Recombinant prostate-specific antigen proaerolysin shows selective protease sensitivity and cell cytotoxicity

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Native proaerolysin is a channel-forming bacterial protoxin that binds to cell-surface receptors and then is activated by furin or furin-like proteases. We genetically engineered proaerolysin by replacing the furin-cleavage sequence with a prostate-specific antigen-selective sequence. The recombinant modified proaerolysin was expressed and purified from *Aeromonas salmonicida* in good yields and purity. Recombinant modified proaerolysin had no furin sensitivity and markedly increased prostate-specific antigen sensitivity relative to wild-type proaerolysin. Human prostate cancer cells were significantly more sensitive to recombinant modified proaerolysin in the presence of active prostate-specific antigen when compared with the absence of prostate-specific antigen or the presence of potent prostate-specific antigen inhibitors. Most normal human cells with the exception of prostate and renal epithelial cells showed very low sensitivity to recombinant modified proaerolysin. Our results suggest that recombinant modified proaerolysin is a potent prostate-specific antigen-sensitive protoxin that deserves further development for regional therapy of benign and malignant prostate growths. *Anti-Cancer Drugs* 18:809–816 © 2007 Lippincott Williams & Wilkins.

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Introduction

Locally advanced prostate cancer and benign prostatic hyperplasia (BPH) are extremely common illnesses in men in the US [1,2]. BPH affects 50% of men between the ages of 50 and 60, and 90% of men in their 80s. Prostate cancer occurs in one in six men and a third of these develop locally advanced disease. As the number of elderly men is increasing in the US population, these disorders are becoming more frequent. Furthermore, because these ailments produce morbidity but are not rapidly fatal, their prevalence is also increasing [3]. The current standard treatments include surgery, radiation therapy, androgen ablation therapy and chemotherapy for malignancy, and α-adrenergic blockers, 5-α-reductase inhibitors or surgery for BPH. These treatments are associated with significant side effects and are frequently ineffective. Novel agents for these conditions with unique mechanisms of action are needed.

One target associated with differentiated prostate epithelium is prostate-specific antigen (PSA). PSA is a 33–34-kDa single-chain, chymotrypsin-like, serine protease of the human glandular kallikrein family. PSA is synthesized in prostate epithelium and secreted into the seminal fluid. The protein liquifies semen by degrading the major coagulum-forming proteins in semen – semenogelins I and II. The PSA substrates include the semenogelin peptide, HSSKLQ [4–6]. The liquefaction releases spermatozoa, promoting fertility [7]. PSA is also present in prostate interstitial spaces and the bloodstream [8–10]. The vast majority of circulating PSA is complexed to the 100 000-fold excess of α_2-macroglobulin and α_1-antichymotrypsin [11–13]. This complexed PSA is enzymatically inactive. Catalytically active PSA is found almost exclusively in normal and malignant prostate tissue [14]. Hence, prodrugs dependent upon PSA activation should show excellent specificity.

PSA-activated prodrugs have been made with doxorubicin and thapsigargin [15–17]. Tissue-selective release of the cytotoxic compounds has been demonstrated. We sought to adopt the same approach by modifying proaerolysin, a highly potent bacterial toxin, to a form that was activated by the serine protease activity of PSA [18].

Proaerolysin secreted by the bacterial pathogen, *Aeromonas hydrophila*, is the inactive precursor of the pore-forming toxin aerolysin [19]. The protein is released as a soluble dimer that can bind to glycosylphosphatidylinositol-anchored proteins on the surface of target cells

[20]. Proaerolysin is subsequently activated through proteolytic cleavage by cell surface furin-like proteases [21], which release the C-terminal inhibitory peptides producing aerolysin. Activation enables the next step in channel formation, which is the generation of a heptameric oligomer. The heptamer then inserts into the cell membrane producing well-defined channels. Depending upon the number of channels formed, cells may undergo a number of changes that result in cell death, such as loss of small molecules and ions through the aerolysin channels, vacuolation of the endoplasmic reticulum or apoptosis [22].

In this study, we describe the protease sensitivity and cell toxicity of genetically engineered proaerolysin – recombinant modified proaerolysin (PRX302), in which the furin cleavage sequence KVRRAR has been replaced with the PSA-selective cleavage sequence HSSKLQ [18,23]. The recombinant PSA-activated proaerolysin (PRX302) was produced in *Aeromonas* bacteria and was purified from supernatants. The purified PRX302 protein was characterized by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and size-exclusion high-pressure liquid chromatography. Protease sensitivity was assessed by coincubation with protease, followed by sodium dodecyl sulfate–polyacrylamide gel electrophoresis and densitometry. Cell cytotoxicity to human prostate cancer and normal human cells was assessed by thymidine incorporation inhibition assays. The results suggest that PRX302 is a potent PSA-selective toxin that deserves further development for regional therapy of benign and malignant prostatic disease.

