Production of multiple cytokines and induction of cachexia in athymic nude mice by a new anaplastic thyroid carcinoma cell line

J W-C Chang¹, K-Y Yeh¹, Y-C Shen^{1,2}, J-J Hsieh¹, C-K Chuang^{3,4}, S-K Liao⁴, L-H Tsai⁵ and C-H Wang^{1,2}

¹Department of Hematology/Oncology, Chang Gung Memorial Hospital, Taoyuan 333, Taiwan

²Department of Internal Medicine, Chang Gung Memorial Hospital, Keelung 204, Taiwan

³Department of Urology, Chang Gung Memorial Hospital, Taoyuan 333, Taiwan

⁴Graduate Institute of Clinical Medicine, Chang Gung University, Taoyuan 333, Taiwan

⁵Research Division, Show Chwun Memorial Hospital, Changhua 500, Taiwan

(Requests for offprints should be addressed to C-H Wang, Department of Internal Medicine, Chang Gung Memorial Hospital, 222 Mai Chin Road, Keelung 204, Taiwan; Email: chwang@adm.cgmh.org.tw)

Abstract

An anaplastic thyroid cancer cell line, Thena, was recently established in our laboratory following radical thyroidectomy of a patient with anaplastic thyroid cancer. Microscopically, Thena cells were spindle-shaped or small round cells. Thena cells were reactive with cytokeratin AE1/AE3 antibodies, epithelial membrane antigen, interleukin (IL)-6, epithelial growth factor receptor, transforming growth factor (TGF)- α , vascular endothelial growth factor, and vimentin. Thena cells secreted high levels of IL-6, leukemia inhibitor factor (LIF), tumor necrosis factor (TNF)- α , and TGF- β 1 in the culture supernatants, as

determined by enzyme-linked immunosorbent assay. When subcutaneously injected with Thena cells, athymic nude mice developed tumor masses in the skin within 2 weeks. Furthermore, Thena cells induced cachexia in these tumor-bearing mice. High levels of human IL-6, LIF and TGF- β 1 were detected in the mouse sera. To our knowledge, the Thena cell line is the first thyroid cancer cell line reported to induce cachexia in nude mice. This cachectic animal model is worthy of further study to explore the treatment of thyroid cancer-induced cachexia.

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Introduction

Anaplastic thyroid carcinoma (ATC) is one of the most aggressive neoplasms in humans. The median survival is less than 1 year after initial diagnosis. It commonly occurs in the elderly. Patients suffering from ATC often have a long history of goiter. Dysphagia, dysphonia, respiratory tract compression and cervical pain are the most common complaints of ATC patients. The common presence of admixed foci of differentiated thyroid cancer in ATC suggests that it arises through the mechanism of 'dedifferentiation'. The expression of keratin by immunohistochemical staining further supports this argument (see Lampertico 1993 for review). Multimodality treatment combining chemotherapy, radiotherapy and surgery for patients with anaplastic carcinoma have not been efficient in terms of disease-free and overall survival. The disease also causes cachexia and airway compression, resulting in patient morbidity and mortality. Therefore, a better understanding of the tumor biology of ATC may lead to novel treatment of this devastating disease.

Several growth factors were reported to stimulate growth of normal thyroid epithelial cells. De-regulation of these factors and/or their receptors may lead to the growth

of thyroid tumors. Cultured normal thyroid follicular cells constitutively produce high levels of interleukin (IL)-6 and IL-8 (Weetman *et al.* 1992). Reduced IL-6 expression has been demonstrated in anaplastic thyroid carcinomas (Basolo *et al.* 1998). Over-expression of transforming growth factor- α (TGF- α) has been implicated in thyroid tumors. By binding to its receptor, epidermal growth factor receptor (EGFR), TGF- α acted to stimulate tumor growth by an autocrine mechanism (Haugen *et al.* 1993). Aberrant activation of EGFR leading to activation of the Met gene has also been indicated in association with the malignant phenotype of anaplastic thyroid carcinomas (Bergstrom *et al.* 2000). Transforming growth factor- β (TGF- β) inhibits the growth of most epithelial cells including cancer cells. Irresponsible to negative regulation by TGF- β has been implicated in the progression of epithelial tumors (Blaydes & Wynford-Thomas 1996). Loss of TGF- β type II receptor has also been demonstrated to contribute to the progression of well-differentiated thyroid carcinomas toward undifferentiated anaplastic ones (Lazzereschi *et al.* 1997).

