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# NEUROENDOCRINE AND IMMUNE MEDIATORS IN PROSTATE CANCER PROGRESSION

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## 1. ABSTRACT

Cytokines constitute a diverse group of intercellular signaling proteins that regulate local and systemic, immune and inflammatory responses as well as wound healing and hematopoiesis. The proliferation and maturation of cells of the immune system, both normal and malignant, is regulated by cytokines such as the interleukins. Such cytokines may also influence the proliferation and differentiation of other cell types. Prostate epithelial cells differentiate along two pathways, exocrine or neuroendocrine. Elevation in the exocrine marker prostate-specific antigen and/or the neuroendocrine marker chromogranin A in serum has been associated with prostate cancer progression. Interleukin-1 (IL-1) mRNA is expressed by two androgen-insensitive (AI) but not by three androgen-sensitive prostate cancer cell lines. IL-1 inhibits while IL-2 stimulates the growth of the androgen-sensitive LNCaP cell line. Neither affects growth of AI PC-3 or DU-145 cell lines. IL-1 promotes the neuroendocrine phenotype and IL-2 promotes the exocrine phenotype in prostate cancer. The influence of the immune mediators IL-1 and IL-2 on the growth and differentiation of prostate cancer cells and its implication in tumor progression is described herein. Relationship of IL-1 with bone metastasis and the involvement of ß-2 microglobulin in the development and progression of prostate cancer are also discussed.

## 2. INTRODUCTION

The glandular epithelium of the normal human prostate consists of three cell types (1, 2). Columnar cells of the lumen form the majority, have a secretory function and express proteins such as prostate-specific antigen (PSA) and prostatic-acid phosphatase (PAP) which are useful markers of exocrine differentiation. Underlying the luminal cells is the basal cell layer containing stem cells and few scattered neuroendocrine (NE) cells. Substances such as chromogranin A (CGA), serotonin and neuron-specific enolase (NSE) are expressed by NE cells and serve as markers of NE differentiation (1). Exocrine as well as NE cells are probably derived from the pluripotent stem cells of the basal layer (2).

Study of the behavior of prostatic small cell carcinoma (SCC), a tumor with profound NE differentiation, over a 23-year period indicated an aggressive clinical course (3,4). NE differentiation in prostatic carcinoma (PCa) with the morphological characteristics of SCC occurs with low frequency (<1%). However, NE cells lacking overt SCC or carcinoid-like morphological features but positive for immunoreactivity of NE markers such as CGA and serotonin, are often present in typical adenocarcinomas of the prostate. Serum CGA levels in PCa patients correlate well with the extent of tissue CGA immunostaining (5 ) and in our experience are elevated with a frequency of about 35% in advanced PCa (6). NE cells and their products, often found intimately associated with prostatic adenocarcinoma, have been implicated in tumor growth and invasiveness (reviewed in 7).

## 3.THE NEUROENDOCRINE PHENOTYPE AND ANDROGEN-INDEPENDENT PROSTATE CANCER

Several lines of evidence implicate NE factors in the emergence of androgen-independence in PCa. Immunohistochemical double-label methods to evaluate the nuclear androgen-receptor status of NE cells have revealed a lack of the androgen receptor in cells staining for CGA in normal, hyperplastic as well as neoplastic human prostate glands (8). The widespread absence of androgen-receptor immunoreactivity in NE tumor cells suggests that these cells may represent the population that is initially AI and therefore refractory to androgen-deprivation therapy (8). In a recent extensive study, the number of NE cells increased with time in repeated biopsy specimens of 60 hormone-treated patients and paralleled tumor progression (9).

There has been a surge of interest in prostatic NE cells after a 1990 report of significantly shortened patient survival associated with positive immunostaining of CGA and NSE in primary PCa tissue (10). A study found elevated plasma CGA levels in 48% of 25 patients with stage D2 PCa (11). All 12 patients with elevated plasma CGA had AI disease. In another clinical investigation of 130 patients at various stages of PCa, the mean serum CGA level was highest in patients with stage D3 PCa (hormone escape) (12). We have reported that serum CGA was elevated in 1 of 15 (7%) androgen-dependent (AD) patients and 11 of 21 (52%) AI PCa patients (6). Six of the 12 patients with elevated CGA in our study had low serum PSA (< 7 ng/mL).

