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RESEARCH HIGHLIGHT

Mechanisms of p53-mediated mitochondrial membrane permeabilization

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The p53 protein is mutated or inactivated in more than 50% of human cancers, underscoring its cardinal importance as an oncosuppressor. p53 is expressed in all nucleated cells and can be activated by a plethora of post-transcriptional modifications (in particular by the phosphorylation of critical serine residues), as well as by the inhibition of its degradation (mainly mediated by the E3 ubiquitin ligase MDM2). p53 was first characterized as a transcription factor that, once activated, drives the expression of gene programs causing a transient cell cycle arrest linked to DNA repair, a permanent and irreversible cell cycle arrest (senescence) or programmed cell death by apoptosis. Moreover, p53 stimulates mitochondriogenesis and inhibits glycolysis. In cancer cells, p53 inactivation has multiple effects including metabolic reprogramming (with reduced oxidative phosphorylation and enhanced glycolysis), unrestricted proliferation (due to the inactivation of senescence programs), genomic instability (due to the subversion of essential cell cycle checkpoints) and apoptosis resistance. Thousands of articles have reported on the genes that are induced or repressed by p53, and on the mechanisms through which p53 interacts with other nuclear cofactors to transactivate specific sets of cell cycle-arresting or proapoptotic genes. Lethal gene products induced by p53 include proteins that mediate the generation of reactive oxygen species, notably within mitochondria, and proapoptotic proteins of the Bcl-2 family, in particular Bax and Puma. These proapoptotic proteins induce mitochondrial outer membrane permeabilization (MOMP), which represents the molecular "point-of-no-return" of the intrinsic pathway of apoptosis [1].

Although p53 has been prominently characterized as a transcription factor, accumulating evidence indicates that p53 exerts important extranuclear effects. For example, in response to cellular stress induced by genotoxic agents, inhibition of transcription and hypoxia, p53 accumulates not only in the nucleus but also in the cytoplasm, where it interacts with mitochondria to stimulate MOMP, as first discovered by Ute Moll's group [2]. This effect is so strong that adenoviruses encoding a p53 mutant targeted to mitochondria are nearly as efficient as adenoviruses that encode wild-type p53 in inducing MOMP and in killing human cancer cells, both *in vitro* and *in vivo* [3]. It has also been found that basal levels of p53 in the cytoplasm mediate a tonic inhibition of autophagy. This means that deletion, depletion or pharmacological inhibition of p53 induce macroautophagy, a cytoprotective process ensuring cell survival in adverse metabolic conditions. In response to multiple stressful stimuli (such as nutrient depletion, inhibition of the mammalian target of rapamycin (mTOR) kinase or endoplasmic reticulum stress), p53 is depleted from the cytoplasm, which allows for the induction of autophagy. Although the exact mechanisms through which p53 inhibits autophagy remain unclear, these findings point to the importance of cytoplasmic p53 levels in the control of major cellular functions [4].

Several groups have characterized the molecular determinants of the proapoptotic effects of cytoplasmic p53 [5]. In the present issue of *Cell Research*, Ute Moll and collaborators report new important insights into the mechanisms of MOMP induction by p53 [6]. MOMP is regulated by pro- (MOMP-inducing) and antiapoptotic (MOMP-inhibiting) members of the Bcl-2 protein family, as well as by components of the permeability transition pore complex (PTPC). Bcl-2-like proteins are characterized by the presence of 1 to 4 Bcl-2 homology (BH) domains. Antiapoptotic Bcl-2 family proteins (such as Bcl-2, Bcl-X_L and Mcl-1) carry the BH1, BH2, BH3

Abbreviations: ANT, adenine nucleotide translocase; BH, Bcl-2 homology; IMS, mitochondrial intermembrane space; MOMP, mitochondrial outer membrane permeabilization; PTPC, permeability transition pore complex; VDAC, voltage-dependent anion channel

