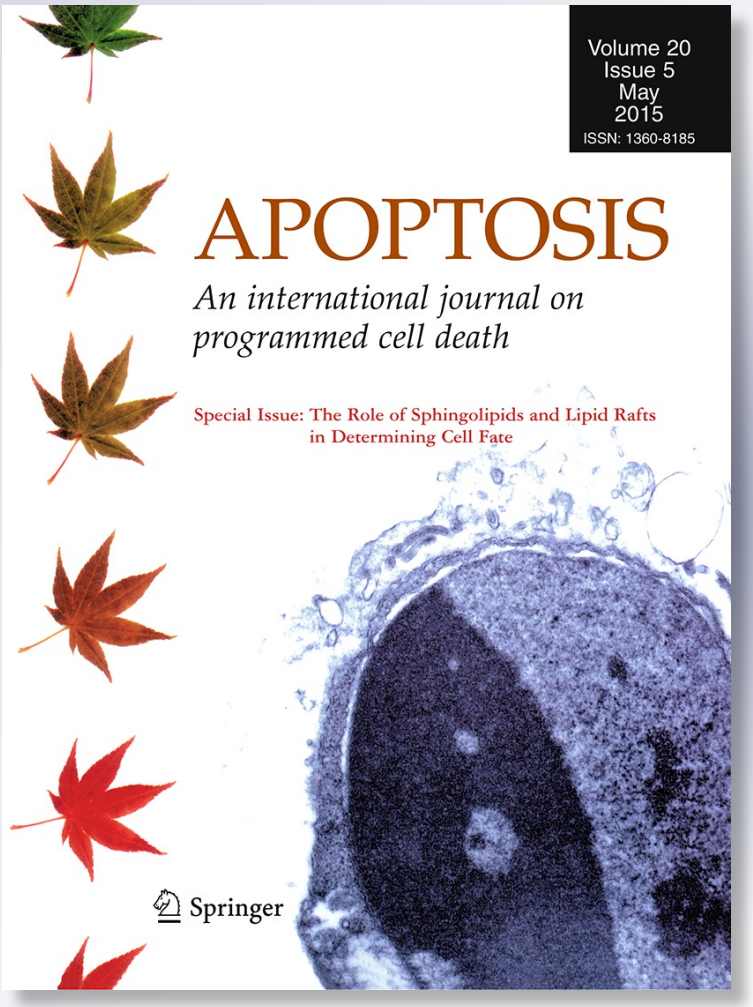
# *Role of mitochondrial raft-like microdomains in the regulation of cell apoptosis*

**Tina Garofalo, Valeria Manganelli, Maria Grasso, Vincenzo Mattei, Alberto Ferri, Roberta Misasi & Maurizio Sorice**

**Apoptosis**
An International Journal on Programmed Cell Death

ISSN 1360-8185
Volume 20
Number 5

Apoptosis (2015) 20:621-634
DOI 10.1007/s10495-015-1100-x




Springer

Apoptosis (2015) 20:621–634
DOI 10.1007/s10495-015-1100-x



# Role of mitochondrial raft-like microdomains in the regulation of cell apoptosis

**Tina Garofalo · Valeria Manganelli · Maria Grasso · Vincenzo Mattei · Alberto Ferri · Roberta Misasi · Maurizio Sorice**

Published online: 5 February 2015


**Abstract** Lipid rafts are envisaged as lateral assemblies of specific lipids and proteins that dissociate and associate rapidly and form functional clusters in cell membranes. These structural platforms are not confined to the plasma membrane; indeed lipid microdomains are similarly formed at subcellular organelles, which include endoplasmic reticulum, Golgi and mitochondria, named raft-like microdomains. In addition, some components of raft-like microdomains are present within ER–mitochondria associated membranes. This review is focused on the role of mitochondrial raft-like microdomains in the regulation of cell apoptosis, since these microdomains may represent preferential sites where key reactions take place, regulating mitochondria hyperpolarization, fission-associated changes, megapore formation and release of apoptogenic factors. These structural platforms appear to modulate cytoplasmic pathways switching cell fate towards cell survival or death. Main insights on this issue derive from some pathological conditions in which alterations of microdomains structure or function can lead to severe alterations of cell activity and life span. In the light of the role played by raft-like microdomains to integrate apoptotic signals and in regulating mitochondrial dynamics, it is conceivable that these membrane structures may play a role in the mitochondrial alterations observed in some of the most common human neurodegenerative diseases, such as Amyotrophic lateral sclerosis, Huntington's chorea and prion-related diseases. These findings introduce an additional task for identifying new molecular target(s) of pharmacological agents in these pathologies.

**Keywords** Lipid rafts · Microdomains · GD3 · Mitochondria · Apoptosis

**Abbreviations**

| | |
|---|---|
| ALS | Amyotrophic lateral sclerosis |
| Bax | Bcl-2-like protein 4 |
| Bid | BH3 interacting-domain death agonist |
| CL | Cardiolipin |
| CLIPR-59 | Cytoplasmic linker proteins-59 |
| DAMP | Danger-associated molecular pattern |
| DLP1/ Drp1 | Dynamin-like protein-1 |
| [D]-PDMP | (±)-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol hydrochloride |
| ER | Endoplasmic reticulum |
| fALS | Familial form of ALS |
| GSK-3b | Glycogen synthase kinase-3b |
| HD | Huntington disease |
| hFis1 | Mitochondrial fission 1 protein |
| Htt | Huntingtin |
| IP3R-1 | Inositol-1,4,5-tris-phosphate receptor |
| MAM | Mitochondria-associated membrane |
| MßCD | Methyl ß-cyclodextrin |
| Mfn2 | Mitofusin-2 |
| OMM | Outer mitochondrial membrane |
| OPA1 | Optic Atrophy 1 |

T. Garofalo · V. Manganelli · M. Grasso · R. Misasi · M. Sorice (✉)
Department of Experimental Medicine, Sapienza University of Rome, Viale Regina Elena 324, 00161 Rome, Italy
e-mail: maurizio.sorice@uniroma1.it

V. Mattei · M. Sorice
Laboratory of Experimental Medicine and Environmental Pathology, University Consortium "Sabina Universitas", Rieti, Italy

A. Ferri
Institute of Cellular Biology and Neurobiology, CNR, Rome, Italy

| | |
|---|---|
| $PrP^{C}$ | Cellular prion protein |
| $PrP^{Sc}$ | Conformationally altered isoform of prionic protein |
| PTPIP51 | Protein tyrosine phosphatase interacting protein 51 |
| Sig1R | Sigma1 receptor |
| SOD1 | Superoxide dismutase 1 |
| t-Bid | Truncated bid |
| TDP43 | Transactive response DNA binding protein 43 |
| VAPB | Vesicle-associated membrane protein-associated protein B |
| VDAC-1 | Voltage-dependent anion channel-1 |

## Lipid rafts and cell apoptosis

Lipid rafts are a dynamic assemblage of sphingolipids, cholesterol and proteins that dissociate and associate rapidly and form functional clusters in cell membranes [1]. These clusters provide highly efficient lipid–protein modules, which operate in membrane trafficking and cell signaling. Indeed, lipid rafts are thought to function as platforms that sequestrate specific proteins, thus introducing and modulating cell signaling [2, 3]. A general function of lipid rafts in signal transduction may be to allow the lateral segregation of proteins within the plasma membrane, providing a mechanism for the compartmentalization of signaling components, concentrating certain components in lipid rafts, including those of importance in apoptosis, and excluding others [4–6].

They have been associated with several cell functions [4–7], including cell death. In particular, the possibility that lipid rafts could be involved in the complex framework instructing the apoptotic cascade has been investigated in a series of works carried out in diverse cell systems [8–10].

In fact, it has been suggested that lipid rafts could play a key role in receptor-mediated apoptosis of T cells [11, 12]. This is apparently due to two events that follow the receptor engagement: (i) the recruitment of CD95/Fas [8, 12, 13] as well as tumor necrosis factor-family receptors [14] to plasma membrane lipid rafts, and (ii) the recruitment of specific proapoptotic Bcl-2 family proteins to mitochondrial "raft-like microdomains" [15, 16].

As a matter of fact, main insights on this issue derive from some pathological conditions providing useful clues on the fact that alterations of microdomains structure or function can lead to severe alterations of cell activity and life span. A good example in this context is represented by the field of cancer research and the development of new therapeutic strategies. In fact, the need to overcome the rather poor outcomes of current cancer chemotherapy stimulated the development of novel targets and drugs. Since apoptotic triggering clearly represents the goal of cancer therapy, the development of drugs that target lipid rafts leading to the formation of clusters of apoptotic signaling molecule-enriched rafts could offer new opportunities for therapeutic intervention in cancer therapy [17, 18].

## Intracellular scrambling to mitochondria

The concept of "organelle scrambling" has emerged to describe the intermixing of membranes that normally belong to different types of organelles, but apparently merge together following apoptosis-mediated changes in membrane traffic [19]. An example of this concept is the global alteration in the organelle membrane traffic induced by the prototypic death receptor CD95/Fas in physiologically sensitive cells, such as activated T lymphocytes. Then, an initial wave of enhanced endocytosis is followed by a caspase-dependent movement of internal membranes toward the cell periphery, that primarily involves secretory and endosomal membranes, partially intermixed with mitochondria [20] and presumably enhancing intercellular communication [21].

Indeed, scrambling among different cell organelles, including plasma membrane, endoplasmic reticulum (ER), and ER–mitochondria associated membranes, as well as lysosomal vesicles and Golgi apparatus, was also hypothesized after triggering of death receptors [22]. The importance of ER in the apoptotic cascade has been recently investigated [23, 24]. For example, under ER stress conditions, ER transmembrane receptors initiate the unfolded protein response [25] and, if the adaptive response fails, apoptotic cell death ensues. This response is associated with organelle remodeling and intermixing and is implicated in the pathophysiology of several neurodegenerative and cardiovascular diseases [26].

It is well known that depletion of ER lumenal $Ca^{2+}$ is a known inducer of ER stress; however, following the depletion of ER $Ca^{2+}$, calreticulin, PDI, BiP/GRP78, and GRP94 escape ER retention and may translocate to the cell surface [27]. The appearance of ER chaperones and oxidoreductases on the plasma membrane corresponds to a danger-associated molecular pattern (DAMP). Their presence on the cell surface leads to the recruitment of innate inflammatory cells, following the interaction of surface DAMPs with pattern-recognition receptors. Upon formation of a complex between these proteins, a potent "eat-me" signal is generated and phagocytosis of calreticulin or GRP94-bearing stressed cells is initiated, thus representing an important element of the immunological response to cancer.

