Abundant thyroid hormone is produced by human thyroid cells in culture

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Permanent hypothyroidism following surgical resection in a patient with Graves' disease requires levo-thyroxine replacement therapy for the rest of the patient's life. We mentioned\(^1\) one therapeutic regimen for this patient in our first report on the autotransplantation of cryopreserved thyroid tissue in a patient with irreversible hypothyroidism.

In this second report, we present the findings of our efforts to evaluate the tissue-cultured living cells from surgical specimens from the Graves' patient. Specific emphasis in the study was placed on whether or not these cells synthesize and secrete thyroid hormone and whether or not the transplantation of these living cells is capable of supplementing enough thyroid hormone to the permanent hypothyroid patient.

Surgical specimens of normal thyroid around the nodular region and from the Graves' disease patient were minced and washed three times with phosphate buffer saline (PBS) at room temperature. The tissues were vigorously agitated in Dulbecco's Minimum Essential Medium (DMEM) containing 0.1% collagenase for 20 min. at room temperature and then centrifuged. 4.0 × 10^5 cells were harvested and a cell culture was started in DMEM containing 10% fetal bovine serum (FBS) and 10 mU/ml of thyroid stimulating hormone (TSH). The medium was changed every two or three days. Hormone synthesis was detected by immunohistochemical staining of the thyroglobulin in the cultured cells prepared from cell blocks which were in formalin-fixed paraffin sections. Hormone secretion was evaluated by measuring the thyroid hormone in the cultured medium.

The cultured cells continued growing with colony formation. Immunohistochemical thyroglobulin staining of the cultured cells obtained from normal thyroid tissue showed a strong positive reaction as depicted in Photo. 1. The concentrations of thyroid hormone in cultured media 1 and 2 were apparently elevated compared with the control medium, which did not contain cultured thyroid cells, as indicated in Fig. 1. The thyroid hormone concentration of the cultured medium from the Graves' disease patient also clearly increased to 7.8 ng/day compared with the control one week after culturing was begun. These results indicate that Graves' disease cultured cells also synthesize and secrete thyroid hormone.

We previously reported\(^1\) on our experimental study of autotransplantation of cryopreserved thyroid tissue from Graves's disease patients as a treatment for patients with permanent

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Phot. 1 Immunostaining of the thyroglobulin using SAB method. Four micron of deparaffinized formalin-fixed specimen from cultured thyroid cells was preincubated with 3% H$_2$O$_2$ to prevent from the reaction with the endogenous peroxidase. Anti-thyroglobulin antibody was then incubated with the section, followed by incubation with biotin-conjugated anti-mouse antibody. The peroxidase-conjugated streptavidin was incubated with the section. The specimen was then coloured by diaminobenzidine. The strong positive reaction to thyroglobulin is recognized in the cytoplasms of the cultured thyroid cells.

hypothyroidism following surgery. The characteristics of this study compared with previous study are focused on autotransplantation of living cells that can also be cryopreserved and functioning after thawing according the results of our further study (data; not shown$^2$). The advantages using living cells can be described that the amount of thyroid hormone which should be supplied depending upon the severity of the hypothyroid state can be regulated by adjusting the cell number. Further in vivo study will be needed to clarify exact amount of thyroid cells for hormone supplementation and are now underway.

References


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