



A Prostate-Specific Antigen (PSA) Activated Channel-Forming Toxin as Therapy for Prostatic Disease

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Introduction

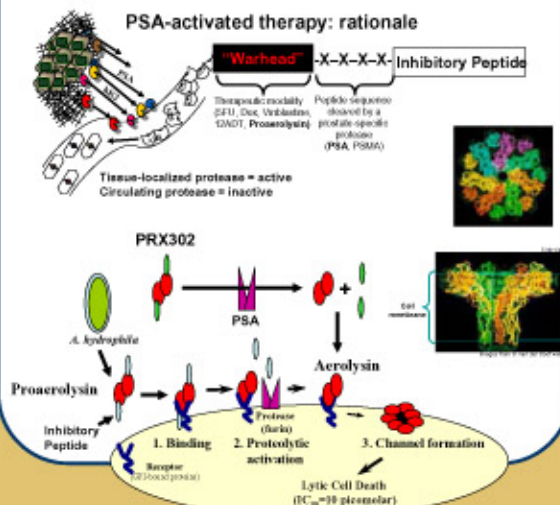
Previous studies have demonstrated that prostate cancer cells are very slow-growing. This low proliferative index could explain the relative unresponsiveness of prostate cancer to men to standard chemotherapy, which is better suited to treat fast-growing cancers. Therefore, novel therapies are required that can kill cancer cells in a proliferation-independent manner, while avoiding toxicity to normal tissues.

Our group is actively developing anti-prostate cancer agents that rely on tissue and cancer-specific proteins for their toxicity. These agents kill the prostate tissue and the prostate-derived cancer while sparing other tissues. Prostate specific antigen (PSA), in addition to being an effective screening tool, is a tissue-specific protease. We have previously demonstrated the effectiveness of chemotherapeutic agents and other compounds converted to prodrugs that become activated by the proteolytic activity of PSA. In this study we present the application of proeroerolysin (PA), converted to a form that is cleaved to activation by PSA (PSA-PAH1). PA is secreted by the bacterial pathogen, *Aeromonas hydrophila*. PA binds to target cells and is cleaved to activation by a variety of proteases. Activated PA forms pores in the cell membrane, which makes the cell permeable, leading to death. Our modified version of proeroerolysin (PSA-PAH1) is designed to be activated by PSA, with effects specific to prostate tumors.

In our characterization of PA and PSA-PAH1, we show that PA is toxic to a variety of cells at picomolar concentrations with no preference for killing prostate cancer cells. PSA-PAH1 was dramatically more effective at killing a variety of cancer cells in the presence of PSA than without the protease. While we had previously shown that PSA-producing tumor volumes were reduced when treated with a series of intratumoral injections of PSA-PAH1, here we show dose-dependent anti-tumor effects after a single treatment. By four weeks after therapy, treated tumor volumes remained as much as 40% smaller than their untreated counterparts. In contrast, no such response was observed in tumors that do not produce PSA. To assess the effect of the toxin in a PSA-producing prostate system, primates were treated with 1, 5, and 25ug of the toxin. The prostates were removed at 3 and 15 days for analysis. Analysis of the organ revealed a dose-dependent effect on the prostate tissue that remained localized to the prostate even at the highest dose of treatment. Immunohistochemistry revealed further the distribution and nature of the damage, the toxin, and that PSA is abundantly expressed in the organ.

In summary, this study confirms the application of a modified pore-forming toxin for prostate cancer therapy. We demonstrated PSA-dependent killing of prostate cancer cells by PSA-PAH1 in a variety of cells. The anti-tumor effect of PSA-PAH1 was seen in an animal model with a single dose at a variety of concentrations. Finally, we employed a PSA-expressing primate model to further display the intended application of this strategy, and show that the toxin and its effects are successfully localized to the intended organ.

PSA-Activated Proeroerolysin: Rationale and Mechanism of Action



Studies with Native Proeroerolysin (PA) reveal Lack of Therapeutic Index

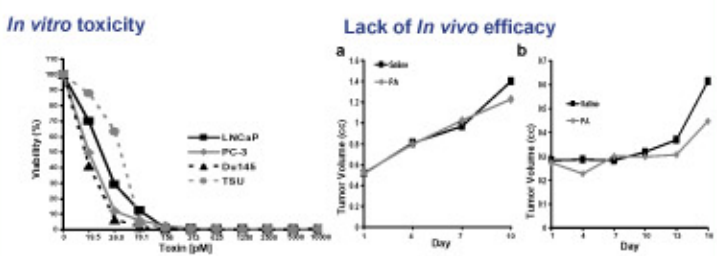
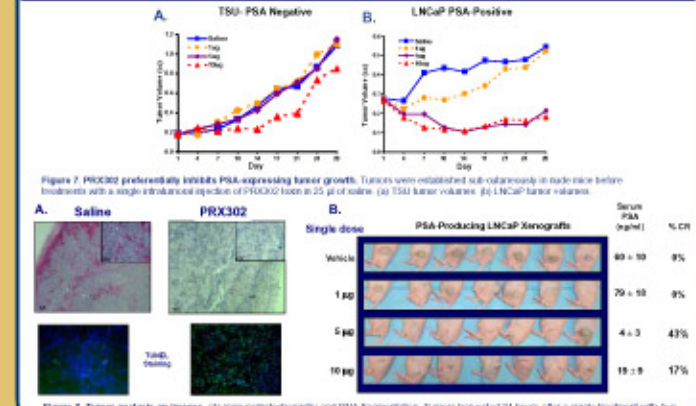


Figure 1. PA displays potent in vitro toxicity. Cells were treated for 48 hours with PA at various doses in serum-free conditions, before being subjected to the MTT assay of viability. Results were normalized to growth without toxin.

