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Immunohistochemical study of chromogranin in 100 cases of pheochromocytoma, carotid body tumour, medullary thyroid carcinoma and carcinoid tumour

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Summary. Neuroendocrine cells have histologically common features represented by argyrophilic cytoplasm containing neuroendocrine granules. Neuroendocrine granules are composed of various kinds of peptide hormones, amines, carrier proteins and ATP. Although various kinds of peptide hormones have been detected in neuroendocrine tumours, a peptide hormone has not been required as a standard marker for these tumours. Chromogranin is a purified protein which binds catecholamines specifically and is recognized as a carrier protein. We carried out an immunohistochemical study of chromogranin immunoreactivity in 100 neuroendocrine tumours including pheochromocytomas, carotid body tumours, medullary thyroid carcinomas and carcinoid tumours. Marked immunoreactivity was observed in 85% of carcinoid tumours and 100% of the other tumour types. A non-functioning paraganglioma and a malignant carcinoid tumour without any other detectable marker also showed strong immunoreactivity to chromogranin. Chromogranin immunoreactivity is a useful tool for neuroendocrine tumours.

Key words: Chromogranin – Pheochromocytoma – Medullary thyroid carcinoma – Carcinoid tumour – Immunohistochemistry

Introduction

Chromogranin is a soluble protein found in greatest quantity in the electron dense granules of the adrenal medulla. While its exact function is not known, studies of bovine adrenal medulla showed that chromogranin stabilizes the soluble portion of the secretory granules through interaction with ATP and catecholamines (Depurda et al. 1971).

Chromogranin has been detected in most polypeptide hormone-producing tissues by radioimmunoassay and immunohistochemistry (O'Connor et al. 1983). A variety of neuroendocrine tumours including pituitary adenomas (De Stephano et al. 1984, Lyoyd et al. 1985), islet cell tumours (Lyoyd et al. 1984), pheochromocytomas, medullary thyroid carcinomas and parathyroid adenomas (O'Connor et al. 1983) have been found to contain chromogranin. A large series of pheochromocytomas, medullary thyroid carcinomas and carcinoid tumours of various sites were evaluated for chromogranin immunoreactivity and its usefulness as a general marker for tumours of neuroendocrine origin was confirmed.

Materials and methods

Thirty-six surgically excised pheochromocytomas (27 of adrenal and 9 of extra-adrenal origin) were examined. Preoperative diagnosis was based on elevated serum catecholamine levels in all patients, except for a case with a non-functioning paraganglioma of the urinary bladder. Three were malignant and showed distant metastases. Three cases with Sipple's syndrome (multiple endocrine adenomatosis, type II) were included.

Serum catecholamine levels were not available in the four carotid body tumours studied. These showed a typical alveolar pattern with a vasculized stroma.

Five cases of Sipple's syndrome were included in the fifteen cases of MCT. In one case, a large amount of melanin production was observed in the tumour cells. Amyloid was present in most of the cases.

There were 45 carcinoid tumours including 38 of gastrointestinal tract origin, 3 from the bronchus, 2 from the ovary and one each from the kidney and middle ear. Seven tumours were obtained at autopsy and were 1 gastric, 4 rectal and 2 of bronchial origin associated with distant metastases. The histological pattern in the carcinoid tumours was mainly of mixed trabecular and insular type. A glandular pattern was present in five tumours of gastric and duodenal origin. All of the above tumours were argyrophilic.

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Tissues were fixed in 10% formalin, embedded in paraffin and serial sections were cut at 2.5 μm. Sections were stained with the avidin-biotin peroxidase complex (Hsu et al. 1981). After blocking of endogeneous peroxidase with methanol-hydrogen peroxide for 20 min and incubation with normal goat serum, sections were incubated for 30 min with primary rabbit antiserum diluted at 1:100. Antibody for chromogranin was producd by rabbits immunized with chromogrann of the pig adrenal medulla in which chromogranin A was the major component (Yamada et al. 1978). Simultaneous immunostaining for serotonin (Sera-lab Ltd. Co., Sussex, England), gastrin and glicentin (Yanaihara, Shizuoka), somatostatin (Mouri, Sendai), pancreatic polypeptide (IBL, Takasaki, Japan), calcitonin (DAKO patts, Copenhagen, Denmark) and carcinoembryonic antigen (Mochida, Tokyo, Japan) was carried out on occasional cases. Specificity of the antibodies except for those obtained commercially were confirmed by radioimmunoassay. Control studies were performed by replacement of primary antisera by non-immune rabbit serum. Biotinylated goat anti-rabbit IgG and avidin biotin peroxidase complex (Vector Lab., USA) were incubated for 30 min in each. Diaminobenzidine (Dojin Kagaku, Kumamoto, Japan) was used as a chromogen.