Cultured cells and cell lines provide a useful means to study the molecular event and regulatory mechanism of

cell growth and also the neoplastic progression of this type of cancer. Recently, we established a new thyroid carcinoma cell line, Thena, following thyroidectomy of a patient with anaplastic thyroid carcinoma. In this report, we analyze the morphological features, immunophenotypic characteristics, and cytokine production of this cell line. A cachectic model has also been established in nude mice bearing Thena cells. To our knowledge, Thena is the first thyroid carcinoma cell line reported to induce cachexia in athymic nude mice. Further studies using Thena cells to explore the mechanisms and treatment in cancer-induced cachexia are warranted.

Materials and Methods

Patient

A 75-year-old woman presented with a thyroid mass and was admitted to Chang Gung Memorial Hospital. She had had the mass for more than 10 years. The mass had enlarged rapidly in the 6 months before admission to the hospital, leading to neck pain, erythema, and airway compression. Poor appetite and body weight loss was also noted. Palliative resection of the tumor to relieve symptoms was performed. The tissue was frozen in liquid nitrogen or fixed in formalin for histopathological diagnosis and immunohistochemical staining. Part of the specimen was transferred to our laboratory in complete medium for subsequent processing.

Cell line establishment and culture conditions

The thyroid tumor specimen, obtained by thyroidectomy with the prior informed consent of the patient, was minced mechanically with a pair of scissors. Single cells were seeded into a T-25 flask (Nunc, Naperville, IL, USA) in RPMI 1640 medium (Life Technologies, Gaithersburg, MD, USA) supplemented with 10% fetal bovine serum (FBS; HyClone, Logan, UT, USA), 1% L-glutamine, 1% sodium pyruvate, 100 U/ml penicillin, and 50 µg/ml streptomycin (Life Technologies). Cells were incubated at 37 °C in an incubator supplied with 5% humidified CO₂. Tumor colonies were observed 1 week after seeding, surrounded by fibroblasts. Occasionally, fibroblasts were depleted by light trypsin-EDTA (0.02%, Life Technologies). Intermittently, cells were resuspended in complete medium containing 10% dimethyl sulfoxide (DMSO; Sigma, St Louis, MO, USA) and frozen in liquid nitrogen for further use. Four thyroid carcinoma cell lines, ARO, CGTH-w1, SW579 and WRO were maintained in RPMI complete medium as described above. ARO, SW579, WRO (all from the American Type Culture Collection, Rockville, MD, USA) and CGTH-w1 (Lin *et al.* 1998) cells were kindly provided by Dr Jen-Der Lin, Department of Metabolism, Chang Gung Memorial

Hospital. ARO is an anaplastic thyroid carcinoma cell line and SW579 is a poorly differentiated papillary thyroid carcinoma cell line; CGTH-w1 and WRO are follicular thyroid carcinoma cell lines.

Morphological analysis by electron microscopy

Cells grown in 3-cm dishes were fixed with 2.5% glutaraldehyde in phosphate-buffered saline (PBS) for 2 h at 4 °C. After being washed with PBS, cells were post-fixed with 1% osmium tetroxide in 0.1 mol/l cacodylate buffer and embedded in epoxy resin (Nacalai Tesque, Kyoto, Japan). They were cut into thin sections with a Reichert ultracutter (Leica, Germany), stained with uranyl acetate and lead citrate (Electron Microscopy Sciences, Fort Washington, PA, USA) and examined with an H-7500 electron microscope (Hitachi, Tokyo, Japan).

