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Research paper

Lysosomal Ca^{2+} release channel TRPML1 regulates lysosome size by promoting mTORC1 activity

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ABSTRACT

Lysosomal Ca^{2+} release channel TRPML1 has been suggested to regulate lysosome size by activating calmodulin (CaM). To further understand how TRPML1 and CaM regulate lysosome size, in this study, we report that inhibiting mTORC1 causes enlarged lysosomes, and the recovery of enlarged lysosomes is suppressed by inhibiting mTORC1. We also show that lysosome vacuolation induced by inhibiting TRPML1 is corrected by mTORC1 upregulation, and the facilitating effect of TRPML1 on the recovery of enlarged lysosomes is suppressed by inhibiting mTORC1. In the meantime, lysosome vacuolation induced by inhibiting CaM is corrected by mTORC1 upregulation, and mTORC1 overexpression corrects the inhibitory effect of CaM antagonist on the recovery of enlarged lysosomes. Conversely, the vacuolation induced by suppressing mTORC1 is not corrected by upregulating CaM. These data suggest that mTORC1 functions downstream of TRPML1 and CaM to regulate lysosome size. Together with our recent finding showing that TRPML1, CaM and mTORC1 form a macromolecular complex to control mTORC1 activity, we suggest that TRPML1 and CaM control lysosome fission through regulating mTORC1, identifying an mTORC1-dependent molecular mechanism for lysosomal membrane fission.

1. Introduction

Lysosome membrane fission is an important cellular process involved in many human diseases (Luzio et al., 2007; Saftig and Klumperman, 2009; Cao et al., 2017). It is suggested that intraluminal Ca^{2+} release is required for lysosome fission (Pryor et al., 2000; Piper and Luzio, 2004; Treusch et al., 2004; Campbell and Fares, 2010; Miller et al., 2015; Cao et al., 2017). Recently, we together with other groups further suggested that TRPML1 might be the Ca^{2+} release channel that controls lysosome fission (Treusch et al., 2004; Campbell and Fares, 2010; Miller et al., 2015; Li et al., 2016b; Cao et al., 2017) through activating calmodulin (CaM) (Li et al., 2016a; Cao et al., 2017; Sun et al., 2018). However, how activation of TRPML1-CaM pathway triggers fission remains elusive.

The mammalian target of rapamycin (mTORC1) is a ubiquitous protein kinase that is involved in autophagy regulation. Inhibition of mTORC1 by depleting energy or nutrients initiates autophagy whereas reactivation of mTORC1 (Yu et al., 2010; Michaillat et al., 2012; Roczniak-Ferguson et al., 2012; Settembre et al., 2012; Sun et al., 2018) is required for autophagic lysosomal reformation or lysosome biogenesis, the cellular process requires lysosome fission. In agreement with this, emerging evidence suggests that mTORC1 regulates phagosome and entotic vacuole fission (Michaillat et al., 2012; Krajcovic et al., 2013). Given that mTORC1 activity is increased by TRPML1 via activating CaM (Li et al., 2016a; Sun et al., 2018), we postulated that TRPML1-CaM may regulate lysosome fission (Cao et al., 2017) through activating mTORC1.

To explore the mechanism of lysosome membrane fission, we adopted the recovery of enlarged vacuoles as an indicator of lysosome fission as before (Cao et al., 2017). In this study, we showed that enlarged vacuoles/lysosomes induced by vacuolin-1 were suppressed by mTORC1 overexpression, and upregulating mTORC1 facilitates the recovery of enlarged vacuoles induced by vacuolin-1. The facilitation of enlarged vacuole/lysosome recovery by TRPML1 upregulation was eliminated by inhibiting mTORC1 but not vice versa, suggesting that mTORC1 functions downstream of TRPML1. In addition, enlarged lysosomes induced by inhibiting CaM was rescued by mTORC1 but not vice versa. This is in agreement with our previous data showing that CaM functions linking TRPML1 and mTORC1 (Sun et al., 2018). Furthermore, the interaction of TRPML1-CaM-mTORC1 was increased

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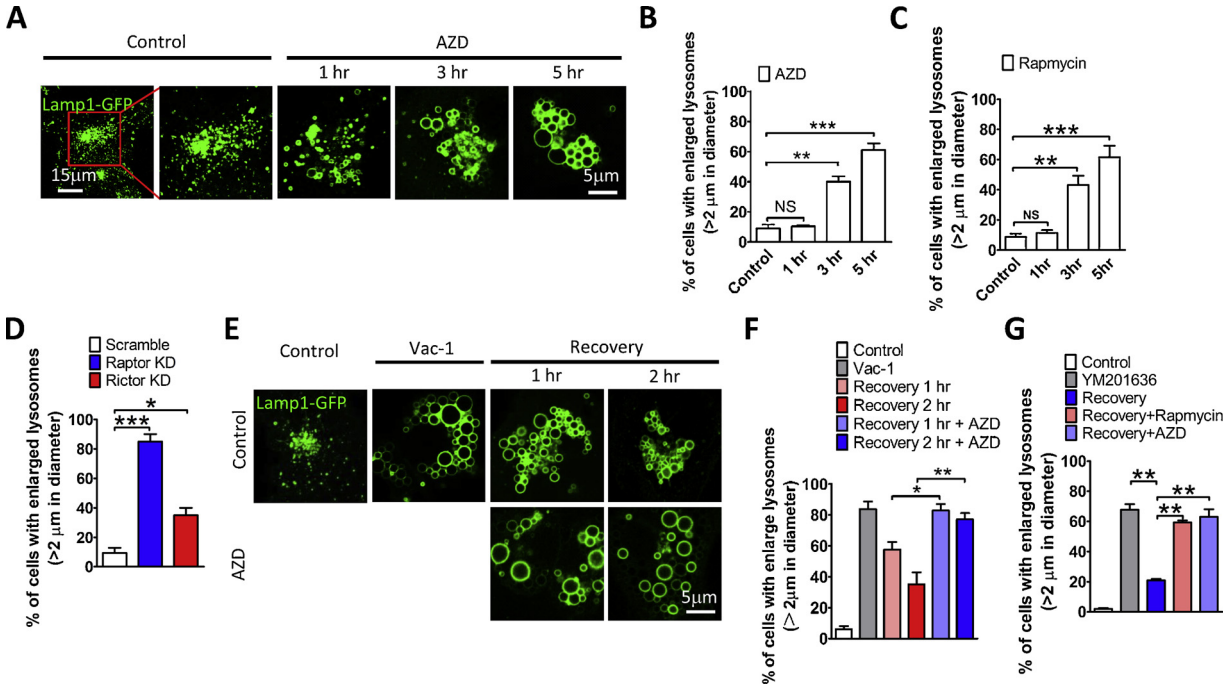

