PathHunter™ β-Arrestin Assays as a Universal Tool For Orphan GPCR Analysis

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Abstract

Orphan GPCRs continue to represent a target class with immense potential for the discovery of novel therapeutic compounds. In spite of the proven track record of GPCRs as druggable targets, over 100 non-odorant GPCRs remain to have their cognate ligands identified. Here we present the PathHunter Arrestin assay as a facile screening approach to orphan GPCR drug discovery. In this system, a small peptide is fused to the GPCR target, and the complementing fragment is fused to β-Arrestin2. Upon activation, the GPCR binds to arrestin forcing complementation of the enzyme fragments. The activity is detected with a single addition, HTS friendly, chemiluminescent reagent and is analyzed without waiting for reporter gene activity, or without knowing the second messenger signaling of the target receptor. The PathHunter assay is also unique in that only activation of the tagged GPCR will yield signal, providing a system that will not be affected by endogenous receptors. This eliminates many of the common false positives associated with other assays and makes it an ideal format for primary HTS screening. DiscoveRx has created an industry leading portfolio of orphan GPCR stable cell lines that are available for surrogate ligand discovery, de-orphanization, and profiling. This presentation will focus on the validation of the PathHunter system for orphan GPCR research and screening, the development of a cell line pane for profiling, and proof data from a focused library that receptors can in fact be de-orphanized with our system. Furthermore, we will present exciting examples where orphan GPCRs appear to regulate the function of characterized GPCR, highlighting the potential for orphans to heterodimerize with other GPCR partners and modulate their activities.

Cell-Based PathHunter β-Arrestin Assays

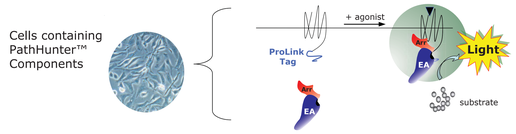


Figure 1. The PathHunter β-Arrestin assays monitor the interaction of β-Arrestin with activated GPCRs using Enzyme Fragment Complementation. A small 42 AA enzyme fragment, ProLink is appended to the C-terminus of the GPCR. Arrestin is fused to the larger enzyme fragment, EA (Enzyme Acceptor). Activation of the GPCR stimulates binding of arrestin and forces complementation of the two enzyme fragments. This action results in an increase in enzyme activity that is measured using chemiluminescent PathHunter Detection Reagents.

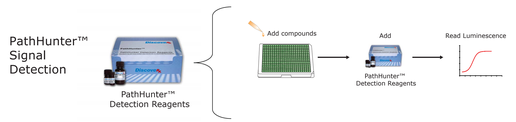


Figure 2. The PathHunter Detection Reagents consist of a lysis buffer and substrate mixture that is added as a single solution to stimulated cells. After a short incubation, the PathHunter chemiluminescent signal can be detected using any standard luminometric plate reader.

DiscoveRx Orphan GPCR Biosensor Cell Lines

PathHunter oGPCR Cell Pool Creation

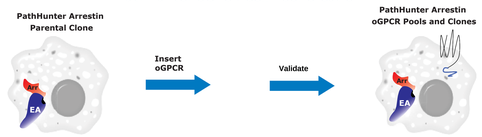


Figure 3. Starting from a clonal PathHunter β-Arrestin cell line, the oGPCR-ProLink fusion is transduced into the cells to make a stable population. This population is then validated for expression of the oGPCR and measured for baseline activity. Several PathHunter parental cell lines are available including: CHO, HEK, U2OS, PC-12, C2C12, 1321N1, and TM4. The oGPCR panel was made from CHO, HEK, and U2OS parentals.

PathHunter oGPCR cell pool Validation

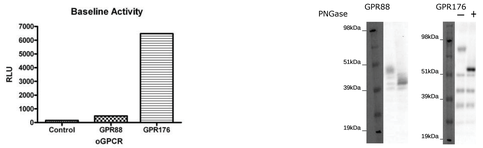


Figure 4. A) Each stable pool containing the Arrestin-EA and the oGPCR-ProLink is tested for baseline interaction of the GPCR and arrestin. The "tone" of orphan GPCRs varies widely and is shown in panel A. **B)** To ensure expression of the fusion at the appropriate size, cell lysates are prepared in the presence and absence of PNGase. Most oGPCRs are glycosylated, and removal of these glycosylations with PNGase causes the oGPCRs to run at the appropriate molecular weight.

