Biochemical and Biophysical Research Communications 463 (2015) 7–12

Contents lists available at ScienceDirect

Biochemical and Biophysical Research Communications

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

Regulation of basal autophagy by transient receptor potential melastatin 7 (TRPM7) channel

Hyun Geun Oh [a], Yoon Sun Chun [a], Chul-Seung Park [b], Tae-Wan Kim [c], Myoung Kyu Park [a], Sungkwon Chung [a, *]

[a] Department of Physiology, Samsung Biomedical Research Institute, Sungkyunkwan University School of Medicine, Suwon 440-746, South Korea
[b] School of Life Sciences, Gwangju Institute of Science and Technology, Gwangju 500-712, South Korea
[c] Department of Pathology and Cell Biology, Taub Institute for Research on Alzheimer's Disease and the Aging Brain, Columbia University Medical Center, New York, NY 10032, USA

ARTICLE INFO

Article history:
Received 17 April 2015
Available online 15 May 2015

Keywords:
Autophagy
TRPM7 channel
AMP-activated protein kinase
CaMKKβ
Parkinson's disease

ABSTRACT

Macroautophagy (hereafter referred to as autophagy) is a catabolic process for the degradation and recycling of cellular components. Autophagy digests intracellular components, recycling material subsequently used for new protein synthesis. The Ca^{2+}- and Mg^{2+}-permeable transient receptor potential melastatin 7 (TRPM7) channel underlies the constitutive Ca^{2+} influx in some cells. Since autophagy is regulated by cytosolic Ca^{2+} level, we set out to determine whether Ca^{2+} influx through the TRPM7 channel regulates basal autophagy. When TRPM7 channel expression was induced from HEK293 cells in a nutrient-rich condition, LC3-II level increased indicating the increased level of basal autophagy. The effect of TRPM7 channel on basal autophagy was via Ca^{2+}/calmodulin-dependent protein kinase kinase β, and AMP-activated protein kinase pathway. In contrast, the level of basal autophagy was decreased when the endogenous TRPM7 channel in SH-SY5Y cells was down-regulated using short hairpin RNA. Similarly, an inhibitor for TRPM7 channel decreased the level of basal autophagy. In addition, the inhibitory effect of channel inhibitor on basal autophagy was reversed by increasing extracellular Ca^{2+}concentration, suggesting that Ca^{2+} influx through TRPM7 channel directly links to basal autophagy. Thus, our studies demonstrate the new role of TRPM7 channel-mediated Ca^{2+} entry in the regulation of basal autophagy.

1. Introduction

Autophagy is a conserved process for degradation and recycling of cytoplasmic components and organelles using the lysosomal machinery [1–4]. Once the isolated autophagic double membrane is formed, it engulfs the cytosolic components making autophagosomes [5], which fuse to lysosomes for the degradation and recycling of its cargo. Nutrient starvation potently induces autophagy, which digests intracellular components, recycling material subsequently used for synthesis of new proteins that are essential for survival [6–8]. Even in normal growing conditions, however, the basal level of autophagy is maintained, securing cellular homeostasis in all eukaryote cells [9,10].

Many different signaling pathways control autophagy. Beclin-1 initiates autophagy, while mammalian target of rapamycin (mTOR) is the key regulator. mTOR is endogenously inhibited by AMP-activated protein kinase (AMPK), which detects the cellular metabolic status [11]. Increase in the phosphorylated form of AMPK (pAMPK) and decrease in mTOR activity activate autophagy. The upstream regulator of AMPK is another kinase, Ca^{2+}/calmodulin-dependent protein kinase kinase β (CaMKKβ), which is regulated by cytosolic Ca^{2+} [12,13]. Thus, changes in Ca^{2+} influx are recognized as an important regulator for autophagy [14,15]. However, the source of Ca^{2+} influx regulating autophagy is unknown yet.

The transient receptor potential melastatin 7 (TRPM7) is ubiquitously expressed in most tissues, which is non-selective conducting Na^{+}, Mg^{2+} and Ca^{2+}. TRPM7 channel activity is very low at physiological conditions due to tonic inhibition by intracellular Mg^{2+}, MgATP, and MgGTP [16,17]. Since TRPM7 channel underlies the spontaneously constitutive Ca^{2+} influx in some cells [18,19], we hypothesize that Ca^{2+} influx through TRPM7 channel may regulate basal autophagy via CaMKKβ-AMPK pathway. We found that increasing TRPM7 channel expression increased the level of basal

* Corresponding author. Fax: +82 31 299 6129.
E-mail address: schung@skku.edu (S. Chung).

http://dx.doi.org/10.1016/j.bbrc.2015.05.007
0006-291X/

autophagy via the CaMKKβ-AMPK pathway. Down-regulating the endogenous TRPM7 channel decrease the level of basal autophagy. Similarly, a specific inhibitor for TRPM7 channel decreased the level of basal autophagy. These results indicate that Ca^{2+} influx through TRPM7 channel regulates the basal autophagy.