Fig. 1. Lysosome enlargement was induced by inhibiting mTORC1. (A) AZD8055 (0.5 μM) induced enlarged lysosomes which were labeled with Lamp1-GFP in Cos1 cells. Images were taken at 0, 1, 3 and 5 hs after AZD8055 treatment. The enlargement was in a time dependent manner. (B) Histogram summary of percentage of cells containing enlarged lysosomes at indicated time from AZD8055 treatment as in (A). (C) Histogram summary of percentage of enlarged lysosomes induced by rapamycin (0.1 μM). (D) Histogram summary of percentage of enlarged lysosomes in BHK cells that infected by lentivirus carrying shRNA against Raptor and Rictor, respectively. (E) The recovery of vacuolin-1 induced lysosome enlargement was inhibited by AZD8055 (0.5 μM) in Cos1 cells. (F) Summary of percentage of cells containing enlarged lysosomes as in D. (G) The recovery of enlarged lysosomes induced by PIKfyve inhibitor YM206136 (0.5 μM, 2.5 h) was blocked by rapamycin (0.1 μM) and AZD8055 (0.5 μM). Images were taken at 30 min. after recovery. Cells with at least three lysosomes of > 2 μm in diameter were counted. Data were pooled from three independent experiments and > 250 cells were counted for each experiment. Error bars represent Mean ± SEM. NS: no significance, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

during the recovery of enlarged lysosomes. Altogether, these data suggest a TRPML1-CaM-mTORC1-dependent molecular mechanism for lysosomal membrane fission.

2. Material and methods

Cell culture-Cos1 cells were obtained from ATCC (Manassas, VA) and maintained in Dulbecco's Modified Eagle's Medium: Nutrient Mixture F-12 (DMEM/F12) supplemented with 10% fetal bovine serum (FBS), Invitrogen, Carlsbad, CA, USA). HEK293 T cells were maintained in Dulbecco's Modified Eagle's Medium supplemented with 10% fetal bovine serum (FBS). Cells were cultured at 37 °C in a 5% CO_2 atmosphere. For some experiments, cells were seeded on 0.1% poly-lysine coated coverslips and cultured for 24 h before further experiments. Cells from passage numbers 5–25 were used for subsequent assays.

Antibodies and reagents-Antibodies used for Western blotting were rabbit anti-CaM (1:2000; Abcam), mouse anti-GFP (1:1000; Thermo Scientific), mouse anti-Flag (1:1000; Thermo Scientific), mouse anti-p-p70S6K (1:1000; Cell Signaling Technology), rabbit anti-p70S6K (1:1000; Cell Signaling Technology). HRP-conjugated goat anti–rabbit and goat anti–mouse secondary antibodies were purchased from Thermo Fisher Scientific and Bio-Rad Laboratories, Inc. and used at 1: 5000 and 1: 10,000 dilutions, respectively. The following chemicals were used in the present study: Vacuolin-1 (3 μM, Santa Cruz Biotechnology, Inc.), W7 (15 μM, Sigma), BAPTA-AM (15 μM, Invitrogen), ML-SA1 (15 μM, Tocris Bioscience), ML-SI1 (25 μM, Enzo Life Sciences Inc), AZD8055 (0.5 μM, Sigma), Rapamycin (0.1 μM), and YM201636 (0.5 μM).

Confocal microscopy-Confocal fluorescent images were taken using an inverted Zeiss LSM510 Axiovert 200 M confocal microscope with a 40× or 63× oil-immersion objective at room temperature. Sequential excitation wavelengths at 488 nm and 543 nm were provided by argon and helium-neon gas lasers, respectively. Emission filters BP515-565 and LP590 were used for collecting green and red images in channels one and two, respectively. After sequential excitation, green and red fluorescent images of the same cell were saved with ZEN2012 software. Images were analyzed by Zeiss software. The term colocalization refers to the coincident detection of above-background green and red fluorescent signals in the same region.

Molecular biology and biochemistry- Plasmids rCaM-myc-his and its mutant are generous gifts from L. Mei (Georgia Regents University, Augusta, GA). In brief, the cDNA for rat CaM (450 bp) was cloned into pcDNA3.1/myc-his vector with a T7 promoter. Cloning sites are BamH1 and Kpn1 at the 5′ and 3′ ends. Lamp1-GFP and mTOR-Flag were homemade and employed as described before (Cao et al., 2015; Sun et al., 2018). Raptor shRNA, Rictor shRNA, mTOR-S2035 T (active) and mTOR-S2035 T/D2357E (kinase dead) were purchased from Addgene (Cang et al., 2013). Cos1 cells were transiently transfected using Lipofectamine 2000 or 3000 (Invitrogen) which usually reach > 85% transfection efficiency.

