# Characterization of MRGPRX2 as a Novel Target to Address Mast-Cell Mediated Disorders

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## INTRODUCTION

**Mast cells are immune cells that have been implicated in the pathophysiology of many diseases including allergy, gastrointestinal disorders and pain:**

- Mast cells can be activated through the crosslinking of the immunoglobulin E (IgE)-Fc epsilon RI (FcεRI) receptor or through activation of G-protein coupled receptors (GPCRs) such as mas-related G protein-coupled receptor X2 (MRGPRX2).[1]
- MRGPRX2, a receptor that is highly expressed in mast cells, induces non-histaminergic, IgE-independent mast cell activation and degranulation upon binding to a wide variety of agonists including endogenous neuropeptides (eg, substance P, pituitary adenylate cyclase-activating polypeptide [PACAP], and cortistatin), anti-microbial peptides (eg, LL-37 and β-defensins), cationic proteins (eg, major basic protein 1 and 2), a number of pseudo-allergic drugs (eg, fluroquinolone antibiotics, phenothiazines, neuromuscular blocking agents and hormone receptor modulators) and natural remedies.[2]
- Mouse Mrgprb2 and Mrgpra1 are putative orthologs of the human MRGPRX2.[3,4]
- Activation of MRGPRX2 triggers the release of proinflammatory mediators, including tryptase, and a multicellular signaling cascade that likely plays a key role in multiple human diseases.

## AIM

To characterize the biology of MRGPRX2 *in vitro* and *in vivo*, including the ability of structurally diverse MRGPRX2 agonists to induce mast cell degranulation and the interaction between the MRGPRX2 and IgE pathways, to support the development of MRGPRX2 antagonists.

## MATERIALS AND METHODS

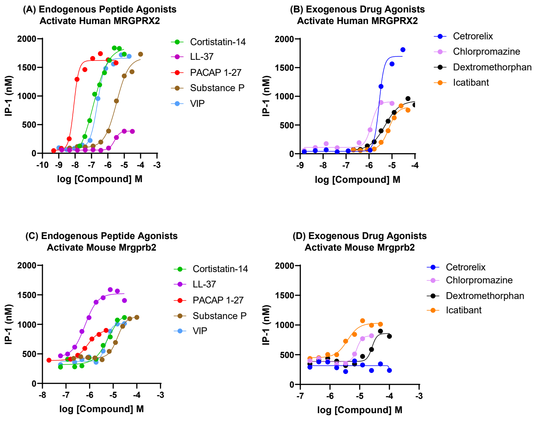
***In Vitro* Studies:** Chinese hamster ovary (CHO) cells stably expressing human MRGPRX2 and human embryonic kidney (HEK) cells stably expressing mouse Mrgprb2 were generated by Eurofins (San Diego, CA). Inositol monophosphate-1 (IP-1) standards and homogeneous time resolved fluorescence (HTRF) detection reagents were added according to an IP-One – Gq Kit purchased from Cisbio. IP-1 assay plates were read on a BMG ClarioStar plate reader or on a Molecular Devices SpectraMax iD5 plate reader. The HTRF ratio was calculated from the raw data and graphed using GraphPad Prism to calculate half maximal effective concentration ($EC_{50}$) values. Laboratory of Allergic Diseases type 2 (LAD2) mast cells (National Institutes of Health)[5] and human peripheral stem cell-derived mast cells (PSCMCs)[6] were treated with agonist compounds for 30 minutes at 37°C and evaluated for β-hexosaminidase[7] or tryptase release.[8] For IgE induced degranulation, cells were incubated overnight with 400 ng/mL biotin-IgE followed by stimulation with streptavidin to determine the IgE stimulation dose response. The percentage degranulation (percent β-hexosaminidase release) was calculated, followed by analysis using GraphPad Prism software to calculate $EC_{50}$ values. For LAD2 knockout cell lines, cells were transfected with clustered regularly interspaced short palindromic repeats (CRISPR) single guide ribonucleic acid (sgRNA) against either MRGPRX2 or FcεR1α and CRISPR associated protein 9 (Cas9) 2NLS Nuclease (Synthego). The cells were then sorted by flow cytometry. For cytokine secretion, LAD2 cells were treated for 24 hours followed by collection of supernatant and assessment of cytokine levels by Luminex multiplexed cytokine assays with Rules-Based Medicine HumanMAP Biomarker Panels.

