Cellular Signalling 63 (2019) 109358

Contents lists available at ScienceDirect

Cellular Signalling

journal homepage: www.elsevier.com/locate/cellsig

Store-operated calcium entry (SOCE) contributes to phosphorylation of p38 MAPK and suppression of TNF-α signalling in the intestinal epithelial cells

Junsuke Uwada[a,*], Takashi Yazawa[a], Hitomi Nakazawa[b], Daisuke Mikami[c], Susanne M. Krug[d], Michael Fromm[d], Kiyonao Sada[e], Ikunobu Muramatsu[f], Takanobu Taniguchi[a]

[a] Division of Cellular Signal Transduction, Department of Biochemistry, Asahikawa Medical University, Asahikawa 078-8510, Japan
[b] Department of Functional Anatomy and Neuroscience, Asahikawa Medical University, Asahikawa 078-8510, Japan
[c] Department of Nephrology, Faculty of Medical Sciences, University of Fukui, Fukui 910-1193, Japan
[d] Institute of Clinical Physiology, Charité – Universitätsmedizin Berlin, 12203 Berlin, Germany
[e] Department of Genome Science and Microbiology, University of Fukui, Fukui 910-1193, Japan
[f] Department of Pharmacology, Kanazawa Medical University, Kanazawa 920-0293, Japan

ARTICLE INFO

Keywords:
Store-operated calcium entry
p38 MAPK
Tumor necrosis factor
Muscarinic acetylcholine receptor
Protease-activated receptor-2

ABSTRACT

Calcium influx via store-operated calcium entry (SOCE) has an important role for regulation of vast majority of cellular physiological events. MAPK signalling is also another pivotal modulator of many cellular functions. However, the relationship between SOCE and MAPK is not well understood. In this study, we elucidated the involvement of SOCE in $G\alpha_{q/11}$ protein-mediated activation of p38 MAPK in an intestinal epithelial cell line HT-29/B6. In this cell line, we previously showed that the stimulation of M3 muscarinic acetylcholine receptor (M3-mAChR) but not histamine H1 receptor (H1R) led to phosphorylation of p38 MAPK which suppressed tumor necrosis factor-α (TNF-α)-induced NF-κB signalling through ADAM17 protease-mediated shedding of TNF receptor-1 (TNFR1). First, we found that stimulation of M3-mAChR and protease-activated receptor-2 (PAR-2) but not H1R induced persistent upregulation of cytosolic Ca^{2+} concentration through SOCE. Activation of M3-mAChR or PAR-2 also suppressed TNF-α-induced NF-κB phosphorylation, which was dependent on the p38 MAPK activity. Time course experiments revealed that M3-mAChR stimulation evoked intracellular Ca^{2+}-dependent early phase p38 MAPK phosphorylation and extracellular Ca^{2+}-dependent later phase p38 MAPK phosphorylation. This later phase p38 MAPK phosphorylation, evoked by M3-mAChRs or PAR-2, was abolished by inhibition of SOCE. Thapsigargin or ionomycin also phosphorylate p38 MAPK by Ca^{2+} influx through SOCE, leading to suppression of TNF-α-induced NF-κB phosphorylation. Finally, we showed that p38 MAPK was essential for thapsigargin-induced cleavage of TNFR1 and suppression of TNF-α-induced NF-κB phosphorylation. In conclusion, SOCE is important for p38 MAPK phosphorylation and is involved in TNF-α signalling suppression.

1. Introduction

Store-operated calcium entry (SOCE) is ubiquitous calcium entry pathway which is mainly studied in non-excitable cells. SOCE is induced by depletion of calcium stores from endoplasmic reticulum (ER). Stromal interaction molecule 1 (STIM1), localized on the ER membrane, detects reduction of ER Ca^{2+} store, and then translocates to plasma membrane to activate Ca^{2+} release-activated Ca^{2+} (CRAC) channels including Orai1 [1]. Through this process, SOCE plays a homeostatic role in providing Ca^{2+} from extracellular sources to refill the ER with Ca^{2+}. Furthermore, calcium influx via SOCE exerts many signalling cascades including kinase signalling and gene regulatory pathways [2]. SOCE is known to associate with tumor development, growth and metastasis [3,4]. Therefore, the relationship between SOCE and the mitogen-activated protein kinase (MAPK) pathway is of interest. For example, thapsigargin-induced SOCE exerts CAMKII-

Abbreviations: 2-f-LIGRLO, 2-furoyl-Leu-Ile-Gly-Arg-Leu-Orn-NH_2; ADAM17, A disintegrin and metalloproteinase 17; CCh, Carbachol; CRAC channel, Ca^{2+} release-activated Ca^{2+} channel; DAG, Diacylglycerol; GPCRs, G Protein-coupled receptors; H1R, Histamine H1 receptor; IBD, Inflammatory bowel disease; IP_3, Inositol triphosphate; M3-mAChR, M3 Muscarinic acetylcholine receptor; MAPK, Mitogen-activated protein kinase; PKC, Protein kinase C; PLCβ, Phospholipase Cβ; PAR-2, Protease-activated receptor-2; SOCE, Store-operated calcium entry; STIM1, Stromal interaction molecule 1; TNF-α, Tumor necrosis factor-α

* Corresponding author.
E-mail address: uwada@asahikawa-med.ac.jp (J. Uwada).

https://doi.org/10.1016/j.cellsig.2019.109358
Received 15 March 2019; Received in revised form 25 June 2019; Accepted 6 July 2019
Available online 08 July 2019

mediated ERK1/2 phosphorylation in melanoma [5]. However, the contribution of SOCE on MAPK family including p38 MAPK is still largely unknown.

G protein-coupled receptors (GPCRs), especially $G\alpha_{q/11}$ protein-associated receptors are known to mediate the induction of SOCE. Activation of $G\alpha_{q/11}$-coupled receptors leads to phospholipase Cβ (PLCβ)-mediated hydrolysis of phosphatidyl-inositol 4,5-bisphosphate, resulting in generation of inositol tri-phosphate (IP_3) and diacylglycerol (DAG). DAG directly activates protein kinase C (PKC). On the other hand, IP_3 binds to the IP_3 receptor which exerts Ca^{2+} release from ER, and then the emptiness of this Ca^{2+} storage induces SOCE. $G\alpha_{q/11}$-coupled receptors are also known to activate MAPKs including p38 MAPK. Many signalling factors, including PKC, src tyrosine kinase, Rho, Rac and ROS, are reported to act upstream of p38 MAPK phosphorylation [6–9]. However, contribution of $G\alpha_{q/11}$-mediated intracellular calcium upregulation, especially Ca^{2+} influx by SOCE to p38 MAPK activation has still remained unclear.

In colonic epithelial cells, several $G\alpha_{q/11}$-coupled receptors were expressed, including M3-subtype of muscarinic acetylcholine receptor (M3-mAChR), histamine H1 receptor (H1R) and protease-activated receptor-2 (PAR-2) [10–12]. p38 MAPK is involved in several events in intestinal epithelium such as Cl^- secretion and wound closure through up-regulation of cell migration [13,14]. Previously, we have reported that M3-mAChR activates p38 MAPK in a colonic epithelial cell line, HT-29/B6 cells. Interestingly, activation of p38 MAPK suppressed tumor necrosis factor-α (TNF-α)-induced NF-κB signalling through ADAM17 protease-mediated shedding of TNF receptor-1 (TNFR1) [15,16]. TNF-α and subsequent NF-κB signalling associate with disruption of intestinal barrier function, which could cause inflammatory bowel diseases (IBD) like Crohn's disease and ulcerative colitis [17,18]. Therefore, downregulation of TNF-α signalling by p38 MAPK might contribute to maintain the intestinal barrier function. However, the upstream signalling event on M3-mAChR-induced p38 MAPK phosphorylation in intestinal epithelium remained unclear so far.

In this study, we revealed that M3-mAChR and PAR-2 but not H1R phosphorylated p38 MAPK through Ca^{2+} influx by SOCE in HT-29/B6 cells. SOCE induced by thapsigargin or ionomycin also phosphorylated p38 MAPK. Finally, SOCE-mediated phosphorylation of p38 MAPK suppressed TNF-α-induced NF-κB phosphorylation.

2. Materials and methods

2.1. Materials

SB203580 from AdooQ BioScience (CA, USA). YM-58483 from Cayman Chemical (MI, USA). *O*,*O*′-Bis(2-aminophenyl)ethyleneglycol-N,N,N′,N′-tetraacetic acid, tetraacetoxymethyl ester (BAPTA-AM) from DOJINDO (Kumamoto, Japan). Fetal bovine serum (FBS) and Fluo-4 AM from Invitrogen (CA, USA). Thapsigargin and gadolinium chloride from Nakalai Tesque (Kyoto, Japan). Recombinant human TNF-α from PEPROTECH (NJ, USA). 2-furoyl-Leu-Ile-Gly-Arg-Leu-Orn-NH_2 (2-f-LIGRLO) and TNF-α protease inhibitor-0 (TAPI-0) from Peptides International (KY, USA). Ionomycin calcium from LKT Laboratories (MN, USA). Carbachol (CCh) and ethylene glycol-bis(2-aminoethylether)-N,N,N′,N′-tetraacetic acid (EGTA) from Sigma Aldrich (MO, USA). Trypsin from porcine pancreas, histamine and YM-254890 from Wako pure chemical (Osaka, Japan).

2.2. Cell culture

HT-29/B6 cells are selected from the human colon cancer derived HT-29 cells [19]. Cells were grown in a humidified atmosphere with 5% CO_2 at 37 °C in RPMI 1640 medium supplemented with 2 mM glutamine, 15 mM HEPES (pH 7.2), 10% FBS, 100 U/ml penicillin and 100 μg/ml streptomycin. Culture medium was changed every 2 days. For the knock-down experiments, siRNA for human STIM1 gene (HSS110309, Invitrogen), STIM2 gene (HSS183972) or control siRNA were introduced to the HT-29/B6 cells. Each oligonucleotide was transiently transfected at a final concentration of 10 nM using Screen-Fect siRNA (Wako), according to the manufacturer's instruction, and then cells were cultured for 3 days.

