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Review

p53-targeted cancer pharmacotherapy: move towards small molecule compounds

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Abstract

Objectives For the past three decades of research, p53 has been identified as one of the most targetable molecules for developing anticancer treatments. This tumour suppressor protein is involved in apoptosis, cell cycle arrest and senescence. A wide range of pharmaceutical drugs and radiotherapy treatments activate this protein and rely on p53 signalling for therapeutic outcome. Promising small molecular weight compounds, some of which are undergoing clinical trials, are discussed in this review.

Key findings The spectrum of potential therapeutic approaches trialled for p53 stretch from gene therapy to the more recent development of small molecules capable of activating wild-type p53 or reactivating mutant p53.

Summary Our ever-growing knowledge leads us to better understand this protein, from its structure and activities to its potential therapeutic application, firstly for cancer and then for other diseases and maybe even for reversal of ageing.

Keywords apoptosis; cancer; chemotherapy; drug; p53; tumour

Introduction

In 1993, the tumour suppressor gene for p53 was hailed as the 'molecule of the year' by *Science* magazine.[1] Even in those early days of research into this fascinating protein, it was known that p53 was mutated in a large percentage of tumours. With the advent of gene therapy in the 1980s, scientists switched from conventional forms of therapeutics using proteins and peptides to using gene manipulation techniques. It was for cancer that the majority of gene therapy clinical trials were undertaken, and this remains the case today.[2] One of the major gene targets was p53, and this has since led to some significant achievements, even to a few marketed products such as Gendicine® and Oncorine™.[3–5] Despite this, however, in the past decade emphasis has shifted back to using small molecule agents to modulate p53 function in cells, and this is largely the case for research and development into cancer pharmacotherapeutics. This study highlights the biological functions of p53 and p53-targeted cancer therapy. In particular, it focuses on the recent trend of p53 research by outlining the promising small molecule compounds identified to date. It also suggests some future directions of p53 research, some of which may widen the potential application of p53-targeted pharmacotherapy. These include targeting p53 for diseases other than cancer, such as Parkinson's disease, ischaemia and Alzheimer's disease, and reducing the side effects of conventional chemotherapy and radiotherapy by temporarily inactivating p53 in cancer cells.

The Tumour Suppressor Gene, p53, and its Family Genes

Arguably, p53 is the most widely and intensively studied gene of the last quarter century, with much evidence that shows a close association between p53 and tumour development. Donehower *et al.*[6] showed that p53-deficient mice were normal in development and growth but most of them developed cancer. Even more evidence that backs the importance of p53 in tumours is provided by families with Li–Fraumeni syndrome, which is defined by the development of multiple cancers. Many studies identified that germ-line p53 mutations are associated with this inherited syndrome.[7–9]

p53 is frequently mutated in about 50% of human tumours, and the remainder seem to have malfunctions in its pathways.[10] This strongly suggests that most cancer cells are

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defective either in p53 or in its pathways and p53 malfunction is considered one of the most common mechanisms in tumour development.[11] p53 has therefore been a key target for developing novel cancer therapies.

In addition to p53's most recognised functions of eliciting apoptosis, cell cycle arrest and senescence, more recent studies have discovered it can also limit angiogenesis,[12] regulate autophagy[13] and directly influence survival proteins in the mitochondria, mRNA processing and DNA repair pathways.[14]

In 1997, two p53-related genes, p63 and p73, were identified.[15,16] These proteins share homology with p53, particularly in the DNA binding domain.[17] Despite this similarity in normal, non-stressed conditions, their biological functions differ from p53 and each has its own biological functions.[18] In certain conditions, however, p63 and p73 can function as tumour suppressors. As these genes are less frequently mutated in tumours than is p53, it would be efficient to use the p53-like activities of these genes in tumours in which p53 is defective or null.[19–21]

p53 and DNA Damage Response

p53 is activated in response to DNA damage and other cellular stress signals (hypoxia, nutrient deprivation or oncogenic activation). While being normally expressed in low levels in normal (healthy) cells, p53 rapidly accumulates in cells undergoing stress as a result of the protein being stabilised. In cells undergoing stress, the physical interaction between p53 and Mdm2 (an E3 ubiquitin ligase that binds and targets p53 for proteasomal degradation) is perturbed,[22] leaving p53 unchecked to perform its biological functions.

