is only found in primates and humans, PRX302 was subsequently injected into monkey prostates and toxicity evaluated.
Results: PA is cytotoxic to prostate cancer cells in vitro but no therapeutic index could be achieved by intratumoral injection into prostate cancer xenografts. Substitution of the PSA-selective activation site for the furin site neutralized furin activation. PRX302 is activated by purified PSA and produces PSA-dependent cell killing at picomolar concentrations in vitro. PRX302 was stable to activation in human plasma containing high levels of PSA (10,000 ng/ml). Single dose intratumoral injections of 1–10 micrograms of PRX302 produced significant and often complete tumor and biochemical (i.e. no measurable serum PSA) regression of PSA-secreting human prostate cancer xenografts. Injection of a single 25 μl dose of PRX302 into non-PSA producing dog prostates produced no effect at doses up to 400 μg of PRX302. In contrast, injection of 0.35 μg and 4.1 μg of PRX302 into PSA-producing prostates of cynomolgus monkeys resulted in destruction of ~25% and 50% of the prostate gland respectively. This extensive damage was confined to the prostate with no toxicity observed in any other normal tissue including those tissues adjacent to the prostate (e.g. urinary bladder, urethra, rectum, seminal vesicles).
Conclusions: Our observations demonstrate the potential for intraprostatic application of this engineered PSA-activated protoxin for the treatment of locally recurrent or advanced prostate cancer and for benign prostatic hyperplasia (BPH). A phase I clinical trial for men with locally recurrent prostate cancer after definitive radiation therapy is currently in progress.

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Fibroblast Activation Protein (FAP) activated anti-stromal prodrug therapy for cancer

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Background: The tumor stroma compartment represents a major component of the mass of most carcinomas. Reactive tumor stromal fibroblasts differ from fibroblasts of normal adult tissues in regards to morphology, gene expression and production of important biological mediators such as growth factors and proteases. A highly consistent trait of tumor stromal myofibroblasts is the induction of fibroblast-activation protein (FAP), a membrane-bound serine protease that has dipeptidase and gelatinase/collagenase activity. FAP is not expressed in normal adult tissues, but has been shown to be selectively expressed by stromal myofibroblasts in >90% of epithelial cancers examined in one study with minimal expression in either cancerous epithelial or adjacent normal tissues. Therefore, a FAP-activated prodrug strategy is proposed that takes advantage of FAP's enzymatic activity to selectively activate a highly potent cytotoxin thapsigargin (TG) in the peritumoral fluid leading to death of FAP positive tumor stromal cells while at the same time generating a bystander effect that also results in death of tumor and endothelial cells.
Material and Methods: A map of FAP cleavage sites within recombinant gelatin was generated using LC-MS-MS and from this map a series of peptides were tested as FAP substrates. The best FAP substrate was coupled to a TG analog to create a FAP-activatable prodrug that was screened for plasma stability and for in vitro toxicity against FAP-positive fibroblast and negative cancer cell lines. The FAP prodrug was then tested for efficacy against human breast cancer xenografts.
Results: Analysis of the gelatin cleavage sites following FAP digestion showed preferred cleavage after proline, but also cleavage after alanine and glycine. Glycine was the preferred in the P2 position, while alanine was preferred in P'1. Seven amino acid fluorescence quenched peptides from the cleavage map were readily cleaved by FAP with Km values of 5–50 micromolar. A consensus peptide was also readily cleaved by FAP. This peptide was coupled to a potent 8-O-12 aminododecanoyl analog of TG (12ADT) to produce the FAP activated prodrug. This prodrug was stable to hydrolysis in human and mouse plasma and was selectively toxic to FAP positive cells in vitro. Preliminary in vivo studies demonstrated significant antitumor activity against MDA-MB-231 human breast cancer xenografts with no significant toxicity to treated animals. Further studies are underway to evaluate in vivo toxicity, pharmacokinetics, biodistribution and efficacy against a panel of breast cancer xenografts producing varying amounts of stroma.
Conclusions: Advantage can be taken of FAP's selective expression by reactive fibroblasts within human tumors and its unique enzymatic activity to generate prodrugs that are selectively activated within human cancers with minimal side effects to normal host tissues.

