Dynamic Mass Redistribution phenotypic assay for identifying ligands active at GPR35 receptors

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Introduction

GPR35 is an orphan receptor reported to be involved in inflammatory disorders (HU H. 2012), CNS disfunction (Shore DM 2015), pain (Alkondon M 2015), diabetes (Tanoguchi Y 2006) and immunologycal diseases such as asthma (Yenkins L 2010). Kinurenic acid (HU H. 2012), 2-oleil lisophosphatidic acid and recently chemokine CXCL17 (Berlinguer-Palmini R 2013) have been described as potential endogenous ligands but without confirmation; showing differences in efficacy between human and rat orthologues (Yenkins L 2010). Phenotypic assays based on Dynamic Mass Redistribution (DMR) revealed as powerful tool screening libraries in orphan receptors which signalling pathways and biology remain unknown. We aimed to develop a miniaturized phenotypic assay based on a DMR label-free technology that enables the detection of new ligands for human and rat GPR35 receptors.

Material and Methods

HT-29 cell line and IEC-6 cell line were seeded in LFC-384 well microplates (PerkinElmer 6057408). 24 hours after seeding, the culture medium was replaced with medium or HBSS buffer to optimize the assay conditions. Plates were incubated before reading a base line and later standard compounds were added. Measurements were done using an EnSpire (PerkinElmer) reader with Corning ® Epic® Label-free technology.

Results

A miniaturized phenotypic assay based on a DMR was developed to find new ligands for hGPR35 and rGPR35. For both cell lines, 15000 cells per well were selected as a suitable concentration. McCoy´s 5A medium supplemented with 25mM HEPES pH=7.4 was selected as Buffer Assay for HT-29 whereas HBSS buffer was selected for IEC-6. To validate the feasibility of the method, we obtained concentration-response curves of a synthetic agonist of GPR35 (Taniguchi Y 2008), Zaprinast, with values of EC_{50}=0.50±0.25µM for human GPR35 and values of EC50=4.2±0.30µM for rat GPR35.

Conclusions

We have developed a miniaturized phenotypic assay based on a DMR technology to measure the activity of compounds in human and rat cell lines expressing the orphan receptor GPR35.