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The AIF/XIAP Axis in Prostate Cancer

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13. SUPPLEMENTARY NOTES

14. ABSTRACT
We have made substantial progress over the past year towards completion of the project goals both outlined in and extending beyond the original Statement of Work. We have made slow progress in establishing restoration cells expressing AIF variants with defined properties, in part due to difficulties associated with achieving successful lentiviral infection of target cells. In other studies, we have determined that AIF ablation has a significant impact on the ability of PC3 cells to grow and develop macroscopic clusters when plated in Matrigel. Further, the invasive characteristics of PC3 cells are substantially attenuated when AIF expression is suppressed. These data correlate well with studies presented in year 1, and suggest that not only is AIF necessary for the tumorigenic growth of prostate cancer cells in vivo, but also that the mechanism by which AIF affects tumor growth is through supporting the invasive growth of cells in 3D cultures. Our experiments to assess those properties of AIF that are most critical for prostate tumor growth continue to be underway; these new data suggest a possible mechanism for AIF support of prostate cancer through augmenting the ability of prostate cancer cells to grow invasively.

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

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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 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).** As described in our annual report last year, we had 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). For reasons that remain unclear, we have been unsuccessful at using these lentiviruses to stably infect AIF deficient cells in order to generate "restoration" cells lines with these AIF variants. While preliminary results suggest viral infection was successful, we have been unable to achieve stable incorporation of AIF cDNA contained within these viruses. Our current hypothesis is that the lentiviral backbone is tool large for efficient viral packaging, and we are currently in the process of moving our AIF cDNA sequences into an alternative lentiviral plasmid.

- **Assessment of the PC3-derived tumor progression (Months 18-27).** Experiments are awaiting establishment of cells described in the section above. Due to delays in establishment of these cells, these experiments are currently behind schedule by approximately 6 months.

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

- **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.

In addition to the progress described above relating to the original statement of work, we have made additional progress related to understanding the role of XIAP and AIF in the control of prostate cancer pathogenesis. Data presented in our annual summary report last year suggested that whereas AIF deficient cells displayed no differences in cell growth characteristics when cultured in vitro, these cells are significantly attenuated in their ability to grown in vivo. These data led us to investigate the ability of AIF to support the ability of prostate cancer cells to grow in 3D culture and invade basement membrane. When plated in culture dishes coated with Matrigel basement membrane, parental and control PC3 cells grow well and display macroscopic structures (Figure 1). AIF-deficient cells not only fail to display

macroscopic structures seen with parental and control cells, they appear round up and detach from the dish, indicative of cell death (Figure 1). When the invasive properties of these cells were investigated, we observed that control cells displayed substantial invasive growth (Figure 2). However, AIF-deficient cells were completely repressed in their ability to invade through Matrigel (Figure 2). These data suggest that the mechanism by which AIF supports prostate cancer tumorigenesis is through allowing cells to survive and invade the extracellular milieu.

Key Research Accomplishments

- It was demonstrated that PC3 cells deficient in AIF are attenuated in their ability to growth in three dimensional Matrigel cultures
- PC3 cells deficient in AIF were shown to be substantially reduced in their ability to grow invasively in culture.

Reportable Outcomes

None to date

Conclusions

Our current conclusions are 1) AIF directly contributes to the ability of PC3 cells to grow and survive in the context of three dimensional cultures, similar to results observed in our in vivo tumor growth studies and 2) AIF additionally supports the invasive growth of PC3 cells. Overall these conclusions further confirm our overall hypothesis that in androgen-independent prostate cancer cells AIF is critical for establishing tumorigeneic potential, and that this is achieved through support of invasion. Studies currently in progress are designed to determine which properties of AIF are necessary for support of tumor growth in vivo.

