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Dedifferentiation of differentiated thyroid carcinoma cell line FTC-133 is enhanced by ^{131}I pretreatment☆

Fang Feng, Hui Wang*, Hongliang Fu, Shuqi Wu, Zhiyi Ye, Suyun Chen, Jianing Li

Department of Nuclear Medicine, Xin Hua Hospital, Shanghai Jiao Tong University School of Medicine, Shanghai 200092, China

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Abstract

Introduction: Differentiated thyroid carcinoma (DTC) usually has a high iodine uptake. However, dedifferentiation of DTC with decreased or no radioiodine (^{131}I) uptake is observed in clinical practice, with poor prognosis. The aim of this study was to investigate the effects of ^{131}I radiation on radioiodine uptake (RAIU) and the expression of thyroid-specific molecules.
Methods: FTC-133 cells were treated with ^{131}I, the dosage dictated by methylthiazol tetrazolium test results and preliminary experiments. The experimental cell group was incubated with ^{131}I for 48 h and then cultured for 3 months in ^{131}I-free medium. The control group was set without ^{131}I. Primary cells were defined as the blank group. Following treatment, RAIU was measured with a gamma counter as the counts/cell number. Na^+/I^- symporter (NIS), thyroid-stimulating hormone receptor (TSHR), thyroid peroxidase (TPO) and thyroglobulin (Tg) levels were detected by Western blotting and radioimmunoassay, and their mRNAs were detected by real-time polymerase chain reaction.
Results: RAIU of FTC-133 cells decreased gradually after coincubation with ^{131}I and did not recover even if ^{131}I was removed. The relative RAIU of the control and experimental groups was 0.567 and 0.182, respectively, a statistically significant difference ($P<.01$). Expression of NIS, TSHR, TPO and Tg decreased in the experimental group to a statistically significant degree compared to that of controls ($P<.05$).
Conclusion: Changes in the mRNA levels were in accordance with the expression of thyroid-specific proteins. Thus, FTC-133 cells undergo dedifferentiation during long-term culture in vitro, and ^{131}I may promote this progress.

Keywords: Differentiated thyroid carcinoma; Dedifferentiation; Radioiodine; Follicular thyroid carcinoma; Nuclear medicine

1. Introduction

Thyroid cancer is the most common endocrine malignant tumor. The majority of thyroid cancers are differentiated thyroid carcinomas (DTCs), including papillary thyroid carcinomas (PTCs), follicular thyroid carcinomas (FTCs) or a combination of these. The metastases of DTCs can retain their ability to concentrate iodine, so radioiodine (^{131}I) therapy is effective, and most treated DTC patients experience long-term disease-free survival [1]. However, about one third of thyroid cancer metastases can lose their ability to concentrate iodine, a phenomenon called dedifferentiation [2,3], which indicates a poor prognosis. In recent in vivo studies, some researchers have demonstrated that well-differentiated PTCs and FTCs may develop into anaplastic thyroid cancers (ATCs), as they share the same genetic alterations as mutations of *RAS*, *BRAF* and *PIK3CA* genes [4–6].

The dedifferentiation of DTC is usually observed during ^{131}I therapy [7], when metastases appear negative in a ^{131}I whole-body scan, although they definitely exist. We therefore hypothesized that radiation with ^{131}I may affect the dedifferentiating progress of DTC cells, and we established an in vitro model to confirm this. Some researchers have reported that ATCs arise more often from FTCs [6], and therefore, we chose FTC cell lines to study.

Iodide trapping is a mechanism regulated by thyroid-stimulating hormone (TSH) and involves an energy-dependent transport mediated by the Na^+/I^- symporter (NIS). Iodide is organized by thyroid peroxidase (TPO)

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* Corresponding author. Tel.: +86 21 65156486; fax: +86 21 65156486.
E-mail address: wanghuishanghai@hotmail.com (H. Wang).

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and conjugated to tyrosine residues on thyroglobulin (Tg) [8]. Na^+/I^- symporter, the key molecule responsible for the iodide concentration, has been frequently studied, and the down-regulated expression in DTC resistance to ^{131}I therapy has been observed [9]. Some techniques have been tried to regulate NIS expression in order to enhance or reestablish radioiodine uptake (RAIU). These methods utilize agents such as retinoic acids and NIS gene transfection, but the results have not been encouraging [3,10,11]. Therefore, it is important to further investigate changes at the molecular level that are involved in dedifferentiation of DTC in order to find new methods to reestablish iodide uptake.