2.3. Western blot

Cells were maintained in serum free medium for 2 h prior to drug treatment. Cells were stimulated with 100 μM CCh, 1 μM 2-f-LIGRLO, 100 μM histamine, 1 μM thapsigargin or 1 μM ionomycin for 5 min with or without the pretreatment of 1 μM YM-254890, 5 mM EGTA, 20 μM BAPTA-AM, Gadolinium or 10 μM YM-58483 for 15 min. To examine TNF-α-induced NF-κB phosphorylation, cells were treated with 10 ng/ml TNF-α for 5 min just before harvesting. In Figs. 4D and 5D, culture medium were replaced with Ca^{2+} free Krebs-Hepes Buffer (140 mM NaCl, 5.4 mM KCl, 1.2 mM $MgCl_2$, 10 mM glucose, and 10 mM HEPES, pH 7.4). After 30 min incubation, cells were stimulated with CCh, 2-f-LIGRLO or thapsigargin for 2 min, and then 2 mM $CaCl_2$ were replenished to medium. At the end of reaction, the medium was removed and cells were lysed by adding a SDS sample buffer (50 mM Tris-HCl, pH 6.8, 10% glycerol, 1% SDS, 1% β-mercaptoethanol, 0.1% bromophenol blue). Cell lysates were collected into tubes and heated for 10 min at 96 °C. Proteins were separated by SDS-PAGE and transferred to polyvinylidene fluoride membranes (Wako). Membranes were probed with appropriate concentrations of primary antibody against NF-κB p65 (#4764), phospho-NF-κB p65 (Ser536, #3033), p38 MAPK (#9212), phospho-p38 MAPK (Thr180/Tyr182, #4511), (Cell Signaling Technology, MA, USA), β-actin (clone AC-74, Sigma Aldrich, MO, USA) and STIM1 (610954, BD Transduction Laboratories, CA, USA). The immunoreactive proteins were detected by horseradish-peroxidase-labeled secondary antibody with Clarity Western ECL substrate (BioRad, CA, USA). The signal intensity was calculated using Image J software.

2.4. Measurement of intracellular Ca^{2+} concentration

HT-29/B6 cells were cultured in glass bottom dishes (Greiner Bio-One, Frickenhausen, Germany). Just before Fluo-4 loading, medium was replaced with Krebs-Hepes Buffer (140 mM NaCl, 5.4 mM KCl, 1.2 mM $MgCl_2$, 2.0 mM $CaCl_2$, 10 mM glucose, and 10 mM HEPES, pH 7.4) containing 0.7 mM probenecid. Then cells were loaded with 2 μM Fluo-4 AM (solubilized in 10% Pluronic F-127 just before use) for 30 min at 25 °C and washed with probenecid containing Krebs-Hepes buffer. Fluorescent images were obtained using confocal laser microscopy (FV1000-D, Olympus, Tokyo, Japan). During imaging, cells were maintained at 37 °C. Fluo-4 was excited using a diode laser (473 nm). Data are given as fluorescence ratio F/F0, indicating relative cytosolic Ca^{2+} concentrations.

2.5. Enzyme-linked immunosorbent assay (ELISA)

HT-29/B6 cells were cultured after seeding of 1.0×10^5 cells per well of a 24-well plate for 3 days. After 10 min incubation with 600 μl of fresh medium in presence or absence of SB203580, cells were stimulated with thapsigargin for 30 min. Then, cell culture supernatants were collected and assayed by using human sTNFR1 Quantikine ELISA Kit (R &D Systems, Abingdon, UK). Absorbance was measured at 450 nm using a spectrophotometer (SpectraMax PLUS; Molecular Devices, CA, USA). sTNFR1 concentration of culture medium without cells was also measured and subtracted.

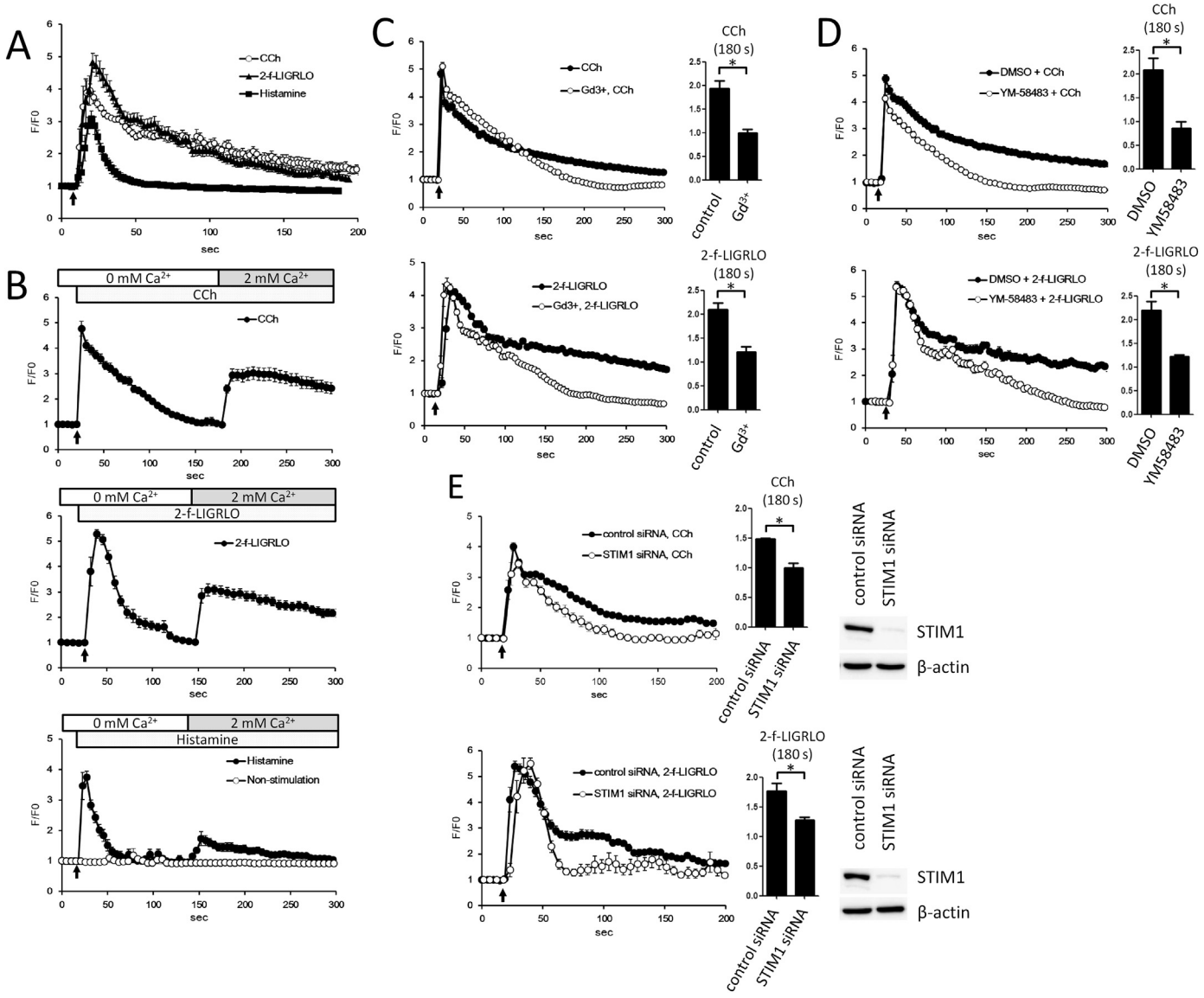

Fig. 1. M3-mAChR and PAR-2 but not H1R could evoke persistent Ca^{2+} influx through SOCE.
Changes in cytosolic Ca^{2+} concentration are expressed as fluorescence ratio F/F0. (A) Representative traces of cytosolic Ca^{2+} levels in response to CCh (100 μM), 2-f-LIGRLO (1 μM) or histamine (100 μM) in HT-29/B6 cells. Each agonist was administrated at 10–15 s as indicated by the arrow. Each trace of cytosolic Ca^{2+} levels were averaged of 6–8 cells (mean ± S.E.M.) and representative of 3 similar experiments. (B) Cells were stimulated with CCh (100 μM, upper panel), 2-f-LIGRLO (1 μM; middle panel) or Histamine (100 μM; lower panel) in extracellular Ca^{2+}-depleted condition. After that, 2 mM Ca^{2+} was added to the extracellular pool in the indicated time. Each trace of cytosolic Ca^{2+} levels were averaged of 10–17 cells (mean ± S.E.M.) and representative of 3 similar experiments. (C) (D) Cells were stimulated with CCh (100 μM, upper panels) or 2-f-LIGRLO (1 μM, lower panels) in the presence or absence of Gd^{3+} (10 μM) (C), or YM-58483 (10 μM) or DMSO (1:100, YM-58483 solvent) (D). Each trace of cytosolic Ca^{2+} levels were averaged of 17–24 cells (mean ± S.E.M.) Cytosolic Ca^{2+} levels of 180 s after agonist stimulation were compared with bar graphs in each right panel. Values represent the means ± S.E.M. of 4 independent experiments. *p < .05 (two-tailed Student's *t*-test). (E) Cells were incubated with STIM1-targeted siRNA for 3 days. Then cells were stimulated with CCh (100 μM, upper panel) or 2-f-LIGRLO (1 μM, lower panels). Each trace of cytosolic Ca^{2+} levels were averaged of 15–20 cells (mean ± S.E.M.). Cytosolic Ca^{2+} levels of 180 s after agonist stimulation were compared with bar graphs in each right panel. Values represent the means ± S.E.M. of 4 independent experiments. *p < .05 (two-tailed Student's *t*-test). After Ca^{2+} experiments, cell lysates were subjected to immunoblot by using STIM1 antibodies to conform the effect of siRNA.

