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Invited critical review

# Chromogranin A in gastrinomas: Promises and pitfalls

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ABSTRACT

Patients with neuroendocrine tumors are found with increasing frequency. Accordingly, knowledge about relevant tumor markers and assays for diagnosis and control has become essential. Neuroendocrine tumors release one or more granin proteins. Of these, chromogranin A (CgA) has so far become the most widely used general marker. The CgA protein is, however, extensively cleaved and otherwise modified during the biosynthetic processing. In addition, the CgA-processing in individual tumors varies considerably. But only few CgA-assays have taken the processing into account and characterized the assays with respect to precise epitope-specificity. Consequently, we do not know which fragments most CgA-assays measure. It is therefore at present difficult to compare CgA-measurements from tumor patients. Some tumors, however, release – in addition to granins – also a specific hormone that causes a clinical syndrome. This review uses gastrinomas (gastrin-producing tumors) as a starting point for discussion of CgA versus peptide hormone as tumor marker. Data available so far indicate that well-defined assays for gastrin have significantly higher diagnostic sensitivity than CgA measurements in gastrinomas. But the review suggests that CgA-quantitation using processing-independent analysis (PIA) may provide an equally high diagnostic sensitivity and in addition offer a simple possibility for estimation of the tumor-burden.



**Contents**



## 1. Introduction

Granins (chromogranins and secretogranins) constitute a family of phylogenetically old proteins, which are highly acidic in spite of several basic cleavage sites. Their structure is further modified by amino acid derivatizations (α-amidations, glycosylations, phosphorylations, and sulfations (Table 1)). Granins are neuroendocrine, i.e., they are primarily expressed in neurons and endocrine cells, although with a cell- and species-specific pattern [for reviews, see refs. 1–5 and Table 1]. Occasionally, however, granins are also expressed in non-neuroendocrine cells such as cardiomyocytes [6] and in non-endocrine carcinomas [7,8].

Functionally, the granins are chaperones for peptides and amines in the intracellular biogenesis and transport of secretory granules and vesicles in which the granins are packed together with peptide hormones, neuropeptides and/or monoamines [1–4]. As suggested by the multiple cleavage sites, they are also subject to extensive posttranslational endoproteolytic cleavages and subsequent exoproteolytic trimmings. As a result, the granins are released from neuroendocrine cells as a mixture of fragments, some of which have been supposed to exert specific

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**Table 1**
Structural characteristics of the granins.

| | CgA | CgB | SgII | SgIII (1B1075) | SgIV (HISL-19) | SgV (7B2) | SgVI (NESP55) |
|---|---|---|---|---|---|---|---|
| Amino acids (n) | 439 | 653 | 586 | 468 | | 185 | 241 |
| Calculated Mr (kDa) | 48 | 76 | 67 | 57 | | 21 | 28 |
| Apparent Mr (kDa) | 75 | 110 | 86 | 57 | 35 | 23 | 41 |
| Acidic residues (%) | 25 | 24 | 20 | 19 | | 16 | 21 |
| pI | 4.9 | 5.2 | 5.0 | 5.1 | 5.6 | 5.0 | 5.0 |
| Dibasic sites (n) | 9 | 16 | 9 | 5 | | 4 | 5 |
| Sulfation | + | + | + | + | | + | − |
| Phosphorylation | + | + | + | − | | + | + |
| Glycolysation | + | + | + | − | | − | − |

hormone-like regulatory functions. This functional issue, however, still remains to be settled in terms of receptor mechanisms and physiological significance [2,5,9].

Neurons and endocrine cells may become neoplastic and grow into neuroendocrine tumors. Some of the tumors are associated with clinical syndromes caused by hypersecretion of a particular peptide hormone and/or monoamine (i.e., Cushing tumors, insulinomas, glucagonomas, gastrinomas, VIPomas, somatostatinomas, CCKomas, carcinoid tumors with flushing). But the majority of neuroendocrine tumors are functionally silent and associated only with less specific tumor or cancer symptoms such as weight loss, diarrhea, pain, and metastases [9–13]. The phenotypic pathology of a neuroendocrine tumor may also vary during its course, for instance from non-functional to insulinoma and further to somatostatinoma; from VIPoma to CCKoma; from gastrinoma to ACTH-producing tumors with Cushing's syndrome. Moreover, immunohistochemistry often reveals expression of multiple hormones in a single tumor [14,15]. Irrespective of hormone release and clinical symptoms, the tumors also release granins. And some granins have turned out to be used as markers in the diagnosis and the often long-term control of treatment of neuroendocrine tumor patients [3,11–13]. So far, CgA (Chromogranin A) has been the most widespread and popular tumor marker, partly because it has been reported to be expressed in most neuroendocrine tumors, and partly because CgA-expression has been included in the pathology definition of a neuroendocrine tumor [1,9–13,16–21].

