RAPID COMMUNICATION Jpn. J. Cancer Res. 82, 12–18, January 1991

A Pulmonary Large Cell Carcinoma Cell Line Expressing Neuroendocrine Cell Markers and Human Chorionic Gonadotropin α-Subunit

Kiyoshi Kasai,[1] Toru Kameya,[1] Kuniko Kadoya[1] and Chieki Wada[2]

Departments of [1]Pathology and [2]Clinical Pathology, School of Medicine, Kitasato University, Kitasato 1-15-1, Sagamihara, Kanagawa 228

A cell line producing the neuroendocrine cell surface antigen and human chorionic gonadotropin (hCG) α-subunit, designated as KTA7, was established from human large cell carcinoma using a serum-free medium, ACL-3. KTA7 continued to grow in the ACL-3 medium, showing the morphological characteristics of large cell undifferentiated carcinoma. The KTA7 cells reacted with antibodies such as 6H7 and MOC1 directed against the cell surface antigens and PGP9.5 directed against a cytoplasmic protein of neuroendocrine cells but did not possess either most epithelial markers other than low-molecular-weight keratin (Cytokeratin) or neuron-specific enolase. The KTA7 cells, by immunostaining with anti-hCG subunit antibodies, were shown to produce hCG α- but not β-subunit. Northern blot analysis showed KTA7 RNA to synthesize hCG α-subunit mRNA but not that of the hCG β-subunit. Thus, the hCG α-subunit alone was independently expressed in KTA7. Chromosome analysis showed loss of alleles of chromosome 3p and 17 in KTA7 cells but not loss of 13q. KTA7 was considered to be derived from large cell undifferentiated carcinoma with neuroendocrine differentiation (large cell neuroendocrine tumor) and thus may fine use in studies on the pathobiology of large cell-type neuroendocrine tumors since it expresses at the same time marker substances of neuroendocrine differentiation and the hCG α-subunit.

Key words: Pulmonary large cell carcinoma cell line — Neuroendocrine tumor — hCG α-subunit — Cluster I antigen of small cell carcinoma — Serum-free medium

Small cell carcinoma of the lung (SCCL) has neuroendocrine properties; neuroendocrine features in non-SCCL have also been observed and multidifferentiations such as mixed neuroendocrine and epithelial features in lung cancers have been described.[1-3] Various neuroendocrine markers including peptide hormones,[1,4] enzymes of the APUD cell system[5] and monoclonal antibodies against surface antigens of small cell carcinomas[6-8] have provided clarification of the neuroendocrine features in lung cancers. Although cultured SCCLs in serum-free medium showed morphological, biochemical and neuroendocrine properties, along with the ability to differentiate *in vitro*,[9] the neuroendocrine features of non-SCCL cell lines have yet to be described in detail. A cell line derived from non-SCCL was established using ACL-3, which was developed as a serum-free medium for a non-SCCL cell culture,[10] and its neuroendocrine features were determined. The present study was conducted to establish its morphological and molecular characteristics.

Tumor tissue was obtained at surgical operation on a 46-year-old man with lung cancer. The histology of the original lung tumor was adenocarcinoma mixed with foci of large cell carcinoma. The tumor specimen was cut into small pieces with fine scissors. Cells of the lung tumor and the small tumor pieces were placed in 25-cm^2 tissue culture dishes and cultured in ACL-3 medium supplemented with RPMI1640, 20 μg/ml insulin, 10 μg/ml transferrin, 50 n*M* hydrocortisone, 25 n*M* sodium selenite, 10 μg/ml epidermal growth factor, 100 p*M* triiodothyronine, 2 m*M* glutamine and 5 mg/ml bovine serum albumin. Tumor cells were examined by phase contrast microscopy and cytology. Two milliliters of the medium was added to each culture twice a week. The tumor cells continued to grow in ACL-3 medium in 25 cm^2 culture flasks (Corning, New York) and the established line was designated as KTA7. They were then transplanted subcutaneously into the backs of 5- to 8-week-old BALB/c female nude (*nu/nu*) mice (Clea Japan Inc., Tokyo). Serial transplantations of the tumor were similarly carried out. The transplanted tumors were fixed in 10% formalin and embedded in paraffin. Cultured KTA7 cells were cytocentrifuged and fixed immediately for 5 min in cold acetone or 10% formalin. Paraffin-embedded sections or cytocentrifuged KTA7 cells were stained with hematoxylin and eosin or immunostained by the avidin-biotin-complex method as described previously.[3] The following antibodies were used for immunohistochemistry. Antibodies reactive with epithelial membrane antigen (EMA), carcinoembryonic antigen (CEA), secretory component (SC), keratin (polyclonal antibody), α-fetoprotein (AFP) and

