

Immunohistochemical studies with region-specific antibodies to chromogranins A, B and C in pheochromocytomas.

Guida Maria Portela-Gomes^{a,b}, Lars Grimelius^b, Mats Stridsberg^c, Ursula Falkmer^d and Sture Falkmer^e

a. Centre of Nutrition, Lisbon University, Portugal.

b. Department of Genetics and Pathology, Unit of Pathology, Uppsala University, Sweden.

c. Department of Medical Sciences, Clinical Chemistry, Uppsala University, Sweden.

d. Department of Cancer Research and Molecular Medicine, Unit of Oncology, St.Olav's University Hospital, Trondheim, Norway.

e. Department of Laboratory Medicine and Children's and Women's Health, Unit of Morphology/Pathology, St.Olav's University Hospital, Faculty of Medicine, Norwegian University of Science and Technology, Trondheim, Norway.

Correspondence: Dr. Guida Maria Portela-Gomes, Rua Domingos Sequeira 128, 2765-525 S. Pedro do Portugal, Portugal

Phone: 351-214683600; **Fax:** 351-214683600; **Email:** portela_gomes@yahoo.com

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Chromogranins (Cgs) are a family of glycoproteins that occur in the secretory granules of neuroendocrine (NE) cells and NE tumours. Post-translational processing of Cgs has been reported in various human NE cell types and NE tumours. This processing appears to differ in various human NE cell types and NE tumours^{1,2,3}. The plasma concentrations of CgA and CgB, and of a fragment of CgC (secretoneurin) have been reported to be increased in patients with pheochromocytomas^{4,5}.

The aim of the present investigation was to study the immunoreactivity to various fragments of the molecules of CgA, B and C in pheochromocytomas in order to analyse if the expression patterns differed between benign and malignant tumours.

MATERIALS AND METHODS

Antibodies raised in rabbits against specific regions of the molecules of CgA (CgA 116–130, chromostatin; 176–195, chromacins; 361–372, catestatin), CgB (CgB 244–255; 312–331; 542–552, PE-11; 568–577, chrombacin; 647–657, CCB) and CgC (CgC 154–165, N-terminal secretoneurin; 172–186, C-terminal secretoneurin) were used. The CgA and CgB region-specific antibodies have been described before^{1,2}. Peptides corresponding to the N-terminal and C-terminal regions of secretoneurin were synthesised and rabbit polyclonal antibodies were raised as described earlier for the CgA and CgB region-specific antibodies. For comparison, a commercial antibody raised against the C-terminal half of CgA was also used (code no. A0430, DakoCytomation, Glostrup, Denmark). Tumour specimens from 25 patients operated on for clinico-pathologically benign pheochromocytomas and 4 for metastasizing ones were routinely fixed in 10% buffered neutral formalin and processed to paraffin. Adrenal medulla from 3 patients operated on for cortical adenomas were used as 'non-neoplastic' controls. Sections were immunostained with the streptavidin–biotin complex technique using diaminobenzidine as chromogen.

RESULTS AND DISCUSSION

All adrenal chromaffin cells from the 'non-neoplastic' controls were immunoreactive to all eleven region-specific antibodies. In the

pheochromocytomas, one or more fragments were lacking, but variations in the expression pattern occurred both in benign and malignant pheochromocytomas. The frequency of immunoreactive cells to the various region-specific antibodies did not differ significantly between benign and malignant Pheos. CgA 176–195 (chromacins), CgB 647–657 (C-terminal CgB) and CgC 175–186 (C-terminal secretoneurin) were expressed in all cells in both benign and malignant Pheos. As earlier reported³, this investigation confirmed that CgA 176-195 is a good NE cell marker for both benign and malignant pheochromocytomas. In the malignant pheochromocytomas the antibodies raised against the C-terminal fragments of both CgB (CgB 647–657) and secretoneurin (CgC 175–186), visualized a distinct neoplastic chromaffin spindle cell population, characterized by elongated processes, a cell type which was demonstrated in all malignant pheochromocytomas, but only in one of the benign tumours. These spindle cells did not display S-100 immunoreactivity, *i.e.* were different from sustentacular cells which occur in some pheochromocytomas. This study shows that the use of antibodies to epitopes in the C-terminal regions of CgB and secretoneurin may facilitate the differential diagnosis of a malignant pheochromocytoma.

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