p53 exerts its biofunctions at two levels of molecular regulation. The most recognised of these is its function as a transcriptional regulator, where it binds to the promoter of a large number of genes, resulting in their upregulation.[23] Alternatively, p53 can downregulate the expression of target genes at the transcriptional level.[24] The second type of regulation is at the post-transcriptional level, one which entails p53 physically interacting with other proteins and thereby regulating the function of the latter.[25] Despite some evidence that supports the existence of post-transcriptional p53 functions – for example, that transactivation-deficient mutant p53 can induce apoptosis[26] and that p53-dependent apoptosis can be achieved without new RNA or protein synthesis[27] – the transcription-independent (cytoplasmic) functions of p53 have not been investigated as intensively as the transcriptional function of p53 in the early days. In this transcription-independent manner, the p53 protein moves to mitochondria in response to cell stress – in particular, acute cell-death-inducing stress – and interacts with the Bcl-2 homology domain proteins such as Bax and Bak to release cytochrome c from the mitochondrial intermembrane space.[28]

Owing to its multipotent role in cell biology, the ultimate outcome of p53 activation may vary widely (see Figure 1) and is dependent not only on its interacting partners, but other factors such as cell type, type of cellular insult and the location of the injured cells.[24] These are summarised in Table 1,[29–50] and include cell cycle arrest, apoptosis, DNA repair and senescence, and may influence cell differentiation, metabolism and the more recently implicated events of autophagy and angiogenesis. For example, the fatty acid synthase (FAS) gene, involved in biogenesis of cellular membranes in rapidly proliferating cells and during embryonic development, is a conserved target of the p53 family (p53, p63 and p73).[51] In most normal human tissues, FAS is generally expressed at low levels. However, many human cancers show high levels of FAS expression, for example breast, prostate, colon, ovary, endometrium and thyroid.[52,53] It has therefore been suggested that FAS is a target gene for cancer therapy. p53 serves as an important 'command centre' that directs the cell to commit to one of the possible endpoints as a result of genotoxic stress.

The sensing of DNA damage in the form of single-strand DNA and double-strand DNA breaks is an intricate process that requires much more study to better understand the underlying mechanisms. Phosphorylation of kinases such as Chk1 and Chk2, involved in DNA damage response, in turn leads to phosphorylation of various downstream targets, including the transcription factors p53, p73 and E2F-1[54] and may result in the phenomena of DNA repair, cell cycle arrest or, in extreme cases, apoptosis. p53 may serve as a direct DNA damage sensor.

However, not all apoptotic pathways require activation of p53. For instance, the E2F class of transcription factors determine the timely expression of genes involved in the S phase of the cell cycle.[55] More recently, it has been reported that E2Fs function in mitosis, DNA replication, DNA damage checkpoints, DNA repair, development and differentiation. One member of this transcription factor family, E2F1, is capable of inducing apoptosis via both p53-dependent and -independent pathways.[56] In such cases the efficacy of a number of cancer cytotoxics depends on their ability to activate the p53 pathway[57] or, in the absence of p53, p73 signalling.[58] Intriguingly, Dz13 activates a potent apoptotic response in the absence of both of these critical mediators.

A Case in Point – The Role of p53 in Osteosarcoma

In many osteosarcoma cell lines it has been noted that rearrangement of the first intron of the p53 gene occurs consistently, and leads to altered protein expression.[59] A number of p53 point mutations have also been identified in osteosarcomas,[60] and a small proportion of osteosarcomas are associated with either germ-line p53 mutations or the Li–Fraumeni syndrome.[61] This syndrome involves an autosomal dominant mutation in the p53 gene and is associated with the development of multiple neoplasms such as soft tissue sarcomas, osteosarcoma, breast cancer, brain tumours and leukemias. The risk of developing a second malignancy is over double that of the rest of the population.[62]

