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Targeting a major hub of cell fate decisions – the mitochondrial-associated membrane

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Great progress has been made in the treatment of human cancer but, unfortunately, there remains an abundance of treatment failures due to primary therapy resistance and/or the emergence of drug refractory clones. This is certainly true for acute leukemias, which are the most common malignancies in children and while they make up a smaller fraction of cancer in adults, their impact is substantial given the poorer outcome. Furthermore, the high doses of cytotoxic agents that are often used in therapy are associated with short- and long-term side effects. Thus, there is an urgent need to develop novel therapeutic approaches to improve outcome and decrease side effects. One such strategy, taken by Koczian and colleagues and described in this issue of *Haematologica*, is to augment the effectiveness of conventional agents.[1] They report that the use of the small molecule inhibitor of protein disulfide isomerase (PDI), PS89, has a significant impact on the effectiveness of cytostatic agents used routinely in the therapy of acute leukemias. The model that emerges is that PS89 amplifies the apoptotic stimulus induced by cytotoxic therapy, thereby allowing for increased efficacy at lower doses, through modulation of proteins at the mitochondrial-endoplasmic reticulum (ER) interface. The agent itself has poor pharmacokinetic properties limiting *in vivo* examination, but the results indicate substantial benefit and a wide therapeutic index. Much work remains to be done but the results emphasize the opportunity to target a unique intracellular sub-compartment that plays a key role in cell fate decisions: the interface between the ER and mitochondria.

Mitochondria are multifaceted organelles responsible for an array of cell functions critical for energy production, redox balance, adaptation to cell stress, and activation of the intrinsic apoptotic pathway. They make up 20% of the cytoplasmic volume of a cell and are dynamic, motile structures constantly altering shape through fission and fusion. These alterations involve two lipid bilayers that make up the inner membrane forming cristae (containing membrane-bound enzymes involved in oxidative phosphorylation) which enclose the matrix, and the smooth outer membrane. Mitochondria make important contact with other organelles, particularly the ER, which is in direct contacts with 20% of the mitochondrial surface. Changes in energy metabolism related to cancer, the so-called Warburg effect, have received renewed interest, especially with the discovery of "oncometabolites", but changes that occur at the mitochondrial-ER interface are also critical in controlling mitochondrial metabolism and cell fate decisions.[2]

The mitochondrial-ER interface, commonly referred to as the mitochondria-associated membrane (MAM), is a proteinaceous tether facilitating bidirectional communication between the two organelles controlling the balance between survival and death.[3,4] The exchange of metabolites and contact at the interface controls energy produc-

