Elevated Serum Chromogranin A is Detectable in Patients with Carcinomas at Advanced Disease Stages

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Abstract. Chromogranin A (CgA), a marker of neuroendocrine cells and an indicator for neuroendocrine differentiation, is associated with a poor prognosis when detected in tumor tissue, based on immunohistochemical techniques. We sought to determine whether it is possible to detect elevated serum CgA in patients with commonly occurring carcinomas of non-neuroendocrine origin. CgA was measured in both random and serial serum specimens, using a serum CgA assay developed in our laboratory. Elevated levels of serum CgA were detected in patients with carcinoma of the prostate, breast, ovary, pancreas, and colon. Serum CgA levels in patients with all types of carcinoma appeared to parallel the changes of serum dominant tumor markers and were found in sera containing highly elevated tumor markers. Based on these preliminary findings, perhaps we should monitor CgA, in addition to the routinely used tumor markers, during the treatment of patients with carcinomas to determine if CgA is useful as a prognostic marker in carcinomas other than prostatic cancer.

Keywords: Chromogranin A, carcinoma antigens CA-15-3, CA-19-9, CA-125, carcinoembryonic antigen (CEA), prostatic specific antigen (PSA)

Introduction

Serum chromogranin A (CgA) has been recognized as a valuable marker for neuroblastoma, pheochromocytoma, small cell lung carcinoma, and carcinoid tumors—a group collectively known as the neuroendocrine tumors [1,2]. Even though plasma and urine catecholamines and serotonin have been traditionally used for the diagnosis and management of these tumors, CgA has been found to be more stable and thus more easily applied clinically [3]. Serum CgA has been reported to have a sensitivity of 91% and a specificity of 100% for detection of neuroblastomas [4]. Moreover, CgA may be a better marker than neuron-specific enolase in neuroendocrine tumors [5] and is considered by some as the preferred marker for neuroendocrine cells [6].

CgA has been used to indicate neuroendocrine differentiation, which is a sign of poor prognosis. Neuroendocrine differentiation has recently been studied intensively in prostatic cancer [7], despite the fact that prostate tumors are not considered to be related to neuroendocrine tumors. Because of the relatively large number of neuroendocrine cells present in the prostate and urethra [8], neuroendocrine differentiation is frequently observed in prostate carcinomas, particularly in patients with distant metastases (such as stage D2). Detection of CgA in prostate tumor tissue is also an indication of poor prognosis. An increasing number of reports in the recent literature suggest that monitoring plasma or serum CgA levels might reflect neuroendocrine differentiation of prostatic tumors [9,10].

Other than prostatic cancer, most carcinomas have not been investigated to any great extent with respect to CgA. As with prostatic cancer, carcinomas that display neuroendocrine differentiation usually arise in organs in which the epithelium normally contains neuroendocrine cells. However, Bosman [11] recently
noted that neuroendocrine differentiation can occur in carcinomas that lack neuroendocrine cells in their normal epithelial counterparts; such carcinomas include mucinous cystadenocarcinoma of the ovary, ovarian teratoma, and hepatocellular carcinoma. Most of these observations have been based on immunohistochemical techniques. Since an unfavorable prognosis has frequently been found to be associated with neuroendocrine differentiation in epithelial tumors [6,8], and since positive correlation has been reported between the serum levels of CgA and the number of CgA-staining cells in carcinoma of the prostate [12], we were interested to know whether serum CgA would be a prognostic marker in those commonly occurring carcinomas. We suspected that, in addition to being useful for prognosis, detection of elevated CgA might indicate the proliferation of neuroendocrine cells in the tumor. In addition to targeting epithelial tumor cells for treatment, therapeutic strategies should take into consideration the presence or absence of neuroendocrine cells.

In this study, we measured CgA levels in both random and serial serum specimens that had been collected from patients with various carcinomas, taking advantage of a sensitive serum CgA immunoassay that has been developed in our laboratory. Elevated serum CgA levels were detected in patients with carcinomas of the prostate, breast, ovary, pancreas, and colon, and appeared to be associated with serum specimens that contained highly elevated tumor markers. This suggests that elevated serum CgA usually appears at advanced stages of disease progression.

Materials and Methods

Polyclonal rabbit anti-human CgA antibody and HRP-conjugated monoclonal mouse anti-human CgA antibody were both purchased from Dako (Carpinteria, CA). These antibodies were against the C-terminal, 20 kDa fragment of the CgA molecule. Immulon-4 Removawell strips were obtained from Dynatech Laboratories (Chantilly, VA). K-blue substrate-TMB reagent was obtained from Neogen Corp. (Lexington, KY). Assays were performed on random and serial serum specimens that had been sent to ARUP laboratories for determination of tumor markers. Some of the sera had been frozen at -70°C for a few years.