3. Results

3.1. M3-mAChR and PAR-2 but not H1R could evoke persistent Ca^{2+} influx through SOCE

In HT-29/B6 cells, activation of M3-mAChR but not H1R leads to suppression of TNF-α-mediated NF-κB signalling [16]. Therefore, it is indicated that different intracellular signalling was evoked through M3-mAChR from that through H1R respectively. First, we evaluated Ca^{2+} response which was induced by M3-mAChR, H1R or other $G\alpha_{q/11}$-coupled receptor PAR-2. PAR-2 was expressed in HT-29, a parental cell line of HT29/B6 cells, which can be activated by a specific agonist, 2-f-LIGRLO [20]. Intracellular Ca^{2+} concentration was monitored by a fluorescent Ca^{2+} indicator Fluo-4. In HT-29/B6 cells, treatment with CCh or 2-f-LIGRLO induced transient release of Ca^{2+} followed by moderate reduction of Ca^{2+} concentration which continued for over 200 s to return to basal levels (Fig. 1A). In contrast, histamine evoked transient Ca^{2+} upregulation and turned to basal level rapidly (within 60 s). Next, these receptors were activated in Ca^{2+} free condition (Fig. 1B). Without extracellular Ca^{2+}, M3-mAChRs and PAR-2 upregulated intracellular Ca^{2+} concentration, though which the concentration returned to basal level within 150 s. Then, supplementation

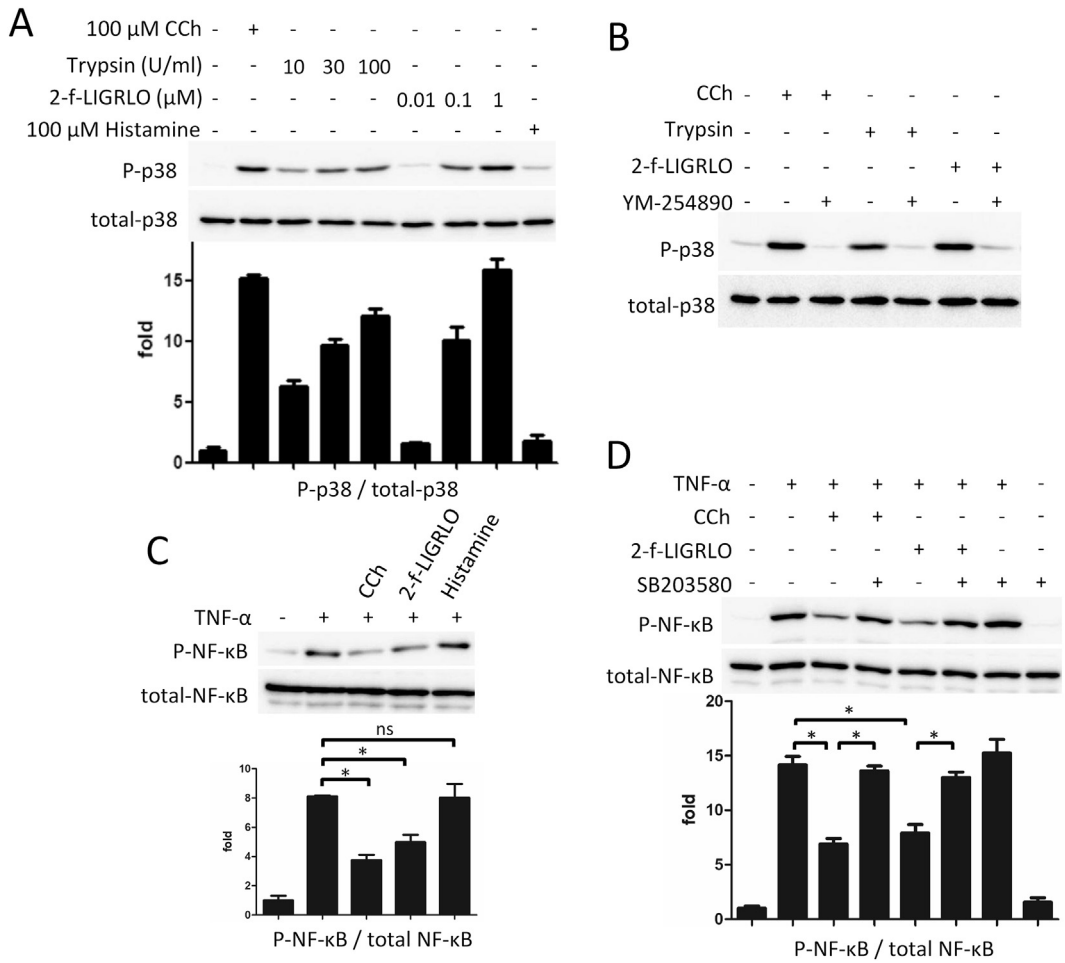

Fig. 2. Stimulation of M3-mAChRs and PAR-2 but not H1R evoked p38 MAPK phosphorylation and suppression of TNF-α signalling. (A) HT-29/B6 cells were treated with CCh (100 μM), Trypsin (10, 30, 100 U/ml), 2-f-LIGRLO (0.01, 0.1, 1 μM) or histamine (100 μM) for 5 min. Cell lysates were subjected to immunoblot by using phosphorylated p38 MAPK and p38 MAPK antibodies. The ratio of intensities of signal was quantified by densitometry (bellow). Values represent the means ± S.E.M. of 3 independent experiments. (B) Cells were treated with CCh (100 μM), trypsin (100 U/ml) or 2-f-LIGRLO (1 μM) for 5 min with or without the pretreatment of $G\alpha_{q/11}$ inhibitor YM-254890 (1 μM) for 15 min. (C) HT-29/B6 cells were treated with TNF-α (10 ng/ml) for 5 min with or without the pretreatment of CCh (100 μM), 2-f-LIGRLO (1 μM) or histamine (100 μM), and YM-254890 (1 μM). Cell lysates were subjected to immunoblot by using phosphorylated NF-κB, NF-κB antibodies. *p < .05, (one-way ANOVA with Tukey's post hoc test). ns, not significantly different. Values represent the means ± S.E.M. of 3 independent experiments. (D) Cells were treated with SB203580 (10 μM) for 15 min, prior to the addition of CCh (100 μM) or 2-f-LIGRLO (1 μM), and TNF-α (10 ng/ml). *p < .05, (one-way ANOVA with Tukey's post hoc test) of 3 independent experiments.

of extracellular Ca^{2+} evoked persistent Ca^{2+} upregulation. In contrast, H1R stimulation induced little Ca^{2+} influx after Ca^{2+} addition. These results suggested that M3-mAChR and PAR-2 cause more sustained Ca^{2+} influx compared to H1R, through the SOCE mechanism. To demonstrate the involvement of SOCE for persistent Ca^{2+} upregulation, gadolinium ion (Gd^{3+}) and YM-58483 were used as SOCE inhibitors. As shown in Fig. 1C and D, pretreatment of Gd^{3+} or YM-58483 abolished the persistent Ca^{2+} upregulation. In addition, acute application of YM-58483 at persistent phase of Ca^{2+} upregulation decreased cytosolic Ca^{2+} concentration (Supplementary Fig. 1A and B). The relevance of SOCE was further evaluated by knock-down of a SOCE component STIM1. Silencing of STIM1 led to suppression of M3-mAChR- and PAR-2-mediated persistent Ca^{2+} upregulation (Fig. 1E). We also checked the contribution of STIM2, which is another member of STIM family and is a relatively weaker activator of CRAC channels compared to STIM1 [21]. Expression of STIM2 proteins were suppressed by gene silencing, however, which had no effect on the persistent Ca^{2+} upregulation (Supplementary Fig. 2A and B). Thus, STIM1 preferentially participates to continuous Ca^{2+} influx by M3-mAChR or PAR-2 stimulation. These results indicated that M3-mAChR and PAR-2 effectively induced persistent Ca^{2+} influx through SOCE compared to H1R.

3.2. Stimulation of M3-mAChRs and PAR-2 but not H1R evoked p38 MAPK phosphorylation and suppression of TNF-α signalling

PAR-2 evoked SOCE-mediated persistent Ca^{2+} upregulation like M3-mAChR. Next, we examined whether PAR-2 could phosphorylate p38 MAPK and suppress TNF-α signalling as M3-mAChR did. Stimulation of PAR-2 with trypsin or PAR-2-specific agonist 2-f-LIGRLO phosphorylated p38 MAPK in a concentration-dependent manner (Fig. 2A). M3-mAChR- and PAR-2-induced p38 phosphorylation was sensitive to the $G\alpha_{q/11}$ protein inhibitor YM-254890 (Fig. 2B). In HT-29/B6 cells, M3-mAChRs activation led to p38 MAPK-mediated suppression of TNF-α-induced NF-κB phosphorylation [16]. As shown in Fig. 2C, activation of PAR-2 or M3-mAChRs, but not H1R, attenuated TNF-α-induced NF-κB phosphorylation. Furthermore, this effect was prevented by p38 MAPK inhibitor SB203580 (Fig. 2D). Thus, in these $G\alpha_{q/11}$-coupled receptors, M3-mAChR and PAR-2, but not H1R, could phosphorylate p38 MAPK and suppress TNF-α signalling.

