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THE RELEASE OF CHROMOGRANIN A AND B LIKE ACTIVITY FROM HUMAN LUNG CANCER CELL LINES

A potential marker for a subset of small cell lung cancer

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Abstract

Human small cell lung cancer (SCLC) is *in vivo* and *in vitro* characterized by a heterogeneous spectrum of neuroendocrine markers. The non-SCLC group is deprived of these markers, or expresses them in low quantities. In this paper we report on the release to the culture medium of neuroendocrine associated proteins, chromogranin A and B like activity (CABLA). The culture medium from three out of five SCLC cell lines and in one/five non-SCLC cell line contained significant levels of CABLA. Normal diploid foreskin fibroblasts and a histiocytic lymphoma cell line were deprived of CABLA production. The presence of CABLA in both SCLC and non-SCLC further stress their common histogenetic origin. The CABLA values were partly unrelated to other neuroendocrine markers. Determinations of CABLA could thus be a potential and valuable marker for a subset of SCLC.

Key words: Lung cancer, chromogranin A and B, cell lines.

Human small cell lung cancer (SCLC) is both *in vivo* and *in vitro* characterized by a wide spectrum of neuroendocrine markers (1–5). The production of these enzymes (creatine-kinase BB, l-DOPA decarboxylase, neuron specific enolase) and hormones (most frequently adrenocorticotropic hormone, bombesin, calcitonin, etc.) in large amounts distinguishes SCLC from the non-SCLC group (squamous cell, adeno- and large cell carcinoma). The latter group is instead defined by a 'plain' epithelial phenotype and the expression of the above mentioned markers at considerably lower levels.

We have used a radioimmunoassay (RIA) to study the release of chromogranin A and B like activity (CABLA) from human lung cancer cell lines representing both the SCLC and non-SCLC group. Chromogranin A is a glycoprotein with an apparent molecular weight of approximately 75 000 dalton, while chromogranin B has an approximate molecular weight of 100 000 dalton. Both forms are closely associated with the chromaffin granules in a wide variety of neuroendocrine cells and tumors (6–13). However, the presence of chromogranin A and B in established human lung cancer cell lines has not been studied. In this paper we demonstrate significant CABLA release to the culture medium in 3 out of 5 SCLC cell lines, while only 1/5 non-SCLC released CABLA.

Material and Methods

Cell lines and culture conditions. A panel of well-characterized SCLC and non-SCLC lung cancer cell lines were seeded at 2×10^5 cells/ml in RPMI-1640 supplemented with 10% foetal calf serum (FCS) and antibiotics (Penicillin 100 IU/ml and streptomycin 50 µg/ml). The SCLC cell lines were defined by a spectrum of neuroendocrine markers (1, 14, 15). The non-SCLC cell lines were characterized by epithelial markers as outlined in Table 1 (1, 14, 16). The histiocytic lymphoma cell line U-937 (17, 18) and a short-term culture of human neonatal diploid foreskin fibroblasts denoted AG-1523 were used as controls (AG 1523 was purchased from the Human Mutant Repository, Camden, NJ, USA). Double samples of each cell line were analyzed. The cells were harvested at day 4 and the cell number was counted in a Bürker chamber. The cells were washed 3 times in PBS and the supernatant was immediately frozen at −70°C until the time of analysis.

Radioimmunoassay for chromogranin A and B. Antibodies directed against CABLA were obtained by repeated immunizations of rabbits by chromogranin A- and B-fractions from a human pheochromocytoma. These frac-

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tions were obtained by ion exchange chromatography followed by reverse phase high performance liquid chromatography (Eriksson et al., submitted). Amino acid sequencing and composition data revealed identities with the mid portions of both chromogranin A and B (Eriksson et al., submitted). Furthermore, chromogranin A and B standards were analyzed in conjunction with the CABLA radioimmunoassay. The chloramine-T-method was used for preparation of the tracer. Aliquots of culture medium, cell homogenates and standards (200 μl) were incubated with 100 μl of the antiserum diluted 1/2000 and 100 μl tracer for 48 h at +4°C. The antibody-bound fraction was separated by addition of solid phase second antibodies, linked to μSepharose (Pharmacia, Uppsala, Sweden).

Results

Three out of 5 SCLC cell lines examined contained elevated levels as compared to the non-SCLC group (Table 2). The CABLA values for the SCLC group ranged from 21 to 546 ng/ml corresponding to 3 to 67 ng if calculated per 10^5 cells at the day of harvest (Table 2). The non-SCLC group presented values between 21 and 47 ng/ml, equivalent to 2–9 ng per 10^5 cells (Table 2). The control cell line from a histiocytic lymphoma (U-937) and the human fibroblasts gave estimates of less than 3 ng/ml medium, equivalent to <0.4 to 0.6 ng/10^5 cells.

