
Restoring Function to a Variant of p53 in Solid Tumors

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In this issue of the *Journal*, Dumbrava et al.[1] report the results of a Phase I trial of rezatapopt, a reactivator of the p53 protein, in patients with solid tumors harboring a specific *TP53* mutation.

What is p53?

In 1979, Lloyd Old's group at Memorial Sloan Kettering identified a tumor-specific antibody recognising a tumor antigen of 53 kDa,[2] and duly dubbed it p53. In the same year, two other groups independently reported the identification of a 53–54 kDa host protein as the binding partner of the large T antigen oncoprotein in cells transformed by the oncovirus SV40.[3,4] We now know that the p53 tumor antigen and the host protein that binds the large T antigen of SV40 virus are one and the same. Over the following decade, the precise role of p53 in tumorigenesis remained ambiguous, but in 1989, Bert Vogelstein's group at Johns Hopkins University discovered that *TP53* (which encodes p53) is often mutated in human cancers.[5,6,7] Persons born with a mutated *TP53* allele have Li-Fraumeni syndrome and develop cancer at a young age,[8] and p53-knockout mice also develop early-onset tumors. These findings established the tumor-suppressive function of p53 in cancer, and research over subsequent decades established *TP53* as the most commonly mutated gene in human cancers.

As for its function, p53 is a transcription factor. It regulates the expression of hundreds of target genes to maintain cell and tissue homeostasis and to suppress tumor growth through various pathways that include but are not limited to its ability to inhibit cell growth, induce cell

death in response to stress, and maintain genomic integrity and metabolic homeostasis (**Figure 1A**). It has a transactivation domain at one end, a DNA-binding domain that controls target gene expression in a sequence-specific manner in the middle, and a tetramerization domain at the other end (which binds DNA non-specifically). Most mutations affect the central DNA-binding domain (which contains a zinc-figure domain that is important for its structural stability) (**Figure 1B**): the majority of these mutations are missense mutations, replacing an existing amino acid with a new one, disrupting the structure of p53 or preventing its binding to DNA. Some amino acids are more frequently mutated than others and they are called “hotspot” mutations (**Figure 1B**).

Restoring Tumor Suppressive Function to p53

Worldwide, there are an estimated ~20 million people who are diagnosed with cancer and the cancers in about half of these persons harbor mutated *TP53* on one or both alleles.[10] However, in the other half (persons whose tumors are *TP53*-wildtype), the tumor suppressive function of p53 is usually compromised. Restoring the tumor-suppressive function of p53 (regardless of the status of *TP53*) therefore represents enormous potential in cancer therapy. Thus, there have been many attempts to restore p53 function in tumors containing wildtype and/or mutant p53.

Suppressing MDM2-mediated Degradation of p53

A key transcriptional target of p53 is *MDM2,* a gene that encodes a specific type of enzyme (called a ubiquitin ligase) that binds and tags the N-terminus of p53, thus slating it for ubiquitin-mediated degradation. The

transcriptional activation of *MDM2* by p53 is therefore part of a p53-autoregulation loop. Extensive efforts have been made to develop small molecules that can enhance wildtype p53 function by preventing MDM2 binding to it.

How to Reactivate Pan-mutant p53?

Nonsense mutations are common in tumor suppressor genes. They usually result in a loss of function of the encoded protein. However, only 8% of *TP53* mutations are nonsense. More than 73% are classed as missense: they are predicted to result in the substitution of one amino acid residue by another. For example, the mutant Y220C features a cysteine (C) at residue 220 in the place of tyrosine (Y). The high prevalence of missense mutations in *TP53* has spurred the identification of small molecules able to restore the tumor-suppressive function in mutant p53.

