Aus der Klinik für Dermatologie, Venerologie und Allergologie
der Medizinischen Fakultät Charité – Universitätsmedizin Berlin

DISSERTATION

The MRGPRX2-dependent pseudo-allergic/neurogenic route in human skin mast cells: functional programs, signal transduction, and regulation by cytokines

Die MRGPRX2-abhängige pseudo-allergische/neurogene Route in humanen Hautmastzellen: funktionelle Programme, Signaltransduktion und Regulation durch Zytokine

zur Erlangung des akademischen Grades

Medical Doctor - Doctor of Philosophy (MD/PhD)

vorgelegt der Medizinischen Fakultät

Charité – Universitätsmedizin Berlin

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Datum der Promotion: 17.09.2021

Table of Contents

List of abbreviations

AC: Adenylyl Cyclase

ARRB: Arrestin Beta

C3aR: Complement Component 3a Receptor

C48/80: Compound 48/80

CRAC: Ca^{2+} Release Activated Channel

DAG: Diacylglycerol

ERK: Extracellular Signal-Regulated Kinases

FcεRI: High affinity IgE Receptor

GPCR: G Protein-Coupled Receptor

GRKs: G Protein-Coupled Receptor Kinases

Ig: Immunoglobulin

IL: Interleukin

IP3: Inositol-(1,4,5)-trisphosphate

JNK: C-Jun N-Terminal Kinase

KD: Knockdown

LAD2: Laboratory of Allergic Diseases 2

La^{3+}: Lanthanum (III) chloride heptahydrate

MAPK: Mitogen-Activated Protein Kinase

MCs: Mast Cells

MC_T: Mast Cells Expressing Tryptase

MC_{TC}: Mast Cells Expressing Tryptase And Chymase

MRGPRX2: MAS-Related G Protein–Coupled Receptor X2

NFAT: Nuclear Factor of Activation Of T-Cells

PI3K: Phosphoinositide 3 Kinase

PIP2: Phosphatidylinositol 4,5-Bisphosphate

PKA: Protein Kinase A

PKC: Protein Kinase C

PLC: Phospholipase C

PTx: Pertussis Toxin

RBL-2H3: Rat Basophilic Leukemia 2H3

SCF: Stem Cell Factor

siRNA: Small Interfering RNA

SP: Substance P

TF: Transcription Factor

TLRs: Toll-Like Receptors

Abstract

Mast cells (MCs) are critical effector cells of cutaneous hypersensitivity reactions and inflammatory dermatoses alike. Apart from immunologic activation by aggregation of the high-affinity IgE receptor (FcεRI), MCs can be stimulated via a pseudo-allergic/neurogenic route. Antigen independent MC activation occurs mainly by ligation of the MAS-related G protein-coupled receptor-X2 (MRGPRX2). The recent discovery of MRGPRX2 explains multiple findings which could not be explained beforehand. Using MCs isolated directly from human skin, the major producer cells of MRGPRX2, the present study aims to fill in some gaps by exploring MRGPRX2-mediated activation, and to conduct a comparison with FcεRI-triggered events.

It was found that both receptor systems degranulate skin MCs and elicit similar cytokine patterns. Signaling intermediates provoked by both pathways included ERK1/2, p38 and AKT, while JNK-phosphorylation was weaker. In the kinetic resolution, phosphorylation events triggered by MRGPRX2 were swift, whereas FcεRI triggered signals were delayed. ERK was the most influential kinase involved in cytokine synthesis with some contribution also from other kinases, while PI3K and ERK were both implicated in the degranulation response triggered by both pathways. MRGPRX2-mediated degranulation could be inhibited by pertussis toxin (PTx), YM-254890 and 2-Aminoethyl diphenylborinate (2-APB), which indicated Gi and Gq coupling as well as involvement of inositol-(1,4,5)-trisphosphate receptors (IP3R). Trying to connect the early events with downstream outcomes, it was found that p38, JNK and ERK1/2 depended on Gi, Gq, and IP3R, while AKT was only Gq-dependent. In addition to G protein activation, the β-arrestin pathway was also triggered by c48/80 leading to receptor internalization. By using RNA interference, β-arrestin-1 was detected as the dominant isoform in this scenario. MC modulating factors SCF, IL-4 and IL-33 were selected to study the impact from the micromilieu on MC functionality. SCF and IL-4 reversely regulated MRGPRX2 and FcεRI receptor expression and MC activation, whereby the MRGPRX2 pathway was dampened and the FcεRI pathway increased. IL-33's modulation was time dependent but consistent between the two pathways: Long-term IL-33 decreased expression of both receptors and degranulation mediated by their ligands, while short-term IL-33 priming led to enhanced degranulation.

Collectively, MRGPRX2- and FcεRI-mediated routes are independent in terms of kinetics and regulation by extracellular stimuli, yet they also share similarities regarding functional outputs and the signaling intermediates underlying these outputs. Research regarding MRGPRX2 is still at its beginning, and a more comprehensive understanding of MRGPRX2 biology will provide a renewed understanding of MCs' role in skin pathophysiology.

Zusammenfassung

Mastzellen (MZ) stellen die wesentlichen Effektorzellen bei kutanen Hypersensibilitätsreaktionen und entzündlichen Hauerkrankungen dar. Über die immunologische Aktivierung durch Aggregation des hochaffinen IgE-Rezeptors (FcεRI) hinaus können MZ auch über eine pseudo-allergische/neurogene Route stimuliert werden. Diese Antigen-unabhängige MZ-Aktivierung erfolgt hauptsächlich durch Bindung spezifischer Liganden an den „MAS-related G protein-coupled receptor-X2“ (MRGPRX2). Seine jüngste Entdeckung erschließt zahlreiche Befunde, die zuvor unerklärlich waren. Unter Verwendung direkt aus der Haut isolierter Mastzellen, den Hauptproduzenten des MRGPRX2, zielt die vorliegende Studie darauf ab, einige der Lücken der MRGPRX2-vermittelten Aktivierung zu schließen, und einen direkten Vergleich zu den FcεRI-induzierten Vorgängen zu ziehen.

Dabei wurde ermittelt, dass beide Rezeptorsysteme Hautmastzellen degranulieren und auch ähnliche Zytokinmuster stimulieren können. Die Signalkomponenten, die über beide Wege aktiviert wurden, umfassten ERK1/2, p38 und AKT, während die JNK-Phosphorylierung schwächer ausfiel. In der kinetischen Auflösung ließ sich erkennen, dass MRGPRX2-induzierte Phosphorylierungsereignisse prompt auftraten, wohingegen FcεRI-ausgelöste Signale verzögert waren. ERK stellte sich als die einflussreichste Kinase heraus, die maßgeblich in die Zytokinproduktion involviert war – mit zusätzlicher Unterstützung durch andere Kinasen –, während sowohl PI3K als auch ERK für die über beide Routen stimulierte Degranulation erforderlich waren. Die MRGPRX2-vermittelte Degranulation wurde zudem durch Pertussistoxin (PTx), YM-254890 and 2-Aminoethyldiphenylborinat (2-APB) inhibiert, was auf eine Kopplung an Gi und Gq ebenso wie auf die Beteiligung von Inositol-(1,4,5)-trisphosphat-Rezeptoren (IP3R) hinweist. Bei dem Versuch, die frühen mit den späteren Ereignissen zu verknüpfen, ließ sich zeigen, dass die Aktivierung von p38, JNK und ERK1/2 von Gi, Gq und IP3R gesteuert wird, während die AKT-Aktivierung einzig auf Gq beruht. Zusätzlich zur G-Protein-Aktivierung wurde auch der β-Arrestin-Weg durch den MRGPRX2-Liganden c48/80 angeschaltet, was die Internalisierung des Rezeptors zur Folge hatte. Die Verwendung von RNA-Interferenz konnte zeigen, dass β-Arrestin-1 die dominante Isoform in dem Szenario darstellt. Die MZ-modulierenden Faktoren SCF, IL-4 und IL-33 wurden gewählt, um den Einfluss des Mikromilieus auf die MZ-Funktionalität zu studieren. SCF und IL-4 regulierten die MRGPRX2- und FcεRI-Expression und -Aktivierung in umgekehrter Weise, wobei der MRGPRX2-Weg abgeschwächt, die FcεRI-Route hingegen verstärkt wurde. Die Modulation durch IL-33 war zeitabhängig, aber konsistent für beide Aktivierungswege: Eine Langzeitbehandlung mit IL-33 verminderte die Expression beider Rezeptoren und durch ihre Liganden vermittelte Degranulation, während ein kurzes „IL-33-Priming“ eine Erhöhung der Degranulation zur Folge hatte.

Zusammengefasst stellen MRGPRX2 und FcεRI unabhängige Systeme dar, wobei sich die ausgelösten Ereignisse in Bezug auf Kinetik und Regulation durch extrazelluläre Stimuli

unterscheiden. Sie zeigen jedoch auch Ähnlichkeiten bezüglich funktioneller Outputs und der Signalkomponenten, die diesen Outputs zugrunde liegen. Die MRGPRX2-Forschung ist erst an ihrem Beginn, und ein besseres Verständnis der MRGPRX2-Biologie wird neue Einsichten in die pathophysiologische Rolle von MZ in der der Haut erbringen.

1. Introduction

Mast cells (MCs) are of hematopoietic origin, which mature and become resident in terminal tissues. Mast cells are mostly found at the interface between tissue and environment, including skin, respiratory tract and intestinal mucosa [1]. The tissue localization of MCs favors their function as sentinels in the immune system. MC activation can initiate or contribute to a wide range of disorders, such as urticaria, angioedema, atopic dermatitis, anaphylaxis, mastocytosis, conjunctivitis, rhinitis and asthma [2]. MC-activation induced manifestations are prominent in skin, as skin is one of the largest organs and has the highest MC density across the body.

1.1. Mast cell functional programs and activating receptors

MCs are characterized by their quick and late-phase mediator exocytosis in response to various endogenous and exogenous stimuli [3]. Frist, the acute MC activation happens in the first 30 min, which results in rapid granule exocytosis with release of preformed mediators, including histamine, heparin and MC proteases (e.g. tryptase, chymase). Generation of lipid mediators is a swift process, as well, yet behind the release of pre-formed granule mediators. The late-phase response is mediated by *de novo* generation of cytokines (including chemokines) by upregulation of transcription. Generation of cytokines is a delayed process, usually takes hours to detect elevated mRNA [1,4].

MC function is modulated through a variety of receptors expressed on the cell surface. These include: stem cell factor (SCF) receptor, KIT; high affinity IgE receptor, FcεRI; IL-33 receptor, ST2; pathogen recognizing Toll-Like Receptors (TLRs); as well as several G protein-coupled receptors (GPCRs) such as adenosine receptors, prostaglandin E2 receptors, C3a/C5a receptors and especially MAS-related G protein–coupled receptor X2 (MRGPRX2). The significance of degranulation is highlighted by its participation in the acute-phase allergic reaction. However, not all the receptors are able to trigger both degranulation and cytokine generation. For example, IL-33 and TLR ligands can only stimulate cytokine production but not degranulation in MCs [3].

1.2. Immunological mast cell activation via High Affinity IgE Receptor-FcεRI

Hypersensitivity responses are typically considered as the consequence of IgE/antigen induced aggregation of FcεRI. FcεRI aggregation includes three components: 1) IgE; 2) IgE specific antigen; 3) high affinity IgE receptor (FcεRI) on the MC surface [2,5]. The role of FcεRI in MC activation has been exhaustively studied in the past decades. Aggregation of FcεRI upon binding of antigen elicits downstream signaling cascades, which mainly encompass phosphorylation of signal transducing FcεRI β and γ chain induced Syk recruitment/activation; the LAT-PLCγ1-protein kinase C (PKC)-Ca^{2+} axis; phosphoinositide 3 Kinase (PI3K) dependent signaling; Mitogen-activated protein (MAP) kinase pathways induced transcriptional regulation [3,5].

