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p53 and DNA methylation suppress the TRAIN to cell death

Omid Tavana and Wei Gu*

Institute for Cancer Genetics and Department of Pathology and Cell Biology; College of Physicians and Surgeons; Columbia University; New York, NY USA

Hallmark to most cancers is the mutation or complete loss of function of the tumor-suppressor gene p53. p53 functions by transcriptionally activating or repressing putative downstream targets to elicit a network of tumor-suppressive functions—transient cell cycle arrest allowing for DNA repair and, upon irreparable damage, senescence or apoptosis.[1] Recently, our group has shown that p53-meidated transactivation of metabolic targets can suppress tumorigenesis in the absence of both cell cycle arrest and apoptosis.[2] Notably termed the "guardian of the genome," many therapies have been targeted to reactivate p53 or the downstream targets to initiate apoptosis or cell cycle arrest in cells that have escaped these properties, yet in some instances, p53-proficient tumors have a worse response to therapy.[3] An opposite and ever-attractive chemotherapeutic approach has been to selectively target attributes of p53-mutant and -deficient cells that are absent in p53 proficiency.

In 2004, Manuel Serrano's group characterized a demethylating agent with the highest selective toxicity toward p53-deficient cells compared with other popular DNA-damaging agents.[4] This demethylating agent, 5-aza-2'-deoxycytidine (5-aza-dc), also called decitabine and marketed as Dacogen, has been recently FDA approved for the treatment of chronic myelomonocytic leukemia (CMML) and myelodysplastic syndrome (MDS) as well as showing promise with other tumor types.[5-7] In the most recent issue of *PNAS*, Andrie Gudkov's group elegantly elucidates a mechanism of action for p53-deficient cell death after 5-aza-dc treatment, highlighting potential markers to determine increased drug efficacy for different tumor types.[8]

Initially, Leonova et al, confirmed the selective toxicity of the demethylating agent 5-aza-dc in proliferating p53-mutant and -deficient fibroblasts compared with wild-type controls[8] as previously described.[4] Microarray gene expression profiling using murine embryonic fibroblasts (MEFs) with and without p53 and drug treatment uncovered 55 genes normally repressed by p53 in a methylated state that were upregulated 5-fold upon hypomethylation. For the most part, these target mRNA only increased to the level of untreated $p53^{-/-}$, suggesting that only in the presence of p53, DNA methylation helps suppress these targets. Interestingly, 5-aza-dc treatment of p53-deficient MEFs strongly upregulated 124 genes, remaining silent in drug-treated WT MEFs. The majority of these targets were classified as part of or downstream to the type I interferons (INF-α and -β).[8] Classically, type I INFs signal through the INF cell-surface receptor (IFNAR) to initiate a cellular cascade to enhance the immune response upon viral infections as well as regulate tumor cell survival.[9] To confirm the dependency of a "suicidal" INF-stimulated p53-deficient cell death after 5-aza-dc treatment, $IFNAR^{-/-}$ MEFs were generated that lose the ability of stimulating a type I INF response. After hypomethylation, knocking down p53 in $IFNAR^{-/-}$ MEFs reversed the cell death seen in p53 deficiency alone, validating the hypothesis of INF-dependent cell death.

Leonova et al, next sought to pinpoint the trigger activating the "suicidal" INF response. Since the gene-expression profiling did not uncover any plausible explanation for the INF activation and is solely a read out of protein-coding mRNA, researchers intuitively utilized RNA sequencing.[8] Moreover, INFs are classically activated upon double-stranded RNA typically from viral infections, increasing the likelihood that the candidate of the INF response would not be from a protein coding transcript. Intriguingly, 5-aza-dc-treated $p53^{-/-}$ MEFs showed a significant abundance of three specific types of RNA transcripts produced 150-fold greater than β-actin mRNA.[8] Once referred to as "junk DNA," these repetitive elements are gaining more attraction in the recent years. Comprising two-thirds of the RNA transcripts produced from drug-treated $p53^{-/-}$ MEFs, are gamma satellite repeats (GSATs) transcribed from large tandem repeats of non-coding DNA near the centromeres and in heterochromatin as well as short interspersed elements (SINEs), which are short DNA sequences interspersed throughout the genome. The other highly abundant classification of transcript is termed non-coding RNAs (ncRNA), simply characterized as an RNA that does not produce a protein product. The high abundance of these RNA transcripts has been proposed to form large amounts of dsRNA characteristic of a viral infection resulting in the activation of an INF response.

