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Secretogranin IV immunoreactivity in medullary thyroid carcinoma: an immunohistochemical study of 62 cases

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Abstract. The presence and intracellular distribution of secretogranin IV (Sg IV) was determined on light microcop by the avidin-biotin peroxidase complex method with the monoclonal antibody (mAb) Hisl-19 in normal and hyperplastic C-cells, in 62 primary medullary thyroid carcinomas (MTCs) and in 17 MTCs in tissue from synchronous and/or metachronous lymph node metastases and in one liver metastasis. Sg IV immunoreactivity was present in almost all normal-looking and hyperplastic C-cells, in 59 of 62 (96%) of the primary tumours, in 18 of 26 (69%) lymph node metastases and in distant mestastasis. Sg IV reactivity ranged from small foci of positive tumour cells to a reaction in virtually every malignant cell. Two different staining patterns were obvious: a granular cytoplasmic reactivity and a perinuclear cluster-type signal. Normal-appearing and hyperplastic C-cells were characterized by a uniform granular staining often coexisting with discrete cluster-type immunoreactivity. Various combinations of these staining patterns were observed in C-cell carcinomas. The pure cluster-type reactivity was restricted to malignant C cells and was not detected in normal-appearing and hyperplastic C-cells. In serial sections immunohistochemical results for Sg IV, calcitonin (Ct) and chromogranin A (Cg A) showed only partial correlation. Depending on the area of the tumour chosen, immunohistochemical reactivity for Ct and Cg A might not be demonstrated in neoplastic C-cells, while staining for Sg IV was retained. The amout and type of Sg IV reactivity of MTCs was not correlated with the biological behaviour of the tumours. These results indicate that mAb Hisl-19 is an excellent marker for normal, hyperplastic and neoplastic C-cells. MAb Hisl-19 is especially useful in cases with weak or questionable reactivity for Ct and Cg A. The switch from the granular pattern to the perinuclear distribution seems to indicate a malignant transformation of C-cells and might prove useful as an additional diagnostic clue.

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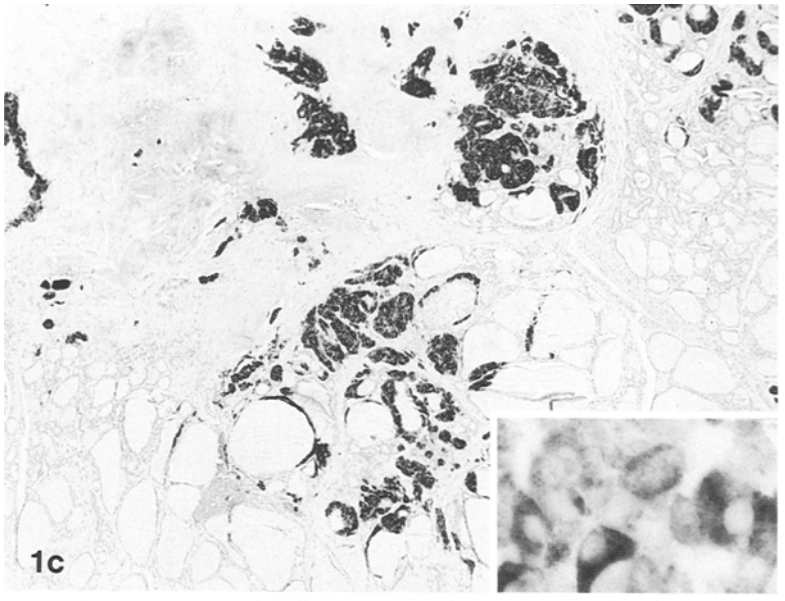
Introduction