neuron-specific enolase (NSE) were purchased from DAKOpatts, Copenhagen. Anti-Cytokeratin monoclonal antibody reacting with low-molecular-weight keratin was purchased from Becton Dickinson, Mountain View, CA.[11] Monoclonal antibodies (MAbs) reacting with sialosylated Lewis^x (SLEX) or sialosylated Lewis^a (SLEA) were from UCLA Tissue Typing Laboratory, Los Angeles, CA.[12,13] MAb against a surface antigen of neuroendocrine tumors and tissues, 6H7, was developed in our laboratory.[8] MOC1 reactive with small cell carcinoma was purchased from Daiichi Seikagaku Kogyo, Tokyo.[14] Anti-hCG α monoclonal antibody was purchased from ICN Immunobiologicals, Lisle, USA. Anti-hCG β antibody was purchased from Ortho Diagnostic Systems, Raritan, NJ. Antibody reacting with protein gene product (PGP) 9.5 was purchased from Ultra Clone, Isle of Wight, England.[15] Anti-chromoranin monoclonal antibody (CG) was purchased from Lipshaw, Detroit, MI.[16] For electron microscopic studies, the specimens were fixed in a 2.5% glutaraldehyde solution followed by 1% osmium tetroxide, then embedded in Epon, and ultrathin sections were stained with uranyl acetate and lead citrate, and observed under an electron microscope. For the assay of hCG in the KTA7 culture supernatants, time-resolved fluoroimmunoassay (TR-FIA) was conducted by Kitasato Biochemical Laboratories.[17] Chemical assay for NSE of KTA7 cells was performed by Dr. K. Kato of the Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony.[18] Extraction of total cellular RNA was performed according to the guanidinium/hot phenol method and polyadenylated RNA was isolated by oligo (dT)-cellulose chromatography. Northern transfer, hybridization with ^{32}P-labeled cloned cDNA-hCG α- and β-subunits gene probes (provided by Dr. John C. Fiddes, California Biotechnology Inc., CA)[19,20] and postwashes

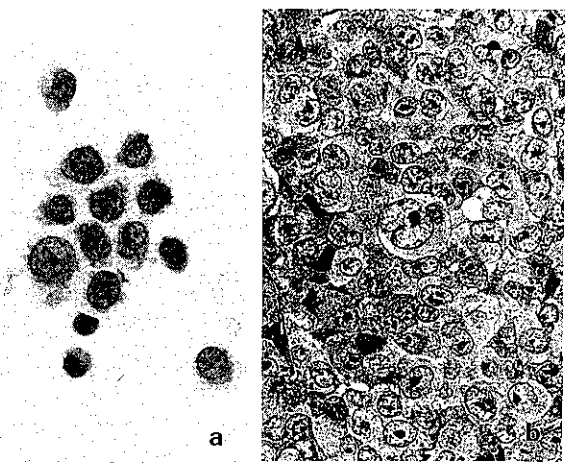

Fig. 1. Hematoxylin and eosin staining of KTA7 cultured cells (a) and a nude mouse transplanted tumor (b). a, b; ×200.

