Review Article

Chromogranin A in Physiology and Oncology

(chromogranin / neuroendocrine tumours / cleavage fragments / extracellular functions / intracellular functions / tumour marker)

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Abstract. Chromogranin A (CgA) is a hydrophilic acidic one-chain peptide containing 439 amino acids, preceded by NH$_2$-terminal 18-amino-acid signal peptide; the complete pre-chromogranin A molecule thus encompasses 457 amino acids. It is a member of the chromogranin family that comprises several proteins. The *CgA* gene is a single-copy gene localized in the locus 14q32. Chromogranin A is produced by endocrine and neuroendocrine cells. The largest amount of CgA occurs in chromaffin granules of adrenal medulla and in the dense-core vesicles of sympathetic nerves. Its biological functions have not been completely elucidated, but it is known that it acts as a precursor of many biologically active peptides generated by cleavage at specific sites. It is the major soluble protein co-stored and co-released along with resident catecholamines and polypeptide hormones or cell-specific neurotransmitters. Because of its widespread distribution in neuroendocrine tissue, it can be used both as immunohistochemical marker and serum marker of neuroendocrine tumours. CgA has been used as a rather reliable tumour marker because its level is significantly increased in neuroendocrine tumours and changes of its level reflect the tumour response to therapy or tumour recurrence.

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Abbreviations: ACTH – adrenocorticotropic hormone, BAM-1745 – 1745-dalton pyroglutamyl peptide, CgA – chromogranin A, CgB – chromogranin B, CgC – chromogranin C (= secretogranin II), DNES – diffuse neuroendocrine system, ECL – enterochromaffin-like, ELISA – enzyme-linked immunosorbent assay, FSH – follicle-stimulating hormone, GAWK – chromogranin B 420–493, GH – growth hormone, IRMA – immunoradiometric assay, LH – luteinizing hormone, MEN 1 – multiple endocrine neoplasia, type 1, NET – neuroendocrine tumour, RIA – radioimmunoassay, SgI – secretogranin I, SgII – secretogranin II, SgIII – secretogranin III, SgIV – secretogranin IV, NESP55 – neuroendocrine secretory protein 55, TSH – thyroid-stimulating hormone.

Introduction

The chromogranin (or granin) family comprises water-soluble acidic glycoproteins stored in the matrix of large dense secretion granules, containing protein hormones in a wide variety of endocrine and neuroendocrine cells. Chromogranin A is the major member of the chromogranin family. Chromogranins are rich in acidic amino acids; they exhibit aggregation at low pH and high capacity for calcium binding. The exact function(s) of these proteins is not yet settled but it is supposed that granins function as pro-hormones. They share the sequences of several dibasic amino acids acting as cleavage sites for proteolytic enzymes, thus giving rise to many peptide fragments with biological, endocrine activity.

1. Chromogranin family

According to Feldman et al. (2003) the chromogranin family includes the following members:

Chromogranins	Synonym
Chromogranin A (CgA)	parathyroid secretory protein 1
Chromogranin B (CgB)	secretoneurin, secretogranin I (SgI)
Chromogranin C (CgC)	secretogranin II (SgII), secretoneurin-included (SN, included)
Secretogranin III/1B1075 (SgIII)	
Secretogranin IV/HISL-19 antigen (SgIV)	
Secretogranin V	neuroendocrine protein 7B2
Secretogranin VI	NESP55

These hydrophilic proteins share some overall properties, such as the above-mentioned abundance of acidic amino acid residues and a number of basic amino acid residues as potential positions for posttranslational

processing and common distribution in the secretion granules. They also share thermostability, acidic isoelectric point (pI 4.5–5.0), high content of glutamic and aspartic acid, ability to bind calcium with mild affinity but high capacity, and ability to aggregate in the presence of calcium ions.

Granins as a whole family do not share too many structural similarities. Just one short region, located in the C-terminal motif, is conserved in all these proteins. However, chromogranins A and B share a region of high similarity localized in their N-terminal sections; this region includes two cysteine residues involved in a disulphide bond. This structure does not occur in secretogranin II (CgC).

With respect to the abundance of chromogranins in the neuroendocrine tissue, they are supposed to be involved in numerous intracellular and extracellular biological processes, even though their biological role has not yet been fully elucidated. Among others, they play multiple roles in storage and release of hormone peptides. Chromogranins are proteolytically processed in the endocrine tissues and turn into biologically active peptides controlling the function of the respective tissues.