2. Materials and methods

2.1. Constructs and mutagenesis of TRPM7

Wild-type murine TRPM7 was modified and subcloned into the tetracycline-inducible mammalian expression vector, pcDNA4-TO. The kinase domain of TRPM7 was removed downstream of position 1599 including whole kinase domain sequences of TRPM7. They were stably transfected into modified HEK293 cells, T-RE$_X$™-293 (Invitrogen) [16,20]. For inducing TRPM7 expression, cells were treated with 1 μg/ml tetracycline for 2 h. SH-SY5Y cells were grown in F12/DMEM (1:1) supplemented with 10% fetal bovine serum and 1% penicillin/streptomycin in a humidified incubator at 37 °C under 5% CO_2 atmosphere.

2.2. Western blotting and immunocytochemistry

Cell lysates were prepared using lysis buffer and were centrifuged at 600 × g for 10 min. Proteins were separated by SDS-PAGE and transferred to PVDF membranes, which were used to detect LC3. Nitrocellulose membrane was used to transfer other proteins. The primary antibodies used were TRPM7 (Abcam), TRPM6 (Sigma–Aldrich), LC3 (Thermo Scientific), AMPK (Cell Signaling Technology), phospho-AMPK (Cell Signaling Technology), p62 (Abnova), WIPI2 (Thermo Scientific) and β-actin (Sigma–Aldrich). Secondary antibody conjugated peroxidase activity was visualized with enhanced chemiluminescence. The Multi Gauge software using a LAS-3000 system (Fuji Film) was used to quantify the immunoblots.

For immunocytochemistry, SH-SY5Y cells were grown on glass coverslips with normal growth media and then, fixed using 4% paraformaldehyde and permeabilized by incubation with 0.1% Triton-X100 at room temperature for 1 min. After fixation, non-specific sites were blocked with 5% bovine serum albumin. LC3 and WIPI2 puncta were stained using the primary antibodies and AlexaFluor 488 (Sigma–Aldrich). LSM 710 confocal microscope (Carl Zeiss) was used and images were analyzed off-line using NIH ImageJ™ software.

2.3. Patch clamp

TRPM7 currents were recorded using whole-cell mode of patch-clamp method. The currents were filtered at 5 KHz, and recorded using an Axonpatch-1D amplifier. TRPM7 current was elicited by using ramp pulse of 200 ms-long from −100 mV to +100 mV from a holding voltage of 0 mV. Command potentials and data acquisition was controlled by pClamp 7.0 software (Axon Instrument). The bath solution contained (in mM) 150 Na•methansulfonate, 10 HEPES, 10 EGTA, 0.1 EDTA and 0.5 $CaCl_2$ (pH was adjusted to 7.2 with NaOH). Pipette solution contained (in mM) 135 Cs•methansulfonate, 10 BAPTA, 10 HEPES and 3.17 $CaCl_2$ (pH was adjusted to 7.4 with CsOH).

2.4. Cytoplasmic Ca^{2+} measurements

Cytoplasmic Ca^{2+} levels ($[Ca^{2+}]_i$) were measured using Fura2-AM from SH-SY5Y cells as previously described [21]. Approximately 1 × 10^6 cells were prepared in 1 ml HEPES Ringer solution, and recorded by using fluorescence spectrophotometer (CAF-110, Jasco). For the calibration of $[Ca^{2+}]_i$, the recording data was calculated by substituting a dissociation constant of 150 nM for Ca^{2+}-Fura2 at room temperature.

2.5. Silencing TRPM7 and TRPM6 expression

The TRPM7-specific shRNA ($shRNA_{TRPM7}$) and control shRNA ($shRNA_{SCR}$) were prepared as described previously [19]. shRNAs were designed based on accession numbers in GenBank (NM_017672.3, NM_017662.4), and transfected into SH-SY5Y cells using RNAiFect transfection reagent (Qiagen).

3. Results

3.1. Induction of TRPM7 channel expression increases basal autophagy

We used a tetracycline-inducible expression system in HEK293 cells to determine whether TRPM7 channel expression regulates basal autophagy. Since TRPM7 is endogenously expressed in HEK293 cells, further expression of TRPM7 was induced by treating cells with tetracycline in nutrient-rich culture medium. However, TRPM7 is implicated in apoptotic and necrotic signaling [21,22]. We found that treating cells with 1 μg/ml tetracycline for more than 3 h increased the level of cleaved form of caspase 3, an apoptosis and necrosis mediator (Supplementary Fig. 1). For this reason, we limited the incubation time with tetracycline to less than 2 h to avoid the activation of cell death signaling.

First, non-selective TRPM7 currents were measured using whole-cell voltage-clamp method. Ramp voltage pulse from −100 mV to +100 mV was applied, and the resulting current–voltage curves were obtained (Fig. 1A). TRPM7 current density measured at −100 mV was significantly increased in tetracycline-treated cells (tet; 33.5 ± 2.1 pA/pF, n = 6) compared to control cells (control; 20.9 ± 1.2 pA/pF, n = 6). A typical Western blotting result showed that TRPM7 expression level was increased in tetracycline-treated cells (Fig. 1B). When the expression of TRPM7 was quantified relative to β-actin levels, it increased significantly in tetracycline-treated cells (Fig. 1C, n = 6). As a key regulator for autophagy, pAMPK level was increased by TRPM7 channel induction, while the total AMPK level was not changed (Fig. 1B). Thus, the pAMPK/AMPK ratio was significantly increased by TRPM7 channel induction (Fig. 1D, n = 6). Autophagosomal formation was measured by detecting LC3 using Western blotting (Fig. 1B). When the LC3-II/β-actin ratio was quantified, it was increased approximately 30% in tetracycline-treated cells compared to control cells (Fig. 1E, n = 6). In serum-free starvation condition, autophagy level was greatly increased as expected (Supplementary Fig. 2). However, the starvation-induced autophagy level was not affected by TRPM7 channel expression, suggesting that TRPM7 regulates basal autophagy in normal culture condition, but not the starvation-induced autophagy.

