

## New Insights into P53 Signalling and Cancer: Implications for Cancer Therapy

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### ABSTRACT

The tumor suppressor p53 is one of the most highly studied proteins in the field of cancer research because of its role in tumor cell survival and apoptosis. Research over the past three decades has identified p53 as a multifunctional transcription factor, which regulates the expression of >2,500 target genes. p53 impacts myriad, highly diverse cellular processes, including regulation of the cell cycle, maintenance of genomic stability and fidelity, apoptosis, senescence and longevity, metabolism, angiogenesis, cellular differentiation, and the immune response<sup>[1]</sup>.

It is one of the most important and extensively studied tumor suppressors. Approximately half of human cancers have inactivating mutations in the p53 gene (known as TP53 in human) and most of the remaining malignancies deactivate the p53 pathway by increasing its inhibitors, reducing its activators or inactivating its downstream targets. Activated by various stresses, including genotoxic damage, hypoxia, heat shock and oncogenic assault, p53 blocks cancer progression by inducing transient or permanent growth arrest, by enabling DNA repair or by activating cellular death programs<sup>[2]</sup>.

In addition to the indisputable importance of p53 as a tumor suppressor, an increasing and sometimes bewildering number of new roles for p53 have recently been reported, including the ability to regulate metabolism, fecundity, and various aspects of differentiation and development<sup>[3]</sup>.

It is impossible to cover all aspects of p53-associated biology in one review and so we have reluctantly passed over many fascinating topics and we will focus on current strategies and challenges to restore p53 tumor suppressor function in established tumors and the

therapeutic approaches designed to promote or deliver wild-type p53 function to cancer cells (i.e. adenoviral gene transfer and small molecule activator of p53, to inactivate p53 inhibitors and to restore wild-type function to mutant p53).

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**Key words:** p53; Complex biology; Signalling network; Cancer therapy

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### INTRODUCTION

The tumour suppressor TP53 (MIM# 191170), located on chromosome 17p13.1 is known as 'the guardian of the genome' or 'the cellular gatekeeper of growth and division'. The gene contains 11 exons and transcribes a 2.8 kb mRNA, which is translated into a 53 kDa phosphoprotein containing 393 amino acids. p53 is a key regulator of cellular growth control and plays a central role in the induction of genes that are important in cell cycle arrest and apoptosis following DNA damage<sup>[4]</sup>.

Whilst the tumor suppressor functions of p53 have long been recognized, so that p53 is one of the most highly studied tumor suppressor genes in the field of cancer research, the contribution of p53 to numerous other aspects of disease and normal biology and physiology is only now being appreciated. This ever increasing range of responses to p53 is reflected by an increasing variety of mechanisms through which p53 can function, although the ability to activate transcription remains key to p53's *modus operandi*.

p53 is a central hub in a molecular network controlling cell proliferation and death in response to potentially oncogenic stress conditions. A wide array of covalent post-translational modifications and protein interactions regulate its stability and sub-cellular localisation and modulate the nuclear and cytoplasmic activities of p53<sup>[3]</sup>.

The p53 relatives p73 and p63 are entangled in the same regulatory network, being subject at least in part to the same

modifications and interactions that convey signals on p53. Other p53 interactors exert an effect further downstream to directly modulate p53 biological effects, for example, at the mitochondria<sup>[5]</sup>.

One recent review lists 129 such p53 transcriptional targets that were identified as a result of either single gene discoveries or multigene screens<sup>[6]</sup>. There are likely to be many more genes specifically bound and activated by wild-type p53 (wtp53). Furthermore, the number of genes whose expression is altered indirectly upon induction of p53 is likely to be in the thousands, both with canonical<sup>[6]</sup> and non-canonical<sup>[7]</sup> p53 response elements (REs).

This complex set of molecular events actively contribute to the resulting cellular output and lead to growth restraining responses. Control of p53's transcriptional activity is crucial for determining which p53 response is activated, a decision we must understand if we are to exploit efficiently the next generation of drugs that selectively activate or inhibit p53<sup>[4]</sup>.

With the molecular elucidation of p53 signalling continuing to unravel novel concepts and broaden our horizon of p53 function and the importance of p53 signalling for the pathogenesis of cancer, drug development programs have begun to target the p53 signalling pathway.

In this review, we describe the multi-faceted spectrum of p53 activities, discuss current strategies to active p53 in tumors and will conclude with an outlook on future strategies and challenges to translate p53-targeting therapies into clinical practice.

### The complex biology of p53

Three decades of p53 research have produced more than 50,000 publications, which characterized p53 as a transcription factor orchestrating transcriptomic changes in response to a broad spectrum of cellular stresses. In more recent years, it has become clear that p53 function extends beyond canonical cell cycle, senescence and cell death signalling<sup>[2]</sup>.

Gene expression microarrays have revealed that p53 associated-gene clusters impact additional, highly diverse biological processes such as metabolism, aging, energy metabolism, angiogenesis,

immune response, cell differentiation, motility and migration and cell-cell communication<sup>[2]</sup>. Recent studies have demonstrated how p53-dependent activation of microRNA genes can participate in the modulation of various biological activities<sup>[7]</sup>.

The complex repertoire of p53 regulated genes further highlights the need to understand how p53 is regulated and how selects its targets.

