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Excess iodine promotes apoptosis of thyroid follicular epithelial cells by inducing autophagy suppression and is associated with Hashimoto thyroiditis disease

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ABSTRACT

The incidence of the autoimmune thyroid disease Hashimoto thyroiditis (HT) has increased in recent years, and increasing evidence supports the contribution of excess iodine intake to thyroid disease. In this study, we examined the status of autophagy and apoptosis in thyroid tissues obtained from patients with HT, and we determined the effects of excessive iodine on the autophagy and apoptosis of thyroid follicular cells (TFCs) in an attempt to elucidate the effects of excess iodine on HT development. Our results showed decreases in the autophagy-related protein LC3B-II, and increases in caspase-3 were observed in thyroid tissues from HT patients. Interestingly, the suppression of autophagy activity in TFCs was induced by excess iodine *in vitro*, and this process is mediated through transforming growth factor-β1 downregulation and activation of the Akt/mTOR signaling pathway. In addition, excess iodine induced autophagy suppression and enhanced reactive oxygen species (ROS) production and apoptosis of TFCs, which could be rescued by the activation of autophagy. Taken together, our results demonstrated that excess iodine contributed to autophagy suppression and apoptosis of TFCs, which could be important factors predisposing to increased risk of HT development.

1. Introduction

Iodine is an essential microelement necessary to synthesize thyroid hormones, which play important roles in human development. Iodine deficiency is often the underlying reason for developmental delays, endemic goiter, and other issues [1]. To prevent iodine deficiency disorders, salt iodization has been implemented in many countries. However, according to epidemiological investigations, excess iodine intake could lead to hypothyroidism, hyperthyroidism, and thyroid cancer, among others [2,3]. For example, excess iodine has been linked to increased subclinical hypothyroidism and autoimmune thyroiditis [4]. The NOD.H-2h4 mouse is widely used as an autoimmune thyroiditis-prone non-obese diabetic animal model to study Hashimoto thyroiditis (HT), and the results from these mice indicate that excess iodine affects the autoimmunity of the thyroid [5].

HT, a chronic thyroid inflammation, is a very common organ-specific autoimmune disease that is pathologically characterized by varying degrees of lymphocytic infiltration, obliteration of thyroid follicles, and elevated thyroid antibody concentrations [6,7]. In general, moderate iodine supplementation helps prevent thyroid diseases; however, excess iodine plays a role in the genesis of autoimmune thyroiditis in humans [8]. Although the pathogenesis of HT remains unclear, initiation of iodine-induced autoimmune thyroiditis in NOD.H-2h4 mice has shown explicit evidence that iodine excess is associated with the autoimmunity of the thyroid

Abbreviations: HT, Hashimoto thyroiditis; TFCs, thyroid follicular cells; NAC, *N*-acetyl cysteine; FCM, flow cytometry; IHC, immunohistochemistry.

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gland. Excess iodine induces thyroid follicular cell (TFC) injury, apoptosis, and necrosis and precedes infiltration of lymphocytes and production of unbalanced redundant reactive oxygen species (ROS) [9–11].

A number of works in recent years indicate that ROS production correlates with the occurrence of autophagy in a more intimate and coordinated manner than by a simple ON/OFF signal [12]. In some studies, ROS can induce autophagy by stimulating the proteolytic activity of ATG4 and other mechanisms [13,14]. In addition, autophagy has been shown to protect cells from DNA damage by reducing mitochondrial ROS production [15]. A previous study has demonstrated that autophagy protects chondrocytes from apoptosis by decreasing the ROS level in chondrocytes [16]. It is noteworthy that inhibition of autophagy may result in a bioenergetic shortage and favor oxidative reactions that trigger apoptosis [17]. Our pilot studies have also shown that decreased autophagy and enhanced apoptosis levels can be observed in thyroid tissues of HT patients, and that excess iodine could induce autophagy suppression in TFCs. Until now, there had been no research performed to determine whether iodine concentration could affect the autophagy activity of TFCs. In the present study, we found that excess iodine induced ROS-dependent apoptosis of TFCs and inhibited autophagy in a transforming growth factor (TGF)-β1-dependent manner promote apoptosis of TFCs. These results suggest that excess iodine serves as a pathogenic factor in HT development and that proper iodine administration is important to protect against autoimmune thyroiditis development.

2. Materials and methods

2.1. Cell culture and samples

Thyroid cell line (human thyroid follicular epithelial) Nthy-ori 3–1 from the European Collection of Animal Cell Cultures was cultivated in RPMI-1640 (Gibco, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum and 2 mM L-glutamine (Biological Industries, Kibbutz Beit Haemek, Israel). Thyroid glands were obtained from 10 patients with HT who underwent thyroidectomy. HT diagnosis was made based on clinical evaluations and Japanese guidelines as described previously [18,19]. Thyroid tissues from 5 patients with a simple goiter were used as controls based on clinical evaluations and laboratory findings. All samples were obtained in accordance with the regulations and approval of the Institutional Review Board of the Affiliated Hospital of Jiangsu University; for all cases, written informed consent was obtained from the patients. This study was approved by the Ethics Committee of the Affiliated Hospital of Jiangsu University and conducted in accordance with the guidelines of the Declaration of Helsinki.

