

## Hormone-dependent cancers: new approaches to identification of potential diagnostic and/or therapeutic biomarkers

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*Proceedings Asia Pacific Conference on Plant Tissue and Agribiotechnology (APaCPA) 17-21 June 2007*

**Abstract.** Hormone-dependent cancers of the prostate and breast are the most common cancers in men and women, respectively, in the Western world and are major causes of illness and death. Current diagnostic/prognostic tests are invasive or lack specificity and “certainty” with respect to detecting these cancers early and in differentiating between different phenotypes. More specific diagnostic/prognostic markers are needed to better define treatment options for individuals. Classical approaches have focussed on candidate genes/proteins that are involved in tumour cell growth, invasion and metastasis. Using genomic, proteomic, molecular and cell biology approaches *in vitro*, we have identified additional proteins – the ghrelin growth factor axis and variants within the kallikrein family of serine proteases – that may serve as potential candidate markers and therapeutic targets. However, with recent advances in high through-put technologies (gene tissue microarrays, 2D gel and mass spectrometry proteome analyses, bioinformatics) relevant genes/proteins can now be more efficiently screened, identified and validated. These technologies can readily be coupled with proof-of-principle experiments – *in vitro* and *in vivo* assays using gain and loss of function models, targeted drug design and pharmacological/toxicological studies and subsequent clinical trials – to develop a fully integrated, translational research model which has the capacity to deliver clinical outcomes more rapidly.

**Keywords:** Biomarkers; Breast cancer; Prostate cancer.

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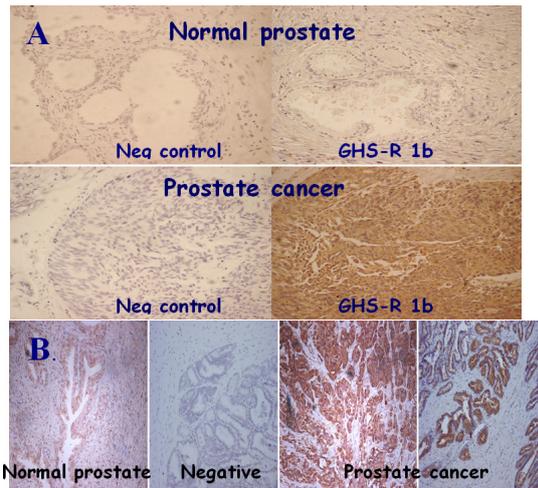
### INTRODUCTION

Hormone-dependent cancers are classically those of the reproductive tract – prostate and testis cancer in men and breast, ovarian and endometrial cancer in women. The sex steroid hormones, androgens, estrogens and progestins are the primary modulators of both normal development and maintenance of these organs, as well as malignant growths. These cancers are some of the most commonly diagnosed cancers in men and women and the second most common cause of cancer deaths in the Western world, and represent a lifetime risk of between 1:6 and 1:10 for prostate and 1:7 and 1:11 for breast cancer, in the USA and Australia (Jemal *et al.*, 2006 and The Cancer Council, 2006). Ovarian and endometrial cancers represent the 4th and 5th most common cancers in Western women (Jemal *et al.*, 2006). Early stages of these diseases are often asymptomatic and not diagnosed until the cancer cells have begun to migrate from their primary tumour site and metastasised to secondary sites. Current diagnostic tests are either generally invasive (eg digital rectal examination [prostate] or biopsy) or not specific enough to: a) detect these cancers early, b) to differentiate between slow growing and aggressive tumours (which is particularly relevant for prostate cancer) or c) to

detect different genotypes/phenotypes (except for breast cancer where estrogen and progesterone receptors and Her2 receptor status are informative) (Speirs and Walker, 2007). Serum prostate specific antigen (PSA) is the basis for initial screening for prostate cancer, despite it being subject to significant false positives and false negatives (Amling, 2006). Current treatments for these cancers include anti-hormone therapy in the early stages of disease, surgery, radiotherapy and chemotherapy but these frequently affect quality of life and have significant side effects (e.g. impotence and incontinence for prostate cancer). The incidence of familial-linked cancers is small but significant (5-10% for prostate; up to 20% for breast) (Klein, 2002 and Peto *et al.*, 1999) which provides a known level of risk (e.g. BRCA 1 and 2 for breast cancer), but there remains no test that is predictive of sporadic cancer. This is due partly to the fact that the underlying causes of these cancers are multi-factorial and involve both genetic and environmental factors (eg diet) (Kolonel, 2001). Current research is aimed at identifying:-

