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Production of Chromogranin A and B Derived Peptides in Human Small Cell Lung Carcinoma Cell Lines

Haruo Iguchi, Satoko Bannai, Naoki Takanashi and Yutaka Tsukada

Production of chromogranin (Cg)A and B derived peptides [pancreastatin (PST), GAWK, CCB] was studied using human lung carcinoma derived cell lines. PST-like immunoreactivity (LI) was detected in the culture medium in 3 of 6 small cell lung carcinoma (SCLC) cell lines, while GAWK- and CCB-LIs were detected in 5 of 6 and all the 6 SCLC cell lines, respectively. CCB-LI was produced in large amounts in SCLC cell lines as compared to PST- and GAWK-LIs. In non-SCLC cell lines, on the other hand, PST- and GAWK-LIs were not detected. CCB-LI was detected in 1 of 7 non-SCLC cell lines, but not detected in the remainder. PST, GAWK and CCB-LIs, secreted by these cell lines, consisted of several peaks, and these peaks were different among cell lines. This suggests that processing of CgA and B is different in the cell lines. Production of CgA and B derived peptides seems to be a characteristic feature of SCLC, and among them, CCB LI may be a useful marker for SCLC.
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INTRODUCTION

A CHROMOGRANIN (Cg) and secretogranin (Sg) family [CgA, CgB (SgI), SgII (CgC)] shares characteristic biochemical features and is distributed in secretory granules of neuroendocrine (NE) tissues of several species [1]. The primary structures of these proteins were recently described, and the aminoacid sequences deduced from cDNAs reveal several sites of paired or more adjacent basic aminoacids, which are potential proteolytic cleavage sites in the processing of precursor proteins [2–4]. Thus, a Cg/Sg family is considered to be a precursor for biologically active peptides. Pancreastatin was initially isolated from porcine pancreas [5], and its sequence has been shown to be located in the CgA molecule [6]. Similarly, GAWK and CCB were initially isolated from human pituitary glands [7, 8]. These names were derived from the first four aminoacids of the initially isolated fragment of a GAWK molecule [Gly(G)-Ala(A)-Trp(W)-Lys(K)] and the abbreviation of *C*-terminal region of chromogranin *B*, respectively. The aminoacid sequences of GAWK and CCB are entirely homologous to human CgB 420–493 and 597–653, respectively [8]. Therefore, these peptides could be originated from CgA and CgB through processing.

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Small cell lung carcinoma (SCLC), on the other hand, expresses multiple markers for NE differentiation, which distinguish SCLC from non-SCLC [9]. Among the Cg/Sg family, CgA has been well documented to be expressed in a variety of NE tumours, including SCLC [10–13]. However, little is known about expression of Cg B or SgII in these tumours.

In this paper, we studied production of pancreastatin (PST)-like, GAWK-like and CCB-like immunoreactivity (LI), presumed processing products of CgA and CgB, using human SCLC cell lines and characterized molecular forms of these peptide-LIs in gel permeation chromatography.

MATERIALS AND METHODS

Cell Culture

SCLC cell lines (Lu130, Lu134, Lu135, Lu139 and Lu140) were established at the National Cancer Center (Tokyo). The SCLC cell line (MS-1) and non-SCLC cell lines (Ma-1, Ma-2) were established at Osaka Prefectural Habikino Hospital (Habikino). Non-SCLC cell lines (PC-3, A-549, RERF-LC-OK, EBC-1 and PC-13) were obtained from Japanese Cancer Research Resources Bank (Tokyo). The cell lines were maintained in RPMI1640 (Flow) or MEM (Gibco) supplemented with 10% fetal bovine serum (FBS) (Flow), 200 U/ml penicillin (Gibco) and 200 μg/ml streptomycin (Gibco) in 5% CO_2 in air at 37°C.

Gel permeation chromatography

Gel permeation chromatography of the culture medium of the SCLC cell lines were performed on a Sephadex G-50 column (95 × 1.4 cm) equilibrated with 1 mol/l acetic acid. Two ml of the medium were layered onto the column and eluted with 1 mol/l acetic acid at a rate of 15 ml/h at 4°C. Fractions (1.3 ml) were collected, dried with a centrifugal concentrator and reconstituted with radioimmunoassay (RIA) buffer before assay. The column was calibrated with protein markers (Vo, catalase; 13.7k, ribonuclease; 6k, human insulin; 1.6k, human GRP1-16).

