In vitro and in vivo characterizations of established human follicular carcinoma cell line derived from thyroid cancer: A novel model for well-differentiated thyroid malignant tumor

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A continuous cell line, named SMC R86 F1, was established from a surgically resected primary thyroid lesion. The cell grew as an adhering monolayer with a doubling time of about 25 hours in modified Eagle's medium supplemented with fetal bovine serum. When the cells were transplanted into athymic nude mice, tumors developed at the site of inoculation. The cells not only showed epithelial origin upon light and electron microscopic examination but also possessed a biosynthetic marker human thyroglobulin (hTg). In order to examine the iodide trapping ability of the xenografts, radioiodine at doses of 3.7 MBq was injected into the peritoneum of 131I treated nude mice bearing xenografts at about 4 weeks after the cell inoculation. Judging from the results of scintigraphic, autoradiographic and biodistribution studies, viable tissue of the xenografts in the treated mice had the ability to trap radioiodine. Histological sections of the xenografts resected from the treated mice consisted of follicle-like and trabecular growing structures, and immunohistochemically the cytoplasm of the tissues was hTg positive. The cells possessed the ability to trap radioactive iodine in vitro under the control of TSH. In addition, the expression of iodinated 19S Tg in the cell cytoplasmic in the monolayer cultures was revealed by immunoblotting and autoradiographic assays. These observations provide strong evidence that the SMC R86 F1 cell line possesses well-differentiated properties of the malignant thyroid follicular epithelial cells.

Key words: well-differentiated thyroid carcinoma cell line, follicular formation, iodine organization

INTRODUCTION

It is well recognized that certain endocrine tumors may be subject to selective hormonal control. Thyroid cells are regulated by thyrotropin (TSH), which is a glycoprotein hormone produced in the anterior pituitary. TSH exerts its effects without entering the cell by interacting with a specific cell surface receptor. This interaction results in activation of the adenylate cyclase system. The inner membrane-bound enzyme system catalyzes the production of adenosine 3',5'-cyclic monophosphate (cAMP) which is thought to be the intracellular mediator for the hormone. The growth of some well-differentiated thyroid cancers may be dependent on the presence of TSH. In other tumors, TSH deprivation is followed by no regression. In order to explain differences in the relative susceptibility of these thyroid neoplasms to hormonal manipulation, many investigators have evaluated the primary site of TSH action in experimentally generated thyroid tumors, as well as in human thyroid tumors.

Thus, permanent cell lines established from thyroid cancer could provide a clue to a better understanding of this disease. However, the difficulty in establishing well differentiated thyroid tumor cell lines in vitro is reflected by the limited number of successful reports in this field. A follicular carcinoma cell line (SMC R86 F1) has recently been established as a model for thyroid cancer research.
In this article, the establishment and in vitro and in vivo characterization of this cell line are described.

MATERIALS AND METHODS

Thyroid carcinoma
Thyroid carcinoma was resected from a 61-year-old Japanese female patient, who was referred to the Sapporo Medical College Hospital in 1986. Metastasis was found in the right ischiatic bone. Thyroidectomy was performed without any previous therapies. In the tumor, the presence of a primary follicular carcinoma was confirmed. Administration of 74 MBq of $^{131}$I and total body scintigraphy in the supine position were performed 4 weeks after thyroid hormone withdrawal. The metastatic lesion showed intense radioiodine activities (Fig. 1). At this time, serum thyroglobulin (hTg) and serum TSH levels were 32 µg/ml and 65 µIU/ml, respectively.

Cell culture
The tissue was cut as finely as possible, and the tumor pieces were then dispersed in 20 ml of Eagle’s medium (Nissui Pharma, Tokyo, Japan) supplemented with 10%, fetal bovine serum (FBS) (HyClone, Logan, Utah), immediately dispersed into 25 cm$^2$ flasks (Falcon 3013, Falcon Plastics, Oxnard CA), and incubated at 37°C in a humidified atmosphere of 5% CO$_2$ in air. During the subsequent 4 weeks of primary culture, sparsely populated colonies of small epithelioid cells attached to grew slowly, and they were surrounded by a considerable number of fibroblastic cells. During a further 5 weeks of primary culture, it became apparent that a homogeneous population of small epithelioid cells with dark cytoplasm had propagated and reached confluence. The cell line was passaged by treatment with 0.25% trypsin and 0.02% EDTA in PBS (—).

