TP53 mutations in cancer: Molecular features and therapeutic opportunities (Review)

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Abstract. The tumour suppressor factor p53 plays an essential role in regulating numerous cellular processes, including the cell cycle, DNA repair, apoptosis, autophagy, cell metabolism and immune response. TP53 is the most commonly mutated gene in human cancers. These mutations are primarily non-synonymous changes that produce mutant p53 proteins characterized by loss of function, a dominant negative effect on p53 tetramerisation and gain of function (GOF). GOF mutations not only disrupt the tumour-suppressive activities of p53 but also endow the mutant proteins with new oncogenic properties. Recent studies analysing different pathogenic features of mutant p53 in cancer-derived cell lines have demonstrated that restoring wild-type p53, rather than removing GOF mutations, reduces cancer cell growth. These findings suggest that therapeutic strategies for reactivating wild-type p53 function in cancer cells may bring a greater benefit than approaches halting mutant p53. This approach could involve the use of small molecules, gene therapy and other methods to re-establish wild-type p53 activity. This review describes the complexity of the biological activities of different p53 mutants and summarizes the current therapeutic approaches to restore p53 function.

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1. Introduction

The p53 protein was first described in 1979 by several research groups as a cellular factor forming a complex with the simian virus 40 large tumour antigen (1,2). Subsequent studies demonstrated excessive accumulation of p53 both in cells expressing viral tumour antigens and in cancer cells negative for viral infections, whereas p53 levels were low in normal, uninfected cells (3,4). In the early 1980s, p53 was recognised as an oncoprotein whose upregulation by tumour viruses or other mechanisms could contribute to cellular transformation (5,6). Indeed, the TP53 cDNA cloned from various cancer cell lines was shown to immortalise primary cells, induce multilayer cell growth and promote tumourigenicity in animal models, thereby experimentally substantiating the oncogenic role of p53 in tumour development (7,8). In the late 1980s, DNA sequencing of the TP53 gene isolated from tumour cells revealed frequent missense mutations conferring oncogenic features to the mutant p53 proteins (9,10). Conversely, the expression of wild-type p53 in transformed cells was shown to suppress the transformed phenotype without inducing damaging effects in non-transformed cells (11,12). The critical role of p53 as a tumour suppressor was further demonstrated in patients with Li-Fraumeni syndrome, associated with monoallelic germline TP53 mutations and characterized by a high predisposition to various cancer types, including breast cancer, sarcomas, brain tumours, leukaemia and adrenal gland cancers (13,14). The observation that tumours developing in patients with Li-Fraumeni syndrome have lost the wild-type TP53 allele definitively established p53 as a tumour suppressor factor (15).

The scientific evidence accumulated over the past 40 years since the discovery of p53 has shown that this oncosuppressor is involved in regulating a broad range of cellular processes (16,17). These include cell cycle control, activation of DNA repair mechanisms in response to genetic damages, programmed cell death in response to severe cellular stress, induction of cell senescence and regulation of metabolic pathways (Fig. 1) (18,19). Therefore, mutations within the TP53 gene and expression of mutant p53 proteins can be considered to be involved in virtually all hallmarks of cancer (20).

TP53 is the most commonly mutated gene in human cancers, with mutation frequencies exceeding 50% in at least 20 tumour types, including colorectal, ovarian and oesophageal carcinoma and lung cancer (Fig. 2). Certain nucleotide

changes in the TP53 gene have been recognized as molecular signatures of carcinogen exposure in tumours developing in specific organs, such as aflatoxin B1 and G to T transversion at codon 249 in hepatocellular carcinoma, tobacco smoking and G to T transversion at specific G:C base pairs in lung cancer, and ultraviolet light irradiation leading to CC to TT tandem mutations in skin cancers (21,22). In addition, a significant proportion of TP53 mutations consist of G:C to A:T transitions at CpG sites, possibly originating from spontaneous deamination of DNA bases (23). However, numerous aspects regarding the cause of TP53 mutations, their diverse activities in cancer cells and therapeutic opportunities in tumours harbouring p53 mutations remain mostly unknown.

2. Mutations in TP53 gene

The human TP53 gene is ~20 kilobases long, contains 11 exons and encodes a zinc-coordinated protein composed of 393 amino acids (24). In normal cells, p53 protein is present at low levels, with an unstable conformation and short half-life due to continuous degradation mediated by its negative regulator mouse double minute 2 (MDM2) (25,26).

