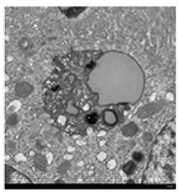
Autophagy



Autophagy

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REVIEW

MCOLN1/TRPML1 in the lysosome: a promising target for autophagy modulation in diverse diseases

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ABSTRACT

MCOLN1/TRPML1 is a nonselective cationic channel specifically localized to the late endosome and lysosome. With its property of mediating the release of several divalent cations such as Ca^{2+}, Zn^{2+} and Fe^{2+} from the lysosome to the cytosol, MCOLN1 plays a pivotal role in regulating a variety of cellular events including endocytosis, exocytosis, lysosomal biogenesis, lysosome reformation, and especially in Macroautophagy/autophagy. Autophagy is a highly conserved catabolic process that maintains cytoplasmic integrity by removing superfluous proteins and damaged organelles. Acting as the terminal compartments, lysosomes are crucial for the completion of the autophagy process. This review delves into the emerging role of MCOLN1 in controlling the autophagic process by regulating lysosomal ionic homeostasis, thereby governing the fundamental functions of lysosomes. Furthermore, this review summarizes the physiological relevance as well as molecular mechanisms through which MCOLN1 orchestrates autophagy, consequently influencing mitochondria turnover, cell apoptosis and migration. In addition, we have illustrated the implications of MCOLN1-regulated autophagy in the pathological process of cancer and myocardial ischemia-reperfusion (I/R) injury. In summary, given the involvement of MCOLN1-mediated autophagy in the pathogenesis of cancer and myocardial I/R injury, targeting MCOLN1 May provide clues for developing new therapeutic strategies for the treatment of these diseases. Exploring the regulation of MCOLN1-mediated autophagy in diverse diseases contexts will surely broaden our understanding of this pathway and offer its potential as a promising drug target.

Abbreviation: CCCP:carbonyl cyanide3-chlorophenylhydrazone; CQ:chloroquine; HCQ: hydroxychloroquine;I/R: ischemia-reperfusion; MAP1LC3/LC3:microtubule associated protein 1 light chain 3; MCOLN1/TRPML1:mucolipin TRP cation channel 1; MLIV: mucolipidosis type IV; MTORC1:MTOR complex 1; ROS: reactive oxygenspecies; SQSTM1/p62: sequestosome 1.

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Autophagy

Macroautophagy (hereinafter referred to as autophagy) is a highly conserved catabolic process that maintains the quality of the cytoplasm by eliminating unnecessary proteins and dysfunctional organelles [1]. The process of autophagy is initiated when the double-membraned phagophores engulf cytosolic damaged or unwanted organelles, aggregate-prone proteins, or pathogens. The mature autophagosome, which originated from the phagophore, subsequently delivers its engulfed cargo to the lysosome for ultimate degradation by various lysosomal hydrolases (Figure 1).

Due to its degradative capability in eliminating damaged organelles and protein aggregates, autophagy maintains cellular homeostasis and facilitates bioenergetic balance. The dysfunction of the autophagic process has been linked to a variety of human diseases including neurodegenerative diseases, cardiovascular disorders, inflammation conditions, and cancer [2,3]. Hence, autophagy modulation presents itself as a promising avenue for developing therapeutic strategies to combat these diseases. Current autophagy modulators include pharmacological and nutritional interventions that either induce or inhibit autophagy at the initiation, nucleation, fusions and degradation phase [4]. Among these, the available modulators aimed at inhibiting autophagy by targeting autophagosomes/lysosomes fusion or lysosome functions include bafilomycin A_1, chloroquine (CQ), hydroxychloroquine (HCQ) and Lys05 [5]. These small molecule inhibitors of autophagy can suppress tumor growth both *in vitro* and

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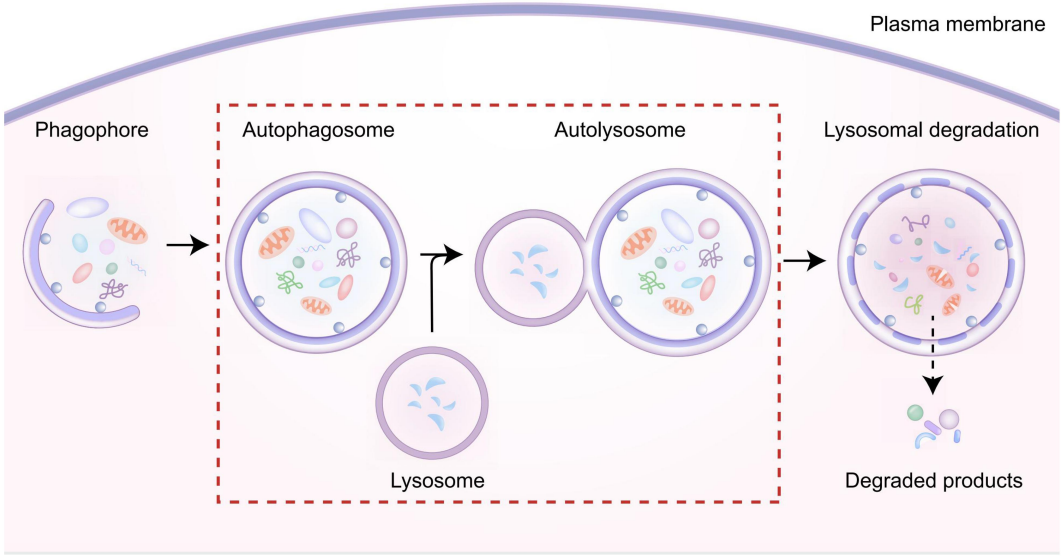

Figure 1. The process of autophagy in mammalian cells.

in vivo [6]. However, it is worth noting that bafilomycin A_1 is rather toxic, while CQ and HCQ, although possessing antineoplastic effects, do so independently of modulating autophagy. The newly developed Lys05 is still under investigation. The adverse and off-target effects of autophagy modulators limit their usage to combat cancer in the clinical settings. As such, there is a growing demand for developing autophagy modulators with well-defined mechanisms and specific targets.

In this review, we introduce an emerging target of autophagy modulation, the MCOLN1 channel, which plays a pivotal role in the development of dysfunctional autophagy associated with cancer and cardiovascular disorders. This review not only broadens our current understanding of how ion channels influence the regulation of the cellular autophagy process but also opens the possibilities of targeting at MCOLN1 as a mean to restore dysfunctional autophagy in diseases linked to this pathway.