2. Chromogranin derivatives

As mentioned, all chromogranins share the sequences of several dibasic amino acids that act as cleavage sites for proteolytic enzymes. Chromogranin A is a precursor for various biologically active peptides such as pancreastatin (Gonzalez-Yanez et al., 2001) and vasostatin-1 (CgA 1–76), vasostatin-2 (CgA 1–113), parastatin (CgA 357–428), catestatin (CgA 352–372), chromostatin (CgA 124–143) and others. The details are beyond the scope of this article (see Fig. 1). CgB is a precursor of two peptides: GAWK (chromogranin-B 420–493) and BAM-1745. Secretoneurin is a derivative of CgC.

3. Chromogranin A

3.1 Basic characteristic

CgA (synonym: secretory protein I) is the major member of the chromogranin family of neuroendocrine secretory proteins. This acidic hydrophilic protein encompasses 439 (Degorce et al., 1999) amino acids arranged into one-chain peptide, preceded by NH_2-terminal 18-amino-acid signal peptide. Thus, the whole pre-chromogranin A molecule encompasses 457 amino acids, see Table 1 showing the scheme of pro-chromogranin A.

Its molecular weight is 48 kDa and isoelectric point 4.9. CgA molecules undergo posttranslational modification (Barbosa et al., 1991), which includes carboxymethylation, glycosylation, phosphorylation and sulphation. CgA is produced by endocrine and neuroendocrine cells, where it is co-expressed together with other polypeptide hormones and neurotransmitters. CgA is localized in the dense secretion granules that store peptide hormones, as well as in the vesicles containing catecholamines (Ferrari et al., 1999).

A few regions of the CgA molecule are homologous to calcium-binding proteins, such as calmodulin. CgA molecules share structural homology among different species, the sequence of amino-terminal 76 amino acids shares 72–80 % identity with corresponding regions of mammalian chromogranin A molecules.

3.2 Gene expression regulation of CgA

The human *CgA* gene is a single-copy gene, it spans 12,194 base pairs in the locus 14q32.12 (Deftos et al., 1991) and it consists of 8 exons giving rise to 2043 nucleotide transcripts. Because many polypeptide hormones and specific neurotransmitters are co-expressed with chromogranin A, chromogranin A seems to be capable of responding to the wide scale of modulation signals influencing CgA expression in various tissues (Rosa
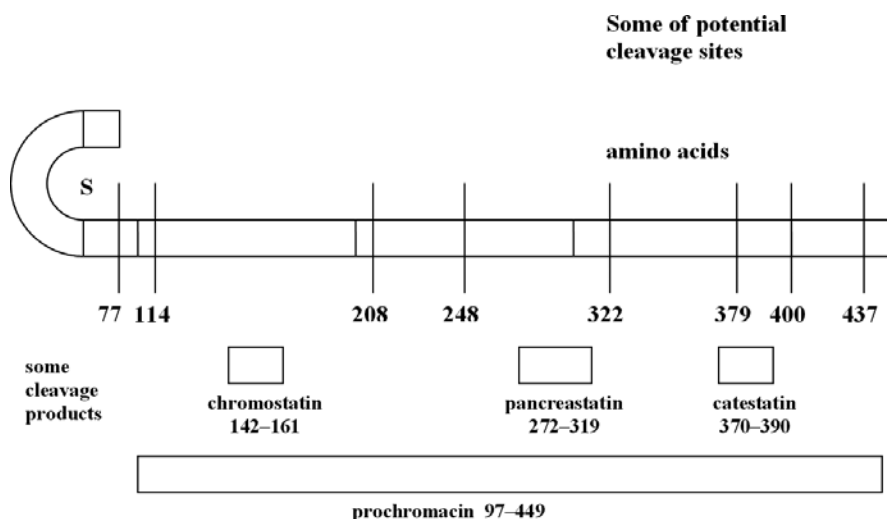

Fig. 1. Scheme of the chromogranin A molecule and some cleavage fragments

Table 1. Molecule of pro-chromogranin A: 457 amino acid residues

MRSAAVLALL	LCAGQVTALP	VNSPMNKGDT	EVMKCIVEVI	40
SDTLSKPSPM	PVSQECFETL	RGDERILSIL R	HQNLLKELQ	80
DLALQGAKER	AHQQKKHSGF	EDELSEVLEN	QSSQAELKEA	120
VEEPSSKDVM	EKREDSKEAE	KSGEATDGAR	PQALPEPMQE	160
SKAEGNNQAP	GEEEEEEEA	TNTHPPASLP S	QKYPGPQAE	200
GDSEGLSQGL	VDREKGLSAE	PGWQAKREEE	EEEEEEAEAG	240
EEAVPEEEGP	TVVLNPHPSL	GYKEIRKGES	RSEALAVDGA	280
GKPGAEEAQD	PEGKGEQEHS	QQKEEEEEMA	VVPQGLFRGG	320
KSGELEQEEE	RLSKEWEDSK	RWSKMDQLAK	ELTAEKRLEG	360
QEEEEDNRDS	SMKLSFRARA	YGFRGPGPQL	RRGWRPSSRE	400
DSLEAGLPLQ	VRGYPEEKKE	EEGSANRRPE	DQELESLSAI	440
EAELEKVAHQ	LQALRRG			457

