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Activation of TRPV1 mediates thymic stromal lymphopoietin release via the Ca^{2+}/NFAT pathway in airway epithelial cells

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ABSTRACT

The airway epithelium is exposed to a range of irritants in the environment that are known to trigger inflammatory response such as asthma. Transient receptor potential vanilloid 1 (TRPV1) is a Ca^{2+}-permeable cation channel critical for detecting noxious stimuli by sensory neurons. Recently increasing evidence suggests TRPV1 is also crucially involved in the pathophysiology of asthma on airway epithelium in human. Here we report that in airway epithelial cells TRPV1 activation potently induces allergic cytokine thymic stromal lymphopoietin (TSLP) release. TSLP induction by protease-activated receptor (PAR)-2 activation is also partially mediated by TRPV1 channels.

1. Introduction

Inflammatory airway diseases such as asthma are a prevailing global public health problem [1,2]. The airway epithelium is a frontline barrier responding to environmental stimuli including cold air, pollution, viral infection, allergens and such stimuli are well known to be associated with the risk of developing asthma [2]. These stimuli damage the lung epithelium by activating specific receptors on the cellular surface [3,4] like transient receptor potential (TRP) ion channels, which will induce inflammatory responses [5–8].

The transient receptor potential vanilloid type 1 (TRPV1) channel is a polymodal transducer activated by noxious physical and chemical stimuli [9,10]. TRPV1 channel is a temperature- and ligand-sensitive Ca^{2+}-permeable ion channel highly expressed in dorsal root ganglia (DRG) nociceptor neurons to detect pungent extracts, proton, heat and membrane depolarization [11–13]. However, the expression profile of TRPV1 channels is broad, particularly, these channels are functionally expressed in non-sensory tissues including the human epidermal keratinocyte and airway epithelium [14–16]. Recently, population-based genetic epidemiological studies provided evidence for the involvement of TRPV1 in symptoms typically associated with inflammation such asthma [17]. For instance, expression of TRPV1 is significantly up-regulated in the airway epithelia of patients with refractory asthma [18]. Moreover, environmental risk factors such as airborne 2- and 10-μM particulate matter and coal fly ash particulate matter cause lung epithelial toxicity through activation of TRPV1 channel. In addition, respiratory rhinovirus up-regulates TRPV1 expression level to trigger airway hypersensitivity [19–21]. Herein, we sought to elucidate the mechanisms how airway inflammation such as asthma is regulated by TRPV1 channel in airway epithelium.

TSLP is a protein belonging to the cytokine family, whose expression is linked to human allergic airway disease including asthma [22,23]. Mice overexpressing TSLP in the lungs showed spontaneous airway inflammation and hyperreactivity with features similar to asthma, suggesting TSLP is necessary and sufficient for the development of airway inflammation [22,24]. TSLP is also implicated to play a key role in a number of other disorders, such as atopic dermatitis, allergic rhinitis and autoimmunity [23,25]. The factors that can promote TSLP release include intracellular Ca^{2+} increase and protease-activated receptor-2 (PAR-2) activation [26,27]. PAR-2 is a protease-sensing G protein-coupled receptor and has been found to involve in airway inflammation and airway hyperresponsiveness, both are the prominent features of asthma. To date, the molecular mechanisms that control the expression and release of TSLP are still not unveiled.

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In this report, we provide evidence that TRPV1 channel mediates an asthma-associated physiological response via inducing TSLP production. TRPV1 activation increases intracellular Ca^{2+} and then triggers nuclear factor of activated T-cells (NFAT) action to further enhance cytokine TSLP secreted from BEAS-2B cells. Our results suggest that TSLP release by PAR-2 activation also depends on Ca^{2+} influx and is partially dependent on TRPV1 channel activation. These findings suggest that TRPV1 and other Ca^{2+}-permeable ion channels are potential therapeutic targets for the treatment of airway inflammation such as asthma.

2. Materials and methods

2.1. Reagents

The PAR-2 agonist peptide, SLIGKV-NH_2 was purchased from Alomone Labs. Cyclosporin A was purchased from Santa Cruz. Ruthenium red (RR), capsaicin and capsazepine were purchased from Sigma–Aldrich. GCaMP6s-encoding plasmid was obtained from Addgene (Plasmid 40753). The siRNA against human TRPV1 (sc-36826A, F: gaagaccugucugcugaaatt, R: uuucagcagacaggucuuctt) and negative control siRNA were designed and synthesized by Santa Cruz.

2.2. Cell culture

BEAS-2B human bronchial epithelial cells were cultured in RPMI-1640 medium (Invitrogen) supplemented with 10% FBS (Gibco) at 37 °C with 5% CO_2.

