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Regulated Apoptosis and Immunogene Therapy for Prostate Cancer

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Purpose: The central tenet of this proposal is that methods to effectively trigger apoptosis within prostate tumors can both reduce tumor burden and elicit adaptive immunity, provided a pro-inflammatory environment can be created.

Scope: Previously created inducible caspases (iCaspases) have been used as the basis of both a prophylactic vaccine and as treatment of pre-existing subcutaneous (sc) and autochthonous TRAMP-derived prostate tumors. While these studies are centered on prostate cancer, they could be extended to other tumor types.

Major findings: The combination of iCaspases and IL-12 can completely eliminate small (≤ 40 mm³) sc tumors and largely eliminate larger (≤ 100 mm³) tumors while IL-12 alone had minimal effect and iCaspase alone had no significant effect. Anti-tumor efficacy mirrored expansion of anti-tumor cytotoxic T lymphocytes and IFN-γ-producing cells from splenocytes of vaccinated animals. Further, orthotopic injections into the prostates of tumor-bearing TRAMP mice trigger apoptosis, secondary necrosis and inflammation. Finally, in transgenic animals, the hK2-E3/F, PSA-E2/F and ARn-PB composite promoters are highly active in prostate epithelial cells and largely prostate specific.

Significance: This work lays the groundwork for an "off-the-shelf" injectable immunogene therapy that could treat prostate cancer as a neoadjuvant therapy or possible less mutagenic treatment for metastatic disease.
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INTRODUCTION:
Currently, there are no effective treatments for men with androgen-independent metastatic prostate cancer. Among systemic therapies, including chemotherapeutic combinations, novel biological targets linked to radiopharmaceuticals, and bone-targeting bisphosphonates, all are palliative at best and usually demonstrate high morbidity associated with their mutagenicity. In contrast, new treatment modalities that target tumor antigens or tumor vasculature may treat disseminated disease with lower side effects. Immunotherapies that require patient-tailored cell culturing or knowledge of tumor antigens would be prohibitively expensive for most men. Therefore, we set out to develop a potentially injectable immunotherapy that could be used as a stand-alone or neoadjuvant therapy. This novel approach is based on tissue-specific expression of pharmacologically activated caspases that can kill slowly dividing prostate cancer cells in the primary (or secondary) tumor in a pro-inflammatory environment. We previously described the proof-of-principal of this method. Herein, we describe further testing of vaccination of pre-existing sc and orthotopic tumors and testing of various prostate-specific promoters.

BODY:
Following is a list of tasks along with a summary of progress to date:

**Task 1: Test the hypotheses that iCaspases can trigger apoptosis in normal and malignant prostate epithelial cells in vivo.**

We previously demonstrated that iCaspases can trigger apoptosis in syngeneic TRAMP-

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**Fig. 1. CID activation of inducible caspases leads to necrosis and lymphocyte infiltration in 20-wk TRAMP prostates.** H&E staining of prostates processed 10 –days after CID injection of ADV-iCasp1 treated prostates (B) or control ADV/c-treated prostates (A). While tumor progression is highly variable in TRAMP model, widespread necrosis was only (and reproducibly (3/3)) seen in iCaspase1-treated ventral prostates.

based tumors[1] and LNCaP xenografts[2]. To demonstrate iCaspase killing in mouse prostates, we have injected ADV/CMV-iCaspase-1 [1] into the ventral prostate lobes of 20-week old TRAMP mice, which should contain primarily poorly differentiated adenocarcinoma in most animals [3]. While control virus led to no obvious increased necrosis over wild-type mice in 3/3 animals, transduction with ADV/CMV-iCaspase-1 followed by intraperitoneal CID injection 3 days later, led to widespread necrosis (viewed after 10 additional days), demonstrating that prostate cancer cells are highly sensitive to caspase-1 activation. (Fig. 1). Due to the high variability of the TRAMP model, we will repeat this experiment with younger mice and with control adenovirus, expressing EGFP to identify cells most efficiently transduced with adenovirus.

To demonstrate the utility of adenoviruses expressing tissue-restricted iCaspases, we are currently injecting adenovirus ADV/ARR3PB-iCaspase9 [2] into the ventral lobes of normal and TRAMP prostates.

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**Fig. 2. Cover art from Mol Endo (3/04) showing tissue-specificity of hK2-E3/P promoter in vivo driving luciferase activity primarily in the prostate.** Transgenic mice were injected with 1 mg D-Luciferin and imaged (for 30") 15' later (left) with an IVIS-imaging system or ex vivo (right) ~45' later.
Moreover, we have made transgenic mice expressing luciferase under the influence of three different prostate-specific promoters, hK2-E3/P [4, 5], ARR2-PB [6] and a composite PSA-based promoter, PSA-E2/P [2]. In addition to the published EZC-Prostate line based on the human kallikrein 2 promoter, hK2-E3/P (Fig. 2) [5], selected founder lines based on the rat probasin-based promoter, ARR2-PB, and PSA-E2/P also show high-level tissue-specific reporter expression. Further, when crossed the hK2-E3/P-Luc mice onto the TRAMP background, we could detect metastatic prostate cancer in intact tissues using luciferase expression as a reporter (Fig. 3). Similar experiments are underway with the PSA and probasin-based promoters. Therefore, it is very likely that adenoviruses using the ARR2-PB promoter should express well in prostate tissue.

Fig. 3. Prostate Metastasis in TRAMP x hK2-E3/P-Luc mice express luciferase. 24-week bigenic mice bearing large tumor burdens were injected with 1 mg D-Luciferin. After 20', organs were dissected and arranged on black paper. 45' later, light (left) or chemiluminescent(right) microcopy was performed on Lung(LG), Liver, (LV), Spleen (SP), Kidneys (K), and other tissues. Note regions of metastasis (white in left panel) correspond to luminescence, which is not seen in control mice (not shown).