2.2. Cell treatments

2.2.1. Potassium iodine

High-concentration iodine treatment was performed by diluting a 1-M stock solution of potassium iodide (KI) with the medium to a final concentration of 0.1–50 mM. Nthy-ori 3–1 cells were treated with 0.1–50 mM KI for 12 h. The control group included cells without any treatment.

2.2.2. Rapamycin and N-acetyl cysteine

Autophagy activation and oxidation resistance studies were performed by adding 10 nM rapamycin and 8 nM *N*-acetyl cysteine (NAC) to the culture medium. Rapamycin and NAC were purchased from Sigma and Beyotime Institute of Biotechnology, respectively. Control cells were treated with the vehicle.

2.2.3. Recombinant TGF-β1

Nthy-ori 3–1 cells were treated for 12 h with exogenous recombinant TGF-β1 (Peprotech, Rocky Hill, NJ, USA) in the culture medium at a dose of 10 ng/mL.

2.3. Reagents and antibodies

The following primary antibodies were used: rabbit anti-human LC3B-II, rabbit anti-human caspase-3, rabbit anti-human mTOR, rabbit anti-human *p*-mTOR, rabbit anti-human Akt, rabbit anti-human *p*-Akt, rabbit *anti*-β-actin (Cell Signaling Technology, Danvers, MA, USA), mouse anti-human TGF-β1 (R&D Systems, Minneapolis, MN, USA), mouse *anti*-β-actin, goat anti-rabbit IgG-HRP, and goat anti-mouse IgG-HRP (Santa Cruz, Santa Cruz, CA, USA).

2.4. Immunohistochemistry

Samples were fixed in 10% neutralized formalin, embedded in paraffin, cut into 4-μm sections, and mounted on slides. After deparaffinization and rehydration, antigen retrieval was performed by boiling samples in 10 mmol/L citrate buffer (pH 6.0) for 10 min and then washing the slides with phosphate-buffered saline (PBS). Sections were blocked with 2% bovine serum albumin in PBS for 30 min and then incubated with rabbit anti-human LC3B-II, caspase-3, mTOR, and TGF-β1 antibodies overnight at 4 °C. After three washes with PBS, the sections were treated with the corresponding streptavidin peroxidase–conjugated secondary antibody (Maixin Biotechnology Co., Ltd.). Tissue sections were then counterstained with 3,3′-diaminobenzidine and hematoxylin and observed under an optical microscope. The results of quantitative analyses of all samples by Image-Pro plus 6.0 software were presented graphically.

2.5. Immunoblot analysis

Total protein was extracted from cell lines using a whole-cell extraction kit (Merck Millipore, Billerica, MA, USA). Protein concentration was determined using a BCA protein concentration kit (Beyotime, Shanghai, China). First, 5 μg of protein was subjected to electrophoresis on 10%–15% acrylamide gel by SDS-PAGE and then transferred onto a polyvinylidenedifluoride membrane (Merck Millipore, Billerica, MA, USA) by electrophoresis. After blocking for 1 h in 5% bovine serum albumin, the membranes were incubated with antibodies against proteins or β-actin (standard controls), followed by HRP-conjugated secondary antibodies. The signals were detected using the Pierce ECL-plus substrate (Thermo Fisher Scientific, Waltham, MA, USA) and scanned with a Fluor Chem FC3 camera system (Protein-Simple, California, USA). Images were analyzed using Alpha View software (AIC, California, USA), and the results of quantitative analyses were presented graphically.

2.6. Autophagy determination

Nthy-ori 3–1 cells were infected with adenoviral-expressing mRFP-GFP-LC3 (Hanbio Biotechnology, Shanghai, China) at a multiplicity of infection of 200 for approximately 2 h. To quantify the number of puncta, mRFP-GFP-LC3B–transfected cells were seeded in a culture plate with 24 wells 1 day before treatment. Images were then recorded by fluorescence microscopy (Olympus). The number of puncta per cell was determined using Image-Pro plus 6.0. (Version X; Media Cybernetics, Silver Springs, MD, USA) More than 10 cells were analyzed for each condition.

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2.7. Measurement of apoptosis by flow cytometry

Apoptosis was measured by flow cytometry (FCM) according to the protocol of the annexinV-FITC/7-AAD kit (BD Biosciences, San Jose, CA, USA). When the cells had grown to 80% confluence after various treatments for 12 h, Nthy-ori 3–1 cells were collected and washed with PBS, gently resuspended in binding buffer, and incubated with annexin V/7-AAD. FCM analysis was performed using cell quest software (FACS Calibur; BD Biosciences, San Jose, CA, USA). For each sample, 10^4 cells were analyzed, and the results were presented as means ± SD of three independent experiments.