One-letter abbreviations of amino acids: A – alanine, C – cysteine, D – asparagine, E – glutamic acid, F – phenylalanine, G – glycine, H – histidine, I – isoleucine, K – lysine, L – leucine, M – methionine, N – asparagine, P – proline, Q – glutamine, R – arginine, S – serine, T – threonine, V – valine, W – tryptophan, Y – tyrosine.

et al., 1994). The gene for this granin may encode individual chains of amino acids, each of them carrying the amino-terminal signal peptide that enables posttranslational modification of proteins that are present in the endoplasmic reticulum and Golgi network.

The *CgA* gene expression is regulated by steroid hormones as well (Hendy et al., 1995). Steroid hormones are bound to their cognate nuclear or cytosolic receptors. Thus, the formed complex may directly associate with *cis*-acting DNA response elements to exert either positive or negative transcriptional control.

3.3 Distribution

The largest amount of CgA occurs in chromaffin granules of adrenal medulla and in the dense-core vesicles of sympathetic nerves. The content of CgA in neuroendocrine cells varies according to the type of the tissue. Central and peripheral nervous system, pituitary gland, and parathyroid glands (Deftos et al., 1991) are also rich in CgA. CgA is present as well in C calcitonin-producing cells of thyroid gland, in exocrine tissue of the pancreas, and in insulin- and glucagon-producing cells or in placenta. CgA is also detectable in diffuse neuroendocrine system (DNES) dispersed in the lungs, spleen, prostate, thymus and in the gastrointestinal tract.

The order of chromogranin A concentrations ranks as follows: adrenal medulla > pituitary gland > pancreas > stomach > small intestine (jejunum and ileum) > brain (frontal cortex) > parathyroid > thyroid gland. Chromogranin A is also expressed in many neuroendocrine tumours. They originate from DNES, especially in the gastrointestinal and bronchopulmonary systems. Their typical representative is carcinoid, originating in small intestine, presenting by flushing, diarrhoea, tachycardia and bronchospasms.

Expression of other granins is not strongly correlated with CgA, which may indicate their different functions. They seem to be functionally redundant and, therefore, their uniform expression in neuroendocrine cells seems to be irrelevant.

3.4 Biological properties

3.4.1 Regulation of biosynthesis and secretion of CgA

Several intracellular messenger systems participate in the regulation of CgA biosynthesis, including protein kinase A, protein kinase C signalling intracellular pathways, and intracellular calcium as well. Phosphorylation events mediated by protein kinase C have been implicated in the regulation of CgA biosynthesis. This regulation is cell-specific, and intracellular calcium plays additional activity in this regulation process. Histamine, cholinergic agonist nicotine, bradykinin, angiotensin II, prostaglandin E2 and potassium ions may also facilitate chromogranin A biosynthesis. CgA is abundant under normal conditions. Thus, an increase in its serum level rather indicates a disorder of breakdown than increased gene transcription.

After biosynthesis, the granins are transported into the rough endoplasmic reticulum and Golgi apparatus. At trans-Golgi network, the granins are accumulated into the newly formed neuroendocrine granules and subsequently proteolytically processed into biologically active peptides (Nobels et al., 1998). Endoproteases split the precursors at dibasic or monobasic sites. Various types and various amounts of CgA's cleavage products are generated according to the type and activity of the endoproteases. Proteolysis proceeds not only in intracellular matrix but also in the granules.

Stimulation of neuroendocrine secretion granules is an impulse for the release of their content. CgA is released together with hormones or neurotransmitters. The proteins are secreted through exocytosis in a constitutive or regulatory manner (Taupenot et al., 2003).

a) Within the constitutive pathway, the proteins are continuously transferred to the cell surface without previous storage.

b) In the regulatory pathways, the secreted proteins are concentrated and stored in secretory granules and subsequently released in response to external stimulation.

The constitutive manner means that the amount of newly secreted peptide is a function of intensity of its synthesis. Specialized cells, such as neuroendocrine cells, prefer the regulatory manner.

Selective aggregation of the regulated secretory proteins at the level of trans-Golgi network (so-called: sorting by retention) proceeds at high calcium ion concentrations and acidic pH. This mechanism separates the regulated material from constitutively secreted proteins and prevents release of the regulated secretory proteins from immature granules.

A specific sorting signal in the secretory protein that binds to a sorting receptor may direct movement of the protein into the regulated secretory pathway; it is the so-called sorting for entry. E.g., chromogranin B contains a hydrophobic loop structure that leads to sorting of CgB from the trans-Golgi network into the regulated secretory granules of the PC12 line of pheochromocytoma cells.