Materials and methods

Construction and purification of PRX302

DNA encoding proaerolysin was isolated and cloned into the vector pTZ18U [18,22,23]. Using a two-step PCR protocol [24,25], the furin-activation sequence of proaerolysin ^{427}KVRRAR^{432} was replaced with a sequence selectively cut by PSA–^{427}HSSKLQ^{432} and His_6 tag was placed at the C-terminus of the modified proaerolysin using a Stratagene Quickchange kit (Stratagene, La Jolla, California, USA) to encode PSA-PAH6 (PRX302) following the instructions of the manufacturer. This construct was cloned into the expression vector pMMB208 and the plasmid was moved into the *A. salmonicida* strain CB3 as described earlier [22], producing CB3γ123PSAPAH6.

PRX302 was purified from culture supernatant of CB3γ123PSAPAH6 grown overnight and induced with isopropylthio-β-D-galactopyranoside. The supernatant was concentrated 30-fold using a tangential flow concentrator and the concentrated sample was loaded onto a nickel-affinity column. After washing, PRX302 was eluted from the column, desalted and loaded onto a diethylaminoethanol column. Peak fractions were pooled and exchanged into phosphate-buffered saline, and were stored as aliquots at −80°C.

Wild-type proaerolysin

Wild-type proaerolysin was obtained from T.J. Buckley and was prepared as described earlier [26].

Enzyme assays

Five micrograms of native proaerolysin or recombinant PRX302 were incubated in 20 mmol/l *N*-2-hydroxyl piperazine-*N*′-2-ethane sulfonic acid buffer (pH 7.4), containing 150 mmol/l NaCl and various amounts of enzyme, at 37°C for 10–420 min in a total volume of 250 µl. The reaction was stopped by addition of 5 µl of 100 mmol/l phenylmethylsulfonyl fluoride in isopropyl alcohol followed by cooling on ice. For the MMP-7 enzyme assay 10 mmol/l $CaCl_2$ was added to the reaction buffer. The reaction was stopped with 2 µmol/l leupeptin and 1 µmol/l phenantrolin monohydrate for the cathepsin B and MMP-7 reactions, respectively. An aliquot of stopped reaction mixture was denatured in BioRad sample-loading buffer (BioRad Laboratories, Hercules, California, USA) containing 0.5% β-mercaptoethanol. The sample was electrophoresed on precast 10% Tris–HCl gels using XT MOPS running buffer (BioRad Laboratories). Coomassie Blue staining (BioRad Laboratories) was performed according to the manufacturer's instructions. Densitometry was performed on an AlphaInnotech CCD densitometer (AlphaInnotech, San Leandro, California, USA) with ImageQuant software (GE Healthcare Biosciences, London, UK). The illumination density was determined for each area of the lane, corresponding to the distance traveled of the intact protein and the major component that appeared after incubation with protease. An additional area outside of the placement of expected bands was used to determine background density measurement. Using an Excel spreadsheet and densitometric measurements, the percent of intact protein remaining was determined for each lane by dividing density of the intact band by the sum of the intact and clipped bands, after correction for background. After graphing the percent intact protein against amount of the enzyme, the three values nearest to 50% intact were used to determine the 50% point by linear regression. This value was corrected for aerolysin-related protein concentration before comparison of enzyme 50% values between proteins.

Cell culture

The human prostate cancer cell line LNCaP was grown in RPMI-1640 medium and the prostate cancer cell line PC-3 was grown in Ham's F12K medium (ATCC, Rockville, Maryland, USA). Both media were supplemented with 10% fetal bovine serum and cells were maintained in a 5% CO_2, 95% air-humidified atmosphere at 37°C. Normal human prostate stromal cells, prostate epithelial cells, bladder microvascular endothelial cells, renal epithelial

cells, renal proximal tubule cells and renal mesangial cells were purchased from Cambrex Life Science (Walkersville, Maryland, USA), and were cultured and maintained in serum-free medium as recommended by the supplier.