2.8. ROS analysis

Levels of intracellular ROS were evaluated using a commercial kit (Beyotime Biotechnology, Shanghai, China). Cells were cultured and treated with KI and/or rapamycin for 6 h and then incubated with serum-free medium containing dichlorofluorescein diacetate for 20 min at 37 °C. After removal of excess dichlorofluorescin diacetate by washing, the cells were fixed with 4% paraformaldehyde and sections were imaged via fluorescence microscopy. Green fluorescence represented the amounts of ROS and fluorescence intensity was analyzed with Image-Pro plus 6.0 software.

2.9. Statistical analyses

Statistical analyses were performed using GraphPad Prism5 software (GraphPad Software, Inc., San Diego, CA, USA). Descriptive data were expressed as mean ± standard deviation (SD), and numerical data between groups were compared using the homogeneity of variance test or Wilcoxon Mann-Whitney test, as appropriate. Differences in the mean values of various groups were analyzed by using one-way ANOVA with the Tukey–Kramer multiple comparison tests. $P < 0.05$ was considered statistically significant.

3. Results

3.1. Decreased autophagy and enhanced apoptosis in thyroid tissues from HT patients

To evaluate the autophagy status in thyroid tissues of HT patients (n = 10), the expression of autophagy-related proteins, LC3B-II and mTOR, were detected by immunohistochemistry (IHC) analysis. The results indicated that HT tissues expressed low levels of LC3B-II and high levels of mTOR. In contrast, healthy control tissues (n = 5) expressed high levels of LC3B-II and low levels of mTOR. We also examined the expression of caspase-3, an indicator of apoptosis, in HT and control thyroid tissues and found that caspase-3 levels were upregulated in HT tissues (n = 10) as compared with those in controls (n = 5). Taken together, our results suggest the occurrence of reduced autophagy and increased apoptosis in the thyroid tissues of HT patients (Fig. 1A). The results of IHC quantification from all samples are shown in Fig. 1B.

3.2. Autophagy activity of TFCs suppressed by excess iodine

It is known that a proper dose of iodine is essential and critical for the health of thyroid tissues; however, too much or too little iodine may cause thyroid dysfunction and pathology such as HT. However, the molecular mechanisms regarding how iodine regulates TFC function and the pathogenesis of HT have not been elucidated. By using KI as the source of the iodine, we assessed the effect of excess iodine on the function of TFCs. Nthy-ori 3–1 cells, a human immortalized thyroid follicular cell line, were treated with different concentration of KI, and the expression of LC3B-II was detected by immunoblot analysis. The results showed that LC3B-II levels significantly decreased in KI-treated Nthy-ori 3–1 cells in a concentration-dependent manner ($P < 0.01$; Fig. 2A). To further confirm the inhibitory effect of excess iodine in autophagy, Nthy-ori 3–1 cells were infected by adenovirals expressing mRFP-GFP-LC3B and the autophagic flux status was analyzed. The GFP signal can be quenched in a lysosomal environment; in contrast, the RFP signal is more stable in an acidic environment [20]. Therefore, autophagosomes and autolysosomes are labeled with yellow (green and red) or red, respectively. By detecting and analyzing these two different fluorescent signals, the autophagic flux can be monitored. The results showed that the numbers of yellow and red puncta in the cells were significantly increased after treatment with rapamycin (an inhibitor of mTOR that positively regulates autophagy). However, the formation of fluorescent puncta was significantly decreased in the cells after treatment with KI as compared with the non-treated cells (Fig. 2B). Taken together, these findings suggested that excess iodine exposure in the thyroid could suppress autophagy activity in TFCs.

3.3. TGF-β1/Akt/mTOR signaling contributes to iodine-induced autophagy suppression

To further determine the mechanism of KI-induced autophagy suppression, Akt/mTOR signaling, an important regulatory pathway of autophagy, was analyzed after KI treatment in Nthy-ori 3–1 cells. Immunoblot analysis was performed to examine the protein expression levels of phosphorylated and total Akt and mTOR in KI-treated cells. The results showed that KI could induce the phosphorylation of Akt and mTOR in Nthy-ori 3–1 cells, and that the phosphorylated protein levels increased along with the increased concentration of KI (Fig. 3A). These findings suggest that excess iodine could activate Akt/mTOR signaling pathway, which may contribute to the autophagy suppression in TFCs.