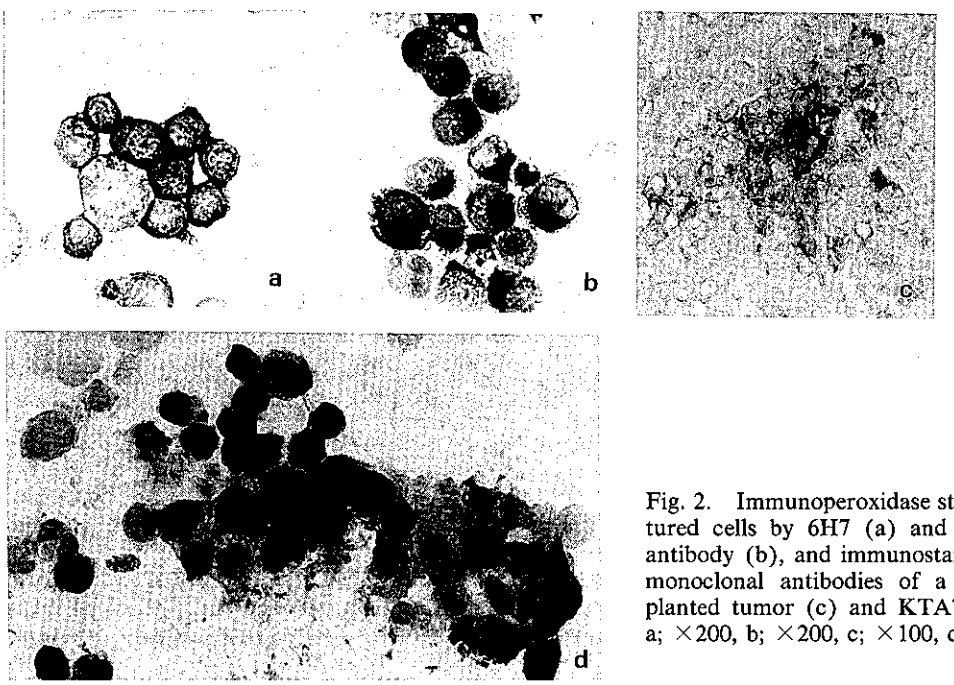

Fig. 2. Immunoperoxidase staining of KTA7 cultured cells by 6H7 (a) and by anti-Cytokeratin antibody (b), and immunostaining by anti-hCG α monoclonal antibodies of a nude mouse transplanted tumor (c) and KTA7 cultured cells (d). a; ×200, b; ×200, c; ×100, d; ×200.

were carried out as reported previously[21] and autoradiography, at −70°C. Chromosome analysis of KTA7 cells was performed at the Special Reference Laboratories, Tokyo.[22]

The established KTA7 cell line continued to grow in the ACL-3 medium in 25 cm^2 flasks, the population doubling time being 28 h. KTA7 grew as floating cell clusters with loose intercellular contact and had a single round or polygonal nucleus with one or more prominent nucleoli and scanty cytoplasm (Fig. 1a). Nude mouse xenografts exhibited undifferentiated large cell carcinoma without formation of acini or keratinization (Fig. 1b). Immunohistochemical studies on both cytocentrifuged KTA7 cells and nude mouse xenografts indicated KTA7 to express 6H7 and MOC1 antigens (Fig. 2a), and Cytokeratin (Fig. 2b), which consists of low-molecular-weight cytokeratin polypeptides of 39,000, 43,000 and 50,000 daltons, but not keratin consisting of polypeptides of 56,000 and 64,000 daltons. KTA7 cells bore no SLEX, SLEA, EMA, CEA, SC, AFP, CG or NSE. NSE content detected by chemical assay of KTA7 cells was 63.3 ng/mg protein, thus being much less than that of SCCL and neurogenic tumors[4, 18] and the cells did not immunostain positively for the anti-NSE antibody. However, KTA7 cells were positive for PGP 9.5, which was a 27,000-molecular-weight soluble protein isolated from brain and a cytoplasmic marker for neurons and some endocrine cells.[23] hCG α-subunit immunoreactivity was noted in nude mouse transplanted KTA7 tumors (Fig. 2c) and KTA7 cultured cells (Fig. 2d), whereas no reaction for hCG β-subunit could be detected in the cells and transplants. Electron microscopic examination showed that adjacent cell membranes of the KTA7 cells were closely apposed to a few long tight junctions and that the cytoplasm of the KTA7 cells contained abundant free ribosomes, scattered dilated rough endoplasmic reticulum and a moderate number of mitochondria, but some cells had glycogen granules in the cytoplasm. No secretory granules were observed in the cells (Fig. 3). No basement membrane which separated the parenchyma from the connective tissue was present in transplants of KTA7 (photograph not shown). hCG was shown to be secreted