Hormone treatment

LNCaP and PC-3 cells were grown in medium containing 10% fetal bovine serum and after 24 h cells were transferred to 10% charcoal-stripped fetal bovine serum, followed by treatment with and without 10 nmol/l of androgen, 5α-androstan-17β-ol-3-one (Sigma, St Louis, Missouri, USA), as per the experiment requirement. Androgen stocks were prepared in 100% ethanol at a concentration 1000-fold higher than the working concentrations.

Prostate-specific antigen levels

PSA levels in the supernatants of both prostate tumor cell lines and normal human cells were determined by enzyme-linked immunosorbent assay (ELISA; Abazyme, Needham, Massachusetts, USA) and the assay was done according to the description provided in the Human PSA ELISA kit. Ten thousand cells were plated in wells of a 96-well Costar plate and were maintained in their respective media as mentioned in the cytotoxicity assay. The harvested supernatants from each well were measured for antigenic PSA levels.

Cytotoxicity assay

Cell-line sensitivity to PRX302 was determined as described earlier [27]. Aliquots of 10^4 cells (LNCaP and PC-3 cells) were plated in 96-well flat-bottomed Costar plates and were grown for 24 h in media with 10% charcoal-stripped fetal bovine serum. The cells were then transferred to the same medium with or without androgen and cells were allowed to grow for 24 h. After 24 h, medium was changed again to the same medium with or without androgen and with or without charcoal-stripped fetal bovine serum. Fifty microliters of wild-type proaerolysin and PSA-activated proaerolysin (PRX302) in matching medium were added to each column to yield concentrations ranging from 10^{-8} to 10^{-13} pmol/l and the cells were then incubated at 37°C/5% CO_2 for 24 h. Then, 1 μCi (0.037 MBq) [^{3}H]thymidine (NEN DuPont, Boston, Massachusetts, USA) in 50 μl of medium was added to each well and incubation was continued for an additional 18 h at 37°C/5% CO_2. Cells were then harvested with the Skatron Cell Harvester (Skatron Instruments, Lier, Norway) onto glass fiber mats and counts per minute of incorporated radiolabel were counted using an LKB-Aallac 1205 Betaplate liquid scintillation counter (Perkin-Elmer, Gaithersburg, Maryland, USA) gated for ^{3}H as described earlier [28]. The percent maximal [^{3}H]thymidine incorporation was then plotted versus the log of the toxin concentration and nonlinear regression with a variable-slope sigmoidal dose–response curve was generated along with IC_{50} using GraphPad Prism software (GraphPad Software, San Diego, California, USA). All assays were performed at least twice with an interassay range of 30% or less for IC_{50}. For cytotoxicity with exogenous PSA, LNCaP or PC-3 cells were plated and maintained as mentioned above. The medium was changed to RPMI-1640 medium with or without 10% charcoal-stripped fetal bovine serum and with or without 1 μg/ml of exogenous PSA (Calbiochem, San Diego, California, USA), and cytotoxicity was determined as mentioned above. Human normal cells were cultured and maintained in their respective media, and cytotoxicity was done as mentioned above.

Results

Production and characterization of PRX302

Approximately 40 mg of PRX302 was recovered per liter of *A. salmonicida* culture supernatant. The 52.6-kDa protein was analyzed by electrophoresis as well as by high-pressure liquid chromatography and determined to be more than 95% pure (Fig. 1).

PRX302 enzyme selectivity

PRX302 is a genetically engineered form of proaerolysin in which the furin-cleavage site (residues 427–432) has been replaced with a PSA-cleavable site. Furin is thought likely to be responsible for the activation of native proaerolysin *in vivo*. The results in Fig. 2a show that PRX302 was totally resistant to furin cleavage and activation, whereas the results in Fig. 2b show that it was much more susceptible to PSA activation than the native protein. We also compared the susceptibility of PRX302 and of native proaerolysin to other proteases that they might encounter. Unlike proaerolysin, PRX302 was totally resistant to thrombin, human kallikrein 1 and human kallikrein 2 activation (data not shown). It also showed dramatically reduced sensitivity to activation by urokinase plasminogen activator (uPA), trypsin and chymotrypsin when compared with native proaerolysin (specific activity = 1.1 and 25.8 fmol/μg/min for uPA, 5.7 and 489 pmol/μg/min for trypsin, and 0.15 and 1.55 pmol/μg/min for chymotrypsin, respectively; Fig. 2b). Both proaerolysin and PRX302 were totally resistant to MMP-7 activation (data not shown).