Determination of PST, GAWK and CCB-LIs

A PST antiserum was raised against a synthetic fragment of porcine PST33–49 (pCgA 272–288) in rabbits. The antiserum cross-reacted with pPST33–49 and hPST1–52, but did not cross-react with pituitary hormones, pancreatic hormones and other commercially available gut hormones. Cross-reaction of the antiserum with human CgA was not tested since human CgA was not available. pPST33–49 (Peninsula Labs) and ^{125}I-pPST 1–49 were used as standard and tracer, respectively. Iodination of pPST1–49 was performed by the Bolton–Hunter method [14]. 100 μl of samples or standards were incubated with the antiserum (1:20 000) for 48 h at 4°C in a volume of 0.25 ml. Then, 50 μl of ^{125}I-pPST 1–49 was added and the incubation was continued overnight at 4°C. Finally, 100μl of 10% goat antirabbit γ-globulin, 100 μl of 1% normal rabbit serum and 50 μl of 25% polyethylene glycol (PEG) were added and further incubated for 10 min at 4°C. Bound and free fractions were separated by centrifugation. Intra- and interassay coefficients of variation were less than 10% (n = 5) and the sensitivity of the RIA was 10 pmol/l.

A GAWK antiserum was raised against a synthetic fragment corresponding to GAWK20–38 (hCgB 439–457) and a CCB antiserum was raised against that corresponding to CCB6–21 (hCgB602–617) in rabbits (gifts from Dr Michel Chretien, Clinical Research Institute of Montreal). These antisera cross-reacted with the synthetic fragments (GAWK20–38, CCB6–21), but did not cross-react with pituitary hormones, pancreatic hormones and other commercially available gut hormones. Cross-reaction of these antisera with authentic human CgB was not tested since human CgB was not available. GAWK20-38 and CCB6-21 were used as standard and ^{125}I-GAWK20-38 and ^{125}I-CCB6-21, iodinated by the chloramin-T method [15], were used as tracer. The assay procedure of GAWK and CCB-LIs were described previously [16]. The intra- and interassay coefficients of variation were less than 11% for GAWK and less than 10% for CCB, and sensitivity of the RIAs were 10 pmol/l for both GAWK and CCB-LIs.

Table 1. Concentrations of PST, GAWK and CCB-LIs in the culture medium of human lung carcinoma derived cell lines

Cell line	PST-LI	GAWK-LI	CCB-LI
SCLC			
Lu130	77(15)	109(11)	918(324)
Lu134	101(16)	65(6)	352(31)
Lu135	N.D.	90(14)	946(135)
Lu139	67(7)	23(8)	1140(153)
Lu140	N.D.	N.D.	395(58)
MS-1	N.D.	277(26)	2500<
Non-SCLC			
PC-3	N.D.	N.D.	N.D.
A-549	N.D.	N.D.	588(70)
RERF-LC-OK	N.D.	N.D.	N.D.
MA-1	N.D.	N.D.	N.D.
MA-2	N.D.	N.D.	N.D.
EBC-1	N.D.	N.D.	N.D.
PC-13	N.D.	N.D.	N.D.

Values represent mean (S.D.) pmol/l in five replicate dishes. N.D., not detected.

RESULTS

Concentrations of PST, GAWK and CCB-LIs in the culture medium after 24 h-incubation were shown in Table 1. PST-LI was detected in 3 of 6 SCLC cell lines and its concentrations ranged from 67 to 101 pmol/l. GAWK-LI was detected in 5 of 6 SCLC cell lines while CCB-LI was detected in all the 6 SCLC cell lines. Concentrations of GAWK and CCB-LIs ranged from 23 to 277 and from 352 to 2500 < pmol/l, respectively. The concentrations of CCB-LI were higher than those of GAWK-LI. In seven non-SCLC cell lines, PST and GAWK-LIs were not detected. CCB-LI was detected in one of seven non-SCLC cell lines, but was not detected in the remainder.

Figure 1 depicts elution profiles of PST-LI in the culture medium of the SCLC cell lines on a Sephadex G-50 column. Multiple peaks of PST-LI with a major peak of an apparent molecular weight of more than 13.7 k were found in Lu134, while only one peak with an apparent molecular weight of more than 13.7 k was found in Lu139. Elution profiles of GAWK-LI revealed multiple peaks with a wide range in the molecular size (Fig. 2), while those of CCB-LI revealed few peaks with a major peak of around 10 k in Lu134 and Lu135 and that of void volume in MS-1 (Fig. 3).

