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ORIGINAL ARTICLE

# Effect of parathyroid hormone on apoptosis of human medullary thyroid carcinoma cells*

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## Abstract

**Objective** The aim of the study was to investigate the effect of parathyroid hormone (PTH) on the apoptosis of human medullary thyroid carcinoma (MTC) cells.

**Methods** *In vitro* cultured medullary thyroid carcinoma cell lines were treated with parathyroid hormone and parathyroid hormone receptor-monoclonal antibody, and the apoptosis of cells was detected by flow cytometry.

**Results** The cell morphology changed significantly after treatment based on the observation using the inverted phase-contrast microscope. Various concentrations of parathyroid hormone and parathyroid hormone receptor-monoclonal antibody effectively induced apoptosis in a time- and concentration-dependent manner. When the concentration of parathyroid hormone was 2.0 μmol/L and that of parathyroid hormone receptor-monoclonal antibody was 1.0 μmol/L, the apoptotic rate was 13.24% and 20.78%, respectively, representing a statistically significant difference from that of the control cells ($P < 0.05$).

**Conclusion** PTH plays a role in inducing apoptosis of human MTC cells.

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**Key words:** parathyroid hormone (PTH); medullary thyroid carcinoma (MTC); cell apoptosis

Parathyroid hormone (PTH) is secreted by the parathyroid, and along with calcitonin it regulates calcium and phosphorus metabolism [1]. PTH and its related peptides have been reported to have a proliferative effect on tumor cells [2–3]. In this study, the human medullary thyroid carcinoma (MTC) cell line TT was treated with PTH and anti-PTH receptor antibody (anti-PTHR1), respectively. The growth status of TT cells was observed and the apoptosis rate was measured. These findings on the effect of PTH and anti-PTHR1 on the apoptosis of MTC cells will provide a new theoretical basis for the treatment of MTC.

## Materials and methods

### Major reagents and equipments

The human MTC cell line TT was obtained from the Shanghai Institute of the Chinese Academy of Sciences (Shanghai, China), F12K medium was obtained from Gibco (USA), and fetal bovine serum, trypsin, ethylenediaminetetraacetic acid, dimethyl sulfoxide, and PTH were purchased from Sigma (St. Louis, MO, USA). Anti-PTHR1 was obtained from Abcam (Cambridge, MA, USA), the flow cytometry kit was purchased from BD (USA), and an Olympus (Tokyo, Japan) inverted phase-contrast microscope was used. Other conventional equipment and reagents were obtained from domestic companies.

### Cell culture and treatment

The human MTC TT cell line was cultured in F12K medium at 37 °C (containing 15% fetal bovine serum, 500 U/mL penicillin, and 100 μg/mL streptomycin) through routine digestion passages. After the logarithmic growth phase of TT cells, three experimental groups were established: (1) control group (without PTH and anti-PTHR1); (2) PTH intervention group; and (3) anti-PTHR1 intervention group. PTH was treated at the various concentrations of 0, 0.5 μmol/L, 1.0 μmol/L, 1.5 μmol/L, and 2.0 μmol/L respectively, and anti-PTHR1 was treated at concentrations of 0, 0.25 μmol/L, 0.5 μmol/L, 0.75 μmol/L, and 1.0 μmol/L, respectively. After 48 h, the cells were washed with phosphate-buffered saline (PBS), the supernatant was discarded, and the cells were detected by

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flow cytometry.

After the cells were cultured for 24 h, the cells in each concentration group were collected, washed with cold PBS, centrifuged, and then the supernatant was discarded. The cell suspension was treated with 1× annexin binding buffer with an average of $10^6$ cells in 1 mL of suspension, to a total volume of 100 μL. Alexa Fluor 488 annexinV (5 μL) and 1 μL propidium iodide reserve liquid (100 μg/mL) were added to the 100-μL cell suspension. The cells were cultured for 15 min at room temperature and then 400 μL of 1× annexin-binding buffer was added, and the sample was gently mixed on the ice and tested as soon as possible.

### Statistical analysis

SPSS 17.0 software was used for statistical analysis. Data between two groups were compared using *t*-tests, and those among groups were compared using analysis of variance; $P < 0.05$ was considered to indicate a statistically significant difference.

