Regulatory Peptides 165 (2010) 5–11

Contents lists available at ScienceDirect

Regulatory Peptides

journal homepage: www.elsevier.com/locate/regpep

Review

Granin-derived peptides as diagnostic and prognostic markers for endocrine tumors

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ARTICLE INFO

Article history:
Received 16 June 2009
Received in revised form 13 November 2009
Accepted 13 November 2009
Available online 26 November 2009

Keywords:
Chromogranin A
Chromogranin B secretogranin II
Pancreastatin
Secretoneurin
Endocrine tumor

ABSTRACT

Chromogranin A-like immunoreactivity (CgA-LI) has been, and remains, the most widely used diagnostic and prognostic marker for endocrine tumors. The availability of assay kits combined with moderately high sensitivity and specificity has meant that there has been no great incentive to develop alternative markers. However, circulating concentrations of CgA-LI are elevated in several non-neoplastic diseases and in patients receiving acid-suppression therapy which may lead to false positive diagnosis. Additionally, certain endocrine tumors, such as rectal carcinoids, do not express the CgA gene so that there is a need for additional markers to complement CgA measurements. Plasma concentrations of the CgA-derived peptide, pancreastatin, measured with antisera of defined regional specificity, have a prognostic value in patients with metastatic midgut carcinoid tumors receiving somatostatin analog therapy or hepatic artery chemoembolization. Other CgA-derived peptides with potential as tumor markers are vasostatin-1, WE-14, catestatin, GE-25, and EL-35 but their value has yet to be fully assessed. Circulating concentrations of chromogranin B-like immunoreactivity (CgB-LI) are not elevated in non-neoplastic diseases and measurements of CCB, the COOH-terminal fragment of CgB, may be useful as a biochemical marker for neuroendocrine differentiation in lung tumors. Antisera to the secretogranin II-derived peptide, secretoneurin detects carcinoid tumors of the appendix with greater frequency than antisera to CgA and are of value in identifying therapy-resistant carcinoma of the prostate (clinical stage D3). Measurement of concentrations of a second secretogranin II-derived peptide, EM-66 in tumor tissue has been used to differentiate between benign and malignant pheochromocytoma. These examples point to a limited although potentially valuable role for granin-derived peptides as tumor markers.

Contents

1. Introduction

The importance of chromogranin A (CgA) in the diagnosis, prognosis, and clinical evaluation of patients with endocrine tumors has been appreciated for more than 20 years [1]. Circulating concentrations of chromogranin A-like immunoreactivity (CgA-LI) are elevated in the plasma or serum of patients with a wide range of endocrine tumors such as pheochromocytoma [2], neuroblastoma and ganglioneuroma [3], carcinoid tumors of the gastrointestinal tract, lung, and ovary [4], pancreatic endocrine tumors [5], medullary thyroid carcinoma [6], and in type 1 [7] and type 2 [8] multiple endocrine neoplasia syndromes. The reader is referred to a revised clinicopathological classification of neuroendocrine tumors of the gastroenteropancreatic tract that has been developed under the auspices of the World Health Organization for definitions of tumor types [9]. In addition, elevated CgA-LI values are found in many so-called 'nonfunctioning' endocrine tumors that do not present any clinical symptoms and are not associated with hypersecretion of a defined hormonal product, such as gastrin in Zollinger–Ellison syndrome [10–12]. Provided that appropriate cut-off ranges are chosen [13], CgA-LI measurements show moderate sensitivity and specificity as a diagnostic marker and plasma concentrations frequently correlate with disease stage and tumor burden [12–14]. An inverse relationship

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0167-0115/$ – see front matter © 2009 Elsevier B.V. All rights reserved.
doi:10.1016/j.regpep.2009.11.013

between prognosis (progression-free survival) and circulating CgA-LI concentrations at the time of diagnosis has been proposed for several tumor types [15–17]. For example, in patients with carcinoid tumors of the midgut the survival at 5 years is 22% with CgA-LI levels >75 nmol/l and 63% in the group with CgA-LI levels less than this value [17]. However, it is clear that further studies are required before the prognostic value of CgA-LI measurements can be assessed.

Despite its undoubted value and widespread adoption for identification of endocrine cancers, CgA-L1 measurements are subject to limitations that can lead to false positive diagnoses. Circulating concentration are raised in several conditions that are not associated with neoplastic disease, notably in patients with renal failure [18], inflammatory bowel disease [19], chronic atrophic gastritis [20] or under treatment with H2 receptor antagonists or proton pump inhibitors [21]. Consequently, determinations of circulating concentration of chromogranin B-like immunoreactivity (CgB-LI) [22,23] and/or secretogranin II-like immunoreactivity (SgII-LI) [24,25] have been advocated to complement CgA-LI measurements, as it is claimed that elevated concentrations of CgB-LI and SgII-LI are more specific for the presence of certain endocrine tumors [22]. A further complication in the interpretation of circulating CgA-LI concentrations in neoplastic disease arises from the fact that the regional specificity of the antiserum used is often unknown so that it is unclear exactly what is being measured in the assays. As readers of this special issue will certainly be aware, the granins are processed to multiple fragments in tumor tissue by the action of the PC1/3 and PC2 proprotein convertases and these fragments circulate along with the intact proteins. Consequently, the concentrations measured in plasma will depend markedly upon the specificities of the antisera used for measurement in radioimmunoassay or ELISA. An antiserum directed against an epitope that is present only in the intact granin will invariably measure different concentrations than one that was raised against a particular granin fragment. In a study of 8 patients with gastric endocrine tumors (ECLomas), it was shown that plasma concentrations of CgA-LI were markedly dependent upon the regional specificity of the antiserum used for measurement [26]. Highest concentrations were measured using antisera directed against the CgA (1–17) and CgA(116–130) epitopes with much lower concentrations measured using antisera directed against the CgA(17–37), CgA(63–76), and CgA(238–247) epitopes. A number of commercially available assays for CgA are available but the regional specificity of the antisera used in the kits has not been precisely defined [27,28]. Monoclonal antibodies directed against well-defined epitopes may improve reproducibility in immunohistochemical studies but their use in RIA or ELISA frequently results in low assay sensitivity.

