Jpn. J. Cancer Res. 85, 718–722, July 1994

Establishment of a Human Small Cell Lung Cancer Cell Line Producing a Large Amount of Anti-diuretic Hormone

Takeo Terasaki,[1] Yoshihiro Matsuno,[1] Yukio Shimosato,[3] Ken Yamaguchi,[2] Hiroshi Ichinose,[4] Toshiharu Nagatsu[4] and Kanefusa Kato[5]

[1]Pathology Division, [2]Growth Factor Division, National Cancer Center Research Institute, [3]Clinical Laboratory Division, National Cancer Center Hospital, 1-1 Tsukiji 5-chome, Chuo-ku, Tokyo 104, [4]Division of Molecular Genetics (II), Institute for Comprehensive Medical Science, Fujita Health University, 1-98 Dengakugakubo, Kutsukake-cho, Toyoake 470-11 and [5]Department of Biochemistry, Institute for Developmental Research, Aichi Prefectural Colony, 713-8 Kamiya-cho, Kasugai 480-03

A new cancer cell line (Lu-165) producing a large amount of anti-diuretic hormone (ADH, 2.8 μg/g protein) was established from a 50-year-old small cell lung cancer patient presenting with a syndrome of inappropriate anti-diuretic hormone secretion. These cells grew well in serum-supplemented medium and during more than 100 passages they continued producing a large amount of this hormone. This cell line will be a useful tool for studies of the biochemistry and pathology of ADH-producing cancer.

Key words: Anti-diuretic hormone — Small cell lung cancer — Cell line

Small cell lung cancer (SCLC) is one of the highly malignant cancers.[1, 2] It responds well to drugs and radiation, but the survival rate is low because of the regrowth of cancer cells after therapies. Many SCLC cell lines have been established, and biological and genetic characteristics of these cells have been studied.[3–7] SCLC produces various peptide hormones such as adrenocorticotropic hormone, calcitonin, gastrin-releasing peptide, growth hormone-releasing hormone, somatostatin, anti-diuretic hormone (ADH) and so on.[8–10] Some studies have dealt with the relationship between the level of ADH and the frequency of metastasis in SCLC.[9, 11] In order to establish the characteristics of ADH-producing tumor cells, studies using cell lines are desirable, but only a few cell lines that produce this hormone have been established.[12–15] Almost all SCLC cell lines that express ADH gene show low levels of ADH immunoreactivity.[14, 15] We have succeeded in establishing a cell line (Lu-165) that produced a large amount of ADH (2.8 μg/g protein) from an SCLC patient with a syndrome of inappropriate anti-diuretic hormone secretion (SIADH). After more than 100 passages, these cells still produced a large amount of ADH.

MATERIALS AND METHODS

Case A 50-year-old Indonesian male was admitted to the hospital presenting with severe nausea and vomiting. Blood chemistry revealed severe hyponatremia; Na 113 mmol/liter and Cl 82 mmol/liter. Serum neuron specific enolase (NSE) level was 31.7 ng/ml, which was higher than the normal upper limit of 15. He complained of headache and sometimes showed convulsional movement. Mediastinoscopic examination demonstrated lymph node enlargement and he was diagnosed as having SCLC presenting with SIADH. He received 5 courses of chemotherapy and 50 Gy of thoracic radiation therapy, and achieved complete remission with recovery of serum Na level to 144 mmol/liter. Later, tumor recurrence at the primary site was found and he received 2 additional courses of chemotherapy with no response. As there was a possibility that the remaining tumor was not small but non-small cell lung cancer, he underwent surgery. Histological diagnosis was pure small cell carcinoma of intermediate cell type. Metastatic small cell carcinoma was found in mediastinal and hilar lymph nodes. The resected tumor tissue contained a large amount of ADH (18 μg/g protein). Serum Na levels were restored to the normal range of 144 mmol/liter 10 days after operation.

Cell culture Resected tumor tissue from lymph node metastasis was used for macroscopic and microscopic diagnoses, and the rest of the tumor tissue was rinsed well in antibiotics-supplemented culture medium, minced using scissors, seeded on dishes containing serum-supplemented medium (SSM, RPMI 1640 + 10% fetal calf serum), and cultured in a humidified atmosphere of 5% CO_2/95% air at 37°C.

Light and electron microscopy Pellets of cultured cells were fixed in 10% formalin, embedded in paraffin, sectioned and stained with hematoxylin and eosin, or immunohistochemically with anti-arginine vasopressin (AVP) antibody (DAKO A-S, Denmark) by using the avidin-biotin-peroxidase complex method.[16] For electron microscopy, cultured cell pellets were fixed in 2% glutar-

aldehyde solution for 60 min and 1% OsO_4 for 60 min and processed routinely. Epon-embedded ultra-thin sections were observed under a Hitachi H-600 electron microscope after double staining with uranyl acetate and lead citrate.

