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p53 orchestrates calcium signaling *in vivo*

Giovanni Sorrentino[1], Anna Comel[1], and Giannino Del Sal[1,2,*]

[1]Laboratorio Nazionale CIB; Trieste, Italy; [2]Dipartimento di Scienze della Vita; Università degli Studi di Trieste; Trieste, Italy

Intracellular calcium (Ca^{2+}) governs several aspects of the homeostasis of living organisms. The levels of Ca^{2+} in the cytosol are maintained at a concentration of about 0.1 μM by the continuous action of ATP-powered Ca^{2+} pumps, which transport Ca^{2+} ions across the plasma membrane to the outside of the cell or into the lumen of the endoplasmic reticulum (ER) and other vesicles.[1] In response to a variety of *stimuli*, Ca^{2+} is released from these storage sites leading to the activation of several Ca^{2+} responsive proteins. Intracellular Ca^{2+} fluxes play fundamental roles in regulating a wide variety of cellular responses, among which programmed cell death has been one of the most studied biological process in the last decade. Several reports have demonstrated that the Ca^{2+} content within the ER determines cell sensitivity to apoptotic stress and that changes in the Ca^{2+} flux from the ER to mitochondria represent a signal capable of triggering the rupture of the mitochondrial membrane as well as the consequent release of several pro-apoptotic factors into the cytosol.[1]

This pathway is inactivated or impaired in cancer progression, with important consequences. Indeed, several tumor suppressor genes, such as PML, PTEN and TP53, have been unexpectedly found enriched at the ER where they regulate the Ca^{2+} crosstalk between the ER and mitochondria thus favoring Ca^{2+}-dependent apoptosis after stress *stimuli*.[2] Particularly intriguing is the role of p53, a stress induced tumor suppressor, able to orchestrate mitochondrial apoptosis acting both in the nucleus via transcriptional regulation of several proteins (e.g. Bcl-xL, Bcl-2, PUMA and others) as well as in the cytoplasm.[3]

Despite its biological importance, the mechanism of Ca^{2+} flux between the ER and mitochondria and its regulation by tumor suppressors is not completely understood. Contributing in filling this gap, Pinton and colleagues found that p53 localizes at ER and at mitochondria-associated membranes (MAMs), and upon activation by a variety of *stimuli*, is able to bind the sarco/ER Ca^{2+}-ATPase (SERCA) pump, leading to an increase of Ca^{2+} release from the ER and a consequent mitochondrial Ca^{2+} overload that profoundly impacts on the ability of the cells to undergo apoptosis.[4] The same group has also recently overcome the technical limitations that slowered so far the acquisition of evidences on the *in vivo* impact of this process, by using a novel strategy that, for the first time, allows the direct monitoring of intracellular Ca^{2+} fluxes within a tumor mass.[5,6] For the first time Giorgi and colleagues, starting from the techniques already used to measure Ca^{2+} *in vivo*,[7] undoubtedly demonstrated a role for p53 in modulating Ca^{2+} homeostasis in tumors. The authors injected murine $p53^{-/-}$ embryo fibroblasts (MEF) transduced with the oncogene $H\text{-}RAS^{V12}$ alone or with wild type (wt) p53 into a skinfold chamber, that had been implanted in the dorsal skin of female athymic mice. Upon tumor growth, the masses originated from these cells have been treated with phthalocyanine, a light-activatable agent used in cancer photodynamic therapy. Photo-activation of the drug with a LED light caused ROS formation in the ER and consequent Ca^{2+} release. The monitoring of pro-apoptotic Ca^{2+} signal with the ratiometric Ca^{2+} indicator Fura-2 by spinning disc confocal microscopy unveiled a massive release of Ca^{2+} ions into the cytoplasm of $p53^{+/+}$ tumors after photo-activation. Instead, Ca^{2+} release was almost absent in the $p53^{-/-}$ counterpart. Of note, the higher Ca^{2+} response evoked by p53 was instrumental for an efficient induction of apoptosis and reduction of tumor mass. More interestingly, restoration of Ca^{2+} fluxes in $p53^{-/-}$ cells by overexpression of the mitochondrial Ca^{2+} uniporter (MCU) or of the sarco/ER Ca^{2+}-ATPase (SERCA) pump, was able to rescue the apoptotic sensitivity of the cells to activated phthalocyanine. On the other hand, pharmacological manipulation of Ca^{2+} signaling in $p53^{+/+}$ tumors efficiently prevented apoptosis *in vitro* and *in vivo*, meaning that a functional p53 is required to trigger an efficient stress-induced Ca^{2+} release able to sustain mitochondrial swelling and apoptosis (**Fig. 1**).

The potential impact of this new technique is wide opening the possibility of studying protein and RNA-based modulators of the Ca^{2+}-mediated apo-

*Correspondence to: Giannino Del Sal; Email: giannino.delsal@lncib.it
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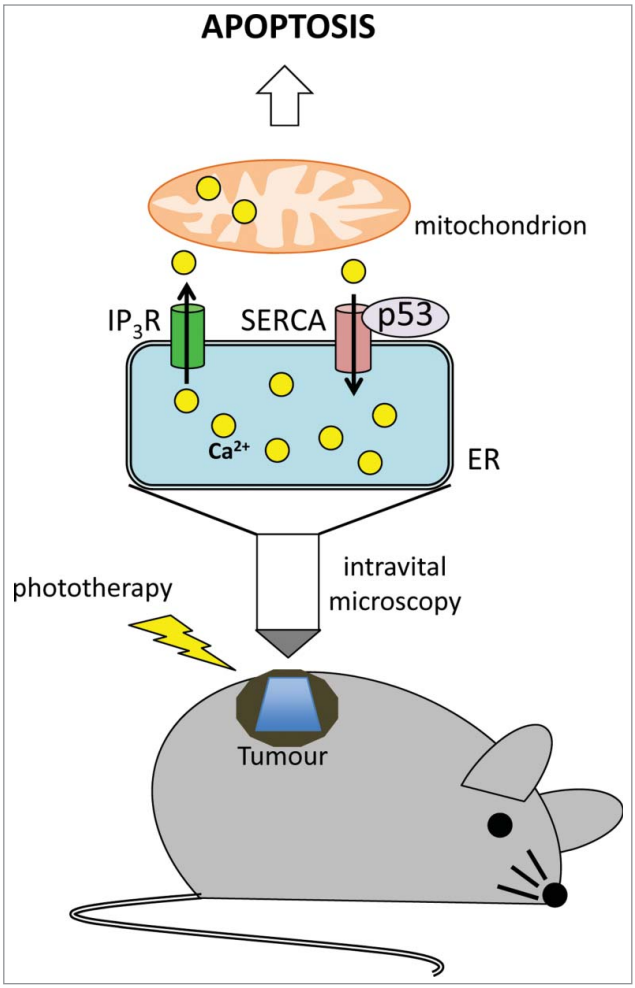

Figure 1. Intravital imaging of ER-mitochondrial Ca^{2+} transfer and its regulation by p53. Ca^{2+} is transferred to the ER by the SERCA pumps and is released by inositol 1,4,5-triphosphate (IP_3)-gated channels (IP_3R). p53 binds and activates SERCA pumps thus increasing the amount of Ca^{2+} storage into the ER. The Ca^{2+} released to the mitochondria after phototherapy leads to mitochondrial swelling with consequent apoptosis induction in $p53^{+/+}$ tumors.

ptotic cascade *in vivo* and also allowing the screening of molecules that could change cancer cell sensitivity to apoptotic signals.

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