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The rise of p53

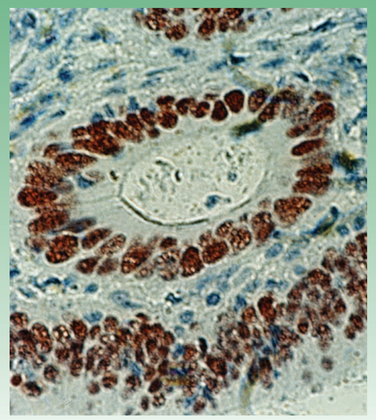
Bert Vogelstein and Carol Prives

It is now clear that p53 inactivation is essential for the formation of nearly all cancers. This clarity was reached through a meandering set of observations that initially seemed entirely unrelated. The gene encoding p53 (*TP53*) was first thought to be an oncogene that mediated the pathological effects of experimental DNA tumour viruses and chemical carcinogens. However, after 10 years of research *TP53* was found to be a tumour suppressor gene that is often inactivated in human cancers. The discovery that p53 controls cell death — as well as cell division — was one of the stimuli that induced an avalanche of research on apoptosis. The volume of work on p53 that resulted from these seminal observations has shown it to be at the centre of a cellular network of feedback and feedforward loops, forming a paradigm for systems biology. Further understanding of this network, and determining how it can be exploited for therapeutic benefit, will keep investigators busy for years to come.

1979–1989

Discovery of p53

In 1979 six groups of investigators independently reported the discovery of a ~53 kDa protein that was present in human and mouse cells. Five of these studies showed that this protein bound to the large T-antigen of SV40 in cells infected with this virus, and the sixth found it expressed in several types of mouse tumour cells. Various investigations carried out on this protein (p53), and later on the gene (*TP53*), suggested that it was an oncogene. This interpretation, which reflected the research climate of that time, was based on apparently compelling experimental evidence: the p53 protein was bound to the major oncogenic protein of SV40, which strongly suggested that it was a downstream effector of the large T-antigen pathway; high levels of p53 expression were found in many cancers; and the overexpression of an apparently wild-type *TP53* gene transformed a normal cell into a cancer cell. Although there were a few experimental observations that did not fit with the idea that *TP53* was an oncogene, the existence of tumour suppressor genes was hypothetical in the mid-1980s and so there was little reason to think otherwise.

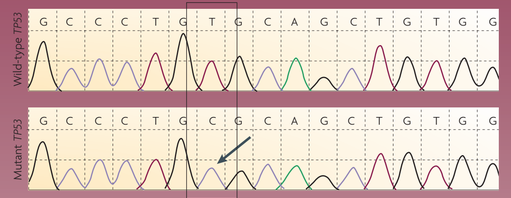


High levels of mutant p53 were detected in human intestinal tumour cells (brown) but not normal cells by immunohistochemistry.

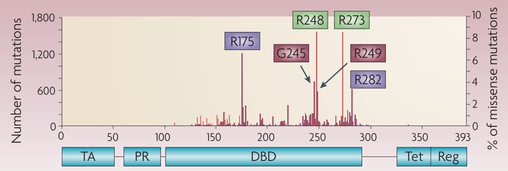
1989 onwards

p53 has a pivotal role in human tumorigenesis

The turning point in p53 research came in 1989. The remaining *TP53* allele sequenced from a tumour that had lost a portion of the p arm of chromosome 17, where *TP53* resides, was found to be mutated. The point mutation (shown in the chromatogram below) was not found in normal tissue samples taken from the patient. This, coupled with the frequent loss of 17p in tumours, indicated that *TP53* was a tumour suppressor gene. It became clear that the 'wild-type' *TP53* gene used to demonstrate that p53 was oncogenic in cell transformation experiments was, in fact, mutated. That p53 is a bona fide tumour suppressor was confirmed in 1990 by the finding that patients with Li–Fraumeni syndrome — which predisposes to diverse tumour types — had inherited *TP53* mutations, and further confirmed in 1992 by experiments showing that *Trp53* (which encodes mouse p53) knockout mice are prone to tumours.