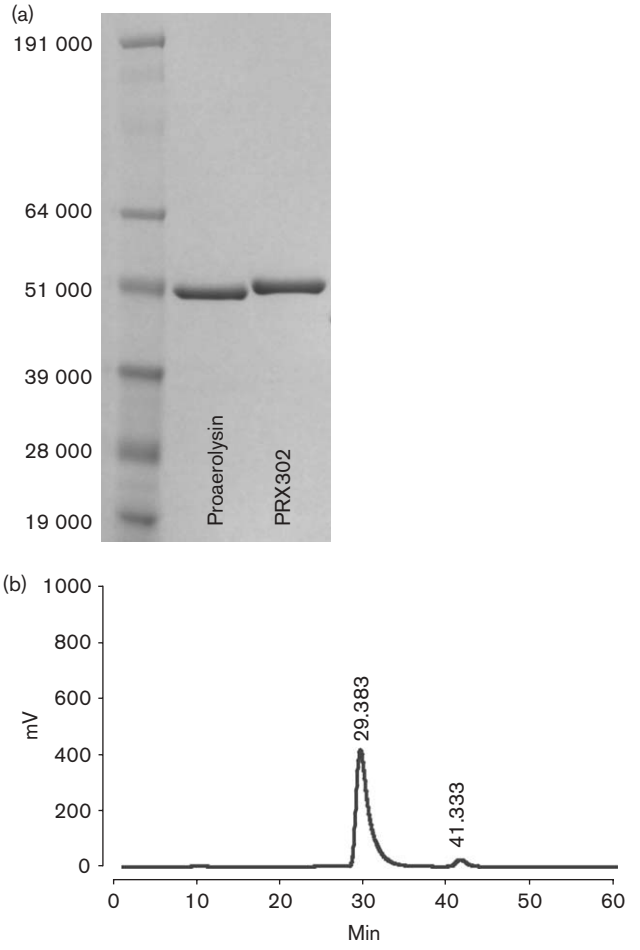
Prostate-specific antigen levels

The PSA expression levels in the two prostate cancer cell lines (LNCaP and PC-3) were determined by ELISA from the supernatants of the cells. No detectable levels of PSA were observed in the PC-3 cell line under all conditions. In contrast, we detected high levels of PSA in the supernatants of LNCaP cells. Exposure of LNCaP cells to androgen alone, to serum alone or to both caused increases in PSA level, compared with native/untreated cells (1.6-, 1.8- and 2.4-fold respectively) after 24 h (Table 1).

PRX302 cell cytotoxicity

We performed four experiments to evaluate PRX302 cytotoxicity and PSA specificity. In the first set of

Fig. 1



(a) Sodium dodecyl sulfate–polyacrylamide gel electrophoresis analysis of purified wild-type proaerolysin and recombinant modified proaerolysin (PRX302) with more than 95% purity. (b) High-pressure liquid chromatography elution profile of purified PRX302 with more than 95% purity.

experiments, native/untreated prostate cancer cell lines LNCaP and PC-3 (−S, −A), were exposed to PRX302. PRX302 was much more potent against LNCaP cells than against PC-3 cells (IC_{50} = 25 and 1130 mol/l, respectively) (Fig. 3). The differential sensitivity of these cells to PRX302 corresponded to the levels of PSA found in the supernatant of these cells (240 ng/ml in LNCaP and no detectable PSA levels in PC-3 cells) (Table 1).

In the second set of experiments, we tested the effects of androgen stimulation on PSA levels and cell sensitivity to PRX302. Androgen-stimulated LNCaP and PC-3 cells (−S, +A) were tested for sensitivity to this toxin. Androgen-stimulated LNCaP cells were still 50-fold more sensitive to PRX302 than androgen-stimulated PC-3 cells (IC_{50} = 23 and 1170 pmol/l). Despite a 1.6-fold increase in PSA levels, however, androgen-stimulated LNCaP cells were still as sensitive as native/unstimulated cells (IC_{50} = 25 and 23 pmol/l; Table 1). This might indicate a maximal activation of the toxin in native cells, which was not affected by the increase in PSA levels.

In the third set of experiments, we tested the effects of serum, which contains high levels of potent PSA inhibitors (α_2-macroglobulin, α_1-anti-chymotrypsin and other small molecules) on cell sensitivity to PRX302. In the presence of serum, LNCaP cells (+S, −A) had increased PSA levels compared with native/untreated (−S, −A) cells, but were 45-fold less sensitive to PRX302 with an IC_{50} = 1222 p mol/l (Fig. 4a). In the presence of serum, PC-3 cells (+S, −A) showed no increase in PSA levels and no increase in cell sensitivity to PRX302 (Table 1).

