Ligand Characterization of Mas-Related GPCR-X2 via Cell-Based Assays: Implications in Safety Liability Tests

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Abstract

Mast cells are principal players in IgE-, and more recently, non-IgE-mediated anaphylactoid reactions or pseudo-allergic Adverse Drug Reactions (ADR). A wide range of ligands can activate mast cells owing to their multiple receptor expression profiles, and their characterization hence warrants closer study, especially into their safety pharmacological roles. Among the receptor classes, orphan GPCRs have emerged as active proponents in non-immunogenic mast cell activation profiles with possible roles in pseudo-allergic or anaphylactoid reactions (McNeil *et al.* 2015). A relatively new subfamily of GPCRs associated with the MAS1 oncogene was identified as Mas-Related genes (MRGs) and their receptors, MAS-related GPCRs (MRGPR). One of its members, MrgprX2 encoded by the gene MRGPRX2, has been identified to play a role in the activation of several synthetic small molecules and peptides including U.S. FDA approved drugs. Studies have identified MrgprX2 receptor-mediated agonism to be implicated in causing adverse reactions associated with clinical candidate molecules during their development (Grimes *et al.* 2019).

In a recently released Application note, with represented data shown here, the characterization of selected agonists, previously identified as MrgprX2 agonists, was performed using calcium mobilization and β-arrestin recruitment assays feasible with the application of the DiscoverX's PathHunter® MrgprX2 cell line. The goal of this study was to show the high-throughput and time-savings value of using engineered, cell-based assays to study Mrgx2 ligand activation through these screening assays.

Targets Needing Safety Liability Screens in Pharmacological Panels

The importance and role of MrgprX2 as an emerging activator of non-IgE mediated mast cell degranulation (MCD) underscores its value in being a top panelist in safety pharmacology. MrgprX2's potential as a drug target for conditions/diseases related to mast cell function and regulation, including anaphylaxis, attests to its position as central to safety liability testing.