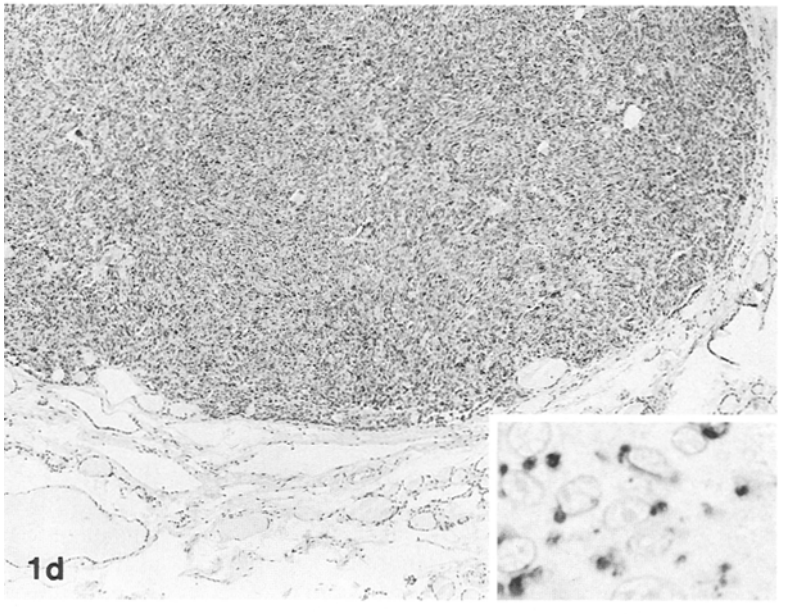
In a recently published classification of acidic proteins associated with secretory granules the protein specifically recognized by monoclonal antibody (mAb) Hisl-19 was referred to as secretogranin IV (Sg IV; Huttner et al. 1991). It shares many biochemical and molecular key features with the chromogranin family, but differs from chromogranin (Cg) A, Cg B and Sg II in tissue distribution, apparent molecular weight and isoelectric point (Krisch et al. 1988). Previous reports have described a widespread Sg IV immunoreactivity in cells and neoplasms of the dispersed neuroendocrine system (Bordi et al. 1988; Krisch et al. 1986; Srikanta et al. 1986; Wirnsberger et al. 1992). Sg IV has also been demonstrated in normal and hyperplastic C-cells (Neuhold et al. 1989) as well as in a small series of C-cell carcinomas (Deftos et al. 1988).

The aim of the present study was to extend the analysis of Sg IV immunoreactivity to a larger series of primary and metastatic neoplastic C-cell lesions (MTCs). Therefore the amount and pattern of Sg IV immunoreactivity were compared with Cg A and calcitonin (Ct) reactivity and to the outcome of disease. Additionally, apparently normal C-cells in extratumoral thyroid tissue in sporadic MTCs and hyperplastic C-cells of familial MTCs were studied.

Materials and methods

The study material consisted of formalin-fixed (8%) paraffin – or paraplast – embedded archival tissue from 62 cases of primary medullary carcinomas, available from the files of the Department of Clinical Pathology, University of Vienna School of Medicine. According to Harach et al. (1992) only tumours exhibiting a positiv-