Furthermore, the potential implication of Golgi apparatus remodeling in apoptosis execution has been analyzed in detail [28]. Notably, the Golgi apparatus participates to the complex framework of subcellular intermixing activities that lead to CD95/Fas-triggered apoptosis [20].

In the same vein, lysosomal organelles have been analyzed in different experimental settings to understand how certain lysosomal cysteinyl proteases, e.g. cathepsins, may contribute to the cross talk between endolysosomes and mitochondria during apoptosis.

In the last decade, intense research has provided a wealth of information on how death signaling engages mitochondria, a process that also involves dynamic aspects, such as fusion and fission of mitochondrial membranes, which requires mitochondrial and cytosolic proteins, including members of the Bcl-2 family [29].

However, mitochondria have been shown to be tightly associated with elements of the ER [30–35] and these membranes have been called ER–mitochondria contact sites [36, 37]. Several observations suggested a connection between ER and mitochondria in mitochondrial dynamics; the most spectacular example being the fact that events of mitochondrial fission are enriched at sites of ER–mitochondria crossovers [38]. For instance, the ER may provide scaffold molecules required for the fission reaction. Alternatively, the ER may physically constrict mitochondria, priming them for division. This observation, although purely phenomenological, unveils a relationship between the ER and mitochondrial division, which can be explained by multiple models. In addition, contacts between ER and mitochondria may facilitate a variety of signalling processes between the two organelles, including $Ca^{2+}$ and phospholipid exchange [37, 39, 40]. Indeed, ER–mitochondria associations may impact on a diverse number of physiological processes, including ATP production, autophagy, protein folding in the ER, mitochondrial biogenesis, transport and apoptosis [37, 40–43].

Therefore, identification of proteins that operate at the interface between mitochondria-associated membrane (MAM) and mitochondria has become an active area of research. The distance between the ER and mitochondria at these contact sites is 10–30 nm [23]. Consequently, a protein bridge, linking the ER/MAM to mitochondria could be accommodated within this space. In this regard, most of the lipid biosynthesizing enzymes that are enriched in MAM, probably do not directly link the apposing membranes. Typical proteins, which are enriched in MAM include the inositol-1,4,5-tris-phosphate receptor-1 (IP3R-1) [44, 45], the sigma-1 receptor (Sigma-1R) [46], the dynamin-like protein-1 (DLP1/Drp1) [47], the oxidoreductase Ero1 [48], the mitochondrial voltage-dependent anion channel-1 (VDAC-1) [49] and calnexin [46, 50, 51]. However, it is not clear whether these proteins directly tether the two organelles, whether they increase the stability of the association between the ER/mitochondria, or just enhance processes mediated by these contacts. For example, the IP3R-1 at the ER face of the MAMs is bridged to VDAC-1 at the OMM (outer mitochondrial membrane) by glucose-regulated-protein 75, a molecular chaperone also present in the mitochondrial matrix [49]. Association of these proteins confers stability to the tethering complex and allows the formation of a pore that favors $Ca^{2+}$ transfer from the ER to the mitochondria [49, 52].

The involvement of MAM in apoptosis process is complex [53]. Cell death mediated by Fas ligand binding to hepatocytes, HeLa cells and Jurkat T lymphoma [54, 55] heavily relies on protein–protein interaction at the MAM interface, thus highlighting the importance of MAM tethers not only for mitochondrial metabolism, but also for programmed cell death.

Mitochondrial dynamics and morphology are modulated by the formation of ER–mitochondria contacts [56]. For instance, disruption of these contact sites and the corresponding increase in mitochondrial fission/fragmentation, are associated with the induction of apoptosis [57]. Apoptosis is also intimately connected to the calcium status of mitochondria. The flow of calcium from the ER into mitochondria promotes the oligomerization of Bax (Bcl-2-like protein 4), a pro-apoptotic mitochondrial outer membrane protein, and causes permeabilization of mitochondrial outer membranes. Consequently, cytochrome *c* is released into the cytosol, where the caspase cascade is activated and, ultimately, apoptosis is induced [58]. The calcium-mediated activation of the mitochondrial fission protein DLP1 also stimulates Bax oligomerization and increases apoptosis [56, 59]. Taken together, these observations indicate that the formation of contact sites between the ER and mitochondria is functionally linked to both mitochondrial $Ca^{2+}$, flow and apoptosis induction [60].

Hence, several actors can contribute to the propagation of death signalling [19, 28, 61, 62] and, among these, are also lipid rafts. These structural platforms seem to be not merely confined to the plasma membrane but, as demonstrated in various cell models, lipid rafts appear to contribute to the propagation of death signalling and to modulate cytoplasmic pathways switching cell fate towards cell survival or death [22].

Indeed, lipid microdomains are similarly formed at subcellular organelles, which include endoplasmic reticulum, Golgi and mitochondria, named lipid raft-like microdomains [15, 63]. In addition, some components of lipid raft-like microdomains have been detected within ER–mitochondria contact sites.

Nevertheless, in brains of $\beta$-galactosidase knock-out mice, GM1 reportedly accumulated in MAM, in detergent-

resistant domains and triggered a calcium-mediated stress response and apoptosis. In fact, treatment of *β-gal*$^{-/-}$ cells with methyl β-cyclodextrin (MβCD), which efficiently extracts GM1 from the MAMs, rescues opening of the permeability transition pore (PTP), dissipation of the potential, and apoptosis [52]. A similar outcome can be obtained by silencing Mitofusin-2 (Mfn2, dynamin-related protein), which underscores the importance of membrane tethering in eliciting the apoptotic process. In particular, Mfn2, a potent effector of mitochondrial fusion, is located not only in the OMM, precisely in the MAMs, but it is also present in the ER, albeit in low amounts [64]. Loss of function or silencing of Mfn2 in MEFs and HeLa cells increases the distance between ER and mitochondria with consequent reduction of $Ca^{2+}$ flux at the MAMs [64], and mitochondria-mediated cell death [52]. Together, these results establish Mfn2 as a physical tether between ER and mitochondrial membranes at the MAMs and emphasize the reciprocal connection between the topology of MAMs and their ability to control $Ca^{2+}$ flux [52, 64, 65].

These findings support the joined role of both proteins and lipids, particularly GM1, in the regulation of $Ca^{2+}$ flux at the MAMs/glycosphingolipid-enriched microdomains and in $Ca^{2+}$-dependent apoptosis. Thus, MAM have been proposed to contain membrane microdomains that are enriched in cholesterol and gangliosides, similar to the detergent-resistant lipid rafts of the plasma membrane [52, 66, 67]. Interestingly, the accumulation of lipid metabolism enzymes on the MAM could be the reason for the formation of a glycosphingolipid enriched microdomain fraction at the MAM [52]. This observation, together with the identification of a special set of proteins associated with these microdomains [63, 68], suggested that lipid synthesis and exchange at the MAM generate an ER subdomain with unique properties. Therefore, the existence of cholesterol- and GM1-enriched microdomains in MAM, and the role of these microdomains in regulating the association between the ER and mitochondria, require further investigation.

In this scenario, raft-like microdomains might participate not only in the organization of interorganelle protein networks, but also exert a role in the intercellular communication associated with cell death, participating in structural and biochemical remodeling in terms of structural modifications, i.e. their curvature changes, as well as their fission process. In particular, previous works, including ours, identified mitochondria as possible targets for raft components and hypothesized that the rearrangement of glycosphingolipids, may be involved in the mitochondrial remodeling leading to the completion of the apoptotic cell death program [15, 69, 70].

Indeed, a trafficking of disialoganglioside GD3, considered as a paradigmatic component of lipid rafts, to mitochondria has been reported [15, 71, 72].

## GD3 traffic to mitochondria

Different trafficking pathways, leading to accumulation of GD3 in mitochondria, were demonstrated in different cells in response to various apoptotic inducers, including C2 ceramide [69], TNF-α [70] or anti-CD95/Fas administration [15].

Giammarioli et al. demonstrated that, during CD95/Fas-mediated apoptosis, ezrin, a cytoskeletal protein, may directly interact with actin-dependent clustering of GD3 distributed in uropods of lymphoblastoid T cells [73]. Similary, actin cytoskeleton might also be implicated in the stabilization of CD95/Fas clusters in lipid rafts [74]. Then, cytoskeletal elements could act as concentrators of death receptors, as well as drivers of GD3 traffic to the mitochondrion. How these proteins may interact with lipids to regulate sorting between intracellular trafficking pathways has been investigated in the last few years.

In this regard, some authors characterized the trafficking of GD3 from the plasma membrane to mitochondria following TNF-α-exposure in rat hepatocytes, indicating that GD3 mitochondrial targeting could depend on endosomal/actin cytoskeleton vesicular trafficking through a Golgi/endosomal network [70]. In fact, actin disruption and microtubule depolymerization by latrunculin A and nocodazole prevented GD3 redistribution and interaction with mitochondria, sparing sensitized hepatocytes to TNF-α-exposure [70].

Although these data suggest the involvement of endosomal vesicle movement in the targeting of GD3 to mitochondria, it cannot discard a direct targeting of GD3 to mitochondria resulting from the continuity and contact between the Golgi/endoplasmic reticulum network with mitochondrial membranes [45, 75].

On the basis of these observations, we hypothesized that GD3 can proceed from the cell plasma membrane and/or from *trans* Golgi network to the mitochondria via a microtubule-dependent mechanism. Thus, during early time points following Fas ligation, microtubules may be used as tracks to direct intracytoplasmic transport of lipid raft glycosphingolipid(s) to mitochondria. The movement of GD3 from plasma membrane may be instructed by its association with cytoplasmic linker proteins-59 (CLIPR-59) [76], a new CLIP-170-related protein, involved in the regulation of microtubule dynamics and associated with lipid rafts by a double palmitoylation on tandem cysteines within the C-terminal domain [77]. Since CLIPR-59 is not only associated with the plasma membrane, but is also targeted to *trans* Golgi network membranes, it may regulate both plasma membrane and *trans* Golgi network interactions via microtubules. Hence, CLIPR-59 may facilitate rafts/microtubules interaction following anti-CD95/Fas treatment, supporting the view that CLIPR-59 is

involved in intracellular trafficking, acting as a chaperone molecule allowing a fast and prompt interaction between GD3 and cytoskeletal proteins. As microtubules are assembled by polymerization of Tubulin hetero-dimers composed of α- and β-tubulin [78], Sorice et al., showed a direct GD3-tubulin interaction depending on its high affinity for α/β-tubulin heterodimers, as confirmed by docking analyses [76]. Microtubular network integrity and function are required for T cell pro-apoptotic signaling, since low doses of demecolcine, a microtubule assembly-disrupting drug, impaired glycosphingolipid trafficking towards mitochondria also hindering apoptosis execution. Thus, microtubules may represent the directional network by which glycosphingolipids, which are key signaling molecules during receptor-mediated apoptosis of T cells, can move and re-distribute inside the cells. Once in the mitochondrial membrane they could contribute to the cascade of events leading to apoptosis execution and cell demise.