In the present study, we examined the effect of ^{131}I uptake in a DTC cell line cultured in vitro. We then investigated whether ^{131}I radiation influenced specific molecular expression, including that of NIS, TPO, Tg and thyroid-stimulating hormone receptor (TSHR) in a cultured FTC-133 cell line, to determine why DTCs lose their ability to concentrate iodine.

2. Materials and methods

2.1. Cell culture

The human DTC cell line FTC-133 was obtained from the Department of Nuclear Medicine, Ruijin Hospital, Shanghai, China. The cells were cultured in Dulbecco's modified Eagle's medium and Ham's F-12 nutrient mixture (DMEM/F12; Invitrogen Life Technologies, Carlsbad, CA, USA) supplemented with 10% fetal bovine serum containing 1% penicillin/streptomycin (Invitrogen Life Technologies) and grown at 5% CO_2 and 37°C.

2.2. Cellular growth analyses

Cellular growth was measured by the 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyl tetrazolium bromide (MTT) assay (Sigma-Aldrich, St. Louis, MO, USA) as previously described [12]. Briefly, cells were seeded in 96-well plates at a density of 2000 cells/well and incubated for 24 h under standard conditions to allow cell attachment. The cells were given 5 to 50 μCi (in 5-μCi increments) $Na^{131}I$ in 20 μl phosphate-buffered saline (PBS) per well except for the control wells, and blank wells were set without cells. All of the wells were incubated for 48 h. The media were then removed, and the cells were incubated with MTT (5 mg/ml) for 4 h at 37°C. After incubation, 100 μl of dimethyl sulfoxide (Sigma-Aldrich) was added to each well and mixed thoroughly. The amount of formazan crystals, reflecting cellular growth and viability, was determined quantitatively by absorbance measurements in spectrophotometric assays at 570 nm.

2.3. ^{131}I treatment

According to the MTT results, cells cultured in 24-well plates were treated with ^{131}I (5–25 μCi), and a control group was set without ^{131}I. Each group consisted of a set of six wells, and the experiment was repeated three times. All of the cells were incubated for 12 h, and the RAIU assay was employed. The dosage of ^{131}I which led to the most significant decrease of RAIU was selected for subsequent experiments.

The experimental group of cells was subsequently incubated for 48 h with ^{131}I at the dosage selected in the last step. The RAIU values were tested at 6, 12, 24 and 48 h. At 48 h, the ^{131}I was removed by washing (×3) with Hank's balanced salt solution (HBSS), and the cells were cultured in activity-free medium for 3 months, and at 1 week or 3 months, the RAIU values were tested. Simultaneously, the control group was set without ^{131}I and cultured in parallel, and RAIU values were tested at the same time as the experimental group.

2.4. RAIU assay

Radioiodine uptake was measured as previously described [13]. By the end of preset incubation period, cells were washed (×3) with 1 ml of HBSS and then incubated at 37°C for 40 min with 500 μl buffered HBSS containing 0.1 μCi carrier-free $Na^{125}I$ and 10 μM sodium iodide. The radioactive medium was aspirated, and cells were washed (×3) 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.

2.5. Western blotting

Three groups of FTC-133 cells were selected for this step. The blank group consisted of cryopreserved primary cells before ^{131}I was administrated. The cells of the experimental group received ^{131}I radiation for 48 h and were then cultured for 3 months. The cells of the control group received no ^{131}I radiation and were cultivated for 3 months. Cells were washed with PBS and harvested by scraping with RIPA lysis buffer. Lysates were quantified spectrophotometrically using the bicinchoninic-acid-based (BCA) method (Pierce Chemical, Rockford, IL, USA). Twenty-five micrograms of each sample was separated by gradient polyacrylamide gels and transferred onto polyvinyldifluoride membranes (Pierce Chemical). Membranes were incubated with one of the following primary antibodies — affinity purified mouse monoclonal anti-human NIS antibody (1:1500; Chemicon International, Hofheim, Germany), monoclonal mouse anti-TSHR (1:1000; Santa Cruz Biotechnology, Santa Cruz, CA, USA), TPO antibody (1:500; Thermo Fisher Scientific Inc., Fremont, CA, USA) or mouse anti-human β-tubulin antibody (1/2000; Santa Cruz Biotechnology) — overnight at 4°C in TTBS/milk. The membranes were then incubated with a horseradish peroxidase conjugated anti-mouse antibody (1:2000; Santa Cruz Biotechnology). The protein