Case Study

Development of a multimodal assay system for GDF15/GFRAL drug discovery

Introduction

As a distant member of the transforming growth factor-beta (TGF-β) superfamily, Growth Differentiation Factor 15 (GDF15) functions through its receptor, Glial cell-derived neurotrophic factor (GDNF) family receptor alpha-like (GFRAL). Recently, the GDF15-GFRAL-RET signalling complex has emerged as a focal point for research, with RET acting as a co-receptor for GFRAL and facilitating downstream signalling pathways.

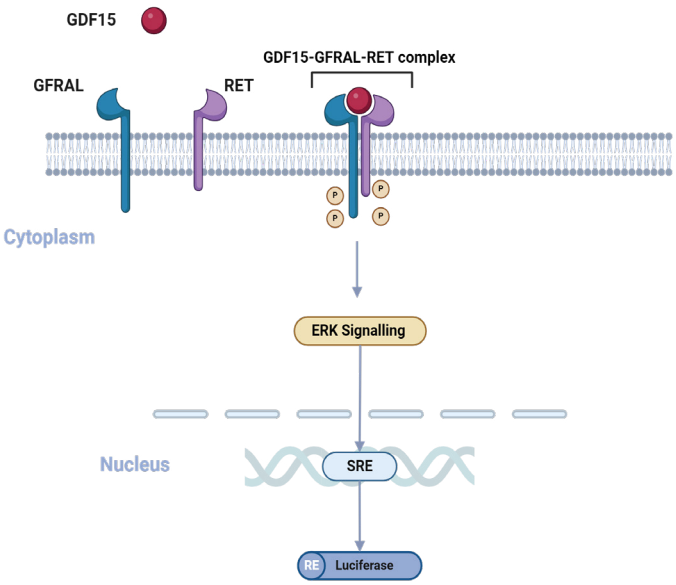

Figure 1. **Schematic representation of GDF15-GFRAL-RET complex signalling pathway.**

This collaboration underscores the multifunctional nature of GDF15-GFRAL-RET, impacting pathways critical for cellular stress response and energy balance. Given its regulatory influence across broad physiological systems, this pathway presents new therapeutic opportunities, particularly in areas such as metabolic disorders and cancer.

Custom Cell-Based Tools for Complex Receptors

- **Cell Line Generation:** Custom cell line generation utilizing advanced technology to ensure hihg-quality. reliable reagents for complex receptor research.
- **Assay Development:** Development of cutting-edge assays tailored to measure key signalling events with precision.
- **High-throughput Screening:** Efficient screening in 96- or 384-well formats for rapid hit identification.
- **Lead Optimization:** Optimizing leads with robust, reproducible assays, integrating custom cell lines into workflows for reliable data.

Supporting Your Research

SB Drug Discovery is at the forefront of innovation specialzing in tailored assays to meet specifc research goals and providing a comprehensive understanding of intricate signalling pathways. Leveraging advanced technology and innovative methodologies to meticulously screen hundreds of cell clones during the cell line generation process to ensure the highest quality and target solutions for your research needs.

Our comprehensive suite of assays, including SRE luciferase reporter assays and pERK ELISA platforms, supports high-throughput screening and hit validation studies. These assays are engineered for efficiency, measuring both GDF15-induced ERK phosphorylation and luciferase activity.

With an innovative multimodal system for GDF15/GFRAL drug discovery and team of experts, we provide comprehensive support for your drug discovery initiatives. Our expertise allows for seamless integration of custom cell lines and assays into existing workflows, optimizing them for reproducibility and delivering high-quality, reliable data.

Stable Cell Line Generation

HEK cells capable of driving inducible target expression were co-transfected with GFRAL and RET expression vectors and maintained in antibiotic containing media to allow isolation of single cell derived colonies.

Clones were first screened for the presence of GFRAL and RET protein expression (data not shown). Expressing (induced) and non-expressing (uninduced) clones were then screened for functional response to GDF15 in the pERK ELISA assay (Figure 2). Selected clones were analysed further and GDF15 concentration dependent effect on pERK was examined across multiple experiments (Figure 3).

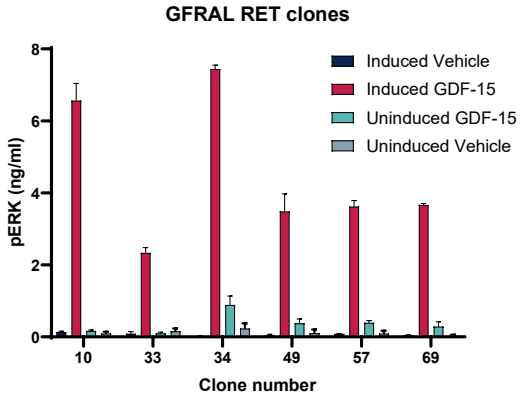

Figure 2. **Screening GFRAL/RET clones in the pERK ELISA assay. Data represents average data for each sample (n=3; ±SEM).**

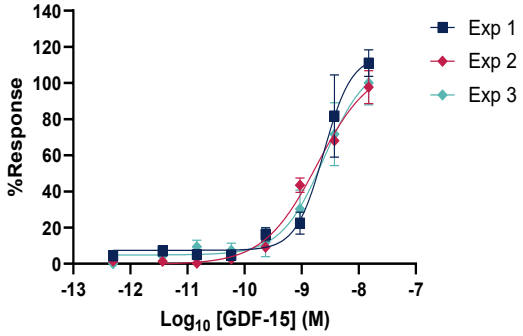

Figure 3. **Concentration response curve showing reproducibility of GDF activation in pERK ELISA assay tested over multiple experiments.**

Whilst the pERK ELISA allows analysis of compound effects against GFRAL, due to the transient nature of the pERK response, the assay is highly time dependent. To develop a more robust HTS assay, it was thought the SRE reporter assay may allow a more robust detectable response.