Since we observed the activation of AMPK pathway by TRPM7 channel induction, we tested the well-known upstream regulator of AMPK, CaMKKβ. A typical Western blot result showed that TRPM7 channel expression was increased by tetracycline even in the presence of 10 μM STO609, a specific blocker for CaMKKβ (STO; Fig. 1B). Consistently, the quantified expression levels of TRPM7 was also increased in this condition (Fig. 1C, n = 6). The activation of AMPK was blocked by STO609 (Fig. 1B, D). Moreover, the effect of TRPM7 channel induction on LC3-II/β-actin ratio was prevented by STO609 (Fig. 1B, E). These results indicate that TRPM7 channel expression regulates basal autophagy via the CaMKKβ-AMPK signaling pathway.

We tested whether the kinase domain of TRPM7 contributes to the effect of TRPM7 expression on the basal autophagy. In HEK293

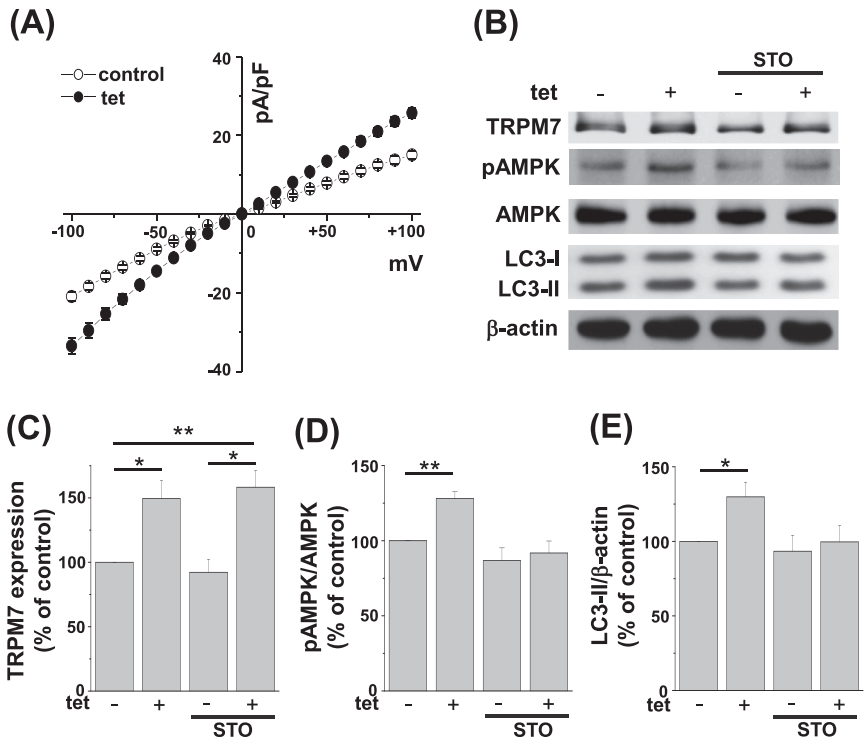

Fig. 1. TRPM7 regulates basal autophagy via CaMKKβ-AMPK signaling pathway. (A) TRPM7 currents were measured by whole-cell mode of patch-clamp from TRPM7-inducible HEK293 cells. Cells were treated with (tet) or without (control) 1 μg/ml tetracycline for 2 h before recordings (n = 6). (B) A typical Western blotting result shows that autophagy level was increased by the induction of TRPM7 channel in HEK293 cells. LC3-II/β-actin, and pAMPK/AMPK ratio were increased. The presence of 10 μM STO609 (STO), a blocker for CaMKKβ, inhibited the effects of TRPM7 induction on autophagy as well as AMPK activation. (C–E) Band densities from 6 different experiments were normalized. *, $p < 0.05$, **, $p < 0.01$.

cells, the expression of TRPM7 kinase deletion mutant (TRPM7-ΔK) was induced by treating cells with tetracycline for 2 h in nutrient-rich normal culture medium (Supplementary Fig. 3). TRPM7 currents were increased by approximately 50% by the induction, which was less than the current levels from cells expressing wild type TRPM7 channel as previously reported [23]. Level of pAMPK and the LC3-II/β-actin ratio were increased similar to what was observed in cells expressing wild type TRPM7 channels. These results indicate that the kinase domain of TRPM7 is not necessary for the regulation of basal autophagy.