Measurement of cellular cyclic AMP (Short-term effect of TSH on cell culture)
Cyclic AMP was measured with a radioimmunoassay kit (E I Du Pont de Memours Inc, N. Billerica, MA). Confluent cell cultures were incubated with 0 to 1 mIU/ml of bTSH with 0.5 mM isobutylmethylxanthine, and control dishes were incubated with 0.5 mM isobutylmethylxanthine for 60 minutes at 37°C. Cells were lysed and the concentration of cAMP in the cytoplasm was measured. The protein content was determined with a Bio-Rad protein assay kit (Bio-Rad Laboratories, Richmond, CA) with BSA as the standard.

Fig. 1 Scintigrams of the patient following administration of 74 MBq of $^{131}$I. Radioiodine accumulations in the metastatic bone lesions and remnant thyroid gland are seen four weeks after total thyroidectomy (Serum TSH; 65 µIU/ml, Serum Tg; 32 µg/ml). (a): Head and neck (A-P), (b): Thorax and abdomen (A-P), (c): Abdomen and pelvis (A-P), (d): Pelvis (P-A). A-P: Anteroposterior view; P-A: Posteroanterior view
**Determination of cell growth**

A cell suspension of $1 \times 10^5$ cells (12th passaged cell line) was seeded into plastic Petri dishes (Falcon 3001, 35 x 10 mm) with 2 ml of the growth medium, which was replaced every day. A cell count was taken every 2 days in three dishes. The effect of TSH on cell growth was studied by the addition of 1.0 mU/ml of bovine TSH (bTSH Sigma Chemical Co, St Louis, MO).

**Radiolabeling of proteins in thyroid cell culture: preparation of cell extracts**

[$^{35}$S]methionine (41.8 TBq/mmol; E I Du Pont de Nemours Inc, N. Billerica, MA) was added to the basal medium (10 μCi/ml; Modified Eagle’s MEM) 2 hours before the end of the culture with bTSH (100 μU/ml) or without bTSH. Culture cells were extensively but quickly washed with PBS, then isolated by centrifugation and stored at -80°C. They were homogenized (10 x 10⁶ cells/0.6 ml) in PBS and mixed with hot trichloroacetic acid (TCA). Then the mixture was centrifuged for 5 minutes at 27,000 g. The precipitated protein fractions were trapped on a 0.5 μm glass filter, washed with cold 10% TCA solution and their radioactivity measured.

**Iodine uptake assay in vitro**

Radioactive iodine uptake studies were carried out in 50 ml plastic tubes containing 1.0 x 10⁶ SMC R86 F1 cells or an identical number of rat medullary carcinoma cells line (ATCC CRL1670). Varying amounts (cpm) of radioiodine were added to the cell culture medium and the cells were incubated for 24 hours. After gentle shaking of the tubes, 400 μl of the medium was transferred to microfuge plastic tubes. The experimental tubes were then centrifuged for 3 minutes at 1,000 rpm at room temperature. The supernatant was aspirated and discarded, and the pellets were counted in an auto-well gamma spectrometer (Shimadzu Co, Japan). The radioactivity in the pellet was expressed as a percentage of the total activity in the control tubes. At the end of each experiment the viability of the thyroid cells was more than 90% when judged by the Trypan Blue dye exclusion technique. In one experiment, thyroid cells were studied after the addition of 1 mIU/ml bTSH to the culture medium for 24 hours. In order to radioiodinate proteins of the cultured cells, $^{131}$I was added to the culture medium 24 hours before the end of the culture with bTSH (1 mIU/ml) for 10 days. The radioiodinated proteins were obtained as described below.

**Preparation of cellular soluble protein**

The cells (5.5 x 10⁶ cells) cultured with 1.0 mIU/ml bTSH for 10 days were suspended in 100 μl of PBS (pH 7.4) buffer containing 10 mM Tris-HCl (pH 7.4), 0.1% bovine serum albumin (BSA) and 1 mM phenylmethylsulfonyl fluoride, and then treated with a homogenizer (Sonifier 250, Branson Ultrasound Corporation). The homogenate was centrifuged at 800 g for 10 minutes and the pellets were discarded. The supernatant was centrifuged at 10,000 g for 20 minutes. The supernatant (30 μl; protein concentration: 450 μg/ml) was resuspended in the tracking buffer (15 μl; 1% glycerol, 10% 2-mercaptoethanol, 0.025% bromophenol blue and 50 mM Tris-HCl, pH 6.8) and incubated at 60°C for 15 minutes.

