Marijana C. Jandric-Kocic[1], Mario Mizdraric[2, 3]

CHROMOGRANIN A

Summary: Chromogranin A (CgA) is the most important member of the granin family. It is stored with hormones and neurotransmitters in large dense core vesicles or chromaffin granules and together with them it is released constitutively or in a controlled way.

In this review are presented the role of CgA and biologically active peptides derived from it, as well as its use as a marker of certain diseases.

CgA plays a significant role in sectorial granulogenesis (sorting and processing of secretory protein content, maturation and condensation of secretory granules) and maintaining calcium homeostasis. Glycosylation, phosphorylation, sulfation, and/or proteolytic processing of CgA results in biologically active peptides that exhibit hormonal, autocrine, and paracrine activity. In the cardiovascular, metabolic, immune and diffuse neuroendocrine system, CgA and biologically active peptides derived from it have numerous, sometimes conflicting roles. CgA has been identified as an important immunohistochemical tumor marker of neuroendocrine tumors, prostate cancer and microcellular lung cancer with neuroendocrine differentiation. Its credibility is conditioned by the tumor itself (localization, size, density of secretory granules and secretory activity), dissemination of the tumor and application of the diagnostic technique. Elevated CgA values cannot be used to assess the progression of tumors that are not of neuroendocrine origin. In chronic heart failure and acute coronary syndrome, CgA is an independent prognostic factor of the disease.

Key words: chromogranin A, diffuse neuroendocrine system, tumor, disease

INTRODUCTION

The granin family consists of chromogranin A, chromogranin B, secretogranin II (or chromogranin C), secretogranin III (or 1B1075 gene product), secretogranin IV (or

[1] Marijana C Jandric-Kocic, Health care Center Krupa na Uni, Republic of Srpska, Bosnia and Herzegovina, marijanajandrickocic@gmail.com

[2] University Clinical Center of the Republic of Srpska

[3] University of Banja Luka, Faculty of Medicine

HISL-19 antigen), secretogranin V (or 7B2 antigen), secretogranin VI (or neuroendocrine secretory protein 55) and secretogranin VII (or subtilisin/kexin type 1 proprotein convertase inhibitor) (1,2). Granins are proteins with a molecular weight of 27 to 10 kilodaltons (KDa) (1). They are built from single polypeptide chains with at least 10% acid residues (glutamic or aspartic acid) and multiple single and dibasic amino acid residues (3). They are stored in secretory granules of endocrine, neuroendocrine and neuronal cells (1). Granins play a significant role in sectorial granulogenesis, sorting and processing of secretory protein content, maturation and condensation of secretory granules (3). They maintain calcium homeostasis and exhibit hormonal, autocrine and paracrine activity (1,4,5).

Granins are produced in the granular endoplasmic reticulum and enter the inner space of the reticulum via the signal peptide at the N terminal end of the molecule (3,4). From there, via transport vesicles, they enter the Golgi complex (3). Then, with the remaining contents of the vesicles, they are packed into immature secretory granules in the trans network of the Golgi complex (1,3,4). There, they are partially processed into various biologically active peptides (3). Immature granules mature by subsequent acidification of the granule contents and removal of the clathrin envelope, synaptotagmin IV and membrane protein 4 (1,3). Mature dense-core secretory granules (DCG), known as large dense-core vesicles (LDCV) or chromaffin granules (CG) are stored in the medulla of the adrenal gland and their contents are released after stimulation (3).

Granins facilitate DCG formation when an immature granule is released from the trans network of the Golgi complex (4,5). They enable the recruitment and condensation of the soluble protein content of the secretory granules into dense nuclei (5). The presence of granins in secretory granules, as well as their high calcium binding capacity at low pH, ensures calcium storage in cells (4). Proteolytic degradation of granins produces proteins that have hormonal, autocrine and paracrine activity (2,4,5).

Granins and bioactive fragments derived from them contribute to the release of certain hormones, as well as glucose homeostasis, neuronal excitability, autoimmunity and smooth muscle contractility (4). In this way, they modulate metabolism, pain pathways, mood and blood pressure (4). Granins represent a marker of specific diseases, including neurodegenerative diseases, psychiatric diseases and cancers (4).

METHODS

The literature was searched using keywords: chromogranin A, diffuse neuroendocrine system, tumor, disease. The search was conducted within the following databases: PubMed, Emabase, Scopus, SCIn-dex and Hrčak. Due to the limited number of available studies, no available filters were used in the database search.