MCOLN1/TRPML1 channels

MCOLN1/TRPML1 is a member of the transient receptor potential (TRP) superfamily, which encodes multiple complex transmembrane proteins and functions as ion channels [7]. The TRP superfamily is subdivided into seven subfamilies based on their sequence homology: TRPC (canonical), TRPV (vanilloid), TRPM (melastatin), TRPA (ankyrin), PKD/TRPP (polycystin), MCOLN/TRPML (mucolipin) and TRPN (No mechanoreceptor potential C, nompC) [8,9]. Among these TRP families, the mucolipin TRP subfamily comprises MCOLN1, MCOLN2, and MCOLN3. MCOLN1 is ubiquitously located on lysosomes in all cell types, whereas the other two members are found exclusively in specific cell types. Human MCOLN1, the founding member of the MCOLN/TRPML subfamily, is encoded by the *MCOLN1* gene located on chromosome 19 (19p13.2–13.3; base pair positions 7,587,496–7,598,895) [10]. Loss-of-function mutations in *MCOLN1* gene cause mucolipidosis type IV (MLIV), an inherited autosomal recessive disorder that is characterized by delayed motor and mental development, achlorhydria, and retinal degeneration [11]. With advances in lysosome patch-clamp recording, it has been proven that MCOLN1 functions as nonselective cationic channel that mediates the release of divalent cations such as Ca^{2+}, Zn^{2+}, and Fe^{2+} from the lysosomal storage [10,12]. MCOLN1 is permeable to most divalent trace metals such as Ca^{2+}, Zn^{2+}, Fe^{2+}, etc [13]. A functional cationic selectivity assay has revealed the overall sequence of divalent permeability of MCOLN1 channel, with Ba^{2+} exhibiting the highest permeability followed by Mn^{2+}, and Fe^{2+}, Ca^{2+}, and Mg^{2+} having similar permeability levels, while Ni^{2+}, Co^{2+}, Cd^{2+}, and Zn^{2+}exhibit lower permeability, and Cu^{2+} having the lowest (at pH 4.6) [13]. Patch-clamping recordings and calculation of the Goldman – Hodgkin – Katz equation have determined permeability ratios, indicating that the permeability of Zn^{2+} ($P_{Zn}/P_{Ba} = 0.55$) and Ca^{2+} ($P_{Ca}/P_{Ba} = 0.70$) are in close proximity [13]. Therefore, it can be concluded that the selectivity of MCOLN1 channel for Zn^{2+} is comparable to that of either Ca^{2+} or Fe^{2+}. With regard to Zn^{2+} homeostasis, Zn^{2+} transporters, including SLC30/ZnTs (solute carrier family 30) and SLC39/ZIPs (solute carrier family 39), play critical roles in cellular and physiological functions via mobilizing Zn^{2+} across the cellular membrane. ZnTs are specialized in transporting Zn^{2+} from the cytoplasm into intracellular organelles. Notably, within the SLC30/ZnTs family, SLC30A2/ZnT2 and SLC30A4/ZnT4, mainly localized in lysosomes, are responsible for transporting Zn^{2+} from the cytoplasm into the lysosomes [14,15]. Zn^{2+} can also be transported into the lysosomes through the process of endocytosis. Organellar Zn^{2+} export serves to mitigate cytoplasmic Zn^{2+} toxicity, particularly in pathophysiological conditions like neurodegeneration. ZIP transporters, on the other hand, facilitates the transport of

Zn^{2+} into the cytoplasm across cellular membranes, whether through influx from the extracellular space or efflux from intracellular organelles. SLC39A3/ZIP3, SLC39A4/ZIP4 and SLC39A8/ZIP8, have been reported to localize to lysosomes and facilitate the transport of Zn^{2+} from lysosomes to the cytosol in various cell types, including kidney, testis, T cells, and neurons [16].

Structurally, electron cryo-electron microscopy assays have revealed the formation of the canonical homotetrameric assembly of the MCOLN1 channel. In brief, MCOLN1 channel consists of two structural components: one membrane spanning S1-S6 region and another pore region consisting of the S5 and S6 and two pore helices (PH1 and PH2) [17]. Within this arrangement, helices S5, S6, and two pore helices form the conducting pore of the MCOLN1 channel, allowing ions transport. Notably, the MCOLN1 selectivity filter harbors the sequence^{469}Asn-Gly-Asp-Asp-Met, which establishes the lumenal Ca^{2+}-blocking site crucial for modulating channel conductance in response to lumenal pH and Ca^{2+} levels [17,18]. However, the conformational changes accompanying the passage of different ions, such as Zn^{2+} or Fe^{2+} through the MCOLN1 channel, still remain to be fully elucidated. Further structural investigations would definitely provide valuable insights into the functional mechanisms and molecular dynamics governing the MCOLN1 channel. Small-molecule chemical compounds capable of activating or inhibiting MCOLN1 channel have been identified through high-throughput screen [19]. The agonists and antagonists of MCOLN1 channel include ML-SA1, ML-SA5, ML-SI1, and ML-SI3 [19–21]. Structural studies on the MCOLN1 channel have revealed that agonists such as ML-SA1 bind specifically to the pore region between S5 and S6 of MCOLN1 channel without activating those of other TRP subfamilies. Upon binding of ML-SA1, a structural rearrangement of pore helix 1 is triggered, resulting in the formation of a new hydrophilic bond between S6 and the neighboring subunit of pore helix 1. This leads to a slight opening of the selectivity filter, and ultimately stimulates MCOLN1 channel activity [22]. In addition, Schmiege et al. reported that synthetic antagonists of MCOLN1, such as ML-SI3, bind to the same site as the synthetic agonist ML-SA1, subsequently blocking MCOLN1 activity [18].

Functionally, through its capacity to maintaining lysosomal ion homeostasis, MCOLN1 plays a pivotal role in regulating a variety of intracellular processes including endocytosis, exocytosis, lysosome re-formation, lysosomal biogenesis and autophagy [20,23–28]. In addition, reactive oxygen species (ROS) serve as endogenous activator of the MCOLN1 channel [29]. This implies that MCOLN1 May participate in the pathological processes of a range of diseases in which ROS levels are abnormally elevated such as in inflammation responses, neurodegenerative diseases, and myocardial ischemia-reperfusion (I/R) injury [21,30]. Because abnormal ROS is involved in multiple pathophysiological diseases, the role of MCOLN1 as a ROS effector, may represent an underappreciated aspect of the pathogenesis of many diseases that has not received enough attention until now.

