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14. ABSTRACT

In the past year significant progress has been made towards completion of the project goals as outlined in the original statement of work. We have succeeded in establishing PC3-derived cell lines that are deficient in protein expression for apoptosis inducing factor (AIF), X-linked inhibitor of apoptosis (XIAP), and both proteins simultaneously. AIF-deficient cells have been further characterized, and we have determined that these cells display no differences in either sensitivity to death-inducing stimuli or in vitro growth characteristics when compared to control cells. When implanted subcutaneously into immunocompromised mice, AIF-deficient PC3 cells display significant impairment in both the establishment rate and tumor growth characteristics in vivo. These data suggest that while AIF protein expression is not required for cell growth in culture, AIF plays a significant role in supporting the ability of PC3 cells to establish tumors and grow in vivo. Overall these data support the hypothesis that AIF supports prostate cancer pathogenesis, and experiments currently underway will determine those properties of AIF that are most critical for prostate tumor growth.

15. SUBJECT TERMS
Prostate Cancer; X-linked Inhibitor of Apoptosis; Apoptosis Inducing Factor

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Table of Contents

Introduction

As the second most common form of cancer diagnosed among men in the western world prostate cancer represents a significant healthcare threat, and there is great need for new and more effective treatments for this disease. This research project seeks to determine the potential role of two proteins, apoptosis inducing factor (AIF) and X-linked inhibitor of apoptosis (XIAP), in prostate cancer pathogenesis. Both proteins are multifunctional, and one of our goals is to determine which properties of each molecule are important for disease progression. This report summarizes progress made in the past year towards completing these research goals.

Body

We have made significant progress towards completing the tasks outlined in our original Statement of Work, as summarized below:

Task 1a. To determine the effects of AIF and XIAP on tumorigenesis.

- **Generation of PC3-derived cell lines that stably lack expression of endogenous AIF, XIAP, or both (Months 1-9).** PC3 cells have been successfully infected with lentiviruses containing RNAi sequences targeting AIF and XIAP. Stable, clonal cell lines have been isolated using fluorescence activated cell sorting, and suppression of protein expression has been determined by immunoblot analysis (Supporting Data Figure 1). Nearly complete ablation of XIAP and AIF protein expression has been achieved. Control and AIF-deficient cells have been evaluated both for in vitro growth (Figure 2) and for differences in sensitivity to a panel of chemotherapy drugs (Figure 3). No differences in the in vitro growth rates or sensitivity to chemotherapeutics were observed between AIF-deficient and AIF-proficient cells.

- **Assessment of tumor progression (Months 10-18).** Control and AIF-deficient PC3 cells have been implanted subcutaneously into athymic nude mice. Tumor progression of these cells has been followed over time, and we have observed a substantial decrease in the rate of tumor growth of AIF-deficient cells compared to controls (Figure 4). Immunoblot analysis of tumor lysates confirmed that AIF protein expression was not reactivated during tumor growth (Figure 4).

Task 1b. To determine the specific properties of AIF and XIAP required for tumorigenesis.

- **Generation of PC3-derived "restoration" cell lines expressing AIF and XIAP variants lacking defined functions (Months 10-18).** We have succeeded in producing lentiviruses containing sequences to express wildtype AIF and the AIF variants T263A/V300A (TVA mutant) and K255/265/510/518A (K4A mutant). As described in our original proposal, the TVA mutant lacks enzymatic activity but retains death inducing functions, whereas the K4A mutants retains enzymatic activity but fails to induce cell death (Urbano et al., 2005). These viruses have been used to subject AIF-deficient PC3 cells derived above to a second round of lentivirus infection. Cells are currently in the process of drug selection.

- **Assessment of the PC3-derived tumor progression (Months 18-27).** Experiments are awaiting establishment of cells described in the section above, and are currently on schedule.

Task 2. To investigate the contribution of AIF to tumorigenesis in a transgenic murine model of cancer**.**

- **Establish a breeding colony of prostate-specific *Pten*-deficient mice (Pten$^{-/-}$) (Months 18-24).** Experiments are on schedule to begin in year 2 of study.