1.3. Non-Immunological mast cell activation via MRGPRX2

Among non-immunological MC activation, or so called pseudo-allergic MC activation, MRGPRX2 recently entered the scene as an efficient MC degranulator. In fact, non-immunologic MC stimulation in skin has been observed with a long history by skin tests and later also in dermal MC containing suspensions *ex vivo* [6]. Common stimuli known early on are polymer compound 48/80 (c48/80), neuropeptides (e.g. substance P, vasoactive intestinal peptide, cortistatin), antimicrobial peptides (e.g. β defensins and cathelicidin), various FDA-approved cationic drugs (e.g. bradykinin B2 receptor antagonist icatibant, neuromuscular blocking drugs atracurium and mivacurium) [7]. Skin dysfunction via MRGPRX2 encompasses injection-site hypersensitivity reactions, chronic urticaria, atopic dermatitis, red man syndrome and drug anaphylaxis [6-8].

It has been recognized that human MCs are differentiated into 2 subtypes by proteases expressed in their granules. MRGPRX2 is only expressed by the so-called MC_{TC}-type MC characterized by tryptase and chymase expression [6]. MC_{TC} is predominately expressed in skin, while most lung and gut MCs belong to MC_T (MC subset containing only tryptase).

Cells of the MC_{TC} and MC_T category differ in their responses to endogenous and exogenous secretagogues, which indicates that MRGPRX2-mediated route is independent from canonical FcεRI-mediated route [9,10]. This is also underlined by a population-based study performed by our laboratory, which reported no correlation between the two routes [11]. Although the response to MRGPRX2 ligands seems to depend on the amount of receptor expression in the individual subject, the pathway can be universally triggered, e.g. by icatibant causing injection-site effects in nearly every patient [12].

1.4. Signaling regulation of GPCR: activation and termination

MRGPRX2 is a novel GPCR, the present study attempts to explore signaling initiated by MRGPRX2 in skin MCs. Ligands binding to GPCRs lead to conformational changes of the receptor and facilitate the interaction with heterotrimeric G proteins, GPCR kinases (GRKs) and β-arrestins. G proteins activate GPCR signaling, while GRKs and β-arrestins terminate the signal yet can also start alternative cascades on their own.

GPCR activating signals dependent on intracellular coupling of heterotrimeric G proteins [13]. The G protein remains inactive as a heterotrimer until GPCR is bound by an agonist. G proteins consists of a GDP/GTP binding α-subunit and a βγ dimer. G protein α-subunits are differentiated into Gs, Gi/o, Gq/11, and G12/13). Gs and Gi/o modify activity of adenylyl cyclase (AC) which function to converse ATP to cAMP. cAMP is considered as the second messenger for protein kinase A (PKA) and ion channels. Activation of Gi inhibits generation of cAMP and the subsequent responses. Opposite to Gi, Gs activates the above reactions. Coupling of Gq activates phospholipase C-β (PLCβ), which cleaves phosphatidylinositol 4,5-bisphosphate (PIP2) into plasma membrane binding diacylglycerol (DAG) and inositol (1,4,5) trisphosphate (IP3). IP3 binds to its receptor on endoplasmic reticulum, and

thus elicit Ca^{2+} increase in the cytosol. Elevated Ca^{2+} is not only essential for MC degranulation, Ca^{2+} mobilization can also activate calcineurin and calmodulin, which will trigger activation of transcription factor (TF) for cytokine generation, e.g. nuclear factor of activation of T-cells (NFAT) [14].

The loss of receptor sensitivity is termed desensitization. This process serves to protect cells from overstimulation when they are surrounded by high concentrations of agonists [13]. GRKs and subsequent recruitment of β-arrestins are regulatory proteins, that function to terminate the GPCR signal. GRKs recognize serine and threonine residues within the c-terminus of GPCR and its third intracellular loop; the phosphorylation further augments the binding of β-arrestins [13]. β-arrestins binding on GPCR will competitively hinder G protein coupling and significantly promote the decoupling of G proteins from their coupled GPCR as well. Furthermore, β-arrestins activate clathrin-adapter protein-2 complex, which promotes receptor internalization. Depending on the length of over-activation time, receptors can be temporarily internalized or downregulated by lysosomal degradation.

1.5. Mast cell influencing extracellular factors: SCF, IL-4 and IL-33

MCs phenotype and function are influenced by their micromilieu. Factors affecting MCs form a complicated network, which includes the biological factors secreted by other skin cells. Here SCF, IL-4 and IL-33 were selected to study their modulation of human skin MCs.

SCF is a MC growth factor, which modulates survival, maturation, cytokine production and chemotaxis. What's more, SCF amplifies degranulation induced by FcεRI-aggregation [1-3]. IL-4 is as well an important MC regulator known to elicit cytokine release and promotes IgE-mediated degranulation [15]. For MC culture, IL-4 is regularly applied to synergize with SCF for MCs proliferation.

IL-33 is secreted by endothelial cells, epithelial cells and fibroblast-like cells, and acts as an alarmin and pro-inflammatory factor when it is released into the extracellular space upon infection or cell injury [16]. Due to its inflammatory and Th2-skewing potential, IL-33 is critical in the induction and maintenance of allergic disorders, such as asthma and atopic dermatitis [16,17]. MCs are among the primary target cells of IL-33 [18], also owed to their abundant expression of the IL-33 receptor ST2 in comparison with a multitude of primary cells, as uncovered by the comprehensive body-wide expression atlas FATOM5 [19]. IL-33 influences MC biology at various levels and modulates their phenotype, synthetic capacity and mediator release, but this occurs in a MC-subset dependent manner. The question of how extracellular cues impact on MRGPRX2 function is waiting to be answered.

1.6. Aim of the study

This thesis aimed to shed light on the MRGPRX2 mediated cutaneous MC activation. Functionally it is focused on (acute) degranulation and (delayed) cytokine synthesis, FcεRI was studied as a counterpart to analyze the distinctiveness between two pathways. To study the mechanism behind

the functional regulation, MRGPRX2-triggered signaling was explored both at the level of receptor-proximal G protein subunits, Ca^{2+} channels and downstream events like MAPKs and PI3K. Selective inhibitors were employed to confirm the contribution of signaling intermediates in MRGPRX2-mediated MC degranulation and cytokine generation. Agonist-triggered MRGPRX2 internalization was studied in parallel. Another part of the thesis was focused on the impact of selected extracellular mediators on human skin MCs and their modulation of MC function, in particular of MRGPRX2- versus FcεRI-mediated activation.

2. Methods and materials

2.1. Isolation and culture of human skin MCs

MCs were isolated from human foreskin and breast skin tissue by a technique established nearly 20 years ago by the group [20], and constantly optimized [21-23]. Human skin tissue was obtained from circumcisions or cosmetic breast reduction surgeries, with informed consent provided by the patients or their legal guardians and approval by the university ethics committee. The experiments were performed according to the Declaration of Helsinki Principles. Briefly, skin was cut into strips and treated overnight at 4 °C with 3.5 U/ml dispase (BD Biosciences, Heidelberg, Germany). The epidermis was removed, the dermis was chopped and digested with 1.5 mg/ml collagenase type 1 (Worthington, Lakewood, NJ, USA), 0.75 mg/ml hyaluronidase type 1-S (Sigma, Deisenhofen, Germany), and DNase I at 10 µg/ml (Roche, Basel, Switzerland) at 37 °C in a shaking water bath for 75 min. The cells were filtered from remaining tissue. MC purification was achieved with anti-human c-Kit microbeads and the Auto-MACS separation device (both from Miltenyi-Biotec, Bergisch Gladbach, Germany). MC purity, as assessed by acidic toluidine-blue staining was >98%. Viability by trypan blue exclusion was >99%.

Skin MCs were used either ex vivo (within 18 h after isolation) or cultured until reaching optimal proliferation in Basal Iscove medium, supplemented with 10% FCS (Biochrom, Berlin, Germany), SCF (Peprotech, Rocky Hill, NJ, USA, at 100 ng/ml) and IL-4 (Peprotech, 10 ng/ml) [21-23]. Cytokines were provided twice a week and cultures were automatically counted (by CASY-TTC, Innovatis/Casy Technology, Reutlingen, Germany) and re-adjusted to $5x10^5$/ml.

For long-term culture experiments, MCs were kept in SCF alone (100 ng/ml) with or without IL-33 (20 ng/ml) for 5 weeks [22,23]. Here, cytokines were provided once a week.

For study with inhibitors, cells were deprived of SCF and IL-4 for 16 h prior to the experiment. For kinase inhibition [22,23], cells were pre-incubated with SP600125 (JNK inhibitor, 5µM, ApexBio, Houston, TX, USA), SB203580 (p38 inhibitor, 5 µM, ApexBio), Pictilisib (PI3K inhibitor, 5µM, Selleckchem, Munchen, Germany), SCH772984 (ERK1/2 inhibitor, 10 µM, BioVision, California, USA), or Vx-11e (ERK2 inhibitor, 2µM, BioVision) for 15 min prior to stimulation as above. For G

protein inhibitor and Ca^{2+} channel inhibition, cells were pre-treated 16 h with pertussis toxin at 200 ng/ml (PTx, Gi inhibitor, List Biological Labs, Campbell, California, USA), 5 min with YM-254890 at 10 μM (Gq inhibitor, Fujifilm, Osaka, Japan), 5 min with 2-Aminoethyl diphenylborinate at 100 μM (2-APB, a dual inhibitor of inositol 1,4,5-triphosphate receptor and Orai-1/Orai-2, Santa Cruz Biotechnology, Dallas, TX, US), 5 min with Lanthanum(III) chloride heptahydrate at 1μM (La^{3+}, a Ca^{2+} release activated channel blocker, Sigma).

2.2. Accell® mediated RNA interference

RNA interference in MCs was performed by using the Accell® siRNA technology (Dharmacon, Lafayette, CO, USA) as described [22,23]. Briefly, MCs were washed with Accell siRNA medium (supplemented with Non-Essential Amino Acids and L-Glutamine), then plated at $1x10^6$/ml in Accell siRNA medium with 1 μM c-Jun N-terminal kinase (JNK)-targeting siRNA (E-003514-00-0010), MRGPRX2-targeting siRNA (E-005666-00-0050), ARRB1-targeting siRNA (E-011971-00-0050), ARRB2-targeting siRNA (E-007292-00-0050) or non-targeting siRNA (D-001910-10-50, serving as control) for 48 h. After incubation, cells were harvested for downstream experiments.

2.3. Flow cytometry

For cell surface staining, MCs were blocked for 15 min at 4 °C with human AB-serum (Biotest, Dreieich, Germany) and incubated with specific antibodies for 30 min at 4 °C. The antibodies were as follows: anti-human FcεRIα-FITC (clone AER-37, eBioscience, San Diego, CA, USA), Mouse IgG2b, κ-FITC served as isotype control (clone MPC-11, Biolegend, San Diego, CA, USA); PE-labelled anti-human MRGPRX2 (clone K12H4, Biolegend), mouse IgG2b-PE (clone eBWG2b, eBioscience) served as isotype control.

For intracellular staining [22,23], the primary antibodies from Cell Signaling Technology (Frankfurt am Main, Germany) were as follows: anti-pp38 (Thr180/Tyr182, #9211), anti-p38 (#9212), anti-pJNK (T183/Y185, #9251), anti-JNK (#9252), anti-pAKT (S473, #9271), anti-AKT (#9272), anti-pERK1/2 (T202/Y204, #9101), anti-ERK1/2 (#9102). FITC-goat anti rabbit IgG was used as secondary antibody (Jackson, #111-095-003).

Cells were measured on the Facscalibur (BD Biosciences, San Jose, CA, USA) and analyzed with the FlowJo analysis software (FlowJo LLC, Ashland, OR, USA). Net mean fluorescence intensity (MFI), i.e. “MFI specific antibody – MFI isotype control” served for receptor quantification. Negative values after applying the equation were set as 0.

