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Inhibition of Thyroid-Restricted Genes by Follicular Thyroglobulin Involves Iodinated Degree

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ABSTRACT

Follicular thyroglobulin (TG) reflects the storage of both iodine and thyroid hormone. This is because it is a macromolecular precursor of thyroid hormone and organic iodinated compound in follicular lumen. Thus, it may have an important feedback role in thyroid function. In this study, monolayer cells were cultured and follicles were reconstituted with primary pig thyroid cells in vitro. Reconstituted follicles were treated with iodine and methimazole (MMI), a drug that blocks iodine organification and reduces the degree of TG iodination in follicular lumen. The high degree of iodinated TG in follicular lumen was observed to inhibit thyroid-restricted gene expression. To confirm this finding, monolayer thyroid cells were treated with a different degree of TG iodination at the same concentration. These iodinated TG were extracted from reconstituted follicles of different groups. In this manner, this study provides firsthand evidence suggesting that follicular TG inhibits the expressions of thyroid-restricted genes NIS, TPO, TG, and TSHr. J. Cell. Biochem. 112: 971–977, 2011.

KEY WORDS: IODINATED THYROGLOBULIN; RECONSTITUTED THYROID FOLLICLES; THYROTROPIN RECEPTOR; THYROID PEROXIDASE; SODIUM IODIDE SYMPORTER

Thyroglobulin (TG) was synthesized in thyrocytes, secreted outside the cells, and stored in thyroid follicles lumen, a spheroidal closed space composed of anywhere from several to dozens of thyroid cells. Iodinated TG is the macromolecular precursor of thyroid hormone and organic iodinated compound stored in follicle lumen. Thus, it reflected the storage of both thyroid hormone and iodine. Thus, it may play an important feedback role in thyroid function.

A number of studies have revealed that exogenous TG may inhibit thyroid-restricted gene expression, thereby suppressing thyroid function in monolayer thyroid cell in vitro (Suzuki et al., 1999a,b,c; Suzuki and Kohn, 2006; Noguchi et al., 2010). However, no further information has explained the degree of TG iodination used in studies. Moreover, data originating from monolayer cells failed to reflect the effects of follicular TG objectively. Because monolayer cells were incapable of forming follicular lumen structure, it synthesized and secreted iodinated TG directly into the culture medium instead of storing it in follicular lumen (Ambesi-Impiombato et al., 1980; Takasu et al., 1992). These iodinated TG were diluted in culture medium. They rarely affect thyroid function.

Thyroid follicle was a basic morphology functional unit of thyroid gland. A large number of high concentration iodinated TG that accumulated in follicular lumen have a significant impact on thyroid function. Analysis on the level of thyroid follicle provides a better understanding of physiological modulation of thyroid function as well as an opportunity to observe the regulation of iodinated TG stored in follicle lumen.

A recent study revealed that methimazole (MMI), an inhibitor of thyroid peroxidase (TPO) activity, could inhibit iodine organification and abolish the Wolff–Chaikoff effect; the latter is a phenomenon wherein high doses of iodine block iodine organification and uptake (Ferreira et al., 2005). This strongly indicated that the Wolff–Chaikoff effect may be mediated by follicular iodinated compounds, majority of which are iodinated TG. However,

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details on iodinated TG stored in follicular lumen on regulation of thyroid function remain poorly defined.

In this study, reconstituted thyroid follicles and monolayer cells of primary pig thyrocytes are used in vitro as research models. Furthermore, the effect of degree of follicular iodinated TG on thyroid-restricted gene expressions were investigated. The study attempts to explain the feedback regulation mechanism of iodinated TG stored in follicular lumen on the regulation of thyroid function.

MATERIALS AND METHODS

CULTURED THYROCYTE MONOLAYERS AND RECONSTITUTED FOLLICLES

After receiving approval from the institutional research ethics board, a healthy adult pig thyroid was obtained from a local slaughterhouse. Thyroid was extracted within 2 h after the pig expired. It was washed several times with sterilized phosphate buffer solution (PBS) and cut into pieces at a volume of 1 mm^3 under aseptic conditions. Thyroid fragments were digested with 0.125% trypsin (Sigma) for 30 min at room temperature and dispersed into cell suspension. After being filtered in 200 mesh filter, the suspension was centrifuged at 1,000 rpm for 5 min. Cells were cultured in DMEM medium (Hyclone) containing 1 mIU/ml TSH (Merck), 0.05 μmol/L NaI, 10% bovine serum (Hyclone), 200 IU/ml penicillin, and 200 IU/ml streptomycin. These were maintained at 37°C with 5% CO_2.

For reconstituted follicles, 2 ml cell suspension was seeded at a density of 2×10^6 cells/ml on a culture dish. Reconstituted follicle formed after 3 days. For the monolayer, 2 ml cell suspension were seeded at an initiated density of 0.2×10^6/ml and cultured in the same medium, but without TSH on the first day. Cells did not form reconstituted follicles and merely displayed monolayer cells.