3.2. Down-regulation of TRPM7 channel expression attenuates basal autophagy

To confirm that TRPM7 channel regulates basal autophagy, the endogenous TRPM7 channel expression was down-regulated using TRPM7-specific short hairpin RNA (shRNA). For this purpose, SH-SY5Y cells were transfected for 24 h with either control shRNA with random sequence ($shRNA_{SCR}$) or with TRPM7-specific shRNA ($shRNA_{TRPM7}$). A typical Western blot result is shown in Fig. 2A. The presence of $shRNA_{TRPM7}$ reduced approximately 55% of endogenous TRPM7 channel expression (Fig. 2B, n = 5). In the same condition, pAMPK/AMPK ratio was reduced by 25% (Fig. 2C, n = 5) and LC3-II/β-actin ratio was reduced by 50% (Fig. 2D, n = 5). p62 is a maker for basal autophagic activity since it serves as a linker between LC3 and ubiquitinated substrates. Expression of p62 was increased by $shRNA_{TRPM7}$ (Fig. 2A and E, n = 5), which support our conclusion that the down-regulation of TRPM7 expression leads to the attenuation of basal autophagy.

TRPM7 channel may form a heterotetramer complex with its closest homolog, the Ca^{2+}/Mg^{2+}- permeable TRPM6 channel [24]. To test whether the TRPM6 channel affects basal autophagy, the endogenous expression level of TRPM6 in SH-SY5Y cells was down-regulated using TRPM6-specific shRNA ($shRNA_{TRPM6}$). We confirmed that $shRNA_{TRPM6}$ reduced the expression of the endogenous TRPM6 (Supplementary Fig. 4). LC3-II/β-actin ratio was not changed by the down-regulation of TRPM6 expression, indicating the specific effect of TRPM7 channel on the regulation of basal autophagy.

3.3. Ca^{2+} influx through TRPM7 channel regulates basal autophagy

To inhibit TRPM7 channel function, we utilized Waixenicin A (waix), a selective TRPM7 channel blocker [25]. When 5 μM waix was added to the extracellular solution of SH-SY5Y cells, the endogenous TRPM7 current was inhibited by approximately 65% (waix, n = 8), compared to control (control, n = 5) as shown in Fig. 3A. Since TRPM7 channel underlies the spontaneously constitutive Ca^{2+} influx in some cells [18,19], we also tested the effect of waix on Ca^{2+} influx. For this purpose, the intracellular Ca^{2+}concentration ($[Ca^{2+}]_i$) was monitored using Fura-2 [19]. Cells were exposed to a Ca^{2+}-free extracellular solution, and Ca^{2+} was re-added into the extracellular solution. The increase of $[Ca^{2+}]_i$ represented the passive Ca^{2+} influx (Fig. 3B). When $[Ca^{2+}]_i$ was measured at 200 s, 20% of Ca^{2+} influx was blocked by the presence of waix (inset, Fig. 3B), indicating that TRPM7 channel constitutes some, if not all, of the underlying current for the passive Ca^{2+} influx.

Then, we tested the effect of waix on autophagy. The LC3-II/β-actin ratio was effectively decreased when SH-SY5Y cells were treated with 5 μM waix for 24 h with physiological extracellular Ca^{2+}concentration ($[Ca^{2+}]_{ex}$) at 1.8 mM as shown in Fig. 3C for a typical Western blot result. Waix decreased pAMPK/AMPK ratio (Fig. 3D, n = 5), and LC3-II/β-actin ratio (Fig. 3C and E). These results confirm that TRPM7 channel activity regulates basal autophagy. We

