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Review

Granins and granin-related peptides in neuroendocrine tumours

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ABSTRACT

This review focus on neuroendocrine tumours (NETs), with special reference to the immunohistochemical analysis of granins and granin-related peptides and their usefulness in identifying and characterizing the great diversity of NET types. Granins, their derived peptides, and complex protein-processing enzyme systems that cleave granins and prohormones, have to some extent cell-specific expression patterns in normal and neoplastic NE cells. The marker most commonly used in routine histopathology to differentiate between non-NETs and NETs is chromogranin (Cg) A, to some extent CgB.

Other members of the granin family may also be of diagnostic value by identifying special NET types, e.g. secretogranin (Sg) VI was only found in pancreatic NETs and phaeochromocytomas. SgIII has recently arisen as an important NET marker; it was strongly expressed in NETs, with some exceptions — phaeochromocytomas expressed few cells and parathyroid adenomas none.

Some expression patterns of granin-related peptides seem valuable in differentiating between some benign and malignant NETs, some may also provide prognostic information, among which: well-differentiated NET types expressed more CgA epitopes than the poorly differentiated ones, except insulinomas, where the opposite was noted; medullary thyroid carcinomas containing few cells immunoreactive to a CgB antibody were related to a bad prognosis; C-terminal secretoneurin visualized a cell type related to malignancy in phaeochromocytomas.

Further research will probably establish new staining patterns with marker functions for granins in NETs which may be of histopathological diagnostic value.

Contents

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1. Introduction

Neuroendocrine tumours (NETs) synthesize, store and release biogenic amines and/or peptide hormones. These substances are stored, together with granins and prohormone convertases, in membrane-bound large dense core vesicles, usually called secretory granules. NETs usually represent well-defined clinical entities, however, difficulties in diagnosis may sometimes arise. Traditionally non-endocrine (adeno) carcinomas often contain scattered NE cells as a minority cell population. There is usually no reason to modify the original diagnosis on the basis of such observations. The non-endocrine carcinomas containing less than 30% NE cells are named "carcinomas with NE differentiation" [cf. 1]. However, infrequently the number of NE cells may be large (more than 30% of the tumour cell population). The World Health Organization proposed the diagnosis of "mixed exocrine-endocrine carcinomas" and it should be based on at least two major diagnostic parameters:(i) extension of each component (at least 30%) and (ii) structural features of the NE components as well-differentiated organoid or solid/diffuse growth patterns [cf. 1,2]; one example of such a tumour is the goblet cell tumour of the appendix, which is composed of NE cells, intermingled with mucus-positive goblet cells.

Sensitive methods have to be used in histopathology to differentiate between NETs and non-NE tumours [cf. 3], as the diagnosis of NETs, and their degree of differentiation and expression of hormones, have important consequences for the choice of therapy and for the prognosis.

The immunohistochemical demonstration of the biogenic amines or specific NE peptides allows classification of the NETs with regard to clinical symptoms, e.g. insulinomas and glucagonoma. However, it is not always possible to demonstrate specific hormones in NETs.

1.1. General considerations regarding negative immunohistochemical staining

There are several possible reasons for negative immunohistochemical results. Weak or negative immunoreactivity to some peptides may be a result of (i) masking of their epitopes, either by other granule-related proteins or by conformational changes on the peptide molecular structure due to the histological processing of the tissue, preventing the immunohistochemical staining. Microwave pre-treatment is a common method of unmasking antigens in immunohistochemistry [4,5]. However, it might not unmask all protein–protein interactions [6]; on immunostaining with granin region-specific antibodies, it has been found that some demasking occurs, as this pre-treatment influenced the results for some antibodies, mostly by increasing the staining intensity, but not the cell frequency [7–9]. A weak or negative immunoreaction can also occur (ii) if there is rapid synthesis and release of peptide(s), resulting in too low concentrations for visualization with the immunohistochemical techniques available, or (iii) if the peptides in question are produced in an abnormal molecular form. The absence of expression of peptides, for example granins or granin-derived peptides, may result from (iv) abnormal splicing of the molecule, either without cleavage at pairs of basic amino acids, or with cleavage without any relationship with the dibasic sites, so that the antibodies do not bind to the molecule. Another possible reason for negative staining results may be that the cells contain mRNA coding for a granin, but that there is (v) too little or lack of transcription of some exons, or (vi) abnormal splicing of the granin mRNA.