**Polyacrylamide gel electrophoresis**

14% polyacrylamide gel slabs were used for electrophoresis. The 45 μl of the sample (protein concentration: 300 μg/ml) and the mixture of normal hog thyroglobulin (hTg) and bovine serum albumin (BSA) were applied to the gel. The electrophoresis was done at 25 mA until the tracking dye reached the bottom of the gel. The gel slabs that were stained for protein were placed in 0.25% Coomassie blue dissolved in an aqueous solution containing 10% acetic acid and 50% methanol, and were destained with 7.5% acetic acid and 10% methanol. The radioiodinated proteins dialyzed against 1.15 M NaCl for 10 hours at 4°C were also subjected to electrophoresis and autoradiographic examinations. The gel slabs stained with Coomassie blue were applied to Fuji RX X-ray film and exposed for 4 weeks.

**Western blotting**

For western blotting, the protein after electrophoresis was transferred to nitrocellulose filters (Funakoshi Pharma Co Ltd, Tokyo, Japan) with a blotting set. Subsequently, the anti-hTg mouse monoclonal antibody (IgG1) to 19S glycoprotein (Sigma Chemical Co, St Louis, MO) diluted 1: 50 with PBS (–) was placed on the blots for 2 hours. The blots were washed well with PBS (–) and then incubated with horseradish peroxidase (HRP) conjugated goat antimouse immunoglobulin antibodies (Sigma Chemical Co). A 4-CN (chloro-1-naphthol) and hydrogen peroxidase mixture was used to develop the blots.

In vivo characterization of the cells

Inocula of $1 \times 10^6$ cells suspended in PBS were injected subcutaneously into about eight-week-old nude mice (BALB/cAJcl-nu) treated with $^{131}$I and untreated athymic nude mice. The treated mice were administered 3.7 MBq of $^{131}$I about 4 weeks before the cell inoculation for their thyroid ablation. Tumors in nude mice were removed for light microscopical examination. Human Tg levels in the serum of the tumor-bearing mice was measured by radioimmuno-
assay. The assay was carried out at Otsuka Assay Laboratories Ltd, Tokushima. The \textit{in vivo} characteristics of the cells were examined by using the 72nd passaged cell line.

\textbf{Histopathological examination}

The extirpated xenograft was embedded in paraffin, and stained with hematoxylin and eosin (H-E) for light microscopy. For immunohistochemical analysis, 4-micron paraffin-embedded tissue sections were deparaffinized in xylene, washed in PBS (−), and rehydrated. They were then processed for the detection of hTg. Staining was achieved by the avidin-biotin peroxidase complex (ABC) method. Endogenous peroxidase was removed by incubation in a mixture of 100 ml absolute methanol and 1 ml 30% hydrogen peroxide for 30 minutes. The slides were covered with normal horse serum. Subsequently, anti-hTg mouse monoclonal antibody to 27S, 19S, and 12S hTg (Sanbio Bv-biological Products, Uden, The Netherlands) was applied. Then, biotinylated anti-mouse antisera and ABC were successively applied for 30 minutes, according to the instructions with a vectstain ABC kit (Vector Laboratories, Burlingame, CA). Peroxidase activity was revealed in 0.1% deaminobenzidine tetrahydrochloride (Sigma Chemical Co, St Louis, MO) with 0.02% hydrogen peroxide in Tris-HCl buffer (50 mM, pH 7.6). Control sections were incubated with PBS in place of the monoclonal antibodies. For electron microscopy, tissues of the xenograft from the treated nude mice were fixed in 10% formalin PBS, postfixed in 1% osmium tetroxide in Millonig buffer, dehydrated in graded ethanol and embedded in Epon 812. Ultra thin sections were cut with a microtome, stained with uranyl acetate and lead citrate, and studied with an electron microscope.