After reading the abstracts, the papers were studied in more detail, and those that did not correspond to the research objective were excluded.

CHROMOGRANIN A

Chromogranin A (CgA) is the first identified representative of granins (6). Its name comes from the place of primary identification, adrenal medulla chromaffin vesicles containing catecholamines (6).

CgA is a protein with a molecular weight of 48 kDa, made of 439 amino acids (1). Chromaffin cells of the medulla of the adrenal gland represent the main source of circulating CgA (2). Adrenergic nerve endings and neuroendocrine cells secrete CgA in peripheral tissues (2). CgA has been detected in the adenohypophysis, lung, pancreas, stomach, small intestine (jejunum and ileum), frontal cerebral cortex, thyroid gland, parathyroid glands, myocardium and polymorphonuclear leukocytes (2,7-10).

The production of CgA is regulated by several intracellular messenger systems, which include protein kinase A, protein kinase C, as well as intracellular calcium (8). Histamine, nicotine, bradykinin, angiotensin II, prostaglandin E2 and potassium can facilitate the production of CgA (8). Together with hormones or neurotransmitters, CgA is released constitutively or in a controlled pathway (8).

The CgA gene is located on chromosome 14q32.12 and includes 12,192 base pairs (bp) organized in eight exons and seven introns (1). The primary transcript of 2 kilobases (kb) is made into a protein with a molecular weight of 52 kDa, made of 457 amino acids, which undergoes post-translational modifications, i.e. endoproteolysis by prohormone convertases (1). Proteolytic cleavage of CgA produces several biologically active peptides: vasostatins 1 and 2, prochromacin, chromacins 1 and 2, chromofungin, pancreastatin, catestatin, parastatin, serpinin, WE14, EA-92, SS-18, LF-19, GR-44, SS-18, AL-11, ER-37, ES-43, GV 19, WA-8 (1,7).

PHYSIOLOGICAL ROLE OF CHROMOGRANIN A AND ITS CLEAVAGE PRODUCTS

CgA has numerous physiological roles inside and outside the cell (Table 1) (1,7,9). By inhibiting granule degradation mediated by serpinin, it modulates DCG biogenesis (1). Also, it plays an important role in the recovery of secretory granule cells after exocytosis (1). The abundance (~2-4 mM) of CgA within DCG enables the storage of a large amount of calcium in them (1). At the same time, CgA enables the rapid exchange of free and bound calcium within the DCG, as well as the mobilization of calcium into the cytoplasm (through the activation of inositol triphosphate receptors of calcium channels on the DCG membranes) (1).

The role of CgA outside the cell is not completely clear (1,7). CgA inhibits the secretion of proopiomelanocortin in the anterior lobe of the pituitary gland (15). By inducing the production of tumor necrosis factor alpha and diffusible neurotoxic substances in microglia cells, it causes neuronal apoptosis (1). A significant number of roles, originally considered roles of CgA, have been attributed to biologically active peptides resulting from glycosylation, phosphorylation, sulfation and/or proteolytic processing of CgA (7). Most of them consist of direct or indirect inhibitory regulation via autocrine, paracrine or hormonal pathways (7). So far, the role of catestatin, prochromacin, chromacin 1 and 2, chromofungin, pancreastatin, parastatin, vasostatin 1 and 2 and WE 14 has been discovered (1,7). The role of the remaining biologically active peptides derived from CgA remains unknown (1,7).

Table 1. Role of chromogranin A and biologically active peptides derived from it (7)

In the cell	Outside the cell
Granulogenic activity	Inhibition of gastric acid secretion
Protein storage	Inhibition of colonic peristalsis
Storage and regulation of calcium flow	Inhibition of arterial vasoconstriction
Prohormone	Negative inotropic effect
	Regulation of carbohydrate, fat and protein metabolism
	Inhibition of secretion of: insulin, parathyroid hormone, catecholamine, pancreatic amylase, proopiomelanocortin
	Antimicrobial effect
	Anti-inflammatory activity
	Tissue regeneration: regulation of cell adhesion
	Regulation of nociception
	Activation of microglia in the central nervous system
	Apoptotic degeneration of cortical neurons