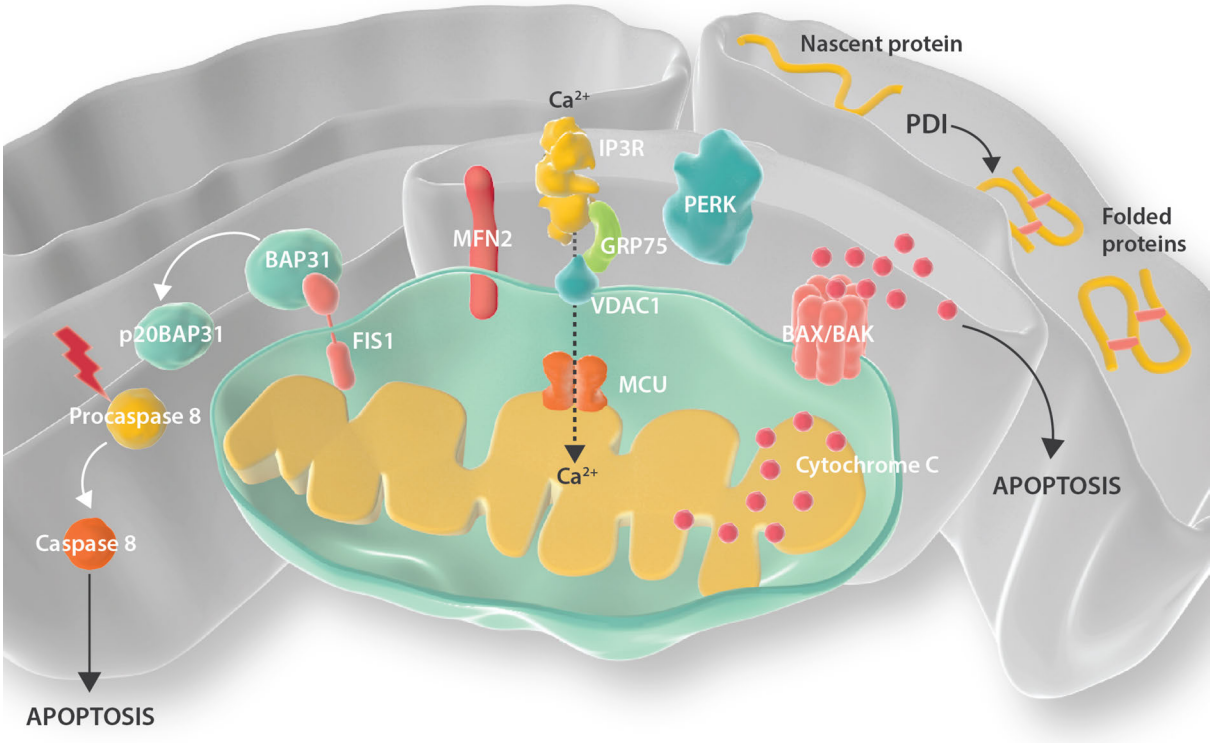

Figure 1. The mitochondrial associated membrane. Hundreds of proteins operate at the mitochodrial-associated membrane (MAM) but the location of proteins discussed in this editorial are illustrated here. A major route of communication between the endoplasmic reticulum (ER) and mitochondria occurs through the release of ER calcium via the inositol 1,4,5-trisphosphate receptor (IP3R), the volatage-gated anion channel (VDAC) and the chaperone GRP75. Calcium gains access to the matrix through the mitochondrial Ca^{2+} uniporter (MCU) leading to membrane depolarization and cytochrome C release as part of the apoptotic pathway. This exchange of calcium is triggered by the interaction of fission protein 1 (FIS1), B-cell receptor-associated protein 31 (BAP31), and caspase 8 within the MAM upon apoptotic stimuli, such as combination treatment with etoposide and PS89.

tion, mitochondrial shape and, importantly, apoptosis. One of the most important regulators of mitochondrial energy production and cell death is the release of ER calcium into the mitochondria thereby facilitating the opening of the permeability transition pore at the inner mitochondrial membrane, depolarization, and cytochrome C release. This process is orchestrated by a vast network of proteins that could serve as potential targets as evidenced by their alteration in cancer cells as a means of escape from chemotherapy-induced apoptosis that relies on Ca2+ signaling.[5] A number of proteins appear to stabilize ER-mitochondria contact thereby prolonging calcium flux including mitofusin-2, phosphofurin acidic cluster sorting protein 2 (PACS-2), and double-stranded RNA-activated protein (PKR)-like ER kinase (PERK) among others. Modulation of such contacts and crosstalk may facilitate apoptosis in cancer cells particularly since the Bcl-2 family of proteins reside and interact at the MAM, highlighted by the increasing interest in BH3-mimetics that alter proteins of this interconnected network. Likewise, a number of oncoproteins and tumor suppressors such as p53, PTEN and AKT function locally. For example, p53 is enriched on the MAM where it interacts with the ER Ca^{2+} pump SERCA to boost ER-mitochondrial Ca^{2+} flux and apoptosis.[6]

In addition to Ca^{2+} storage/signaling, the ER also plays a major role in protein synthesis, post-translational modifications and folding. ER homeostasis is a delicate balance and when the folding machinery can no longer keep up with protein synthesis an adaptive response called the unfolded protein response (UPR) occurs. This response seeks to restore balance by attenuating protein translation, upregulating ER protein degradation and increasing the level of chaperone proteins to inflate protein folding capacity.[7] When the UPR fails to restore ER proteostasis, the pathway shifts to promote cell death primarily though the PERK branch of the UPR. In this way, the ER functions as a sensor of protein stress and perturbations lead to the induction of a variety of survival/death pathways, many of which rely on crosstalk with the mitochondria.