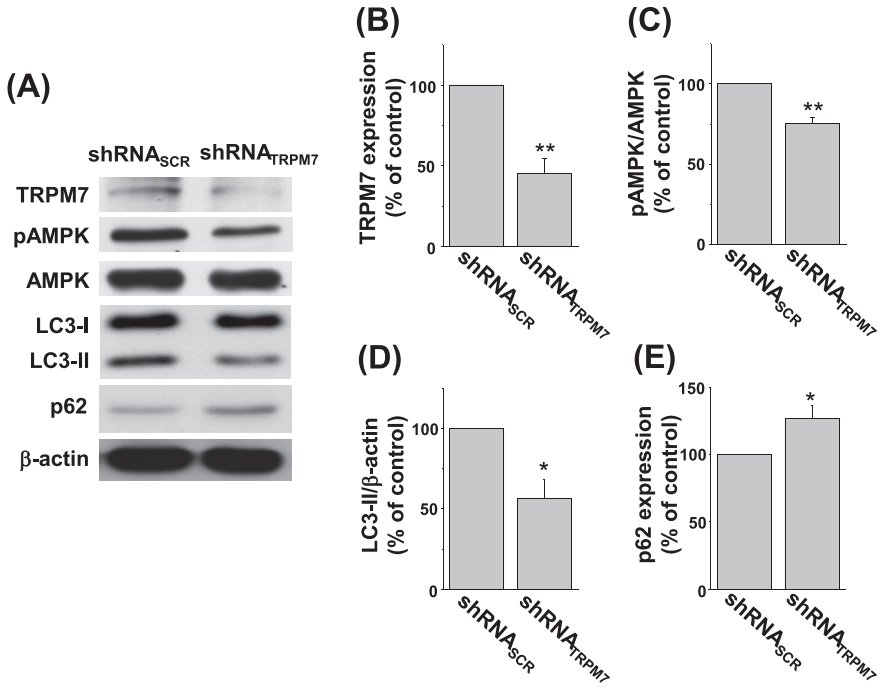

Fig. 2. Basal autophagy is down-regulated by the inhibition of TRPM7 expression. We used shRNA-mediated RNA interference method to test the effect of inhibition of TRPM7 expression on basal autophagy in neuroblastoma SH-SY5Y cells. (A) A typical Western blotting result shows that the endogenous expression level of TRPM7 was significantly attenuated in cells transfected with TRPM7-specific shRNA (shRNA$_{TRPM7}$), compared to cells transfected with control shRNA (shRNA$_{SCR}$). pAMPK level and LC3-II/β-actin ratio were decreased, while p62 expression level was increased when TRPM7 expression was down-regulated. (B–E) Band densities from 5 different experiments were normalized. *, $p < 0.05$, **, $p < 0.01$.

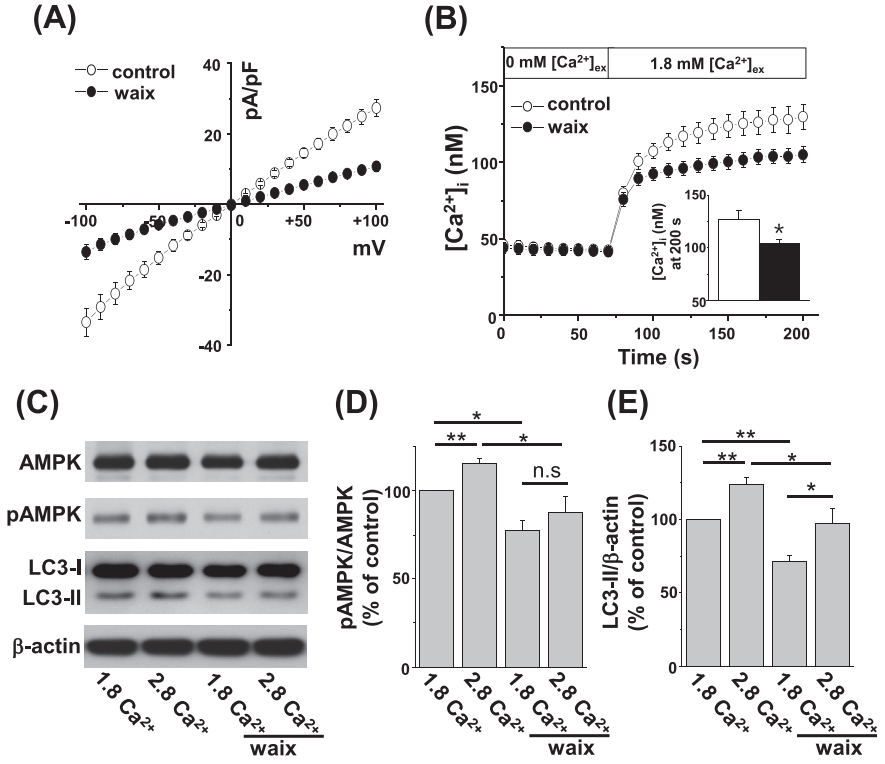

Fig. 3. TRPM7 channel blocker inhibits basal autophagy. (A) A specific TRPM7 channel blocker, Waixenicin A (waix) was used to test the effect of inhibiting TRPM7 channel activity on basal autophagy in SH-SY5Y cells. TRPM7 currents were decreased by 5 μM waix (n = 8), compared to vehicle control (n = 5). (B) Since TRPM7 channel underlies the constitutive Ca^{2+} influx, intracellular Ca^{2+} concentration ($[Ca^{2+}]_i$) was monitored using fura-2AM. $[Ca^{2+}]_i$ was significantly increased by the re-addition of 1.8 mM Ca^{2+} (1.8 mM $[Ca^{2+}]_{ex}$) into Ca^{2+}-free extracellular solution (0 mM $[Ca^{2+}]_{ex}$). Compared to control cells (n = 5), 5 μM waix-treated cells (n = 6) showed decreased $[Ca^{2+}]_i$, indicating the reduced Ca^{2+} influx by waix. Inset shows the measured $[Ca^{2+}]_i$ at 200 s after re-addition of extracellular Ca^{2+}. (C) A typical Western blotting result is shown for the effects of waix on autophagy. Cells were treated with or without 5 μM waix either at 1.8 mM or 2.8 mM $[Ca^{2+}]_{ex}$ for 24 h (D, E) Band densities from 5 different experiments were normalized. *, $p < 0.05$, **, $p < 0.01$.

also tested whether the inhibitory effect of waix on the basal autophagy could be reversed by increasing Ca^{2+} influx. For this purpose, $[Ca^{2+}]_{ex}$ was increased from 1.8 mM to 2.8 mM. Increasing $[Ca^{2+}]_{ex}$ to 2.8 mM by itself increased levels of pAMPK/AMPK and LC3-II/β-actin as shown in Fig. 3C through 3E. Even in the presence of waix, increasing $[Ca^{2+}]_{ex}$ to 2.8 mM recovered LC3-II/β-actin level similar to that of control condition at 1.8 mM $[Ca^{2+}]_{ex}$ (Fig. 3C and E). Thus, the inhibitory effect of waix on the basal autophagy was reversed by increasing Ca^{2+} influx, suggesting that Ca^{2+} influx through TRPM7 channel regulate basal autophagy. The down-regulated pAMPK/AMPK ratio by waix was not fully recovered by increasing $[Ca^{2+}]_{ex}$ (Fig. 3C and D). Treating SH-SY5Y cells with 5 μM waix increased p62 level in a time-dependent manner (Supplementary Fig. 5), consistent with inhibition of autophagy. Together, these results indicate that basal autophagy is inhibited by inhibiting TRPM7 channel activity.

