The Effects of Triptolide on the phenotype of pancreatic cancer cells.

Jessica Furrh[1], Shweta Sharma[2], Dawn Jaquish[2], Andrew M. Lowy[2]
[1]Partnership Scholar,San Diego State University
[2] Department of Surgery, Division of Surgical Oncology
Moore's Cancer Center, University of California San Diego

BACKGROUND

Pancreatic ductal adenocarcinoma (PDAC) is one of the most lethal human malignancies. This may be attributed to several factors including late detection, the propensity for early metastases, and profound resistance to therapies. Due to these factors, PDAC has a grim median survival of less than one year and an overall 5-year survival rate of less than 10% (Kleef et al. 2016).

Triptolide, a novel therapeutic derived from a chinese herb, *Tripterygium wilfordii*, has demonstrated anti-proliferative activity *in vitro* and demonstrated tumor reduction *in vivo* displaying promising anti-cancer capabilities (Meng et al. 2014). The specific mechanism of action is still not understood, but it can affect cells in multiple ways such as reduction in NF-κB activity, iNOS and Cox-2 expressions, and decrease HSP70 expression (Sangwan and Saluja 2012). A clinical trial of the Triptolide prodrug Minnelide recently opened at UCSD.

The objective of these studies was to understand mechanisms of resistance to Triptolide in pancreatic cancer cells using a murine model of pancreatic cancer (KPC) which harbors Kras and p53 mutations.

SPECIFIC AIMS

1. Determine how triptolide treatment modifies the phenotype of KPC cells with particular attention to:
   a. Examining any induction of epithelial-mesenchymal transition (EMT) by characterizing cell morphology as well as specific epithelial and mesenchymal markers.
   2. Evaluating stem cell marker expression in treated versus untreated populations.

METHODS

PDAC cells, derived from a genetically engineered mouse model (KPC), were initially treated with three different concentrations, 250nm, 500nm, and 1000nm over a three day period. Resistant cells were then passaged in increasing triptolide concentrations Analyses of these cells was done through:.

1. Cell Cytotoxicity Assay
   a. determine the activity of triptolide as an anti-cancer agent
2. q-RTPCR
   a. evaluate EMT marker expression
3. Flow Cytometry
   a. observe the presence of stem cell and epithelial markers

RESULTS

Figure 1: Triptolide inhibits growth of KPC tumor derived cells in-vitro.

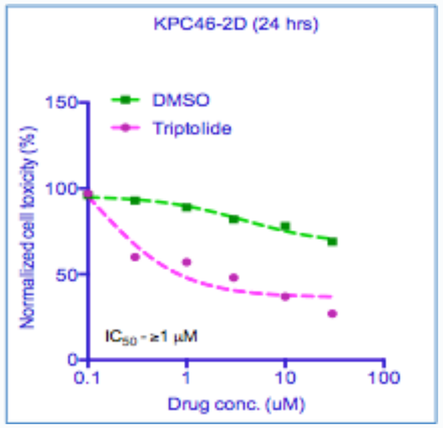
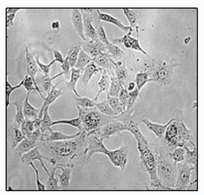
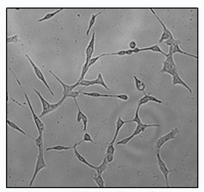


Figure 2: PDAC cells resistant to Triptolide assume a mesenchymal phenotype and express markers of EMT.

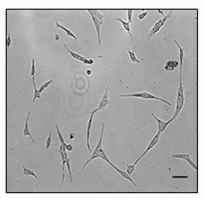
Morphology of TRP Resistant Cells vs. the Parent Line