TGF-β1 has been reported to be an autophagy inducer by inhibiting Akt/mTOR signaling [21]. Interestingly, we found that the TGF-β1 expression in Nthy-ori 3–1 cells was very sensitive to iodine exposure, which significantly inhibits TGF-β1 protein levels after treatment with KI, even under very low concentration (Fig. 3A). Therefore, we speculate that TGF-β1 may be the reason for KI-induced autophagy suppression in TFCs. To test this hypothesis, we examined the possible involvement of TGF-β1 in KI-induced autophagy suppression by using recombinant TGF-β1. Immunoblot results showed obvious inhibition of phosphorylation of Akt and mTOR and increased expression of LC3B-II in the presence of TGF-β1 in Nthy-ori 3–1 cells. In addition, TGF-β1 reversed the suppressive effect of Akt/mTOR phosphorylation and LC3B-II expression induced by KI ($P < 0.001$; Fig. 3B). More importantly, IHC analysis showed that the expression of TGF-β1 in HT thyroid tissues from 10 patients was much lower than that in control thyroid tissues (n = 5). The results of IHC quantification from all samples is shown in Fig. 3C. Taken together, these findings suggest that excess iodine could inhibit TGF-β1 production, which may contribute to the autophagy suppression in TFCs and the pathogenesis of HT.

3.4. Excess iodine enhanced ROS production and apoptosis of TFCs

As shown in Fig. 1, caspase-3 expression is significantly upregulated in HT thyroid tissues; therefore, we tried to determine whether iodine could induce apoptosis in Nthy-ori 3–1 cells. Nthy-ori 3–1 cells were incubated with different concentrations of KI for 12 h, and apoptosis rates were measured by FCM analysis based on annexin V and 7-AAD staining. The results showed that KI

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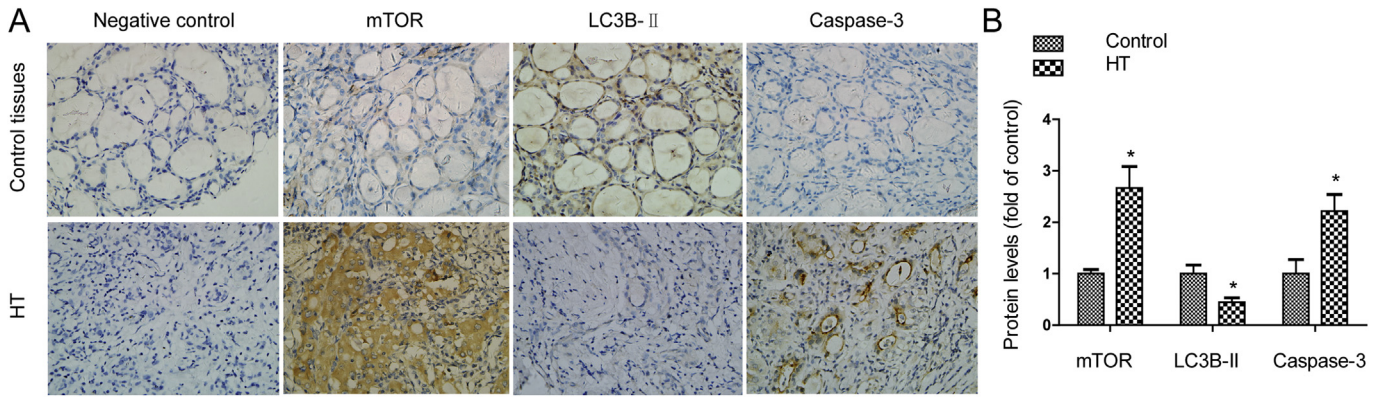

Fig. 1. mTOR, LC3B-II, and caspase-3 expression in Hashimoto thyroiditis (HT) and control tissues. (A) Representative results of immunohistochemical staining for mTOR, LC3B-II, and caspase-3 in HT tissue (n = 10) and control tissue (n = 5) are shown. "Control" indicates tissues from patients with simple goiter of the thyroid. Brown regions represent positive expression (original magnification, 200×). (B) The results of immunohistochemistry (IHC) quantification from all samples are shown. Significant differences and *P* values were calculated by the Mann-Whitney *U* tests. $^{*}P < 0.05$ *vs.* controls.