2.3. Electrophysiology

BEAS-2B cells grown on glass coverslips were used for recording currents. Whole-cell membrane currents were recorded using a HEKA EPC10 amplifier. Patch pipettes were pulled by P-97 puller (Sutter) and then fire polished to a resistance of about 4 MΩ. Membrane potential were held at 0 mV and membrane currents were elicited by a 300-ms step to +80 mV followed by a 300-ms step to −80 mV at 1-s intervals (Fig. S1A). Mean current amplitude was analyzed at ±80 mV. During whole cell recording the series resistance was compensated by 80%. To record TRPV1 currents, both the external and internal solution contained 130 mM NaCl, 3 mM EGTA, and 10 mM HEPES (pH 7.2 with NaOH) as TRPV1 channel is sensitive to divalent cations including Ca^{2+} and Mg^{2+} from the intracellular and extracellular sides [17,28,29]. To record PAR2 activation-induced currents we employed an external solution containing 130 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 10 mM HEPES (pH 7.2 with NaOH); and an intracellular solution containing 135 mM KCl, 1 mM $CaCl_2$, 2 mM $MgCl_2$, 10 mM HEPES (pH 7.2 with KOH). Chemical solutions were applied to the patch with gravity perfusion system (VC3-8C, ALA Scientific Instruments). All experiments were conducted at 22–24 °C.

2.4. GCaMP6s-based Ca^{2+} imaging

BEAS-2B cells were grown on glass coverslips and transfected with GCaMP6s-encoding plasmid using 2 μg GCaMP6s-encoding plasmid at 30–50% confluence. 24 h after transfection, cells were continuously perfused with physiological buffer containing 130 mM NaCl, 2 mM $CaCl_2$, 1 mM $MgCl_2$, 5 mM KCl, 10 mM HEPES (pH 7.2 with NaOH) and images was captured on a Olympus IX-81 inverted fluorescence microscope equipped with a 75-W xenon arc lamp. Fluorophores were excited at 474 nm, and fluorescence intensity was measured through a 515/30-nm bandpass emission filter. Images were collected every 3 s, and exposure time was set to 300 ms. Background correction was performed by subtracting the average pixel intensity of a non-cellular region. The basal response was recorded for 30 s and then chemical solution was applied for 2 min. The fluorescence intensity was proportional to the Ca^{2+} concentration, thus the effect was quantified as a response ratio by dividing the chemical induced response amplitude by the basal response amplitude.

2.5. RNA isolation and Real-time (RT)-PCR

Total RNA was purified from BEAS-2B with TRIzol (Invitrogen). The primers used for RT-PCR amplification were as follows: hTSLP, 5′catggaagtgctgtcgaaga3′, 5′tttccgtgaccaatcctttc3′ (231 bp); hTRP V1, 5′ ggctgtcttcatcatcctgctgct3′, 5′gttcttgctctcctgtgcgatcttgt3′ (117 bp); β-actin, 5′atcatgtttgagaccttcaaca3′, 5′catctcttgctcgaagtcca3′ (322 bp). Reactions were made up to a final volume of 20.0 μl with sterile water. Quantitative RT-PCR analysis were performed using the iQ™5 Multicolor Real-Time PCR Detection System (Bio-Rad).

2.6. ELISA analysis of TSLP released in cell supernatants

The culture supernatants from BEAS-2B cells with or without stimulation were quantitated using hTSLP ELISA Kit according to the manufacturer's instructions (R&D Systems).

2.7. Immunofluorescent microscopy and image analysis

BEAS-2B cells were seeded on sterile slides and cultured in the absence/presence of stimuli for 24 h. In the next day the slides were fixed with poly-formaldehyde for 20 min then treated with 0.1% TritonX-100 for 5 min. The slides were incubated with rabbit anti-NAFT1 antibody (1:200, CST) or Rabbit anti-TRPV1 antibody (1:100, Alomone) overnight at 4 °C. After a second incubation with appropriate secondary antibodies rhodamine-conjugated anti-rabbit IgG (1:200, ZSGB-Bio, China), the slides were counterstrained with 1 mg/ml DAPI (4′-6-diamidino-2-phenylindole, Sigma).

2.8. Western blotting

Protein pellet were isolated with TRIzol Regent (Invitrogen). Proteins were separated by 10% SDS–PAGE and transferred to a PVDF membrane (Bio-Rad). Membranes were probed overnight with rabbit anti-TRPV1 (1:1000, Alomone) and mouse anti-β-actin (1:5000, Sigma) antibodies followed by incubation with HRP-conjugated goat anti-rabbit IgG (1:5000, ZSGB-Bio, China) and HRP-conjugated goat anti-mouse IgG (1:5000, Santa Cruz), membranes were analyzed using a Hitachi Genetic Systems (Hitachi software Engineering).

2.9. Statistical analysis

Data are presented as means ± S.E.M. Two-sided differences between two samples were analyzed with Student t test. Values of $P < 0.05$ were considered significant.