The expression of genes such as *bcl-2* which function to prevent programmed cell death is increased after androgen-ablation therapy suggesting that BCL-2 may have a role in androgen-resistance (13). All 6 cases of prostatic SCC displayed high BCL-2 immunoreactivity (13). A propotional relationship between tissue levels of the NE marker NSE and BCL-2 was found in 11 of 13 primary PCa by Western analysis (14). Double immunostaining showed proximity between BCL-2 and CGA- or NSE-containing cells, although in no case were the NE markers co-expressed with BCL-2 in the same cell (14).

In addition there is evidence from studies of PCa cells in-vitro, supporting a correlation between the development of AI PCa and increased NE involvement (15-17). Androgen-withdrawal from the androgen-sensitive LNCaP cell line either by change to serum-free, phenol-red free, defined medium (15, 16) or to a charcoal stripped, serum-containing medium (17) results in increased activities of NE peptides such as neurotensin (15, 16), NE markers such NSE and S-100 as well as morphological changes such as the presence of numerous dense-core secretory granules and elongated neuronal-type processes (17).

Interestingly, androgen-deprivation of LNCaP cells results in down-regulation of the neutral endopeptidase 24.11 (NEP), a cell-surface enzyme expressed by a wide range of tissues including normal prostatic epithelial cells (16). NEP expression (protein and mRNA) is lost in AI PCa cell lines TSU-Pr1, PC-3 and DU-145 (16). NEP protein expression is commonly low in metastatic PCa specimens of AI but not androgen-dependent (AD) patients (16). Overexpression of NEP in AI cell lines or their incubation with recombinant NEP results in reduced growth (16). In the absence, but not in the presence of androgens, LNCaP cells secrete neurotensin and respond to exogenous neurotensin with growth stimulation (15). These data show that decreased NEP expression in AI PCa contributes to AI growth by permitting uncleaved neuropeptides to substitute for androgens as stimulators of cellular proliferation (16).

Taken together these clinical as well as laboratory data suggest the selection of, or adaptation to, the NE phenotype after hormonal therapy and a role for NE factors in tumor progression.

## 4. IMMUNE MEDIATORS CAN MODULATE PROSTATE CANCER PROLIFERATION

We have experimental evidence supporting the idea that AI cells in the tumor population gain growth advantage by suppressing the proliferation of androgen-sensitive cells (18). Conditioned medium (CM) from AI, human PC-3 and DU-145 PCa cell lines inhibited the growth of androgen-sensitive, human LNCaP cells in-vitro. Under similar conditions, LNCaP CM did not inhibit the proliferation of DU-145 or PC-3 cells, but had a small (~20%) stimulatory effect on DU-145 growth. Also, inoculation of 100,000 PC-3 cells near LNCaP tumors, growing subcutaneously in nude mice for over 3 months, resulted in a dramatic inhibition of LNCaP tumor growth (18). These data suggested that AI cells secrete factor(s) that inhibit(s) the growth of androgen-sensitive cells, resulting in the domination of AI cells within the tumor. From its physicochemical characteristics and use of neutralizing antibodies, we identified this growth-inhibitory activity secreted by AI cells as interleukin-1 (IL-1).

The growth-inhibitory effect of the immune mediators were confirmed by addition of authentic IL-1 alpha and IL-1 beta to LNCaP cells (18). Exogenous IL-1 beta at low concentrations (3-500 units per mL) caused a 60% reduction in LNCaP growth. At doses higher than 500 units/mL IL-1 alpha reduced LNCaP growth by 30%-40%. Thus, IL-1 could be responsible for the clonal dominance of AI PCa cells. Exogenous IL-1 alpha or -beta had no effect on the growth of AI DU-145 or PC-3 cells (18), suggesting that AI cells have lost sensitivity to the growth-suppressive effects of IL-1. In contrast to IL-1, IL-2 enhanced the growth of the androgen-responsive LNCaP cells (18). In similarity to IL-1, IL-2 had no effect on the proliferation of the AI PC-3 or DU-145 cells (18).