In vitro cell growth

Cells were seeded into wells of 6-well plates (Nunc) at 2×10^5 cells/well in 2 ml complete RPMI medium. Triplicate wells were trypsinized every other day. Cell numbers were counted using the Trypan Blue exclusion method. Culture supernatants were collected and frozen at -80 °C for the cytokine production assay. The population doubling time was estimated at the log phase of the growth curve.

Immunohistochemical staining

Cells were seeded into wells of Lab-Tek chamber slides (Nunc) at 5×10^4 cells per well and incubated for 24 h. Immunohistochemical staining was performed according to the manufacturer's instructions (Vector, Burlingame, CA, USA). In brief, cells were fixed with glacial acetone and air-dried. The following antibodies, AE1/AE3 (Signet, Dedham, MA, USA), carcinoembryonic antigen (CEA; Novacastra, Newcastle, UK), cytokeratin (CK) -7, -8, -18, -19, and -20 (all from Dako, Glostrup, Denmark), CD40 (BD Pharmingen, San Jose, CA, USA), CD44 (Bender MedSystems, Vienna, Austria), desmin (Dako), E-cadherin (Biodesign, Saco, ME, USA), EGFR (Santa Cruz Biotechnology Inc., Santa Cruz, CA, USA), epithelial membrane antigen (EMA; Dako), human chorionic gonadotropin (β -hCG; Signet), intracellular adhesion molecule (ICAM-1; Dako), proliferating cell nuclear antigen (PCNA; Dako), TGF- α (Santa Cruz), thyroglobulin (Dako) thyroid-stimulating hormone receptor (TSH-R; Santa Cruz) and vascular endothelial growth factor (VEGF) (BD Pharmingen), were added and incubated for 1 h after blocking the wells with 1:5000 diluted normal horse serum. Anti-HLA-ABC antibody (W6/32, Dako) and normal mouse serum served as the positive and negative controls respectively. After three washes, the appropriate biotin-conjugated secondary antibodies were

added and further incubated for 1 h. An avidin-biotin complex (ABC Elite, Vector) was applied followed by the addition of 3-amino-9-ethylcarbazole (AEC; Sigma) to achieve a permanent color change in the reactive cells. Cells were counterstained with hematoxylin (Gill's formula #3, Electron Microscopy Sciences).

Cytokine production

Levels of cytokines secreted by Thena cells in *in vitro* culture, including IL-6, IL-10, leukemia inhibitor factor (LIF), TGF- α , TGF- β 1, and tumor necrosis factor- α (TNF- α), were determined by an enzyme-linked immunosorbent assay (ELISA) method, following the instructions of the manufacturer. All the assay kits were from R&D Systems (Minneapolis, MN, USA) except for TGF- α , which was from Oncogene (Boston, MA, USA). The sensitivities of these assay kits were as follows: IL-6, 0.2 pg/ml; LIF, 8 pg/ml; TGF- α , 2.1 pg/ml; TGF- β 1, 7 pg/ml; and TNF- α , 4.4 pg/ml. In brief, culture supernatants collected from 72-h cultures were added to a 96-well plate pre-coated with anti-cytokine antibodies and incubated for 2 h. After washing three times with wash buffer, horseradish peroxidase-conjugated secondary antibody was added and further incubated for 2 h. A chromogen (tetramethylbenzidine and hydrogen peroxide mixed at 1:1) was then added for color formation. The reaction was stopped by the addition of 1 M H₂SO₄. Results were read with a Dynatech MR5000 ELISA reader (ThermoLabsystems, Chantilly, VA, USA) at 450 nm with correction at 570 nm.