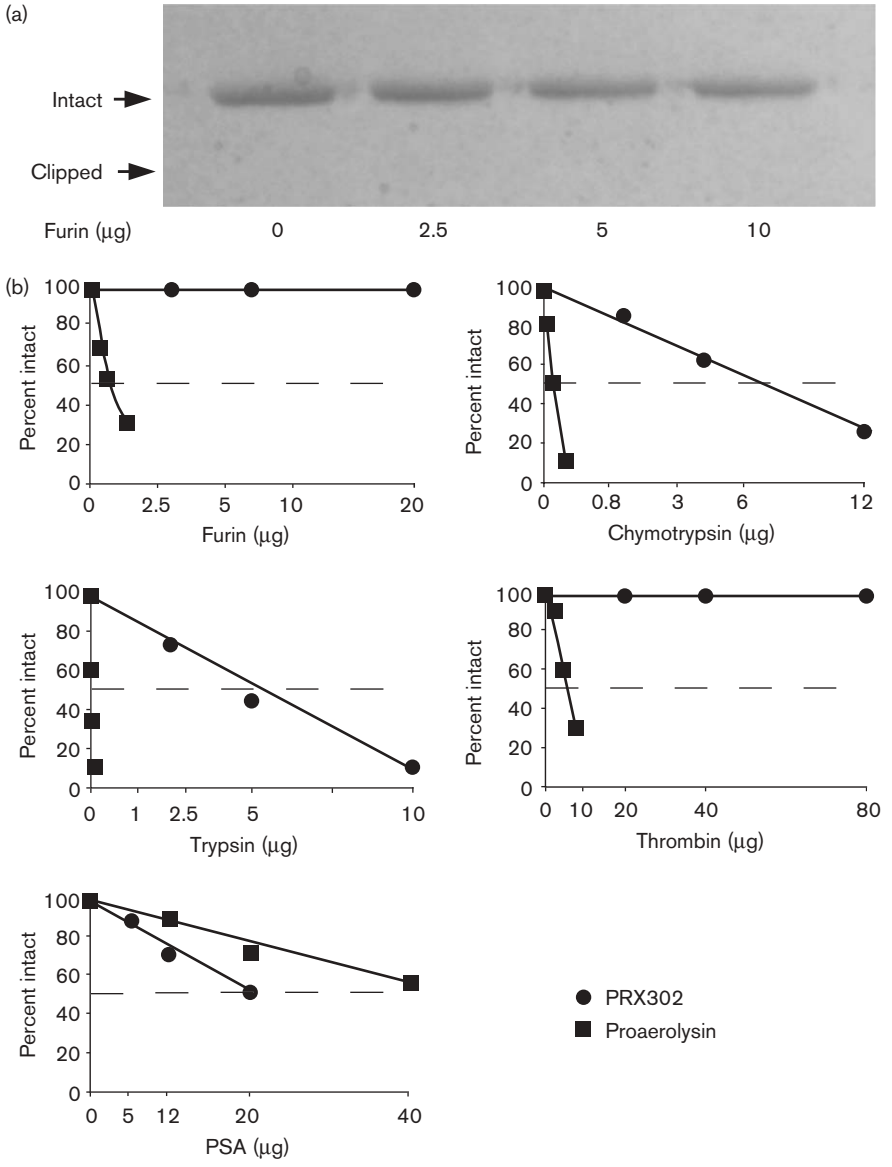
In the fourth set of experiments, we tested the effects of the addition of both androgen (increase PSA levels) and serum (inhibit PSA activity) on the sensitivity of cells to PRX302. LNCaP cells treated with both serum and androgen (+S, +A) had intermediate sensitivity to PRX302 (IC_{50} = 104 p mol/l), despite a 2.4-fold increase in PSA levels. They were more sensitive to LNCaP cells treated with serum alone (+S, −A) (IC_{50} = 1222 pmol/l) but were less sensitive to native/untreated LNCaP cells (−S, −A) (IC_{50} = 23 pmol/l). PC-3 cells treated with both serum and androgen (+S, +A) were not affected and remained not sensitive to PRX302.

Both LNCaP and PC-3 cells were sensitive to wild-type proaerolysin in all conditions with IC_{50} values below 10 pmol/l.

To test further the PSA specificity of this toxin, we sought to determine the effects of the addition of exogenous PSA on PRX302 resistance in both LNCaP and PC-3 cells. The addition of exogenous PSA increased the sensitivity of serum-treated LNCaP cells (+S, −A) to PRX302 by 8- to 10-fold, overcoming the resistance of these cells to this protoxin (Fig. 4b). The addition of exogenous PSA also reversed the resistance of native PC-3 cells (−S, −A) to PRX302 by increasing its sensitivity by 5- to 6-fold.