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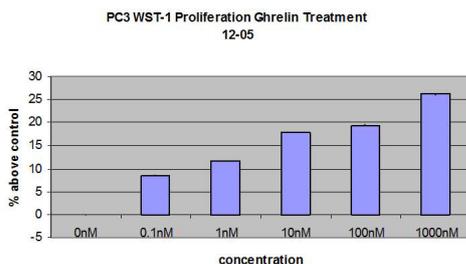
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**Figure 1.** Immunohistochemistry in normal prostate and prostate cancer tissue sections showing immunoreactivity for GHS-R 1b (A) and the exon 3-deleted variant of preproghrelin

- better predictive markers for cancer risk
- new diagnostics that are more specific, provide earlier diagnosis and differentiate between slow growing and aggressive disease
- prognostic markers to determine the type of disease and therefore predict outcome
- new treatments with fewer side effects
- genotype/phenotype-specific and individualised treatments.

While recent approaches have included large scale genome-wide association studies to successfully identify single nucleotide polymorphisms that associate with particular cancers (e.g. breast) (Easton, *et al.*, 2007), many contemporary studies of the hormone-dependent cancers remain focussed on identifying single candidate genes/proteins that are likely to play a role in tumour cell growth, cell migration, invasion and/or metastasis. Using a combination of hormone regulation studies, molecular and cellular biology and protein structure/function modelling, under-pinned by use of modern bioinformatics analyses, our laboratory has identified several new candidate genes/proteins for both prostate and breast cancers that have potential as diagnostic/prognostic biomarkers and/or therapeutic targets. These include the novel growth factor ghrelin/ghrelin receptor axis (Jefferey *et al.*, 2003) and the kallikrein family of serine proteases (Clements *et al.*, 2004).



**Figure 2.** Effect of ghrelin on the proliferation (WST-1 assay) of PC3 prostate cancer cells in culture.

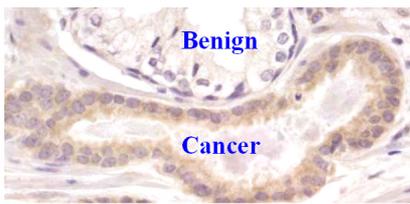
## RESULTS

*The Ghrelin Axis.* Both ghrelin and its receptor, the growth hormone secretagogue receptor (GHS-R), are expressed in prostate cancer cell lines and in prostate tumour tissue (data not shown) (Yeh *et al.*, 2005 and Jefferey *et al.*, 2002). Of particular interest was the differential expression (low/absent in normal tissue; high in cancer tissue sections) of two variants identified by us in prostate and breast cancer tissue – the truncated and presumed non-functional GHS-R 1b isoform (Figure 1A) and an exon 3 deleted form of the preproghrelin molecule (Figure 1B). This latter protein still yields mature ghrelin, but also produces a novel C-terminal peptide (Jefferey *et al.*, 2003), the potential function of which is not yet determined. While expression has been examined in just small numbers of tissue sections, to date, these data suggest that these variants may be potential diagnostic markers.

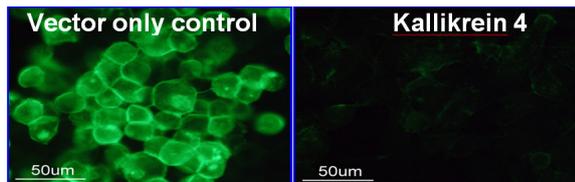
The ghrelin axis is also a potential therapeutic target based on its potential for autocrine / paracrine function - i) ghrelin and the GHS-R are co-expressed in prostate and breast tumour tissue, ii) ghrelin is secreted into conditioned medium of prostate cancer cells in culture (data not shown) and iii) ghrelin stimulates both prostate and breast cancer cell line proliferation (Figure 2).

