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EDITORIAL

Mitochondria in the line of fire

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In this issue of *Cell Death & Differentiation*, two papers (a review and a research article) discuss and investigate the importance of targeting mitochondria to arrest cancer cell proliferation and overcome cell death resistance. The review by Sainero-Alcolado et al. summarizes the main metabolic derangements originating from specific mitochondrial defects and presents the mitochondrial enzymes that can be targeted by potential antineoplastic compounds [1]; Quarato et al. explored the mechanistic link between mitochondrial physiology, intracellular Ca^{2+} unbalance, and cell death [2] (Fig. 1).

The paper by Douglas R. Green's group provided insights on the mechanism of action of the 2,4,6-trimethyl-N-(m-3-trifluoromethyl-phenyl) benzenesulfonamide (m-3M3FBS) compound, which was initially described as a phospholipase c (PLC) activator, as well as an inducer of intracellular Ca^{2+} rising through the generation of inositol 3-phosphate (IP3) [3]. However, the ability of m-3M3FBS to evoke Ca^{2+} signals is not dependent on the PLC targeting [4], but it is required to promote cell death in multiple cancer cell types [5]. Quarato et al. confirmed that the Ca^{2+}-related functions of m-3M3FBS are PLC-independent and demonstrated that m-3M3FBS triggers a rapid Ca^{2+} efflux from the Endoplasmic Reticulum (ER), culminating in mitochondrial Ca^{2+} overload and collapse of the normal mitochondrial activity, identified with a drastic depolarization and respiratory failure [2].

The authors elegantly showed how these effects are accompanied by mitochondrial inner membrane permeabilization (MIMP), which is followed by mitochondrial outer membrane permeabilization (MOMP), the release of internal (both matrix and intermembrane space) factors into the cytosol, and execution of cell death [2]. Of note, if MOMP is tightly controlled by the Bcl-2 family members [6] and triggers a rapid spillage of mitochondrial intermembrane space proteins, MIMP displays distinct features in both kinetic and molecular regulation, evidenced by the slower release of mitochondrial factors, fast mitochondrial Ca^{2+} uptake, and total independence from Bcl-2 proteins. This latter m-3M3FBS-related property is intriguing since the antiapoptotic Bcl-2 members, including BCL-2 itself as well as BCL2-like 1 (BCL2L1, best known as BCL-XL), protect from cell death also by reducing the IP3-mediated Ca^{2+} transfer from the ER to mitochondria [7–9], thus suggesting an alternative molecular route that allows mitochondrial Ca^{2+} accumulation after ER Ca^{2+} discharge. Accordingly, inhibition of the major ER Ca^{2+} release systems, such as inositol 1,4,5 trisphosphate receptors (IP3Rs) or ryanodine receptors (RyRs), as well as blocking the mitochondrial Ca^{2+} uptake machinery, by genetic manipulation of both the Mitochondrial Calcium Uniporter (MCU) and Leucine Zipper And EF-Hand Containing Transmembrane Protein 1 (LETM1) [10], have no impact on the m-3M3FBS-related Ca^{2+} signals generation and cell death induction. Although non-canonical molecular pathways coordinating ER-mitochondria Ca^{2+} fluxes may exist or emerge, especially during non-physiological conditions, the morphological defects observed upon m-3M3FBS addiction might indicate permeabilization of both ER and mitochondrial membranes, thus allowing Ca^{2+} leak from the ER and uncontrolled Ca^{2+} entry inside the matrix. Cell death induction by m-3M3FBS is blocked by chelating intracellular Ca^{2+} or using cyclosporine A (CsA), a known inhibitor of mitochondrial permeability transition (MPT) [11]. However, Quarato et al. demonstrated that CsA also acts on the ER by preventing Ca^{2+} efflux, but if it is due to the inhibition of an unconventional Ca^{2+} signaling pathway or preserving the integrity of membranes (thus avoiding Ca^{2+} leak) remains to be established [2].