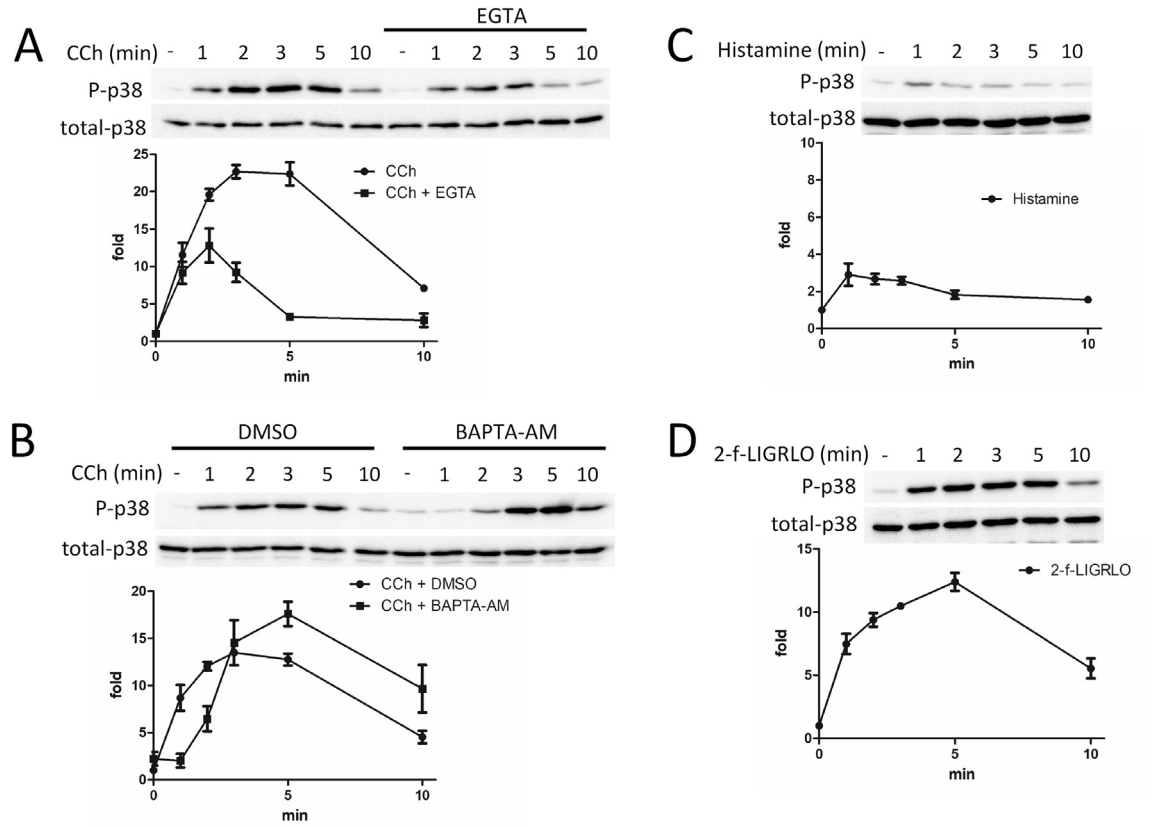

Fig. 3. Intracellular and extracellular Ca^{2+}-dependent phase of p38 MAPK phosphorylation. HT-29/B6 cells were stimulated with 100 μM CCh (A, B), 100 μM Histamine (C) or 1 μM 2-f-LIGRLO (C) for indicated times, with or without 5 mM EGTA, 20 μM BAPTA-AM or DMSO (1:100, BAPTA-AM solvent). Densitometries of immunoblot from three independent experiments are shown in each lower panel.

3.3. Intracellular and extracellular Ca^{2+}-dependent phase of p38 MAPK phosphorylation

M3-mAChR and PAR-2, which can evoke SOCE-mediated persistent Ca^{2+} upregulation, could also induce p38 MAPK-mediated suppression of TNF-α signalling. We, thus, postulated that Ca^{2+} influx is implicated in the phosphorylation of p38 MAPK. Time course of p38 MAPK phosphorylation by M3-mAChR activation was examined with or without EGTA or BAPTA-AM treatment (Fig. 3A and B). p38 MAPK phosphorylation was initiated within 1 min and persisted at least 10 min. Depletion of extracellular Ca^{2+} with EGTA predominantly downregulated p38 MAPK phosphorylation at later phase (3 to 5 min from agonist stimulation). On the other hand, chelation of intracellular Ca^{2+} with BAPTA-AM didn't disturb the later phase of p38 MAPK phosphorylation whereas early phase (1 to 2 min) was suppressed by this treatment. These results indicated that the M3-mAChR stimulation evoked intracellular and extracellular Ca^{2+}-dependent p38 MAPK phosphorylation. Histamine stimulation evoked weak p38 MAPK phosphorylation with a peak around 2 min (Fig. 3C). PAR-2 activation induced robust p38 MAPK phosphorylation which persisted at least 5 min as in the case of, M3-mAChR activation (Fig. 3D).

3.4. SOCE contributes to M3-mAChR- and PAR-2-mediated p38 MAPK phosphorylation

Next, we investigated the role of SOCE on M3-mAChR and PAR-2 mediated p38 MAPK phosphorylation. Effect of SOCE inhibitor YM-58483 on the time course of M3-mAChR-mediated p38 MAPK phosphorylation was evaluated (Fig. 4A). As same as EGTA treatment, YM-58483 primarily inhibited later phase of p38 MAPK phosphorylation. In addition, suppression of SOCE with Gd^{3+} inhibited p38 MAPK phosphorylation elicited 5 min after M3-mAChR and PAR-2 activation (Fig. 4B). To verify the involvement of SOCE on p38 MAPK phosphorylation, STIM1 was down-regulated by siRNA (Fig. 4C). Silencing of STIM1 suppressed M3-mAChR- and PAR-2-induced p38 MAPK phosphorylation at 5 min after the stimulation. Furthermore, to demonstrate the importance of Ca^{2+} influx, cells were stimulated with CCh or 2-f-LIGRLO in Ca^{2+} free condition followed by with or without Ca^{2+} replenishment. As shown in Fig. 4D, Ca^{2+} replenishment induced p38 MAPK phosphorylation. Thus, SOCE contributes to M3-mAChR- and PAR-2-mediated p38 MAPK phosphorylation.

3.5. Ca^{2+} influx by thapsigargin could induce p38 MAPK phosphorylation

To demonstrate that the Ca^{2+} influx is sufficient for p38 MAPK phosphorylation, we used thapsigargin, a sarco/endoplasmic reticulum Ca^{2+} ATPase inhibitor. Time course experiments showed that thapsigargin initiated p38 phosphorylation at 2–3 min and peaked around 5 min (Fig. 5A). Depletion of extracellular Ca^{2+} with EGTA significantly suppressed p38 MAPK phosphorylation excepting a small peak around 2 min. In contrast, BAPTA-AM had no suppressive effect on the peak intensity at 5 min (Fig. 5B). Thapsigargin induces two mode of Ca^{2+} upregulation, firstly, leakage of Ca^{2+} from ER, secondly, Ca^{2+} influx by SOCE mechanism through ER Ca^{2+} depletion. In Ca^{2+} free condition, thapsigargin induced transient Ca^{2+} upregulation derived from internal-stored Ca^{2+}, then, supplementation of Ca^{2+} to medium increased intracellular Ca^{2+} concentration again through Ca^{2+} influx through SOCE (Fig. 5C). Thapsigargin alone could not induce p38 phosphorylation in Ca^{2+} free medium, in contrast, thapsigargin treatment followed by Ca^{2+} supplementation led to phosphorylation of p38 MAPK (Fig. 5D). Thus, thapsigargin induce p38 phosphorylation through Ca^{2+} influx but not intracellular Ca^{2+} release.

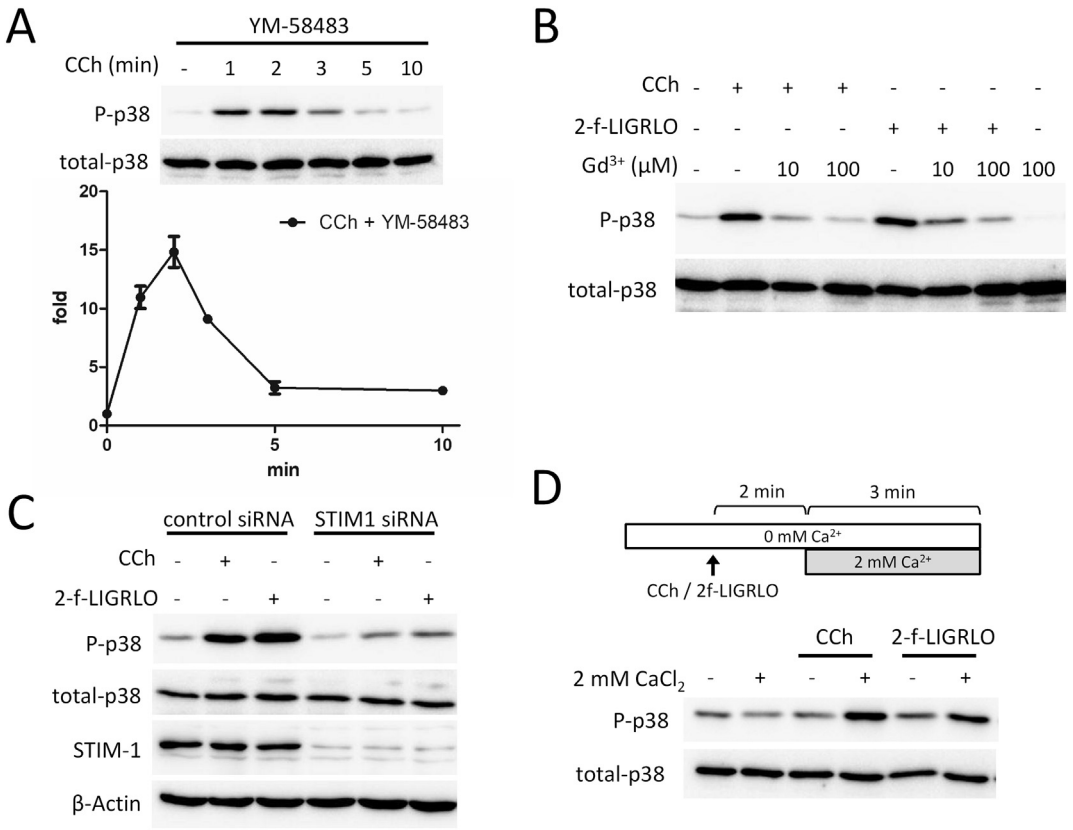

Fig. 4. SOCE was essential for M3-mAChR- and PAR-2-mediated p38 MAPK phosphorylation. (A) HT-29/B6 cells were stimulated with 100 μM CCh for indicated times with 10 μM YM-58483. Densitometry of immunoblot from three independent experiments is shown in below. (B) Cells were stimulated with 100 μM CCh or 1 μM 2-f-LIGRLO for 5 min with the indicated concentration of Gd^{3+}. (C) Cells were incubated with STIM1-targeted siRNA for 3 days. Then cells were stimulated with CCh (100 μM) or 2-f-LIGRLO (1 μM) for 5 min. Effect of STIM1 siRNA was shown by immunoblot with STIM1 antibody. (D) Cells were stimulated with CCh (100 μM) or 2-f-LIGRLO (1 μM) for 2 min in extracellular Ca^{2+} free condition. After that, 2 mM Ca^{2+} was added to the extracellular pool, followed by 3 min incubation. Top panel shows experimental design. Results are representative of 3 independent experiments.