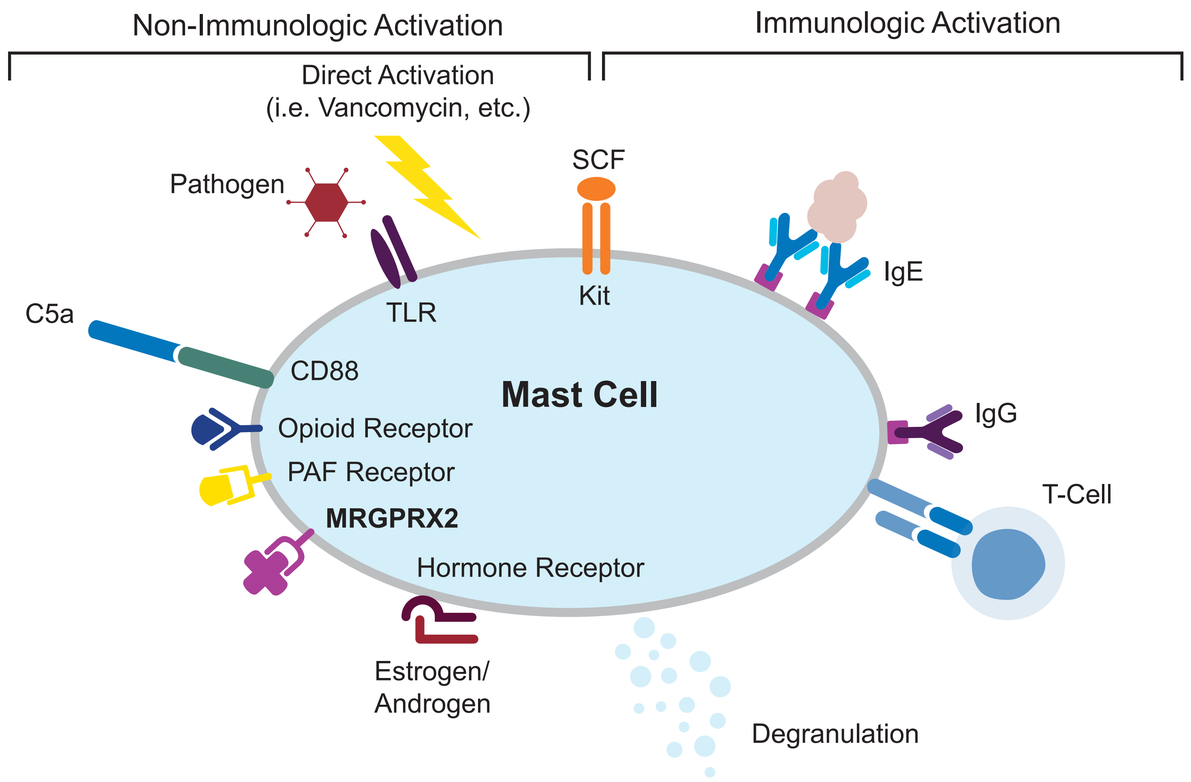

Figure 1. Mast cell degranulation including non-immunological activation route (modified from Spoerl *et al.* 2017). Mast cells can be activated by immunogenic effectors and by several types of receptors including Toll-like receptors (TLR), protease-activated receptors (PARs), opioid receptors, c5a complement, and T-cells depending on localization and type of mast cell (Hennino *et al.* 2006, Spoerl *et al.* 2017) opening up routes along non-immunogenic activation related pathways.

Receptor activation towards regulating mast cell degranulation has a purported key safety liability, as observed in studies such as that of Lafleur *et al.* 2020. Of particular mention is a study led by Dr. Andrew Brown's team at GlaxoSmithKline (GSK), which identified a series of orthologue receptors, typically of pharmacological interest during drug development, with a bioinformatics approach (Grimes et al. 2019). Subsequently, recombinant expression studies and compound profiling, including Eurofins DiscoverX's β-arrestin recruitment and calcium signaling assays, provided a rigorous characterization platform of compound pharmacology.

Ligand-Induced Cell-Based MrgprX2 Activation Assays

Several agonists (cortistatin-14, indolicidin, compound 48/80, mastoparan, octreotide, and vancomycin) were assessed in PathHunter MRGPRX2 cell lines for β-arrestin recruitment and calcium release using Eurofins DiscoverX assays.