CgA is processed, specifically according to the tissues, at dibasic sites into peptides that possess different biological properties. This processing proceeds at amino- and carboxy-terminals, where there are most dibasic cleavage sites. It is in accordance with the fact that chromogranin A is a precursor of various active, tissue-specific polypeptides. Among others, CgA is the precursor of pancreastatin (Gonzalez-Yanez et al., 2001), vasostatin/betagranin, chromostatin, and others.

CgA is closely related to CgB. They both contain a disulphide-bonded loop structure with a highly homologous sequence of amino acids localized in the proximity of their amino (NH_2-) terminus. This structure may play a role in directing chromogranin into pertinent secretory vesicles. A disulphide bridge is located near the aminoterminal part of the molecule and RGD sequence (sequence consisting of arginine-glycine-asparagine) that is probably involved in intracellular protein transport and/or has membrane-binding function for CgA (Takiyyuddin et al., 1990). Proteolytic processing of CgA proceeds both intracellularly and extracellularly, i.e. after secretion into the extracellular space, and extracellular processing shows to be particularly important for generation of catecholamine release-inhibitory activity from the CgA molecule in the vicinity of the chromaffin cells. Recent studies suggest a major role for the plasminogen/plasmin protease system in CgA processing.

3.4.2 Intracellular and extracellular functions of chromogranin A

CgA is ubiquitous in the neuroendocrine tissues and is involved in many intracellular and extracellular processes (Strub et al., 1996). It is the major soluble protein co-stored and co-released along with resident catecholamines and polypeptide hormones or cell-specific neurotransmitters (Koeslag et al., 1999) within chromaffin cell secretory granules such as adrenal medulla and sympathetic neuronal vesicles during exocytosis. CgA is a high-capacity, but low-affinity, calcium-binding protein. Calcium binding may be influenced by pH and may change during the process of maturation of secretory granules. The calcium concentration and hydrogen ion are capable of inducing marked conformational changes of the CgA molecule and may enhance association of this protein with membranes (Hendy et al., 1995). These processes contribute to CgA's ability to sort neurotransmitters and peptide hormones and package them into secretory granules. Because of CgA's association with other hormones, CgA participates in exocytotic secretory activity.

CgA modulates processing of proteolytic hormones during their transport in neuroendocrine vesicles, and is probably involved in the process of storage and release of hormone peptides. Granins facilitate creation of storage complexes and function as chaperones in sorting of other regulated secretory proteins (Taupenot et al., 2003).

The high content of acidic amino acids may be important, among others, for endocrine secretion in (a) packaging peptide hormones, (b) generating precursors of peptide hormones.

In summary, CgA exerts the following intracellular functions:

(a) granulogenesis – formation of immature structures via "trans-Golgi net" and formation of secretory vesicles together with nucleotides, neurotransmitters and cations within the granules
(b) co-storage and co-release with other co-resident hormones, regulation of packaging and processing hormone molecules
(c) stabilization of secretory vesicles through diminishing effective osmotic pressure in the intact chromaffin vesicle with subsequent stabilization of the vesicles against rupture
(d) calcium binding and regulation of calcium flow
(e) binding of catecholamines
(f) precursor of several peptides generated through proteolytic processing

Extracellular and intracellular proteolytic processing of CgA is probably the most important event within its biological activity. The resulting peptides may exert many biological responses. The review of the processed peptides is beyond the scope of this paper. They exert a number of autocrine, endocrine and paracrine functions. Moreover, chromogranins inhibit catecholamine secretion by adrenal medulla, cholecystokinin-induced amylase secretion by the exocrine pancreas, pro-opiomelanocortin secretion in various neuroendocrine tissues and acid secretion by parietal cells of stomach.

3.4.3 CgA and catecholamines

Chromogranins are known to inhibit catecholamine secretion by adrenal medulla. During sympathoadrenal activation, CgA is released by exocytosis along with catecholamines out of the cell vesicles into adrenal medulla and sympathetic nerve endings. The CgA level correlates with the norepinehrine level during sympa-

thetic stimulation and with the epinephrine level during adrenal medulla stimulation; however, this correlation is not significant at rest and may be afflicted by various tissue factors. The level of CgA increases during intensive stimulation, e.g. during strenuous exercise or stress, but the CgA level here fails to exceed double the normal upper border compared to the NETs in which the pathological CgA value is usually much higher (typically in small intestine carcinoids). The CgA level is also high in pheochromocytoma (D'Herbomez et al., 2001; Giovanella et al., 2006; Bílek et al., 2008), i.e. neuroendocrine tumour (NET) growing in adrenal medulla, presenting with high blood pressure within so-called secondary hypertension. However, it must be kept in mind that some NET subtypes may cause only slight CgA level elevation.