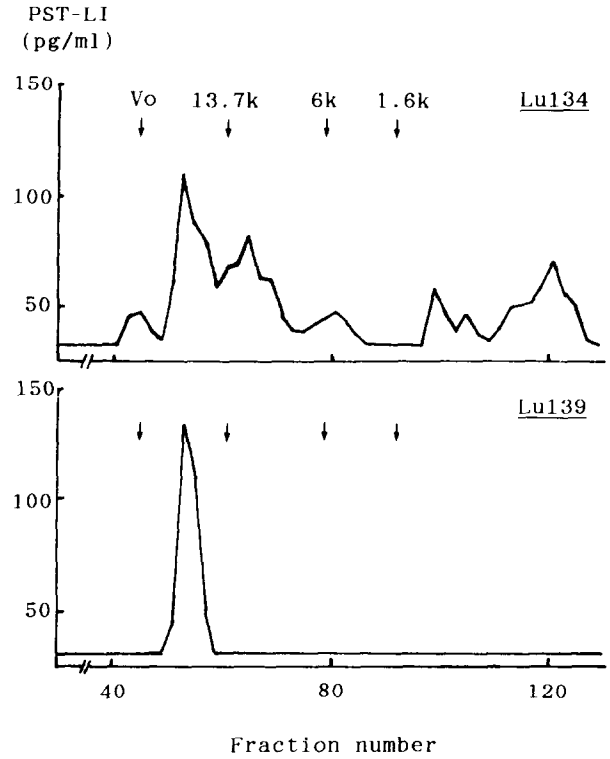

Fig. 1. Elution profile of PST-LI in the culture medium of human SCLC-derived cell lines (Lu134, Lu139) on Sephadex G-50 gel permeation chromatography. Two ml of the medium were layered onto a column (95 × 1.4 cm) and eluted with 1 mol/l acetic acid. Fractions were collected, dried, reconstituted with RIA buffer and assayed for PST-LI. The column was calibrated with protein markers (Vo, catalase; 13.7 k, ribonuclease; 6 k, human insulin; 1.6 k, human GRP1-16).

DISCUSSION

The Cg/Sg family is distributed in the NE tissues and secreted into the bloodstream by these tissues [1]. Expression of CgA and/or CgB in various endocrine tumours was immunohistochemically demonstrated [10, 17–20], and elevation of plasma CgA was also noted with high frequency in patients with these tumours [21–24]. Regarding SCLC, expression of CgA mRNA was noted with high frequency in the cell lines, as well as tumours [11]. However, immunohistochemical studies revealed the presence of CgA in about 50% of SCLC [10], and Sobol *et al.* [21] observed elevation of plasma CgA concentrations in 65% of SCLC patients. In the present study, production of PST LI, a CgA-derived peptide, was noted in 3 (50%) of 6 SCLC cell lines. This value is similar to those of the immunohistochemical and plasma studies although the number of cell lines studied was quite few. These values are lower than that of the expression for CgA mRNA in SCLC. Such a difference may depend on different processing of CgA and/or rapid secretion of CgA, as well as CgA derived peptides.

Information regarding CgB in SCLC, on the other hand, is limited to date. Weiler *et al.* [10] observed no expression of CgB in SCLC using immunoblot and immunohistochemical techniques. Sekiya *et al.* [25] reported low content of CgB-derived peptide (GAWK) and negative staining of GAWK in SCLC specimens. In contrast to these findings, production of CgB-derived peptides (GAWK, CCB) were noted in high frequency in SCLC cell lines. In particular, production of CCB-LI was remarkable as compared to that of GAWK-LI. Discrepancy between the present data and previous reports is unknown. However, production of GAWK-LI was relatively low even in the present study. This is consistent with low content of GAWK-LI in SCLC described by Sekiya *et al.* [25]. One possibility is that most of CgB in SCLC is processed into small fragments like GAWK and/or CCB-LIs, therefore CgB is not detected by immunoblot or immunohistochemical techniques. However, the present data do not allow us to draw conclusions of expression of CgB in SCLC. Further studies, especially about the expression of CgB mRNA in SCLC are necessary.