We have determined the presence of IL-1 alpha and IL-1 beta mRNA in five PCa cell lines by RT-PCR (manuscript in preparation). We found IL-1 beta transcripts in the PC-3 cell line but not in DU-145, LNCaP, MDA-PCA-2B and MDA-PCA-2A lines. IL-1 alpha message was found in the AI PC-3 and DU-145 lines but not in the androgen-sensitive LNCaP, MDA-PCA-2B and MDA-PCA-2A cell lines. IL-2 message was absent and that of GAPDH was present in all the five PCa lines examined. Thus, IL-1 is expressed only in AI cell lines. Results of the IL-1 mRNA expression in PCa cell lines are in agreement with our immunohistochemical findings of the presence of IL-1 beta in both stromal as well as epithelial compartments in human PCa specimens but the presence of IL-2 only in the stroma (manuscript in preparation).

## 5. IMMUNE MEDIATORS CAN MODULATE EXPRESSION OF EXOCRINE AND NEUROENDOCRINE MARKERS

Death from carcinoma of the prostate is preceded by AI growth of the tumor. Dominance of the tumor by hormone-insensitive cells with disease progression, may occur by selection of androgen-insensitive clones (viz. androgen-receptor negative NE cells) and/or by adaptation by hormone-sensitive cells (as discussed above, androgen-deprivation causes down-regulation of a neuropeptidase (16) allowing neuropeptides such as bombesin (6, 7, 20, 21) and neurotensin (15) to promote growth and metastasis). NE factors such as bombesin can induce the expression of IL-1 beta in other systems (22).

IL-1 mRNA is expressed by two androgen-insensitive PCa cell lines PC-3 (both IL-1 beta and IL-1 alpha) and DU-145 (only IL-1 alpha) but not by three androgen-sensitive lines (LNCaP, MDA-PCA-2B and

**Table 1.** Influence of interleukin-1, -2 and -6 on prostate cancer progression

| |
|---|
| **IL-2 PROMOTES THE EXOCRINE PATHWAY** |
| ❑ IL-2 stimulates growth of LNCaP cells (androgen-responsive cells expressing PSA) |
| ❑ IL-2 increases PSA secretion in LNCaP cells after normalization for cell number |
| ❑ IL-2 decreases CGA secretion in LNCaP and DU-145 cells |
| **IL-1 AND IL-6 PROMOTE THE NEUROENDOCRINE PATHWAY** |
| ❑ IL-1 beta and IL-6 increase CGA expression and secretion |
| ❑ IL-1 alpha decreases LNCaP PSA synthesis and secretion |
| ❑ IL-1 beta inhibits LNCaP growth (no effect on androgen-independent DU-145 and PC-3 proliferation) |
| ❑ IL-1 beta decreases LNCaP PSA expression (after normalization for cell number). |
| ❑ IL-1 mRNA present in two androgen-independent cell lines, DU-145 and PC-3, but absent in three androgen-sensitive cell lines including LNCaP cells. |

MDA-PCA-2A). PC-3 and DU-145 cells display strong NE features such as presence of abundant bombesin receptors, CGA and serotonin and are relatively aggressive in their growth and invasive behavior compared to LNCaP cells. Exogenously added IL-1 suppresses LNCaP growth and PSA expression but in similarity to IL-6 stimulates CGA expression in LNCaP as well as DU-145 cells (18, 23). Thus, IL-1 and IL-6 expression in PCa appear to correlate with the NE phenotype and with tumor progression (6, 23). In contrast, IL-2 appears to promote the exocrine pathway of PCa progression (18). IL-2 increases LNCaP growth and PSA secretion (18) and decreases LNCaP as well as DU-145 CGA levels (23). These findings are summarized in table 1.

