MTC in vivo was examined according to the methods described by Ning et al. (26). TT-CHERRY or TT-NDRG2 cells at 5×10^6 per mouse were subcutaneously injected into nu/nu mice ($n = 3$ per group; Fourth Military Medical University Animal Research Center, Xian, China). The experimental pairs (TT-CHERRY vs. TT-NDRG2) were done in different mice. The development and growth of solid tumors were monitored by measuring tumor size using a vernier caliper in a blinded fashion every 5 days for a 30-day period. The tumor volume was calculated using a standard formula (27): tumor volume $(mm^3) = $ width $(mm)^2 \times$ length $(mm) \times 0.5$. At the end of the experiment, all mice were sacrificed, and individual tumor weights were measured using a balancer.

$^{99m}TcO_4^-$ Radionuclide SPECT Imaging

After the solid tumors grow up to 10 mm in diameter, mice (two from TT-CHERRY group and two from TT-NDRG2) were intraperitoneally injected with 1 mCi $^{99m}TcO_4^-$. SPECT images were taken 10 min after injection.

Statistical Analysis

SPSS 13.0 software was used to perform statistical analysis. Data are presented as mean ± SD, and statistical comparisons between groups were made using one-way ANOVA followed by Student's *t*-test. A value of $p < 0.05$ was considered statistically significant.

RESULTS

Effect of Upregulated NDRG2 Expression on TT Cell Growth

Our previous study (18) showed that significant staining of NDRG2 was observed in normal thyroid tissues, while the expression of NDRG2 was almost undetectable or at a significantly low level in all histotypes of thyroid carcinoma. In the current study, following infection and selection of blasticidin on human MTC TT cells with the lentivirus, the protein and mRNA transcript levels of NDRG2 in those cells were determined by Western blotting and RT-PCR (Fig. 1A). The results showed that the levels of NDRG2 protein and mRNA transcripts in TT-NDRG2 cells increased significantly, and infection with control lentivirus did not affect the expression of NDRG2 as the levels of protein and mRNA transcripts in TT-CHERRY cells were similar to that in parent TT cells. The successful establishment of NDRG2 gene overexpression TT cell lines provided a useful tool for investigating the function of NDRG2 in the growth of MTC cells in vitro.

To investigate the possible function of NDRG2 in the growth of TT cells, MTT assays were performed. Following a 10-day period, the growth of TT-CHERRY cells was indistinguishable from its parent TT cells and showed strong proliferation. On the contrary, the growth of TT-NDRG2 cells was much slower, compared with that in control groups on day 10 (Fig. 1B). A similar pattern of inhibitory effect of NDRG2 overexpression in TT cells was achieved in colony formation assay. Following

incubation for 3 weeks, 86, 76, or 35 colonies were generated from TT, TT-CHERRY, or TT-NDRG2 cells, respectively (Fig. 1C). Therefore, the low MTT activity and fewer numbers of cell colonies from TT-NDRG2 cells demonstrate that upregulation of NDRG2 expression inhibits the growth of MTC cells in vitro.

Effect of Upregulated NDRG2 Expression on the Migration of TT Cells

Metastasis to distant sites is a great character of MTC. Thus, we examined the impact of NDRG2 overexpression on the migration of MTC cells in vitro by the wound healing assay. Following incubation of physically wounded cells for 48 h, the TT and TT-CHERRY cells migrated at 98.633 ± 5.684 μm and 90.333 ± 6.067 μm, respectively. In contrast, the TT-NDRG2 cells only migrated about 44.667 ± 3.066 μm, which was significantly shorter than control groups ($p < 0.05$) (Fig. 2A). Further analysis of the impact of NDRG2 on TT cells revealed that upregulation of NDRG2 expression reduced the invasiveness of MTC cells in vitro, determined by the Matrigel invasion assay. While average 75 ± 6.7 TT and 81.0 ± 7.8 TT-CHERRY cells per high power field had migrated onto the filter surface, only 37.0 ± 4.7 TT-NDRG2 cells reached on the filter ($p < 0.05$) (Fig. 2B). The significantly shortened distance of migration and fewer numbers of TT-NDRG2 cells on the filter surface indicated that upregulation of NDRG2 expression mitigates the migration of MTC cells in vitro.