In response to DNA damage, functional p53 induces apoptosis via upregulation of p21 and bax. p53 is regulated by Mdm2, which degrades p53 and in turn Mdm2 is regulated by the tumour suppressor p19Arf, which facilitates sequestration of Mdm2 to the nucleoli.[63] Not surprisingly, there is an association between Mdm2 overexpression and the presence of recurrence or metastatic disease.[64]

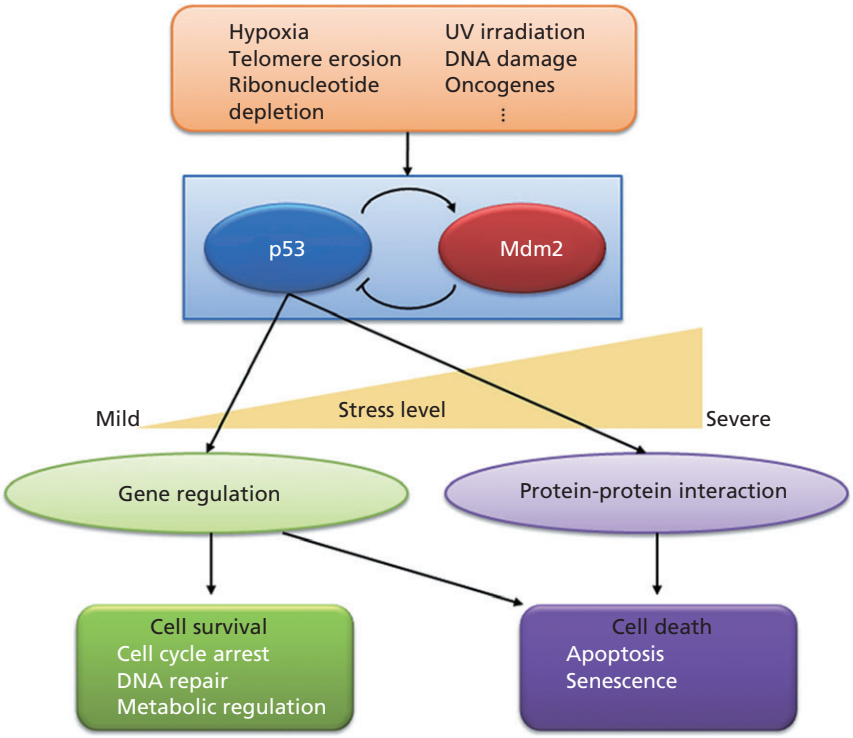

Figure 1 The ultimate outcome of p53 activation. p53 is activated in response to cell stress signals such as hypoxia, UV irradiation, DNA damage and oncogenes. This simplified scheme looks straightforward, but the actual process that decides whether cells die (apoptosis) or survive (cell cycle arrest) is complicated, and strongly dependent on various factors, including the interacting partners of p53, damaged cell type and location, and the intensity of stress. In this model, mild stress induces cell survival responses, that is, reversible cell cycle arrest combined with efforts to deal with the damages that are caused by the stress, whereas severe and acute stress lead to extreme, apoptotic cell death, thereby removing the injured cell. Alternatively, in response to non-repairable cell damage, the outcome can be irreversible cell cycle arrest, namely senescence. In general, the downstream pathways of p53 largely depend on its transcriptional functions. However, the transcription-independent pathway of p53 also plays an important role in apoptotic cell death.

Table 1 Biological functions of p53

Function	Target genes
Cell cycle arrest in G1 and G2/senescence	$p21^{W,af1}$[29] BTG2,[30] GADD45,[31] 14-3-3-σ,[32] PAI-1[33]
DNA repair	$p21^{CDKN1A}$,[34] p53R2,[35] p48[36]
Apoptosis	Bax,[37] IGF-BP3,[38] KILLER/DR5,[39] Puma,[40] Noxa,[41] FAS[42]
Metabolism	TIGAR,[43] SCO2,[44] PGM[44]
Angiogenesis	TSP-1,[45] α(II)PH[46]
Autophagy	DRAM,[47] mTOR[48]
Differentiation	Myocd,[49] Osterix,[50] Runx2[50]

