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

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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% (Klee 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. Its mechanism of resistance to Triptolide and the related prodrug Minneldie 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.

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 concentrations, 250nm, 500nm, and 1000nm, to determine the activity of Triptolide in pancreatic cancer cells under a variety of conditions. Analyses of these cells were done through:

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

RESULTS

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.
   b. Evaluating stem cell marker expression in treated versus untreated populations.

2. Evaluating stem cell marker expression in treated versus untreated populations.

REFERENCES


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 vulnerabilities.

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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