### Regulate the regulator

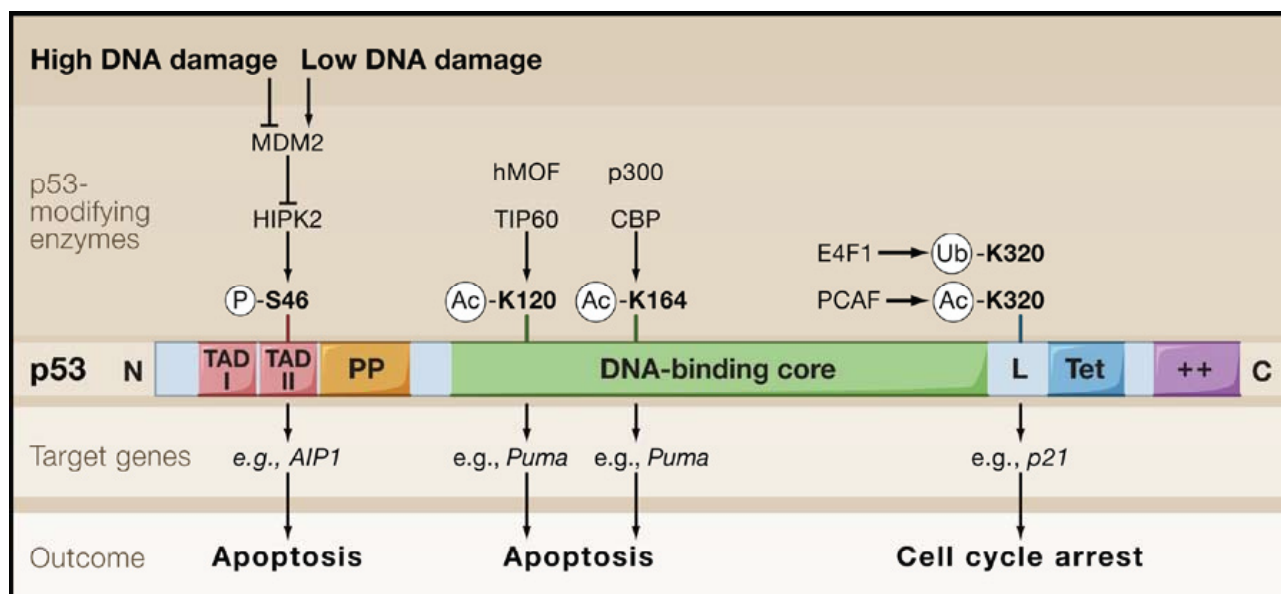
p53 is regulated by an array of posttranslational modifications both during normal homeostasis and in stress-induced responses. More than 36 different amino acids within p53 have been shown to be modified in various biochemical and cell culture studies<sup>[8]</sup>.

Since the first discoveries showing that p53 undergoes stress induced phosphorylation or acetylation, there have been numerous complicated studies describing the modifications to p53 and deciphering how they affect p53 function as a transcriptional regulator. Phosphorylation of p53 is classically regarded as the first crucial step of p53 stabilization, but the tight control of cellular p53 levels is primarily achieved through its ubiquitin-mediated proteasomal degradation. A number of excellent reviews cover these aspects of p53 regulation<sup>[8-11]</sup>.

### p53 mutations and human cancers

As a tumour suppressor, the major p53 functions are to regulate growth arrest and apoptosis (see the review by Vousden and Prives<sup>[3]</sup>) (Figure 1) and the balance of these two cellular events can determine the fate of individual cells. Unlike other tumour suppressor genes, most TP53 mutations in tumours are of the missense type and lead to single amino acid changes that predominantly affect residues in the DNA binding domain of the protein, strongly suggesting that targeted sequence-specific DNA binding is crucial for the escape of tumours from p53 suppressor activity<sup>[7]</sup>.

More than 26,000 somatic mutation in TP53 appear in the international agency for research on cancer (IARC) TP53 database version R14 (<http://www-p53.iarc.fr/>; the International Agency for Cancer Research TP53 Mutation Database; TP53 Website).



**Figure 1** (from Vousden and Prives<sup>[3]</sup>). Selective impact of p53 modifications. p53 protein domains include the transcriptional activation domain I (TAD I, residues 20-40), the transcriptional activation domain II (TAD II, residues 40-60), the proline domain (PP, residues 60-90), the sequence-specific core DNA-binding domain (DNA-binding core, residues 100-300), the linker region (L, residues 301-324), the tetramerization domain (Tet, residues 325-356), and the lysine-rich basic C-terminal domain (++ , residues 363-393). A few examples are depicted of residues that when modified by phosphorylation (P), acetylation (Ac), or ubiquitination (Ub), result in a specific cellular outcome in response to p53 activation that depends on preferential activation of the indicated target genes.

The frequency of TP53 mutation varies from around 10% (hematopoietic malignancies) to 50-70% (ovarian, colorectal, and head and neck malignancies)<sup>[4,12]</sup>. Germline mutations of TP53 cause Li-Fraumeni syndrome, which is a familial cancer syndrome including breast cancer, soft tissue sarcoma, and various other types of cancer<sup>[13]</sup>. Most TP53 mutations in human cancers result in mutations within the DNA binding domain, thus preventing p53 from transcribing its target genes. However, mutant p53 (mutp53) not only loses normal function of the wild-type protein but also gains new abilities to promote cancer<sup>[14]</sup>.

Somatic mutations at individual residues have been associated with specific clinical phenotypes in different type of cancer<sup>[15]</sup>. In addition, the spectrum of p53 deletions or mutations observed among tumor cells suggests that the mutations vary in their prognostic power. Disruptive p53 mutations in tumor DNA are reported to be associated with reduced survival following surgical treatment of Head and Neck Squamous Cell Carcinoma (HNSCC)<sup>[16]</sup>.