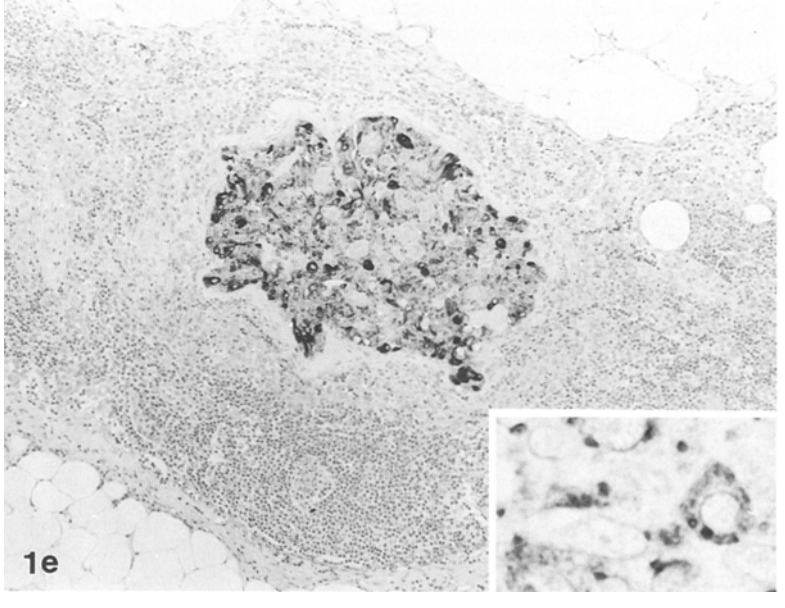


Fig. 1. C-cell hyperplasia: double immunostaining for secretogranin (Sg) IV (**a**) and calcitonin (**b**): most of the calcitonin-positive cells showed Sg IV immunoreactivity; only very few cells remained unstained (×120). Sg IV staining patterns: intense granular staining in an occult, familial medullary thyroid carcinoma (MTC; **c**). Exclusively Golgi-related paranuclear signal in a primary MTC (**d**). Combined cluster and granular pattern in the accompanying lymph node metastasis (**e**). Avidin-biotin peroxidase complex technique (**c, d** ×40; **e** ×100)

ity to Ct or Cg A of at least 1% of the cells were accepted as C-cell carcinomas and included in this study. Forty-six tumours were of the sporadic type and 16 tumours represented the familial form. Six MTCs were smaller than 1 cm (stage pT1). Twenty-six lymph node metastases from 17 cases were studied. Tissue from one liver metastasis was also used. The patients were followed up until September 1992, and in all cases with a fatal outcome deaths from MTC were identified.

Tumour blocks were cut serially and stained with haematoxylin and eosin and the alkaline congo-red stain for the identification of amyloid. Immunohistochemical staining for Ct, Cg A and Sg IV was performed by the avidin-biotin peroxidase complex technique (Hsu et al. 1981). The anti-Ct antibody used was purchased from Chemicon (Germany; preabsorbed with thyroglobulin); the anti-Cg A antibody MU 137–UC was from Biogenex (USA); and the Hisl-19 antibody was kindly supplied by Dr. G.S. Eisenbarth, Joslin Diabetes Center, Boston, Massachusetts. As a negative control non-immune serum was substituted for the primary antibody, while C-cells from tumour-free areas and normal human pancreas served as positive control. The results with each antibody were assessed semiquantitatively and graded as (−)=absent, (+)=isolated tumour cells positive, (++)=moderate to strong focal positivity and (+++)=majority of tumour cells positive.

Double immunostaining for calcitonin and Sg IV was carried out in two cases with C-cell hyperplasia as described by Scopsi (1990) to determine whether all C-cells express Sg IV immunoreactivity. Briefly, the sections were first stained for Sg IV with Hisl-19 antibody and the PAP procedure using 3-amino-9-ethylcarbazole as substrate. After selected areas were photographed the red reaction product was removed with alcohol, and Ct immunoreactivity was shown by avidin-biotin/alkaline phosphatase complex and development with fast blue BB/naphtol AS–BI phosphate. Identical fields that had been previously recorded were photographed (Fig. 1a, b).

Results

Sg IV immunoreactivity was seen in various combinations of the cluster-type and the granular pattern as described by Bordi et al. (1988). Coarse immunoreactive aggregates in a perinuclear position representing the cluster-type pattern can be differentiated from fine-granular cytoplasmic staining. The two patterns were often found to coexist within the same cell and the cluster type tended to be masked in cells with strong granular cytoplasmic positivity.

Almost all normal-looking C-cells in extratumoral areas of sporadic carcinomas as well as most of the hyperplastic C-cells in familial cases showed a more or less marked granular cytoplasmic Sg IV immunoreactivity. Concomitant cluster-type staining was observed in a considerable percentage of normal looking C-cells, but a pure cluster-type never appeared. The follicular epithelium was consistently negative.

Fifty-nine of the 62 primary tumours, 18 of 26 lymph node metastases and the liver metastasis showed variable degrees and patterns of Sg IV immunoreactivity ranging from a few to virtually all cancer cells. In 45 of the primary tumours and in 10 lymph node metastases a mixed granular and cluster type Sg IV signal was seen. Predominant granular staining was present in 11 of the 16 familial cases, in 17 of the 46 sporadic ones and in 8 of 26 nodal metastases. MTCs of stage pT1 expressed marked granular immunostaining in 5 of 6 cases, while one tumour measuring less than 1 cm in diameter showed the cluster type only. A pure cluster type was observed in 14 primary and in 7 metastatic lesions. In 9 of 17 cases the extent and pattern of Sg IV immunostaining of the lymph node metastases differed from the respective primary tumour. In contrast with exlusively cluster-type positive primary lesions, an intense granular staining pattern was recognized in their lymph node metastases (2 cases).