p53-Targeted Therapy for Cancer: Emphasis on Small Molecules

Pharmaceutical cancer therapy relies on the process of apoptosis, a programmed form of cell death, for efficacy. p53, with its ability to induce apoptosis and being central to pro-apoptotic signalling in cancer therapy, has been used as a target for cancer therapy for the past two decades.[65] As mentioned above, all types of cancers have p53 that is inactivated in some way, either by the p53 itself being mutated or by a malfunction in the p53 pathways. This strongly suggests that it is critical for cancer therapy to make p53 active. Two elegant mice studies showed that restoring p53 function alone is sufficient for the clearance of liver tumour, autochthonous lymphomas and sarcomas,[66,67] and this may be significant for potential therapies to target human cancers. Martins *et al.*[68] showed the therapeutic efficacy of p53 restoration but also gave the sobering insight that tumours might find other ways to interfere with p53 pathways, which might regenerate tumours.

As interactions related to p53 are very complex, so are the strategies to target p53 for cancer therapy. These multi-faceted strategies are summarised in Table 2.[4,5,69–87] p53 gene therapy mainly relies on adenovirus-based gene delivery. Ad-p53 (brand name Gendicine) is engineered as an adenovirus that encodes human wild-type p53, and is capable of delivering wild-type p53 into tumour cells. Another gene therapy, named ONYX-015 or Oncorine, is an E1B-defective adenovirus. The strategy of this treatment is to selectively replicate in and destroy tumour cells that carry mutant p53.

Gendicine and Oncorine were approved for head and neck cancer therapy by the State Food and Drug Administration of China and both are now being used for clinical therapy in China.[3,70,88] However, the adverse immunological effects from viral vectors are a caveat of this approach, which might reduce the effect of the therapy and thereby its use. If severe, this immune response might lead to critical side effects

Table 2 Strategies for targeting p53

Mechanism	Agents
Gene therapy	
Deliver wild-type p53 into tumour cells	Ad-p53 (Gendicine[4]/ Advexin[69])
Selectively eliminate mutant p53-carrying cancer cells	ONYX-015,[70] Oncorine[5]
p63/p73 transduction into tumour cells	Ad-p63,[71] Ad-p73[72]
Wild-type p53 activation	
Inhibit Mdm2–p53 interaction (Mdm2 binding)	Nutlins,[73] MI-219,[74] MI-319,[75] Benzodiazepinedione (BDA)[76]
Inhibit Mdm2–p53 interaction (p53 binding)	RITA[77]
Inhibit Mdm2 E3 ubiquitin ligase	HLI98[78]
Inhibit CRM1	Leptomycin B[79]
Inhibit SirT1 and SirT2 deacetylation	Tenovin-1 (Tenovin-6)[80]
Mutant p53 reactivation	
Restore DNA binding and change p53 conformation into wild type	CP-31398,[81] PRIMA-1,[82] Ellipticine,[83] CDB3[84]
Thermally stabilise p53	PhiKan083[85]
Inhibit mutant p53–p73 interaction	RETRA,[86] SIMP[87]

and, in extreme cases, even to death.[89,90] Potential immune responses of adenovirus-based gene therapy are excellently reviewed by Hartman *et al*.[91]

More recently, the research focus has shifted back to finding another mode of therapy that will target p53: small molecules. These have been identified by either protein assays or cellular assays. The cellular approach involves screening to identify compounds with desired phenotypic effects such as apoptosis. An advantage of this approach is that the compounds identified – for example, PRIMA-1 – have a desired biological outcome and rarely exhibit genotoxicity, but researchers may have difficulties illustrating their exact mechanisms.

