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Mitochondrial Ca^{2+} homeostasis in lysosomal storage diseases

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Abstract

Lysosomal storage diseases (LSDs) are a class of genetic disorders in which proteins responsible for digestion or absorption of endocytosed material do not function or do not localize properly. The resulting cellular “indigestion” causes buildup of intracellular storage inclusions that contain unprocessed lipids and proteins that form macromolecular complexes. The buildup of storage material is associated with degenerative processes that are observed in all LSDs, albeit the correlation between the amount of storage inclusions and the severity of the degenerative processes is not always evident. The latter suggests that a specific mechanism set in motion by aberrant lysosomal function drives the degenerative processes in LSDs. It is becoming increasingly clear that in addition to their function in degrading endocytosed material, lysosomes are essential housekeeping organelles responsible for maintaining healthy population of intracellular organelles, in particular mitochondria. The present review surveys the current knowledge on the lysosomal-mitochondrial axis and its possible role as a contributing factor to mitochondrial Ca^{2+} homeostasis and to cell death in LSDs.

1. The endocytotic machinery and lysosomal storage diseases

Endocytosis is a complex process that allows cells to internalize small fragments of the plasma membrane and extracellular medium [1]. Much like the food in our digestive tract, the endocytosed material passes through a series of intracellular compartments that sort it into the lysosomes for digestion and absorption. Endocytosis starts with invagination of the plasma membrane that is followed by fission of the endocytic vesicles and their consolidation into early endosomes (Fig 1). At this point, some membrane proteins are recycled back to the cell surface, while those destined for degradation are tagged by mono-ubiquitin and together with fluid-phase proteins, are delivered to late endosomes through early endosomes [2]. The lumens of late endosomes contain vesicular structures formed by invagination of the late endosomal membrane and it is likely that lipids destined for degradation are sorted into these inclusions. The inclusions may also improve digestion efficacy by increasing the membrane surface area that is accessible to digestive enzymes.

Lysosomes are small vesicular organelles that fuse with late endosomes and absorb and digest their content [3,4]. In the lysosomes, degradation is performed by a variety of lipid and protein hydrolases. Mutations that inactivate lysosomal hydrolases result in storage of endosomal material in the lysosomes (Fig 2). There is a range of lysosomal storage conditions that occur due to mutations in different hydrolases, but all of them are characterized by a buildup of

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undigested lipids, proteins or sugars; examples include mucopolysaccharidoses [5] and gangliosidoses [6].

Soluble lysosomal hydrolases are delivered to lysosomes via late endosomes due to the interaction of hydrolases with mannose 6-phosphate receptors that shuttle between the Golgi and late endosomes [7]. Mutations in lysosomal hydrolases that render them unable to bind to the mannose 6-phosphate receptors result in their secretion into the extracellular space instead of being targeted to lysosomes. Examples of diseases with this type of aberrant trafficking of lysosomal hydrolases are Mucopolysaccharidoses II and IIIc [8].

Lysosomal hydrolases are themselves subject to proteolytic degradation. Some hydrolases are protected by binding to a scaffold that contains the protective protease cathepsin A [9,10]. Such a protective mechanism likely involves cleavage and inactivation of nearby proteases before they can inactivate the cathepsin A-protected proteases. It has been estimated that binding to the cathepsin A scaffold increases the half life of a protease from minutes to several hours [9,10]. No such protective mechanism has been demonstrated for lipases. Cathepsin A deficiency leads to inactivation of several lysosomal hydrolases. This results in a lysosomal storage disease (LSD) known as galactosialidosis [11].

Excretion of hydrolytic products through the lysosomal membrane is mediated by specialized transporters. When the extrusion process fails, specific digestion products accumulate in the lysosomal lumen. Several of these products are chemically active and their buildup may lead to formation of high molecular weight complexes inside the lysosomes. This was shown to occur in cystinosis, which result from impaired cystine excretion from the lysosomes to the cytoplasm [12,13].