NDRG2 Induces the Cell Cycle Arrest and Apoptosis of TT Cells

To investigate the potential mechanism underlying the action of NDRG2 in the growth of MTC, cell cycle was characterized by FACS analysis (Fig. 3A and B). The results indicated that the frequency of TT-NDRG2 cells at the G_0/G_1 phase reached 60.50 ± 3.83%, while lower frequency of cells progressed at the S phase, compared
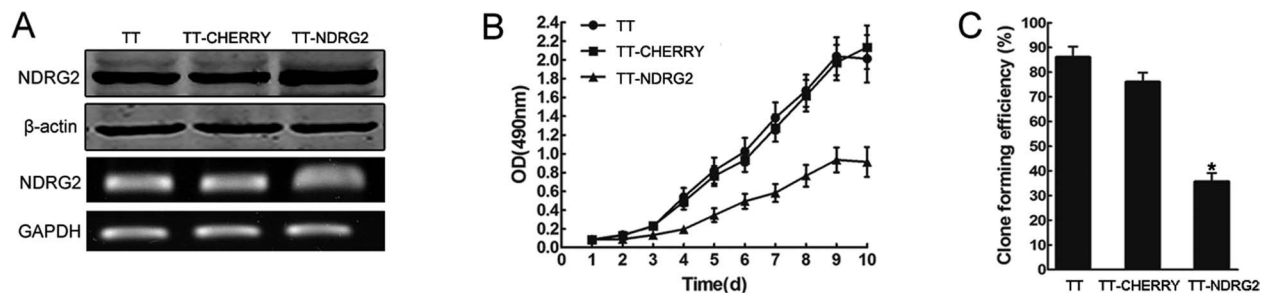

Figure 1. Effect of NDRG2 overexpression on MTC cell growth. (A) Western blot analysis and RT-PCR were performed to detect NDRG2 expression in TT cells after being infected by NDRG2 lentivirus. β-Actin and GAPDH were used as loading controls, respectively. (B) MTT assay was performed to detect the growth dynamics of TT, TT-CHERRY, and TT-NDRG2 cells. Data are the OD (490 nm) values. (C) Quantitative analysis of colony formation of TT, TT-CHERRY, and TT-NDRG2 cells in vitro after being cultured for 3 weeks and stained with H&E. Data are expressed as the efficiency of colony formation (%) and expressed as mean ± SD of three separate experiments. Experimental and control groups were simultaneously tested. *$p < 0.05$ versus TT or TT-CHERRY.
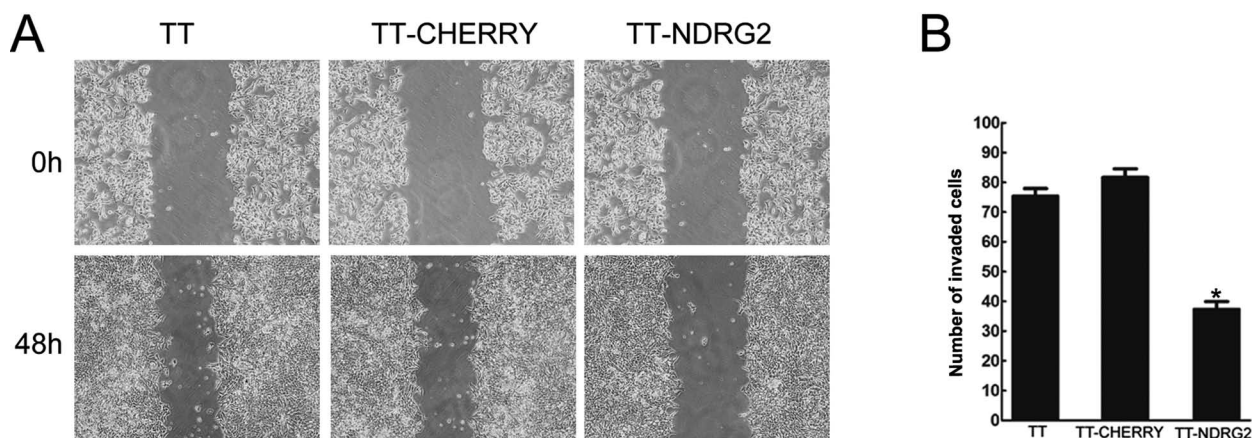
Figure 2. Effect of NDRG2 overexpression on MTC cell migration and invasion. TT, TT-CHERRY, and TT-NDRG2 cells were subjected to migration (A) and invasion assays (B), respectively. (B) The histograms represent the cells having invaded through Matrigel. Data represent three independent experiments. *$p < 0.05$ versus TT or TT-CHERRY.

