Identification of Cobra Venom Actives as Potential Novel Pancreatic Cancer Therapeutics
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Abstract
Pancreatic cancer is an aggressive form of cancer which has a particularly poor prognosis. Currently only 3% of patients survive more than five years and just 1% of patients survive for more than ten years after diagnosis1. This highlights the urgent need for development of novel treatments for pancreatic cancer and the importance of early diagnosis.

Animal venom contains a complex mixture of proteins, peptides, enzymes and small molecules. In addition to their negative effects on human health, components from venoms have been utilised as treatments for conditions such as hypertension, angina and even cancer2. This research investigates potential use of cobra venom as a treatment for pancreatic cancer.

Resazurin, a blue weakly fluorescent redox dye was used as an indicator of cell viability. Conversion of resazurin to resorufin, a pink, highly fluorescent dye is proportional to cell viability and may be seen visually or measured through changes in fluorescence values. A panel of 19 cobra venoms were screened against MIA PaCa-2 and BxPC-3 pancreatic cancer cell lines at different venom concentrations in order to identify potentially toxic venoms. Following venom exposure, fluorescence values were measured, allowing assessment of cell viability. Venoms from five phylogenetically and geographically related cobras were identified to have selective toxic activity at low concentration against MIA PaCa-2 cells. These five venoms were fractionated using RP-HPLC to separate out the venom components and the fractions were screened for activity. The identified active fractions were further fractionated using size exclusion chromatography in order to identify the single entities responsible for cell toxicity.

Methodology
- MIA PaCa-2 and BxPC-3 pancreatic cancer cell lines were maintained in culture with DMEM and RPMI media respectively, each supplemented with 10% FCS and 2mM L-glutamine (plus pen/strep in RPMI only).
- Resazurin sodium salt was prepared at a concentration of 160 μM in appropriate cell media.
- 96-well clear U-bottom plates were plated with 2x10^4 MIA PaCa-2 cells or 1x10^5 BxPC-3 cells per well.

Cobra Screen and Fraction Assays
- Lyophilised cobra venoms were prepared in 0.9% NaCl and applied to cells for two hours, after which resazurin was applied.
- Fluorescence readings (excitation 544 nm, emission 590 nm, Fluostar Omega plate reader (BMG LABTECH, Ortenberg, Germany)) were taken two hours after exposure to resazurin.
- Z’ values of 0.91 (MIA PaCa-2) and 0.85 (BxPC-3) were obtained for this methodology.

HPLC
- Identified active venoms were separated using reverse phase HPLC (Vydac 218TP54 Sum 250x4.6mm column on Agilent 1100 Series HPLC).
- Active fractions were further separated using size exclusion chromatography (Agilent Bio SEC 3 100A 7.8x150mm column on Agilent 1100 Series HPLC).

Results

![Cobra Screen](image1)

![Size Exclusion](image2)

![Fraction Assay](image3)

Conclusion and Future Work
Nineteen cobra venoms were screened against the MIA PaCa-2 and BxPC-3 pancreatic cancer cell lines. Venoms from five phylogenetically and geographically related cobra species were identified as causing selective reduction in cell viability in MIA PaCa-2 cells but not BxPC-3 cells measured by resazurin reduction assay. These venoms were fractionated using reverse-phase HPLC and the fractions screened again. Each venom contained between 1-3 active fractions. Each of these fractions were further fractionated using size exclusion chromatography to give discrete proteins/peptides.

Planned future work includes screening the size exclusion fractions against MIA PaCa-2 and BxPC-3 cells and identification of active components by mass spectrometry. In order to better understand the effect of these venom constituents, the up/down regulation of genes involved in pancreatic cancer when exposed to these fractions will be investigated by qPCR.

Assessment of toxicity to healthy cells is also planned to further analyse selectivity of the identified compounds and viability as a potential drug lead.

References