Parent Line

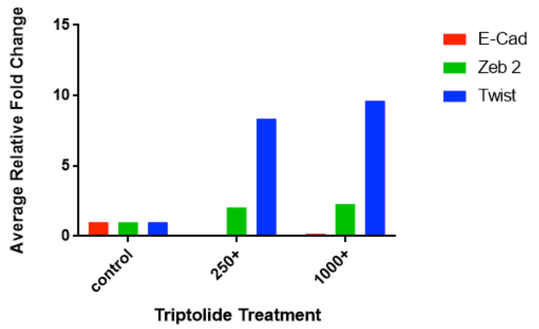


250nm TRP-Resistant line after 10 Tx cycles



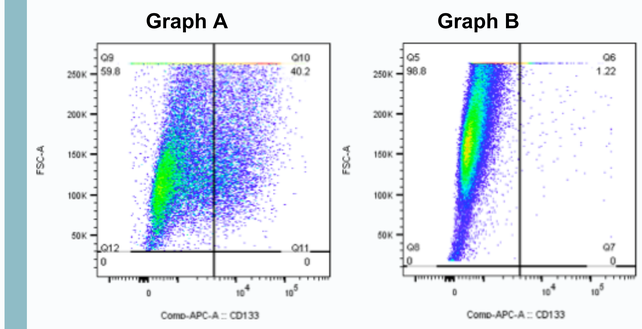
1000nm TRP-Resistant line after 10 Tx Cycles

Expression of EMT Markers in Treated Population



RESULTS

Figure 3: flow cytometry results for CD133 stem cell marker in KPC cells.
Graph A represents the no treatment group.
Graph B represents the treated group.



The graphs below analyze the epithelial characteristic of the treated and untreated populations.

Figure 4: Untreated Cell Population

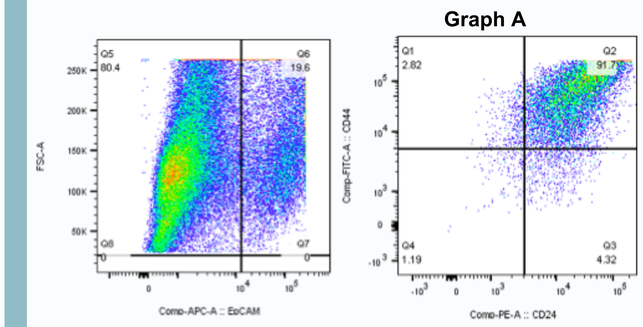
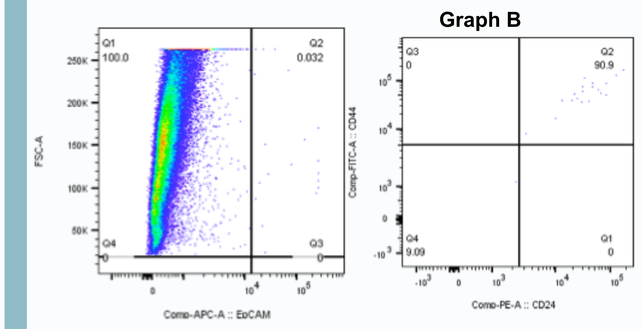


Figure 5: Triptolide Treated Cell Population



Graphs A and B show the population of cells that exhibit the combination of EpCAM+CD24+CD44 for each group.

CONCLUSIONS

Our experiments suggest that PDAC cells that are resistant to triptolide undergo EMT as evidenced by a decrease in epithelial markers in conjugation with an increase in mesenchymal morphology and changes in gene expression. Studies are ongoing to determine if the EMT transition is permanent or if cells have the capability to revert back to their previous state. Further understanding of the transition could provide insight into how PDAC cells could be re-sensitized or whether this transition is associated with new therapeutic vulnerabilties.

Overall, triptolide exhibits anti-cancer activity, but in PDAC derived cancer cells, however after prolonged exposure to drug, resistance was observed and was associated with expression of genes consistent with epithelial to mesenchymal transition.

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ACKNOWLEDGEMENTS

Special thanks to Dawn Jaquish for helping with the procedural aspects of this experiment and teaching us how to do q-RTPCR and flow cytometry. Also, special thanks to Andrew Lowy for opening up the Lowy Lab to the Partnership Scholars Program.

Additional funding from Stand Up to Cancer grant number:

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Research reported in this poster was supported by the National Cancer Institute of the National Institutes of Health under award numbers:
U54CA132384 & U54CA132379