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Inhibition effects of parathyroid hormone on human medullary thyroid carcinoma cells*

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Abstract ***Objective:*** The purpose of the study was to investigate the effects of parathyroid hormone and parathyroid hormone receptor monoclonal antibody on *in vitro* growth and proliferation of human medullary thyroid carcinoma cell lines. ***Methods:*** The medullary thyroid carcinoma cell line was cultured *in vitro*, with parathyroid hormone and parathyroid hormone receptor monoclonal antibody treatment intervention, the growth of the cells was observed under an inverted contrast microscope, the MTT assay was used to detect the cell growth inhibition rate. ***Results:*** Under the inverted contrast microscope, the cells changed significantly, the parathyroid hormone and parathyroid hormone receptor monoclonal antibodies can effectively inhibit the proliferation of medullary thyroid cancer cells in a time and dose dependent. When parathyroid hormone concentration reached a concentration of 2.0 μmol/L, the parathyroid hormone receptor monoclonal antibody reached a concentration of 1.0 μmol/L, the cell growth was most significantly inhibited ($P < 0.05$). ***Conclusion:*** Parathyroid hormone and parathyroid hormone receptor monoclonal antibody were able to inhibit the proliferation of medullary thyroid carcinoma cells and significantly reduce the proliferation index.

Key words parathyroid hormone (PTH); medullary thyroid carcinoma (MTC) cell line; parathyroid hormone receptor monoclonal antibody

Medullary thyroid carcinoma (MTC) is an malignancy originated from parafollicular cells (C cells), it is accounted for 8%–10% of thyroid cancer, it has a high degree of malignancy, the calcitonin is the special marker [1, 2]. The parathyroid hormone (PTH) is secreted by the parathyroid glands, it can regulate calcium and phosphorus metabolism with calcitonin (CT) in the body [3]. Reports showed that PTH related peptide has proliferation effects on tumor cells [4–6]. However, studies about the effects of PTH on MTC and the association between the two have not been reported. In this study, the MTC cell line (TT cell line) was the research object, PTH and anti-PTH receptor 1 antibody (anti-PTHR1) were given for intervention. The growth of tumor cells were observed, the cell growth inhibition rate was detected, the impact of PTH and anti-PTHR1 on MTC cells and its mechanism were studied. It was expected to open up new approaches for the treatment of MTC.

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Materials and methods

Reagents and equipments

Fetal bovine serum (Sigma, USA), F12k medium (Gibco, USA), trypsin, EDTA, MTT, DMSO, PTH (Sigma, USA), anti-PTHR1 (Abcanm, USA), cells cycle detection kit (Invitrogen, USA) were purchased. The inverted phase contrast microscope was purchased from Olympas (Japan), the clean benches and CO_2 incubators were produced by domestic companies.

Methods

TT cell culture

Human MTC cell line (TT cell line) was purchased from Cell Bank of Shanghai Science Institute (China). It was cultured in F12K medium containing 15% fetal bovine serum, 500 U/mL penicillin, 100 μg/mL streptomycin. It was then cultured in 37 ℃ incubator with 5% CO_2, the 0.5% trypsin digestion was used for passage.

TT cells morphological changes under inverted phase contrast microscope

TT cells were taken at logarithmic growth phase, after counting, they were seeded in 25 cm^2 flasks according to 1×10^6 /mL concentrations and 2 mL/flasks. Twenty-four hours later, the PTH and anti-PTHR1 were added

after that the cells adhered to the wall. The PTH concentrations were 0, 0.5, 1.0, 1.5, and 2.0 μmol/L. The anti-PTHR1 concentrations were 0, 0.25, 0.5, 0.75, and 1.0 μmol/L. Twenty-four hours later, the cell morphology changes were observed under inverted phase contrast microscope.