Subsequent studies have demonstrated that *TP53* is more frequently mutated in human tumours than any other gene in the genome, and >25,000 *TP53* mutations have been reported to date. Of these mutations, 75% occur as missense mutations that predominantly occur in the DBD. The figure below shows the distribution of tumour-associated missense mutations relative to the domains of p53 and highlights the six most common mutations. These mutations can affect the structure of p53 (distorting or unfolding the native conformation) and they can impair DNA binding. Experimental models have revealed that such tumour-derived mutations can have dominant-negative properties and, in some cases, can confer gain-of-oncogenic function properties in the absence of wild-type p53.

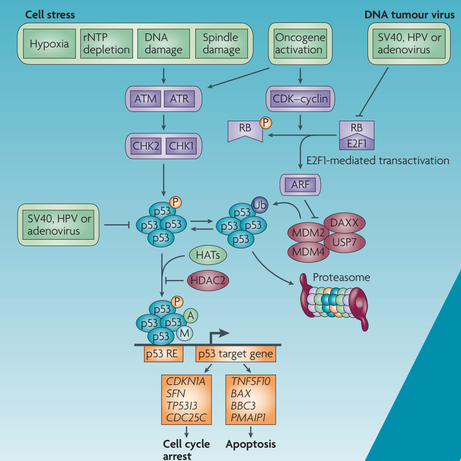


Mutations in green affect DNA contacts, those in red cause local distortions and those in purple cause global denaturation. The x-axis indicates amino acid number.

1991 onwards

The biological effects of p53

The first clue into the outcome of activating p53 was revealed when it was shown that the expression of wild-type p53 arrests the cell cycle in a subset of cell lines. It was subsequently shown that the expression of wild-type p53 in other cells results in cell death rather than arrest. These findings were major stimulants to the developing field of apoptosis as they showed that the regulation of apoptosis and the regulation of cell proliferation were equally important to tumour suppression. In the early 1990s two seminal observations were made: that p53 has a transactivation domain, and that it can bind to specific DNA sequences. The crystal structure of the p53 core domain bound to DNA showed exactly which p53 residues make contact with DNA and revealed how different tumour-associated mutations abrogate this binding. It is now understood that wild-type p53 functions as a tetrameric transcription factor that regulates net cell growth, inhibiting the cell cycle in some circumstances and promoting apoptosis in others, through the activation or repression of key target genes. It has also been suggested that p53 promotes apoptosis in the cytoplasm through mechanisms that do not involve transcription. Both apoptosis and cell cycle arrest have since been shown to be important for limiting the propagation of mutations following many types of cellular stress, especially DNA damage. Moreover, p53-regulated genes that function in other cellular processes, such as senescence, metabolism, autophagy, angiogenesis and DNA repair, have also been identified and these might have a role in its tumour suppressive activities (not shown in the figure).



Cellular stress or DNA tumour virus infection triggers mediating proteins to activate p53 by phosphorylating it or inhibiting its ubiquitylation by MDM2. Following further post-translational modifications, the p53 tetramer regulates transcription by binding to p53 response elements and recruiting cofactors, and the protein products lead to cell cycle arrest or apoptosis.

1999

Translating p53 research to the clinic

Much remains unknown about this multi-talented tumour suppressor. For example, which changes in the microenvironment favour the selection of cells with *TP53* mutations? Does continuous or unrepairable DNA damage or the presence of reactive oxygen species, perhaps in association with alternating cycles of hypoxia and normoxia, influence the survival of cells with *TP53* mutations? We also do not understand why the expression of wild-type p53 results in apoptosis in some cells and cell cycle arrest in others, or how the various p53 post-translational modifications might regulate this switch. Finally, and perhaps most importantly, we do not yet know how to use our knowledge of p53 for therapeutic purposes. Approaches to reactivate mutant p53 or remove inhibition of wild-type p53 are being developed and some show great promise (see the TABLE). However, the field is wide open to new, creative approaches that effectively translate p53 to the clinic. p53 research lives on.

