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Channel Function of TRPML1 Prompts Lipolysis in Mature Adipocytes

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Increased intracellular levels of Ca^{2+} are generally thought to negatively regulate lipolysis in mature adipocytes, whereas store-operated Ca^{2+} entry was recently reported to facilitate lipolysis and attenuate lipotoxicity by inducing lipophagy. Transient receptor potential mucolipin1 (TRPML1), a Ca^{2+}-permeable non-selective cation channel, is mainly expressed on the lysosomal membrane and plays key roles in lysosomal homeostasis and membrane trafficking. However, the roles of TRPML1 in lipolysis remains unclear. In this study, we examined whether the channel function of TRPML1 induces lipolysis in mature adipocytes. We found that treatment of mature adipocytes with ML-SA1, a specific agonist of TRPML1, solely upregulated extracellular glycerol release, but not to the same extent as isoproterenol. In addition, knockdown of TRPML1 in mature adipocytes significantly reduced autophagic flux, regardless of ML-SA1 treatment. Our findings demonstrate that the channel function of TRPML1 partially contributes to lipid metabolism and autophagic membrane trafficking, suggesting that TRPML1, particularly the channel function of TRPML1, is as therapeutic target molecule for treating obesity.

Key words: Transient receptor potential mucolipin1, lipolysis, ML-SA1, adipocyte

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Introduction

Adipocytes are known to play key roles in energy homeostasis by mediating lipid metabolism [1]. Most triacylglycerol in vertebrates are deposited in white adipose tissue, and subset of lipases and hydrolases in white adipose tissue are responsible for degrading triacylglycerol into free fatty acids and glycerol, a process known as lipolysis. Until recently, most studies of lipid metabolism have examined cytosolic lipolysis, in which upregulation of cAMP was regarded as a main factor in the hydrolysis of free fatty acids from glycerol [2]. In contrast, autophagy of lipid droplets (LD), known as lipophagy, is thought to be restricted to the catabolism of endocytosed lipoproteins. Increasing evidence has indicated that cytosolic lipolysis and lipophagy communicate and crosstalk during lipid metabolism [2]. Thus, store-operated Ca^{2+}entry (SOCE) was reported to facilitate lipolysis by regulating adenylyl cyclase-dependent signaling and lipase expression, and a deficiency of SOCE in cells leads to increased lipophagy, resulting in compensation for the loss of cytosoliclipolysis [3]. However, the correlation

between lipolysis and Ca^{2+}signaling is unclear.

Transient receptor potential mucolipin1 (TRPML1), a Ca^{2+}-permeable non-selective cation channel, has diverse roles in lysosomal trafficking and functions [4, 5]. Mutations in TRPML1 causes mucolipidosis type IV, a lysosomal storage disorder [6]. TRPML1-deficient cells show impaired membrane trafficking between late endosomes and lysosomes, lysosomal degradation, and lysosomal secretion [7, 8]. A previous study indicated that TOR kinase directly inactivates TRPML1 to suppress autophagy [9]. Moreover, lysosomal Ca^{2+} release resulted in the induction of autophagy in a TFEB-dependent manner [10]. Based on these studies, we hypothesized that TRPML1 contributes to lipolysis through either cytosolic lipolysis or lipophagy. In this study, we examined whether activation of the channel function of TRPML1 in mature adipocytes affects lipid metabolism without activation the β -adrenergic receptor. We further examined whether ML-SA1 mediates lipophagy.

Materials and Methods

Materials and cell culture

ML-SA1, isoproterenol, 3-isobutyl-1-methylxanthine, dexamethasone (DEXA), insulin, and polyclonal Mcoln1 antibody were purchased from Sigma Aldrich (St. Louis, MO, USA). Polyclonal LC3 antibody and monoclonal β-actin antibody were obtained from Cell Signaling Technology (Danvers, MA, USA) and Santa Cruz Biotechnology (Dallas, TX, USA), respectively. To silence TRPML1, recombinant siRNA (5′-agattggcattgaggtaaagaacct-3′, 5′-aggttctttgcctcaatgcc aatcttc-3′) were delivered into cells using transfectamine following the manufacturer's protocol. OP9 cells, which are murine bone marrow-derived stromal cells, were from ATCC (Manassas, VA, USA) and maintained in minimum essential medium α supplemented with 20% fetal bovine serum and antibiotics at 37°C in a 5% CO_2 incubator.

In vitro adipogenesis assay

OP9 cells were seeded onto designated culture dishes at 80% confluence. To induce differentiation into mature adipocytes, the cells were incubated in adipogenic medium supplemented with 10% fetal bovine serum, 0.5 mM 3-isobutyl-1-methylxanthine, 0.25 μM DEXA, 175 nM insulin, and 2 mM L-glutamine for 5 days.

