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THE GENE FOR HUMAN CHROMOGRANIN A (CgA) IS LOCATED ON CHROMOSOME 14

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Chromogranin A (CgA) is a protein that is present in most neuroendocrine tissues and is co-secreted with their resident hormones. We have assigned the CgA gene to human chromosome 14 by hybridization of a CgA cDNA probe cloned from a cDNA library of human medullary thyroid carcinoma cells to spots of individual human chromosomes flow-sorted onto nitrocellulose filters. Southern analysis of human genomic DNA with the same probe revealed only 1-3 restriction bands. These studies indicate that the CgA gene is probably single copy and not a member of a dispersed, multigene family. The CgA gene is not co-localized with the genes of any of the CgA-associated hormones. © 1987 Academic Press, Inc.

Chromogranin A (CgA) is a protein that is found in most hormone-producing tissues, including the pancreas, pituitary gland, parathyroid glands, C- cells of the thyroid gland, and the neuroendocrine cells of the lung and gut (1). It was originally described as the major soluble protein of adrenal chromaffin granules and in retrospect is indistinguishable from parathyroid secretory protein (2). In hormone-producing tissues it is co-localized and co-secreted with resident hormones of the tissue (3,4). It is found in elevated concentrations in the serum of patients with tumors of neuroendocrine tissues, including medullary thyroid carcinoma and small cell lung cancers (4,5). cDNA clones have been used to demonstrate the neuroendocrine specificity of CgA mRNA (6,7) and to predict the amino acid sequence of the bovine form (7,8).

Materials and Methods

The CgA-specific cDNA probe used in these studies is 339 base-pairs in length and encodes the first 113 amino acids of human CgA; its cloning from a lambda gt11

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cDNA library of human medullary thyroid carcinoma cells has been described previously (6,9). For the hybridization studies the probe was labeled with ^{32}P-dCTP using the method of Feinberg and Vogelstein (10).

For Southern blot analysis, total cellular DNA was extracted from peripheral mononuclear cells using standard methods (11). The mononuclear cells were separated from the blood of normal human subjects by centrifugation with Histopaque-1077 (Sigma Chemical Co., St. Louis, Missouri) according to the method of Boyum (12). DNA from cultured human lymphoblastoid cells (GM130-A) was extracted using the same methods. Aliquots of the DNA samples were digested with the indicated restriction endonucleases, fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized with the CgA-specific probe (13).

Human chromosomes were isolated and sorted as previously described (14-16) from diploid human lymphoblastoid cells (GM 130A) grown in suspension culture in RPMI medium supplemented with 10% fetal calf serum. Sorting was accomplished using an EPICS V flow cytometer (Coulter Electronics, Inc., Hialeah, FL). The chromosomes were sorted directly onto nitrocellulose discs. Each chromosomal type can be sorted with greater than 90% purity, with the exception of numbers 9-12 and 14 and 15. Chromosomes 9-12 overlap in one peak; 14 and 15 can be separated from each other, but with approximately 15 percent contamination of 15 with 14 (16). Thirty thousand chromosomes of each type were sorted onto the nitrocellulose discs, resulting in individual spots of 3×10^4 chromosones for 1-8, 13-22, x,y and 1 spot of 1.2×10^5 chromosomes for 9-12. The DNA was denatured by washing the discs in .5 M NaOH, 1.5 M NaCl for 5 minutes, then neutralized in 0.5 M Tris, pH 7.5, 3.0 M NaCl, and baked at 80°C for 90 minutes under vacuum. The filter discs were hybridized with the CgA-specific probe for 24 hours (6,13).

Results

The results of the Southern blot analysis of DNA from mononuclear cell DNA digested with various restriction endonucleases are shown in Figure 1. The appearance of a single dominant band of hybridization in some of the lanes suggests that the CgA gene occurs as a single copy; however, multiple bands are present in 2 of the 4

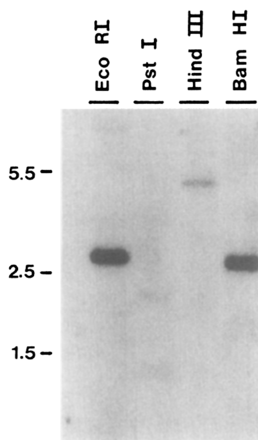

Figure 1. Southern blot analysis of DNA extracted from normal human mononuclear blood cells. Aliquots of 10 μg of DNA were digested with the restriction endonucleases shown, fractionated by agarose gel electrophoresis, transferred to nitrocellulose, and hybridized with the CgA-specific cDNA probe. DNA size markers in kilobases are shown at the top.

