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Fatty Acid Synthesis and control of caspase 2 in Prostate cancer

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14. ABSTRACT Although prostate cancers are initially responsive to androgen deprivation therapy, castration resistant prostate cancer demands alternative treatment options. It has been reported that the apoptotic protease, caspase 2 (C2) is critical for prostate cancer cell death in several settings. Several reports have also indicated that fatty acid synthesis is critical for maintaining prostate cancer cell viability and that inhibition of fatty acid synthesis can lead to apoptosis. Finally, C2 is suppressed by high intracellular NADPH and this is not due to the redox effects of NADPH, suggesting that the synthetic role of NADPH (e.g., in fatty acid synthesis) may account for its ability to suppress apoptosis. Our new data suggest that manipulating metabolism, particularly inhibiting fatty acid synthesis, can alter chemosensitivity in prostate cancer cells.					
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Narrative

This proposal was based on the hypothesis that manipulation of metabolism could enhance cell death of prostate cancer cells in response to chemotherapeutic agents, thereby rendering these agents more effective. As described in our original proposal, although prostate cancers are initially responsive to androgen deprivation therapy, castration resistant prostate cancer (CRPC) demands alternative treatment options. Docetaxel treatment has increased survival of men with metastatic CRPC, but clinical benefits are not enduring. Our proposal was based on the observation that the apoptotic protease, caspase 2 (C2) is critical for cell death in many cancers in response to chemotherapeutic agents. We had reported that C2 is suppressed by high intracellular NADPH and that this is not due to the redox effects of NADPH, suggesting that the synthetic role of NADPH (e.g., in fatty acid synthesis) may account for its ability to suppress apoptosis. Regulation of C2 in response to NADPH levels is exerted through phosphorylation at a key site of negative regulation (Ser 164 in human C2), mediated by the calcium dependent kinase, CaMKII. Our proposal joined these strands of investigation in an effort to determine whether C2 phosphorylation might serve as a biomarker in prostate cancer treatment and to determine whether manipulating metabolism to alter C2 phosphorylation could enhance prostate cancer cell killing by chemotherapeutics, in cultured cells and mouse models of prostate cancer. We proposed 2 tasks, but were only able to work successfully on aspects of Task 1 in the one year time frame due to technical issues with our antibodies. We show data below that establishes the validity of our approach, which we will now continue with other resources. To some extent, this was successful in that the work was meant to establish the validity of the hypothesis to enable further exploration.

Body of report

Task 1: Determine whether manipulation of metabolism enhances the chemoresponsiveness of prostate cancer cells in culture (months 1-5)

Task 1A. Culture PC3, LNCaP, DU145, MDA, PCa 2a and MDA PCa 2b cells and assay cell death in response to various doses of docetaxel, doxorubicin, vinblastine, paclitaxel, estramustine phosphate, and etoposide by flow cytometry and caspase activity assays (month 1)

As shown in Fig. 1, we were able to demonstrate death of prostate cancer cells following treatment with various chemotherapeutic agents and, importantly, the cell death was at least partially dependent upon caspase 2, consistent with the notion that manipulation of caspase 2 responsiveness