Our finding of promotion of NE differentiation in PCa by IL-6 (23 ) has also been reported by others (24). IL-6 treatment of LNCaP induced neurite extension and enhanced expression of neuronal markers in their study (24). Etk/Bmx a new member of Btk tyrosine kinase family was shown to have a pivotal role in IL-6 signaling in LNCaP. The NE phenotype could be abrogated by the over-expression of a dominant-negative Etk, indicating Etk is required for this differentiation process (24). The same group also demonstrated a requirement of ErbB2 for signaling by interleukin-6 in prostate carcinoma cells (25). Of note, the positive staining for EGFR and C-erb B-2 in NE cells present in PCa specimens (26). IL-1 is known to induce IL-6 expression in many cell types (27, 28). We and others (6, 29) have reported the elevation of serum IL-6 levels in patients with advanced prostatic carcinoma and a lack of correlation between serum PSA and serum IL-6 levels.

## 6. RELATIONSHIP BETWEEN INTERLEUKIN-1 AND BONE METASTASIS

IL-1 has been previously implicated in the growth and metastasis of other malignancies such as melanoma, ovarian and breast carcinoma (30-32). Five different human ovarian epithelial tumor cell lines and tumor cells isolated from the ascitic fluid of four ovarian cancer patients express IL-1 alpha and IL-1 beta genes constitutively (31). IL-1 inhibited the in-vitro proliferation of hormone-dependent breast cancer cell lines MCF-7 and ZR-75-B but not of hormone-independent HS-578-T and MDA-231 cell lines (32). These results with the breast cancer cell lines (32) are in similarity to our findings of differences in the response of AI and AD PCa cell lines to IL-1.

The adhesion of cancer cells to the endothelial lining of blood vessels which is important for metastasis is promoted by the action of interleukin 1 (IL-1) and other cytokines (30). Also, IL-1 induces collagenase expression in certain cell types (33). Liver, lung and bone marrow have been reported as being favorable "soils" for IL-1 mediated metastasis (30). Mouse melanoma metastasis of IL-1 expressing B-16 cells to bone marrow, spleen, liver, lung was dependent on IL-1 (30) and was blocked by rhuIL-1 RA (recombinant human IL-1 receptor antagonist).

IL-1 beta can modulate several aspects of the activity of bone cells suggesting its potential role as a physiological and pathological modulator of bone metabolism. This cytokine is a potent stimulator of bone-resorption via stimulation of osteoclasts (34, 35). In addition, IL-1 beta influences several activities of osteoblasts (35). rhIL-1beta stimulated cellular proliferation and synthesis of prostaglandin $E_2$ as well as plasminogen activator activity in osteoblast-like cells derived from human trabecular bone.

The propensity of PCa to metastasize to bone and affect bone cell metabolism is well-known. The PC-3 cell line which expresses IL-1 beta was isolated from a bone metastasis of PCa. Also, we have found IL-1 betaimmunoreactivity in PCa specimens as discussed above. Since IL-1 beta is expressed by PCa, it may be responsible at least in part for the bone lesions commonly observed in PCa. Targeting of tumor IL-1 beta production as well as its cellular release and effects may prove to be a useful therapeutic strategy in PCa.

## 7. INCREASED ß-2 MICROGLOBULIN (ß-2M) SHEDDING IN METASTATIC PROSTATE CANCER

ß-2m is another immune substance altered in prostate cancer. It is the 11.8 kDa light chain moiety of the major histocompatibility complex class 1 (MHC I) present on the surface of nearly all nucleated cells. MHC I functions immunologically in antigen presentation to cytotoxic T cells. In addition, the MHC I complex has been implicated in non-immunological functions associated with hormone/growth factor (EGF, insulin, IGF-I, IGF-II) receptor interactions and cell proliferation (36). A mitogenic action of ß-2m on bone osteoblasts (37) and human PC-3 PCa cells as well as rat PS-1, normal prostatic stromal cells has been reported (36).

