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The Inhibition of the Paracrine Progression of Prostate Cancer as an Approach to Early Therapy of Prostatic Carcinoma

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Abstract The prevalence of neural elements in prostatic carcinoma and their effects on the behavior of the lesion have recently been recognized. Recent reports suggest that chromogranin-A- and neuron-specific enolase-expressing tumors have an earlier progression and a lower response rate to hormonal therapy. The extreme presentation of this tumor is presumed to be small cell carcinoma of the prostate. This bombesin-secreting tumor, which has a characteristic clinical picture of early visceral involvement, wide-ranging metastases, and a relatively low rate of expression of PSA and PAP, is highly responsive to chemotherapy.

The relatively high rate of expression of neural elements in primary prostatic carcinoma is discordant with the low frequency of clinical small cell carcinoma of the prostate. In order to account for these differences, one can assume that neural elements may play a role in the progression of this disease by either developing their own neoplastic process (small cell carcinoma of the prostate) or, in the majority of cases, causing paracrine progression of the tumor.

Bombesin is typically secreted by small cell carcinoma of the lung and possibly by the prostate. It has been shown to be a growth factor mediating the progression of this disease in a number of experiments. Preclinical data demonstrate increased invasiveness and increased proliferation associated with bombesin in the treatment of prostatic carcinoma.

Based on the hypothesis that neural peptides may be important mediators of androgen-independent growth of prostatic carcinoma as well as predicting poor prognosis, inhibition of these factors may represent a therapeutic strategy of relevance for the treatment of patients with prostatic carcinoma.

Key words: bombesin, neural peptides, paracrine progression, prostate, small cell carcinoma

Strategies for the chemoprevention of prostatic carcinoma are complicated by the prevalence of indolent prostatic carcinoma. Indolent prostatic carcinoma occurs at a higher frequency than clinically significant prostatic carcinoma; only a minority of patients with the diagnosis of prostatic carcinoma will succumb to the disease. Current chemotherapy strategies used in other tumor types are justified by the high mortality associated with these tumors once they develop (head and neck, lung, and GI tract). Such strategies may not necessarily be suited for the prevention of prostatic carcinoma where only a portion of the tumors are of clinical relevance. Approaches used to direct the treatment of prostatic carcinoma could include the refinement of the population by selecting patients at a unique risk for developing prostatic carcinoma based on epidemiologic data (familial risk, environmental). This approach limits the number of patients who would require intervention and enhances the increased "yield" of the intervention strategies. An alternate approach would be to select patients with established carcinomas who have tumor types that possess a clinically virulent phenotype. Such therapy strategies would be more appropriately labeled as inhibiting progression of prostatic carcinoma rather than as chemoprevention. It is our hypothesis that the presence of neural elements may serve as a marker of tumor progression. Their presence in a tumor may indicate the existence of a clinically relevant subtype and may also be a target for therapeutic intervention. In this manuscript, we outline clinical and preclinical data which support our hypothesis and the future approach that we will adopt to study the role of neural elements.

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CLINICAL DATA

Neural elements in prostatic carcinoma have been reported to be expressed at a frequency of 30-50% [1-4]. These elements are very important predictors of survival. In the manuscript by Cohen *et al.* [5], a direct relationship was shown between survival and the presence of the neuro-endocrine cells as documented by immunoperoxidase staining of neuron-specific enolase (NSE) and chromogranin. This correlation appeared to be independent of other known prognostic variables. Although this data has not yet been clinically confirmed, ongoing studies are currently being performed to confirm the relevance of these findings. At the University of Texas M. D. Anderson Cancer Center (UTMDACC) the significance of neural elements was recognized when patients with metastatic prostatic carcinoma of the small cell subtype were treated [6]. Such tumors have been known to express neural peptides which are potent mitogens for prostatic carcinoma *in vitro*. The clinical phenotype of small cell carcinoma (SCC) is thought to represent clonal expansion of the neural elements which we believe possess the ability to achieve androgen-independent growth. In support of this, the majority of SCC of the prostate that we treated became clinically apparent after castration of the patient for what initially was thought to represent an adenocarcinoma of the prostate. Patients with SCC of the prostate can be viewed as having a tumor which is comprised of an enriched population of androgen-independent cells whose growth is sustained through alternate paracrine or autocrine pathways. The clinical phenotype of the acquired form of SCC of the prostate is characterized by a short duration of response to castration, early visceral metastases, and a disproportionate amount of tumor volume in relationship to the degree of elevation of the PSA or a complete absence of over-expression of the PSA level. In our experience and the experience of others, tumor markers presumed to be unique to this subset of tumor were measured at a high frequency [6]. These markers correlated with the rate of tumor growth and represent valuable markers measuring the progression of the disease. Forty-seven percent of patients treated at UTMDACC with a demonstrated androgen-independent growth of a classic adenocarcinoma of the prostate were found to have a significant elevation in serum bombesin level (Unpublished data, Logothetis *et al.*). The degree of elevation of the serum bombesin level did not correlate with clinical stage disease or expression of other markers. All the patients in this study were previously found to have a castrate level of serum testosterone, which is objective evidence of progression while maintaining a castrate serum testosterone. The relatively high frequency of elevated serum bombesin implies that this may be an important mediator in the progression of classic adenocarcinoma of the prostate.