- **Breed Pten$^{-/-}$ with AIF-deficient (Hq) mice (Months 24-30).** Experiments are on schedule to begin in year 3 of study.

- **Evaluation of tumor progression/burden in Pten$^{-/-}$/Hq mice (Months 30-36).** Experiments are on schedule to begin in year 3 of study.

Key Research Accomplishments

- PC3 cells deficient in AIF, XIAP, and both proteins have been established
- It has been determined that the loss of AIF does not affect the in vitro growth characteristics of PC3 cells
- AIF protein ablation has no effect on the sensitivity of PC3 cells to a wide range of cell death-inducing stimuli
- It has been shown that loss of AIF dramatically slows the growth of PC3 cells when examined in a mouse xenograft tumor growth model
- Lentiviruses containing expression sequences for the AIF mutants T263A/V300A and K255/265/510/518A have been created
- AIF-deficient PC3 cells have been infected with lentiviruses containing the AIF variants T263A/V300A and K255/265/510/518A

Reportable Outcomes

None to date

Conclusions

Based on the progress so far, current conclusions include: 1) loss of AIF in PC3 cells has no affect upon the in vitro growth characteristics of these cells, 2) AIF does not contribute, either positively or negatively, to the death of PC3 cells induced by a variety of cell death stimuli, 3) loss of AIF severely compromises the ability of PC3 cells to form and grow tumors in a mouse xenograft model. Overall these conclusions suggest that in androgen-independent prostate cancer cells (PC3) AIF is not required for growth in vitro but is critical for normal tumor growth in vivo, and are consistent with the hypothesis that AIF is a relevant target for molecular therapeutic intervention. Studies currently in progress are designed to determine which properties of AIF are necessary for support of tumor growth in vivo.

References

Urbano, A., Lakshmanan, U., Choo, P.H., Kwan, J.C., Ng, P.Y., Guo, K., Dhakshinamoorthy, S. and Porter, A. (2005) AIF suppresses chemical stress-induced apoptosis and maintains the transformed state of tumor cells. *Embo J*, **24**, 2815-2826.