2.4. β-hexosaminidase release assay

Cells were washed and resuspended in PAG-CM buffer (Piperazine-N,N-bis[2-ethanesulfonic acid]-Albumin-Glucose buffer containing 3 mM $CaCl_2$ and 1.5 mM $MgCl_2$, pH 7.4). Then aliquots of 100 μl were seeded into 96-well-plates ($5×10^4$/well) and stimulated by anti FcεRIα-Ab AER-37 (eBioscience,

at 0.1 µg/ml), c48/80 (Sigma, at 10 µg/ml), SP (Bachem, Budendorf, Switzerland at 30 µM) or with no stimulus (spontaneous release). After 60 min incubation, supernatants (SNs) were collected by centrifugation at 500 g, 4 °C for 3 min, and the pelleted MCs rapidly frozen with 100 µl H_2O at -80 °C. After thawing, aliquots of 50 µl of 5 µM 4-methyl umbelliferyl-N-acetyl-β-D-glucosaminide (4-MUG) (Sigma) in citrate buffer (pH 4.5) were mixed with the same volume of supernatant or cell lysate and incubated for 60 min at 37 °C. The reaction was stopped by adding 100 mM sodium carbonate buffer (pH 10.7). Liberated 4-MUG was determined fluorometrically at an emission wavelength of 460 nm after excitation at 355 nm. Percentage of β-hexosaminidase release was calculated as: [fluorescence intensity supernatant / (fluorescence intensity supernatant + fluorescence intensity lysate)] × 100. Net release was calculated by subtracting spontaneous release (i.e. release in the absence of stimulus).

2.5. Histamine release assay (HRA)

MCs in PAG-CM buffer were stimulated by the stimuli specified above under "β-hexosaminidase release assay" for 30 min at 37 °C. For total histamine content, MCs were lysed in 1% perchloric acid for 30 min at 37 °C. Cells were centrifuged and supernatants were stored at -20 °C until measurement. Histamine content was measured by an automated fluorescence method (Alliance Instruments, Salzburg, Austria). Histamine concentration was calculated according to a standard curve. Percentage of net histamine release was calculated as [(stimulated release–spontaneous release)/total histamine in the MC preparation] x100.

2.6. Reverse transcription-quantitative PCR (RT-qPCR)

Total RNA was isolated with the Nucleo spin RNA Kit (Macherey-Nagel, Düren, Germany), then digested with RNase-free DNase (Qiagen, Hilden, Germany). Total RNA was reverse-transcribed with a first-strand synthesis kit (Invitrogen, Darmstadt, Germany). PCR was carried out with the LC Fast Start DNA Master SYBR Green kit (Roche Applied Science). All of the above steps were carried out as per instructions by the respective manufacturers. The primer pairs are as follows: 5'-GGATCAGGAAGACCGGGATCA and 5'-CGGCCTGGGGAACAGAAAGT for MRGPRX2. 5'-GCGCACCGTCTTCTTCTTAG and 5'-CGTCCCTTCAGTCTGTCAGT for HDC. 5'-CCAGGTCTTCACGGCCATAG AND 5'-AGTCGAGCCCTAACTGCAAG for ARRB2. 5'-CAAAGGGACCCAGTGTTCA, 5'-TTGGCCACAAACAGGTCCTT for ARRB1. 5'-ATGACTTCCAAGCTGGCCGTGGCT and 5'- TCTCAGCCCTCTTCAAAAACTTCTC for IL-8, 5'-TCTCGAACCCCGAGTGACAA and 5'-TCAGCCACTGGAGCTGCC for TNF-α, 5'-TTGCGGAGCAAGAGATTCCC and 5'-GGCAGTGCCTCAGCATTTTT for CCL1, 5'-CCCCAAGCAGAAGTGGGTTC, and 5'-TTGGGTTGTGGAGTGAGTGTT for CCL2, 5'-CTCACACACACACAACCAGG and 5'- GAAGAAGCAGCCCATGACAG for VEGFA, 5'-CACACAGACAGCCACTCACCTC and 5'- CTCAGGCTGGACTGCAGGAAC for IL-6, 5'-CATCCGCTCCTCAATCCTCT and 5'- GATGCTCCATACCATGCTGC for IL-13, 5'-

CCCGTCCGTTTACTACGACC and 5'- TTGAGATATGCCCGGATGGC for IL-31, 5'-CTGGAACGGTGAAGGTGACA and 5'-AAGGACTTCCTCTAACAATGCA for β actin. 5'-AAGATGTCCCTGTGCCCTAC and 5'-ATGGCAAGCATGTGGTGTTT for Cyclophilin B. 5'-ATCTCGCTCCTGGAAGATGG and 5'-AGGTCGGAGTCAACGGATTT for GAPDH. The values of target genes were normalized to the housekeeping genes β actin, cyclophilin B, and GAPDH, each ratio contrasted against control conditions (set as 1) and the mean of the three determinations was used for the analysis and depicted in the figures.

2.7. Immunoblot analysis

Detection of total and phosphorylated JNK, ERK1/2, AKT and p38 was performed exactly as described [22,23]. In brief, MCs were stimulated as described in the figures, then boiled in Laemmli buffer (Bio-Rad, Feldkirchen, Germany). Lysates were resolved by SDS-PAGE (NuPAGE™ Bis-Tris 4 to 12% gel, Invitrogen, Carlsbad, CA, USA). After transferring to a PVDF membrane (iBlot™ Transfer Stack, Invitrogen), membranes were blocked with 5% Non-Fat Dry Milk (Sigma) and incubated with antibodies. Finally, proteins were visualized by a chemiluminescence assay (Weststar Ultra 2.0, Cyanagen, Bologna, Italy) and bands were recorded on a chemiluminescence imager (Fusion FX7 Spectra, Vilber Lourmat, Eberhardzell, Germany). The blots were stripped and reprobed to visualize several proteins. The following primary antibodies were used: anti-pp38 (1:1000 dilution, Thr180/Tyr182, #9211), anti-p38 (1:1000 dilution, #9212), anti-pJNK (1:500 dilution, T183/Y185, #9251), anti-JNK (1:1000 dilution, #9252), anti-pAKT (1:500 dilution, S473, #9271), anti-AKT (1:1000 dilution, #9272), anti-pERK1/2 (1:1000 dilution, T202/Y204, #9101), anti-ERK1/2 (1:1000 dilution, #9102), and anti-Cyclophilin B (1:25000 dilution, #43603), all from Cell Signaling Technology (Frankfurt am Main, Germany). Goat anti-rabbit IgG peroxidase-conjugated antibody was administered as the detection antibody (1:10000 dilution, Merck, #AP132P).

Densitometry of the band intensity was detected by Image J (National Institutes of Health, Bethesda, MD, USA). Then the degree of phosphorylated signal was normalized against its representative total signal and Cyclophilin B. The value was then used for downstream analysis depending on the purpose of the experiment.

For quantification of the phosphorylation kinetics upon c48/80 and IgER-CL stimulation, to minimize the variation caused by exposure length and reactivity across different cultures, the mean of every particular blot (sum of signals from all lanes/number of lanes) was calculated, then this mean was used to normalize the intensity of each single sample on this blot.

For G protein and Ca^{2+} channel inhibitor study, to have a precise view of the inhibition extent, cells with inhibitor were normalized against their respective no inhibitor control. Intensity of the protein of interest was finally defined as the mean value (± SEM) of all blots (given as arbitrary units).

2.8. Enzyme-linked immunosorbent assay (ELISA)

Supernatants were harvested 24h after stimulation. Concentrations of TNF-α (Invitrogen, Vienna, Austria), CCL1 and CCL2 (from R&D Systems, Wiesbaden, Germany) were detected by ELISA according to the manufacturers' instructions.

2.9. Statistics

For 2-sample comparisons, differences between groups were assessed by paired t-test (when normally distributed) or by Wilcoxon matched-pairs signed rank test (when not normally distributed) or by one-sample t-test (when normalized to control). For more than two groups RM one-way ANOVA with Holm-sidak's multiple comparisons test (when normally distributed) or Kruskal-Wallis-test with Dunn's multiple comparisons test (when not normally distributed) were employed. Statistical analyses were performed with GraphPad-Prism 8 (San Diego, CA, USA). $p < 0.05$ was considered as statistically significant.

3. Results

3.1. MRGPRX2 and FcεRI triggered activation of human skin MCs show comparable outcomes but are independently regulated

Degranulation of human skin derived cultured MCs triggered by MRGPRX2 ligands c48/80 (10 µg/ml) and SP (30 µM) versus IgER-crosslinking (AER-37, 0.1 µg/ml) was detected, and both FcεRI and MRGPRX2 activation were able to induce cutaneous MC degranulation (Fig. 1a). In skin MCs ex vivo, i.e. directly after isolation, degranulation by MRGPRX2-ligands was even slightly more pronounced than by FcεRI-aggreation [11]. Then MRGPRX2-target siRNA was employed to knockdown (KD) MRGPRX2 expression (Fig. 1b). Degranulation triggered by c48/80 and SP were effectively decreased in MRGPRX2 KD cells, while IgER-CL triggered degranulation remained intact (Fig. 1c).

To study cytokine generation, candidate cytokine genes were selected based on their upregulation by FcεRI-aggregation [19,24]. CCL1 and CCL2 were additionally included as they were selectively and potently induced in MCs after FcεRI-aggregation in the FANTOM5 body-wide expression atlas [25]. Cytokine mRNA activated by MRGPRX2 and IgER-CL were compared side-by-side (Fig. 1d). TNF-α, CCL1, CCL2, IL-8 were effectively elicited via both pathways. IL-31 was only induced by FcεRI-aggregation. IL-13 and VEGFA were slightly increased, while IL-6 was unaffected by either FcεRI- or MRGPRX2-mediated activation.

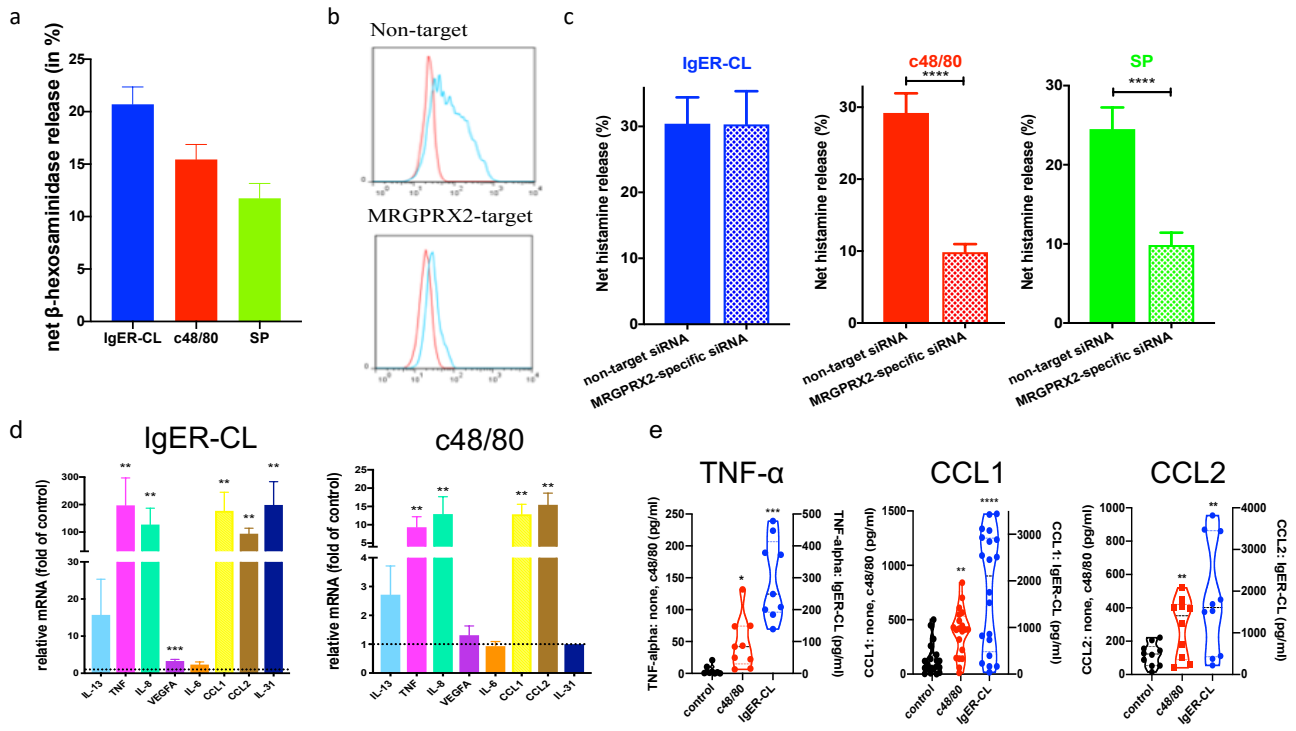

Figure 1: Degranulation and cytokine patterns by MRGPRX2- versus FcεRI-triggering in human skin-derived MCs

Cultured skin-derived MCs were stimulated by c48/80 (10 µg/mL), SP (30 µM) or IgER-crosslinking (CL) (AER-37, 0.1 µg/mL). a) Net β-hexosaminidase release (n=12). b, c) Cells were incubated for 48 h with MRGPRX2-specific siRNA or non-target siRNA, then cells were challenged with the indicated stimuli. b) Representative histograms: blue–MRGPRX2, red–isotype. c) Net histamine release (n=7-8). d) Cells were harvested after 2.5 h stimulation for mRNA quantification by RT-qPCR (n=5-12). e) Supernatants were harvested after 24h and cytokine proteins were quantified by ELISA. Control and c48/80 were plotted on the left y axis, IgER-CL was plotted on the right axis, respectively. Each dot represents an independent MC preparation and the data are shown with median and interquartile range. n=9-20 shown as mean ± SEM, * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

TNF-α, CCL1 and CCL2 were selected to study at protein level and found to be augmented by both allergic and pseudo-allergic activation (Fig. 1e). However, the canonical FcεRI-aggregation induced higher cytokine production than MRGPRX2 triggering. This finding was consistent with cytokine mRNA fold change of control, whereby the allergic route induced higher cytokine generation than the pseudo-allergic route. Despite the variation in quantity, the two routes elicited comparable cytokine profiles.

