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RESEARCH HIGHLIGHT

p53 REEPs to sow ER–mitochondrial contacts

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Cell Research (2018) 28:877–878; https://doi.org/10.1038/s41422-018-0073-z

The intrinsic pathway of apoptosis requires mitochondrial outer membrane permeabilization, a process that can be facilitated by mitochondrial Ca^{2+} influx. In a recent paper published in *Cell Research*, Zheng and colleagues now show that p53-mediated changes in ER morphology, specifically in response to DNA damage, contribute to the induction of apoptosis.

The tumor suppressor p53 is a master regulator of cell fate determination in response to DNA damage.[1] As a transcription factor, p53 induces the upregulation of thousands of transcripts, which have various roles in the cellular response to the sustained damage. A fatal outcome of genotoxic stress is apoptosis, the controlled destruction of the cell if the damage is too severe. p53 is directly responsible for the transcription of a number of genes essential in this process, most importantly the BH3-only protein Puma.[2,3] It can inactivate the pro-survival members of the Bcl-2 family of proteins, which then leads to activation of the pro-apoptotic proteins BAX and BAK. They are capable of promoting mitochondrial outer membrane permeabilization (MOMP), which subsequently leads to the release of soluble mitochondrial intermembrane space proteins (most importantly cytochrome *c*).[4] These proteins can then activate proteolytic caspases, which cleave specific target proteins and therefore execute apoptosis.

Changes in a mitochondrial function, via permeabilization, is therefore a crucial event in apoptosis. However, other organelles impact on programmed cell death as well. For example, lysosomes can also permeabilize and release proteases of the cathepsin family into the cytoplasm to process their substrates.[5] Additionally, the Golgi has been shown to be dispersed in response to DNA damage,[6] a process that may promote survival.

In the current study, Zheng et al. now identify remodeling of the endoplasmic reticulum (ER) shape as being involved in DNA damage-induced apoptosis.[7] They noted that cells that have undergone acute DNA damage show profound morphological changes in the ER structure, with an increase in the total ER area. High-resolution imaging methods revealed extended ER tubules as well as an increase in tubule junctions, and that these changes were specific to DNA damage (Fig. 1). This effect was absent in cells with defective p53 function, and additional experiments supported the hypothesis that tubular ER extension was dependent on p53 transcriptional activity. Given its role as a transcription factor, the authors performed a focused screen of ER-shaping proteins, finding the genes *REEP1* and *REEP2* to be direct transcriptional targets of p53. Loss-of-function studies indicated that both REEP1 and REEP2 are required for tubular ER extension in response to DNA damage, however, their loss could not prevent it completely. This suggested an additional factor involved in the process, and the authors identified *EI24* as another p53 target and the main gene responsible for DNA damage-induced tubular ER extension.

So how do changes in ER morphology regulate DNA damage-induced apoptosis? While tubular extension on its own (by overexpression of REEP1/2 or Rtn4a) is not sufficient to promote apoptosis, it is required for apoptosis induced by DNA damage. Interestingly, co-immunoprecipitation studies showed an interaction between EI24 and VDAC2, an ion channel localized to the outer mitochondrial membrane. The conundrum of the interaction between EI24, an ER protein, and VDAC2, a mitochondrial protein, was resolved by showing that DNA damage enhances the number of ER–mitochondrial contact sites, a process facilitated by the EI24–VDAC2 interaction. This subsequently promotes uptake of Ca^{2+} from the ER into the mitochondria via VDAC2, mitochondrial permeability transition pore (MPTP) opening followed by mitochondrial swelling, outer membrane rupture, and cell death.[8] While untested, p53-induced mitochondria–ER contacts may also facilitate mitochondrial apoptosis in other ways, e.g., via transfer of lipids that support BAX/BAK activation.[9]

The involvement of mitochondria in apoptotic cell death is well established, and other cellular organelles have been described to contribute to cell death. Zheng et al. now provide intriguing data on the interplay between mitochondria and the ER during DNA damage-induced apoptosis. Given the major role of p53 in transducing this stress towards cellular fate decisions, it is also involved in this instance by upregulating several genes required to promote morphological changes and organelle contacts. One interesting aspect of this study is the specificity for DNA damage in the involvement of these ER remodeling activities. The present study suggests that this mechanism is different between cells undergoing apoptosis in response to DNA damage compared to other apoptotic stimuli. It will be interesting to see why, in this setting, DNA damage-induced apoptosis uses a unique mechanism to promote calcium import to the mitochondria.

While the authors focused on DNA damage-induced activation of p53, it can also be activated by other stresses like oncogenes, ribosomal stress, loss of cell–cell contacts (anoikis), or hypoxia.[10] Not all of these stimuli lead to the same post-translational modifications of p53 and could therefore have different outcomes in relation to transcriptional induction of REEP1/2 and EI24 and the involvement of tubular ER extension in apoptosis. One very intriguing observation was the inability of Bcl-2 to prevent DNA damage-induced apoptosis under conditions of EI24 overexpression. Together with other data presented, this suggests that, rather than being via canonical BAX/BAK-mediated mitochondrial permeabilization, the DNA damage-induced apoptosis described herein may be due to calcium overload, enabling MPTP opening and mitochondrial rupture. This is of potential clinical importance,

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Published online: 24 July 2018

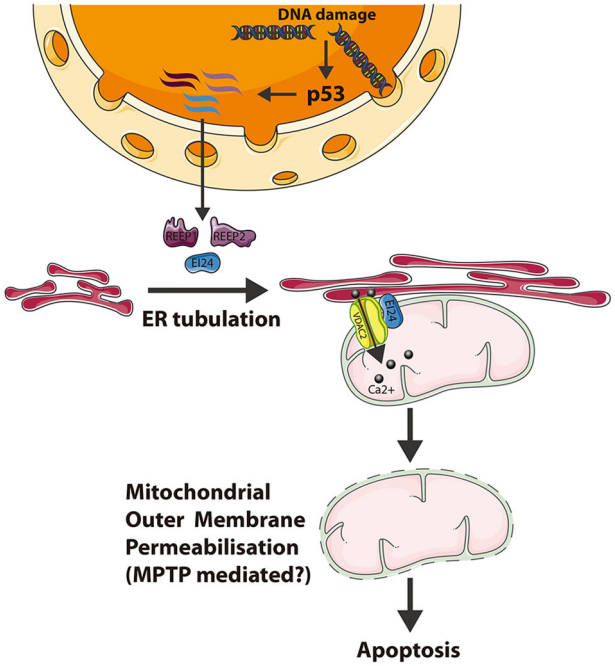

Fig. 1 DNA damage induces activation of p53, which directly binds to the promoters of *REEP1/2* and *EI24* to increase their transcription. REEP1/2 and EI24 then promote tubular ER extension which leads to enhanced numbers of mitochondria–ER contact sites. By interacting with VDAC2 on the mitochondria, EI24 then facilitates the transfer of Ca^{2+} into the mitochondria, which contributes to mitochondrial permeabilzation and subsequently apoptosis of the damaged cell. The figure was generated using Servier Medical Art

since overexpression of Bcl-2 is a common mechanism of cancer cell resistance to chemotherapeutic drugs. Activation of EI24 (or tubular ER extension as a general mechanism) may therefore prove useful in overcoming this resistance and re-sensitize cancer cells to DNA damaging therapies.

In summary, the report by Zheng et al. provides another facet of the multiple roles that p53 plays during cell stress, namely by promoting a connection between two cellular organelles in order to facilitate apoptosis.

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