*The Kallikrein family.* The kallikreins (KLKs) are a family of 15 serine proteases, clustered on human chromosome 19q13.4, which are known to activate proteases (e.g. urokinase-type plasminogen activator, uPA) and growth factors, degrade growth factor binding proteins (insulin-like growth factor binding proteins, IGFBP) and degrade extracellular matrix proteins (Clements *et al.*, 2004). In this context they are obvious candidates for a role in cell growth, motility, invasion and metastasis. Several of the kallikreins are expressed in hormone-dependent cancers. Indeed, prostate specific antigen, PSA, the current serum marker for prostate cancer, is KLK3. We have examined expression and function of several of the kallikreins in prostate and ovarian cancers, in particular, and have shown that KLK4 and/or alternatively spliced KLK4 variants are over-expressed at both mRNA and protein levels in serous epithelial ovarian tumours compared to normal tissue and benign, granulocytic and mucinous tumours (data not shown) (Dong *et al.*, 2003) and in prostate cancer (Figure 3) (Veveris-Lowe *et al.*, 2005).

PC3 prostate cancer cells transfected and over-expressing KLK4 also show increased cell migration in a Transwell TM assay (data not shown) and a complete loss of both E-cadherin mRNA and protein (Figure 4) (Veveris-Lowe *et al.*, 2005). Coupled with a concomitant rise in vimentin expression, this indicates that these KLK4-PC3 cells have undergone an epithelial to mesenchymal transition (EMT), a hallmark of cancer progression (Savager, 2001).



**Figure 3.** Immunohistochemistry of KLK4 expression in benign and cancerous prostate glands



**Figure 4.** Immunostaining of E-cadherin in and vector-only and KLK4-transfected PC3 cells.

## DISCUSSION

The above data indicate that components of both the ghrelin growth factor axis and the kallikrein family of serine proteases represent potentially important diagnostic/prognostic biomarkers and therapeutic targets for several hormone-dependent cancers. This conclusion is based on the over-expression of exon 3 deleted proghrelin, the ghrelin receptor variant GHS-R 1b, and wild type and alternatively spliced forms of KLK4 in prostate, ovarian and breast cancers, together with the functional roles of these entities in stimulating cell proliferation, cell migration and, in the case of KLK4, the induction of an epithelial to mesenchymal transition in prostate cancer cells. Coupled with an observed increase in migration of prostate cancer cells in the presence of osteoblast conditioned medium, increased expression of KLK4 in co-cultures of prostate cancer cells and osteoblasts and the expression of KLK4 within bone metastases of prostate cancer (Gao *et al.*, 2007), these data suggest that KLK4 may play a particularly key role in the well-known selectivity of prostate cancer cells to metastasise to bone (Vela *et al.*, 2007).

The fundamental aim of such studies is to identify robust and informative biomarkers and therapeutic targets that can be subsequently validated and translated into clinical use. Our studies represent a classical approach to the initial stages of the complete “translational pipeline” which involves the discovery of candidate genes/proteins, their validation in a disease context, assessing their functional impact in cell and animal models, development and testing of assays or therapeutic drugs, followed by clinical testing in human trials. Commonly, these stages have been carried out in isolation and have led to significant time restraints in getting new biomarkers/therapeutics into clinical practice. With recent advances in high through-put technologies

(gene microarrays, 2D gel and mass spectrometry proteome analyses, bioinformatics, disease-focussed tissue microarrays, molecular imaging) relevant genes/proteins can now be more efficiently screened, identified and validated. Through creation of integrated, multi-disciplinary teams of researchers and clinicians the opportunity now exists to readily couple these technologies with proof-of-principle experiments – *in vitro* and *in vivo* assays using gain- and loss-of-function models, targeted drug design and pharmacological/toxicological studies and subsequent clinical trials – to develop a fully integrated, translational research model which has the capacity to deliver clinical outcomes more rapidly.

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