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A CLONED CHROMOGRANIN A (CgA) cDNA DETECTS A 2.3Kb mRNA IN DIVERSE NEUROENDOCRINE TISSUES

L. J. Deftos, S. S. Murray, D. W. Burton, R. J. Parmer, and D. T. O'Connor

University of California, San Diego and San Diego VA Medical Center, San Diego, California

A.M. Delegeane and P. L. Mellon

Regulatory Biology Laboratory, Salk Institute, San Diego, California

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SUMMARY CgA is a 72Kd protein of unknown function that is present in many neuroendocrine tissues and co-secreted with their resident hormones. We prepared a cDNA library to the mRNA from CgA-producing human medullary thyroid carcinoma (MTC) cells in the expression vector lambda gt11. The library was screened with a panel of one polyclonal and two monoclonal antibodies to CgA. The specificity of the antibodies for CgA was demonstrated by immunoassay, immunohistology, and immunoprecipitation of the in vitro translation products of mRNA from CgA-producing tissues. A chromogenic second antibody identified five immunoreactive clones. Their cDNA inserts were isolated after EcoRI digestion and agarose gel electrophoresis. These cDNAs were ^{32}P-labelled and used as probes in Northern hybridization studies. An mRNA of approximately 2.3Kb was detected with the cDNA probes in human cell lines from MTC and lung cancers that were shown to produce CgA and in human pheochromocytoma and bovine adrenal medulla tissue. To confirm its identity, one of the putative CgA cDNAs was subcloned into a plasmid and was used to hybridization-arrest the in vitro translation of CgA mRNA. These studies demonstrate the cloning of cDNAs which hybridize with CgA mRNA from diverse neuroendocrine tissues.

Chromogranin A (CgA) is a soluble protein originally isolated from the chromaffin vesicles of the adrenal medulla (1,2). Its major species is 72Kd by SDS-polyacrylamide gel electrophoresis, although size heterogeneity has been described (3-5). The function of CgA is not known but it is present in a variety of eutopic and ectopic neuroendocrine sites and in the parathyroid gland where it was originally described as secretory protein I (6-9). It is not yet clear if chromogranins from various tissues are identical or members of a family of proteins (10). CgA is secreted by its resident tissues and its blood concentration is increased in patients with a wide variety of neuroendocrine and related tumors ranging from medullary thyroid carcinoma to small cell lung cancer (11-14).

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MATERIALS AND METHODS

Human CgA (hCgA) was purified as previously described and used for the production of antibodies and for radioiodination (2,12,15). Three human neuroendocrine cell lines were established in culture, one from a human medullary thyroid carcinoma (MTC-1) and two from lung cancer (M103 and NCI-1)(13-17). Radioimmunoassay and immunohistology procedures were performed for each of these cell lines as previously described (8,9,11,12). A cDNA library was made from mRNA of the MTC-1 cells in the bacteriophage lambda gt11 according to the method of Young and Davis (17). The library was screened with a mixture of one rabbit polyclonal (137B-3) and two mouse monoclonal (3H12,8A5) antibodies to CgA and a chromogenic second antibody (avidin-biotin). Restriction digests, Northern hybridizations, and in vitro translations were performed according to standard procedures (19, 20). Library rescreening and Northern hybridizations were accomplished with cDNAs labelled by nick-translation or random primed labelling (19,21). Cloned cDNAs were subcloned into the EcoRI site of a 3.4Kb PBR322-based plasmid vector constructed by one of us (PLM) and designated PM2 (19). This construct was also used for Northern hybridizations and hybridization-arrested in vitro translation studies (19-21).

RESULTS

We have demonstrated the production of CgA by diverse neuroendocrine tissues and have identified CgA cDNAs using the expression vector lambda gt11 with specific antibodies. The polyclonal and monoclonal antibodies directed against hCgA were identified by the following criteria: (a) They specifically bound and immunoprecipitated ^{125}I-hCgA under radioimmunoassay conditions and the binding of ^{125}I-hCga was specifically displaced by serial dilutions of unlabeled human CgA (11,12,15). (b) The antibodies immunohistologically stained CgA-containing cells in adrenal medulla, thyroid, parathyroid, pituitary, and pancreas (8,9). (c) The antibodies immunoprecipitated CgA in vitro translation products of adrenal medullary mRNA. These antibodies also demonstrated CgA in the three cultured cell lines (MTC-1, M103, and NCI-1) by immunohistology and by radioimmunoassay (14) (Table 1).

A mixture of the three antibodies demonstrated five positive clones during two screenings of $0.5X10^6$ placques from the MTC-1 cDNA library. EcoRI digestion of the DNA from these phages revealed single bands on agarose gel electrophoresis ranging from 300-400 base pairs. When ^{32}P-labeled, these cDNAs hybridized with a predominant 2.3Kb mRNA in Northern analysis (Figure 1) of RNA from five CgA-positive tissues: the three

Table 1. Radioimmunoassay and immunohistology studies of Chromogranin A (CgA) in cultured cell lines

	--Cg A---	---------Cg A*---------	
Cell Designation	Immuno-histology	Cell Pellet ug/mg protein	Medium ng/ml
MTC-1	+	1.7	237
NCI-1	+	0.84	156
M103	+	0.47	210
UMR	-	ND	ND

*72 hours of growth and expressed as mean of triplicate radioimmunoassay determinations for cell pellet and cell culture medium, respectively (12).
ND = not detectable (<10 ng per tube).
MTC-1 = medullary thyroid carcinoma. NCI-1 and M103 = lung cancer.
UMR = osteogenic sarcoma (control).