It has also been evidenced (Jiang et al., 2001) that the plasminogen/tissue-plasminogen activator (t-PA) system plays a major role in catecholaminergic activity by means of interaction with CgA. It is suggested that a specific mechanism must exist as well as a discrete CgA peptide fragment through which this effect is mediated. These interactions may have important implications for cardiovascular regulation under normal and pathologic conditions, including regulation of catecholamine release during stress. In this respect, CgA levels are also increased in untreated hypertension (Takiyyudin et al., 1990).

3.4.4 Antimicrobial activities of chromogranins

Chromogranins and their cleavage products may play a role in systemic infection (Strub et al., 1996). Antimicrobial activity of chromogranin A and of its fragments, such as chromacin I, chromacin II and prochromacin, results from the ability of these peptides to create ion channels through the cell membranes. Chromogranin B fragment secretolytin possesses bacteriolytic properties as well.

3.5 Chromogranin A in oncology

CgA is an important tumour marker used both in immunohistochemical examination of biopsied tumour tissue and as a serum tumour marker. Its presence along with synaptophysin is necessary for confirmation of diagnosis of neuroendocrine tumours.

3.5.1 Pathology

CgA and its fragments may play an interesting role in the regulation of cell adhesion. N-terminal fragments of chromogranin A correspond with amino acid residues 1–78 and 1–115 and may induce adhesion and spreading of fibroblasts. These fragments could be important for the local control of cell adhesion by stimulated cells (Gasparri et al., 1997), which are dispersed in endocrine tissues, so the activity of the peptides may be important for the regulation of development and remodelling of the neuroendocrine tissue. Therefore, it is supposed that CgA is somehow involved in the process of progression and dissemination of NETs.

Expression of circulating CgA is a complex function of the density of secretory granules, total tumour burden in well-differentiated tumours with constant granule density per unit of tissue, and capacity for cellular secretion.

3.5.2 Chromogranin A in immunohistochemistry

In the tissue samples, CgA stain positivity allows confirmation of the neuroendocrine character of the tumours. CgA may be used as an immunohistochemical tissue marker for NET as sensitive and specific as argyrophil reaction (see Figs. 2, 3).

3.5.3 Circulating tumour marker

Because of its co-storage and co-expression together with other peptide hormones produced by neuroendocrine cells, chromogranin A may serve as a serum marker of functions of various endocrine and neuroendocrine cells and tumours. The examination is more feasible than examination of individual respective hormones (Rosa et al., 1994; Giovanella, 2000; Zatelli et al., 2007). Chromogranin examination is convenient especially if the tumour fails to produce sufficient amounts of other hormones or if the serum levels of other markers, e.g. catecholamines and serotonin, rapidly vary and fluctuate (Öberg et al., 1999). In most CgA-producing tumours, the levels of this peptide markedly exceed the normal upper margin, and false positive and false negative results are not too frequent.

CgA may be examined in the serum or plasma using radioimmunoassays (RIA, CgA-RIACT), ELISA method (enzyme-linked immunosorbent assay) and immunoradiometric assay (IRMA). E.g., the RIACT technique is able to detect the intact molecule and most (almost 100 %) of all circulating fragments (total CgA).

CgA as tumour marker is a suitable diagnostic tool for three situations: (a) basic diagnosis within other diagnostic procedures, (b) evaluation of the course of the disease, i.e. tumour regression or progression, (c) evaluation of the response to treatment (Eriksson et al., 2000). CgA is a useful diagnostic tool for detection of neuroendocrine tumours, growing mainly in the gastrointestinal tract. Moreover, CgA is secreted by endocrine neoplasms producing ACTH, FSH, GH, LH, TSH. No CgA secretion has been detected in prolactinoma so far.

3.5.4 CgA as tumour marker in gastro-enteropancreatic NET

Carcinoids and pancreatic islet cells produce biologically active substances capable to bring about clinical symptoms. The highest levels of CgA have been reported in gastrointestinal NETs, in particular in small intestine carcinoids and pancreatic islet cells.