Immunoprecipitation and Western Blot-Cell lysates (2–5 mg/ml) were incubated with 30 μl 50% Protein A/G-agarose beads in PBS for 15 min at 4 °C to reduce background proteins that non-specifically bound to the beads. After centrifugation at 12,000 × g for 15 min to remove the beads, aliquots of cell lysates (1–2 mg protein) were incubated with the desired antibodies (3–4 μg) or control IgG at 4 °C overnight in a final volume of 1 ml RIPA-PBS buffer with constant rocking. After antibody incubation, protein A/G-agarose beads were added and the samples were incubated at 4 °C for 4 h, followed by centrifugation at 1500 rpm for 10 min at 4 °C. The beads were then washed three times with pre-cooled RIPA without proteinase inhibitors and each time centrifuged at 1500 rpm for 10 min at 4 °C. Immune complexes were resolved by

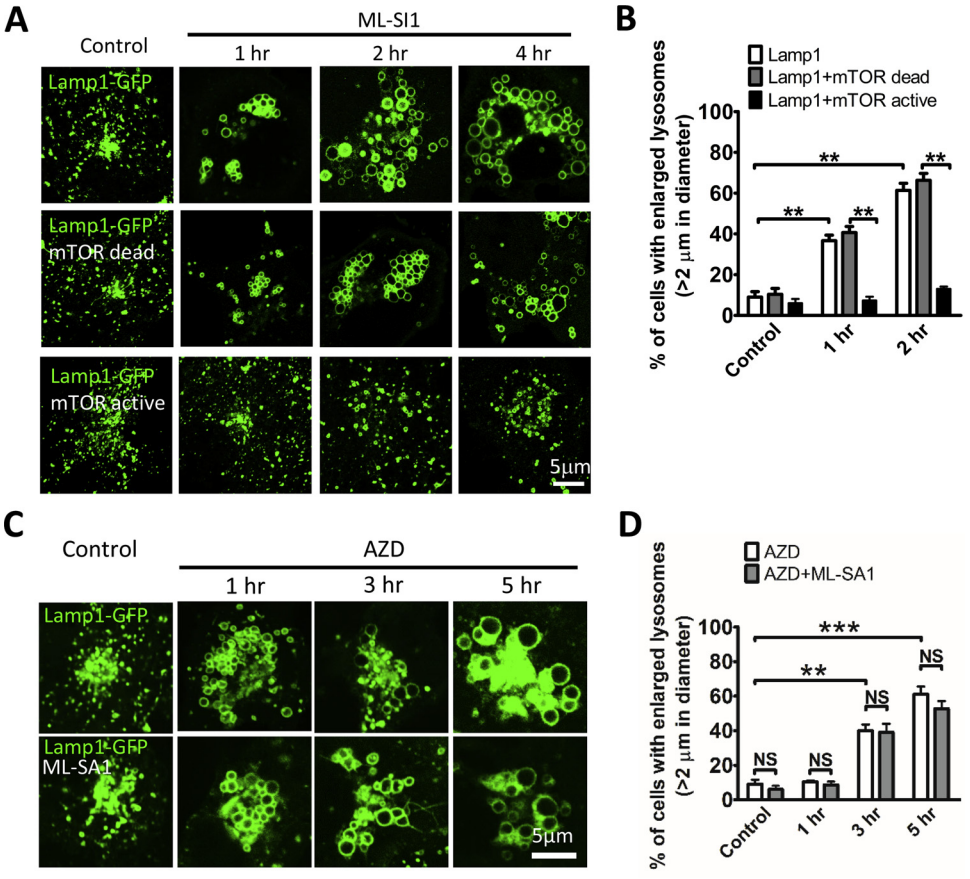

Fig. 2. TRPML1 functions upstream of mTORC1 to regulate lysosomal size. (A) Cos1 cells were transiently transfected with Lamp1-GFP, together with kinase active mTOR or kinase dead mTOR. ML-SI1 (25 μM) treatment caused lysosome enlargement in a time-dependent manner. Kinase active mTOR overexpression rescued ML-SI1 induced lysosome enlargement. (B) Summary of percentage of cells containing enlarged lysosomes as in A. (C, D) AZD8055 (0.5 μM) treatment caused lysosome enlargement, and this was not corrected by activating TRPML1 with ML-SA1 (15 μM). (D) Summary of percentage of cells containing enlarged lysosomes as in C. Cells were counted as described in Fig. 1. Error bars represent Mean ± SEM. NS: no significance, **: $P < 0.01$, ***: $P < 0.001$.

SDS–PAGE and subjected to immunoblotting. Proteins were analyzed by standard Western analysis methods.

Vacuole assay-Cos1 cells were transiently transfected with desired DNAs. Non-transfected cells or cells transfected with Lamp1 were used as controls. At 24 h after transfection, cells on coverslips were treated with various chemicals as indicated. After the treatment, they were rinsed and fixed with 4% PFA and coverslips mounted to glass slides and viewed immediately. All images were taken using a Zeiss Meta510 confocal microscope. Normally, the majority of LEL have sizes with diameters less than 0.5 μm, which are hard to resolve with light microscopy. For quantification, cells were counted as vacuolated if there were more than 3 enlarged (> 2 μm or > 4 μm in diameter) cytoplasmic vacuoles, with the vacuole sizes determined using ZEN 2012 program. Percentage of vacuolated cells in each experiment was calculated from counting at least 250 cells from randomly chosen fields and the experiments were repeated three times (n = 3).

Data analysis-Data are presented as mean ± SEM. Statistical comparisons were made using analysis of variance (ANOVA) and Student's *t*-test P values of < 0.05 were considered statistically significant. *: $P < 0.05$; **: $P < 0.01$; ***: $P < 0.001$.