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Gemcitabine prodrug has efficacy when dosed orally in a human colon tumor xenograft model

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Clinically, Gemzar® (gemcitabine hydrochloride) shows good efficacy as an anticancer drug used in the treatment of pancreatic, NSLC, and breast cancer when dosed intravenously. When gemcitabine is dosed orally to mice, the drug causes gastrointestinal toxicity. In an effort to improve oral delivery of gemcitabine, the current study examines a gemcitabine prodrug (LY) for its properties in the treatment of a human colon tumor xenograft model. LY is a prodrug that has little to no cytotoxic activity in 3-day proliferation assay using two cell lines, the acute promyelocytic leukemia HL-60 cells and the human colon carcinoma HCT-116 cells. LY was stable in mouse and human small intestinal epithelial homogenates, but was susceptible to hepatic hydrolysis in vitro. When dosed orally to mice, high plasma concentrations of intact prodrug were detected in the portal vein. The prodrug was then hydrolyzed systemically to release gemcitabine. LY demonstrated significant anti-tumor activity in the HCT-116 colon tumor xenograft model when administered on three dose schedules (qd × 14, q2d × 7, q3d × 4). A dose response was observed in all studies and a margin of safety of 2 on at least one dose schedule. LY may be clinically efficacious after oral administration and may provide important new applications for this drug.

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An albumin-binding prodrug of doxorubicin that is cleaved by prostate-specific antigen: development and biological evaluation in an orthotopic mouse model

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Metastatic prostate cancer is difficult to treat, and to develop better compounds, good models mimicking the characteristics of human disease are required. Such a model was developed using the PSA-positive prostate cell line LnCAP implanted orthotopically into SCID mice. Transduction of the cell line with a retrovirus encoding a luciferase-neomycin resistance fusion protein, generating LNCaP LN, was used to monitor the growth of the tumors in the mouse prostate via in vivo bioluminescence. Luciferase assays from organ homogenates allowed the detection of metastasizing tumor cells, identifying the lung as a prime target.
Prostate-specific antigen (PSA), a serine protease that is over-expressed in prostate carcinoma, represents a molecular target for selectively releasing an anticancer agent from a prodrug formulation. An albumin-binding prodrug, EMC-Arg-Arg-Ser-Ser-Tyr-Tyr–Ser-Gly-DOXO [EMC: ε-maleimidocaproic acid; DOXO: doxorubicin], was efficiently cleaved by PSA at the P_1–P'_1 scissile bond releasing the doxorubicin dipeptide Ser-Gly-DOXO. Albumin is a suitable carrier of cytostatic agents due to passive accumulation in solid tumors. EMC-Arg-Arg-Ser-Ser-Tyr-Tyr–Ser-Gly-DOXO showed no in vivo activity in the PSA-negative PC-3 model, but good activity in the CWR22 PSA-positive model [1]. However, cleavage with PSA released the less active doxorubicin dipeptide DOXO-Gly-Ser, and not doxorubicin. Thus, to improve the compound, a spectrum of amino acids was tested that allow free doxorubicin to be released. An arginine residue proved effective and the doxorubicin dipeptide Ser-Arg-DOXO was degraded to doxorubicin in PSA positive xenografts (CWR22, LNCAP). The new prodrug EMC-Arg-Arg-Ser-Ser-Tyr-Tyr–Ser-Arg-DOXO bound rapidly to the cysteine-34 position of albumin, and was efficiently cleaved by PSA, releasing Ser-Arg-DOXO.
We thus used our orthotopic LNCaP model to test this novel targeted compound. SCID mice were injected orthotopically with the LNCaP LN cell line. Mice bearing tumors were readily randomized according to the in vivo luciferase signal, and treated with the novel formulation. Results from the treatment, analysed in vivo using bioluminescence, and at necropsy via primary tumor size, as well as metastatic burden, will be presented.

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