In addition to the presence of somatic mutations, polymorphic features of the gene may also contribute to alteration of normal p53 function and variants, mainly in the form of single nucleotide polymorphisms, can be expected to impact tumor susceptibility to cytotoxic drugs, radiation and chemo-radiation<sup>[4,17,18]</sup>. Elucidation of the effect of TP53 polymorphisms is a challenge, which is attracting an interest in the recent years. In fact, no uniform conclusion can be drawn for roles of polymorphisms and mutations in the TP53 gene as results are so far inconsistent.

#### **p53 as a Sensor of DNA Damage: apoptosis or cell cycle arrest and senescence**

Environmental hazards (e.g., UV sunlight, chemical mutagens, and oncogenic pathogens), and cell-intrinsic metabolic processes can damage DNA. Such damage can alter DNA structure and consequently gene transcription, or can cause mutations that impact function. If left unrepaired, DNA damage can cause neoplastic growth. There are very sophisticated systems for detecting DNA damage and repairing the genome. p53 is normally in 'standby' mode and plays an important role in such "caretaker" systems. This is why p53 is the so called "guardian of the genome"<sup>[16]</sup>. p53 acts as an internal sentinel for DNA damage, and other cellular stresses, including hypoxia, oncogene activation, starvation, altered mitochondrial and ribosomal biogenesis, spindle poisons, or denuded telomeres. Depending on the level of cellular compromise, p53 can either promote the repair and survival of damage cells or promote the permanent removal of irreparable damage cells through apoptosis or autophagy<sup>[19]</sup>.

Many of our models for p53 function suppose that induction of programmed cell death is the key mechanism by which p53 eliminates cancer cells. During cell cycle arrest, p53-regulated pathways, including those involving growth arrest and DNA damage inducible 45 (Gadd45) and the p53 ribonucleotide reductase small subunit 2 (p53R2), are significant in the repair of damaged DNA<sup>[16]</sup>. In the absence of competent repair activity, DNA damage induces apoptosis (a) by transcriptional activation of critical apoptosis regulators of the extrinsic, i.e. death receptor-dependent, and the intrinsic, i.e. mitochondria-mediated apoptosis signalling pathway, and (b) by directly impacting mitochondrial membrane physiology via the intricate interplay with mitochondrial membranes and Bcl-2 family proteins<sup>[2]</sup>.

p53-controlled apoptosis involves transcriptional induction of components of the death receptor and mitochondrial pathways including CD95, Puma, Noxa, Bax and others, which cooperatively

promote cell death. In addition, p53 protein can directly promote mitochondrial outer membrane permeabilization (MOMP) to trigger apoptosis by modulating the MOMP governing Bcl-2 family<sup>[20]</sup>.

Early studies showed that wtp53, functioning mainly as a transcription factor, can bind the Bax gene promoter region and regulate Bax gene transcription. Bax is a member of the Bcl-2 family, which forms heterodimers with Bcl-2, inhibiting its activity. The Bcl-2 protein family plays an important role in apoptosis and cancer<sup>[21]</sup>. For example, Bcl-2 controls the release of cytochrome c from the mitochondria, which activates the apoptotic pathway by activating caspase 9. Caspase 9 then activates executioner caspase 3. Both caspases play key roles in the apoptotic pathway.

Moreover, upon stress, a p53-protein based mitochondrial apoptosis program may be activated and a cytoplasmic pool of p53 rapidly translocates to the mitochondrial surface, where it physically interacts with both anti- and pro-apoptotic Bcl-2 family members to inhibit or activate their respective functions, leading to MOMP and apoptosis. In this role, p53 acts like a BH3-only protein, either as direct activator of the Bax/Bak effectors, or as sensitizer/de-repressor of Bcl-xL/2 and Mcl1<sup>[20]</sup>.

More in details, PUMA (p53-upregulated modulator of apoptosis) is a key mediator of the apoptotic pathway induced by p53. When PUMA is disrupted in colon cancer cells, p53-induced apoptosis is prevented. Thus, PUMA may play a pivotal role in determining cell fate (programmed cell death versus cell cycle arrest) in response to p53 activation<sup>[22]</sup>. CD95 (also called Fas and Apo-1) is a "death receptor" indicating its major role in apoptosis. Several reports have indicated the CD95 pathway to play an important role in apoptosis induced by cytotoxic agents, and that this system involves the activation of wtp53<sup>[23]</sup>. Therefore, the p53 status may influence chemosensitivity via CD95 signalling. However, a recent report indicated that CD95 could promote tumor growth<sup>[24]</sup>.

By contrast, the p53-regulated Ring domain E3 ubiquitin ligase MDM2 (murine double minute 2) functions to produce negative feedback, which regulates p53 activity<sup>[25]</sup>. MDM2 recognizes a short region in the TA domain of p53 and interferes with its transcriptional activity; at the same time, MDM2 interacts with the DBD region and ubiquitinates p53, promoting its proteasomal degradation. As MDM2 is a transcriptional target of p53, inhibition by MDM2 is part of a negative feedback loop on p53 activation<sup>[26]</sup>.

In addition, depending on the type of cellular stress, p53 can induce G1 arrest through activation of transcription of the cyclin-dependent kinase inhibitor p21. The p21/WAF1 (wtp53 activated fragment 1) gene product, a p53 target gene, inactivates the proliferating cell nuclear antigen (PCNA), which can regulate DNA replication, and induce a p53-dependent G1 arrest through the inhibition of cyclin/CDK activity<sup>[16]</sup>.