ß-2m is elevated in several lymphoproliferative disorders including chronic lymphocytic leukemia, lymphomas and multiple myeloma and is prognostically important in these conditions (38). Elevation in tissue/serum levels of ß-2m in breast, lung, gastrointestinal antigens are critical for the cellular immune by which

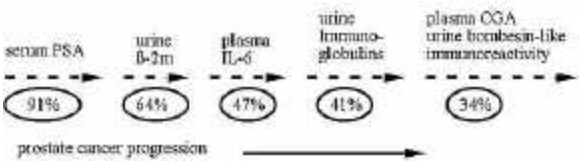


**Figure 1.** Frequency of elevation of soluble markers in advanced prostate cancer (~100 patients)

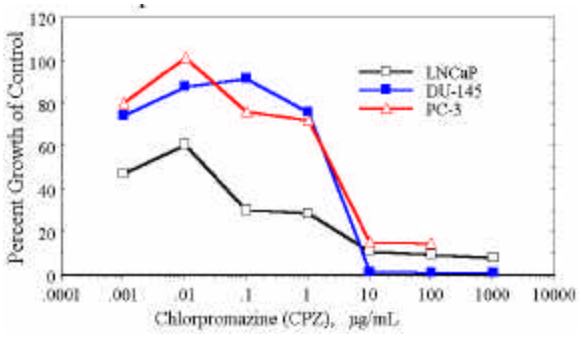


**Figure 2.** Effect of chlorpromazine on the *in-vitro* growth of prostate cancer cell lines. Cells were plated at 10K/well in 96-well plates. 2 days later cells were treated with CPZ in 1% serum-supplemented growth medium. Cell numbers were determined 5 days later.

cancer cells escape immune recognition.

Defective class I MHC assembly in metastatic PCa cell lines has been reported (42). Class I expression is also altered in PCa specimens (43). It has been suggested that analysis of HLA haplotypes may prove to be useful in determining the men with a higher risk of developing PCa (44). We have reported that urine B-2m levels are elevated with a frequency of 64 percent in patients with advanced PCa (figure 1) and are associated with shorter patient survival (45, 46).

Older PCa patients have higher urine ß-2m levels. A correlation between advancing age and increased ß-2m shedding has also been found in breast and lung cancer patients and also in normal controls. We have noted increased ß-2m release from the cell-surface of PCa cells in primary culture as well as PCa cell lines derived from distant metastasis compared to those from local/regional extensions of the disease (manuscript in preparation). The above studies together indicate a role for ß-2m in the etiology as well as progression of PCa.

## 8. PERSPECTIVE

Malignant prostate tumors are initially composed of predominantly androgen-responsive cells. After androgen-deprivation, tumor cells can adapt by using growth factors other than androgens. Adaptation may involve a change from an exocrine to a neuroendocrine phenotype which is androgen-independent (AI). Immune mediators such as IL-1 and IL-2 could participate in the adaptation process and in tumor-progression. In the absence of androgens, IL-2 may support the growth of androgen-sensitive cells. IL-2 also promotes PSA secretion by androgen-responsive cells, and could be responsible in part for the elevated serum PSA levels often observed upon tumor progression after hormonal therapy. IL-1 expressed by androgen-independent cells promotes the neuroendocrine phenotype. Clonal dominance of the tumor by the androgen-independent sub-population, which precedes death from prostatic carcinoma, may be mediated by IL-1.

During adaptation an intermediate cell type may exist: amphicrine cells that have both exocrine and neuroendocrine characteristics (47, 48). PCa cells with overt NE differentiation are sensitive to chemotherapeutic agents active against other NE tumor types such as small cell lung cancer (49). In order to prevent the adaptation process, which likely occurs after androgen-withdrawal, strategies to concomitantly inhibit growth of androgen-responsive and -unresponsive cells should be explored. One approach would be to find agents that are active against both the tumor sub-populations. For example, we find that androgen-responsive as well as androgen-resistant PCa cell lines display sensitivity to the growth-inhibitory effects of phenothiazines such as chlorpromazine (figure 2) (50).

In this review we have discussed the influence of immune mediators on the growth and differentiation of PCa cells. The cellular effects of immune mediators such as IL-1, IL-6 and IL-2 on prostate cancer warrant further investigation as potential targets for therapy.

