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BRIEF REPORT

Expression of Pax8 is decreased and bortezomib does not increase the iodine uptake in thyroid carcinoma cells

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Abstract

Fundamental treatment for papillary thyroid carcinoma (PTC) involves total or subtotal thyroidectomy. Iodine-131 (^{131}I) is routinely utilized to target remnant thyroid cancer and metastasis after thyroidectomy. The effectiveness of other therapeutic modalities remains unsatisfactory; thus, these patients have a poor prognosis. The manner in which the ability of ^{131}I uptake can be improved is vital for their prognosis. Bortezomib has been used as a re-differentiation agent for the treatment of patients with multiple myeloma; however, little is reported about the role of bortezomib in thyroid cancer. To evaluate the therapeutic potential of bortezomib in a human PTC cell line, expression of paired-box 8 (Pax8) protein was determined using Western blot in PTC, normal thyroid, and anaplastic/undifferentiated thyroid carcinoma (ATC) cells. The expression of Pax8 protein in PTC cells pretreated with bortezomib was determined using the same method. Iodine uptake was determined using ^{131}I radioactivity assay. The level of Pax8 protein in normal thyroid cells was significantly higher than in PTC ($P < 0.05$) and ATC cells ($P < 0.05$); its expression in PTC cells was also significantly higher than in ATC cells ($P < 0.05$). The PTC cells in the bortezomib-treated group showed a higher expression of Pax8 protein than the control group ($P < 0.05$). These findings indicate that bortezomib can increase the expression of Pax8, but does not significantly increase the iodine uptake of PTC cells.

Introduction

Thyroid carcinoma is the most common endocrine tumor. It is classified as well-differentiated thyroid carcinoma (WDTC) and anaplastic/undifferentiated thyroid carcinoma (ATC). Papillary thyroid carcinoma (PTC) is estimated to account for 80% of WDTC.[1] Its fundamental treatment involves total or subtotal thyroidectomy. Beta-emitting radioactive iodine, also called iodine-131 (^{131}I), is routinely utilized to target remnant thyroid cancer and metastasis after thyroidectomy. Unfortunately, about 30% of patients have either a poor ability or an absence of iodine uptake.[2] The effectiveness of other therapeutic modalities, including chemotherapy and radiation, also remains unsatisfactory; thus, these patients have a poor prognosis. The manner in which ^{131}I uptake in these patients can be improved is vital for their prognosis.

The unique function of thyroid follicular epithelial cells involves utilizing iodine to synthesize thyroid hormones; the process involves thyroid-specific iodine handing.[3] The efficacy of ^{131}I to destroy target tumors is dependent on the tissue-selective induction of the sodium-iodine symporter (NIS). ^{131}I is transported into cancer cells via the NIS expressed on the basal membrane, where it exerts a local

destructive effect. Thyroid-stimulating hormone (TSH) can increase the expression of the NIS gene and improve the efficacy of ^{131}I therapy.[4,5]

Paired-box 8 (Pax8) is a member of the paired-box-containing protein family and is expressed in the thyroid.[6] It plays a significant role in the differentiation of thyroid cells and, based on a study of Pax8 knockout mice, it seems to be significant for the formation of follicles of polarized epithelial thyroid cells.[7] It has recently been reported that reduced expression of the NIS gene in human papillary thyroid cancer cells is the partial reason for absent or reduced DNA binding of transcription factors, such as Pax8.[8] Pax8 protein regulates several genes involved in the production of thyroid hormones.

Bortezomib, an inhibitor of chymotrypsin-like activity of the 26S proteasome, has been used as a re-differentiation agent for the treatment of patients with multiple myeloma. It has been shown that proteasome inhibitors interfere with important steps of tumor cell regulation, leading to the death of neoplastic cells both in vitro and in vivo, thus emerging as a new class of powerful anticancer drugs.[9] However, little is reported about the role of bortezomib in thyroid cancer. Therefore, the present study was designed to evaluate the expression of Pax8 and the therapeutic potential of bortezomib in human PTC.

Materials and methods

Human thyroid anaplastic carcinoma cell line

Anaplastic/undifferentiated thyroid carcinoma cell line C643 and PTC cell line K1 were obtained from the First Affiliated Hospital, Chinese Medical University, Shenyang, China. Normal thyroid cells were obtained from Shandong Qianfoshan Hospital, Jinan, China.

Cell cultures

Anaplastic/undifferentiated thyroid carcinoma, PTC, and normal thyroid cells were respectively cultured in Dulbecco's Modified Eagle's Medium (DMEM) 1640 and DMEM-F12 containing 10% (v/v) fetal bovine serum (Gibco, San Francisco, CA, USA), 1 μM L-glutamine, 100 IU/mL penicillin, and 100 μg/mL streptomycin solution at 37°C in a humidified atmosphere of 5% CO_2 in air. Human recombinant TSH (Sigma, Rimini, Italy) was added at a concentration of 2 mU/mL. Bortezomib standard substance was dissolved in dimethyl sulfoxide (DMSO) (Sigma). Bortezomib or the same volume of DMSO (0.1%, control) was added to the cultures. Bortezomib-containing medium was refreshed every 48 hours.