3.6. SOCE was also important for thapsigargin- and ionomycin-induced p38 MAPK phosphorylation

Next, the role of SOCE on thapsigargin-induced p38 MAPK phosphorylation was also examined. We also evaluated the effect of a calcium ionophore ionomycin as another chemical agent for intracellular Ca^{2+} upregulation. As shown in Fig. 6A, thapsigargin-induced p38 MAPK phosphorylation was inhibited by YM-58483. Interestingly, YM-58483 also suppressed ionomycin-induced phosphorylation of p38 MAPK. YM-58483 inhibited the persistent Ca^{2+} upregulation by not only thapsigargin but also by ionomycin (Fig. 6B). Although ionomycin acts as calcium ionophore, by which extracellular Ca^{2+} is directly transported to cytosol, a previous report had revealed that ionomycin also induces Ca^{2+} influx through SOCE [22]. To demonstrate that the ionomycin-induced p38 MAPK phosphorylation is mediated by ER Ca^{2+} depletion followed by Ca^{2+} influx, cells were treated with thapsigargin for 1 h to deplete ER Ca^{2+}, and then stimulated with ionomycin. As shown in Fig. 6C, long term thapsigargin treatment suppressed ionomycin-mediated p38 MAPK phosphorylation, while oxidative stress-induced p38 MAPK phosphorylation was not inhibited. Thus, ER Ca^{2+} store was also critical for ionomycin-induced p38 MAPK phosphorylation. STIM1 knock-down downregulated thapsigargin- or ionomycin-induced p38 MAPK phosphorylation and persistent Ca^{2+} upregulation (Fig. 6D and E). Therefore, SOCE had also crucial role for thapsigargin- and ionomycin-induced p38 MAPK phosphorylation.

3.7. SOCE-mediated p38 MAPK phosphorylation was enough to suppress TNF-α signalling

Finally, we elucidated the role of SOCE on TNF-α signalling. As shown in Fig. 7A, thapsigargin suppressed TNF-α-induced NF-κB phosphorylation. This effect was attenuated by depletion of extracellular Ca^{2+} or inhibition of CRAC channel (Fig. 7A and B). Inhibition of p38 MAPK with SB203580 also attenuated thapsigargin-induced suppression of TNF-α signalling (Fig. 7C). Previously, we reported that p38 MAPK-induced downregulation of TNF-α signalling is mediated by shedding of TNFR1. Thapsigargin treatment led the augmentation of cleaved soluble TNFR1, which was inhibited by SB203580 (Fig. 7D). To demonstrate that SOCE-induced suppression of TNF-α signalling is mediated by downregulation of TNFR, the activity of ADAM17, which can cleave TNFR, was inhibited with the ADAM17 inhibitor TAPI-0. In Fig. 7E, pretreatment of TAPI-0 abolished thapsigargin-induced suppression of NF-κB phosphorylation by TNF-α. Taken together, SOCE is sufficient to suppress TNF-α signalling through p38 MAPK-mediated downregulation of TNFR1.

4. Discussion

In this study, we have shown that M3-mAChR and PAR-2 but not H1R evoke persistent Ca^{2+} upregulation through SOCE in HT-29/B6 cells. M3-mAChR and PAR-2 also suppress TNF-α-induced NF-κB phosphorylation through p38 MAPK. Activation of M3-mAChR and PAR-2 shows early intracellular Ca^{2+}-dependent and late extracellular Ca^{2+}-dependent p38 MAPK phosphorylation. Later phase p38 MAPK phosphorylation is mediated by Ca^{2+} influx through SOCE. p38 MAPK

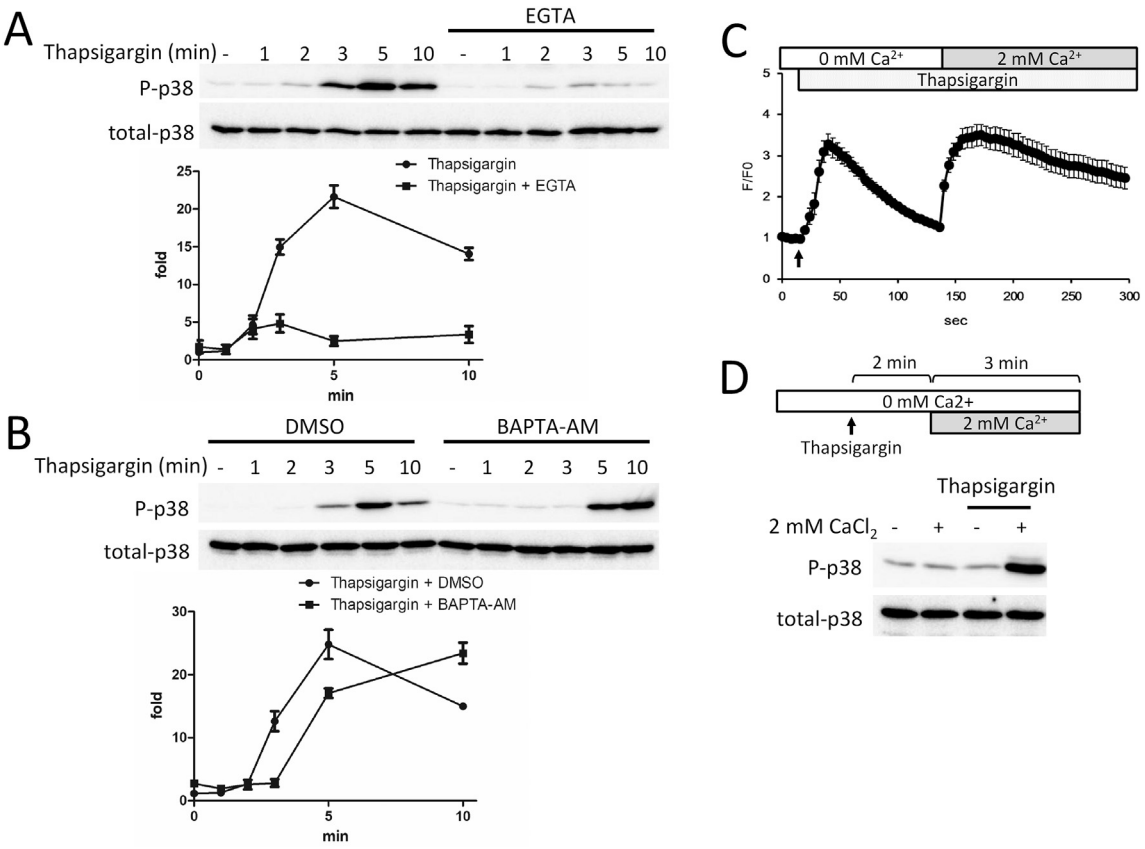

Fig. 5. Ca^{2+} influx by thapsigargin could induce p38 MAPK phosphorylation. (A) (B) HT-29/B6 cells were stimulated with 1 μM thapsigargin for indicated times, with or without 5 mM EGTA (A), 20 μM BAPTA-AM or DMSO (1:100, BAPTA-AM solvent) (B). Densitometries of immunoblot from 3 independent experiments are shown in each lower panel. (C) Cells were stimulated with thapsigargin (1 μM) in extracellular Ca^{2+}-depleted condition. After that, 2 mM Ca^{2+} was added to the extracellular pool in the indicated time. The trace of cytosolic Ca^{2+} levels were averaged of 21 cells (mean ± S.E.M.) and representative of 3 independent experiments. (D) Cells were stimulated with thapsigargin (1 μM) for 2 min in extracellular Ca^{2+}-depleted condition. After that, 2 mM Ca^{2+} was added to the extracellular pool, followed by 3 min incubation. Top panel shows experimental design. Data is a representative of 3 independent experiments.

phosphorylation is also induced by thapsigargin or ionomycin in a SOCE-dependent manner. Finally we have shown that SOCE-mediated p38 MAPK phosphorylation is sufficient to suppress TNF-α signalling.