Lysosomal pH is in the 4.5–5 range. Most lysosomal hydrolases display a bell shaped pH dependence with a pH optimum around 5; hence the term acid hydrolases [14,15]. Under- and over-acidification, both inactivate the acidic hydrolases. Lysosomes are acidified by the vacuolar-type H^+ ATPase that pumps H^+ into the lysosomes and against their concentration gradient. Lysosomal acidification is determined by the balance between the rates of H^+ pumping into the lysosomal lumen and H^+ leak out of the lysosomes. The molecular identity of a lysosomal H^+ efflux or leak pathway that prevents over-acidification is not known at present. We suggested that TRPML1 the lysosomal that is defective in the LSD mucopolipidosis type IV (MLIV), provides the lysosomal H^+ leak pathway [16]. However, the original assertion that TRPML1 is a H^+ channel may not be correct. A recent study failed to see any channel function of wild-type TRPML1, and analyzed the activity of TRPML1 that was converted to a spontaneously active channel by the TV423P mutation [17]. The V423P mutation is equivalent to the gain-of-function A419P mutation in TRPML3 [18,19], that causes the varitint-waddler phenotype. TRPML1(V423P) functions as a inward rectifying nonselective cation channel that is activated by H^+ [17]. We were able to confirm the behavior of TRPML1(V423P) and thus the outwardly rectifying conductance previously reported by us and others [16,20–22] likely reflects misidentification of TRPML1 conductance or upregulation of endogenous channel by expression of TRPML1. It should be noted that the activity of wild-type TRPML1 may not be identical to that of TRPML1(V423P) and a way to activate wild type TRPML1 has to be found so that the function of this channel can be characterized towards understanding its role in lysosomal function and MLIV.

The H^+ ATPase is electrogenic and its ability to pump H^+ is limited by the buildup of positive charge inside the lysosomes. The positive charge buildup is neutralized by passive anion flow through Cl^- channels, which allows H^+ pumping to continue [23]. Inhibition of the Cl^- conductance in the endocytic pathway by pharmacological and genetic means inhibits

lysosomal acidification. Loss of the lysosomal Cl^- channels CIC-6 and CIC-7 leads to a lysosomal storage phenotype in mice and humans [24,25].

Another vitally important cellular process in which lysosomes play a key role is autophagy, the act of digestion by the cell of its own organelles and cytoplasmic content (Fig 1) [26,27]. This highly complex process is associated with a *de-novo* formation of autophagic vesicles that envelope the doomed organelles or regions of the cytoplasm. The autophagic process is coordinated by a system of several protein complexes that we are only beginning to enumerate and define their composition and roles. Some of the markers of autophagic vesicles that are indispensable for this process include the autophagic light chain 3 (LC3) and lysosome-associated membrane protein 2 (LAMP2) [28,29]. Ablation of elements of the autophagic machinery disrupts the autophagy and results in neurodegeneration [30,31]. The basal autophagy is responsible for utilization of old and damaged organelles. Autophagy is triggered by cell stress such as starvation, which restricts substrates availability for energy production, leading to supplements of energy needs by degrading organelles and cytoplasmic content [32–34]. Whilst undeniably having a major housekeeping function, autophagy also is thought to mediate a specialized form of cell death, that is distinct from necrosis and apoptosis [35].

Several aspects of the aberrant lysosomal function in lysosomal storage diseases (LSDs) await clear identification, and the products of several genes mutated in LSDs have not been ascribed a specific function. The former include the complex machinery responsible for the fusion and fission events in the endocytic pathway. The fusion of organelles within the endocytic pathway propels the endocytosed material along the cellular digestive tract and is also responsible for the delivery of enzymes to the endocytic organelles. The fusion seems to require members of the Rab family of small GTP binding proteins and components of the SNARE complexes [36,37]. Like all SNARE-driven processes, fusion in the endocytic pathway is mediated by several Ca^{2+} -dependent reactions. This information taken together with the observations of lipid traffic delays in fibroblasts from MLIV patients [38] and the reports that TRPML1 may have Ca^{2+} permeability [39], led to the suggestion that TRPML1 releases lysosomal Ca^{2+} and drives the fusion of lysosomes with late endosomes. However, lysosomal Ca^{2+} content appears to be the same in wild-type and TRPML1 deficient MLIV patient fibroblasts [16] and divalent ions appear to inhibit the current of TRPML1(V423P) [17]. In addition, the same lipid traffic delays were reported in LSDs whose main causes have not been directly linked to membrane traffic [40].

