The newly discovered functions of RECS1 in the regulation of lysosomal calcium and cell death.

To directly study the regulation of cell death triggered by different conditions, we conducted an analysis of cell death pathways. RECS1 overexpression or starvation results in decreased lysosomal pH (∼4.5). Under stress conditions, LMP leads to the cytosolic translocation of lysosomal enzymes causing cell death. Lysosomes are also intracellular calcium reservoirs. Lysosomal calcium signaling is mediated by at least three different processes of the cell. For their optimal function, calcium signaling is involved in the etiology of many diseases, including lysosomal storage disorders, neurodegenerative diseases, and cancers. Since RECS1 is a member of the TMBIM family of apoptosis regulators and localizes to the lysosome, we assessed its function in the regulation of cell death triggered by different stress conditions. To this end, we conducted an

![Figure 1](image-url)

**Figure 1** The newly discovered functions of RECS1 in the regulation of lysosomal calcium and cell death.

(A) Lysosomes are membrane-enclosed cytoplasmic organelles, functioning as the main intracellular catabolic compartment. In the lysosomal lumen, biomolecules are degraded by hydrolase enzymes that function at very acidic pH (∼4.5). Under stress conditions, LMP leads to the cytosolic translocation of lysosomal enzymes, lysosomal biogenesis, and cell death. RECS1 overexpression or starvation results in decreased lysosomal pH (∼4.5). However, the lysosomal membrane comprises dozens of integral and peripheral proteins of unknown functions. The identification of new regulators of lysosomal biology is essential to understand the role of lysosomes in the global regulation of adaptive and pro-death responses, and their close connection to cell metabolism.

RECS1 (responsive to centrifugal force and shear stress 1) [also known as transmembrane bcl-2-associated X protein (BAX) inhibitor motif containing 1 (TMBIM1)] is a member of the TMBIM superfamily, which consists of at least six evolutionarily conserved proteins with homologs in viruses, bacteria and plants. The proteins of the TMBIM family localize to different intracellular membranes, including the endoplasmic reticulum, Golgi apparatus, and mitochondria, and they regulate stress-induced cell death and lysosomal homeostasis (Rojas-Rivera and Hetz, 2015). The resolution of the crystal structure of the TMBIM ortholog from *Bacillus subtilis*, Bset1, together

with additional functional studies in human cells, have revealed that the proteins of the TMBIM family are seven-transmembrane, pH-sensitive calcium channels (Chang et al., 2014). RECS1 localizes to endosomes and lysosomes, suggesting that it may be involved in the regulation of lysosomal calcium homeostasis. RECS1 also regulates apoptosis in response to external stimuli; raising the possibility that its location at the lysosome membrane is implicated in the regulation of cell death. In our recent study (Pihan et al., 2021a), we have uncovered a novel function for RECS1 in the regulation of lysosomal pH, calcium homeostasis, and cell death. RECS1 overexpression triggered cell death through a crosstalk with the canonical mitochondrial pathway of apoptosis. These results suggested the identification of the first proapoptotic component of the TMBIM family. One of the main problems to accurately measure lysosomal intraluminal calcium concentration is that the affinity of commonly used calcium fluorescent probes (i.e. Fura-2) varies non-linearly with proton concentration at pH below 6. To circumvent this issue, calcium and pH must be determined simultaneously in each lysosome, allowing the precise assessment of the effects of luminal pH on the affinity of the calcium probe (K$_d$) (Christensen et al., 2002). Using a simplified version of this method (Pihan et al., 2021b), we measured lysosomal pH and calcium in cells overexpressing RECS1. We found that RECS1 overexpression increased lysosomal acidification, correlating with increased lysosomal intraluminal calcium concentration. These results suggest that RECS1 may be a novel lysosomal calcium channel, regulating resting lysosomal Ca$^{2+}$ and H$^+$ concentrations (Figure 1B, left). To directly determine RECS1’s channel conductance, we expressed the protein in *Xenopus laevis* oocytes and carried out electrophysiological studies at different pH values. Interestingly, we found that RECS1 exhibited spontaneous single-channel current spikes at neutral pH, which decreased dramatically when the pH was lowered to 6.5. Our results showed that RECS1 is permeable to Ca$^{2+}$ and Na$^+$, identifying a new lysosomal cation channel that regulates intraluminal pH and calcium concentration. Interestingly, as also shown in other members of the TMBIM family, the calcium conductivity of RECS1 is regulated by a conserved di-aspartyl pH sensor motif contained in its C-terminal domain. Ablation of this motif by mutagenesis significantly reduces the conductivity of the RECS1 channel. What cellular processes are regulated by RECS1 through calcium signals? We found that RECS1 overexpressing cells showed an increased accumulation of autophagosomes and a higher susceptibility to stress.