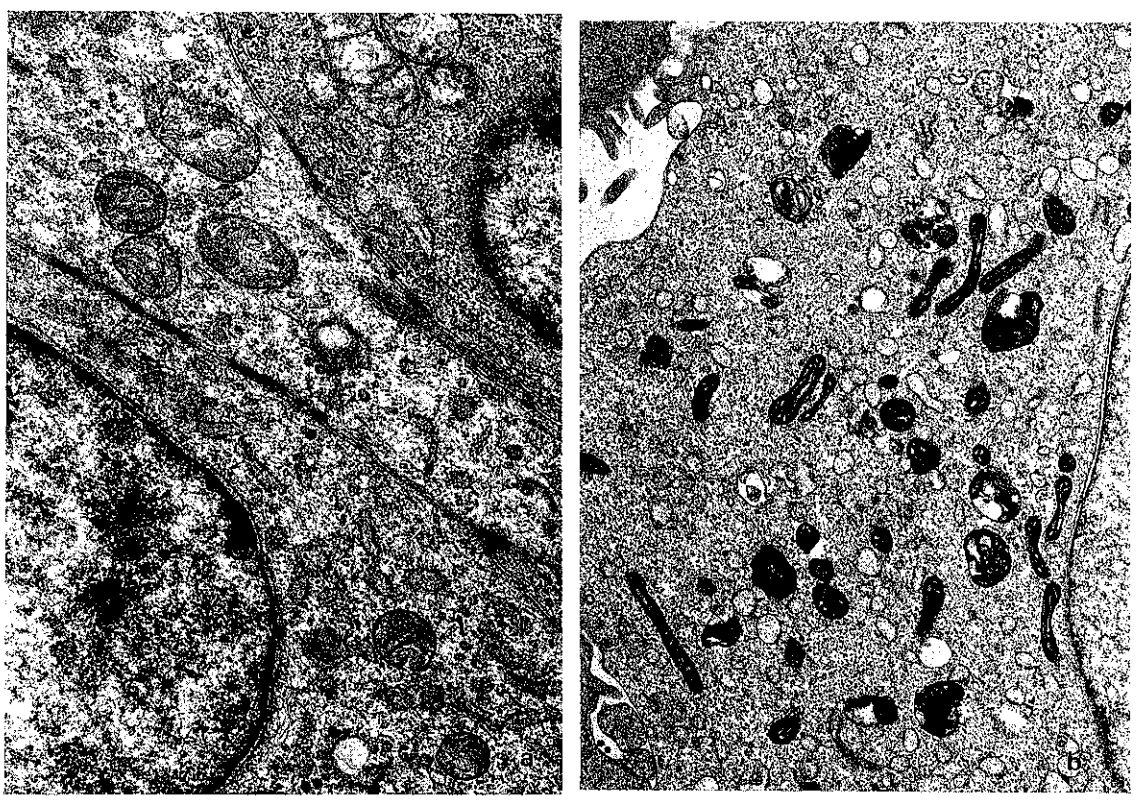

Fig. 3. Electron microscopic examination of cultured KTA7 cells. Adjacent cell membranes of KTA7 cells were closely apposed to a few long tight junctions. The cytoplasm contained abundant free ribosomes, scattered dilated rough endoplasmic membranes and a moderate number of mitochondria (a). Some KTA7 cells had abundant glycogen granules in the cytoplasm (b). a; ×15,400, b; ×10,000

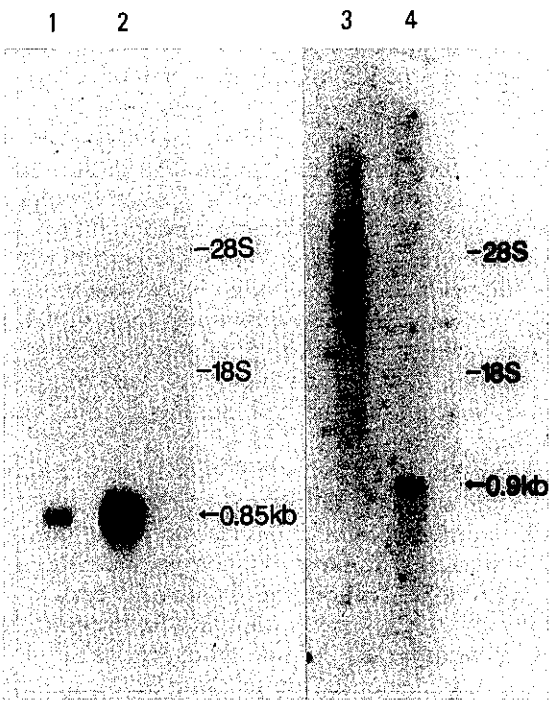

Fig. 4. Northern hybridization analysis. Total cellular and poly $(A)^+$ RNA (3 μg) was denatured and separated in a 1% agarose gel containing formaldehyde and hybridized with hCG α- and β-subunit gene cDNA probes, which were the 621 bp *Hind* III genomic fragment containing the hCG α-subunit gene and the 491 bp of *Hind* III-*Apa* I genomic fragment containing the hCG β-subunit gene, respectively. Lane 1, total RNA of KTA7 cells hybridized with the hCG α-subunit gene probe; lane 2, poly $(A)^+$ RNA of KTA7 cells hybridized with hCG α-subunit gene probe; lane 3, poly $(A)^+$ RNA of KTA7 cells hybridized with the hCG β subunit gene probe; lane 4, poly $(A)^+$ RNA of the placenta hybridized with the hCG β-subunit gene probe. The exposure times were 15.5 h in lanes 1 and 2, and 44 h in lanes 3 and 4.