Numerous studies detected some components of lipid raft-like microdomains within ER–mitochondria contact sites [52]. In this respect, we recently identified $PrP^C$, a GPI-anchored glycoprotein, as a new interacting protein component of mitochondrial raft-like microdomains in lymphoblastoid T cells undergoing CD95/Fas-mediated apoptosis. It indicates that raft components can undergo intracellular re-localization via ER–mitochondria associated membranes and microtubular network [79, 80]. Hence, we hypothesized that, following CD95/Fas triggering, microtubules could play key roles in the intracellular directional redistribution, as well as in the recruitment to the mitochondrial compartment, of both glycosphingolipids and protein raft components [79].

The importance of glycosphingolipids–protein molecular interactions has already been suggested. In particular, it has been hypothesized that ganglioside–protein interactions could act as regulators of membrane organization, leading to the creation of a platform for the recruitment of specific effectors for membrane-trafficking events, including autophagosome biogenesis [81]. During the autophagic process, membrane trafficking and intermixing leads to the formation of autophagosomes, but the origin of autophagosomal membranes appears still unclear. Recently, it has been suggested that autophagosomes form at the ER–mitochondria contact site [82]. In this vein, our recent studies provide evidence that gangliosides, i.e. GD3, could participate to this intermixing activity thanks to their molecular interaction with key molecules involved in autophagosome biogenesis and maturation [83]. Hence, considering the fact that several studies have shown that classic apoptotic regulators can regulate autophagy [84], our findings suggest that ganglioside GD3 may also play a pivotal role in morphogenetic remodeling of autophagosomes during the autophagic process.

## Raft-like microdomains in mitochondria

Raft-like microdomains are envisaged as lateral assemblies of specific lipids and proteins in mitochondrial membrane, enriched in gangliosides and cholesterol (although with a content lower as compared to plasma membrane), but with a relatively low content of phospholipids. They have been proposed to function in changes of mitochondrial membrane potential, in mitochondrial network remodeling and in mitochondrial fusion/fission processes. In addition, they may contribute to cell polarization, mitochondrial oxidative phosphorylation and ATP production and release of apoptogenic factors. Thus, these dynamic structures contribute to apoptotic process and represent preferential sites on the mitochondrial membrane where some key reactions can be catalyzed [13, 15]: indeed some molecules, including VDAC-1 and the fission protein hFis1, are constitutively present, whereas Bcl-2 family proteins, including truncated Bid, t-Bid, and Bax, are recruited following CD95/Fas triggering [15]. Furthermore, during apoptosis, cardiolipin (CL) may be present in raft-like microdomains, where it acts as an activation platform for caspase-8 traslocation on mitochondria, at contact sites between inner and outer membranes, facilitating its self-activation [16]. In addition, CL acts as the mitochondrial receptor for Bid [85], providing specificity for targeting of t-Bid to mitochondria, regulating the oligomerization of Bax [86] and mobilization of cytochrome *c*.

Other groups have also suggested that proapoptotic members of the Bcl-2 family associate with mitochondrial fission sites and mitochondrial fission proteins during apoptosis [87]. Since mitochondria fission includes a profound mitochondria remodeling and changes of mitochondrial membrane curvature, it was suggested that changes of lipid and glycolipid moieties could play a role in the morphologic changes of mitochondria in cells undergoing apoptosis. We found that, following apoptotic stimulation, the molecules involved in mitochondrial fission processes are recruited to the mitochondrial raft-like microdomains. In particular, as reported above, hFis1 appears to be constitutively included in these microdomains, whereas DLP1 is recruited to raft-like microdomains only after CD95/Fas triggering. Interestingly, the disruption of rafts, for example, by MβCD, by fumonisin B1 (an inhibitor of ceramide synthase) or by [D]-PDMP (an inhibitor of glucosyltransferase), leads to an impairment of fission molecule recruitment to the mitochondria, a reduction of mitochondrial fission and a significant reduction of apoptosis [88]. The process of mitochondrial fission has been recently analyzed extensively. Proteins that are involved in the fission process (e.g. DLP1, hFis1), are associated either with cell proliferation, when daughter cells need their own energy factories, or with cell death by apoptosis, when

fission process precedes the release of apoptogenic factors by mitochondria. For instance, it is well known that, following apoptotic stimulation, DLP1 is recruited to the mitochondrial outer membrane, where it colocalizes with Bax and Mfn2 at the fission sites [89, 90]. For example, DLP1 function is required for apoptotic mitochondrial fission and cytochrome *c* release [91]. In addition, it has also been suggested that Optic Atrophy-1 (OPA1), a profusion dynamin-related protein involved in cristae remodeling, could represent a further actor participating in mitochondrial changes during apoptosis [92–95]. However, the submitochondrial localization of this protein appears still controversial. Indeed, a differential localization of different OPA1 isoforms has been observed. It has been suggested that the 88 kDa isoform preferentially associates with the outer membrane [96]. We found that this isoform is present in raft-like microdomains and coimmunoprecipitates with hFis1 after apoptotic stimulation; we cannot rule out the possibility that further unidentified molecules could participate in the interaction between OPA1 and hFis1 proteins. Finally, as mitochondria fission includes a profound mitochondrial remodeling and changes of mitochondrial membrane curvature, it was suggested that changes of lipid and glycolipid moieties could play a role in the morphogenetic changes of mitochondria in cells undergoing apoptosis. On this basis, lipid rafts may be included in the complex molecular framework leading to mitochondrial fission: their presence can provide catalytic domains where unknown molecular associations and/or cleavages could occur. The role of the ganglioside in this multimolecular system could be to facilitate the transient and local formation of inverted hexagonal structures in mitochondrial membrane that undergo the fission process, for example, modifying mitochondrial membrane curvature and fluidity [88].

Mitochondria depolarization and cytochrome *c* release are also dependent on raft-like microdomain integrity, since the disruption of raft-like microdomains in isolated mitochondria by MβCD prevented mitochondria depolarization, cytochrome *c* release and apoptosis execution after treatment with GD3 or t-Bid, pro-apoptotic protein [15]. MβCD-induced disruption of mitochondrial raft-like microdomains impairs mitochondrial bioenergetics, increasing mitochondria resistance to $Ca^{2+}$-inducing swelling [97].

It is conceivable that GD3, by interacting with mitochondrial raft-like microdomains [13], may trigger specific sequential events involved in the apoptotic program [98], including changes of mitochondrial membrane potential (MMP), mitochondrial fission associated changes and the subsequent release of apoptogenic factors [13, 15, 99, 100]. Regarding the first, i.e., the modifications of MMP, both hyperpolarization (early event) and depolarization (late event) have been shown to occur in T cells [101–104]. It has, in fact, been hypothesized that MMP alterations could be associated with relocalization of GD3 to the mitochondria [15, 105]. The role of GD3 could be to enhance the generation of reactive oxygen species (ROS) from the complex III of the mitochondrial electron transport chain, which triggers the mitochondrial permeability transition pore opening, leading to the cyt c-dependent caspase 3 activation. Furthermore, selective depletion of GSH in mitochondria sensitizes intact hepatocytes to GD3-induced apoptosis by enhancing the oxidative stress caused by GD3 [106]. Furthermore, it could suppress the proton pumping activity and/or the increase in small-conductance channels [107], thus modifying mitochondrial membrane fluidity and MMP.

Mitochondrial lipid microdomains may thus be considered essential activating platforms, where specific key reactions can take place and can be catalyzed, representing smart and compelling interactors in determining cell fate.

## Role of raft-like microdomains in neurodegenerative diseases

Mitochondrial dysfunction is a common hallmark of aging-related neurodegenerative diseases in humans, indeed, neurons, characterized by high-energy demands, are highly dependent on mitochondrial metabolism, being unable to switch to glycolysis when mitochondrial oxidative phosphorylation is impaired [108].

In the light of the role played by raft-like microdomains to integrate apoptotic signals and in regulating mitochondrial dynamics, both in physiological and in pathological conditions [72], it is conceivable that this membrane structures may play a role in the mitochondrial alterations observed in the most common human neurodegenerative diseases. Indeed, proteins involved in neurodegeneration mechanisms that affect apoptotic processes and mitochondrial functionality, have been described as component of lipid raft structures [109]. Among these, VDAC-1 is an interesting example. This protein, imbedded in the outer mitochondrial membrane, was described as a resident protein of lipid rafts [110] and our previous data revealed the localization of VDAC-1 in the mitochondrial raft-like microdomains as reported above [15]. The four VDAC isoforms were described as lipid sensors, since regulate cholesterol content in the outer membrane of mitochondria and, in turn, membrane lipid composition affects their functionality [111 and references therein]. In addition, it can also participate to mitochondrial membrane permeabilization, an apoptotic checkpoint in stress and pathological conditions [111].

An in depth analysis performed on purified mitochondria from copper, zinc superoxide dismutase (SOD1)-

linked animal models for the familial form of amyotrophic lateral sclerosis (fALS) indicated that misfolded forms of active and inactive mutant SOD1s are localized onto the cytoplasmic face of the outer membrane of spinal cord mitochondria [112] and here bind directly to VDAC-1, inhibiting its conductance and activity [113].

The direct interaction with VDAC-1 is a property only of spinal cord mitochondria and no association of mutant SOD1 was seen with purified brain mitochondria from the same animals. This latter finding is consistent with previous data demonstrating that mutant SOD1 associates with the cytoplasmic face of the outer membrane of mitochondria in spinal cord, but not in other tissue types [112]. Moreover, mutant SOD1 binding to VDAC-1 is inversely correlated with the level of hexokinase-I, a known partner that binds to VDAC-1 exposed on the cytoplasmic mitochondrial surface [113], with hexokinase-I accumulating to much higher level in brain than in spinal cord mitochondria.

