News and Commentary

Novel function of cytoplasmic p53 at the interface between mitochondria and the endoplasmic reticulum

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Germline mutations in the gene coding for tumor protein p53 (TP53, best known as p53) are associated with the Li-Fraumeni syndrome, a dominant hereditary disorder characterized by an increased predisposition of patients to the development of various tumors relatively early in life. In addition, TP53 is affected by somatic loss-of-function mutations in a large fraction (>50%, according to recent estimates) of human cancers all confounded.1 Finally, several other molecular defects causally associated with malignant transformation or tumor progression result in the functional inactivation of the p53 system. As a notable example, multiple neoplasms express increased levels of MDM2 proto-oncogene, E3 ubiquitin protein ligase (MDM2), resulting in an accrued degradation of p53 by the proteasome.2 These observations indicate that the loss of p53 functions favors the establishment and/or progression of various malignancies.

Throughout the past 25 years, p53 has been the subject of intense investigation, revealing a wide panel of mechanisms by which this protein exerts robust oncosuppressive functions.3 Initially, p53 was recognized for its ability to respond to DNA damage by transactivating several genes that regulate cell cycle progression (e.g., CDKN1A) and apoptotic cell death (e.g., BAX), hence preventing the propagation of potentially transforming genetic defects.4 Later, stress-activated p53 turned out to participate in the activation of mitochondrial apoptosis by physically interacting with pro- and antiapoptotic members of the Bcl-2 protein family (such as BAX, BCL-2 and BCL-XL), thereby favoring the elimination of potentially dangerous cells via transcription-independent mechanisms.5 More recently, several studies demonstrated that p53 mediates oncosuppressive effects not only when cells are confronted with sources of stress, but also in physiological conditions. In particular, p53 has been attributed with a key role in the preservation of physiological bioenergetic metabolism, mostly linked to its potential implication of the ER in this process.6,7 Given the implication of Ca2+ fluxes between the ER and mitochondria in the control of mitochondrial apoptosis,5,6,11 Giorgi and colleagues investigated the effects of the absence of p53 on reticular Ca2+ homeostasis. They found that Trp53−/− MEFs exhibit lower steady-state reticular Ca2+ levels than their Trp53+/+ counterparts, resulting in decreased Ca2+ mobilization and mitochondrial accumulation in response to ATP (a purinergic receptor agonist that is commonly employed to trigger cytosolic Ca2+ waves) or hydrogen peroxide. Similar

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results were obtained with HCT 116 cells, p53-overexpressing human cervical carcinoma HeLa cells and Trp53<sup>−/−</sup> MEFs reconstituted with wild-type p53. Moreover, Trp53<sup>−/−</sup> MEFs, but not their Trp53<sup>+/−</sup> counterparts, responded to hydrogen peroxide with a fragmentation of the mitochondrial network, a phenomenon that could be drastically exacerbated by the preadministration of doxorubicin (which *per se* failed to do so).<sup>9</sup> Importantly, the authors excluded the involvement of the transcriptional activity of p53 in this process by several experimental strategies, including (1) the pharmacological blockade of transcription with a-amanitin, alone or combined with the p53 inhibitor pifithrin α, to Trp53<sup>−/−</sup> MEFs; (2) the reconstitution of Trp53<sup>−/−</sup> MEFs with p53 mutants lacking the nuclear localization signal (NLS); and (3) the reconstitution of Trp53<sup>−/−</sup> MEFs with an NLS-deficient p53 variant specifically addressed to the ER. Moreover, Giorgi and colleagues demonstrated that various naturally occurring p53 mutants, such as p53<sup>R175H</sup> and p53<sup>R273H</sup>, are unable to restore reticular Ca<sup>2+</sup> homeostasis in Trp53<sup>−/−</sup> MEFs, while the WT protein efficiently does so. Accordingly, p53<sup>WT</sup>, but not p53<sup>R175H</sup> and p53<sup>R273H</sup>, increased the sensitivity of Trp53<sup>−/−</sup> MEFs to oxidative stress back to the levels of their Trp53<sup>−/−</sup> counterparts.<sup>9</sup> These data suggest that the cytoplasmic pool of p53 regulates the accumulation of Ca<sup>2+</sup> ions within the ER, a process that influences the sensitivity of the mitochondrial network to the induction of apoptosis.