The paper by Sainero-Alcolado et al. well illustrates the metabolic heterogeneity of cancers, which often stems from aberrations in key mitochondrial enzymes [1]. In this context, Ca^{2+} signaling can be crucial for shaping the metabolic profile of malignant cells, and mitochondrial Ca^{2+} homeostasis influences neoplastic transformation, progression, and response to therapy by regulating cell death susceptibility, metabolic rewiring, and reactive oxygen species (ROS) production [12]. For example, a superior capacity for mitochondrial Ca^{2+} uptake stimulates mitochondrial respiration by activating key dehydrogenases involved in the tricarboxylic acid (TCA) cycle [13], culminating in augmented ATP and ROS generation that boosts cancer cell proliferation. On the other hand, a low mitochondrial Ca^{2+} entry could promote a switch of the tumor bioenergetics towards glycolysis and endow resistance to cell death stimuli, including multiple chemotherapeutics [12]. Therefore, different cancer types could rely on a proper ER-mitochondria Ca^{2+} connection to sustain mitochondrial respiration and functions or acquire alterations that minimize Ca^{2+} accumulation, thus eluding mitochondrial Ca^{2+} overload and conferring cell death resistance. A key example of such diametrically opposed phenotypes is the role played in cancer by the type 3 IP3R (IP3R3), one of the most studied IP3R subtypes that supervises the connection between ER-mitochondria. In gastric cancers, IP3R3 is expressed at high levels and supports cancer bioenergetics by favoring mitochondrial Ca^{2+} accumulation, thus hastening malignant progression [14, 15]. Conversely, IP3R3 downregulation enables resistance to a wide range of chemotherapeutics [16, 17]. If in the first case, the inhibition of IP3R3 triggers an energetic crisis that leads to tumor regression, in the latter, restoration of the normal IP3R3 levels (for example, by inhibiting its degradation [16]) increases the sensitivity to cell death.

In conclusion, the two articles discussed here point out the importance of (i) identifying the alterations that affect the mitochondrial homeostasis and metabolism, resulting in malignant transformation and progression; (ii) comprehending the molecular mechanisms and the mitochondrial modifications involved in the different types of cell death. Focusing on the therapeutic potential of targeting mitochondria metabolism to arrest cancer development, Sainero-Alcolado et al. argued for using standard chemotherapeutics or immunotherapy in combination with mitochondria-targeted drugs as an innovative

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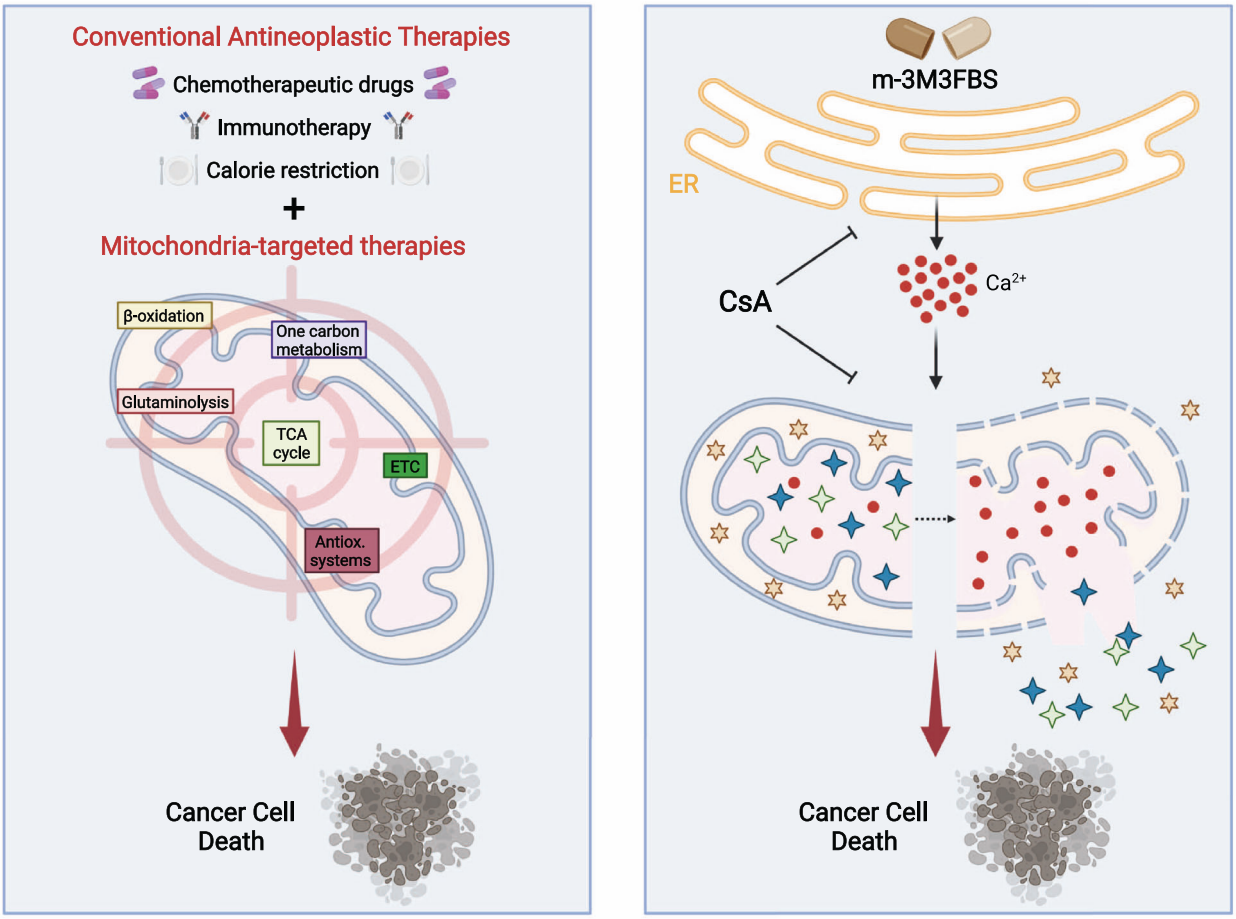