The mutant SOD1–VDAC-1 interaction, as mentioned, compromises VDAC-1 conductance and this mutant SOD1 property may offer a mechanistic explanation for alterations of mitochondrial electron transfer chain complexes activity and the capacity to consume oxygen and synthesize ATP previously reported in both ALS patients and in experimental models for the pathology [114 and references therein].

DLP1, as above described, is recruited in mitochondrial lipid raft-like microdomains specifically after apoptotic stimuli [88], where interacts with GD3, regulating the mitochondrial morphogenetic changes during fission processes. In this regard, in literature are present several data indicating a disregulation of DLP1 in ALS experimental models that could partially justify mitochondrial fragmentation observed in the same models [114].

In particular, several works describe morphological alterations that are reminiscent of fragmentation in mitochondria of cultured cells and mice expressing fALS-linked mutant SOD1 [112, 115, 116]. Moreover, changes in the expression levels of proteins modulating mitochondrial dynamism have been observed in a cellular model of the disease [116]. Bossy–Wetzel's group described mitochondrial fragmentation induced by mutant SOD1 in primary spinal cord motor neurons co-cultured with spinal cord astrocytes [117]. Furthermore, the same authors rescued the neurons from mutant SOD1-induced mitochondrial fragmentation and cell death restoring the fission and fusion balance by the expression of a dominant-negative mutant of DLP1 [117].

Raft like-microdomains are also present in the close contact and juxtaposition sites through which mitochondria and ER communicate [118 and references therein]. These contact sites are involved in the collaborative production of lipids and calcium homeostasis [37, 39, 40]. Indeed, close associations between both organelles enhance the interorganelle phospholipid transport [53], but also the transport of other lipids, such as cholesterol [119] and sphingolipids, supporting the metabolism of ceramide in cell cycle, cell differentiation and apoptosis [120]. In addition to promote lipid transfer, MAMs are also involved in $Ca^{2+}$ ions exchange between ER and mitochondria [118]. Recently, several ER or mitochondria bound proteins have been described to play an important role in maintaining the close relationship between ER and mitochondria [118]. Intriguingly, these proteins include proteins involved in the pathogenesis of ALS, such as Sigma1 Receptor (Sig1R), Erlin2 and vesicle-associated membrane protein-associated protein B (VAPB) [121, 122], that have been described as MAM residents [63, 68].

Indeed, mutations in the genes coding for the proteins Sigma1R and Erlin2 are related to a juvenile form of ALS and mutation in gene coding for VAPB is responsible of type-8 ALS [121, 122].

Loss of Sig1R leads to abnormal ER morphology and mitochondrial abnormalities and depletion of Sig1R destabilizes lipid rafts and calcium mobilization in a motor neuronal cell line, confirming the crucial role of Sig1R in lipid rafts and intracellular calcium homeostasis [123]. Moreover, recent papers from Miller's group described the physical interaction between VAPB and protein tyrosine phosphatase interacting protein 51 (PTPIP51) localized on the outer mitochondrial membrane and how this interaction impacts on cytosolic and mitochondrial calcium handling. Recently, the same authors reported that both wild type and fALS-linked mutant transactive response DNA binding protein 43 (TDP43) alter ER-Mito cross talk, perturbing specifically VAPB–PTPIP51 interaction [124] both in vivo and in vitro. TDP43 perturbs VAPB–PTPIP51 interaction modifying the activity of glycogen synthase kinase-3β (GSK3β), a kinase involved in ALS pathology [125] and recruited in the active form within neuronal lipid rafts [126]. Inhibition of GSK3β activity prolongs survival and improves motor performances of SOD1-linked fALS transgenic mice, partially blocking the CD95/FAS-mediated extrinsic apoptotic pathway [125].

GSK3β is also recruited within neuronal lipid rafts in a mouse model for Huntington disease and, as in ALS transgenic mice, treatment of primary neurons with GSK3β inhibitors reduces neuronal death [127]. This kinase is implicated in regulating mitochondrial dynamics and metabolism, affecting DLP1 phosphorylation and VDAC-1 function [128]; several papers showed mitochondrial loss, abnormal mitochondrial dynamics in HD patients and models for the disease [129, 130].

Huntingtin (Htt) has been described associated with lipid rafts isolated from mouse brains and mutant Htt from

presymptomatic Huntington's disease knock-in mice is more strongly associated with rafts than wild type. This association, as mentioned, is accompanied by a significant increase in raft-associated GSK-3β, which coincides with apoptotic damages [127]. Interestingly, other authors described the segregation of the mutant Htt in the membrane lipid raft in an in vitro HD model, where polyQ-expanded protein interacts with NADPH oxidase membrane subunit, gp91, and facilitates the activation of the enzyme [131]. These data were confirmed in vivo by Valencia and colleagues [132]. The authors described the relevance of the mutant Htt-induced activation of NADPH oxidase at lipid raft in generating mitochondrial oxidative stress.

The polyQ-expanded protein triggers mitochondrial fragmentation in different models for the disease [133 and references therein]. This toxic property comes out from the increased activity of DLP1 triggered by the direct interaction of this protein with mutant Htt [134]. The recent data by Guo and colleagues [135] confirmed the key role of this aberrant interaction, indeed, the authors showed that the selective inhibition of DLP1 in cells from HD patients and in mouse models for the pathology hampers the mutant Htt induced neurodegeneration.

Our group has described in lymphoid cells from HD patients the localization of mutant Htt in the mitochondrial raft-like microdomains [136]. The presence of mutant Htt in mitochondrial raft-like microdomains is closely related to the recruitment in these areas of apoptotic factors belonging to the Bcl-2 family, such as Bak, Bax, and t-Bid and this condition evokes in HD cells an increased susceptibility to apoptotic stimuli. Furthermore, mutant Htt colocalizes with DLP1 and interestingly this colocalization takes place in absence of apoptotic stimuli [136], therefore, mutant Htt is able to attract DLP1 onto outer mitochondrial membrane. Once again the mitochondrial raft-like microdomains play a key role in integrating the toxicity of proteins responsible for neurodegenerative diseases with key factors for mitochondrial function. Indeed, the treatment of lymphoid cells from HD patients with compounds that perturb raft composition, such as fumonisin B1 or methyl-β-cyclodextrin, inhibited mitochondrial apoptotic pathway induced by staurosporin as well as metabolic and morphological damages of mitochondria observed in this experimental model [136].

In the light of these several evidences, being central the metabolism of cholesterol in regulating the physiology of rafts, this metabolic pathway might play a key role in neurodegenerative processes described in both ALS and HD, and indeed in both pathologies are described disturbances in lipid metabolism [137] and, at least in animal models, the high-energy diets have a relevant impact in the progression of the disease [138].

Another relevant example of a key role of lipid rafts in neurodegenerative diseases is represented by prion-related diseases, also know as trasmissible spongiform encephalopathies. Prions are infectious pathogens that cause a group of invariably fatal neurodegenerative diseases affecting humans and other mammalian species, mediated by a novel mechanism [139]. They are able to acquire alternative conformations that become self-propagating [140]. In fact, prion protein (PrP) consists of two isoforms, one is a host-encoded cellular isoform ($PrP^{C}$) and the other is an abnormal protease-resistant pathogenic isoform ($PrP^{Sc}$), of which the latter is a causative agent of prion disease [140].

**Table 1** Functions of lipid-enriched microdomains related to cell apoptosis

| Microdomains | Function | Functional example |
|---|---|---|
| Plasma membrane rafts | • Signal transduction processes | • Death receptor signaling |
| | • Catalyze molecular interactions | • Apoptosis signaling compartmentalization |
| Golgi microdomains | • Organelle scrambling | • Trafficking and sorting |
| | | • Organelle reshaping |
| ER microdomains | • Organelle scrambling | • ER stress |
| | • Organelle cross talk | • Unfolded protein response |
| MAM microdomains | • Recruitment of $Ca^{2+}$ channels | • $Ca^{2+}$ signalling |
| | • Lipid homeostasis | • PtdSer and PtdEtn enzymes regulation |
| | | • Cholesterol esterification |
| Mitochondrial raft-like microdomains | • Changes of curvature | • Mitochondrial network remodeling |
| | • Recruitment of proapoptotic factors | • Changes of mitochondrial membranes potential |
| | • Recruitment of fission molecules | • Cyt *c* release, Bak/Bax oligomerization |
| | | • Cardiolipin exposure on outer membrane, Caspase-8 recruitment |
| | | • Mitochondrial fusion/fission process |

*ER* endoplasmic reticulum, *MAM* mitochondria-associated membrane, *PtdSer* phosphatidylserine, *PtdEtn* phosphatidylethanolamine, *Cyt c* cytochrome *c*