Results

All pheochromocytomas including both those of adrenal and extra-adrenal origin were strongly positive for chromogranin in most of the tumour cells (Fig. 1). The cells showed immunoreactive brown granules, diffusely in the cytoplasm. Cells in the metastatic sites also showed diffuse immunoreactivity. The hyperplastic region and the normal adrenal medulla adjacent to pheochromocytoma showed stronger immunoreactivity than in the tumour. There was no difference in the intensity or frequency of immunoreactivity between the epinephrine dominant type and norepinephrine dominant type of pheochromocytoma. A malignant paraganglioma of the urinary bladder in which catecholamine levels of serum and urine were normal also showed strong immunoreactivity to chromogranin.

Carotid body tumours had sporadically positive cells for chromogranin. The number and intensity of the immunoreactive cells varied case by case (Fig. 2).

All medullary thyroid carcinomas were positive for chromogranin. The tumour cells showed heterogeneity in staining; some cells reacted weakly but the other cells reacted strongly (Fig. 3). In the cases of Sipple' syndrome, the clusters of chromograinin positive cells were remarkable around the tumour (Fig. 4). They were consistent with hyperplastic parafollicular cells. Thyroid follicular cells were usually negative, however, several follicles adjacent to the medullary thyroid carcinomas were composed of cells obviously positive for chromogranin (Fig. 5). Simultaneous staining of chromogranin, calcitonin and carcinoembryonic antigen (CEA) in the serial sections of the tumours and hyperplastic regions revealed that those positive cells were identical.

Thirty seven out of 44 cases (85%) of carcinoid tumours were positive for chromogranin. Cases immunoreactive to chromogranin were summarized in the Table 1. The immunoreactive site was localized in the infranuclear region which was very similar to the immunoreactive site of prostate-specific acid phosphatase and Grimelius' argyrophilic reaction (Fig. 6). All malignant carcinoid tumours with distant metastases were positive, even in the metastatic sites (Fig. 7). Only 7 carcinoid tumours (3 rectal, 3 small intestin and one stomach) were negative for chromogranin.

Simultaneous immunostaining of peptide hormones such as gastrin, glicentin, somatostatin, pancreatic polypeptide and serotonin in the serial sections revealed that chromogranin and several peptide hormones were contained in the same cells of the most cases. However, 9 carcinoid tumours comprising 1 gastric, 7 rectal and 1 of bronchial origin were positive only for chromogranin and were negative for the hormones mentioned above. In contrast, among 7 carcinoid tumours which were negative for chromogranin, a gastric carcinoid was positive for serotonin, gastrin and somatostatin, and a small intestinal carcinoid reacted to serotonin, gastrin and pancreatic polypeptide. The remaining tumours had no immunoreactive cells for any peptides examined. Relationships between chromogranin and peptide hormones in the carcinoid tumours is summarized in Table 2.

There was no correlation between the biological behavior of the tumours and the intensity or frequency of the immunoreactivity of chromogranin.

Discussion

The composition of chromaffin granules of the adrenal medulla is 42% protein, 20% lipid, 19% catecholamines and 17% nucleotides (Winkler 1976). The soluble proteins are composed of 40% chromogranin, 5% dopamine-β-hydroxylase and methionine-enkephalin, leucine-enkephalin, pro-enkephalin (Mizobe F, 1984) and glycoprotein III (Fischer-Colbrie et al. 1984). Human chromaffin granules of the adrenal medulla contain three immunologically distinct groups of acidic proteins; chromogranin A, B and C (Hagn et al. 1986). Those chromogranins are also found in other endocrine tissues but are not always stored together (Hagn et al. 1986).

