Serum Chromogranin A: Early Detection of Hormonal Resistance in Prostate Cancer Patients

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We monitored both chromogranin A (CgA) and neuron specific enolase (NSE) in serial serum specimens from 14 patients with prostate cancer (CAP patients) showing resistance to hormonal treatment. Elevated serum CgA was detected in 10 out of these 14 patients (71%) during treatment, and an early appearance of elevated serum CgA was found in 6 of 14 (43%) of these patients when serum tPSA levels were still in the normal range. If patients with radical prostatectomy were not included, the percentage of patients showing an early appearance of elevated serum CgA would have been much higher. Elevated serum CgA levels also were found in patients not subject to hormonal therapy. Serial specimens from two out of three prostate cancer patients, randomly selected, contained elevated serum CgA. Serum NSE was

not detectable in any of the serial specimens we studied, suggesting that CgA, not NSE, should be used as a marker for neuroendocrine differentiation. We also compared the serum CgA in random serum specimens between patients with BPH (benign prostate hyperplasia) and with prostate cancer in the concentration range of serum tPSA between 3–15 ng/mL. Although serum CgA concentrations in BPH patients overlapped considerably with those levels in patients with prostate cancer, levels > 100 ng/mL should suggest prostate cancer. The early appearance of elevated serum CgA allows an early change of therapy to be made and can lead to the effective prevention of any further development of metastases. *J. Clin. Lab. Anal.* 12:20–25, 1998. © 1998 Wiley-Liss, Inc.

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INTRODUCTION: A STUDY OF MARKERS

Neuroendocrine cell (or endocrine-paracrine cells, or amine precursor uptake and decarboxylation cell) are intraepithelial cells with hybrid neuroendocrine and epithelial characteristics. These cells are widely distributed within the urethroprostatic region with cells in the prostatic and penile urethra, prostatic ducts, and peripheral prostate. The prostate neuroendocrine cell is a regulatory cell, and many of them contain a biogenic amine, most often serotonin, as well as one or more of the neuropeptides, including chromogranin A, neuron specific enolase (NSE), substance P, calcitonin, somatostatin, neurotensin, bombesin, and even parathyroid hormone-related protein and thyroid-stimulating hormone-like peptide with β chain homology (1). Some subpopulations of neuroendocrine cells also were found to contain epidermal growth factor receptor and *c-erbB-2*. This type of prostate cell coproliferates as a malignant epithelial component in most, if not all, prostatic carcinomas, which has been overlooked until very recently.

Normal neuroendocrine cells of the prostate do not contain androgen receptor and, therefore, are not regulated by androgen. As a result, neuroendocrine differentiation usually is associated with the increase of the NSE and CgA markers

and increases with the increase of hormonal resistance in prostate cancer. Apparently, neuroendocrine cells in the prostate tumor gain growth advantage during hormonal deprivation therapy. They not only proliferate after androgen ablation therapy, but also have a tendency to become malignant. Consequently, prostatic tumors with extensive neuroendocrine differentiation were found to behave aggressively and are usually associated with a poor prognosis (2). Nearly all, if not all, prostatic carcinomas have some degree of neuroendocrine differentiation.

Pure neuroendocrine malignancies of the prostate also can be found in small cell carcinoma and carcinoid-like tumors. The malignancies in both cases are highly aggressive and malignant, but also rare. Understandably, it is critical that neuroendocrine differentiation in prostatic tumor be detected early. Patients with prostate cancer ideally should be evaluated for new treatment strategies before tumors become malignant. Chemotherapeutic regimens similar to those used in

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small cell carcinoma of the lung have been found to be beneficial also for small cell carcinoma of the prostate (1,3).

