

Dynamics of mitochondrial raft-like microdomains in cell life and death

Maurizio Sorice,^{1,2,*} Vincenzo Mattei,¹ Paola Matarrese,^{3,4,5} Tina Garofalo,² Antonella Tinari,^{3,6} Lucrezia Gambardella,^{3,4} Laura Ciarlo,^{3,4} Valeria Manganelli,² Vincenzo Tasciotti,¹ Roberta Misasi² and Walter Malorni^{3,4,7}

¹Laboratory of Experimental Medicine and Environmental Pathology; Sabina Universitas; Rieti, Italy; ²Department of Experimental Medicine, Sapienza University; Rome, Italy; ³Istituto Superiore di Sanità; Rome, Italy; ⁴Section of Cell Aging and Degeneration; Department of Therapeutic Research and Medicine Evaluation; ⁵Center of Integrated Metabolomics; Rome, Italy; ⁶Department of Technology and Health; ⁷San Raffaele Institute Sulmona; L'Aquila, Italy

On the basis of the biochemical nature of lipid rafts, composed by glycosphingolipids, cholesterol and signaling proteins, it has been suggested that they are part of the complex framework of subcellular intermixing activities that lead to CD95/Fas-triggered apoptosis. We demonstrated that, following CD95/Fas triggering, cellular prion protein (PrP^C), which represents a paradigmatic component of lipid rafts, was redistributed to mitochondrial raft-like microdomains via endoplasmic reticulum (ER)-mitochondria associated membranes (MAM) and microtubular network.

Raft-like microdomains appear to be involved in a series of intracellular functions, such as: (1) the membrane “scrambling” that participates in cell death execution pathways, (2) the remodeling of organelles, (3) the recruitment of proteins to the mitochondria; (4) the mitochondrial oxidative phosphorylation and ATP production.

In conclusion, we suggest that lipid raft components can exert their regulatory activity in apoptosis execution at three different levels: (1) in the DISC formation at the plasma membrane; (2) in the intracellular redistribution at cytoplasmic organelles, and, (3) in the structural and functional mitochondrial modifications associated with apoptosis execution.

Subcellular organelles including mitochondria, endoplasmic reticulum and the Golgi complex are involved in the progression of cell death program. Recent evidence suggests that CD95/Fas-mediated apoptosis induces scrambling of mitochondrial

and secretory organelles by alteration of membrane traffic. Scrambling among different cell organelles include plasma membrane, endoplasmic reticulum as well as lysosomal vesicles.¹ For instance, the importance of endoplasmic reticulum (ER) in the apoptotic cascade has recently been investigated.^{2,3} Under ER stress, ER transmembrane receptors initiate the unfolded protein response.⁴ If the adaptive response fails, apoptotic cell death ensues. This response is associated with organelle remodeling and intermixing. In the same vein, lysosomal organelles have been analyzed in different experimental settings, precisely devoted to the understanding of how certain lysosomal cysteinyl proteases, e.g. cathepsins, can contribute to the cross talk between endolysosomes and mitochondria during apoptosis.⁵ More recently, the possible implications of the Golgi apparatus remodeling in the apoptosis execution has also been analyzed in detail. It was found that also this organelle participates to the complex framework of subcellular intermixing activities that lead to CD95/Fas-triggered apoptosis.⁶

On the basis of the biochemical nature of lipid rafts, composed by glycosphingolipids, cholesterol and signaling proteins, it has been suggested that they are part of this traffic and can participate in cell remodeling, contributing to cell death program execution.⁷⁻⁹ Although detected in various cell types, the role of lipid rafts in apoptosis has been mostly studied in T cells, where the physiological apoptotic program occurs through CD95/Fas. Previous works, including ours, identified mitochondria as possible targets for raft components and hypothesized that the

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*Correspondence to: Maurizio Sorice;
Email: maurizio.sorice@uniroma1.it

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

Microdomains	Putative function	Functional example	Contribute to
Plasma membrane rafts	√ Catalyze molecular interactions	√ Death receptor signaling	√ Receptor mediated apoptotic triggering
Golgi, ER, MAM microdomains	√ Membrane Scrambling	√ Organelle reshaping √ Trafficking √ Molecular interplay √ ER stress √ Lysosomal fusion	√ Cell physiology √ Organelle-organelle cross-talk √ Organelle fusion processes in autophagy √ Calcium signaling pathway
Mitochondrial raft-like microdomains	√ Changes of curvature √ Recruitment of fission molecules	√ Mitochondrial fusion/fission process √ Mitochondrial network remodeling √ Changes of mitochondrial membranes potential √ Signaling tags	√ Respiration √ Fission √ Cell polarization √ Release of apoptogenic factors √ Mitophagy?