In Table 1 the intensity of Sg IV immunostaining and the number of Sg IV positive tumours are compared with the results of the immunohistochemical detection of Ct and Cg A. The evaluation of serial sections only showed colocalization of Sg IV, Cg A and Ct in significat fractions of the neoplastic cells in about one third of all cases. In some MTCs complementary results for Sg IV, Cg A and Ct were observed (Fig. 2a–c). Generally Cg A and Ct immunostaining was present in a greater proportion of the neoplastic cells than Sg IV immunoreactivity. Five primary tumours and two lymph node metastases showed intense Sg IV immunoreactivity in the majority of the tumour cells while immunostaining for Cg A and Ct yielded only a few scattered, weakly positive cells (Fig. 2d–f).

By the end of this study 12 of 62 patients had died as a direct consequence of MTC. The comparison of the type and/or score of Sg IV immunoreactivity with the biological of behaviour of MTCs revealed no correlation.

Discussion

The immunohistochemical results of the present study confirm that Sg IV immunoreactivity, detected by the mAb Hisl-19, is present in C-cells and in most MTCs

Table 1. Comparison of secretogranin (Sg) IV, chromogranin (Cg) A and calcitonin (Ct) immunoreactivity in primary and metastatic C-cell carcinomas

	Sg IV				Cg A				Ct			
Intensity	−	+	++	+++	−	+	++	+++	−	+	++	+++
Familial $n=16$	1	3	1	11	0	1	4	11	0	1	5	10
Sporadic $n=46$	2	14	18	12	1	15	17	13	1	13	15	17
Metastases $n=26$	8	6	7	5	0	8	9	9	0	9	9	8