cell lines, MTC-1, M103, and NCI-1, as well as bovine adrenal medulla and human pheochromocytoma. These five cDNA's also cross-hybridized with each other. CgA-negative cells showed no hybridization. One of these cDNAs, designated 73, subcloned into the plasmid PM2 and ^{32}P-labelled, gave the

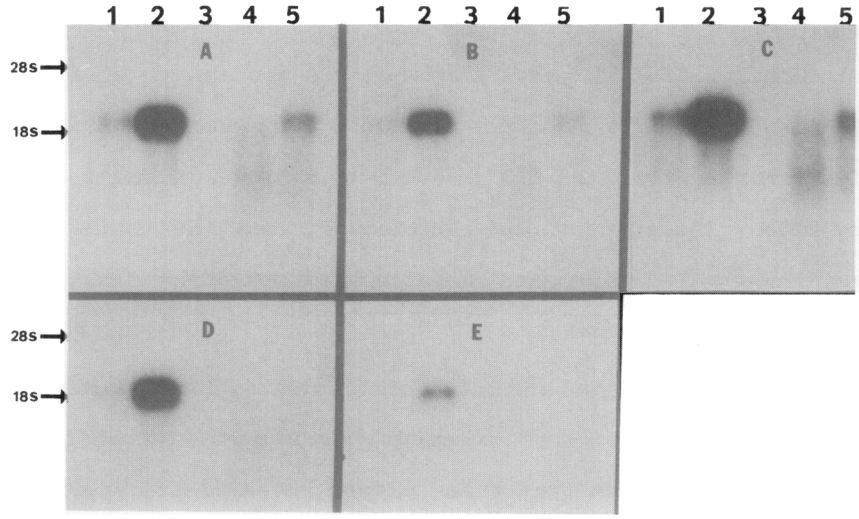

Figure 1. Northern hybridization with five ^{35}P-labelled cDNAs (A-E), including one plasmid subclone PM273 (E), of RNA from CgA-producing cells and tissues (1-MTC, 2-BAM, 4-M103, 5-NCI-1) and control cells (3-UMR - osteogenic sarcoma. The cDNAs ranged in size from 300-400 BP. 28S and 18S designate the mobility of the eukaryotic ribosomal RNA bands.

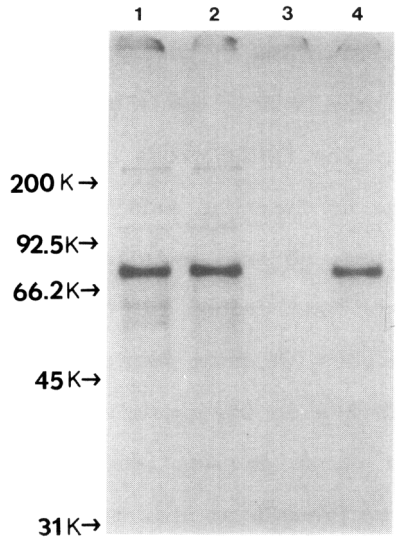

Figure 2. Hybridization-arrested in vitro translation of mRNA from bovine adrenal medulla (BAM) in a rabbit reticulocyte lysate system performed according to the protocol of Patterson et al. (20). 1:BAM mRNA hybridized with plasmid PM2 (plasmid control), 2: BAM mRNA alone, 3:BAM mRNA hybridized with PM273 (CgA cDNA 73 cloned in plasmid PM2). 4:same as 3 except that the hybrid was first "melted" by boiling for 5 minutes. After in vitro translation, CgA products immunoprecipitated with monoclonal antibodies 3H12 and 8A5 were electrophoresed by SDS-PAGE and the dried gels were autoradiographed (22). Molecular weight standards are given on the left. The dark bands in lanes 1,2, and 4 migrate to the position of CgA, 72 kilodaltons

same hybridization pattern. The in vitro translation of CgA mRNA isolated from adrenal medulla was arrested by hybridization with this plasmid (Figure 2). Rescreening of the original library has identified a 2.1Kb cDNA with the appropriate Northern hybridization characteristics for CgA.

DISCUSSION

Our studies show that diverse neuroendocrine tissues produce CgA. We have isolated several cDNAs for this ubiquitous neuroendocrine protein, (1-3). The cDNAs were isolated from a lambda gt11 library made from CgA-positive cells with a mixture of antibodies specific for the protein. The antibodies were made against pure CgA and their specificity for CgA was further documented by radioimmunoassay, immunohistology, and in vitro mRNA translation studies (Table 1). Each of the cDNAs identified the same 2.3Kb mRNA on hybridization of Northern transfers of RNA from

normal and malignant CgA-positive cells and tissues (Figure 1). The strongest hybridization signal was in the RNA from cells most abundant in CgA, adrenal medullary tissue, and it was absent in mRNA from CgA-negative cells (Figure 1). The cDNAs cross-hybridized with each other and a plasmid subclone of one of them was able to arrest the in vitro translation of CgA mRNA from adrenal medullary tissue (Figure 1). These observations identify our cDNA probes for CgA (22).

Using these cDNA probes, we have identified in Northern hybridizations a single 2.3Kb RNA in CgA-positive cells (Figure 1). However, we do occasionally see a 2.3Kb doublet band and other larger and smaller species. We cannot be certain at this time if these latter observations represent true multiple RNAs or are artifacts of RNA preparations (3,4). The presence of multiple species of chromogranin A does have experimental support (3,4,22). Our recent identification of a 2.1Kb cDNA along with further studies should help to elucidate the molecular structure and regulation of CgA (23).

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