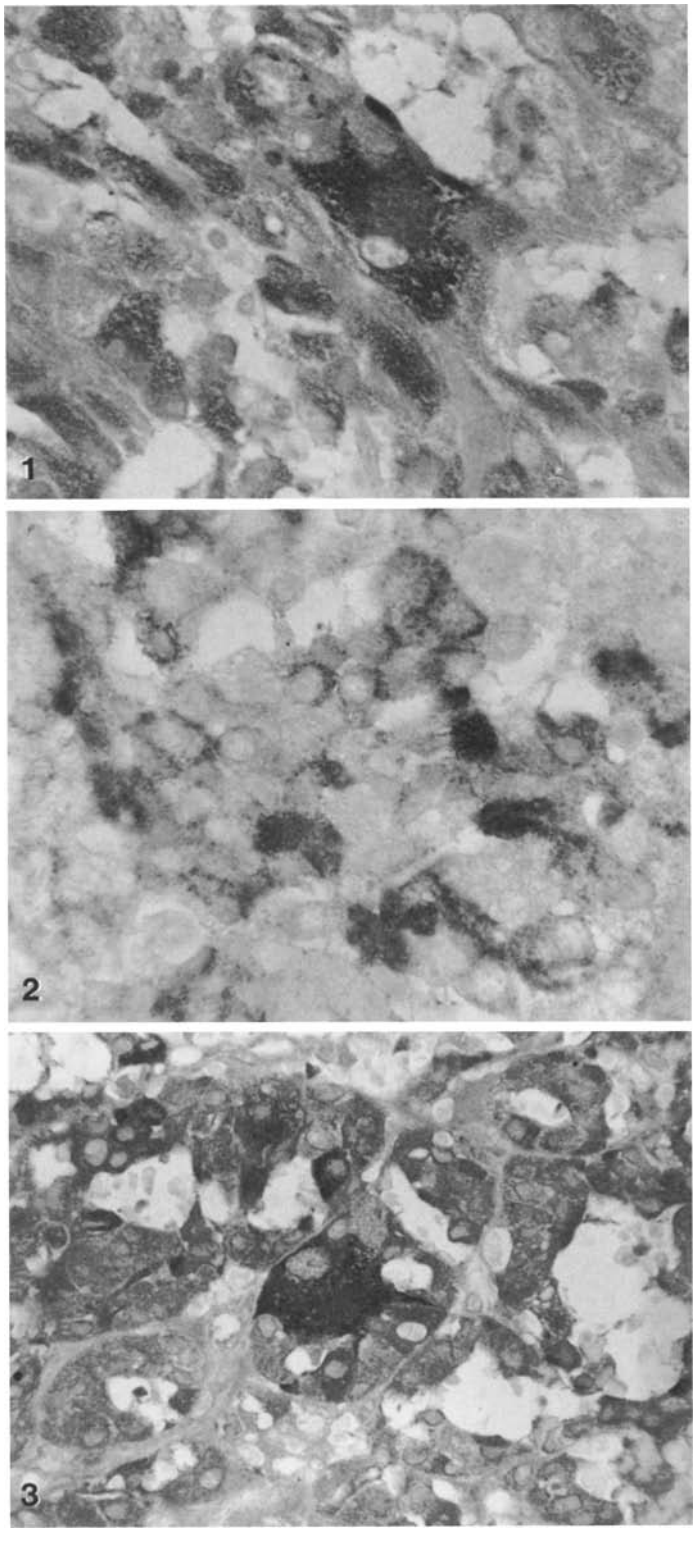
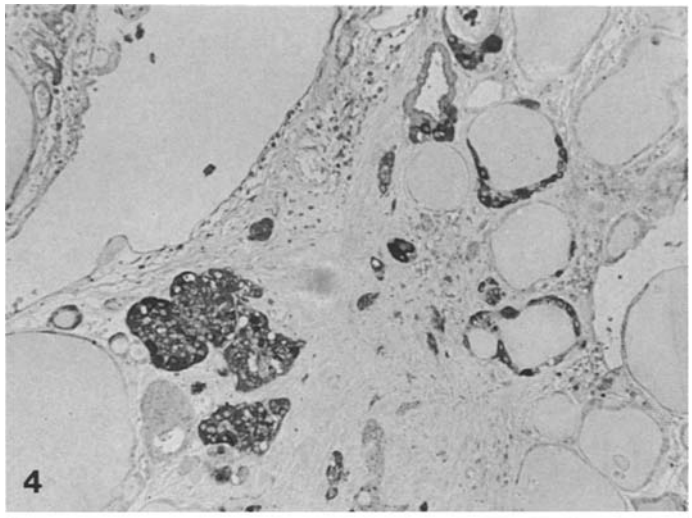


Fig. 1. Pheochromocytoma: Most of the tumor cells are strongly positive for chromogranin (ABC ×400)

Fig. 2. Carotid body tumor shows heterogeneity of immunoreactivity for chromogranin. Some cells are strongly positive but others are negative (ABC ×400)