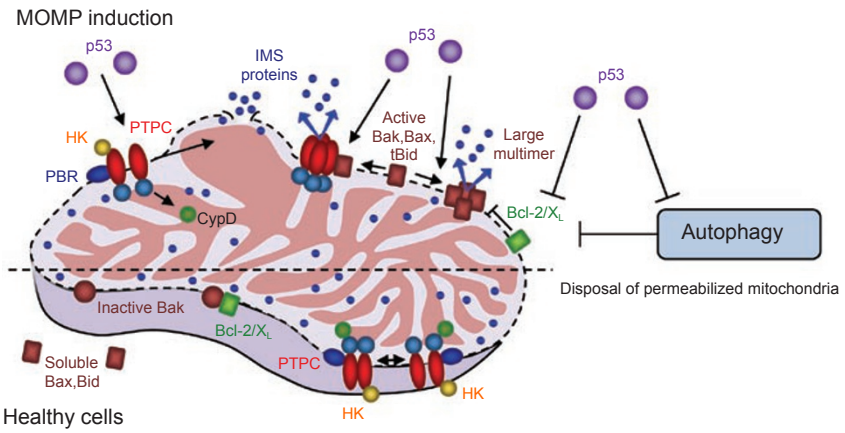

Figure 1 Cytoplasmic effects of p53. p53 has a number of extranuclear, transcription-independent proapoptotic effects. For instance, p53 can interact with and trigger the opening of the permeability transition pore complex (PTPC), which results in osmotic swelling of mitochondria and eventually mitochondrial outer membrane permeabilization (MOMP). Cytosolic p53 can also activate multidomain proapoptotic members of the Bcl-2 family of proteins (*e.g.* Bax, Bak), thereby favoring the generation of multimeric pores (which allow for the liberation of cytotoxic mitochondrial intermembrane space proteins, *i.e.* IMS) and/or MOMP-inducing interactions among Bax/Bak and PTPC components. Moreover, p53 can prevent the function of antiapoptotic Bcl-2-like proteins (*e.g.* Bcl-2/Bcl-X_L), which normally keep under control their proapoptotic relatives (*e.g.* Bax, Bak, Bid). Finally, p53 has been shown to inhibit autophagy, a cytoprotective process that is also responsible for the removal of permeabilized (and hence unfunctional) mitochondria. CypD, cyclophilin D; HK, hexokinase; PBR, peripheral-type benzodiazepine receptor.

and BH4 domains, while their proapoptotic relatives either carry three BH domains (BH1, BH2 and BH3) or just a single BH3 domain. Multidomain proapoptotic Bcl-2 proteins comprise Bax and Bak, which can insert in the outer mitochondrial membrane and oligomerize, thereby creating pores that allow for the release of toxic mitochondrial intermembrane space (IMS) proteins. More than one dozen of so-called "BH3-only" proteins are thought to induce apoptosis by two distinct effects. On one hand, they can directly trigger the oligomerization of Bax/Bak and hence act as "activators" (prototype: Bid). On the other hand, they can neutralize antiapoptotic Bcl-2 proteins (which usually maintain in check Bax, Bak and "activators" BH3-only proteins), thereby functioning as "derepressors" (prototype: Puma). The PTPC is composed by proteins from the outer mitochondrial membrane (*e.g.* the voltage-dependent anion channel, VDAC), the inner mitochondrial membrane (*e.g.* the adenine nucleotide translocase, ANT) and the mitochondrial matrix (*e.g.* cyclophilin D). Although the mechanisms of MOMP induced by the PTPC and Bcl-2 family members may be different, there have been multiple reports on functional and molecular interactions among these two systems [7-9].

Ute Moll and colleagues [6] have now carried out an investigation on the minimal requirements of MOMP induction by p53 protein one step further. It had previously been reported that p53 might act as a sort of "super" BH3-only protein that can (i) neutralize antiapoptotic proteins from the Bcl-2 family (*e.g.* Bcl-X_L), (ii) convert antiapoptotic proteins (*e.g.* Bcl-2) into proapoptotic ones, (iii) liberate proapoptotic Bcl-2-like proteins (*e.g.* Bak) from the inhibition by their antiapoptotic counterparts (*e.g.* Mcl-1), (iv) directly activate the pore-forming function of multidomain proapoptotic proteins (*e.g.* Bax), and (v) functionally cooperate with BH3-only proteins (*e.g.* Puma) to trigger MOMP [2, 5, 6, 10].

Using a set of isogenic human colon cancer HCT116 cell lines that lack different proapoptotic genes and cell-free systems in which purified mitochondria are confronted with recombinant p53 protein, Moll and collaborators [6] now demonstrate that, surprisingly, neither Bax nor Puma are required for p53 to induce MOMP, at least in this particular paradigm. Moreover, p53 can induce the oligomerization not only of Bax and Bak but also of VDAC, pointing to (direct or indirect) effects of p53 on the PTPC (Figure 1). In line with this notion, the group of Ute Moll [6] now reports that p53 co-immunoprecipitates with cyclophilin D, yet another protein of the PTPC. By directly comparing p53 and Bid, Moll and collaborators [6] conclude that the specific mode of membrane permeabilization mediated by p53 is different from that promoted by Bid. Indeed, while truncated Bid (tBid) requires Bax to induce MOMP and causes the liberation of cytochrome *c* and Smac/DIABLO (but not that of the caspase-independent death effectors AIF and endonuclease G) from mitochondria, p53 triggers MOMP independently of Bax and stimulates the release of all IMS proteins, indicating superior MOMP capabilities. Moreover, p53 promotes the generation of low molecular weight complexes containing Bax or Bak, while tBid induces an higher-order oligomerization state, pointing to additional differences in the mechanisms of MOMP induction by p53 and tBid.

In conclusion, the report by Moll and colleagues [6] suggests that p53 does not only act as a mere Bid-like BH3-only protein, but that it induces MOMP to higher extents and through a distinct molecular pathway, which involves functional and physical interactions with PTPC proteins. In line

with this interpretation, Tang *et al.* [11] demonstrated that the pharmacological reactivation of mutant p53, which causes its translocation to mitochondria, induces a MOMP modality that can be inhibited by cyclosporine A, an inhibitor of cyclophilin D. These results underscore the existence of a novel crosstalk between p53 and the PTPC. Future studies will have to elucidate how p53 can trigger PTPC opening. Moreover, it will be important to know how MOMP induction is linked to autophagy inhibition by cytoplasmic p53. Autophagy can counteract the toxic activity of permeabilized mitochondria while improving the adaptation of cells to stress, and it appears hence (teleo)logical that p53 simultaneously stimulates apoptosis (by inducing MOMP) and subverts cellular defense (by inhibiting autophagy).

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