Follicular Reconstruction and Hormone Production by Human Adenomatous Goiter Cells in Culture

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GOTO, K., SASANO, N. and MATOBA, N. Follicular Reconstruction and Hormone Production by Human Adenomatous Goiter Cells in Culture. Tohoku J. exp. Med., 1982, 136 (3), 285-290 — Morphological and functional properties of dispersed cells of human adenomatous goiter and those of the thyroid tissue adjacent to and distant from the nodular lesion (normal control) were investigated. In the presence of TSH, reconstructive arrangement of adenomatous goiter cells into a three-dimensional follicular structure occurred in a similar manner to that of normal controls. An addition of thyrotropin (TSH) to culture resulted in the secretion of thyroid hormones in control cells, but adenomatous goiter cells showed no response to TSH. It was found that the ratio of rT₃ to T₃, the ratio of an inactive to an active form, was about three times higher in adenomatous goiter than in normal control. These findings suggested that conversion from T₄ to rT₃ is increased in adenomatous goiter.

Thyroid cells dispersed by an enzymatic treatment grow in culture as a monolayer, while TSH added to the medium from the onset of the culture induces a follicular reorganization (Kerkof et al. 1964). Such cells reorganized exhibit the same polarity and hormone-producing activity as those of the normal thyroidal follicles (Lissitzky et al. 1971). The follicular structure and cell polarity are closely related to endocrine activities of the thyroid (Inoue et al. 1980).

In thyroid tumors, the follicles, morphological and functional units of the thyroid gland, have been considered to change in morphological characteristics, biological functions and the reactivity to TSH.

In the present study, follicular reconstruction and hormone production by human adenomatous goiter cells cultured with or without TSH were investigated in comparison with those by the normal thyroid cells.

MATERIALS AND METHODS

Culture. Materials was obtained from surgical specimens of adenomatous goiter. Apparently normal thyroid tissues adjacent to and distant from the nodular lesion were used for normal controls. Thyroid tissue obtained from a patient with hyperparathyroidism was also included in normal controls. Altogether 9 cases were examined.

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The tissues weighing 0.3 to 3 g were cut into small pieces less than 0.5 mm³, dissociated with gentle swirling in a solution of Ca²⁺- and Mg²⁺-free phosphate buffered salt solution (PBS) containing 0.2% collagenase (Sigma) at 37°C for 1 hr. The medium was centrifuged three times at 1,000 × g for 1 min each and then resuspended in a medium supplemented with 10% calf serum. Then cells centrifuged were explanted into 30 mm-plastic Petri dishes (Falcon) and incubated in a moist atmosphere of 95% air and 5% CO₂ at 37°C in 1.5 ml of Eagle’s MEM (Gibco) supplemented with 2 mM L-glutamine, 0.2 mM serine, 2mM pyruvate, penicillin (100 units/ml), 10% calf serum (Flow Laboratory) and 100 µU/ml bovine TSH (Tokyotanabe). Cultures without bovine TSH were used for controls. Calf serum was not heat-treated in order to avoid denaturation of serum components, because the presence of complement is a prerequisite for radioimmunoassay for T₃ and reverse T₃ and T₄. Culture medium was changed every day.

Electron microscopy. The cultured cells were fixed in situ with 2.5% glutaraldehyde in PBS for 10 min followed by 1% osmium tetroxide for 10 min, and were dehydrated in a graded ethanol series. The culture dish was placed down on a gelatin capsule containing epoxy resin (Epon) prepared immediately before use. Then the culture dish with a gelatin capsule was reverted to the upright position and thus polymerization of Epon was allowed to proceed. Thereafter thin sections were prepared and poststained with uranyl acetate and lead citrate, and examined with an electron microscope (JOEL 100C).

Incorporation of ¹³¹I and radioimmunoassay of T₃, rT₃, and T₄. Twenty-four hr before the harvest of cultured cells, carrier-free ¹³¹I (N.E.N.), 5 µCi, was introduced into each culture dish. Uptake of ¹³¹I by cultured cells was counted by a well-type scintillation counter. Concentrations of thyroid hormones were measured using commercial RIA kits for T₃ and T₄ (Eiken. Tokyo) and rT₃ (Tokyo Dinavot).