TRPML1 was also suggested to play a role in the NAADP-induced Ca^{2+} release from lysosomes [41]. This possibility definitely warrants further investigation in the light of the failure of a previous report to detect an increase in NAADP binding in TRPML1 enriched membrane fractions [42]. Whether such discrepancy indicates that NAADP binding and the Ca^{2+} release function resides in different molecules is one of the many interesting questions raised by these findings.

Examples of genes with unknown function that have been implicated in LSDs include members of the transmembrane proteins of the CLN family whose dysfunction causes a set of diseases collectively called Batten disease or ceroid lipofuscinoses [43]. CLN3 has been suggested to regulate lysosomal pH [44], while the function of CLN5 through 9 is unknown. Other examples of LSDs whose mechanisms have not been completely settled include Niemann-Pick disease [45].

2. Lysosomal storage diseases and neurodegeneration

Most LSDs are associated with neurodegenerative processes that affect some regions of the brain with remarkable specificity. For example, mice with deletion of NPC1 (a model of Niemann-Pick C) display little neuronal loss in the prefrontal cortex or the thalamus, while

there is a severe loss of Purkinje cells in the cerebellum [46]. Similar profile was detected in the sialidosis and the galactosialidosis mouse models [47]. A pronounced Purkinje cell loss was also detected in a mouse model of late infantile neuronal ceroid lipofuscinosis (a variant of Batten disease) [48].

The region specificity of the neurodegeneration in LSDs can be explained by postulating that a vitally important biochemical pathway specific to the affected regions of the brain is specifically inhibited by the disease. Such an explanation does not account for the similarities in the neurodegenerative profiles of diseases whose basic causes are different (e.g. Niemann-Pick and late infantile neuronal ceroid lipofuscinosis). It is, therefore, likely that the specificity of neurodegeneration in LSDs is determined by the affected brain tissue and by its ability to withstand a dual insult from defective lysosomal function and stimulation history. There are indications in several LSDs that such dual insults converge on vitally important organelles, the mitochondria.

3. The mitochondria-lysosome axis

Mitochondria are unique in that they integrate a number of vitally important cellular functions. Their membranes harbor the enzymes chain responsible for the oxidative reactions and energy production; liberation of cytochrome C from the mitochondrial matrix through the transition pore triggers the caspase 9-dependent branch of apoptosis; and Ca^{2+} uptake by the mitochondrial Ca^{2+} uniporter buffers cytoplasmic Ca^{2+} and negates the pro-apoptotic effects of the Ca^{2+} spikes. These topics are extensively discussed in several reviews in this special issue.

Perturbations of the mitochondrial architecture or number dramatically affect cell function. Mitochondrial shape, size and number are defined by a balance between fusion and fission of mitochondria. The fission is driven by a complex, or complexes of proteins that include the dynamin related protein Drp1, Fis1, Sumo and others [49,50]. Their inhibition induces formation of abnormally long mitochondria. Mitochondrial fragmentation driven by Drp1 and Fis1 precedes apoptosis [51,52]. Mitochondrial fusion is driven by Opa1, Mitofusin and possibly other proteins [51,53,54].

Whether mitochondrial lability that affects apoptosis directly affects their Ca^{2+} buffering function has not been systematically explored. Our findings show that mitochondrial fragmentation in cells from patients with LSDs and mitochondrial fragmentation brought about by treatment with the inhibitors of lysosomal or autophagic activity suppresses Ca^{2+} uptake by the mitochondria [55]. Similar reduction in mitochondrial Ca^{2+} uptake due to mitochondrial fragmentation was reported in cells treated with hepatitis B virus X protein [56]. Drp1-mediated mitochondrial fragmentation blocked mitochondrial Ca^{2+} wave propagation [57,58].

Since mitochondria are involved in apoptosis as well as in Ca^{2+} buffering and Ca^{2+} wave propagation, it is not surprising that the relation between mitochondrial fragmentation and apoptosis is complex. Thus, mitochondrial fragmentation induced by Drp1 overexpression specifically protected the cells against Ca^{2+} -dependent apoptosis [57,58]. At the same time, suppression of mitochondrial fission retards apoptosis induced by several signals in, apparently, Ca^{2+} -independent manner [51,52] and mitochondrial fusion driven by Opa1, Mitofusin and other proteins that elongates mitochondria has anti-apoptotic effect [51,53,54]. Therefore, the protective effect of both mitochondrial fission and fusion suggest that the extent to which mitochondrial fragmentation enhances pro-apoptotic insults depends on the nature of the pro-apoptotic stimulus as well as the cell type.