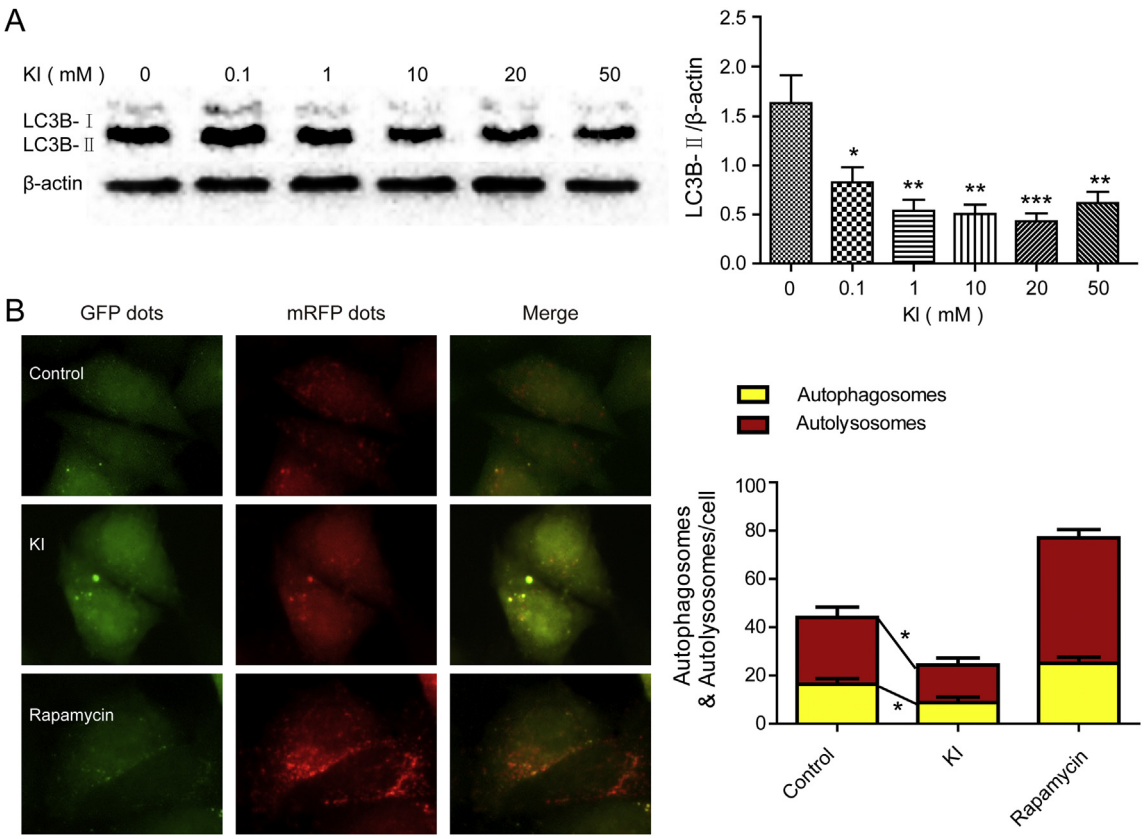

Fig. 2. Effects of potassium iodide (KI) exposure on the expression of LC3B-II in Nthy-ori 3–1 cells. Nthy-ori 3–1 cells were treated with gradient concentrations of KI. (A) Cell lysates were harvested after 12 h of KI treatment. The images presented are immunoblots (left upper panel) probed for LC3B-II (β-actin served as the loading control). The mean values of all three replicates are shown (right upper panel). (B) Representative fluorescent images of Nthy-ori 3–1 cells transiently transfected with mRFP-GFP-LC3; the cells were left non-treated, treated with rapamycin, or treated with KI. Images and quantification of mean red and green fluorescent puncta are shown in the left and right lower panels, respectively (original magnification, 400 ×). The results shown are representative of three replicates. Significant differences and *P* values were calculated by the one-way ANOVA. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* controls.

treatment induced remarkable increased apoptosis of Nthy-ori 3–1 cells in a dose-dependent manner (Fig. 4A). In addition, exposure of Nthy-ori 3–1 cells to KI (10 mM) led to a notable increase in intracellular ROS as compared with the control, and either mTOR inhibitor rapamycin (10 nM) or ROS inhibitor NAC (8 mM) reversed KI-induced ROS production (Fig. 4B). Consistently, KI-induced apoptosis of TFCs was further suppressed by rapamycin or NAC (Fig. 4C), suggesting that excess iodine-induced ROS production may be the reason for the apoptosis of TFCs and that the activation of autophagy could rescue cells from ROS-dependent apoptosis.

4. Discussion

Adding iodine to salt is an important component of preventive health care aiming to reduce the prevalence of thyroid disorders

