ELRIG DD 2021
POSTER NUMBER
46

Novel GPCR Ligands: GPR120 Case Study

Danielle McCullough, Steven A Trim, Stuart Baker and Paul Grant

Affiliation – Venomtech Ltd, Discovery Park, Sandwich, Kent, UK.

Abstract

GPR120 (FFAR4) is a free fatty acid receptor that stimulates incretin hormone release from colonic endocrine cells and is implicated in macrophage and adipocyte function. GPR120 activation has been linked with inhibition of inflammation, modulation of hormone secretion from the pancreas and gastrointestinal tract, and the regulation of lipid and/or glucose metabolism in adipose, liver, and muscle tissues. GPR120 agonism correlates with prevention of the occurrence and development of metabolic disorders such as obesity and diabetes. Natural ligands to GPR120 are free fatty acids, which do not make good drug candidates. Previous published attempts to identify agonists from compound libraries failed, making this a challenging and interesting receptor for drug discovery and novel drug candidates.

Venom peptides have evolved in many venomous genera to enable them to undertake both effective predation of prey and defence against their own predators. Venom peptides are secreted into the lumen of the venom gland and stored ready for rapid delivery in under seconds and so, have evolved to be exceptionally stable. They have evolved to naturally act as ligands for a large variety of receptors and ion channels. These naturally occurring properties also make them ideal for ligand-receptor interactions in drug discovery.

In this study a Targeted-Venom Discovery Array^{GPCR} containing 956 venom fractions was screened with the DiscoverX PathHunter® eXpress GPR120S CHO-K1 β-Arrestin GPCR Assay Kits to identify peptides with agonistic effects against GPR120. Luminescent signal was measured using a CLARIOstar+ plate reader (BMG LabTech). Using a mini Z' on each assay plate we confirmed an acceptable assay robustness (Z' = 0.697) and identified 24 hit fractions (2.5% hit rate) all from the venom of true cobras. Dose response follow up confirmed the agonistic effects of these hits against GPR120S.

Results

Figure 1. Hit screen data

Figure 1: Initial hit finding screen identified 24 hits spread evenly across one of the three screened T-VDA© plates. From these the best 19 were selected for dose response confirmation. Eighteen fractions from elapid venoms and one viper venom fraction was selected. The mini Z' performed with the control edges of the plates confirmed the expected assay robustness of Z'=0.697.

Figure 2. Dose response confirmation of hits

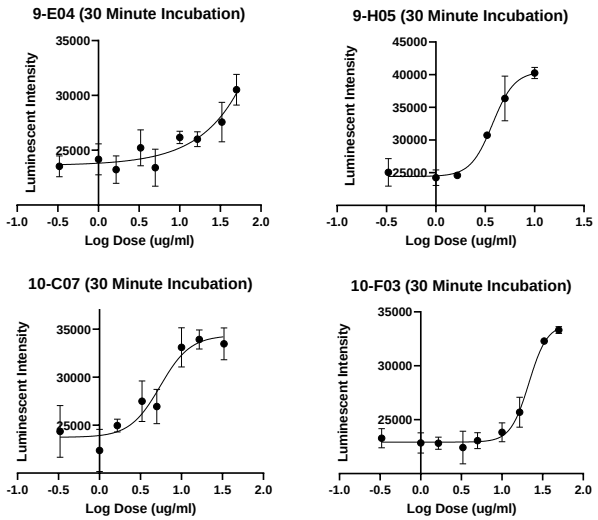

Figure 2: Complete and partial agonism of GPR120 by venom fractions in a dose responsive manner. Fractions Displayed varying potencies. All hits for follow up were from the venom of *Naja siamensis.* EC50: **9-E04 -** 4.098mM (27mg/ml), **9-H05 -** 0.559µM (3.75ug/ml), **10-C07 -** 0.81µM (5.5ug/ml), **10-F03 –** 3.176µM (21.4ug/ml)

