Transcriptional profiling of primary human-skin derived mast cell activation by various MRGPRX2 agonists and inhibition with EVO756

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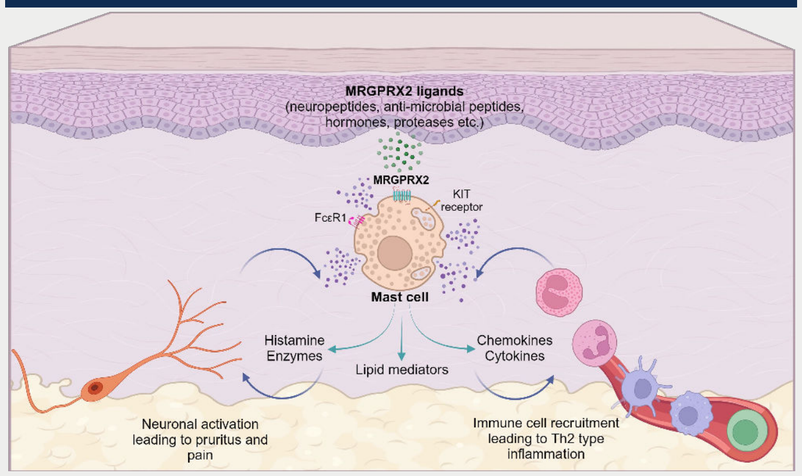
Abstract

Mas-related G-protein coupled receptor X2 (MRGPRX2) is a GPCR expressed predominantly by mast cells and has been implicated to play an important role in diseases such as chronic spontaneous urticaria, atopic dermatitis, and asthma. Cationic ligands, either endogenous or exogenous, can activate the receptor and induce IgE-independent mast cell activation, resulting in allergic responses and non-histaminergic itch. We have previously shown that EVO756, a novel small molecule antagonist of MRGPRX2, demonstrates potent, concentration dependent inhibition of MRGPRX2 mediated activation of transfected CHO-cells, ROSA and LAD2 mast cell lines, and primary human skin-derived mast cells. Furthermore, EVO756 does not affect mast cell viability. Here, we describe, for the first time, the transcriptional landscape of primary human skin-derived mast cells cultured in the presence of several MRGPRX2 agonists and analyze how the mast cell phenotype is altered in response to MRGPRX2 inhibition by EVO756.

Background



Method

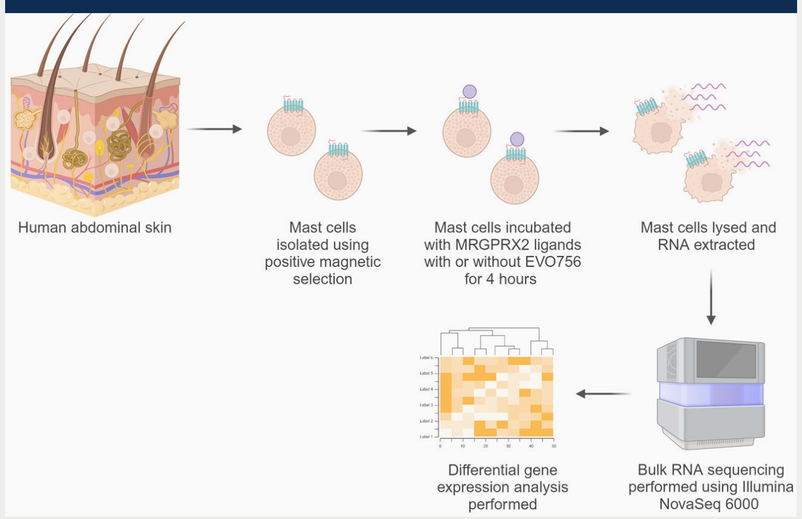


Figure 2. Primary human mast cells were isolated from human abdominal skin. Briefly, punch biopsies derived from the skin were incubated overnight in dispase II and the epidermis separated from the dermis. Dermal tissue was cut into fine pieces and incubated with collagenase, hyaluronidase and DNaseI for 90 minutes. Subsequently, single cells were filtered and incubated with an anti-CD117 antibody conjugated to magnetic beads. The cell suspension was passed through a magnetic column to isolate a pure population of mast cells. For RNA isolation, mast cells were treated with either CST-14 (with and without EVO756) or SP (with and without EVO756) for 4 hours. Mast cells were then collected, lysed and RNA extracted using a Qiagen Rneasy micro kit. RNA was then sent to MedGenome for library preparation and sequencing. EVO756 was used at a concentration of 10 mM.

EVO756 blocks MRGPRX2 mediated signaling in the presence of various agonists

MRGPRX2 Agonist		FLIPR	Mast cell degranulation assay			
		hMRGPRX2-CHO	LAD2	ROSA	Primary human mast Cell	
Endogenous					β-hex	CD63/CD107a
Neuropeptides	Substance P	5.3	24.9	3.6	63.2	29/27
	Cortistatin-14		-	7.6	82.3	44/67
	PACAP		-	-	-	
Hormones	PAMP	9.2	-	-	-	
Antimicrobial peptides	LL-37	-	-	7	-	39.8/62.7
Exogenous						
Drugs	Codeine	-	-	-	65.8	-
	Icatibant	12.9	-	-	51.3	43.23/95.16
Antibiotics	Ciprofloxacin	16.6	-	-	-	-
Other	Compound 48/80	6.7	-	-	-	-

Table 1. IC50 values (nM) for EVO756 in the presence of various MRGPRX2 agonists in different cell types.