The robust oxidative processes accompanied by production of reactive oxygen species cause, with time, an extensive damage to the mitochondria. Reactive oxygen species are generated

by several components of the oxidative chain [59] and may also include extra-mitochondrial elements such as the 66 KDa isoform of the growth factor adapter Shc (p66Shc), that when phosphorylated translocates into the mitochondria [60]. In the mitochondria, electron transfer between p66Shc and cytochrome c generates reactive oxygen that damages the mitochondria and triggers mitochondria dependent apoptosis [61]. The reactive oxygen species damage the mitochondrial membrane by modifying its lipid and protein components [59]. The damaged mitochondria have to undergo remodeling. Mitochondrial remodeling is driven by autophagy. Inhibition of autophagy is associated with accumulation of fragmented mitochondria [32,55], which suggests that autophagy of the mitochondria is preceded by fragmentation, perhaps to facilitate the engulfment of the mitochondria by autophagic vacuoles.

The role of the lysosomes in mitochondrial renewal has been known for a while. The first complete model that established the mitochondria-lysosome axis was formulated using aged post-mitotic cardiac myocytes [62]. Lysosomal deficiency in such cells is caused by lysosomal iron buildup. The intestinal brush border and hepatocytes uptake ferrous iron directly by the divalent ion transporter NRAMP2 (SLC12A2) [63,64]. However, most other cells endocytose iron bound to proteins such as transferrin and uncage the endocytosed iron in the lysosomes. The liberated iron is transported out of the lysosomes by lysosomal NRAMP2 [63,64]. While in the lysosomal lumen, the chemically active iron catalyzes the Fenton reactions. This results in the peroxidation of lipids in the lysosomal lumen with the ensuing accumulation of an inert pigment called lipofuscin [65]. Lipofuscin deposits were thought to only induce lysosomal deficiency. However, the original mitochondria-lysosome axis model established a connection between lipofuscin buildup and mitochondrial dysfunction in aged cells. It is now established that lipofuscin buildup is associated with mitochondrial fragmentation, likely because lipofuscin-filled lysosomes disrupts autophagy of the mitochondria [62]. By analogy with these findings, we postulated that lysosomal deficiency in LSDs should also bring about mitochondrial fragmentation [55,66]. This was tested using human skin fibroblasts from patients with several unrelated LSDs: MLIV, mucopolidoses II and III and late infantile neuronal ceroid lipofuscinosis. Indeed, all cell lines affected by LSD showed increased number of shorter mitochondria and decreased number of long mitochondria [55].

That the observed mitochondrial fragmentation is a result of impaired lysosomal function and autophagy was established by testing the effects of inhibiting autophagy and lysosomal function by pharmacological means. In cardiac myocytes, HeLa cells and fibroblasts, inhibition of lysosomal function and of autophagy causes mitochondrial fragmentation similar to that observed in aged cell or cells affected by LSDs [32,55]. These findings suggest similarity between the mitochondrial phenotype in aged cells and cells with LSD that may stem from aberrant lysosomal function that, in turn, affect autophagy and result in a buildup of fragmented effete mitochondria (Fig 3).

Mitochondrial abnormalities have been shown in many LSDs. Aberrant mitochondrial appearance and energy production have been reported in several models of Batten disease (reviewed in [33]), the cystine over-load model [67], Niemann-Pick disease [68] and neurodegenerative diseases like Parkinson's disease, Alzheimer's disease and amyotrophic lateral sclerosis [69]. The commonality of mitochondrial abnormalities across the spectrum of LSDs suggests a shared cause that translates lysosomal deficiencies into mitochondrial abnormalities. Such a common cause may be impaired autophagy, a deficit of molecules used to build mitochondria due to inefficient utilization of endocytosis products, or it may be the result of pre-apoptotic state of cells affected by LSDs. In support of the former notion is the fact that suppression of autophagy by genetic ablation of the major regulators of autophagy in mice causes a neurodegenerative phenotype similar to that of LSDs [30,31]. Obviously, further investigation of the connection between lysosomal storage and mitochondria is warrant.