Xenotransplantation and tumor cachectic model in nude mice

Nude mice (Balb/c nu/nu) were purchased from the Animal Facility of the National Science Council (NSC), Taiwan. They were housed in an isolated pathogen-free cage and were fed with UV-sterilized pellets and autoclaved distilled water. Thena cells were harvested by trypsinization and were washed twice with PBS. Mice were subcutaneously injected with 5×10^6 Thena cells resuspended in 0.1 ml PBS at the right hind leg. Tumor growth was monitored every other day and the sizes of tumors were measured with a caliper. Body weight was measured using a digital scale once a week after mice had been anesthetized with ketamine (5 mg/kg). All procedures used in this animal study were performed in accordance with institutional guidelines for animal care at Chang Gung Memorial Hospital.

Statistical analysis

All values are expressed as the mean $\pm$ S.E. Student's *t*-test was used to compare the differences in all categories. *P* values less than 0.05 were considered statistically significant.

Figure 1 (A) Hematoxylin and eosin staining of primary tumor specimen. The tumor mass was composed of some papillary tumor cells (arrows) with a majority of anaplastic components. (B) Phase contrast microphotograph of Thena cells *in vitro*. Thena cells displayed polygonal or spindle-shaped epithelial cell morphology. Osteoclast-like giant cells were occasionally seen in early culture. However, they were eliminated following several passages (original magnification, $\times 100$).

Results

Isolation and characterization of Thena cells

Hematoxylin and eosin (H&E) staining showed that the tumor mass was composed of some well-differentiated papillary cancer cells, with a majority of anaplastic cancer cells (Fig. 1A). Intensive infiltration of granulocytes was observed. Extensive necrosis and areas of trabecular pattern were seen. Numerous osteoclast-like giant cells were also observed. Immunohistochemical staining of the primary tumor was strongly positive for cytokeratin AE1/AE3, but negative for leukocyte common antigen (LCA); thyroglobulin was negatively stained (data not shown).

Single cells obtained by mincing the tumor specimen mechanically were seeded into a T-25 flask in RPMI complete medium. Following the observation of tumor colonies, contaminated fibroblasts were depleted by light trypsinization (0.02%). After five passages, pure cancer cells were obtained. This anaplastic thyroid carcinoma cell

Figure 2 Electron microphotograph of Thena cells. (A) Abundant electron-dense material (inset, $\times 50\,000$) was found along the intercellular junction (original magnification, $\times 3\,500$). (B) Plenty of microvilli and mitochondria, well-developed endoplasmic reticulum, and cytoplasmic invagination were observed (original magnification, $\times 20\,000$)

line was named Thena. Morphologically, Thena cells showed a polygonal or spindle-shaped epithelial cell morphology under phase contrast microscopy and the nucleus to cytoplasm ratio was large, as illustrated in Fig. 1B. Thena cells firmly attached to the bottom of the flask. A longer period of trypsinization was required (>5 min) to harvest the cells. Thena cells were maintained *in vitro* for more than 40 passages. The population doubling time was 29.7 h.

Electron microscopic study revealed well-developed endoplasmic reticulum, numerous mitochondria, and cytoplasmic invagination in Thena cells. Plenty of microvilli were observed on the luminal surface of the tumor cells (Fig. 2B). Electron-dense materials were found along the cell membrane of the intercellular junction (Fig. 2A and insert). These morphological characteristics of Thena cells are similar to those of undifferentiated thyroid carcinoma cells.

Immunohistochemical staining showed that Thena cells were positively reactive to antibodies against AE1/AE3, CK-7, CK-8, CK-18, CD40, CD44, EGFR, EMA,

β -hCG, ICAM-1, PCNA, TGF- α and VEGF, but stained negatively with antibodies to CEA, CK-19, CK-20, desmin, E-cadherin, thyroglobulin and TSH-R. Some staining patterns are illustrated in Fig. 3.