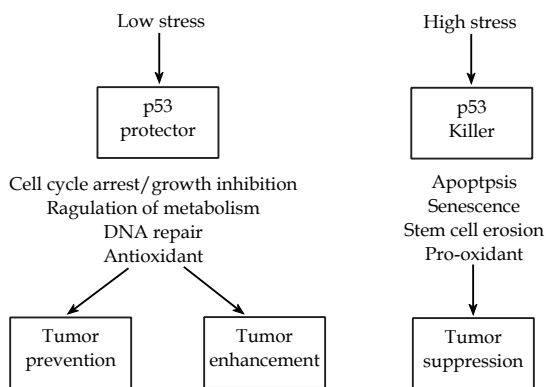
In the presence of cellular stresses, p53 is subjected to a complex and diverse array of covalent post-translational modifications. These include phosphorylation, acetylation, poly (ADP-ribose)1 ation, ubiquitylation and sumoylation. In response to cellular stress, Ser15/Ser20 in p53 are phosphorylated and MDM2 is separated from the phosphorylated p53, leading to the stabilization and activation of p53. Therefore, p53 can bind to the promoter of the p21 or p53R2 genes associated with DNA repair, and induce their expression<sup>[16]</sup>.

It is currently unknown whether p53 can also activate oxidative stress-induced necrosis. Intriguingly, recent findings provide genetic, biochemical and pharmacological evidence that fundamentally expands our understanding of p53-mediated cell death networks into necrosis. An unexpected critical role of stress-accumulated mitochondrial p53 protein in directly regulating permeability

transition pore (PTP) at the inner membrane has been described. Upon oxidative stress, p53 triggers PTP opening by engaging in a physical interaction with Cyclophilin D (CypD), thereby inducing necrotic cell death in mouse and human cells<sup>[20]</sup>.

However, transient cell cycle may not lead to tumor eradication, because a cell with oncogenic potential that cannot be repaired may resume proliferation<sup>[27]</sup>. Therefore, the other mechanism, cellular senescence, may play an important role in p53-mediated tumor suppression. Cellular senescence is permanent cell cycle arrest. There are many reports regarding the correlation between tumor development, p53 and senescence<sup>[28]</sup>. The inactivation of p53, as is present in most human cancers, allows cells to evade cellular senescence, thus resulting in tumor development. How precisely p53 determines whether or not the activation of the senescence program or the apoptosis program occurs still remains to be elucidated. This question is especially important for the development of p53-based cancer therapy, including approaches in combination with conventional chemotherapy. Most conventional chemotherapeutic agents achieve elimination of cancer cells by killing them. Therefore, if p53 induces senescence rather than apoptosis, a conflict will emerge.

Vousden and Prives<sup>[3]</sup> proposed a model wherein the decision between life and death can be determined by the extent of damage or the duration of stress. In their model, a low level of stress which can be repaired elicits a DNA repair/survival response, while a high level of stress that cannot be repaired induces an apoptotic or senescence response. This dual nature of p53, killer and protector, indicates the possibility that p53 may also act as tumor promoter. The anti-apoptotic function of p53 may lead to the survival of damaged cells, which may increase the possibility for malignant transformation (Figure 2).



**Figure 2** (from Vousden and Prives<sup>[3]</sup>). Model of the dual mechanism of p53 function in tumors. The result of p53 activation depends on multiple variables. In this model, the p53 response to low stress results in cell cycle arrest, growth inhibition and DNA repair. This response enables cells to restore the damage induced by the cellular stress. But when the cells receive high stress that cannot be restored, p53 acts as killer that can induce apoptosis or senescence, preventing proliferation of defective cells. If p53 mistakenly responds as a protector when cells receive a high stress that cannot be repaired, the cells keep the genetic damage, which can lead to or contribute to cancer progression (dotted line).

### p53 and cell survival

In addition to eliminating damaged cells, p53 can also contribute to cell survival through a surprisingly large number of mechanisms<sup>[3]</sup>. Numerous p53 target proteins function to inhibit apoptosis, including p21, decoy death receptors such as DcR1 and DcR2, the transcription factor SLUG (which represses the expression of PUMA), and several activators of the AKT/PKB (protein kinase B) survival pathways<sup>[29]</sup>. Another group of p53-inducible genes have recently also been shown to act as antioxidants by decreasing the levels of intracellular

reactive oxygen species<sup>[30,31]</sup>. Although this function for p53 would help inhibit tumor progression by protecting cells against DNA damage and genome instability, down-regulation of reactive oxygen species through these p53-dependent mechanisms can also result in decreased susceptibility to apoptosis<sup>[32]</sup>.

### p53 and autophagy

An emerging non-nuclear function of p53 is in regulation of autophagy, a process that allows removal of damaged cytoplasmic organelles and adaptation of cells to metabolic stress. Although p53 can transactivate genes that induce autophagy under stress conditions (e.g. DRAM, TSC2, Sestrin1 and 2, PTEN, and IGFBP3), depletion or mutation of p53 actually increases autophagy, suggesting that p53 constitutively limits this process in normal growing cells<sup>[33,34]</sup>. Even if the mechanism remains unknown, the autophagy-inhibitory activity is ascribable to the cytoplasmic pool of p53, as degradation of cytosolic p53 by MDM2 promotes autophagy after nutrient depletion, endoplasmic reticulum stress, or treatment with rapamycin<sup>[5,35]</sup>. In fact, p53 can be activated by metabolic adversity (such as starvation) - a response that can be mediated through the action of AMP-activated protein kinase (AMPK), a key component of the cell's response to bioenergetic stress<sup>[36]</sup>. p53 then promotes a program of gene expression (including the induction of AMPK expression) to negatively regulate the kinase mTOR (mammalian target of rapamycin), a central node in the control of protein synthesis<sup>[3,33,37]</sup>.

