# Prostate secretory protein of 94 amino acids (PSP-94) and its peptide (PCK3145) as potential therapeutic modalities for prostate cancer

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**This review focuses on the promising roles of prostate secretory protein of 94 amino acids (PSP-94) and one of its derived peptides (PCK3145) as potential therapeutic modalities for prostate cancer and its associated complications. Evaluation of these compounds was carried out *in vitro* and *in vivo* using syngeneic models of rat prostate cancer. Overproduction of parathyroid hormone-related protein (PTHrP) results in the development of hypercalcemia of malignancy in several malignancies including prostate cancer. In order to evaluate the effect of PSP-94 and PCK3145 on prostate cancer progression, the rat Dunning R3227 MatLyLu cell line transfected with full-length cDNA encoding PTHrP (MatLyLu-PTHrP) was used. As the main pathogenetic factor of hypercalcemia of malignancy, overexpression of PTHrP was aimed at mimicking the hypercalcemic nature seen in patients suffering from late-stage cancer. *In vitro* studies showed that PSP-94 and PCK3145 can cause a dose-dependent inhibition in the growth of MatLyLu-PTHrP cells. For *in vivo* studies, male Copenhagen rats were inoculated either s.c. into the right flank or directly into the left ventricle via intracardiac (i.c.) inoculation with MatLyLu-PTHrP cells. In these models, s.c. injection of MatLyLu cells results in the development of primary tumor growth, whereas i.c. inoculation routinely results in the development of experimental skeletal metastases in the lumbar vertebrae causing hind-limb paralysis. Administration of PSP-94 and PCK3145 into tumor-bearing animals resulted in a dose-dependent inhibition of primary tumor growth, and tumoral and plasma PTHrP levels, and in the reduction of plasma calcium levels. Additionally, treatment with PSP-94 or PCK3145 caused an inhibition of skeletal metastases resulting in a significant delay in the development of hind-limb paralysis. Interestingly, equimolar concentrations of PCK3145 were shown to be more effective in delaying the development of experimental skeletal metastases as compared to PSP-94. One of the possible mechanisms of action of these modalities is through the induction of apoptosis which was observed by both in-vitro and in-vivo analyses of MatLyLu-PTHrP cells and tumors. Several intracellular mechanisms can also be involved in inhibiting PTHrP production and anti-tumor effects of PSP-94 and PCK3145. Collectively, these studies warrant the continued clinical development of these agents as therapeutic agents for patients with hormone-refractory prostate cancer.**


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## Background

Prostate cancer is one of the most commonly diagnosed cancers in men, especially in the western world, and is a leading cause of cancer morbidity and mortality following lung cancer [1]. Advances in screening and detection methods have led to an increasing number of men being diagnosed with prostate cancer [2,3]. Adenocarcinoma of the prostate starts out as an asymptomatic androgen-dependent disease confined within the prostate gland which eventually acquires a highly invasive, highly metastatic and androgen-independent phenotype [4,5]. Advances in therapeutic modalities such as surgery, radiation therapy and hormone replacement therapy have led to great success rates in treating prostate cancer in its early stage [6,7]. However, limited success has been obtained in blocking progression or cure of late-stage, hormone-refractory prostate cancer [8–10].

The ability of late-stage prostate cancer to metastasize to both skeletal and non-skeletal sites is the main reason for the limited success achieved thus far in curing the disease at its late stages [11]. Although skeletal metastases can be either of the osteolytic or osteoblastic phenotype, those observed in late-stage prostate cancer patients tend to be of the osteoblastic variety [11–13]. This is due to the ability of prostate cancer cells which

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have metastasized to the bone to produce several factors involved in osteoblast proliferation. These factors include endothelin-1, bone morphogenetic proteins and urokinase (uPA) [14–19]. In previous studies, we have shown abundant production of uPA by human prostate cancer cells [20]. Both uPA and its N-terminal fragment were shown to act as selective mitogens for cells of the osteoblast phenotype [20,21].

A common observation seen in patients suffering with late-stage, highly advanced prostate cancer is the elevated levels of parathyroid hormone-related protein (PTHrP) [22,23]. Studies have shown that PTHrP is indeed the major pathogenetic factor involved in hypercalcemia of malignancy, which is commonly observed in about 15–20% of patients with different cancers [24,25]. PTHrP has also been shown to be involved in promoting the growth of prostate cancer due to its ability to inhibit the apoptotic machinery, resulting in increased prostate cancer cell survival [26–28]. In addition, PTHrP's role in promoting osteoblast proliferation and differentiation has been well documented, contributing to the skeletal metastases seen in patients with late-stage, hormone-refractory prostate cancer [29–31].