3. Results

3.1. Activation of TRPV1 increases intracellular Ca^{2+} level in BESA-2B cells

First, we investigated if TRPV1 channels are expressed on BESA-2B cells. BEAS-2B cells are an SV40-transfected, immortalized, human bronchiolar epithelial cell line that is frequently used to study the mechanisms of airway inflammation in vitro [30]. Consistent with previous reports, Immunofluorescence studies

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revealed that TRPV1 protein was abundantly expressed in BEAS-2B cells [19,31] (Fig. 1A). We further characterized the TRPV1 channel function in BEAS-2B cells by using patch clamp technique and GCaMP6s (an ultrasensitive Ca^{2+} fluorescent protein) imaging [32]. TRPV1-mediated currents were recorded by whole-cell patch clamp in BEAS-2B cells. Capsaicin (1 μM) elicited noticeable currents and capsaicin-induced currents were blocked completely by non-selective TRP channel blocker RR (1 μM) (Fig 1B). Ca^{2+} influx through activated TRPV1 channels was assessed by GCaMP6s-based Ca^{2+} imaging. Capsaicin (1 μM) induced a significant increase in intracellular Ca^{2+} levels (fold change 1.25 ± 0.04) in BEAS-2B cells. In the presence of RR (1 μM), capsaicin-induced elevation of intracellular Ca^{2+} levels was completely blocked (Fig. 1C and D). In summary, our results suggested that the presence of functional TRPV1 channel in BEAS-2B cells and TRPV1 activation enhanced Ca^{2+} influx.

3.2. TRPV1 activation induces TSLP release from BEAS-2B cells

Recently, the major role of the epithelial cell-derived cytokine TSLP is highlighted in the induction of airway allergic inflammation [22]. TSLP release is controlled by intracellular Ca^{2+} level [27], suggesting TRPV1 channel may be involved in this process. Thus we investigated the ability of TRPV1 agonist capsaicin to induce TSLP overexpression and release in BEAS-2B cells. TSLP mRNA quantification revealed that the transcription of this cytokine was concentration-dependently enhanced by capsaicin stimulation at the relatively low concentrations (Fig. 2A), whereas RR (1 μM) and capsazepine (10 μM) caused significantly down-expression of TSLP mRNA, indicating the TRPV1 channel has a role in maintaining the basal level of TSLP (Fig. 2B). For ELISA assay BEAS-2B cells were stimulated with capsaicin (50 nM) for 20 min and TSLP release was measured. As shown in Fig. 2C, capsaicin also significantly stimulated TSLP secretion 1.6-fold compared to basal levels. This stimulation was completely blocked by capsazepine and RR. Finally, to further confirm the TSLP induction by TRPV1 channel in BEAS-2B cells, we knocked down TRPV1 channel by transfecting BEAS-2B cells with siRNA for TRPV1 channel or control siRNA and then measured them at basal level or stimulated with capsaicin (50 nM) for 20 min. Expression TRPV1 mRNA and protein were suppressed significantly by TRPV1 siRNA but not by control siRNA (Fig. 2D). The basal level of TSLP was almost abolished by TRPV1 siRNA and capsaicin-induced TSLP induction was also significantly reduced by TRPV1 siRNA (Fig. 2E). These data showed that TRPV1 channel plays a critical role in maintaining the basal level of TSLP and TRPV1 activation further triggers TSLP release.

3.3. TRPV1-mediated TSLP release depends on Ca^{2+}/NFAT pathway

To evaluate the contribution of Ca^{2+} signaling pathway in capsaicin stimulated TSLP release, we chelated extracellular Ca^{2+} by 2 mM EGTA. In the presence of EGTA, capsaicin (50 nM) did not stimulate TSLP release, indicating that capsaicin-induced TSLP release was dependent on the influx of extracellular Ca^{2+} (Fig. 3C). In immune cells and DRG neurons, Ca^{2+} signaling activates NFAT transcription factor to trigger cytokine expression and secretion and modulate neuronal excitation-transcription coupling [33,34]. The Ca^{2+}/NFAT pathway also plays a similar role in keratinocytes to promote the TSLP-induced itch [35]. An increase in intracellular Ca^{2+} causes NFAT dephosphorylation by the Ca^{2+}-dependent phosphatase calcineurin and translocation from the cytosol to the nucleus. Our immunofluorescence studies demonstrated that treatment of BEAS-2B cells with capsaicin (50 nM) for 30 min induced NFAT translocation to the nucleus (Fig. 3A).