Normal human cells were tested for their sensitivity to wild-type proaerolysin and PRX302 (Table 2 and Fig. 4c). Prostate epithelial cells and to, a lesser extent, prostate stromal cells and normal epithelial cells (renal epithelial cells and renal proximal tubule cells) showed some sensitivity to PRX302. Human prostate epithelial cells were equally sensitive to both proaerolysin and PRX302 with IC_{50}s of 7 and 12 pmol/l, respectively, whereas renal epithelial cells, renal proximal tubule epithelial cells and prostate stromal cells were 10-fold

Fig. 2



Activity of serine proteases toward recombinant modified proaerolysin (PRX302) and proaerolysin. Various serine proteases were incubated with PRX302 and proaerolysin (5 μg) for 10 min. Aliquots of incubates were subjected to electrophoresis, Coomassie stained and quantified with a densitometer to determine the amount of enzyme necessary to produce cleavage of 50% of the compound. (a) Picture of a gel illustrating the resistance of PRX302 to cleavage by furin proteases. (b) Graphs comparing the cleavage of both PRX302 and proaerolysin by several serine proteases. The prostate-specific antigen (PSA) used in panel 5 of (b) is of commercial origin (Calbiochem, San Diego, California, USA) and might be contaminated with human kallikrein 2, which explains its ability to cleave proaerolysin. No activation of proaerolysin by PSA was seen when highly purified PSA was used (Buckley *et al.*, unpublished observations).

less sensitive to PRX302 compared with proaerolysin. Renal mesangial cells and bladder microvascular endothelial cells were the most resistant to PRX302, and were more then 25-fold less sensitive to this toxin compared with wild-type proaerolysin. All these cells had PSA levels that were below the lower limit of detection (2 ng/ml in the supernatants). Sensivity of the normal cells to PRX302 may represent effects of non-PSA proteases.

Discussion

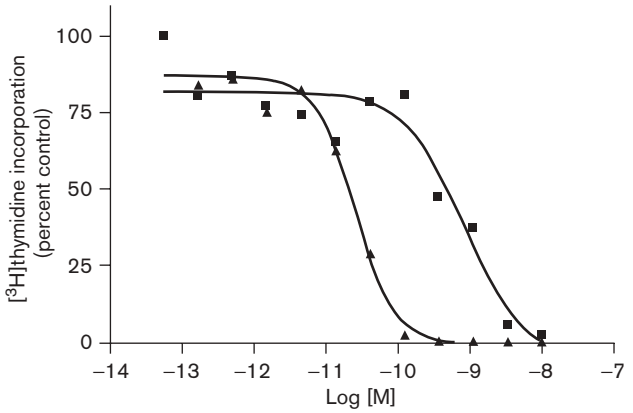
Locally advanced prostate cancer is defined as tumor extending beyond the prostatic capsule without evidence of nodal or distant metastatic spread. No standard treatment for this disease exists. Options include hormonal therapy, radiation therapy, cryotherapy, radical prostatectomy or chemotherapy [29]. Although the initial response is usually favorable, the majority of patients develop androgen-independent carcinoma with multiple metastases.

Table 1 The expression levels of PSA in LNCaP and PC-3 cells in different cell-culture conditions and their cytotoxic effects

Cell lines treated	PSA level (ng/ml)	PSA (fold increase)	Cytotoxicity (IC_{50}; pmol/l)
LNCaP (−S, −A)	240	1	25
LNCaP (−S, +A)	396	1.6	23
LNCaP (+S, +A)	576	2.4	104
LNCaP (+S, −A)	447	1.8	1222
PC-3 (−S, −A)	0	–	1130
PC-3 (−S, +A)	0	–	1170
PC-3 (+S, +A)	0	–	3700
PC-3 (+S, −A)	0	–	3800

S, 10% Charcoal-stripped fetal bovine serum; A, 10 nmol/l 5α-androstan-17β-ol-3-one (androgen); PSA, prostate-specific antigen.