Prostate secretory protein of 94 amino acids (PSP-94), commonly known as prostatic inhibin or β-microseminoprotein, is a cysteine-rich, non-glycosylated protein initially discovered due to its ability to inhibit follicle-stimulating hormone [32]. It has since been realized that PSP-94 is one of three predominant proteins found in human seminal fluid along with prostate-specific antigen (PSA) and prostatic acid phosphatase [33]. Although the conventional prostate-specific protein has been PSA, several studies have been carried out determining the higher degree of specificity of PSP-94 as being a prostate-specific entity compared to PSA. Immunohistological analyses of several male tissues using antibodies directed against PSP-94 showed positive staining indicative of the presence of PSP-94 only in the prostate gland [34–37]. Additionally, through the use of transgenic models, it has been shown that PSA transgenic mice where the PSA promoter was used to drive the expression of a mutant RAS oncoprotein develop non-prostatic tumors; specifically, tumors in the salivary glands and gastrointestinal tracts [38]. In contrast, use of the PSP-94 promoter to drive the expression of the SV-40 Tag oncoprotein resulted in mice developing tumors only in the prostate gland [39]. Collectively, these studies provide the rationale for the use of PSP-94 to target therapeutic modalities specifically to the prostate gland.

Immunohistological studies have demonstrated the differential expression of PSP-94. It is abundantly expressed in normal prostatic epithelium and the levels of PSP-94 decrease as prostate cancer advances from early to late stages, with complete lack of PSP-94 expression in late-stage, hormone-refractory prostate cancer [40]. Furthermore, RT-PCR analyses of mRNA extracted from normal prostatic epithelial cells, and early- and late-stage prostate cancer cells showed similar differential expression of PSP-94 in different stages of prostate cancer. Recently, epigenetic regulation of the PSP-94 gene has been attributed as a potential mechanism for the downregulation of PSP-94 in prostate cancer [41]. This differential expression of PSP-94 allows its use as a diagnostic marker for prostate cancer in the clinical setting [42]. The added advantage of PSP-94's regulation being androgen independent as compared to that of PSA provides for a higher degree of sensitivity in tumors that have been exposed to androgen-ablating agents [43].

A great deal of information is known pertaining to the biological function and regulation of PSP-94 [44–47]. However, little information is currently available as to how PSP-94 exerts its effects at the cellular level. This stems from the fact that a PSP-94 receptor has yet to be identified and characterized. It is nevertheless possible that PSP-94 can exert its effects without a biological receptor through perturbation of other signaling molecules. However, several studies have been carried out since the discovery of PSP-94 that indicate the presence of specific binding proteins on the surface of the prostate gland itself as well as prostate cancer cells *in vitro* [48–50]. It was not until recently that this binding protein was identified, purified and characterized [51]. Nevertheless, several additional studies have to be undertaken to validate that this newly characterized binding protein is indeed the *bona fide* receptor for PSP-94 [52]. Several of the criteria that are needed to consider this binding protein as the PSP-94 receptor have already been met, particularly the presence of this binding protein on the prostate gland and its ability to bind to PSP-94 with high specificity and affinity. However, the final and most important criterion of identifying the specific intracellular signaling molecules and the signaling cascade which is initiated upon PSP-94 binding to its binding protein has yet to be met. The recent characterization of this binding protein will facilitate the identification process [51].

## Therapeutic potential of PSP-94

Evaluation of the anti-tumor effects of PSP-94 was carried out in syngeneic models of rat prostate cancer. These models provide several advantages over xenograft models of prostate cancer. There is no species barrier to overcome since both the tumor cells and host are of the same species, allowing for an increased tumor intake and growth rate, and resulting in increased efficiency and reproducibility. Furthermore, use of a syngeneic model of prostate cancer permits evaluation of the anti-tumor effects while allowing full interaction between the growth

factors and proteases secreted by the tumor cells and the surrounding stroma. Using these syngeneic models, a series of in-vitro and in-vivo studies were carried out to evaluate the efficacy and possible mechanism of action of PSP-94 and its peptide (PCK3145) to block prostate cancer growth and metastases.

### PSP-94 and PCK3145 inhibit the rat Dunning R3227 MatLyLu cell line transfected with full-length cDNA encoding PTHrP (MatLyLu-PTHrP) growth *in vitro* and *in vivo*

In-vitro analyses showed that PSP-94 was able to inhibit the growth of MatLyLu-PTHrP cells in a dose-dependent manner [53]. The most effective dose of PSP-94 was able to significantly inhibit the growth of MatLyLu-PTHrP cells by up to 40%. To further evaluate the effect of PSP-94 *in vivo*, male Copenhagen rats were inoculated with MatLyLu-PTHrP cells via the s.c. route into the right flank. Animals were treated with different doses of PSP-94 (0.1–10 μg/kg) via s.c. injection at the site of tumor cell inoculation. Treatment with PSP-94 resulted in a dose-dependent inhibition in MatLyLu-PTHrP tumor growth with no systemic side-effects observed (Fig. 1a) [53].

