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Hypothesis

Cardiolipin-enriched raft-like microdomains are essential activating platforms for apoptotic signals on mitochondria

Maurizio Sorice [a,b,*], Valeria Manganelli [a], Paola Matarrese [c], Antonella Tinari [d], Roberta Misasi [a], Walter Malorni [c,1], Tina Garofalo [a,b,1]

[a] Department of Experimental Medicine, "Sapienza" University of Rome, viale Regina Elena 324, 00161 Rome, Italy
[b] Laboratory of Experimental Medicine and Environmental Pathology, "Sapienza" University, Rieti, Italy
[c] Section of Cell Aging and Degeneration, Department of Drug Research and Evaluation, Istituto Superiore di Sanita', Rome, Italy
[d] Section of Ultrastructural Infectious Pathology, Department of Technology and Health, Istituto Superiore di Sanita', Rome, Italy

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ABSTRACT

Cardiolipin (CL) has recently been shown to provide an anchor and an essential activating platform for caspase-8 on mitochondria. We hypothesize that these platforms may correspond to "raft-like" microdomains, which have demonstrated to be detectable on mitochondrial membrane of cells undergoing apoptosis. The role for CL in "raft-like" microdomains could be to anchor caspase-8 at contact sites between inner and outer membranes, facilitating its self-activation, Bid cleavage and apoptosis execution. The role played by "raft-like" microdomains in the apoptotic program could introduce a new task in the pathogenetic studies on human diseases associated with cardiolipin dismetabolism.

1. The role of lipid rafts in cell apoptosis

Apoptosis represents a genetically defined program of cell death that controls development and remodeling of tissues. Different mechanisms are involved in the regulation of this process. An external stimulus may engage a cell surface receptor, thus triggering an intracellular signal transduction pathway. In this case, following the activation of a death receptor, the so-called death-induced signaling complex (DISC) is assembled within specialized platforms of the cell plasma membrane [1]. In these domains, named lipid rafts [2], glycosphingolipid molecules, including gangliosides, are concentrated [3] and complexed with several glycoproteins implied in signal transduction pathways [4]. These include tyrosine kinase receptors, mono- or heterotrimeric G proteins, glycosyl phosphatidylinositol-anchored proteins, Src-like tyrosine kinase and protein kinase C isozymes [5]. During apoptosis the DISC is assembled within lipid rafts, where proenzymes of the apical caspases, mainly procaspase-8, are recruited [6] and undergo activation by proximity. When enough DISCs are formed, active apical caspases directly process and turn effector caspases on. Alternatively, in type II cells, additional amplificatory mechanisms are required [7]. In these cells, caspase-8 cleaves a BH3 member of the Bcl-2 family of proteins, Bid, triggering its translocation to mitochondria leading to cytochrome *c* release [7].

Very recently, Gonzalvez et al. [8] showed a novel role for the mitochondrial phospholipid cardiolipin (CL) in the activation of the apical caspase-8 in type II cells, which requires the mitochondrial amplificatory loop. These authors showed that in these cells, following an external stimulus, caspase-8 translocates to the mitochondrial membrane, where it binds to CL. Thus, CL provides an anchor and an activating platform for caspase-8 translocation to, and embedding in, the mitochondrial membrane, where it oligomerizes and is activated, leading to the proteolytically active p43 and p10 fragments [9].

2. Raft-like microdomains on mitochondria

We recently demonstrated that under pro-apoptotic stimulation, raft-like microdomains can be detected on mitochondria, where they contribute to apoptosis-associated modifications and to late apoptogenic events [10]. In fact, they could represent preferential sites where some key reactions can be catalyzed,

* Corresponding author. Address: Department of Experimental Medicine, "Sapienza" University of Rome, viale Regina Elena 324, 00161 Rome, Italy. Fax: +39 6 4454820.
E-mail address: maurizio.sorice@uniroma1.it (M. Sorice).
[1] They equally contributed to this work.