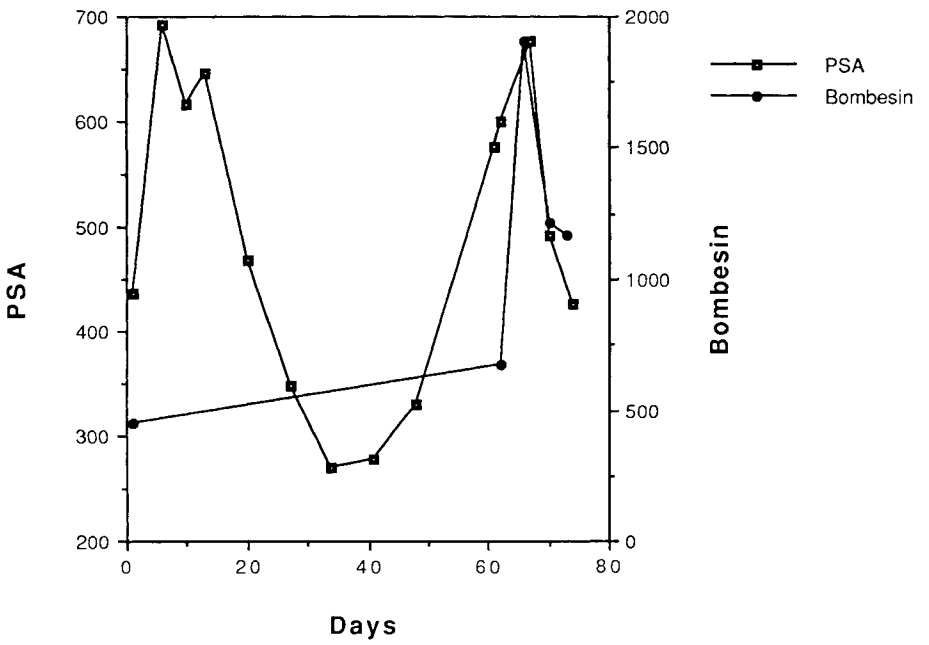

Figure 1. Anecdotal case of co-expression of the serum levels of PSA and bombesin in a patient with androgen-independent growth of prostatic carcinoma.

Sequential analyses of the serum bombesin levels among patients with prostatic carcinoma has not been routinely performed. In individual cases we have been able to find coexpression of serum PSA and serum bombesin levels among patients with typical adenocarcinoma (Fig. 1).

Such cases emphasize the potential importance of serum bombesin. In addition to the coexpression of the serum bombesin levels, we were able to find a crude correlation between the degree of elevation of serum bombesin and survival; the degree of elevation of the serum bombesin was inversely related to survival (Fig. 2).