Two markers expressed by tumor tissues, CgA and NSE, have been used for the detection of neuroendocrine differentiation. Positive immunostain for both markers in the tumor tissue biopsies were found to be associated with poor prognosis and a short survival interval (1,4). However, whether their serum or plasma concentrations might also have proved useful as prognostic markers is not entirely clear. As pointed out by Cussenot et al. (5), the serum marker is a more global indicator and a more objective quantifier of the neuroendocrine differentiation of tumors. The serum concentration of CgA, for example, reflects the activity of not only the primary tumor, but also its associated metastases. Cussenot et al. (5) found both elevated plasma CgA and NSE for prostate cancers showing hormonal resistance. They found that the determination of neuroendocrine differentiation by NSE or CgA immunoassays was not helpful in the prediction of progressive localized prostatic carcinoma. Kadmon et al. (6) found elevated plasma CgA in 48% of 25 patients with stage D2 prostate cancer. Angelsen et al. (7) could not find any of their patients with carcinoma of the prostate who had elevated serum values of NSE, despite the presence of NSE-positive tumor cells in 77% of the tumors. However, they not only found elevated serum values of CgA in 59% of their patients, but also a positive correlation between the number of CgA-staining cells and the serum values of CgA. They believe that CgA is a more useful serum marker in predicting the extent of neuroendocrine differentiation in prostatic tumors.

Neuroendocrine cells are present not only in prostate tumor. Cockett et al. (8) showed that small proliferating nodules of BPH also contain abundant serotonin positive neuroendocrine cells and that 10.7% of patients with BPH had abnormally high NSE levels. In fact, they also found that many of those D2 prostate cancer patients containing low tPSA (<4 ng/mL) had elevated CgA in the serum. Therefore, CgA may complement the tPSA assay in order to reveal the presence of cancer. It was also interesting to learn from the study by Kadmon et al. (6) that even though there were elevated serum CgA in BPH, the plasma CgA levels in BPH patients usually fell below 100 ng/mL, whereas 50% of their cancer patients (D2) had CgA > 100 ng/mL. CgA is also the major soluble protein in catecholamine-storage vesicles, which is stored and released by exocytosis with catecholamines and has been a useful index of sympathoadrenal catecholamine release in laboratory animals.

Continued progression of prostate cancer during endocrine therapy is a major clinical problem. Many studies suggested that prostatic cell lines may be stimulated to invade with neuropeptides secreted by neuroendocrine cells. Hoosein et al. (9) suggest that certain neuroendocrine peptides can increase the invasive potential of prostatic carcinoma cells and may thereby contribute to the rapid progression and aggressive clinical course of prostate tumors containing neuroendocrine

elements. Clearly, detecting resistance during the hormonal treatment of prostate cancer is important for proper therapy and to prevent further development of malignant tumor. In this study we measured both serum NSE and CgA in the serial specimens collected from 14 prostate cancer patients showing hormonal resistance. These measurements were then compared to the tPSA levels of the same samples to determine which marker would provide the early detection of hormonal resistance in these patients. We also compared the serum CgA between random specimens containing tPSA levels in the concentration range from 3–15 ng/mL from BPH and prostate cancer patients. We wanted to know whether the determination of serum CgA level would help differentiate prostate cancer from BPH. Our results suggested that in a large number of the cases, measuring serum CgA appeared useful for the early detection of hormonal resistance in patients with prostate cancer.

MATERIAL AND METHODS

CGA-RIACT, a solid-phase, two-site immunoradiometric assay for the measurement of human chromogranin A, was provided by CIS Bio International (Bagnols-sur-Céze, France). According to the kit insert, if serum specimens are used, the upper normal limit is 54 ng/mL (mean + 1 SD). The serum PSA (or tPSA) was performed on Abbott IMx using Abbot kit.

Serum NSE (neuron specific enolase) concentrations were determined using the ELSA-NSE kit, also from CIS Bio International. This is a solid phase, two-site immunoradiometric assay using two monoclonal antibodies prepared against sterically remote antigenic sites on the NSE molecule.

Serum Specimens

All serum specimens were obtained from the Department of Urology, University of Utah School of Medicine. Diagnoses associated with the majority of the samples, whether from patients with BPH or with prostate cancer, were confirmed by clinical biopsies. All specimens had Abbott PSA measured immediately after blood drawn and were then kept frozen at -70°C for different lengths of time, several months to several years, before they were subjected to other measurements.