\textbf{In vivo biodistribution study and radioiodine trapping of xenografts in nude mice}

For the scintigraphic and autoradiographic examinations, and the biodistribution study, radioiodine at doses of 3.7 MBq was injected into the peritoneum of \textsuperscript{125}I treated nude mice bearing xenografts at about 4 weeks after the cell inoculation. Under light anesthesia with ether or pentobarbital, scintigrams were obtained with a gamma camera equipped with a pinhole collimator 24 hours after the intraperitoneal injection of \textsuperscript{125}I. At that time, the mice were killed and viable parts of the xenografts and selected organs were removed, weighed, and counted for radioactivity. Biodistribution data were expressed as percentage of the injected dose per gram of tissue normalized to that of a 20-gram mouse ($\%$ ID/g). 8-micron tissue sections of the xenografts and the thyroid glands were applied to Fuji RX X-ray film, exposed for 4 weeks and then stained with hematoxylin and eosin (H-E).

\textbf{Statistical analysis}

In this study, data were analyzed by Student's t-test. For statistical comparisons, a p value of 0.05 or less was considered significant.

\begin{figure}[h]
\centering
\includegraphics[width=\textwidth]{fig2.pdf}
\caption{Long-term effect of TSH on cell growth. (a) The doubling time of the cell population was about 25 hours in the logarithmic growth phase without TSH. (b) TSH had no effect on cell division.}
\end{figure}
RESULTS

Growth characteristics
Growth curves of the cultured cells are shown in Figs. 2a and b. From the curves, the population doubling time of both TSH treated and untreated cells was estimated to be about 25 hours. TSH had no effect on cell division or cellular morphology.

Effect of TSH on cAMP, total protein synthesis and iodine uptake in vitro
Basal intracellular levels of cAMP in confluent cell culture were 7 to 10 pmol per mg of cell protein. The addition of 100 μIU to 1 mlU of bTSH per ml for 60 minutes caused a 30- to 50-fold increase in the intracellular content of cAMP (Table 1). [35S]methionine incorporation and 131I% uptake of the thyroid tumor cells lines are also summarized in Table 2. The mean methionine incorporation and iodine uptakes of the cells treated with bTSH were about twice that of the cells without bTSH (p<0.01 and p<0.05 respectively).

hTg production by and tumorigenicity of SMC R86 F1 cells
Electrophoretic analysis of soluble cellular proteins
The immunoprecipitate assays with anti 19S hTg MoAb show expressions of Tg in the cells cultured

<table>
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<th>Table 1 Effect of TSH on cellular cAMP accumulation</th>
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<td>Cyclic AMPa) (pmoles cAMP/mg protein)</td>
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<tr>
<td>SMC R86 F1 cell</td>
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<td>8.52±0.59</td>
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Mean±SD for triplicate cultures
b) Significantly different from the value for cells incubated in medium alone (p<0.01)

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<th>Table 2 Effect of bTSH on [35S]methionine incorporation into protein and iodine 131 uptake determined in monolayer culture</th>
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<td>[35S]methionine incorporationa) (c.p.m./106)</td>
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<tr>
<td>Cell line</td>
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<td>Treatment</td>
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<tr>
<td>Basal</td>
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<td>TSH</td>
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(N: 5)
MD: Rat medullary carcinoma cell line (ATCC CRL1670), a) Mean±SD, b) Significantly different from the value for cells incubated in medium alone (p<0.01), c) Significantly different from the value for cells incubated in medium alone (p<0.05), d) Not significant difference from the value for cells incubated in medium alone.
with 1.0 mIU/ml bTSH for 10 days (Fig. 3A). An autoradiogram (Fig. 3B) showed that the radioiodinated band agrees well with the protein band which indicates hTg.

Transplantation into nude mice
Subcutaneous transplantation of the cells into athymic nude mice led to the production of tumors that were clearly visible at 3 to 4 weeks. The larger tumors had a tendency to develop necrosis and degenerative changes. The solid tumors obtained

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Fig. 4  Light micrographs of the transplanted tumors in the $^{131}$I treated nude mouse. Trabecular growth pattern and microfollicular formation are evident in the sections. Intense immunoreaction with hTg in the cytoplasm of cells of the microfollicles is observed. The staining is weak and irregularly distributed in the poorly differentiated structure. (original magnification; ×190) (a): HE stain, (b): Immunostain for hTg (dil. 1:5).
from the $^{131}$I treated nude mice showed signs of multiple small follicle formation and immunoreactive hTg in the follicle formation tumor cells (Figs. 4a, b). The xenografts from untreated mice had a trabecular growth pattern not accompanied by structural differentiation, which was different from that of the original tumor. And that only weak cytoplasmic positivity for Tg was seen in some tumor cells of the tissues (data not shown). Electron-microscopic studies of the xenografts revealed prominent nuclei with large nucleoli and the presence of microvilli with interdigitations and follicular lumen within the cytoplasm. Many mitochondria and endoplasmic reticula were present (Fig. 5). The levels of serum hTg of tumor-bearing $^{131}$I treated mice were higher than those of the untreated mice (Table 3).