3.2. Phosphorylation of ERK1/2, p38, AKT is rapidly induced by MRGPRX2 activation, while FcεRI elicited signals are delayed

Compared to the exhaustively investigated FcεRI-mediated route, signaling via MRGPRX2 activation was poorly studied previously. PI3K/AKT and MAP kinases including ERK, p38, JNK were selected to be studied as they are the major components involved in multiple responses of MCs, including degranulation and cytokine generation [1,3,4].

Phosphorylation signals were detected 0, 1, 2, 8, 30 min after stimulation by c48/80, then band intensity was quantified by densitometry analysis (Fig. 2a, b). The kinetics for the distinct kinases were similar. Indeed, signaling triggered by MRGPRX2 activation was rapid, the strongest signal

appeared after 1 min, then signal decreased but still remain detectable after 30 min. Overall, ERK1/2 was the most strongly elicited signal, followed by p38 and AKT, the p-JNK signal was the weakest.

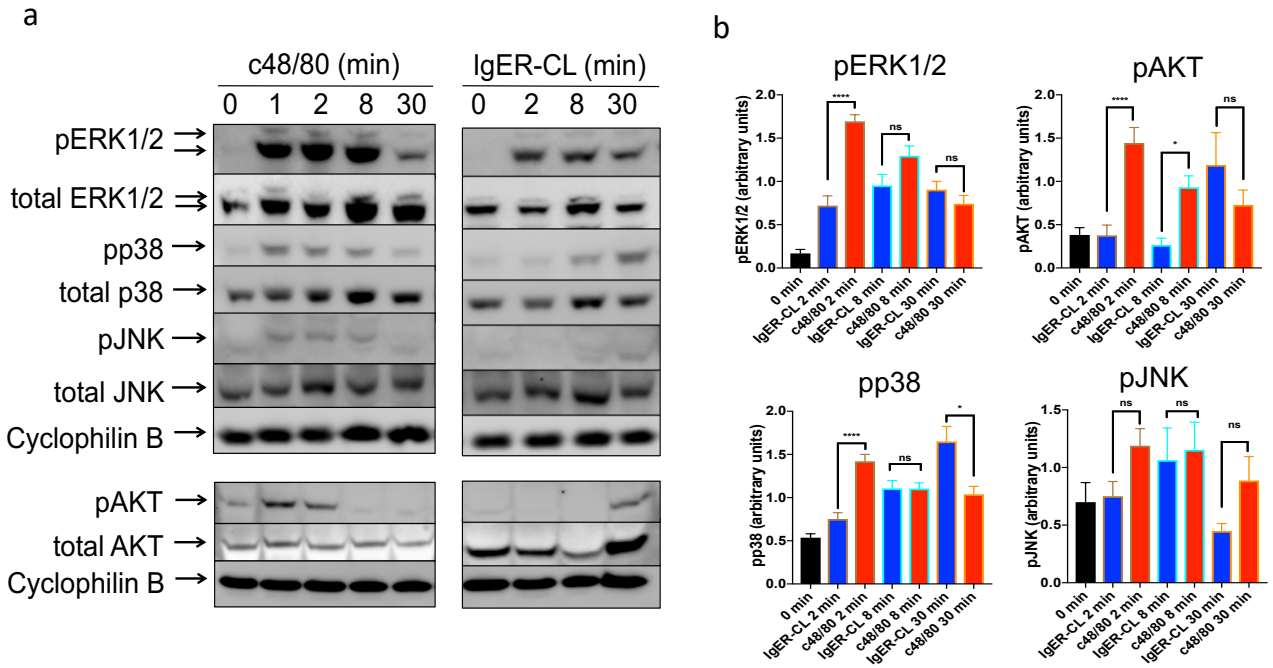

Figure 2. c48/80 induces more rapid phosphorylation of ERK1/2, p38 and AKT than FcεRI aggregation

Kinase phosphorylation elicited by c48/80 (10 µg/mL), or IgER-CL (AER-37, 0.1 µg/mL) in skin-derived MCs at the times indicated. pERK1/2, pp38, pJNK, and pAKT were visualized, then membranes were stripped and re-probed with antibodies against total ERK1/2, p38, JNK, and AKT, and finally with anti-cyclophilin B to monitor loading. a) Representative immunoblots. b) Band intensity of pERK1/2, pp38, pJNK, and pAKT. The data are the mean ± SEM (arbitrary units, calculated as explained in Methods) of 13-18 independent experiments. ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

Signaling by FcεRI-aggregation was studied in parallel. Interestingly, the phosphorylation dynamic triggered by FcεRI was delayed vis-à-vis MRGPRX2 triggering (Fig. 2). Despite the delay, ERK1/2 was also the most prominently activated kinase upon FcεRI-aggregation.

3.3. ERK1/2 activity is dominantly involved in the production of TNF-α, CCL1 and CCL2 by both routes, while p38, JNK and AKT are of weaker and cytokine-dependent significance

To explore and compare the mechanisms of cytokine generation stimulated via the allergic and pseudo-allergic routes, pharmacological inhibitors of ERK1/2 (i.e. inhibiting both ERK1 and ERK2), ERK2 (i.e. ERK2 isoform specific), JNK, p38 and AKT were included in the study. TNF-α, CCL1 and CCL2 mRNA and protein after IgER-CL and c48/80 triggering were detected after pre-incubation with the inhibitors.

ERK was found to be involved in the production of TNF-α, CCL1 and CCL2 upon both FcεRI-aggregation and MRGPRX2 activation (Fig. 3). In fact, induction of their mRNAs was almost abolished upon pretreatment with either ERK1/2 inhibitor or ERK2 inhibitor. It is thus noteworthy that the inhibition of ERK2 was of similar potency as that of ERK1/2. This result is in accordance with the stronger phosphorylated (and total) signal of ERK2 over ERK1 (Fig. 2).

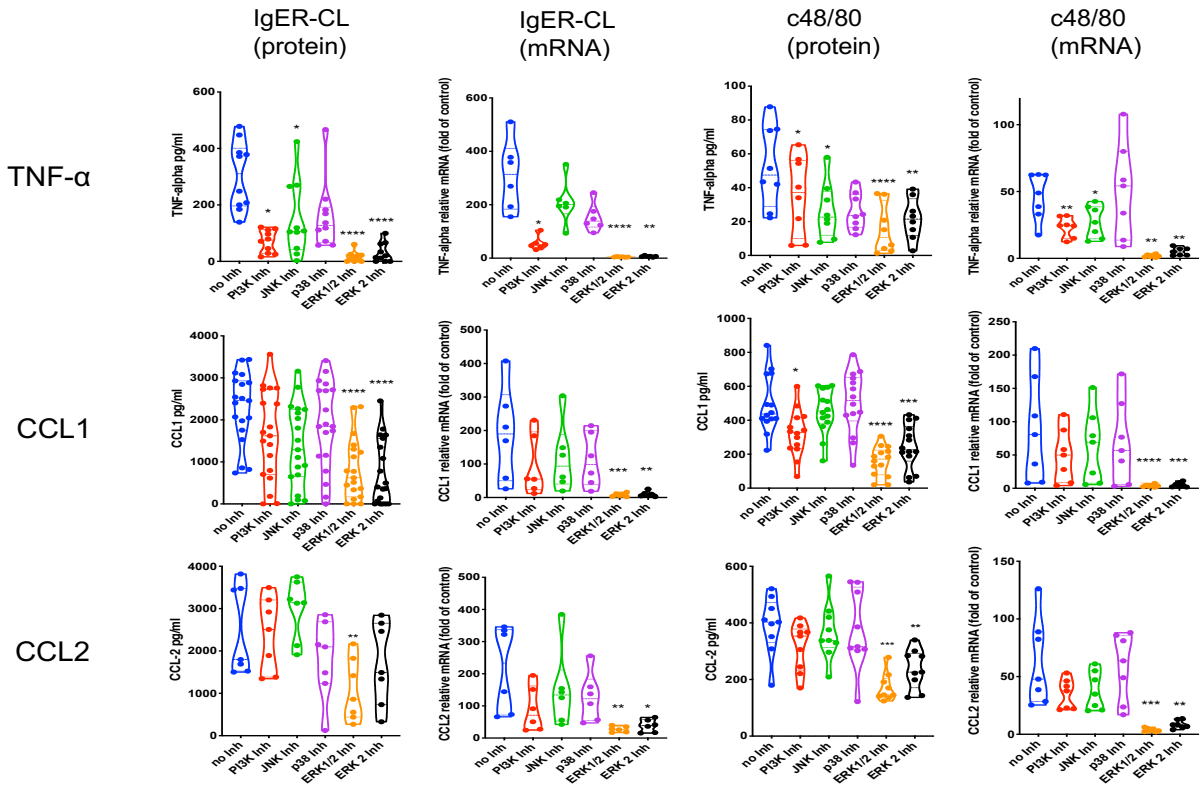

Figure 3: Cytokine production of skin MCs is primarily ERK-dependent, TNF-α additionally requires PI3K and JNK

Cells were SCF and IL-4 deprived for 16h, then pretreated with the inhibitors Pictilisib (PI3K), SP600125 (JNK), SB203580 (p38), SCH772984 (ERK1/2) or Vx-11e (ERK2) for 15 min, and finally stimulated by c48/80 (10 µg/mL) or IgER-CL (AER-37, 0.1 µg/mL). Cells treated without inhibitors served as control. Cytokine proteins were determined by ELISA after 24 h stimulation, mRNA was quantified after 2.5 h by RT-qPCR. The results were from 6-19 independent experiments, plotted with median and interquartile range. Each dot represents an independent experiment (MC culture). * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$, **** $p < 0.0001$.

PI3K and JNK were involved in the regulation of TNF-α by both pathways, PI3K also participated in the upregulation of CCL1 after c48/80. The p38 inhibitor gave a tendency to decrease TNF-α generation but the effect was below significance (Fig. 3). Noteworthy, the inhibitory effect on cytokine mRNA was typically stronger than on their corresponding proteins.

Together, ERK is the most influential kinase implicated in the generation of cytokines by FcεRI aggregation and MRGPRX2 ligation, of which the ERK2 isoform is likely the major component. Other kinases contribute to cytokine generation but to a lower extent than ERK and chiefly limited to TNF-α.

3.4. Receptor-proximal signaling by MRGPRX2: activation of Gi and Gq subunits is upstream of ERK and PI3K activation - significance to degranulation

To study the mechanism of how MRGPRX2 elicits MC degranulation, receptor-proximal events were focused on, i.e. the induced coupling of this GPCR to G proteins. In addition, Ca^{2+} release, which is absolutely essential to MC degranulation, was embarked upon. For this purpose, the Gi inhibitor PTx, the Gq inhibitor YM-254890, the dual inhibitor 2-APB (targets both IP3R and Orai-1/Orai-2), and the Ca^{2+} release activated channel (CRAC) blocker La^{3+} were included.