with control groups ($p < 0.01$). These data indicated that upregulation of NDRG2 expression arrests TT-NDRG2 cell cycle at the G_0/G_1 phase, which may inhibit the growth of MTC cells. We then investigated the mechanism by which NDRG2 induced cell cycle arrest in TT cells. Cell cycle effectors were examined by Western blot analysis (Fig. 3C). Our results indicated that upregulation of NDRG2 protein was associated with a reduction in cyclin D1, cyclin E proteins, whereas Cdk2 was not affected. In addition, FASC also revealed that there were much more apoptotic cells in TT-NDRG2 cells than that in the control cells ($p < 0.05$) (Fig. 3D).

Impact of Upregulated NDRG2 Expression on the Tumor Development of Inoculated TT Cells In Vivo

To determine the role of NDRG2 in the MTC development, TT, TT-CHERRY, or TT-NDRG2 cells were injected subcutaneously into nu/nu mice. The development of solid MTC was monitored for 30 days. As shown in Figure 4A, the developed tumors were first visible about 15 days postinoculation and rapidly grew later in TT and TT-CHERRY groups. In contrast, the development of TT-NDRG2 cell-related solid tumors grew slowly, and the mean volume of the tumors in the TT-NDRG2 group decreased by about 60%, compared with that in control groups (Fig. 4B). As a result, the mean weight of tumors in the TT-NDRG2 group was significantly lighter than that in control groups ($p < 0.05$) (Fig. 4C). These data indicated that upregulation of NDRG2 expression mitigated the development of MTC in vivo.

Relation Between NDRG2 and NIS

MTC remains difficult to treat because of its unresponsiveness to radioiodine therapy. To testify whether

NDRG2 could induce NIS expression and increase the uptake of iodine by MTC cells, we enhanced the expression of NDRG2 following infection and selection of blasticidin on human MTC TT cells with the lentivirus. The protein and mRNA levels of NIS in those cells were then determined by Western blotting and RT-PCR (Fig. 5A and B). Results showed that NIS in TT-NDRG2-transfected cells increased significantly, while the expression of NIS in TT-CHERRY-transfected cells was similar to that in parent TT cells. To further investigate whether the NDRG2-induced NIS was functional or not, the iodine uptake test was performed (Fig. 5C). Data showed that TT-NDRG2 cells significantly restored the ability of iodine uptake compared to the TT-CHERRY group and the TT group.

In vivo, after the solid tumors grow up to 10 mm in diameter, mice were injected with $^{99m}TcO_4^-$, and then SPECT images were taken. The radionuclide imaging of the solid tumors indicated that MTC acquired the ability of $^{99m}TcO_4^-$ accumulation after tumor-specific NDRG2 gene expression (Fig. 5D). Collectively, these data indicated that enhanced NDRG2 expression restored the function of NIS and radionuclide uptake in MTC cells.