### p53 and the regulation of ribosome biogenesis

In a growing cell, ribosome biogenesis is a major consumer of cellular energy and resource. Thus, as growth conditions change, cells must rapidly rebalance ribosome production with the availability of resources. It has been shown that serum starvation activates p53 and induces cell cycle arrest in an RPL11 (L11)-dependent manner through a mechanism involving translocation of L11 of from the nucleolus to the nucleoplasm<sup>[38]</sup>. The model derived from a myriad of *in vitro* data suggested that when cells sense nutrient-shortage stress, ribosomal proteins are released from the nucleolus to the nucleoplasm where they can bind to MDM2 and inhibit its E3 ligase activity, leading to activation of p53<sup>[39]</sup>. The relevance of these findings was further substantiated by the discovery of MDM2 mutations in the region that binds to L11 in human cancers. As a result, the MDM2 mutants are refractory to inhibition by ribosomal proteins and are maintained in a p53-suppressive mode<sup>[40]</sup>.

### p53, tumor glycolysis and fatty acid metabolism

Recent observations show that many tumor suppressor genes play important roles in metabolic regulation, in addition to their established roles in cell survival and apoptosis<sup>[19]</sup>. Cancer cells are characterized by aerobic glycolysis with the use of glucose and production of lactate. Several biologic functions of p53 decrease the glycolysis pathway in cells. p53 induces TP53-induced glycolysis and apoptosis regulator (TIGAR) expression via transcriptional activation. Moreover, wtp53 downregulates phosphoglycerate mutase (PGM), and mutation of p53 can enhance PGM activity and glycolytic flux<sup>[41,42]</sup>.

In addition, p53 can reduce intracellular glucose levels by inhibiting the expression of glucose transporters. For example, p53 directly represses the transcriptional activity of the GLUT1, GLUT3 and GLUT4 gene promoters<sup>[43]</sup>.

### p53, aging and stem cells

Cancer is an age-related disease. Consequently, genes that stop cancer

progression promote longevity by restraining genomic instability, and inhibiting the growth and expansion of genetically aberrant cells. p53 has a prominent - and controversial - role in the regulation of ageing and longevity. Excessive p53 tumor suppressive activity, however, can be detrimental to organism homeostasis by promoting certain aspects of aging<sup>[2]</sup>. Maintenance and regeneration of adult tissues, and consequently longevity depend on the continuous proliferation and differentiation of resident stem cells<sup>[44,45]</sup>. In line with a tumor suppressive, pro-death and anti-proliferative role of p53, deficiency in p53 was shown to attenuate profound defects in tissue homeostasis caused by mutations in DNA repair genes. Some studies suggest that moderate p53 activation is beneficial for tissue homeostasis, as chronic hyperactivation of p53 decreases longevity, while moderate, physiological enhancement of p53 activity with intact regulatory mechanisms to control p53 stability, induces an anti-aging phenotype. It appears that duration and extent of stress and consequently levels p53 activity determine cell and organism fate; high p53 activation restricts proliferation, low levels induce cell survival, and decrease oxidative damage via induction of an antioxidant gene signature<sup>[46,47]</sup>.

**p53 and miRNA**

Protein-encoding genes are not the only transcription targets of p53. Several groups independently reported that p53 can directly regulate the expression of specific microRNAs (miRNAs), most dramatically the miR-34 locus consisting of miR-34a, miR-34b, and miR-34c<sup>[48-51]</sup>. Several reports showed that p53 can bind directly to response elements within the miR-34a and miR-34b/c promoters to stimulate transcription from this locus. Certainly, miR-34a expression is physiologically relevant to the impact of p53 activity on cells: it can induce cell cycle arrest and senescence, as well as facilitate cell death<sup>[3]</sup>.

It has now been reported that miR-192 and miR-215 are also induced by p53 and promote increased p21 expression<sup>[49]</sup>. Moreover, miR-145 has been implicated as a p53 target that can repress c-myc expression<sup>[52]</sup>.

**CURRENT STRATEGIES TO TARGET THE P53 PATHWAY**

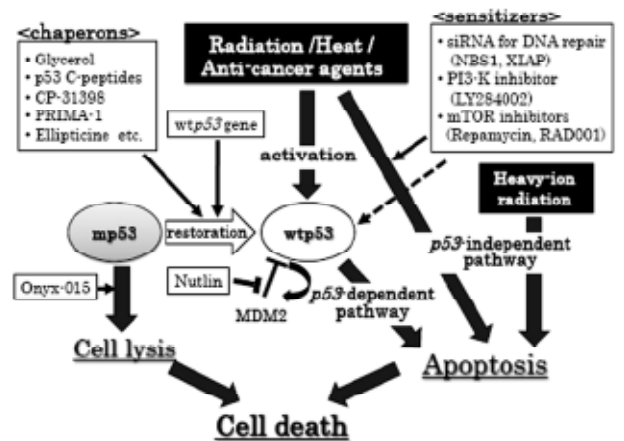
In tumor cells, the p53 pathway is often disrupted. Therefore, recovering the function of wtp53 and its targets in tumor cells is a key therapeutic objective; the obvious goal is to try to reestablish the growth-inhibitory functions of p53 in cancer cells.

