Investigation of a Panel of Snake Venoms for EGFR Phosphorylation Reducing Capabilities in EGFR Over-Expressing Cancers

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Introduction

Cancer is currently one of the biggest health problems facing modern medicine. Cancers result from changes in the expression or activation of proteins fundamental to the normal functioning of cells within the body.

Epidermal growth factor receptor (EGFR) is a crucial player in cell signalling, responsible for many cellular processes, such as DNA replication, cellular division, differentiation and apoptosis.

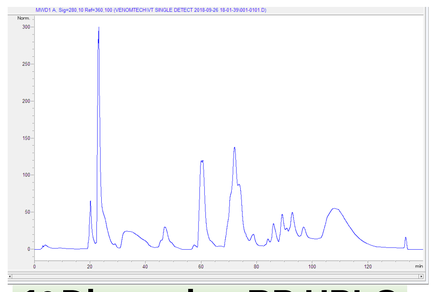
Aberrant signalling in EGFR has been linked to the development and progression of many cancer types, including breast, lung, gastric, pancreatic and colorectal cancers[1]. Increased activity (phosphorylation) through EGFR is often linked to rapid cancer growth and progression, leading to poor patient prognosis.

Current targeted treatments for EGFR including small molecule tyrosine kinase inhibitors (SM-TKIs) and antibody-based therapies are failing as cancer cells develop resistance to their mechanistic actions[2].

Due to the complex nature of their biological make-up, venoms have been shown to display a range of anti-microbial, analgesic, anti-coagulant and anti-cancer properties. Venoms-derived peptides have shown the capacity to become FDA-approved drugs[3]