There is an association between CgA and concentration of circulating serum gastrin in the patients with hyperparathyreoidism and multiple endocrine neoplasia (MEN 1).
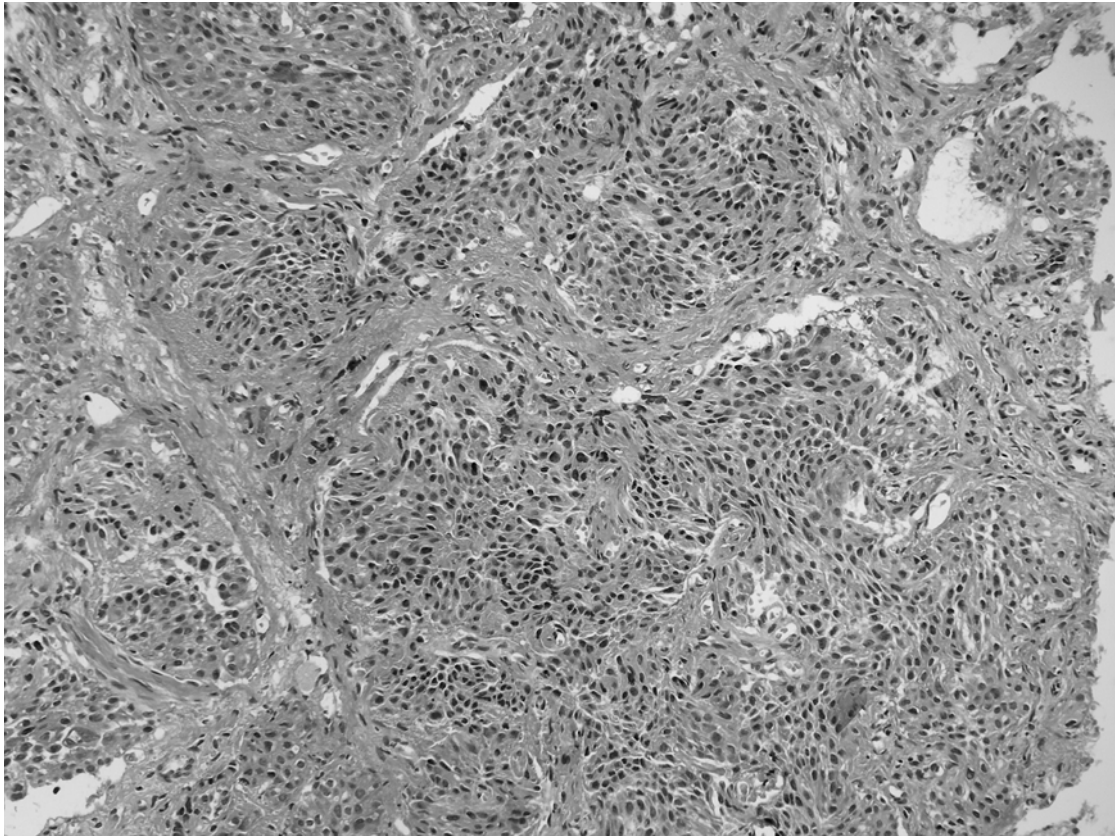
Fig. 2. Histologic biopsy specimen of liver metastases of well-differentiated neuroendocrine carcinoma stained with haematoxylin and eosin. Magnification 200×.
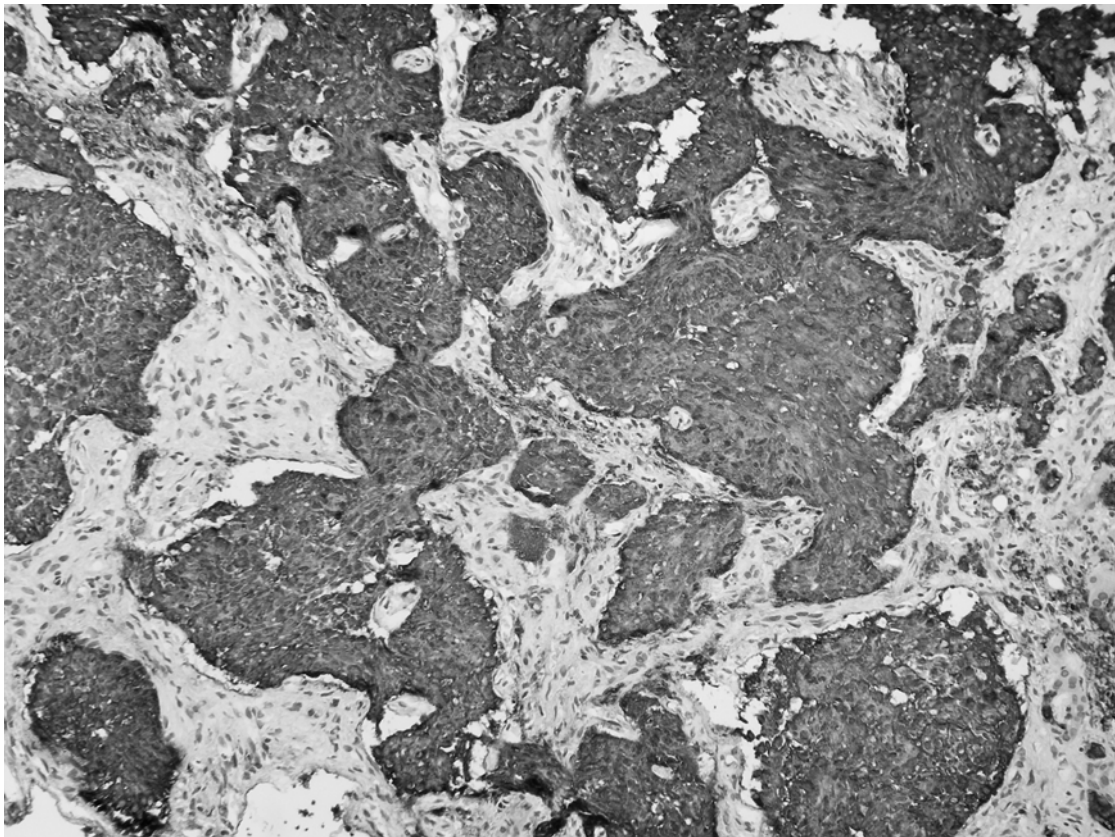

