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*Tumoricidal effects of a PSA-activated pore-forming toxin*

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Successful prostate cancer therapy must utilize unique properties of the organ and subsequent tumors. Our group is actively developing anti-cancer agents processed to toxicity by prostate-specific proteases. This strategy relies on the localization of the tissue-specific enzyme's activity to the tumor microenvironment.

Previously, we used the serine protease and common marker of disease progression, prostate-specific antigen (PSA), to activate prodrugs. In this study, we present the therapeutic utilization of the first bacterial toxin, proaerolysin (PA), activated by PSA. PA is a pore-forming cytotoxic agent secreted by the bacterial pathogen, *Aeromonas hydrophila*. Once bound to the target cell and activated by the ubiquitous cell-surface protease furin, aerolysin forms pores in the cell membrane, causing death. Our mutated version of proaerolysin (PSA-PA) is designed to be activated by PSA, with effects specific to prostate tumors.

In our characterization of PA and PSA-PA, we show that PA has broad-based toxicity in vitro at Picomolar (pM) concentrations. PA caused an approximate 90% loss in viability to a panel of prostate cancer cell lines. The PSA-PA variant caused red blood cell lysis in a PSA-dependent fashion that is inhibited by serum. PSA-PA was 10-fold more toxic to PC-3 cells when PSA was in the media with an IC50 of 250 pM following 48 hours of exposure. To confirm that the therapeutic window was due to PSA-dependent

toxin processing, treated PC-3-derived whole cell lysates were immunoblotted for aerolysin. The PSA-PA variant was selectively cleaved, generating pore-forming heptamers by 48 hours. PSA-producing LNCaP xenografts were treated with a series of 1mg intratumoral injections of PSA-PA. By the end of the treatment series, PSA-PA-treated LNCaP tumor volumes were 50% of their saline-treated counterparts and the mice showed no signs of toxicity. The difference in tumor volume was confirmed by tumor weight. Mouse blood PSA levels showed a 3-fold difference between treatment conditions, suggesting an under representation of toxin effect by volume and weight. TUNEL staining revealed that the PSA-PA-treated LNCaP tumors contained large areas of fragmented DNA, suggestive of cell death, whereas no such staining was seen in the saline-treated tissue. In contrast, PSA-null TSU tumor xenografts displayed no reduction in tumor volume as a result of PSA-PA treatment.

In summary, this study establishes for the first time the use of a modified pore-forming toxin for prostate cancer therapy. PSA-dependent killing of prostate cancer cell lines by PSA-PA was due to selective toxin processing by PSA. The antitumor effect of PSA-PA was also seen in a xenograft model, and was the result of cell killing. This strategy may be employed to develop other biological agents that kill by disrupting the cell surface in a PSA-dependent fashion. We hope to adapt PA and similar agents for systemic therapy.