The p53 protein contains two transcriptional activation domains (TAD), namely TAD1 comprising amino acids (aa)21-28 and TAD2 comprising aa47-55, and a proline-rich sequence (aa62-94) at the N-terminal region, a DNA-binding domain (DBD, aa94-292) and a tetramerisation domain (TET, aa318-355) in the central region, as well as a nuclear localization signal and sites of post-translational modifications at the C-terminal domain (RD, aa363-393) (Fig. 3) (27-29). The p53 TAD1 induces the transcriptional activation of genes involved in cell cycle arrest, such as cyclin dependent kinase inhibitor 1A CDKN1A), and regulates apoptosis by activating the BCL2 associated X, apoptosis regulator (BAX), BCL2 binding component 3 (PUMA), NADPH oxidase activator (NOXA) and BCL2 associated agonist of cell death genes. Both TAD1 and TAD2 participate in tumour suppression and binding to MDM2 (Fig. 1) (30,31). The p53 DBD domain interacts with specific p53-responsive elements consisting of two copies of 5'-RRRCWWGYYY-3' DNA sequences that are located in the regulatory regions (enhancers and promoters) of genes regulated by the p53 protein (32). The TET domain, involved in p53 tetramerisation, allows the appropriate conformation of p53 and interaction with target DNA sequences (33). The RD region regulates the binding of DBD to p53-responsive elements in the p53-regulated genes, depending on post-translational modifications such as acetylation, phosphorylation and sumoylation (34-40). The p53 C-terminal domain also contains nuclear export and localisation signals, which are important for its vehiculation to the nucleus as well as for transferring p53 into the cytoplasm for degradation (41,42).

Mutations in the TP53 gene have been shown to confer to cells new biological features, such as increased proliferation, deregulated metabolism, metastatic potential and an altered tumour microenvironment, as well as resistance to chemotherapy and radiotherapy (43). Importantly, >90% of tumours carrying a mutant TP53 gene show loss of the second allele by mutation, chromosomal deletion or loss of heterozygosity, strongly supporting the tumour suppressor model (44).

The main genetic alterations of the TP53 gene include truncating and splice sites mutations, in-frame insertions/deletions (indels), frameshift indels and missense mutations arising from diverse mechanisms, playing specific roles in tumour development (45,46). Missense mutations, resulting in single amino acid changes, represent the vast majority of mutations occurring in >190 different codons of the TP53 gene (>80%), with the highest frequency in the DNA-binding domain (Fig. 2). Post-translational modifications of mutant p53 proteins, such as phosphorylation and acetylation, contribute to the stabilization of mutant proteins, leading to their accumulation in cancer cells (44,47). For instance, phosphorylation on Ser15 and Ser37 in mutant p53 has been shown to stabilise the protein and to enhance the oncogenic activity in ovarian cancer (48).

Different missense mutations in p53 may confer new biological activities to the mutant oncosuppressor, which can be grouped into three possible mechanisms: i) Loss of function (LOF) in transcriptional regulation of p53-dependent genes; ii) dominant negative effect (DNE) on the activity of wild-type p53 via the formation of mixed heterotetramers; and iii) gain of function (GOF) in terms of oncogenic activity (Fig. 3) (49-52). While LOF and DNE of mutant p53 have been proven crucial for cell proliferation and malignant transformation, the processes activated by mutant p53 characterised by GOF remain to be fully elucidated (52-56).

Approximately 30% of missense mutations in the TP53 gene occur at six hot spots and produce eight diverse p53 mutants, such as R175H, G245S, R248Q, R248W, R249S, R273C, R273H and R282W (57-59). These have been reported to possess GOF activity, although the molecular mechanisms behind their novel oncogenic functions have not been fully elucidated. The hot spot mutations determine amino acid changes in p53 that have been classified as contact mutants, which occur in the DNA-binding domain (R248Q, R248W, R273H and R273C), and conformational mutants, which cause abnormal protein folding (R175H, G245S, R249S and R282H) (60,61). Contact mutants directly affect the ability of mutant p53 to control the transcription of target genes, while conformational mutants cause the loss of zinc coordination and DNA-binding activity (62).

Mutant p53 has been shown to bind nuclear transcription factors, such as nuclear transcription factor Y (NF-Y), tumour protein p73, nuclear factor erythroid 2-related factor 2 and protein C-ets-1, to activate the transcription of their target genes and promote malignant transformation by inducing overexpression of cell cycle genes (63,64). Complexes formed by mutant p53 and the transcriptional co-regulator tyrosine-protein kinase Yes-associated protein and the nuclear transcription factor NF-Y were shown to promote the aberrant expression of cell cycle-related genes, such as cyclin A, cyclin B and cyclin-dependent kinase 1 (65). In addition, mutant p53 has been shown to antagonise the tumour suppression activity mediated by p63/p73 via the Notch1 signalling pathway in colorectal and pancreatic cancers (66). The p53 mutations causing nuclear delocalization are also involved in regulating oncogenic activity. Indeed, in colon cancer cells, the p53 P151H and R282W mutants located in the nucleus were shown to hinder autophagy, while p53 E258K, R273H and R273L