RESULTS

Serum CgA in Prostate Cancer Patients Showing Hormonal Resistance

Early detection

We have identified serial serum specimens from 14 patients with prostate cancer showing hormonal resistance. All of the frozen specimens with known tPSA values, determined

early with the Abbott assay on the IMx instrument, were retrieved from the freezer and subjected to CgA measurements. Elevated serum CgA concentrations were found in 10 out of 14 patients (71%) (Figs. 1, 2, 3). Particularly in six of these 14 patients (43%), the serum CgA elevated much earlier than the elevation of serum tPSA. As shown in Figure 1, in the majority of the cases, serum CgAs were elevated, whereas tPSA remained below 4 ng/mL (upper normal limit). Five patients in Figure 1 had already received hormonal ablation. Patient 188 had hormonal ablation 500 days later as indicated. Patient 155 received both radiation therapy and hormonal treatment alone. According to the kit insert, the upper normal limit for serum specimens is 54. Although this upper normal limit needs to be further verified (horizontal dotted line in Fig. 1), the value serves as an indication as to how highly elevated most of the CgA levels were. It was surprising to find such a large proportion of hormonal resistant patients showing elevated serum CgA, especially appearing at such an early stage

Fig. 1. Early appearance of elevated serum CgA in prostate cancer patients showing hormonal resistance. Hormonal resistance was demonstrated by the rise of serum tPSA after hormonal ablation following hormonal treatment. In all these six patients, elevated serum CgA was detected when most serum tPSA were still < 4 ng/mL. All patients except patient 188 had ablation performed a few years before the zero time shown in these figures. Patient 188 did not start hormonal treatment until after 500 days. The arrow in patient 188's plot indicates orchietomy was performed at that time. The dotted square in patient 188 indicates elevated serum CgA despite low tPSA after orchietomy.

Fig. 2. Absence of early appearance of serum CgA in prostate cancer patients showing hormonal resistance. Serum CgA concentrations rose at later stage of the disease course and coincided with the increase of tPSA levels. Patients 224 and 37 did not show elevation of serum CgA at all. The arrow indicates orchietomy was performed.

when the tPSA level was still within the normal range. This early appearance of elevated CgA, if detected, would allow earlier changes in treatment to be made sooner, increasing the probability of intervention before the tumor progresses to metastasis. For instance, for patient 72 the first tPSA value was only slightly elevated at 8.3 ng/mL. The serum CgA con-

Fig. 3. Effect of prostatectomy on the serum levels of CgA in prostate cancer patients showing hormonal resistance. These patients did not have highly elevated serum CgA, as compared to cases shown in Figure 1. Also, there was an absence of early appearance of elevated serum CgA. The presence of much fewer tumor cells to produce CgA after prostatectomy may account for the lack of highly elevated CgA. The arrow indicates orchietomy was performed at that time.

centration, meanwhile, > 100 ng/mL even at this initial point and continually climbing, would have more accurately established the prognosis. The highly elevated serum CgA concentration in patient 72 would suggest the presence of neuroendocrine differentiation and the likely development of hormonal resistance. The slow rising of the tPSA concentrations over time at the early stages, by contrast, might not give the impression that the tumor was aggressive. Based on the tPSA values, we would have concluded that the tumor was still organ confined. In sum, the monitoring of serum CgA during hormonal treatment may provide a means of early prediction. Monitoring serum CgA enables therapy to be adjusted in the crucial early stages leading to more effective measures which can be taken to prevent the development of malignant tumors.

Absence of early appearance of serum CgA

The plots of four prostate cancer patients with hormonal resistance in Figure 2 also show elevation of serum CgA at some point during the course of treatments, although the appearance of elevated serum CgA was no more heightened than the concentration of tPSA. None of them displayed the benefit of early detection as demonstrated in Figure 1. However, if one undertakes a close, careful examination of the plots, especially of patients 119 and 136, one finds that the tPSA levels of these patients were not highly elevated, whereas the chromogranin A serum levels were. These results consistently support the importance of monitoring serum CgA during hormonal treatment. Both patients 119 and 136 have received hormonal ablation earlier.

After prostatectomy

All patients shown in Figure 3 had received radical prostatectomy a few years earlier. The low and only moderately elevated serum CgA levels found in this group of prostate cancer patients could have been affected by the operation of radical prostatectomy. After prostatectomy it would be understandable if there were few tumor cells left to produce CgA. If tPSA were monitored during recurrence, we would not expect highly elevated serum CgA to accompany the appearance of tPSA. Therefore, there would not be any advantages to measuring both CgA and tPSA, nor much reason to hope for early detection with CgA while monitoring for recurrence. However, it would be beneficial to start measuring serum CgA as soon as treatment begins. As demonstrated with patient 176, the presence of elevated serum CgA following orchiectomy would indicate that patient was going to develop resistance to hormonal treatment. The much slower rise of tPSA confirmed that this was the case, but this would not have been clear until several months later.