On the other hand, a protein-based approach enables researchers to identify compounds – for example, CP-31398 – that directly affect a target protein and to determine a clear molecular mechanism. However, the compounds may be toxic or may not have adequate bioavailability. More recently, detailed structural data that were obtained using advanced computer technologies such as X-ray crystallography have enabled rational molecular modelling. For example, PhiKan083, which is shown to improve p53 stability by elevating the p53 melting temperature, was identified by the Fersht group using in-silico modelling as targeting the Cys220 mutant of the tumour suppressor.[85]

Strategies for targeting p53 using small molecules are divided, in a broad sense, into two: reactivation of mutant p53 and activation of wild-type p53. Although there have been more than 2000 mutation types reported to date (these can be seen on the International Agency for Research on Cancer (IARC) TP53 database at http://www-p53.iarc.fr),[92] most mutations have common features that would make restoring wild-type p53 functions feasible. An absolute majority of all mutations target the core DNA-binding domain, approximately 75% of which are missense point mutations with a single amino-acid residue substitution. These point mutations are overexpressed in tumours and result in disrupted tetramerisation, reduced thermostability and destabilised core domain folding and DNA binding.[93,94]

Early studies showed that peptides derived from the p53 C-terminal domain restore the DNA-binding ability of mutant p53, inducing apoptosis in cancer cells.[95,96] CP-31398 is the first molecule identified to reactivate mutant p53 functions. A murine study demonstrated that the compound restores active conformation in mutant p53 and represses tumour growth.[81] Further research showed that CP-31398 is capable of restoring the DNA-binding function of mutant p53 without affecting its homologues p63 and p73.[97] Interestingly, CP-31398 was shown to function in both a p53-dependent and -independent manner,[98] and this suggests that it may interact with target genes other than p53.

Bykov and colleagues identified a compound named PRIMA-1 using a phenotypic screen of a chemical library. It is reported that PRIMA-1 restores the DNA binding to several p53 mutants and induces apoptosis in a mutant p53-dependent manner.[82] However, the molecular mechanism of this fascinating compound is not yet clear. RETRA, a small molecule that has been identified more recently, has also been shown to enhance p53 activation in a mutant p53-dependent manner. Interestingly, unlike other compounds that reactivate mutant p53, RETRA releases p73, a p53 family member, from being inhibited by mutant p53 and thereby suppresses tumours.[86] How RETRA interferes with the mutant p53–p73 complex is currently unclear, so this observation might suggest a promising potential subject for further studies.

The other approach to p53 targeting uses tumours that retain wild-type p53. In these types of tumours, the wild-type p53 activity is usually rendered latent by p53 inhibitors and modification proteins such as Mdm2, sirtuins and CRM1. In wild-type p53 activation strategies, researchers' key target has been to regulate the p53–Mdm2 negative feedback loop, which has long been shown to be the key regulator of p53. Mdm2 directly binds to the amino terminal transactivation domain of p53. This physical interaction prevents p53 from interacting with its transcription factors.[22] Mdm2 also induces p53 degradation by the ubiquitylation function of the E3 ubiquitin ligase.[99] Meanwhile, Mdm4, Mdm2's interacting partner, provokes Mdm2-mediated ubiquitylation of p53 by forming heterodimers, leading to p53 degradation.[100]

Of the small molecules that inhibit the protein–protein interaction between p53 and Mdm2, the first reported was nutlins. These are designed to bind to the p53 pocket of Mdm2, resulting in the reactivation of p53 by displacing Mdm2.[73] Another hit discovered by a cell-based assay, RITA (also known as NSC 652287), directly binds to p53 to inhibit the p53–Mdm2 interaction.[77] Intriguingly, RITA also induces apoptosis. One possible explanation for this is that Mdm2 released from p53 by RITA degrades p21, which is a cyclin-dependent kinase inhibitor that plays a critical role in G1 cell cycle arrest, and thereby induces apoptosis.[101] Other mechanisms of RITA in suppressing cancer are yet to be discovered.

Yang *et al*.[78] identified a family of small molecules, named HLI98, that attack the E3 ubiquitin ligase activity of Mdm2. In-vitro assays showed that HLI98-compounds inhibit Mdm2-mediated ubiquitylation, leading to the restoration of wild-type p53 activity. Small molecules that target proteins

other than Mdm2 do so by targeting p53 modification proteins, such as CRM1 and the sirtuin family. Tenovin-6 (which is a water-soluble analogue of Tenovin-1) and Leptomycin B are inhibitors that target the sirtuin family (SirT1 and SirT2) and the nuclear export protein CRM1, respectively.[79,80]