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contributing to cell death execution steps. For instance, raft-like domains are enriched in gangliosides (GD3, GM3), but show a relatively low content of cholesterol. In these domains some molecules, including the voltage-dependent anion channel-1 (VDAC-1) and the fission protein hFis1, are enriched, whereas Bcl-2 family proteins such as truncated Bid (t-Bid) and Bax are recruited, following CD95/Fas triggering. Both mitochondria depolarization and cytochrome *c* release are dependent on raft-like microdomain integrity, since the disruption of raft-like microdomains by methyl-β-cyclodextrin prevented mitochondria depolarization, cytochrome *c* release and apoptosis execution [11]. It was suggested that these microdomains could bolster mitochondrial subcompartmentalization hijacking cells towards a more apoptosis-prone phenotype.

3. Presence of cardiolipin in raft-like microdomains on mitochondria

Till now, CL, a specific mitochondrial phospholipid, was found predominantly in the inner membrane, but it was also found at the contact sites formed between the inner and outer membranes [12].

To verify whether CL is present in mitochondrial raft-like microdomains, we analyzed the distribution of cardiolipin in Triton X-100-insoluble fractions from isolated mitochondria [13] of CEM lymphoblastoid T cells, either in the absence or in the presence of stimulation through CD95/Fas. Our observations revealed that CL is present mainly in the detergent-insoluble fraction of both untreated and anti-CD95/Fas treated cells (Fig. 1). Hence, CL, as well as cholesterol, is constitutively present in GD3-enriched raft-like microdomains of mitochondria. On the contrary, caspase-8, as well as tBid and Bax, are recruited to this fraction only upon CD95/Fas ligation. Bak, which forms oligomers with Bax on the outer mitochondrial membrane, also partition in the proposed raft-like microdomains fraction (Fig. 1); this distribution was also observed in Bax-negative primary cells, such as hepatocytes.

The observation that CL may be associated with mitochondrial raft-like microdomains is not surprising, since it contains four acyl chains, most of which highly unsaturated and two negative charges on the head group. Mature CL is produced by the combined action of phospholipase A, which removes one saturated acyl chain to generate monolysocardiolipin (MCL), and by tafazzin, a mitochondrial enzyme that catalyzes the addition of an unsaturated chain to MCL [14]. Moreover, Kutik et al. [15] identified Tam41 (translocator and maintenance protein 41) as a regulator of CL biosynthesis, suggesting that this protein plays a dual role in maintaining mitochondrial structural integrity by regulating both protein and phospholipid composition. A fascinating new dimension of CL regulation is seen in the study of Osman et al. [16], which shows that the biosynthesis of CL and PE is coordinately regulated and tied to the prohibitins (Phbs), which are also involved in cell proliferation, cristae morphogenesis and functional integrity of mitochondria [16]. Based on these data, Gohil et al. [17], proposed the presence of defined "lipid clusters" in the mitochondrial membrane facilitated by the ringlike Phbs. Since their role in the maintenance of mitochondrial morphology has been proposed, a possible role of Phbs and CL in mitochondrial fusion and fission is conceivable [17].

We previously reported a direct Bid-CL interaction following CD95/Fas stimulation [18]. This was demonstrated by the observation that CL and its metabolite MCL coimmunoprecipitated with Bid especially after CD95/Fas triggering, indicating a dynamic interaction of the protein with CL and its metabolites. In addition, it was shown that CL acts as the mitochondrial receptor for Bid