## Results

Under the microscope, the cells of the control group were distributed as a monolayer, showing good growth; the cells were transparent and the boundaries were clear. After treatment with PTH and anti-PTHR1 for 24 h, nuclei concentration was initiated, the nuclear membrane gradually disintegrated, and the chromatin showed a great division with an increase in concentration of PTH and anti-PTHR1, and time. Therefore, these cells demonstrated the hallmark changes that occur during apoptosis.

Flow cytometry demonstrated no significant changes in the control group cells, whereas apoptosis of TT cells was clearly detected under all tested concentrations of PTH and anti-PTHR1. The apoptotic effect increased with an increase in their concentration and with increase in time for a given concentration, indicating time- and concentration-dependent effects. Indeed, the apoptosis rate was positively correlated with concentration and time ($P < 0.05$). When the concentration of PTH was 2.0 μmol/L, the apoptosis rate of TT cells was significantly increased at 13.24% ($P < 0.05$). When the concentration of anti-PTHR1 was 1.0 μmol/L, the apoptosis rate of TT cells was significantly increased, at 20.78% ($P < 0.05$) (Fig. 1 and 2).

## Discussion

MTC is a malignant tumor originating from follicular side cells (C cells), and calcitonin is a specific marker of MTC cells [4–5]. Surgical resection is still the preferred radical approach for treatment. MTC is distinct from