MCs were pre-incubated with the inhibitors then challenged with MRGPRX2 agonists or IgER-CL. PTx and YM-254890 significantly decreased degranulation elicited by c48/80 and SP, but not by IgER-CL (Fig. 4a). Inhibition by PTx and YM-254890 suggests that MRGPRX2 coupling to both Gi and Gq is involved in mediating granule exocytosis, whereby Gi seems to have a stronger effect. 2-APB significantly decreased degranulation via both pathways, indicating that Ca^{2+} influx via IP3R and/or other Ca^{2+} channels were required in both FcεRI and MRGPRX2 triggered degranulation. Conversely, the CRAC blocker La^{3+} only had a similar effect as 2-ABP in the case of c48/80 but was strikingly less effective in the case of SP (2-ABP > La^{3+}). This hints at some differences in the signaling cascades triggered by distinct ligands of the same receptor, i.e. MRGPRX2. IgER-CL showed a tendency yet did not reach significance.

For kinase inhibition, MAPKs ERK1/2, ERK2, JNK and PI3K inhibitors were included. It was clearly reported by the group that p38 has no impact on skin MC degranulation by both routes in the absence of IL-33 [23] and was therefore omitted. The PI3K inhibitor significantly decreased degranulation by both pathways (Fig. 4b). Intriguingly, ERK1/2 inhibitor likewise impeded degranulation triggered by both FcεRI and MRGPRX2, while dominance of ERK2 could only be detected for FcεRI-mediated degranulation. JNK did not participate in the signaling cascade underlying degranulation in both pathways. Collectively, both PI3K and ERK seem to be crucial components to drive degranulation by both receptor systems.

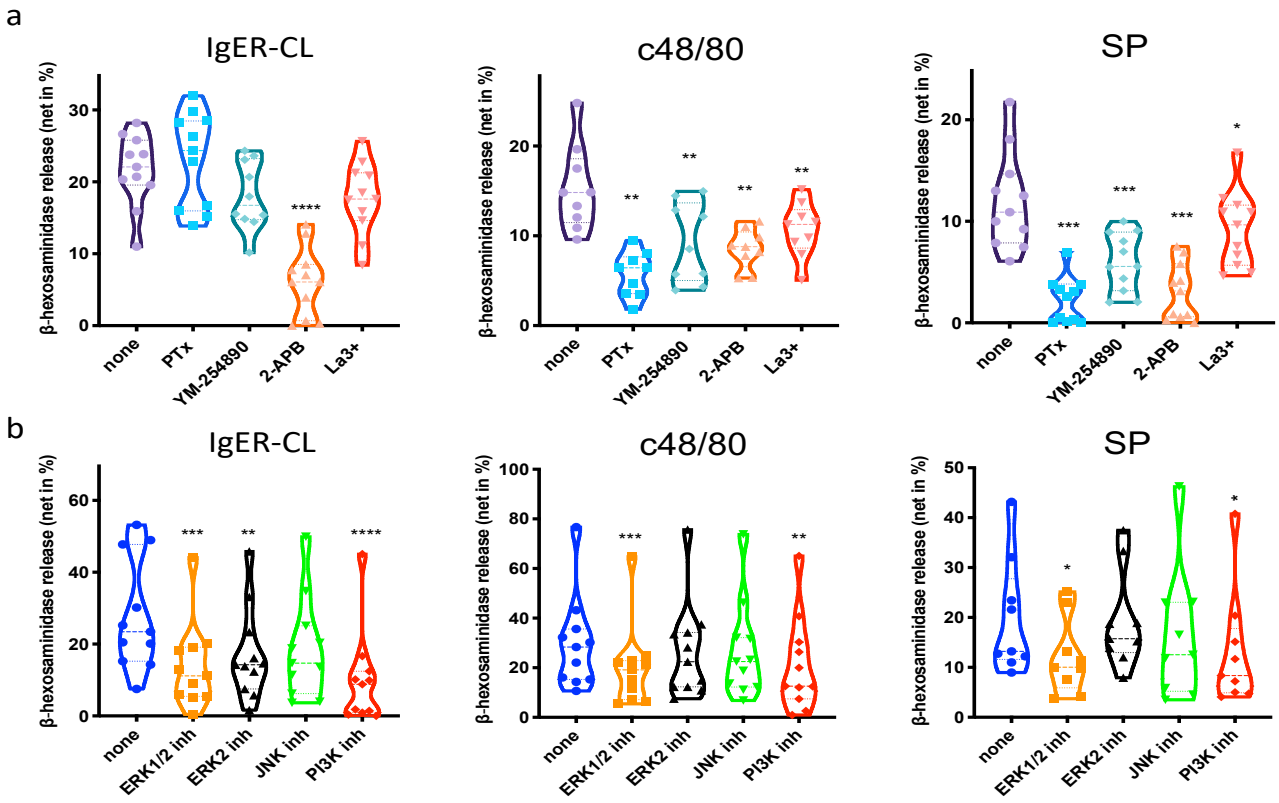

Figure 4: Signaling underlying MC degranulation triggered via FcεRI and MRGPRX2

MCs were pre-treated with inhibitors, then β-hexosaminidase release triggered by c48/80 (10 µg/mL), SP (30 µM) or IgER-CL (AER-37, 0.1 µg/mL) was measured and the net release was calculated. a) MCs were pre-treated for 16 h with pertussis toxin (PTx), or for 5 min with YM-254890, 2-APB and La^{3+}. b) MCs were pre-treated with the inhibitors SCH772984 (ERK1/2), Vx-11e (ERK2), SP600125 (JNK) and Pictilisib (PI3K) for 15 min. Data are from 9-11 independent experiments and plotted with median and interquartile range. Each dot represents an independent MC culture. * p < 0.05, ** p < 0.01, *** p < 0.001, **** p < 0.0001.

3.5. p38, JNK, ERK1/2 are phosphorylated following both Gi and Gq coupling by MRPGRX2 activation, AKT is only Gi-dependent

MRGPRX2 activation is initiated by conformational changes of the receptor and coupling with one or more classes of G protein subunits, then activate downstream signaling intermediates to finally elicit functional programs. To associate the early events of G protein activation with MAP kinases and AKT phosphorylation at a later stage, Gi, Gq and IP3R inhibitors were applied to decipher the potential interconnections.

To this end, cells were pre-treated with PTx, YM-254890 or 2-APB, followed by a 1-min- stimulation with c48/80 (10 µg/ml). According to the time-course of kinase phosphorylation (Fig. 2), the peak activation following c48/80 occurs after 1 min. Phosphorylation of ERK1/2, JNK, p38 and AKT were visualized by western blot (Fig. 5a), and the band intensities were quantified (Fig. 5b).

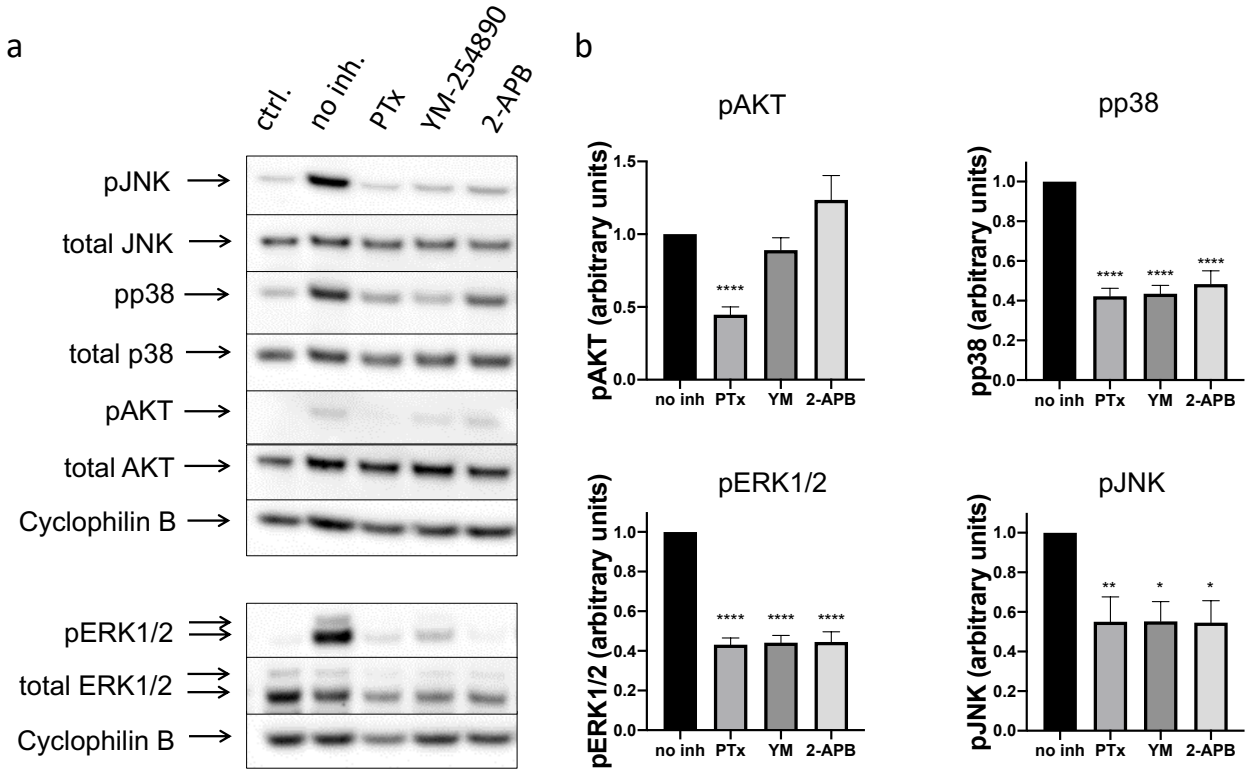

Figure 5: pJNK, pp38 and pERK1/2 are decreased by PTx, YM-254890 and 2-APB, pAKT is only attenuated by PTx

Cells were pretreated with Pertussis Toxin (PTx, 16 h at 200ng/ml), YM-254890 (5 min at 10 µM) and 2-APB (5 min at 100 µM). The phosphorylation of kinases was elicited by c48/80 (10 µg/mL) for 1min. Cells not treated with inhibitors or stimulus were set as control (ctrl.). pERK1/2, pp38, pJNK, and pAKT were visualized, then membranes were stripped and re-probed with antibodies against total ERK1/2, p38, JNK, and AKT, and finally with anti-cyclophilin B to monitor loading. a) Representative immunoblots. b) Band intensities of pERK1/2, pp38, pJNK, and pAKT presented as arbitrary units detailed in Methods. The data are the mean ± SEM of 10 independent experiments. ** p < 0.01, **** p < 0.0001.

For the MAPKs ERK1/2, p38 and JNK, all the inhibitors employed impeded kinase activation (Fig. 5). The extent of inhibition was similar across Gi, Gq and IP3R/Ca^{2+} mobilization inhibitors. AKT phosphorylation was only perturbed by PTx, but not YM-254890 or 2-APB. Indicating that Gi, rather than Gq, is required for AKT activation. In addition, 2-ABP slightly promoted AKT phosphorylation (yet did not reached significance), which suggests elevated Ca^{2+} might be a negative regulator of AKT activation.

3.6. MRGPRX2 agonist c48/80 causes receptor internalization by β-arrestin activation

C48/80 was observed to act through Gi and Gq activation, and the question arose whether it can also initiate β-arrestin signaling leading to MRGPRX2 internalization. In fact, human skin MCs stimulated with c48/80 drastically diminished cell surface MRGPRX2 expression even after 0.5 h and reached the maximum effect at 1 h (Fig. 6a, b).

Both Arrestin β (ARRB) 1 and 2 are expressed in human skin MCs [25], which both encode β-arrestin proteins. To assess the contribution of β-arrestins in agonist-mediated MRGPRX2 sequestration, ARRB1 and ARRB2-specific siRNA were employed to perturb the respective β-arrestin protein expression (Fig. 6c). ARRB1-specific siRNA significantly reversed the internalization triggered by c48/80, while ARRB2-specific siRNA had no significant effect (Fig. 6d, e). In conclusion, the β-arrestin pathway mediates internalization of MRGPRX2 upon c48/80 stimulation in skin MCs. The ARRB1 subtype seems to be chiefly responsible for MRGPRX2 endocytosis.