Fig. 3. Strongly positive immunohistochemical staining for chromogranin, the same neuroendocrine carcinoma, liver metastases (67-year old female, liver metastases of neuroendocrine carcinoma of unknown primary origin). Magnification 400×.

Pheochromocytoma

CgA is released together with chromaffin granules of pheochromocytoma. CgA corresponds with total tumour burden and local catecholamine production. Therefore, CgA is a suitable complementary method for the specific laboratory tests. Examination of CgA is an important tool in differential diagnosis of pheochromocytoma as a cause of secondary hypertension versus essential hypertension (D'Herbomez et al., 2001; Giovanella et al. 2006).

CgA level in other endocrine and non-endocrine tumours

CgA levels are often higher in atypical lung carcinoids; in small-cell lung cancers CgA correlates with the stage and total tumour burden. CgA may also be increased in medullary carcinoma of the thyroid and in neuroblastomas. CgA could be slightly elevated as well in some non-neuroendocrine tumours because neuroendocrine tumour cells may be dispersed in original neoplasia, or they may create small clusters in their tissues. For example, prostate adenocarcinomas may express both CgA and prostate-specific antigen. Neuroendocrine differentiation of prostate carcinoma tissue heralds resistance to therapy and worse prognosis.

Serum chromogranin A validity as tumour marker

(1) The main remark concerns the own 52 cases evaluated for serum chromogranin A concentration by the author. This sample served for the evaluation of sensitivity and specificity of chromogranin A estimation in the serum. More information would be therefore important (which tumors have been enrolled and what was the difference between controls and patients in chromogranin A concentrations). This could improve and strenghten the information on autenticity of serum chromogranin A values. Serum chromogranin A validity may be illustrated by the following experimental data. Control sample included 50 patients of the 4th Department of Medicine of the First Faculty of Medicine and General University Hospital of Charles University in Prague, 28 females and 22 males. The patients with all types of cancers were strictly excluded as well as the persons with all diseases and medications known from the literature to interfere with CgA levels. Renal functions were normal in all controls. The laboratory tests were performed within routine clinical examination. Median age was 61.5 years, range 35–90 years. The arithmetic mean of CgA values was 64.6 ng/ml, median was 55.8 ng/ml, range 14.3–188.0 ng/ml (the normal range is 19.4–94.1 ng/ml, according to CgA-RIACT laboratory kit producer, Cisbio Bioassays, Codolet, France).

(2) Sample of patients with neuroendocrine tumours included 44 patients with generalized NETs and 8 locally advanced NETs (i.e. radically inoperable but without distant metastasis), thus 52 patients with neuroendocrine carcinomas in total.

Localization of primary tumours

NET of the caecum, rectal NET, NET of the biliary tract and NET of adrenal gland – in singles, thus four cases in total, two ovary carcinoid cancers, three retroperitoneal NETs, three gastric NETs, four bronchial NETs, eight cases of NETs of unknown primary origin, 15 pancreatic NETs and 13 NETs of small intestine, thus 52 patients in total. All tumours were malignant, endocrine non-functional tumours were represented by 39 (75 %) cases and endocrine functional cases occurred in 13 persons (25 %). Endocrine functional tumours were represented by carcinoids (11 subjects) and by two pancreatic gastrinomas.

Increased levels of CgA were found in 43 patients, normal values occurred in nine persons. CgA arithmetic mean in generalized NETs was 617.3 ng/ml, median 673.7 ng /ml, range 27.1–1384 ng/ml. In locally advanced cases, arithmetic mean was 389.3 ng /ml, median was 236.5 ng /ml and range 72.6–1100.9 ng /ml.

Mann-Whitney non-parametric test proved a highly significant difference of CgA values in normal controls compared to the patients with NETs, at P level = 0.000001.