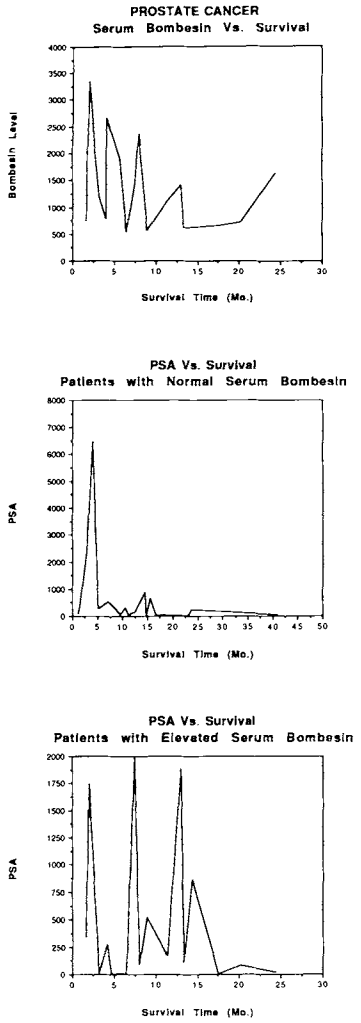

Figure 2. Independent prognostic value of serum bombesin level. Top - Correlation between degree of elevation of serum bombesin level and survival in patients with androgen-independent prostatic carcinoma. Middle - Correlation between the degree of elevation of serum PSA and survival of patients with androgen-independent growth of prostatic carcinoma and normal serum bombesin level. Bottom - Significant elevation in PSA and lack of correlation with survival in patients with elevated serum bombesin and PSA.

A similar relationship was found for the degree of elevation of the PSA which is attributed to its crude correlation with tumor volume. Patients with an elevated serum bombesin level who also had an elevated PSA showed no clear relationship between the degree of elevation of the serum PSA and survival (Fig. 2). We interpret this preliminary data as suggesting that bombesin is a marker of a distinct clone of prostatic carcinoma which is of clinical relevance and may be responsible for androgen-independent growth and poor prognosis in a relevant subset of patients. This will need to be confirmed in larger numbers of patients prospectively. In addition, the frequency of expression of these neural elements and secretion of bombesin should be studied in patients with earlier stage disease.

A significant disparity exists between the frequency of expression of neural elements in patients initially presenting with prostatic carcinoma, its prognosis, and the very low frequency of the development of SCC of the prostate. SCC of the prostate which is clinically evident may represent an expansion of the epithelial cells possessing neural properties. This rare occurrence accounts for the clinically recognizable SCC, but would not account for the potential prognostic influence of neural elements among patients with prostatic carcinoma in general. In view of the peptides secreted by the neural elements, it is possible that the negative influence on survival of these elements may be mediated through a paracrine stimulation of the adenocarcinoma compartment of the tumor. Such a hypothesis could account for the disparity between the frequency of expression of neural elements and the small frequency of SCC.

Therefore, we hypothesize that an important mechanism of the development of androgen-independent growth in prostatic carcinoma may be mediated through the expression of neural peptides. Such sustained androgen-independent growth could be responsible for the short survival associated with SCC patients treated by castration alone or those patients with an elevated serum bombesin level at the time of relapse. Clinically studied therapeutic approaches to the inhibition of these pathways could be useful in early intervention if further studies confirm these preliminary findings.

PRECLINICAL DATA

An autocrine progression of SCC via the bombesin/gastrin releasing peptide (GRP) pathway appears to be an essential component of progression in small cell lung cancer [7]. The observation that antibodies to GRP, the mammalian analog of bombesin, can be used to block the growth of SCC of

the lung *in vivo* is the basis for preclinical studies of patients treated with an immunological approach [8]. Autocrine/paracrine growth stimulation of human prostatic carcinoma cell lines by bombesin/GRP has been recently demonstrated [9,10]. Proliferation of human prostatic carcinoma cell line PC-3 and of the epithelial cell strain PMU 23 (derived from a primary culture of a stage III prostatic carcinoma) was enhanced in a dose-dependent manner by adding 0.1 to 10.0 nM bombesin to the culture medium [9]. Saturable, specific binding sites for bombesin/GRP on PC-3 cells numerically comparable to those measured on small cell lung cancer cell lines have been identified [9]. Bombesin antiserum suppressed growth of androgen-independent, human prostatic carcinoma cells DU-145 and PC-3 in culture as well as of transplanted DU-145 cells in nude mice [10]. Also, bombesin/GRP immunoreactivity was detected on DU-145 and PC-3 cells [10]. These data suggest that bombesin/GRP may be an important growth factor involved in the progression of prostate tumor cells to the androgen-independent state.