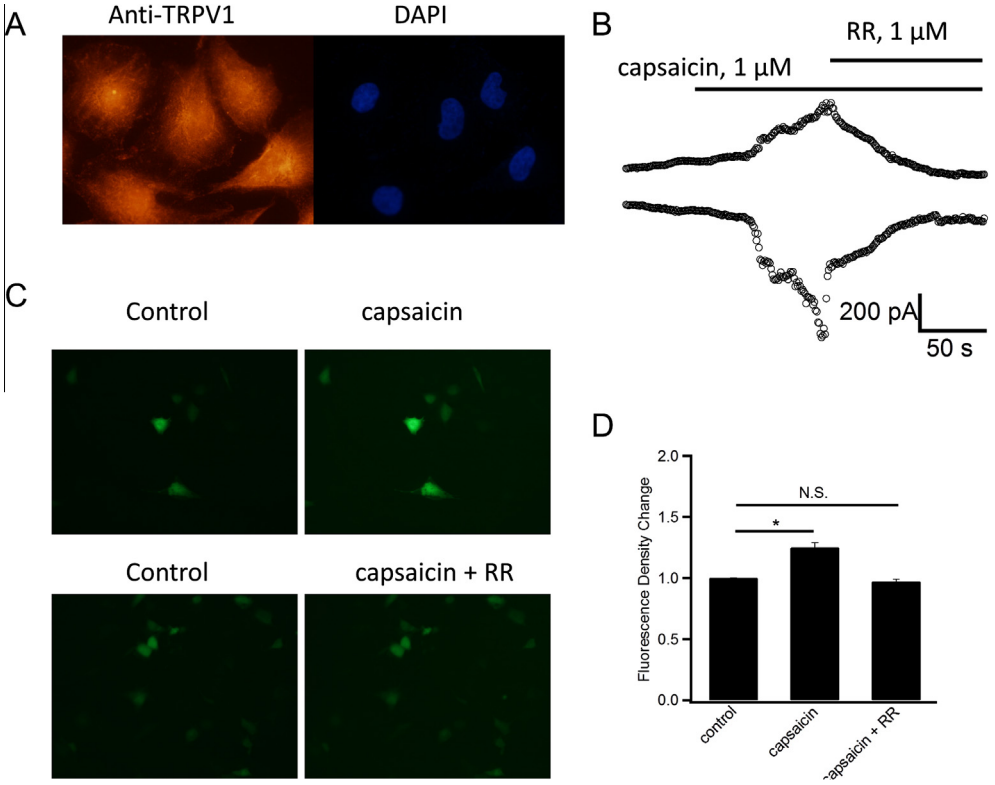

Fig. 1. (A) Expression of TRPV1 channel in BEAS-2B cells. Cells were immunostained for TRPV1 (orange) and loaded with DAPI (nucleus, blue). (B) The mean whole cell current was measured at ±80 mV in BEAS-2B cells. The cells were stimulated with 1 μM capsaicin and then exposed to 1 μM RR. (C) Fluorescent images showing GCaMP6s-transfected BEAS-2B cells (green). Capsaicin increased fluorescent density in GCaMP6s-transfected BEAS-2B cells and RR blocked this increase. (D) Comparison of the fluorescent density changes in (C). $^{*}P < 0.05$. ($n = 4$).

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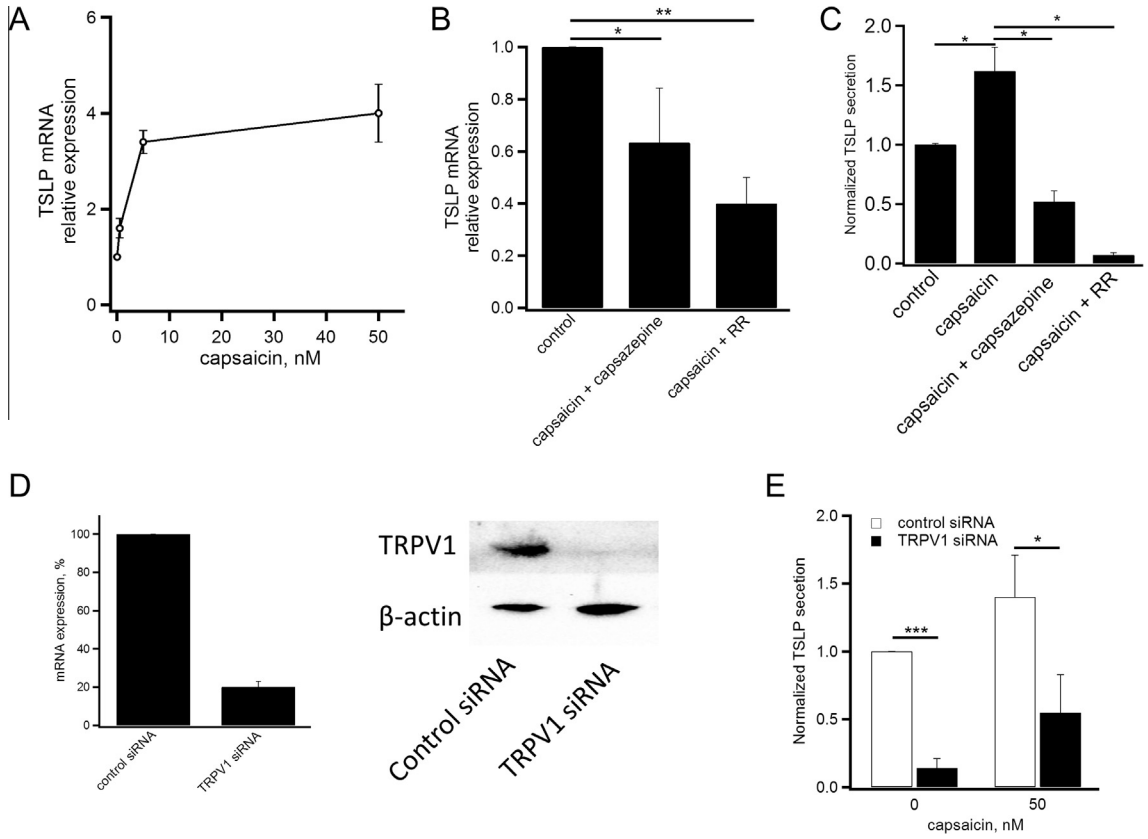