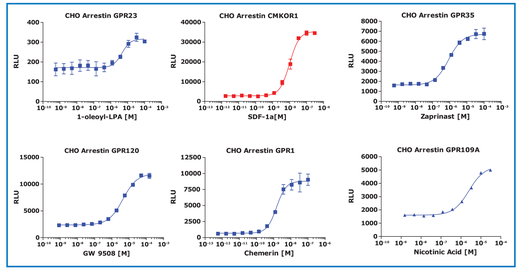
DiscoveRx Class A Orphan Panel

Class A PathHunter oGPCR Offerings

Family	Ligand	Hu Gene	DRX Product	Family	Ligand	Hu Gene	DRX Product
Class A Orphans		CCRL2	X	Class A Orphans		GPR45	X
Class A Orphans	RARRES2, resolvin E1, TIG2	CMKLR1	X	Class A Orphans		GPR50	X
Class A Orphans	SDF1a	CMKOR1	X	Class A Orphans		GPR52	X
Class A Orphans	alpha ketoglutarate	EBI2	X	Class A Orphans	LPI	GPR55	X
Class A Orphans	Chemerin	GPR1	X	Class A Orphans	sphingosine 1-phosphate	GPR6	X
Class A Orphans		GPR30	X	Class A Orphans		GPR61	X
Class A Orphans		GPR31	X	Class A Orphans		GPR62	
Class A Orphans	NF amide	GPR103 (AXOR)	X	Class A Orphans		GPR63	
Class A Orphans		GPR107	X	Class A Orphans	(lyso) phospholipid mediators	GPR65	X
Class A Orphans	oleoylethanolamide	GPR119	X	Class A Orphans	(lyso) phospholipid mediators	GPR68	X
Class A Orphans	sphingosine 1-phosphate	GPR12	X	Class A Orphans		GPR75	X
Class A Orphans	free fatty acids	GPR120	X	Class A Orphans		GPR78	X
Class A Orphans	(lyso) phospholipid mediators	GPR132	X	Class A Orphans		GPR79	X
Class A Orphans		GPR135	X	Class A Orphans		GPR82	
Class A Orphans		GPR137	X	Class A Orphans		GPR83	X
Class A Orphans		GPR139	X	Class A Orphans		GPR84	
Class A Orphans		GPR141	X	Class A Orphans		GPR85	X
Class A Orphans		GPR142	X	Class A Orphans		GPR87 (FKSG78)	
Class A Orphans		GPR146	X	Class A Orphans		GPR88	X
Class A Orphans		GPR148	X	Class A Orphans	1-oleoyl LPA	GPR92	X
Class A Orphans		GPR149	X	Class A Orphans		LGR4 (GPR48)	X
Class A Orphans		GPR15	X	Class A Orphans		LGR5	X
Class A Orphans		GPR150	X	Class A Orphans		LGR6	X
Class A Orphans		GPR151	X	Class A Orphans		MAS1	
Class A Orphans		GPR152	X	Class A Orphans		MAS1L	
Class A Orphans		GPR153		Class A Orphans	beta;-alanine	MRGPRD	X
Class A Orphans		GPR156	X	Class A Orphans		MRGPRE	X
Class A Orphans		GPR160		Class A Orphans		MRGPRF	X
Class A Orphans		GPR161		Class A Orphans		MRGPRG	
Class A Orphans		GPR162	X	Class A Orphans		MRGPRX1	X
Class A Orphans		GPR17	X	Class A Orphans		MRGPRX2	
Class A Orphans		GPR171	X	Class A Orphans		MRGPRX3	X
Class A Orphans		GPR173	X	Class A Orphans		MRGPRX4	X
Class A Orphans		GPR174		Class A Orphans		OPN3	
Class A Orphans		GPR18		Class A Orphans		OPN5	
Class A Orphans		GPR182	X	Class A Orphans	alpha;-ketoglutarate	OXGR1	X
Class A Orphans		GPR19	X	Class A Orphans		P2RY10	
Class A Orphans		GPR20	X	Class A Orphans	LPA	P2RY5	
Class A Orphans		GPR21		Class A Orphans	P2RY8		X
Class A Orphans		GPR22		Class A Orphans	succinate	SUCNR1 (GPR91)	X
Class A Orphans	lysosphosphatidic acid	GPR23	X	Class A Orphans		TAAR2	
Class A Orphans		GPR24	X	Class A Orphans		TAAR3	
Class A Orphans		GPR25		Class A Orphans		TAAR5	
Class A Orphans		GPR27	X	Class A Orphans		TAAR6	
Class A Orphans	sphingosine 1-phosphate	GPR3	X	Class A Orphans		TAAR8	
Class A Orphans		GPR31		Class A Orphans		TAAR9	
Class A Orphans		GPR32	X	Class A Orphans	nicotinic acid (high affinity)	GPR109A	X
Class A Orphans		GPR33		Nicotinic acid receptor family	nicotinic acid (low affinity)	GPR109B	
Class A Orphans		GPR34		Nicotinic acid receptor family	lactate, niacin	GPR81 (FKSG80)	X
Class A Orphans	ziprinast	GPR35	X	Nicotinic acid receptor family		CCBP2	
Class A Orphans	neuropeptide head activator	GPR37		Non-signalling 7TM CBP		CCRL1	
Class A Orphans		GPR37L1	X	Non-signalling 7TM CBP		DARC	
Class A Orphans	obestatin	GPR39	X	Non-signalling 7TM CBP		GPER (GPR30)	X
Class A Orphans	(lyso) phospholipid mediators	GPR4	X	Estrogen Receptor			