Table | **Drugs targeting the p53 pathway**

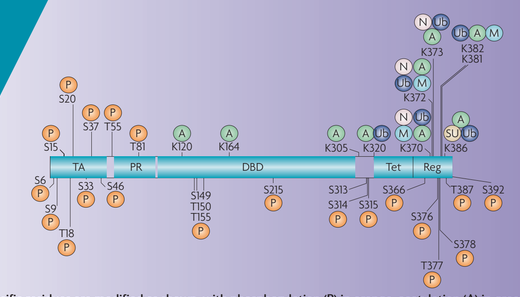
Drug	Action
Advexin, Gendicine*, SCH-58500	Non-replicating adenovirus that carries wild-type *TP53*
ONYX-015	Adenovirus that was designed to preferentially replicate in cells without normal p53 function
PRIMA-1, MIRA-1, CP-31398, STIMA-1	Small molecules that reactivate mutant p53
HL198C	Ubiquitin ligase inhibitor
Nutlin, NU8354, chlorofusin, RITA, MI-219, BDP 23	Inhibitors of p53–MDM2 interaction
GEM240	Antisense oligonucleotide that inhibits MDM2 expression
Tenovin-6	Deacetylation inhibitor that activates p53

*Gendicine is already being given to cancer patients in China, following approval by the Chinese Food and Drug Administration in 2004.

1993 onwards

The p53 regulatory network

It was known as early as 1981 that in normal, unstressed cells the level of p53 protein is low owing to its rapid turnover, whereas high levels of p53 are observed in tumours. In 1993 MDM2 was found to be a transcriptional target of p53 and was subsequently shown to be an E3 ubiquitin ligase that targets p53 for degradation, defining a negative feedback loop. Then, in 2001 MDM4, an MDM2 homologue that was first identified in 1996, was also shown to be important for the regulation of p53 activity, although its precise role remains unclear. In addition to ubiquitylation by MDM2, p53 is subject to many other post-translational modifications in response to stress, as shown in the figure. These modifications modulate the activity of p53 and its binding partners and are thought to determine the resulting p53-dependent cellular response.



Specific residues are modified as shown, with phosphorylation (P) in orange, acetylation (A) in green, ubiquitylation (Ub) in dark blue, neddylation (N) in pink, methylation (M) in blue and sumoylation (SU) in yellow.

2000 onwards

The Timeline arrow represents a graph of the number of publications on p53 each year since 1979 (not to scale).

2009

1979

1989

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Further information
IARC *TP53* database: http://www-p53.iarc.fr
IARC Mouse models with targeted p53 alterations: http://www-p53.iarc.fr/MouseModelView.asp
Nature Reviews Cancer Focus on p53 – 30 years on: http://www.nature.com/nrc/focus/p53.html
Pathway Interaction Database for p53: http://pid.nci.nih.gov/search/pathway_landing.shtml?what=graphic&jpg=on&pathway_id=p53regulationpathway

Abbreviations
ATM, ataxia telangiectasia mutated; ATR, ataxia telangiectasia and Rad3 related; BAX, BCL2-associated X protein; BBC3, BCL2 binding component 3 (also known as PUMA); BDP, benzodiazepine; CDC25C, cell division cycle 25 homolog C; CDK, cyclin-dependent kinase; CDKN1A, cyclin-dependent kinase inhibitor 1A; CHK, checkpoint kinase; DAXX, death-domain associated protein; DBD, DNA-binding domain; HAT, histone acetyltransferase; HDAC2, histone deacetylase 2; HPV, human papillomavirus; MIRA-1, mutant p53 reactivation and induction of rapid apoptosis; PMAIP1, phorbol-12-myristate-13-acetate-induced protein 1 (also known as NOXA); PR, proline-rich domain; PRIMA-1, p53 reactivation and induction of massive apoptosis; RB, retinoblastoma; RE, response element; Reg, carboxy-terminal regulatory domain; RITA, reactivation of p53 and induction of tumour cell apoptosis; rNTP, ribonucleotide triphosphate; SFN, stratifin; SV40, simian virus 40; TA, transactivation domain; Tet, tetramerization domain; TNFSF10, tumour necrosis factor (ligand) superfamily, member 10 (also known as TRAIL); TP53I3, tumour protein p53 inducible protein 3; USP7, ubiquitin carboxyl-terminal hydrolase 7.

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