Several other peptides have been identified in resected human prostate tumors. Immunoreactivities for adrenocorticotropic hormone (ACTH), corticotropin-releasing factor (CRF), parathyroid hormone (PTH), antidiuretic hormone (ADH), as well as somatostatin, calcitonin and endorphins, among others, were found [1,2,11,12]. Also, receptors for somatostatin and growth inhibitory effects of this peptide have been described in the rat Dunning prostate cancer model [13]. The effect of each of these neuropeptides, including somatostatin, on human prostate cancer cells, and their possible involvement in tumor progression remain undetermined. Several bioactive peptides, such as calcitonin, GRP, somatostatin, thyrotropin-releasing hormone (TRH) and thyroid-stimulating hormone (TSH), were found in neuroendocrine cells present in the normal human prostate [3,4,14,15]. These peptides normally occur in only a small portion (less than 5%) of total neuroendocrine cells within the prostate [3]. Malignant transformation could involve clonal expansion of cells producing these peptides.

Also, peptidergic neurons innervating the normal human prostate gland have been shown to be positive for vasoactive intestinal polypeptide (VIP), neuropeptide Y and enkephalins [16-18]. The presence of serotonin was common in most neuroendocrine cells within the normal prostate [3]. Serotonin content is increased in the hyperplastic [19] and neoplastic human prostate gland [1]. Increased serotonin production may stimulate tumor growth in an autocrine or paracrine manner [20,21]. Catecholamines have been reported to be involved in regulating gene expression and functional differentiation of the rat ventral prostate gland in addition to androgens [22]. Receptors for dopamine and VIP as well as catecholamines have been demonstrated in rat prostate epithelial cells [23]. Reduced response of the catecholamine-sensitive adenylate cyclase system of the rat prostate has been observed after androgen deprivation and in aging animals [24,25]. Whether aberrant expression and/or response of prostatic cells to one or more of these hormones plays a role in prostatic neoplasia is not known and requires further investigation.

We have recently investigated the possible role of polypeptide hormones, associated with neuroendocrine cells, in enhancing the invasive potential of human prostate tumor cells in culture. The effects of bombesin, VIP and a somatostatin analog (RC-160) on the *in vitro* invasion of reconstituted basement membrane (Matrigel) by two human prostatic carcinoma cell lines (PC-3 and LNCaP) were examined. Tumorigenic, androgen-unresponsive PC-3 cells of high metastatic potential were found to be invasive in this assay, in contrast to the relatively indolent, androgen-responsive LNCaP cells: $6.5\pm0.5\%$ and $0.3\pm0.2\%$, respectively, of total tumor cells (2×10^5) had penetrated Matrigel after 72 h incubation under serum-free conditions (Fig. 3).

Under similar conditions, treatment with 5 nM bombesin resulted in an increase in maximum invasion by both PC-3 ($14.4\pm1.0\%$) and LNCaP ($2.5\pm0.3\%$) cells. While VIP had no significant effect on invasion by PC-3 cells, it enhanced invasion by LNCaP cells in a dose-dependent manner with a maximum at 0.1 uM VIP ($2.5\pm0.3\%$ invasion) (Fig. 3). In agreement with these results, 0.1 uM VIP increased cAMP levels from a basal level of 0.2 ± 0.1 pmol to 8.5 ± 0.6 pmol/4 x 10^5 cells/10 min. in LNCaP cells alone (data not shown). In contrast to bombesin and VIP, RC-160 did not alter invasion of Matrigel by either cell line (Fig. 3). These results indicate that certain peptide hormones 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.

The increase in cellular invasiveness by VIP is of interest. Other neuroendocrine peptides found in prostate tumors (ACTH and calcitonin, for example) increase intracellular cAMP concentration similarly to VIP. Also, isoproterenol, a beta-2 adrenergic agonist, caused an approximately 5-fold elevation in LNCaP cellular cAMP levels at a concentration of 0.1 uM (not shown). Therefore we tested the effect of exogenously added cAMP analog (dibutyryl cAMP, dbcAMP) on cellular invasiveness. Penetration of Matrigel by both LNCaP as well as PC-3 cells was enhanced (Fig. 3). Studies by Korman *et al.* [26] on small cell lung cancer (SCLC) cell lines have shown that the addition of secretin and VIP to SCLC cells *in vitro* stimulated the secretion of bombesin-like peptides. It is conceivable that a similar mechanism is responsible for the effects of VIP and dbcAMP in our invasion assay (Fig. 3). Thus, neuroendocrine peptides and classical neurotransmitters, whose second messenger is cAMP, may affect the metastatic potential of prostate tumor cells.