The relation between lysosomal deficiencies and mitochondrial aberrations in LSDs discussed above may explain the specificity of the neurodegenerative impact of LSDs. Indeed, several studies have shown that the most metabolically active tissues of the brain are the most prone to neurodegeneration in LSDs, exactly because such tissues are the most sensitive to abnormal mitochondrial function (reviewed in [33]). An exacerbating and complimentary cause for the cell death in LSDs is the involvement of the mitochondria in cytoplasmic Ca^{2+} signaling and regulation of Ca^{2+} spiking.

4. Mitochondrial Ca^{2+} in lysosomal storage diseases

According to the dual insult model, lysosomal dysfunctions in LSDs cause cells to be more sensitive to stress. Abnormal mitochondrial function that takes place in LSDs is one component of the dual insult. The second component is oxidative stress or cytoplasmic Ca^{2+} spiking during cell stimulation. This idea is supported by solid evidence that were reviewed before [33].

The role of the mitochondria in Ca^{2+} signaling that confers the uniquely important status to the mitochondria in cell signaling and cell death is their ability to uptake cytoplasmic Ca^{2+} and function as cellular Ca^{2+} buffers. The close to 200 mV negative membrane potential of healthy mitochondria drives Ca^{2+} from the cytoplasm into the mitochondrial lumen through a Ca^{2+} uniporter that resides in the inner mitochondrial membrane. It is due to the activity of this uniporter that mitochondria serve a protective role by taking up Ca^{2+} that enters the cells through Ca^{2+} channels (reviewed in [70], [71]) during large cytoplasmic Ca^{2+} spikes [72]. In polarized cells, mitochondria are located in critical cellular zones to protect these zones against Ca^{2+} tides. This is well documented in secretory cells where mitochondria confine the Ca^{2+} spikes to the apical part of the cell [73] and prevents flooding the nucleus with Ca^{2+} [73,74]. In neurons mitochondria are clustered in active Ca^{2+} signaling zones along axons and dendrites [75–77]. These examples emphasize the importance of the mitochondria in shielding the cells from pro-apoptotic effects of sustained elevated cytoplasmic Ca^{2+} . This role of the mitochondria is supported on a functional level by our observation that cells affected by LSDs are significantly more sensitive to pro-apoptotic effects of Ca^{2+} stimulation [55]. It is possible that the mitochondrial fragmentation in cells affected by LSDs and the increased sensitivity of the cells to the pro-apoptotic effects of Ca^{2+} are coincidental rather than consequential. However, the finding that the same results were observed in unrelated LSDs argues in favor of consequential effects.

Once again, the complex role played by mitochondria in cell function seems to underlie a seemingly conflicting set of effects of mitochondrial Ca^{2+} . Thus, mitochondrial Ca^{2+} overload is known to induce apoptosis by destabilizing the mitochondrial membrane and releasing cytochrome C. This has been documented in many cell types and is thought to be a major contributing factor to cytotoxicity in neurons. Accordingly, the uncoupler FCCP that collapses mitochondrial membrane potential and prevents mitochondrial Ca^{2+} overload has a protective effect [78]. Although these findings appear to conflict with the model discussed above, in which the lack of cytoplasmic Ca^{2+} clearance by the mitochondria enhances the pro-apoptotic effects of Ca^{2+} stimulation, it is likely that the two scenarios coexist and come into effect under various modes of stimulation. For example, the “ Ca^{2+} overload” scenario may be responsible for cell death during acute intense stimulation, while the predisposition of the cells to apoptosis due to the “lack of Ca^{2+} uptake” scenario may be responsible for chronic processes in which cells deteriorate as a result of repetitive stimulation at physiological intensity. These scenarios may explain the different profiles of cell death during acute insults, such as stroke, and the slow onset “fading away” reported in LSDs. As such, the two models are probably complimentary, rather than conflicting. The role of both, reduced mitochondrial Ca^{2+} uptake and of mitochondrial Ca^{2+} overload in cell death is well established and is the subject of several

reviews in this issue and elsewhere. Therefore the discussion here is limited to aberrant mitochondria Ca^{2+} handling in Ca^{2+} dependent excitotoxicity in LSDs.