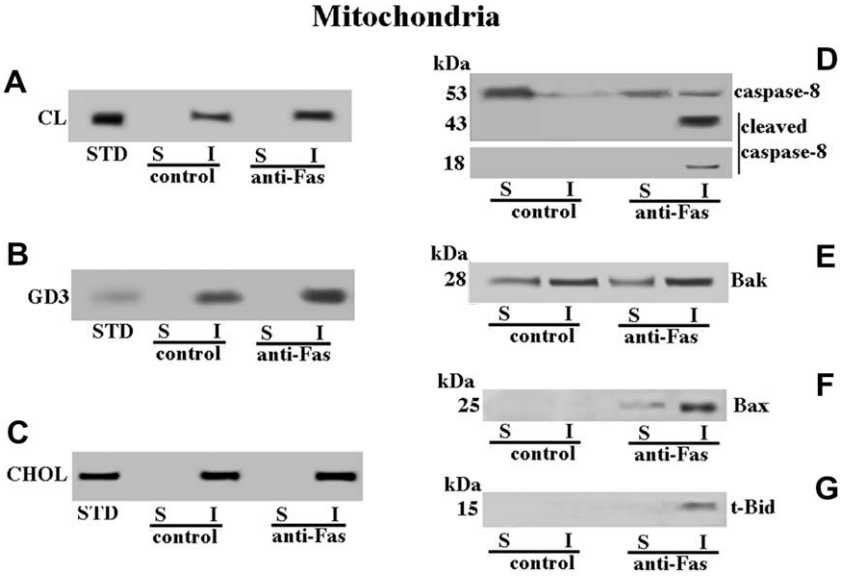

Fig. 1. (A) Raft-like lipid microdomains localization of cardiolipin. Isolated mitochondria from CEM cells either untreated or treated with CD95/Fas for 1 h were detergent solubilized, according to Ref. [13]. Both Triton X-100-soluble and -insoluble fractions were subjected to phospholipid extraction and analyzed by monodimensional HPTLC analysis, by using a solvent system of chloroform/methanol/acetic acid/water (100:75:7:4, v/v/v/v), followed by staining by exposure to iodide vapours. (B) Raft-like lipid microdomains localization of GD3. Isolated mitochondria from CEM cells, treated as above, were subjected to ganglioside extraction and analyzed by HPTLC analysis, by using a solvent system of chloroform/methanol/0.25% aqueous KCl (5:4:1, v/v/v). GD3 localization was detected by TLC immunostaining using an anti-GD3 MoAb (GMR19). (C) Raft-like lipid microdomains localization of cholesterol. Isolated mitochondria from CEM cells, treated as above, were subjected to neutral lipids extraction and analyzed by HPTLC analysis by using a solvent system of hexane/diethylether/acetic acid (70:30:1, v/v/v). (D) Caspase-8 oligomerizes into mitochondrial raft-like lipid microdomains after CD95/Fas triggering. Isolated mitochondria from CEM cells, treated as above, were analyzed by Western blot. Caspase-8 localization and autoprocessing was detected using the anti-caspase-8 MoAb. The different cleaved products of caspase-8 are indicated on the right. (E) Raft-like lipid microdomains localization of Bak. Isolated mitochondria from CEM cells, treated as above, were analyzed by Western blot. Bak localization was detected using the anti-Bak polyclonal Ab. (F) Raft-like lipid microdomains localization of Bax. Isolated mitochondria from CEM cells, treated as above, were analyzed by Western blot. Bax localization was detected using the anti-Bax MoAb [11]. (G) Raft-like lipid microdomains localization of Bid. Isolated mitochondria from CEM cells, treated as above, were analyzed by Western blot. Bid localization was detected using the anti-Bid polyclonal Ab [11]. The purity of the mitochondrial preparations was checked as reported in Ref. [11].