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## 10. REFERENCES

1. Di Sant'Agnese PA: Neuroendocrine differentiation in carcinoma of the prostate. Diagnostic, prognostic, and therapeutic implications. *Cancer* 70, 254-267 (1992)

2. Bonkhoff H, Stein U & Remberger K: Multidirectional differentiation in the normal, hyperplastic and neoplastic human prostate: simultaneous demonstration of cell specific epitheleal markers. *Human Pathology* 25, 42-46 (1994)

3. Tetu B, Ro JY, Ayala AG, Johnson DE, Logothetis CJ & Ordonez NG: Small cell carcinoma of the prostate Part I, A clinicopathologic study of 20 cases. *Cancer* 59, 1803-1809 (1987)

4. Ro JY, Tetu B, Ayala AG & Ordonez NG: Small cell carcinoma of the prostate Part II, Immunohistochemical and electron microscopic study of 18 cases. *Cancer* 59, 977-982, (1987)

5. Deftos LJ, Nakada S, Burton DW, di Sant'Agnese PA, Cockett ATK, Abrahamsson PA: Immunoassay and immunohistology studies of chromogranin A as a neuroendocrine marker in patients with carcinoma of the prostate. *Urology* 48, 58-62 (1996)

6. Hoosein NM, Abdul M, McCabe R, Gero E, Deftos L, Banks M, Hodges S, Finn L & Logothetis CJ : Clinical significance of elevation in neuroendocrine factors and interleukin-6 in metastatic prostate cancer. *Urologic Oncology* 1, 246-251 (1995)

7. Hoosein NM, Abdul M & Logothetis CJ: Significance of neuroendocrine differentiation in prostatic carcinoma. *The Cancer Journal* 9, 291-295 (1996)

8. Bonkhoff H, Stein U & Remberger K: Androger receptor status in endocrine-paracrine cell types of the normal, hyperplastic and neoplastic human prostate. *Virchows Archiv A Pathol Anat* 423, 291-294 (1993)

9. Jiborn T, Bjartell A & Abrahmsson PA: Neuroendocrine differentiation in prostatic carcinoma during hormonal treatment. *Urology* 51, 801-804 (1998)

10. Cohen RJ, Glezerson G, Haffejee Z & Afrika D: Prostatic carcinoma: histological and immunohistological factors affecting prognosis. *Brit J Urol* 66, 405-410 (1990)

11. Kadmon D, Thompson TC, Lynch GR & Scardino PT: Elevated plasma chromogranin A concentrations in prostatic carcinoma. *J Urol* 146, 358-361 (1991)

12. Cussenot O, Villette JM, Valeri A, Cariou G, Desgrandchamps F Cortesse A, Meria P, Teillac P, Fiet J & Le Duc A: Plasma neuroendocrine markers in patients with benign prostatic hyperplasia and prostatic carcinoma. *J Urol* 155, 1340-1343 (1996)

13. McDonnell TJ, Troncoso P, Brisbay SM, Logothetis CJ, Chung LW, Hsieh JT, Tu SM & Campbell ML: Expression of the protooncogene bcl-2 in the prostate and its association with emergence of androgen-independent prostate cancer.Cancer Research. 52, 6940-6944 (1992)

14. Segal N, Cohen RJ, Haffejee Z & Savage N: BCL-2 Proto-oncogene expression in prostatic carcinoma and its relationship to the prostatic neuroendocrine cell. *Arch Pathol Lab Med* 118, 616-618 (1994)

15. Sehgal I, Powers P, Huntley B, Powis G, Pittelkow M & Maihle NJ: Neurotensin is an autocrine trophic factor stimulated by androgen-withdrawal in human prostate cancer. *Proc Natl Acad Sci USA* 91, 4673-4677 (1994)

16. Papandreou CN, Usmani B, Geng Y, Bogenreider T, Freeman R, Wilk S, Finstad CL, Reuter VE, Powell CT, Scheinberg D, Magill C, Scher HI, Albino AP & Nanus DM: Neutral endopeptidase 24.11 loss in metastatic human prostate cancer contributes to androgen-independent progression. *Nature Medicine* 4, 50-57 (1998)

