CDDpress

www.nature.com/cdd

EDITORIAL

Targeting p53 gain-of-function activity in cancer therapy: a cautionary tale

Cell Death & Differentiation (2024) 31:133–135; https://doi.org/10.1038/s41418-023-01253-7

TP53 is renowned for being the most commonly mutated gene in sporadic human cancers; over half of all human cancers, of a variety of types, sustain mutations in *TP53* [1]. *TP53* encodes a transcriptional activator, p53, which binds DNA as a tetramer and transactivates a host of downstream target genes involved in its anti-tumor responses [1]; Fig. 1. The importance of this transcriptional function of p53 is underscored by the observation that most cancer-associated mutations in *TP53* are in the central, sequence-specific DNA-binding domain and disrupt the ability of p53 to bind to DNA [1]. Curiously, ~75% of these mutations are missense mutations, rather than nonsense or frameshift mutations that typify other tumor suppressor genes [2]. Although loss of function (LOF) of p53 clearly promotes cancer, as demonstrated by the universal predisposition of *p53* knockout mice to cancer [3], the unusual accumulation of *TP53* missense mutations in cancers long ago led to the idea that there is some significance to retaining mutant p53 protein during cancer development. Originally, this phenomenon was attributed to mutant p53 acting as a dominant negative protein, hetero-tetramerizing with wild-type p53 and inhibiting it [4]; Fig. 1. Shortly thereafter, it was suggested that mutant p53 may be preserved in tumors because it carries neomorphic, gain-of-function (GOF) properties that confer a selective advantage to tumor cells [5]. Numerous studies showed that mutant p53 can enhance cell proliferation and survival, metastasis, resistance to cancer therapy, and other phenotypes important for cancer progression, relative to simple deletion of *p53* [6]. With a couple of studies in mouse models in 2004 (See references in [6]), the GOF model took hold, and putative mechanisms were elaborated. The most common model centered on the notion that mutant p53 acts with other transcription factors to reprogram patterns of gene expression to promote tumorigenesis (Fig. 1).

One key implication of these findings was that targeting mutant p53 might be useful in cancer therapy. Akin to eradicating tumors addicted to oncogenic proteins such as activated Kras, it was suggested that tumors might become addicted to mutant p53 and that knockdown of mutant p53 might therefore provide a therapeutic strategy for tumors expressing p53 GOF mutants. Indeed, knockdown of mutant p53 showed some therapeutic benefit in mouse models [7].

However, GOF mechanisms of mutant p53 action in cancer did not remain undisputed. Two studies, one in human AML cells and one in mouse lymphoma models, emphasized that the primary effect of mutant p53 expression is to inhibit wild-type p53 through dominant negative mechanisms [8, 9]. Moreover, a comprehensive screen using a lentiviral library expressing all possible p53 variants (with substitutions of every amino acid with all other possible residues) indicated that >80% of full length p53 DNA-binding domain missense mutants that exhibit LOF also display dominant negative activity [10].

With an eye on seriously evaluating the potential of targeting mutant p53 in cancer therapy, Wang et al. have now comprehensively and systematically evaluated how knocking out mutant *p53* affects cancer cell fitness using a battery of assays in an array of different cancer models [11]. First, using 16 cell lines derived from diverse cancers (e.g. breast, liver, colon, lung), and carrying 12 different missense mutant *p53* variants, they assayed the consequences of mutant *p53* knockout by CRISPR. They assessed cell proliferation in standard conditions and under stress conditions (with nutrient deprivation or chemotherapy treatment), as well as cell survival. They found no difference between cells expressing p53 mutants and their isogenic counterparts lacking those mutants. Their analysis then extended to in vivo contexts, where they implanted human breast cancer cells into mouse mammary fat pads and tracked metastasis. They observed no effect of mutant *p53* knockout in metastasis in vivo or in migration assays in vitro. Using human colon cancer organoids grown in culture or in mice, they found that the growth, gene expression profiles, and response to 5-FU were similar whether mutant p53 was present or absent. Upon transplanting mouse lymphoma or breast cancer cells into syngeneic hosts with intact immune systems, there was again no difference in tumor growth between cells expressing or lacking mutant p53. Finally, by mining human Cancer Dependency Map data, with 391 cell lines of diverse origins and 158 p53 mutants, the authors showed that there was no effect of mutant *p53* knockout on cell fitness. Thus, in a wide range of settings, the team found no direct evidence for p53 GOF activity.