Fig. 3

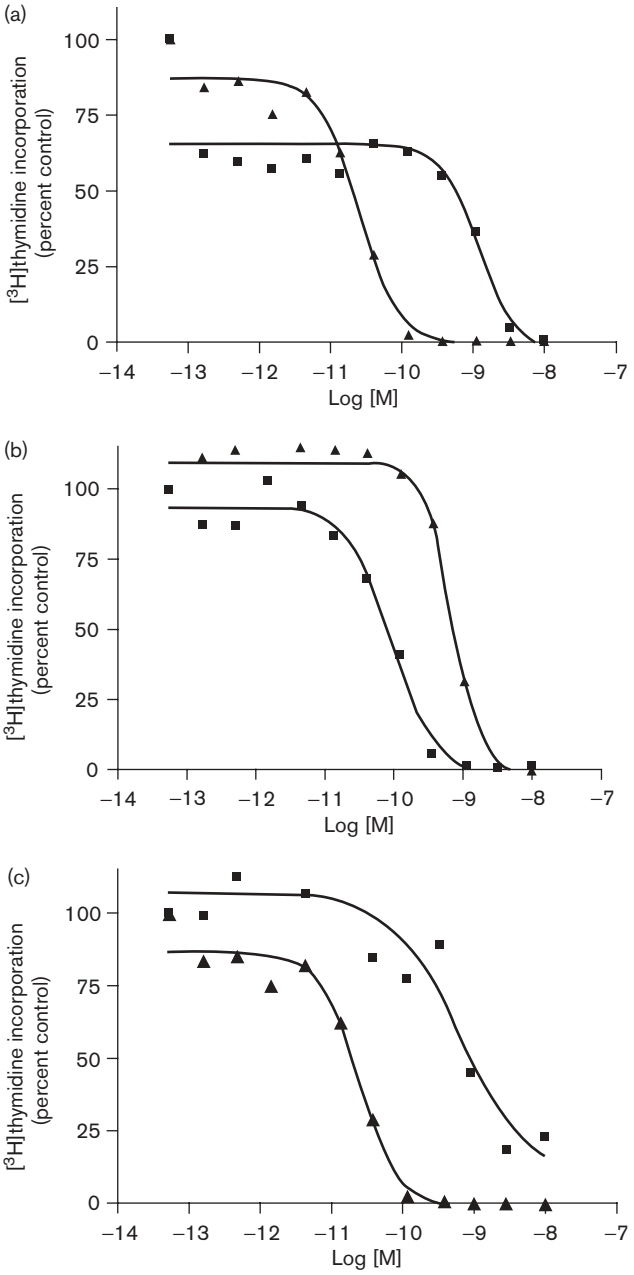


Cytotoxic effect of recombinant modified proaerolysin (PRX302) on prostate-cancer cell lines LNCaP (triangles) and PC-3 (squares) using a [^{3}H]thymidine incorporation inhibition assay. The *x*-axis represents the log of the molar concentration of toxin and the *y*-axis represents cell viability expressed as percent control of [^{3}H]thymidine incorporation in counts per minute. LNCaP cells (triangles) were highly sensitive to PRX302 and were 50-fold more sensitive to this toxin when compared with PC-3 cells (squares), IC_{50} = 25 and 1130 pmol/l, respectively.

PSA-activated prodrugs made with doxorubicin and thapsigargin could be given systemically and had minimal toxicity. The maximal response, however, was tumor-growth inhibition [15–17]. As peptide toxins are generally more potent, we sought to design a prodrug with one of these molecules. Proaerolysin was an attractive candidate because it naturally requires a protease-activation step and because it does not require cell entry, intracellular processing or translocation to accomplish cell death, as do most peptide toxins [30].

By replacing the furin-specific sequence on proaerolysin with a PSA-specific sequence, the activation of PRX302 by furin was totally abolished and its activation by PSA was increased significantly. This demonstrates that the new cleavage sequence that we introduced shifted the activation specificity of this toxin from furin-like proteases to PSA. The introduction of this new sequence also reduced activation of the protoxin by other serine proteases. PRX302 became totally resistant to thrombin cleavage, and significantly less susceptible to activation by trypsin and chymotrypsin than native proaerolysin.

Fig. 4



Prostate-specific antigen specific activation of PRX302 on LNCaP cells as determined by [^{3}H]thymidine incorporation inhibition. The *x*-axis represents the log of PRX302 concentration and the *y*-axis represents cell viability as expressed by percent of [^{3}H]thymidine incorporation in counts per minute. (a) Sensitivity of LNCaP cells treated with charcoal-stripped fetal bovine serum (IC_{50} = 1222 pmol/l) (squares) compared with native/untreated LNCaP cells (triangles) (IC_{50} = 25 pmol/l). (b) The addition of exogenous active prostate-specific antigen (squares) increases LNCaP cell sensitivity to PRX302 8 to 10-fold compared with native/untreated cells (triangles). (c) Comparison of the sensitivity of normal human bladder microvascular endothelial cells (squares) and LNCaP cells (triangles) to PRX302 (IC_{50} = 643 and 25 pmol/l, respectively).