Next, Giorgi and colleagues set out to investigate the molecular mechanisms by which cytoplasmic p53 influences reticular Ca<sup>2+</sup> homeostasis. Pull-down assays in human non-small cell lung carcinoma H1299 cells engineered to overexpress p53 as well as co-immunoprecipitation experiments in Trp53<sup>−/−</sup> MEFs revealed that p53<sup>WT</sup>, but not p53<sup>R175H</sup> and p53<sup>R273H</sup>, physically binds to SERCA, an interaction that relies on the C-terminal fragment of p53 (aa 294-393).<sup>9</sup> This domain of p53 is known to accommodate several post-translational modifications,<sup>18</sup> which, at least theoretically, can modulate its ability to bind (and hence regulate the activity of) SERCA. However, the C-terminal fragment of p53 was unable to influence reticular Ca<sup>2+</sup> homeostasis and sensitivity to oxidative stress *per se*, indicating that this function resides in another domain of the protein. Of note, the overexpression of SERCA was sufficient to rescue the sensitivity of Trp53<sup>/−</sup> MEFs to hydrogen peroxide. This is in agreement with the hypothesis that SERCA operates downstream of p53 in the cascade of events that connects oxidative stress to apoptosis, although it does not formally exclude that these proteins operate independently from each other. Finally, Giorgi *et al.* checked whether p53 would modulate the activity of SERCA by altering its oxidative status. Indeed, p53<sup>WT</sup> turned out to respond to doxorubicin by limiting the inhibitory sulfenylation of cysteine residues in SERCA, an activity that was not displayed by p53<sup>R273H</sup>. Thus, cytosolic p53 influences reticular Ca<sup>2+</sup> homeostasis by regulating the pump activity of SERCA.

To test the relevance of their findings in vivo, Giorgi and collaborators developed a novel technological platform for the intravital imaging of Ca<sup>2+</sup> waves, based on skinfold chambers and the ratiometric Ca<sup>2+</sup> probe Fura-2.<sup>8</sup> Using this approach, Giorgi *et al.* were able to monitor Ca<sup>2+</sup> waves elicited by photodynamic therapy (PDT), an anticancer regimen relying on the administration of an ER-targeted photosensitizer coupled to the exposure of neoplastic lesions to visible light (which promotes oxidative stress), in tumor masses developing *s.c.* in mice. In particular, they tested the ability of neoplastic lesions formed by HRAS<sup>G12V</sup>-expressing Trp53<sup>+/−</sup> or Trp53<sup>−/−</sup> MEFs to respond to PDT by generating Ca<sup>2+</sup> fluxes that ignite the intrinsic pathway of apoptosis. Confirming their *in vitro* observations, the authors found that Trp53<sup>+/−</sup>, but not Trp53<sup>−/−</sup>, tumors respond to PDT by accumulating Ca<sup>2+</sup> ions within the mitochondrial matrix and, as a consequence, initiate the apoptotic program. Moreover, they confirmed that the overexpression of SERCA rescue the sensitivity of Trp53<sup>−/−</sup> tumors to PDT-elicited oxidative stress, as does the overexpression of the mitochondrial calcium uniporter (MCU),<sup>9</sup> the protein that is responsible for the uptake of cytosolic Ca<sup>2+</sup> by mitochondria.<sup>19</sup> Finally, they demonstrated that intercepting intracellular Ca<sup>2+</sup> ions with the cell-permeant chelator BAPTA-AM significantly reduces the sensitivity of Trp53<sup>−/−</sup> cancers to PDT. Taken together, these data indicate that the regulation of reticular Ca<sup>2+</sup> homeostasis by p53 determines the response of established neoplasms to clinically employed inducers of oxidative stress.
The recent papers from Paolo Pinton’s laboratory add yet another entry to the ever growing list of p53 functions, the direct control of reticular Ca$^{2+}$ homeostasis. However, several questions remain to be addressed. First, which domain of p53 is responsible for this functional effect (and not just for the interaction between p53 and SERCA)? Second, do compounds that transcriptionally reactivate mutant p53 variants, such as thiosemicarbazone derivatives, also restore its ability to activate SERCA? Third, what is the role of anti-apoptotic members of the Bcl-2 protein family, which (at least in part) localize to the ER and modulate Ca$^{2+}$ homeostasis, in this process? Shedding light on these and other incognita may drive the development of novel strategies for resensitizing p53-deficient tumors to therapy based on the restoration of Ca$^{2+}$ fluxes. Now, also p53 surfs the Ca$^{2+}$ wave.

Conflict of Interest
The authors declare no conflicts of interest

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