DISCUSSION

Although surgical resection is the most common therapy for MTC, approximately 50% to 80% of patients have already metastasis at the time of initial diagnosis, and more than 50% of patients will recur or progress after operative resection (28). Therapeutic drugs specifically targeting to tumor-related molecules for MTC are therefore urgently required. Earlier reports have shown that NDRG2 plays a variety of roles in cell proliferation and differentiation (10,29,30). We also demonstrated that low levels of NDRG2 are expressed in MTC tissues by using
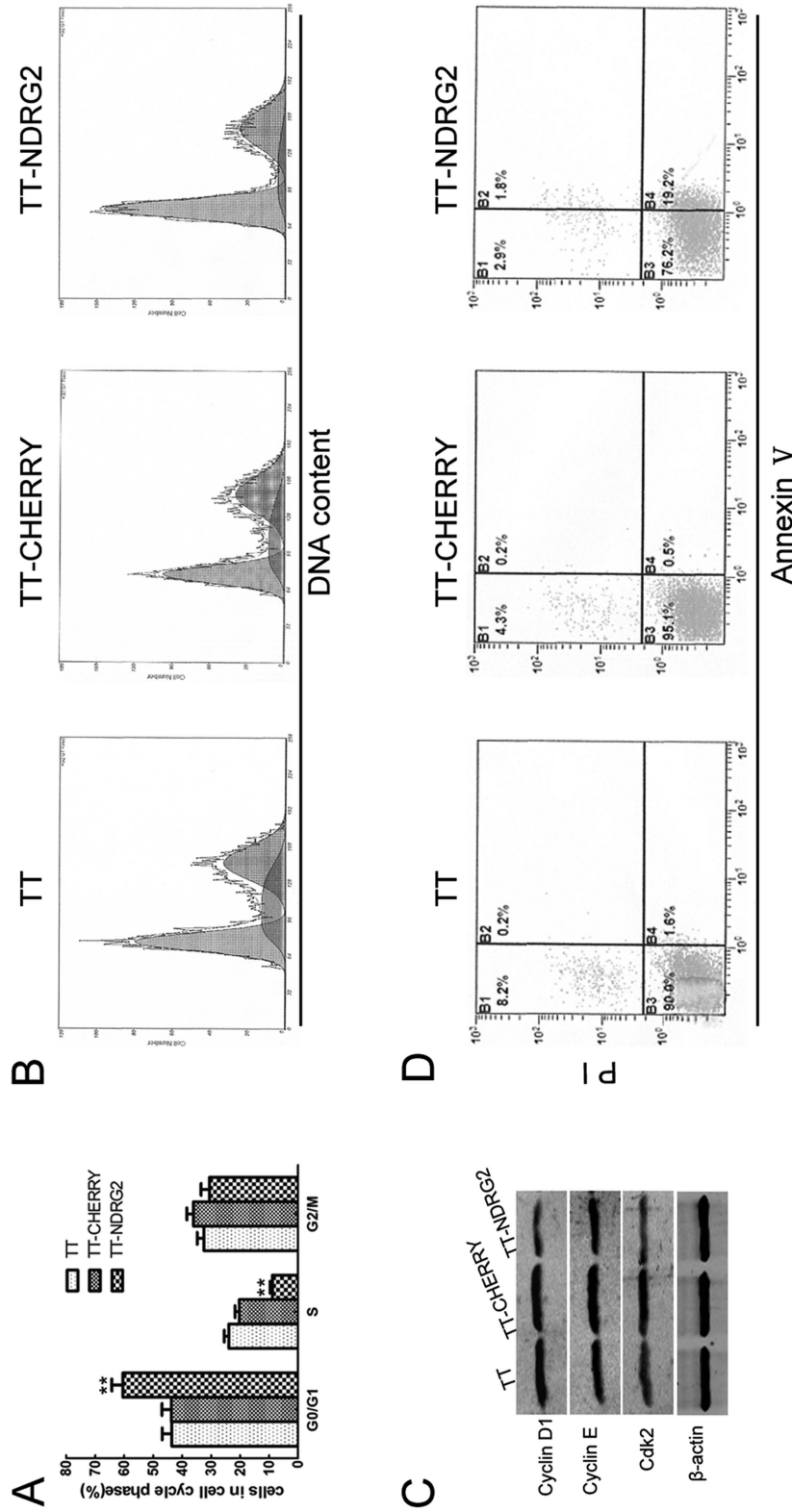
Figure 3. NDRG2 overexpression induces MTC cell G_1/S arrest and apoptosis, changes cell cycle regulators. TT, TT-CHERRY, and TT-NDRG2 cells were collected, fixed, and stained for FACS analysis. Proteins were harvested for cell cycle regulator detection by Western blot analysis. (A) and (B) Representative results of cell cycle distributions. (C) The levels of cyclin D1, cyclin E, and Cdk2 proteins. β-Actin was used as a loading control. (D) Representative plots of apoptosis analysis. The percentages of apoptotic cells are indicated as Annexin V^+ cells. Data are expressed as mean±SD of three independent experiments. Experimental and control groups of cells were analyzed simultaneously. $^{**}p < 0.01$ versus TT or TT-CHERRY.
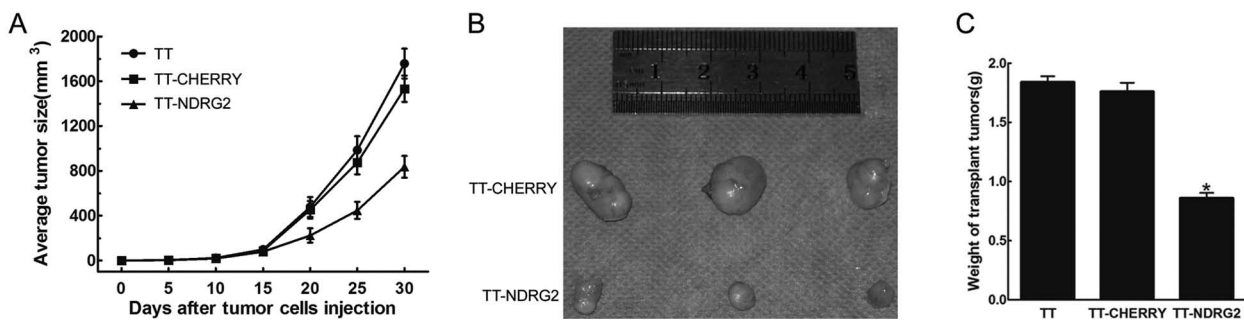
Figure 4. Effect of NDRG2 overexpression on the development and growth of inoculated TT cells. Groups of nu/nu mice ($n = 3$ per group) were inoculated with 5×10^6 TT, TT-CHERRY, or TT-NDRG2 cells, and the development of solid MTC tumors was monitored every 5 days. The mice were sacrificed 30 days postinoculation, and their tumor weights were measured. (A) The dynamics of MTC tumor growth. (B) The image of individual tumors. (C) Quantitative measurement of tumor weights. Data are expressed as mean ± SD of each group. *$p < 0.05$ versus TT or TT-CHERRY.

