Purified Venom Components Inhibit EGFR Phosphorylation in Triple Negative Breast Cancer

EACR 2022 | 0963

Danielle McCullough[1,2], Stuart Baker[1], Steven A Trim[1], Carol Trim[2]
[1] Venomtech Ltd. Sandwich, UK. [2] Canterbury Christ Church University, Canterbury, UK

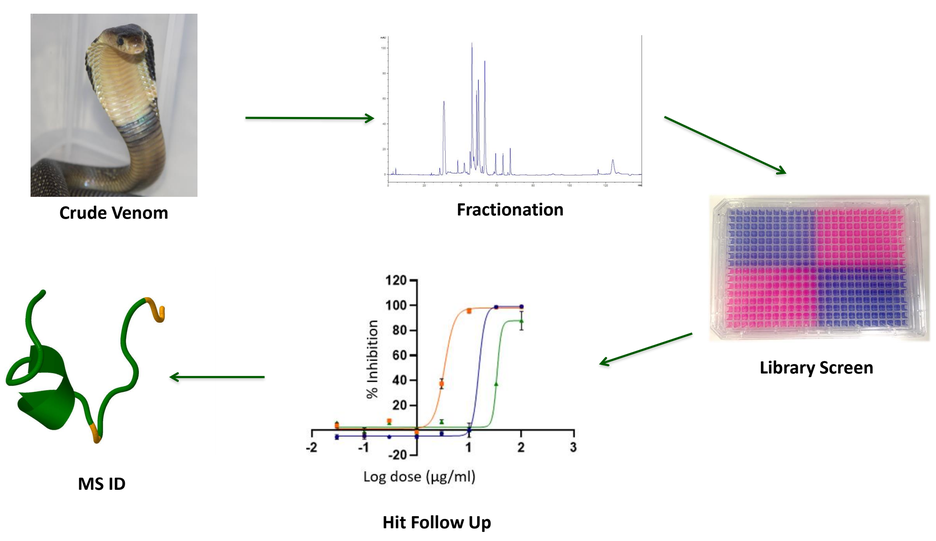
1 Overview

- Breast cancer remains the most commonly diagnosed cancer in women. EGFR is expressed in 15-45% of all breast tumours, including triple negative breast cancer (TNBC). Thus, EGFR is an attractive target for drug development.
- T-VDA^{EGFR} (Venomtech) containing 320 2-dimensional uHPLC fractions, purified from the venom of seven snake species, was screened for inhibition of phosphorylation at EGFR Y1086 in TNBC cell line MDA-MB-468
- The screens identified four reproducible hits that caused reductions to phosphorylation at Y1086, providing a hit rate of 1.25%.
- Hits resulting in partial and full antagonism of EGFR pY1806 were identified using MS/MS

2 Introduction

- Breast cancer remains the most commonly diagnosed type of cancer in both menopausal age women and adolescent/young adults. 10-20% of diagnosed breast cancers are deemed to be triple negative (TN), lacking expression of hormone receptors and HER2. Triple negative breast cancers (TNBC) present with poor patient prognosis, through their lack of effective treatment options.
- Studies report that epidermal growth factor receptor (EGFR) is expressed in 15–45% of all breast tumors and that its expression is inversely related to hormone receptor expression. Activation of EGFR requires homo- or hetero-dimerization and phosphorylation at Y1086, making the inhibition of pY1086 a potential target for treatment in both TN and receptor expressing cancer subtypes.
- Development of resistance to current EGFR-targeted therapeutics is common, leading to treatment failure and patient relapse. Thus, novel compound classes are needed. Venom peptides naturally act as ligands for a large variety of receptors and ion channels, making them a rich source of potentially new drug like molecules.

