Development of a Compound Library from Cobra Venom and the Results of Cytotoxicity Screening in SW620 Colorectal Cancer Cells

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Introduction

It has been demonstrated that animal venom provides a rich library of potential novel therapeutics and is an excellent source of drug discovery tools[1]. 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].

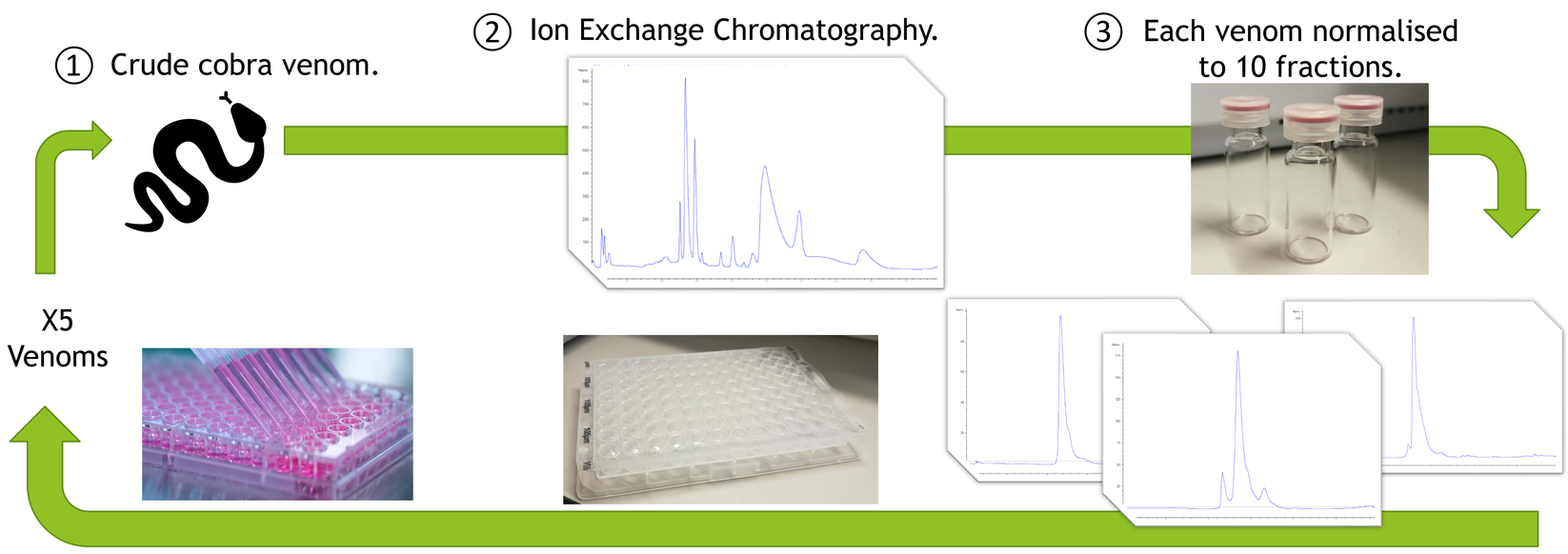
Colorectal cancer has the third highest global incidence and is the fourth leading cause of cancer related death. The global burden is anticipated to increase by 60% by 2030[3]. Despite advances in treatment options, chemotherapeutic resistance presents as one of the main challenges faced by patients, making the development of novel therapeutic options urgently required.

This research investigates potential use of cobra (*Naja spp.*) venom as a treatment for colorectal cancer.

Objectives

- To develop a 96-well format Cytotoxin Targeted Venom Discovery Array™ (T-VDA^{ctx}) to be screened against colorectal cancer cells.
- To utilise a fluorescence based cell viability assay to accurately estimate cell viability of SW620 colorectal cancer cells in 96-well format.
- To identify 'hit' fractions as potential lead compounds for further development by mass spectrometry.