Cytokine production

Culture supernatants of Thena were tested for the release of IL-6, IL-10, LIF, TGF- α , TGF- β 1, and TNF- α . Three independent experiments were performed. A set of representative data is depicted in Table 1. LIF and TNF- α were only detected in Thena cells. TNF- α secretion reached ng/ml concentrations. Both ARO and Thena cells released mild amounts of TGF- α into the culture supernatants. The production of IL-6 by Thena cells was extremely high, with 20-fold more than that of other cell lines tested. All cell lines secreted TGF- β 1. Cytokine secretion by ARO cells was the lowest among these cell lines, with the exception of TGF- α . IL-10 was not detected in any of the cell lines.

Induction of cachexia in nude mice

Tumor growth ($>3 \times 3$ mm) was observed within 2 weeks (10–14 days) in nude mice injected subcutaneously with Thena cells. Rapid tumor growth was seen in the first month, followed by a slow growth. Cachexia syndrome was observed in 4 of 4 Thena-injected mice 4 weeks after the inoculation. Weight loss proceeded after tumor growth reaching plateau. Mice were killed 1 month after the observation of cachexia syndrome (Fig. 4A). At this point, the average of the largest diameter of tumors was 13.8 mm (12–15 mm); however, the average body weight of the mice had decreased by 23% compared with that of age-matched controls (21.75 ± 1.69 g, $n=4$ vs 28.28 ± 0.86 g, $n=3$, $P<0.01$). High levels of human IL-6 (502.4 ± 82.5 pg/ml), LIF (764.5 ± 138.7 pg/ml) and TGF- β 1 (82.7 ± 12.4 ng/ml) were detected in the serum of the cachectic mice. H&E staining of tumor sections revealed the appearance of anaplastic thyroid cancer cells (Fig. 4B). Cells derived from nude mouse xenografts (Thena-Nu) secreted even higher levels of cytokines than their parental counterparts, except for TGF- β 1 (Table 1) ($P>0.05$ for TGF- β 1; $P<0.05$ for TGF- α ; $P<0.01$ for IL-6, LIF, and TNF- α).

Discussion

We established an anaplastic thyroid carcinoma cell line, Thena. Thena cells produced high levels of growth factors in culture supernatants, including IL-6, LIF, TGF- β 1, and TNF- α (Table 1). Thena cells exhibited a very aggressive growth in nude mice, accompanied by the induction of cancer cachexia (Fig. 4A). Small tumor burden indicated soluble factors secreted by Thena cells might contribute to

Figure 3 Immunohistochemical staining of Thena cells. Thena cells were positively reactive with antibodies against (A) AE1/AE3, (B) EGFR, and (C) ICAM-1 but were negatively stained with antibody to thyroglobulin (D) (original magnification, $\times 100$).

this paraneoplastic syndrome. High levels of IL-6, LIF and TGF- β 1 were detected in the serum of these cachectic mice, indicating that these three cytokines might play an important role in the induction of cachexia in this mouse model.

The expression of cytokines in the development and progression of human epithelial tumors is only partially defined. In thyroid carcinoma, the existence of an inverse relationship between IL-6 production and tumor aggressiveness was demonstrated (Basolo *et al.* 1998).

Table 1 Production of soluble growth factors by thyroid carcinoma cell lines. Supernatants were collected from 72-h cultures. Results for IL-6, TGF- β 1, LIF, and TNF- α are expressed as ng/ 10^6 cells; results for TGF- α are expressed as pg/ 10^6 cells. Experiments were performed in triplicate

	Cell line					
	ARO	SW579	CGTH-w1	WRO	Thena	Thena-Nu*
IL-6	<0.1	5.28 $\pm$ 0.32	2.32 $\pm$ 0.21	4.61 $\pm$ 0.51	110.53 $\pm$ 3.62	>160
TGF- β 1	2.00 $\pm$ 0.21	4.91 $\pm$ 0.63	3.43 $\pm$ 0.32	1.21 $\pm$ 0.12	8.12 $\pm$ 1.20	6.73 $\pm$ 0.92
LIF	UD	UD	UD	UD	0.71 $\pm$ 0.06	2.68 $\pm$ 0.19
TGF- α	99.0 $\pm$ 10.5	UD	UD	UD	115.7 $\pm$ 10.2	162.3 $\pm$ 15.3
TNF- α	UD	UD	UD	UD	1.06 $\pm$ 0.10	1.82 $\pm$ 0.21

*Cells derived from nude mouse xenografts.