Western blotting

Equal amounts of protein from cell lysates were loaded in each well of a 12% sodium dodecyl sulfate-polyacrylamide gel electrophoresis. After electrophoresis, proteins were transferred to polyvinylidene difluoride membranes, blocked for one hour with 5% fat-free milk at room temperature, and blotted with the indicated primary antibodies (monoclonal rabbit anti-phospho-p44/42 extracellular signal-regulated kinase [ERK]1/2 antibody and polyclonal rabbit anti-pan-ERK[c-14] antibody, 1:1000; Cell Signaling Technology, Boston, MA, USA) overnight at 4°C in a humidified atmosphere of 5% CO_2 in air. Human recombinant TSH (Sigma) was added with gentle agitation. The membranes were incubated with horseradish peroxidase-conjugated secondary antibody for one hour at room temperature. The immune complexes were detected using an enhanced chemiluminescence (ECL) plus kit (Hyclone Company, Logan. UT, USA) and visualized on a Storm 860 Gel and Blot Imaging System (Amersham Biosciences, Shanghai, China).

In vitro iodine uptake assay

Cells were treated with or without 112 ng/mL bortezomib for 48 hours, and the iodine uptake was determined. PTC cells were incubated for 48 hours in the presence of 350 μmol/L bortezomib, and then the medium was removed and washed with 1 mL Hank's balanced salt solution (HBSS) containing 10 μmol/L Na ^{125}I. After 30 minutes at 37°C in a humid atmosphere, cells were washed with ice-cold HBSS. Radioactivity was measured in a γ-counter. The number of cells was also determined and iodine uptake was expressed as counts per minute per 10^6 cells.

Statistical analysis

All data were expressed as mean ± standard error of the mean. Differences between groups were examined using Student's *t*-test or analysis of variance test using SPSS 17.0 software (SPSS Inc., Chicago, IL, USA). $P < 0.05$ was considered statistically significant.

Results

Expression of paired-box 8 (Pax8) in FRO cells, papillary thyroid carcinoma PTC cells, and normal thyroid cells

As shown in Figure 1, the expression of Pax8 protein in normal thyroid cells was significantly higher than in PTC ($P < 0.05$) and ATC cells ($P < 0.05$). Its expression in PTC cells was also significantly elevated compared with ATC cells ($P < 0.05$).

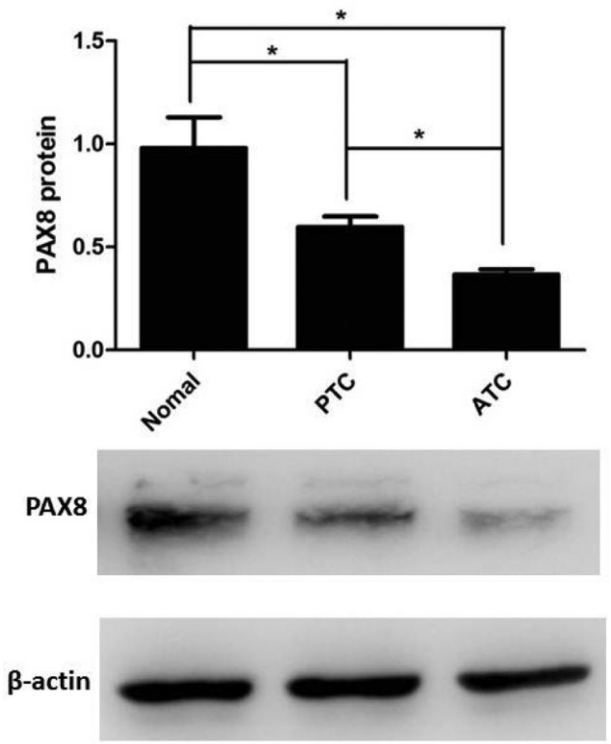

Figure 1 The expression of paired-box 8 (Pax8) protein in normal thyroid cells was significantly higher than in papillary thyroid carcinoma (PTC) cells ($P < 0.05$) and anaplastic/undifferentiated thyroid carcinoma (ATC) cells ($P < 0.05$). Its expression in PTC cells was also significantly elevated compared with ATC cells ($P < 0.05$).

