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Perspective

Novel frontiers in calcium signaling: A possible target for chemotherapy

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ABSTRACT

Intracellular calcium (Ca^{2+}) is largely known as a second messenger that is able to drive effects ranging from vesicle formation to muscle contraction, energy production and much more. In spite of its physiological regulation, Ca^{2+} is a strategic tool for regulating apoptosis, especially during transmission between the endoplasmic reticulum and the mitochondria. Contact sites between these organelles are well-defined as signaling platforms where oncogenes and oncosuppressors can exert anti/pro-apoptotic activities. Recent advances from in vivo investigations into these regions highlight the role of the master oncosuppressor p53 in regulating Ca^{2+} transmission and apoptosis, and we propose that Ca^{2+} signals are relevant targets when developing new therapeutic approaches.

Calcium (Ca^{2+}) is a fundamental second messenger that is involved in a variety of cellular processes, such as proliferation or apoptosis, and therefore is of potential interest in cancer biology. The modulation of the Ca^{2+} signal can change cells' sensitivity to signals, such as those from chemotherapeutic agents, which induce cell death. Ca^{2+} is stored at high concentrations in the endoplasmic reticulum (ER) and is kept at very low levels in the cytoplasm and mitochondrial matrix [1–3]. While rapid release of Ca^{2+} from the ER generates transient waves in the cytoplasm and mitochondria to stimulate pro-survival events, stimuli that elevate the mitochondrial Ca^{2+} concentration for a sustained period of time induce a phenomenon called mitochondrial permeability transition (MPT), which in turn triggers apoptotic or necrotic cell death [4–6]. The Ca^{2+} is transferred to the mitochondria from the endoplasmic reticulum via specialized domains where the two organelles make contact, called mitochondria-associated membranes (MAMs) [7].

Important evidence that points to a key role for Ca^{2+} in regulating cancer comes from the data showing that oncogenes protect against cell death and perturb intracellular Ca^{2+} homeostasis. A critical link between Ca^{2+} and apoptosis was established while studying the oncoprotein B cell lymphoma 2 (Bcl-2) and its mechanism of action. Bcl-2 is a central regulator of apoptosis that is able to block or delay apoptosis in different cell types, from hematopoietic to neural cells [8]. Our group demonstrated that Bcl-2 over-expression was able to reduce steady-state Ca^{2+} levels within the ER, reducing Ca^{2+} transfer to the mitochondria during apoptotic stimulation and inhibiting apoptosis initiation [9,10]. Other groups have reported similar data, confirming that Bcl-2 could mediate an augmented leak from the compartment without affecting the activity of ER Ca^{2+} ATPases [11,12]. Moreover, it has been demonstrated that Bcl-2 in the ER acts via its N-terminal BH4 domain, which directly binds and inhibits the inositol 1,4,5-trisphosphate receptor (IP3R) [13,14]. Some years later, another master regulator of tumor growth, the mitogenic kinase Akt, was linked to Ca^{2+} homeostasis control. This protein was found to modulate the phosphorylation state of IP3R to inhibit its Ca^{2+} channel activity and then reduce the transfer of Ca^{2+} from the ER to the mitochondria [15,16]. Conversely, the tumor suppressors PML and PTEN, in cooperation with protein phosphatase 2A (PP2A), support the Ca^{2+} transfer between the ER and mitochondria by reducing the phosphorylation state of IP3R [17]. The loss of these regulators inevitably reduces the probability of correctly transmitting Ca^{2+} during the initiation of apoptosis. Apparently, tumor progression is supported by the accumulation of a series of alterations in the Ca^{2+} signal that inhibits its cytotoxic activity (Fig. 1). In particular, deregulating the Ca^{2+} signal has been associated with each cancer hallmark [18].

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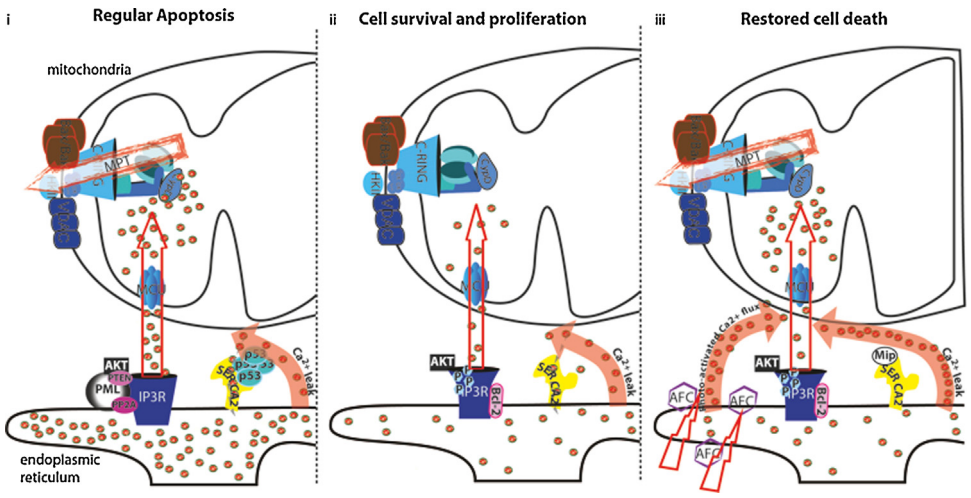

Fig. 1. (i) In normal tissue oncosupressors PML and PTEN antagonize the kinasic activity of AKT on the IP3R allowing a sustained overload of Ca^{2+} into mitochondria that induce MPT and cell death. Contemporary p53 stimulate the activity of SERCA2 promoting an increased Ca^{2+} refilling of endoplasmic reticulum. (ii) Loss of oncosuppressors or oncogene activation leads to inhibition of Ca^{2+} transfer between endoplasmic reticulum to mitochondria. This results in a consequent inhibition of apoptosis. (iii) Photodynamic therapy, by the use of aluminum ftalocyanine (AFC), is able to generate localized Ca^{2+} transfer "on-demand" in a context with inhibited IP3R. Alternatively the massive inhibition of SERCA2 activity by targeted inhibitors (Mip) can also engage an alternative Ca^{2+} signal-like event.