3. Results

mTORC1 regulates lysosome size-Both TRPML1 and mTORC1 have been suggested to be involved in lysosome biogenesis or reformation (Treusch et al., 2004; Yu et al., 2010; Medina et al., 2015; Li et al., 2016b; Sun et al., 2018). We recently found that TRPML1 might regulate lysosome fission via activating CaM (Cao et al., 2017). We (Sun et al., 2018) together with others (Li et al., 2016a) further suggested TRPML1 interacts with and regulates mTORC1 activity through CaM in the lysosome. Therefore, we predicted that TRPML1 may regulate lysosome fission via increasing mTORC1 activity. To test our hypothesis, we first evaluated whether inhibiting mTORC1 caused enlarged lysosomes as shown in TRPML1 deficient cells (Dong et al., 2008, 2010b; Cao et al., 2017). Indeed, suppressing mTORC1 using either the dual mTORC1 and mTORC2 inhibitor AZD8055 (0.5 μM, at 3 h and 5 h) (Fig. 1A, B) or mTORC1 inhibitor Rapamycin (0.1 μM) (Fig. 1C) induced enlarged lysosomes that were labeled with Lamp1-GFP in Cos1 cells, suggesting that mTORC1 may play a major role in lysosome fission. To validate this, we specifically disrupted mTORC1 and mTORC2 by knocking down Raptor and Rictor (Cang et al., 2013), respectively. We found that although knockdown of both Raptor and Rictor induced enlarged lysosomes, Raptor knockdown had a larger effect (Fig. 1D). Next, we assessed the effect of AZD8055 on the recovery of enlarged lysosome that was adopted as an indicator of lysosome fission (Cao et al., 2017). We found that the recovery of enlarged lysosomes induced by either vacuolin-1 (3 μM) (Fig. 1E, 1 F) or PIKfyve inhibitor YM201636 (0.5 μM) (Fig. 1G) was suppressed by AZD8055 and/or Rapamycin. Altogether, these data suggest that mTORC1 may control lysosome size by promoting lysosome fission.

mTORC1 functions downstream of TRPML1 to regulate lysosome size-If both TRPML1 and mTORC1 are involved in lysosome fission and TRPML1 activates mTORC1, we expected that mTORC1 upregulation rescued enlarged vacuoles induced by inhibiting TRPML1 but not the visa versa. Indeed, inhibiting TRPML1 using ML-SI1 (Cao et al., 2017) induced enlarged lysosomes in Cos1 cells expressing Lamp1-GFP, and this was inhibited by overexpressing mTOR-S2035 T (kinase active) but not mTOR-S2035 T/D2357E (kinase inactive) (Cang et al., 2013) (Fig. 2A, 2B, S1). On the contrary, enlarged lysosomes induced by inhibiting mTORC1 with AZD8055 (0.5 μM) (Chresta et al., 2010) were not affected by activating TRPML1 with ML-SA1 (15 μM) (Cao et al., 2017) (Fig. 2C, D). These data suggest that mTORC1 functions downstream of TRPML1 to regulate lysosome size.