***In Vivo* Studies:** CRISPR-mediated Mrgprb2 knockout (Mrgprb2 KO) and MRGPRX2 knock-in (MRGPRX2 KI) mice were custom generated by Applied StemCell for Escient Pharmaceuticals, Inc. (Escient). MRGPRX2 KI mice were confirmed for expression by polymerase chain reaction (PCR) as well as at the protein level by flow cytometry analysis of MRGPRX2+ peritoneal mast cells. 8- to 10-week-old C57BL/6J (Jackson Laboratories) or Mrgprb2 KO (Escient) and MRGPRX2 KI mice (Escient) were restrained and injected intravenously (IV) with 1% Evans Blue (Fisher Chemical) prior to intradermal treatment with vehicle phosphate-buffered saline (PBS), cortistatin-14 (Tocris Bioscience), substance P (Tocris Bioscience), LL-37 (Tocris Bioscience), icatibant (HOE-140; Tocris Bioscience) or goat anti-mouse IgE (Abcam). Evans Blue dye was quantitated from the skin tissue of sacrificed animals and results expressed as μg dye per mg tissue.

## RESULTS

### 1. A wide variety of structurally diverse agonists activate both human MRGPRX2 and mouse Mrgprb2 in overexpressing cell lines with differing efficacy and potency

**Figure 1: Activation of human MRGPRX2 (A-B) and mouse Mrgprb2 (C-D) by selected endogenous and exogenous agonists**




### 2. MRGPRX2 agonists potently induce degranulation of LAD2 mast cells and PSCMCs as measured by β-hexosaminidase release

**Figure 2: Degranulation of LAD2 mast cells (A) and PSCMCs (B) by selected MRGPRX2 endogenous and exogenous agonists**

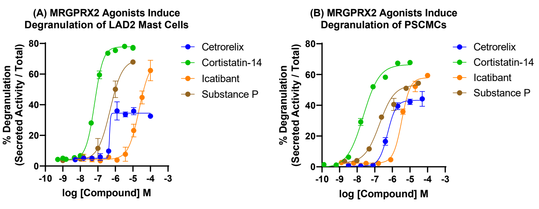


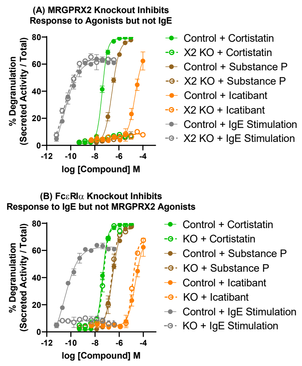

**Table 1: Select MRGPRX2/Mrgprb2 agonist potencies in overexpressing cell lines and mast cells**

| Agonist | MRGPRX2 IP-1 $EC_{50}$ (nM) | Mrgprb2 IP-1 $EC_{50}$ (nM) | LAD2 $EC_{50}$ (nM) | PSCMC $EC_{50}$ (nM) |
|---|---|---|---|---|
| **Endogenous Agonists** | | | | |
| Cortistatin-14 | 91 | 4200 | 59 | 57 |
| LL-37 | 2700 | 390 | 980 | nd |
| PACAP (1-27) | 52 | 3700 | 27 | nd |
| Substance P | 3200 | 18000 | 500 | 190 |
| VIP | 440 | 9500 | 450 | nd |
| **Exogenous Agonists** | | | | |
| Cetrorelix | 5300 | >60000 | 320 | 540 |
| Chlorpromazine | 3000 | 3800 | 4600 | nd |
| Dextromethorphan | 3600 | 18000 | 51000 | nd |
| Icatibant | 26000 | 7600 | 27000 | 3200 |

nd = no data

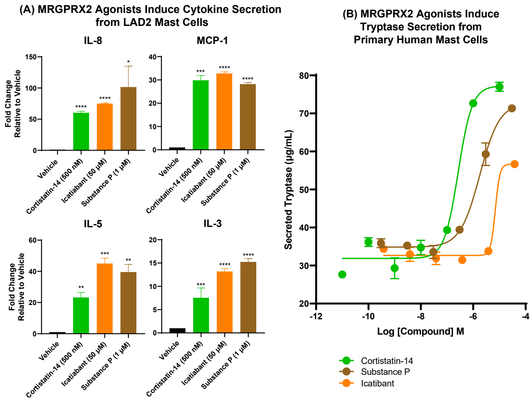
### 3. The MRGPRX2 and IgE pathways act independently to induce mast cell degranulation in LAD2 mast cells