Table 2 Cytotoxic activities of PRX302 and proaerolysin on normal cells

Cells	Origin	Proaerolysin (IC_{50}; pmol/l)	PRX302 (IC_{50}; pmol/l)
Prostate epithelial cells	Normal prostate	7	12
Renal epithelial cells	Normal renal	3	37
Renal proximal tubule cells	Normal renal	13	112
Prostate stromal cells	Normal prostate	14	158
Renal mesangial cells	Normal renal	20	531
Bladder microvascular endothelial cells	Normal bladder	4	643

PRX302, recombinant modified proaerolysin.

PRX302 was highly potent against native/untreated LNCaP cells and this potency correlated with high PSA levels produced by these cells. Earlier it had been demonstrated that LNCaP cells growing in culture produce much lower levels of PSA than those produced by human prostate cancers [23,31]. In addition, even in the absence of serum, the majority of PSA produced by LNCaP cells (i.e. $>80\%$) is enzymatically inactive primarily owing to lack of processing of the inactive proPSA precursor to active PSA [23,31]. In contrast, the majority of PSA (i.e. $>75\%$) produced by the normal prostate and in the extracellular fluid surrounding human prostate cancers is enzymatically active [31]. Therefore, although LNCaP cells are a useful model for testing PSA-activated agents *in vitro*, results obtained with these cells may not necessarily correlate with results obtained *in vivo* with PRX302-treated human prostate tissues. Native/untreated PC-3 cells on the other hand, did not produce PSA and had reduced sensitivity to PRX302. This implies that PSA is a significant contributor to PRX302 activation and potency. The addition of serum to LNCaP cells abolished their sensitivity to PRX302 and slightly increased PSA levels. This indicated that the inhibition of PSA activity through PSA inhibitors present in the serum markedly decreases the PRX302 activation and potency. This inhibition was partially reversed by the addition of androgen, which increased the PSA levels and, therefore, alleviated some of the inhibition caused by the presence of serum. The serum-induced inhibition of PRX302 potency was also reversed with the addition of exogenous PSA. Additionally PC-3 cells, not sensitive under all conditions, were rendered sensitive to PRX302 by the addition of exogenous PSA. This demonstrates the significant contribution of PSA in the activation and potency of PRX302.

Among the normal cells tested, prostate epithelial cells were the only normal cells that were highly sensitive to PRX302. This is expected because of the known production of PSA by prostate epithelial cells and because of our detection of PSA in the supernatants of these cells. Epithelial cells of renal origin and non-epithelial prostate cells (stromal) showed some sensitivity to PRX302, in spite of the absence of or greatly reduced levels of PSA, thus suggesting a possible contribution of other proteases to the activation of PRX302. This observation is supported by the fact that non-PSA-producing PC-3 cells still showed limited activation of the toxin associated with some degree of sensitivity.

The limited sensitivity of normal cells to PRX302 associated with the nature of the target disease renders it optimal for regional therapy, which would further improve its therapeutic index. Animal studies in rats and monkeys confirm the safety of local therapy with this agent [18]. In fact, despite the limited in-vitro sensitivity of certain normal cells to PRX302, no significant systemic toxicity to any organ or tissue was observed in any of the animal models with intraprostatic administration of PRX302. Furthermore, no gross injury was observed in the urethra, urinary bladder or rectum of cynomolgus monkeys treated intraprostatically with PRX302 [18]. Ultrasound-guided stereotactic delivery of PRX302 will take advantage of the extensive experience of brachytherapy techniques with locally advanced prostate cancer [32]. The material has since been produced with sufficient yield and purity to obtain US Food and Drug Administration Investigational New Drug approval (IND #12866; to be published). A clinical study of regional therapy of locally recurrent prostate cancer following radiation therapy with PRX302 has begun and no treatment-related toxicities have been observed to date at dose levels of 0.6 μg/gm prostate (Coffield, unpublished observations).

In conclusion, results suggest that PRX302 is a potent PSA-sensitive protoxin that deserves further development for regional therapy of benign and malignant prostate growths. PRX302, a member of a new class of prostate-targeted agents composed of PSA-specific protein toxins, is an addition to our armamentarium for locally advanced prostate cancer and BPH.

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