Method

- GPCR Targeted-Venom Discovery Array© (T-VDA^{GPCR}) designed
- Venoms collected from nine elapid snakes, four pit vipers and a lizard
- Venoms two dimensionally fractionated, using in-line system on UltiMate 3000
- T-VDA^{GPCR} standardized, assembled and lyophilized in echo qualified 384-well plates
- DiscoverX PathHunter® eXpress GPR120S CHO-K1 β-Arrestin GPCR Assay Kits Screened
- GPR120S CHO-K1 β-Arrestin expressing Cells plated out in 384 well plates and incubated for 24h at 37ºC, 5% CO_2
- T-VDA^{GPCR} dissolved in 15µl assay media, 2.5µl aliquoted into each well of cells and incubates for 90minutes at 37ºC, 5% CO_2 (N=2).
- 13.8ul of substrate reagent was added to all wells and plates incubated in the dark for 60mins at RT
- Plates read at RT in the CLARIOstar+ plate reader (BMG Labtech) using the quick luminescence settings
- Data was analysed as relative luminescence. Hits followed up in DRC
- Selected hits identified by intact mass and peptide mapping mass spectrometry at Peak Proteins Ltd

Visual Method

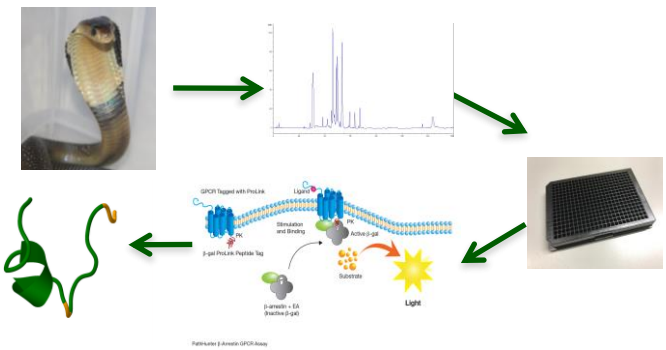

Figure 3. Mass Spec Identification of GPR120 Agonist Hits

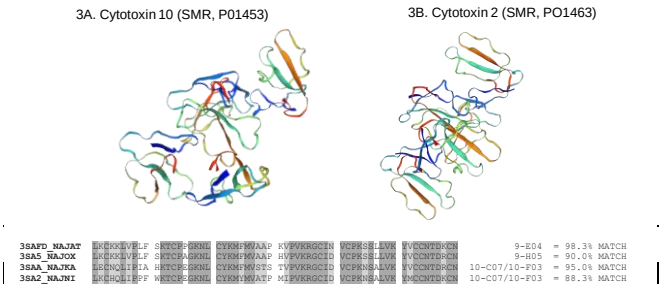

Figure 3: Venom peptides from *Naja siamensis* identified as a three finger cytotoxins. Using intact mass and peptide mapping from trypsin/chymotrypsin digests the top 4 hits were identified as cobra three-finger cytotoxins. Venom peptide from 9-E04 matched 98.3% to Cytotoxin SP15d from *Naja atra,* 9-H05 matched 90% to Cytotoxin Vc-5 from *Naja oxiana* and 10-C07 and 10-F03 matched two peptides, Cytotoxin 10 from *Naja annulifera* and Cytotoxin 2 from *Naja nivea* with 95% and 88.3% match respectively

Sequence analysis identified that the four cytotoxins have a highly conserved structure, sharing a 68.3% exact sequence match across all four peptides. 19aa are variable amongst the four sequences and are therefore the key residues responsible for the differences observed in potency

Conclusions

- Venom peptide libraries can deliver novel hits when screened against challenging targets
- GPCR receptors can be modulated by venom peptides
- Agonists for GPR120 don't have to be free fatty acids
- This shows further evidence of 3-finger toxins acting as agonists for novel GPCR receptors
- Analysis of the sequences from 3-finger toxins could aid drug design of smaller peptides

Acknowledgements

- Thanks to DiscoverX for use of their images
- Thank you to Rachel Rowlinson and the team at Peak Proteins for the MS ID service.