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Editorial

Ferroptosis in p53-dependent oncosuppression and organismal homeostasis

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Tumor protein p53 (TP53, hereafter referred to as p53) is an oncosuppressive transcription factor that mediates critical homeostatic functions. For a long time, the oncosuppressive activity of p53 was attributed to its capacity to initiate cell cycle arrest (be it temporary or permanent), and/or regulated cell death (RCD) in response to stress, via transcriptional and transcription-independent mechanisms. Thus, p53 was thought to operate in favor of organismal homeostasis by keeping under check or eliminating potentially dangerous cells.[1] More recent data have challenged this notion by suggesting that the oncosuppressive functions of p53 mainly originate from its ability to regulate metabolism in baseline conditions.[2–4] Now, investigators from the Columbia University (New York, NY, USA) identified ferroptosis, an iron-dependent form of necrotic RCD, as an additional mechanism through which stress-activated p53 may maintain organismal homeostasis.[5]

Using a cell line stably transduced with a tetracycline-inducible p53-encoding construct, Jiang *et al.* identified solute carrier family 7 (cationic amino-acid transporter, *y+* system), member 11 (*SLC7A11*), as a novel transcriptional target of p53. Accordingly, the promoter of *SLC7A11* contains a p53-responsive element, and p53 activation by tetracycline resulted in the time-dependent repression of *SLC7A11*, along with the expression of other well-known p53-regulated genes.[5] This effect was found to directly rely on p53, as it could not be appreciated in the presence of a p53-specific short-hairpin RNA. However, it did not relate to the ability of p53 to transactivate cell cycle-arresting and RCD-inducing genes, as it was retained by a p53 mutant ($p53^{3KR}$) that is deficient for these functions (as it bears R → K substitutions in three lysine residues that are critical for the post-translational regulation of p53 by acetylation).[5,6] SLC7A11 is a component of the X_C^- system, a plasma membrane multiprotein transporter that mediates the Na^+-independent uptake of extracellular cystine in exchange of cytosolic glutamate.[7] In line with this notion, $p53^{3KR}$ expression caused a reduction in intracellular cystine concentrations, whereas the homologous knockout of *Trp53* resulted in a 60% increase in baseline cystine levels.[5] Importantly, inhibition of the X_C^- system has previously been mechanistically linked to the ability of one peculiar molecule, namely, erastin, to cause ferroptosis in cancer cells expressing mutant RAS.[8,9]

At odds with their $Trp53^{-/-}$ counterparts, wild-type (WT) and $p53^{3KR}$-expressing mouse embryonic fibroblasts (MEFs) were found to be sensitive to cell death induction by erastin.[5] Importantly, such a lethal response was completely prevented by the ferroptosis inhibitor ferrostatin-1 (Fer-1), but it was not influenced by the wide-spectrum caspase inhibitor Z-VAD-fmk,[10,11] the autophagy inhibitor 3-methyladenine[11,12] or the necroptosis inhibitor necrostatin-1.[13,14] Additional inhibitors of ferroptosis including deferoxamine, *N*-acetyl-cysteine, *β*-mercaptoethanol and 1,4-diamino-2,3-dicyano-1,4-bis[2-aminophenylthio]butadiene (U0126) all inhibited erastin-induced RCD in $p53^{3KR}$-expressing MEFs. Moreover, the transgene-enforced overexpression of SLC7A11 quenched the loss of colony-forming capacity exhibited by $p53^{3KR}$-expressing human cancer cells (in the absence of overt RCD). These findings suggest that ferroptosis can ensue the p53-dependent transcriptional repression of SLC7A11 in response to stress. However, the authors did not test whether cyclosporine A, an inhibitor of mitochondrial permeability transition (MPT)-dependent regulated necrosis (a major, physiologically relevant form of nonapoptotic RCD), or the absence of *Ppif* (coding for the key MPT regulator cyclophilin D),[15] would influence the ability of erastin to kill p53- or $p53^{3KR}$-expressing cells. As chemical inhibitors of ferroptosis (as well as cystine) share a robust antioxidant activity, which precise RCD modality is activated in this context remains unclear (although previous data support the notion that MPT-driven regulated necrosis and ferroptosis are mechanistically distinct).[7]

