GPR75 and GPR151: NOVEL METABOLIC ORPHAN GPCR THERAPEUTIC TARGETS FOR DRUG DISCOVERY IN OBESITY

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INTRODUCTION

Orphan G-protein coupled receptors (orGPCRs) – receptors without known natural ligands – offer a promising but largely untapped source for new therapies across various diseases. Developing drugs for these approximately 100 uncharacterized receptors is challenging due to the lack of validated ligands and limited insight into their signaling, making drug screening assay design difficult.

Recent genetic studies, including whole exome sequencing, have linked two orGPCRs, GPR75 and GPR151, to obesity and metabolic disorders (see Figure 1). Leveraging Kainova's bioSens-All® platform and GPCR expertise, we are addressing these challenges by creating high-throughput screening assays using our enhanced bystander bioluminescence resonance energy transfer (ebBRET) technology.

Our approach exploits the receptors' constitutive activity to identify agonists, inverse agonists, and antagonists. For instance, we performed a screening campaign to target GPR75 and discovered that GPR151's constitutive activity can trans-inhibit a surrogate receptor's activity, providing a novel assay for antagonist screening in the absence of known ligands.

This work not only provides new screening tools for orGPCRs but further expands potential therapeutic targets for obesity beyond the traditional incretin receptors.

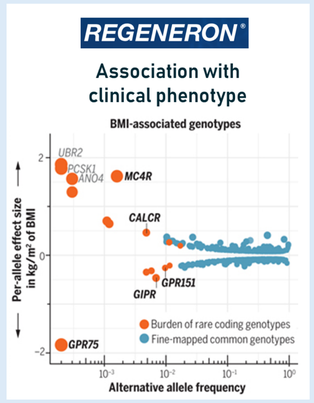

Figure 1: BMI-related gene discovery via exome sequencing. (*Akbari, P., et al., Science 2021*)

MATERIAL AND METHODS

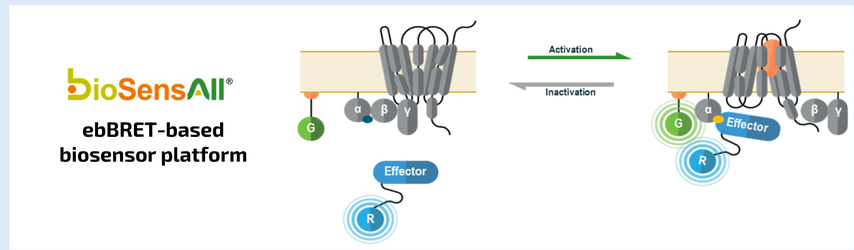

Figure 2: Assay principle underlying our ebBRET-based biosensors.
Renilla luciferase (RlucII)-tagged (**R**) G protein-specific effector proteins are recruited to activated (GTP-bound) **untagged** G alpha subunits following activation of **untagged** receptor. This event brings RlucII in close proximity to the plasma membrane-anchored *Renilla* green fluorescent protein (**G**), leading to an increase in ebBRET. The same assay principle is used to detect βarrestin recruitment to the plasma membrane following receptor activation.

GPR75 – ASSAY DEVELOPMENT HTS

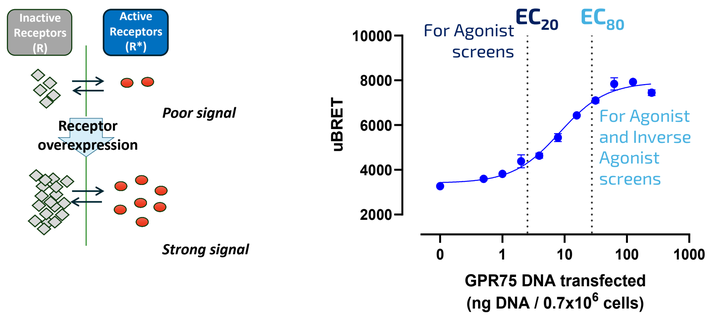

Figure 3: GPR75 displays receptor dose-dependent constitutive recruitment of βarrestin-2.
Left: GPCRs naturally fluctuate between inactive (R) and active (R*) conformations, establishing a dynamic equilibrium that underlies constitutive activity. Even in the absence of agonists, a small fraction of receptors spontaneously adopt active states capable of coupling to signaling proteins. Constitutive activity occurs when R* populations are sufficient to drive measurable signaling. In order to achieve R* population levels sufficient to promote measurable signaling in our assay system, increasing amounts of GPCR plasmid DNA are transfected along with one of various signaling pathway-specific ebBRET biosensors. Signals can be measured at 24 to 72 hours post-transfection. **Right:** HEK293 cells were co-transfected with increasing amounts (0-500 ng/ 0.7x10⁶ cells) of human GPR75-encoding plasmid DNA and the βarrestin-2 biosensor. Constitutive receptor activity was assessed at 72h post-transfection and resulting curves were fitted using the (agonist) vs. response - Variable slope (four parameters) non-linear regression model (GraphPad 10). Constitutive activity was detected on the βarrestin-2 pathway, providing assay windows sufficient for the screening for agonist and inverse agonists.

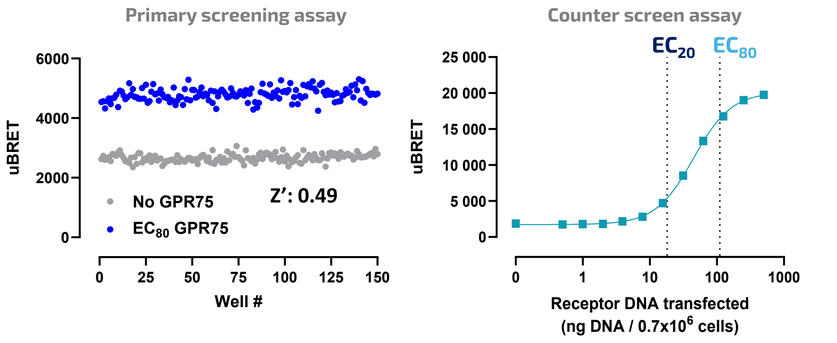

Figure 4: High-throughput screening of GPCR75. For the primary assay (*left*), HEK293 cells were co-transfected with an EC_{80} of GPR75-encoding plasmid DNA and the βarrestin-2 biosensor. BRET was recorded at 48h post-transfection, resulting in a robust **Z' of 0.49**. A secondary counter screen assay (*right*) was developed by using GPR17, a non-related GPCR that exhibits constitutive activity on βarrestin-2. HEK293 cells were co-transfected with increasing amounts (0-500 ng/ 0.7x10⁶ cells) of a GPCR-encoding plasmid and βarrestin-2 biosensor. Constitutive activity of the counter screen receptor was determined at 72h post-transfection, revealing a sizeable assay window offering a robust dynamic range to distinguish receptor-driven signals from non-specific compound effects.

GPR75 - SECONDARY ASSAY

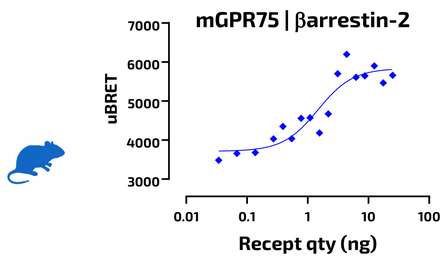

Figure 6: Development of mouse assay for hit characterization. HEK293 cells were co-transfected with increasing amounts (0-500 ng/ 0.7x10⁶ cells) of mouse GPR75-encoding plasmid DNA and the βarrestin-2 biosensor. Constitutive activity was detected on the βarrestin-2 pathway, providing assay windows sufficient for the screening of agonist and inverse agonists and enabling the evaluation of hits in rodent models. *Constitutive* receptor activity was assessed between 24h and 72h post-transfection and resulting curves were fitted using the (agonist) vs. response - Variable slope (four parameters) non-linear regression model (GraphPad 10).

CONCLUSIONS

- Leveraging our unique biosensor platform, we successfully developed an HTS assay for the intractable GPR75
- We also identified a unique trans-inhibition MoA for GPR151 and developed a screening assay
- Screening of Kainova's proprietary library led to the identification of drug-like ago/inverse ago GPR75 hit compounds
- Early analoging efforts revealed initial SAR of GPR75 inverse agonists

✓ Kainova is open to partnership to continue its GPR75 program
✓ Kainova is open to partnership to exploit its platform for other challenging orGPCRs

kainova THERAPEUTICS

RESULTS

GPR75 - ACTIVITY PROFILE OF SELECTED HITS & ANALOGUES

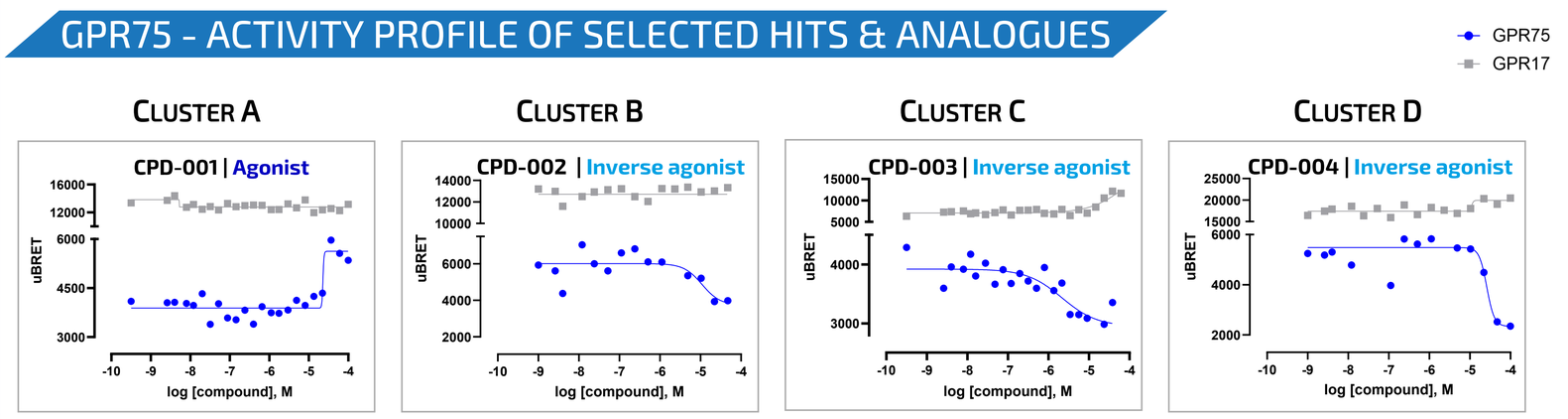

Figure 5: Summary of HTS hits and analogues. Agonist and inverse agonists hits of GPR75 have been identified from different chemical series (**Top**). Compounds from the same chemical series as the GPR75 inverse agonist CPD-004 demonstrate different potencies and profiles showing the beginning of SAR on this series. Similarly, Compounds from the same chemical series as the GPR75 inverse agonist CPD-002 show increased activity enabling a second series for SAR studies (**Bottom**). HEK293 cells were co-transfected with an EC_{80} of GPR75- or GPR17-encoding plasmid DNA and the βarrestin-2 biosensor. BRET was recorded at 72h post-transfection. Resulting curves were fitted using the (agonist) vs. response - Variable slope (four parameters) non-linear regression model (GraphPad 10).

ID	MW	clogP	TPSA	HBD	HBA	Profile on GPR75	EC50 (µM)
CPD-001	325	3.7	30	0	4	Agonist	22.8
CPD-002	485	5.4	92	2	7	Inverse agonist	10.9
CPD-003	329	2.2	58	0	3	Inverse agonist	2.16
CPD-004	361	4.1	72	1	5	Inverse agonist	26.1

ID	Profile on GPR75	EC50 (µM)
CPD-020	Inverse agonist	6.39
CPD-023	Inverse agonist	>30
CPD-027	Inverse agonist	0.95
CPD-028	Inactive	>30

Key molecular properties of hits from each cluster are summarized providing early insight into identification of compounds with favorable drug-like properties (**left**). Activity profile and potencies of hits and their corresponding analogues (**right**) support evidence of early SAR on these series. MW: molecular weight (g/mol), clogP: calculated octanol-water partition coefficient, TPSA: topological polar surface area, HBD: number of hydrogen bond donor, HBA: number of hydrogen acceptor.

GPR151

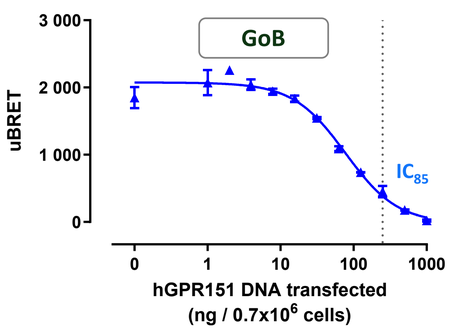

Figure 7: Human GPR151 dose-dependently decreases basal GoB activity. HEK293 cells were co-transfected with increasing amounts (0-1000 ng/ 0.7x10⁶ cells) of hGPR151-encoding plasmid DNA and the GoB biosensor. BRET was recorded at 48h post-transfection and resulting curves were fitted using the (agonist) vs. response - Variable slope (four parameters) non-linear regression model (GraphPad 10). hGPR151 exhibited an unconventional mode of constitutive activity, characterized by a receptor dose-dependent decrease in basal GoB activity.