17. Shen R, Dorai T, Szaboles M, Katz AE, Olsson CA & Buttyan R: Transdifferentiation of cultured human prostate cancer cells to a neuroendocrine cell phenotype in a hormone-depleted medium.*Urologic Oncology* 3, 67-75 (1997)

18. Abdul M, Diaz M & Hoosein NM: Influence of interleukin-1 and -2 on cellular proliferation, exocrine and neuroendocrine markers in prostate cancer. *Seminars in Oncology* in press (1999)

19. Abdul M, Hsieh, JT, Logothetis CJ & Hoosein NM: Secretion of prostate-specific antigen (PSA)-suppressing activity by two human prostate carcinoma cell lines.*Urologic Oncology* 1, 38-41 (1995)

20. Hoosein NM, Logothetis CJ & Chung LWK: Differential effects of neuroendocrine peptides bombesin, vasoactive intestinal polypeptide and somatostatin on the invasiveness of human prostate tumor cell lines. *J Urol* 149, 1209-1213 (1993)

21. Aprikian AG, Han K, Guy L, Landry F, Begin, LR & Chevalier S: Neuroendocrine differentiation and the bombesin/gastrin-releasing peptide family of neuropeptides in the progression of human prostate cancer. *The Prostate* Supplement 8, 52-61 (1998)

22. Lemaire I: Bombesin-related peptides modulate interleukin-1 production by alveolar macrophages. *Neuropeptides* 20, 217-223 (1991)

23. Diaz M, Abdul M & Hoosein NM: Modulation of neuroendocrine differentiation in prostate cancer by interleukin-1 and -2. *The Prostate* Supplement 8, 32-36 (1998)

24. Qiu Y, Ravi L & Kung HJ: Requirement of ErbB2 for signalling by interleukin-6 in prostate carcinoma cells. *Nature* 393, 83-85 (1998)

25. Qiu Y, Robinson D, Pretlow TG & Kung HJ: Etk/Bmx, a tyrosine kinase with a pleckstrin-homology domain, is an effector of phosphatidylinositol 3'-kinase and is involved in interleukin 6-induced neuroendocrine differentiation of prostate cancer cells. *Proc Natl Acad Sci USA* 95, 3644-3649 (1998)

26. Iwamura M, Koshiba K & Cockett ATK: Receptors for BPH growth factors are located in some neuroendocrine cells. *The Prostate* Supplement 8, 14-17 (1998)

27. Jones TH, Justice SK, Timperley WR & Royds JA: Effect of interleukin-1 and dexamethasone on interleukin-6 production and growth in human meningiomas. *Journal of Pathology* 183, 460-468 (1997)

28. Beasley D: Phorbol ester and interleukin-1 induce interleukin-6 gene expression in vascular smooth muscle cells via independent pathways. *J Cardiovas Pharmacol* 29, 323-330 (1997)

29. Sanda MG, Restifo NP, Walsh JC, Kawakami Y, Nelson WG, Pardoll DM & Simons JW: Molecular characterization of defective antigen processing in human prostate cancer. *J Natl Can Inst* 87, 280-285 (1995)

30. Anasagasti MJ, Olaso E, Calvo F, Mendoza L, Martin JJ, Bidaurrazaga J, Vidal-Vanaclocha F: Interleukin 1-dependent and -independent mouse melanoma metastases. *J Natl Can Inst* 89, 645-651 (1997)

31. Li BY, Mohanraj D, Olson MC, Moradi M, Twiggs L, Carson LF & Ramakrishnan S: Human ovarian epithelial cancer cells cultures in vitro express both interleukin 1 alpha and beta genes. *Cancer Res* 52, 2248-2252 (1992)

32. Paciotti GF & Tamarkin L: Interleukin-1 directly regulates hormone-dependent human breast cancer cell proliferation in vitro. *Molec Endocrin* 2, 459-464 (1988)