MCOLN1 channels and autophagy

The activation of MCOLN1 inhibits the process of autophagy by mediating the release of lysosomal Zn^{2+} into the cytosol

Intracellular Ca^{2+} originates from both extracellular space and intracellular storages reservoirs such as the ER and lysosomes. Its hemostasis is meticulously regulated by functional ion channels, pumps, and organelles. Recognized as a key second messenger, Ca^{2+} has long been established as a major player in autophagy since the autophagy field came to the spotlight. Numerous studies have shown that cytosolic Ca^{2+} signals can trigger autophagy, whereas, in certain contexts, they may also exhibit the capability to suppress autophagy [17]. Serving as the primary lysosomal Ca^{2+} release channel, MCOLN1-mediated lysosomal Ca^{2+} signaling regulates a myriad of crucial cellular events, encompassing exocytosis, phagocytosis, autophagy and the replenishment of Ca^{2+} in the lysosomal lumen [12,31]. Numerous studies have reported the involvement of MCOLN1 in the regulation of autophagy. To name a few, fibroblasts from patients with MLIV display significantly elevated levels of lipidated MAP1LC3/LC3 (LC3-II) and SQSTM1/p62 [32], both of which are commonly utilized markers to monitor autophagic flux [1]. These observations indicate that the autophagic flux is impaired in fibroblasts obtained from MLIV patients, as evidenced by the significant accumulation of cellular autophagosomes.

In 2015, Medina et al., reported that MCOLN1-mediated Ca^{2+} release from the lysosomes, promotes lysosomal biogenesis and autophagy by regulating the PPP3/calcineurin-TFEB (transcription factor EB) pathway. TFEB is a master transcriptional regulator of genes involved in lysosomal biogenesis and autophagy [33]. Mechanistically, MCOLN1-mediated Ca^{2+} release activates PPP3/calcineurin, leading to the nuclear translocation of TFEB. This event promotes the transcription of autophagy-related genes to induce autophagy [33]. These results suggest that MCOLN1-mediated Ca^{2+} release from the lysosomes activates autophagy by modulating TFEB pathway. However, a subsequent study published in 2016 proposed an alternative perspective on the role of lysosomal Ca^{2+} through MCOLN1 channel in autophagy. Li et al., reported that lysosomal Ca^{2+} through MCOLN1 channel activates MTOR (mechanistic target of rapamycin kinase) complex 1 (MTORC1) by inducing association of calmodulin with MTOR [34]. It has been widely accepted that activation of MTORC1 results in the inhibition of autophagy by suppressing the phosphorylation of various autophagy-related proteins, including ULK1 (unc-51 like autophagy activating kinase 1), ATG13 (autophagy related 13), and ATG14 (autophagy related 14). These proteins are crucial for autophagy initiation and autophagosome nucleation [1]. Thus, it is conceivable to suggest that lysosomal Ca^{2+} through the MCOLN1 channel inhibits the process of autophagy, rather than promoting autophagy as described in the paper by Medina et al. [33]. Shortly after the publication of the paper by Li et al., Sun et al., proposed that lysosomal Ca^{2+} signaling mediated by the MCOLN1 channel indeed serves as a negative feedback regulation of MTORC1, preventing excessive loss of MTORC1

function and the induction of autophagy during starvation [35]. In particular, Sun et al., meticulously monitored the changes in the autophagic flux following the opening of MCOLN1. Their findings revealed that overexpression of the MCOLN1 channel blocks autophagosome-lysosome fusion, consequently disrupting the autophagic flux. However, the precise underlying mechanism behind this phenomenon remains elusive. In a recent study, Scotto-Rosato et al. demonstrated that MCOLN1-mediated lysosomal Ca^{2+} signaling promotes autophagosome biogenesis independent of TFEB transcription pathway [36]. In light of these discrepancies, the Ca^{2+}-dependent mechanism alone fails to fully explain the regulation of MCOLN1 channel in autophagy. As a result, the mechanism by which the activity of the MCOLN1 channel influences the process of autophagy remains enigmatic.

In alignment with this puzzle, a series of recent studies have proposed a novel role for MCOLN1 in autophagy regulation, suggesting that it mediates the release of lysosomal Zn^{2+} rather than lysosomal Ca^{2+} [20,21,27,28]. Rather than indirectly investigated the effect of MCOLN1 on autophagy, Qi et al., dissected the direct effect of MCOLN1 by investigating the cellular integrity of the autophagic influx process without an intermediary, either by using specific MCOLN1 agonists such as ML-SA1, ML-SA5, MK6–83, or by genetically increasing its protein expression levels. They demonstrated that the activation of MCOLN1 indeed inhibits autophagic flux in conjunction with blocked fusion between autophagosomes and lysosomes. Specifically, the lysosomal Zn^{2+} influx, following MCOLN1's opening, perturbs the fusion of autophagosomes and lysosomes by eliminating the interaction between STX17 (syntaxin 17) in the autophagosome and VAMP8 (vesicle associated membrane protein 8) in the lysosome. These two proteins, both of which are soluble N-ethylmaleimide-sensitive-factor attachment protein receptor/SNARE proteins, facilitate the fusion status of autophagosomes and lysosomes. Furthermore, the autophagy inhibition resulting from the activation of MCOLN1 channels has been observed in a wide range of cell lines, including HeLa, HEK 293T, HAP1, normal rat kidney, as well as virous human cancer cell lines such as pancreatic cancer, breast cancer, gastric cancer, malignant melanoma, and glioblastoma cells [20,27,28]. The conclusion, to some extent, deviates from the initial hypothesis, suggesting that the lysosomal Ca^{2+} efflux through MCOLN1 promotes autophagy by triggering the activation of PPP3/calcineurin and subsequent dephosphorylation of TFEB [36]. Qi et al., reasoned that since autophagy is a dynamic process, the accumulation of autophagosomes could be the result of either enhanced initiation or blocked autophagic flux, manifesting as elevated LC3-II levels or LC3 puncta structures. Therefore, it becomes essential to take additional approaches such as monitoring SQSTM1 level and assessing the tandem GFP-RFP-LC3 status to provide a more comprehensive understanding of the regulation of cellular autophagic influx. To be more precise, Yoshii et al., outlined a schematic illustration for the detection of the autophagic flux using fluorescence microscopy in details in their 2017 publication [37]. This methodology aligns with the approach, findings and explanations taken by Qi et al.