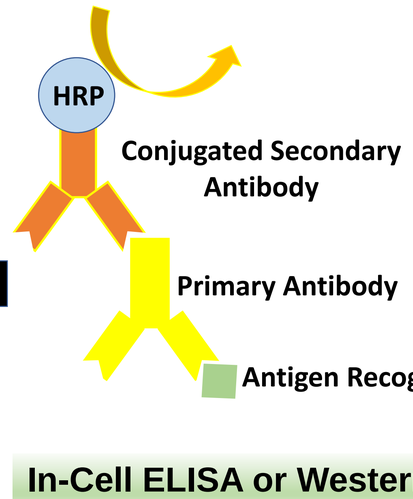
Methodology

Plate Cells

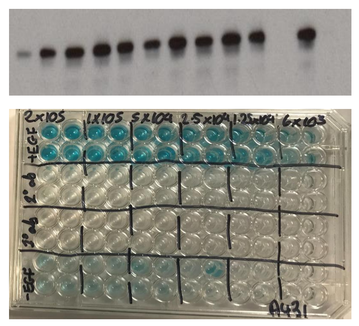


1º Dimension RP HPLC

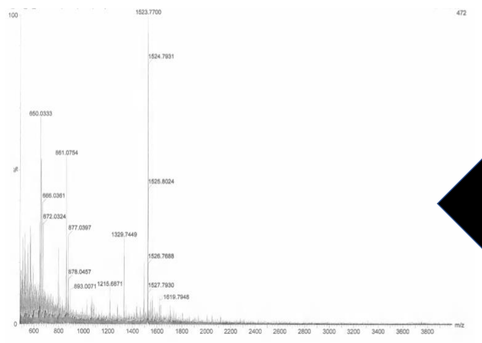
Venom treatment and EGF Stimulation



In-Cell ELISA or Western Blot



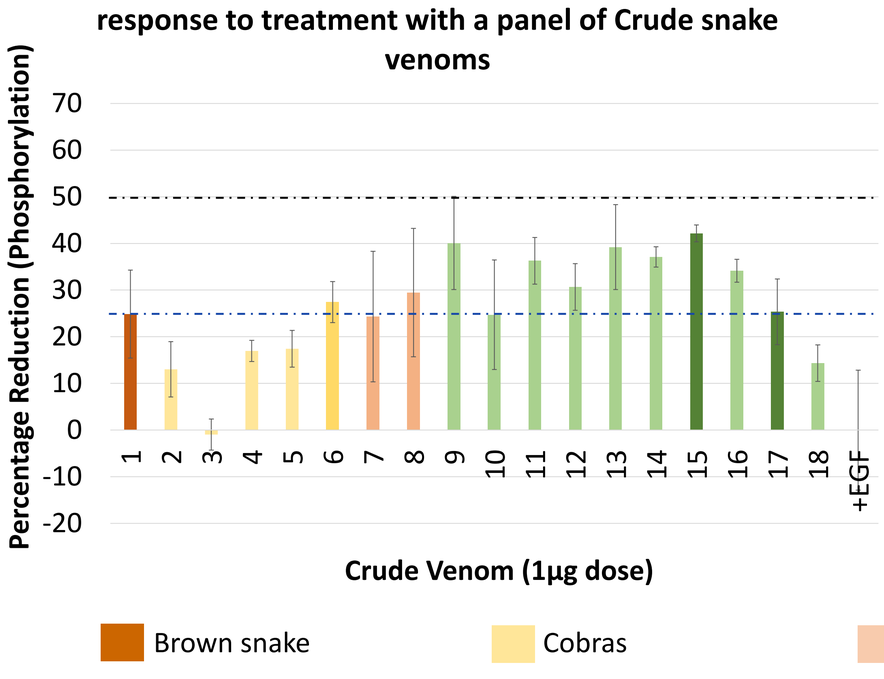
Development of TMB or ECL Signals



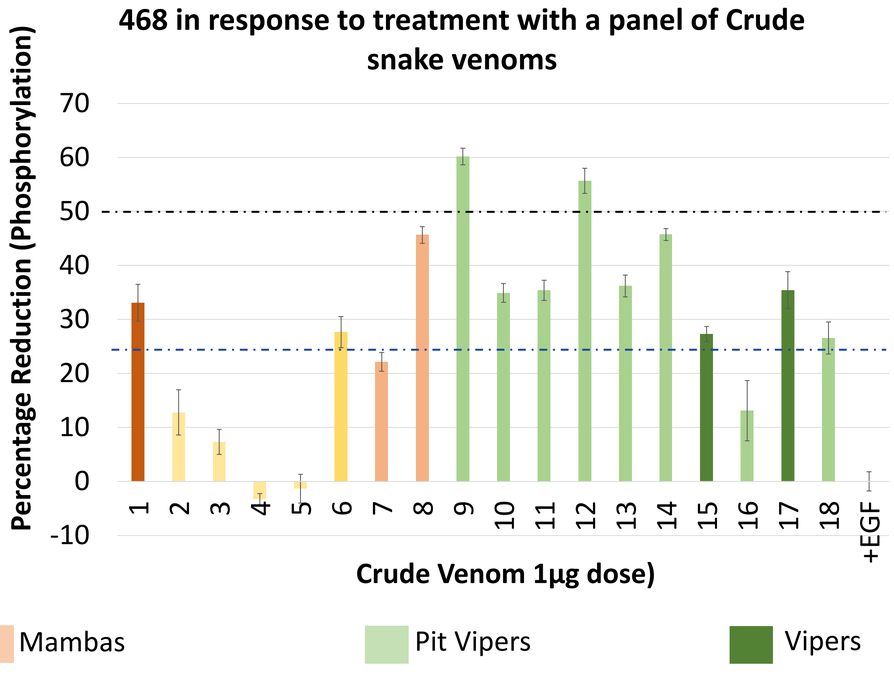
2º Dimension SEC HPLC & Mass Spec

Results and Interpretation

1A. % Reduction in EGFR Phosphorylation in A431 cells in response to treatment with a panel of Crude snake venoms



1B. % Reduction in EGFR Phosphorylation in MDA-MB-468 in response to treatment with a panel of Crude snake venoms



Brown snake | Cobras | Mambas | Pit Vipers | Vipers

1C. Western Blot analysis of MDA-MB-468 cells treated with a 20µg/ml dose of a panel of snake venoms