TT cell growth inhibition rate detected with MTT assay

TT cells were taken at logarithmic growth phase, after digestion, the cell suspension concentration was adjusted to 1×10^6 /mL, they were seeded in 96-well plates with 100 μL/plate in 37 ℃ incubator with 5% CO_2 for 24 h, the cell fusion was 80%–90%. (1) Blank group (no cells); (2) Control group (without PTH and anti-PTHR1); (3) Experimental groups: with PTH and anti-PTHR1 intervention, PTH concentrations were 0, 0.5, 1.0, 1.5, and 2.0 μmol/L, anti-PTHR1 concentrations were 0, 0.25, 0.5, 0.75, and 1.0 μmol/L. There were four wells in each group, after further incubation for 48 h, 4 h before termination of culture, the MTT was added according to 40 μL/well, the final concentration of MTT was 1 g/L. When the culture was finished, the culture supernatant was sucked out, 150 μL DMSO was added, the OD value was measured by enzyme-linked detection. The mean OD value was taken as the vertical axis, the time was taken as the horizontal axis, growth curve of cells were drawn, four times were repeated in each experiment group. According to the above grouping, the cells changes in different time periods were measured. The absorbance OD values (λ = 492 nm) were detected on days 1, 2, 3, 4, and 5. The cell growth inhibition rate was calculated as follows: inhibition rate (%) = [(control group OD – blank group OD) – (experimental group OD – blank group OD)] / (control group OD – blank group OD) × 100%.

Statistical analysis

The mean were expressed as $\bar{\chi} \pm s$ and inhibition rate, SPSS 17.0 statistical software was used for data analysis. The means between the two groups were compared using *t* tests. The means among groups were compared using ANOVA, $P < 0.05$ was consider as statistically significant difference.

Results

Effects of PTH and anti-PTHR1 on TT cell morphology

Inverted phase contrast microscope revealed that cells in control group showed single layered, spindle-shaped adherent growth, the growth was in good condition, the cells were transparent. The cells contained less intracellular granules, boundaries between cells were clear. When different concentrations of PTH and anti-PTHR1 were used on TT cells 24 h later, with the increase of drug concentration, the cell gradually shrink and became round, the number of cells with floating growth state significantly increased, the internal structure of the cell was damaged and fragmented. However, the membrane was still intact, the survival cells gradually decreased (Fig. 1 and 2).

Growth inhibition effects of PTH and anti-PTHR1 on TT cell

MTT colorimetric assay displayed that various concentrations of PTH and anti-PTHR1 had significant inhibition effect on TT cell proliferation activity of MTC, the difference was statistically significant ($P < 0.05$). The inhibitory effects showed concentration and time dependent manner. The inhibitory effects on TT cell line were the most significant when the PTH concentration reached 2.0 μmol/L, it reached the peak 72 h later, the difference was statistically significant ($P < 0.05$). The concentration 1.0 μmol/L of anti-PTHR1 had most significant inhibitory effects on TT cell line, it reached the peak 96 h later, the difference was statistically significant ($P < 0.05$). It suggested that PTH and anti-PTHR1 had inhibition effects of MTC *in vitro* (Fig. 3 and Table 1).

Discussion

Thyroid stimulating hormone (TSH) is hormone secreted by the pituitary to promote the thyroid growth and function, it mainly acted on the thyroid gland. TSH and thyroid follicular cell specific receptor binding promoted the release of thyroid hormone and synthesis of T3, T4. On one hand, TSH is promoted by hyrotropin-releasing hormone secreted by the hypothalamus, on the other hand, it is feedbackly inhibited by thyroid T3, T4 secreted by the thyroid. After the binding of TSH and the receptor, the growth and proliferation of thyroid follicular cells were stimulated. Endocrine therapy for differentiated thyroid carcinoma was based on feedback inhibition of thyroid-stimulating hormone secretion, then the therapeutic effect can be achieved. MTC cells originated from C cells, they were not affected by TSH, they did not express thyrotropin receptor and uptake iodine, TSH suppression therapy and I^{131} radionuclide therapy were invalid [7–9]. To explore new therapeutic approaches and to develop new therapeutic drugs to MTC was of great significance.