A consistent proportion of human neoplasms exhibit increased SCL7A11 levels, irrespective of *TP53* mutational status, suggesting that SCL7A11 expression may support oncogenesis. In support of this hypothesis, the oncosuppressive functions of $p53^{3KR}$ in xenograft tumor models were significantly impaired upon SLC7A11 re-expression. Moreover, the lethal phenotype associated with the homologous

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knockout of transformed mouse 3T3 cell double minute 2 (*Mdm2*, coding for a key p53 inhibitor) appeared to rely, at least in part, on the capacity of p53 to stimulate ferroptosis. Indeed, the intraperitoneal administration of Fer-1 to pregnant mice partially relieved the lethal developmental defects associated with the *Trp53*$^{3KR/3KR}$ *Mdm2*$^{-/-}$ genotype.[5] This suggests that the activation of p53 induced by the absence of *Mdm2* may be embryonically lethal owing to the activation of ferroptosis. Jiang *et al.*, however, did not test whether their observations also apply to *Mdm2*$^{-/-}$ mice expressing WT p53. Thus, the suggested link between the *Mdm2*$^{-/-}$ genotype and ferroptosis-mediated embryonic lethality remains to be formally demonstrated.

Given the implication of ferroptosis in oxidative stress responses, Jiang *et al.* tested the sensitivity of p53^{3KR}-expressing cells to RCD induction by tert-butyl hydroperoxide (TBH).[5] In the presence of p53^{3KR}, cancer cells were hypersensitive to TBH, a lethal response that could be blocked by Fer-1, but not by Z-VAD-fmk, 3-methyladenine or necrostatin-1. Again, the authors did not include cyclosporine A in their experimental determinations. Sublethal TBH concentrations strongly synergized with the p53-activating molecule nutlin-3 in killing human osteosarcoma U2OS cells (which express WT p53), and this synergistic interaction could be completely blocked by Fer-2 and deferoxamine. Finally, the cytotoxic activity of TBH and erastin was significantly reduced in MEFs obtained from BAC transgenic mice that express increased amounts of SLC7A11. Taken together, these data point to the existence of a p53 → SLC7A11 signaling axis that mediates lethal effects in the context of failing antioxidant defenses.

The molecular cascade highlighted by Jiang *et al.*[5] may contribute to the oncosuppressive functions of p53. However, there are at least two major questions that await urgent clarification. First, does Fer-1 inhibit embryonic degeneration in *Trp53*$^{+/+}$*Mdm2*$^{-/-}$ mice as it does in *Trp53*$^{3KR/3KR}$*Mdm2*$^{-/-}$ animals? Second, what is the contribution of CYPD-dependent MPT-driven regulated necrosis in this setting? Irrespective of these incognita, the findings discussed above lend fresh support to the notion that the oncosuppressive activity of p53 involves the elimination of potentially oncogenic cells by senescence or RCD (depending on the experimental model). This said, a large body of evidence indicates that p53 mediates oncosuppressive functions also by avoiding, rather than responding to, the potentially oncogenic degeneration of cellular functions.[2–4] Most likely, the robust tumor-suppressive activity of p53 reflects its unique ability to preserve both cellular and organismal homeostasis (Figure 1).

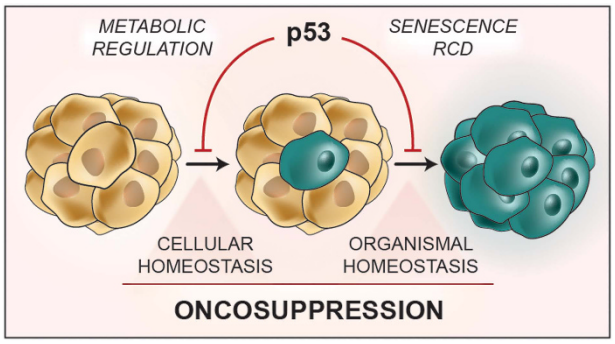

Figure 1 Oncosuppressive functions of p53. For a long time, the oncosuppressive activity of p53 was mainly ascribed to its capacity to keep under check or eliminate damaged, and hence potentially oncogenic, cells. Thus, p53 was viewed as a guardian of organismal homeostasis that operates at the expenses of individual cells. More recently, it has become clear that p53 mediates prominent oncosuppressive effects also by preventing (rather than simply responding to) the potentially tumorigenic degeneration of cellular functions. Thus, p53 appears to suppress tumorigenesis by preserving both cellular and organismal homeostasis. RCD, regulated cell death

Conflict of Interest

The authors declare no conflict of interest.

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