**Figure 3: Knockout of MRGPRX2 inhibits degranulation induced by selected MRGPRX2 agonists but not IgE (A), whereas knockout of FcεRIα inhibits degranulation induced by IgE but not MRGPRX2 agonists (B)**




### 4. Downstream inflammatory cytokines and tryptase are released after MRGPX2 activation from mast cells

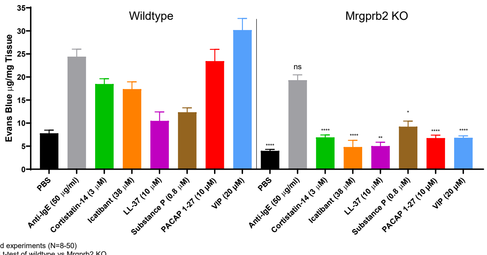
**Figure 4: MRGPRX2 agonists induce cytokine secretion from LAD2 mast cells (A) and tryptase secretion from peripheral stem cell-derived primary mast cells (B)**




(A) Unpaired t-test of agonist vs vehicle, n = 3 experiments
*p<0.05, **p<0.01, ***p<0.001, ****p<0.0001

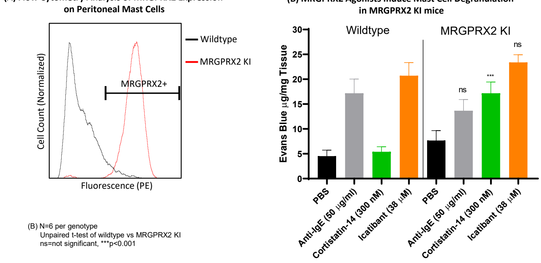
### 5. MRGPRX2/Mrgprb2 is the primary receptor mediating agonist-induced mast cell degranulation and vascular leakage in our proprietary transgenic knockout and knock-in animals

**Figure 5: Mast cell activation induced by a wide variety of MRGPRX2/Mrgprb2 agonists is inhibited in Mrgprb2 KO mice whereas IgE mediated degranulation is not inhibited**




Combined experiments (N=8-50)
Unpaired t-test of wildtype vs Mrgprb2 KO
ns=not significant, *p<0.05, **p<0.01, ****p<0.0001

**Figure 6: Flow cytometry analysis confirmed expression of MRGPRX2 on peritoneal mast cells isolated from MRGPRX2 KI mice (A); MRGPRX2 agonists induce mast cell degranulation in MRGPRX2 KI mice, and cortistatin-14 induced degranulation with elevated potency in KI mice (300 nM vs 3 μM), which is consistent with increased potency at human MRGPRX2 compared to mouse Mrgprb2 (B)**




(B) N=6 per genotype
Unpaired t-test of wildtype vs MRGPRX2 KI
ns=not significant, ***p<0.001

## CONCLUSIONS

- A wide variety of structurally diverse agonists activate both human MRGPRX2 and mouse Mrgprb2 in overexpressing cell lines with differing efficacy and potency.
- MRGPRX2 agonists potently induce degranulation of LAD2 mast cells and PSCMCs as measured by β-hexosaminidase release with potencies comparable to that observed in the MRGPRX2 overexpressed cell lines.
- MRGPRX2 and IgE act through independent pathways to induce mast cell degranulation as demonstrated by ablation of MRGPRX2 and the IgE receptor (FcεR1α) in the CRISPR knockout cell lines.
- Downstream inflammatory mediators tryptase and cytokines were significantly elevated after agonist treatment of LAD2 and PSCMCs *in vitro*.
- Acute mast cell degranulation and vascular permeability induced by MRGPRX2/Mrgprb2 agonists were significantly attenuated in the Mrgprb2 KO animals compared to wildtype controls, demonstrating dependence on Mrgprb2. IgE-mediated degranulation was not inhibited and is independent of the Mrgprb2 pathway *in vivo*.
- MRGPRX2 KI mice express human MRGPRX2 on mast cells, show agonist-induced mast cell degranulation and vascular leakage comparable to wildtype animals and will be a useful tool to investigate antagonism of this receptor *in vivo*.

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## DISCLOSURES

JW, MS, CV, AK, SA, CP, DF, AV, BC, AV, JN, LD, RP, VV and MB are employees of and hold stock in Escient.

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