Figure 2. PA displays poor anti-tumor effect in vivo at maximally tolerated intravenous dose. TSI (a) and LNCaP (b) tumors established subcutaneously on nude mice were subjected to a series of intra-tumoral injections of 50ng of PA and volume measurements at 72 hour intervals.

Selective Anti-Tumor Effect of PRX302 on PSA-expressing Tumors



Converting Proeroerolysin to a PSA-Activated Toxin (PRX302)

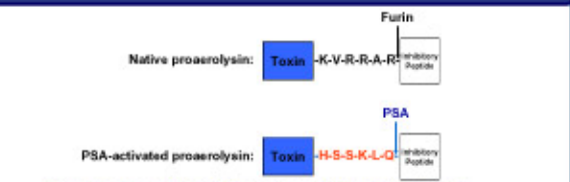


Figure 3. Conversion of PA to a PSA-targeted agent PRX302. The furin-recognition sequence of the native proeroerolysin gene was subjected to site-directed mutagenesis, converting it to a PSA-cleavable sequence.

PSA-Dependent Activation of PRX302 Causes Cell Lysis

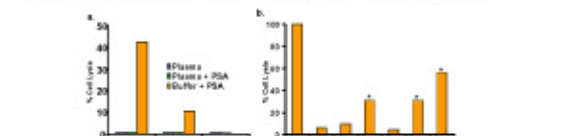
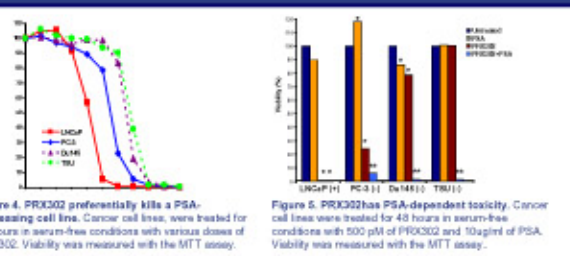


Figure 4. PRX302 preferentially kills PSA-expressing cell lines. Cancer cell lines, were treated for 48 hours in serum-free conditions with various doses of PRX302. Viability was measured with the MTT assay.

Figure 5. PRX302 has PSA-dependent toxicity. Cancer cell lines were treated for 48 hours in serum-free conditions with 500 pM of PRX302 and 10ug/ml of PSA. Viability was measured with the MTT assay.

Figure 6. PSA-dependent PRX302-induced cell lysis. (a) Red blood cells (RBC) were treated with various doses of PSA-PAH1 with 10ug/ml of PSA and 50% plasma for an hour before measures of absorbance at 540nm were made of the supernatant. (b) Media conditioned with tissue from human (human prostate conditioned media, HPCM), cynomolgus monkey (NPCM), and dog (DPCM) prostates normalized to PSA levels were expressing and subjected to the RBC lysis assay. Activity was compared to isolated PSA at an equal level.

Use of PSA-Producing Primate Prostate Model Demonstrates Ability of Intraprostatic PRX302 to Selectively Destroy Prostate Tissue

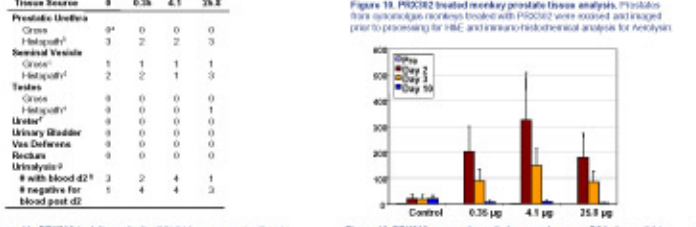


Figure 9. Human and monkey prostatic tissue compared. Normal human and cynomolgus prostatic sections were stained for morphology (hematoxylin red stain, left panels) and PSA (right panels).

Figure 10. PRX302 treated monkey prostatic tissue analysis. Prostatic tissue sections were treated with PRX302 and analyzed (prior to processing for IHC and tissue histological analysis for Androgen).

Figure 11. PRX302 toxicity analysis. PRX302 causes no significant toxicity to adjacent normal tissues compared to vehicle control.

Figure 12. PRX302 causes dramatic increase in serum PSA. Serum PSA levels in monkeys treated with increasing doses of PRX302.

Conclusions

PSA-activated proeroerolysin toxin (PRX302) demonstrates:

- PSA-dependent activation, cell lysis, and cell killing in vitro.
- Selective anti-tumor effect on PSA-expressing xenografts.
- Significant destruction of the PSA-expressing prostate with minimal effect on adjacent normal tissues.
- PRX302 is currently under evaluation in a Phase I study in the US as intraprostatic therapy for men with recurrent prostate cancer following radiation therapy to the prostate.