Future Directions

It has been two decades since p53 was first described in the scientific literature. Ample evidence accumulated in the last 20 years has made it obvious that targeting p53 is an extremely attractive strategy in cancer therapy and more recent studies have added to this promise. At the same time, however, our ever-growing knowledge base also makes things much more complex, in that novel conduits of p53 signalling have been uncovered but are not fully understood. Biologically, relatively recently discovered p53 activities related to metabolism, autophagy, antioxidation and differentiation have added further complexity. Conversely, however, this may widen the potential uses of p53-targeting therapy, rather than having it limited to cancer alone. p53 might be an attractive therapeutic goal for some diseases, for example, Parkinson's disease,[102] ischaemia[103] and Alzheimer's disease.[104] These still remain unexplored in terms of therapeutic research, so would be an attractive research area in coming years.

To make matters even more complex, it was recently discovered that p53, as well as its family members p63 and p73, are not single proteins. Recent studies have identified many different isoforms of the p53 family, implying that these various isoforms may be associated with the modulation of p53 activities and may have their own biological functions.[105] For example, Aoubala *et al*.[106] showed that Δ133p53α, one of nine p53 isoforms identified to date, antagonises p53-dependent apoptosis and G1 cell cycle arrest, as well as regulating p53 target genes such as p21, Mdm2 and Bcl-2 by forming a protein complex with p53. However, the precise mechanisms of these isoforms still remain unknown. This will therefore be one of the main subjects in further research on this fascinating protein.

Meanwhile, some novel compounds have been identified in recent years, some of which have produced hopeful results in clinical trials, and it is notable that all of these drugs influence not only tumour cells but also normal cells. From this point of view, a necessary criteria for future p53 drug development is that the activation of p53 function in tumour cells be selective for neoplastic cells, and that remains a significant challenge.

Temporarily preventing p53 activation, instead of promoting it, would be helpful for conventional cancer therapy. Many of the side effects of chemotherapy and radiotherapy are due to p53 activation in response to their genotoxicity. If p53 in normal cells can be inactivated for a short time so that p53-induced apoptosis is prevented, a patient may tolerate a higher – that is, more effective – dose. In this respect, a p53-inhibiting molecule named pifithrin (PFT) suggests the prospective application of p53 inhibitors in cancer therapy. Liu *et al*.[107] showed that PFTα inhibits doxorubicin-induced apoptosis in cardiac cells in mice, while Zhang *et al*.[108] demonstrated that PFTα protects against cisplatin-induced apoptosis in hair cells, in addition to potentially inhibiting other cisplatin-induced side effects such as ototoxicity, vestibulotoxicity and neurotoxicity. These results suggest that temporal inactivation of p53 may relieve the adverse effects of chemotherapy. A mice study by Strom *et al*.[109] also showed that PFTμ inhibited the mitochondrial pathway of p53 so that mice were more tolerant of gamma radiation.

Summary

p53 has been an attractive target for novel cancer therapy over the last 30 years, and we have come to better understand this fascinating protein based on ever-growing knowledge. Recent studies have identified p53 activities other than the widely known apoptosis, cell cycle arrest and senescence activities, for example anti-angiogenesis, autophagy and metabolism. Although the very first commercialised p53-target therapy was gene therapy, more recent emphasis has shifted to using small molecules to activate wild-type p53 or reactivate mutant p53. Many compounds have shown their potential antitumour effects *in vivo* and *in vitro* and some of these, such as CP-31398, PRIMA-1 and MI-219, are going through clinical trials. The prospective study subjects in the fourth decade of p53 research may include potentiality of application of p53-target therapy for diseases other than cancer, such as Parkinson's disease and ischaemia, the exact roles of the isoforms of p53 and its homologues p63 and p73, methods to make compounds selective for neoplastic cells, and the therapeutic potentiality of p53 inhibitors. One thing that is clear about p53 research is that many things are still unclear. However, it is also clear that our ever-growing knowledge leads us to better understand this protein, from its structure and activities to its therapeutical application. Thus, in the near future we might see novel drugs not only for cancer but also for other pathologies, or even for ageing.

Declarations

Conflict of interest

The Author(s) declare(s) that they have no conflicts of interest to disclose.

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None to declare.

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