RECONSTITUTED FOLLICLE TREATED WITH IODINE AND MMI

When reconstituted follicles were formed on the third day, they were treated with drugs by being directly added to serum-containing culture medium. Group HI was treated with final concentration of 10 μmol/L sodium iodine (Sigma) containing 10 uci ^{125}I (China National Nuclear Corporation); HI + MMI was treated with final concentration of 10 μmol/L sodium iodine (Sigma) containing 10 uci ^{125}I and final concentration of 2 mmol/L MMI (Sigma). Reconstituted follicles were not treated with any drug, thus serving as control group. All groups were cultured continuously for 72 h.

EXTRACTION OF IODINATED TG IN LUMEN FROM RECONSTITUTED FOLLICLES

After removing the culture medium, both HI and HI + MMI groups were washed twice with ice-cold PBS. This was performed to extract iodinated TG in follicular lumen from reconstituted follicles. For incubation, 0.5 ml PBS with 0.02% ethylene diamine tetraacetic acid (EDTA) was added for 5 min. PBS with 0.02% EDTA can chelate Ca^{2+} and loosen connection between cells, which eases the structure dispersion of reconstituted follicles. After this solution was discarded, cells again were washed twice with ice-cold PBS to ensure the absence of exogenous substances in reconstituted follicles. After reconstituted follicles were dispersed with 0.1 ml deionized water, follicular TG entered the suspension from follicular lumen. The suspension was centrifuged at 1,200 rpm for 5 min at 4°C to remove cells and exclude the effect of intracellular TG.

TG synthesized by reconstituted follicles was enclosed in airtight follicular lumen instead of being directly secreted into the culture medium. Therefore, after the culture medium was decanted, reconstituted follicles were washed with PBS and resuspended with deionized water, the enclosed structure of follicles was destroyed and TG was released in deionized water. After the above series of manipulations were applied, pure TG solution without any contaminants was finally obtained, such as cell culture medium, cell secretion, and administrated drugs. We then detected concentration and iodination degree of these TG solution and used them to treat monolayer cells.

MEASUREMENT OF THE CONCENTRATION AND IODINATION DEGREE OF TG EXTRACTED FROM RECONSTITUTED FOLLICLES

To measure the degree of iodinated TG and detect TG concentration in the extraction, enzyme-linked immunosorbent assay (ELISA) was performed following the manufacturer's instructions (TG test kit, R&D company). TG concentration in the extraction was determined, and the quantity attracted to the ELISA tube by coated TG antibody was identified. RIA γ counter was used to determine γ counting in iodinated TG labeled with ^{125}I in the ELISA tube. TG iodination degree was defined as γ counting (cpm)/TG (μg).

MONOLAYER CELLS TREATED WITH IODINATED TG EXTRACTED FROM RECONSTITUTED FOLLICLES

Iodinated TG was prepared by extraction from different groups of reconstituted follicles (for details on extraction and measurement method, see Measurement of the Concentration and Iodination Degree of TG Extracted From Reconstituted Follicles Section and Monolayer Cells Treated With Iodinated TG Extracted From Reconstituted Follicles Section). The degree of iodination of TG from HI groups was considerably higher compared with that from HI + MMI groups. These high and low degree of iodination of TG solution were then used to treat monolayer cells respectively, for 3 days at a final concentration of 500 μg/ml.

LASER CONFOCAL MICROSCOPY AND PHASE-CONTRAST MICROSCOPY OBSERVATION

Thyrocytes were attached to cover slips coated with 1% rat tail collagen. On the third day after seeding, cells were observed under phase-contrast microscopy to observe follicular structure. For laser confocal microscopy, cells were fixed with 4% paraformaldehyde for 15 min and permeabilized using 0.25% Triton X-100 for 15 min. Subsequently, they were incubated with primary monoclonal antibody of GAPDH generated in rabbits (Beijing Biosynthesis Biotechnology Co., Ltd.) in a humidified chamber for 2 h at 37°C. Immune complexes were detected with fluorescein isothiocyanate (FRITC)-labeled anti-rabbit monoclonal antibody.

WESTERN BLOTTING ANALYSIS

Cell lysates were centrifuged at 12,000*g* for 30 min. Protein concentration was determined by BCA assay (Sigma). Cell lysates were separated electrophoretically in 12% polyacrylamide gels and transferred onto PVDF membranes (Beijing Biosynthesis Biotech-

TABLE I. Sequence of the Primer and Amplified products

gene	Sequence of the primer (5′ → 3′)	Amplified products (bp)
TSHr	Forward: CTTTTACGCCCTCTCAGCAC Reverse: GAGCCTGGCGTTTACAGAAG	188
NIS	Forward: AGTGATGCTGACGGTTTCTGGGTT Reverse: AGGTTGATCCGGAGTGGTTCTT	105
TPO	Forward: TTCGCCTGCATCATCGGAAAG Reverse: CAGATGACGCGGGACATAGAGTG	131
TG	Forward: TCGCTTCCTCCTTCACTGAGACCA Reverse: CAAACCGCTCCACTTCGCACTTC	120
GAPDH	Forward: GAAGGTCGGAGTGAACGGAT Reverse: CATGGGTAGAATCATACTGGAACA	200

nology Co., Ltd.). Membranes were blocked for 1.5 h at room temperature in 5% nonfat milk and incubated overnight at 4°C with thyrotropin receptor (TSHr) antibodies (Beijing Biosynthesis Biotechnology Co., Ltd.). After being washed thrice in Tris-buffered saline Tween-20 (TBST) for 30 min, the membranes were incubated with horseradish peroxidase-conjugated secondary antibodies for 45 min. Afterward, they again were washed thrice in TBST.