Western blotting

OP9 cells seeded on a 60-mm dish at 80% confluence were lysed with RIPA buffer (Pierce Biotechnology, Rockford, IL, USA) containing protease inhibitors. Following incubation under the stated conditions, whole cell lysates were collected and cleared by centrifugation at 14,000 ×*g* for 10 min at 4°C. Proteins in total lysates were separated by SDS-PAGE and transferred onto a polyvinylidene fluoride membrane. The membrane was incubated with antibodies against TRPML1 (1:1000), LC3 (1:1000) and β-actin (1:1000) overnight. On the following day, immunoreactive proteins were evaluated using an electrochemiluminescence detection system.

Glycerol release assay

Mature adipocytes were treated with isoproterenol (200 nM) and various concentrations of ML-SA1 for 16 h. Glycerol in the culture medium was quantified using Free Glycerol Reagent (Sigma Aldrich) following the manufacturer's protocol. Briefly, the collected culture medium was reacted with Free Glycerol Reagent and then incubated for 30 m at room temperature. Glycerol in each well was quantified by measuring the OD_{540}.

Statistical analysis

The data were expressed as the mean ± SEM of at least three independent experiments. Statistical analysis was conducted using SPSS version 14.0 (SPSS, Inc., Chicago, IL, USA). Differences were evaluated by one-way analysis of variance followed by Tukey's post hoc test, and $P < 0.05$ was considered statistically significant.

Results

Treatment of ML-SA1 without activation of β-adrenergic receptor upregulates extracellular glycerol release

Previous reports have clearly shown that cytosolic lipolysis and lipophagy crosstalk and interact each other [2, 11, 12]. Notably, SOCE positively regulates cytosolic lipolysis by controlling cAMP-dependent signaling and lipase expression, whereas SOCE-deficiency increases lipophagy to compensate for impaired lipolysis [3]. Because TRPML1 has been reported to play crucial roles in autophagic membrane trafficking, we hypothesized that activation of TRPML1 affects lipid metabolism by either cytosolic lipolysis and lipophagy. To confirm this, we employed an *in vitro* adipogenesis model. OP9

cells, as a precursor of adipocytes, differentiated into mature adipocytes following induction by adipogenic stimuli for 5 days. Following differentiation, the cells were subjected with isoproterenol, a nonselective β-adrenergic receptor agonist, and different doses of ML-SA1 (20, 50, 100 μM). Each sample was incubated for 16 h, and the culture medium was collected to measure glycerol release. Interestingly, our data showed that treatment with ML-SA1 (100 μM) in the absence of isoproterenol significantly increased glycerol release compared to in controls (not treated with both isoproterenol and ML-SA1). In addition, ML-SA1 (100 μM) did not increase glycerol release to the same extent as isoproterenol. Considering that TRPML1 mediates lysosomal membrane trafficking, our data strongly suggest that activation of the channel function of TRPML1 can partially stimulate lipolysis, which contributes to receptor-mediated lipolysis.

Deletion of TRPML1 in mature adipocytes results in reduction of LC3 cleavage

A previous report indicated that adipogenic stimulation of 3T3-L1 cells elicits autophagic flux depending on CCAAT/enhancer-binding protein β [13]. Furthermore, LDs can be a target of autophagy, and this induction of autophagy in adipocytes is critical for maintaining cellular energy homeostasis [12]. To determine the roles of TRPML1 during lipolysis, TRPML1 expression was acutely knocked down in mature adipocytes and the cells were incubated under the indicated conditions. Figure 2 shows that TRPML1 expression was efficiently repressed, and upregulated LC3 cleavage in the control, treated with vehicle only in mature adipocytes, was not affected by isoproterenol and ML-SA1. However, deletion of TRPML1 significantly reduced LC3 cleavage compared to in control cells. This indicates that autophagy is in a fully activated state during adipogenesis, and TRPML1 is necessary for inducing autophagic flux.

Discussion

In terms of intracellular lipolysis, it is generally accepted cAMP-dependent signaling and activated neutral lipases are responsible for hydrolysis of triacylglycerol [11]. In contrast, the autophagy of LDs, also known as lipophagy, has been reported as a protective mechanism against lipotoxicity [14]. Recently, Maus et al. demonstrated that Ca^{2+} influx via store-operated Ca^{2+} channels participates in lipolysis by regulating lipase expression and cAMP-dependent signaling [3]. Notably, SOCE-deficiency in cells resulted in upregulation of autophagy of LDs, suggesting that increased lipophagy compensates for the loss of cytosolic lipolysis. In contrast, a previous study indicated that lysosomal Ca^{2+} release enhances autophagy in a TFEB-dependent manner [10]. These two studies showed different results, possibly because the pathophysiological roles of cytosolic Ca^{2+} depend on the Ca^{2+} signaling tool kit involved in specific lipolysis processes. In this study, we pharmacologically activated TRPML1 in mature adipocytes in the absence of the agonist of β-adrenergic receptor and evaluated intracellular lipolysis, which is measured by glycerol release. As shown in Fig. 1, 100 μM of ML-SA1, a relatively higher concentration than that used in other experiments, significantly induced lipolysis. Interestingly, the amount of released glycerol was significantly lower than that in the isoproterenol-treated group. As described above, TRPML1, a Ca^{2+}-permeable non-selective cation channel, plays key roles in lysosomal membrane trafficking, including in autolysosome formation [15], lysosomal secretion [16], and secretory organelle fusion [17]. Consistent with this, our data indicates that activation of the channel function of TRPML1 alone can induce partial lipolysis.