Fig. 2. (A) BEAS-2B cells were incubated with 0.5–50 nM capsaicin. (B) BEAS-2B cells were incubated with 50 nM capsaicin with 10 μM capsazepine or 1 μM RR. The TSLP transcription was normalized in each sample and expressed as a ratio to the BEAS-2B cells cultured in absence of chemicals. (C) TSLP release in response to treatment with control, 50 nM capsaicin, and 50 nM capsaicin with 10 μM capsazepine or 1 μM RR. The basal TSLP level was 78 pg/ml and the TSLP release in response to treatment with chemicals was normalized to the basal level and expressed as a ratio. (D) siRNA-induced inhibition of TRPV1 mRNA (left) and protein (right) expression. (E) The effect of TRPV1 siRNA on TSLP release at basal level or elicited by 50 nM capsaicin. The basal TSLP level from BEAS-2B cells transfected with control siRNA was 82 pg/ml and the TSLP release was normalized to the basal level and expressed as a ratio. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ($n = 4–10$).

This translocation was attenuated by blocking TRPV1 channel with capsazepine (10 μM), or by inhibiting NFAT activity with cyclosporine A (1 μM), an inhibitor of calcineurin (Fig. 3A). To rule out the possibility that cyclosporine A may also function as a TRPV1 channel blocker, we expressed TRPV1 channel in HEK293 cells and tested the effect of cyclosporine A on TRPV1 activity in excised inside-out patches. Interestingly, we observed cyclosporine A increased TRPV1 open probability (Fig. S1B) and it is consistent with previous report that showed cyclosporine A can sensitize the potency of TRPV1 agonists [36], hence cyclosporine A should inhibit NFAT nucleus translocation via a classical calcineurin inhibition instead of TRPV1 channel blocker. Furthermore capsaicin-stimulated TSLP expression and secretion was completely blocked by cyclosporine A (Fig. 3B and C). These results showed that TRPV1 activation induced Ca^{2+}-dependent NFAT translocation, leading to NFAT-dependent changes in TSLP expression.

3.4. TRPV1 contributes to PAR2-induced TSLP release

Several studies have identified PAR2 as a major effector of the airway inflammatory response through its proteolytic activation by serine proteases [37]. Proteases can induce changes in intracellular Ca^{2+} level in cells by cleaving and activating PAR2. PAR2 have been shown to be expressed and induce TSLP production in BEAS-2B cells [26]. Here we directly tested if PAR2 activation in BEAS-2B cells resulted in changes in Ca^{2+} signaling using the PAR2-specific peptide agonist SLIGKV-NH_2. In GCaMP6s transfected BEAS-2B cells, the SLIGKV-NH_2 peptide elicited a much brighter green fluorescence indicating an increasing intracellular Ca^{2+} levels (fold change 1.65 ± 0.14). These data established that direct activation of PAR2 can lead to robust Ca^{2+} signaling (Fig. 4A). Immunofluorescence studies demonstrated that treatment of BEAS-2B cells with SLIGKV-NH_2 for 30 min also induced robust NFAT translocation to the nucleus, suggesting that PAR2 activation may induce TSLP production via a similar Ca^{2+}-dependent NFAT pathway. Interestingly, this Ca^{2+}-dependent NFAT translocation was abolished by RR, indicating TRP channels sensitized by PAR2 activation may participate in this process (Fig. 4B). Indeed studies had suggested that PAR2 coupled with several TRP channels including TRPV1, TRPA1 and TRPV4 to maintain sustained inflammatory signaling [38–41]. We found that PAR2 activation induced currents were partially inhibited by capsazepine and completely blocked by RR (Fig. 4C). Subsequently, capsazepine, RR and cyclosporine A also significantly attenuated SLIGKV-NH_2-evoked TSLP production and release in BEAS-2B cells (Fig. 4D and E), and TRPV1 siRNA also attenuated SLIGKV-NH_2-induced TSLP secretion (Fig. 4F). These results suggested that TRPV1 activation contributes to PAR2-evoked TSLP secretion in airway epithelium.