33. Kusano K, Miyaura C, Inada M, Tamura T, Ito A, Nagase H, Kamoi K & Suda T: Regulation of matrix metalloproteinases (MMP-2, -3, -9, and -13) by interleukin-1 and interleukin-6 in mouse calvaria: association of MMP induction with bone resorption. *Endocrinol* 139, 1338-1345 (1998)

34. Dewhirst FE, Stashenko PP, Mole JE & Tsurumachi T: Purification and partial sequence of human osteoclast activating factor: identity with Interleukin-1ß. *J Immunol* 135, 2564-2568 (1985)

35.Evans DB, Bunning RAD & Russell RGG: The effects of recombinant human interleukin-1ß on cellular proliferation and production of prostaglandin E2, plasminogen activator, osteocalcin and alkaline phosphatase by osteoblast-like cells derived from human bone. *Biochem Biophys Res Comm* 166, 208-216 (1990)

36. Rowley DR, Dang TD, McBride L, Gerdes MJ, Lu B & Larsen M: ß-2 Microglobulin is mitogenic to PC-3 prostatic carcinoma cells and antagonistic to transforming growth factor ß1 action. *Cancer Res* 55, 781-786 (1995)

37. Canalis E, McCarthy T & Centrella M: A bone derived growth factor isolated from rat calvariae is ß-2 microglobulin. *Endocrinol* 118, 2002-2008 (1987)

38. Jacobs EL & Haskell CM: Clinical use of tumor markers in oncology. *Curr Probl Cancer* 15, 301-321 (1991)

39. Klein T, Levin I, Niska A, Koren R, Gal R, Schachter J, Kfir B, Narinski R,Warchaizer S & Klein B: Correlation between tumour and serum beta 2m expression in patients with breast cancer. *Eur J Immunogenetics* 23, 417-423 (1996)

40. Auer IO, Watzel C & Greulich M: The plasma concentration of beta 2-microglobulin in the diagnosis of malignancy of the gastrointestinal tract . *Medizinische Klinik* 74, 1581-1583 (1979)

41. Hallgren R, Nou E, & Lundqvist G: Serum beta 2-microglobulin in patients with bronchial carcinoma and controls. *Cancer* 45, 780-785 (1980)

42. Sanda MG, Restifo NP, Walsh JC, Kawakami Y, Nelson WG, Pardoll DM & Simons JW: Molecular characterization of defective antigen processing in human prostate cancer. *J Natl Canc Inst* 87, 280-285 (1995)

43. Bander NH, Yao D, Liu H, Chen YT, Steiner M, Zuccaro W & Moy P: MHC class I and II expression in prostate carcinoma and modulation by interferon-alpha and -gamma. *The Prostate* 33, 233-239 (1997)

44. Akdas A, Turkeri L, Alican Y, Simsek F, Akoglu T: Human leukocyte antigen subtype analysis in patients with advanced adenocarcinoma of the prostate. *The Prostate* 24, 111-113 (1994)

45. Abdul M, Banks M & Hoosein NM: Urinary markers for prostate cancer. *Int J Onc* 8, 735-739 (1996)

46. Hoosein NM, Finn L, Hodges S, Diaz M & Abdul M: Soluble markers of PCa progression. *Cancer Research (Therapy and Control)* 28: 1- 5, 1997.

47. Bonkhoff H: Neuroendocrine cells in benign and malignant prostate tissue: morphogenesis, proliferation and androgen receptor status. *The Prostate* Suppl 8, 18-22 (1998)

48. Xue Y, Smedts F, Verhofstad A, Debruyne F, de la Rosette J & Schalken J: Cell Kinetics of prostate exocrine and neuroendocrine epithelium and their differential interrelationship: new perspectives. *The Prostate* Supplement 8, 62-73 (1998)

49. Amato RJ, Logothetis CJ, Hallinan R, Ro JY, Sella A, & Dexeus FH: Chemotherapy for small cell carcinoma of prostatic origin. *J Urol* 147, 935-937 (1992)

50. Jones GRN: Successful cancer therapy with promethazine: the rationale. *Medical Hypotheses* 46, 25-29 (1996)

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