Mainly, the variation in interpretation leads to the disparity in conclusions. In support of their observations, several other research groups have also reported similar evidence, suggesting that overexpressed MCOLN1 blocks autophagosome-lysosome fusion, resulting in disrupted autophagic flux. This was confirmed by the RFP-GFP-LC3 tandem plasmid [35]. In addition, Qi et al., thought that the dynamic changes of the autophagic flux might not be adequately captured by monitoring autophagy parameters at the limited observed timing points. Consequently, the prolonged disruption of fusion, leading to accumulation of autophagosomes, could be misinterpreted as changes in autophagy initiation. Furthermore, the variations in observations could be explained by the differences in the choice of cell types selected, experimental conditions applied, or the levels of MCOLN1 expressed in the various cell lines used by the different research groups. These discrepancies highlight the importance of exercising caution when interpreting the changes in autophagic flux. In a later study, another esteemed research group have made a similar discovery, demonstrating that the Zn^{2+} through MCOLN1 channel triggers cell death in melanoma cells [38].

Given the analogous selectivity of the MCOLN1 channel for both Ca^{2+} and Zn^{2+}, it is reasonable to infer that upon the opening of MCOLN1, both lysosomal Ca^{2+} and Zn^{2+} would be concurrently released into the cytosol. Further, it has been well acknowledged that intracellular Ca^{2+} signaling plays a pivotal role in promoting various membrane trafficking processes, including endocytosis, exocytosis and phagocytosis [39]. Therefore, lysosomal Ca^{2+} theoretically is beneficial for the fusion between autophagosomes and lysosomes, exerting an opposite effect to that of Zn^{2+}. Additionally, it has been observed that cytosolic application of Ca^{2+} promotes the interaction between STX17 and VAMP8 (data not shown), consequently leading to enhanced autophagy. As a result, MCOLN1-mediated Zn^{2+} influx takes precedence over Ca^{2+} in inhibiting the process of autophagy. Qi et al., hypothesize that the ultimate outcome of autophagy inhibition following MCOLN1 stimulation is primarily due to the predominant regulatory influence of Zn^{2+} over Ca^{2+} on the fusion between autophagosomes and lysosomes. This hypothesis gains support from the findings wherein the inhibition of autophagy caused by ML-SA5 treatment remained unaffected by the application of Ca^{2+} chelator BAPTA-am, yet was entirely reversed upon treatment with Zn^{2+} chelators N,N,N',N'- tetrakis-(2-pyridylmethyl)ethylenediamine (TPEN) or 1,10 phenanthroline [20]. These results revealed that Zn^{2+} can counter the effects of Ca^{2+} on the interaction of STX17 and VAMP8 when both Zn^{2+} and Ca^{2+} are simultaneously released from the lysosome following MCOLN1 activation. However, it is unlikely to isolate the individual effects of Zn^{2+} or Ca^{2+} on regulating autophagy separately within a physiological context. It is also possible that the distinct environmental cues may trigger MCOLN1 to release the corresponding cations tailored to regulate various cellular events. However, this needs to be elucidated experimentally. In addition, the fundamental role of Zn^{2+} in membrane trafficking is strongly supported by findings demonstrating that Zn^{2+} influx from the extracellular fluid inhibits autophagy through the same

mechanism as lysosomal Zn^{2+} [20]. These results collectively indicate that Zn^{2+} indeed regulates the fusion process between autophagosomes and lysosomes.

Furthermore, corroborating the findings regarding the role of Zn^{2+} influx in regulating autophagy, Xing et al., have recently reported that the activation of intracellular TRPM7 channel also inhibits the autophagic flux by mediating the release of Zn^{2+} into the cytosol [40]. TRPM7, a cationic channel belonging to the TRPM family, is ubiquitously expressed on the plasma membrane and also on the membranes of unidentified intracellular vesicles where large quantities of zinc are stored [41,42]. TRPM7 channel also exhibits a notably high permeability to Zn^{2+} and Mg^{2+} ($P_{Zn}/P_K = 1.21 \pm 0.13$ and $P_{Mg}/P_K = 0.94 \pm 0.08$), as determined by planar lipid bilayers assay [43]. Activation of TRPM7 mediates the release of Zn^{2+} from these unknown intracellular vesicles, which subsequently eliminates the interaction between STX17 in the autophagosome and VAMP8 in the lysosome. The disruption of the interaction between STX17 and VAMP8 caused by activation of TRPM7 leads to the stalling of autophagic flux during the phase when autophagosomes and lysosomes are supposed to fuse. As a result, the activation of TRPM7 produces the same outcome in terms of inhibiting the autophagy process by disrupting the fusion between autophagosomes and lysosomes similar to the effects observed with the activation of MCOLN1 [20]. Serving as pivotal gatekeepers in regulating the organismal balance of Zn^{2+}, TRPM7 triggers Zn^{2+} influx from unidentified intracellular vesicles where a large amount of zinc accumulates, whereas MCOLN1 mediates the release of Zn^{2+} from the lysosome into the cytosol. Whether originating from unidentified intercellular vesicles or from lysosomes, the Zn^{2+} flux into the cytosol exerts an equivalent inhibitory effect on autophagy. Moreover, the use of clioquinol, a Zn^{2+} ionophore that transports extracellular Zn^{2+} into the cytosol, similarly results in autophagy inhibition [20]. Altogether, these observations collectively illustrate that Zn^{2+} influx, whether it originates from the lysosome, other unidentified intracellular vesicles, or extracellular fluid, consistently results in autophagy inhibition within cells [20,40].

When considered together, these findings have established an emerging role for MCOLN1 in the regulation of the autophagy process in different cell types. This regulation is achieved through the mediation of the release of lysosomal Zn^{2+} into the cytosol. This study has transformed the conventional thinking that Ca^{2+} ion is not the exclusive ion responsible for regulating the cellular autophagy process. Instead, divalent ions such as Zn^{2+} is the central player in this regulation.