3.4. TRPM7 channel regulates the autophagic formation

The formation of functional basal autophagosome was visualized from SH-SY5Y cells using immuno-cytochemistry. Staining with LC3 antibody showed the puncta formation as shown in Fig. 4A for a typical result. In waix-treated cells, however, the numbers of puncta were significantly reduced. It was about 2.5/cell in control cells (n = 15), and reduced to less than one in waix-treated SH-SY5Y cells (Fig. 4B, n = 14). In addition, only one cell out of 15 cells in control group was without puncta. However, 6 out of 14 cells in waix-treated cells were without puncta. As another way to monitor the puncta formation, we used antibody against WD repeat domain of phosphoinositide-interacting protein 2 (WIPI2). WIPI2 puncta were also down-regulated in waix-treated as shown in Fig. 4C for a typical result. Control cells had 7.5 puncta/cell (n = 16), and waix-treated cells had about 4.5 puncta/cell (n = 19) (Fig. 4D). These results suggest that inhibiting the endogenous TRPM7 channel activity effectively inhibits the puncta formation.

Autophagic flux represents the dynamic process from the synthesis of autophagosome to the degradation of autophagic substrates in the lysosome. Autophagic flux was measured by the blocking autolysosomal degradation using lysosomal proteolysis inhibitors [26]. To determine whether the regulation of basal autophagy by TRPM7 is via regulating autophagic flux, SH-SY5Y cells were treated with 5 μM waix in the presence or absence of lysosomal proteolysis inhibitors, 100 nM bafilomycin A1, 100 μM chloroquine or 20 mM NH_3Cl. Following the waix pre-treatment, proteolysis inhibitors were treated for the last 1 h. In the presence of these inhibitors, autophagic flux was not affected by the presence of waix (Supplementary Fig. 6). These results suggest that TRPM7 channel may regulate the autophagic formation, but not the autophagic flux.

4. Discussion

Cytosolic Ca^{2+} is recognized as an important regulator of autophagy. In this study, we showed that Ca^{2+}-permeable TRPM7 channel plays an important role as the regulator of the basal autophagy. Ca^{2+} influx through ubiquitously expressed TRPM7 channel increased the level of basal autophagy by activating CaMKKβ-AMPK signaling pathway.

Høyer-Hansen et al. reported that autophagy is activated via increased cytosolic Ca^{2+} level, which affects CaMKKβ-AMPK-mTOR signaling and Beclin1-ATG7 signaling [12]. Law et al. showed that Alisol B, an inhibitor of the sarco/endoplasmic reticulum Ca^{2+}-ATP pump, leads to the induction of autophagy via the CaMKKβ-AMPK-mTOR pathway [27]. In addition, resveratrol, an AMPK activator, reduced amyloid-β peptide via increasing Ca^{2+} influx and inducing autophagy [28]. These results are consistent with our finding that the increased cytosolic Ca^{2+} level through the ubiquitously expressed TRPM7 channel leads to the activation of basal autophagy. Since TRPM7 channel underlies the constitutive inward Ca^{2+} influx in some cell types [18,19], increasing or decreasing TRPM7 channel activity may affect cytosolic Ca^{2+} level, thereby, modulating the basal autophagy.

Even though we demonstrated that Ca^{2+} regulates the basal autophagy via the AMPK-dependent signaling pathway, the AMPK-