Cell growth Growth curves and doubling time of Lu-165 cells were determined by protein measurement according to the methods of Oyama and Eagle[17] as described elsewhere.[18]

Assays for aromatic L-amino acid decarboxylase (AADC), NSE and creatine kinase, brain isoenzyme (CK-BB) Cultured cells in the exponential growth phase were washed with phosphate-buffered saline, and the washed pellet was frozen at −80°C and stored until use. For AADC activity, the amount of dopamine generated was determined by high-performance liquid chromatography with electrochemical detection.[19] Highly sensitive enzyme immunoassay systems were used to test for NSE[20] and CK-BB.[21]

Assays for ADH The contents of ADH in fresh SCLC tissues and cultured cells were determined by a radioimmunoassay (RIA)[22] after extraction of ADH with boiling water using rabbit antibody (R-0073, Mitsubishi Petrochemical Co., Ltd.), which recognizes the C-terminus of AVP. [^{125}I]AVP (purchased from New England Nuclear) was used as a tracer.

Cell lines Fifteen more SCLC cell lines were used for the determination of ADH contents and levels of biochemical activities. Five of them, H-69, H-82, N-230, N-231 and N-417 were kindly supplied by Dr. A. F. Gazdar, Bethesda, Maryland, USA.[4] The remaining ten, Lu-24-H, Lu-24-V, Lu-130, Lu-134-A, Lu-134-B, Lu-135, Lu-139, Lu-140, Lu-141 and Lu-143 were established in our laboratory from tissues obtained at surgery or from xenotransplanted tumors in nude mice.[23]

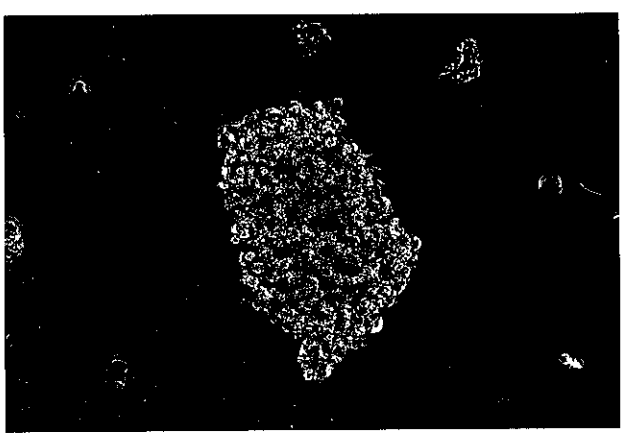

Fig. 1. Morphological features of Lu-165 cells. Cells grew floating, and growth pattern was classified as type 2 according to the classification of Carney *et al.*[3] ×100.

Transplantation into nude mice Cultured cells (approximately 5×10^7 cells) were transplanted into the subcutaneous tissues of 3 athymic BALB/c mice.

RESULTS

Growth pattern and morphology of cultured cells A continuous cell line (Lu-165) was obtained in SSM. Lu-165 cells grew in suspended clumps as shown in Fig. 1, and this growth pattern was classified as type 2 according to the classification of Carney *et al.*[3] Cells stained with hematoxylin and eosin possessed round or ovoid nuclei with nucleoli and a relatively increased amount of cytoplasm (Fig. 2A), resembling the cells of lymph node metastasis from which cultured cells were established. Immunocytochemically, cytoplasm in some cells reacted with anti-ADH antibody, showing production of ADH by these cells (Fig. 2B). Electron microscopically, cells possessed comparatively well-developed organelles in-