Vasostatins 1 and 2 represent N-terminal fragments of CgA (1). They inhibit vasoconstriction of arteries mediated by endothelin-1 and have a negative inotropic and lusitropic effect on the heart, predominantly in the presence of intense adrenergic stimuli (1,7). Furthermore, they inhibit proliferation and migration of endothelial cells

induced by vascular endothelial growth factor (1). Thanks to their cardiotropic and vasoactive properties, in conditions of excessive sympathetic stimulation, they can play the role of a homeostatic stabilizer of the cardiovascular system (1). Vasostatin 1 and 2 inhibit the secretion of gastric acid and parathyroid hormone and have an antimicrobial and anti-inflammatory effect (7). Namely, they inhibit the growth of yeasts, bacteria and fungi and stimulate the migration of macrophages (1). They protect the integrity of the endothelial barrier against pro-inflammatory agents and modulate the adhesion of fibroblasts (7). Ultimately, vasostatins 1 and 2 induce neuronal apoptosis and control nociception (7).

Pancreastatin is the first identified biologically active peptide derived from CgA (1). It inhibits the exocrine activity of the pancreas and stomach as well as the release of parathyroid hormone (1,7). It modifies the insulin:glucagon ratio by stimulating the secretion of glucagon and inhibiting the secretion of insulin conditioned by physiological stimuli (7). It increases the production of nitric oxide which inhibits insulin (9). Released with catecholamines from the sympathetic nervous system in stressful situations, it participates in the modulation of energy metabolism (1). Namely, with unbalanced sympathetic activation, excess catecholamines together with increased values of pancreastatin reduce glucose intake (by ~50%) and increase the release of free fatty acids (by 4.5 to 6.4 times) (1). Individual studies indicate that pancreastatin (by a mechanism that is not entirely clear) plays a significant role in the pathogenesis of various forms of diabetes mellitus (9).

Catestatin acts on nicotinic acetylcholine receptors as a strong autocrine inhibitor of catecholamine secretion (1,7). It is believed that catestatin deficiency plays a significant role in the development of hypertension caused by long-term overactivity of the sympathetic nervous system (1). By inducing the release of histamine from mast cells, it achieves a vasorelaxant and antihypertensive effect (1). Catestatin has pronounced angiogenic and vasculogenic activity, since it induces the migration and proliferation of endothelial and smooth muscle cells of blood vessels (1). It stimulates chemotaxis activity and the activity of pro-inflammatory cytokines, and inhibits the growth of fungi, yeasts and bacteria (Gram-positive and Gram-negative) (1).

Parastatin was discovered in the parathyroid gland of a pig (1). In the presence of low levels of calcium in the blood, it inhibits the secretion of parathyroid hormone (1).

Prochromacin and the biologically active peptides derived from it, chromacins 1 and 2 inhibit the growth of fungi and bacteria (7,10).

WE 14 can modulate the value of gonadotropic hormones (7).
In addition, research by eminent authors identified WE 14 as an autoantigen of the pancreas that plays a significant role in the pathogenesis of various forms of diabetes mellitus (9).

METHODS OF DETERMINING THE VALUE OF CHROMOGRANIN A IN THE BLOOD

There are a number of commercially available kits for the determination of CgA in blood (6). They are based on three diagnostic techniques: enzyme linked immunosorbent assay (ELISA), immunoradiometric assay (IRMA) and radioimmunoassay (RIA) (6). Although a significant number of studies recommend RIA, there is no universally accepted diagnostic technique and no standardization of any of the mentioned diagnostic techniques (6,11). Therefore, caution is recommended when trying to compare the results of different diagnostic centers (6). CgA can be determined in plasma or serum (6). The value of CgA in plasma is often significantly higher than the value determined in serum (6). Research by certain authors recommends the determination of CgA in saliva (1).

CHROMOGRANIN A AS A DIAGNOSTIC AND PROGNOSTIC MARKER

An increase in the value of CgA in the blood occurs in certain benign diseases and conditions, malignant diseases as well as iatrogenic (Table 2) (12).

CgA is elevated in individuals using proton pump inhibitors, histamine H2 receptor antagonists, and selective serotonin reuptake inhibitors (12). Therefore, it is recommended to stop using drugs (histamine H2 receptor antagonists for 24 hours, proton pump inhibitors for 14 days) before determining the CgA value in the blood (12). Intense physical activity as well as food intake immediately before testing results in an increase in CgA values in the blood (12). Slightly elevated CgA values were found in menopause (due to increased sympathetic tone) and pregnancy (1).