ER, endoplasmic reticulum; MAM, mitochondria-associated membrane

rearrangement of raft-like microdomains may be involved in the mitochondrial remodeling leading to apoptosis execution phase.^{7,10,11}

Several of these insights have been provided by the analysis of the behavior of the ganglioside GD3, which can be considered as a paradigmatic component of rafts.¹² On this point, we demonstrated 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 cell apoptosis, microtubules may be used as tracks to direct intracytoplasmic transport of lipid raft glycosphingolipid(s) to mitochondria. This transport may be regulated by the Cytoplasmic linker proteins-59 (CLIPR-59) a new CLIP-170-related protein, involved in the regulation of microtubule dynamics.¹³ 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. 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. Since microtubules are assembled by polymerization of Tubulin hetero-dimers composed of α - and β -Tubulin,¹⁴ we showed by FRET and co-immunoprecipitation analyses that a direct GD3-tubulin interaction can effectively take place.⁸ Thus, microtubules may represent the directional network by which glycosphingolipids,

which represent 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.

Further insight in the knowledge of the dynamics of mitochondrial raft-like microdomains in cell apoptosis derives from the analysis of traffic of the cellular prion protein (PrP^C), an ubiquitous GPI-anchored protein, which represents a further paradigmatic component of lipid rafts.¹⁵ In particular, we demonstrated that, following CD95/Fas triggering, PrP^C was redistributed to raft-like microdomains at endoplasmic reticulum (ER)-mitochondria associated membranes (MAM) as well as at mitochondrial membrane.¹⁶ Thus, our data identified PrP^C as a new component of mitochondrial raft-like microdomains in cells undergoing CD95/Fas-mediated apoptosis, suggesting that PrP^C could undergo intracellular re-localization via mitochondria-associated membrane (a sub-region of the endoplasmic reticulum that facilitates crosstalk between the ER and mitochondria) and microtubular network. Usually, PrP^C is anchored at the cell surface via a GPI moiety.¹⁷ However, this protein has also been found associated with many intracellular compartments. In fact, recent evidence revealed the association of PrP^C with lipid microdomains on the ER¹⁸ as well as with the cytoskeleton network.¹⁹ Since the ER-mitochondria associated membranes (MAM) represent an ER subcompartment connected to the mitochondria, and since they display the

characteristics of lipid rafts,²⁰⁻²² we investigated whether PrP^C may be present in this compartment. Our immunoelectron microscopy observations revealed that PrP^C was actually present in both MAM and mitochondrial membranes. Moreover, 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. Hence, we hypothesize that microtubules could play key roles in the intracellular directional re-distribution of PrP^C as well as in the recruitment of this small polypeptide to the mitochondrial compartment following CD95/Fas triggering.¹⁶

On the basis of these findings, including results obtained by using agents capable of disrupt lipid rafts, we can suggest that raft-like microdomains can actually exert a role in the trafficking pathways associated with cell death and actively participate to the structural and biochemical remodeling leading to injury and apoptotic cell death program execution (Table 1). In particular, a part from their well known role at the plasma membrane as chambers with catalytic functions, these microdomains appear to be involved in a series of intracellular functions, such as: (1) the membrane “scrambling” that participates in cell death execution pathways, (2) the remodeling of organelles, e.g. changes of curvature in mitochondria, (3) the recruitment of proteins to the mitochondria, including molecules associated with mitochondrial fission;⁹ (4) the mitochondrial oxidative phosphorylation and ATP production,²³ and, furthermore, (5) they seem

to act as signaling tags in cytoplasmic directional movements and remodeling, contributing to cell polarization and to the intracytoplasmic redistribution of organelles, including mitochondria.²⁴

On these bases, we suggest that lipid raft components could exert three different regulatory activities in apoptosis execution: (1) in the DISC formation at the plasma membrane;^{25,26} (2) in

the intracellular redistribution at cytoplasmic organelles,^{16,22} and, (3) in the structural and functional mitochondrial modifications associated with apoptosis execution.^{7,11}

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