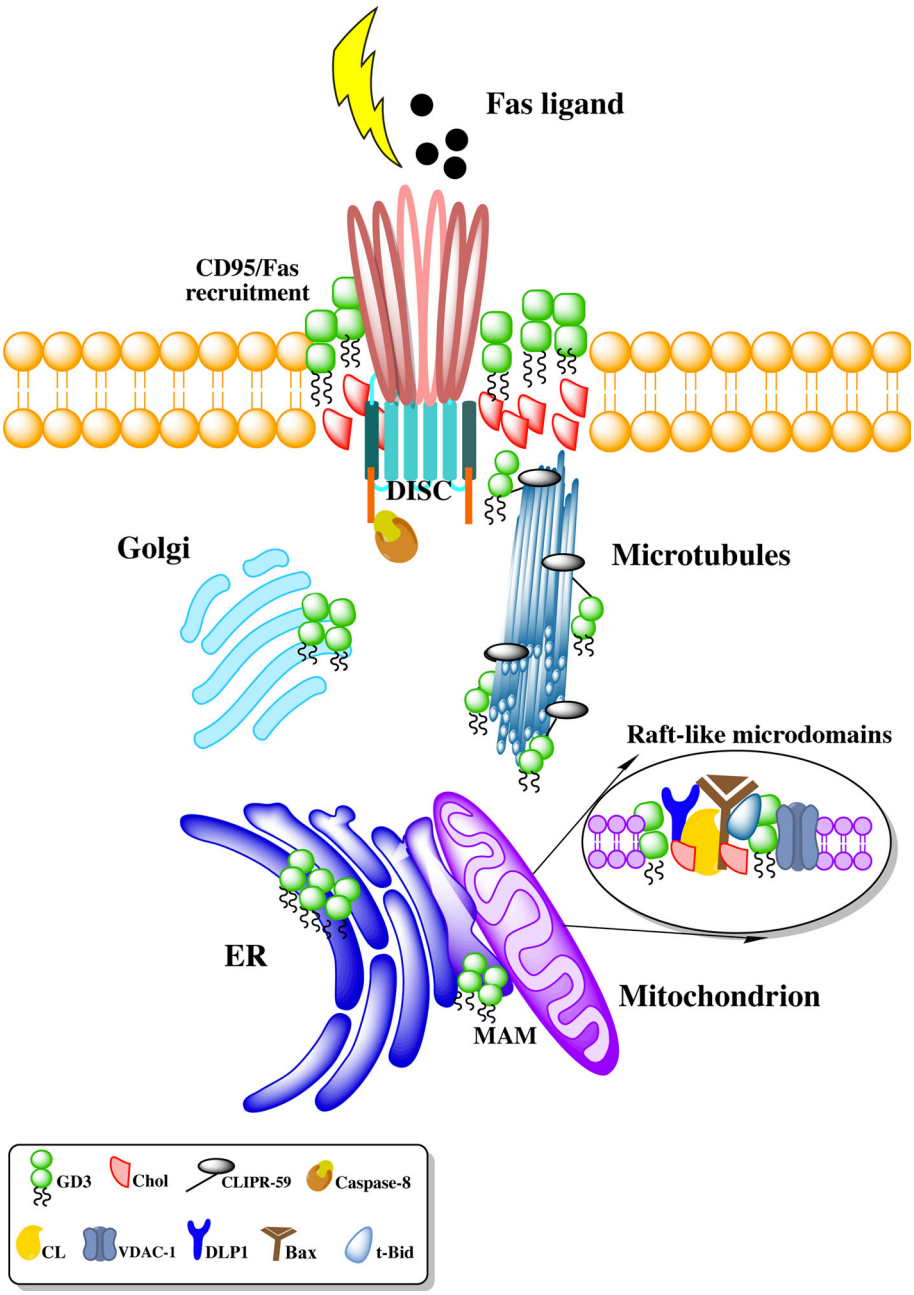



**Fig. 1** Schematic drawing depicting the intracellular traffic of raft-like microdomain components to mitochondria and their possible role in the apoptotic cascade. *Bax* Bcl-2-like protein 4, *CL* cardiolipin, *CLIPR-59* cytoplasmic linker proteins-59, *Chol* cholesterol, *DISC* death-induced signaling complex, *DLP1* dynamin-like protein-1, *ER* endoplasmic reticulum, *MAM* mitochondria-associated membrane, *t-Bid* truncated Bid, *VDAC-1* voltage-dependent anion channel-1

Like other GPI-anchored proteins, most of $PrP^{C}$, as well as $PrP^{Sc}$, were found in lipid rafts of the neural plasma membrane and lymphocytes [141], which are also enriched in several cytoplasmic proteins, including tyrosine kinases [142]. This localization is required for conversion of $PrP^{C}$ to the transmissible spongiform encephalopathy-associated protease-resistant isoform [143, 144]. The refolding process of $PrP^{C}$ into $PrP^{Sc}$ is generally accompanied by increase in β-sheet structure and a propensivity to aggregate into oligomers [140].

Several studies suggested that $PrP^{C}$ is also involved in the regulation of presynaptic copper concentration, intracellular calcium homeostasis, lymphocyte activation, astrocyte proliferation, and cellular resistance to oxidative stress [145, 146]. Recent studies show the involvement of $PrP^{C}$ in apoptotic signaling pathways [147]. In this context Mattei et al. [79] showed that CD95/Fas triggering induced a redistribution of $PrP^{C}$ to the mitochondria of T cells via a mechanism that brings into play microtubular network integrity and function. Indeed, when we analyzed the $PrP^{C}$

intracytoplasmic trafficking in CD95/Fas treated cells, we found that microtubular network-perturbing agent demecolcine impaired either mitochondrial re-localization of $PrP^C$ or apoptosis induction. $PrP^C$ was redistributed to mitochondrial raft-like microdomains, as well as at ER–mitochondria associated membranes, as revealed by immunoelectron microscopy observations. The analysis of the mitochondria swelling profile pointed to a dose-dependent effect of $recPrP^C$ in inducing the increase of mitochondrial membrane potential (i.e., hyperpolarization), followed by the "typical" loss of mitochondria membrane potential (i.e., depolarization) and the release of apoptogenic factors, such as cytochrome *c*. In particular, it was suggested that lipid rafts could contribute to define the metabolic fate of $PrP^C$. Indeed, if raft-embedded $PrP^C$ is part of the complex framework normally contributing to the death of the cell, a defective trafficking of $PrP^C$ from and toward lipid rafts could also affect normal $PrP^C$ catabolism, also leading to the formation of the 17-kDa polypeptide hydrolysis to form the PrP 27-30 scrapie isoform.

## Conclusion remarks

Raft-like microdomains are small dynamic components of mitochondrial membrane, which may trigger specific key events involved in the apoptogenic program, including mitochondria hyperpolarization, fission-associated changes, megapore formation and release of apoptogenic factors (Table 1).

ER–mitochondria associated membranes have been also proposed to contain membrane microdomains that are enriched in cholesterol and gangliosides, similar to the detergent-resistant lipid rafts of the plasma membrane. We suggest that these microdomains at the ER–mitochondria contact site may play a pivotal role in trafficking of key molecules involved in the organization of interorganelle protein networks as well as in the intercellular communication associated with cell death.

Thus, lipid raft components can exert their regulatory activity in apoptosis execution at three different levels: (i) in the DISC formation at the plasma membrane; (ii) in the intracellular redistribution at cytoplasmic organelles, and, (iii) in the structural and functional mitochondrial modifications associated with apoptosis execution (Fig. 1).

In the light of the role played by raft-like microdomains to integrate apoptotic signals and in regulating mitochondrial dynamics, both in physiological and in pathological conditions, it is conceivable that these membrane structures may play a role in the mitochondrial alterations observed in some of the most common human neurodegenerative diseases, such as ALS, Huntington's chorea and prion-related diseases. All these findings may introduce an additional task for identifying new molecular target(s) of pharmacological agents in these pathologies. For instance, non-toxic semisynthetic cyclodextrins, which are widely used in vitro and in vivo as drug carriers, could be taken into consideration for their raft-targeted activity. Hence, on the basis of the results reported above, molecularly targeted therapy, inhibiting the interaction of key molecules with mitochondrial raft-like microdomains, may be proposed.

## References


1. Sonnino S, Prinetti A (2012) Membrane domains and the "lipid raft" concept. Curr Med Chem 20:4–21
2. Simons K, Toomre D (2000) Lipid rafts and signal transduction. Nat Rev Mol Cell Biol 1:31–39
3. Barbat C, Trucy M, Sorice M, Garofalo T, Manganelli V, Fischer A, Mazerolles F (2007) p56lck, LFA-1 and PI3K but not SHP-2 interact with GM1- or GM3-enriched microdomains in a CD4-p56lck association-dependent manner. Biochem J 402:471–481
4. Simons K, Ikonen E (1997) Functional rafts in cell membranes. Nature 387:569–672
5. Pizzo P, Giurisato E, Bigsten A, Tassi M, Tavano R, Shaw A, Viola A (2004) Physiological T cell activation starts and propagates in lipid rafts. Immunol Lett 91:3–9
6. Pizzo P, Viola A (2004) Lipid rafts in lymphocyte activation. Microbes Inf 6:686–692
7. Pizzo P, Viola A (2003) Lymphocyte lipid rafts: structure and function. Curr Opin Immunol 15:255–260
8. Garofalo T, Misasi R, Mattei V, Giammarioli AM, Malorni W, Pontieri GM, Pavan A, Sorice M (2003) Association of the death-inducing signaling complex with microdomains after triggering through CD95/Fas. Evidence for caspase-8-ganglioside interaction in T cells. J Biol Chem 278:8309–8315
9. Grassmè H, Jekle A, Riehle A, Schwarz H, Berger J, Sandhoff K, Kolesnick R, Gulbins E (2002) CD95 signalling via ceramide-rich membrane rafts. J Biol Chem 22:207–222
10. Scheel-Toellner D, Wang K, Singh R, Majeed S, Raza K, Curnow SJ, Salmon M, Lord JM (2002) The death-inducing signaling complex is recruited to lipid rafts in Fas-induced apoptosis. Biochem Biophys Res Commun 297:876–879
11. Hueber AO, Bernard AM, Herincs Z, Couzinet A, He HT (2002) An essential role for membrane rafts in the initiation of Fas/CD95-triggered cell death in mouse thymocytes. EMBO Rep 3:190–196
12. Scheel-Toellner D, Wang K, Singh R, Majeed S, Raza K, Curnow SJ, Salmon M, Lord JM (2002) The death-inducing signaling complex is recruited to lipid rafts in Fas-induced apoptosis. Biochem Biophys Res Commun 297:876–879
13. Malorni W, Giammarioli A, Garofalo T, Sorice M (2007) Dynamics of lipid raft components during lymphocyte apoptosis: the paradigmatic role of GD3. Apoptosis 12:941–949
14. Legler DF, Micheau O, Doucey MA, Tschopp J, Bron C (2003) Recruitment of TNF receptor 1 to lipid rafts is essential for TNF alpha-mediated NF-kappaB activation. Immunity 18:655–664
15. Garofalo T, Giammarioli AM, Misasi R, Tinari A, Manganelli V, Gambardella L, Malorni W, Sorice M (2005) Lipid microdomains contribute to apoptosis-associated modifications of mitochondria in T cells. Cell Death Differ 12:1378–1389
16. Sorice M, Manganelli V, Matarrese P, Tinari A, Misasi R, Malorni W, Garofalo T (2009) Cardiolipin-enriched raft-like