Indeed, reintroduction of wtp53 using a replication-defective adenoviral vector showed efficacy in reversing the growth of several human tumor types, demonstrating that restoration of p53 activity is a viable anticancer therapeutic approach. However, as regulation of p53 activity becomes better understood, approaches that exploit our deeper understanding of the biochemistry of p53 activation have led to the identification of small molecules that can manipulate the endogenous non-functional protein that is so often expressed in tumor cells. As a result, strategies have focused on restoring wild-type activity to the mtp53 protein, restoring functionality of the p53 pathway or activating one of the p53 family members.

In this review, we have described the multi-faceted spectrum of p53 activities; now we will discuss current strategies to activate p53 in tumors, with an outlook on future strategies and challenges to introduce p53-targeting therapies into clinical practice.

In particular, cancer therapies aimed at targeting signalling pathways controlled by p53 include p53-gene therapy, chemical chaperones, p53 C-terminal peptides and small molecules that can

target p53. Some therapeutic strategies are independent of p53 status in cancer cells, including high-linear energy transfer (LET) heavy-ion radiation; others involve enhancement of cancer therapies with different strategies, including an RNA-silencing therapy targeted at DNA repair pathways and a molecular-targeting therapy for the survival pathway Akt-mTOR (Ota 2012) (Figure 3).



**Figure 3** (from Ota 2012<sup>[16]</sup>). p53-dependent and -independent therapeutic strategies for cancer cells. Circles, p53 status of cancer cells; black squares, cancer therapeutic tool; white squares, enhancer for cancer therapeutic tool; thin arrows, enhancement; dashed arrows, partial enhancement; thick arrows, therapeutic pathway. MDM2, murine double minute 2; XIAP, X-chromosome-linked inhibitor of apoptosis protein; wtp53, wild type p53; mtp53, mutant p53; siRNA, small interference RNA.

**p53 gene therapy**

In recent years, one line of attack that has been successful in the clinic is the introduction of exogenous wtp53 into cancer cells, either by gene delivery or by direct protein delivery<sup>[16,53]</sup> has been explored.

The first p53-based gene therapy was reported in 1996. A retroviral vector containing the wild-type p53 gene under the control of an actin promoter was injected directly into tumors of non small cell lung cancer patients<sup>[54]</sup>. After development of a replication-defective recombinant p53 virus (Ad5CMV-p53)<sup>[3]</sup>, many clinical subsequent trials have been performed.

Although preliminary studies in cell cultures and in animal models have indicated the potential viability and low toxicity of these approaches<sup>[55-58]</sup>, their efficacy in clinical trials is currently controversial. Clinical studies in lung, bladder, ovarian and breast cancer showed no beneficial effects compared to conventional treatments. On the other hand, encouraging results were reported in HNSCC, where p53 mutations are frequent, and their incidence increases with progression (for review: Ota *et al.*, 2012<sup>[16]</sup>; Shen *et al.*, 2012<sup>[19]</sup>).

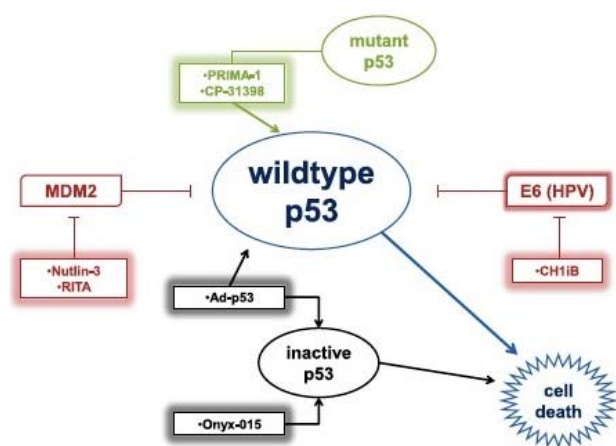
Because of its affinity for the cells of the upper aerodigestive tract, a modified adenovirus has been the most widely-used vector for p53 gene therapy in HNSCC (AdCMV5-p53; INGN 201)<sup>[59,60]</sup>. Therefore, a recombinant human adenovirus that expresses functional wtp53 has been approved by the Chinese government for the treatment of HNSCC<sup>[25,61]</sup>. Treatments showed that antitumor efficacy was associated with the expression and activity of functional p53, and adverse effects were also significant<sup>[54,62-64]</sup>. For a complete review on the current p53-based therapeutics for HNSCC, refer to Tassone *et al.*<sup>[65]</sup> (Figure 4).

Although such results are encouraging, further improvements in methods are required to accomplish the safe and effective delivery of wtp53 *in vivo*<sup>[16,66]</sup>.

Another way to eliminate cells with mtp53 is to deliver a virus



that preferentially target cells that lack functional p53. Adenoviruses contain an E1B gene producing a 55 kD protein that inactivates host p53 to promote host cell survival. Extensive *in vitro* and *in vivo* studies showed that Onyx-015, an adenovirus lacking the E1B gene, developed to selectively eliminate cells without functional p53, is capable of replicating in and promoting the lysis of carcinoma cells. In the absence of wtp53 activity in cancer cells, the generation of a mutated viral vector for tumor cell lysis (as Onyx-015) was exploited<sup>[16,64,65,67]</sup>. Accordingly, the Onyx 015 reagent, a p53-targeting oncolytic mutant adenovirus, has been developed for clinical application. However, evaluation of numerous clinical trials performed thus far have indicated that the administration of Onyx-015 as a single agent produces only marginal benefit, whereas its administration in combination with conventional therapy is more effective<sup>[68]</sup>.