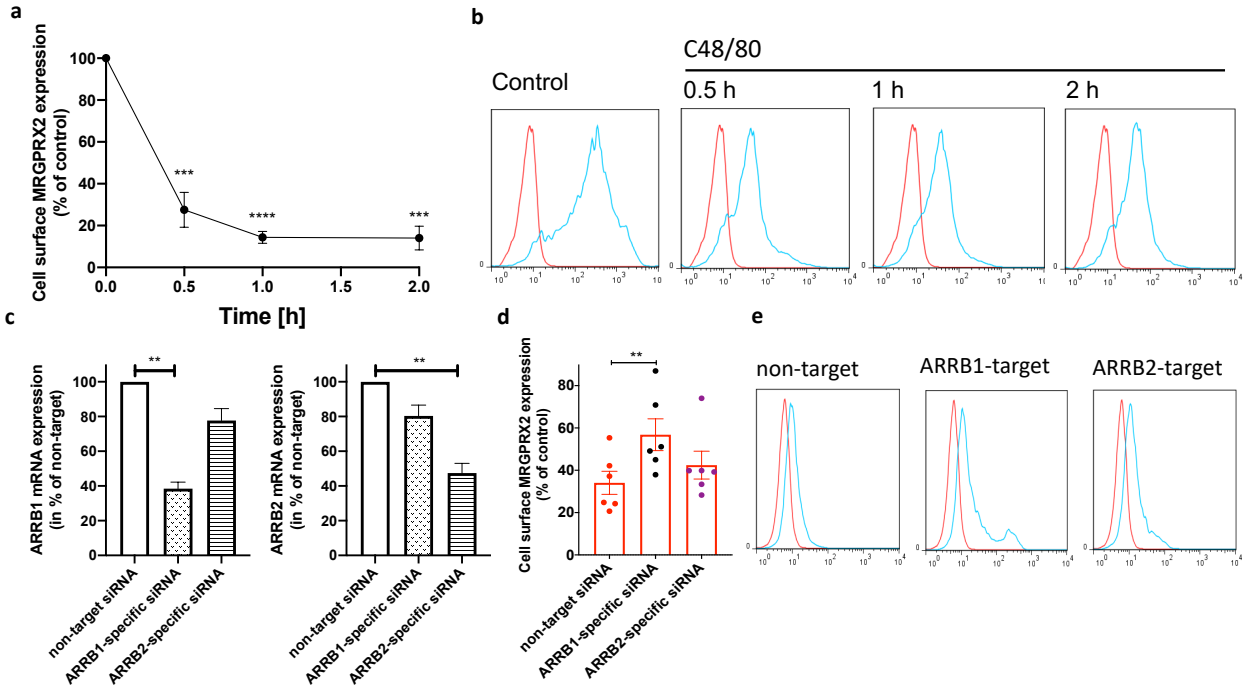

Figure 6. c48/80 triggers rapid downregulation of MRGPRX2 cell surface expression in skin-derived MCs by β-arrestin activation

a) Cells were triggered by c48/80 (10 µg/ml) for the times indicated, and MRGPRX2 cell surface expression was detected by flow cytometry, data are the mean ± SEM of net MFI (MFI MRGPRX2 antibody - MFI isotype control) from 5 independent experiments. b) Representative histograms of a); blue – MRGPRX2, red – isotype. c) ARRB1 and ARRB2 mRNA expression after knockdown by the indicated siRNAs. The data are shown as mean ± SEM from 6 independent experiments. d) MRGPRX2 cell surface expression after triggering with or without

c48/80 (10 µg/ml) for 1 h. net MFI of c48/80 treated cells normalized to the non-stimulated control, data are shown as mean ± SEM (n=6). e) Representative histograms of d); blue – MRGPRX2, red – isotype.

3.7. MRGPRX2 modulation by extracellular cues: Long-term stimulation with SCF, IL-4 and IL-33 synchronically downregulate MRGPRX2 expression and degranulation, while IL-33 priming reversely upregulates MRGPRX2 triggered exocytosis

Long-term culture of MCs depends on SCF to maintain viability, IL-4 is normally added to increase yield [21-23]. However, it is noticed that cultured MCs decrease MRGPRX2 expression substantially. In order to investigate the modulation of MRGPRX2 and FcεRI expression by the two MC growth factors, cells were deprived or not of IL-4 and SCF for 16 h. Re-add of SCF and IL-4 individually or in combination reduced MRGPRX2 mRNA, protein expression and histamine release triggered by MRGPRX2 ligands (see Fig. 2A-C in P1 [21]). Conversely, SCF increased FcεRI protein expression, IL-4 slightly increased the FcεRI transcript, both SCF and IL-4 reinforced degranulation triggered by IgER-CL (see Fig. 2D-F in P1 [21]). Therefore, SCF and IL-4 had opposite effects on the two routes by dampening the MRGPRX2-mediated pathway on one hand, but increasing the allergic route involving FcεRI on the other.

IL-33, another influential modulator of MCs, was selected to study its impact on FcεRI mediated allergic and MRGPRX2 mediated pseudo-allergic pathways. Beforehand, IL-33 stimulated signaling in skin MCs was addressed, since distinct routes can be triggered by the cytokine depending on the cell type. Here, MCs were harvested for immunoblotting and flow cytometry following IL-33 stimulation. IL-33 triggered pronounced JNK and p38 phosphorylation. In contrast, no p-ERK1/2 or p-AKT could be detected upon IL-33 stimulation (see Fig. 4 in P2 [22]).

Functionally, IL-33 exhibited opposite effects depending on the length of exposure, i.e. in a chronic versus acute scenario, but effects were consistent for the two pathways. Short-term 30 min IL-33 priming augmented both MRGPRX2 and FcεRI mediated MC degranulation while receptor expression remained unaltered over the short period of time (see Fig. 4 in P3 [23]). It was therefore likely that IL-33 triggered signaling positively influenced the signaling underlying degranulation (note that IL-33 does not elicit degranulation on its own). Based on the above findings of IL-33 activated JNK and p38 phosphorylation [22], cells were pretreated with JNK and p38 inhibitors to study the mechanism of IL-33 promoted degranulation. The p38 inhibitor basically abolished the priming effect of IL-33 for both routes (i.e. MRGPRX2- and FcεRI-mediated), while the JNK inhibitor had no significant effect (see Fig. 5 in P3 [23]).

Long-term (5 weeks) culture with IL-33 diminished FcεRI expression and attenuated FcεRI-triggered exocytosis (see Fig. 2 in P2 [22]). With regard to the alternative pseudo-allergic/neurogenic route, IL-33 almost eliminated MRGPRX2 expression and MRGPRX2-triggered degranulation (see Fig. 1 in P3 [23]). A time-course study into this effect revealed that MRGPRX2 downregulation was fairly rapid and did not require prolonged culture with IL-33 to come into force (see Fig. S2 in P3 [23]). To address

the mechanism behind the notable downregulation of MRGPRX2, specific inhibitors of JNK and p38 were used. JNK inhibition partially reversed IL-33 driven downregulation of MRGPRX2 expression, while inhibition of p38 had no effect (see Fig. 3 in P3 [23]). The role of JNK was further ascertained by specific siRNA mediated knockdown.

In conclusion, SCF and IL-4 attenuated MRGPRX2 function by decreasing receptor expression, while augmenting the FcεRI-mediated route. Chronic treatment with IL-33 attenuated FcεRI and MRGPRX2 expression and degranulation, while short term IL-33 primed for boosted degranulation. Thus, MRGPRX2- and FcεRI-mediated routes can be subject to opposite or accordant modes of regulation depending on the cytokine and precise conditions.

4. Discussion

4.1. MRGPRX2: a paradigm shift away from canonical IgE-mediated hypersensitivity

Mast cells are effector cells of various cutaneous disorders and mediate innate and adaptive immune responses [26]. They can play either detrimental or beneficial roles when they respond to exogenous and endogenous stimuli. It has been noticed for decades that MCs can be activated in the absence of IgE, but the mechanisms of non-IgE secretagogues operated MC activation have remained a mystery.

The discovery of MRGPRX2 provides an explanation for numerous findings which could not be explained in the past, emphasizing the significance of MRGPRX2 in the activation of MCs. For example, the confined expression of MRGPRX2 in the MC_{TC} subset over MC_T elucidates the reason behind a number of exogenous (icatibant, c48/80) and endogenous (SP, cortistatin) ligands only activating certain populations of MCs [6]. Although there are other receptors, which are also able to trigger MC degranulation independently of FcεRI, e.g. CD88 (C5AR1) and C3AR1 [27], they are not only expressed by MCs, but also by other cells like macrophages [25]. Moreover, MRGPRX2 responds to a wider spectrum of stimuli than the other non-IgE pathways. The above makes MRGPRX2 a distinctive molecule in the case of non-IgE mediated MC activation.

The list of substances acting as MRPGRX2 ligands is constantly growing, now comprising the polymer compound 48/80, numerous drugs like neuromuscular blocking agents, fluoroquinolones and icatibant, host defense peptides (e.g. cathelicidin), and neuropeptides like Substance P (SP), and catestatin [9,10]. There are several studies suggesting the participation of MRGPRX2 in the pathogenesis of cutaneous disorders as reviewed recently [6], e.g. atopic dermatitis, contact dermatitis, chronic idiopathic urticaria and drug induced anaphylaxis. As reported by a recent study, MRGPRX2 partakes in the itch amplifying loop formed by MCs, neurons and SP: Tryptase released from MCs activates PAR-2 on neurons to produce SP, SP then triggers MRGPRX2 (or Mrgprb2 for mouse) mediated MCs

activation [28]. The importance of tryptase for MRGPRX2-triggered activation is also marked by the ineffectiveness of Histamine H1 receptor blocker in some chronic disorders such as AD [6].

4.2. MRGPRX2 versus FcεRI: induction of degranulation and cytokine production

As reported by a previous study from our laboratory, MC activation via FcεRI- and MRGPRX2-mediated routes seems to be independently controlled [11]. In the current thesis, degranulation and cytokine synthesis by MRGPRX2 triggering were studied and compared side by side with FcεRI-aggregation. It was found that the activation of the two routes had similar biological consequences, but several differences were also detected. FcεRI-aggregation induced greater MCs degranulation than MRGPRX2-induced in cultured skin MCs. This may due to the boosted FcεRI expression in culture, while MRGPRX2 is reversely dampened. The opposite receptor regulation is at least partially due to the presence of SCF and IL-4, which are typically added to the culture to maintain survival and to initiate cell cycle progression [21-23]. Different MRGPRX2 ligands have varied ability to induce MC activation. As GPCR agonists are classified into full and partial agonists, a partial agonist is not able to achieve maximal activation even at the highest concentration. In line with that, it was found that the degranulation induced by SP was less pronounced than by c48/80 (Fig. 1a).

Cytokine synthesis by human skin MCs via MRGPRX2 is of particular interest within the scope of the present thesis. MRGPRX2-triggered cytokine synthesis is controversial. It was reported that depending on the agonist applied, MRGPRX2 ligands SP, VIP and c48/80 triggered VEGF, TNF, GM-CSF and IL-3 with primary human peripheral blood–derived cultured MCs [29,30]. SP, VIP, c48/80 and LL-37 can induce multiple cytokines and chemokines in LAD2 cells, e.g. TNF-α, GM-CSF, IL-6, IL-8, IL-31, CCL2, CCL5 [30,31].

The LAD2 cell line has several chromosomal aberrations and shows higher degranulation by MRGPRX2 than primary skin MCs [32], studies with the cell line therefore cannot fully represent primary human skin MCs. With human skin derived MCs in the present study, a similar pattern of cytokines and chemokines were induced by FcεRI-aggregation and MRGPRX2 elicitation, although cultured MCs were more responsive to FcεRI-aggregation triggering (Fig. 1d, e). However, the capacity as such to trigger cytokine generation endows MRGPRX2 to also contribute to the late-phase clinical responses, i.e. the chronic and delayed clinical manifestation of hypersensitivity reactions.