MCOLN1-mediated autophagy inhibition blocks the mitophagic flux in cells, leading to impaired mitochondrial turnover

It has been well accepted that autophagy is one of the most crucial mechanisms for damaged mitochondria to be eliminated [44]. When the fusion between autophagosome and lysosome is disrupted by the activation of MCOLN1, the proper delivery of the mitochondria-engulfed autophagosome to the lysosome for elimination becomes compromised. Thus, it is conceivable that the MCOLN1-induced autophagy inhibition results in a blockade of the mitophagic flux in cells. Liu et al., subsequently investigated the changes in mitophagic flux and the quality of mitochondria following MCOLN1's activation [28]. Their findings revealed that the fusion between mitochondria-engulfed autophagosomes and lysosomes in A-375 (a human melanoma cell line) and U-87 MG (a human glioblastoma cell line) cells is indeed blocked [27,28], as assessed by using mito-Keima, a probe for tracing mitophagic flux [45]. In detail, a chemical inhibitor of oxidative phosphorylation, carbonyl cyanide 3-chlorophenylhydrazone (CCCP), was selected as a positive control. CCCP is known to depolarize mitochondrial membranes and activates mitophagy. Without CCCP treatment, Mito-Keima-labeled mitochondrial emitted predominantly green fluorescence, indicating the physiological status of mitochondria turnover in A-375 and U-87 MG cells. By contrast, when treated with CCCP, Mito-Keima displayed a notable increase in the proportion of red fluorescence, a phenomenon also observed under starvation conditions. These results showed active mitophagic flux under either CCCP or starvation condition in A-375 and U-87 MG cells. In comparison, the activation of MCOLN1 abolished the active mitophagic flux under starving conditions, which demonstrated the blockade of mitophagic flux to lysosomes caused by MCOLN1 activation. These evidences have confirmed that MCOLN1-mediated autophagy inhibition blocks the mitophagic flux by disrupting the fusion between mitochondria-engulfed autophagosomes and lysosomes (Figure 2).

The mitochondrial respiratory chain is the major sources of cellular ROS. As such, it is conceivable that the blockade in mitophagic flux resulting from the activation of MCOLN1 disrupts the turnover of mitochondria, potentially culminating in to the accumulation of damaged mitochondria and excessive ROS release [28]. Notably, the damaged mitochondria and elevated ROS in these cancer cells caused by the activation of MCOLN1 can be rescued by pre-treatment with Wortmannin, an autophagy inhibitor mainly targeting the initiation step of the autophagy process [1]. These observations indicate that mitophagy, as a form of selective autophagy, may partially share the common initiation pathway with nonselective autophagy, even though the initiation step of the machinery of mitophagy involving PINK1-PRKN and BNIP3-BNIP3L/NIX pathways is different from nonselective autophagy [46].

What is even more significant is that serving as an oxidative stress sensor, MCOLN1 is activated in response to the elevated ROS during the pathological progression of various diseases [29], subsequently blocking mitophagic flux and severely interfering mitochondria turnover. As a result, the buildup of damaged mitochondria not only leads to more ROS release but also theoretically activates MCOLN1 channels, exacerbating mitochondrial damage. Therefore, MCOLN1 becomes a critical factor that links ROS and mitophagic flux regulation. In response to abnormal ROS, the activation of MCOLN1 initiates a detrimental feedback loop between these two biological processes, and thereby playing a partial role in the pathogenesis ofdiseases, such as myocardial I/R injury [21,30].

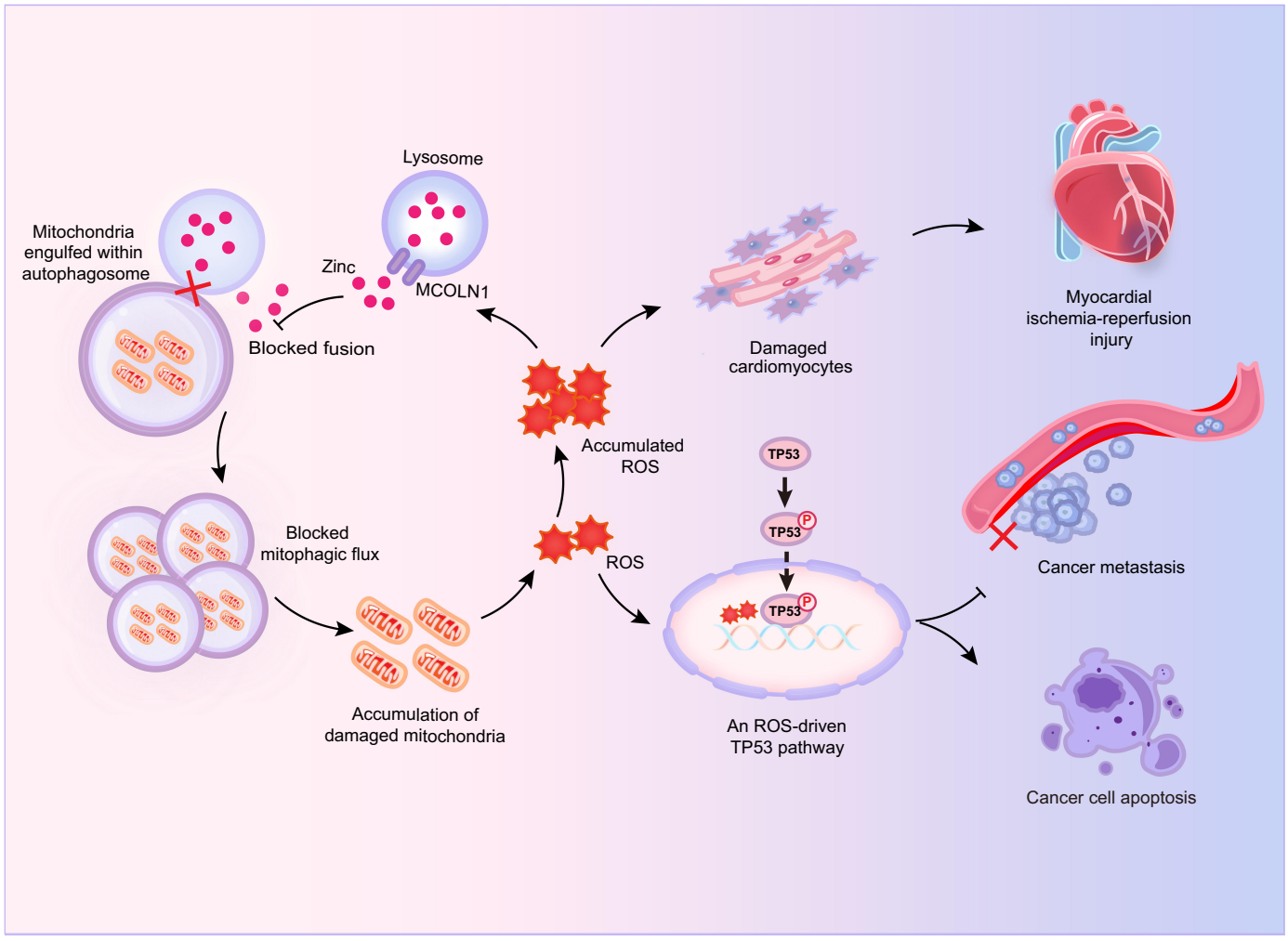

Figure 2. The mechanism by which MCOLN1-mediated autophagy inhibition contributes to the pathogenesis of cancer and myocardial I/R injury.