In vivo biodistribution study of radiiodine ($^{131}$I)
In scintigrams obtained after administrations of $^{131}$I, it was seen that the tumor trapped radioactive iodine (Fig. 6A). Autoradiogram (Fig. 6B) and biodistribution study revealed that viable tissue of the xenografts was able to trap radioactive iodine. The results of the in vivo localization of the injected radioiodine are shown in Table 4. However, scintigraphic examinations revealed insufficient thyroid ablation and the larger tumors with extensive

<table>
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<th>Table 3</th>
<th>Plasma hTg levels in nude mice bearing xenografts</th>
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<tr>
<td><strong>Nude mice</strong></td>
<td>Plasma hTg levels (ng/ml)</td>
</tr>
<tr>
<td>Untreated mice</td>
<td>$&lt;10.0$</td>
</tr>
<tr>
<td>(N=4)</td>
<td></td>
</tr>
<tr>
<td>Treated mice</td>
<td>$14.0\pm2.0$</td>
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<tr>
<td>(N=4)</td>
<td>$(10.5–18.6)$</td>
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a) Mean±SD of the value for four nude mice bearing xenografts

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<th>Table 4</th>
<th>Biodistribution of radiiodine in seven tumor bearing nude mice</th>
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<td>(24 hours after administration)</td>
<td>Organ</td>
</tr>
<tr>
<td></td>
<td>Thyroid</td>
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<tr>
<td>%injected dose/g$^a$</td>
<td>$6.75\pm3.7$</td>
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a) Mean±SD of the value for seven nude mice

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**Fig. 5** A transmission electron micrograph of a cell. Tumor cells with well-developed organelles characteristic of thyroid epithelial cells are surrounded by microvilli in apical membrane (Original magnification: $\times3300$). N: Nucleus, Nu: Nucleoli, M: Mitochondria, Mv: Microvilli, RER: Rough endoplasmic reticulum, D: Desmosome.
necrosis resulted in sending a lower radiiodine concentration into the xenografts.

**DISCUSSION**

It has been clearly established that patients with well-differentiated thyroid carcinoma and metastases have thyroid tissue which remains responsive to endogenous TSH and that this stimulation is manifested by the production of Tg\(^7\)\(^{-10}\) and the concentration of iodine.\(^{11,12}\) The proliferative effect of TSH on its targets cells and thyroid follicular epithelium in the intact animal is largely undisputed. However, the biological response to TSH on epithelial cells from abnormal tissue is controversial.\(^{13}\) The previous observations, in which freshly prepared tumor cells\(^{14,15}\)
retained the ability to respond to TSH in vitro, suggest that the adenylate cyclase system is involved in this stimulatory process. Only one report on the establishment of a well differentiated human thyroid carcinoma cell line has apparently been published to date. The tumor cell (UCLA RO 82 W-1) retained its capacity to secrete hTg in vivo, however, the cell lost the ability to trap radioactive iodine even after TSH stimulation in vitro. We have established a thyroid follicular adenocarcinoma cell line (SMC R86 F1) for thyroid cancer research and examined its in vitro and in vivo characteristics.

It is well established that cAMP appears to be a mediator of thyroid cell functions including stimulation or inhibition, depending upon the protein involved under the control of TSH. Using dog thyroid cells Rapoport showed that the net cAMP response to TSH represented a single function over the physiological TSH concentration. SMC R86 F1 cell cultures responded with a marked increase in cAMP and by increasing in the protein synthesis after the addition of bTSH. This indicates that SMC R86 F1 cells have a TSH-responsive adenylate cyclase system. Generally, TSH is considered important for the expression of differentiated functions of the normal and morbid thyroid tissues and also generally believed to be a trophic factor for the thyroid tissue in vivo. However, TSH does not stimulate the growth of the cells in nonconfluent cell culture and is considered important for the expression of differentiated function of the cells.