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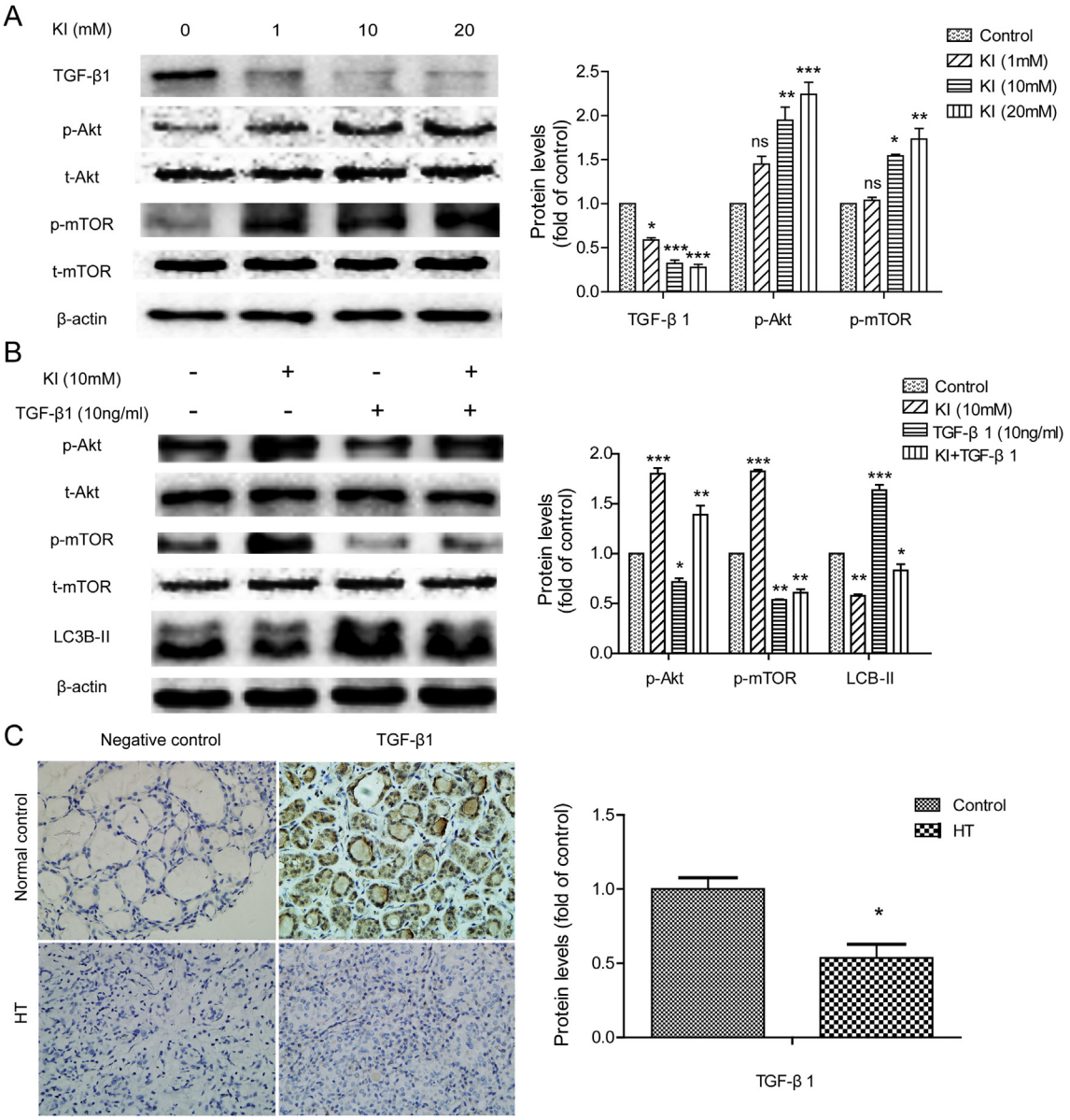

Fig. 3. Excess iodine-induced autophagy suppression in Nthy-ori 3–1 cells through the transforming growth factor (TGF)-β1/Akt/mTOR signaling axis. (A) Nthy-ori 3–1 cells were incubated with gradient concentrations of potassium iodide (KI) for 12 h, and proteins were harvested for Western blot analysis. The images presented are immunoblots (left upper panel) probed for TGF-β1, *p*-mTOR, mTOR, *p*-Akt, and Akt expression (β-actin served as the loading control). The results shown are representative of three replicates (right upper panel). (B) Nthy-ori 3–1 cells were treated with 10 mM KI, 10 ng/ml TGF-β1, or KI plus TGF-β1 for 12 h. The images presented are immunoblots (left middle panel) probed for *p*-mTOR, mTOR, *p*-Akt, Akt, and LC3B-II expression (β-actin served as the loading control). The results shown are representative of three replicates (right middle panel). (C) Representative images of the expressions of TGF-β1 in Hashimoto thyroiditis (HT) and control tissues were measured by immunohistochemical staining (left lower panel). Brown regions represent positive expression of TGF-β1 (original magnification, 200×). The results of immunohistochemistry (IHC) quantification from all samples of 5 healthy controls and 10 patients are shown in the right lower panel. Significant differences and *P* values were calculated by the one-way ANOVA and the Mann-Whitney *U* tests. $^{*}P < 0.05$, $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* controls.

because the strategy is simple, effective, safe, and inexpensive [22]. According to epidemiological investigations, however, levels of iodine intake are highly correlated with thyroid diseases, such as goiter and nodularity, hyperthyroidism, hypothyroidism, thyroid autoimmunity, and thyroid cancer [23]. Some studies have noted that increased concentrations of serum thyroid antibodies were detected in people with excessive iodine intake; for example, rapid increases in iodine intake promote the risk of thyroid autoimmunity and HT is more common after salt iodization than before [24–26]. However, the underlying molecular mechanism of thyroid disorders induced by inappropriate iodine intake remains unclear.