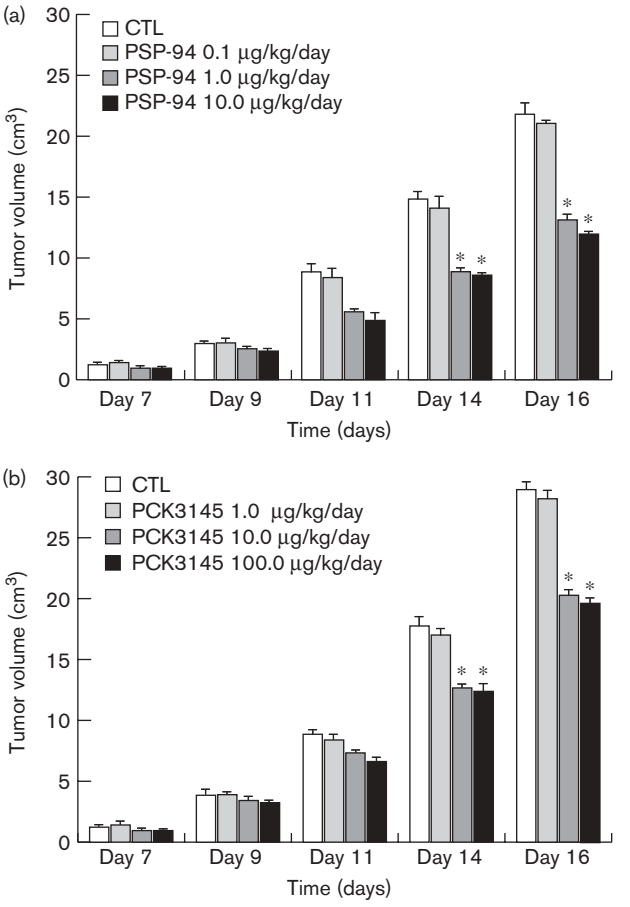
As a follow-up, structure–function studies were carried out to determine the region that might be responsible for the anti-tumor effects of PSP-94 [54]. Different peptide analogs spanning different amino acid regions of PSP-94 were synthesized and tested for their ability to inhibit MatLyLu-PTHrP tumor cell growth *in vitro*. Only peptides corresponding to regions spanning amino acids 7–21, 21–45 and 76–94 were effective at inhibiting MatLyLu-PTHrP tumor cell growth. Further evaluation of these peptides revealed that only the peptide spanning amino acids 31–45 (PCK3145) was effective at reducing MatLyLu-PTHRP primary tumor growth *in vivo*. Similar to PSP-94, administration of PCK3145 (1.0–100 μg/kg) resulted in a dose-dependent inhibition of MatLyLu-PTHrP tumor growth (Fig. 1b) [54]. Equimolar concentrations of PSP-94 and PCK3145 were similarly effective in reducing MatLyLu-PTHrP tumor growth.

Both in-vitro and in-vivo analyses revealed that a possible mechanism for the anti-tumor effects of PSP-94 and PCK3145 is due to the induction of tumor cell apoptosis as analyzed by DNA fragmentation and TUNEL assay [53].

### PSP-94 and PCK3145 decrease tumoral and plasma PTHrP and calcium levels

A complication seen in patients with late-stage, highly advanced prostate cancer is hypercalcemia of malignancy which can be attributed to increased production of PTHrP [22]. In order to evaluate the effects of PSP-94 and PCK3145 on tumoral and plasma PTHrP levels, primary tumors and plasma were collected from animals

**Fig. 1**

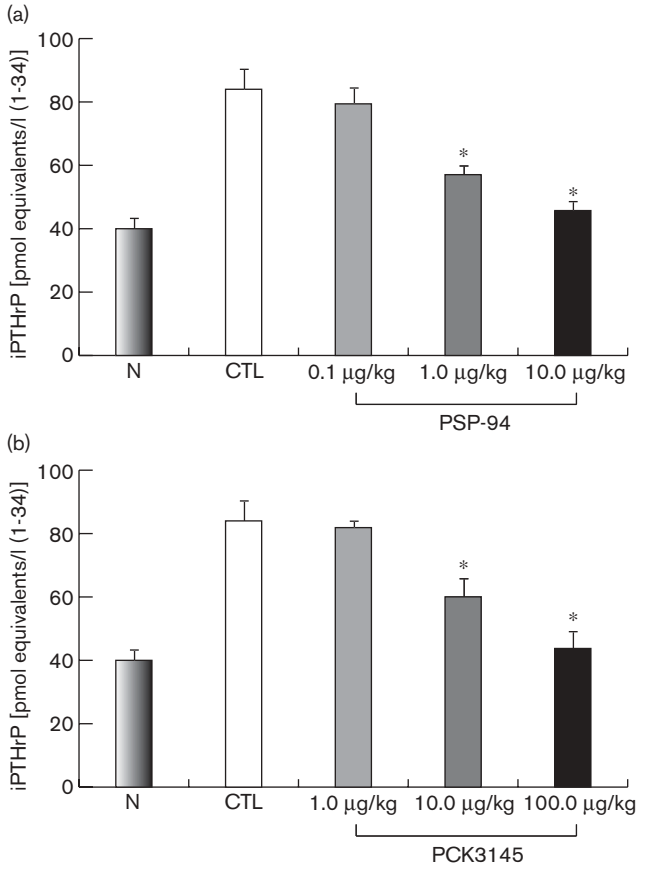



Effect of PSP-94 and PCK3145 on MatLyLu-PTHrP tumor volume. Male Copenhagen rats were injected with $5 \times 10^5$ MatLyLu-PTHrP cells s.c. into the right flank. Animals were infused daily with different doses of PSP-94 (a) or with PCK3145 (b) starting on the day of tumor cell inoculation for up to 15 consecutive days. Tumor volumes were measured at timed intervals and comparison was made with tumor-bearing animals receiving vehicle alone as control (CTL). Results represent the mean ± SEM of five animals in each group in three different experiments. Significant differences from control tumor-bearing animals receiving vehicle alone are represented by asterisks ($P < 0.05$).