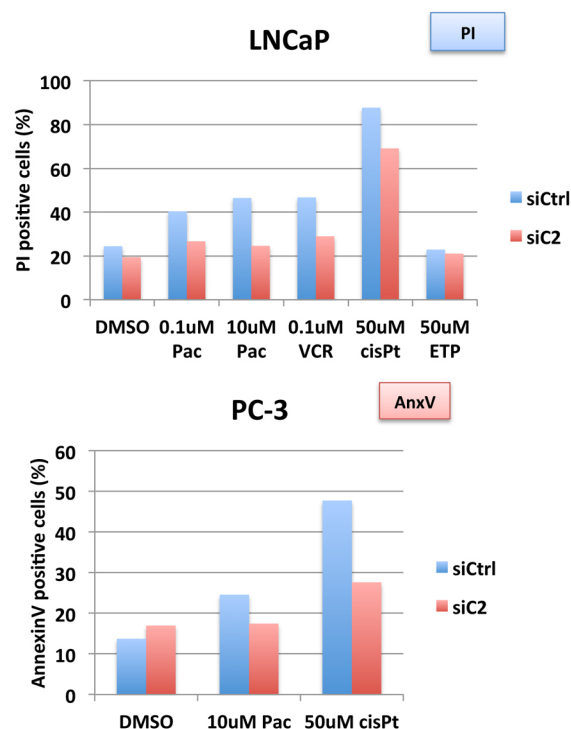


Fig. 1. LNCaP or PC3 prostate cancer cells were treated with the indicated concentrations of Paclitaxel (Pac), Vincristine (VCR), cisplatin (cisPt) or etoposide (ETP) and were stained either with propidium iodide or annexin V. Cells were quantitated by flow cytometry.

might, indeed, enhance chemotherapeutic response.

Task 1B. Assess the phosphorylation status of caspase 2 in cells treated as in 1A (month 2).

This aim caused significant delay in completion of our tasks as we had to regenerate high quality phospho-caspase 2 antibody, which required 5 repeated rounds of affinity purification. We also have had to work around the significant complication (that we had not anticipated) that a short anti-apoptotic form of caspase 2 is phosphorylated on S164 as well. This would not be problematic except for the fact that the natural proteolytic cleavage product co-migrates with the short caspase 2 isoform. Therefore, we have engineered a variant of caspase 2 with catalytic activity that lacks internal cleavage sites. We have also been using cells transfected with just the caspase 2 prodomain (which contains S164) to assess phosphorylation status. We will now proceed to assess caspase 2 phosphorylation by immunoblotting as proposed in the original application.

Before we can proceed with tissue staining (as described in the original proposal for task 2), we need to figure out whether phosphorylation of the short isoform is pro- or anti-apoptotic. If it is anti-apoptotic as is long form phosphorylation, staining will be informative. If phosphorylation of the short and long isoforms produces different apoptotic responses, this will confound the analysis and we will have to develop another way to monitor endogenous caspase 2 phosphorylation (or subtract out the signal from the short isoform in some way; this may be possible if their subcellular localizations are distinct).

Task 1C. Repeat does responses in 1A in combination with inhibitors of NADPH production (DHEA), fatty acid synthesis, (C75, C93, cerulenin) and CaMKII (KN93) (month 3).

As shown in Fig. 2, we were able to enhance docetaxel-induced death in DU145 prostate cancer cells through manipulation of metabolism. Interference with flux through the pentose phosphate pathway (DHEA and 2DG) enhanced the death of these cells over that induced by treatment with docetaxel alone. In addition, we found that inhibition of fatty