Fig. 3. Medullary thyroid carcinoma showing strong immunoreactivity to chromogranin (ABC ×400)

Pheochromocytomas contain abundant chromaffin granules in both epinephrine and norepinephrine-dominant types (Kimura et al. 1984). Chromaffin reaction is sometimes negative in carotid body tumours and non-functioning paragangliomas, however, formaldehyde-induced fluorescence demonstrates formaldehyde conjugated catecholamines. Electron-microscopy revealed numerous electron dense granules in the tumour cells (Glenner and Grimlay 1974). Thus it seems reason-



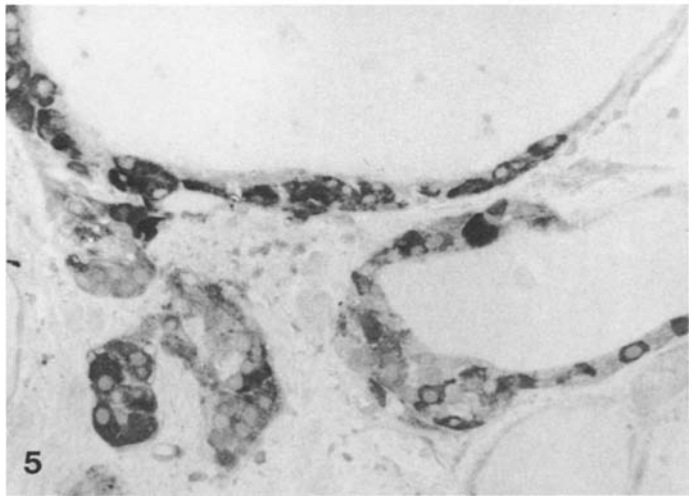


Fig. 4. Clusters of chromogranin positive cells consistent with C-cell hyperplasia in Sipple's syndrome (ABC ×160)

Fig. 5. Some of the thyroid follicular cells adjacent to MCT are composed of chromogranin positive cells (ABC ×250)

Table 1. Immunoreactive cases to chromogranin

	Positive Cases
Pheochromocytoma ($n=36$)	36 (100%)
Carotid body tumor ($n=4$)	4 (100%)
Medullary thyroid carcinoma ($n=15$)	15 (100%)
Carcinoid tumor ($n=45$)	38 (85%)
Rectum ($n=27$)	24 (89%)
Appendix ($n=1$)	1 (100%)
Small Intestine ($n=6$)	3 (50%)
Stomach ($n=4$)	3 (75%)
Bronchus ($n=3$)	3 (100%)
Ovary ($n=2$)	2 (100%)
Kidney ($n=1$)	1 (100%)
Middle Ear ($n=1$)	1 (100%)

able that all pheochromocytomas and carotid body tumours showed immunoreactivity for chromogranin. It is especially noteworthy that a malignant paraganglioma of the urinary bladder showed strong immunoreactivity to chromogranin in spite of normal levels of catecholamines in serum and urine.

Numbers of peptides are known to be produced by pheochromocytomas, methionine-enkephalin is the most readily detectable peptide in pheochromocytomas. However, 3 out of 14 tumours failed to react (Hassoun et al. 1984).

Medullary thyroid carcinoma also produces multiple peptide hormones and amines, such as calcitonin, gastrin-releasing peptide (Kameya et al. 1983), serotonin, dopamine (Baylin et al. 1979) and CEA. Chromogranin was demonstrated in all medullary thyroid carcinomas together with calcitonin and CEA.

Carcinoid tumours are known to produce multiple hormones, amines and sometimes prostate-specific acid phosphatase, probably produced from cells of hindgut origin (Kimura and Sasano 1986). Serotonin is most often detected in mid gut origin carcinoids. Chromogranin, however, has no predi-