Supporting Data

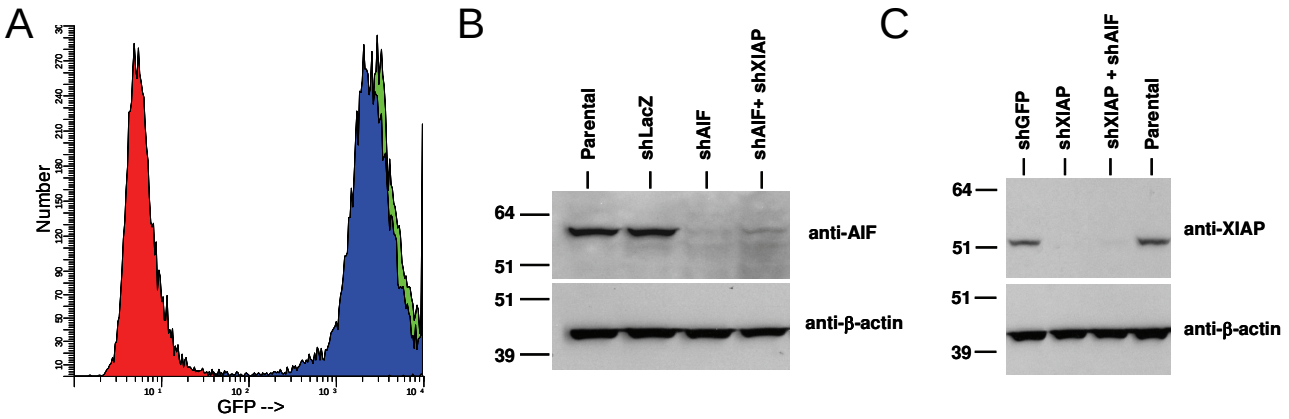

Figure 1. Establishment of AIF-Deficient PC3 Cells. PC3 cells were infected with control (shLacZ, shGFP), shAIF containing, shXIAP containing, or both shAIF and shXIAP containing lentiviruses. GFP (shLacZ, shAIF) and dsRed (shGFP, shXIAP) positive cells were then sorted by flow cytometry. A) Representative flow cytometry histograms for parental (red), shLacZ (blue) and shAIF (green) PC3 cells. B) Anti-AIF immunoblot analysis of sorted cells demonstrating near complete ablation of AIF protein in cells infected by shAIF lentivirus. C) Anti-XIAP immunoblot analysis of sorted cells demonstrating near complete ablation of XIAP in cells infected by shXIAP lentivirus.

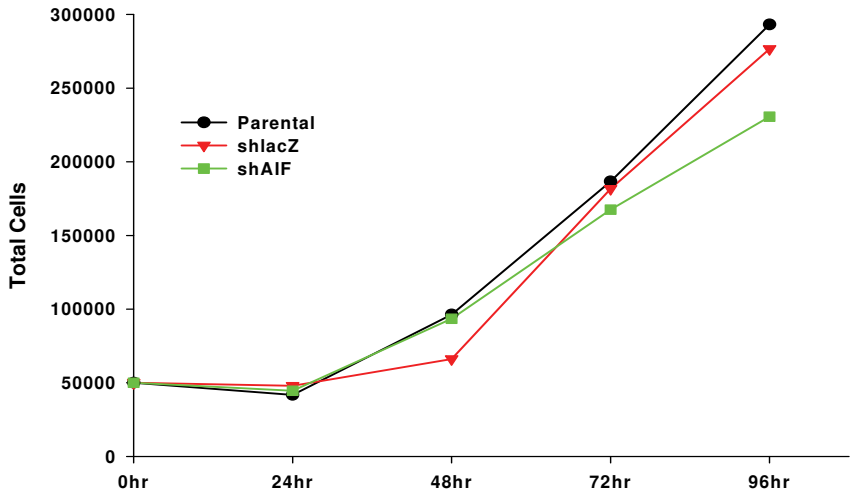

Figure 2. Loss of AIF does not affect growth rate of PC3 cells in vitro. Parental, shLacZ, and shAIF PC3 cells were seeded in replicate wells at identical densities in six well plates. Beginning at 24 hours and continuing daily, cells were harvested and total cell number was determined by Coulter counting. Data shown is representative of four independent experiments. As indicated by the data, AIF deficient PC3 cells grow at the same rate in vitro as parental and shLacZ control cells, suggesting that AIF protein is not required for normal growth in culture.