Gene signatures in primary human mast cells associated with CST-14 and SP treatment

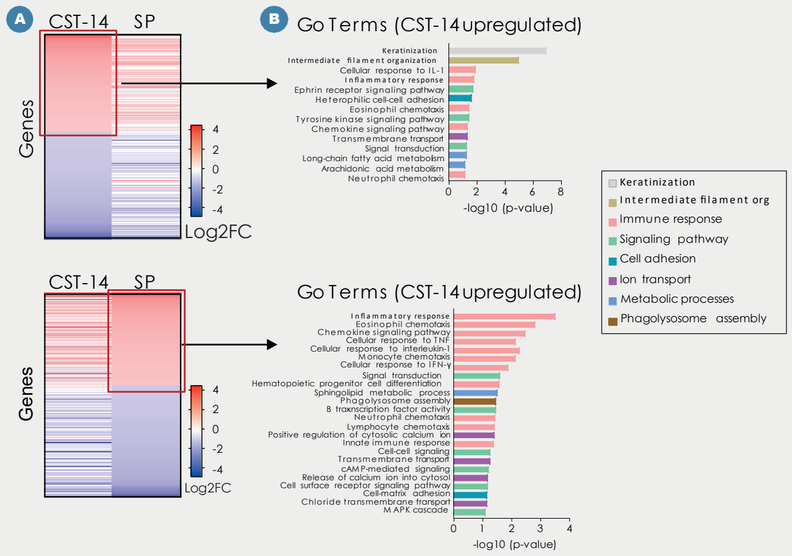


Figure. 3. A) Upregulated and downregulated genes (at least two-fold compared to untreated controls) in mast cells upon treatment with CST-14 and SP. B) Go terms associated with upregulated genes. Data representative of mast cells isolated from 3 donors.

Immune response and signal transduction pathways upregulated in primary human mast cells upon treatment with CST-14 and SP

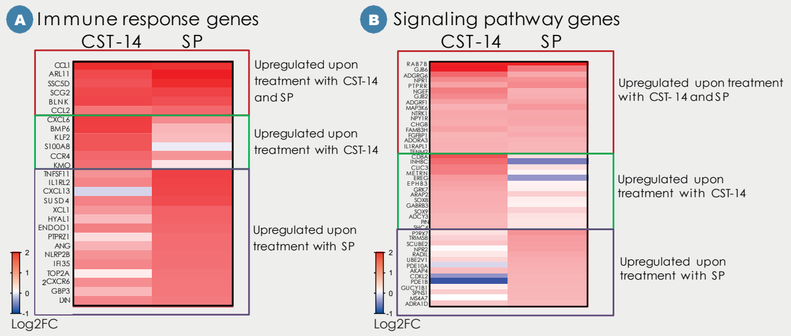


Figure. 4. A) Immune response genes that are upregulated upon treatment with both CST-14 and SP, upregulated only upon treatment with CST-14 and upregulated upon treatment with only SP. B) Signaling pathway genes that are upregulated upon treatment with both CST-14 and SP, upregulated only upon treatment with CST-14 and upregulated upon treatment with only SP. Data are pooled from 3 donors.

EVO756 inhibits biological processes activated by CST-14 and SP

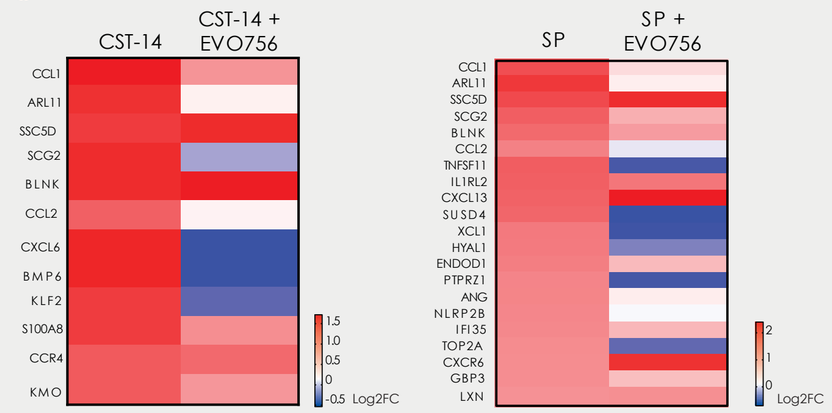


Figure. 5. A) CST-14 and SP induced immune response genes were largely inhibited in the presence of EVO756. B) CST-14 and SP induced signaling pathway genes were mostly inhibited in the presence of EVO756. Data are pooled from 3 donors.

Conclusions

1. CST-14 and SP induce gene expression changes in primary human mast cells within hours of being stimulated by the MRGPRX2 agonists.
2. Upregulated genes are associated by biological processes like immune response, signaling, cell adhesion, ion transport, metabolic processes and phagolysosome assembly.
3. While some upregulated genes are shared between CST-14 and SP, several upregulated genes are specific to the ligands.
4. EVO756 can inhibit upregulation of genes associated with immune response and signaling pathways.

Acknowledgements

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Disclosures

SB, JP, LRB and JLH are employees of, and hold stock in, Evommune.