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## Protective role of p53 in doxorubicin-induced cardiomyopathy as a mitochondrial disease

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### ABSTRACT

Doxorubicin is widely used against cancer but carries the risk of a progressive cardiomyopathy associated with mitochondrial loss. Using genetic models, our recent study demonstrates that mitochondrial genomic DNA regulation by tumor protein p53 (TP53, best known as p53) prevents the cardiotoxicity of low dose doxorubicin which does not activate the p53-dependent cell death pathway.

### ARTICLE HISTORY

Received 31 December 2019  
Revised 24 January 2020  
Accepted 28 January 2020

### KEYWORDS

doxorubicin;  
cardiomyopathy; p53;  
mitochondria; mtDNA;  
TFAM; p53R2; NAD<sup>+</sup>

Doxorubicin is an effective chemotherapeutic agent used against a wide spectrum of cancer types. Total lifetime exposure above a threshold dose is associated with increased risk of progressive heart failure, often years after treatment. To date, the mechanisms and genetic susceptibilities underlying this disease still remain unclear. The role of tumor protein p53 (encoded by human *TP53* and mouse *Trp53* genes) in doxorubicin-induced cardiomyopathy has been investigated previously with studies showing that p53-induced cell death promotes this cardiotoxicity.<sup>1–3</sup> However, the majority of these studies used mouse models treated with bolus injections of high dose doxorubicin (~20 mg/kg) that cause acute cardiac dysfunction and high mortality within days after treatment.<sup>2</sup> In the clinics, doxorubicin is given to cancer patients in divided lower doses over weeks due to its toxicity. Thus, the acute cell death caused by a bolus dose injection of doxorubicin in mice may not reflect the progressive dilated cardiomyopathy observed in patients. Furthermore, the time course of doxorubicin induced cardiomyopathy contrasts from the early onset heart failure caused by acute processes such as myocardial infarctions where ischemia-induced cardiomyocyte cell death clearly mediates pathogenesis.

Given this background of information, it was notable that an interesting study using mice treated with low divided doses of doxorubicin showed that p53 might have a protective role in preventing the late-onset cardiomyopathy.<sup>4</sup> Consistent with its interference of DNA replication and transcription in cancer cells, doxorubicin has also been reported to cause mitochondrial DNA (mtDNA) damage, which can impair mitochondrial function and result in cardiac dysfunction.<sup>5</sup> Besides inducing cell death as one way of maintaining nuclear genomic integrity, there is growing evidence that p53 can also act as guardian of the mitochondrial genome.<sup>6</sup> Thus, p53 potentially has a dual nature: its acute cell death activity under severe genotoxic stress by high bolus doses of doxorubicin; and its mtDNA maintenance function

that protects against late onset cardiomyopathy associated with doxorubicin given in low divided doses as in the clinics.

Using 3 different *Trp53* genotype mice with unique profiles of cellular activity, our recent study dissected the role of cell death versus non-death, including mitochondrial, activities of p53 in the later onset cardiomyopathy model (Figure 1).<sup>7</sup> Mice with knockin of the *Trp53* R172H mutation (*p53*<sup>R172H/H</sup>), homologous to the human *TP53* R175H that causes early-onset cancer disorder Li-Fraumeni syndrome, retains mtDNA maintenance activity while losing cell death/cell cycle arrest activities as in the *Trp53* null (*Trp53*<sup>-/-</sup>) state.<sup>8</sup> Unlike the high bolus dose of doxorubicin, the low divided doses of doxorubicin did not increase the mortality of treated mice, permitting longer term measurement of their cardiac function. In contrast to both wild-type *Trp53*<sup>+/+</sup> and knockin mutant *Trp53*<sup>R172H/H</sup> mice, *Trp53*<sup>-/-</sup> mice began to show left ventricular (LV) dilatation and systolic dysfunction 4 wk after doxorubicin treatment in association with reduced mitochondrial metabolism. Mechanistically, both wild-type and R172H mutant *Trp53* can promote the expression of *Mitochondrial transcription factor A (TFAM)* and *p53-inducible ribonucleotide reductase 2 (RRM2B or p53R2)*, which are critical for mtDNA transcription and maintenance.<sup>6,8</sup> In contrast to the cardiac tissue of *Trp53*<sup>+/+</sup> mice acutely exposed to bolus dosing of doxorubicin, samples from mice treated with low divided doses of doxorubicin did not show any detectable apoptosis, regardless of their *Trp53* genotypes. Therefore, these 3 different *Trp53* genotypes with unique cellular activities indicated that p53-regulated cell death pathways are unlikely to be involved in the development of late-onset cardiomyopathy induced by doxorubicin administered in a more clinically relevant manner.