We also tested whether AZD8055 affected the facilitating effect of

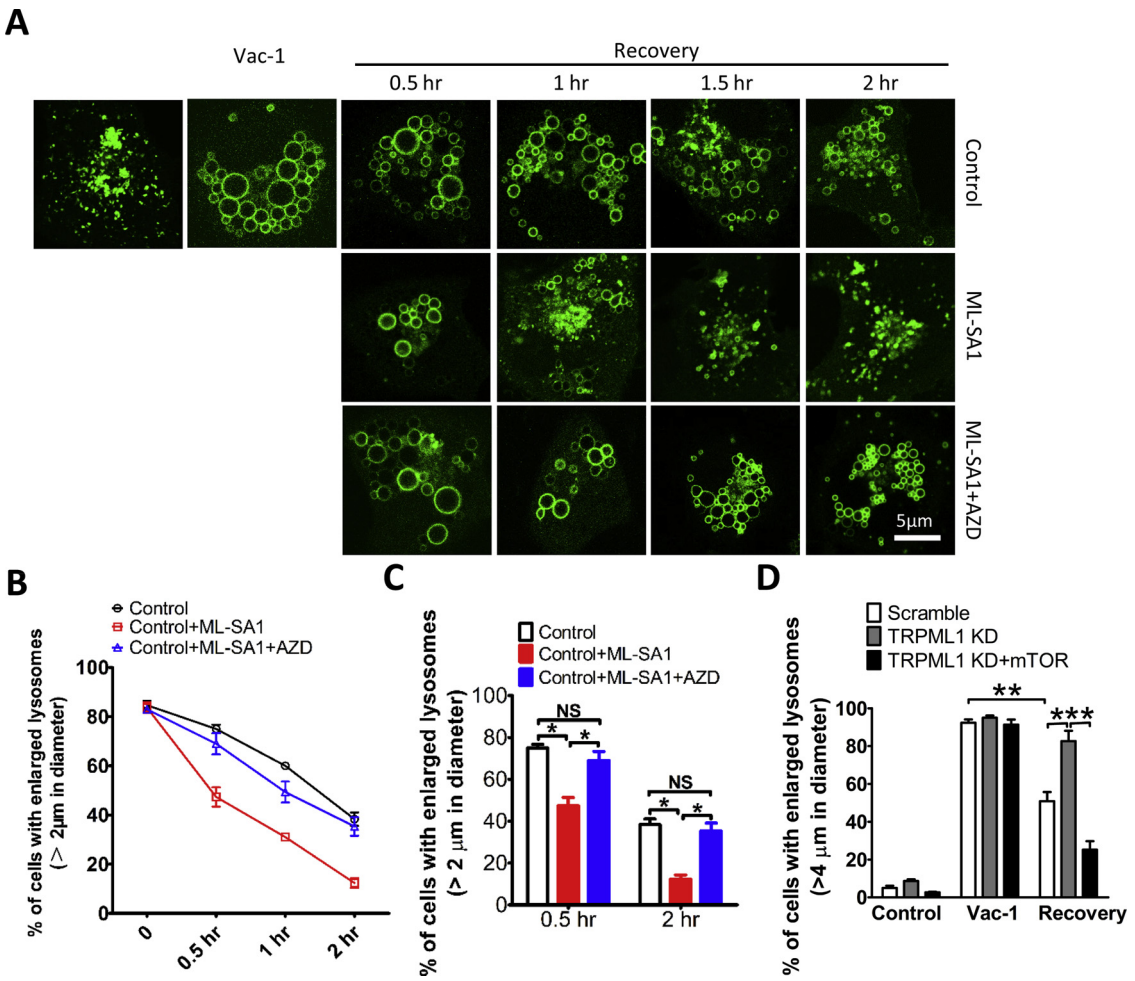

Fig. 3. TRPML1 activation enhances the recovery of enlarged lysosomes and this is inhibited by suppressing mTORC1. (A) Cos 1 cells transfected Lamp1-GFP were treated with vacuolin-1 (3 μM, 2.5 h) to induce enlarged lysosomes. The recovery of enlarged lysosomes was facilitated by ML-SA1 (15 μM). This was blocked by AZD8055. (B, C) Summary of percentage of vacuolated cells (with at least three vacuoles of > 2 μm in diameter) at different time. (D) mTOR overexpression significantly facilitated the recovery of enlarged lysosomes in TRPML1-KD BHK cells. Lamp1-GFP transfected cells were treated with vacolin-1 (3 μM) overnight to induce vacuoles with comparable size, and then let recover for 3 h. Histogram summary of percentage of cells containing enlarged lysosomes. Cells were quantitated as described in Fig. 1. Error bars represent Mean ± SEM. NS: no significance, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

ML-SA1 (15 μM) on the recovery of vacuolation induced by vacuolin-1. Consistent with previous report (Cao et al., 2017), activating TRPML1 with ML-SA1 facilitated the recovery of enlarged lysosomes (Fig. 3A–C). However, the facilitating effect of ML-SA1 on vacuolation recovery was dramatically inhibited by AZD8055 (0.5 μM), suggesting that TRPML1 mediated lysosome fission requires mTORC1 activation. To exclude the potential off-target effect of ML-SI1 and to test whether mTOR overexpression corrected the effect of TRPML1 reduction on enlarged lysosome recovery, we generated TRPML1 knockdown (TRPML1-KD) cells. We first synchronized the size of enlarged lysosome by increasing the treatment time of vacuolin-1 (3 μM) for 12 h, and then let the enlarged lysosomes to recover for 3 h. In agreement with the ML-SI1 data (Fig. 2), the recovery of enlarged lysosomes was inhibited by TRPML1-KD, and this was rescued by mTOR overexpression (Fig. 3D).

TRPML1-CaM-mTORC1 complex regulates lysosome fission-Because TRPML1 regulates mTORC1 via activating CaM (Li et al., 2016a; Sun et al., 2018), if both TRPML1 and mTORC1 regulate fission, we would expect that TRPML1, CaM and mTORC1 form a macromolecular complex to regulate lysosome fission. Indeed, inhibiting CaM with W7 (15 μM) induced enlarged lysosomes, and this was corrected by mTOR overexpression (Fig. 4A, B). In contrast, AZD8055 (0.5 μM)-induced lysosome enlargement was not rescued by overexpressing CaM (Fig. 4C, D). Additionally, ML-SI1 (25 μM) induced lysosome enlargement was rescued by overexpressing both CaM (Fig. 4E, F) and mTORC1 (Fig. 2A).

To consolidate our conclusions, we synchronized the size of enlarged lysosome by increasing the treatment time of vacuolin-1 (3 μM) for 12 h, and then let the enlarged lysosomes to recover for 3 h. We found that inhibition of vacuole recovery by W7 (15 μM) (Fig. 5A, B) or CaM dominant negative (CaM-DN) (Cao et al., 2015) (Fig. 5C) was rescued by mTOR overexpression, whereas inhibition of vacuole recovery by AZD8055 (0.5 μM) was not rescued by CaM overexpression (Fig. 5D, E). In addition, the inhibition of enlarged vacuole recovery by TRPML1-KD was also rescued by CaM overexpression (Fig. 5F). Altogether, these data suggest that TRPML1, CaM and mTORC1 form a macromolecular complex, i.e. TRPML1-CaM-mTORC1, to regulate lysosome fission. CaM functions upstream of mTORC1 but downstream of TRPML1.

TRPML1, CaM and mTORC1 form a macromolecular complex in lysosomes-Previously, we have shown stronger association of TRPML1 and CaM during the recovery phase of enlarged lysosome (Cao et al., 2017). To strengthen our conclusion, we tested whether the association of the TRPML1-CaM-mTORC1 complex is stronger during the recovery phase of the enlarge vacuole. As we expected, the recovery from vacuolin-1 treatment significantly increased the association between TRPML1 and mTOR, and this was suppressed by inhibiting CaM with W7 (15 μM)