Methodology



Ion Exchange Chromatography

Column: Tosoh Bioscience TSK SP-5PW 10um 7.5x75mm column on Agilent 1100 Series HPLC.
Buffers: **A**: 17 mM NaH_2PO_4, 3 mM $Na_2HPO_{4.}$; **B**: 15 mM NaH_2PO_4, 4.9 mM Na_2HPO_4, 1 M NaCl.

Reverse Phase HPLC

Column: Vydac 218TP54 5um 250x4.6mm column on Agilent 1100 Series HPLC.
Buffers: **A**: 0.05% TFA in H_2O. **B**: 80% ACN plus 0.045% TFA in H_2O.

SW620 cell viability assay

SW620 cells were maintained in DMEM + 10% FCS and 2 mM L-glutamine. Cells were plated at a density of $2x10^5$ cells per well in 96-well format and allowed to adhere. T-VDA^{CTX} fractions were tested across triplicate plates at a dose of 0.5 µg of venom protein per well. Fractions were incubated with cells for 120 minutes, then removed and discarded. 50 µl of 160 µM resazurin concentration was pipetted into each well. Fluorescence readings (excitation 544 nm, emission 590 nm, Fluostar Omega plate reader (BMG LABTECH, Ortenberg, Germany)) were taken 120 minutes post resazurin application.

A Z'-factor of 0.91 was previously established using this assay methodology for SW620 cells[4].