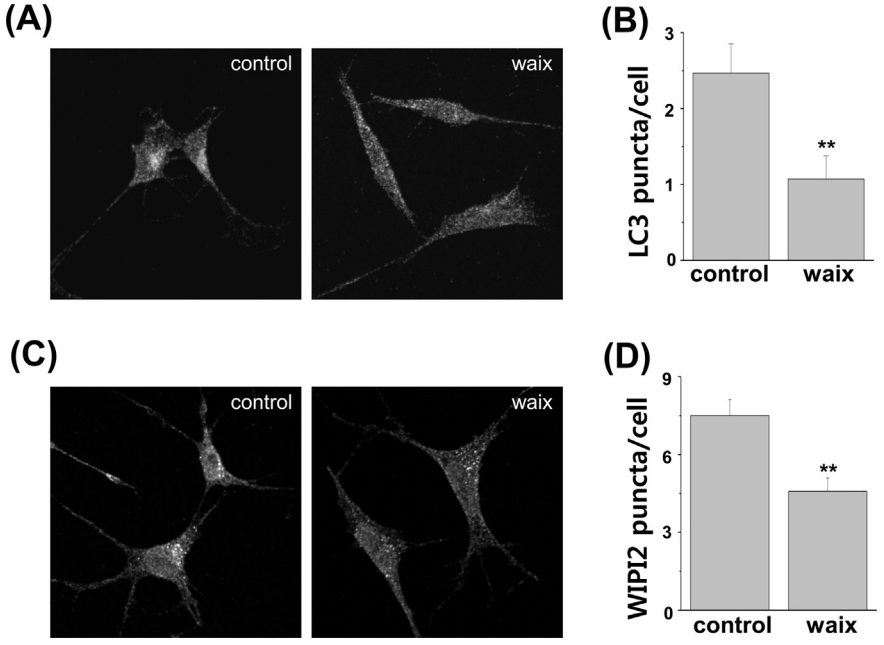

Fig. 4. TRPM7 channel blocker inhibits puncta formation. (A, B) Immunocytochemistry was performed using LC3 antibody to measure the puncta formation from control (n = 15) or from waix-treated SH-SY5Y cells (n = 14) for 24 h. A typical result is shown in (A), and the number of puncta was counted using Image J software in (B). (C, D) WIPI2 puncta were immunostained and counted using Image J software. WIPI2 puncta were also reduced in waix-treated cells (n = 16), compared to control cells (n = 19).

independent pathway may also regulate autophagy. The increase of cytosolic Ca^{2+} induces autophagy from AMPK knockout fibroblasts with minimal changes in mTOR activity [29]. Sasaki et al. established that the activation of protein kinase Cθ by cytosolic Ca^{2+} elevation was required for ER stress-induced autophagy [30]. In this report, we showed that increasing $[Ca^{2+}]_{ex}$ to 2.8 mM from physiological 1.8 mM by itself increased basal autophagy. Also, the down-regulated basal autophagy by the TRPM7 channel inhibitor was recovered by increasing $[Ca^{2+}]_{ex}$ (Fig. 3C, E), indicating that Ca^{2+}influx through TRPM7 channel regulate basal autophagy. However, increasing $[Ca^{2+}]_{ex}$ was not able to fully recover the down-regulated pAMPK/AMPK ratio by waix (Fig. 3C, D), which suggest that the effect of $[Ca^{2+}]_{ex}$ could be via the AMPK-independent and mTOR-dependent pathway [29].

TRPM7 contributes to anoxic neuronal death during prolonged oxygen-glucose deprivation (OGD) in mouse cortical neurons [21]. Neuronal injury associated with OGD is lowered by nonspecific TRPM7 blockers (Gd^{3+} and 2-APB) and TRPM7-specific siRNA. Similarly, overexpression of TRPM7 channel in HEK-293 cells led to increase in intracellular Zn^{2+} accumulation and Zn^{2+}-mediated cell death [31]. In addition, TRPM7 channel participates in cell membrane depolarization, intracellular Ca^{2+} accumulation, and cell swelling during the initial period of acute brain ischemia in hippocampal CA1 neurons [32]. Since autophagy is closely associated with neuronal cell death, changes in TRPM7 channel activity in various conditions may affect the basal autophagy.

In summary, we show that TRPM7 channel regulates autophagy via AMPK signaling in a nutrient-rich condition. This is the first report showing that the constitutive inward Ca^{2+} influx through TRPM7 channel control and maintain the basal autophagy level. Since TRPM7 channel is implicated in many autophagy-related neurodegenerative diseases, the regulation of basal autophagy by TRPM7 channel may serve as possible therapeutic targets.

Conflict of interest

None.

Acknowledgments

This work was supported by Basic Science Research Program through the National Research Foundation of Korea (NRF) funded by the Ministry of Education, Science and Technology (2013R1A1A2006475), Samsung Biomedical Research Institute (SMX1132661) to S.C.

Appendix A. Supplementary data

Supplementary data related to this article can be found at http://dx.doi.org/10.1016/j.bbrc.2015.05.007.

Transparency document

Transparency document related to this article can be found online at http://dx.doi.org/10.1016/j.bbrc.2015.05.007.