First, we evaluated Ca^{2+} responses which induced by $G\alpha_{q/11}$ protein-coupled receptors, M3-mAChR, PAR-2 and H1R in HT-29/B6 cells. Transient Ca^{2+} upregulation was observed by activation of all of each receptor which was released from internal Ca^{2+} store since it was not suppressed by extracellular Ca^{2+} depletion (Fig. 1A and B). In these receptors, M3-mAChR and PAR-2 but not H1R induced SOCE-mediated persistent Ca^{2+} upregulation. SOCE could be elicited by the activation of $G\alpha_{q/11}$ protein-coupled receptors through production of IP_3 which evokes ER Ca^{2+} release. Sufficient downregulation of ER Ca^{2+} concentration is required for Ca^{2+} influx. In extracellular Ca^{2+} depleted condition, it took about 100 s after M3-mAChR or PAR-2 activation to return the cytosolic Ca^{2+} concentration to the basal level. In contrast, activation of H1R evoked short term Ca^{2+} upregulation which took about 30 s to return to basal concentration (Fig. 1B). Therefore, H1R might fail to evoke sufficient Ca^{2+} release to deplete ER Ca^{2+} store, although the underlying mechanism has remain unclear.

In our previous study, stimulation of M3-mAChR but not H1R prevented TNF-α-induced NF-κB signalling through p38 MAPK-mediated shedding of TNF-α receptor by ADAM17 [15,16]. In this study, we showed that PAR-2 stimulation also suppressed TNF-α-induced NF-κB phosphorylation, which was dependent on p38 MAPK activation. PAR-2 expressed in many cell types like epithelium, endothelium, immune cells and neurons in gut [23]. For example, PAR-2 activation lead to release of TNF-α in mast cells thereby which might contribute to the pathogenesis of IBD [24]. However, the role of PAR-2 in TNF-α signalling in epithelial cells had not been well understood. Our results suggest that PAR-2, in addition to M3-mAChR, has protective role to pro-inflammatory action of TNF-α in intestinal epithelium. Thus, activation of PAR-2 might have cell type specific effects on inflammatory processes.

We revealed that p38 MAPK is important for M3-mAChR and PAR-2 to suppress TNF-α signalling. p38 phosphorylation by $G\alpha_{q/11}$ protein-coupled receptors had been revealed in many previous works. $G\alpha_{q/11}$ protein-mediated p38 MAPK phosphorylation had been demonstrated by transfection of dominant active form of $G\alpha_{q/11}$ protein [25]. On the other hand, there are several signalling factors which had been reported as the downstream of $G\alpha_{q/11}$ protein to phosphorylate p38 MAPK. For example, transfection of $G\alpha_{11}$ protein to HEK293 cells leads to p38 MAPK phosphorylation through PKC and src family kinase [6]. PKC is also involved in gonadotropin-releasing hormone-induced phosphorylation of p38 MAPK in αT3–1 cells [26]. $G\alpha_{q/11}$ protein had also been shown to activate Rho and Rac, which could contribute to phosphorylation of p38 MAPK [7]. Ca^{2+} is also involved in p38 MAPK signalling. In human polymorphonuclear neutrophils, p38 MAPK phosphorylation by pituitary adenylate cyclase-activating protein (PACAP) is inhibited by EGTA but not by BAPTA-AM [27]. Endothelin and noradrenaline-induced p38 MAPK phosphorylation in rat small arteries is sensitive to depletion of extracellular Ca^{2+} [28]. Interestingly, these studies indicated that extracellular Ca^{2+} is important rather than intracellular Ca^{2+}. In this study, we showed that there are two phase of p38 MAPK phosphorylation in HT-29/B6 cells, the early phase peaked at 2 min from agonist stimulation and the later phase peaked at 3 to 5 min. There was a different Ca^{2+} dependency for p38 MAPK phosphorylation, the

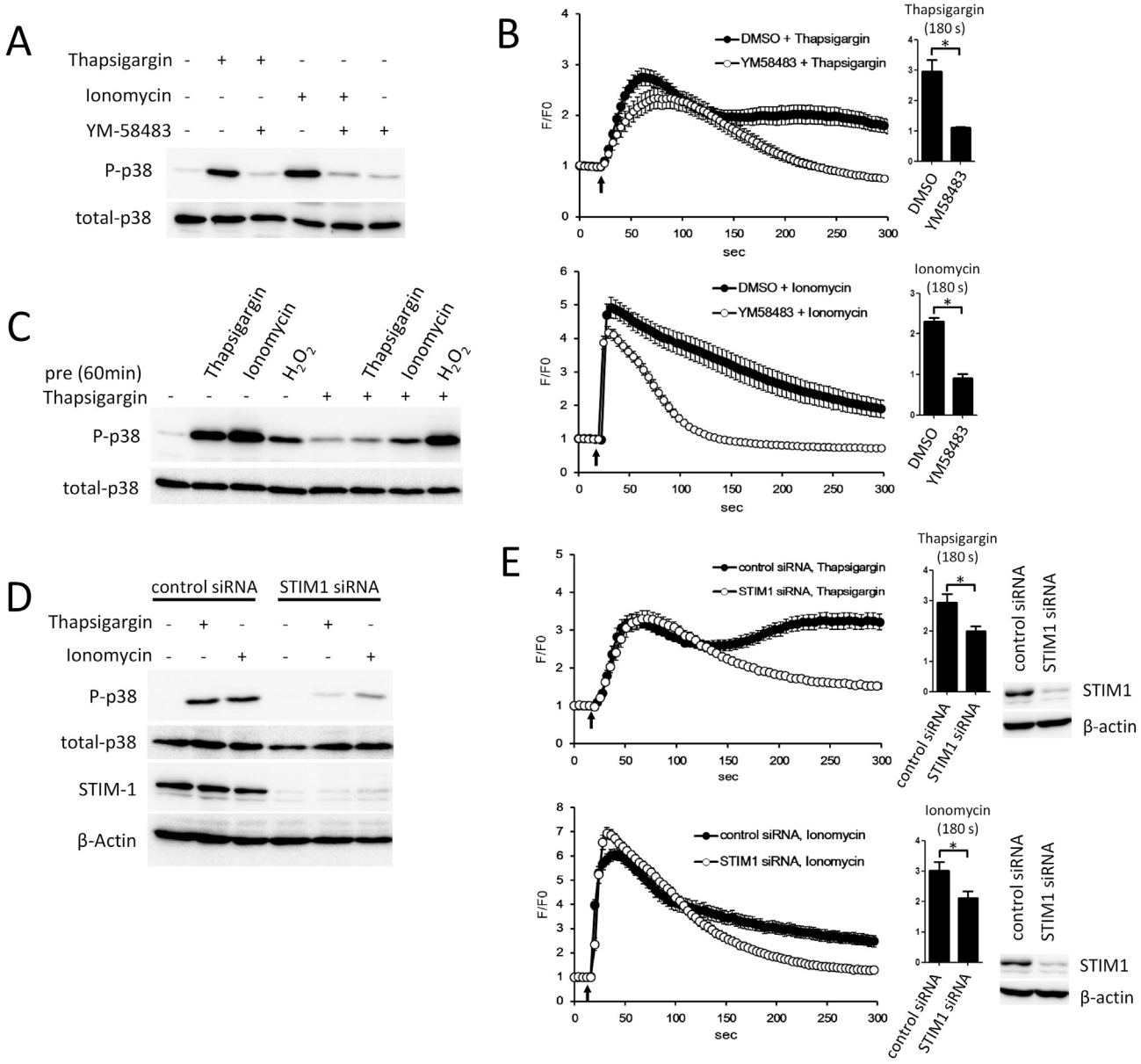

Fig. 6. SOCE was also important for thapsigargin- and ionomycin-induced p38 MAPK phosphorylation. (A) HT-29/B6 cells were stimulated with 1 μM thapsigargin or 1 μM ionomycin for 5 min with or without 10 μM YM-58483. (B) Representative traces of cytosolic Ca^{2+} levels. Cells were stimulated with thapsigargin (1 μM, upper panels) or ionomycin (1 μM, lower panels) in the presence or absence of YM-58483 (10 μM). Each trace of cytosolic Ca^{2+} levels were averaged of 10–14 cells (mean ± S.E.M.). Cytosolic Ca^{2+} levels of 180 s after drug stimulation were compared with bar graphs in each right panel. Values represent the means ± S.E.M. of 3 to 4 independent experiments. *p < .05 (two-tailed Student's *t*-test). (C) Cells were treated with thapsigargin (1 μM) for 60 min to deplete internal Ca^{2+} stores. Then cells were stimulated with thapsigargin (1 μM), ionomycin (1 μM) or H_2O_2 (1 mM). (D) Cells were incubated with STIM1-targeted siRNA for 3 days. Then cells were stimulated with 1 μM thapsigargin or 1 μM ionomycin for 5 min. Effect of STIM1 siRNA was shown by immunoblot with STIM1 antibody. (E) Cells were incubated with STIM1-targeted siRNA for 3 days. Then cells were stimulated with thapsigargin (1 μM, upper panels) or ionomycin (1 μM, lower panels). Each trace of cytosolic Ca^{2+} levels were averaged of 15–20 cells (mean ± S.E.M.). Cytosolic Ca^{2+} levels of 180 s after drug stimulation were compared with bar graphs in each right panel. Values represent the means ± S.E.M. of 4 to 5 independent experiments. *p < .05 (two-tailed Student's *t*-test). After Ca^{2+} experiments, cell lysates were subjected to immunoblot by using STIM1 antibodies to conform the effect of siRNA.

early phase is intracellular and the later phase is extracellular Ca^{2+}-dependent (Fig. 3). This later phase p38 MAPK phosphorylation was suppressed with the blockage of SOCE (Fig. 4). This result is consistent with the calcium experiments in which Ca^{2+} influx by M3-mAChR or PAR-2 activation is dependent on SOCE (Fig. 1). Furthermore, SOCE induced by chemical agents thapsigargin or ionomycin also evoked p38 MAPK phosphorylation (Fig. 6). These results demonstrate that SOCE is sufficient for p38 MAPK phosphorylation. The underlying molecular mechanism of SOCE-induced p38 MAPK remain unclear. One possible hypothesis is that the local upregulation of Ca^{2+} concentration contributes to p38 MAPK phosphorylation. Ca^{2+} which is transported by plasma Ca^{2+} channels rapidly diffuses and is diluted into cytosolic space. However, Ca^{2+} concentration in the vicinity of Ca^{2+} channel is much higher, and this compartmentalized Ca^{2+} has special roles [29]. For example, postsynaptic caldendrin protein acts as a calcium sensor for NMDA receptor-induced transient calcium influx for actin re-modeling in dendritic spines [30]. L-type Ca^{2+} channels localized to caveolae microdomains in cardiac myocytes specifically associate with NFAT-mediated hypertrophic signalling [31]. STIM1 organizes clusters in plasma membrane with CRAC channels, and form the puncta which