The solid tumors obtained from the 131I treated nude mice showed multiple small follicle formation and intense immunoreactive Tg in the follicle formation tumor cells. A transmission electron micrograph of the xenograft tissues revealed that the most individual cells with well-developed organelles characteristic of thyroid epithelial cells are surrounded microvilli in apical membrane, and were characterized by interdigitations and follicular lumen within the cytoplasm. In addition, the levels of serum hTg of tumor-bearing 131I treated mice were higher than those of the untreated mice. These observations provide strong evidence that the origin of this cell is the thyroid follicular epithelium and the cell retains well-differentiated properties. It may be considered that the effect of endogenous murine TSH on cell differentiation appeared.

Recently, Estour B and associates were the first to establish a differentiated thyroid tumor cell line (UCLA RO 82 W-1) derived from metastatic tissue from a patient with a microfollicular carcinoma of the thyroid. Although their established cells retained the capacity to synthesize Tg, the cells lacked the ability to trap radioactive iodine, indicating a certain degree of dedifferentiation. SDS-PAGE, western blotting and autoradiographic examinations of the soluble cellular proteins revealed that our established cells possessed the ability to trap and organize radioactive iodine in vitro. Scintigrams obtained 24 hours after the administrations of 131I and autoradiograms also revealed that the xenografts in 131I treated mice concentrate radioiodine. The biodistribution study revealed that the percentage of the injected dose per gram of viable tissues was 12.0%. It seems to be quite all right to consider that SMC R86 F1 xenografts are able to trap and organize radioactive iodine.

The tumors of the follicular epithelium of the thyroid gland represent various stages of tumor progression. Benign follicular adenomas are considered to progress at low frequency to follicular carcinomas. These carcinomas may progress to rarely occurring undifferentiated carcinomas, and thyroid differentiated cancer sometimes shows anaplastic transformation during its course. Thus, thyroid neoplasms show a wide range of lesions varying from slowly progressive well-differentiated tumors to anaplastic highly malignant neoplasms. Valenta and associates assayed Tg of surgically resected tissue samples in order to investigate possible abnormalities in Tg in carcinomas of the thyroid by electrophoresis and ultracentrifugation methods. They reported that 12S and components heavier than 19S were not detectable in functioning carcinoma and that the 12S and heavier than 19S components were mainly present in adenomas and functioning carcinomas. In the cytoplasm of the TSH treated monolayer cultured cells, expression of 19S Tg was also found unrelated to the cell proliferation. Higher expression of 12S, 19S and 27S Tg occurred in the cell cytoplasm of the tissues with differentiated structures than in the cell cytoplasm of those with dedifferentiated structures. Therefore, it is considered that the established cell line possesses highly differentiated characteristics under the control of TSH in vitro and in vivo and it is suggested that the viable and morphologically differentiated tissue was able to trap and organize iodine. The results of in vitro and in vivo studies given above are consistent with the idea that Tg in differentiated thyroid cancer may differ quantitatively from Tg in poorly differentiated tissue20,21 under the control of TSH. Moreover, these results also indicate the possibility that well-differentiated properties of the cell are induced by TSH action in the malignant thyroid follicular epithelium, but the cell growth is not.

Difficulty in establishing a differentiated thyroid tumor cell line has prevented us from obtaining a new interpretation of the behavior of thyroid malignancy. Therefore, permanent well-differentiated cell lines established from human thyroid cancer could provide clues to a better understanding of this dis-
case. In addition, the animal model presented in this article would be useful for the quantification of the radiation absorbed dose delivered by the therapeutic activities of administered radiiodine to lesions in well-differentiated thyroid carcinoma and of radiiodinated immunoglobulin to lesions in various malignant disease.

In conclusion, the investigation of in vitro and in vivo characterization of the established follicular carcinoma cell line is summarized above. Under the control of TSH, well-differentiated properties of the malignant thyroid follicular cell may be induced by TSH action. The original cell line, designated SMC R86 F1, has been continuously propagated by serial subcultures during the past 60 months and possesses highly-differentiated characteristics.

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REFERENCES

7. Van Herle AJ, Uller RP: Elevated serum thyro-


Takatoshi Tsuda

Annals of Nuclear Medicine