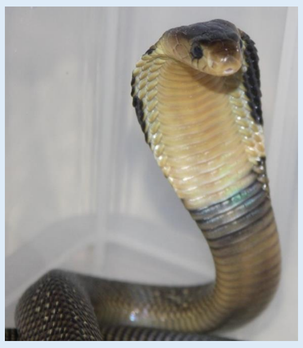

Identification of Cobra Venom Actives as Potential Novel Pancreatic Cancer Therapeutics

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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 diagnosis[1]. 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 cancer[2]. 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 $2x10^5$ 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 5um 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

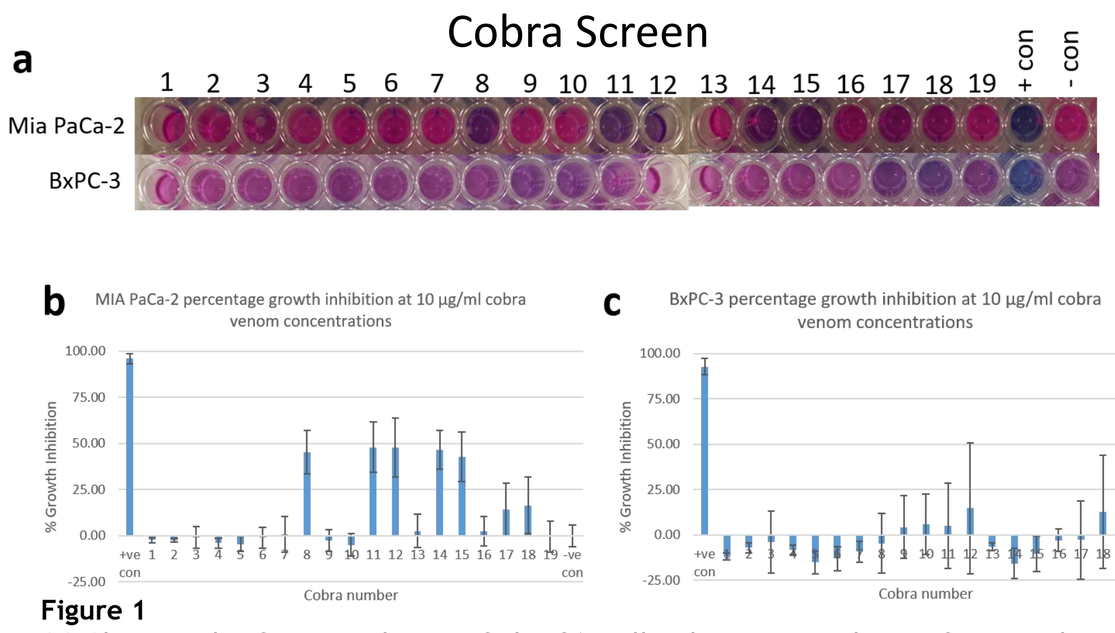

Figure 1

(a) Photograph of one triplicate of the 96-well cobra screen plates after two hours of resazurin exposure. Cobras given numbers 1-19. Graphs to show cell viability of **(b)** MIA PaCa-2 cells and **(c)** BxPC-3 cells exposed to 10 µg/mL cobra venoms. Error bars show standard deviation based on n=4.

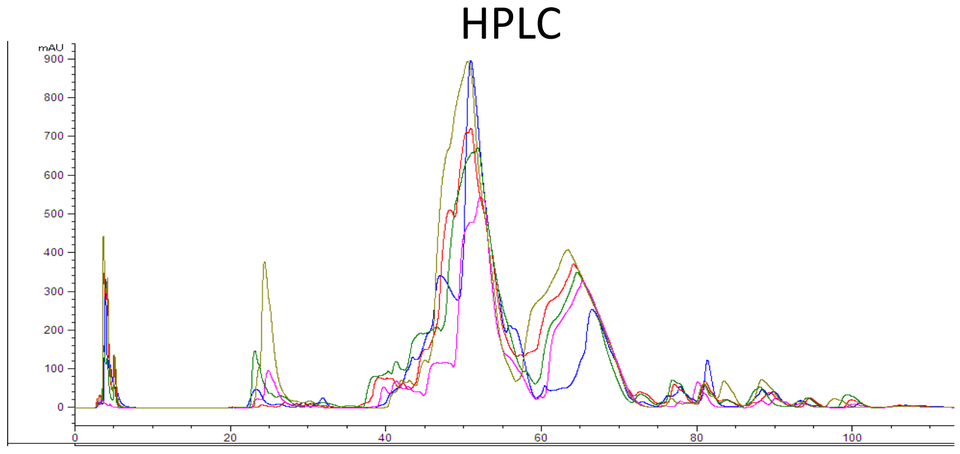

Figure 2

Overlay of the HPLC spectra for the five active venoms discovered during the MIA PaCa-2 cobra screen.