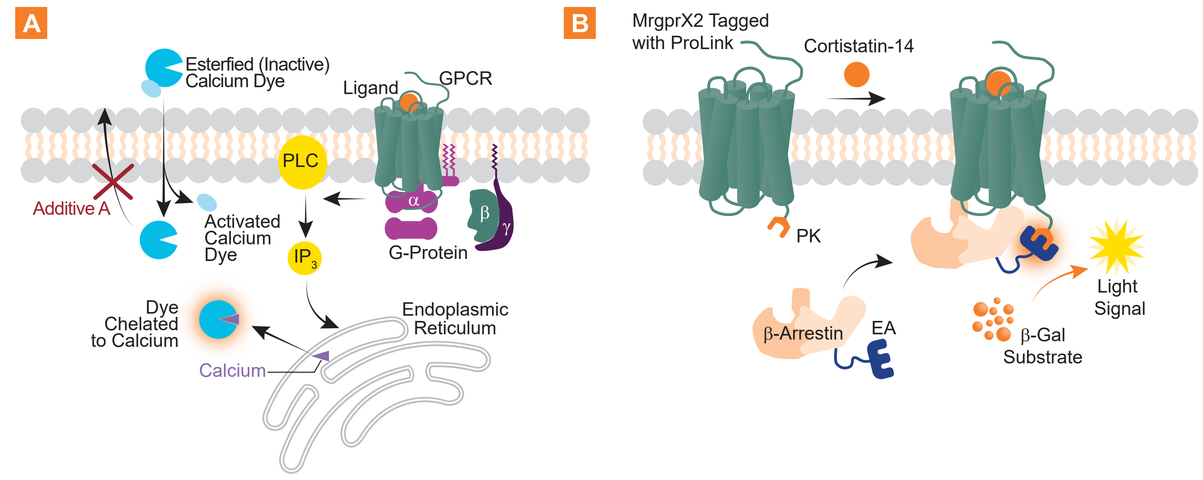

Figure 2. Calcium mobilization and β-arrestin recruitment assays. A. Measuring intracellular calcium mobilization based on the activation status of specific GPCRs can provide information that uncovers pharmacological rank-orders of lead agonists and calcium signaling flux. The calcium mobilization assay, Calcium NoWash^{PLUS} Assay, uses an esterified (inactive) calcium dye (probe) that penetrates the cell membrane and becomes active once inside the cell. The active form of the dye becomes fluorescent after binding to intracellular calcium. Additive A is added to prevent the dye from being released out from the cell. Ligand binding stimulates GPCR activation, resulting in the release of intracellular calcium stores from the endoplasmic reticulum (ER), which leads to an increase in fluorescence in the presence of the activated calcium dye. B. β-Arrestin recruitment was measured using the PathHunter β-arrestin assay, which is based on DiscoverX's proprietary Enzyme Fragment Complementation technology. This technology involves two recombinant β-galactosidase (β-gal) enzyme fragments that act as an enzyme acceptor (EA) and an enzyme donor (ED). Separately, the fragments are inactive, but when combined, they form an active enzyme. In this assay, MrgprX2 cell lines were engineered to co-express the ED, called ProLink™ (PK), tagged to the receptor, MrgprX2,and the EA tagged to β-arrestin. Activation of the MrgprX2-PK by agonist treatment (cortistatin-14) induces β-arrestin-EA recruitment, forcing complementation of the two β-gal enzyme fragments. The resulting functional enzyme hydrolyzes substrate to generate a chemiluminescent signal.

Dose Response and Rank Order Potency for Calcium Mobilization and β-Arrestin Recruitment Assays

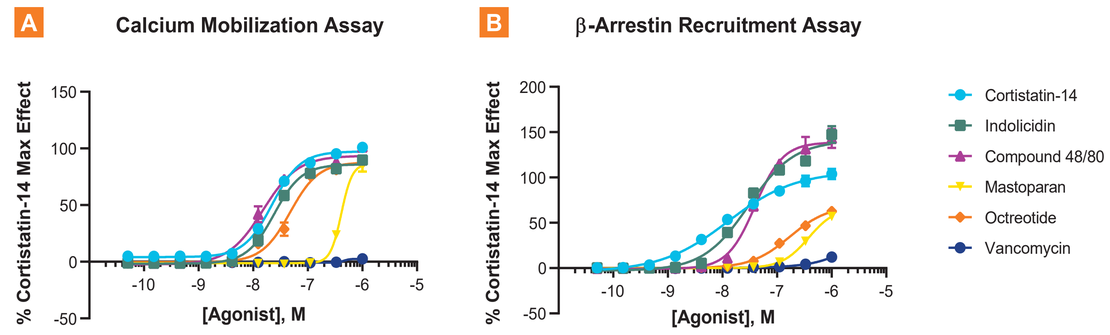

Figure 3. Dose response and rank order potency for calcium mobilization and β-arrestin recruitment assays. Readouts showing select agonists effect on (A.) calcium mobilization of intracellular calcium and on (B.) β-arrestin recruitment upon binding to MrgprX2 receptors. Both dose response curves are the mean of two replicates and show normalized data calculated from the maximum effect obtained with cortistatin-14.

Potency and Efficacy of Agonists

	Calcium Mobilization		β-Arrestin Recruitment	
	EC_{50} µM	% Max Cortistatin-14	EC_{50} µM	% Max Cortistatin-14
Cortistatin-14	0.22	100	0.11	100
Indolicidin	0.26	92	0.3	139.1
Compound 48/80	1.69	100	3.88	136
Mastoparan	4.34	89	3.28	60
Octreotide	4.84	90	15	65
Vancomycin	> 100	3	>100	12

Table 1. Potency and efficacy of agonists relative to Cortistatin-14.

Results herein are in good agreement with those previously published with respect to rank orders of potency agonists (Grimes *et al.* 2019) maintained relative to cortistatin-14 used as reference agonist for both assays (Figure 3. & Table 1.). Vancomycin activity is negligible with the concentration range used in these assays, but consistent with its expected low potency. These studies also highlight the potential for the β-arrestin assay to report higher levels of efficacy relative to cortistatin-14 that was not observed in the calcium mobilization assay.

Summary

In an effort to support the characterization and possible role of MrgprX2 in non-classical mast cell-mediated anaphylactoid reactions, Eurofins DiscoverX collaborated with Andrew Brown (GSK) and helped identify compounds/peptides that activated MrgprX2 via calcium mobilization and β-arrestin recruitment assays (Grimes *et al.* 2019).

Further, assessing MrgprX2 activation in an engineered cell model can provide value as a rapid, high-throughput, economical, mechanism-based screening tool for early mast cell degranulation or hazard identification during preclinical safety evaluation of therapeutics.

Learn more about the Eurofins DiscoverX GPCR Assays at discoverX.com/gpcrs

References

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