A powerful approach to directly compare the fitness of cells expressing mutant p53 or lacking p53 is to perform a competition experiment. To this end, Wang et al. mixed BFP-labeled, mutant p53-expressing cell lines and their *p53* knockout derivatives, labeled with GFP, at a 50:50 ratio and measured competition over time in vitro by flow cytometry [11]. They observed no competitive advantage for either cell line, suggesting that loss of the p53 point mutant does not compromise cell fitness and casting doubt on a GOF effect. This recalls experiments in a pancreatic cancer mouse model, where the effect of Cre-mediated expression of mutant *p53* on tumor development was compared to *p53* deletion [12]. Interestingly, tumor latency was not decreased nor was metastasis increased in $p53^{R172H/-}$ or $p53^{R270H/-}$ mice relative to $p53^{-/-}$ mice, indicating no clear GOF effect of the p53 mutants. Additionally, there was not even consistent Cre-driven expression of the p53 mutants in all tumors, indicating an absence of strong selection for mutant p53 expression in this context.

How do we reconcile the current findings with previous work reporting GOF activity for mutant p53? Wang et al. emphasize the importance of the isogenic systems they use in this work. Moreover, to understand differences between their findings and previous work, the authors strove to repeat select previously

Received: 24 November 2023 Revised: 8 December 2023 Accepted: 15 December 2023
Published online: 27 December 2023

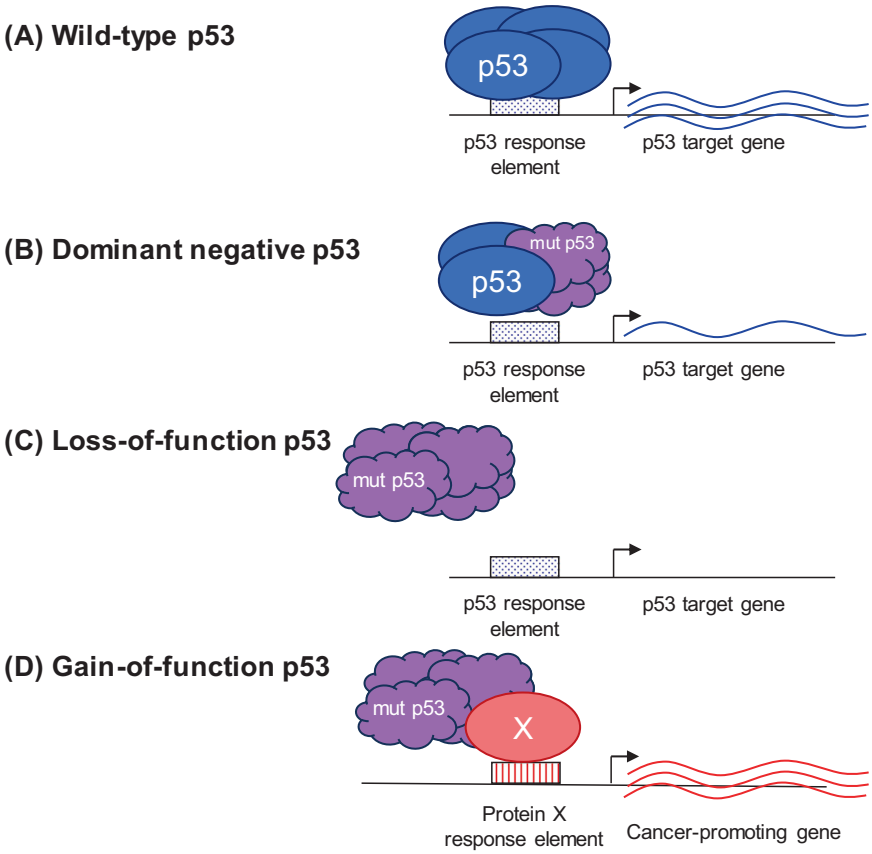

Fig. 1 Models for how *p53* alleles affect p53 function. A The wild-type p53 protein forms a tetramer that binds to specific response elements and transcriptionally activates p53 target genes. **B** p53 missense mutant proteins can have dominant negative effects by hetero-tetramerizing with wild-type p53 and interfering with wild-type p53 transcriptional activation function. This could be a partial effect, as there may still be some binding of the mixed tetramer to DNA and some gene activation, depending on the gene. **C** Loss of p53 function occurs when all subunits of missense mutant p53 homo-tetramerize and are incapable of binding to p53 response elements and transactivating target genes. **D** Gain of p53 function can occur when p53 missense mutants interact with other transcription factors (denoted as "X") to enhance expression of cancer-promoting genes.

published experiments. Using previously described shRNAs and cell lines in which p53 mutants were proposed to display GOF activity, the authors observed nonspecific toxic effects of these shRNA that reduced fitness not only of mutant p53-expressing cells but also of isogenic *p53* null cells [11]. Moreover, their analyses of cell lines in DepMap recapitulated the observation that *p53* targeting RNAi has off-target toxicity. Hence the authors provide a cautionary note about using certain techniques, like RNAi.