into the culture medium by immunoassay of KTA7 culture supernatant (4.8 ng/ml per 10^6 cells per 48 h). The hCG secretion of KTA7 cells was that of hCG α-subunit because the polyclonal anti-hCG antibody used in the present immunoassay could react with not only whole hCG molecule but also either of the hCG subunits. Its level was quite low compared to those of gestational choriocarcinoma cell lines.[24] Northern blot analysis was conducted on total cellular and poly-adenylated RNA to examine the transcription of hCG α- and β-subunits genes in the KTA7 cells. hCG α-subunit transcripts of 0.85 kb were demonstrated in the KTA7 cells (Fig. 4, lane 1 and lane 2). However, no transcript of the hCG β-subunit could be detected in the cells, although hCG β-subunit transcript of 0.9 kb was detected in the placenta as a positive control (Fig. 4, lane 3 and lane 4). Chromosome analysis of KTA7 cells showed that its modal chromosome number was 55 with a narrow distribution. Loss of alleles of chromosome 3p and translocations of alleles of chromosomes 9p, 17p and 20q were observed in the KTA7 cells. Chromosomes 6, 9, 17 and 20 were found to be monosomic. Chromosomes 21 and 22 were trisomic. Deletions of chromosome 13q were not found in the KTA7 cells, although loss of heterozygosity was reported in 3p, 13q and 17p in small cell lung carcinomas and may be involved in tumorigenesis in SCCL.[25, 26]

In summary, a new non-small cell lung cancer cell line, KTA7, which was established in serum-free media, ACL-3, was found to produce and secrete the hCG α-subunit by immunohistochemistry, immunoassay and northern hybridization analysis. hCG consists of α- and β-subunits, and the α-subunit is almost identical with that of the pituitary glycoprotein hormones. Although hCG is a pregnancy-associated hormone produced and secreted by the trophoblast of the placenta, the immunoreactivity was demonstrated in various extraplacental tissues and tumors.[27] The hCG α-subunit-containing cells were found to be present not only in small cell carcinomas of the lung (SCCL) and carcinoid tumors but also in non-SCCLs including adenocarcinomas, squamous cell carcinomas and large cell carcinomas.[28] hCG α-containing cells have also been reported in most cases to lack hCG β-subunit immunoreactivity in lung tumors.[28] The hCG subunits are thus considered to be expressed through independent genetic mechanisms in KTA7 cells as well as most hCG α-subunit-positive lung tumors. Lung cancer cell lines producing hCG subunits have already been reported. Belper *et al.* described the features of 3 lung cancer cell lines producing hCG subunits but did not mention their molecular characteristics.[29] The molecular mechanisms of hCG production and secretion of two clonal strains from the ChaGo cell line, derived from lung cancer and possessing the morphological features of trophoblastic tumor, have been examined in detail.[30, 31] Production of α-hCG has been shown to be controlled pretranslationally in ChaGo cells.[30] However, the relationship between production of α-hCG and expression of other neuroendocrine markers in non-SCCL was not described.

While 6H7 was reported to recognize a polypeptide of 128,000 daltons,[8] it was found to react with the same molecule recognized by MOC1,[32] and thus is considered to belong to the cluster I antigen of small cell carcinoma (SCC) since the MOC1 antigen is classified as the cluster I antigen of SCC.[33] Recently, the antigenic specificity of the cluster I antigen was found to be quite similar to that of the neural cell adhesion molecule (N-CAM).[34] Monoclonal antibodies reactive with cluster I antigen of SCC such as MOC1 and 6H7 react with a small population of

non-SCCL and are capable of detecting the neuroendocrine differentiation of non-SCCL, while other neuroendocrine features of non-SCCL expressing the cluster I antigen have yet to be described in detail.[7, 8, 33] Recently, the cluster I antigen expression in non-SCCL was suggested to represent an oncofetal form of expression since it was found to be expressed in fetal bronchi without accompanying co-expression of other endocrine cell markers.[32] However, the cluster I antigen expression in non-SCCL is considered to represent neuroendocrine features when other neuroendocrine markers are co-expressed in the tumors, because it is expressed in not only neurons and neurogenic tumors but also endocrine cells and tumors to a high degree.[8, 14]