Immunoreactive bands were revealed using an enhanced chemiluminescence detection system. Meanwhile, 2% BSA was used in the negative group instead of the primary antibodies. GAPDH (Beijing Biosynthesis Biotechnology Co., Ltd.) and internal control were detected. The X-ray film was scanned, and band density was calculated using ImageJ software (Sheffield, 2007).

PREPARATION OF TOTAL RNA AND FLUORESCENT QUANTITATIVE REAL-TIME RT-PCR

Total RNA was isolated from cells using RNAiso Plus Kit (TaKaRa). RNA precipitate was dissolved in 10–15 μl of RNAse-free water and analyzed for quantity and quality using a spectrophotometer. Total RNA integrity was determined through 1% formaldehyde agarose gel electrophoresis. A two-step reverse transcription-PCR procedure was performed using the PrimeScriptTM RT Reagent Kit (TaKaRa) following the manufacturer's instructions. cDNA was then used in real-time PCR. For PCR amplification, 2 μl cDNA was used in a 20 μl reaction mixture. PCR primers were used as follows (Table I).

Hot-start real-time PCR was initiated at 95°C for 30 s. Mixtures were subjected to 40 cycles of a two-step PCR, comprising 5 s

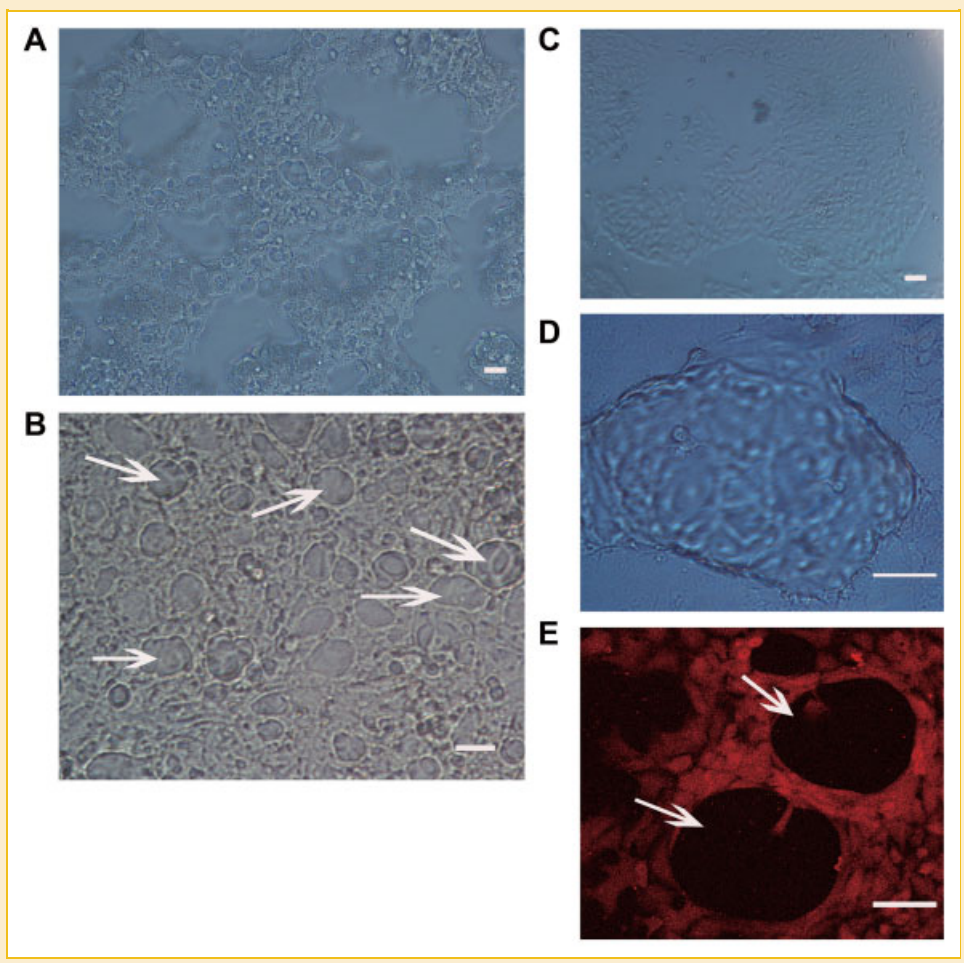

Fig. 1. Morphological observation of reconstituted thyroid follicles are shown. Reconstituted thyroid follicles are presented in hemisphere and spherical shape (A,B,D). The follicular lumen of spherical shaped reconstituted follicles are displayed (E); monolayer cells are shown (C). A, B, C, and D are obtained by phase contrast microscope with amplification times of 10 × 10 or 10 × 20, and E is obtained by laser confocal microscope with amplification times of 10 × 20 (bar, 50 μm). Arrow indicates thyroid follicles.

denaturation at 95°C and 30 s annealing/elongation phase at 60°C. GAPDH was amplified as internal control. Data were analyzed using the relative expression software.

