Communicative & Integrative Biology 4:3, 334-335; May/June 2011; ©2011 Landes Bioscience

Mitochondria associated membranes (MAMs) as critical hubs for apoptosis

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Apoptosis is a process of major biomedical interest, since its deregulation is involved in the pathogenesis of a broad variety of disorders (neoplasia, autoimmune disorders, viral and neurodegenerative diseases, to name a few). It is now firmly established that variations in cellular calcium (Ca^{2+}) concentration are pivotal in the control of a variety of cellular functions. Strong evidence has been accumulated supporting a central role of Ca^{2+} in the regulation of cell death. In particular, in the context of the biochemical mechanisms of apoptosis, increasing evidence support a role for endoplasmic reticulum (ER)-mitochondria Ca^{2+} cross talk as a crucial regulator of several pathways of apoptosis. Recent data highlight as also the promyelocytic leukemia protein (PML), by modulating the ER machinery at the contact sites between ER and mitochondria (the mitochondria associated membranes, MAMs), regulates cell survival through the ER-cytosol/mitochondria Ca^{2+} signaling.

Key words: apoptosis, promyelocytic leukemia protein (PML), endoplasmic reticulum (ER), mitochondria associated membranes (MAMs), calcium (Ca^{2+}), cell death, oncosuppressor, Akt, inositol-1,4,5-trisphosphate receptor (IP3R)

Submitted: 01/30/11

Accepted: 01/30/11

DOI: 10.4161/cib.4.3.15021

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Addendum to: Giorgi C, Ito K, Lin HK, Santangelo C, Wieckowski MR, Lebiedzinska M, et al. PML regulates apoptosis at endoplasmic reticulum by modulating calcium release. Science 2010; 330:1247–51; PMID: 21030605; DOI: 10.1126/science.118.

Apoptosis is an essential, genetically regulated and finely tuned process of cell elimination required for embryogenesis, development and tissue homeostasis of multicellular organisms.[1] Apoptosis takes part in the normal development and functions of organisms as different as nematodes, insects or humans. Deregulation or impairment of apoptosis can therefore have deleterious consequences. In humans, important pathological conditions such as neurodegenerative and autoimmune diseases, cancer or AIDS to name a few have in defective apoptosis the main cause.[2] Cell death by apoptosis is accompanied by a stereotyped and interconnected series of events among which cell collapse, formation of membrane blebs, chromatin condensation, DNA degradation and mitochondrial damage are well recognized.[3]

The possibility that apoptosis is critically regulated by alterations in intracellular Ca^{2+} homeostasis is now supported by a number of experimental evidence.[4-7] When the systems responsible for the regulation of cellular Ca^{2+} homeostasis are irreversibly compromised a cell is condemned to die. Cell death can occur either in a disordered manner, by necrosis (i.e., through activation of Ca^{2+}-activated hydrolysing enzymes) or in a more controlled way, by apoptosis.[8]

Important evidence pointing to a key role of Ca^{2+} in apoptotic cell death comes from the demonstration that oncogenes that protect from cell death perturb intracellular Ca^{2+} homeostasis. The first example was provided by the study of the prototype of this class of oncogenes, *bcl-2*. The *bcl-2* product shows a unique intracellular distribution, as it is localized in organelles, i.e., ER and mitochondria having an important role in controlling Ca^{2+} signaling and homeostasis.[9] It has been demonstrated that Bcl-2 can induce a decrease in the Ca^{2+} concentration of the ER by increasing the leak of the cation from this organelle.[10] Specifically, Bcl-2

(and related antiapoptotic proteins) controls the phosphorylation state of IP3R[11] that in turn regulates the leak rate through the channel.

A few recent papers have drawn the attention on the possibility that IP3R is also a target of Akt, a serine/threonine kinase.[12] This is an interesting observation, because Akt is involved in the control of apoptosis.[13] In particular, through this phosphorylation Akt inhibits Ca^{2+} release from IP3R, after Ca^{2+}-dependent apoptotic stimuli. In turn, this alteration of ER Ca^{2+} release prevents mitochondrial Ca^{2+} overload (and the consequent release from mitochondria of proapoptotic signaling molecules) and reduces significantly cellular sensitivity to Ca^{2+}-mediated apoptosis.[14,15] Vice versa, others' papers have drawn the attention on the possibility that activation of proapoptotic proteins such as Bax[16] and FHIT[17] induce an increased mitochondrial Ca^{2+} uptake after ER Ca^{2+} release induced by apoptotic stimuli.

Recent observations indicate as also the PML protein, encoded by a tumor suppressor gene implicated in the pathogenesis of leukemia and cancer,[18] plays a critical role in the Ca^{2+} cross talk between ER and mitochondria during apoptotic stimulation.[19] Within the cell PML isoforms display both nuclear and cytosolic distribution. At the nucleus PML epitomizes a multiprotein nuclear structure, the PML-nuclear bodies (PML-NBs), which depends on PML for its formation and function.[20] Interestingly, in the cytosol PML is now found to localize at the ER and the MAMs.[19] MAMs are the specialized domains selectively enriched of mitochondrial Ca^{2+} signaling elements, where Ca^{2+} transfer between ER and mitochondria takes place. In particular, on the ER side MAMs are enriched in IP3R.[21]

Pml$^{-/-}$ mice and cells are protected from apoptosis triggered by a number of stimuli such as Fas ligand, tumor necrosis factor α and type I and II interferons. These effects depend in part on the PML modulation of nuclear transcriptional pro-apoptotic pathways.[22] However, PML regulates apoptosis also induced by Ca^{2+} dependent stimuli in a transcription-independent way through its ER/MAMs localization. Indeed, in these hot signaling sites, PML is shown to regulate the phosphorylation of IP3R by controlling the activity of Akt through the recruitment of the PP2A phosphatase at the ER/MAMs. In so doing, PML is able to regulate Ca^{2+} mobilization into the mitochondrion, which then triggers the cell death program. Conversely, in the absence of PML, PP2A does not accumulate in the complexes with IP3R and Akt, and this results in an accumulation of activated Akt (phospho-Akt). Once activated Akt can hyper-phosphorylate IP3R thus inhibiting the ER Ca^{2+} release towards the mitochondria.[19]

This new apoptogenic mechanism, which appears to operate in parallel to those regulated at other sites such as the PML-NBs, demonstrates that the role of PML in apoptosis is broader than previously believed inasmuch as it does modulate apoptosis both in the nucleus as well as at the MAM. In turn, these new findings further emphasize the role of MAMs as critical hubs for the apoptotic response.

Acknowledgements

We thank members of Pinton, Wieckowski and Pandolfi laboratories for critical discussion and suggestions. This research was supported by: the Italian Association for Cancer Research (AIRC), Telethon (GGP09128), local funds from the University of Ferrara, the Italian Ministry of Education, University and Research (COFIN), the Italian Cystic Fibrosis Research Foundation and Italian Ministry of Health to P.P.; the Polish Ministry of Science and Higher Education under grant NN407 075 137 to MRW as well as to NIH grants to P.P.P.

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