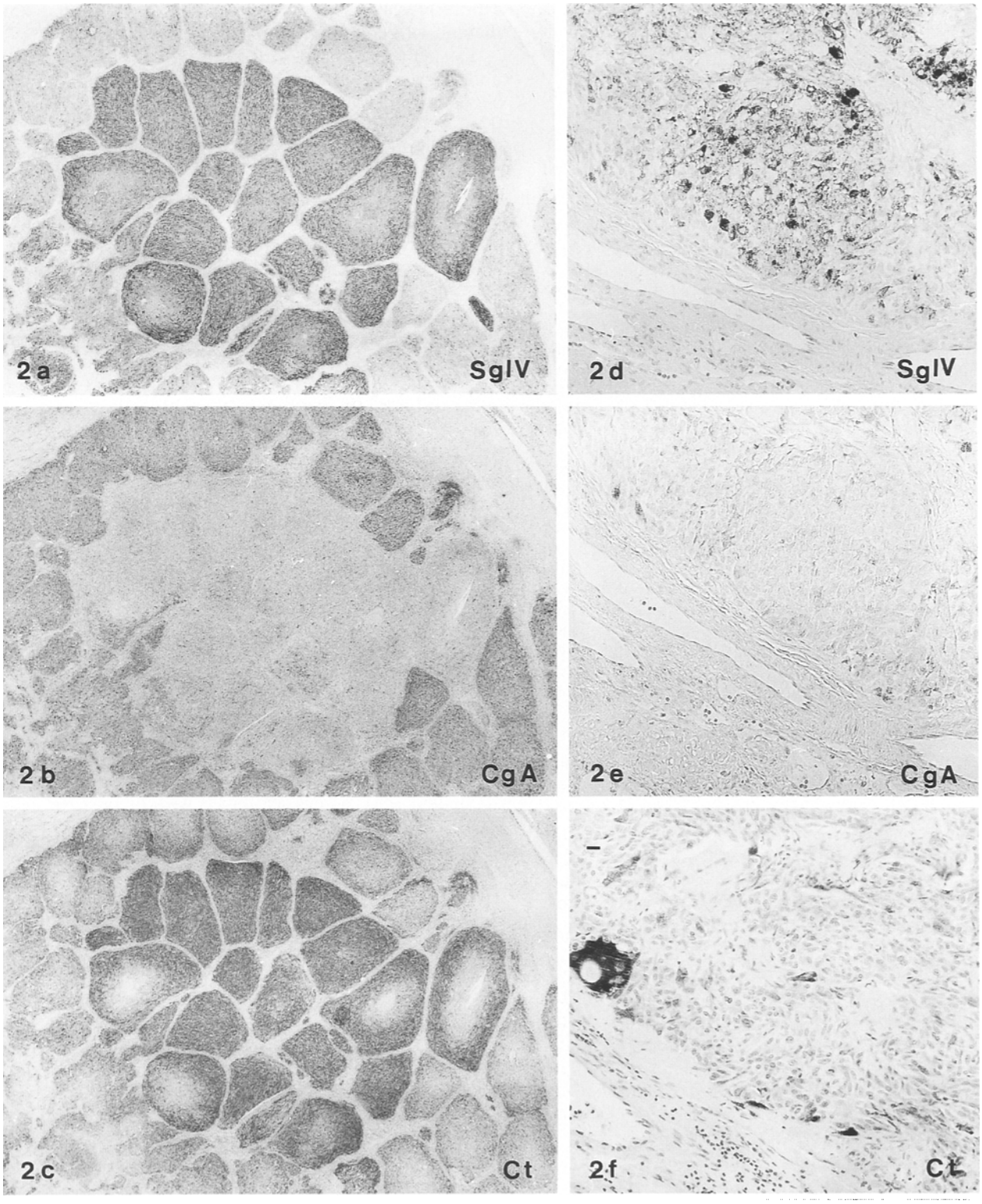


Fig. 2. Serial sections of a primary medullary thyroid carcinoma (**a–c** × 25) with almost complementary distribution of secretogranin (Sg) IV (**a**) and chromogranin (Cg) A (**b**) and calcitonin (Ct; **c**). Accompanying lymph node metastasis (**d–f** × 160) with intense Sg IV (**d**) immunostaining. Only a few neoplastic cells were weakly reactive for Cg A (**e**) or Ct (**f**). Avidin-biotin peroxidase complex technique

(Deftos et al. 1988; Neuhold et al. 1989). The granular Sg IV immunostaining is assigned to cytoplasmic secretory granules similar to those demonstrated previously for Cg A (Varndell et al. 1985). Our colocalization experiments with Cg A are indirect evidence that the absence of the granular staining pattern of Sg IV is not commonly associated with degranulation of the tumour cells, but is consistent with a deficient or considerably reduced aggregation in the secretory granules of neoplastic C-cells.

A peculiar feature of Sg IV is the cluster-type im-

munoreactivity corresponding mainly to the Golgi region at the ultrastructural level (Bordi et al. 1988; Krisch et al. 1988). A combination of the two basic staining patterns was observed in normal-appearing and neoplastic C-cells. Loss of granular staining with Sg IV in MTCs was in part combined with a retained Golgi-type signal. In C-cells this phenomenon seems to be associated with neoplastic transformation. We detected no normal-looking or hyperplastic C-cells that solely exhibited the cluster type of Sg IV immunoreactivity, while 14 of 62 MTCs demonstrated a pure cluster-type staining pattern.

In our series of MTCs neither the staining pattern nor the cell differentiation correlated with the biological behaviour of the carcinomas, contrasting previous findings in endocrine pancreatic tumours (Bordi et al. 1988). Comparison of the results for Sg IV, Cg A and Ct confirms that these substances are regular constituents of normal looking and hyperplastic C-cells. Nevertheless, colocalization studies showed appreciable differences in the distribution of these peptides in C-cell carcinomas. Our findings parallel immunohistochemical data obtained for Cg A, Cg B and Sg II in MTCs (Schmid et al. 1992).

The observed heterogeneities may reflect different biosynthesis and intracellular processing of distinct members of the chromogranin/secretogranin family (Cetin and Grube 1990) but other reasons such as masking or post-translation modification of the immunoreactive epitopes cannot be excluded. Recent data suggest that chromogranins/secretogranins and their proteolytic products are involved in the regulation of packaging, processing and secretion of peptide hormones (for reviews see Huttner et al. 1991). The biological function of the chromogranins/secretogranins in normal and neoplastic C-cells remains to be established by further studies.