STATISTICAL ANALYSIS

Statistical differences were determined using Student's *t*-tests. A *P*-value of <0.05 was considered significant.

RESULTS

MORPHOLOGY OBSERVER OF RECONSTITUTED FOLLICLES AND MONOLAYER CELLS

Two patterns of reconstituted follicles were observed under phase-contrast microscope. One was hemispherical in shape, composed of scores of cells (Fig. 1A and B), while the other was spherical (Fig. 1D), whose follicular lumen was clearly displayed when observed under laser confocal scanning microscopy (Fig. 1E). Meanwhile, the monolayer growth cells did not exhibit any of these morphological characteristics (Fig. 1C).

DIFFERENT DEGREES OF TG IODINATION IN VARIOUS TREATED RECONSTITUTED FOLLICLES

Although iodine concentration in HI + MMI group was the same as that in group HI, its MMI inhibited TPO activity, resulting in the suppression of organification reaction. Therefore, degree of TG iodination in HI + MMI group was significantly lower compared with that in group HI (Fig. 2A).

EXPRESSION OF THYROID-RESTRICTED GENES IN RECONSTITUTED FOLLICLES

Biosynthesis of thyroid hormone depends on the presence of sodium iodine symporter (NIS), TPO, and TG. In this research, fluorescence quantitative real-time PCR revealed that mRNA levels of NIS, TPO, and TG were all significantly lower in group HI compared with that in group HI + MMI. This suggests that expression of these genes was inhibited by high degree of TG iodination in follicular lumen (Fig. 2B–D).

EFFECTS OF IODINATED TG ON THYROID-RESTRICTED GENES IN MONOLAYER CELLS

Expressions of NIS, TPO, TG, and TSHr decreased when monolayer cells were treated with follicular iodinated TG from HI group, in