The Cg/Sg molecules contain multiple sites of paired or more adjacent basic aminoacids [1–4], suggesting that various biologically active peptides are generated through processing. In fact, several peptides including PST, GAWK and CCB were isolated from adrenal medulla, pituitary gland, or endocrine tumours of several species [5, 7, 26–30]. The aminoacid sequences of these peptides were homologous to certain parts of the Cg/Sg molecules, indicating that these peptides could be processing products. The present chromatographic study revealed multiple peaks of PST, GAWK and CCB-LIs in the culture medium of SCLC cell lines and the elution profiles were different among them. This suggests that the CgA and CgB molecules are processed into PST, GAWK and CCB-LIs in SCLCs and the processing is different among SCLCs.

In conclusion, production of PST, GAWK and CCB-LIs,

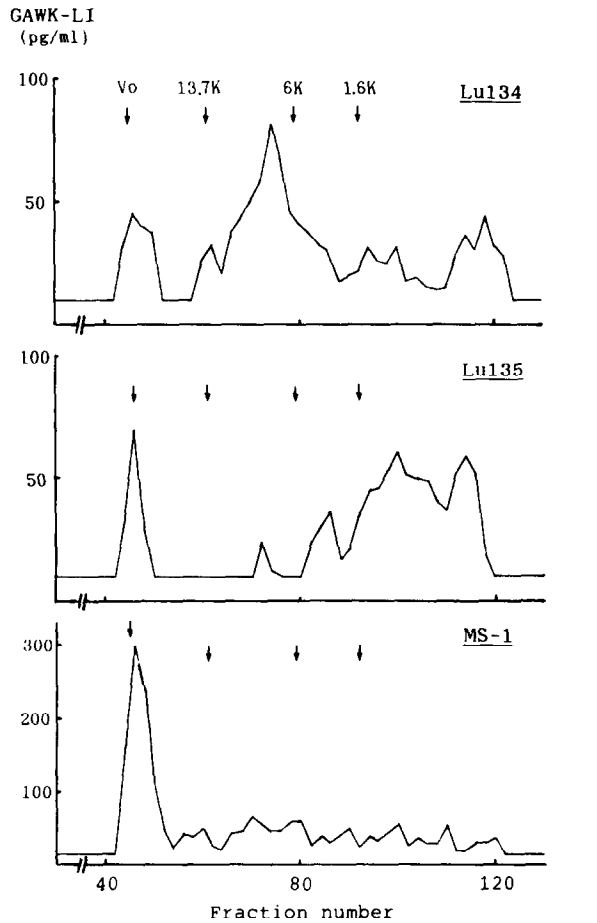

Fig. 2. Elution profile of GAWK LI in the culture medium of human SCLC-derived cell lines (Lu134, Lu135, MS-1) on Sephadex G-50 gel permeation chromatography. Two ml of the medium were layered onto a column (95 × 1.4 cm) and eluted with 1 mol/l acetic acid. Fractions were collected, dried, reconstituted with RIA buffer and assayed for GAWK-LI. The column was calibrated with protein markers (Vo, catalase; 13.7 k, ribonuclease; 6 k, human insulin; 1.6 k, human GRP1—16).