Medullary thyroid cancer secreted calcitonin, however, serum calcium concentrations were within the normal range, the main reason was associated with the regulation of PTH. From this perspective, the PTH may play a regulatory role in the occurrence and development process of thyroid medullary carcinoma. PTH may have feedback inhibition effects on the MTC cells. Research has shown that protein kinase C could bate MTC cell proliferation by increasing apoptosis *in vitro* [10]. However, the effects of

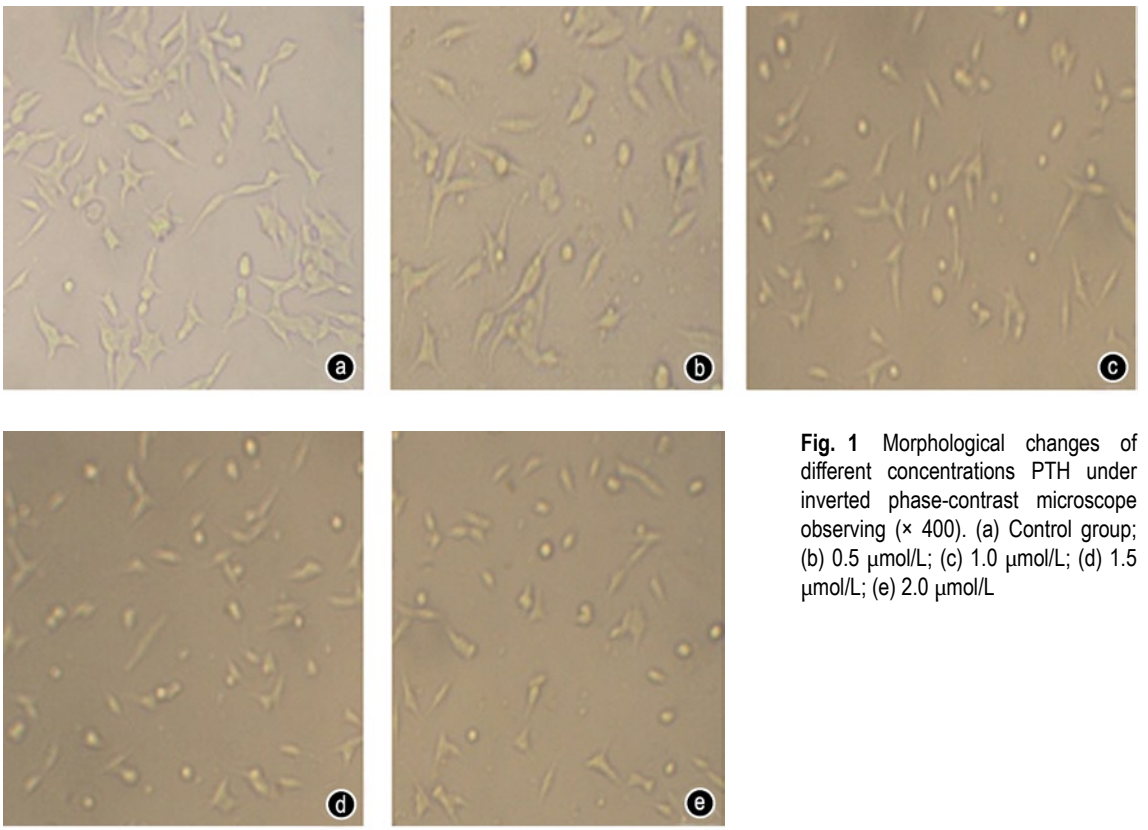

Fig. 1 Morphological changes of different concentrations PTH under inverted phase-contrast microscope observing (× 400). (a) Control group; (b) 0.5 μmol/L; (c) 1.0 μmol/L; (d) 1.5 μmol/L; (e) 2.0 μmol/L