3 Methods



- Venoms were fractionated using UltiMate 3000 in-line 2D uHPLC (Thermo Scientific) and standardised at 20µg/ml
- MDA-MB-468 cells were plated at 1x10^5 cells/well and incubated overnight to adhere. Cells were dosed for 2h with each fraction at 37^{o}C, 5% CO_2. After 2h cells were stimulated with 1x10^{-7} M EGF for 5 minutes. Cells were washed with PBS and lysed using 100µl of the Abcam (ab126441) - EGFR (pY1086) + total EGFR Human ELISA Kit lysis buffer (+ protease/phosphatase inhibitors)
- Lysates were diluted in 100µl of assay diluent buffer and the ELISA assay kits performed and developed as via the manufacturer's instructions
- ELISAs were read at 450nm using a BMG ClarioStar^{PLUS} (Labtech) plate reader
- Dose response curves were performed on hit fractions to assess potency.

4 Results

Table 1. Summary of TVDA^{EGFR} fraction screen

Cell Line	Format	Z'	Venom fractions screened	Screening conc. (µg/ml)	Active fractions	Hit rate (%)	Max potency (IC_{50} (µg/ml))	
							2-D07	4-G03
MDA-MB-468	96	0.707	320	20	4	1.25%	64.77	19.73

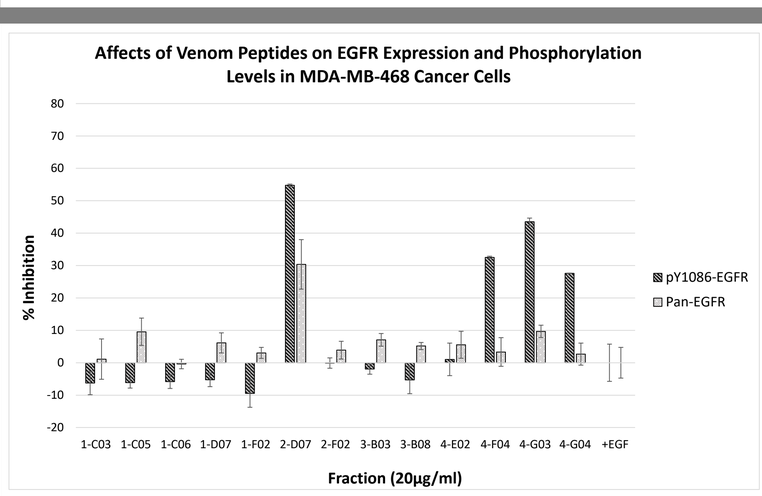


Figure 1. Hit Confirmation and Pan-EGFR effects

Confirmation of EGFR pY1086 inhibitory effects of fractions identified in original screen. Four fractions were identified to cause reductions to EGFR Y1086 phosphorylation levels.

Assessment of overall EGFR expression levels in response to treatment identified 2-D07 as causing a partial reduction in overall receptor expression levels

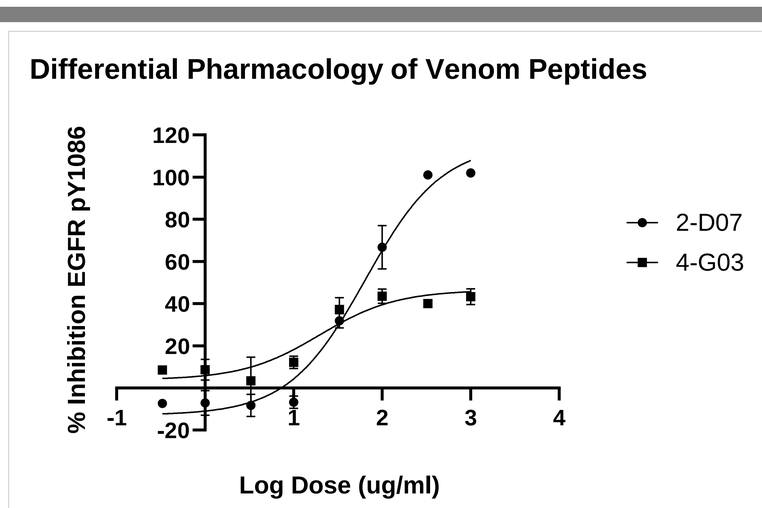


Figure 2. DRC of Lead Two Fractions

Log dose response curves of two lead hits from TVDA^{EGFR} library screen.