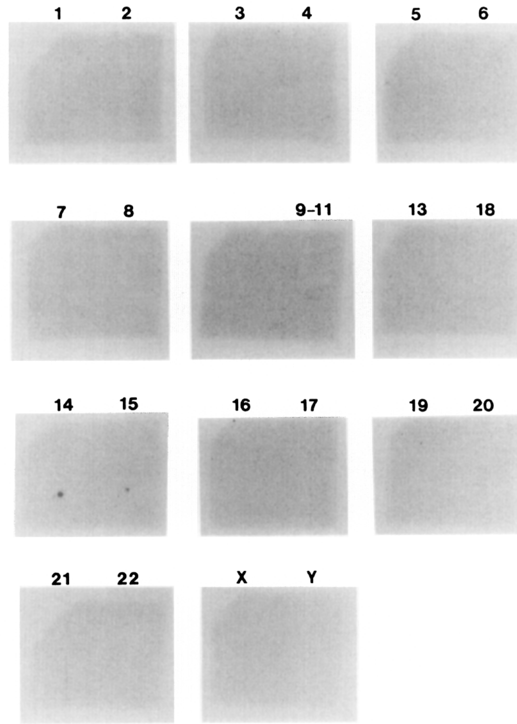

Figure 2. Autoradiograph of flow-sorted human chromosomes hybridized with the CgA-specific cDNA probe. The intense signal in compartment 14 indicates that the CgA gene is located on chromosome 14. The faint signal in compartment 15 likely represents hybridization to chromosome 14 that commonly contaminates preparations of chromosome 15.

lanes. The cDNA did not contain restriction sites for any of the endonucleases used. Similar results were obtained from Southern blot analysis of DNA extracted from GM130-A cells (data not shown).

Figure 2 shows the results of hybridization with the cDNA probe of human chromosomes sorted by flow cytometry and blotted on nitrocellulose (14-17). A strong hybridization signal is seen over the blot corresponding to chromosome 14. A much weaker signal is seen over the blot corresponding to chromosome 15. This is most likely due to the small amount of chromosome 14 DNA in the chromosome 15 spot because of the close proximity of chromosome 14 and 15 peaks in the flow histogram. It is also possible that a weakly cross-hybridizing species, such as a pseudogene to CgA, exists on Chromosome 15.

Discussion

CgA is a protein found in many neuroendocrine tissues (6-8). It is co-secreted with many peptide hormones and with catecholamines and its serum concentration is in-

creased in patients with eutopic and ectopic endocrine tumors (4). For example, serum CgA levels are increased in patients with medullary thyroid carcinoma and small cell lung cancer where its co-secreted hormone is calcitonin (4,5). Although its function is not known, CgA may play a role in the process of hormone secretion (18). There is some evidence to suggest that there may be multiple species of CgA and CgA-like proteins (19). However, our results suggest that the CgA gene is not a member of a dispersed, multi-gene family (Figures 1 and 2). Two of the peptides most clearly associated with CgA, calcitonin and parathyroid hormone, are encoded by genes of chromosome 11 (20,21). Furthermore, the genes for none of the other hormones or genetic abnormalities, such as Multiple Endocrine Neoplasia, that may be associated with CgA are assigned to chromosome 14 (20-30). Thus, it is not likely that cis acting factors account for the co-regulation of CgA and its associated hormones (31). Further studies are in progress to define the function of CgA in neuroendocrine cells.

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References

1. O'Connor DT, D Burton, and LJ Deftos: Chromogranin A: Immunohistology reveals its universal occurrence in normal polypeptide hormore producing endocrine glands. Life Sciences 33:1657-1663, 1983.
2. Cohn DV, R Zangerle, R Fisher-Colbrie, LLH Chu, JJ Elting, JW Hamilton and H Winkler: Similarity of secretory protein I from parathyroid gland to chromogranin A from the adrenal medulla. PNAS USA 79:6056-6059, 1982.
3. O'Connor DT, D Burton and LJ Deftos: Immunoreactive human chromogranin A in diverse polypeptide hormone producing tumors and normal endocrine tissues. J Clin Endo Metab 57(4):1084-1086, 1983.
4. O'Connor DT and LJ Deftos: Secretion of chromogranin A by peptide-producing endocrine neoplasms. New Engl. J. Med., 314(18):1145, May 1, 1986.
5. Sobol RE, DT O'Connor, J Addison, K Suchocki, I Royston and LJ Deftos: Elevated serum chromogranin A concentration in small-cell lung carcinoma. Annals of Internal Medicine, in press.
6. Deftos LJ, SS Murray, DW Burton, RJ Parmer, DT O'Connor, AM Delegeane and PL Mellon: A cloned chromogranin A (CgA) cDNA detects a 2.3Kb mRNA in diverse neuroendocrine tissues. Biochem Biophys Res Comm 137:418-423, May 29, 1986.

7. Benedum UM, PA Baeurle, DS Konecki, R Frank, J Power, J Mallet and WB Huttner: The primary structure of bovine chromogranin A, a representative of a class of acidic secretory proteins common to a variety of peptidergic cells. EMBO J. 5:1495-1502, 1986.

8. Iacangelo A, HU Affolter, LE Eiden, E Herbert and M Grimes: Bovine chromogranin A sequence and distribution of its messenger RNA in endocrine tissues. Nature 323:82-86.Sept.4, 1986

9. Huynh TV, RA Young and RW Davis: Constructing and screening cDNA libraries in lambda gt10 and lambda gt11. In: Glover DM (ed); DNA Cloning. Volume I. IRL Press, Oxford, 1985.