receiving vehicle alone and the different doses of either PSP-94 or PCK3145. These tumors were analyzed by immunohistochemical analyses using an antibody directed against PTHrP for the levels of tumoral PTHrP production. Whereas tumors excised from animals treated with vehicle alone exhibited a high degree of positive PTHrP staining, a dose-dependent inhibition was observed in tumors from experimental animals receiving different doses of either PSP-94 or PCK3145. The most pronounced effects in reducing PTHrP production were seen following administration of the highest doses of PSP-94 or PCK3145 [53,54].

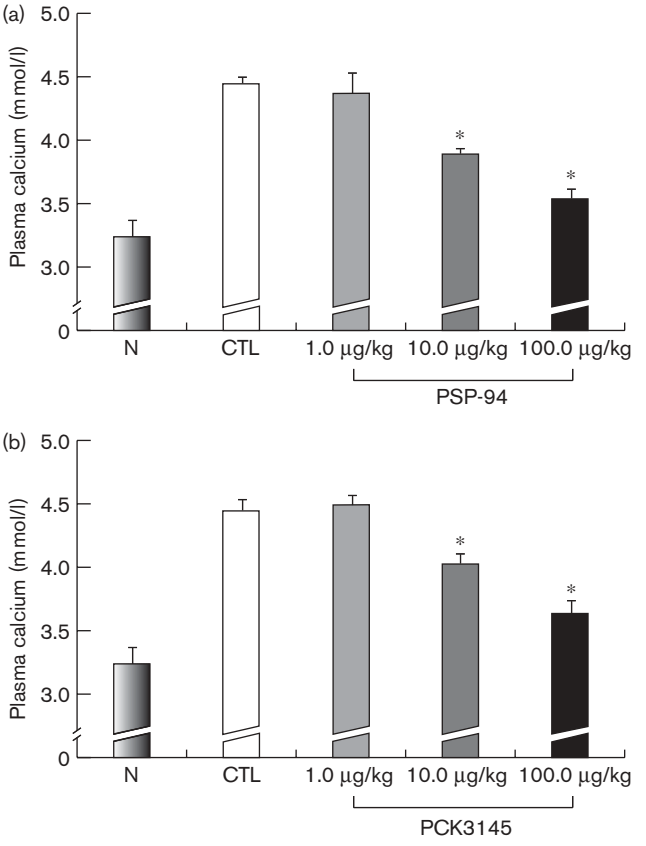
At the end of these studies, normal and experimental animals were sacrificed, and plasma was collected to determine the levels of plasma PTHrP using a

**Fig. 2**




Effect of PSP-94 and PCK3145 on plasma PTHrP levels. Male Copenhagen rats were inoculated with $5 \times 10^5$ MatLyLu-PTHrP cells s.c. into the right flanks. Animals were treated with different doses of either PSP-94 (a) or PCK3145 (b) starting on the day of tumor cell inoculation for up to 15 consecutive days. All animals were sacrificed at the end of the study, and plasma was collected and analyzed for immunoreactive plasma PTHrP (iPTHrP). Results represent the mean ± SEM of five animals in three different experiments. Significant differences from control tumor-bearing animals receiving vehicle alone as control (CTL) are represented by asterisks ($P < 0.05$). Plasma PTHrP levels from normal non-tumor-bearing animals are also shown (N).

