Validation Of The DiscoveRx PathHunter™ Assay Using Human S1P3 Receptors At Different Expression Levels

Darren Riddy1, Craig Stamp1,2, Mark Dowling1
1Novartis Institutes of Biomedical Research, Horsham, West Sussex, United Kingdom, 2University of York, Yorkshire, United Kingdom

Sphingosine-1-phosphate (S1P) is a bioactive lipid involved in a wide range of cellular responses. S1P binds to five G-protein coupled receptors (GPCRs), S1P1-5, with nanomolar potencies. The receptors signal differentially via numerous G-proteins, including Goi, Goq/11 and Gao12/13 hinders the development of a single uniform assay difficult. Recently a G-protein independent assay technology, that utilises the recruitment of β-arrestin, has been made available. DiscoveRx’s PathHunter™ assay, which is G-protein independent, utilises β-galactosidase, where upon receptor activation complementation of two β-gal fragments causes formation of a functional enzyme that converts added substrate into a detectable signal. Use of this assay format is increasing for both uHTS and HTS campaigns; however, no thorough investigation into the pharmacological parameters has been performed.

Using the commercially available agonists, S1P, CYM-5442 and FTY720-P, we describe a direct comparison of traditional assays including radioligand binding and a functional calcium assay with the PathHunter™ assay. Furthermore, we investigated changes in compound pharmacology using cell lines with different expression levels of the humanS1P3 receptor. Four clones were selected (F6, B3, E5 and B6) with varying Emax levels indicating different expression levels (RLU ranging from 4,500 to 37,000). These were confirmed using radioligand binding with a mean pEC50 of 8.80 ± 0.07 (8.73 – 8.90). We profiled the agonists in the binding assay and determined their pKi’s. There was no significant change in the calculated pKi’s for FTY720-P (8.50 ± 0.14) and CYM5442 (6.87 ± 0.00). However, there was a significant change between F6 (9.51 ± 0.10) and B6 (8.76 ± 0.15) and B3 (9.63 ± 0.06) and B6 when using S1P. After showing that these compounds bind to the receptor we next investigated whether this could translate into a functional response. Using the PathHunter™ assay we determined pEC50 for the agonists. There was no significant change in the pEC50 of FTY720-P (7.82 ± 0.10) or CYM5442 (5.68 ± 0.06) with increasing expression levels, however, we did identify changes in pEC50 to S1P (8.15 ± 0.14 to 8.61 ± 0.61) with a significant difference between clones F6 and B6. Agonist function can be determined by both potency (Ka) and efficacy (Ta); therefore, we fitted the PathHunter™ data to the Operational model. No meaningful values were generated indicating a nonlinear stimulus coupling between agonist binding and β-arrestin recruitment. This correlation between approximate pEC50 and calculated pKi, suggests that the PathHunter™ assay could be used to measure potency and affinity. Next we profiled the agonists in a G-protein mediated calcium assay. All agonists showed increases in Emax with increasing receptor expression levels, whilst the pEC50 value decreased with increasing receptor expression, S1P (9.01 to 9.82) and CYM5442 (6.30 to 6.84). FTY720-P only showed a partial response in the two highest expressing clones (F6 and B3). Using the Operational model we show that the calcium data increases in logτ with increasing receptor expression for S1P (0.45 ± 0.21 to 1.38 ± 0.27) and CYM5442 (-1.12 ± 0.22 to 1.36 ± 0.27). However, FTY720-P gives ambiguous data with values of < 1 suggesting its partial agonist effect.

Data generated in the PathHunter™ lays to the right of both the binding (Ka) and calcium data, therefore, we hypothesise that the PathHunter™ assay only detects the approximate low affinity conformation (R). As the PathHunter™ only measures affinity and potency, and does not give a good indication of efficacy; caution must be taken when screening for agonists.