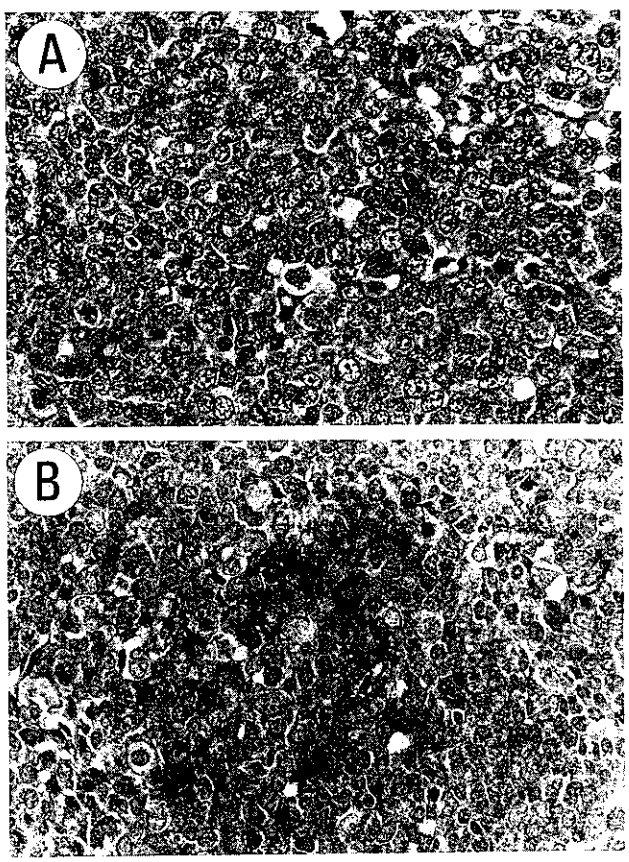

Fig. 2. Lu-165 cells stained with hematoxylin and eosin (A) possess round or ovoid nuclei with nucleoli and have comparatively well developed cytoplasm. Lu-165 cells immunostained for ADH (B) show a positive reaction in the cytoplasm. ×350.

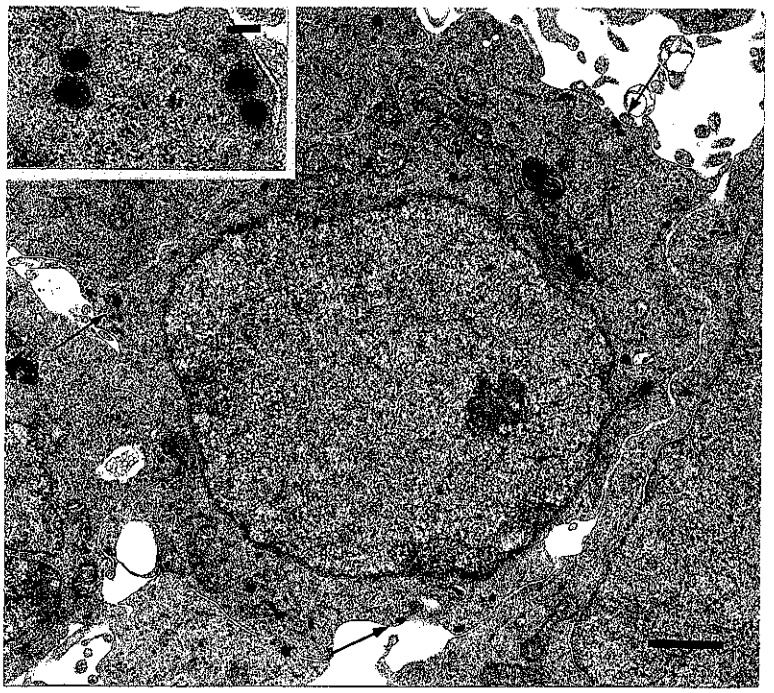

Fig. 3. Electron micrographs of Lu-165 cells showing comparatively well developed cytoplasm with free ribosomes, mitochondria, Golgi apparatus and neurosecretory granules (arrows). The bar represents 1 μm. Inset, higher magnification of neurosecretory granules. The bar represents 0.1 μm.

cluding free ribosomes, mitochondria, Golgi apparatus and a moderate number of neurosecretory granules (mean diameter; 112 nm, Fig. 3).

Doubling time Doubling time of Lu-165 cells was calculated from growth curves of the cells, and was 142 h.

Transplantation into nude mice Cultured SCLC cells were transplanted to 3 nude mice and tumors were found in all 3 mice 4 weeks after transplantation. The histological characteristics were the same as those of resected tissue from lymph node metastasis.

ADH production The contents of ADH in 16 SCLC cell lines and that of xenotransplanted nude mice tumor are shown in Table I. Lu-165 cells after 121 passages contained 2.8 μg ADH/g protein (2,600 pmol/g protein), and nude mouse tumor contained 0.17 μg/g protein (160 pmol/g protein). The other 15 cell lines contained no detectable ADH.

Biochemical analysis Biochemical activities of SCLC cell lines are shown in Table I. The levels of AADC, CK-BB and NSE were high in Lu-165 cells, showing that these cells were classic-type small cell cancer cells. Four (Lu-24-V, Lu-135, H-82 and N-417) of 16 SCLC cell lines had low AADC activity, showing that these cells were variant-type small cell cancer cells.