Autophagy is a critical pathway that leads to programmed cell death in a number of circumstances [27]. It is an essential and homeostatic process by which cells break down their own components, thus avoiding necrosis-induced detrimental effects. Several researchers have revealed the crucial role of the autophagy pathway and its related proteins in immunity and inflammation. The autophagy process balances the beneficial and detrimental effects of immunity and inflammation and may protect against the development of infectious, autoimmune, and inflammatory diseases [28]. In this study, we detected autophagy-related proteins (i.e., LC3B) in the thyroid tissues of patients with thyroid disorders and found that autophagy activities were decreased and apoptosis levels were increased in HT patients. Recently, it has been reported

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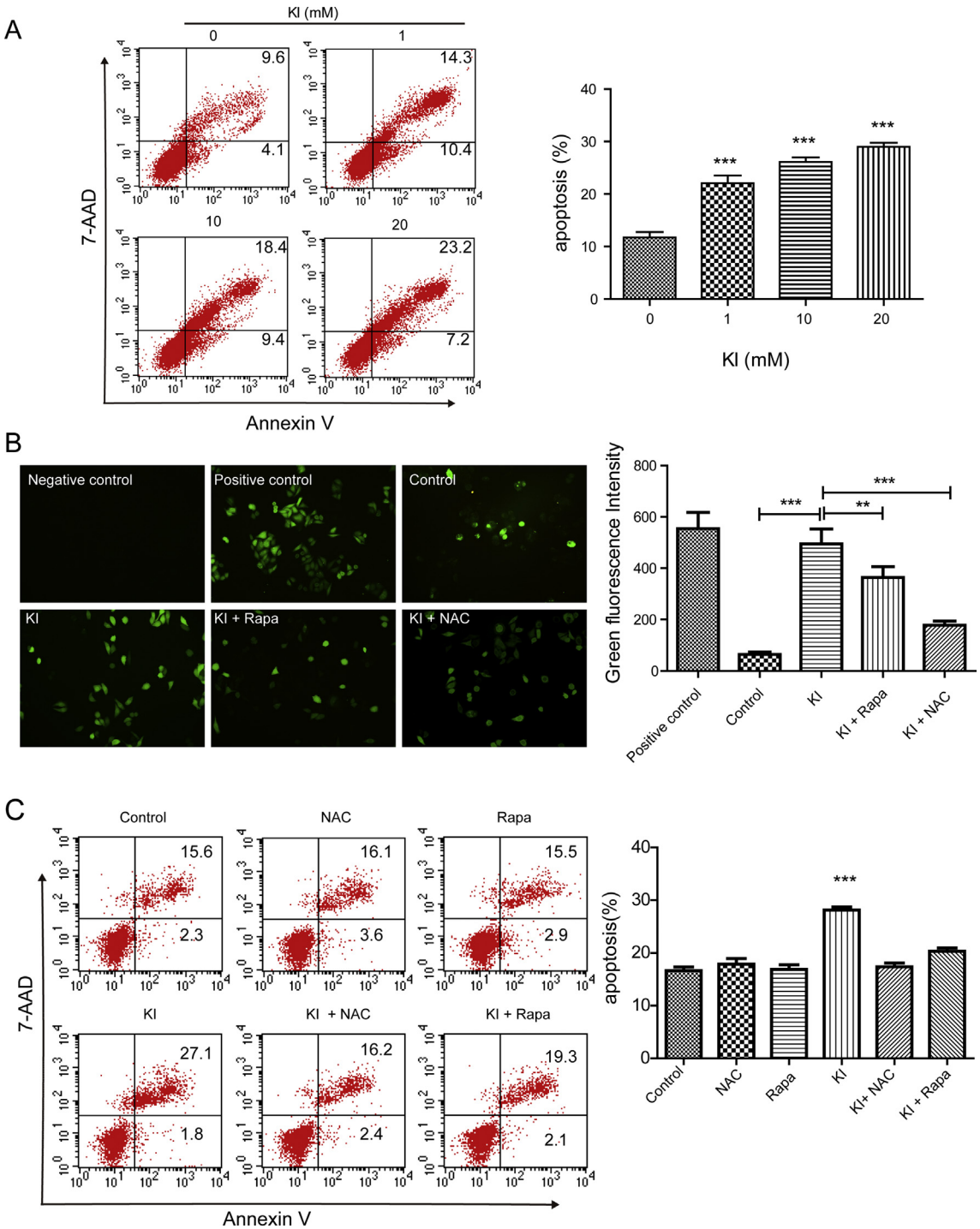