Fig. 1 Mitochondria as key targets of anticancer therapies. Left panel: targeting mitochondrial metabolic pathways, including fatty acid oxidation, the electron transport chain (ETC), glutamine metabolism, the tricarboxylic acid (TCA) cycle, reactive oxygen species (ROS) generation, and one-carbon metabolism, could support standard antineoplastic therapies to arrest tumor progression. Right panel: treatment with m-3M3FBS culminates with massive cell death, through a series of events that comprehends: (1) Ca^{2+} efflux from the ER; (2) mitochondrial Ca^{2+} accumulation; (3) inner mitochondrial membrane (IMM) permeabilization; (4) outer mitochondrial membrane (OMM) rupture; (5) release of mitochondrial factors into the cytoplasm. Created with BioRender.com.

approach for cancer cure [1]. On the other hand, Quarato et al. describe a new relationship between intracellular Ca^{2+}, mitochondrial derangements, and cell death [2]. Notably, the idea that stimuli capable of inducing mitochondrial Ca^{2+} overload and massive cell death might be employed as anticancer agents was formulated more than 20 years ago. The most concrete attempt consisted of engineering thapsigargin, a potent inhibitor of the sarco/endoplasmic reticulum Ca^{2+} ATPase (SERCA), with the prostate-specific membrane antigen (PSMA), a peptidase highly expressed in various tumor types but not in normal tissues [18]. Such a cancer-selective, Ca^{2+}-based prodrug, named G-202 or mipsagargin, obtained promising results in phase I clinical trials, but its employment and development as an anticancer drug seem to have stuck (http://clinicaltrials.gov; May 2022). Although thapsigargin promotes intracellular Ca^{2+} mobilization like m-3M3FBS, the kinetics and intensity of Ca^{2+} spikes are completely different. Moreover, thapsigargin does not induce MIMP, indicating distinct mitochondrial modifications underlying cell death, compared to m-3M3FBS. It will be interesting to design m-3M3FBS variants to circumvent the broad cytotoxicity, as done with thapsigargin, and test their efficacy as novel antineoplastic agents, especially towards those tumors resistant to conventional therapies and characterized by reduced ER-mitochondria Ca^{2+} connection.

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AUTHOR CONTRIBUTIONS

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COMPETING INTERESTS

The authors declare no competing interests.

ADDITIONAL INFORMATION

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