Modified serum CgA assay. The enzyme immunoassay used in this study was a slightly modified version of our previous serum CgA assay [13]. The modified assay has a wider concentration range and reduced risk of "hook" effects. In the modified assay, 150 μl (instead of 100 μl) of polyclonal rabbit anti-human CgA (4 μg/ml) is coated onto each well, and less serum (only 20 μl) is added. Only one incubation (4 hr) is performed. The serum sample (20 μl), the detecting antibody (50 μl), and the BSA-PBS reagent (80 μl) are incubated together in the antibody-coated well. The BSA-PBS reagent contains phosphate buffer (pH 7.2, 10 mmol/L) and bovine serum albumin (10 g/L). Sera with highly elevated CgA levels from patients with renal insufficiency were pooled to prepare the calibrator.

Results

CgA in random specimens. Elevated CgA was detected in random serum specimens from patients with prostatic, breast, ovarian, colon, and pancreatic malignancies (Fig. 1). Weak correlations between the
Chromogranin A levels in serums of carcinoma patients

Serum CgA levels and tumor marker levels appeared to exist. It should be noted that prostatic specific antigen (PSA), CA-15-3, CA-125, CA-19-9, and carcinoembryonic antigen (CEA) are dominant tumor markers of the prostate, breast, ovary, pancreas, and colon, respectively. Extremely elevated serum CgA levels (eg, >500 ng/ml) were only detectable in sera containing highly elevated serum tumor markers, suggesting that the elevation of serum CgA is more likely at advanced stages of these malignancies.

**CgA in serial specimens.** The appearance of elevated serum CgA levels at advanced stages of carcinoma progression was corroborated by levels of serum CgA in serial specimens from individual patients with various carcinomas. Elevated serum CgA was associated with dominant tumor markers in carcinomas (Fig. 2). To compare the serum CgA levels and the levels of dominant tumor markers, the data were normalized by dividing the observed levels by the upper limits of their respective normal ranges. After such adjustment, values >1 indicated an abnormal or elevated serum level for the marker; values <1 indicated that the serum level was within the normal range of concentrations. In each of the cases shown in Fig. 2, serum CgA became elevated at a later stage of the malignant disease or at a time when the dominant tumor markers were already greatly increased. In the patients with pancreatic, prostatic, or ovarian cancer, serum levels of CgA and their predominant tumor markers changed in parallel with each other, whereas in the patients with colon or breast cancer, concordance was less evident.

The degree and frequency of serum CgA elevation varied among the various carcinomas. Breast cancer patients showed the lowest frequency of serum CgA elevation; ovarian cancer patients exhibited the highest frequency and highest levels of CgA elevation (Figs. 1 & 2). Only 1 of 5 breast cancer patients showed elevated serum CgA, but 9 of 9 ovarian cancer patients showed elevated serum CgA. Extremely elevated levels of CgA were detected in sera from ovarian cancer patients whose CA-125 levels were only moderately above the upper limit of the normal range.

**Breast tumor cytosol.** As shown in Fig. 3, CgA was detected in 50% of the receptor-negative and 44% of the receptor-positive cytosols. The highest CgA levels (ng/ml) in tumor cytosol samples are shown in parentheses.
detected in several specimens of human breast tumor cytosol that had been retained after estrogen receptor (ER) and progesterone receptor (PgR) measurements. CgA was detected in 50% (39/78) of tumor cytosols that were ER- and PgR-negative, and in 44% (7/16) of tumor cytosols that were ER- and PgR-positive. The results did not indicate that the presence of detectable CgA was associated with positive or negative expression of ER and PgR in breast tumor cytosols.

Discussion

It was hardly surprising to find elevated serum CgA levels in patients with prostatic cancer, because normal prostate contains neuroendocrine cells. On the other hand, the elevated serum CgA levels in patients with non-endocrine carcinomas (breast, ovarian, colorectal, and pancreatic) were unexpected. The dominant serum tumor marker for each type of carcinoma represents a product of epithelial tumor cells, whereas serum CgA is considered a marker of neuroendocrine differentiation. Changes in serum levels of the two classes of markers may indicate if one or both types of cells are proliferating, which might aid in designing the cancer treatment strategy.

Neuroendocrine differentiation has generally been associated with poor prognosis in prostatic cancer. Neuroendocrine cells are known to be regulatory cells capable of exerting a paracrine influence on the growth and proliferation of surrounding cells [1,14]. One can speculate that inhibition of CgA production, or removal of CgA from the tumor or blood circulation, such as might occur after administration of anti-CgA antibody, could slow tumor progression. It is unclear whether production of CgA causes a malignancy to progress, or whether the malignant tumor itself is responsible for the elevation of serum CgA.

Although serum CgA levels are oftentimes elevated in patients with various carcinomas, the serum CgA levels are generally much lower than in patients with neuroendocrine tumors, such as pheochromocytoma. Serum CgA levels close to 1,000 ng/ml are frequently encountered in patients with neuroendocrine tumors.

References