RESULTS

Normal thyroidal cells cultured in the absence of TSH grew as a two-dimensional layer with microvilli and desmosomes faced on medium (Fig. 1a). In contrast, cells cultured in the presence of 100 µU/ml of TSH were rearranged into typical three-dimensional follicles as evidenced by the appearance of desmosomes, differentiated apical poles with microvilli, and of a follicular lumen containing occasionally electron dense material. (Fig. 1b). Such rearranged follicles were observed on the second day of culture. A gradual increase in size of follicles was also noticed.

The cultured normal thyroidal cells reacted strongly to TSH. The cells of adenomatous goiter cultured in the absence of TSH grew as a two-dimensional layer in the similar manner as normal cells (Fig. 2a). Microvilli and desmosomes of the cultured goiter cells faced on the medium. In contrast, the addition of 100 µU/ml of TSH caused the cultured goiter cells to undergo follicular rearrangement as shown by the presence of desmosomes, microvilli, and a follicular lumen containing material which was relatively electron lucent (Fig. 2b). The polarity of cultured goiter cells after follicle reorganization was similar to that of cultured normal thyroid cells, which indicated morphological evidence of reactivity to TSH.

Fig. 3 shows the rate of secretion of T₃, rT₃ and T₄ by cultured cells. Cultured normal thyroid cells secreted T₃, rT₃, and T₄ even in the absence of TSH. Moreover, TSH caused a 1.6-fold increase of T₃, rT₃ and T₄. Cultured cells from adenomatous goiter in the absence of TSH secreted a small quantity of T₃, rT₃ and T₄. The cells from two goiters alone showed a weak reaction to the addition of 100 µU/ml of TSH.
The above results indicated that adenomatous goiter reacted to TSH when evaluated by morphological properties. However, the activity to produce thyroid hormones has remained unchanged even after the addition of TSH. For understanding the properties of adenomatous goiter, the amounts of reverse T₃ produced by the cultured cells from adenomatous goiters and the normal tissues adjacent to
and distant from goiters were examined. Cultured cells from the normal thyroid gland secreted only a small amount of rT₃ and those from adenomatous goiter secreted less (Fig. 3). However, the ratio of rT₃ to T₃, the ratio of the inactive to the active form (Chopra 1974) was about three times higher in

**Fig. 3.** Tri-iodothyronine (T₃), reverse T₃ and thyroxine (T₄) released by the cultured cells in the presence and absence of TSH. G, adenomatous goiter cells; AG, cells adjacent to goiter; DG, cells distant from goiter.

**Fig. 4.** Molar ratio of reverse T₃: T₃ released by the cells of goiter, adjacent to and distant from goiter cultured in the absence of TSH.
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adenomatous goiter than in the normal thyroid (Fig. 4). Thus the rate of conversion from T₄ to rT₃ was regarded to increase in adenomatous goiter.

DISCUSSION

The response of goiter cells in culture to TSH was revealed by the morphological evidence of follicular reorganization. After the addition of TSH the cultured goiter cells were reorganized into a follicular structure containing colloid-like substance light in electron density. The low electron density of colloid-like substance in the case of goiter compared with that of normal cells seems to reflect a slow rate of thyroid hormone production. The cultured goiter cells showed no response to TSH in the secretory activities of T₃, rT₃, and T₄ but a higher conversion ratio of T₄ to rT₃ as compared with cultured normal cells. The above findings indicate that, in adenomatous goiters, the capability to form a follicular structure is maintained, but that to produce thyroid hormones became lost.

An increase in the conversion ratio from T₄ to rT₃ was reported in patients with liver cirrhosis (Chopra et al. 1975), uremia (Lim et al. 1977), and chronic consumptive diseases, in all of which the increased conversion ratio of T₄ to rT₃ was considered to result from a decreased conversion ratio of T₄ to T₃ (Chopra 1974). In other words, the increase of rT₃ production in chronic consumptive diseases seems to play a mechanism of decelerating rapid consumption. Adenomatous goiter is usually a focal lesion and the increase of in vitro rT₃ production seems to play a different role.

Reorganization of cultured cells is a useful criterion for the evaluation of the properties of thyroidal tumors. Our study applying these morphological criteria to cultured cells from thyroid adenoma and carcinoma is now in progress.

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References

5) Lim, V.S., Fang, V.S., Katz, A.I. & Refettof, S. (1977) Thyroid dysfunction in chronic