microdomains are essential activating platforms for apoptotic signals on mitochondria. FEBS Lett 583:2447–2450
17. Mollinedo F, Gasate C (2010) Lipid rafts and clusters of apoptotic signaling molecule-enriched rafts in cancer therapy. Future Oncol 6:811–821
18. Dimanche-Boitre MT, Rebillard A, Gulbins E (2011) Ceramide in chemotherapy of tumors. Recent Pat AntiCancer Drug Discov 6:284–293
19. Degli Esposti M (2008) Organelle intermixing and membrane scrambling in cell death. Methods Enzymol 442:421–438
20. Ouasti S, Matarrese P, Paddon R, Khosravi-Far R, Sorice M, Tinari A, Malorni W, Degli Esposti M (2007) Death receptor ligation triggers membrane scrambling between Golgi and mitochondria. Cell Death Differ 14:453–461
21. Eda S, Yamanaka M, Beppu M (2004) Carbohydrate-mediated phagocytic recognition of early apoptotic cells undergoing transient capping of CD43 glycoprotein. J Biol Chem 279:5967–5974
22. Degli Esposti M, Tour J, Ouasti S, Ivanova S, Matarrese P, Malorni W, Khosravi-Far R (2009) Fas death receptor enhances endocytic membrane traffic converging into the Golgi region. Mol Biol Cell 20:600–615
23. Csordas G, Renken C, Varnai P, Walter L, Weaver D, Buttle KF, Balla T, Mannella CA, Hajnoczky G (2006) Structural and functional features and significance of the physical linkage between ER and mitochondria. J Cell Biol 174:915–921
24. Faitova J, Krekac D, Hrstka R, Vojtesek B (2006) Endoplasmic reticulum stress and apoptosis. Cell Mol Biol Lett 1:488–505
25. Lai E, Teodoro T, Volchuk A (2007) Endoplasmic reticulum stress: signaling the unfolded protein response. Physiology (Bethesda) 22:193–201
26. Szegezdi E, Logue SE, Gorman AM, Samali A (2006) Mediators of endoplasmic reticulum stress-induced apoptosis. EMBO Rep 7:880–885
27. Gutierrez T, Simmen T (2014) Endoplasmic reticulum chaperones and oxidoreductases: critical regulators of tumor cell survival and immunorecognition. Front Oncol 4:291. doi:10.3389/fonc.2014.00291
28. Siegel RM, Muppidi JR, Sarker M, Lobito A, Jen M, Martin D, Straus SE, Lenardo MJ (2004) SPOTS: signaling protein oligomeric transduction structures are early mediators of death receptor-induced apoptosis at the plasma membrane. J Cell Biol 167:735–744
29. Karbowski M, Youle RJ (2003) Dynamics of mitochondrial morphology in healthy cells and during apoptosis. Cell Death Differ 10:870–880
30. Lewis LA, Tata JR (1973) A rapidly sedimenting fraction of rat liver endoplasmic reticulum. J Clin Sci 13:447–459
31. Wanson JC, Drochmans P, May C, Penasse W, Popowski A (1975) Isolation of centrolobular and perilobular hepatocytes after phenobarbital treatment. J Cell Biol 66:23–41
32. Shore GC, Tata JR (1977) Two fractions of rough endoplasmic retuiculum from rat liver. J Cell Biol 72:714–725
33. Pickett CB, Montisano D, Eisner D, Cascarno J (1980) The phsyical association between rat liver mitochondria and rough endoplasmic reticulum. Exp Cell Res 128:343–352
34. Meier PJ, Spycher MA, Meyer UA (1981) Isolation and characterization of rough endoplasmic reticulum associated with mitochondria from normal rat liver. Biochim Biophys Acta 646:283–297
35. Katz J, Wals PA, Golden S, Raijman L (1983) Mitochondrial–reticular cytostructure in liver cells. Biochem J 214:795–813
36. Raturi A, Simmen T (2013) Where the endoplasmic reticulum and the mitochondrion tie the knot: the mitochondria-associated membrane (MAM). Biochim Biophys Acta 1833:213–224
37. Kornmann B (2013) The molecular hug between the ER and the mitochondria. Curr Opin Cell Biol 25:443–448
38. Friedman JR, Lackner LL, West M, DiBenedetto JR, Nunnari J, Voeltz GK (2011) ER tubules mark sites of mitochondrial division. Science 334:358–362
39. Hayashi T, Rizzuto R, Hajnoczky G, Su TP (2009) MAM: more than just a housekeeper. Trends Cell Biol 19:81–88
40. Rowland AA, Voeltz GK (2012) Endoplasmic reticulum-mitochondria contacts: function of the junction. Nat Rev Mol Cell Biol 13:607–625
41. Simmen T, Lynes EM, Gesson K, Thomas G (2010) Oxidative protein folding in the endoplasmic reticulum: tight links to the mitochondria-associated membrane (MAM). Biochim Biophys Acta 1798:1465–1473
42. Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, Oomori H, Noda T, Haraguchi T, Hiraoka Y, Amano A, Yoshimori T (2013) Autophagosomes form at ER-mitochondria contact sites. Nature 495:389–393
43. Korobova F, Ramabhadran V, Higgs HN (2013) An actin-dependent step in mitochondrial fission mediated by the ER-associated formin INF2. Science 339:464–467
44. Rizzuto R, Brini M, Murgia M, Pozzan T (1993) Microdomains with high Ca2+ close to IP3-sensitive channels that are sensed by neighboring mitochondria. Science 262:744–747
45. Rizzuto R, Pinton P, Carrington W, Fay FS, Fogarty KE, Lifshitz LM, Tuft RA, Pozzan T (1998) Close contacts with the endoplasmic reticulum as determinants of mitochondrial Ca2+ responses. Science 280:1763–1765
46. Hayashi T, Su TP (2007) Sigma-1 receptor chaperones at the ER–mitochondrion interface regulate Ca(2+) signaling and cell survival. Cell 131:596–610
47. Pitts KR, Yoon Y, Krueger EW, McNiven MA (1999) The dynamin-like protein DLP1 is essential for normal distribution and morphology of the edoplasmic reticulum and mitochondria in mammalian cells. Mol Biol Cell 10:4403–4417
48. Gilady SY, Bui M, Lynes EM, Benson MD, Watts R, Vance JE, Simmen T (2010) Ero1alpha requires oxidizing and normoxic conditions to localize to the mitochondria-associated membrane (MAM). Cell Stress Chaperones 15:619–629
49. Szabadkai G, Bianchi K, Varnai P, De Stefani D, Wieckowski MR, Cavagna D, Nagy AI, Balla T, Rizzuto R (2006) Chaperone-mediated coupling of endoplasmic reticulum and mitochondrial Ca2+ channels. J Cell Biol 175:901–911
50. Myhill N, Lynes EM, Nanji JA, Blagoveshchenskaya AD, Fei H, Carmine Simmen K, Cooper TJ, Thomas G, Simmen T (2008) The subcellular distribution of calnexin is mediated by PACS-2. Mol Biol Cell 19:2777–2788
51. Lynes EM, Raturi A, Shenkman M, Ortiz Sandoval C, Yap MC, Wu J, Janowicz A, Myhill N, Benson MD, Campbell RE, Berthiaume LG, Lederkremer GZ, Simmen T (2013) Palmitoylation is the switch that assigns calnexin to quality control or ER Ca2+ signaling. J Cell Sci 126:3893–3903
52. Sano R, Annunziata I, Patterson A, Moshiach S, Gomero E, Opferman J, Forte M, d'Azzo A (2009) GM1-ganglioside accumulation at the mitochondria-associated ER membranes links ER stress to Ca(2+)-dependent mitochondrial apoptosis. Mol Cell 36:500–511
53. Vance JE (2014) MAM (mitochondria-associated membranes) in mammalian cells: lipids and beyond. Biochim Biophys Acta 1841:595–609
54. Steinmann C, Landsverk ML, Barral JM, Boehning D (2008) Requirement of inositol 1,4,5 trisphosphate receptors for tumor-mediated lymphocyte apoptosis. J Biol Chem 283:13506–13509
55. Wozniak AL, Wang X, Stieren ES, Scarbrough SG, Elferink CJ, Boehning D (2006) Requirement of biphasic calcium release from the endoplasmic reticulum for Fas-mediated apoptosis. J Cell Biol 175:709–714