4. Discussion

The airway epithelium is exposed to a range of physical and chemical irritants in the environment that are known to trigger inflammation, and recent reports have emphasized the importance role of airway epithelia in airway inflammation including asthma [42,43]. The identified environmental asthma triggers include secondhand smoke, dust mites, wood smoke, chemical irritants, cold

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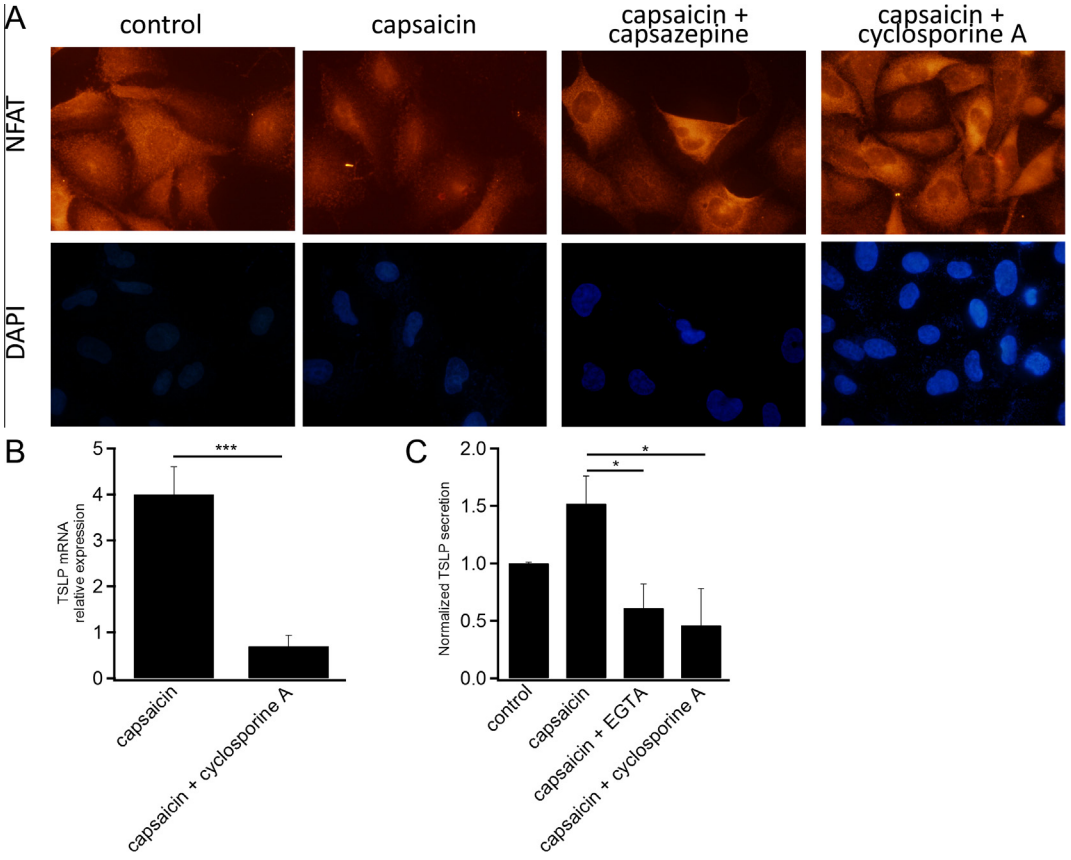

Fig. 3. (A) Representative images displaying cytosolic and nuclear localization of NFAT (orange) and DAPI (blue) in BEAS-2B cells incubation with control, 50 nM capsaicin, 50 nM capsaicin with 10 μM capsazepine or 1 μM cyclosporine A. (B) BEAS-2B cells were incubated with 50 nM capsaicin with 1 μM cyclosporine A. The TSLP transcription was normalized in each sample and expressed as a ratio to the BEAS-2B cells cultured in absence of chemicals. (C) TSLP release in response to treatment with control, 50 nM capsaicin, and 50 nM capsaicin with 2 mM EGTA or 1 μM cyclosporine A. The basal TSLP level was 78 pg/ml and the TSLP release in response to treatment with chemicals was normalized to the basal level and expressed as a ratio. $^{*}P < 0.05$; $^{***}P < 0.001$. ($n = 4–5$).

air and outdoor air pollution [2]. Recent studies suggest these risk factors mediate asthmatic response via activating distinct receptors such as GPCRs and TRP channels [3,22]. Some members of TRP channels, particularly TRPV1, TRPV4, TRPA1 and TRPM8, have been suggested to mediate adverse effects after exposure to these risk factors and be important in the pathophysiological mechanisms responsible for asthmatic patients [8,44]. Here, we are particularly interested in the TRPV1 channel that can be activated by a diverse range of environmental risk stimuli including reactive oxygen species, outdoor particulate matter and coal fly ash particulate matter, and its expression level can be up-regulated by some other risk factor such as respiratory rhinovirus [11,27,45]. Clinically patients affected by severe asthma showed increased expression of bronchial epithelial TRPV1 channels [18]. Moreover, respiratory inflammation like asthma is the result of complex interactions between genetic and environmental mechanisms [46]. Genetical studies also suggest TRPV1 activity is directly relevant for asthma pathophysiology [14]. Thus it is crucial to elucidate the role of TRPV1 channel in airway epithelia.

The airway epithelia maintain airway homeostasis and stressed epithelia release crucial cytokines such as TSLP [42,47,48]. TSLP is an IL-7-like cytokine that is necessary and sufficient for the development of allergic asthma [14,22]. Current research and therapeutic strategies for asthma focus on preventing release of potentially toxic mediators. In this context, understanding how TSLP is regulated in airway epithelium is important.