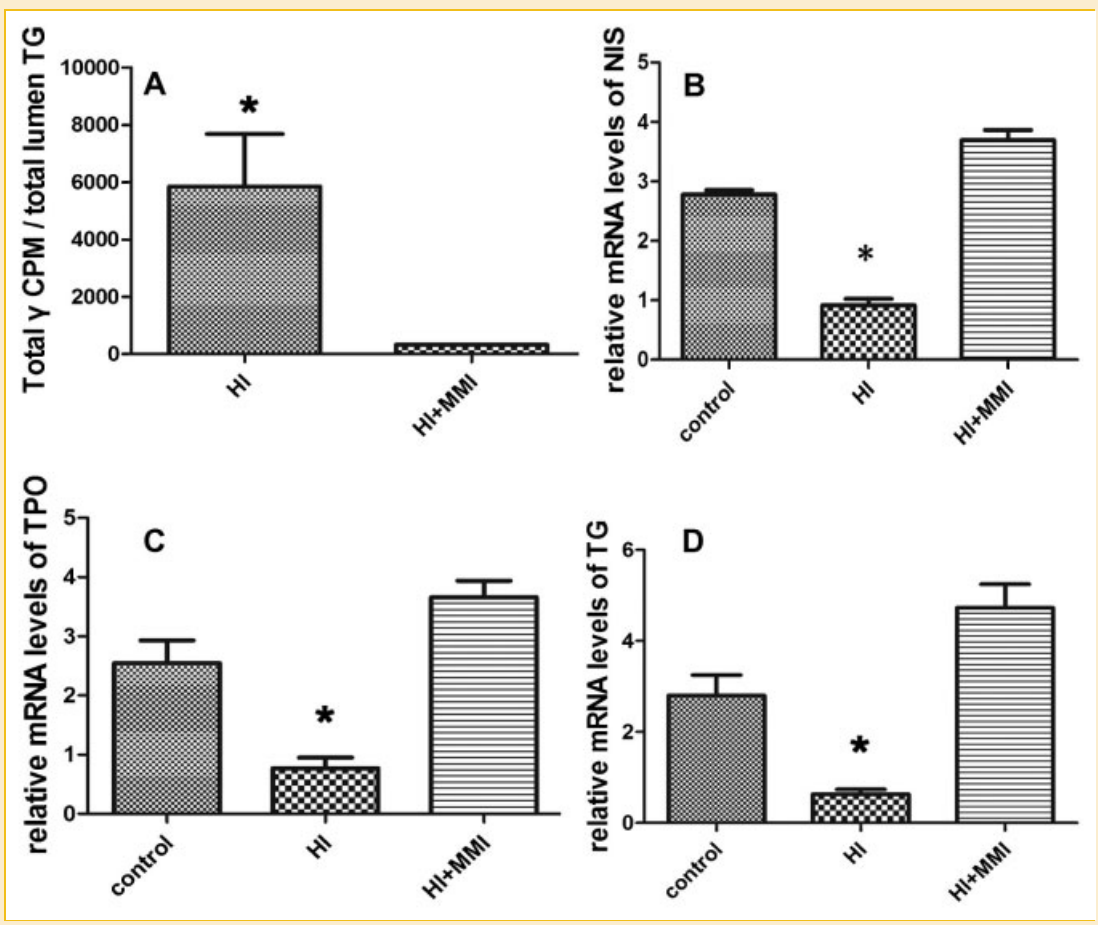

Fig. 2. Degree of TG iodination and expression of thyroid-restricted genes in all reconstituted follicles groups. Degree of TG iodination in HI group is significantly increased compared with that in HI + MMI group, as illustrated in (A). mRNA changes in NIS, TPO, and TG in all groups are determined by real-time quantitative PCR, and all these mRNA are significantly decreased in group HI compared with that in the other groups, as illustrated in B–D. (All mRNA are normalized to GAPDH, degree of TG iodination is expressed in γCPM/μg, and data are mean ± SD of three different experiments in triplicate. $^{*}P<0.05$ compared with HI + MMI and control groups.)

which the degree of TG iodination was higher compared with that in HI + MMI group. In this manner, it was further confirmed that the high degree of TG iodination in follicular lumen was responsible for inhibiting the expression of NIS, TPO, TG, and TSHr (Fig. 4).

EFFECT OF IODINATED TG ON TSHR BOTH AT MRNA AND PROTEIN LEVELS IN RECONSTITUTED FOLLICLES

Fluorescence quantitative real-time PCR and Western blot assays revealed that TSHr expression was significantly decreased in HI groups that underwent a high degree of iodinated TG in its follicular lumens. Therefore, high iodinated TG in follicles was relative to the down-regulation of TSHr (Fig. 3A,B). Time-dependent TSHr protein in HI + MMI group exhibited a gradual increase to the maximum at 3 days (Fig. 3C).

DISCUSSION

Thyroid follicle, the most basic unit of structure and function of thyroid gland is a spheroidal structure formed by polarized cells surrounding a closed lumen where iodinated TG accumulates. Primary pig thyrocytes can reconstitute into follicle-like structures in vitro, possessing most parameters characteristic of thyroid function. In research, they have been widely employed in regulating thyroid function (Mauchamp et al., 1998; Langer et al., 2003; Morand et al., 2003; Bernier-Valentin et al., 2006; Li et al., 2010). Taking advantage of these properties, a new mechanism of thyroid function regulated by follicular iodinated TG is provided, which was the main component of organic iodine. PBS with 0.02% EDTA was used in lieu of trypsin to disperse the follicle structure and prevent TG degradation when extracted from the lumen of reconstituted follicles. TG concentration was measured using ELISA, and iodine incorporation in TG was determined through detection of the I^{125} γ quantity on the wall of ELISA tube. Here, TG was attracted by coated TG antibody, allowing calculation of TG iodination degree. In this manner, TG could be extracted effectively, and its concentration and degree of TG iodination in follicular lumen could be determined for subsequent research.

After treating reconstituted thyroid follicles with a high dose of iodine (HI) for 3 days, expressions of NIS, TG, and TPO mRNA were observed to decrease significantly compared with the group treated with high doses of iodine and MMI (HI + MMI), an inhibitor of TPO activity; the latter could inhibit iodine organification. Thus, this study suggests that iodinated TG in follicular lumen inhibited the expression of these genes. This finding supports the idea that iodinated TG but not TG was responsible for regulating thyroid function, as previously suggested (Grollman et al., 1986).

To confirm this result, monolayer thyroid cells were treated with iodinated TG at the same concentration, but at a different iodinated degree. These iodinated TG were extracted from both HI and HI + MMI groups. Because the monolayer thyroid cells failed to form follicular lumen, the synthesized iodinated TG were secreted into the culture medium directly instead of being stored in follicular lumen. Interference of this part of iodinated TG could be eliminated for extremely low concentration due to dilution by culture medium. Expressions of NIS, TPO, TG, and TSHr were observed to be lower in