4.3. Different signaling kinetics by MRGPRX2 versus FcεRI

The signaling intermediates involved in both pathways were studied to have a comprehensive picture of the downstream events. While FcεRI activation has been studied extensively in the last decades, the signaling triggered by MRGPRX2 has been rarely explored. There are studies to suggest the involvement of p38, ERK, JNK and PI3K phosphorylation after MRGPRX2 activation [7]. Thus, to gain knowledge regarding the signaling cascades triggered by MRGPRX2 versus FcεRI, a direct comparison of the two major MC activation routes was conducted. MRGPRX2 activated by c48/80

led to a fast activation, while signals induced by FcεRI were rather slow but prolonged (Fig. 2). The opposite signaling kinetics are in line with the distinct degranulation modes reported by Gaudenzio et al., where FcεRI triggers delayed but prolonged granule secretion after granule-to-granule fusion in a process termed compound exocytosis, while MRGPRX2-mediated degranulation is rapid and involves single granules [29]. They are also in line with our own data on CD107a exteriorization (an activation marker), which occurs within a few minutes after stimulation with MRGPRX2 ligands, but reaches the maximum after half an hour after FcεRI-aggregation (Babina et al., 2020, under revision). Despite the reversed kinetics, the signal that was most strongly elicited was consistent between MRGPRX2- and FcεRI-triggering, as ERK1/2 was the most prominent signal in both pathways.

4.4. Signaling mediators regulating TNF-α, CCL1 and CCL2 synthesis triggered by MRGPRX2

It is well known that, MAP kinases and PI3K are involved in multifold regulatory programs of MCs [3,4]. Activation of MAPKs and PI3K/AKT and the increase in intracellular Ca^{2+} can induce transcription factors (TFs) like NFAT, NFκB, and AP-1 (fos/c-jun) to interact with the promoter regions of their encoding genes, which will consequently initiate the generation of cytokines [3,4].

Considering the similar cytokine pattern and shared signaling intermediates between allergic and pseudo-allergic activation, it was hypothesized that the routes share signaling components to regulate cytokine production. Utilizing specific kinases inhibitors, the major role of ERK1/2 accompanied by a more diverse impact of PI3K, JNK and p38 in both pathways was demonstrated in the present thesis. This was in line with its strongest phosphorylation induced via MRGPRX2 and FcεRI-aggregation. More precisely, the comparable degree of inhibition of ERK2 versus ERK1/2 substantiated that ERK2 was probably the dominant ERK isoform underlying cytokine generation in human skin MCs.

4.5. G protein subunits, MAPKs and PI3K in the regulation of MRGPRX2 mediated degranulation

Consistently with previous reports showing that the MRGPRX2 ligand LL-37 can induce MC degranulation by activation of PTx-sensitive G proteins [33], the present study confirmed that degranulation triggered by c48/80 and SP in human skin MCs was also inhibited by PTx (Fig. 4). PTx inhibits Gi coupling to its cognate GPCR by catalyzing the ADP-ribosylation of a cysteine residue of Gi. Inactivation of Gi further leads to elevated adenylyl cyclase, cAMP and cAMP mediated signaling. Thus, Gi coupling is implicated in the degranulation triggered by MRGPRX2.

An interesting phenomenon is that some GPCRs appear to exclusively couple with one class of G protein (e.g., “Gs-coupled receptor” or “Gq-coupled receptor”), while others can activate two or more classes of G proteins. In the current thesis, a specific Gq inhibitor (YM-254890) was employed and found to decrease degranulation triggered by MRGPRX2 ligands. Chompunud et al. reported in MRGPRX2 transfected rat basophilic leukemia (RBL-2H3) cells that inhibition of Gq by YM-254890

in a setting of SP-mediated MRGPRX2-stimulation caused a similar decreased degranulation accompanied with reduced Ca^{2+} mobilization [34].

Gq activation is followed by PLC-β activation, which cleaves PIP2 into IP3 and DAG. Then IP3 binds on IP3R of the endoplasmic reticulum and subsequently induces Ca^{2+} release [13]. Coupling of Gq by MRGPRX2 activation was further confirmed by the use of La^{3+} inhibiting only CRAC and dual inhibitor 2-APB which targets IP3R and Orai-1/Orai-2. Although both La^{3+} and 2-APB perturbed MRGPRX2-triggered degranulation, inhibition by 2-APB was more potent than by La^{3+} (Fig. 4a).

The above evidence indicated both Gi and Gq coupling to MRGPRX2 in skin MCs. Moreover, PTx inhibited degranulation more strongly than YM-254890 in response to c48/80 and SP (Fig. 4a). One possible explanation might be that the GPCRs could have varied affinities for certain G protein types. Regarding MRGPRX2 it might be speculated that Gi is preferred over Gq coupling.

There is consensus that PI3K serves to maintain and amplify the signal initiated by PLC, which leads to Ca^{2+} mobilization and degranulation. However, the role of MAPKs in degranulation has been rarely reported. Only a few studies described that ERK activation by FcεRI-aggregation was involved in driving degranulation in mouse bone marrow derived MCs [35,36]. In contrast, MAPKs are considered to be more relevant for cytokines [3,7]. Hence, the involvement of MAPKs in degranulation in human skin MCs is a new finding, and it will be of great interest to identify ERK downstream effectors in this scenario. We found that ERK and PI3K inhibitors decreased degranulation by FcεRI-aggregation and both MRGPRX2 ligands, i.e. SP and c48/80.

4.6. Divergent kinase phosphorylation by Gi and Gq activation

Considering that ERK/PI3K inhibitors and G protein inhibitors both downregulated MC degranulation triggered by MRGPRX2 ligands, it was speculated that MAPKs and PI3K/AKT are downstream of Gi and/or Gq activation. There was a lack of direct support of this assumption in the literature, though, albeit some studies substantiated the involvement of both G protein and MAPKs in the regulation of MC activation. For example, Aung et al. demonstrated that the neuroendocrine antimicrobial peptide catestatin induces MC degranulation and cytokine release, which was decreased by Gi, PLC and ERK inhibition [37]. Moreover, cytokines including various chemokines elicited by the host defense peptide AG-30/5C were reversed by ERK-, JNK-, p38-specific inhibitors as well as G protein and PLC inhibitors [38]. However, those studies were all conduced with LAD2 cells. Here, a link between the upstream G proteins with downstream MAPKs and AKT in human skin derived MCs is demonstrated for the first time to the candidate's knowledge.

Phosphorylation and activation of AKT is commonly regarded as the consequence of PDK recruitment followed by PI3K activation. The Ca^{2+} signaling pathway in MCs is initiated by PLC mediated production of IP3, which leads to Ca^{2+} release from endoplasmic reticulum and Golgi. Intriguingly, in the present study, Ca^{2+} inhibition by 2-APB slightly increased AKT phosphorylation in c48/80 activated

MCs. This finding indicated some convergence point between Ca^{2+} signaling and AKT, whose identification awaits to be revealed.

In conclusion, activation of the Gi subunit triggered the phosphorylation of ERK1/2, JNK, p38 and AKT, while Gq activated only the MAPKs, or in other words, AKT phosphorylation required the action of Gi. This might explain the more extensive degranulation inhibition following PTx rather than 2-APB or Gq inhibitor.

4.7. C48/80 is a balanced ligand that incurs β-arrestin initiated internalization

An important property of GPCRs is that they selectively trigger cell activation via G protein and/or undergo GRK/β-arrestin mediated internalization and desensitization, but β-arrestin can also trigger signaling on its own encompassing cytosolic ERK for example (while G protein triggered ERK is mainly nuclear) [13]. Depending on the preference signaling pathway triggered by agonist binding, a ligand can be classified as G protein biased, β-arrestin biased or balanced; in the latter case the G protein and the β-arrestin routes are similarly elicited.

In terms of MRGPRX2, not all ligands are able to induce receptor sequestration. For example, with transfected RBL-2H3 cells, AG-30/5C and Icatibant do not activate the β-arrestin pathway, while c48/80 leads to internalization of MRGPRX2 [39]. The present study confirmed the balanced activation of both β-arrestin and G protein for c48/80 in human skin MCs and showed that internalization occurs much more rapidly in skin MCs than in RBL-MRGPRX2 cells. The role of β-arrestins in the process of receptor sequestration was further confirmed with the employment of ARRB siRNAs. It was demonstrated that, ARRB1 was mainly responsible for the internalization, while ARRB2 had no significant effect.

4.8. Regulation of MRGPRX2 expression and function by SCF, IL-4 and IL-33

The interaction of MCs with their surrounding habitats have a profound impact on MCs survival, differentiation, phenotype and function [6]. MCs are influenced directly or indirectly by the products from cells in their vicinity like cytokines or neuropeptides. There are also numerous reports of MC influencing factors, which regulate FcεRI-responsiveness. However, the modulation of MRGPRX2 by MC extracellular cues is rarely studied. Therefore, the regulatory role of SCF, IL-4 and IL-33 on the pseudo-allergic system was attempted to be clarified.

SCF is the most well-defined MC maturation, survival and differentiation factor [3]. In MC culture with SCF and IL-4, FcεRI and MRGPRX2 expression and receptor-triggered degranulation are reversely regulated, whereby FcεRI is boosted and MRGPRX2 is decreased. As reported by a previous study from our group, also acute SCF priming augments FcεRI-elicited histamine release but attenuates degranulation triggered by MRGPRX2 [11]. This suggests an instantaneous interference between KIT- and MRGPRX2-triggered signaling. However, it does not answer the question which factor is

responsible for the inverse regulation when the incubation time is prolonged. By depriving SCF and IL-4 and re-adding them either separately or in combination, it was found that SCF potently decreased MRGPRX2 expression, while IL-4 only showed a slight downregulation tendency [21]. These evidenced that SCF was the dominant factor for the downregulation of MRGPRX2 in culture, even though IL-4 contributed.

MCs are principal target cells of IL-33 due to their abundant expression of ST2, which is the IL-33 receptor. Research into IL-33 reported in this thesis was chronologically divided into two parts: long-term culture with IL-33 and SCF (versus SCF alone) and priming effects of short-term (30 min) IL-33. The 5-week-culture with IL-33 aimed to mimic the micromilieu of chronic skin disorders. The long-term exposure to IL-33 basically eliminated the MRGPRX2 elicited degranulation and receptor expression, which indicated that dampened stimulability was brought about by a lack of MRGPRX2 at the cell surface. It was subsequently found, however, that downregulation was already observable 2 hours after contact with IL-33 at transcript level and the maximum decrease was reached after 4 hours [22], allowing to study the mechanism behind this event. In order to understand how IL-33 regulates MRGPRX2 receptor expression and function, signaling elicited by IL-33 in skin MCs was studied. A robust activation of p38 and intermediate-level of pJNK were noticed, but p-ERK1/2 or p-AKT were not detected. Then JNK and p38 signaling transduction was perturbed by means of pharmacological inhibitors and siRNA-mediated knockdown, whereby a major role of JNK but not p38 in the effect of IL-33 on MRGPRX2 expression and function was revealed. JNK acted as a negative regulator of MRGPRX2 when MCs were challenged with IL-33.

Surprisingly, interference with JNK by siRNA in the absence of IL-33 led to reduced MRGPRX2 expression (see Fig. 2 in P3 [23]), identifying JNK as a positive regulator in the absence of IL-33. This indicated JNK's impact even in the steady-state despite the fact that phosphorylated JNK at steady-state was almost below detection by *Flow cytometry* and *Western blot*. The low baseline pJNK indicated that low JNK activity may be sufficient to maintain the function of transcription factors responsible for MRGPRX2 expression. However, the enhanced JNK activity triggered by IL-33 may lead to the phosphorylation of a widened (and/or altered) set of effectors to mediate downregulation of the receptor.

In contrast to the long- or intermediate-term effect of IL-33, a 30-min-priming promoted MRGPRX2 and FcεRI-triggered degranulation. Receptor expression over the short period remained unaltered. It seems that IL-33 is the first mediator which uniformly regulates allergic and pseudo-allergic/neurogenic degranulation pathways, as well as the first MRGPRX2 supportive factor in this study. The acute priming was found to be mediated by p38, which was of interest because p38, even though activated by FcεRI is not relevant to degranulation in the absence of IL-33 in contrast to cytokine production (especially TNF-α), lipid mediator generation, migration, proliferation, chemotaxis

and adhesion [3,7]. This is further supported by the lacking effect of p38 on degranulation elicited by the two routes in the absence of IL-33 (see Fig. S5 in P3 [23]). pp38 was almost under detection in unstimulated cells, but boosted after IL-33 (see Fig. 4 in P2 [22]). Together, p38 only contributes to degranulation in the presence of IL-33.