For clinical chemistry, CgA-assays appear on first sight to be an obvious choice for biochemical diagnosis and control of patients with neuroendocrine tumors [1,3,10–12,18]. There are, however, analytical and diagnostic problems to be considered: First, CgA is as mentioned extensively processed and modified to fragments, the plasma pattern of which varies individually and with tumor and tissue origin [1,3,17]. Second, the precise epitope-specificity of the CgA-immunoassays available on the market is generally unknown or poorly defined [22]. Third, the inclusion of CgA-expression in the definition of neuroendocrine tumors creates a circle argument which excludes neuroendocrine tumors that express other granins. Fourth, measurement of some of the hormones that cause the symptoms provides a better diagnostic specificity and sensitivity [23]. Finally, CgA-concentrations in plasma may be elevated in non-tumorous renal and cardiac diseases, in non-neuroendocrine malignances and during treatment with proton pump inhibitors, as well as decreased by treatment with somatostatin analogues [6–8,20,21,24,25].

The following review will discuss CgA-measurements in diagnosis and control of the specific tumor syndrome, the Zollinger–Ellison syndrome caused by gastrinomas. The case of CgA in gastrinomas is also an illustration of general analytical and diagnostic problems in the handling of patients with neuroendocrine tumors.

## 2. Chromogranin A

CgA was the first granin to be discovered, and it was in extracts from the bovine adrenal medulla [for reviews, see refs. 2, 5 and 26]. Human CgA is an acidic protein with a length of 439 amino acids. The N-terminal sequence contains a disulfide bridge between the cysteinyl residues in position 17 and 38 [27,28], which seems important for the intracellular sorting. The intact human CgA-protein also contains nine dibasic and other basic cleavage sites (Table 1), which are processed to a variable extent. The processing is cell- and tissue-specific and highly individual in neuroendocrine tumors. Some of the fragments (Fig. 1) have been suggested to exert hormonal biological activities [for review, see ref. 5]: Fragments 1–115, 1–76, and 1–40 may inhibit vasoconstriction ("vasostatins"); fragments 79–439 and 176–197 can be bacteriolytic and antifungal ("prochromacin" and "chromacin", respectively); the carboxyamidated fragment 250–301 has in high doses been shown to inhibit insulin secretion from the pancreas ("pancreastatin"); fragment 357–428 inhibits also in high doses parathyroid hormone secretion ("parastatin"); and fragment 352–372 seems to inhibit catecholamine secretion from the adrenal medulla ("catestatin"). Notably, the effects are all inhibitory, and specific receptor-mechanisms of the fragments remain to be identified. The dose–response correlations between physiological plasma concentrations and the suggested target-effects also remain to be described before the CgA-fragments can be considered

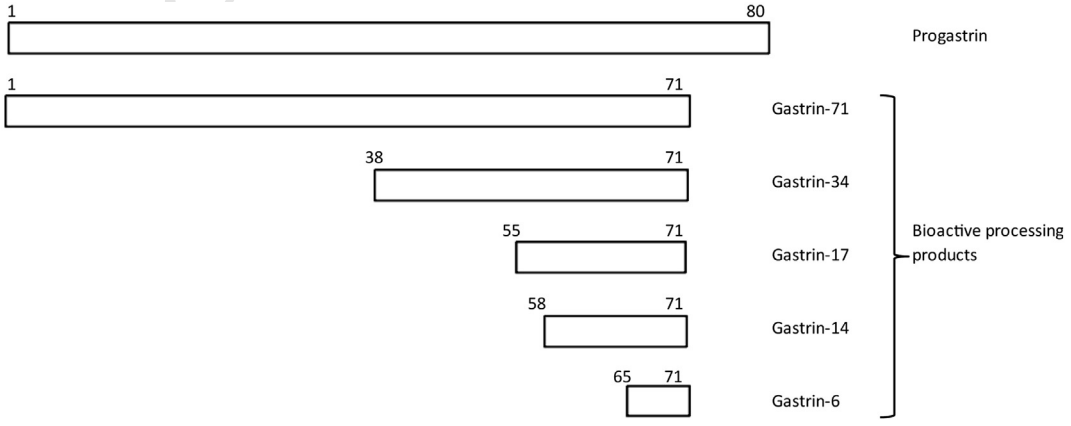


**Fig. 1.** Human progastrin is a protein of 80 amino acid residues containing three dibasic processing sites, which are cleaved to release the major bioactive processing products: gastrin-71, -34 and -17. In addition, gastrin-17 is N-terminally truncated to gastrin-14. The four gastrins circulate in pairs as the tyrosyl residue in half of each gastrin is O-sulfated. Beyond these eight bioactive gastrins, progastrin is processed to a number of biologically inactive processing-intermediates and degradation fragments (for review, see ref. 54).

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