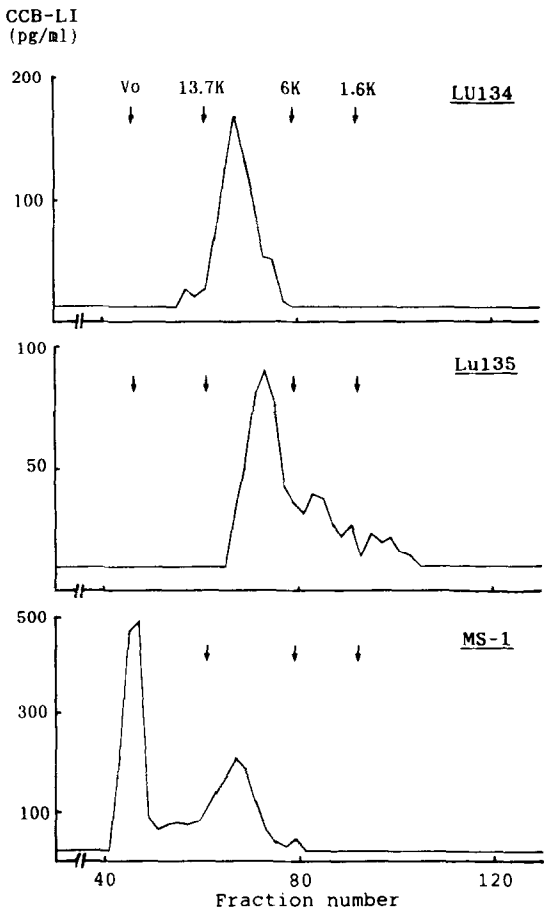

Fig. 3. Elution profile of CCB-LI in the culture medium of human SCLC-derived cell lines (Lu134, Lu135, MS-1) on Sephadex G-50 gel permeation chromatography. Two ml of the medium were layered onto a column (95 × 1.4 cm) and eluted with 1 mol/l acetic acid. Fractions were collected, dried, reconstituted with RIA buffer and assayed for CCB-LI. The column was calibrated with protein markers (Vo, catalase; 13.7 k, ribonuclease; 6 k, human insulin; 1.6 k, human GRP1-16).

presumed processing products of CgA and CgB, were noted in SCLCs. Among them, CCB-LI was produced in quantity and consisted of few components. CCB-LI may be a good marker for SCLC. Further studies to develop a good assay system for human plasma will be necessary.

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Inhibition of Gastrin-stimulated Growth of Gastrointestinal tumour cells by Octreotide and the Gastrin/Cholecystokinin Receptor Antagonists, Proglumide and Lorglumide

Susan A. Watson, David L. Morris, Lindy G. Durrant, John F. Robertson and Jack D. Hardcastle

The rat pancreatic cell line, AR42J possessed high-affinity gastrin and somatostatin receptors and its growth was stimulated by physiological gastrin-17 concentrations between 5×10^{-11} mol/l and 10^{-9} mol/l as measured by [^{75}Se]selenomethionine uptake. The somatostatin analogue, octreotide (2×10^{-7} to 2×10^{-11} mol/l), reduced this stimulated growth. Gastrin-stimulated AR42J growth was also inhibited by proglumide (3×10^{-4} mol/l) and lorglumide (3×10^{-5} mol/l) at maximal G17 concentrations of 5×10^{-11} and 10^{-10} mol/l, respectively, and the analogues competed with [^{125}I] gastrin-17 (5×10^{-10} mol/l) for binding to gastrin receptors on AR42J (50% inhibitory concentrations, $\leq 10^{-3}$ mol/l and 4×10^{-6} mol/l, respectively. Octreotide reduced the basal growth of the human gastric cell line, MKN45G, (which is associated with intracellular gastrin immunoreactivity) in serum-free medium to 73% of control at a concentration of 2×10^{-8} mol/l, which was reversed by gastrin-17 (10^{-10} mol/l). Lorglumide (3×10^{-5} mol/l) also reduced the basal growth to 30% of control, which was reversed to 78% by 10^{-5} mol/l gastrin. Proglumide had no effect on the basal growth of MKN45G.
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INTRODUCTION

THE POLYPEPTIDE hormone, gastrin, has both endocrine [1, 2] and paracrine/autocrine [3–6] growth modulatory effects on human gastrointestinal (GI) adenocarcinomas. Thus potential therapies of such hormone-responsive tumours need to inhibit both mechanisms of gastrin-stimulated growth. Gastrin/cholecystokinin (CCK) receptor antagonists have been described, which include glutamic acid derivatives such as proglumide [7] and benzodiazepam-like compounds such as L-365 260 [8]. For such antagonists to be effective they must bind with high affinity to gastrin receptors (GR) or be non-toxic so they can be administered at high enough concentrations to compete with gastrin for receptor occupation. Receptor antagonists may have to compete with both circulating gastrin, (which may be elevated in GI cancer patients [9]) and unknown concentrations of tumour-associated gastrin.

The hormone, somatostatin, is known to suppress several endocrine functions. These include inhibition of release of peptide hormones [10, 11] and direct effects on the growth of GI mucosa which is partly due to the effect of somatostatin on gastrin release [12, 13]. Long-acting derivatives of somatostatin have been derived, such as octreotide [14] and RC-160 [15].

The purpose of this study is to compare the abilities of the CCK/GR antagonists; proglumide and lorglumide (CR1409) and the somatostatin analogue; octreotide to inhibit GI tumour growth stimulated by gastrin firstly in an endocrine and secondly in a paracrine/autocrine manner.

MATERIALS AND METHODS

Cell lines

AR42J is a rat pancreatic acinar cell line [16]. MKN45G was derived from a human gastric adenocarcinoma, MKN45 [17] and was found to be associated with production of a gastrin-like peptide [4, 5]. The cell lines were maintained in RPMI culture

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