The sensitivity of 96% for the Sg IV immunoreactivity in MTCs based on the present study confirms the usefulness of the mAb Hisl-19. It is an excellent histopathological marker for C-cell carcinomas both in their primary and metastatic stages. The different distribution of Sg IV compared to Cg A and Ct suggests a practical significance for the classification of thyroid tumours with weak or questionable immunoreactivity. The mAb Hisl-19 should be added to the standard antibody panel for the immunohistochemical characterization of MTCs.

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References

Bordi C, Krisch K, Horvat G, Srikanta S (1988) Immunocytochemical patterns of islet cell tumors as defined by the monoclonal antibody Hisl-19. Am J Pathol 132:249–257

Cetin Y, Grube D (1990) Immunoreactivities for chromogranin A and B, and secretogranin II in the guinea-pig pancreas. Histochemistry 94:479–484

Deftos L, Woloszcuk W, Krisch I, Horvat G, Ulrich W, Neuhold N, Braun O, Reiner A, Srikanta S (1988) Medullary thyroid carcinomas express chromogranin A and a novel neuroendocrine protein recognized by monoclonal antibody Hisl-19. Am J Med 85:780–784

Harach HR, Willander E, Grimelius L, Bergholm U, Westermark P, Falkmer S (1992) Chromogranin A immunoreactivity compared with argyrophilia, calcitonin immunoreactivity and amyloid as tumor markers in medullary (C-cell) thyroid carcinoma. Pathol Res Pract 188:123–130

Hsu S-M, Raine L, Fanger H (1981) Use of the avidin-biotin complex method (ABC) immunoperoxidase technique: a comparison between ABC and unlabelled antibody (PAP) procedures. J Histochem Cytochem 29:575–580

Huttner WB, Gerdes HH, Rosa P (1991) The granin (chromogranin/secretogranin) family. Trends Biochem Sci 16:27–30

Krisch K, Buxbaum P, Horvat G, Krisch I, Neuhold N, Ulrich W, Srikanta S (1986) Monoclonal antibody Hisl-19 as an immunocytochemical probe for neuroendocrine differentiation. Its application in diagnostic pathology. Am J Pathol 123:100–108

Krisch K, Horvat G, Krisch I, Wengler G, Alibeik H, Neuhold N, Ulrich W, Braun O, Hochmeister M (1988) Immunochemical characterization of a novel secretory protein (defined by monoclonal antibody Hisl-19) of peptide hormone producing cells which is distinct from chromogranin A, B, and C. Lab Invest 58:411–420

Neuhold N, Wimmer M, Braun O, Horvat G, Krisch I (1989) Reactivity of monoclonal islet cell antibodies on normal, hyperplastic and neoplastic thyroid C-cells. Cell Tissue Res 257:437–443

Schmid KW, Kirchmair R, Ladurner D, Fischer-Colbrie R, Böcker W (1992) Immunohistochemical comparison of chromogranins A and B and secretogranin II with calcitonin and calcitonin gene-related peptide expression in normal, hyperplastic and neoplastic C-cells of the human thyroid. Histopathology 21:225–232

Scopsi L (1990) Non-calcitonin genes-derived neurohormonal polypeptides in normal and pathologic thyroid C-cells. In: Fenoglio-Preiser C, Wolff M, Rilke F (eds) Progress in surgical pathology vol XI. Springer, Berlin Heidelberg New York, pp 185–229

Srikanta S, Krisch K, Eisenbarth GS (1986) Islet cell proteins defined by monoclonal islet cell antibody Hisl-19. Diabetes 35:300–305

Varndell IM, Lloyd RV, Wilson BS, Polak JM (1985) Ultrastructural localization of chromogranin–a potent marker for the electron microscopical recognition of endocrine cell secretory granules. Histochem J 17:981–992

Wirnsberger GH, Becker H, Ziervogel K, Höfler H (1992) Diagnostic immunohistochemistry of neuroblastic tumours. Am J Surg Pathol 16:49–57