Serum CgA Level in Nonhormonal Resistant Patients

The importance of neuroendocrine differentiation does not appear to be limited to prostate cancer with hormonal resistant. Elevated serum CgA was detected in specimens from patients with prostate cancer not undergoing hormonal treatment (Fig. 4 patients, 94, 181, 242). Although orchiectomy was performed for patients NM and MJF (indicated by dramatic decrease of tPSA concentrations), elevated CgAs were detected prior to that procedure. Elevated serum CgA was also detected in a patient with prostatitis (Fig. 4, patient 97). Whether the elevation of CgA accounted for the elevation of tPSA in that patient was not known. Again, we also have no explanation for the decrease of serum CgA observed in Figure 4 (patients 94, 181, 242). More studies will be needed to understand what causes the fluctuation of CgA in the serum in non-hormonal resistant patients. The rise and fall of serum CgA may provide additional information leading to the improved treatment of prostate cancer patients.

Fig. 4. Serum CgA in patients without hormonal treatment. Apparently the elevation of serum CgA also may account for the increase of tPSA levels and thus metastases for patients not subjected to hormonal treatment. Patient 94 and 181 showed an increase of tPSA following the earlier elevation of serum CgA; both had prostatectomy a few years earlier. The clinical situation presented in patient 242 is not clear. The significance of elevated serum CgA in patients 97 with prostatitis is also unknown. Arrow indicates orchiectomy was performed. CAP stands for carcinoma of the prostate, and RP refers to prostatectomy.

Comparison Between Serum CgA and NSE

In the present study we measured both CgA and NSE in sera from prostate cancer patients showing hormonal resistance, since NSE and CgA were both believed markers for neuroendocrine differentiation (5). However, we could not find any elevation of NSE in the serum specimens that were included in this study. Our results are similar to what was reported by Angelsen et al. (7) that they also could not find any of their patients with elevated serum values of NSE. Apparently NSE, which was frequently detected during neuroendocrine differentiation by immunohistochemical technique, was not released into the bloodstream. Our results indicate that serum CgA, not serum NSE, should be monitored for detecting neuroendocrine differentiation in prostate cancer.

Serum CgA in BPH and Prostate Cancer

We also measured CgA in random serum specimens from patients with either BPH or prostate cancer. The results are shown in Figure 5. The idea was to find out whether prostate cancer patients were associated with higher concentrations of serum CgA or not. It has been repeatedly demonstrated that when tissue section of prostate tumor immunostain positive for CgA, poor prognosis was predicted. Therefore, it is reasonable to anticipate a finding of elevated serum CgA in prostate cancer patients. However, the relatively high serum CgA concentrations found in BPH specimens did not make serum CgA as effective as a prognostic marker as it proved

for tumor tissue. Finding serum CgA in BPH specimens was not a total surprise since it was reported by Cockett et al. (8) that small proliferating nodules of BPH contain abundant serotonin positive neuroendocrine cells.

Kadmon et al. (10) measured plasma CgA concentrations in patients with BPH, prostatitis and prostatic cancer. They found that the plasma CgA concentration in patients with benign prostate diseases were all below 100 ng/mL, whereas 48% of D2 prostatic cancer patients contained plasma CgA above that level. We found a similar relationship between serum specimens from BPH and prostate cancer patients (Fig. 5), even though the specimens we selected in Figure 5 only contained tPSA in the concentration range between 3–15 ng/mL, whereas the specimens studied by Kadmon et al. (10) had much wider and higher concentrations of tPSA. It is reasonable to assume that more elevated and higher CgA concentrations would have been found in specimens containing highly elevated tPSA. Moreover, because we used a different kit, values produced by our kit may not have been totally compatible with the values produced by kits used by Kadmon et al. (10). Nevertheless, as shown in Figure 5, the majority of the serum CgA concentration in patients with BPH, in general, were < 100 ng/mL; only three specimens showed CgA slightly above 100 ng/mL. However, 12% of the prostate cancer patients had serum CgA concentration > 100 ng/mL. Apparently, the measurement of serum CgA concentration is not very useful for the differentiation of prostate cancer from BPH in the concentration range of 3–15 ng/mL. However, it is also probably true that when the serum CgA concentration is > 100 ng/mL it is likely that the patient has prostate cancer.

tPSA between 3 to 15 ng/mL

Fig. 5. Comparison of serum CgA concentrations of random specimens from patients with BPH and prostate cancer. Only specimens containing tPSA between 3–15 ng/mL were included in this study.