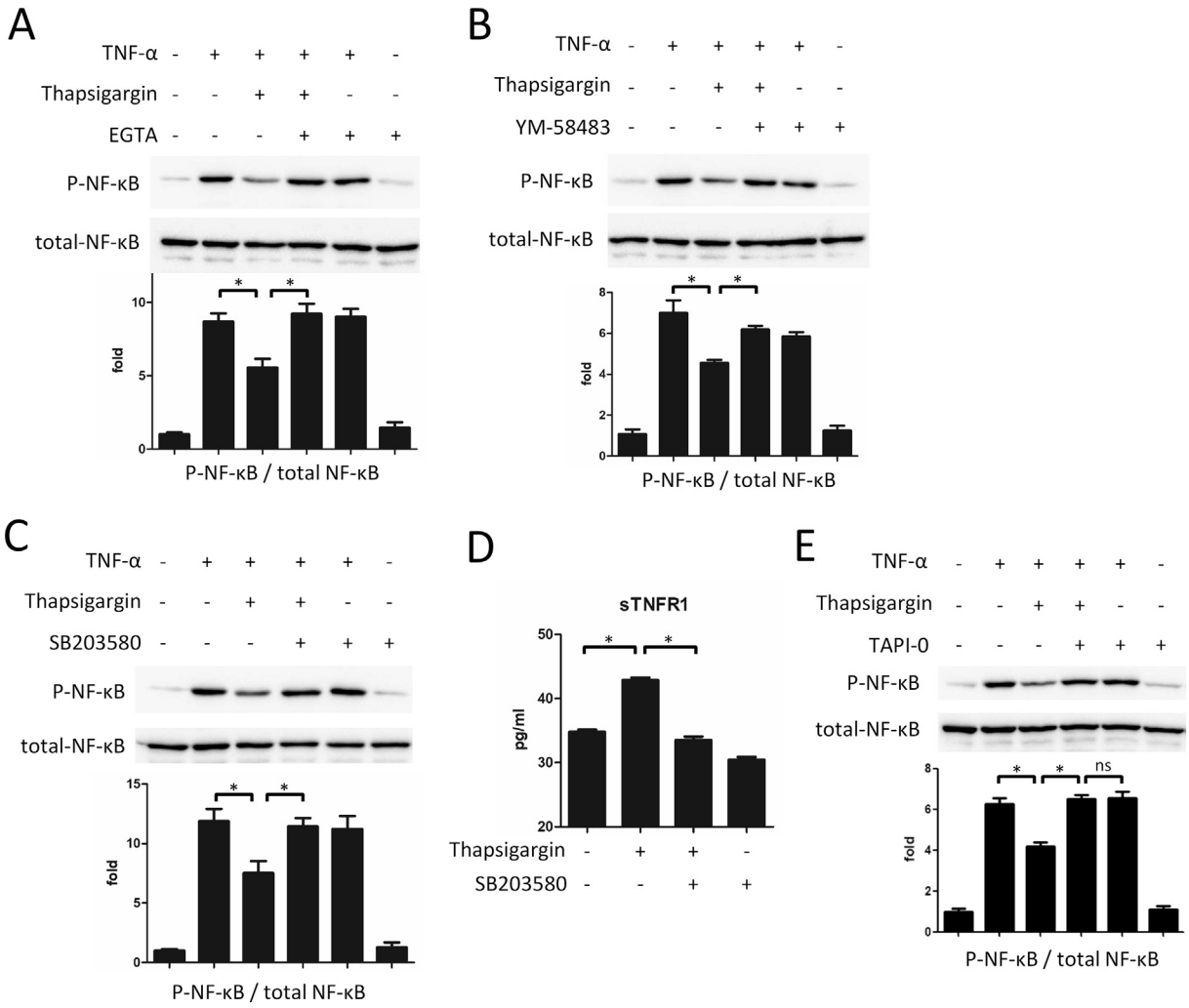

Fig. 7. SOCE-mediated p38 MAPK phosphorylation was enough to suppress TNF-α signalling. (A) (B) (C) HT-29/B6 cells were treated with TNF-α for 5 min with or without the pretreatment of thapsigargin (1 μM) and EGTA (5 mM) (A), YM-58483 (10 μM) (B), or SB203580 (10 μM) (C). The ratio of intensities of signal was quantified by densitometry (bellow). *p < .05, (one-way ANOVA with Tukey's post hoc test). Values represent the means ± S.E.M. of 3 independent experiments. (D) Cell monolayers were treated with thapsigargin (1 μM) and SB203580 (10 μM). After 30 min incubation, the cell supernatants were collected and applied to ELISA kit for sTNFR1. *p < .05, (one-way ANOVA with Tukey's post hoc test). n = 3–4 for each condition. Values represent the means ± S.E.M. (E) HT-29/B6 cells were treated with TNF-α for 5 min with or without the pretreatment of thapsigargin (1 μM) and TAPI-0 (10 μM). *p < .05, (one-way ANOVA with Tukey's post hoc test). ns, not significantly different. Values represent the means ± S.E.M. of 3 independent experiments.

is the hot spot of Ca^{2+} influx [32]. In our study, M3-mAChR or PAR-2 activation-induced later phase p38 MAPK phosphorylation was suppressed by SOCE inhibition by specific inhibitors or STIM1 knock-down, whereas cytosolic Ca^{2+} depletion with BAPTA-AM could not suppress the later phase p38 MAPK phosphorylation. Furthermore, Ca^{2+} release from internal store by histamine or thapsigargin treatment has low potency to phosphorylate p38 MAPK. These results suggest that the p38 MAPK phosphorylation machinery closely associate with SOCE components in plasma membrane. For example, the upstream regulator of p38 MAPK, which has relatively low Ca^{2+} affinity not to react with ER Ca^{2+} release, might associate with STIM1 or CRAC channels and respond to Ca^{2+} influx by GPCR- or thapsigargin-induced SOCE.

p38 MAPK could directly activate ADAM17, which is a potent sheddase for TNF-α receptors [33,34]. Therefore, activation of p38 MAPK contributes to maintain barrier function of intestinal epithelium against TNF-α-induced inflammatory injury [16]. In this study, we showed that SOCE, which was induced by thapsigargin, suppressed TNF-α-induced NF-κB phosphorylation. This suppression was diminished by treatment with p38 MAPK inhibitor. Furthermore, thapsigargin induced shedding of TNFR1 in p38 MAPK-dependent manner (Fig. 7). Immunological regulatory function of SOCE has been studied mainly in immune cells like T cells. Since functional loss of STIM1 causes immunodeficiency, SOCE has been known to contribute to the immunological function of immune cells [35]. For instance, SOCE is required for CD8+ T cells to produce inflammatory cytokines such as TNF-α and IFN-γ [36]. By contrast, our results indicate that SOCE has an immune suppressive effect through downregulation of TNF-α action in intestinal epithelial cells. Therefore, SOCE might have opposite roles in inflammatory response depending on the cell types.

In conclusion, activation of M3-mAChR or PAR-2, or treatment with thapsigargin phosphorylates p38 MAPK through Ca^{2+} influx through SOCE machinery in HT-29/B6 cells, a cell line which is a good model for the intestinal epithelium. SOCE-dependent p38 MAPK activation suppresses TNF-α signalling. TNF-α is a major pro-inflammatory factor and a clinical target for IBD, especially for Crohn's disease [37,38]. Therefore, regulation of SOCE activity in intestinal epithelial cells might be a potential new approach for the treatment of IBD.

Declaration of Competing Interest

The authors declare no conflict of interest.

Acknowledgements

This work was supported in part by JSPS KAKENHI, Japan Grant Number 16K19012 (Grant-in-Aid for Young Scientists (B)) and 18K06946 (Grant-in-Aid for Scientific Research (C)) and granted by Akiyama Life Science Foundation, Japan and the Smoking Research Foundation of Japan. The authors thank to Ms. S.Tsunoda for the efficient secretarial assistance.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.cellsig.2019.109358.