DISCUSSION

The patient from whom the Lu-165 cell line was established was diagnosed as having typical SIADH induced by ADH-producing SCLC, based on the following observations. First, he developed severe hyponatremia with symptoms due to the electrolyte imbalance. Clinically, the signs and symptoms of this patient met the criteria of SIADH. Second, the tumor of this patient could explain the SIADH. Hyponatremia disappeared when he entered complete remission, and it reappeared at the time of tumor recurrence. When the tumor was surgically resected, serum Na levels increased again to the normal range. Third, tumor tissue concentration of ADH was found to be extremely high (16,000 pmol/g protein, 17 μg/g protein). We have previously examined production of ADH in 50 SCLC[8]; ADH was detected in 4 cases (8%), and the contents ranged from 15 to 150 pmol/g protein.[8]

Using a metastatic lymph node of this patient, we established a cell line, Lu-165. Biochemical and morphological characteristics were consistent with the fact that this cell line was derived from SCLC. High levels of AADC, CK-BB and NSE activities of Lu-165 cells were observed, indicating that the biochemical properties of these cells were similar to those of other classic-type SCLC cell lines. Electron-microscopically, neurosecretory granules were found in Lu-165 cells, as in classic-type SCLC cell lines, and their size (mean diameter, 112 nm) was almost the same as that observed in other classic-type SCLC cell lines.

Hormonal studies revealed that Lu-165 cells, even after 121 passages, produced a large amount of ADH; by

Table I. ADH Production and Biochemical and Morphological Characteristics of SCLC Cell Lines and a Xenotransplanted Tumor

Cell line	ADH[a] (μg/g protein)	AADC[b] (pmol/min/mg protein)	CK-BB[c] (ng/mg protein)	NSE[c] (ng/mg protein)	Type
Lu-24	<0.06	4,170	NT[d]	5,350	C[e]
Lu-24-V	<0.06	9	1,330	2,050	V[f]
Lu-130	<0.02	4,400	4,580	2,270	C
Lu-134-A	<0.02	2,477	5,590	1,520	C
Lu-134-B	<0.05	3,639	4,020	604	C
Lu-135	<0.02	174	5,865	3,180	V
Lu-139	<0.03	9,600	6,475	1,875	C
Lu-140	<0.05	4,200	5,520	3,690	C
Lu-141	<0.03	29,100	2,590	3,030	C
Lu-143	<0.03	29,800	3,200	1,940	C
Lu-165	2.8	6,080	2,200	4,100	C
H-69	<0.02	4,900	5,120	1,120	C
H-82	<0.03	900	2,255	573	V
N-230	<0.03	2,930	3,185	1,090	C
N-231	<0.02	4,020	2,210	867	C
N-417	<0.02	6	6,625	495	V
Lu-165 (nude mouse tumor)	0.17	NT	NT	NT	

a) Determined by a radioimmunoassay method after extraction of ADH with boiling water.
b) Dopamine was assayed by high-performance liquid chromatography.
c) Determined by ultrasensitive immunoassay methods.
d) Not tested. *e*) Classic. *f*) Variant.

RIA, the level of ADH in an extract of Lu-165 cell pellet was found to be 2.8 μg/g protein (2,600 pmol/g protein). We have analyzed 15 SCLC cell lines by the same method, and found that none of them produced a detectable amount of ADH. ADH-producing SCLC cell lines have rarely been reported. Pettengill *et al.*[12] first established an SCLC cell line, DMS-44, that produced ADH in 1977. They showed immunologically that the cell line was positive for ADH, but they did not report the content of ADH in these cells. In 1992, Verbeeck *et al.*[14] reported that only one cell line (GLC-8) among 26 SCLC cell lines examined expressed ADH mRNA and that the cell line contained low levels of ADH immunoreactivity. In 1993, Gross *et al.*[15] reported 7 cell lines that expressed mRNA of ADH, established from 11 SCLC patients who showed low serum Na contents, though only one (NCI H711) of the seven cell lines showed positive ADH in cell pellets as determined by RIA (1,400 pmol/g protein). It is worth noting that Lu-165 cell line produced the highest amount of ADH of any SCLC cell line so far reported. Immunocytochemical staining of the cell line with anti-ADH antibody revealed that only a limited number of cells were positive (Fig. 2B). It may be that the competent cells were stimulated to produce ADH by yet unidentified cells in the cell line, or they might do so at a specific stage of the cell cycle.

Our study demonstrated that Lu-165 cells could be transplanted into nude mice, but serum Na levels in Lu-165-bearing nude mice were the same as in controls (data not shown). The ADH in xenotransplanted tumor tissue extracts was detectable but the concentration was rather low, compared to those in fresh tumor extracts obtained from patients and in cultured tumor cells. The reason why ADH contents in nude mice tumor were lower than those in cultured cells or in the primary tumor is not clear, but this phenomenon could explain the disappearance of SIADH in Lu-165-bearing nude mice. We have previously reported that ADH contents in xenotransplanted nude mice tumors were lower than that in the primary tumor.[24]

Further studies will be required to elucidate the mechanism by which ADH production is controlled in cancer cells.

ACKNOWLEDGMENTS

We are indepted to Dr. E. Araki for useful discussions and to Miss K. Otsubo for the measurement of ADH contents in cells and tissues.

(Received February 1, 1994/Accepted March 11, 1994)

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