UD, undetectable, below the minimum detection limit of the kit.

Figure 4 (A) Induction of cachexia by Thena cells. Athymic nude mice were injected s.c. with Thena cells at 5×10^6 in 0.1 ml PBS. Tumor growth was observed 10 days after the injection (arrow). Four weeks later, mice started to lose weight. This photo was taken 8 weeks after tumor inoculation. A normal age-matched mouse was included for comparison. (B) Histochemical staining of a tumor section revealed the typical morphology of undifferentiated tumor cells (original magnification, $\times 200$).

Nonetheless, our results did not correlate with such observations. Thena cells secreted an extraordinarily high level of IL-6 in the culture supernatants, whereas the follicular thyroid cancer cell lines CGTH-w1 and WRO secreted much lower levels of IL-6 (Table 1). Additionally, cells derived from the nude mouse xenograft (Thena-Nu), which were interpreted as fully anaplastic thyroid carcinoma cells histochemically, secreted an even higher amount of IL-6 (>160 ng/ 10^6 cells) than that of their parental cells. The discrepancies between these studies may be due to the different cell lines employed. For example, the poorly differentiated papillary thyroid carcinoma cell line, SW579, produced higher IL-6 than CGTH-w1, a well-differentiated follicular thyroid carcinoma cell line ($P < 0.01$).

TGF- α is not expressed in normal thyroid tissues. Increased TGF- α expression has been associated with malignant transformation of thyroid cells (Haugen *et al.* 1993). TGF- α was detected in Thena cells. Cells were also stained positively with antibody to EGFR in the membrane and cytoplasm (Fig. 3B). Cells derived from nude mouse xenografts secreted an even higher level of TGF- α in the culture supernatants (162.3 ± 15.3 pg/ 10^6 cells vs 115.7 ± 10.2 pg/ 10^6 cells, $P < 0.05$) and exhibited a shorter population doubling time (26.8 h vs 29.7 h) compared with that of their parental cells. These results indicate that the TGF- α /EGFR autocrine loop is in operation in Thena cells and this autocrine mechanism may result in the progression of the disease (Nilsson *et al.* 1995).

Thena cells exhibited a short lag phase, with rapid tumor growth in nude mice as compared with other well-differentiated thyroid carcinoma cell lines described in the literature. Thena cells produced measurable tumors in nude mice (>3 mm in diameter) within 2 weeks, whereas WRO, a follicular thyroid carcinoma cell line, produced tumors 4 months after the injection (Estour *et al.* 1989). B-CPAP, a papillary thyroid carcinoma cell line, developed tumors of 0.5 mm in diameter 8 weeks after the inoculation (Fabien *et al.* 1994). Direct comparisons between Thena and these cell lines would be more ideal to conclude these results. The *in vivo* growth of Thena cells was comparable to other established anaplastic thyroid carcinoma cell lines (Dahlman *et al.* 2002). Nevertheless, none of the published thyroid carcinoma cell lines have been reported to induce cancer cachexia in nude mice.

Cancer-induced cachexia may be due to endogenous or tumor-associated factors. Several cytokines are involved in the process of this paraneoplastic syndrome, such as TNF- α (Yoneda *et al.* 1991), TGF- $\beta 1$ (Zugmaier *et al.* 1991), interferon- γ (Matthys *et al.* 1991), IL-1 (Gelin *et al.* 1991), IL-6 (Tamura *et al.* 1995) and LIF (Mori *et al.* 1991). Specific neutralization of these mediators with antibodies in animal models of cachexia confirmed the involvement of these cytokines in cancer cachexia.