Pairing of oGPCRs with Adopted Ligands

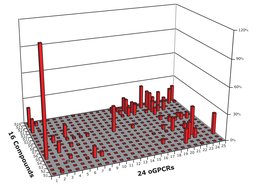
The following PathHunter oGPCRs have been paired with their adopted ligands. Agonist stimulation with the adopted ligands results in binding of arrestin to the ProLink tagged GPCR and an increase in enzyme activity. These results demonstrate the funcitonality of the assay with recently de-orphanized receptors.



oGPCR profiling with a Focused Compound Set

Panel:

45 of the Pathhunter oGPCR cell pools were screened against a panel of 275 GPCR-focused compounds. The cells were screened in 384-well dishes with a library concentration of 10uM. Example screening data is shown below.



Results:

4 confirmed hits were identified using a cutoff of 35% receptor activation across 3 different oGPCRs.

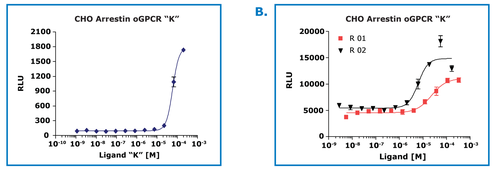


Figure 5. Hits from the small scale oGPCR screen were confirmed and used to select clones from the PathHunter oGPCR pools. Shown are dose response curves for two of the GPCRs with the DiscoveRx identified ligands. For oGPCR "R", two ligands were identified in the library that activate the receptor. Further characterization of these target is ongoing.

oGPCR Expression can Modulate Target Function

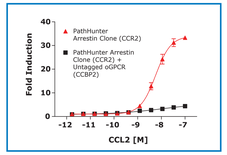


Figure 6. Several oGPCRs are suspected to modulate the function of other GPCRs co-expressed in the cell. Here we expressed an untagged oGPCR, CCBP2, in a PathHunter Arrestin cell line, CCR2. Co-expression of CCBP2 resulted in a decrease in the responsiveness of the cell line to the CCR2 ligand indicating that CCBP2 negatively regulates CCR2 signaling.

Conclusions

- Arrestin binding occurs in the majority of GPCRs tested making the PathHunter Arrestin platform an ideal solution for de-orphanization programs.
- Interaction of the Arrestin-EA fusion with the ProLink tagged GPCR is required for signal generation yielding a highly specific assay with far fewer false positives than second messenger assays.
- DiscoveRx offers a panel of over 80 characterized oGPCR cell lines for research and drug discovery.
- A small scale screen yielded 4 confirmed hits on 3 different GPCRs. Validating PathHunter Arrestin Assays as valid approaches for GPCR de-orphanization.

Technology Access

Cell Lines — Finished cell lines containing the Arrestin-EA and oGPCR-ProLink fusions as heterogeneous pools, or clonal cell lines. > 80 available

Tool Box — A clonal PathHunter parental cell line and a ProLink vector are available for build-your own cell lines (7 cell backgrounds available)

Kits — Cells, detection reagents, and plates ready to go right out of the freezer. Each kit contains enough cells for 2 X 96 well plates

Discovery Partnerships — Custom screening and profiling against PathHunter oGPCRs

DiscoveRx
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