Fig. 4. Excess iodine promotes apoptosis of thyroid follicular cells (TFCs) with increased reactive oxygen species (ROS) production. Nthy-ori 3–1 cells were seeded onto 24-well plates at a density of 1×10^5 cells/well. (A) Nthy-ori 3–1 cells were treated with gradient concentrations of potassium iodide (KI) for 12 h. The percentage of apoptosis of TFCs was measured by flow cytometry (FCM) and the results are shown. (B) Nthy-ori 3–1 cells were pretreated with rapamycin (10 nM) or *N*-acetyl cysteine (NAC; 8 mM) for 2 h in the presence or absence of KI (10 mM) for 6 h. Images and quantification of mean green fluorescent are shown in the left and right lower panels, respectively (original magnification, 200×). (C) Nthy-ori 3–1 cells were pretreated with rapamycin (10 nM) or NAC (8 mM) for 2 h in the presence or absence of KI (10 mM) for 12 h. Pictures and quantifications per condition are shown in the left and right middle panels, respectively. The results shown are representative of three replicates. Significant differences and *P* values were calculated by the one-way ANOVA. $^{**}P < 0.01$, $^{***}P < 0.001$ *vs.* controls.

that LC3B expression levels were increased in homogenized thyroid tissues of HT patients compared with that in healthy controls [29], which is contradictory to our results. The reason for this contradiction may be due to the different methods used to examine LC3B expression. Because we directly detected the LC3B expression by using the thyroid tissues from the HT patients, the results can represent the LC3B expression level in the inflammation site. However, the proteins from HT patients include a large amount of non-inflammatory tissue, which may interfere with the detection of LC3B expression. During evaluation of the effect of iodine on

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autophagy of TFCs *in vitro*, we found that iodine suppressed the expression of autophagy-related proteins and promoted the expression of apoptosis-related proteins in a dose-dependent manner. Interestingly, the changes in autophagy-related and apoptosis-related proteins in iodine-treated Nthy-ori 3–1 cells were similar to those in HT tissue, suggesting that excessive iodine intake may be associated with the development of HT.

Our findings suggest that excess iodine inhibited autophagy activity, although the molecular mechanism of this process was not clear. We further found that excess iodine treatment induced activation of the Akt and mTOR pathway, which has been identified as a common pathway to inhibit autophagy initiation [30,31]. Therefore, we speculate that excess iodine may inhibit the autophagy process through the activation of the Akt/mTOR signaling pathway. A previous study showed that TGF-β1 induces autophagy in a number of cancer cell lines, including breast cancer [32]. In addition, the serum levels of TGF-β1 were lower in HT patients than in controls [33]. Consistently, we found that TGF-β1 expression decreased in thyroid tissues of HT patients, and that iodine treatment dramatically inhibited the production of TGF-β1 in TFCs *in vitro*, which means that excess iodine may inhibit TFC autophagy by reducing the synthesis of TGF-β1. Therefore, overdoses of iodine may be the reason for the decreased serum levels of TGF-β1. According to this hypothesis, exogenous recombinant TGF-β1 may rescue iodine-induced autophagy suppression in TFCs. Our findings support our hypothesis that iodine-inhibited LC3B conversion was rescued by exogenous recombinant TGF-β1.

Excess ROS can cause cell injury via oxidation of cell components, which causes cell injury beyond repair and induces cell apoptosis [34]. Our studies revealed that ROS production could be promoted by excess iodine. A recent study also demonstrated that TFCs in NOD.H-2h4 mice generate higher levels of ROS after treatment with sodium iodide *in vitro* [35]. In the present study, we demonstrated that excess iodine also inhibited autophagy activity and promoted enhanced ROS production. Therefore, the relationship between autophagy and oxidative stress under the circumstances of excess iodine is interesting. Recently, it has been demonstrated that the intracellular levels of ROS influence the activation of autophagy. For instance, ROS may regulate autophagy by modulating the thiol redox state of proteins that are involved in autophagy. ROS has been reported as an inducer of autophagy under nutrient deprivation conditions [36]. Notably, autophagy induction by rapamycin could partly attenuate cell injury driven by ROS generation by KI, indicating that ROS level and, consequently, TFC apoptosis were at least partly dependent on autophagy activity. Consistent with this, a number of reports suggest that induction of autophagy may have a general apoptosis-inhibitory effect by removing potentially harmful protein aggregates [13]. It is possible that excess iodine-induced ROS production exerted an effect on the autophagy activity; however, in this study, the effect may be masked by excess iodine-induced autophagy suppression.

In conclusion, we showed that iodine-induced autophagy suppression may contribute to the induction of apoptosis by activating ROS, which is regulated by the downregulation of TGF-β1 in TFCs. Decreased autophagy and enhanced apoptosis levels were observed in the thyroid tissues of HT patients, suggesting that excess iodine may initiate HT development by inhibition of autophagy and promotion of apoptosis in TFCs. Therefore, optimization of iodine intake is an important component of preventive health care aiming to reduce the prevalence of thyroid disorders [23].

Conflict of interest

The authors declare that they have no conflict of interests.

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