Taken together, the activity of MCOLN1 effectively controls the turnover of mitochondria bymanipulating the mitophagic flux within cells (Figure 2).

The increase in ROS levels resulting from MCOLN1-mediated mitophagy inhibition inflicts damage to cellular DNA, subsequently triggering TP53/p53 activity

It is wildly acknowledged that autophagy and TP53 are closely interconnected. TP53 activity regulates autophagy through both its transcriptional and post-transcriptional regulation mechanisms. However, the precise manner in which autophagy regulates TP53 remains an uncharted territory [47]. Furthermore, it is well known that a complex, yet tightly intertwined relationship exists between ROS and TP53. It is commonly accepted that TP53, in response to intracellular stress and DNA damage stimuli, induces apoptotic mitochondrial changes through a transcription-dependent mechanism [48]. Therefore, it is speculated that autophagy may reciprocally regulate TP53 through mechanisms related to intracellular stress. To probe this theory experimentally, Liu et al., investigated whether the increased ROS level by the MCOLN1-mediated autophagy inhibition triggers TP53 activity in various cancer cells such as A-375 and U-87 MG cells. They found that MCOLN1-mediated autophagy inhibition leads to the accumulation of damaged mitochondria via blocking of the mitophagic flux in cancer cells. The impaired mitochondria turnover then leads to elevation in ROS, which, in turn causes severe damage to the DNA in these cancer cells. The DNA damage, as illustrated by comet assay, induced by MCOLN1-mediated autophagy inhibition subsequently activates TP53 [28]. Furthermore, down-regulating MCOLN1 with ML-SI3, an antagonist of MCOLN1, disrupting autophagy initiation through pretreatment with wortmannin, chelating Zn^{2+} with TPEN, or applying N-acetyl cysteine/NAC, an antioxidant, significantly abolished the DNA damage and consequent elicited TP53 activity caused by the activation of MCOLN1 in cancer cells. To validate the well-recognized phenomenon that ROS act as the upstream effector of TP53 activity following the opening of MCOLN1, it was observed that ROS levels increased rapidly and peaked at 8 h, while TP53 activity showed an increase 4 h after the elevation of ROS elevation. This indicates that elevation of ROS serves as the upstream effector responsible for activating the TP53 signaling pathway in these cancer cells.

In their combined findings, Liu et al., revealed that the activation of MCOLN1 triggers TP53 activity by regulating

the "lysosomal Zn^{2+}—autophagy inhibition – disrupted mitochondria turnover – ROS elevation – DNA damage" axis in cancer cells experimentally (Figure 2). In fact, aberrant regulation of TP53 activity plays a key role in a broad spectrum of pathological conditions including neurological dysfunction and myocardial infarction. Even more important is that although these experiments were performed on cancer cells, the MCOLN1-mediated TP53 transactivity may also apply to the physiological and pathological functions in neurons and cardiomyocytes.

MCOLN1-induced autophagy inhibition triggers mitochondrial mediated apoptosis by regulating a ROS-driven TP53 signaling pathway

Conceptually speaking, autophagy and apoptosis are two distinct self-destructive processes, each representing fundamental physiological mechanisms essential for maintaining cellular and tissue homeostasis. Apoptosis and autophagy both belong to programmed cell death, and many shared effectors simultaneously regulate these two cellular processes [49]. Despite significant differences between these two processes, they are highly interconnected and play a crucial role in determining the ultimate fate of a cell. There have been several theories proposed regarding the functional relationship between autophagy and apoptosis, each with potential underlying mechanisms [49,50]. For instance, some studies indicate that the negative regulation in autophagy by apoptosis is caused by the sequestration of BECN1 (beclin 1), a key factor required to initiate autophagy [1]. Key regulators of apoptosis, such as the BCL2 family or BCL2 homology 3/BH3-only proteins, bind with BECN1 to form a BECN1-BCL2 complex, which prevents BECN1 from inducing the autophagy process [51,52]. Additionally, cytosolic TP53, a tumor suppressor, suppresses autophagy by interfering with the activity of ULK1 complex [53]. These are proposed mechanisms by which apoptosis regulates autophagy. Nevertheless, the underlying mechanism whereby autophagy regulates apoptosis remains unclear. It has been speculated that the elimination of damaged mitochondria through autophagy may suppress apoptosis by restricting the release of pro-apoptotic factors from the mitochondria [54]. Still, this theory lacks robust experimental evidence at this point.

Given that the MCOLN1-mediated autophagy inhibition triggers TP53 activity in cancer cells, Liu et al., sought to elucidate the relationship between MCOLN1-mediated autophagy inhibition and apoptosis in cancer cells [28]. In light of the elicited TP53 activity induced by MCOLN1-mediated autophagy inhibition, the transcription and protein levels of BCL2 and BAX, two major members of the BCL2 family that play a key role in intrinsic apoptotic pathway within tumors [55], were repressed and increased in A-375 and U-87 MG cells, respectively. Subsequently, the changes in the expressions of BCL2 and BAX within the mitochondria result in the release of cytochrome *c* into the cytosol. This event triggers the activation of apoptosomes, resulting in the cleavages of CASP9 (caspase 9), CASP3 (caspase 3), and PARP (poly (ADP-ribose) polymerase) in these cancer cells. These observations demonstrate that MCOLN1-elicited TP53 activity triggers mitochondrial mediated apoptosis in cancer cells. It is worth emphasizing that these findings not only provide experimental evidence supporting the assumption that autophagy negatively regulates apoptosis, but also represents significant breakthroughs in elucidating the underlying mechanism by which MCOLN1-induced autophagy inhibition triggers mitochondrial-mediated apoptosis via the regulation of a ROS-driven TP53 pathway in melanoma and glioblastoma cells (Figure 2). Consequently, a "autophagy inhibition – mitochondria turnover disruption – ROS elevation – DNA damage – TP53 activity-apoptosis" axis has been established that dynamically integrates the process of autophagy and apoptosis. This pathway could potentially be applied to the autophagy inhibition induced by other means, such as CQ and bafilomycin A_1 treatment [56]. This finding provides compelling evidence regarding the mechanism by which autophagy regulates apoptosis.