Lysosomal-mediated cell death pathways are poorly understood. However, lysosomes are considered an attractive therapeutic target for cancer, because the induction of LMP represents an effective strategy against a variety of different cancers. Since RECS1 is a member of the TMBIM family of apoptosis regulators and localizes to the lysosome, we assessed its function in the regulation of cell death triggered by different stress conditions. To this end, we conducted an

**Philippe Pihán, Mateus Milani, Claudio Hetz**

For a long time since their discovery by Christian de Duve in the 1950s, lysosomes have been referred to almost exclusively as passive garbage bags; the endpoint in the degradation of intra- and extracellular cargo. The catalytic function of lysosomes is accomplished by an array of more than 60 acid hydrolases, which together break down a wide variety of biological macromolecules, including proteins, lipids, carbohydrates, and nucleic acids, for reutilization in the metabolic processes of the cell. For their optimal function, these enzymes require an acidic intraluminal pH of ∼4.5, which is maintained by the joint action of a proton pump, the vacuolar H$^+$-ATPase, and several ion channels embedded in the lysosomal limiting membrane. Nowadays, lysosomes are envisioned as complex signaling hubs, integrating diverse stimuli about the cell’s metabolic status to coordinate different adaptive responses (Ballabio and Bonifacino, 2020). The lysosome can also induce cell death signals in response to certain conditions, such as infections and treatment with lysosomotropic drugs, which leads to lysosomal membrane permeabilization (LMP) and the release of cathepsins, resulting in lysosomal-mediated cell death (Figure 1A, left). Lysosomes are also important intracellular calcium reservoirs. Lysosomal calcium plays essential functions in several cellular processes, such as lysosomal fusion with other vesicles, lysosomal biogenesis, and exocytosis (Figure 1A, right). In addition, lysosomal calcium is critical for lysosomal acidification, probably through the establishment of physical contacts with the endoplasmic reticulum. As a signaling molecule, calcium release from the lysosome through the transient receptor potential cation channel, mucolipin subfamily member 1 (TRPML1) activates the autophagic signaling pathway through the transcription factor EB (TFEB), which upregulates genes involved in autophagy and lysosomal biogenesis. Only three main types of lysosomal Ca$^{2+}$ channels have been identified: the transient receptor potential channels of the TPC family; the trimeric Ca$^{2+}$-two-transmembrane channel P2X$_7$. However, the lysosomal membrane comprises dozens of integral and peripheral proteins of unknown functions. The identification of new regulators of lysosomal biology is essential to understand the role of lysosomes in the global regulation of adaptive and pro-death responses, and their close connection to cell metabolism.
unbiased screening with a library of commonly used chemotherapeutic compounds. We found that RECS1 overexpression sensitized cells to a variety of different drugs, including microtubule destabilizers, while inhibiting the effects of DNA damaging agents. However, the most potent sensitizing compounds were chloroquine (CQ) and hydroxychloroquine (HCQ), two lysosomotropic agents that accumulate in the lysosome and lead to increased lysosomal swelling, membrane rupture, and cell death (Figure 1B). This showed that overexpressing RECS1 were sensitized to these drugs, while wild-type cells were completely resistant. To gain mechanistic insights into RECS1’s function, we investigated whether CQ treatment triggers LMP in RECS1 overexpression cell lines. We found that RECS1 induced LMP in response to CQ treatment. Of note, we observed the translocation of the proapoptotic protein BAX to the lysosomal membrane. BAX is a member of the BCL-2 family of apoptotic regulators that forms pores in the mitochondrial outer membrane during the intrinsic pathway of apoptosis, and may also trigger LMP (Bové et al., 2014). These results indicated that RECS1 triggers lysosomal-mediated death in cells treated with lysosomotropic agents and chemotherapeutic compounds. Interestingly, though, we observe that the expression of RECS1 at the levels of RECS1 – and possibly other lysosomal calcium channels may determine the sensitivity thresholds of cancer cells to lysosomotropic agents, a hypothesis we are currently exploring. Currently, CQ and HCQ are the only clinically available drugs used to inhibit autophagy and are being used as co-adjuvant in combination with a wide variety of chemotherapeutic compounds for various types of cancers such as glioblastoma, melanoma, and pancreatic adenocarcinoma ( Levy et al., 2020). The discovery of RECS1 chemicals as direct drivers of pathological features in several neurological diseases, probably through the modulation of the autophagic pathway. The accumulation of abnormal lysosomes and their permeabilization has also been reported in Parkinson’s disease (Bové et al., 2014). Thus, research on RECS1 calcium effects in cancer cells, in particular, indicating or blocking LMP with pharmacological approaches has the potential to reverse several pathophysiological features of neurodegenerative diseases. The design and identification of RECS1 channel inhibitors represent a new putative therapeutic strategy in cancers previously reported for BAX (Hetz et al., 2005).

In conclusion, aberrations in lysosomal calcium signaling are implicated in the etiology of many types of neurological and autoimmune diseases. For example, aberrant lysosomal calcium signaling has been implicated as a pathological driver of Alzheimer’s disease, which leads to impairments in autophagy, amyloid precursor protein, yielding amyloid β peptides –leads to impairments in autophagy, causing the accumulation of incompletely degraded substrates in the lysosome. In fact, PS1 is believed to promote the maturation and lysosomal localization of the vacuolar H⁺-ATPase V0a1 subunit, which is essential for proper lysosomal acidification. However, mutations in PS1 lead to V0a1 instability and degradation, resulting in decreased luminal calcium (FM (2008) Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium and pH by dextran-conjugated fluorescent dyes. Nat Med 14:1247-1255.)