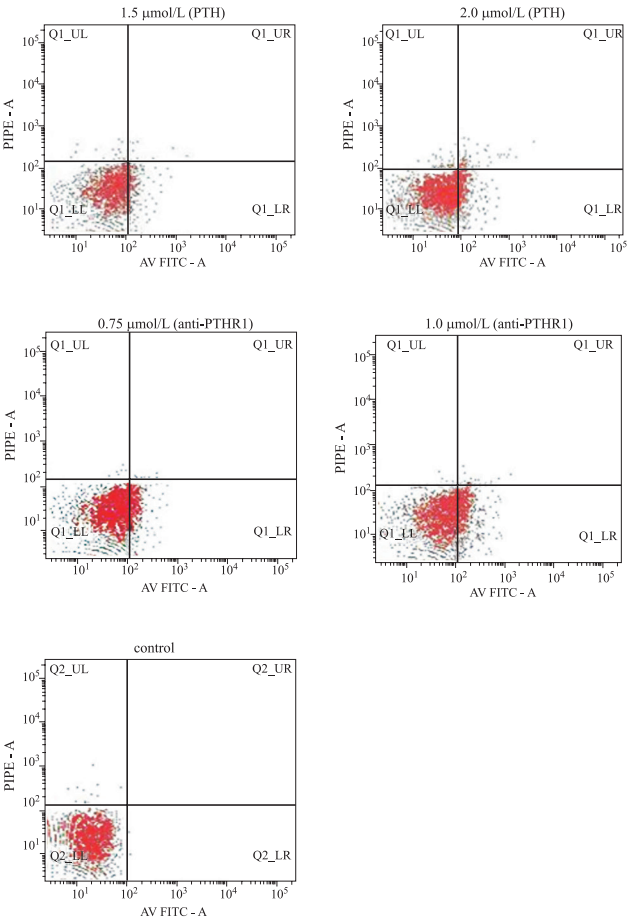


**Fig. 1** Apoptosis of TT cells exposed to different concentrations of PTH and anti-PTHR1.

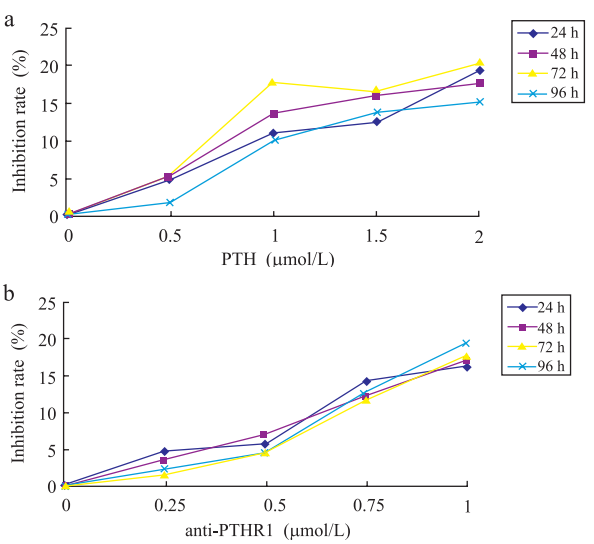


**Fig. 2** Inhibitory effects of (a) PTH and (b) anti-PTHR1 on TT cells over time and at different concentrations

differentiated thyroid cancer, as it originates from C cells, it is not affected by thyroid stimulating hormone (TSH), and it does not express sodium/iodide symporter (NIS); therefore, TSH inhibitory therapy and $I^{131}$ radionuclide irradiation treatment is ineffective for MTC, and the 10-year survival rate is much lower than that of patients with differentiated thyroid cancer [6–8]. To date, a variety of immunotherapy strategies, molecular targeted drugs, and tumor vaccines have shown some good effects in preclinical tests; although some of these drugs have started to be used clinically, the therapeutic effect is still not ideal [9–11]. Therefore, the search for new effective molecular therapeutic targets for MTC remains one of the most difficult and hot issues in the field of thyroid cancer research. Thus, further study of the MTC development mechanism and tumor cell growth can help to provide major breakthroughs for developing new therapeutic drugs.

PTH is an endocrine hormone that regulates calcium and phosphorus metabolism. However, its involvement in the development of MTC and its effect on the growth and proliferation of MTC cells have not been reported. Dexamethasone was shown to inhibit the proliferation of TT cells, mainly by inhibiting the G1 phase of the cell cycle to induce apoptosis [12]. Further studies have shown that protein kinase C (PKC) inhibits the proliferation of MTC cells by increasing apoptosis rate *in vitro* [13]. PTH receptor (PTHR) is a member of the G protein-coupled receptor (GPCR) family. After PTH activates the receptor, G protein decomposes into the α, β, and γ subunits, including the Gs, Gi, Gq, and G12/13 subunit, with different signal transduction functions. The Gs and Gi α subunits respectively activate and inhibit adenylate cyclase (AC), and affect cAMP generation and protein kinase A activity. PLC-independent PKC activation pathways (PTH/non-PLC/PKC). Among the above pathways, cAMP/PKA and PLC are the main signaling pathways that mediate the biological effects of PTH. PTHR is expressed in the normal thyroid C membrane surface, and it shows higher expression in MTC. PTHR activates AC by GPCR, enables a higher concentration of cAMP in cells, which activates PKA to initiate the AC-cAMP-PKA pathways. However, there has been no in-depth study on how these signaling pathways are regulated in MTC or on the correlation among the proliferation, apoptosis, and pathogenesis of MTC [14].

In this study, TT cells were treated with PTH and anti-PTHR1, and light microscopy revealed that TT cells were distributed as a monolayer, in spindle or polygonal shape, the cell contour was clear, the cell morphology was complete, and the cells were well grown. After treatment with PTH and anti-PTHR1, the nuclei appeared to be concentrated, marginalized, and the nuclear membrane gradually disintegrated. The chromatin was divided into several blocks. The apoptosis of the cells was increased with an increase of the drug concentration and with the increase in treatment time. The results of flow cytometry confirmed that all concentrations of PTH and anti-PTHR1 could induce the apoptosis of TT cells, and the difference was significant ($P < 0.05$) compared to the control. The apoptotic rate was positively correlated with concentration and time ($P < 0.05$). The apoptosis of TT cells was most significant when the PTH concentration was 2.0 μmol/L and that of anti-PTHR1 was 1.0 μmol/L. These results suggested that PTH and anti-PTHR1 can inhibit the growth of TT cells and play important roles in the induction of apoptosis in a concentration- and time-dependent manner. Some studies have shown that the addition of exogenous PTH1-34 can promote PTH formation in TT cells, induce and activate PTHR1, increase the cAMP level of TT cells, activated the AC-cAMP-PKA pathway, inhibits the growth of TT cells, and ultimately promotes apoptosis. These results are consistent with those of our study.

The effects of PTH and anti-PTHR on the morphology and structure of human MTC TT cells are consistent with cell apoptosis. These results suggest that PTH and anti-PTHR1 have a dual biological effect by inhibiting the proliferation and inducing apoptosis of human MTC TT cells. These findings provide a theoretical basis and important reference for further studies of the mechanism of PTH on MTC.

In summary, this study confirmed that PTH and anti-PTHR1 can effectively induce the apoptosis of human MTC TT cells. This result provides very important information and presents a practical application value for exploring the mechanism of apoptosis induction and the role of PTH on human MTC TT cells. With further in-depth research and understanding of PTH, PTH and anti-PTHR1 may become new anti-tumor therapy targets, providing a new strategy for the early diagnosis and treatment of MTC.

### Conflict of interest

The authors indicated no potential conflicts of interest.

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