**Figure 4** (from Tassone *et al.*<sup>[65]</sup>). Current p53-based therapeutics for HNSCC. Adenoviral p53 gene therapy, Ad-p53, increases wtp53 levels in HNSCC cells. Onyx-015 selectively eliminates inactive p53 HNSCC cells. PRIMA-1 restores the ability of mtp53 to bind to the p53 transcriptional response elements of target genes and CP-31398 stabilizes the DNA binding domain of mt and wtp53. Nutlin-3 and RITA restore p53 function by blocking the p53-MDM2 interaction. CH11B disrupts the HPV16 E6-p300 interaction to reactivate p53 in HPV16-positive HNSCC.

### Small molecules and chaperones

**p53 Stabilization:** Our growing understanding of how p53 is regulated has also led to the development of small molecule drugs that stabilize and activate the p53 protein.

As can be envisaged, the MDM2-p53 interplay is a particularly attractive target for therapeutic intervention in cancer. Increasing the expression and activity of wtp53 is the ultimate goal in most treatment strategies. MDM2 is an E3 ubiquitin ligase which controls p53 degradation via ubiquitylation<sup>[19,69,70]</sup>. Many tumors overexpress MDM2, even tumors without p53 mutations<sup>[71]</sup>. In fact, due to the importance of the MDM2-p53 interaction, inhibition of this event with small molecules is regarded as having therapeutic potential. A number of different strategies have been employed to screen for and develop small molecules that bind specifically to the N-terminal region of MDM2 that interacts with p53<sup>[3,25,65,72]</sup>.

In particular, MDM2 inhibitors HLI98 and Nutlin 3A can, respectively, stabilize p53 and rescue tumor suppression function in solid tumors<sup>[19,73-75]</sup> and in hematological malignancies<sup>[76]</sup>.

The nutlins are cis-imidazole compounds that act as antagonists of the MDM2-p53 interaction. Analysis of the crystal structure showed that nutlin binds in the pocket of MDM2 to prevent the p53-MDM2 interaction. Nutlin can activate the p53 pathway, thereby inducing cancer cells and xenograft tumors in mice to undergo cell

cycle arrest, apoptosis and growth inhibition<sup>[71,77]</sup>. For a review highlighting recent advances in the development of small-molecule MDM2 antagonists as potential cancer therapeutics, with special emphasis on Nutlin-3, refer to Shen *et al.*<sup>[78]</sup>.

MI-219 binds to the p53 binding pocket in MDM2 and disrupts the MDM2-p53 complex, which leads to activation of p53, induction of growth arrest and apoptosis and suppression of xenograft tumor growth<sup>[75]</sup>. MI-219 also activates the p53 pathway in cells with wtp53. Apoptosis and cell cycle arrest were observed in xenograft tumors which resulted in tumor regression<sup>[71,79]</sup>. Unfortunately, this approach carries the risk of enhancing the pro-survival adaptation functions of p53 in some tumors<sup>[80,81]</sup>. Clarifying the mechanism(s) by which p53 coordinates adaptation could lead to the discovery of new therapeutic targets in cancer expressing wtp53.

Tenovin was found by a cell-based drug screen to activate p53. Tenovin acts as an inhibitor of the NAD<sup>+</sup>-dependent class III histone deacetylating activities of SirT1 and SirT2, two important members of the sirtuin family. Intra-peritoneal administration of tenovin-6 has been demonstrated to induce a regression of xenograft tumors in a mouse model<sup>[82]</sup>.

Issaeva *et al.* (2004) screened a chemical library and found the small molecule RITA (reactivation of p53 and induction of tumor cell apoptosis), which binds to p53 and inhibits the p53-MDM2 interaction both *in vitro* and *in vivo*. RITA is reported to inhibit glycolytic enzymes and, therefore, induce robust apoptosis in various cancer cells, including leukemic cells, that retained wtp53<sup>[55,65,83,84]</sup>. They also found that the p53 released from MDM2 by RITA promotes p21 and hnRNP K (a p53 cofactor), thus implying that p21 plays a major role in regulating cancer cell fate after p53 reactivation<sup>[85]</sup>.

**p53 Restoration:** Another approach in preclinical development involves restoring tumor-suppressing function to mp53. Studies have demonstrated that glycerol, as a chemical chaperone, can restore normal p53 function in mtp53 HNSCC<sup>[16]</sup>.

There is class of small molecules that reactivate the wild-type functions of mtp53. PhiKan083 is a carbazole derivative found from *in silico* screening of the crystal structure of p53. By binding mtp53, PhiKan083 raises the melting temperature of mtp53, which results in the reactivation of its function<sup>[86]</sup>.

PRIMA-1 (or “p53 reactivation and induction of massive apoptosis”) is another small molecule identified by cell-based screening which restored sequence-specific DNA binding and the active conformation of p53. It is known to induce apoptosis through the p53-dependent c-Jun-NH2-kinase pathway<sup>[85,87]</sup>.

CP-31398 is also a small quinazoline based molecule that was found to produce an active p53 in cancer cells and can restore the protein folding of mtp53 to a more natural conformation that permits a wild-type function<sup>[16,88]</sup>. CP-31398 stabilizes the DNA-binding domain of p53 for both wt and mt (V173A and R249S) p53<sup>[89]</sup>.

**Molecules that disrupt exogenous p53 inhibitors:** The causative role of human papillomavirus-16 (HPV16) in HNSCC is largely attributed to two HPV16 oncogenes, E6 and E7. Since inactivation of p53 by HPV16 E6 is critical for HPV-mediated tumorigenesis, reactivation of p53 may be an efficient strategy to eliminate HPV16-positive HNSCC cells. Recent work has identified CH11B as a small molecule that disrupts the interaction between HPV16 E6 and p300 in HPV 16-positive UMSCC47 and UPCI-SCC090 HNSCC cells<sup>[65,90]</sup>. CH11B increased total and acetylated p53 levels, enhanced p53 transcriptional activity, and increased the expression of p53-

regulated genes, p21, miR-34a, and miR-200c.