56. Hoppins S, Nunnari J (2012) Cell biology. Mitochondrial dynamics and apoptosis—the ER connection. Science 337:1052–1054
57. Simmen T, Aslan JE, Blagoveshchenskaya AD, Thomas L, Wan L, Xiang Y, Feliciangeli SF, Hung CH, Crump CM, Thomas G (2005) PACS-2 controls endoplasmicreticulum mitochondria communication and Bid-mediated apoptosis. EMBO J 24:717–729
58. Orrenius S, Zhivotovsky B, Nicotera P (2003) Regulation of cell death: the calcium-apoptosis link. Nat Rev Mol Cell Biol 4: 552–565
59. Montessuit S, Somasekharan SP, Terrones O, Lucken-Ardjomande S, Herzig S, Schwarzenbacher R, Manstein DJ, Bossy-Wetzel E, Basanez G, Meda P, Martinou JC (2010) Membrane remodeling induced by the dynamin-related protein Drp1 stimulates Bax oligomerization. Cell 142:889–901
60. Soriano ME, Scorrano L (2010) The interplay between BCL-2 family proteins and mito-chondrial morphology in the regulation of apoptosis. Adv Exp Med Biol 687:97–114
61. Austin CD, Lawrence DA, Peden AA, Varfolomeev EE, Total K, De Maziere AM, Klumperman J, Arnott D, Pham V, Scheller RH, Ashkenazi A (2006) Death-receptor activation halts clathrin-dependent endocytosis. Proc Natl Acad Sci USA 103:10283–10288
62. Matarrese P, Manganelli V, Garofalo T, Tinari A, Gambardella L, Ndebele K, Khosravi-Far R, Sorice M, Degli Esposti M, Malorni W (2008) Endosomal compartment contributes to the propagation of CD95/Fas-mediated signals in type II cells. Biochem J 413:467–478
63. Hayashi T, Su T (2003) Sigma-1 receptors (sigma(1) binding sites) form raft-like microdomains and target lipid droplets on the endoplasmic reticulum: roles in endoplasmic reticulum lipid compartmentalization and export. J Pharmacol Exp Ther 306: 718–725
64. de Brito OM, Scorrano L (2008) Mitofusin 2 tethers endoplasmic reticulum to mitochondria. Nature 456:605–610
65. Annunziata I, d'Azzo A (2013) Interorganellar membrane microdomains: dynamic platforms in the control of calcium signaling and apoptosis. Cells 2:574–590
66. Hayashi T, Fujimoto M (2010) Detergent-resistant microdomains determine the localization of sigma-1 receptors to the endoplasmic reticulum—mitochondria junction. Mol Pharmacol 77:517–528
67. Lynes EM, Bui M, Yap MC, Benson MD, Schneider B, Ellgaard L, Berthiaume LG, Simmen T (2012) Palmitoylated TMX and calnexin target to the mitochondria-associated membrane. EMBO J 31:457–470
68. Browman DT, Resek ME, Zajchowski LD, Robbins SM (2006) Erlin-1 and erlin-2 are novel members of the prohibitin family of proteins that define lipid-raft-like domains of the ER. J Cell Sci 119:3149–3160
69. Rippo MR, Malisan F, Ravagnan L, Tomassini B, Condò I, Costantini P, Susin SA, Rufini A, Todaro M, Kroemer G, Testi R (2000) GD3 ganglioside directly targets mitochondria in a bcl-2 controlled fashion. FASEB J 14:2047–2054
70. Garcia-Ruiz C, Colell A, Morales A, Calva M, Enrich C, Fernandez-Checa JC (2002) Trafficking of ganglioside GD3 to mitochondria by tumor necrosis factor-alpha. J Biol Chem 277:36443–36448
71. Sorice M, Garofalo T, Misasi R, Manganelli V, Vona R, Malorni W (2012) Ganglioside GD3 as a raft component in cell death regulation. Anticancer Agents Med Chem 12:376–382
72. Sorice M, Mattei V, Matarrese P, Garofalo T, Tinari A, Gambardella L, Ciarlo L, Manganelli V, Tasciotti V, Misasi R, Malorni W (2012) Dynamics of mitochondrial raft-like microdomains in cell life and death. Commun Integr Biol 5:217–219
73. Giammarioli AM, Garofalo T, Sorice M, Misasi R, Gambardella L, Gradini R, Fais S, Pavan A, Malorni W (2001) GD3 glycosphingolipid contributes to Fas-mediated apoptosis via association with ezrin cytoskeletal protein. FEBS Lett 506: 45–50
74. Gajate C, Mollinedo F (2005) Cytoskeleton-mediated death receptor and ligand concentration in lipid rafts forms apoptosis-promoting clusters in cancer chemotherapy. J Biol Chem 280:11641–11647
75. Rusinol AE, Cui Z, Chen MH, Vance J (1994) A unique mitochondria-associated membrane fraction from rat liver has a high capacity for lipid synthesis and contains pre-Golgi secretory proteins including nascent lipoproteins. J Biol Chem 269:27494–27502
76. Sorice M, Matarrese P, Manganelli V, Tinari A, Giammarioli AM, Mattei V, Misasi R, Garofalo T, Malorni W (2010) Role of GD3-CLIPR-59 association in lymphoblastoid T cell apoptosis triggered by CD95/Fas. PLoS One 5:e8567
77. Lallemand-Breitenbach V, Quesnoit M, Braun V, El Marjou A, Pous C, Goud B, Perez F (2004) CLIPR-59 is a lipid raft-associated protein containing a cytoskeleton associated protein glycine-rich domain (CAP-Gly) that perturbs microtubule dynamics. J Biol Chem 279:41168–41178
78. Burns RG (1991) Alpha-, beta-, and gamma tubulins: sequence comparisons and structural constraints. Cell Motil Cytoskeleton 20:181–189
79. Mattei V, Matarrese P, Garofalo T, Tinari A, Gambardella L, Ciarlo L, Manganelli V, Tasciotti V, Misasi R, Malorni W (2011) Recruitment of cellular prion protein to mitochondrial raft-like microdomains contributes to apoptosis execution. Mol Biol Cell 22:4842–4853
80. Sorice M, Mattei V, Tasciotti V, Manganelli V, Garofalo T, Misasi R (2012) Trafficking of $PrP^c$ to mitochondrial raft-like microdomains during cell apoptosis. Prion 6:354–358
81. Dall'Armi C, Devereaux KA, Di Paolo G (2013) The role of lipids in the control of autophagy. Curr Biol 23:R33–R45
82. Hamasaki M, Furuta N, Matsuda A, Nezu A, Yamamoto A, Fujita N, Oomori H, Noda T, Haraguchi T, Hiraoka Y, Amano A, Yoshimori T (2013) Autophagosomes form at ER-mitochondria contact sites. Nature. doi:10.1038/nature11910
83. Matarrese P, Garofalo T, Manganelli V, Gambardella L, Marconi M, Grasso M, Tinari A, Misasi R, Malorni W, Sorice M (2014) Evidence for the involvement of GD3 ganglioside in autophagosome formation and maturation. Autophagy 10:750–765
84. Young MM, Kester M, Wang HG (2013) Sphingolipids: regulators of crosstalk between apoptosis and autophagy. J Lipid Res 54:5–18
85. Lutter M, Fang M, Luo X, Nishijima M, Xie X, Wang X (2000) Cardiolipin provides specificity for targeting of tBid to mitochondria. Nat Cell Biol 2:754–761
86. Kuwana T, Mackey MR, Perkins G, Ellisman MH, Latterich M, Schneiter R, Green DR, Newmeyer DD (2002) Bid, Bax, and lipids cooperate to form supramolecular openings in the outer membrane. Cell 111:331–342
87. Brooks C, Wei Q, Feng L, Dong G, Tao Y, Mei L, Xie ZJ, Dong Z (2007) Bak regulates mitochondrial morphology and pathology during apoptosis by interacting with mitofusins. Proc Natl Acad Sci USA 104:11649–11654
88. Ciarlo L, Manganelli V, Garofalo T, Matarrese P, Tinari A, Misasi R, Malorni W, Sorice M (2010) Association of fission proteins with mitochondrial raft-like domains. Cell Death Differ 17:1047–1058
89. Parone PA, Martinou JC (2006) Mitochondrial fission and apoptosis: an ongoing trial. Biochim Biophys Acta 1763:522–530
90. Martinou JC, Youle RJ (2006) Which came first, the cytochrome c release or the mitochondrial fission? Cell Death Differ 13:1291–1295

91. Suen DF, Norris KL, Youle RJ (2009) Mitochondrial dynamics and apoptosis. Genes Dev 22:1577–1590
92. Cipolat S, Rudka T, Hartmann D, Costa V, Serneels L, Craessaerts K, Metzger K, Frezza C, Annaert W, D'Adamio L, Derks C, Dejaegere T, Pellegrini L, D'Hooge R, Scorrano L, De Strooper B (2006) Mitochondrial rhomboid PARL regulates cytochrome c release during apoptosis via OPA1-dependent cristae remodeling. Cell 126:163–175
93. Frezza C, Cipolat S, de Brito OM, Micaroni M, Beznoussenko GV, Rudka T, Bartoli D, Polishuck RS, Danial NN, De Strooper B, Scorrano L (2006) OPA1 controls apoptotic cristae remodeling independently from mitochondrial fusion. Cell 126:177–189
94. de Brito OM, Scorrano L (2008) Mitofusin 2: a mitochondria-shaping protein with signaling roles beyond fusion. Antioxid Redox Signal 10:621–633
95. Cereghetti GM, Scorrano L (2006) The many shapes of mitochondrial death. Oncogene 25:4717–4724
96. Satoh M, Hamamoto T, Seo N, Kagawa Y, Endo H (2003) Differential sublocalization of the dynamin-related protein OPA1 isoforms in mitochondria. Biochem Biophys Res Commun 300:482–493
97. Wieslaw Z, Michał S, Artur N, Takashi W, Jan JK, Robert AO, Narcyz K, Jedrzej A, Mariusz RW, Michal W (2010) Methyl-beta-cyclodextrin induces mitochondrial cholesterol depletion and alters the mitochondrial structure and bioenergetics. FEBS Lett 584:4606–4610
98. Scorrano L, Petronilli V, Di Lisa F, Bernardi P (1999) Commitment to apoptosis by GD3 ganglioside depends on opening of the mitochondrial permeability transition pore. J Biol Chem 274:22581–22585
99. Yoon Y, Krueger EW, Oswald BJ, McNiven MA (2003) The mitochondrial protein hFis1 regulates mitochondrial fission in mammalian cells through an interaction with the dynamic-like protein DLP1. Mol Cell Biol 23:5409–5420
100. Lee YJ, Jeong SJ, Karbowski M, Smith CL, Joule RJ (2004) Roles of the mammalian mitochondrial fission and fusion mediators Fis1, Drp1, and Opa1 in apoptosis. Mol Biol Cell 15:5001–5011
101. Cossarizza A, Franceschi C, Monti D, Salvioli S, Bellesia E, Rivabene R, Biondo L, Rainaldi G, Tinari A, Malorni W (1995) Protective effect of N-acetylcysteine in tumor necrosis factor-alpha-induced apoptosis in U937 cells: the role of mitochondria. Exp Cell Res 220:232–240
102. Zurgil N, Schiffer Z, Shafran Y, Kaufman M, Deutsch M (2000) Fluorescein fluorescence hyperpolarization as an early kinetic measure of the apoptotic process. Biochem Biophys Res Commun 268:155–163
103. Banki K, Hutter E, Gonchoroff NJ, Perl A (1999) Elevation of mitochondrial transmembrane potential and reactive oxygen intermediate levels are early events and occur independently from activation of caspases in Fas signaling. J Immunol 162:1466–1479
104. Matarrese P, Tinari A, Mormone E, Bianco GA, Toscano MA, Ascione B, Rabinovich GA, Malorni W (2005) Galectin-1 sensitizes resting human T lymphocytes to Fas (CD95)-mediated cell death via mitochondrial hyperpolarization, budding, and fission. J Biol Chem 280:6969–6985
105. Ferri KF, Kroemer G (2001) Organelle-specific initiation of cell death pathway. Nat Cell Biol 3:255–263
106. Garcia-Ruiz C, Colell A, Paris R, Fernandez-Checa JC (2000) Direct interaction of GD3 ganglioside with mitochondria generates reactive oxygen species followed by mitochondrial permeability transition, cytochrome c release, and caspase activation. FASEB J 14:847–858
107. Higuchi Y, Miura T, Kajimoto T, Ohta Y (2005) Effects of disialoganglioside GD3 on the mitochondrial membrane potential. FEBS Lett 579:3009–3013
108. Bossy-Wetzel E, Barsoum MJ, Godzik A, Schwarzenbacher R, Lipton SA (2003) Mitochondrial fission in apoptosis, neurodegeneration and aging. Curr Opin Cell Biol 15:706–716
109. Schengrund CL (2010) Lipid rafts: keys to neurodegeneration. Brain Res Bull 82:7–17
110. Herrera JL, Diaz M, Hernández-Fernaud JR, Salido E, Alonso R, Fernández C, Morales A, Marin R (2011) Voltage-dependent anion channel as a resident protein of lipid rafts: post transductional regulation by estrogens and involvement in neuronal preservation against Alzheimer's disease. J Neurochem 116:820–827
111. Martel C, Wang Z, Brenner C (2014) VDAC phosphorylation, a lipid sensor influencing the cell fate. Mitochondrion 19 Pt A:69–77
112. Vande Velde C, Miller TM, Cashman NR, Cleveland DW (2008) Selective association of misfolded ALS-linked mutant SOD1 with the cytoplasmic face of mitochondria. Proc Natl Acad Sci USA 105:4022–4027
113. Israelson A, Arbel N, Da Cruz S, Ilieva H, Yamanaka K, Shoshan-Barmatz V, Cleveland DW (2010) Misfolded mutant SOD1 directly inhibits VDAC1 conductance in a mouse model of inherited ALS. Neuron 67:575–587
114. Cozzolino M, Ferri A, Valle C, Carrì MT (2012) Mitochondria and ALS: implications from novel genes and pathways. Mol Cell Neurosci 55:44–49
115. Cozzolino M, Pesaresi MG, Amori I, Crosio C, Ferri A, Nencini M, Carrì MT (2009) Oligomerization of mutant SOD1 in mitochondria of motoneuronal cells drives mitochondrial damage and cell toxicity. Antioxid Redox Signal 11:1547–1558
116. Ferri A, Fiorenzo P, Nencini M, Cozzolino M, Pesaresi MG, Valle C, Sepe S, Moreno S, Carrì MT (2010) Glutaredoxin 2 prevents aggregation of mutant SOD1 in mitochondria and abolishes its toxicity. Hum Mol Genet 19:4529–4542
117. Song W, Song Y, Kincaid B, Bossy B, Bossy-Wetzel E (2013) Mutant SOD1G93A triggers mitochondrial fragmentation in spinal cord motor neurons: neuroprotection by SIRT3 and PGC-1α. Neurobiol Dis 51:72–81
118. Naon D, Scorrano L (2014) At the right distance: ER-mitochondria juxtaposition in cell life and death. Biochim Biophys Acta 1843:2184–2194
119. Fujimoto M, Hayashi T, Su TP (2012) The role of cholesterol in the association of endoplasmic reticulum membranes with mitochondria. Biochem Biophys Res Commun 417:635–639
120. Stiban J, Caputo L, Colombini M (2008) Ceramide synthesis in the endoplasmic reticulum can permeabilize mitochondria to proapoptotic proteins. J Lipid Res 49:625–634
121. Nishimura AL, Mitne-Neto M, Silva HC, Richieri-Costa A, Middleton S, Cascio D, Kok F, Oliveira JR, Gillingwater T, Webb J, Skehel P, Zatz M (2004) A mutation in the vesicle-trafficking protein VAPB causes late-onset spinal muscular atrophy and amyotrophic lateral sclerosis. Am J Hum Genet 75:822–831
122. Al-Saif A, Bohlega S, Al-Mohanna F (2012) Loss of ERLIN2 function leads to juvenile primary lateral sclerosis. Ann Neurol 72:510–516
123. Vollrath JT, Sechi A, Dreser A, Katona I, Wiemuth D, Vervoorts J, Dohmen M, Chandrasekar A, Prause J, Brauers E, Jesse CM, Weis J, Goswami A (2014) Loss of function of the ALS protein SigR1 leads to ER pathology associated with defective autophagy and lipid raft disturbances. Cell Death Dis 12:e1290
124. Stoica R, De Vos KJ, Paillusson S, Mueller S, Sancho RM, Lau KF, Vizcay-Barrena G, Lin WL, Xu YF, Lewis J, Dickson DW, Petrucelli L, Mitchell JC, Shaw CE, Miller CC (2014) ER-mitochondria associations are regulated by the VAPB-PTPIP51 interaction and are disrupted by ALS/FTD-associated TDP-43. Nat Commun 5:3996
125. Ahn SW, Kim JE, Park KS, Choi WJ, Hong YH, Kim SM, Kim SH, Lee KW, Sung JJ (2012) The neuroprotective effect of the