immunohistochemistry analysis in our previous study (18). To further characterize the role of NDRG2 in MTC cells, we generated NDRG2 overexpression lentivirus to directly investigate the effect of NDRG2 on the tumor biological characteristics of MTC.

Our in vitro results illustrated that the proliferation of MTC TT cells was greatly inhibited by NDRG2 over-expression. Furthermore, to evaluate the in vivo effects of NDRG2 on MTC cells, we performed a nude mouse

assay according to the methods described by Ning et al. (26). We observed that the average volume and weight of tumors in the TT-NDRG2 group was significantly reduced compared to control groups. Subsequently, when exploring the regulative mechanism of NDRG2 on MTC proliferation, we revealed that overexpression of NDRG2 results in G_1/S phase arrest and an increase in apoptosis of MTC cells. The G_1/S phase arrest was caused by the decreased expression of cyclin D1 and cyclin E. These
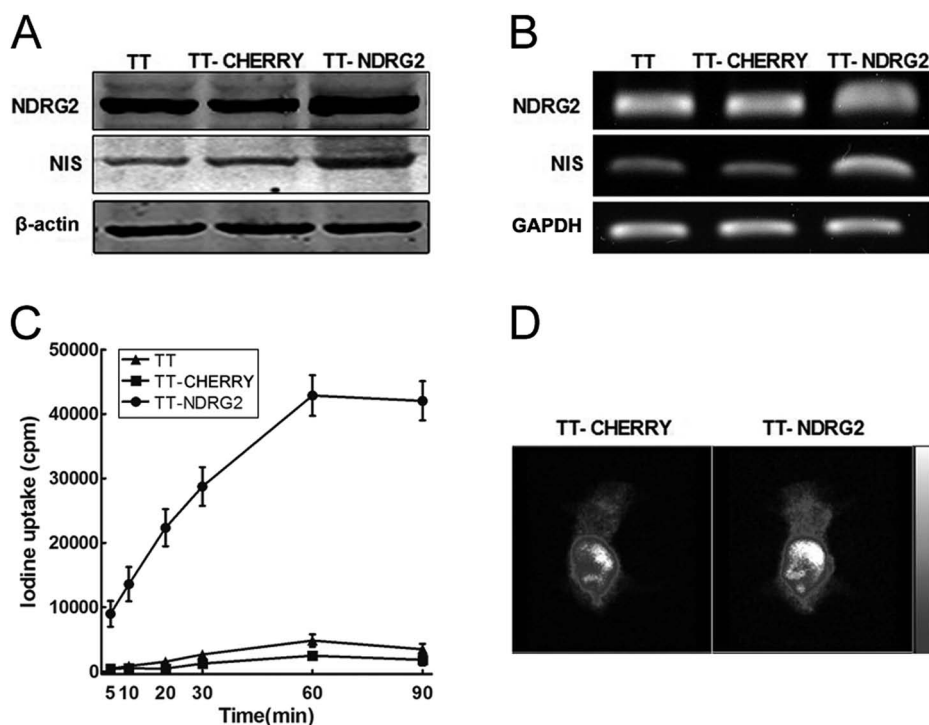