In the present study, the expression of the cluster I antigen of SCC in non-SCCL was confirmed and its relation to neuroendocrine features in non-SCCL was clarified since the cluster I antigen expression in KTA7 cells was accompanied with co-expression of an endocrine cell marker, hCG α-subunit and a cytoplasmic marker for neurons and some endocrine cells, PGP9.5. Although immunoreactivity of the anti-NSE antibody could not be detected in KTA7 cells, immunochemical assay for NSE of KTA7 cells showed NSE to be present at a very low concentration in them and the enzymes of APUD cell systems such as NSE have been shown to have low activity in certain non-SCCL cell lines that produce hCG.[29]

The relationships among morphologic features, expression of the cluster I antigen and production of hCG α-subunit in non-SCCL were not determined in previous reports. A small proportion of large cell undifferentiated carcinomas has been reported to express neuroendocrine properties and such tumors have consequently been designated as large cell undifferentiated carcinomas with neuroendocrine differentiation or large cell neuroendocrine tumors.[35] The original lung tumor of KTA7, however, was comprised of adenocarcinoma and large cell carcinoma and the KTA7 cell line was thus considered to be derived from the large cell component of the original tumor. Large cell neuroendocrine tumors have been reported to take a more aggressive clinical course than most other large cell carcinomas and thus are high-grade tumors. Adjuvant chemotherapy appropriate for SCCL may thus be recommended for all large cell neuroendocrine tumors regardless of the clinical tumor stage.[35] KTA7 may serve as an *in vitro* model for a broad range of studies on the pathobiology of large cell neuroendocrine tumors, since the cell line was derived from a large cell component and has the neuroendocrine features mentioned above.

Chromosomal analysis of KTA7 cells showed chromosome 3p deletion and monosomy of chromosome 17 but not chromosome 13q deletion. Chromosomal deletion at 3p was suggested to be a common pathogenetic step to most types of lung cancers since such deletion was found in not only SCCL but also non-SCCL.[36] Simultaneous loss of heterozygosity for loci on chromosomes 3p, 13q and 17p was demonstrated in most SCCL patients by restriction fragment length polymorphism (RFLP) analysis, although no cytogenetic abnormalities of chromosomes 13 and 17 have been reported.[26] Thus, further studies of KTA7 cells by RFLP analysis will be performed in order to look for a pathogenetic step common to pulmonary neuroendocrine tumors including SCCL and large cell neuroendocrine tumors.

This work was supported in part by a Grant-in-Aid for Cancer Research from the Ministry of Health and Welfare of Japan, and in part by a Grant-in-Aid from the Ministry of Education, Science and Culture of Japan.

(Received August 16, 1990/Accepted October 31, 1990)

REFERENCES

1) Kameya, T., Shimosato, Y., Kodama, T., Tsumuraya, M., Koide, T., Yamaguchi, K. and Abe, K. Peptide hormone production by adenocarcinomas of the lung; its morphologic basis and histogenetic considerations. *Virchows Arch. A*, **400**, 245–257 (1983).
2) Shimosato, Y. Pulmonary neoplasms. *In* "Diagnostic Surgical Pathology," ed. S. S. Sternberg, pp. 785–827 (1989). Raven Press, New York.
3) Kasai, K., Kameya, T., Okuda, T., Terasaki, P. I. and Iwaki, Y. Immunohistochemical examination of lung cancers using monoclonal antibodies reacting with sialosylates Lewis^x and sialosylated Lewis^a. *Virchows Arch. A*, **410**, 253–261 (1986).
4) Abe, K., Kameya, T., Yamaguchi, K., Kikuchi, K., Adachi, I., Tanaka, M., Kimura, S., Kodama, T., Shimosato, Y. and Ishikawa, S. Hormone-producing lung cancer — endocrinologic studies. *In* "The Endocrine Lung in Health and Disease," ed. K. I. Becker and A. E. Gazdar, pp. 549–595 (1984). Saunders, Philadelphia.
5) Negatsu, T., Ichinose, H., Kojima, K., Kameya, T., Shimase, J., Kodama, T. and Shimosato, Y. Aromatic L-amino acid decarboxylase activities in human lung tissues: comparison between normal lung and lung carcinoma. *Biochem. Med.*, **34**, 52–59 (1985).
6) Watanabe, J., Okabe, T., Fujisawa, M., Takaku, F., Hirohashi, S. and Shimosato, Y. Monoclonal antibody that distinguishes small-cell lung cancer from non-small-cell lung cancer. *Cancer Res.*, **47**, 826–829 (1987).