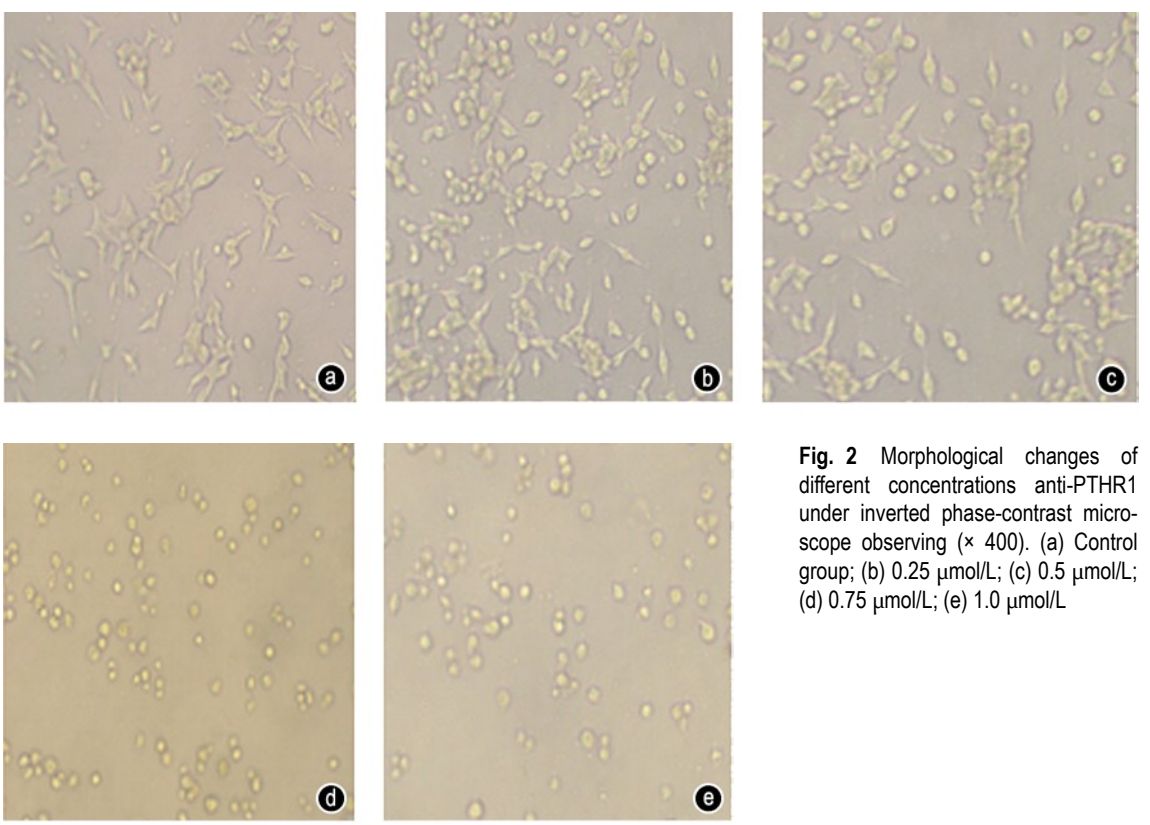

Fig. 2 Morphological changes of different concentrations anti-PTHR1 under inverted phase-contrast microscope observing (× 400). (a) Control group; (b) 0.25 μmol/L; (c) 0.5 μmol/L; (d) 0.75 μmol/L; (e) 1.0 μmol/L

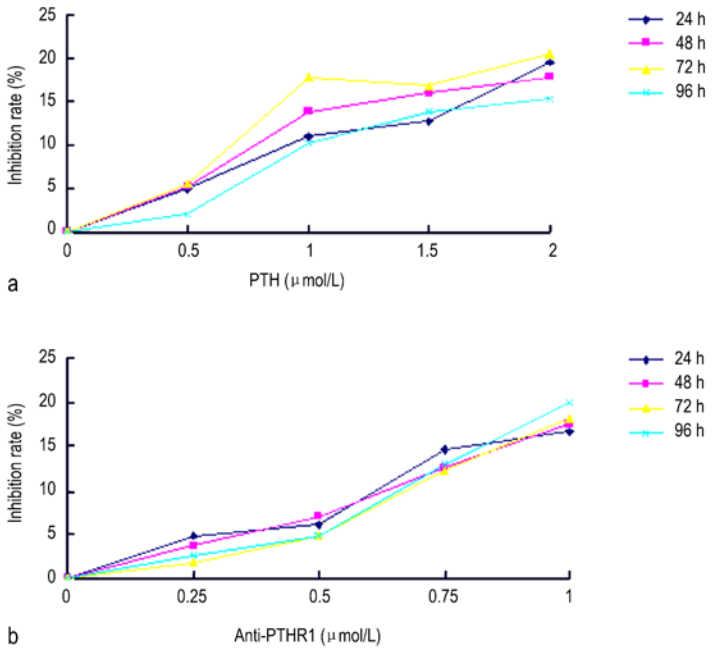

Fig. 3 Inhibitory effects of PTH and anti-PTHR1 on the proliferation of medullary thyoid carcinoma TT cells detected by MTT assay. (a) Treatment by PTH at different concentrations for different time (n = 4); (b) Treatment by anti-PTHR1 at different concentrations for different time (n = 4)