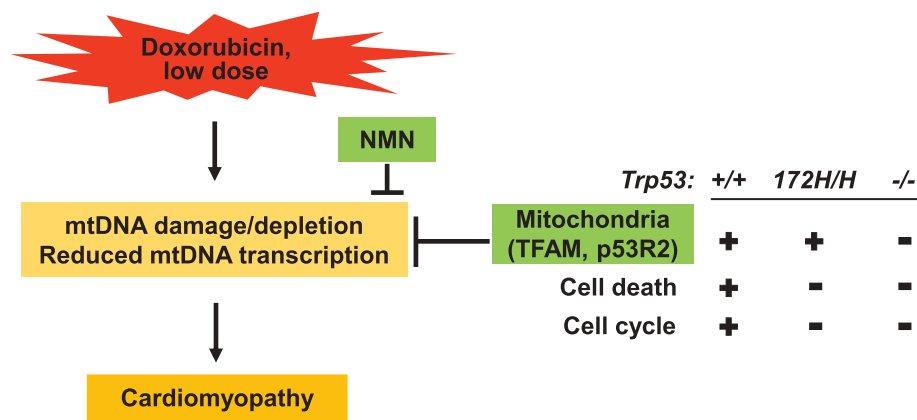
The *Trp53* genotype-dependent responses to doxorubicin treatment in the mouse models were also replicated in human iPS cell-derived cardiomyocytes. Both wild-type and mutant R175H *TP53* human iPS cell-derived cardiomyocytes showed

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AUTHOR'S VIEWS on: Jie Li, Ping-yuan Wang, Nathaniel A. Long, Jie Zhuang, Danielle A. Springer, Jizhong Zou, Yongshun Lin, Christopher K. E. Bleck, Ji-Hoon Park, Ju-Gyeong Kang, and Paul M. Hwang (2019) p53 prevents doxorubicin cardiotoxicity independently of its prototypical tumor suppressor activities. *Proc Natl Acad Sci U S A* 116(39):19626-19634.

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**Figure 1.** Potential mechanism by which p53 regulates mtDNA maintenance in doxorubicin cardiotoxicity. Wild-type (+/+) and mutant (172H/H) *Trp53* genotype states protect mtDNA via TFAM and p53R2, which prevent doxorubicin-induced cardiomyopathy. Note the absence of cell death and cell cycle regulatory activities in both the *Trp53* mutant (172H/H) and null (-/-) states. NMN treatment can also promote the maintenance of mtDNA after doxorubicin treatment. TFAM, mitochondrial transcription factor A; p53R2, p53-inducible ribonucleotide reductase 2; NMN, nicotinamide mononucleotide; mtDNA, mitochondrial DNA.

increased expression of *TFAM* and *p53R2* while apoptosis was only evident with supra-clinical concentrations of doxorubicin in the wild-type state. On the other hand, lower concentrations of doxorubicin were sufficient to increase the transcript levels of mitochondrial respiratory subunits encoded by mtDNA in both wild-type and mutant *TP53* cardiomyocytes while these increases were absent in the *TP53* null state. Similar results were observed in doxorubicin treated human skeletal muscle myoblasts, suggesting their possible contribution to the mechanism of fatigue and peripheral muscle myopathy reported in patients treated with doxorubicin.

Prevention of mitochondrial and LV systolic dysfunction by the concurrent treatment of *Trp53* null mice with nicotinamide mononucleotide (NMN) further supported the mechanism whereby mtDNA depletion underlies doxorubicin-induced cardiomyopathy. At the cellular signaling level, increasing tissue nicotinamide adenine dinucleotide (NAD<sup>+</sup>) content by NMN has been reported to increase the expression of *TFAM* for mtDNA homeostasis via NAD<sup>+</sup>/sirtuin 1 (SIRT1)-mediated deacetylation and activation of PGC-1 $\alpha$ , but this could also involve other SIRT family members.<sup>9</sup>

In summary, the present study provides genetic evidence that p53 has a protective effect on the mitochondrial genome of the heart after doxorubicin exposure and that its regulation of mtDNA transcription may be critical for preventing cardiotoxicity (Figure 1). Chromatin immunoprecipitation (ChIP) analysis in human myoblasts demonstrated that mutant p53 R175H can be recruited to an ETS2-binding site of *TFAM* via protein-protein interaction to increase its expression, analogous to a mechanism previously reported for the *p53R2* gene.<sup>10</sup> In this regard, the higher stability and levels of mutant p53 protein may contribute to the observed gain-of-function in mtDNA encoded respiratory subunit gene expression. Additionally, the decreased levels of mtDNA-encoded transcripts in peripheral blood cells of *Trp53*<sup>-/-</sup> compared with *Trp53*<sup>+/+</sup> and *Trp53*<sup>172H/H</sup> mice suggest that mtDNA transcripts could potentially serve as biomarkers for susceptibility to doxorubicin-induced cardiomyopathy.

The interpretation of our current work has limitations in that the doxorubicin-induced cardiomyopathy was studied in

*Trp53* null mice which is not the *TP53* genetic background in the human disease. It is also well-known that small animals like mice are not ideal for modeling human cardiac pathophysiology, but nonetheless, they represent simple genetic models for gleaning mechanistic insights. The use of larger animals may be necessary to more closely model the human disease. Also, as expected based on the low incidence of the human disease, using divided doses of doxorubicin did not cause significant heart failure in mice with wild-type *Trp53* within the time limits of our study due to early cancer development in *Trp53* mutant states. Cardiac-specific *Trp53* gene disruption may not only permit increasing the duration of follow up after doxorubicin treatment but also allow further investigations into whether mtDNA regulation by p53 in cardiomyocytes plays a cell-autonomous role in preventing doxorubicin-induced cardiomyopathy. Investigating the potential utility of prognostic blood markers and therapeutic interventions to promote mitochondrial biogenesis in future translational studies may be fruitful.

## Disclosure of potential conflicts of interest

No potential conflicts of interest were disclosed.

## Funding

This work was supported by the Division of Intramural Research, NHLBI, NIH.

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