**References**

Ballabio A, Bonifacino JS (2020) Lysosomes as dynamic regulators of cell and organismal homeostasis. Nat Rev Mol Cell Biol 21:101-118.

Bové J, Martínez-Vicente M, Dehay B, Perier C, Racens A, Bombrun A, Antonsson B, Vila M (2014) BAX channel activity mediates lysosomal disruption linked to Parkinson disease. Autophagy 10:889-900.

Coen G, Maragos RS, Baron S, Grano-Lacroix LR, Wang D, Vermeire W, Michiels C, Munk S, Baert Y, Supta S, Wyuat F, Hiesinger PR, Grinstein S, Annaert W (2012) Lysosomal calcium homeostasis defects, not proton pump defects, cause endo-lysosomal dysfunction in PS1 deficient cells. J Cell Biol 198:23-35.

Chang Y, Bruni R, Kloss A, Klippelmann E, Rost B, hendrickson WA, Lu A (2014) Structural basis for a pH-sensitive calcium leak across membranes. Science 345:112-115.

Christensen KA, Myers JT, Swanson JA (2002) pH-dependent regulation of lysosomal calcium in macrophages. J Cell Sci 114:609-617.

Hetz C, Vitte PA, Bombrun A, Rostovtseva TV, Montessuit S, Hiver A, Schwarz MK, Church DJ, Korsmeyer SJ, Martuñez JC, Antonsson B (2005) Bax channel inhibitors prevent mitochondrial-mediated apoptotic cell death in a model of global brain ischemia. J Biol Chem 280:42960-42970.

Lloyd-Evans E, Morgan AI, He X, Smith DA, Elliott-Smith E, Silence DJ, Churchill GC, Schuchman EH, Gallione A, Platt FM (2008) Niemann-Pick disease type C1 is a sphingosine storage disease that causes deregulation of lysosomal calcium. Nat Med 14:1247-1257.

Lloyd-Evans E, Waller E (2020) Lysosomal Ca²⁺ homeostasis and signaling in health and disease. Cold Spring Harb Perspect Biol 12:amr0646.

Pereira RM, Stykovskaya S, Nicolay BN, Ross KN, Fitamant J, Boukhali M, Lengrand J, Despande V, Selig MK, Ferrone C, Cottell MMP, Stevens J, Sastre J, Dyson N, Zhou F, Ramsawamy S, Haas WA, Bardeesy N (2015) Transcriptional control of autophagy-lysosome function drives pancreatic cancer metabolism. Nature 524:361-365.

Phan P, Lisson F, Borgonovo J, Edwards-Jorquera S, Nunes-Hasperl P, Castillo K, Kepp O, Urra H, Saarimäki S, Vihinen H, Carreras-Suárez A, Forfelli S, Savoia A, De Giorgi D, Pupo A, Rodriguez DA, Quarto G, Sagedo A, Lourido F, Letai F, et al. (2021a) Control of lysosomal-mediated cell death by the pH-dependent calcium channel RECS1. Sci Adv 7:eabe4569.

Phan P, Nunes-Hasperl P, Demaurex N, Hetz C (2012b) Lysosomal-to-autophagic calcium and pH by dextran-conjugated fluorescent dyes. Methods Cell Biol 165:199-208.

Rojas-Rivera D, Hetz C (2013) TMBIM protein family: ancestral regulators of cell death. Oncogene 34:269-280.

P-Reviewer: Martino S; C-Editors: Zhao M, Liu WJ, Wang Lu; E-Editor: Jia Y

Program of Cellular and Molecular Biology, Institute of Biomedical Sciences, University of Chile, Santiago, Chile (Phihan P, Milani M, Hetz C) Buck Institute for Research on Aging, Novato, CA, USA (Hetz C)

**Correspondence to:** Claudio Hetz, Ph.D., chetz@uchile.cl.

https://orcid.org/0000-0003-1120-7966 (Claudio Hetz)

**Date of submission:** November 19, 2021

**Date of decision:** January 7, 2022

**Date of acceptance:** January 18, 2022

**Date of web publication:** April 29, 2022

https://doi.org/10.4103/1673-5374.339487

**How to cite this article:** Phihan P, Milani M, Hetz C (2022) Lysosomes: RECS1: a required gateway to lysosomal dysfunction and death. Neural Regen Res 17(12):2695-2696.

**Open access statement:** This is an open access journal, and articles are distributed under the terms of the Creative Commons AttributionNonCommercialShareAlike 4.0 License, which allows others to remix, tweak, and build upon the work non-commercially, as long as appropriate credit is given and the new creations are licensed under the identical terms.

**Open peer reviewer:** Sabato Martino, University of Perugia: Università degli Studi di Perugia, Italy.