Indeed, over the past quarter-century, 11 small molecules have been reported to reactivate mutant p53.[11] The first of these, PRIMA, was shown to restore structure, transcriptional activity and cell growth inhibition to mutant p53 through binding to cysteine residues in the DNA-binding domain[12]. Subsequently, APR-246, a methylated and more potent form of PRIMA, was developed and tested in phase I/II clinical trials.[11]

Recently, combined *in silico* and chemical library screening identified arsenic trioxide as a potent reactivator of mutant p53. Crystal structures of mutant p53 (R175H) in complex with arsenic trioxide showed that arsenic trioxide binds cysteines in the zinc finger domain of p53. This binding refolds many structurally-defective p53 mutants into the

“wildtype” conformation, restoring transcriptional activity.[13] Arsenic trioxide is a first-line clinical drug in the treatment of acute promyelocytic leukemia and phase I–III trials have been registered. [11]

Allelic-specific p53 Reactivators

Among the p53 mutant proteins resulting from *TP53* “hotspot” mutations, the Y220C mutant is attractive from the drug-targeting perspective because the mutant residue creates a structural pocket amenable to small-molecule binding with potential to alter conformation. This variant is present in about 1% of mutant-p53 cancers: there are about 100,000 persons with cancers containing it.[14]

In 2008, Alan Ferscht’s group at the University of Cambridge reported combining *in vitro* thermal denaturation and *in silico* screening approaches to identify a small molecule (with 140 μM binding affinity) able to restore wildtype p53 structure to Y220C p53. Over the following 12 years, at least 11 Y220C p53 reactivators were developed. Of these, rezatapopt binds mutant p53 with greatest affinity (2.5nM). It has shown evidence of selective restoration of transcriptional activity of *TP53* and tumor suppression in mouse models.[15]

In their first-in-class Phase I clinical trial, Dumbrava et al. observed a response to rezatapopt in 14 of 71 patients with p53Y220C-harboring metastatic tumors that could be measured at the beginning of the study: an exciting initial finding that supports further clinical studies.

Disclosure forms provided by the authors are available with the full text of this article at NEJM.org.

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Figure Legend

Figure 1. The Function and Structure of p53.

The protein p53 is a transcription factor. In response to cellular stress signals, p53 becomes activated and its levels increase. Only correctly-folded p53 has transcriptional activity, which results in myriad downstream effects including but not limited to those shown in Panel A. Genetic variation in *TP53* includes missense mutations and hotspots: amino-acid substitutions predicted by the hotspot missense mutations are indicated in Panel B. The height of each vertical line is approximately proportionate to the frequency of the underlying mutation in the ICGC/TCGA Pan-Cancer Analysis of Whole Genomes dataset.[9] Transaction domain (TAD; amino acids 1-61); DNA-binding domain (amino acids 96-292); C-terminal domain (CTD; amino acids 364-393).

New KCs

TP53

The gene that encodes the tumour suppressor protein p53. It is commonly mutated in human cancers. Wildtype p53 protein is short-lived and expressed at low levels. Mutant p53 often acquires an extended life span and accumulates in cancer cells. The p53 protein is a transcription factor that controls the expression of hundreds of target genes by binding and activating specific target genes that, once transcribed and translated, suppress tumorigenesis. Mutant p53 proteins in cancer cells lose their tumor-suppressive function, usually because they cannot bind and activate target genes.

Related terms

for *TP53*: Tumorigenesis, Hereditary Cancer Syndrome, Tumor Heterogeneity

Reciprocally add *TP53* as a related term to: Tumorigenesis, Hereditary Cancer Syndrome, Tumor Heterogeneity.

Tumorigenesis

A biological process that results in the formation of tumors. At the cellular level, it involves the transformation of normal cells to dysplastic cells, which can progress through a multistep process involving changes, including the acquisition of mutations, to full malignant transformation. Cancer cells have high rates of proliferation and migration, a low death rate, and are relatively dedifferentiated. At the molecular level, tumorigenesis is controlled by genes with opposing functions; it can be enhanced or promoted by oncoproteins such as *RAS* and *MYC* or suppressed and inhibited by tumour suppressor proteins such as p53.

Related terms

for Tumorigenesis: Liquid biopsy, tumor heterogeneity, genome instability.

add Tumorigenesis as a related term to: Liquid biopsy, tumor heterogeneity, genome instability.

KCs already in the IG:

Tumor Antigen

Ubiquitin-mediated degradation

Small molecule

Nonsense mutation

Missense mutation