Correct folding of many proteins (e.g. 80% of secretory proteins) requires disulfide (S-S) bonds between cysteine residues. The PDI family of proteins is responsible for the formation and rearrangement of protein disulfide bonds and these ER-resident enzymes also function as chaperones independently of their role in disulfide bond formation.[8] Therefore, these proteins are essential in maintaining protein homeostasis at baseline and during the UPR. Numerous studies have indicated that *PDIA1* (gene name,

aka *PDI*), encoding the archetype PDI protein, and other members of the family are upregulated in many human tumors, correlate with invasiveness (metastasis) and, in some cases, may confer therapy resistance.[8,9] These findings, along with the fact that the interplay between the ER and mitochondria is critical for survival *versus* death, motivated the authors and many other investigators to examine the role of PDI inhibitors in cancer treatment.

PS89 is a derivative of a lead compound discovered by the authors using a screen for chemosensitization of etoposide-induced apoptosis in a variety of cell lines.[10] This initial work showed that PS89 is a reversible inhibitor of PDI and induced the UPR. However, in the current investigation, genetic silencing of PDI failed to recapitulate PS89 activity seen in leukemia cell lines and overexpression did not rescue the chemosensitizing effect. This launched the search for additional targets. It should be noted however that these experiments do not rule out an impact of the UPR in mediating PS89's effect or its modulation of other PDI family members. Indeed, activity-based protein profiling performed by these investigators did show that other PDI family proteins are targets of PS89 as are other resident ER proteins.

B-cell receptor-associated protein 31 (BAP31) was one of the most prominent proteins identified as a PS89 binding partner and is known to operate at the MAM. BAP31 is located at the ER membrane and is tethered to mitochondria through mitochondrial fission protein 1 (FIS1), which appears to serve as a platform for procaspase 8. Previous work has shown that apoptotic signals originating from the mitochondria lead to cleavage of BAP31 into the pro-death p20BAP31 fragment, thereby activating caspase 8 and launching/amplifying apoptosis.[11] Kozcian and colleagues showed that etoposide or PS89 alone had only a modest impact on activation of caspase 8 and other downstream effector pathways, but the combination was highly effective at inducing activity. Moreover, they showed that the combination triggered increased ER-associated calcium influx, loss of mitochondria membrane integrity, cytochrome C release, and increased levels of reactive oxygen species. Other studies have shown that BAP31 plays a critical role in mediating ER stress-related apoptosis through interaction with cell death inducing p53 target 1 (CDIP) resulting in sequestration of BCL-2 and activation of BAX and caspase 8.[12] Thus, BAP31 emerges as a hub for integrating a variety of apoptotic signals from both organelles. Furthermore, their work supports continued efforts to target this interconnected network with the aim of amplifying apoptotic signals.

The authors emphasize the concept of "network pharmacology" in developing potential strategies for the development of novel cancer therapeutics. Given the myriad roles of PDI family members in cell homeostasis, inhibitors of these enzymes are likely to operate through multiple targets in orchestrating their impact in a variety of model systems. For example, two members of the family have opposing effects on the activation of PERK which, as mentioned, plays a key role in the UPR.[13] PERK inhibitors have been associated with unacceptable side effects and PDI inhibitors might provide an alternative, less toxic way to inhibit the ER's ability to maintain homeostasis under stress. Likewise, a recent paper also published in *Hematologica* described that a thioredoxin inhibitor, SK053, promoted differentiation and apoptosis in acute myeloid leukemia cells.[14] As in the experiments by Koczian *et al.*, knock down of the target failed to replicate the phenotype also motivating the authors to search for other targets. In fact, they showed that SK053 binds to and inhibits PDI (i.e., the product of the *PDIA1* gene). Previous work had shown that PDI interacts with a stem loop structure of the CCAAT enhancer-binding protein α (C/EBPα) and blocks its translation. Expression of C/EBPα is critical for myeloid differentiation and its activity is often corrupted in acute myeloid leukemia. PDI inhibition overcomes the translational block leading to increased C/EBPα levels, differentiation and apoptosis in acute myeloid leukemia.

Thus, the observations by Koczian and others highlight the potential utility of targeting the MAM, a dynamic scaffold that plays a critical role in a variety of biological processes, using PDI inhibition or other approaches to augment a variety of cancer therapies.

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