In this study we confirmed that there are functional TRPV1 channel in BEAS-2B cells and proved that the TRPV1 channel plays an important role in basal TSLP release and TRPV1 activation can enhance TSLP production. At human body temperature, the thermo-sensitive TRPV1 channel is tonically activated [49], and it may explain our observation that the TRPV1 channel maintains the constitutive TSLP release. Recent evidence implicates TSLP is not only playing a pivotal role in the pathobiology of allergic asthma but also in atopic dermatitis [50,51]. Similar to the TRPV1 channel in airway epithelium, the TRPV3 channel plays an essential role for basal TSLP release in keratinocyte and up-regulates TSLP level in the skin of patients with atopic dermatitis [52]. TRPV1 agonist capsaicin increases TSLP production and secretion in a concentration-dependent manner, whereas TRPV1 antagonist capsazepine and TRPV1 siRNA strongly inhibits this process. TSLP production relies on Ca^{2+} influx through the opening pore of TRPV1 channel and subsequently NFAT translocation from cytosol into nucleus. Thus elevated intracellular Ca^{2+}-induced NFAT action may be a common pathway to cause TSLP production and secretion, implying other Ca^{2+} permeable channel would also contribute to asthma pathophysiology. Indeed, several other ion channels also participate in the control of intracellular Ca^{2+} and the production of epithelium-based secretion [53]. Moreover, PAR2 as a sensor for active extracellular serine proteases also uses Ca^{2+}-induced NFAT pathway to induce TSLP production and secretion in airway epithelium. Previous studies have showed that PAR2 regulates several channels and stimulates TRPV1 channel by a PKC-dependent pathway [31,54]. Here we showed that TRPV1 channel contributes to the elevated Ca^{2+} level and TSLP production induced by PAR2 activation.

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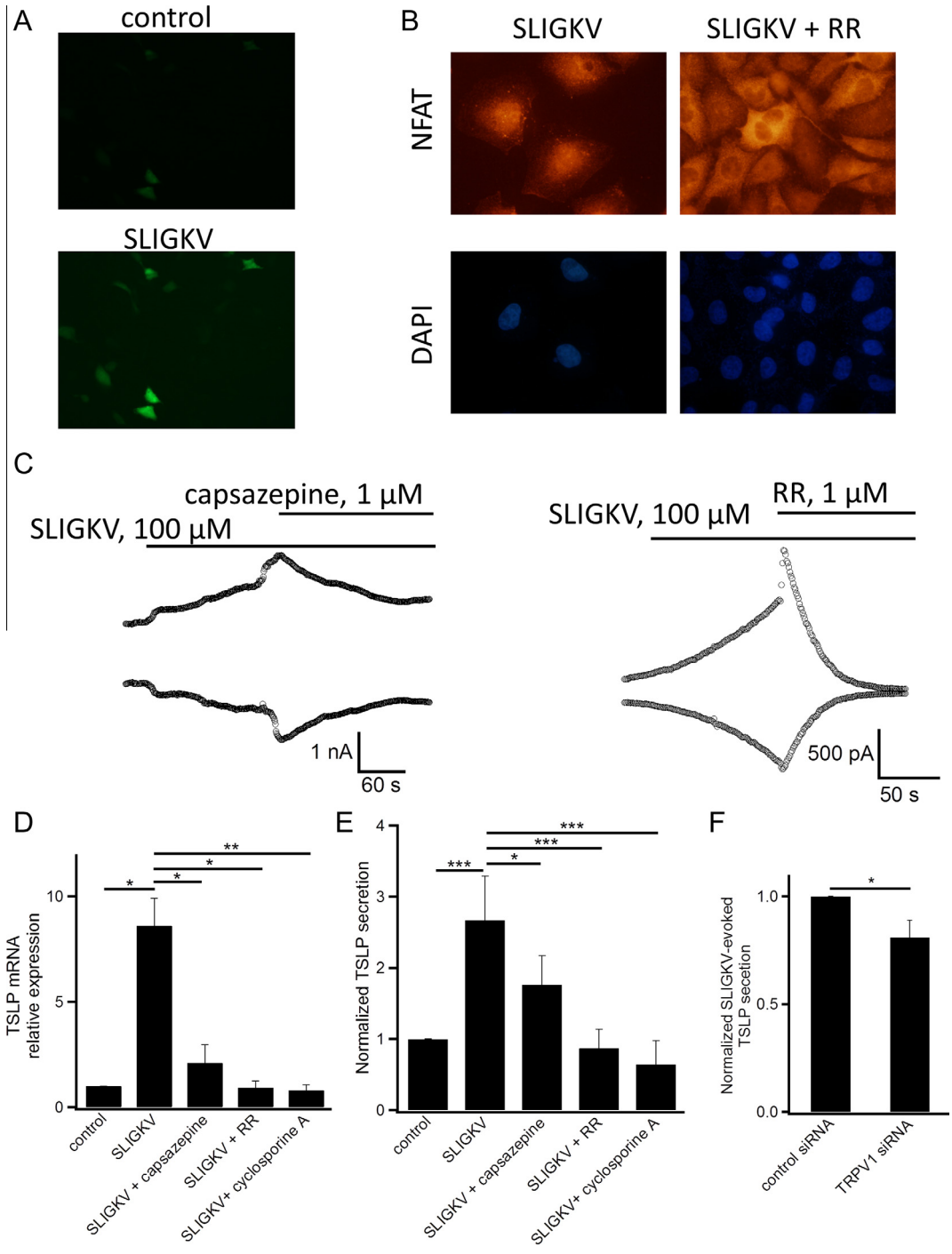