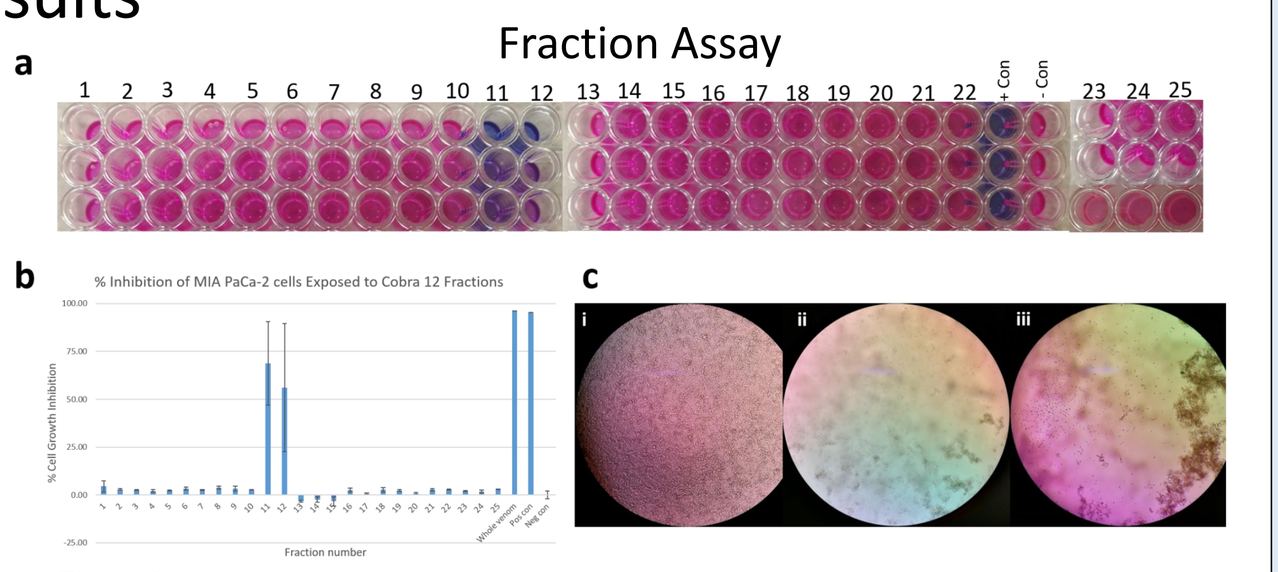

Figure 3

(a) Photograph of the resazurin plate showing colour changes from blue to pink after exposure to cobra 12 HPLC venom fractions. **(b)** Graph to show percentage inhibition of growth of MIA PaCa-2 cells exposed to each of the cobra 12 HPLC venom fractions. Error bars show standard deviation based on n=3 **(c)** Photograph of the MIA PaCa-2 cells down a microscope at 50X magnification *i*. negative control (healthy cells), *ii*. cells exposed to fraction 11, *iii*. cells exposed to fraction 12.

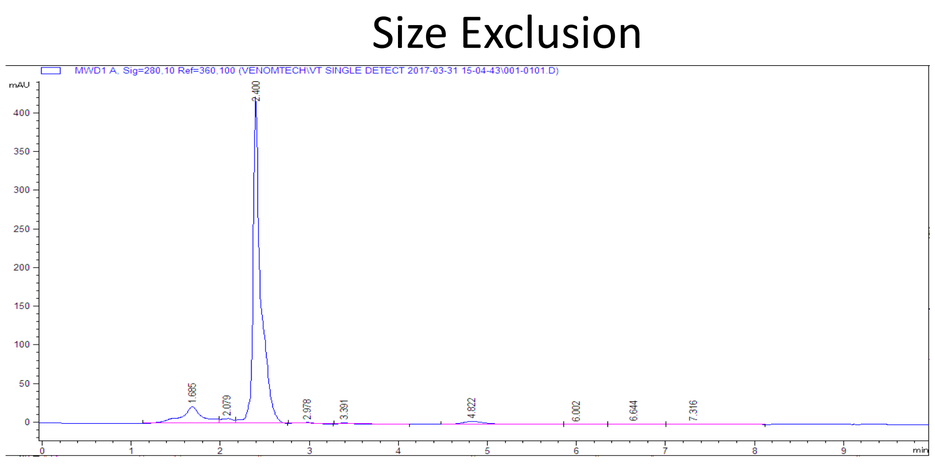

Figure 4

Size exclusion chromatography spectrum from cobra 12, HPLC fraction 12.

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 active constituents 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

(1) Cancer Research UK, http://www.cancerresearchuk.org/health-professional/cancer-statistics/statistics-by-cancer-type/pancreatic-cancer/survival, [Accessed April 2017].

(2) Calderon, L. A., Sobrinho, J. C., Zaqueo, K. D., et al. (2014) Antitumoral Activity of Snake Venom Proteins: New Trends in Cancer Therapy. *BioMed Research International*, 2014(203639), pp. 1-19

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