### Alternative targets

The discovery that the p53 family members p63 and p73 have similar structures and have similar biological activities has provided an additional anti-tumor strategy. Both p63 and p73 can induce apoptosis and do so by activation of some pro-apoptotic targets as wtp53<sup>[91]</sup>. Importantly, mutational inactivation of p63 and p73 is rare in human tumors and they are widely expressed, making these proteins attractive chemotherapeutic targets. Indeed, results from recent studies demonstrate that targeting these proteins may be a useful anticancer approach<sup>[75]</sup>.

Importantly, screens for proteins that interact with p53 have identified a family of proteins, termed ASPP, which can augment the ability of p53 to stimulate the expression of proapoptotic genes. One of these family members, iASPP, suppresses the activity of p53, p63 and p73 by interacting with their DNA binding domains<sup>[92]</sup>. Characterization of the activity of 37AA, a p53-derived peptide termed 37AA which could drive cell death through activation of p73, demonstrated that it functioned by interfering with iASPP binding with p73 and promoted its ability to stimulate the expression of proapoptotic genes such as PUMA and NOXA<sup>[93]</sup>.

### p53-Based Immunotherapy

Other strategies to restore wild-type p53 in the cell have been vaccines against mtp53, small mol-ecules that bind to mtp53 to restore normal conformation and/or activity (e.g. ellipticine)<sup>[94]</sup>. p53 protein, especially mtp53, may be a target of tumor antigen specific cytotoxic T lymphocytes that can mediate immune response of host against cancer *in vivo*<sup>[95]</sup>. Some cancer patients have antibodies against p53<sup>[96,97]</sup>, although the frequency and clinical significance are still under debate<sup>[16]</sup>.

Speetjens *et al*<sup>[98]</sup> reported clinical trials of a p53-specific synthetic long peptide (p53-SLP) vaccine for metastatic colorectal cancer patients<sup>[99]</sup>, where ten patients were vaccinated with p53-SLP in a Phase I and Phase II trial. Preclinical phase I/II trial of INGN-225 (Introgen), a p53-modified adenovirus-induced dendritic cell vaccine for small cell lung cancer (SCLC) patients, has been reported<sup>[100]</sup>.

### Targeting tumor metabolism

In the last years, the emerging role of p53 in tumor metabolism has suggested that drugs that mimic the metabolic effect of p53 are able to perturb cancer cell metabolism and inhibit cancer cell proliferation<sup>[19]</sup>. Because tumor cells rely on glycolysis or ATP production for their survival, the molecular targets of p53 in the glycolytic pathway might be potential therapeutic targets in cancer. Indeed, the non-metabolizable glucose analogues 2-deoxyglucose or 3-bromopyruvate can inhibit glycolysis and ATP production<sup>[101,102]</sup>. Moreover, the glucose transporter inhibitor phloretin inhibits glucose uptake and sensitizes tumor cells to the chemotherapeutic drug daunorubicin<sup>[103]</sup>. As p53 repression of GLUT3 expression is mediated by the IKK–NF-κB pathway, inhibition of the activation of the IKK–NF-κB pathway can, thereby, be another target for cancer treatment. R-roscovitine has been shown to inhibit the function of IKK and downregulate NF-κB activation. In addition to the NF-κB pathway, the cyclin-dependent kinase inhibitor roscovitine can also dramatically enhance the expression of p53 and block the degradation of p53 mediated by MDM2, thereby activating the p53 pathway and inhibiting glycolysis in tumors<sup>[104–106]</sup>.

The activation of AMPK induces fatty acid oxidation and mitochondrial respiration and represses fatty acid synthesis and

glycolysis. Thus, AMPK may be a beneficial target for cancer treatment. Recent studies support this finding, showing that pharmacologic AMPK activators, such as metformin, phenformin, and AICAR, attenuate cancer cell growth and inhibit tumorigenesis in animal models<sup>[107,108]</sup>.

## CONCLUSION

Research over the past three decades has identified p53 as a multifunctional transcription factor, which regulates the expression of >2,500 target genes. p53 impacts myriad, highly diverse cellular processes, including the maintenance of genomic stability and fidelity, metabolism, longevity, and represents one of the most important and extensively studied tumor suppressors. Activated by various stresses, foremost genotoxic damage, hypoxia, heat shock and oncogenic assault, p53 blocks cancer progression by provoking transient or permanent growth arrest, by enabling DNA repair or by advancing cellular death programs. This potent and versatile anti-cancer activity profile, together with genomic and mutational analyses documenting inactivation of p53 in more than 50% of human cancers, motivated drug development efforts to (re-) activate p53 in established tumors. Thus it is indisputable that p53 represents an attractive target for the development of anti-cancer therapies. Whether p53 is ‘druggable’, however, remains an area of active research and discussion, as p53 has pro-survival functions and chronic p53 activation accelerates aging, which may compromise the long-term homeostasis of an organism. Thus, the complex biology and dual functions of p53 in cancer prevention and age-related cellular responses pose significant challenges on the development of p53-targeting cancer therapies.

In this paper, we have focused on the functions of p53 and therapeutic approaches targeting p53 for cancer therapy. However, despite recent advances in the research on p53’s function, it appears that various questions still remain to be answered before the full therapeutic of pharmacological modulation of p53 can be harnessed.

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