4.9. Conclusion and outlook

Combining the findings above, the present study concluded that the MRGPRX2- and FcεRI-mediated pathways are independently regulated though the outcomes are similar, which included degranulation and the release of selected cytokines. ERK was the most strongly activated kinase and implicated in the regulation of degranulation and cytokine synthesis triggered by both routes. C48/80, which is one of the most widely used ligands for MRGPRX2 research, activated MRGPRX2 by Gi and Gq coupling on one hand, and recruited β-arrestin for receptor internalization on the other. For the impact of extracellular-cues, MRGPRX2 and FcεRI were oppositely regulated by SCF and IL-4. IL-33 consistently affected both pathways, but its influence depended on the exposure length. Collectively, MC-supportive factors can either promote or dampen the MRGPRX2-dependent pathway, although a negative impact from the most influential factors seems to prevail.

MRGPRX2 acts as a double-edged sword in MC mediated host defense. There are potential interconnections between MRGPRX2 and diseases like anaphylaxis, atopic dermatitis and chronic urticaria, indicating MRGPRX2's role in the pathogenesis. Apart from the noxious impact of MRGPRX2, the receptor has also beneficial facets, e.g. as an antimicrobial receptor to promote bacterial clearance [40,41]. A study of DNA polymorphism in world-wide human populations substantiated that the MRGPRX2 gene has undergone adaptive changes by Darwinian positive selection [42]. So more potential beneficial functions of the receptor will likely be discovered in the future.

Overall, research on MRGPRX2 is still at a very early stage. Emerging ligands are recognized as MRGPRX2 agonists, and accordingly will provide explanations for hypersensitivity or even anaphylaxis as side-effects caused by several drugs, like vancomycin, icatibant and opiates. The molecular regulation of the MRGPRX2 gene expression is awaiting to be studied. The molecular underpinnings will help to explain its confinement to MC_{TC}-MCs and regulation by MC-supportive factors like SCF and IL-33 shown in the present thesis. Moreover, identifying naturally occurring MRGPRX2 variants could give a comprehensive picture of the varied range of responsiveness across the population.

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Statutory Declaration

"I, **Zhao Wang**, by personally signing this document in lieu of an oath, hereby affirm that I prepared the submitted dissertation on the topic: **"The MRGPRX2-dependent pseudo-allergic/neurogenic route in human skin mast cells: functional programs, signal transduction, and regulation by cytokines"** (German translation: Die MRGPRX2-abhängige pseudo-allergische/neurogene Route in humanen Hautmastzellen: funktionelle Programme, Signaltransduktion und Regulation durch Zytokine), independently and without the support of third parties, and that I used no other sources and aids than those stated.

All parts which are based on the publications or presentations of other authors, either in letter or in spirit, are specified as such in accordance with the citing guidelines. The sections on methodology (in particular regarding practical work, laboratory regulations, statistical processing) and results (in particular regarding figures, charts and tables) are exclusively my responsibility.

Furthermore, I declare that I have correctly marked all of the data, the analyses, and the conclusions generated from data obtained in collaboration with other persons, and that I have correctly marked my own contribution and the contributions of other persons (cf. declaration of contribution). I have correctly marked all texts or parts of texts that were generated in collaboration with other persons.

My contributions to any publications to this dissertation correspond to those stated in the below joint declaration made together with the supervisor. All publications created within the scope of the dissertation comply with the guidelines of the ICMJE (International Committee of Medical Journal Editors; www.icmje.org) on authorship. In addition, I declare that I shall comply with the regulations of Charité – Universitätsmedizin Berlin on ensuring good scientific practice.

I declare that I have not yet submitted this dissertation in identical or similar form to another Faculty.

The significance of this statutory declaration and the consequences of a false statutory declaration under criminal law (Sections 156, 161 of the German Criminal Code) are known to me."

Date Signature

Declaration of your own contribution to the publications

Zhao Wang contributed the following to the below listed publications:

Publication 1:

Babina, M.; **Wang, Z.**; Artuc, M.; Guhl, S.; Zuberbier, T. MRGPRX2 is negatively targeted by SCF and IL-4 to diminish pseudo-allergic stimulation of skin mast cells in culture. Exp Dermatol. 2018

Contribution:

- Shared first author
- FACS data from the Fig. 1B, E and Fig. 2B, E.
- Significant contribution to the processing the RT-qPCR for Fig. 1A. D and Fig. 2A. D.

Excerpt from Journal Summary list:

Journal Data Filtered By: **Selected JCR Year: 2016** Selected Editions: SCIE, SSCI

Selected Categories: **"DERMATOLOGY"** Selected Category Scheme: WoS

Gesamtanzahl: 63 Journale

Rank	Full Journal Title	Total Cites	Jounal Impact Factor	Eigenfactor Score
15	EXPERIMENTAL DERMATOLOGY	5.684	2.679	0.11170

Publication 2:

Babina, M.; **Wang, Z.**; Franke, K.; Guhl, S.; Artuc, M.; Zuberbier, T. Yin-Yang of IL-33 in Human Skin Mast Cells: Reduced Degranulation, but Augmented Histamine Synthesis through p38 Activation. J Invest Dermatol. 2019

Contribution:

- Co-authorship
- Collected data of cell counting in Fig.1a
- Performed the RT-qPCR in Fig. 2a and Fig. 5
- Significant contribution to Histamine Release Assay in Fig 2d, E and Fig. 5
- Performed FACS and collected data for Fig. 2b, c and Fig. 4a, b
- Essential contribution to figures visualization
- Contribution to the draft of manuscript

Excerpt from Journal Summary list:

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE, SSCI Selected Categories: **"DERMATOLOGY"** Selected Category Scheme: WoS

Gesamtanzahl: 63 Journale

Rank	Full Journal Title	Total Cites	Jounal Impact Factor	Eigenfactor Score
3	JOURNAL OF INVESTIGATIVE DERMATOLOGY	28.467	6.448	0.038800

Publication 3:

Wang, Z.; Guhl, S.; Franke, K.; Artuc, M.; Zuberbier, T.; Babina, M. IL-33 and MRGPRX2-Triggered Activation of Human Skin Mast Cells-Elimination of Receptor Expression on Chronic Exposure, but Reinforced Degranulation on Acute Priming. Cells. 2019

Contribution:

- First authorship
- Planning and preparation of the project together with Dr. Magda Babina and Prof. Dr. Torsten Zuberbier
- Performed experiments and in all Figures
- Data collection, analysis and visualization
- Developed manuscript together with Dr. Magda Babina
- Revised the manuscript with Dr. Magda Babina and Prof. Dr. Torsten Zuberbier

Excerpt from Journal Summary list:

Journal Data Filtered By: **Selected JCR Year: 2017** Selected Editions: SCIE, SSCI

Selected Categories: **"CELL BIOLOGY"** Selected Category Scheme: WoS

Gesamtanzahl: 190 Journale

Rank	Full Journal Title	Total Cites	Jounal Impact Factor	Eigenfactor Score
55	Cells	1.005	4.829	0.004100

Signature, date and stamp of first supervising university professor / lecturer

Signature of doctoral candidate

Print copies of the selected publications

P1: MRGPRX2 is negatively targeted by SCF and IL-4 to diminish pseudo-allergic stimulation of skin mast cells in culture.

Babina M, Wang Z, Artuc M, Guhl S, Zuberbier T. Exp Dermatol. 2018; 27(11):1298-1303.

DOI: https://doi.org/10.1111/exd.13762

P2: Yin-Yang of IL-33 in Human Skin Mast Cells: Reduced Degranulation, but Augmented Histamine Synthesis through p38 Activation.

Babina M, Wang Z, Franke K, Guhl S, Artuc M, Zuberbier T. J Invest Dermatol. 2019;139(7):1516-1525.e3.

DOI: https://doi.org/10.1016/j.jid.2019.01.013

P3: IL-33 and MRGPRX2-Triggered Activation of Human Skin Mast Cells-Elimination of Receptor Expression on Chronic Exposure, but Reinforced Degranulation on Acute Priming. Cells. 2019

Wang Z, Guhl S, Franke K, Artuc M, Zuberbier T, Babina M. 2019;8(4):341. Published 2019 Apr 11. DOI: https://doi.org/10.3390/cells8040341

Curriculum vitae

My curriculum vitae does not appear in the electronic version of my paper for reasons of data protection.

List of publications

1. Babina M, **Wang Z**, Artuc M, Guhl S, Zuberbier T. MRGPRX2 is negatively targeted by SCF and IL-4 to diminish pseudo-allergic stimulation of skin mast cells in culture. Exp Dermatol. 2018;27(11):1298-1303. doi:10.1111/exd.13762. Impact factor: 2.8.
2. Babina M, **Wang Z**, Franke K, Guhl S, Artuc M, Zuberbier T. Yin-Yang of IL-33 in Human Skin Mast Cells: Reduced Degranulation, but Augmented Histamine Synthesis through p38 Activation. J Invest Dermatol. 2019;139(7):1516-1525.e3. doi:10.1016/j.jid.2019.01.013. Impact factor: 6.29.
3. **Wang Z**, Guhl S, Franke K, Artuc M, Zuberbier T, Babina M. IL-33 and MRGPRX2-Triggered Activation of Human Skin Mast Cells-Elimination of Receptor Expression on Chronic Exposure, but Reinforced Degranulation on Acute Priming. Cells. 2019;8(4):341. Published 2019 Apr 11. doi:10.3390/cells8040341. Impact factor: 4.36.
4. **Wang, Z**. and Babina, M. (2020), MRGPRX2 signals its importance in cutaneous mast cell biology: Does MRGPRX2 connect mast cells and atopic dermatitis?. Exp Dermatol. Accepted Author Manuscript. doi:10.1111/exd.14182. Impact factor: 3.36.
5. Babina M, **Wang Z**, Roy S, Franke K, Guhl S, Artuc M, Ali H, Zuberbier T. MRGPRX2 is the codeine receptor of human skin mast cells: desensitization via β-arrestin and lack of correlation with the FcεRI pathway. J Invest Dermatol. 2020; Under revision.
6. **Wang Z**, Guhl S, Franke K, Zuberbier T, Babina M. Cytokine production elicited via allergic and pseudo-allergic activation in human skin mast cells - dominance of ERK1/2. In preparation.
7. **Wang Z**, Franke K, Zuberbier T, Babina M. ERK and PI3K orchestrate MRGPRX2-triggered degranulation in skin-derived MCs – connections between G-proteins, Ca^{2+} channels and downstream kinase activation. In preparation.

Acknowledgements

I would like to express my gratitude to my supervisors Prof. Torsten Zuberbier, Dr. Magda Babina and Dr. Maja Hofmann who kept encouraging and endorsing me throughout the project. Without you, my years as a MD/PhD student would not have been possible and as successful and inspiring as they have been.

My special thanks go to Dr. Magda Babina, for guiding me into the academia field and sharing her scientific expertise. Her enthusiasm and rigorous attitude towards science will forever be my academic role model. Your mentorship based on a vast scientific knowledge and bright personality helped me to deal with challenges frequently coming up in research. I could not have imagined a better advisor and mentor for my MD/PhD work.

I would like to thank the China Scholarship Council for providing the generous financial support during the first 3 years and also the Charitè - Universitätsmedizin Berlin for funding of my final year. That way I was free to devote all my time with no-worries into my PhD project.

My sincere gratitude goes to my wonderful group members: Dr. Kristin Franke, Annett von Grüner and Melanie Heßler. Thank you for your time and patience teaching me experimental technics since my first day in the lab, as well as all the collaborative scientific work and discussions. I am also thankful for your care and emotional support making my time in the lab a memorable one.

I appreciated the help from my other colleagues, Dennis Ernst, Dr. Davender Redhu, Dr. Shruti Rastogi, Dr. Kristijan Pazur and Dr. Diana Willmes who supported me throughout my lab time.

Last but not the least, I would like to thank my parents, for their eternal and uncondional support as well as their encourage. I would not have made it so far without you! And to my friends with whom I never felt lonely.