DISCUSSION

Neuroendocrine differentiation, expression of bcl-2 oncoprotein (10) (an apoptosis-suppressing oncoprotein), and an increase in the expression of a wild-type androgen receptor gene by prostate tumor (11) can all lead to hormonal resistance in patients with prostate cancer. Therefore, it is peculiar to find elevated serum CgA in so many prostate cancer patients showing hormonal resistance. The importance of neuroendocrine differentiation in prostate cancer apparently had not been recognized in the past. It appears from this study that for many prostate cancer patients, the development of metastases during the hormonal treatment is likely the result of neuroendocrine differentiation (detectable by measuring serum CgA). The ability of neuroendocrine cells in the prostate tumor to grow in an environment of depleted or low concentrations of serum androgen largely accounts for the failure of treatment for prostate cancer patients when subjected to hormonal treatment. The malignant behavior of neuroendocrine cells also explains why hormonal resistance is the major cause of death in patients with prostate cancer. Yet, it is encouraging to find in this study that so many of these patients (43%) showed elevated serum CgA several months

before the serum tPSA became elevated. Early appearance of serum CgA conceivably would create an opportunity for the early adjustment of therapy and the prevention of any further development of metastasis. In fact, among these 14 patients with hormonal resistance, the low serum CgA found in four patients could be due to the effect of earlier prostatectomy. In other words, the percentage of patients with early appearance of elevated serum CgA could be higher. The results of this study led us to believe that monitoring serum CgA should be carried out routinely during treatment, especially during hormonal therapy in addition to the tPSA assay.

It appears that the importance of neuroendocrine differentiation is not limited to the development of hormonal resistance. Even among the few randomly selected serial specimens from prostate cancer patients not subjected to hormonal therapy, two out of three showed elevated serum CgA sometime during the disease course (Fig. 4). It has been suggested that prostatic neuroendocrine cells may promote prostate tumor proliferation by their secreted neuropeptides. An increase in the proliferation of cells surrounding the neuroendocrine cells was also observed (1). In the present study, the frequent detection of elevated serum CgA in prostate cancer patients indicates the importance of monitoring serum CgA in all prostate cancer patients regardless of the treatment they were undergoing. It would be interesting to know how much the prostate cancer patients would benefit if treatment could be developed aiming at lowering the CgA concentration in the serum.

CgA is the major soluble protein stored and released with catecholamines from vesicles in the adrenal medulla (chromaffin granules with chromaffin cell) and sympathetic nerve. It has a widespread neuroendocrine distribution, wherein it may be stored and released with various polypeptide hormones. The association of neuroendocrine differentiation and poor prognosis in prostate tumors reported in the literature were mostly based on studies examining the expression by the tumor tissue and immunostaining biopsies for chromogranin A. It was concluded that neuroendocrine differentiation is a significant predictor of poor survival (1). However, the relatively high levels of serum CgA detected in the BPH patients does not recommend the use of serum CgA to differentiate BPH from prostate cancer or as a prognostic marker during screening to predict risk of cancer. Yet, when serum CgA is highly elevated, > 100 ng/mL, it is very likely that the person has cancer and not BPH.

It has been reported by Tarle et al. (12) that both serum levels of NSE and CgA correlated with resistance to hormone ablation therapy and a poor prognosis in advanced prostate cancer. Like Angelsen et al. (7), we failed to detect any significant serum NSE at all. Our failure to detect elevated NSE indicates that immunostaining may sometimes detect only the

storage of the marker and not active secretion. In this regard, serum levels may be a better marker that correlates with prognosis because only the secreted neuropeptides will exert physiological effects.

The normal reference levels are very different among various kits. Efforts toward the standardization of the calibrator should be considered in the future. The normal reference levels also will be useful for other diagnoses since the serum CgA also can be used as a marker for carcinoid tumors, pancreatic islet cell tumors, medullary carcinoma of the thyroid, and hyperparathyroidism and endocrine neoplasms, such as pheochromocytoma and neuroblastoma.

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