GSK-3βinhibitor and influence on the extrinsic apoptosis in the ALS transgenic mice. J Neurol Sci 320:1–5
126. Sui Z, Kovács AD, Maggirwar SB (2006) Recruitment of active glycogen synthasekinase-3 into neuronal lipid rafts. Biochem Biophys Res Commun 345:1643–1648
127. Valencia A, Reeves PB, Sapp E, Li X, Alexander J, Kegel KB, Chase K, Aronin N, DiFiglia M (2010) Mutant huntingtin and glycogen synthase kinase 3-beta accumulate in neuronal lipid rafts of a presymptomatic knock-in mouse model of Huntington's disease. J Neurosci Res 88:179–190
128. Chou CH, Lin CC, Yang MC, Wei CC, Liao HD, Lin RC, Tu WY, Kao TC, Hsu CM, Cheng JT, Chou AK, Lee CI, Loh JK, Howng SL, Hong YR (2012) GSK3beta-mediated Drp1 phosphorylation induced elongated mitochondrial morphology against oxidative stress. PLoS One 7:e49112
129. Shirendeb UP, Calkins MJ, Manczak M, Anekonda V, Dufour B, McBride JL, Mao P, Reddy PH (2012) Mutant huntingtin's interaction with mitochondrial protein Drp1 impairs mitochondrial biogenesis and causes defective axonal transport and synaptic degeneration in Huntington's disease. Hum Mol Genet 21:406–420
130. Yano H, Baranov SV, Baranova OV, Kim J, Pan Y, Yablonska S, Carlisle DL, Ferrante RJ, Kim AH, Friedlander RM (2014) Inhibition of mitochondrial protein import by mutant huntingtin. Nat Neurosci 17:822–831
131. Bertoni A, Giuliano P, Galgani M, Rotoli D, Ulianich L, Adornetto A, Santillo MR, Porcellini A, Avvedimento VE (2011) Early and late events induced by polyQ-expanded proteins: identification of a common pathogenic property of polYQ-expanded proteins. J Biol Chem 286:4727–4741
132. Valencia A, Sapp E, Kimm JS, McClory H, Reeves PB, Alexander J, Ansong KA, Masso N, Frosch MP, Kegel KB, Li X, DiFiglia M (2013) Elevated NADPH oxidase activity contributes to oxidative stress and cell death in Huntington's disease. Hum Mol Genet 22:1112–1131
133. Ayala-Peña S (2013) Role of oxidative DNA damage in mitochondrial dysfunction and Huntington's disease pathogenesis. Free Radic Biol Med 62:102–110
134. Song W, Chen J, Petrilli A, Liot G, Klinglmayr E, Zhou Y, Poquiz P, Tjong J, Pouladi MA, Hayden MR, Masliah E, Ellisman M, Rouiller I, Schwarzenbacher R, Bossy B, Perkins G, Bossy-Wetzel E (2011) Mutant huntingtin binds the mitochondrial fission GTPase dynamin-related protein-1 and increases its enzymatic activity. Nat Med 17:377–382
135. Guo X, Disatnik MH, Monbureau M, Shamloo M, Mochly-Rosen D, Qi X (2013) Inhibition of mitochondrial fragmentation diminishes Huntington's disease-associated neurodegeneration. J Clin Invest 123:5371–5388
136. Ciarlo L, Manganelli V, Matarrese P, Garofalo T, Tinari A, Gambardella L, Marconi M, Grasso M, Misasi R, Sorice M, Malorni W (2012) Raft-like microdomains play a key role in mitochondrial impairment in lymphoid cells from patients with Huntington's disease. J Lipid Res 53:2057–2068
137. Martín MG, Pfrieger F, Dotti CG (2014) Cholesterol in brain disease: sometimes determinant and frequently implicated. EMBO Rep 15:1036–1052
138. Dupuis L, Oudart H, René F, Gonzalez de Aguilar JL, Loeffler JP (2004) Evidence for defective energy homeostasis in amyotrophic lateral sclerosis: benefit of a high-energy diet in a transgenic mouse model. Proc Natl Acad Sci USA 101:11159–11164
139. Poggiolini I, Saverioni D, Parchi P (2013) Prion protein misfolding, strains, and neurotoxicity: an update from studies on Mammalian prions. Int J Cell Biol 2013:910314
140. Prusiner SB (2013) Biology and genetics of prions causing neurodegeneration. Ann Rev Genet 47:601–623
141. Mattei V, Garofalo T, Misasi R, Gizzi C, Mascellino MT, Dolo V, Pontieri GM, Sorice M, Pavan A (2002) Association of cellular prion protein with gangliosides in plasma membrane microdomains of neural and lymphocytic cells. Neurochem Res 27:743–749
142. Mattei V, Garofalo T, Misasi R, Circella A, Manganelli V, Lucania G, Pavan A, Sorice M (2004) Prion protein is a component of the multimolecular signaling complex involved in T cell activation. FEBS Lett 560:14–18
143. Vey M, Pilkuhn S, Wille H, Nixon R, DeArmond SJ, Smart EJ, Anderson RGW, Taraboulos A, Prusiner SB (1996) Subcellular colocalization of the cellar and scrapie prion proteins in caveole-like membranous domains. Proc Natl Acad Sci USA 93:14945–14949
144. Naslavsky N, Stein R, Yanai A, Friedlander G, Taraboulos A (1997) Characterization of detergent-insoluble complexes containing the cellular prion protein and its scrapie isoform. J Biol Chem 272:6324–6331
145. Watt NT, Taylor DR, Gillot A, Thomas DA, Perera WS, Hooper NM (2005) Reactive oxygen species-mediated beta-cleavage of the prion protein in the cellular response to oxidative stress. J Biol Chem 280:35914–35921
146. Hu W, Kieseier B, Frohman E, Eagar TN, Rosenberg RN, Hartung HP, Stuve O (2008) Prion proteins: physiological functions and role in neurological disorders. J Neurol Sci 264:1–8
147. Zhang Y, Qin K, Wang J, Hung T, Zhao RY (2006) Dividing roles of prion protein in staurosporine-mediated apoptosis. Biochem Biophys Res Commun 349:759–768