Furthermore, in addition to triggering apoptosis, MCOLN1-mediated autophagy inhibition, which relies on the TP53 signaling pathway, suppresses cancer metastasis via inhibiting a broad spectrum of the metastatic cascades, including the process of the epithelial-mesenchymal transition/EMT [57] and the remodeling of extracellular matrix macromolecules [58]. Mechanistically, the TP53 activity elicited by MCOLN1-mediated autophagy inhibition modulates the expression of its downstream targets, including members of the MMP (matrix metallopeptidase) family and TWIST (twist family BHLH transcription factor) families. This modulation leads to the suppression of the epithelial-mesenchymal transition and extracellular matrix remodeling, respectively, ultimately leading to the suppressed metastasis in melanoma and glioma cells [27] (Figure 2).

In summary, these findings have expanded the current understanding of the role of autophagy in regulating apoptosis and metastasis. More importantly, we propose a novel strategy of intervention in cancer treatment by targeting autophagy inhibition to trigger apoptosis and suppress metastasis. MCOLN1, a lysosomal ion channel, emerges as a promising potential target to inhibit cancer progression and could evidently be exploited as a novel approach to treat cancer.

The potential autophagy modulation of MCOLN1 in diverse diseases- exemplification in the context of combating cancer and cardiovascular diseases

MCOLN1-mediated autophagy inhibition and cancer

Autophagy process affects cancer development and progression [59]. Nearly all eukaryotic cells undergo autophagy at a relatively low basal level under physiological conditions. In contrast, basal autophagy is often up-regulated in cancer cells, and in solid tumors, such as pancreatic cancer, breast cancer, and gastric cancer [20], This upregulation of autophagy is thought to be a response to ensure high demand for nutrients required to maintain the growth of these cancer cells. In line with this observation of increase basal autophagy in cancer cells, there is an increasing body of evidence suggesting that autophagy plays a critical role in various cascades involved in tumorigenesis [60]. Specifically, following tumor formation,

malignant cells actively undergo autophagy as a survival mechanism to adapt to limited nutrient supplies. Accordingly, studies have shown that the inhibition of autophagy has been proposed as a strategy to combat cancer [61–63]. However, due to the complexity of cancer biology, the impact of autophagy modulations on cancer and its molecular mechanism still remains subjects of ongoing debate and research.

Strikingly, the inhibition of oncogenic autophagy by targeting at MCOLN1 triggers apoptosis in a broad range of cancer cells, including pancreatic cancer, breast cancer, gastric cancer, melanoma, and glioblastoma cells [27,28]. Even more important, MCOLN1-mediated autophagy inhibition has little effect on non-cancerous cells such as MCF 10A (a normal human mammary epithelial cell line) and GES-1 cells (a normal gastric epithelial cell line) [20]. Qi et al., proposed that the relatively low levels of basal autophagy in non-cancerous cells limit the numbers of autophagosomes that are available for the fusion process with lysosomes. Therefore, targeting at MCOLN1 to inhibit autophagy is expected to have little effect on autophagic flux in non-cancerous cells because of the already limited numbers of autophagosomes [20,27]. Overall, MCOLN1-mediated autophagy inhibition possesses potent anti-neoplastic effects without affecting normal cells, conferring a great potential for MCOLN1 as a clinical therapeutic target in cancer treatment.

In addition to utilizing using several cancerous cell lines *in vitro*, Liu et al., took a step further by establishing a melanoma (A-375 and SK-MEL-2) ×enografted mice model to evaluate the *in vivo* effects of stimulating MCOLN1 on melanoma growth [28]. The final volume of A-375 ×enograft collected from the ML-SA5 group (a selective MCOLN1 agonist) was significantly reduced to half of the size ofA-375 ×enograft in the PBS group. In a sharp contrast, ML-SA5 administration did not yield significant suppressive effects on the SK-MEL-2 ×enograft that bears mutant TP53. This further confirms the dependency of TP53 regulation by the MCOLN1-mediated autophagy inhibition. And it is likely that the administration of ML-SA5 suppressed tumor growth via inhibiting autophagy through MCOLN1 channels. This is supported by the strong autophagy inhibition triggered in the A-375 ×enograft tissues in ML-SA5 group, as compared to that of PBS group, as reflected by the increased LC3 and SQSTM1 puncta structures in the tumor tissues. Furthermore, apoptosis triggered by MCOLN1-mediated autophagy inhibition was revealed by an elevation in TUNEL staining in A-375 ×enograft tissues collected from the ML-SA5 group compared with the PBS group. Although autophagy inhibition was also triggered by ML-SA5 administration in SK-MEL-2 ×enograft, the lack of proper TP53 function impeded autophagy inhibition to trigger apoptosis in the TP53 deficient melanoma xenograft. These results further proved the key conclusion that MCOLN1-mediated autophagy inhibition triggers apoptosis via the ROS-TP53 pathway in cancer cells. And the *in vivo* experimental confirmation demonstrated that targeting MCOLN1 has great potential for treating human melanoma and glioma in the clinical setting.