References

NA

Supporting Data

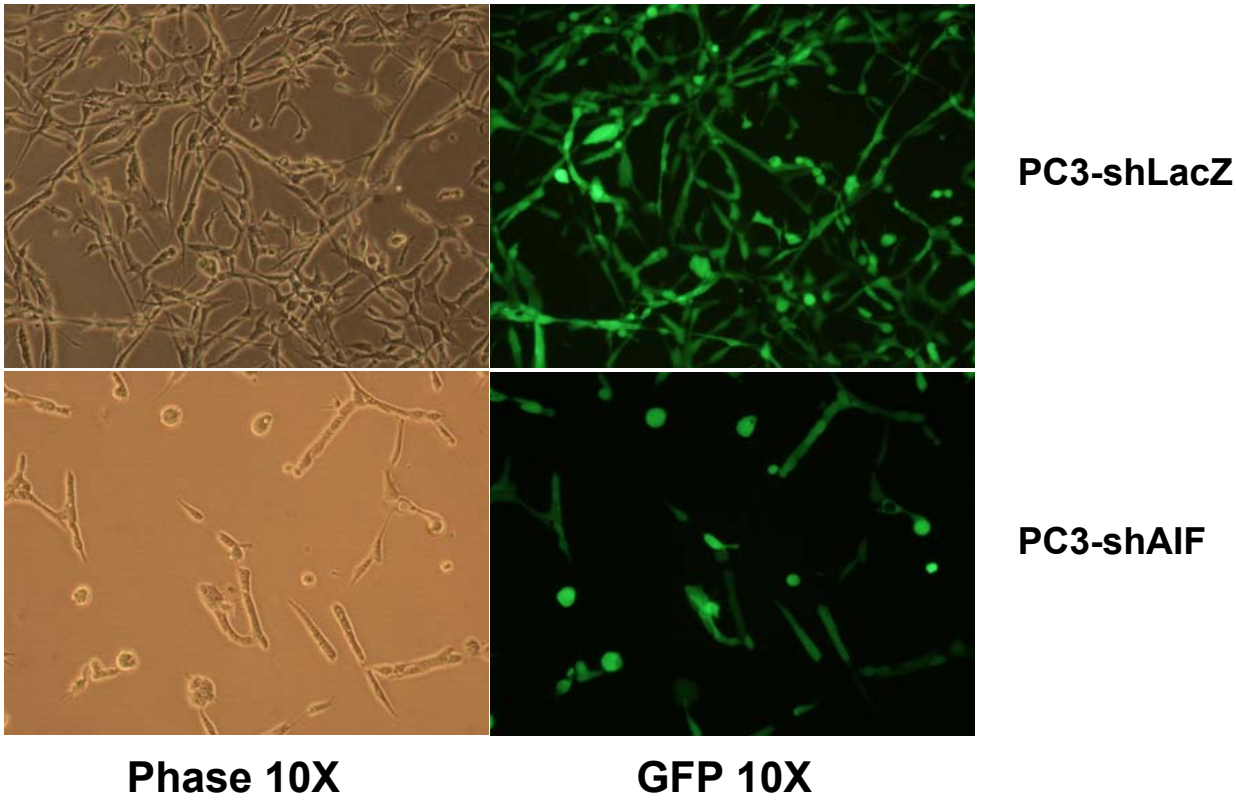
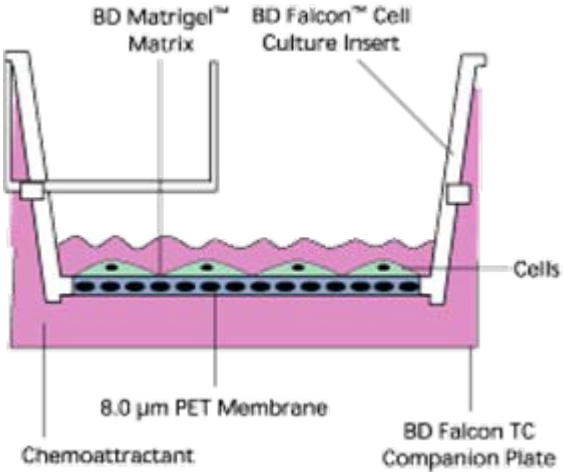


Figure 1. AIF-Deficient PC3 cells grow poorly on Matrigel basement membrane. Control (shLacZ, top row) and AIF deficient (shAIF, bottom row) PC3 cells were plated at 10,000 cells per well in 12-well culture dishes coated with a layer of Matrigel basement membrane. Cells were allowed to grow for 96 h, and then morphological features of cell growth were assessed by phase contrast microscopy (left column, magnification 10X). Since both cell lines express green fluorescent protein as a selectable marker for lentiviral infection, fluorescence microscopy was also employed (right column, magnification 10X. Note that only control cells were capable of effective growth and formation of macroscopic structures whereas AIF deficient cells were fewer in number and appeared apoptotic, based on spherical morphological characteristics.

A



B

	16h	40h	65h
shLacZ	1.65	40.69	43.53
shAIF	0.54	3.88	3.61

Figure 2. Invasive growth of PC3 cells is compromised following AIF protein ablation. A) Schematic representation of the invasion assay. RPMI-1640 growth media with full serum (10%) as chemoattractant was placed in the bottom of 24-well tissue culture plates. 8.0 micron PET membrane inserts either uncoated (as control) or coated with Matrigel Matrix were placed into each well. shLacZ or shAIF cells were harvested, suspended in serum free media, and added to the top of each insert. Following growth for the indicated amounts of time inserts were collected, fixed with paraformaldehyde, and allowed to dry. B) The number of cells present on each insert was then assessed by fluorescence microscopy. Percent invasion for each cell line was determined by the ratio: # cells per Matrigel insert / # cells per control insert. Note that whereas a substantial proportion of control cells displayed invasion after 40h (panel B, top row), virtually no invasion was observed for AIF-deficient cells (panel B, bottom row). These data suggest that AIF is critical for the ability of PC3 cells to grow invasively.