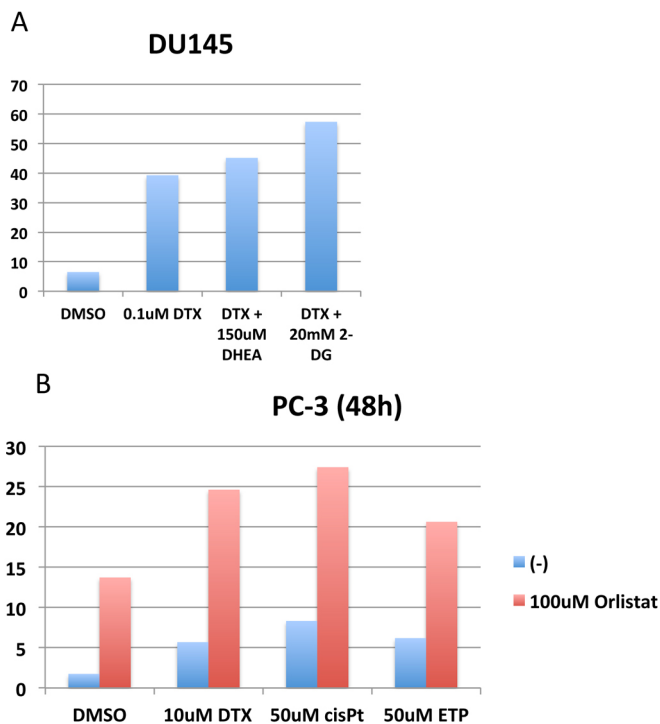


Fig. 2. A. DU145 prostate cancer cells were treated with docetaxel and either DHEA or 2-deoxyglucose and cell were stained with propidium iodide and quantitated by flow cytometry to determine the percentage of cell death. B. PC-3 cells were treated with docetaxel, cisplatin, or etoposide in the presence or absence of the fatty acid synthesis inhibitor, orlistat, stained with propidium iodide and quantitated by flow cytometry to determine the percentage of cell death.

acid synthesis (here with orlistat) could enhance death of prostate cancer cells with docetaxel, cisplatin and etoposide in PC-3 cells.

Although we found some enhancement of death using DHEA and 2-DG, we note that inhibition of the fatty acid synthesis pathway appears to be more promising in terms of enhancing cell death in prostate cancer cells. Indeed, we did not see a particular suppression of death in LNCaP cells treated with a cell permeable analog of malate, which, in other cell types, was able to suppress apoptosis through generation of NADPH (Fig. 3).

Task 1D. Assess the phosphorylation status of caspase 2 in cells treated as in 1C (month 4).

As described above, we were limited in this task by the availability of abundant high quality phosphocaspase 2 antibody, but we are now proceeding with these studies.

Task 1E. For chemotherapeutic agents whose efficacy in cell killing is enhanced by the metabolic inhibitors in 1C, transfect cells with non-phosphorylatable caspase 2 and assess if this is sufficient to enhance chemotherapy-induced cell killing. The cell lines utilized will be determined by the results of the tasks listed above (month 5).

We have generated the relevant mutant caspase 2 variants, including non-phosphorylatable caspase 2 that cannot be cleaved internally (making recognition and quantitation with the phosphoantibody much easier). A third year medical student will be joining my laboratory this summer and will complete this task and task 2 with other resources.

Key research accomplishments

- Established in our hands (though previously reported) that caspase 2 can contribute to cell death of prostate cancer cells in response to chemotherapeutic agents
- Established that manipulation of NADPH levels via blockade of the pentose phosphate pathway could enhance chemoresponsiveness in prostate cancer cells.
- Established that inhibition of fatty acid synthesis could enhance death of prostate cancer cells co-treated with several chemotherapeutic agents.

Reportable outcomes

None yet

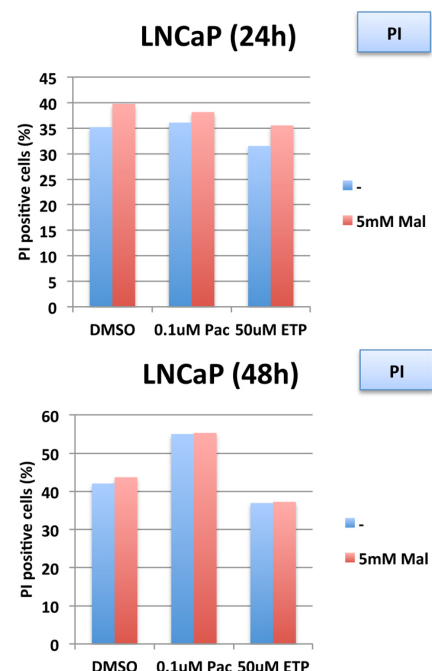


Fig. 3. LNCaP cells were treated for 24 or 48 h with either paclitaxel or etoposide in the presence of a cell permeant analog of malate (to increase generation of NADPH). Cells were then stained with propidium iodide and quantitated for percent PI positivity by flow cytometry.

Conclusion

Although we did not progress as far as we would have liked due to technical issues with the antibodies, we have established the paradigm and a new student coming to the laboratory will build on these findings using other resources. We have found that metabolism does, indeed, regulate prostate cancer death as postulated and this sets the stage for further experimentation in animal models, as originally proposed.