Table 1 Effects of PTH and anti-PTHR1 on proliferation of TT cells

Groups	24 h		48 h		72 h		96 h	
	OD value	Inhibition rate (%)	OD value	Inhibition rate (%)	OD value	Inhibition rate (%)	OD value	Inhibition rate (%)
Control group	0.776 ± 0.002		0.845 ± 0.006		0.895 ± 0.003		0.938 ± 0.005	
PTH 0.5 μmol/L	0.738 ± 0.001	4.900*	0.801 ± 0.003	5.207*	0.846 ± 0.002	5.475*	0.920 ± 0.006	1.919*
PTH 1.0 μmol/L	0.691 ± 0.005	10.954*	0.728 ± 0.002	13.846*	0.763 ± 0.004	17.749*	0.843 ± 0.005	10.128*
PTH 1.5 μmol/L	0.677 ± 0.003	12.758*	0.710 ± 0.001	15.976*	0.754 ± 0.006	16.760*	0.809 ± 0.002	13.753*
PTH 2.0 μmol/L	0.625 ± 0.001	19.459*	0.695 ± 0.004	17.751*	0.711 ± 0.005	20.559*	0.795 ± 0.003	15.245
Control group	0.505 ± 0.001		0.518 ± 0.007		0.526 ± 0.002		0.553 ± 0.003	
anti-PTHR1 0.25 μmol/L	0.481 ± 0.005	4.752*	0.499 ± 0.003	3.668*	0.517 ± 0.004	1.711*	0.539 ± 0.002	2.532*
anti-PTHR1 0.5 μmol/L	0.475 ± 0.003	5.941*	0.482 ± 0.001	6.950*	0.501 ± 0.004	4.753*	0.526 ± 0.006	4.882*
anti-PTHR1 0.75 μmol/L	0.431 ± 0.004	14.653*	0.453 ± 0.002	12.548*	0.462 ± 0.001	12.167*	0.481 ± 0.005	13.020*
anti-PTHR1 1.0 μmol/L	0.421 ± 0.003	16.634*	0.427 ± 0.007	17.568*	0.431 ± 0.005	18.061*	0.442 ± 0.003	20.072

* Compared with control group, $P < 0.05$

PTH on occurrence and development of MTC and growth and proliferation of MTC cells were rarely reported.

In this study, both PTH and anti-PTHR1 were given to MTC TT cells. Through the MTT assay, TT cell growth and proliferation activity were significantly inhibited ($P < 0.05$), and it showed a time and concentration dependent manner at different times and in different concentration gradients. When different concentrations of PTH and anti-PTHR1 acted on the TT cells, the inverted phase contrast microscope revealed that cells in control group showed single layered, spindle-shaped adherent growth, the cells were transparent and in intact shape. It showed good condition in growing. After the treatment of PTH and anti-PTHR1, the TT cell shrank and became round and messy, cytoplasm condensed, nuclei/cytoplasm ratio decreased, some nuclei showed concentration and marginalization, it indicated that TT cell growth and proliferation activity were significantly inhibited, the inhibition effects increased with increasing concentrations of drugs and prolonged duration of action. It also showed that PTH and anti-PTHR1 had the biological effects in inhibiting the growth and proliferation of human medullary thyroid cancer TT cells. It will provide the theoretical basis and important reference for further study of the mechanism of PTH on MTC.

In summary, PTH and anti-PTHR1 had a certain inhibitory effects on MTC TT cells, PTH and anti-PTHR1 may be new therapeutic targets to provide a new way for early diagnosis and treatment of medullary thyroid cancer, the follow-up mechanism remained to be further studied.

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