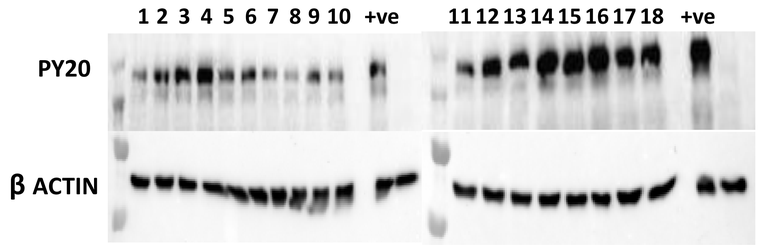


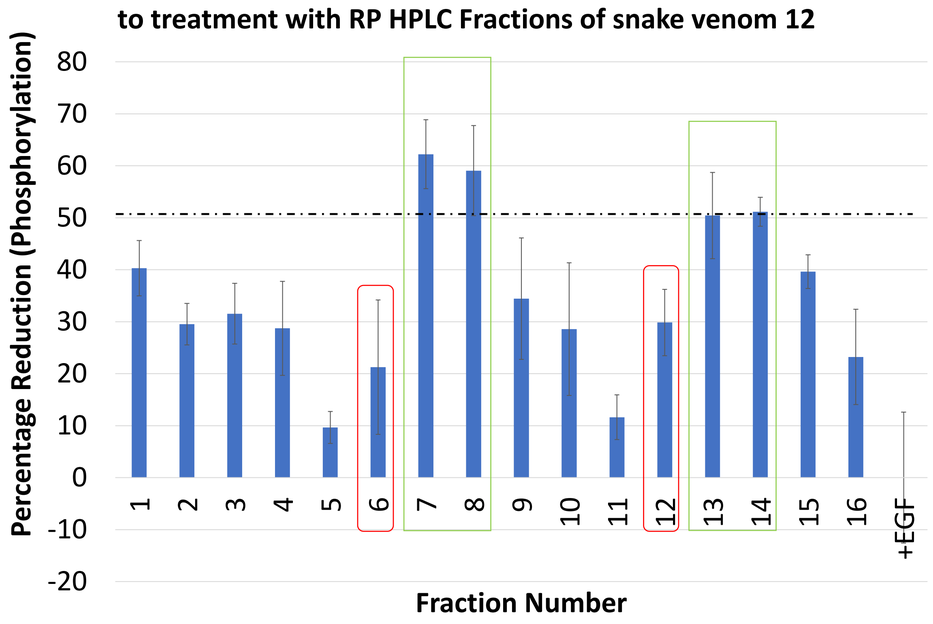
Figure. 1A & 1B

Graphs showing percentage reduction in EGFR Phosphorylation in A431 and MDA-MB-468 cells in response to treatment for 2hrs with 1µg of a panel of 18 snake venoms

Figure. 1C

Western Blot analysis of MDA-MB-468 cells treated with a 20µg/ml dose of a panel of 18 snake venoms

2A. % Reduction Phosphorylation in MDA-MB-468 in response to treatment with RP HPLC Fractions of snake venom 12



2B. % Reduction Phosphorylation in A431 Cells in Response to Treatment with RP HPLC Fractions of Snake Venom 12