Table 2. Causes of increased CgA values in the blood (1.12)

Benign diseases and conditions	Iatrogenic causes	Malignant diseases
Pregnancy	Proton pump inhibitors	Gastric cancer
Menopause	Antagonists of histamine H2 receptors	Pancreatic cancer
Chronic atrophic gastritis	Selective serotonin reuptake inhibitors	Hepatocellular carcinoma
Helicobacter pylori infection	Intense physical activity	Breast cancer
Pancreatitis	Recent food intake	Ovarian cancer
Chronic hepatitis		Prostate cancer
Liver cirrhosis		Neuroblastoma
Irritable bowel syndrome		Small cell lung cancer

Ulcerative colitis		Neuroendocrine tumors
Chron's disease		
Arterial hypertension		
Heart failure		
Acute coronary syndrome		
Myocardial infarction		
Giant cell arteritis		
Renal insufficiency		
Chronic bronchitis		
Obstructive lung diseases		
Rheumatoid arthritis		
Systemic lupus erythematosus		
Hyperthyroidism		
Hyperparathyroidism		
Hypercortisolemia		

Chronic atrophic gastritis and gastritis caused by Helicobacter pylori can cause an increase in the value of CgA, due to a chronic increase in the value of gastrin in the serum (1). In people with these diseases, predominantly in those treated with proton pump inhibitors, the measurement of CgA values in the blood can contribute to the monitoring of enterochromaffin-like gastric cell hyperplasia (1). Ulcerative colitis and Crohn's disease cause a discrete increase in CgA values in the blood (1).

With organ dysfunction, such as kidney, liver and heart failure, CgA values in the blood can also be significantly increased (1). Very high CgA values in people with kidney failure (comparable to CgA values in people with neuroendocrine tumors) support the important role of the kidneys in removing CgA from the body (2). Its moderately elevated values in people with liver failure indicate either the metabolism of CgA in the liver or the activation of the neuroendocrine system in these people (2). Activation of the neuroendocrine system is also present in people with heart failure, acute coronary syndrome and hypertension (1,2). High CgA values in the blood indicate a high risk of death after myocardial infarction, acute coronary syndrome and heart failure (1).

There are high values of CgA in certain inflammatory diseases, such as rheumatoid arthritis and systemic lupus erythematosus (1). Endocrine disorders associated with elevated CgA values in the blood are hypercortisolemia, hyperthyroidism and hyperparathyroidism (1).

CgA is an important marker of neuroendocrine tumors including carcinoid tumor, pheochromocytoma, medullary carcinoma of the thyroid gland, adenoma of the parathyroid gland, neuroblastoma, gastroenteropancreatic neuroendocrine tumors, multiple endocrine neoplasia syndrome type 1 and bronchopulmonary neuroendocrine tumors (13). The highest reliability of this tumor marker was found in disseminated and well-differentiated neuroendocrine tumors that show intense secretory activity (13).

The sensitivity of CgA in people with neuroendocrine tumors is between 10 and 100%, and its specificity is between 68 and 100% (13). In small tumors (except gastrinoma), such as insulinoma, paraganglioma, carcinoid tumor of the bronchus and pituitary tumor, CgA values can be in the reference range (13). The highest sensitivity of CgA was found in gastrinomas (100%), pheochromocytomas (89%), carcinoid tumors (80%), non-functioning tumors of the endocrine pancreas (69%) and medullary carcinoma of the thyroid gland (50%) (13). In patients with metastatic disease, CgA has a sensitivity of 60 to 100% and its blood level can increase by as much as 100-1000 times (13). The highest values are found in carcinoid tumors with liver metastases (13). In addition to diagnosing neuroendocrine tumors, CgA is important in monitoring response to treatment (13).

In patients with small cell lung cancer with neuroendocrine differentiation, CgA can help monitor response to treatment and detect disease recurrence (13). Although the CgA level in the blood indicates the likely degree of neuroendocrine differentiation in the cancer, its low sensitivity makes it unreliable in detecting the disease (13).

In patients with prostate cancer, determining the CgA level in the blood can help in diagnosing and determining the prognosis of the disease (2). Namely, the CgA level in the blood of patients with they can be elevated even if the value of prostate specific antigen s is normal (13). High CgA values in the blood can predict the absence of cancer response to hormone therapy, that is, a poor prognosis of the disease (13).

CgA immunoreactivity has been established in colorectal and breast cancer cells, but its diagnostic value has not been established (2).