**Task 2.3. Test the hypotheses that inducing apoptosis in prostate adenocarcinoma cells will induce a T_H1-biased immune response. Test the hypotheses that triggering apoptosis in the context of a T_H1-induced cytokine milieu will evoke or augment an anti-tumor immune response.**

Tasks 2 and 3 have been combined and are being tested simultaneously in some experiments. To test this hypothesis in sc TRAMP tumors, groups (n =5) of mice bearing small (≤ 35 mm³) and medium (≤100 mm³) tumors were injected intratumorally with adenovirus expressing iCaspase-1 (as above), ADV-IL-12 (expressing IL-12), both or neither (i.e. ADV/c), and tumor sizes were estimated via calipers biweekly. All groups were controlled for total viral particles. Although injection of ADV-IL-12 showed some efficacy in small tumors, the combination of iCaspase-1 (+ CID) and IL-12 led to complete elimination of small tumors. No other group, including non-treated tumor-bearing mice, showed any efficacy (Fig. 4). Further, CTL activity and IFN-γ producing cells in splenocytes from optimally vaccinated mice showed optimum expansion (Fig. 5 and not shown). When medium sized tumors were injected, trends were similar with optimally treated tumors averaging 10% of controls, however only 2/5 mice were completely tumor-free (not shown).

Fig. 4. Treatment of small TRAMP-C2 tumors with Ad-iCasp1 and Ad-IL-12. TRAMP-C2 tumors were established in syngeneic mice following injection of 10⁶ cells. When average tumor size was ~ 35 mm³, tumors were injected with equal numbers of adenovirus, containing IL-12, iCaspase1, both or neither (Ad-c). Control tumors were mock injected. Tumor sizes were estimated with vernier calipers. Average tumor size ± std dev. shown. Fraction of mice (out of 5) with no tumors is also shown.
To test the central hypothesis of this task in autochthonous tumors, we have injected 12-week TRAMP mice intraprostatically with ADV-iCaspase-1 plus ADV-IL-12 or control ADV/C. After two weeks a second injection was performed. Approximately 80% of mice survived the double-survival surgery. Currently, the mice are ~35–weeks old and 3/9 control mice have died (euthanized after heavy tumor burden led to signs of discomfort), while 0/7 immunized mice have died. After 80% of control mice have died, remaining viable animals will be assayed for splenic CTL and IFN-γ producing cell activities.

![Graph showing cytotoxicity](image)

**Fig. 5. Treatment of tumors with Ad-iCasp1 and Ad-IL-12 leads to potent CTL activity.** Splenocytes from vaccinated mice bearing initially medium-sized tumors (≤ 100 mm³ at first ADV injection) were cultured for either 7 days in T-Stim® without re-stimulation or with restimulation with TRAMP-C2-pulsed DCs. % cytotoxicity reflects % specific release of ⁵¹Cr-loaded TRAMP-C2 target cells (pretreated with IFNγ to increase MHC class I expression). Average of 3 mice/group euthanized at conclusion of experiment (~ day 50 after first virus injection) shown.

**Task 4. (Optional) Test the utility of using the EZC-Prostate model to measure tumor growth in vivo following vaccination.** Year 2-3.

We are currently assessing the ability to monitor changes in prostate size in living tumor-prone TRAMP mice and in our inducible prostate cancer model, called JOCK, based on a dimerizer-inducible, prostate-targeted version of FGFR1. Initial results suggest that as the prostate becomes more hyperplastic and dysplastic luciferase activity actually decreases perhaps due to changes in promoter activity, vascularity, or both. We are currently validating Living Image® data with direct anti-luciferase immunohistochemistry. If the promoters are in fact “self-limiting” in transformed tissue, we will proceed with development of a Cre/Lox-based prostate reporter line that converts ARR3PB-based Cre recombinase expression to a constitutive EF1α-based promoter driving luciferase. Final testing of this construct is underway.
KEY RESEARCH ACCOMPLISHMENTS:

- Demonstration that iCaspases can kill prostate adenocarcinoma cells in autochthonous tumors

- Demonstration that combination vaccines with iCaspase-1 and IL-12 can eliminate small sc tumors and greatly retard medium-sized sc TRAMP tumors in syngeneic mice.

- Demonstration that all three composite prostate cell line-specific promotors, hK2-E3/P, PSA-E2/P and hK2-E3/P are active in prostate cells and prostate cancer cells in SC tumors.

REPORTABLE OUTCOMES:

In preparation

CONCLUSIONS:

Our goal is to develop an injectable vaccine for advanced prostate disease that does not depend on antigen characterization or ex vivo culturing of patient tissue. Towards this goal we have demonstrated a combinatorial vaccine based on inducible caspases and the cytokine adjuvant IL-12. These are expressed in an adenoviral vector, which has been injected intratumorally. When these vectors are injected into sc tumors, complete tumor regression is possible in small tumors and significant regression is seen in larger tumors, corresponding to expansion of tumor-specific CTL and IFNγ-expressing cells from splenocytes of vaccinated mice. We have also developed and begun testing delivery of tissue-specific iCaspases to the prostates of TRAMP mice. Initiation of intra-prostatic vaccines is underway.

These studies should be the basis of a novel vaccine approach that should be easily converted to a clinical protocol provided the results show efficacy. Immunotherapy such as this is likely to be more effective and better tolerated than chemotherapies to eliminate disseminated disease, since androgen-independent metastasis often coincides with increased resistance to chemotherapy.
REFERENCES: