The Establishment of Cannabinoid Family Receptors Screening Assays Facilitate Novel Drug Discovery

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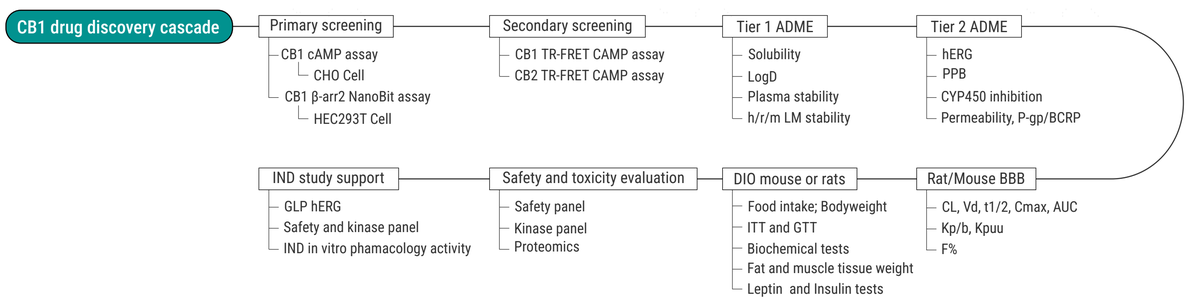
Introduction

The CB1 receptor, a key component of the endocannabinoid system (ECS), is crucial in appetite control, pain perception, mood regulation, obesity, and diabetes. In oncology, the ECS plays a pivotal role in tumors' growth, development, and metastasis, with the activation or inhibition of the CB1 potentially exerting substantial impacts on cancer treatment and prevention. In vitro and in vivo modes are established to understand the signaling pathways of CB1, interactions with other cellular components and the development of more selective novel drugs with fewer side effects. CB1 receptor activation inhibits Forskolin-stimulated adenylate cyclase by G protein (Gαi/o) and increases the phosphorylation of extracellular signal-regulated kinase 1/2 (pERK1/2) via G protein-dependent and β-arrestin-dependent pathways. Here we constructed an integrated experimental cascade, including in vitro HTRF cAMP assay and β-arretin2 NanoBiT assay, to conduct the high throughput hit-to-lead compound screening, agonist, antagonist and inverse agonist validation. Meanwhile, several CDX modes are constructed for promisingly conducting in-vivo experiments and biomarker detection. Thus, ICE supports multiple approaches for helping the drug discovery and development of CB1 to facilitate the treatment of multiple diseases.

CB1 Screening Cascade



GPCR Overexpression Cell Line Construction

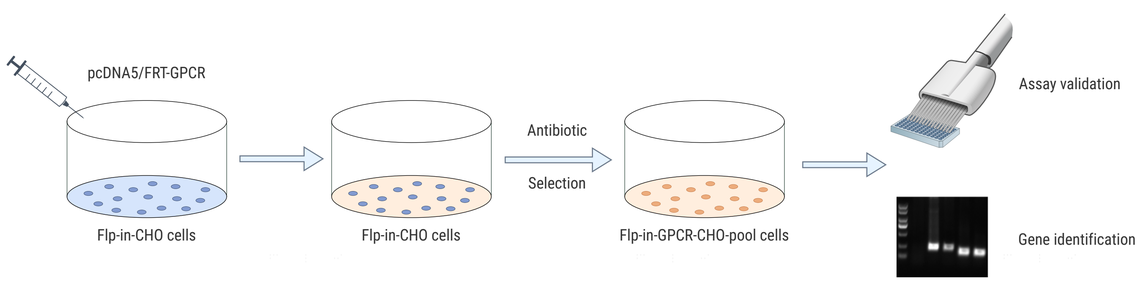


Figure 1. GPCR cell line construction by Flp-in system.

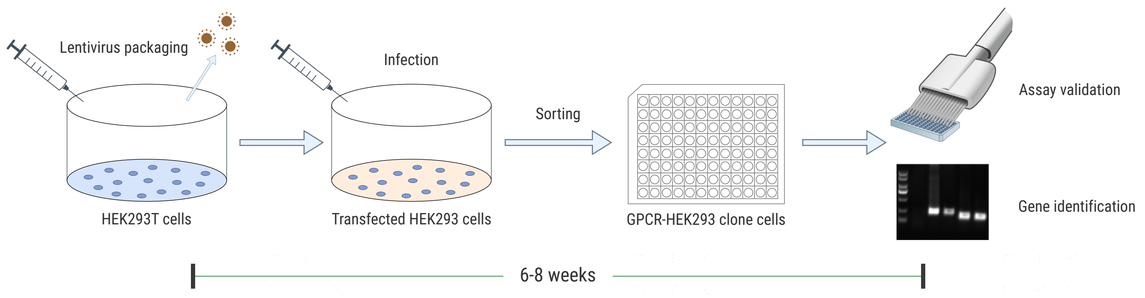


Figure 2. GPCR cell line construction by Lentivirus system.

Results

1. GPCR HTRF cAMP assay

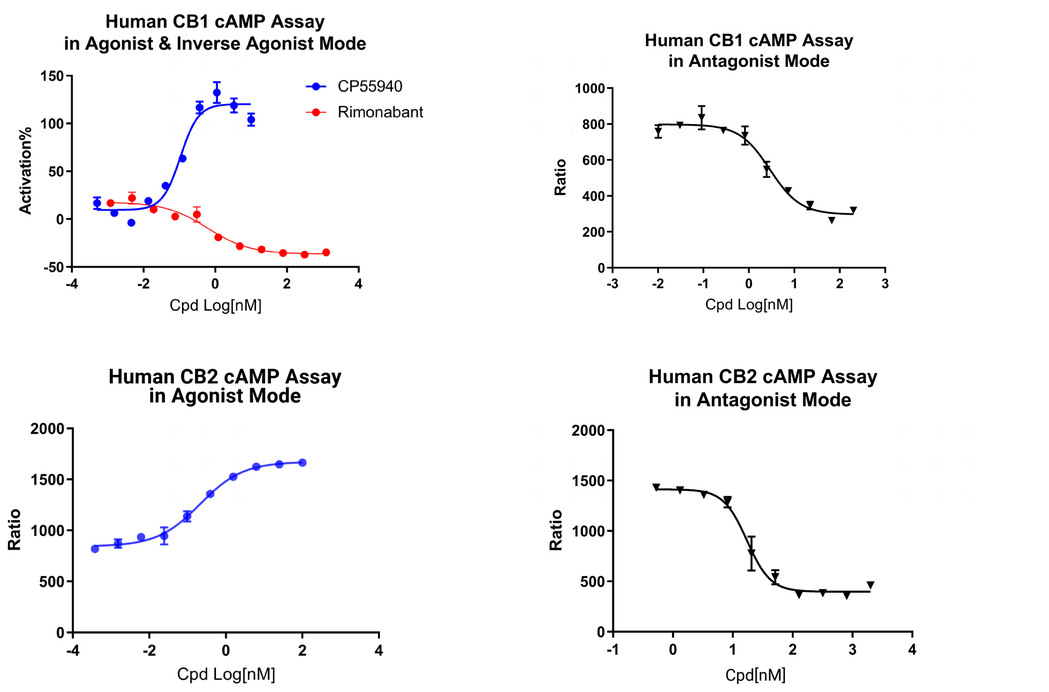
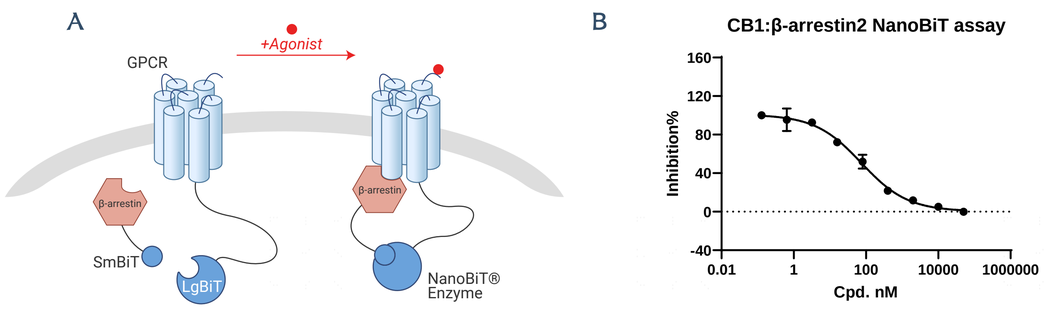


Figure 3. HTRF cAMP assay was constructed for compound screening. The principle of this assay is grounded in HTRF (Homogeneous Time-Resolved Fluorescence) technology. It is formatted as an immunoassay for quantifying cAMP levels, which are generated in response to the modulation of adenylyl cyclase activity by GPCRs: Cannabinoid receptor CB1 and CB2 stable cell lines were constructed for agonist, inverse agonist and antagonist validation.

2. β-arrestin NanoBiT assay



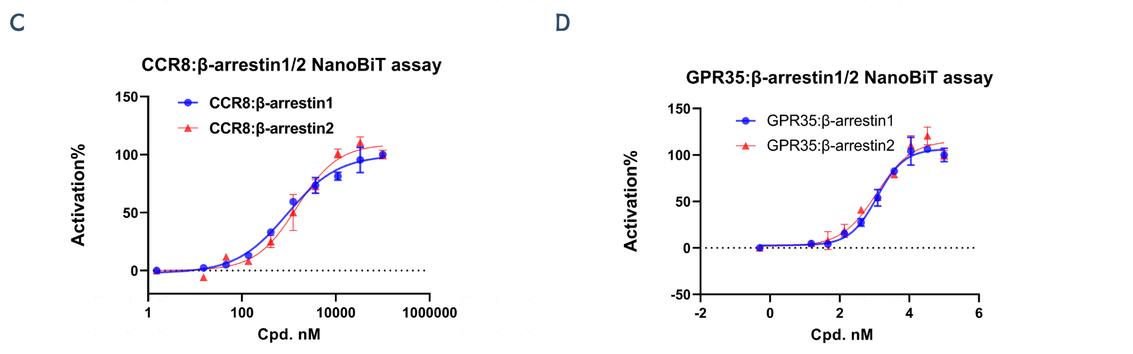


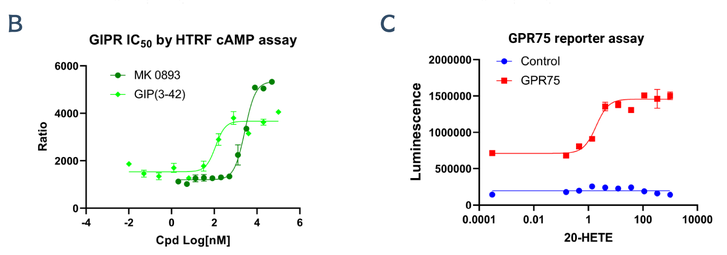
Figure 4. Monitoring recruitment of the GPCR and β-arrestin in cells. A. The NanoBiT® assay operates on the principle that when two protein tags are in proximity to one another, the energy from a bioluminescent luciferase donor can be transferred non-radiatively to an acceptor tag, resulting in the emission of light from the acceptor. Transfer of energy is dependent on the distance and orientation of the tags to each other. Consequently, the assay can be effectively utilized to detect and measure the interactions occurring between proteins. B. The NanoBiT assay was used to characterize the CB1 and β-arrestin recruitment. C. C-C motif chemokine receptor 8 plays an important role in the migration and positioning of immune cells. The CCR8 and beta-arrestin1/2 recruitment was analyzed by NanoBiT assay. D. GPR35 is a multi-functional orphan receptor, a potential target for drug development and the treatment of inflammatory responses and cancer diseases. GPR35and beta-arrestin1/2 NanoBiT assay was developed for dru discovery and compounds screening.