**Fig. 3**



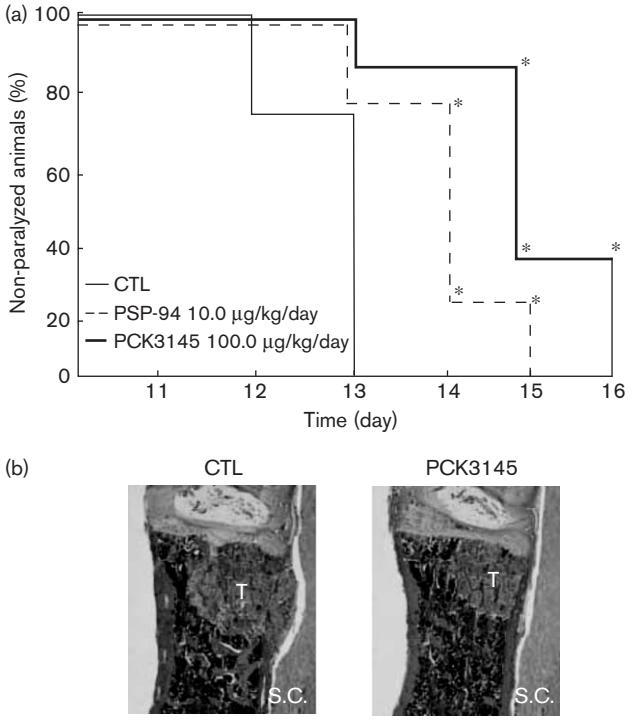

Effect of PSP-94 and PCK3145 on plasma calcium levels. Male Copenhagen rats were inoculated with $5 \times 10^5$ MatLyLu-PTHrP cells s.c. into the right flanks. Animals were treated with different doses of either PSP-94 (a) or PCK3145 (b) starting on the day of tumor cell inoculation for up to 15 consecutive days. All animals were sacrificed at the end of the study, and plasma was collected and analyzed for levels of plasma calcium. Results represent the mean ± SEM of five animals in three different experiments. Significant differences from control tumor-bearing animals receiving vehicle alone as control (CTL) are represented by asterisks ($P < 0.05$). Plasma calcium levels from normal non tumor-bearing animals are also shown (N).

radioimmunoassay. Comparison was made between normal, non-tumor-bearing animals, tumor-bearing animals receiving vehicle alone and tumor-bearing animals receiving different doses of PSP-94 or PCK3145. A marked increase in plasma PTHrP levels was seen following MatLyLu-PTHrP tumor cell inoculation. This rise in PTHrP levels was reduced in a dose-dependent manner following treatment with PSP-94 or PCK3145 (Fig. 2). Both control and experimental animals were also evaluated for levels of plasma calcium. The hypercalcemic nature of tumor-bearing animals was evident when their calcium levels were compared to normal, non-tumor-bearing animals. Administration of PSP-94 or PCK3145 resulted in a dose-dependent inhibition in plasma calcium levels (Fig. 3) [53,54].

### PSP-94 and PCK3145 cause a delay in the development of experimental skeletal metastases

Skeletal metastases are a common development in late-stage, highly advanced prostate cancer, which result in significant tumor-associated morbidity and mortality rates [12]. In order to evaluate the effect of both PSP-94 and PCK3145 on the development of experimental skeletal metastases, male Copenhagen rats were inoculated with MatLyLu-PTHrP cells directly into the left ventricle via intracardiac (i.c.) injections. Inoculation of MatLyLu cells into the left ventricle routinely results in the development of skeletal metastases as represented by hind-limb paralysis [30]. Animals were treated with different dose of PSP-94 and PCK3145 via i.p. injections. Only the highest dose of either PSP-94 or PCK3145 was

**Fig. 4**




Effect of PSP-94 and PCK3145 on experimental skeletal metastases. (a) Male Copenhagen rats were inoculated with $10 \times 10^3$ MatLyLu-PTHrP cells via the i.c. route directly into the left ventricle. Starting from the day of tumor cells inoculation, animals were infused with vehicle alone (CTL) or different doses of PSP-94 or PCK3145. Animals were monitored daily for the development of hind-limb paralysis. The percentage of animals non-paralyzed at different time points is shown. (b) Male Copenhagen rats were inoculated with $10 \times 10^3$ MatLyLu-PTHrP cells via the i.c. route directly into the left ventricle. Animals were infused with either vehicle alone as control (CTL) or PCK3145. All animals were sacrificed before the development of hind-limb paralysis on day 10. Their lumbar vertebrae were excised, paraffin embedded and subjected to histological analysis. A representative micrograph of three such experiments is shown. Original magnification $\times$ 200. Results represent the mean ± SEM of five animals in each group in three different experiments. Significant differences from control are represented by asterisks ($P<0.05$).

able to significantly delay the development of hind-limb paralysis [54]. Interestingly, equimolar concentrations of PCK3145 were more effective at causing a delay in the development of experimental skeletal metastases as compared to that of PSP-94 (Fig. 4a). The ability of PCK3145 to significantly reduce the tumor burden in the lumbar region was evident when animals where sacrificed before the development of hind-limb paralysis, their vertebrae removed and analyzed by immunohistological analyses for the degree of tumor burden. Animals receiving PCK3145 exhibited a significant decrease in tumor burden compared with animals receiving vehicle alone (Fig. 4b). This reduction in tumor burden was directly responsible for the delay previously observed in the development of hind-limb paralysis following administration of PCK3145 [54].

## Future studies

The above-mentioned studies were carried out in syngeneic models of prostate cancer. The use of these models allowed for the opportunity to effectively determine the therapeutic efficacy of PSP-94 or PCK3145 in an environment with full interaction between tumor cells and their microenvironment. However, xenograft models that represent models of human origin mimicking the course of the disease are needed to validate the full clinical benefit of these therapeutic modalities. Further studies evaluating the efficacy of PSP-94 or PCK3145 alone and in combination with other therapeutic agents currently in use or in development for prostate cancer need to be carried out to exploit the full therapeutic potential of these modalities. Careful attention is also required to monitor not only the anti-tumor effects, but also the ability of these therapeutic regimens to block the development or progression of tumor metastases, in general, and skeletal metastases, in particular, which are commonly seen in prostate cancer patients.

Although PCK3145 on its own proved effective at inhibiting tumor growth, several biochemical modifications of this peptide including glycosylation or conjugation to polyethylene glycol or albumin can potentially lead to decreased proteolysis to increase its half-life, which can enhance the biological activity of this agent [55–57].