Discussion

Chromogranin A has previously been demonstrated in sera from patients with lung cancer and, with immunohistochemical techniques, in sections from lung tumors (19–21). O'Connor & Deftos (22) have previously demonstrated chromogranin in a wide variety of neuroendocrine tumors. Nakajima et al. (19) and Said et al. (20) examined tissue sections of SCLC for immunohistochemical demonstrations of chromogranin A. Nakajima et al. found a positive reaction in 5 out of 29 examined biopsies, while Said et al. failed to demonstrate any positive reaction in the 12 examined SCLC cases. Sobol et al. (21) examined sera from 46 patients with SCLC. Fifty-three percent of the patients with limited disease and 72% of those with extensive disease demonstrated elevated levels of chromogranin A. A possible explanation for these partly contradictory results could be that different antisera and methods were used.

The discrepancy in these previous investigations on sera from patients and immunohistochemical examinations tempted us to study a panel of well-characterized cell lines of SCLC and non-SCLC origins. This enabled us to analyze the CABLA release in relation to other well-defined, closely examined, markers for human lung cancers. For this purpose we have used a rabbit antiserum raised against chromogranin A and B (Eriksson et al., submitted). We prefer the designation chromogranin A and B like activity (CABLA) as we have not purified and sequenced the immunoreacting material released by these lung cancer cell lines. However, the very low levels in the control cell lines strongly indicate that CABLA is produced by the lung cancer cell lines. These facts, and previous experiences on human cell lines, make further controls of less importance in this study on human lung cancer cell lines.

Thus we found that 3 out of 5 human SCLC cell lines

Table 1

Phenotypic properties of examined small and non-small lung cancer cell lines

Cell line	Neurosecr. gran.	Growth pattern	Neuron-specific enolase (ng/mg prot.)
U-1285 (SCLC)	+	C-S	380
U-1568 (SCLC)	+	C-S	1 740
U-1690 (SCLC)	+	C-A	2 100
U-1906 (SCLC)	+	C-A	340
U-2050 (SCLC)	+	C-S	850
U-1752 (SQC)	–	A	<100
H-157 (LCC)	NT	A	<100
H-661 (LCC)	NT	A	NT
H-23 (ADC)	NT	A	<100
H-125 (ADC)	NT	A	<100
U-937 (HCL)	–	S	<100
AG-1523 (HFB)	NT	A	NT

Histopathological diagnosis of the cell lines in parenthesis. SCLC: Small cell carcinoma. SQC: Squamous cell carcinoma. ADC: Adenocarcinoma. LCC: Large cell carcinoma. HCL: Histiocytic lymphoma. HFB: Human fibroblasts. S: Suspension growing cell line. C: Cluster-forming cell line. A: Cell line growing with attached cells, monolayer culture. NT: Not tested. Ref: 14, 15, 17, 21, 26.

Table 2

Chromogranin A and B like activity (CABLA) in human lung cancer cell lines

Cell line	CABLA (ng/ml medium)	CABLA (ng/10^5 cells)
U-1285 (SCLC)	546	67
U-1568 (SCLC)	166	53
U-1690 (SCLC)	38	4
U-1906 (SCLC)	21	3
U-2050 (SCLC)	50	9
U-1752 (SQC)	37	5
H-157 (LCC)	21	4
H-661 (LCC)	47	9
H-23 (ADC)	20	2
H-125 (ADC)	25	2
U-937 (HCL)	<3	<0.4
AG-1523 (HFB)	<3	<0.6

For abbreviations and references see Table 1.

released CABLA in higher amounts than the group of non-SCLC cell lines (Table 2). The recorded levels in the SCLC group varied markedly, which is in complete accordance with a number of previous reports on the neuroendocrine marker expression in SCLC, i.e. neurosecretory granules, L-DOPA decarboxylase, bombesin, neuron-specific enolase, creatine kinase BB (3, 10, 14, 15, 23, 24). Gazdar et al. (24) have suggested that the SCLC cell lines can be subgrouped into 'classical' and 'variant' types; the 'classical' type has high levels of all neuroendocrine markers, while the 'variant' type has lower levels of bombesin and L-DOPA decarboxylase and different morphology.

The high CABLA value in the 'variant' SCLC cell line U-1285 was interesting since this cell line expressed other neuroendocrine markers, such as neuron-specific enolase (NSE) in low levels. The SCLC cell lines with low CABLA values (U-1690, U-1906) expressed considerably higher levels of other neuroendocrine markers than the non-SCLC group (Table 1). The cell line U-1690 could be denoted as 'classical type', whereas U-1906 had intermediate characteristics (low NSE values, high bombesin production and variant morphology).

The presence of low levels of CABLA even in non-SCLC cell lines suggests that the SCLC and non-SCLC groups have a common histogenetic origin. This has previously been indicated by Gazdar et al. (25) and by Bergh et al. (1), based on the presence of other neuroendocrine markers and of epithelial markers in low levels in the non-SCLC and the SCLC group respectively, and the fact that SCLC *in vivo* can transform into the non-SCLC type.

In this paper we have demonstrated the CABLA release to the culture-medium from a subset of examined human SCLC cell lines which partly lack some other neuroendocrine markers. CABLA might thus identify a subgroup of SCLC, which otherwise could be considered to belong to the non-SCLC group.

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