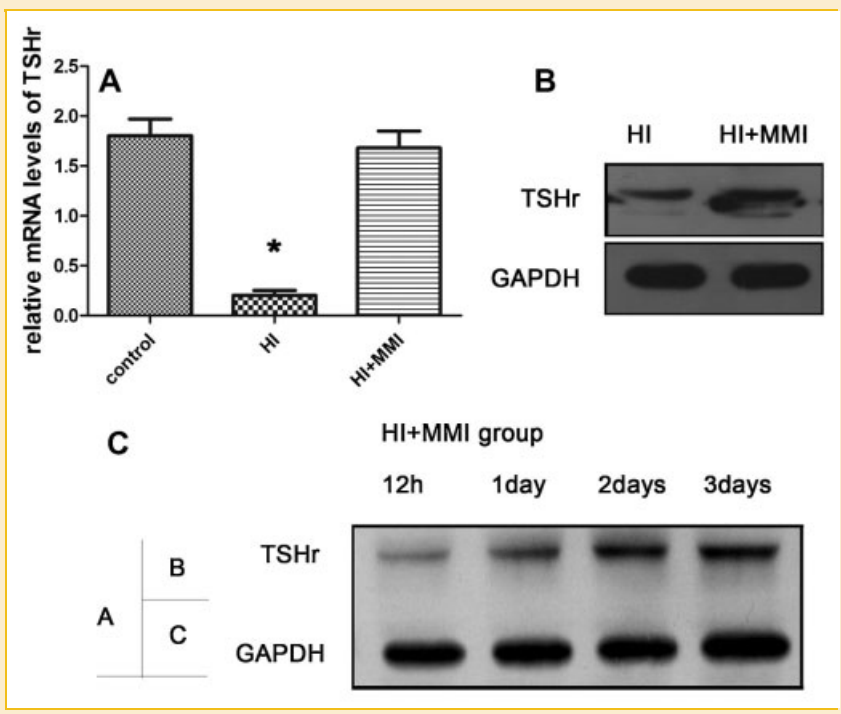

Fig. 3. Expression of both TSHr mRNA (A) and protein (B) levels in all groups and time-dependent TSHr protein expression in HI + MMI group (C) are shown. Both mRNA and protein of TSHr are significantly decreased in HI group compared with that in HI + MMI group (data are the mean ± SD of three different experiments in triplicate. $^{*}P < 0.05$ compared with group (HI + MMI) treated with 10 μmol/L NaI and 2 mmol/L MMI).

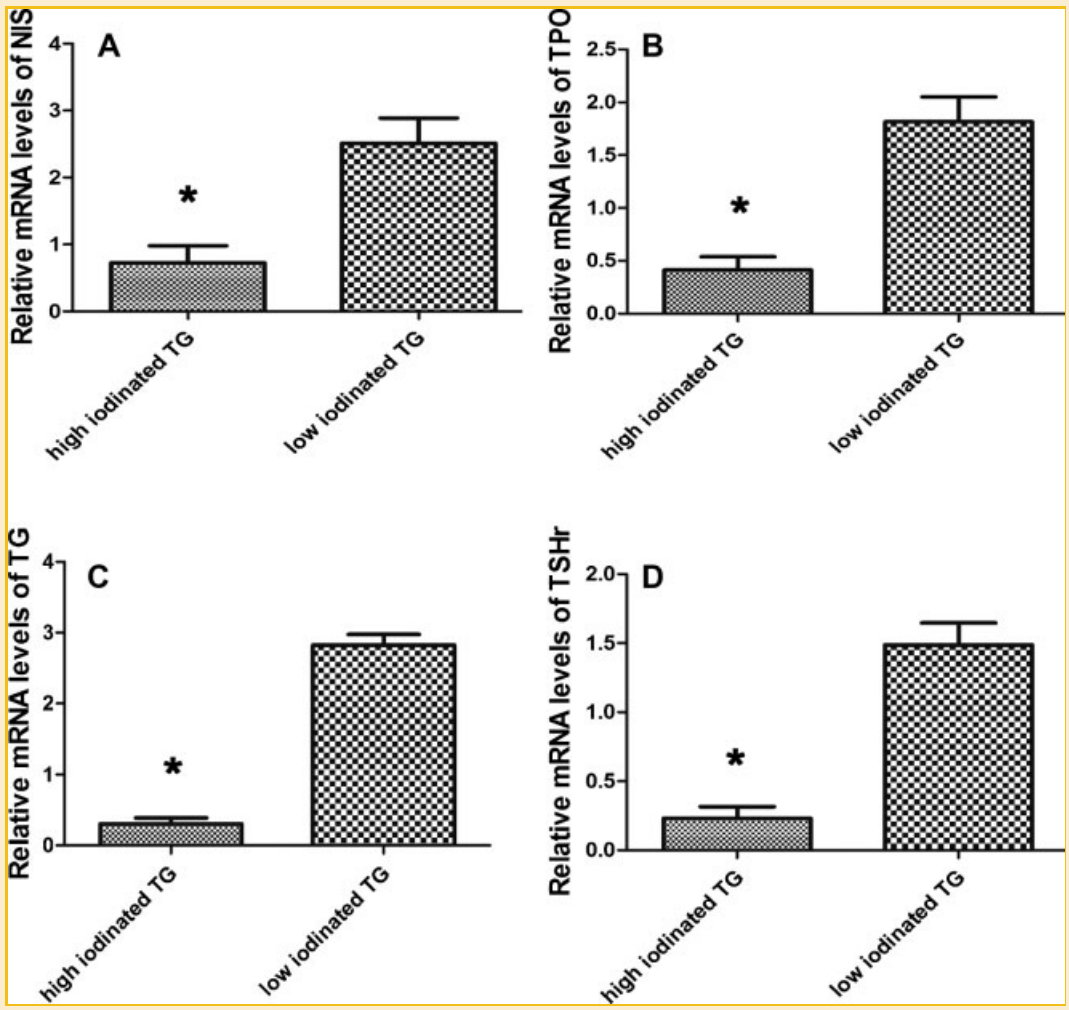

Fig. 4. Effects of iodinated TG on the expressions of thyroid-restricted genes in monolayer cells are shown. The mRNA of NIS, TPO, TG, and TSHr are all decreased when monolayer cells are treated with higher degree of iodinated TG. Results are respectively shown in A–D. (All mRNA were normalized to GAPDH. Data are the mean ± SD of three different experiments in triplicate. $^{*}P < 0.05$ compared with low iodinated TG group.)