10. Feinberg AP and B Vogelstein: A technique for radiolabelling DNA restriction endonuclease fragments to high specific activity. Analyt Biochem 132:6-13, 1983.

11. Maniatis T, EF Fritsch, and J Sambrook: Molecular Cloning. A Laboratory Manual. Cold Spring Harbor Laboratory, Cold Spring Harbor, New York 1982.

12. Boyum A: Separation of leukocytes from blood and bone marrow. Scand J. Clin. Lab.Invest. 21(97):77, 1968.

13. Meinkoth J, and Wahl G: Hybridization of nucleic acids immobilized on solid supports. Anal.Biochem.138:267-284, 1984.

14. Lebo RV, DR Tolan, BD Bruce, MC Cheung and YW Kan: Spot-Blot Analysis of Sorted Chromosomes Assigns a Fructose Intolerance Disease Locus to Chromosome 9. Cytometry 6, 478, 1985.

15. Bartholdi MF, J Meyne, N Albright, M Luedemann, E Campbell, D Chritton, L Deaven, M Van Dilla and LS Cram: Chromosome Sorting by Flow Cytometry. Submitted to Methods in Enzymology, 1986.

16. Deaven LL, MA Van Dilla, MF Bartholdi, AV Carrano, LS Cram, JC Fuscoe, JW Gray, CE Hildebrand, RK Moyzis, and J Perlman: Construction of Human Chromosome-Specific DNA Libraries from Flow Sorted Chromosomes. Cold Spring Harbor Symposium in Quantitative Biology 51, (in press), 1986.

17. Lebo RV and BD Bruce: Gene Mapping with Sorted Chromosomes. Submitted to Methods in Enzymology, January, 1986.

18. O'Connor DT, RJ Parmer and LJ Deftos: Chromogranin A: Studies in the endocrine system. In: "Transactions of the Association of American Physicians", XCVII, Waverly Press Inc., Baltimore, Maryland, pp.242-250, 1984.

19. Settleman J, R Fonesca, J Nolan and H Angeletti: Relationship of multiple forms of chromogranin. J Biological Chem., 260:1645-1651, 1985.

20. Przepiorka D, SB Baylin, OW McBride, JR Testa, AD Bertros and BD Nelkin: The human calcitonin gene is located on the short arm of chromosome 11. Biochem and Biophys Research Comm. 120(2):493-499, April 30, 1984.

21. Naylor SL, AY Sakaguchi, P Szoka, GN Hendy, HM Kronenberg, A Rich and TB Shows: Parathyroid hormone gene is on the short arm of chromosome 11. In: Human gene mapping 7 (1983): Seventh International Workshop on Human Gene Mapping. Cytogenet. Cell Genet. 37:531. 1984.

22. Schneder WT, LC Lopez, ME Harper and GF Saunders. Localization of the human glucagon gene (GCG) to chromosome segment 2q 36--37. Cytogenet.Cell Genetics 38:76-79, 1984.

23. Harper ME, A Ullrich and GF Saunders: Localization of the human insulin gene to the distal end of the short arm of chromosome 11. Proc. Nat. Acad. Sci. (USA) 78:4458-4460, 1981.

24. Naylor SL, AY Sakaguchi, LP Shen, GI Bell, WJ Rutter and TB Shows. Polymorphic human somatostatin gene is located on chromosome 3. Proc.Nat.Acad.Sci.(USA) 80:2686-2689, 1983.

25. Zabel BV, SL Naylor, AY Sakaguchi, GI Bell and TB Shows. High resolution chromosomal localization of human genes for amylase, proopiomelanocortin, somatostatin and a DNA fragment (D3S1) by in situ hybridization. Proc.Nat.Acad.Sci.(USA) 80:6932-6936, 1983.

26. Owerbach D, WJ Rutter, NB Cooke, JA Martial and TB Shows: The prolactin gene is located on chromosome 6 in humans. Science 212:815-816, 1981.

27. Naylor SL, WW Chin, HM Goodman and AY Sakaguchi: Chromosomal locations of human glycoprotein hormone genes: Alpha subunit gene on 6 and chorionic gonadotropin beta-leuteinizing hormone beta gene complex on 19. In: Human gene mapping 7

(1983): Seventh International Workshop on Human Gene Mapping. Cytogenet. Cell Genet. 37:552. 1984.

28. George DL, JA Phillips II, V Francke and PH Seeburg: The gene for growth hormone and chorionic somatomammotropin are on the long arm of human chromosone 17 in region q21→qter. Human Genet. 57:138-141, 1981.

29. Babu VR, DL Van Dyke and CE Jackson: Chromosome 20 deletion in human multiple endocrine neoplasia types 2A and 2B: A double blind study. Proc Natl Acad Sci USA 81:2525-2528, 1984.

30. Krizman DB, S Pathak, NA Samaan, JHC Chong and RC Hickey: Quantitative STudy of a Proposed Interstitial Del (20p 12.2) in Multiple Endocrine Neoplasia (MEN-II). Anticancer Research 6:191 194, 1985.

31. McKnight S and R Tjian. Transcriptional selectivity of viral genes in mammalian cells. Cell 46:795 805, 1986.