As mentioned earlier, a possible mechanism by which PSP-94 and PCK3145 can inhibit tumor growth is by induction of apoptosis. Identification of the receptor to which PSP-94 binds could lead to the identification of the signaling cascade that is initiated upon binding of PSP-94 to its receptors, which can trigger its anti-tumor response. Knowledge of the possible signaling cascade could lead to the identification of other potential mechanisms of actions(s) of PSP-94 that may prove to be useful in developing these agents as therapeutic modalities for prostate cancer.

Many prostate cancer patients undergo androgen ablation as an initial form of prostate cancer therapy [7]. Progression of these tumors tends to be monitored by rising PSA levels. However, the levels of PSA can be affected by circulating levels of androgens, resulting in unreliable readings of PSA levels. Since the regulation of PSP-94 is androgen independent, simultaneous monitoring of PSP-94 levels can prove to be a reliable method to stage and predict the behavior of prostate cancer [43]. Development and use of an ELISA-based assay to detect the levels of circulating PSP-94 levels may also prove to be a powerful diagnostic/prognostic tool for prostate cancer [40,58].

Based on these preclinical and pharmacokinetic studies, clinical evaluation of PCK3145 was carried out in hormone-refractory prostate cancer patients. In phase I/IIa studies, administration of PCK 3145 showed disease stabilization with no cytotoxic effects which could be attributed to PCK3145. Biochemical analyses of several tumor progression markers led to the discovery that treatment with PCK3145 results in a marked decrease in the levels of production of matrix metalloproteinase 9. After completion of these clinical studies, PCK3145 is now being evaluated in phase IIb studies in hormone-refractory prostate cancer patients. Further studies are currently underway to elucidate the exact molecular mechanism of action of PCK3145 and its potential application in the treatment of other common cancers.

## Conclusion

Recent studies have shown the ability of PSP-94 and its analog PCK3145 to act as effective inhibitors of late-stage, hormone-independent prostate cancer without manifesting any noticeable side-effects. Combination of either PSP-94 or PCK3145 with some of the well-known chemotherapeutic agents can lead to additive or synergistic anti-tumor effects, adding to our arsenal of non-toxic therapeutic options which can be made available for patients with late-stage, highly advanced prostate cancer. Development of these novel approaches will have a direct impact on reducing the morbidity and mortality in the prostate cancer patient population.

