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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.