Figure 5. Relation between NDRG2 and NIS. (A) and (B) Western blot and RT-PCR assay showed that the levels of NDRG2 and NIS in TT-NDRG2 group increased significantly compared with that in TT-CHERRY or TT groups. (C) Iodine uptake test showed that TT-NDRG2 cells significantly restored the ability of iodine uptake compared to TT-CHERRY group and TT group. (D) $^{99m}TcO_4^-$ radionuclide imaging of xenografted tumors.

data further revealed the antiproliferative characteristic of NDRG2 in numerous carcinomas.

Our group has demonstrated that there were no significant correlations of NDRG2 expression with different histotypes of thyroid cancers or distant metastases (18). MTC cells have the potential to metastasize in the early stage of its development. When MTC presents with nodal disease, patients are at a very high risk of developing recurrence compared with that of restricted to the thyroid gland and without nodal involvement (31). Thus, in our current study, we conducted experiments to test whether NDRG2 could inhibit MTC migration and invasion. Following physical injury, we observed that the migrated distance of TT-NDRG2 cells was significantly shorter than that of control cells. Similarly, Matrigel invasion assay revealed that upregulation of NDRG2 expression reduced the invasiveness of TT cells. These results demonstrated that upregulation of NDRG2 expression remarkably reduced the migration capacity of TT cells in vitro.

In contrast to differentiated follicular cell-derived thyroid cancer, radioiodine therapy is not successful in treating dedifferentiated MTC because of the reduced ability of iodine uptake by MTC cells. Previous studies have revealed that the level of NDRG2 expression is linked positively to cell differentiation. In addition, our group demonstrated that NDRG2 could increase the stability and extend the half-life of Na^+/K^+-ATPase $\beta1$; the latter is the subunit of Na^+/K^+-ATPase, which generated the electrochemical sodium gradient and drove the NIS-mediated transport of iodide in the thyroid. Taken these into account, we wondered whether NDRG2 is involved in the iodide uptaken in MTC cells.

In the current study, we observed that NIS level was increased after NDRG2 overexpression in TT cells in vitro. Moreover, the increased NIS level restored the iodine uptake by TT cells. After injecting these lentivirus-treated TT cells into nu/nu mice, we applied $^{99m}TcO_4^-$ imaging method to reveal the radionuclide uptaken ability of these solid tumors. Data showed that MTC acquired the ability of $^{99m}TcO_4^-$ accumulation after tumor-specific NDRG2 expression, suggesting the appearance of the functional NIS. These findings demonstrated that upregulation of NDRG2 expression could increase the sensitivity of MTC cells to radioiodine therapy. Although the mechanism by which NDRG2 regulated NIS expression and radionuclide transportation is still not fully understood and merits further investigation, on the basis of our data and previous observations, we may boldly speculate that the relationship between NDRG2 and cell differentiation as well as the protective effect of NDRG2 on Na^+/K^+-ATPase $\beta1$ degradation make radioiodine therapy possible for MTC patients.

Taken together, our data indicated that NDRG2 could suppress the proliferation, migration, and invasion of MTC cells, increase the iodine uptake ability of MTC, and subsequently enhance the sensitivity of MTC to radioiodine therapy. Therefore, it is conceivable that modulating NDRG2 expression may provide a novel therapy for intervention in MTC cases.

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