References

[1] Y. Ohsumi, Molecular dissection of autophagy: two ubiquitin-like systems, Nat. Rev. Mol. Cell. Biol. 2 (2001) 211–216.
[2] N. Mizushima, Y. Ohsumi, T. Yoshimori, Autophagosome formation in mammalian cells, Cell. Struct. Funct. 27 (2002) 421–429.
[3] D.J. Klionsky, Autophagy: from phenomenology to molecular understanding in less than a decade, Nat. Rev. Mol. Cell. Biol. 8 (2007) 931–937.
[4] B. Levine, V. Deretic, Unveiling the roles of autophagy in innate and adaptive immunity, Nat. Rev. Immunol. 7 (2007) 767–777.
[5] N. Mizushima, B. Levine, A.M. Cuervo, et al., Autophagy fights disease through cellular self-digestion, Nature 451 (2008) 1069–1075.
[6] F. Reggiori, D.J. Klionsky, Autophagy in the eukaryotic cell, Eukaryot. Cell. 1 (2002) 11–21.
[7] D.J. Klionsky, S.D. Emr, Autophagy as a regulated pathway of cellular degradation, Science 290 (2000) 1717–1721.
[8] B. Levine, D.J. Klionsky, Development by self-digestion: molecular mechanisms and biological functions of autophagy, Dev. Cell. 6 (2004) 463–477.
[9] T. Kanki, D.J. Klionsky, Mitophagy in yeast occurs through a selective mechanism, J. Biol. Chem. 283 (2008) 32386–32393.
[10] I. Kim, S. Rodriguez-Enriquez, J.J. Lemasters, Selective degradation of mitochondria by mitophagy, Arch. Biochem. Biophys. 462 (2007) 245–253.
[11] W.W. Winder, D.G. Hardie, AMP-activated protein kinase, a metabolic master switch: possible roles in type 2 diabetes, Am. J. Physiol. 277 (1999) E1–E10.
[12] M. Høyer-Hansen, L. Bastholm, P. Szyniarowski, et al., Control of macroautophagy by calcium, calmodulin-dependent kinase kinase-beta, and Bcl-2, Mol. Cell. 25 (2007) 193–205.
[13] M. Høyer-Hansen, M. Jäättelä, AMP-activated protein kinase: a universal regulator of autophagy? Autophagy 3 (2007) 381–383.
[14] J.P. Decuypere, G. Bultynck, J.B. Parys, A dual role for Ca^{2+} in autophagy regulation, Cell. Calcium 50 (2011) 242–250.
[15] D.A. East, M. Campanella, Ca^{2+} in quality control: an unresolved riddle critical to autophagy and mitophagy, Autophagy 9 (2013) 1710–1719.
[16] M.J. Nadler, M.C. Hermosura, K. Inabe, et al., LTRPC7 is a Mg.ATP-regulated divalent cation channel required for cell viability, Nature 411 (2001) 590–595.
[17] J.A. Kozak, M.D. Cahalan, MIC channels are inhibited by internal divalent cations but not ATP, Biophys. J. 84 (2003) 922–927.
[18] T. Hanano, Y. Hara, J. Shi, H. Morita, et al., Involvement of TRPM7 in cell growth as a spontaneously activated Ca^{2+} entry pathway in human retinoblastoma cells, J. Pharmacol. Sci. 95 (2004) 403–419.
[19] H.G. Oh, Y.S. Chun, Y. Kim, et al., Modulation of transient receptor potential melastatin related 7 channel by presenilins, Dev. Neurobiol. 72 (2012) 865–877.
[20] B.C. Lee, S.E. Hong, H.H. Lim, et al., Alteration of the transcriptional profile of human embryonic kidney cells by transient overexpression of mouse TRPM7 channels, Cell. Physiol. Biochem. 27 (2011) 313–326.
[21] M. Aarts, K. Iihara, W.L. Wei, et al., A key role for TRPM7 channels in anoxic neuronal death, Cell 115 (2003) 863–877.
[22] Z. Cai, S. Jitkaew, J. Zhao, et al., Plasma membrane translocation of trimerized MLKL protein is required for TNF-induced necroptosis, Nat. Cell. Biol. 16 (2014) 55–65.
[23] M. Matsushita, J.A. Kozak, Y. Shimizu, et al., Channel function is dissociated from the intrinsic kinase activity and autophosphorylation of TRPM7/ChaK1, J. Biol. Chem. 280 (2005) 20793–20803.
[24] C. Schmitz, M.V. Dorovkov, X. Zhao, et al., The channel kinases TRPM6 and TRPM7 are functionally nonredundant, J. Biol. Chem. 280 (2005) 37763–37771.
[25] S. Zierler, G. Yao, Z. Zhang, et al., Waixenicin A inhibits cell proliferation through magnesium-dependent block of transient receptor potential melastatin 7 (TRPM7) channels, J. Biol. Chem. 286 (2011) 39328–39335.
[26] D.C. Rubinsztein, A.M. Cuervo, B. Ravikumar, et al., In search of an "autophagomometer, Autophagy 5 (2009) 585–589.
[27] B.Y. Law, M. Wang, D.L. Ma, et al., Alisol B, a novel inhibitor of the sarcoplasmic/endoplasmic reticulum Ca^{2+} ATPase pump, induces autophagy, endoplasmic reticulum stress, and apoptosis, Mol. Cancer Ther. 9 (2010) 718–730.
[28] V. Vingtdeux, L. Giliberto, H. Zhao, et al., AMP-activated protein kinase signaling activation by resveratrol modulates amyloid-beta peptide metabolism, J. Biol. Chem. 285 (2010) 9100–9113.
[29] A. Grotemeier, S. Alers, S.G. Pfisterer, et al., AMPK-independent induction of autophagy by cytosolic Ca^{2+} increase, Cell. Signal 22 (2010) 914–925.
[30] K. Sakaki, J. Wu, R.J. Kaufman, Protein kinase ctheta is required for autophagy in response to stress in the endoplasmic reticulum, J. Biol. Chem. 283 (2008) 15370–15380.
[31] K. Inoue, D. Branigan, Z.G. Xiong, Zinc-induced neurotoxicity mediated by transient receptor potential melastatin 7 channels, J. Biol. Chem. (2010) 7430–7439.
[32] J. Lipski, T.I. Park, D. Li, et al., Involvement of TRP-like channels in the acute ischemic response of hippocampal CA1 neurons in brain slices, Brain Res. 1077 (2006) 187–199.