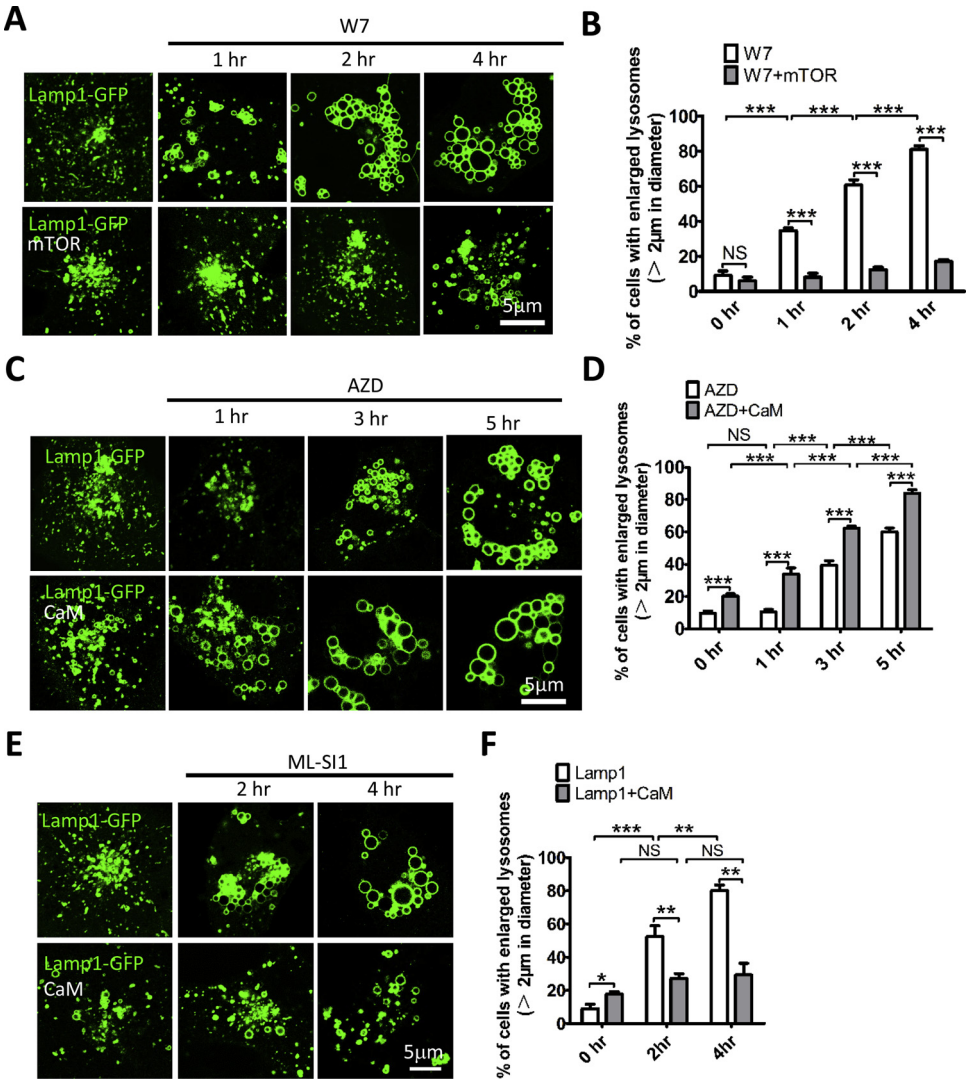

Fig. 4. mTORC1 functions downstream of CaM and TRPML1 to regulate lysosome size. (A, B) Cos1 cells were transiently transfected with Lamp1-GFP and/or mTORC1. W7 (15 μM) treatment caused lysosome enlargement. mTORC1 overexpression rescued W7 induced lysosome enlargement. (C, D) AZD8055 (0.5 μM) treatment caused lysosome enlargement. This was not rescued by CaM overexpression. (E, F) Lysosome enlargement induced by ML-SI1 (25 μM) was rescued by CaM overexpression. Cells were quantitated as described in Fig. 1. Error bars represent Mean ± SEM. NS: no significance, *: $P < 0.05$, **: $P < 0.01$, ***: $P < 0.001$.

(Cao et al., 2015, 2017) or chelating Ca^{2+} with BAPTA-AM (15 μM) (Cao et al., 2015) (Fig. 6A, S2A). This data suggests a stronger association between TRPML1 and mTORC1 during lysosome fission and this requires the activity Ca^{2+}/CaM. A stronger association between CaM and mTOR was also detected during vacuole recovery (Fig. 6B, S2B), and this was suppressed by inhibiting TRPML1 with ML-SI1 (25 μM) and BAPTA-AM (Fig. 6C). Unfortunately, the unavailability of TRPML1 antibody precluded us from assessing the interaction of endogenous TRPML1, CaM and mTORC1. Finally, mTORC1 activity was increased during the recovery phase, and this was suppressed by ML-SI1 and W7 (Fig. 6D). Altogether, we suggest that TRPML1, CaM and mTORC1 associate stronger during vacuole recovery, i.e. TRPML1 release Ca^{2+} to activate CaM and mTORC1, further promoting lysosome fission.

4. Discussion

Lysosomal membrane fusion has been well studied. However, very limited information is available for lysosomal membrane fission. Intralysosomal Ca^{2+} release has been implicated in lysosome fission (Pryor et al., 2000; Morgan et al., 2011). Our recent studies suggest that TRPML1, a lysosomal Ca^{2+} release channel, regulates lysosome fission by activating CaM (Cheng et al., 2010; Dong et al., 2010a). In this study, we further demonstrated that TRPML1 and CaM may regulate lysosome fission by activating mTORC1 although mTORC2 may also play a role.