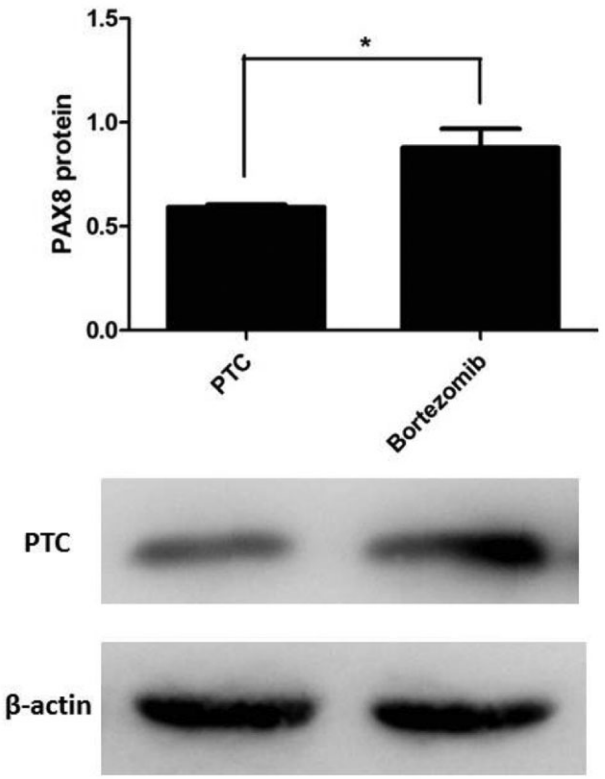

Figure 2 After treatment with bortezomib for 48 hours, the papillary thyroid carcinoma (PTC) cells in the bortezomib-treated group showed a significantly higher expression of Pax8 than in the control group ($P < 0.05$).

Bortezomib upregulated the expression of Pax8 in PTC cells

After treatment with bortezomib for 48 hours, the PTC cells in the bortezomib-treated group showed a significantly higher expression of Pax8 than the control group ($P < 0.05$). As shown in Figure 2, an increased dose of bortezomib increased the expression of Pax8 protein in PTC cells.

Bortezomib had no effect on iodine uptake in PTC cells

The PTC cells not treated with bortezomib showed a low background radioactive count ($[5.34 \pm 0.93] \times 10^3$ cpm/10^6 cells). Bortezomib treatment did not significantly increase the radioactive count ($[5.76 \pm 0.60] \times 10^3$ cpm/10^6 cells) over the background.

Discussion

Thyroid cancer is the most common malignancy of the endocrine system and its incidence has risen gradually in recent years.[10] The main treatment for PTC involves total or subtotal thyroidectomy, ^{131}I ablation, and thyroid hormone inhibitory therapy. After these regimens, most patients have a favorable prognosis and asymptomatic long-term survival. However, patients whose tumor or metastatic foci have no or low ^{131}I uptake (around 30% of thyroid cancer), remain; particularly, patients with distant metastases showed a stronger metastatic spread and poor prognosis.[2] For these patients, the manner in which ^{131}I uptake can be improved is particularly important.

Iodine-131 therapy is an effective approach for the treatment of differentiated thyroid cancers and their metastases. The efficacy of ^{131}I to destroy tumors is dependent on the tissue-selective induction of NIS.[11] Pax8 binds two different sites in the NIS upstream enhancer (rNUE) and has an important role in the expression of the NIS gene.[9] The rNUE contains a cAMP response element-like sequence that mediates thyroid-specific transcription through a novel cAMP-dependent pathway.[8] Recent studies also indicate that Pax8 is involved in the stimulation of NIS gene expression in rats.[8,12] The expression of NIS is controlled by a combination of specific transcription factors with the respective enhancers of

these genes.[11] In thyroid cells, Pax8 has been identified as one of the thyroid-specific transcription factors that regulate the transcriptional activity of thyroglobulin and other thyroid-specific genes. The activity of transcription factors is considered as the main switch to regulate gene expression.[13] Pax8, as an enhancer of NIS, stimulates NIS expression; NIS is the key to iodine intake. It is speculated that the elevation of expression of Pax8 may improve the ability of thyroid cancer cells to intake iodine.

In 2003, the United States Food and Drug Administration approved bortezomib for the treatment of multiple myelopathy because of its strong antitumoral activity. Conticello *et al*. suggested that treatment with bortezomib induced necrosis in the relapsed tumor in patients with ATC.[14] Recently, antitumoral activity of bortezomib in thyroid carcinoma cells has been demonstrated in vitro.[15] It increases the apoptosis of ATC cells and inhibits the proliferation of tumor cells in vitro.[14,15] Putzer *et al*. reported that seven patients with inoperable metastasized thyroid carcinoma received bortezomib intravenously at a dose of 1.3 mg/m^2 on days one, four, eight, and 11. All patients received three therapeutic cycles with intervals of 10 days.[16] Four of the seven patients had stable disease for 27 months after proteasome inhibitor therapy. A study by Altmann *et al*. also showed that bortezomib significantly increased the expression of NIS, and moderately elevated the accumulation of iodine in ATC cells.[17]

The present study shows that Pax8 protein is significantly decreased in PTC and ATC cells compared with normal thyroid cells (Fig. 1), which was consistent with the iodine uptake rates of three types of cancers in clinical studies. In addition, bortezomib did not improve iodine uptake, in spite of the significantly upregulated expression of Pax8 in PTC cells. The mechanism behind this phenomenon still remains unclear. It is likely a result of the translocation dysfunction of NIS; under these circumstances, NIS was retained in the plasma instead of in the cell membrane. In conclusion, bortezomib significantly upregulated the expression of Pax8 in PTC cells. It may be considered a promising therapeutic agent for the treatment of PTC and ATC. Further investigation is needed for better understanding.

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Disclosure

No authors report any conflict of interest.

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