References

[1] M. Fahrner, I. Derler, I. Jardin, C. Romanin, The STIM1/Orai signaling machinery, Channels 7 (5) (2013) 330–343.
[2] M. Prakriya, R.S. Lewis, Store-operated calcium channels, Physiol. Rev. 95 (4) (2015) 1383–1436.
[3] A. Fiorio Pla, K. Kondratska, N. Prevarskaya, STIM and ORAI proteins: crucial roles in hallmarks of cancer, Am. J. Phys. Cell Phys. 310 (7) (2016) C509–C519.
[4] Y.F. Chen, K.F. Hsu, M.R. Shen, The store-operated Ca(2+) entry-mediated signaling is important for cancer spread, Biochim. Biophys. Acta 1863 (6) (2016) 1427–1435 Pt B.
[5] M. Umemura, E. Baljinnyam, S. Feske, M.S. De Lorenzo, L.H. Xie, X. Feng, K. Oda, A. Makino, T. Fujita, U. Yokoyama, M. Iwatsubo, S. Chen, J.S. Goydos, Y. Ishikawa, K. Iwatsubo, Store-operated Ca2+ entry (SOCE) regulates melanoma proliferation and cell migration, PLoS ONE 9 (2) (2014) e89292.
[6] M. Nagao, J. Yamauchi, Y. Kaziro, H. Itoh, Involvement of protein kinase C and Src family tyrosine kinase in Galphaq/11-induced activation of c-Jun N-terminal kinase and p38 mitogen-activated protein kinase, J. Biol. Chem. 273 (36) (1998) 22892–22898.
[7] J.P. Vaque, R.T. Dorsam, X. Feng, R. Iglesias-Bartolome, D.J. Forsthoefel, Q. Chen, A. Debant, M.A. Seeger, B.R. Ksander, H. Teramoto, J.S. Gutkind, A genome-wide RNAi screen reveals a Trio-regulated Rho GTPase circuitry transducing mitogenic signals initiated by G protein-coupled receptors, Mol. Cell 49 (1) (2013) 94–108.
[8] J. Yamauchi, G. Tsujimoto, Y. Kaziro, H. Itoh, Parallel regulation of mitogen-activated protein kinase kinase 3 (MKK3) and MKK6 in Gq-signaling cascade, J. Biol. Chem. 276 (26) (2001) 23362–23372.
[9] M. Mangelus, A. Kroyter, R. Galron, M. Sokolovsky, Reactive oxygen species regulate signaling pathways induced by M1 muscarinic receptors in PC12M1 cells, J. Neurochem. 76 (6) (2001) 1701–1711.
[10] G. Schultheiss, B. Hennig, W. Schunack, G. Prinz, M. Diener, Histamine-induced ion secretion across rat distal colon: involvement of histamine H1 and H2 receptors, Eur. J. Pharmacol. 546 (1–3) (2006) 161–170.
[11] S. Bader, M. Diener, Novel aspects of cholinergic regulation of colonic ion transport, Pharmacol. Res. Perspect. 3 (3) (2015) e00139.
[12] W. Kong, K. McConalogue, L.M. Khitin, M.D. Hollenberg, D.G. Payan, S.K. Bohm, N.W. Bunnett, Luminal trypsin may regulate enterocytes through proteinase-activated receptor 2, Proc. Natl. Acad. Sci. U. S. A. 94 (16) (1997) 8884–8889.
[13] S.J. Keely, K.E. Barrett, p38 mitogen-activated protein kinase inhibits calcium-dependent chloride secretion in T84 colonic epithelial cells, Am. J. Phys. Cell Phys. 284 (2) (2003) C339–C348.
[14] M.R. Frey, A. Golovin, D.B. Polk, Epidermal growth factor-stimulated intestinal epithelial cell migration requires Src family kinase-dependent p38 MAPK signaling, J. Biol. Chem. 279 (43) (2004) 44513–44521.
[15] M.R. Khan, J. Uwada, T. Yazawa, M.T. Islam, S.M. Krug, M. Fromm, S. Karaki, Y. Suzuki, A. Kuwahara, H. Yoshiki, K. Sada, I. Muramatsu, A.S. Anisuzzaman, T. Taniguchi, Activation of muscarinic cholinoceptor ameliorates tumor necrosis factor-alpha-induced barrier dysfunction in intestinal epithelial cells, FEBS Lett. 589 (23) (2015) 3640–3647.
[16] J. Uwada, T. Yazawa, M.T. Islam, M.R.I. Khan, S.M. Krug, M. Fromm, S.I. Karaki, Y. Suzuki, A. Kuwahara, H. Yoshiki, K. Sada, I. Muramatsu, T. Taniguchi, Activation of muscarinic receptors prevents TNF-alpha-mediated intestinal epithelial barrier disruption through p38 MAPK, Cell. Signal. 35 (2017) 188–196.
[17] R. Al-Sadi, S. Guo, D. Ye, M. Rawat, T.Y. Ma, TNF-alpha modulation of intestinal tight junction permeability is mediated by NIK/IKK-alpha axis activation of the canonical NF-kappaB pathway, Am. J. Pathol. 186 (5) (2016) 1151–1165.
[18] G.D. Kalliolias, L.B. Ivashkiv, TNF biology, pathogenic mechanisms and emerging therapeutic strategies, Nat. Rev. Rheumatol. 12 (1) (2016) 49–62.
[19] K.M. Kreusel, M. Fromm, J.D. Schulzke, U. Hegel, Cl- secretion in epithelial monolayers of mucus-forming human colon cells (HT-29/B6), Am. J. Phys. 261 (4) (1991) C574–C582 Pt 1.
[20] V. Iablokov, C.L. Hirota, M.A. Peplowski, R. Ramachandran, K. Mihara, M.D. Hollenberg, W.K. MacNaughton, Proteinase-activated receptor 2 (PAR2) decreases apoptosis in colonic epithelial cells, J. Biol. Chem. 289 (49) (2014) 34366–34377.
[21] X. Wang, Y. Wang, Y. Zhou, E. Hendron, S. Mancarella, M.D. Andrake, B.S. Rothberg, J. Soboloff, D.L. Gill, Distinct Orai-coupling domains in STIM1 and STIM2 define the Orai-activating site, Nat. Commun. 5 (2014) 3183.
[22] A.J. Morgan, R. Jacob, Ionomycin enhances Ca2+ influx by stimulating store-regulated cation entry and not by a direct action at the plasma membrane, Biochem. J. 300 (1994) 665–672 Pt 3.
[23] N. Vergnolle, Clinical relevance of proteinase activated receptors (pars) in the gut, Gut 54 (6) (2005) 867–874.
[24] J.A. Kim, S.C. Choi, K.J. Yun, D.K. Kim, M.K. Han, G.S. Seo, J.J. Yeom, T.H. Kim, Y.H. Nah, Y.M. Lee, Expression of protease-activated receptor 2 in ulcerative colitis, Inflamm. Bowel Dis. 9 (4) (2003) 224–229.
[25] J. Yamauchi, M. Nagao, Y. Kaziro, H. Itoh, Activation of p38 mitogen-activated protein kinase by signaling through G protein-coupled receptors. Involvement of Gbetagamma and Galphaq/11 subunits, J. Biol. Chem. 272 (44) (1997) 27771–27777.
[26] M.S. Roberson, T. Zhang, H.L. Li, J.M. Mulvaney, Activation of the p38 mitogen-activated protein kinase pathway by gonadotropin-releasing hormone, Endocrinology 140 (3) (1999) 1310–1318.
[27] I. Harfi, F. Corazza, S. D'Hondt, E. Sariban, Differential calcium regulation of proinflammatory activities in human neutrophils exposed to the neuropeptide pituitary adenylate cyclase-activating protein, J. Immunol. 175 (6) (2005) 4091–4102.
[28] J. Ohanian, P. Cunliffe, E. Ceppi, A. Alder, E. Heerkens, V. Ohanian, Activation of p38 mitogen-activated protein kinases by endothelin and noradrenaline in small arteries, regulation by calcium influx and tyrosine kinases, and their role in contraction, Arterioscler. Thromb. Vasc. Biol. 21 (12) (2001) 1921–1927.
[29] V. Konieczny, M.V. Keebler, C.W. Taylor, Spatial organization of intracellular Ca2+ signals, Semin. Cell Dev. Biol. 23 (2) (2012) 172–180.
[30] M. Mikhaylova, J. Bar, B. van Bommel, P. Schatzle, P. YuanXiang, R. Raman, J. Hradsky, A. Konietzny, E.Y. Loktionov, P.P. Reddy, J. Lopez-Rojas, C. Spilker, O. Kobler, S.A. Raza, O. Stork, C.C. Hoogenraad, M.R. Kreutz, Caldendrin directly couples postsynaptic calcium signals to actin remodeling in dendritic spines, Neuron 97 (5) (2018) 1110–1125 (e14).
[31] C.A. Makarewich, R.N. Correll, H. Gao, H. Zhang, B. Yang, R.M. Berretta, V. Rizzo, J.D. Molkentin, S.R. Houser, A caveolae-targeted L-type Ca(2)+ channel antagonist inhibits hypertrophic signaling without reducing cardiac contractility, Circ. Res. 110 (5) (2012) 669–674.
[32] R.M. Luik, M.M. Wu, J. Buchanan, R.S. Lewis, The elementary unit of store-operated Ca2+ entry: local activation of CRAC channels by STIM1 at ER-plasma membrane junctions, J. Cell Biol. 174 (6) (2006) 815–825.
[33] P. Reddy, J.L. Slack, R. Davis, D.P. Cerretti, C.J. Kozlosky, R.A. Blanton, D. Shows, J.J. Peschon, R.A. Black, Functional analysis of the domain structure of tumor necrosis factor-alpha converting enzyme, J. Biol. Chem. 275 (19) (2000) 14608–14614.
[34] P. Xu, R. Derynck, Direct activation of TACE-mediated ectodomain shedding by p38 MAP kinase regulates EGF receptor-dependent cell proliferation, Mol. Cell 37 (4) (2010) 551–566.
[35] R.S. Lacruz, S. Feske, Diseases caused by mutations in ORAI1 and STIM1, Ann. N. Y. Acad. Sci. 1356 (2015) 45–79.
[36] C. Weidinger, P.J. Shaw, S. Feske, STIM1 and STIM2-mediated Ca(2+) influx regulates antitumour immunity by CD8(+) T cells, EMBO Mol. Med. 5 (9) (2013) 1311–1321.
[37] S. Zeissig, C. Bojarski, N. Buergel, J. Mankertz, M. Zeitz, M. Fromm, J.D. Schulzke, Downregulation of epithelial apoptosis and barrier repair in active Crohn's disease by tumour necrosis factor alpha antibody treatment, Gut 53 (9) (2004) 1295–1302.
[38] J.P. Terdiman, C.B. Gruss, J.J. Heidelbaugh, S. Sultan, Y.T. Falck-Ytter, A.G.A.I.C. Practice, C. Quality Management, American Gastroenterological Association Institute guideline on the use of thiopurines, methotrexate, and anti-TNF-alpha biologic drugs for the induction and maintenance of remission in inflammatory Crohn's disease, Gastroenterology 145 (6) (2013) 1459–1463.