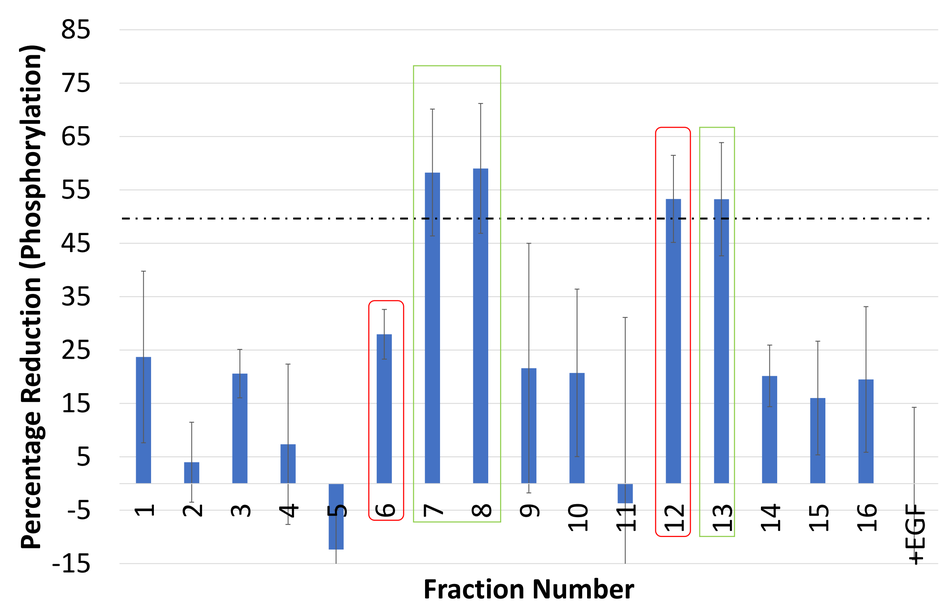


Figure. 2A & 2B

Graphs showing percentage reduction in EGFR Phosphorylation in MDA-MB-468 ***(Fig. 2A)*** *and A431* ***(Fig. 2B)*** *cells in response to treatment for 2hrs with 16 RP-HPLC Fractions from Snake Venom 12 (red: observable cytotoxicity, green: Percentage Reduction (Phosphorylation) ≥50%)*

2C.

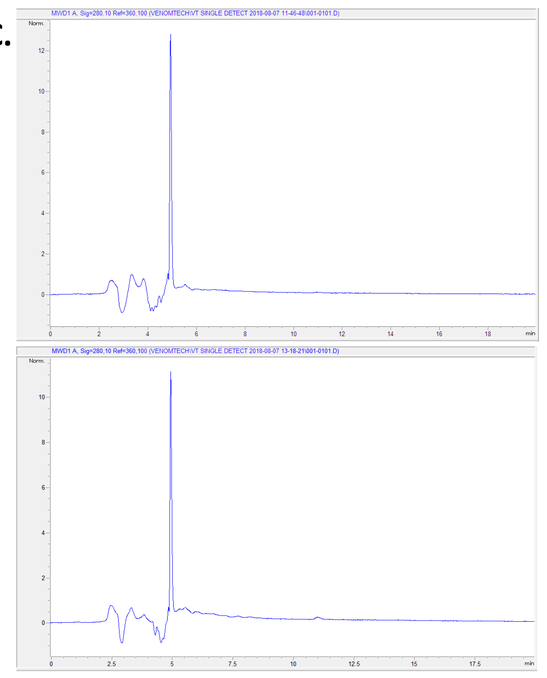


Figure. 2C

2º Dimension Size Exclusion HPLC traces of fractions 7 (Top) & 13 (Bottom) from Snake Venom 12

Discussion

- Crude snake venom ELISAs and western blots identified a range of venoms, predominantly pit vipers, capable of causing reduction in the phosphorylation of EGF receptors in both A431 and MDA-MB-468 cancer cells.
- At least one snake venom from each of the diverse groups (Cobra, Mamba, Viper, Pit Viper) showed capacity for causing a reduction in EGFR activity. Similar patterns of percentage reduction were seen across both cell lines. Cobra venoms showed the least reduction in EGFR phosphorylation, with some showing increases. Greater percentage reductions were seen in MDA-MB-468 cells then in A431 cells, likely to be dose-dependent limitations based on A431 cells expressing a greater level of surface EGFR receptors then MDA-MB-468.
- Snake venom 12 showed fractions which had similar inhibitory effects on EGFR phosphorylation in both the tested cell lines. Fractions 7, 8 and 13 showed similar percentage reductions in both MDA-MB-468 and A431 cells, with inhibition of phosphorylation of greater than 50% in both cell lines.
- 2nd Dimension Size exclusion HPLC showed fractions 7 and 13 contained one large detectible peak and so samples were sent off for mass spec analysis.

References

1) Roskoski Jr R, 'The ErbB/HER family of protein-tyrosine kinases and cancer', Pharm Res, 2014, 79:34-74
2) Roskoski Jr R, 'ErbB/HER protein-tyrosine kinases: structures and small molecule inhibitors', 2014, 87:42-59
3) King G, 'Venoms to drugs: translating venom peptides into therapeutics' Australian Biochemist, 2013, 44(3):13-16