Results and Further Work

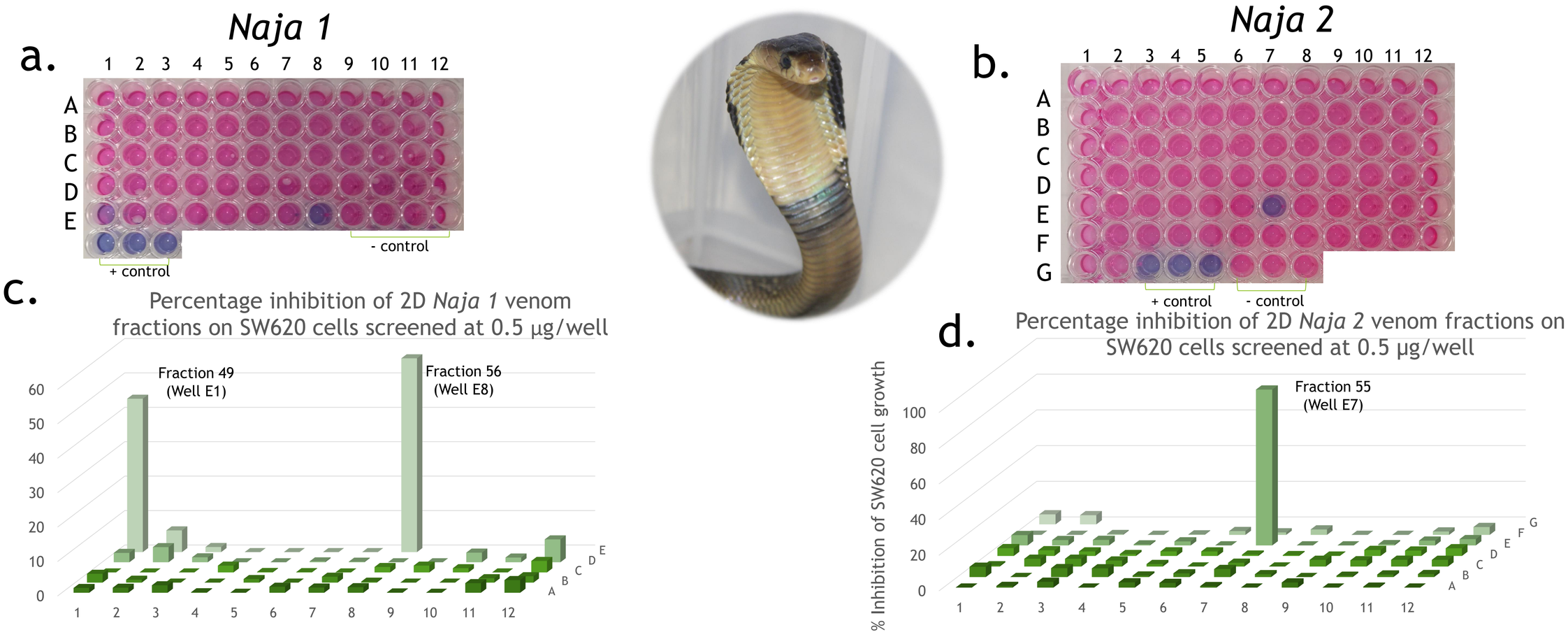


Figure 1. Results of 2D *Naja* venom fraction screening on SW620 cells. Resazurin assay plate photo for a. *Naja 1* and b. *Naja 2*. Samples were repeated across triplicate plates. Graphical representation of % inhibition of SW620 colorectal cells according to position in 96-well plate for c. *Naja 1* and d. *Naja 2 based on n=3 plates*.

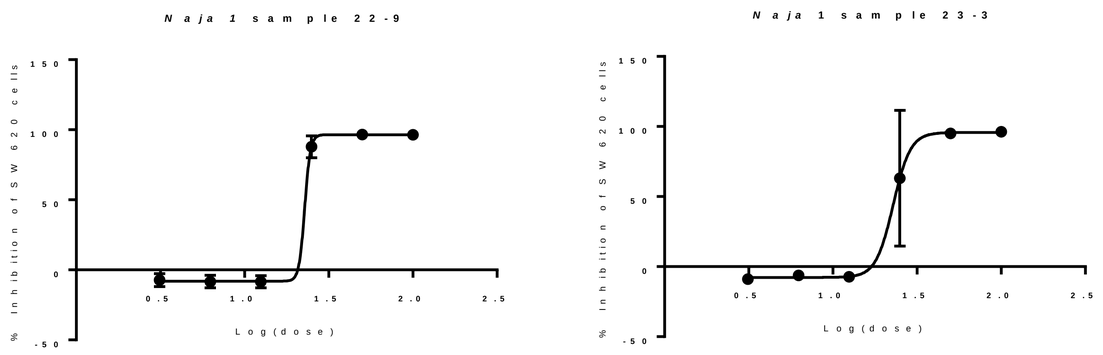


Figure 2. Dose-response curves for two *Naja* 1 2D venom fractions (22-9 and 23-3). LD_{50} values were calculated at 22.77 and 22.83 ug/ml respectively.

303 second-dimension *Naja* venom fractions were compiled into a T-VDA^{CTX} library. Of these, 12 fractions indicated inhibitory activity against SW620 colorectal cancer cells. Shown here is an example of the plate data for three active fractions from two *Naja* species. Active fractions have been sent off for mass spectrometry analysis for identification.

The active components will be analysed using qPCR in order to determine effects on gene regulation in a variety of cancer pathways. Cell viability assays will be performed on other cancers including pancreatic cancer and non-cancerous human cells to determine selectivity of the identified components and suggest suitability as potential lead molecules.

References

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2. Chaisakul, J., Hodgson, W. C., Kuruppu, S. & Prasongsook, N. 2016. Effects of Animal Venoms and Toxins on Hallmarks of Cancer. J Cancer, 7, 1571-8.
3. Arnold, M., Sierra, M. S., Laversanne, M., Soerjomataram, I., Jemal, A. & Bray, F. 2017. Global patterns and trends in colorectal cancer incidence and mortality. *Gut*, 66, 683-691.
4. Knight, E. Trim, S. Trim,C. 2017. Identification of Cobra Venom Actives as Potential Novel Pancreatic Cancer Therapeutics. Poster presented at ELRIG DD 2017.

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