7) Broers, J. V., Rot, M. K., Oostendorp, T., Huysmans, A., Wagenaar, S. S., Wiersma-van Tilburg, A. J. M., Vooijs, G. P. and Ramaekers, F.C. S. Immunocytochemical detection of human lung cancer heterogeneity using antibodies to epithelial, neuronal, and neuroendocrine antigens. *Cancer Res.*, **47**, 3225–3234 (1987).
8) Okuda, T., Kasai, K., Kameya, T., Saito, S. and Takase, N. Monoclonal antibody directed against neuroendocrine properties of both normal and malignant cells. *Hybridoma*, **7**, 569–581 (1988).
9) Terasaki, T., Shimosato, Y., Nakajima, T., Tsumuraya, M., Morinaga, S., Hirohashi, S., Yamaguchi, K., Kato, K., Ichinose, H. and Nagatsu, T. Changes in cell characteristics due to culture conditions in cell lines from human small cell lung cancer. *Jpn. J. Clin. Oncol.*, **16**, 203–212 (1986).
10) Brower, M., Carney, D. N., Oie, H. K., Gazdar, A. F. and Minna, J. D. Growth of cell lines and clinical specimens of human non-small cell lung cancer in a serum-free defined medium. *Cancer Res.*, **46**, 798–806 (1986).
11) Makin, C. A., Bobrow, L. G. and Bodmer, M. F. Monoclonal antibody to cytokeratin for use in routine histopathology. *J. Clin. Pathol.*, **37**, 975–983 (1984).
12) Fukushima, K., Hirota, M., Terasaki, P. I., Wakisaka, A., Togashi, H., Chia, D., Suyama, N., Fukushi, Y., Nudelman, E. and Hakomori, S. Characterization of sialosylated Lewis^x as a new tumor-associated antigen. *Cancer Res.*, **44**, 5279–5285 (1984).
13) Galton, J., Terasaki, P. I., Wakisaka, A., Chia, D., Katz, D. and Hardiwidjaja, S. S. A monoclonal antibody reactive with colonic, gastric and pancreatic adenocarcinomas. Antibodies: protective, destructive and regulatory role. *Proc. 9th Int. Convoc. Immunol.*, 117–125 (1985).
14) De Leiji, L., Poppema, S., Nulend, J. K., Ter Haar, A., Schwander, E., Ebbens, F., Postmas, P. E. and Hauw The, T. Neuroendocrine differentiation antigen on human lung carcinoma and Kultschitzky cells. *Cancer Res.*, **45**, 2192–2200 (1985).
15) Doran, J. F., Jackson, P. J., Kynoch, P. A. M. and Thompson, R. J. Isolation of PGP 9.5, a new human neurone specific protein detected by high resolution two dimensional electrophoresis. *J. Neurochem.*, **40**, 1542–1547 (1983).
16) Wilson, B. S. and Lloyd, R. V. Detection of chromogranin in neuroendocrine cells with a monoclonal antibody. *Am. J. Pathol.*, **115**, 458–468 (1984).
17) Pettersson, K., Siitari, H., Hemmila, I., Soini, E., Lovgren, T., Hanninen, V., Tanner, P. and Stenman, U-H. Time-resolved fluoroimmunoassay of human choriogonadotropin. *Clin. Chem.*, **29**, 60–64 (1983).
18) Ishiguro, Y., Kato, K., Ito, T., Horisawa, M. and Nagaya, M. Enolase isozymes as markers for differential diagnosis of neuroblastoma, rhabdomyosarcoma, and Wilms' tumor. *Gann*, **75**, 53–60 (1984).
19) Fiddes, J. C. and Goodman, H. M. Isolation, cloning and sequence analysis of the cDNA for the α-subunit of human chorionic gonadotropin. *Nature*, **281**, 351–355 (1979).
20) Fiddes, J. C. and Goodman, H. M. The cDNA for the β-subunit of human chorionic gonadotropin suggests evolution of a gene by readthrough into the 3′-untranslated region. *Nature*, **286**, 684–687 (1980).
21) Wada, C., Hashimoto, C., Kameya, T., Yamaguchi, K. and Ono, M. Developmentally regulated expression of the calcitonin gene related peptide (CGRP) in rat lung endocrine cells. *Virchows Arch. B Cell Pathol.*, **55**, 217–223 (1988).