Fraction 2-D07 showed complete antagonism of EGF-induced phosphorylation at EGFR pY1086.

Fraction 4-G03 displayed a partial antagonism of EGF-induced phosphorylation at EGFR pY1086 with a maximum inhibition of 40%

4-G03 :-

```
1) P0DUP2 (PA2H2_CROOA)- Crotalus oreganus abyssus     (Basic Phospholipase A2)
2) C0HM14 (PA2BD_CRODU)- Crotalus durissus terrificus (Phospholipase A2 crotoxin basic subunit CBd)

1) SLVELGKMIL QETGKNAIPS YGFYGCNCGW GGRGKPKDAT DRCCFVHKCC YKKLTDCDPK TDIYSYSWKN KTIICDVNNP - 29.7% Match
   CLKEMCECDK AVAICLRENL DTYNKKYRIY PKFLCKKPDT C

2) HLLQFNKMIK FETRKNAIPF YAFYGCYCGW GGRGRPKDAT DRCCFVHDCC YGKLAKCNTK WDIYRYSLKS GYITCGKGTW - 27.0% Match
   CEEQICECDR VAAECLRRSL STYKYGYMFY PDSRCRGPSE TC
```

2-D07 :-

```
1) P83041 (DID5_CERCE)  - Cerastes cerastes Disintegrin CC5
2) P83043 (DID8A_CERCE) - Cerastes cerastes Disintegrin CC8A
3) P83044 (DID8B_CERCE) - Cerastes cerastes Disintegrin CC8B
4) Q3BK15 (DIDB_CERVI)  - Cerastes vipera   Disintegrin CV-11-beta

1) MNSAHPCCDP VTCKPKRGEH CISGPCCRNCK FLSPGTICK KARGDDMNDY CTGISSDCPR NRYKS- - 92.3% Match
2) MNSAHPCCDP VTCKPKRGEH CISGPCCRNCK FLSPGTICK KARGDDMNDY CTGISSDCPR NRIKK- - 92.3% Match
3) -NSAHPCCDP VTCKPKRGEH CISGPCCENCK FLTAGTVCL PAWGDFDNDL CTGISSDCPR NPWHKS - 78.4% Match
4) -NSAHPCCDP VTCKPKRGEH CISGPCCRNCK FLSPGTICK KARGDDMNDY CTGISSDCPR NPWKD- - 92.2% Match

P01133 EGF_HUMAN          1  -NSDSECPLSHDGYCLHDGVCMYIEALDKYACNCVVGYIGERCQYRDLKWWELR------  53
sp|2-D07                 1  MNSAHPC-------------CDPVTCKPKRGEHCISGPCCRNCKFLSPGTICKKARGDDM  47
                                                 *  : .  * . :: *  *.:: .     :

P01133 EGF_HUMAN         54  ------------------                                          53
sp|2-D07                 48  NDYCTGISSDCPRNRYKS                                          65
```

Figure 3. Mass Spectrometry Identification of Lead Fractions

Alignments of peptide digest sequences of 2-D07 and 4-G03 identified them as matching to disintegrins (92.3% match) and basic PLA2's (29.7% match) respectively. Sequence alignment of Human EGF and 2-D07 identified regions of sequence conservation and similarity

5 Conclusions

- Treatment with purified snake venom peptides caused inhibition of the EGFR phosphorylation at residue Y1086
- Screening of hit fractions in dose response identified both a full and partial inhibitor of phosphorylation at the investigated tyrosine residue
- Intact mass and peptide digestion MS identified the fractions as containing different snake venom peptides (PLA2 and Disintegrin)

12 YEARS ANNIVERSARY

www.venomtech.co.uk
info@venomtech.co.uk

Venomtech