However, no significant difference in medians of CgA was found by Mann-Whitney test between generalized and locally advanced cases (at P level = 0.348).

The following validity data were calculated: 82.7 % specificity, 82 % sensitivity, 82.7 % positive predictive value, 82 % negative predictive value, 18 % false positivity, 17.3 % false negativity and 82.4 % accuracy. These results correspond quite well with previous literary data. Accuracy of serum chromogranin A in NET is as follows, according to various authors: Nobels et al. (1998) report 80 % but Schürman et al. (1990) report 100 % value, Eriksson et al. (2000) about 94 %, and Baudin et al. (2001) present much lower value, just 61 %. As it is apparent, the results differ significantly. The highest accuracy and highest levels have usually been observed in the tumours with intensive secretory activity, mainly in small intestine midgut carcinoids, even if accuracy is satisfactory in non-functional neoplasias (Sobol et al., 1989). There is supposed proportion between the CgA level and biological tumour activity (measured by respective hormones in peripheral circulation), and correlation between density of secretory granules in tumour tissue and serum CgA expression by the tumour tissue.

CgA as prognostic factor

High levels of CgA signalize extensive disease and larger tumour burden. As it has been evidenced by multivariable analysis, the CgA level in the patients with advanced small intestine NETs (also known as midgut carcinoids) is an independent prognostic factor of poor prognosis (Janson et al., 1997). It is supposed that either the CgA molecule or its cleavage products act as growth factor for the NET cells. CgA may rather be associated with proliferation activity than with tumour secretion activity.

This correlation does not hold for gastrinomas because CgA elevation is caused not only by the tumour itself, but also by enterochromaffin-like (ECL) gastric cells being hyperplastic owing to chronic hypergastrinaemia (Nobels, 1998). Thus, it is recommended to use CgA to assess whether surgery is radical, with the exception of gastrinomas.

In summary, chromogranin A is rather a reliable tumour marker for several reasons:

(a) Satisfactory sensitivity and specificity
(b) Increased in functional and non-functional NETs and their metastases irrespective of their location
(c) CgA level roughly correlates with total tumour burden in some NET subtypes (especially so-called midgut, small intestine NETs)
(d) CgA is an independent predictor of survival, high levels portend worse prognosis

3.5.5 CgA levels in non-oncology diseases

The CgA level is falsely elevated in the patients treated with proton pump inhibitors (omeprazole, lanzoprazole, etc.). Falsely elevated CgA levels may also be found in the patients with inflammatory bowel diseases, advanced liver failure, heart failure and atrophic gastritis. Atrophic gastritis causes CgA elevation due to the stimulation of enterochromaffin-like (ECL) cells by gastrin. Mild CgA elevation has also been reported in menopausal women and in inflammatory bowel disease (Crohn disease) (Sciola et al., 2009). Intensive sympathoadrenal stimulation (stress, exercise) is able to increase the CgA level up to twice above the upper margin. However, the most significant increase in CgA has been reported in renal failure due to decreased peptide secretion (Hsiao et al., 1990; Ferrari et al., 1999).

Conclusion

The chromogranin family plays multiple roles in the storage and release of hormone peptides. Chromogranin A is a major representative of this family. It is stored in the large dense granules along with other peptide hormones and released together with them.

Chromogranins are proteolytically processed in the endocrine tissues and turn into biologically active peptides controlling the function of the respective tissues.

Because many polypeptide hormones and specific neurotransmitters are co-expressed with chromogranin A, chromogranin A seems to be capable of responding to the wide scale of modulation signals influencing CgA expression in various tissues.

Cleavage peptides of chromogranins such as pancreastatin, vasostatin and others exert significant biological effects. Some chromogranin fragments exert antimicrobial and antifungal effect.

There is also association between chromogranins and catecholamines. During sympathoadrenal activation, CgA is released along with catecholamines out of the cell vesicles in adrenal medulla and sympathetic nerve endings. Chromogranin cleavage product catestatin inhibits catecholamine secretion by adrenal medulla. Chromogranin A is often elevated in pheochromocytoma, neuroendocrine tumour originating in adrenal gland medulla. In addition, the plasminogen/tissue-plasminogen activator (t-PA) system plays a major role in catecholaminergic activity by means of interaction with CgA.

CgA has been verified as a useful serum and immunohistochemical tumour marker for diagnosis of neuroendocrine tumours. Expression of circulating CgA is a complex function of density of secretory granules, total tumour burden and capacity for cellular secretion. The CgA level is a function of total tumour mass in well-differentiated tumours with constant granule density per unit of tissue. Serum CgA may serve as an independent prognostic indicator in small intestine carcinoids, as an indicator of tumour progression, recurrence or as an indicator of response to therapy.

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