Given that CaM is also involved in lysosome homotypic fusion (Cao et al., 2015), intriguingly, how does inhibiting CaM cause enlarged lysosomes? As we discussed before, segregated subdomains with different pHs must exist in the lysosome (Cao et al., 2015, 2017). Under certain condition, V-ATPase is recruited to the fission subdomain. This causes a decrease of the local pH to active TRPML1-CaM to trigger fission (Xu et al., 2007; Dong et al., 2008). On the contrary, certain stimuli cause the V-ATPase removal from the fusion subdomain, leading to an increase of the local pH and a consequent activation of P2 × 4-CaM to initiate fusion (Huang et al., 2014; Cao et al., 2015). In normal conditions when lysosomes are acidic (with or without W7 or AZD8055) (Fig. S3), the CaM-dependent fusion process is likely inhibited whereas the CaM-dependent fission process dominates due to the differential regulation of P2 × 4 and ML1 by lysosomal pH (Xu et al., 2007; Dong et al., 2008; Huang et al., 2014; Cao et al., 2015). Therefore, inhibiting CaM suppresses lysosome fission whereas lysosome fusion is not largely affected, leading to enlarged lysosomes (Fig. 5A).

Supporting our hypothesis, an increase in PI(3,5)P2, an endogenous

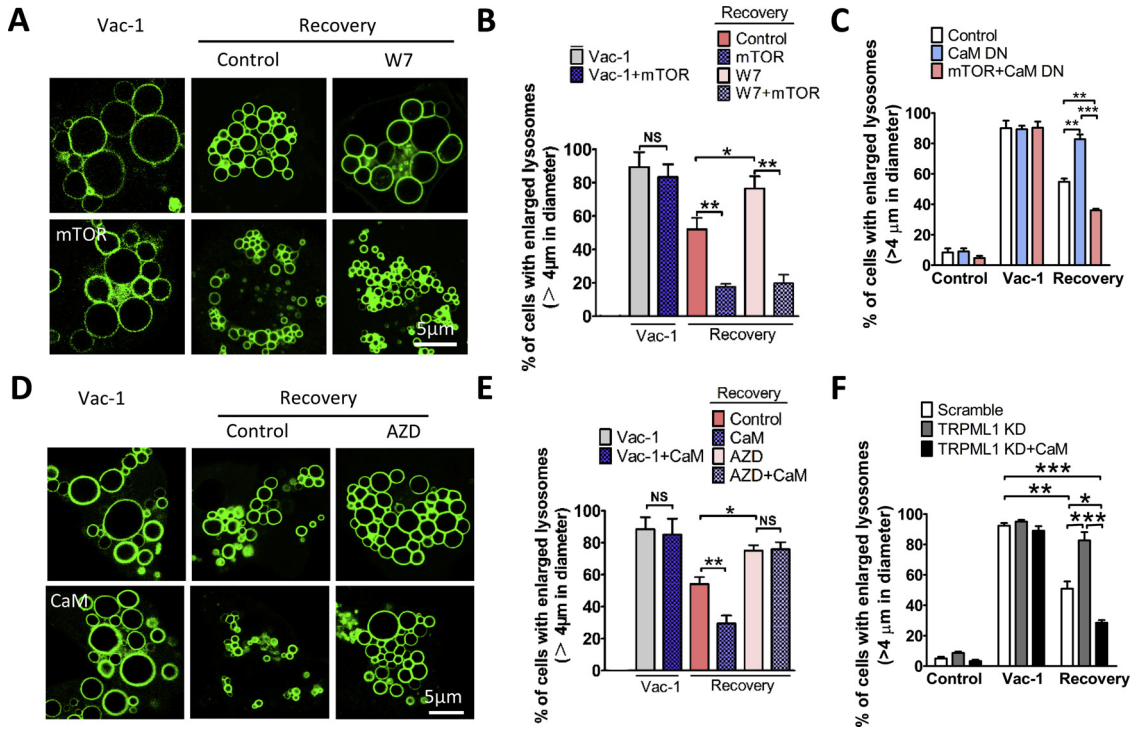

Fig. 5. mTORC1 functions downstream of CaM to regulate lysosome size. (A, B) Cos 1 cells transfected Lamp1-GFP were treated with vacolin-1 (3 μM) overnight to induce vacuoles with comparable sizes, and then let recover for 3 h. W7 (15 μM) blocked vacuole recovery, and this was diminished by mTORC1 overexpression. (C) Enlarged lysosomes induced by CaM-DN was corrected by mTOR overexpression. Cos1 cells were treated with vacolin-1 (3 μM) overnight to induce vacuoles with comparable sizes, and then let recover for 3 h. (D, E) Cos 1 cells expressing Lamp1-GFP were treated with vacolin-1 (3 μM) overnight to induce vacuoles with comparable size, and then let recover for 3 h. AZD8055 (0.5 μM) treatment significantly blocked vacuole recovery, and this was not rescued by CaM overexpression. (F) TRPML1-KD inhibited vacuole recovery, and this was diminished by CaM overexpression in BHK cells. Lamp1-GFP expressing cells were treated with vacolin-1 (3 μM) overnight to induce vacuoles with comparable size, and then let recover for 3 h. Cells with at least three vacuoles of > 4 μm in diameter were counted as described in Fig. 1. Error bars represent Mean ± SEM. NS: no significance, *: $P < 0.05$, **: $P < 0.01$.

