Oustanding Deorphanization Platform to turn Orphan GPCRs in Validated Targets

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Summary

Orphan receptors have been a tremendous source of original targets over the 15 past years since the first success story of reverse pharmacology for nociceptin identification in 1995 (Meunier *et al.,* 1995). Despite the successes obtained in the late nineties with dozens of orphan receptors identified using multiple strategies (reviewed in Civelli *et al.,* 2006; Parmentier & Detheux, 2006), there are still more than 100 orphan receptors awaiting identification of natural ligands and only few deorphanizations reports have been published in the last 5 years. Indeed, use of multiple assays format allowed to circumvent functional selectivity or special coupling of some receptors. In addition the source of ligands is still a limiting step in the process even if heterodimerization or constitutive activity are also possible mechanisms to consider. Here we present results obtained with a library containing hundreds of tissue extracts from different species using protocols specific for various ligands. This strategy enable Euroscreen to deorphanize 10 receptors in the last 10 years and valorize several deorphanized receptors in drug discovery programs.

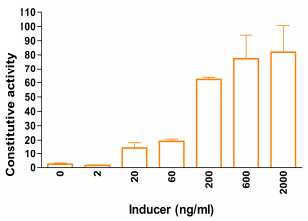
Ligands

Large collections of peptides, lipids, bioamines, natural products, metabolites and small molecules have been successfully used for identification of new ligands for orphan GPCRs (matching). These ligand collections allowed screening at high concentrations in multiple assay formats and identification of different families of receptors. Using this strategy, Euroscreen identified new chemokine for CCR5 receptor (Detheux et al., 2000), purinergic receptor $P2Y_{11}$ (Communi et al., 1997), short chain fatty acid GPR43 receptor (Le Poul et al., 2003), NPFF2 receptor (Kotani et al., 2001) and GPR7 neuropeptide (Brezillon et al., 2003). In parallel, screening of natural products or small molecules libraries allowed identification of surrogate ligands validating specific assay for a given receptor or developing chemical tools to validate an orphan receptor function. Chemoinformatics strategies predicting peptides encoded in the human genome were also evaluated but not very successful. Indeed, this approach does not allow prediction of specific ligand processing or modifications. A promising strategy still relies on the use of tissue extracts to identify a specific activity that can be followed through multiple rounds of HPLC or other purification steps (Step 4) and final identification of the ligand responsible for the activity by mass spectrometry or any other highly sensitive analytical methods. This approach requires using diverse sources of tissues including human pathological samples and different protocols (Step 3) to purify specific natural agonist (Step 4).

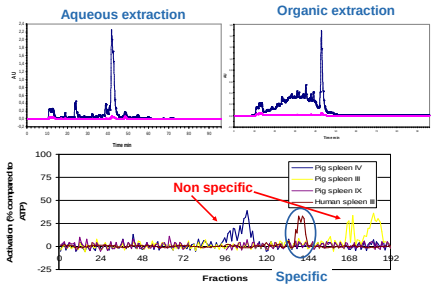
Assays

When a recombinant cell line expressing the orphan receptor at cell surface is validated (Step 1), screening of pure reference ligands, small molecules libraries or natural products can be performed using multiple assays with specific attention for some limitations like fluorescence interference or non specific responses. The sensitivity of the assay is not often an issue because the screening can be performed at high concentration in regard to expected potency, at least for natural ligands. Radioligand binding or functional assays including calcium flux, cAMP, inositol phosphates, GTPγS, β-arrestin, microphysiometer and impedance have been used with success. When considering the screening of tissue extracts, the sensitivity of the assay both for the natural ligand and contaminants found in the extracts is a key factor for success. The selected assay must be sensitive, with low signal background to detect small activation. Robustness is also critical to allow the screening of samples containing high concentrations of various solvents. So far, we have used with success aequorin, cAMP and GTPγS assays and we are still validating the βarrestin assay for screening of tissue extracts (Step 4). Following the identification of a lipid as natural ligand for a ES1 receptor, the different assays were evaluated in dose response curves and showed similar potencies (Step 5), illustrating that sensitivity of an assay is not the only critical factor to be used in tissue extract screening to identify specific activity. Deorphanization allows to validate ES1 receptor and start a drug discovery program (Step 6)

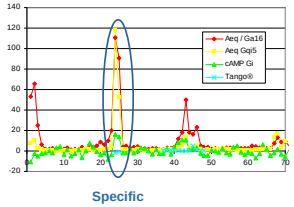
Step 1: Validation of a cellular assay using inducible cell line expressing ES1



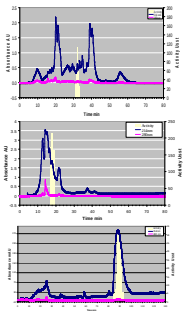
Step 2: Different purifications protocols yield specific profiles and activities for ES1



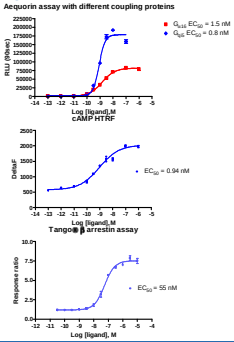
Step 3: Selection of the best assay for ES1 activity purification



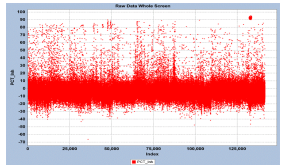
Step 4:: Purification of ES1 activity found in spleen using aequorin



Step 5: Dose response curves of ES1 with identified ligand and various functional assays



Step 6: : Screening of ES1 with Euroscreen library for identification of antagonists



Conclusions

- Chemoinformatics, natural ligand collections and small molecules libraries have been powerful methods to rapidly match a known ligand with an orphan GPCR. However there are still more than 100 orphan GPCRs that were not characterized using such strategies. While constitutively active orphan GPCRs can be used as targets to initiate a drug discovery program, most of the remaining orphan receptors characterizations will rely on tissue extract screening to identify fully processed natural ligand(s) and initiate a target validation and then a drug discovery program.
- As orphan receptor screening is a double-blinded assay with no information for specific assay selection and presence of a specific ligand in the screened samples, Euroscreen has implemented a target discovery platform based on multiple strategies to overcome or decrease these limitations. Use of stable or inducible clonal cell lines validated for complementary assay formats with different sources of ligands allowed identification of natural and surrogate agonists for orphan receptors. Some of these receptors were rapidly validated as novel targets for drug discovery and led to the initiation of hit identification and lead optimization programs, like GPR43 or ES1.