3. ICE_Obesity Panel Establishment

A

Assay Targe	Assay Format
CALCR *	Agonist
CALCR-RAMP *	Agonist
CCKAR *	Agonist
CB1 *	Antagonist
GCGR *	Agonist
GIPR *	Agonist/Antagonist
GLP1R *	Agonist
MC4R *	Agonist
NPY1R *	Agonist
GPR75 *	Agonist/Inverse agonist
HCRTR1 *	Antagonist
HCRTR2 *	Antagonist

* indicates that the protein is available for use in SPR



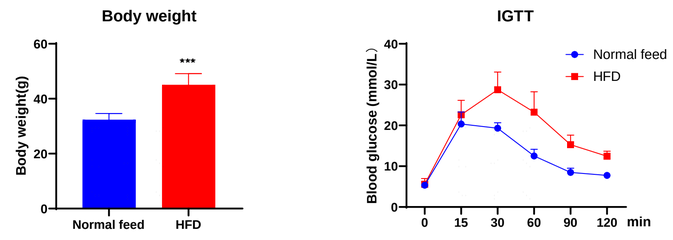
Immobilized ligand	Injection variables Single cycle kinetics 1 Solution	Affinity model	Steady state affinity KD (M)	Rmax (RU)
GPR75	CCL5	Steady state affinity	3.88e-10	5.7

Figure 5. Obesity-Relevant Target Profiling: A. GPCR receptors related to obesity and metabolic disease were chosen to construct the ICE_Obesity Panel. B&C . Different receptors stable cell lines were established for application in multiple screening assays. The GIPR stable cell line was used for HTRF cAMP assay, GPR75 stable cell line was applied in reporter assay to validate its function. D . GPR75 SPR binding assay by Biacore. The signal changes during protein association and dissociation were recorded, and the affinity and kinetic parameters could be fitted.

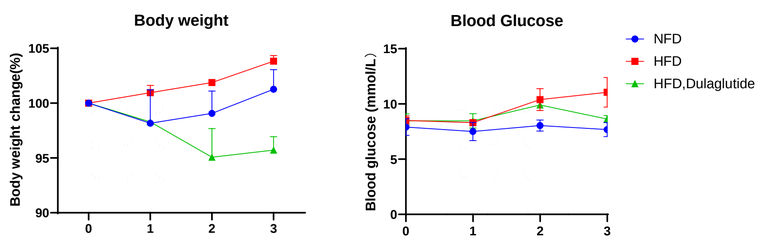
4. DIO MODE in Mouse

HFD/STZ induction conditions

Induction	HFD/STZ (IP)	HFD
Animal	C57BL/6, 4 weeks	C57BL/6, 8 weeks
Criteria	Blood sugar (BS) ≥16.7mM	BW, Blood glucose, IGTT
Treatment	10-12 weeks	16-20 weeks
Routes	PO,IP,IV,SC	PO,IP,IV,SC
Examination	BS, FBS, BW,GTT, AST, ALT etc.	BW, Blood glucose, IGTT
Optional	Pathology, biomarker, PK/PD, in vivo MOA	



The bodyweight (A) and IGTT(B) after 20-weeks normal feed or HFD feed .IGTT : Intraperitoneal injection of glucose tolerance. T-test.***p＜0.001.



The bodyweight change(C) and Blood glucose(D) with 3-weeks dulaglutide treatment after 20-weeks normal feed or HFD feed.

Summary

We have successfully established a stable CB1 cell line that is versatile for various functional assays, ideal for the compound screening process aimed at identifying potential clinical candidates. Additionally, we have developed a β-arrestin2 NanoBiT assay tailored for the validation of oncology-related targets such as CB1, GPR35, and CCR8. This assay is designed to assess the activity of diverse ligands, encompassing agonists, antagonists, and biased agonists. Our CB1 screening cascade can provide comprehensive compound evaluation across in vitro and in vivo platforms, thus serving as an efficient screening platform for new drug discovery.

References

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