CONCLUSION

CgA and the biologically active peptides derived from it have numerous, sometimes conflicting roles in the cardiovascular, metabolic, immune and diffuse neuroendocrine systems. CgA has been identified as an important immunohistochemical tumor marker of neuroendocrine tumors, prostate cancer and microcellular lung cancer with neuroendocrine differentiation. Its credibility is conditioned by the tumor itself (localization, size, density of secretory granules and secretory activity), dissemination of the tumor and application of the diagnostic technique. Elevated CgA values cannot be used to assess the progression of tumors that are not of neuroendocrine origin. In

chronic heart failure and acute coronary syndrome, CgA is an independent prognostic factor of the disease.

LITERATURE

1. D'amico MA, Ghinassi B, Izzicupo P, Manzoli L, Di Baldassarre A. Biological function and clinical relevance of chromogranin A and derived peptides. Endocr Connect. 2014 Apr 29;3(2):R45-54. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC5395093/
2. Taupenot L, Kimberly HL, O'Connor DT. The Chromogranin–Secretogranin Family. NEJM. 2003;348(12):1134-1149. Available from: https://www.nejm.org/doi/full/10.1056/nejmra021405
3. Feldman SA, Eiden LE. The chromogranins: Their roles in secretion from neuroendocrine cells and as markers for neuroendocrine neoplasia. Endocr Pathol. 2003;14:3–23. Available from: https://doi.org/10.1385/EP:14:1:3
4. Bartolomucci A, Possenti R, Mahata SK, Fischer-Colbrie R, Loh YP, Salton SR. The extended granin family: structure, function, and biomedical implications. Endocr Rev. 2011 Dec;32(6):755-97. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC3591675/
5. Estevez-Herrera J, Pardo MR., Dominguez N, Pereda D, Machado JD, Borges R. The role of chromogranins in the secretory pathway. BioMolecular Concepts. 2013;4(6):605-609. Available from:https://www.degruyter.com/document/doi/10.1515/bmc-2013-0020/html?srsltid=AfmBOoqMHFufhcNduEZBODJaF4PL_ddiPBhRUJL34a8zE-ZqRqx-ZKya2
6. Gut P, Czarnywojtek A, Fischbach J, Bączyk M, Ziemnicka K, Wrotkowska E et al. Chromogranin A - unspecific neuroendocrine marker. Clinical utility and potential diagnostic pitfalls. Arch Med Sci. 2016 Feb 1;12(1):1-9. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC4754364/
7. Díaz Pérez JÁ, Currás Freixes M. Cromogranina A y tumores neuroendocrinos. Endocrinol Nutr. 2013;60:386–395. Available from: https://www.elsevier.es/en-revista-endocrinologia-nutricion-412-articulo-chromogranin-a-neuroendocrine-tumors-S2173509313001372#bib0100
8. Louthan O. Chromogranin A in Physiology and Oncology. Folia Biologica (Praha). 2011;57:173-181. Available from: https://fb.cuni.cz/file/5597/FB2011A0026.pdf
9. Herold Z, Doleschall M, Kovesdi A, Patocs A, Somogyi A. Chromogranin-A and its role in the pathogenesis of diabetes mellitus. Endokrynologia Polska. 2018;69(5):598-610. Available from: https://journals.viamedica.pl/endokrynologia_polska/article/view/EP.a2018.0052/45437
10. Scavello F, Kharouf N, Lavalle P, Haikel Y, Schneider F, Metz-Boutigue MH. The antimicrobial peptides secreted by the chromaffin cells of the adrenal medulla link the neuroendocrine and immune systems: From basic to clinical studies. Front Immunol.

2022 Aug 25;13:977175. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC9452953/#B23

11. Stridsberg M, Eriksson B, Oberg K, Janson ET. A comparison between three commercial kits for chromogranin A measurements. J Endocrinol. 2003 May;177(2):337-41. Available from: https://pubmed.ncbi.nlm.nih.gov/12740022/
12. Di Giacinto P, Rota F, Rizza L, Campana D, Isidori A, Lania A et al. Chromogranin A: From Laboratory to Clinical Aspects of Patients with Neuroendocrine Tumors. Int J Endocrinol. 2018 Jul 2;2018:8126087. Available from: https://pmc.ncbi.nlm.nih.gov/articles/PMC6051263/
13. Glinicki P, Jeske W. Chromogranin A (CgA) — the influence of various factors *in vivo* and *in vitro*, and existing disorders on it's concentration in blood. Endokrynologia Polska/ Polish Journal of Endocrinology 2010; 61(4):384-397. Available from: file:///C:/Users/W10/Downloads/25370-31124-1-PB%20(1).pdf