Nonetheless, as Wang et al. state, they cannot rule out some GOF activity for p53 in select contexts, and there is an abundance of data supporting the GOF activity of p53. Indeed, transduction of HCT116 colorectal cancer cells, a line not tested by Wang et al., with a lentiviral library of p53 DNA-binding domain mutants revealed enrichment of so-called hotspot mutant p53 compared to truncation and frameshift mutants only when cells were transplanted into nude mice and not in vitro, suggesting GOF might be context specific [13]. Even Wang et al. observe differences in gene expression profiles between human breast cancer cells that express mutant *p53* and with mutant *p53* knockout. It is logical that cells expressing mutant p53 might differ from cells lacking p53 altogether, at least in some settings. *p53* mutations can result in the expression of unfolded p53 protein that forms aggregates in cells [14], and the accumulation of mutant p53 protein might trigger cellular responses that change the biology of cells.

So where do we stand with respect to the original question posed by the authors about the possibility of mutant p53 ablation for cancer therapy? Given the recent findings from Wang et al., it seems risky to develop general therapies premised on ablating mutant p53. Inactivation of p53 mutants leaves a *p53* null state, which is still a highly malignant state associated with poor prognosis. Instead, efforts would best be focused on alternative approaches, like restoration of wild-type p53 function or synthetic lethality with p53 inactivation [15].

Laura D. Attardi [1,2]✉ and Anthony M. Boutelle[1]
[1]*Division of Radiation and Cancer Biology, Department of Radiation Oncology, Stanford University School of Medicine, Stanford, CA 94305, USA.* [2]*Department of Genetics, Stanford University School of Medicine, Stanford, CA 94305, USA.* ✉*email: attardi@stanford.edu*

REFERENCES

1. Boutelle AM, Attardi LD. p53 and tumor suppression: it takes a network. Trends Cell Biol. 2021;31:298–310.
2. Olivier M, Eeles R, Hollstein M, Khan MA, Harris CC, Hainaut P. The IARC TP53 database: New online mutation analysis and recommendations to users. Hum Mutat. 2002;19:607–14.
3. Donehower LA, Lozano G. 20 years studying p53 functions in genetically engineered mice. Nat Rev Cancer. 2009;9:831–41.
4. Finlay CA, Hinds PW, Levine AJ. The p53 proto-oncogene can act as a suppressor of transformation. Cell. 1989;57:1083–93.

5. Dittmer D, Pati S, Zambetti G, Chu S, Teresky AK, Moore M, et al. Gain of function mutations in p53. Nat Genet. 1993;4:42–6.
6. Oren M, Rotter V. Mutant p53 gain-of-function in cancer. Cold Spring Harb Perspect Biol. 2010;2:a001107.
7. Alexandrova EM, Yallowitz AR, Li D, Xu S, Schulz R, Proia DA, et al. Improving survival by exploiting tumour dependence on stabilized mutant p53 for treatment. Nature. 2015;523:352–6.
8. Boettcher S, Miller PG, Sharma R, McConkey M, Leventhal M, Krivtsov AV, et al. A dominant-negative effect drives selection of TP53 missense mutations in myeloid malignancies. Science. 2019;365:599–604.
9. Aubrey BJ, Janic A, Chen Y, Chang C, Lieschke EC, Diepstraten ST, et al. Mutant TRP53 exerts a target gene-selective dominant-negative effect to drive tumor development. Genes Dev. 2018;32:1420–9.
10. Giacomelli AO, Yang X, Lintner RE, McFarland JM, Duby M, Kim J, et al. Mutational processes shape the landscape of TP53 mutations in human cancer. Nat Genet. 2018;50:1381–7.
11. Wang Z, Burigotto M, Ghetti S, Vaillant F, Tan T, Capaldo BD, et al. Loss-of-function but not gain-of-function properties of mutant TP53 are critical for the proliferation, survival and metastasis of a broad range of cancer cells. Cancer Discov [Internet]. 2023; Available from: https://doi.org/10.1158/2159-8290.CD-23-0402.
12. Flowers BM, Xu H, Mulligan AS, Hanson KJ, Seoane JA, Vogel H, et al. Cell of origin influences pancreatic cancer subtype. Cancer Discov. 2021;11:660–77.
13. Kotler E, Shani O, Goldfeld G, Lotan-Pompan M, Tarcic O, Gershoni A, et al. A systematic p53 mutation library links differential functional impact to cancer mutation pattern and evolutionary conservation. Mol Cell. 2018;71:178–190.e8.
14. Khadiullina R, Mirgayazova R, Davletshin D, Khusainova E, Chasov V, Bulatov E. Assessment of thermal stability of mutant p53 proteins via differential scanning fluorimetry. Life. 2022;13:31.
15. Wang M, Attardi LD. A balancing act: p53 activity from tumor suppression to pathology and therapeutic implications. Annu Rev Pathol Mech Dis. 2022;17:205–26.

AUTHOR CONTRIBUTIONS

LDA and AMB co-wrote the manuscript.

COMPETING INTERESTS

The authors declare no competing interests.