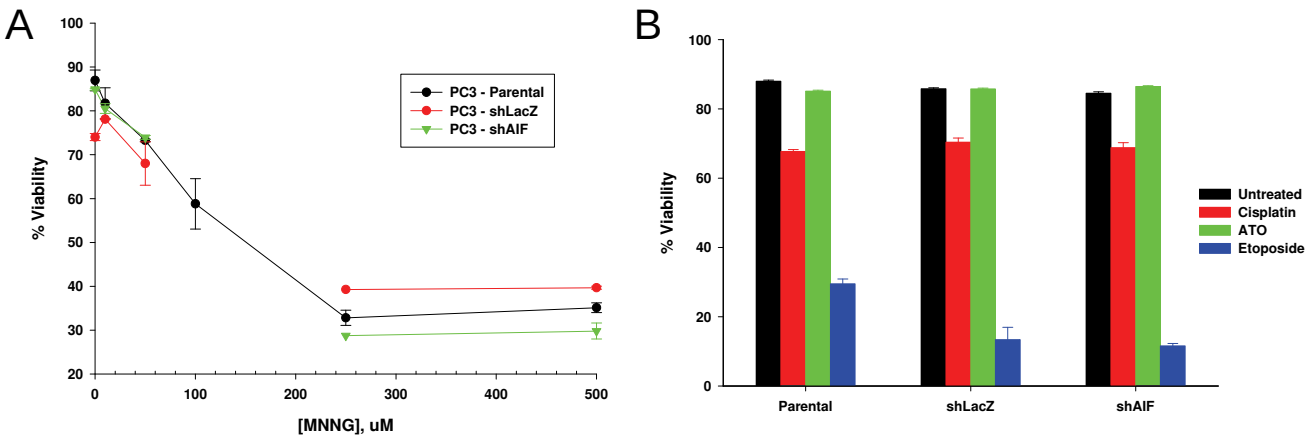

Figure 3. Sensitivity of PC3 cells to death-inducing stimuli is not altered by AIF ablation. A) Parental, shLacZ, and shAIF PC3 cells were treated with increasing concentrations of the DNA alkylating agent MNNG for 15 minutes. Cell were then washed and incubated overnight in normal growth media. Cell viability was then assessed by propidium iodide staining followed by flow cytometry. B) PC3 derived cells were treated overnight with the chemotherapy drugs cisplatin, arsenic trioxide (ATO) and etoposide. Viability was then determined as described in panel A. Note that AIF ablations has no effect on the sensitivity of PC3 cells to any of the treatments employed. These data suggest that AIF is not a critical component either for survival following drug treatment, or for the ability of these drugs to induce cell death.

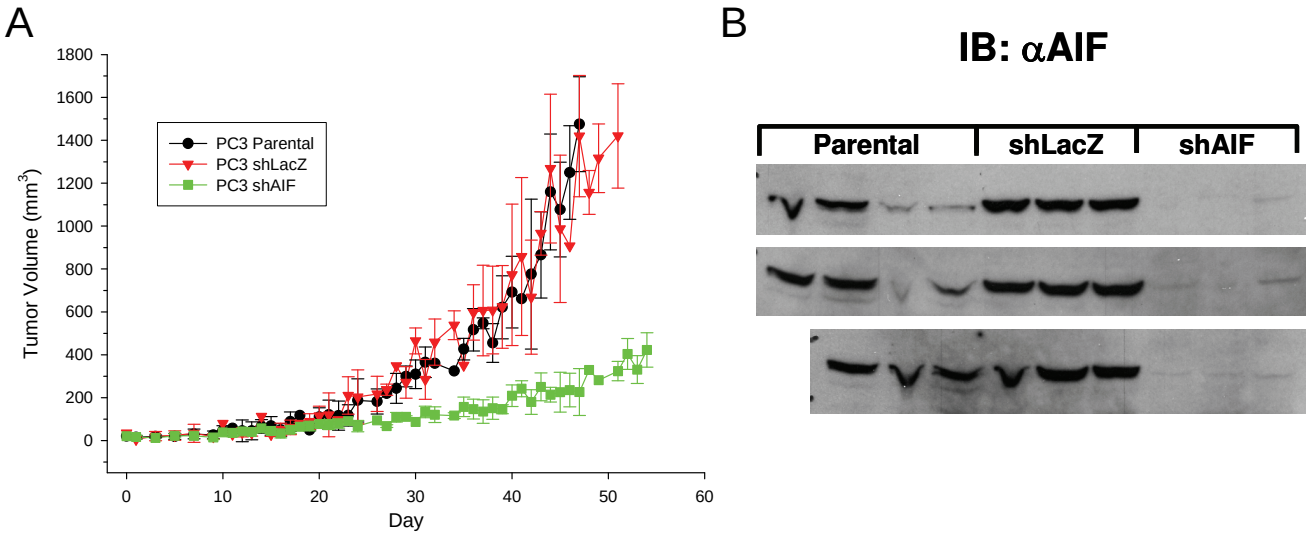

Figure 4. Growth of PC3-derived cells following implantation in nude mice. A) 3 x 10^6 parental (Black), shLacZ (red), or shAIF (green) PC3 cells were injected subcutaneously into the right hind flank of athymic nude mice. Subsequent tumor growth was followed by caliper measurements three times weekly until animals reached criteria for removal from study. Note the substantial decrease in tumor growth observed in AIF-deficient PC3 cells. B) Tumors were harvested, protein extracts were prepared, and AIF protein expression in developed tumors was determined by immunoblot analysis.