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## References

1 Greenlee RT, Hill-Harmon MB, Murray T, Thun M. Cancer statistics. *CA Cancer J Clin* 2001; **51**:15–36.
2 Brawer MK. Screening for prostate cancer. *Semin Surg Oncol* 2000; **18**:29–36.
3 Ryan CJ, Small EJ. Small progress in detection and treatment of prostate cancer. *Curr Opin Oncol* 2005; **17**:257–260.
4 Feldman BJ, Feldman D. The development of androgen-independent prostate cancer. *Nat Rev Cancer* 2001; **1**:34–45.
5 Arnold JT, Isaacs JT. Mechanisms involved in the progression of androgen-independent prostate cancers: it is not only the cancer cell's fault. *Endocr Relat Cancer* 2002; **9**:61–73.
6 Schroder FH. Prostate cancer: natural history and surgical treatment of localised disease. *Eur J Cancer* 2001; **37**(**Suppl 7**):S127–S136.
7 Pirtskhalaishvili G, Hrebinko RL, Nelson JB. The treatment of prostate cancer: an overview of current options. *Cancer Pract* 2001; **9**:295–306.
8 Fusi A, Procopio G, Della Torre S, Ricotta R, Bianchini G, Salvioni R, *et al.* Treatment options in hormone-refractory metastatic prostate carcinoma. *Tumori* 2004; **90**:535–546.
9 Moore CN, George DJ. Update in the management of patients with hormone-refractory prostate cancer. *Curr Opin Urol* 2005; **15**:157–162.
10 Fournier G. Treatment of hormone-refractory prostate carcinoma. *Eur Urol* 1996; **30**(**Suppl 1**):32–37.
11 Koutsilieris M. Skeletal metastases in advanced prostate cancer: cell biology and therapy. *Crit Rev Oncol Hematol* 1995; **18**:51–64.
12 Koutsilieris M. Osteoblastic metastasis in advanced prostate cancer. *Anticancer Res* 1993; **13**:443–449.
13 Bagi CM. Skeletal implications of prostate cancer. *J Musculoskelet Neuronal Interact* 2003; **3**:112–117.
14 Guise TA, Yin JJ, Mohammad KS. Role of endothelin-1 in osteoblastic bone metastases. *Cancer* 2003; **97**:779–84.
15 Guise TA, Mohammad KS. Endothelins in bone cancer metastases. *Cancer Treat Res* 2004; **118**:197–212.
16 Brubaker KD, Corey E, Brown LG, Vessela RL. Bone morphogenetic protein signaling in prostate cancer cell lines. *J Cell Biochem* 2004; **91**:151–160.
17 Thomas BG, Hamdy FC. Bone morphogenetic protein-6: potential mediator of osteoblastic metastases in prostate cancer. *Prostate Cancer Prostatic Dis* 2000; **3**:283–285.
18 Achbarou A, Kaiser S, Tremblay G, St-Marie LG, Brodt P, Goltzman D, *et al.* Urokinase overproduction results in increased skeletal metastasis by prostate cancer cells *in vivo*. *Cancer Res* 1994; **54**: 2372–2377.
19 Goltzman D. Mechanisms of the development of osteoblastic metastases. *Cancer* 1997; **80**:1581–1587.
20 Rabbani SA, Desjardin J, Bell AW, Banville D, Mazar A, Henkin J, *et al.* An amino-terminal fragment of urokinase isolated from a prostate cancer cell line (PC-3) is mitogenic for osteoblast-like cells. *Biochem Biophys Res Commun* 1990; **173**:1058–1064.
21 Rabbani SA, Mazar AP, Bernier SM, Haq M, Bolivar I, Henkin J, *et al.* Structural requirements for the growth factor activity of the amino-terminal domain of urokinase. *J Biol Chem* 1992; **267**:14151–14156.
22 Asadi F, Kukreja S. Parathyroid hormone-related protein in prostate cancer. *Crit Rev Eukaryot Gene Expr* 2005; **15**:15–28.
23 Asadi F, Farraj M, Sharifi R, Malakouti S, Antar S, Kukreja S. Enhanced expression of parathyroid hormone-related protein in prostate cancer as compared with benign prostatic hyperplasia. *Hum Pathol* 1996; **27**: 1319–1323.
24 Suva LJ, Winslow GA, Wettenhall RE, Hammonds RG, Moseley JM, Diefenbach-Tagger H, *et al.* A parathyroid hormone–related protein implicated in malignant hypercalcemia: cloning and expression. *Science* 1987; **237**:893–896.
25 Martin TJ, Suva LJ. Parathyroid hormone-related protein in hypercalcaemia of malignancy. *Clin Endocrinol* 1989; **31**:631–647.
26 Cramer SD, Peehl DM, Edgar MG, Wong ST, Deftos LJ, Feldman D. Parathyroid hormone-related protein (PTHrP) is an epidermal growth factor-regulated secretory product of human prostatic epithelial cells. *Prostate* 1996; **29**:20–29.
27 Dougherty KM, Blomme EA, Koh AJ, Henderson JE, Pienta KJ, Rosol TJ, *et al.* Parathyroid hormone-related protein as a growth regulator of prostate carcinoma. *Cancer Res* 1999; **59**:6015–6022.
28 Deftos LJ, Barken I, Burton DW, Hoffman RM, Geller J. Direct evidence that PTHrP expression promotes prostate cancer progression in bone. *Biochem Biophys Res Commun* 2005; **327**:468–472.
29 Iddon J, Bundred NJ, Hoyland J, Downey SE, Baird P, Salter D, *et al.* Expression of parathyroid hormone-related protein and its receptor in bone metastases from prostate cancer. *J Pathol* 2000; **191**: 170–174.
30 Rabbani SA, Gladu J, Harakidas P, Jamison B, Goltzman D. Over-production of parathyroid hormone-related peptide results in increased osteolytic skeletal metastasis by prostate cancer cells *in vivo*. *Int J Cancer* 1999; **80**:257–264.
31 Blomme EA, Dougherty KM, Pienta KJ, Capen CC, Rosol TJ, McCauley LK. Skeletal metastasis of prostate adenocarcinoma in rats: morphometric analysis and role of parathyroid hormone-related protein. *Prostate* 1999; **39**:187–197.
32 Mundle SD, Sheth NA. *In vivo* suppression of pituitary and circulatory follicle stimulating hormone by human seminal plasma inhibin. *Indian J Exp Biol* 1991; **29**:310–314.
33 Abrahamsson PA, Lilja H. Three predominant prostatic proteins. *Andrologia* 1990; **22**(**Suppl 1**):122–131.
34 Chan PS, Chan LW, Xuan JW, Chin JL, Choi HL, Chan FL. *In situ* hybridization study of PSP94 (prostatic secretory protein of 94 amino acids) expression in human prostates. *Prostate* 1999; **41**:99–109.
35 Kwong J, Xuan JW, Choi HL, Chan PS, Chan FL. PSP94 (or beta-microseminoprotein) is a secretory protein specifically expressed and synthesized in the lateral lobe of the rat prostate. *Prostate* 2000; **42**: 219–229.
36 Thota A, Karajgikar M, Duan W, Gabril MY, Chan FL, Wong YC, *et al.* Mouse PSP94 expression is prostate tissue-specific as demonstrated by a comparison of multiple antibodies against recombinant proteins. *J Cell Biochem* 2003; **88**:999–1011.

37 Brar A, Mbikay M, Sirois F, Fournier S, Seidah NG, Chretien M. Localization of the human prostatic secretory protein PSP94 and its mRNA in the epithelial cells of the prostate. *J Androl* 1988; **9**:253–260.

38 Schaffner DL, Barrios R, Shaker MR, Rajagopalan S, Huang SL, Tindall DJ, *et al.* Transgenic mice carrying a PSArasT24 hybrid gene develop salivary gland and gastrointestinal tract neoplasms. *Lab Invest* 1995; **72**:283–290.

39 Gabril MY, Onita T, Ji PG, Sakai H, Chan FL, Koropatnick J, *et al.* Prostate targeting: PSP94 gene promoter/enhancer region directed prostate tissue-specific expression in a transgenic mouse prostate cancer model. *Gene Ther* 2002; **9**:1589–1599.

40 Liu AY, Bradner RC, Vessella RL. Decreased expression of prostatic secretory protein PSP94 in prostate cancer. *Cancer Lett* 1993; **74**:91–99.

41 Vanaja DK, Cheville JC, Iturria SJ, Toung CY. Transcriptional silencing of zinc finger protein 185 identified by expression profiling is associated with prostate cancer progression. *Cancer Res* 2003; **63**:3877–3882.

42 Sakai H, Tsurusaki T, Kanda S, Koji T, Xuan JW, Saito Y. Prognostic significance of beta-microseminoprotein mRNA expression in prostate cancer. *Prostate* 1999; **38**:278–284.

43 Garde SV, Sheth AR, Venkatesan VM, Panchal CJ, Porter AT, Grignon DJ. Prostate inhibin peptide (PIP) in prostate cancer: a comparative immunohistochemical study with prostate-specific antigen (PSA) and prostatic acid phosphatase (PAP). *Cancer Lett* 1994; **78**:11–17.

44 Mundle SD, Sheth NA. Suppression of DNA synthesis and induction of apoptosis in rat prostate by human seminal plasma inhibin (HSPI). *Cell Biol Int* 1993; **17**:587–594.

45 Vanage GR, Garde SV, Sheth AR, Gopalkrishnan K. Passive immunization against prostatic 'inhibin' peptide as a male contraceptive. *Int J Androl* 1992; **15**:114–126.

46 Shah MG, Sheth AR. Similarities in the modulation of pituitary and prostatic FSH by inhibin and related peptides. *Prostate* 1991; **18**:1–8.

47 Bandivdekar AH, Devasagayam TP, Pradhan DS, Joseph DR, Hurkadli KS. Suppression of lipid peroxidation in human spermatozoa by prostatic inhibin. *Arch Androl* 1989; **22**:217–221.

48 Phadke M, Vijayalakshmi S, Sheth AR. Evidence for the presence of specific receptors for inhibin in human prostate. *Indian J Exp Biol* 1982; **20**:419–420.

49 Yang JP, Finkelman MA, Clarke MW. Detection of PSP94 and its specific binding sites in the prostate adenocarcinoma cell line LNCaP. *J Urol* 1998; **160**:2240–2244.

50 Yang JP, Baijal-Gupta M, Garde SV, Fraser JE, Finkelman MA, Clarke MW. Identification of binding proteins for PSP94 in human prostate adenocarcinoma cell lines LNCaP and PC-3. *Prostate* 1998; **35**:11–17.

51 Reeves JR, Xuan JW, Arfanis K, Morin C, Garde SV, Ruiz MT, *et al.* Identification, purification and characterization of a novel human blood protein with binding affinity for prostate secretory protein of 94 amino acids. *Biochem J* 2005; **385**:105–114.

52 Woodruff TK. Hope, hypothesis, and the inhibin receptor. Does specific inhibin binding suggest there is a specific inhibin receptor? *Endocrinology* 1999; **140**:3–5.

53 Shukeir N, Arakelian A, Kadhim S, Garde S, Rabbani SA. Prostate secretory protein PSP-94 decreases tumor growth and hypercalcemia of malignancy in a syngenic *in vivo* model of prostate cancer. *Cancer Res* 2003; **63**: 2072–2028.

54 Shukeir N, Arakelian A, Chen G, Garde S, Ruiz M, Panchal C, *et al.* A synthetic 15-mer peptide (PCK3145) derived from prostate secretory protein can reduce tumor growth, experimental skeletal metastases, and malignancy-associated hypercalcemia. *Cancer Res* 2004; **64**:5370–5377.

55 Haubner R, Wester HJ, Burkhart F, Senekowitsch-Schmidtke R, Weber W, Goodman SL, *et al.* Glycosylated RGD-containing peptides: tracer for tumor targeting and angiogenesis imaging with improved biokinetics. *J Nucl Med* 2001; **42**:326–336.

56 Dennis MS, Zhang M, Meng YG, Kadkhodayan M, Kirchhofer D, Combs D, *et al.* Albumin binding as a general strategy for improving the pharmacokinetics of proteins. *J Biol Chem* 2002; **277**:35035–35043.

57 Harris JM, Martin NE, Modi M. Pegylation: a novel process for modifying pharmacokinetics. *Clin Pharmacokinet* 2001; **40**:539–551.

58 Girvan AR, Chang P, van Huizen I, Moussa M, Xuan JW, Stitt L, *et al.* Increased intratumoral expression of prostate secretory protein of 94 amino acids predicts for worse disease recurrence and progression after radical prostatectomy in patients with prostate cancer. *Urology* 2005; **65**: 719–723.