IL-6 was reported to reduce adipose lipoprotein lipase activity, leading to the loss of body fat storage in mice (Greenberg *et al.* 1992). High human IL-6 was detected in the serum of Thena-bearing mice, indicating that IL-6 should be involved in this cachectic animal model. However, the presence of IL-6 in the serum of mice bearing non-cachexia-inducing tumors (Fujimoto-Ouchi *et al.* 1995) suggests that other mediators may also participate in the development of cancer cachexia.

LIF was reported to induce cachexia in animal models with human melanoma cell lines (Mori *et al.* 1991, Iseki *et al.* 1995). So far, there are only a few anaplastic thyroid carcinoma cell lines that are reported to secrete LIF. For example, KTC-1 cells (Kurebayashi *et al.* 2000) secreted much lower levels of LIF (152 ± 14 pg/ 10^6 cells/48 h) in the culture supernatants compared with those of Thena

cells. Although both the seeding number and culture period were different in these two studies, KTC-1 cells did not induce cachexia in the nude mouse. LIF was 100-fold more potent than IL-6 for inhibition of lipoprotein lipase activity (Berg *et al.* 1994). High human LIF was detected in Thena-bearing cachectic mouse sera, suggesting that LIF could play a more critical role than IL-6 in this model.

Thena cells also secreted high levels of TGF- β 1 *in vitro*. It is suggested that the de-regulation of TGF- β 1 by well-differentiated thyroid carcinoma cells might result in anaplastic changes (Blaydes & Wynford-Thomas 1996). The involvement of TGF- β 1 in cachexia was demonstrated by injecting recombinant protein into mice instead of TGF- β 1-producing tumor cells (Zugmaier *et al.* 1991). Although high TGF- β 1 content was detected in the serum of Thena-injected nude mice (82.7 ng/ml), whether TGF- β 1 plays a role in this cachectic animal model needs further investigation.

Our data add further support to the suggestion that no single cytokine is responsible for all of the abnormalities contributing to cachexia (Soda *et al.* 1995). Although TNF- α was not detected in the mouse serum, it should not be excluded from the list of possible cachexia mediators in this animal model, given the existence of high TNF- α levels in the culture supernatants of both Thena and Thena-Nu cells (Table 1). The discrepancy may result from rapid clearance by other proteins *in vivo*.

The expression of TNF- α has been demonstrated in thyroid tissues and thyroid carcinoma cell lines (Aust *et al.* 1996); however, it was seldom detected as a soluble factor in thyroid carcinoma cells. Trace amounts of TNF- α (<5 pg/ml) were detected in only two ATC cell cultures (Aust *et al.* 1996, Sato *et al.* 2000). Thena cells might be the first thyroid carcinoma cells reported to produce high TNF- α *in vitro*.

TNF- α may function as an autocrine factor for Thena cells, endowing their resistance to TNF- α (Takeyama *et al.* 1991). The biological functions of TNF- α in Thena cells may also involve the upregulation of adhesion molecules (Bassi *et al.* 1995), which could be reflected in the firm attachment to culture vessels *in vitro*, and factors associated with metastatic potential (Mauceri *et al.* 2002), conferring their proliferative advantages. Further efforts to identify the biological functions and the regulation of TNF- α in Thena cells are presently underway.

While the involvement of TNF- α in our model of cancer cachexia is not verified, it may induce this paraneoplastic syndrome by influencing the expression of other mediators. IL-1 β , although undetectable in the culture supernatants, showed an even greater expression than that of IL-6 by ribonuclease protection assay (data not shown). Since both IL-1 β and TNF- α have been shown to upregulate the expression of IL-6 and LIF (Billingsley *et al.* 1996), they may act indirectly to induce cachexia in our animal model.

In conclusion, we established an anaplastic thyroid carcinoma cell line, Thena. This new cell line may provide a good model to study the interplay of cytokines in the pathogenesis of thyroid cancer and cancer-induced cachexia.

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