Most recently, we identified the master oncosuppressor p53 that participates in regulating Ca^{2+} homeostasis. Furthermore, we identified a portion of p53 that is able to interact with and stimulate the activity of the sarco/endoplasmic reticulum Ca^{2+} ATPase 2 (SERCA2) [19]. SERCA2 is the pump responsible for maintaining high Ca^{2+} levels in the ER lumen [2]. Stabilizing p53 levels results in an increased interaction with SERCA2 and an augmented Ca^{2+} concentration within the ER. This mechanism is completely dependent on the localization of p53 in the ER and is independent of its transcriptional activity. Interestingly, some naturally-occurring p53 mutants lose this effect, suggesting that p53 regulation of Ca^{2+} is at the root of its oncosuppressive activity [20].

Although most of the mechanisms related to intracellular Ca^{2+} responses have been elucidated successfully in vitro, we still know very little about the physiological role of these processes in the context of the actual tumor environment. This limits our comprehension of Ca^{2+}-related mechanisms in tumor biology and reduces the appeal of Ca^{2+} homeostasis studies in cancer research. Recently, through the use of a "skinfold chamber" installed on the back of athymic mice, we were able to generate a "window" that allowed a single-photon fluorescence microscope to investigate the Ca^{2+} signal in a tumor xenograft grown in the derma [21]. Immortalized mouse embryonic fibroblasts were grown in the window to allow the formation of a solid mass. When visible, the mass was stained with a Ca^{2+}-sensitive dye (fura-2) and aluminum chloride phthalocyanine, a photosensitizer commonly used in cancer photodynamic therapy (PDT). This compound accumulates in intracellular organelles, including the mitochondria and ER, and after appropriate photo stimulation, engages the Ca^{2+}-dependent apoptotic pathway (Fig. 1).

Using this technique we were able to confirm in vivo that the tumor suppressor p53 is able to modulate Ca^{2+} homeostasis and its activity is correlated with the ability of PDT to initiate apoptosis. Also, we reported for the first time the measurement of Ca^{2+} signals in a tumor mass at single-cell and millisecond resolution. These observations raise different questions and will lead to new investigations.

First, the use of photodynamic therapies could be reconsidered. In fact, the large Ca^{2+} wave engaged in the clones carrying wild-type p53 was able to induce extensive cell death in the stimulated mass, while the p53 KO clones that displayed smaller Ca^{2+} waves were protected from cell death. This would, at least in part, explain the resistance to photodynamic therapies reported in tumor cells lacking p53 or carrying a mutant p53 compared to cells with wild-type p53 [22,23]. As mentioned previously, due to the ability of Bcl-2 to reduce the Ca^{2+} content in the ER, this idea could be extended to the PDT resistance observed in Bcl-2 overexpressing cells.

Indeed, PDT could have increased success if applied to neoplastic lesions that neither display alterations in their ER Ca^{2+} content nor carry alterations in those oncogenes or oncosuppressor genes that are known to regulate Ca^{2+} homeostasis. Furthermore, the efficiency of these therapies could be improved by pharmacologically increasing the Ca^{2+} content in the ER lumen by, for example, inhibiting the plasma membrane Ca^{2+} ATPase (PMCA). This pump extrudes Ca^{2+} from the cytoplasm to keep its concentration below 500 nM [2]. Due to its higher affinity, the PMCA sequesters Ca^{2+} to the SERCA; thus, PMCA inhibition or silencing increases the Ca^{2+} content in the ER lumen. It has been shown that PMCA inhibition was able to counteract the H-RAS-induced oncogenic transformation and this inhibition was linked to the progressive reduction of Ca^{2+} in the ER [24].

Additionally, PDT could be coupled by using compounds that directly target p53 mutants. Indeed, these are often single point mutations with dominant negative effects. Different compounds have been developed to inactivate the mutant form or to recover p53 folding in those cases where the mutation caused structure-dependent protein inactivation. It is now established that some p53 mutations are able to affect Ca^{2+} homeostasis. Therefore, it would be extremely interesting to test if targeting the p53 mutation could recover Ca^{2+} homeostasis and, in turn, could promote PDT efficiency.

A second major consideration is related to the development of new therapeutic compounds that could restore or selectively induce Ca^{2+}-dependent apoptosis. The most probable consequence of oncogene/oncosuppressor regulation in cancer Ca^{2+} homeostasis is the altered sensitivity to apoptotic stimuli. It could be speculated that pharmacological recovery of Ca^{2+} homeostasis will be sufficient to restore sensitivity to apoptosis. Indeed, overexpression of SERCA2 is sufficient to induce apoptotic cell death in cancer cells [25]. Compounds that are able to modulate expression or regulate activity of Ca^{2+} transporters could be considered a