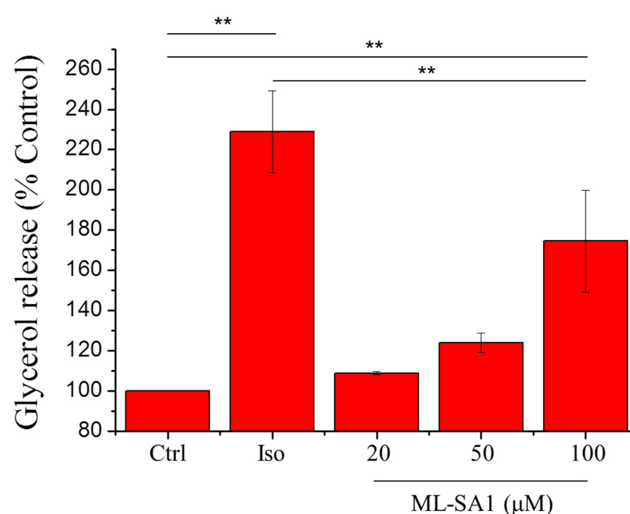

Fig. 1. Effects of ML-SA1 on glycerol release in mature adipocytes. Fully mature adipocytes were incubated for 16 h under the indicated conditions (Ctrl; vehicle only, Iso; 200 nM of isoproterenol, and 20, 50, 100 μM of ML-SA1). Released glycerol in the supernatant was quantified by a colorimetric assay and presented as a percentage of the control (vehicle only). The data are the mean ± SEM of three independent experiments; $**P < 0.05$.

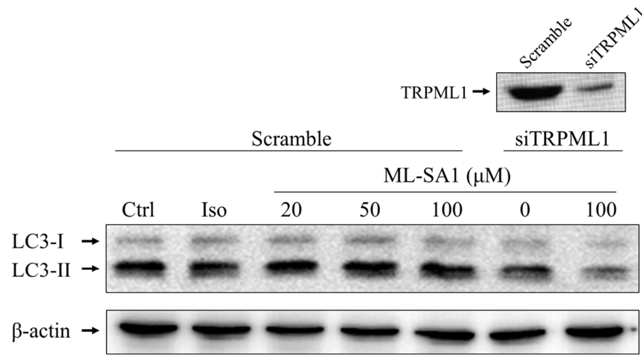

Fig. 2. Effects of ML-SA1 and deletion of TRPML1 on LC3 cleavage. siTRPML1 and scramble RNA were transfected into mature adipocytes for 2 days, followed by additional incubation under the indicated conditions (Ctrl; vehicle only, Iso; 200 nM of isoproterenol, ML-SA1). Total lysates were then collected and used for western blotting of TRPML1, LC3 and β-actin.

As a Ca^{2+} channel in the lysosomal membrane, TRPML1 has been implicated in lysosomal membrane fusion/fission [18]. Notably, TFEB overexpression-induced upregulation of lysosomal exocytosis was abolished by mucolipidosis type IV fibroblasts, which is caused by a loss-of-function mutation in MCOLN1 [19]. Figure 2 shows that autophagic flux was already fully activated during adipogenesis (control) and was not altered by treatment with isoproterenol and ML-SA1. Notably, TRPML1-deficiency led to a significant decrease in autophagic flux. Considering previous reports suggesting that adipogenic stimulation of 3T3-L1 cells mediates the degradation of Klf2 and Klf3, negative which are regulators of adipogenesis, by facilitating autophagic flux [13], this result indicates that autophagy is constantly required for both adipogenesis and lipophgay. We also found that TRPML1 plays crucial roles in mediating autophagy in mature adipocytes. However, further studies are necessary to define the target of autophagy and the molecular mechanisms underlying how TRPML1 mediates lipolysis.

In conclusion, we demonstrated that the channel function of TRPML1 is sufficient for inducing lipolysis and autophagic flux in mature adipocytes. Taken together, our findings indicate that TRPML1 and its channel function are targets for treating obesity and impaired lipid metabolism.

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Conflict of interest

All authors declare no conflict of interest.

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