the group treated with high degree of TG iodination compared with that treated with low degree of TG iodination. This finding provides evidence that iodinated TG in follicular lumen inhibited thyroid-restricted gene expression. Results were supported by data from previous studies on monolayer thyroid cell in vitro. These studies demonstrated that highly iodinated 27S TG was more effctive than low iodinated 19S and 12S TG on inhibition thyroid transcription factor-1 (TTF-1), which promoted the expressions of thyroid-restricted gene (Suzuki et al., 1999a,b,c; Suzuki and Kohn, 2006; Noguchi et al., 2010).

To determine how iodinated TG regulated thyroid function changes in TSHr at mRNA and protein levels were investigated when they were subjected to different degrees of TG iodination. TSHr expressions were observed to decrease noticeably when follicular lumen TG was highly iodinated. However, they significantly increased when follicular TG was low iodinated by blocking iodine organification with MMI. Therefore, it is concluded that iodinated TG, the main component of organic iodine in follicular lumen, was the main reason behind the suppression of TSHr expression.

TSHr located at the basement membrane of thyroid cells was a key protein for controlling thyroid function. Pituitary TSH positively regulated thyroid function by TSH/TSHr via cAMP, both PKA-dependent and -independent pathways (Levy et al., 1997; Saito et al., 1997; Kogai et al., 2000). In the group with high degree of TG iodination, TSHr expression decreased and resulted in low sensitivity of follicle to TSH. Thus, expressions of NIS, TPO, and TG were decreased under TSH stimulation.

Taken collectively, data in this study provided first-time evidence that iodinated TG but not TG in follicular lumen regulated thyroid function by inhibiting TSHr expression, which was responsible for the down-regulation of sensitivity of follicles to TSH. Finally, it decreased the expressions of NIS, TPO, and TG.

Likewise, findings partly clarified the mechanism of follicle heterogeneity, a phenomenon wherein all thyroid follicles in vivo are under identical conditions with the same plasma iodine and TSH concentration, despite differences in their volume and function (Ulianich et al., 1999; Suzuki et al., 1999a,b,c; Faggiano et al., 2004). The different degrees of TG iodination may lead to different follicle

sensitivity to TSH. This feedback regulation of iodinated TG on thyroid follicular function may be attributed to the heterogeneity of thyroid follicles in vivo.

Follicular iodinated TG is a glycoprotein with high molecular mass. It is incapable of passing through thyroid cells freely. Detailed mechanism of iodinated TG regulating the expression of thyroid-restrict genes remains unknown. It was reported that TG is capable of binding to the sialic acid receptor, which localizes at the follicular membrane (Lemansky and Herzog, 1992; Giraud et al., 1997; Ulianich et al., 1999). Whether the sialic acid receptor mediated the regulation of iodinated TG requires further research.

Recently, incidence of thyroid dysfunction and autoimmune thyroid disease caused by excessive iodine has increased dramatically (Laurberg et al., 2001; Teng et al., 2006). Findings on the regulation of iodinated TG may shed light on the pathogenesis of these thyroid diseases.

REFERENCES

Ambesi-Impiombato FS, Parks LA, Coon H. 1980. Culture of hormone-dependent functional epithelial cells from rat thyroids. Proc Natl Acad Sci USA 77:3455–3459.

Bernier-Valentin F, Trouttet-Masson S, Rabilloud R, Selmi-Ruby S, Rousset B. 2006. Three-dimensional organization of thyroid cells into follicle structures is a pivotal factor in the control of sodium/iodide symporter expression. Endocrinology 147:2035–2042.

Faggiano A, Coulot J, Bellon N, Talbot M, Caillou B, Ricard M, Bidart JM, Schlumberger M. 2004. Age-dependent variation of follicular size and expression of iodine transporters in human thyroid tissue. J Nucl Med 45:232–237.

Ferreira AC, Lima LP, Araujo RL, Muller G, Rocha RP, Rosenthal D, Carvalho DP. 2005. Rapid regulation of thyroid sodium–iodide symporter activity by thyrotrophin and iodine. J Endocrinol 184:69–76.

Giraud A, Siffroi S, Lanet J, Franc JL. 1997. Binding and internalization of thyroglobulin: Selectivity, pH dependence, and lack of tissue specificity. Endocrinology 138:2325–2332.

Grollman EF, Smolar A, Ommaya A, Tombaccini D, Santisteban P. 1986. Iodine suppression of iodide uptake in FRTL-5 thyroid cells. Endocrinology 118:2477–2482.