The pathways of post-translational processing of the granins in tumor tissue are markedly dependent upon tumor type and varies appreciable between individual tumors of the same type. The concentration of a particular granin fragment may even change during tumor progression. Consequently, the use of at least two antisera directed against different epitopes on the same granin or antisera directed against epitopes on different granin is more likely to provide a reliable means of monitoring a patient's response to therapy. This article attempts to assess the diagnostic and prognostic value of assays that measure specific granin fragments in plasma and/or tumor tissue of patients with endocrine tumors. The location of these fragments within the granin proteins is illustrated schematically in Fig. 1.

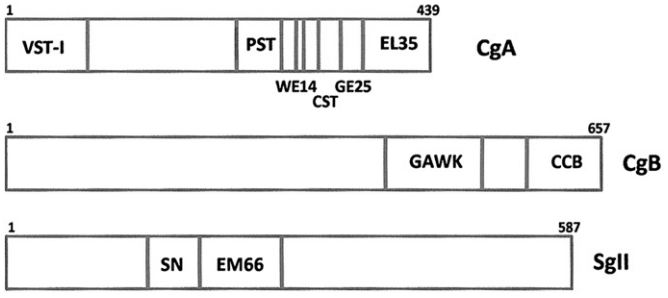

Fig. 1. A simplified representation of the chromogranin A (CgA), chromogranin B (CgB) and secretogranin II (SgII) molecules showing the locations of the granin-derived peptides that have been evaluated as markers for endocrine tumors. VST-I: vasostatin-1, PST: pancreastatin, CST: catestatin, CCB: COOH-terminal fragment of CgB, SN: secretoneurin. Other peptides are named from their N-terminal and C-terminal amino acids.

2. Chromogranin A-derived peptides

Pancreastatin (PST) was first isolated from porcine pancreas as a 49-amino-acid-residue peptide using a chemical assay that detected C-terminally α-amidated amino acid residues [29]. It was regarded as an independent regulatory peptide that inhibited glucose-induced insulin release until it was realized that it was identical to residues (240–288) of porcine CgA [30]. However, the predominant molecular form of pancreastatin-like immunoreactivity (PST-LI) in extracts of human endocrine tumors and non-neoplastic tissues is a 92-amino-acid-residue peptide that represents residues (210–301) of CgA [31,32]. A smaller component corresponding to CgA(250–301) that is orthologous to porcine PST is also found in both tissues and plasma [33]. PST-LI shows potential as a diagnostic marker for endocrine tumors. PST-LI circulates in healthy subjects in very low concentration (<3 pmol/l), does not show changes with age and, like CgA-LI, levels increases only slightly after a meal [28]. The peptide is relatively stable in blood so that measurements can be made in either plasma or serum. Elevated circulating concentrations of PST-LI have been reported for patients with a wide range of endocrine tumors (carcinoid tumors of the gastrointestinal tract, particularly those that have metastasized to the liver [34,35], islet cell tumors [36–38], gastrinoma [39], neuroblastoma and ganglioneuroma [40], and medullary thyroid carcinoma [41]). High concentrations of PST-LI are found in most pituitary adenomas (except prolactinomas) [42] but elevations in plasma values may not be observed because of the small size of the tumors. It is important to know the specificity of the antiserum used to measure PST-LI in order to interpret the data obtained. Antisera directed against an epitope in the N-terminal or central regions of the molecule will generally cross-react with CgA and certain partially processed forms whereas antisera raised against synthetic C-terminal fragments of the peptide require the presence of a C-terminally α-amidated residue in the antigen for reactivity and so are more specific for PST. In the majority of cases where circulating concentrations of PST-LI are high, elevated CgA-LI concentrations are also measured but in groups of patients with renal cell carcinoma [43] and carcinoma of the prostrate that showed neuroendocrine differentiation [44], elevated serum values of CgA-LI were not accompanied by increased concentrations of PST-LI and so it was concluded that PST was not a useful tumor marker in these cases.

PST measurements have been shown to be of value as a prognostic indicator in patients with endocrine tumors. Hepatic artery chemoembolization combined with somatostatin analog (octreotide) therapy has been used in the treatment of patients with disabling symptoms from endocrine tumors that have metastasized to the liver [45]. It was shown that a >20% decrease in serum PST-L1 concentrations after embolization was indicative of a good prognosis whereas a <20% decrease often predicted a poor clinical course. A rising PST-LI value after an initial good response indicated the need for a second chemoembolization of the liver [46]. Unfortunately, no details of the antiserum used in this study were provided. These conclusions were supported by a more recent study involving 122 patients with metastatic carcinoid tumors undergoing hepatic artery chemoembolization [47]. A markedly elevated pre-treatment serum PST-LI concentration was the only parameter associated with poor survival by multivariate analysis. In a retrospective study of patients in the Northern Ireland Tumor Register with highly differentiated, metastasized endocrine tumors of the foregut and pancreas that were receiving somatostatin