Lastly, murine tumor cell lines were treated with 5-aza-dc and probed for transcription of repeats. Samples with a strong induction of GSATs correlated with upregulated INF targets, IRF7 and CXCL10, as well as increased susceptibility to 5-aza-dc, while samples with low expression of these repetitive transcripts did not upregulate INF genes and were modestly resistant to drug treatment.[8] Spontaneous thymic lymphomas arising from p53-deficient mice had increased GSAT and IFN expression when compared

*Correspondence to: Wei Gu; Email: wg8@columbia.edu
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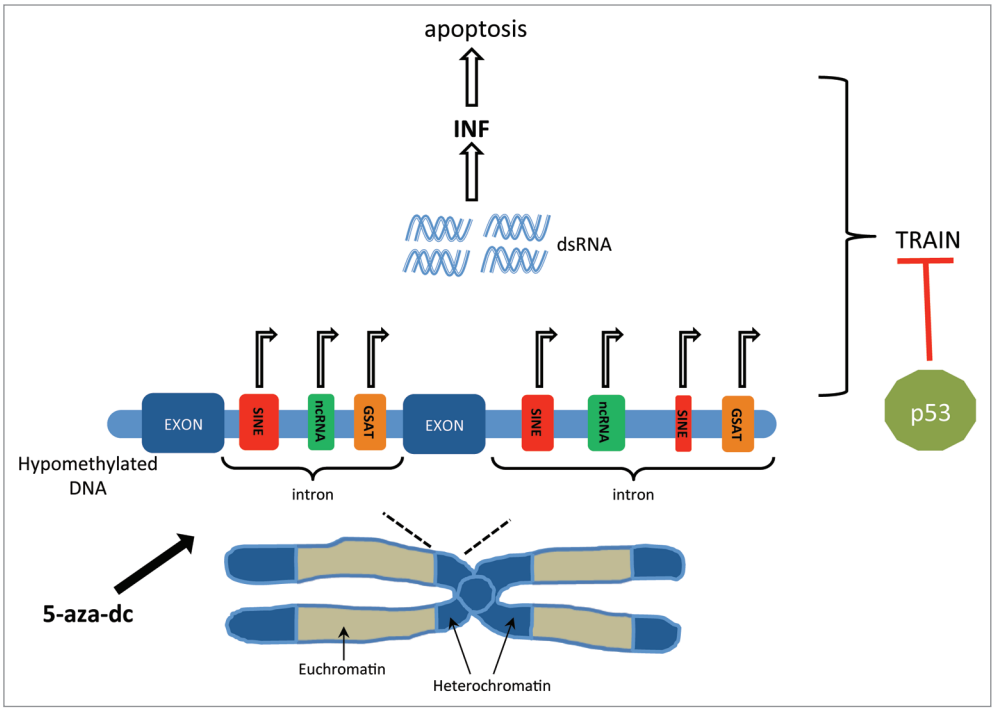

Figure 1. Schematic of TRAIN (transcription of repeats activates interferon)-induced cell death. In a hypomethylated state (caused from 5-aza-dc treatment), p53 suppresses the transcription of repetitive factors (SINE, GSAT, nc-RNA), which presumably form into double-stranded RNA to activate a "suicidal" interferon response leading to apoptosis specifically in a p53-deficient setting.

with $p53^{-/-}$ non-tumorigenic thymi. The authors characterized the phenomenon described here as "TRAIN" (transcription of repeats activates interferon). The model described by Gudkov and colleagues, depicted in **Figure 1**, demonstrates a novel role for p53 as the "guardian of repeats," where, in cooperation with DNA methylation, transcriptionally silences repetitive DNA segments, which otherwise activate a "suicidal" interferon response leading to apoptotic cell death.

With this newly discovered mechanism, 5-aza-dc may become a more attractive therapeutic target for many cancers that have mutated or complete p53 inactivation. Since some tumors exhibit global hypomethylation, taken in concert with the high rate of p53 abrogation, many tumors may have developed resistance to INF-mediated death. Although further investigation is needed to elucidate in vivo efficacy, tumors may be pre-screened for p53 status, increased transcription of repeats and an intact INF response; these criteria will allow a free TRAIN ride to cell death.

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