Fig. 4. (A) Fluorescent images showing GCaMP6s-transfected BEAS-2B cells (green). 100 μM SLIGKV-NH_2 peptide increased fluorescent density in GCaMP6s-transfected BEAS-2B cells. (B) Representative images displaying cytosolic and nuclear localization of NFAT (orange) and DAPI (blue) in BEAS-2B cells incubation with 100 μM SLIGKV-NH_2 peptide, 100 μM SLIGKV-NH_2 peptide with 1 μM RR. (C) The mean whole cell current was measured at ±80 mV in BEAS-2B cells and the cells were stimulated with 100 μM SLIGKV-NH_2 peptide and then exposed to 10 μM capsazepine or 1 μM RR. (D) BEAS-2B cells were incubated with 100 μM SLIGKV-NH_2 peptide, 100 μM SLIGKV-NH_2 peptide with 10 μM capsazepine or 1 μM RR or 1 μM cyclosporine A. The TSLP transcription was normalized in each sample and expressed as a ratio to the BEAS-2B cells cultured in absence of chemicals. (E) TSLP release in response to treatment with control, 100 μM SLIGKV-NH_2 peptide, 100 μM SLIGKV-NH_2 peptide with 10 μM capsazepine or 1 μM RR or 1 μM cyclosporine A. The basal TSLP level was 78 pg/ml and the TSLP release in response to treatment with chemicals was normalized to the basal level and expressed as a ratio. (F) The effect of TRPV1 siRNA on TSLP release elicited by 100 μM SLIGKV-NH_2 peptide. The SLIGKV-evoked TSLP release from BEAS-2B cells transfected with control siRNA was 412 pg/ml. $^{*}P < 0.05$; $^{**}P < 0.01$; $^{***}P < 0.001$. ($n = 4–10$).

In BEAS-2B cells we detected the TRPV1 current induced by capsaicin was relatively slow developed compared with recombinant TRPV1 current in HEK 293 cells. There are several possible explanations for the relatively slow developed TRPV1 current. First, it was previously found that TRPV1 can coassemble with TRPV4 and TRPA1 subunits which are known to be expressed in BEAS-2B cells. Such heteromeric channels have distinct gating properties [55–57]. Particularly, TRPV1-containing heteromultimeric channels in BEAS-2B cells may pharmacologically exhibit altered ligand activation which is similar to the lower efficacy of capsaicin observed on TRPV1/TRPV3 heteromeric channels [58]. Another possibility is that TRPV1 function may be regulated by putative interacting

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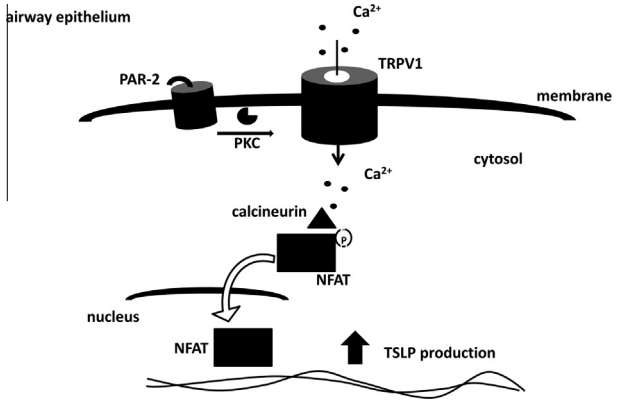

Fig. 5. Schematic diagram depicting the Ca^{2+}/NFAT signaling pathway in airway epithelium that links TRPV1 to TSLP secretion. Ca^{2+} influx via TRPV1 channel activates calcineurin, which dephosphorylates NFAT and causes nuclear translocation, thus inducing transcription of TSLP. Activation of PAR2 triggers PKC and opens TRPV1 channels to promote TSLP production.

proteins in BEAS-2B cells. In sensory neurons there also seems to be a marked difference between TRPV1 and native capsaicin receptors in the pharmacological response profiles to capsaicin. For example, Fas-associated factor 1 constitutively modulates the sensitivity of TRPV1 to various noxious stimuli [59].

In summary, our results identify a signaling pathway by which TRPV1 channel regulates TSLP release to induce airway inflammation. PAR2-induced TSLP production also relies on Ca^{2+}/NFAT pathway and the sensitization of TRPV1 channel contributes to this process. By the mechanism suggested above (Fig 5), our results suggest that TRPV1 channel-induced Ca^{2+}/NFAT pathway is a promising target for the development of novel therapies to treat airway inflammation such as asthma.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.febslet.2014.06.018.

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