Selected GFRAL/RET clones were transfected with SRE reporter vector. This reporter system, coupled with luciferase output, enables screening of small molecule inhibitors targeting the receptor complex in 96 or 384-well format.

GFRAL/RET/SRE clones were initially screened for luciferase activity in response to GDF15 (Figure 5). Selected clones were validated for GDF15 concentration response in the SRE reporter assay (Figure 6).

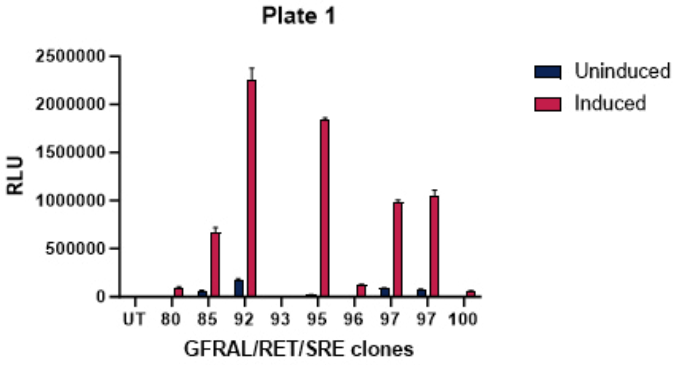

Figure 5. **Selected single cells transfected with SRE reporter vector induced and uninduced clones were expanded and screened for luciferase activity.**

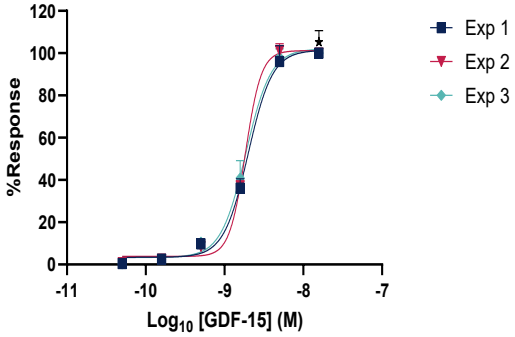

Figure 6. **Concentration response curve showing reproducibility of GDF-15 concentration-dependent increase in luciferase activity.**

Comparison of pERK ELISA and SRE assay

Whilst the reporter assay can allow increased assay processing, it is important to ensure the SRE reporter assay is consistent with pERK response therefore both assays were assessed for GDF15 and inhibitor concentration response curves (Figure 7 and 8 respectively). Comparable EC and IC_{50} values were obtained from both assays. These results confirmed that the SRE reporter assays was a suitable option for agonist or antagonist screening. Hit compounds could be validated using the pERK ELISA.

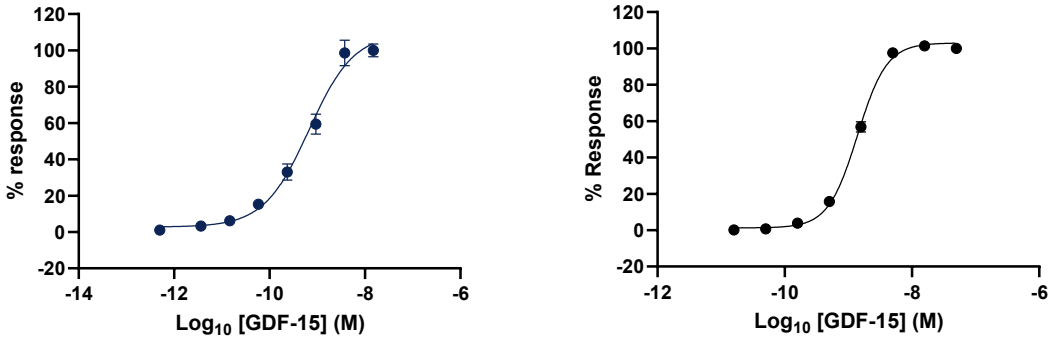

Figure 7. Concentration response curve showing GDF15 concentration-dependent increase in (a) pERK ELISA assay with an EC_{50} value of 6.704e-010 and (b) SRE reporter with an EC_{50} value of 1.391e-009.

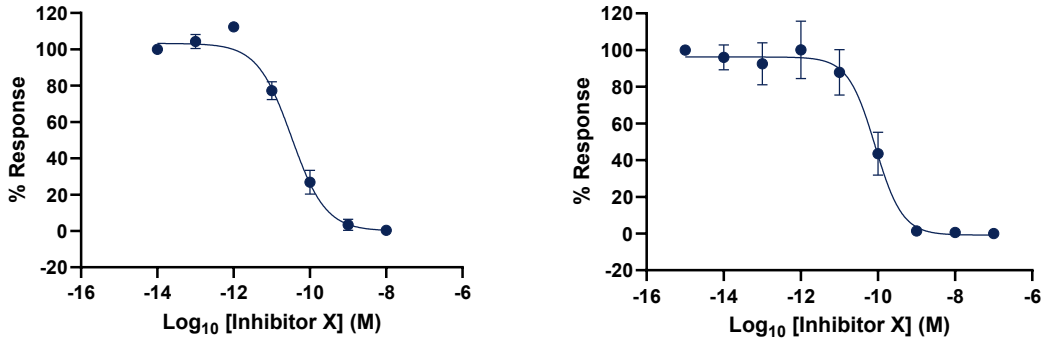

Figure 8. Concentration response curve showing inhibitor X concentration-dependent inhibition in (a) pERK ELISA assay with an IC_{50} value of 3.300e-001 and (b) SRE reporter with an iC_{50} value of 8.477e-011.

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