22) Seabright, M. A rapid banding technique for human chromosomes. *Lancet*, **ii**, 971–972 (1971).
23) Rode, J., Dhillon, A. P., Doran, J. F., Jackson, P. and Thompson, R. J. PGP9.5 a new marker for human neuroendocrine tumours. *Histopathology*, **9**, 147–158 (1985).
24) Sekiya, S., Shirotake, S., Kaiho, T., Iwasawa, H., Kawata, M., Higaki, K., Ishige, H., Takamizawa, H. and Minamihisamatsu, M. A newly established human gestational choriocarcinoma cell line and its characterization. *Gynecol. Oncol.*, **15**, 413–421 (1983).
25) Naylor, S. L., Johnson, B. E., Minna, J. D. and Sakaguchi, A. Y. Loss of heterozygosity of chromosome 3p markers in small-cell lung cancer. *Nature*, **329**, 451–454 (1987).
26) Mori, N., Yokota, J., Oshimura, M., Cavenee, W. K., Mizoguchi, H., Noguchi, M., Shimosato, Y., Sugimura, T. and Terada, M. Concordant deletions of chromosome 3p and loss of heterozygosity for chromosomes 13 and 17 in small cell lung carcinoma. *Cancer Res.*, **49**, 5130–5135 (1989).
27) Tsutsumi, Y. Expression of the alpha subunit of human chorionic gonadotropin in normal and neoplastic neuroendocrine cells. *Acta Pathol. Jpn.*, **39**, 413–419 (1989).
28) Fukayama, M., Hayashi, Y., Koike, M., Hajikano, H., Endo, S. and Okumura, H. Human chorionic gonadotropin in lung and lung tumors. Immunohistochemical study on unbalanced distribution of subunits. *Lab. Invest.*, **55**, 433–443 (1986).
29) Belper, G., Koehler, A., Kiefer, A., Havemann, K., Beisenherz, K., Jaques, G., Gropp, C. and Haeder, M. Characterization of the state of differentiation of six newly established human non-small-cell lung cancer cell lines. *Differentiation*, **37**, 158–171 (1988).
30) Lieblich, J., Weintraub, B. D., Krauth, G. H., Kohler, P. O., Rabson, A. S. and Rosen, S. W. Ectopic and eutopic secretion of chorionic gonadotropin and its subunits *in vitro*: comparison of clonal strains from carcinomas of lung and placenta. *J. Natl. Cancer Inst.*, **56**, 911–917 (1976).
31) Nagelberg, S.B., Burnside, J., Maniatis, A., Lippman, S. S. and Weintraub, B. D. Pretranslational regulation of ectopic hCG α production in ChaGo lung cancer cells by sodium butyrate. *Biochem. Biophy. Res. Commun.*, **133**, 972–980 (1985).
32) Fukayama, M., Funata, N., Hayashi, Y., Maeda, Y., Koike, M., Watanabe, J. and Okabe, T. Brain-associated small-cell lung cancer antigen (BASCA) is expressed in

developing lung: an immunohistochemical and immunoelectron microscopic study. *J. Histochem. Cytochem.*, **38,** 51–57 (1990).
33) Beverley, P. C. L. Souhami, R. L. and Bobrow, L. G. Results of the central data analysis. Proceedings of First International Workshop on Small Cell Lung Cancer Antigens. *Lung Cancer,* **4,** 15–36 (1988).
34) Patel, K., Moore, S. E., Dickson, G., Rossell, R. J., Beverley, P. C., Kemshead, J. T. and Walsh, F. S. Neural cell adhesion molecule (NCAM) is the antigen recognized by monoclonal antibodies of similar specificity in small-cell lung carcinoma and neuroblastoma. *Int. J. Cancer,* **44,** 573–578 (1989).
35) Hammond, E. and Sause, W. T. Large cell neuroendocrine tumors of the lung. Clinical significance and histopathologic definition. *Cancer,* **56,** 1624–1629 (1985).
36) Harbour, J. W., Lai, S-L., Whang-Peng, J., Gazdar, A. F., Minna, J. D. and Kaye, F. L. Abnormalities in structure and expression of the human retinoblastoma gene in SCLC. *Science,* **241,** 353–357 (1988).