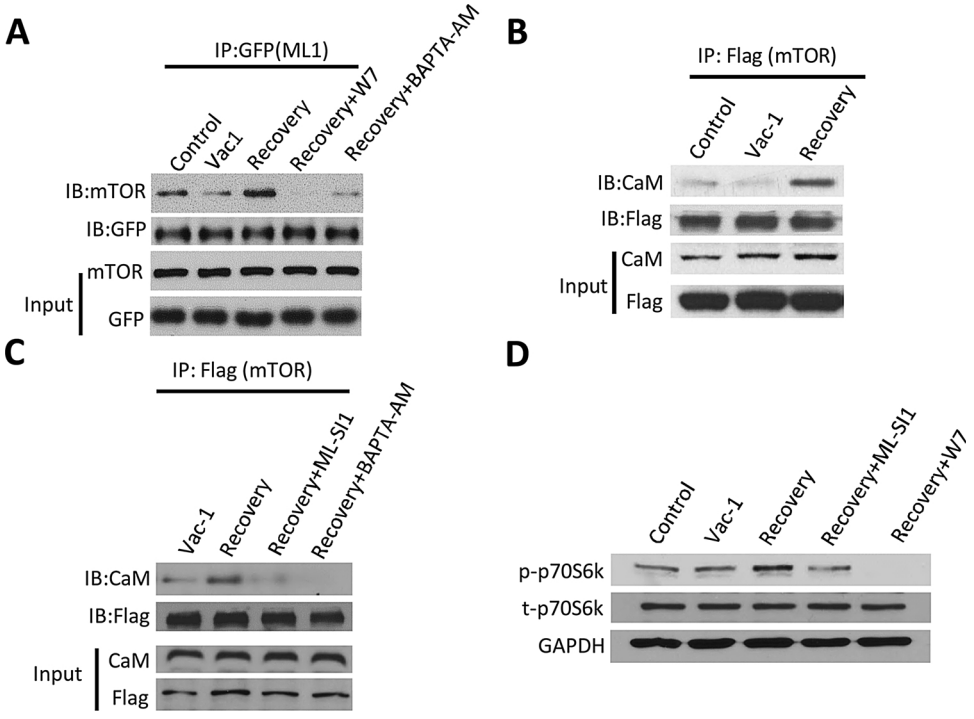

Fig. 6. ML1, CaM and mTOR form a macromolecular complex. (A) Co-IP of mTOR and TRPML1. HEK293 T cells were transfected with TRPML1-GFP. After 24 h, cells were treated with 3 μM vacuolin-1 for 2.5 hs. After wash with PBS twice, cells were allowed to recover for 30 min with or without 15 μM W7 or 15 μM BAPTA-AM. Samples were immunoprecipitated with protein A/G–anti-GFP and blotted with anti-mTOR. The anti-GFP western and anti-mTOR western in immunoprecipitation samples showed similar levels of protein inputs in the immunoprecipitation assays. (B) Co-IP of mTOR and CaM. HEK-293 T cells were transfected with mTOR-Flag. After 24 h, cells were treated with 3 μM vacuolin-1 for 2.5 h. After wash with PBS twice, cells were allowed to recovery 30 min. Samples were immunoprecipitated with protein A/G–anti-Flag and blotted with anti-CaM. The anti-CaM western and anti-Flag western in immunoprecipitation samples showed similar levels of protein inputs in the immunoprecipitation assays. (C) Co-IP of mTOR and CaM. HEK-293 T cells were transfected with Flag-mTOR. After 24 h, cells were treated with 3 μM vacuolin-1 for 2.5 hs. After wash with PBS twice, cells were allowed to recovery 30 min with or without 25 μM ML-SI1 or 15 μM BAPTA-AM. (D) Cos 1 cells were treated with 3 μM vacuolin-1 for 2.5 hs, and then allowed to recover for 30 min in the absence or presence of 25 μM ML-SI1 or 15 μM W7. mTORC1 activation was indicated by the level of phosphorate-p70S6K by western.

activator of TRPML1 (Dong et al., 2010a), has also been associated with the fission of yeast vacuoles, the counterpart of mammalian lysosomes (Rudge et al., 2004; Efe et al., 2005). Deficiency in PI(3,5)P2 leads to defects in the lysosome fission (Zou et al., 2015), lysosomal reformation (Bissig et al., 2017) and enlarged lysosomes (Cheng et al., 2010; Dong et al., 2010a; Bissig et al., 2017; Choy et al., 2018), and activating TRPML1 rescues the defects in PI(3,5)P2 deficient cells (Zou et al., 2015). Our finding is in line with previous reports showing that TRPML1 mutant cells displayed enlarged lysosomes, defects in lysosome biogenesis, retrograde trafficking, and lysosomal exocytosis (Chen et al., 1998; Dong et al., 2009; Cheng et al., 2010; Medina et al., 2011; Samie et al., 2013). Our finding is also in agreement with previous findings showing that both TRPML1 and mTORC1 are involved in autophagic lysosome reformation or biogenesis (Pryor et al., 2000; Treusch et al., 2004). In this regard, the PI(3,5)P2-TRPML1-CaM-mTORC1 pathway is particularly important for cellular homeostasis upon cellular stresses. Because lysosome fission produced small lysosomes that is readily transported along cytoskeleton, we predict that the pathway may be also important for lysosomal exocytosis, thereby playing a role in lysosomal storage removal (Samie and Xu, 2014), membrane repair in response to damage (Reddy et al., 2001; Cheng et al., 2014) and cellular communication by releasing signaling molecules from lysosomes (Zhang et al., 2007; Dou et al., 2012; Imura et al., 2013).

Although this study suggests a potential role of mTORC1 in lysosome fission, two interesting questions remain to be addressed. First, how might Ca^{2+}/CaM modulate mTOR/mTORC1? Second, more interestingly, how might mTORC1 drive lysosome fission? Previous studies suggested that CaM might indirectly interact with mTORC1 via vacuolar protein sorting 34 (Vps34) (Gulati et al., 2008). However, later studies suggested that CaM might directly interact with mTORC1 complex (Li et al., 2016a). Therefore, the precise mechanism of regulation of mTORC1 by Ca^{2+}/CaM remains elusive. For the second question, we speculate that mTORC1 may regulate some molecules such as UVRAG/Vps34 (Munson et al., 2015) that further modulate molecules involved in autophagic lysosome reformation such as adaptor protein 2 (AP2), clathrin (Rong et al., 2012) and dynamin 2 (Schulze et al., 2013) to control lysosome fission. This awaits further investigation.

Declarations of interest

There is no a conflict of interest.

Acknowledgements

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:https://doi.org/10.1016/j.ejcb.2019.05.001.

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