It is thus of diagnostic importance to use initially one or more broad-spectrum NE cell markers to differentiate between NETs and non-NETs, as the cells and the cellular patterns may be similar. Furthermore, it may be difficult to classify NETs into benign or malignant and search for markers characterizing their biological behaviour has been carried out.

In the following, research regarding the occurrence of secretory granule proteins members of the granin family (Table 1), peptides derived from these, as well as prohormone convertases, aiming to find histopathological marker functions, will be reviewed in NETs.

Table 1
Overview of the most relevant immunohistochemical expression patterns of granins — chromogranin (Cg) A–B and secretogranin (Sg) II–VII — in well-differentiated and poorly differentiated neuroendocrine tumours (NETs).

Granins	NETs	
	Well-differentiated	Poorly differentiated
CgA	Most NETs	
	Except: Rectal NETs L-cell type	
	More epitopes	Less epitopes
	Except: Insulinomas,pituitary ACTHomas, rectal NETs	
	Intensity of immunoreactivity:	
	Stronger	Weaker
CgB	Most NETs	
	Except: ECLomas, parathyroid adenomas	
		MTC: Few cells = bad prognosis
SgII: Secretoneurin	Most NETs	
	Except: Parathyroid adenomas	
EM66	Phaeochromocytomas: Spindle cells = malignancy?	
	Phaeochromocytomas	
SgIII	Most NETs	
	Except: Parathyroid adenomas	
SgIV (*HISL-19*)	Immunoreactive staining:	
	Peri-nuclear ring	Peri-nuclear ring
	Basal cytoplasmic	–
SgV (*7B2*)	Insulinomas:	
	Cell frequency related to that of insulin	
SgVI (*NESP55*)	Pancreatic NETs	
	Phaeochromocytomas	
SgVII (*VGF*)	Most NETs	Only lung LCNEC
	Except: Parathyroid adenomas	Other NETs: –

ECL, enterochromaffin-like; MTC, medullary thyroid carcinoma; LCNEC, large-cell neuroendocrine carcinoma.

2. Granins

Granins constitute a family of single-chain glycoproteins consisting of chromogranins (Cgs) and secretogranins (Sgs). Granins are encoded by different genes and share common characteristics, such as a calcium-binding capacity, and possession of several acidic amino acid residues giving rise to acidic isoelectric points, and of various pairs of basic amino acids as well as monobasic residues that are potential cleavage sites for several enzymes, mainly prohormone convertases (PCs). Cgs and Sgs have several biological functions, some of which are related to smaller peptides generated by cleavage from the granins (see below) [cf. 10]. Granins are the major proteins in the core of the secretory granules, where they may account for up to 80% of the total core proteins [cf. 11]; they are released together with hormones, and are involved in the generation or stabilization of the secretory granules and in protein trafficking [cf. 12].

2.1. Chromogranins

The Cg family consists of CgA and CgB, and peptides derived from these. Cgs are glycoproteins that have an N-terminal hydrophobic disulphide-bonded loop [cf. 10].

2.1.1. Chromogranin A

CgA was the first granin to be described and was isolated from bovine adrenal medulla [13,14]. The human CgA molecule consists of 439 amino acids and has 10 pairs of basic amino acids. CgA is a precursor of several peptides resulting from enzymatic cleavage of the molecule. Some of the CgA-derived peptides have several biological functions, including vasostatins (CgA 1–111) [15–20], chromacins (CgA 173–194) [21–23], catestatin (CgA 344–364) [20,24,25], pancreastatin (CgA 250–301) [26], parastatin (CgA 347–419) [27] and chromostatin (CgA 124–147) [28].