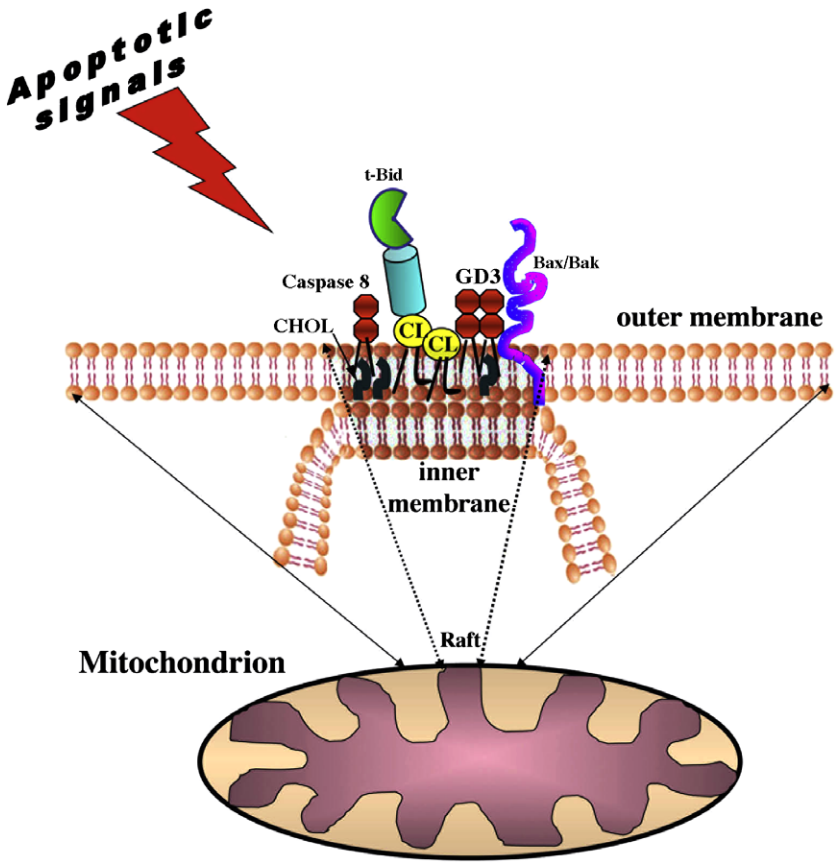

Fig. 2. Schematic drawing illustrating how cardiolipin-enriched raft-like microdomains are essential activating platforms for apoptotic signals on mitochondria. CL, cardiolipin; CHOL, cholesterol. Cardiolipin-enriched microdomains, at the contact sites between inner and outer mitochondrial membranes, represent specialized portions of mitochondrial membrane where t-Bid is recruited and causes Bak/Bax oligomerization.

[19], providing specificity for targeting of tBid to mitochondria and regulating the oligomerization of Bax [20]. In particular, a preferential interaction of tBid with MCL has been observed [21], suggesting that this protein of the bcl family has lipid transfer activity between mitochondria and endoplasmic reticulum [22]. It has been proposed that Bid interaction with MCL "primes" the mitochondrial outer membrane via segregation of lipid domains, facilitating membrane discontinuity and leakage of apoptogenic factors. Since t-Bid and Bax have been shown to be recruited within mitochondrial raft-like microdomains following CD95/Fas triggering [11], all these findings strongly suggest that CL is a constituent of these specialized platforms on mitochondrial membrane.

4. A hypothesis: cardiolipin-enriched platforms correspond to "raft-like microdomains

Cardiolipin has been shown to act as an activation platform for bringing together both the enzyme (caspase-8) and its substrate (Bid). Also, the mobilization of cytochrome *c*, another key apoptotic event, is tightly regulated by its interaction with CL. Thus, CL is an essential constituent of functional domains, localized at contact sites between the inner and outer mitochondrial membranes, from which it orchestrates apoptosis by integrating signals from a variety of death-inducing proteins [23]. Here, we hypothesize that these platforms may correspond to the lipid microdomains that we described on mitochondrial membrane, in which most of these proteins have been identified [10]. The specific role for CL in mitochondrial raft-like microdomains could be to anchor caspase-8 at contact sites between inner and outer membranes, facilitating its self-activation in order to produce active Bid where it is needed (Fig. 2). This, in turn, causes Bak/Bax oligomerization and cytochrome *c* release.

Finally, our findings underline the role played by raft-like microdomains in the apoptotic program, introducing a new task for the study of the pathogenesis of Barth syndrome [24] a rare human genetic disease in which the primary causative factor is an alteration in cardiolipin remodeling.

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