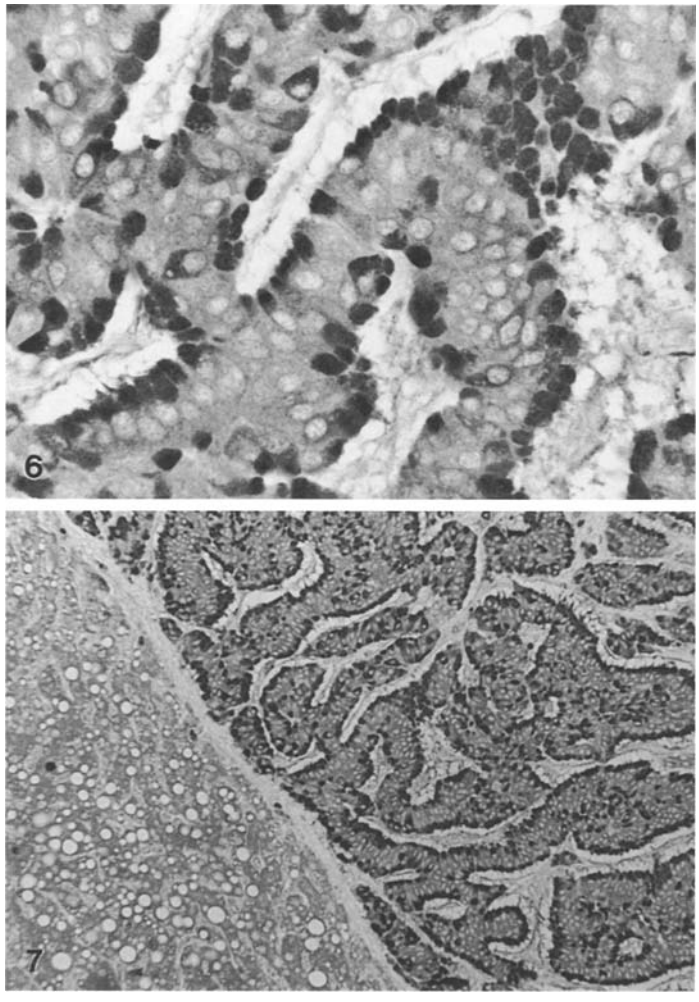


Fig. 6. Strong immunoreactivity to chromogranin is observed mainly in infranuclear area of rectal carcinoid tumor (ABC ×250)

Fig. 7. Chromogranin is strongly positive even in metastatic rectal carcinoid tumor in liver (ABC ×100)

Table 2. Immunohistochemical study of chromogranin and hormones in carcinoid tumors

	Stomach ($n=4$)	Small Intestine ($n=6$)	Appendix ($n=1$)	Rectum ($n=27$)	Others ($n=7$)	Total ($n=45$)
only CMG positive	1	0	0	7	2	10
CMG+5HT	2	1	1	2	1	7
CMG+Gastrin	2	2	0	8	0	12
CMG+Glicentin	0	0	0	5	2	7
CMG+Somatostatin	1	1	0	6	2	10
CMG+pancreatic polypeptide	1	0	1	3	2	7
CMG negative	1	3	0	3	0	7

lective distribution, it does not give information on tumour origin. The fact that chromogranin immunoreactivity is found in 85% of carcinoid tumours suggests that chromogranin is an excellent marker of carcinod tumours. It is useful for the diagnosis of carcinoids derived from unexpected sites, such as the middle ear or kidney. Two of 7 chromogranin negative tumours in the gastrointestinal tract reacted to the other peptide hormones such as gastrin, somatostatin, pancreatic polypeptide and serotonin. Thus, non-immunoreactivity to chromogranin was not dependant on artificial factors such as poor fixation or fixative type. Carcinoids, especially those of rectal origin resemble islet cell tumours of the pancreas in terms of their multiple hormone production (O'Brian et al. 1982). Chromogranin was detected in most of the islet cell tumours, but not in insulinomas

or somatostatinomas (Lyoyd et al. 1984). We could not find any correlation of immunoreactivity with chromogranin and the types of peptide hormone found in carcinoids. In pituitary adenomas, chromogranin was negative in prolactin-, growth hormone and corticotropin-producing adenomas as well as in the cytoplasm of human prolactin, growth hormone and corticotropin producing cells (Lyoyd et al. 1985). We have shown that human ACTH cells, Cushing adenomas and some growth hormone-producing adenomas contained neurophysins which have been considered to be carrier proteins for vasopressin in the hypothalamus and neurohypophysis (Kimura et al. 1986).

Although chromogranin is not necessarily present in all hormone-producing cells, its widespread and highly selective distribution support the suggestion that chromogranin is the most useful marker of this type of cell and tumour. Neuron-specific enolase has been recognized as an excellent marker for neuroendocrine tumours, however, its practical application is not reliable because of its presence in a wide variety of tissues, not restricted to neuronal or neuroendocrine cells (Haimoto et al. 1985).

Chromogranin negative tumours indicate the possibility of the presence of other types of chromogranin, or of completely different proteins.

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