Several observations can explain why the reduction in mitochondrial Ca^{2+} buffering in LSDs kills cells. First, in Batten disease subunit c of the Fo complex of the ATP synthase is released from the mitochondria and found in the lysosomes [33]. The subunit c buildup is associated with a decrease in ATP production capacity that is exacerbated by the loss of Ca^{2+} stimulation of mitochondrial respiratory chain [33]. The “energy linked excitotoxic hypothesis” postulates that the effete mitochondria that accumulate in cells affected by LSDs do not produce enough ATP and it is the lack of ATP production in highly energy dependent tissues that is responsible for the region specific cell death pattern in LSDs observed in the brain. Furthermore, a loss of Ca^{2+} buffering by mitochondria compromise the ability of the cells to protect themselves against large Ca^{2+} tides and Ca^{2+} becomes toxic to the cells. The cells may attempt to adapt by reduction in the activity of Ca^{2+} channels. Recent work showed that CLN3 deficient neurons display reduced Ca^{2+} peak and plateau in response to depolarization [79]. The reduced Ca^{2+} plateau could be attributed to a reduction in the activity of the N-type Ca^{2+} channels, since inhibition of the channels by GVIA resulted in similar plateau in wild-type and CLN3 deficient neurons [79]. The reduced peak may represent reduction in the activity of another Ca^{2+} influx channel or reduction in Ca^{2+} -induced Ca^{2+} release. Reduction in Ca^{2+} influx and in peak Ca^{2+} reduces Ca^{2+} uptake by the mitochondria during fluctuations in cellular Ca^{2+} when energy demand is very high. The deficit in mitochondrial matrix Ca^{2+} that drives the Ca^{2+} dependent activity of ATP synthase will cause further reduction in ATP production. The resulting low cellular ATP may have toxic effects by depolarizing the membrane to cause “slow-onset” neurotoxicity. Membrane depolarization will cause a relief of the Mg^{2+} -block of the NMDA receptors to further increase basal Ca^{2+} and exacerbate cell toxicity by increasing cell stress [80]. Hence, chronically reduced mitochondria Ca^{2+} uptake in LSDs may set a feed-forward scenario that results in increased cell toxicity and cell death.

We directly investigated the mitochondrial Ca^{2+} buffering capacity in cell affected by MLIV and in control cells whose lysosomal function and autophagy were suppressed by pharmacological means. In all systems examined mitochondrial Ca^{2+} buffering capacity measured by two different assays was suppressed [55]. We concluded that the effete mitochondria that accumulate as a result of suppressed autophagy can not effectively buffer Ca^{2+} . The lack of Ca^{2+} buffering capacity was associated with increased sensitivity to the pro-apoptotic effect of stimulation with Ca^{2+} . We did not observe cytochrome C translocation in response to Ca^{2+} -induced apoptosis, which accompanies mitochondrial transition pore opening. Accordingly, Ca^{2+} -dependent apoptosis was not sensitive to caspase 9 inhibition. Based on these findings we concluded that the Ca^{2+} dependent apoptosis in cells affected by LSDs does not involve mitochondrial permeabilization, cytochrome C release and activation of the caspase 9 dependent apoptotic pathway. Rather, the Ca^{2+} dependent cytotoxicity was associated with activation of the “non-mitochondrial” caspase 8 dependent pathway [55].

We note that the mitochondrial apoptotic pathway has been implicated in cytotoxicity in several LSD models. For example, cytochrome C release accompanied progesterone induced excitotoxicity [81]. Although it is unclear to what extent this effect was specific to cells affected by LSDs, it is possible that both, the caspase 9-dependent and caspase 8-dependent, apoptotic pathways contribute to cell death in LSDs and the degree to which each of the pathways is activated depends on the nature of the apoptotic insult. The apoptotic pathways are flexible and appear to adjust in response to lysosomal storage. Comparative global transcript profiling of mRNA expressed in normal and lysosomal storage diseased brains reveals upregulation and downregulation of a number of important regulatory proteins at the mRNA level [46,79,82]. Interestingly, among the upregulated genes, mRNA coding for elements of the apoptotic cascade (notably Caspase 8) seems to have a measurable delay [82]. The delay may reflect

upregulation of the apoptotic machinery in response to the detrimental effects of buildup of undigested material and/or accumulation of dysfunctional mitochondria.