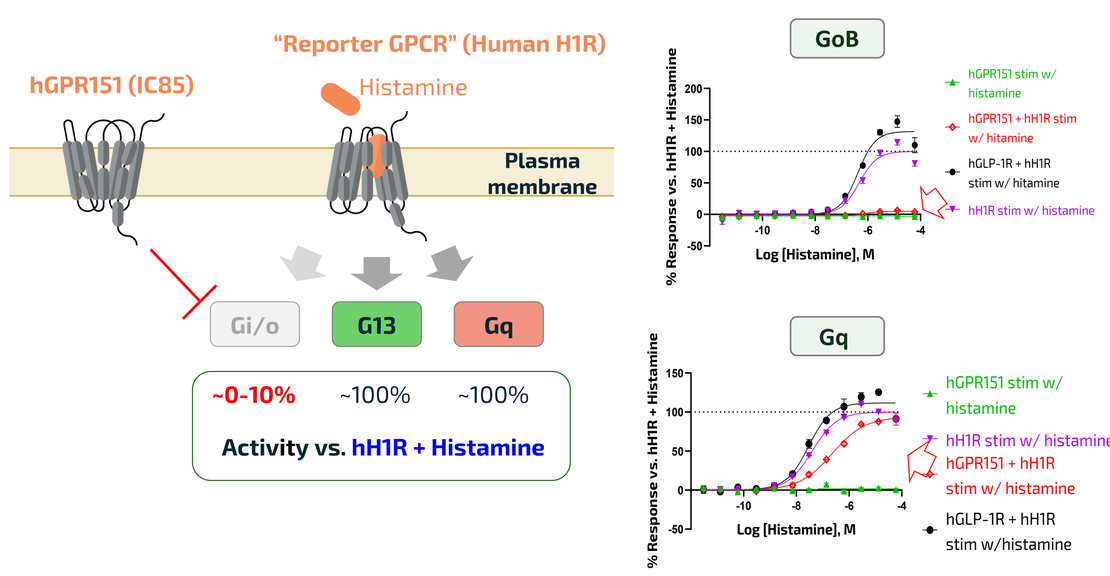

Figure 8: Human GPR151 selectively dampens Gi/o signaling downstream of the human histamine H1 receptor (hH1R).
HEK293 cells were co-transfected with i) an IC_{85} of hGPR151-encoding plasmid DNA (Figure 7) or an equivalent amount of hGLP-1R-encoding plasmid DNA (negative control), ii) hH1R-encoding plasmid DNA and iii) either the GoB or Gq biosensor. At 48h following transfection, cells were stimulated with increasing concentrations of histamine and BRET was recorded 20 minutes later. The resulting curves were fitted using the log(agonist) vs. response - Variable slope (four parameters) non-linear regression model (GraphPad 10). The data revealed that hGPR151 abrogated histamine-induced GoB, but not Gq nor G13 (not shown), signaling by hH1R, thus highlighting the pathway-specificity of this "*trans*-inhibitory" activity.

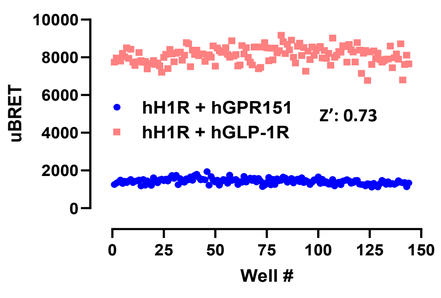

Figure 9: Development of *BASALscreen-trans*-inhibition assay for screening of hGPR151 inhibitors. **Up**: The *BASALscreen- trans*-inhibition assay was successfully miniaturized to 384-well format. The assay is sufficiently robust (**Z': 0.73**) to enable for screening of inhibitors of hGPR151's *trans*-inhibitory action. **Right**: Identification of such inhibitors would result in the reversal of hGPR151's dampening of hH1R GoB signaling, partially or fully restoring activity of this signaling pathway.