Other than the induced apoptosis, targeting at MCOLN1-mediated autophagy inhibition *in vivo* results in the suppression of metastasis, as demonstrated by a significant reduction in the incidence of naïve melanoma in both locoregional axillary lymph nodes and distant lungs and intestines in a subcutaneous xenografts metastatic mice model [27]. Explicitly, Xing et al., utilized a subcutaneous xenografts metastatic mice model to evaluate the effects of ML-SA5 administration on the melanoma metastasis post-development of primary tumors. In more detail, melanomas that were subcutaneously implanted were resected before they reached 2,000 mm^3 and mice were monitored regularly for signs of metastatic outgrowth and distress [27]. The effects of ML-SA5 were then evaluated on the metastatic spread of the melanoma to axillary lymph nodes and distal organs. With the new metastatic mice model, they found that in comparison to the vehicle group (PBS), ML-SA5 administration profoundly suppressed metastasis of melanoma to both locoregional lymph nodes and distant organs like lungs and intestines. Moreover, the extents of apoptosis and proliferation at the site of metastases, either in lymph nodes or in distal lungs, between the vehicle and ML-SA5 group were comparable. This was assessed by immunofluorescence staining with TUNEL for apoptosis and MKI67/ki67 for proliferation. Notably, autophagy inhibition was induced in the metastatic sites, as manifested by the increases in abundance of LC3 and SQSTM1 puncta structures. These results indicate that MCOLN1-mediated autophagy inhibition suppresses cancer metastatic cascades *per se*, without significantly affecting apoptosis or proliferation at the metastatic sites (Figure 2).

Substantial research and pre-clinical evidence have led scientists to think that modulating autophagy process hold great promise as a strategy for anti-cancer drug development [64]. The reality is that there are a small number of autophagy modulators currently available, such as HCQ or Lys05. However, these autophagy modulators have severe adverse and off-target effects. In comparison, MCOLN1 represents a new oncogenic autophagy target that has demonstrated to suppress both cancer growth and metastasis. What is particularly noteworthy is that the property of using MCOLN1 stimulation to trigger cancer cell death with limited effects on normal cells broadens the potential of applying MCOLN1 agonists in cancer therapy.

Blunting MCOLN1 channels protects against myocardial ischemia-reperfusion (I/R) injury by relieving the inhibition of myocardial autophagy

Much like the complex role of autophagy in tumorigenesis, evidence suggests that autophagy dysfunction plays a critical role in myocardial I/R injury. Moreover, there is ongoing controversial debate regarding whether autophagy is beneficial or detrimental to myocardial I/R injury [65]. Previously acknowledged, autophagy is enhanced during the ischemia process through adenosine monophosphate-activated protein kinase (AMPK) [66] or the HIF1A (hypoxia inducible factor 1 subunit alpha) pathway [67–69]. However, discrepancies persist on whether the myocardial autophagic flux is up-regulated or down-regulated during the reperfusion process [70–72].

Meticulously investigating the myocardial autophagic flux post I/R is, therefore, a prerequisite for implementing suitable strategies to manipulate autophagy and prevent I/R injury.

In line with this approach, Xing et al., used both an *in vitro* and an *in vivo* I/R model to monitor the autophagic flux in cardiomyocytes. They achieved this by exposing neonatal rat ventricular myocytes/NRVM to hypoxia-reoxygenation and by subjecting mice to I/R [21,30]. Based on their studies, they found that in contrast to the ischemia process, myocardial autophagic flux is blocked following the reperfusion process during the observed time points. Although these findings may not be aligned with prior understanding, they are consistent with a study reported by Ma et al. [72,73]. These collection of experimental evidence from different research groups, have broadened the previous understanding of autophagy influx dysfunction during cardiac perfusion period. Further investigations are required to identify the dynamic nature of autophagy as well as the contextual factors that comes into play when the myocardium suffers reperfusion stress at a more granular level.

Moreover, Xing et al., determined that MCOLN1 is the key factor resulting in the blocked autophagic flux in cardiomyocytes exposed to I/R. In the heart, secondary to the ROS elevation induced by I/R, MCOLN1 is activated to induce the release of lysosomal Zn^{2+}, which in turn inhibits the myocardial autophagic flux. Autophagy inhibition mediated by MCOLN1 subsequently damages mitochondria turnover and results in further ROS elevation, which ultimately leads to cardiomyocyte death. Taken together, their results uncover a "ROS-MCOLN1 activation-lysosomal Zn^{2+} release-autophagy inhibition" axis, which underlies the pathogenesis of dysfunctional autophagy in the cardiomyocytes subjected to I/R [21,30] (Figure 2).

More importantly, unblocking the inhibited autophagic flux in cardiomyocytes subjected to I/R, by blunting MCOLN1 channels, significantly rescues cardiomyocytes death *in vitro* and greatly improves short-term and long-term function *in vivo* of I/R mouse models. Given the ongoing quest for cardioprotective interventions and the development of therapeutic strategies to prevent myocardial ischemia reperfusion injury, autophagic modulation with MCOLN1 pretreatment could be considered as a promising strategy to provide protection against I/R injury in the clinical setting [21].

The current status of drug development targeting MCOLN1 channel

From the perspective of drug development targeting MCOLN1 channel, it has emerged as a promising therapeutical target for various lysosomal dysfunctional diseases, including Duchenne muscular dystrophy [74], retinal detachment [75], Niemann-Pick disease [19,25]. Calporta Therapeutics has been dedicated to developing synthetic small molecules targeted at the MCOLN1 channel, with the aim of restoring the lysosomal degradation capability within the established research framework. In 2019, Merck Company acquire Calporta Therapeutics, thereby expanding its research efforts to include neurodegenerative diseases such as Parkinson disease, amyotrophic lateral sclerosis, and Alzheimer disease. Despite the early stage of preclinical development, there is a growing demand for the advancement of potent agonists and antagonists targeting the MCOLN1 channel, given its implications in cancer and cardiovascular diseases. This underscores the significance of MCOLN1 channel as a novel therapeutic target for potential treatments.

Concluding remarks

While it has been long acknowledged that autophagy plays a critical role in various human diseases, the development of effective and specific autophagy modulator remains a challenge. Although clinically available medications such as MTOR inhibitors, CQ, HCQ, and several other drugs do have stimulative or suppressive effects on autophagy, it is worth noting that they have various multiple systematic effects and were not developed for the purpose of targeting solely the autophagy process. Part of reason, for the lack of autophagy modulators, stems from a limited understanding of the complexity of the heterogeneity autophagy defects that can occur in any given disease. Thus, there is a pressing need to dissect the exact autophagy status in cells, particularly in the context of specific human diseases. Clearly, MCOLN1 has emerged as an important regulator of autophagy. The regulation of MCOLN1 channels in autophagy is involved in several physiological functions, including mitochondria turnover, cell proliferation and migration. It is also implicated in several human pathophysiological diseases including the progression of cancer and myocardial I/R injury. For this reason, there is a high demand for the development of potent agonists/antagonists for MCOLN1 channel as a novel target for the treatment, drug design and therapeutic approaches of cancer, myocardial I/R injury, and other related diseases.

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