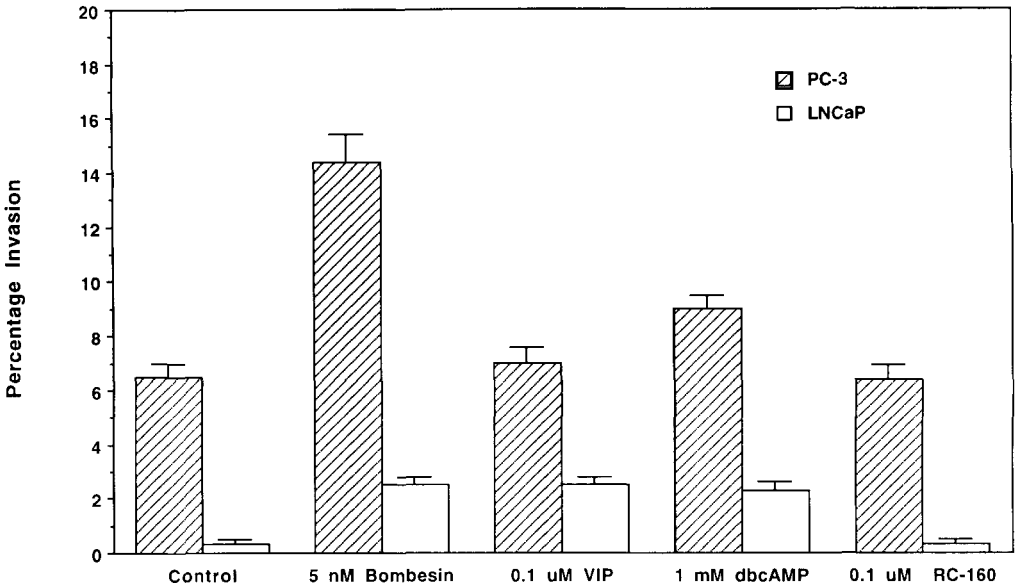

Figure 3: Effect of neuroendocrine peptides on the invasiveness of prostate tumor cells. Both bombesin and dibutyryl cAMP significantly stimulated percent invasion by PC-3 and LNCaP cell lines. VIP selectively stimulated invasiveness in LNCaP alone; however, RC-160 failed to influence the invasiveness of either of the cell lines.

The mitogenic effect of bombesin/GRP on SCLC cancer cells is associated with a rapid but transient increase in intracellular calcium ions. Recently, other neuropeptides, such as bradykinin, neurotensin, cholecystokinin and vasopressin, at nanomolar concentrations stimulated a rapid and transient increase in the intracellular concentration of calcium ions in responsive SCLC cell lines [27]. Importantly, these peptides in the same concentration range also caused a marked increase in colony formation in semisolid medium. These findings support the hypothesis that SCLC cell growth is sustained by an extensive network of autocrine and paracrine interactions involving multiple neuropeptides. The possibility of such interactions in prostatic cells will be studied.

CONCLUSIONS

The clinical and laboratory data suggest that neuroendocrine cells and their peptide products may play a role in the progression of prostatic carcinoma. These elements may be responsible for the rare occurrence of SCC of the prostate with its characteristic clinical picture through clonal expansion, or may be responsible for a mechanism of androgen-independent growth. The second approach may be very significant in a portion of patients with androgen-independent prostatic carcinoma growth.

To confirm the significance of these findings, clinical demonstration of a close relationship between the progression of androgen-independent prostatic carcinoma and the secretion of these peptides *in vivo* will be required. In addition, inhibition of peptide function at the receptor level leading to inhibition of the virulent behavior should be further investigated.

Our data suggests that GRP/bombesin may be a marker of expression of the virulent subtype of prostatic carcinoma, in addition to a mechanism of androgen-independent growth. This potential dual function of GRP/bombesin offers a unique opportunity to select therapy which would be limited to the subset that would benefit from it. In prostatic carcinoma, which has a high frequency of cancers that do not threaten morbidity or mortality, such therapy offers the attraction of limiting the exposure to those patients requiring intervention.

Our future approaches will evaluate the expression of these neural elements in the progression of prostatic carcinoma, confirming the correlation with prognosis and the serum markers in such patients. Prospective trials directed toward the inhibition of these neural elements and their influence on patient outcome will follow.

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