Kogai T, Curcio F, Hyman S, Cornford EM, Brent GA, Hershman JM. 2000. Induction of follicle formation in long-term cultured normal human thyroid cells treated with thyrotropin stimulates iodide uptake but not sodium/iodide symporter messenger RNA and protein expression. J Endocrinol 167:125–135.

Langer R, Burzler C, Bechtner G, Gartner R. 2003. Influence of iodide and iodolactones on thyroid apoptosis. Evidence that apoptosis induced by iodide is mediated by iodolactones in intact porcine thyroid follicles. Exp Clin Endocrinol Diab 111:325–329.

Laurberg P, Bulow Pedersen I, Knudsen N, Ovesen L, Andersen S. 2001. Environmental iodine intake affects the type of nonmalignant thyroid disease. Thyroid 11:457–469.

Lemansky P, Herzog V. 1992. Endocytosis of thyroglobulin is not mediated by mannose-6-phosphate receptors in thyrocytes. Evidence for low-affinity-binding sites operating in the uptake of thyroglobulin. Eur J Biochem 209:111–119.

Levy O, Dai G, Riedel C, Ginter CS, Paul EM, Lebowitz AN, Carrasco N. 1997. Characterization of the thyroid Na+/I symporter with an anti-COOH terminus antibody. Proc Natl Acad Sci USA 94:5568–5573.

Li H, Ganta S, Fong P. 2010. Altered ion transport by thyroid epithelia from CFTR−/− pigs suggests mechanisms for hypothyroidism in cystic fibrosis. Exp Physiol. 95:1132–1144.

Mauchamp J, Mirrione A, Alquier C, Andre F. 1998. Follicle-like structure and polarized monolayer: Role of the extracellular matrix on thyroid cell organization in primary culture. Biol Cell 90:369–380.

Morand S, Chaaraoui M, Kaniewski J, Deme D, Ohayon R, Noel-Hudson MS, Virion A, Dupuy C. 2003. Effect of iodide on nicotinamide adenine dinucleotide phosphate oxidase activity and Duox2 protein expression in isolated porcine thyroid follicles. Endocrinology 144:1241–1248.

Noguchi Y, Harii N, Giuliani C, Tatsuno I, Suzuki K, Kohn LD. 2010. Thyroglobulin (Tg) induces thyroid cell growth in a concentration-specific manner by a mechanism other than thyrotropin/cAMP stimulation. Biochem Biophys Res Commun 391:890–894.

Saito T, Endo T, Kawaguchi A, Ikeda M, Nakazato M, Kogai T, Onaya T. 1997. Increased expression of the Na+/I-symporter in cultured human thyroid cells exposed to thyrotropin and in Graves' thyroid tissue. J Clin Endocrinol Metab 82:3331–3336.

Sheffield JB. 2007. ImageJ, a useful tool for biological image processing and analysis. Microsc Microanal 13:200–201.

Suzuki K, Kohn LD. 2006. Differential regulation of apical and basal iodide transporters in the thyroid by thyroglobulin. J Endocrinol 189:247–255.

Suzuki K, Mori A, Lavaroni S, Miyagi E, Ulianich L, Katoh R, Kawaoi A, Kohn LD. 1999a. In vivo expression of thyroid transcription factor-1 RNA and its relation to thyroid function and follicular heterogeneity: Identification of follicular thyroglobulin as a feedback suppressor of thyroid transcription factor-1 RNA levels and thyroglobulin synthesis. Thyroid 9:319–331.

Suzuki K, Mori A, Lavaroni S, Ulianich L, Miyagi E, Saito J, Nakazato M, Pietrarelli M, Shafran N, Grassadonia A, Kim W.B, Consiglio E, Formisano S, Kohn LD. 1999b. Thyroglobulin regulates follicular function and heterogeneity by suppressing thyroid-specific gene expression. Biochimie 81:329–340.

Suzuki K, Mori A, Saito J, Moriyama E, Ullianich L, Kohn LD. 1999c. Follicular thyroglobulin suppresses iodide uptake by suppressing expression of the sodium/iodide symporter gene. Endocrinology 140:5422–5430.

Takasu N, Ohno S, Komiya I, Yamada T. 1992. Requirements of follicle structure for thyroid hormone synthesis; Cytoskeletons and iodine metabolism in polarized monolayer cells on collagen gel and in double layered, follicle-forming cells. Endocrinology 131:1143–1148.

Teng W, Shan Z, Teng X, Guan H, Li Y, Teng D, Jin Y, Yu X, Fan C, Chong W, et al. 2006. Effect of iodine intake on thyroid diseases in China. N Engl J Med 354:2783–2793.

Ulianich L, Suzuki K, Mori A, Nakazato M, Pietrarelli M, Goldsmith P, Pacifico F, Consiglio E, Formisano S, Kohn LD. 1999. Follicular thyroglobulin (TG) suppression of thyroid-restricted genes involves the apical membrane asialoglycoprotein receptor and TG phosphorylation. J Biol Chem 274: 25099–25107.