It is quite remarkable that the mitochondria in cells affected by LSDs do not take up Ca^{2+} during cell stimulation with Ca^{2+} mobilizing agonists such as bradykinin [55]. Since Ca^{2+} uptake into the mitochondria requires close interaction between mitochondria and plasma membrane and between mitochondria and endoplasmic reticulum (ER) boundaries [71,83], the lack of Ca^{2+} uptake by mitochondria of cells affected by LSDs suggests that Ca^{2+} clearance at the plasma membrane/mitochondria and ER/mitochondria domains may be dysregulated. Hence, the important relationships between the mitochondria and the ER and the plasma membrane may be disrupted in cells with LSDs, which may play key role in Ca^{2+} dependent excitotoxicity. The unbuffered high Ca^{2+} microdomains near mitochondria can activate many Ca^{2+} -dependent enzymes, including harmful proteases such as μ -calpains that cleave and activate caspase 8 [84]. Further probing of the relationship between the mitochondria and the ER and plasma membrane may further clarify the abnormal mitochondria Ca^{2+} signaling and its consequences in LSDs.

5. Summary

The present review makes a case for a connection between lysosomal function and mitochondrial status in cells affected by LSDs. Several unrelated LSDs are associated with mitochondrial dysfunction. This, together with the tissue specific degeneration observed in LSDs suggest that a common mechanism is responsible for cell death in most LSDs. Strong evidence implicates abnormal mitochondrial function in this process, in particular the loss of mitochondrial Ca^{2+} buffering. The applicability of this mechanism to tissues other than the brain and skin fibroblasts and to all LSDs requires further investigation.

LSDs lead to cell toxicity and cell death. Identifying and characterizing the mechanisms of cell death in these diseases may have clinical implications. Finding the pathway that links lysosomes and mitochondria will underscore the central role that lysosomes play in maintaining healthy population of organelles. Identifying the cell death pathways responsible for degeneration of cells with LSDs may lead to formulating means to slow down the degenerative processes. Such approaches could be used in combination with enzyme replacement therapy and virus based gene delivery that are currently being developed for the treatment of these groups of diseases.

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Fig 1. The endocytic pathway, lysosomes and autophagy

Endocytosed material first enters endocytic vesicles, which then consolidate into early endosomes. After sorting, the material destined to be degraded is delivered to late endosomes, with which lysosomes fuse in a transient manner to pick up the material for final digestion and absorption. Lysosomes also drive autophagy and eventually fuse with the autophagic vacuole.

Fig 2. Common dysfunctions that lead to lysosomal storage diseases

1: miss-sorting of enzymes may lead to their secretion instead of targeting to lysosomes (e.g. mucopolipidosis II). 2: Non-functional enzymes or supporting proteins do not break down the endocytosed material (e.g. galactosialidosis, Batten (?), Niemann-Pick (?); question marks indicate that the exact mechanism of lysosomal dysfunctions in these disease has not been completely settled). 3: Defective excretion of hydrolyzed products out of the lysosomes (e.g. sialidosis). 4: Defective regulation of lysosomal pH (Mucopolipidosis IV, mutations in CLC and CLN3 (?)).

Fig 3. The lysosome-mitochondria axis

In normal cells, the fragmented aged or damaged mitochondria are delivered by autophagy to the lysosomes for degradation. Aberrant autophagy in LSDs compromise degradation of effete mitochondria.

Fig 4. Possible consequences of the loss of Ca^{2+} buffering in lysosomal storage diseases
 Red arrows represent Ca^{2+} influx, red “blasts” show the “hotspots” of abnormal Ca^{2+} regulation in cells with affected mitochondrial function. 1, 2: Inefficient Ca^{2+} buffering underneath the plasma membrane or in proximity of the mitochondria at the endoplasmic reticulum/mitochondria boundaries. 3: Inhibition of Ca^{2+} dependent processes in the mitochondria with ensuing lack of ATP production. 4: Activation of perimitochondrial Ca^{2+} dependent proteases.