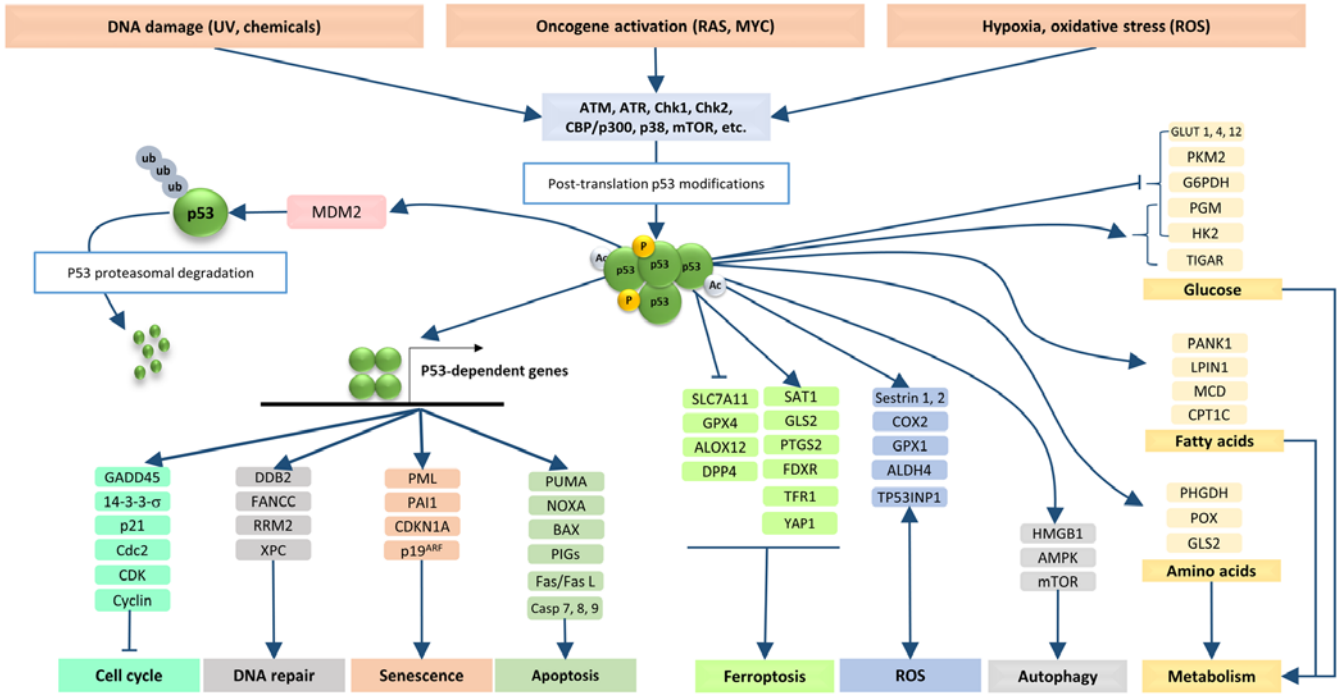

Figure 1. Under physiological conditions, cell stress promotes the activation and stabilization of p53 by post-translational modifications (phosphorylation, acetylation, etc.). The stabilized p53 forms tetramers, which bind the p53-dependent promoters and activate the expression of genes involved in different biological functions, such as cell cycle control, DNA repair, senescence and apoptosis. The p53 protein directly interacts with numerous other proteins and regulates cellular pathways such as ferroptosis, ROS, autophagy and metabolism. The level of p53 protein is regulated by a p53-MDM2/MDMX feedback loop via proteasomal degradation of p53. 14-3-3-s, 14-3-3 protein sigma; ALDH4, aldehyde dehydrogenase 9 family member A1; ALOX12, arachidonate 12-lipoxygenase, 12S type; AMPK, protein kinase AMP-activated catalytic subunit alpha 2; ATM, ataxia telangiectasia mutant; ATR, ataxia telangiectasia related; BAX, BCL2 associated X, apoptosis regulator; Casp 7, caspase 7; CBP/p300, CREB-binding protein/p300; Cdc2, cyclin-dependent kinase 1; CDK, cyclin-dependent kinase; CDKN1A, CDK inhibitor 1; CHK1, checkpoint kinase 1; COX2, prostaglandin-endoperoxide synthase 2; CPT1C, carnitine palmitoyltransferase 1C; DDB2, damage specific DNA binding protein 2; DPP4, dipeptidyl peptidase 4; FANCC, Fanconi anemia complementation group C; Fas/Fas L, Fas cell surface death receptor/Fas cell surface death receptor ligand; FDXR, ferredoxin reductase; G6PDH, glucose-6-phosphate dehydrogenase; GADD45, growth arrest and DNA damage-inducible 45; GLS2, glutaminase 2; GLUT 1, solute carrier family 2 member 1; GPX1, glutathione peroxidase 1; HK2, hexokinase 2; HMGB1, high mobility group box 1; LPIN1, lipin 1; MCD, malonyl-CoA decarboxylase; mTOR, mechanistic target of rapamycin kinase; NOXA, NADPH oxidase activator; $P19^{ARF}$, CDK inhibitor 2A; p38, p38 kinase; PAI1, serpin family E member 1; PANK1, pantothenate kinase 1; PGM, phosphoglucomutase 1; PHGDH, phosphoglycerate dehydrogenase; PIGs, phosphatidylinositol glycan anchor biosynthesis class S; PKM2, pyruvate kinase M1/2; PML, promyelocytic leukemia protein; POX, proline dehydrogenase 1; PTGS2, prostaglandin-endoperoxide synthase 2; PUMA, BCL2 binding component 3; RRM2, ribonucleotide reductase regulatory subunit M2; ROS, reactive oxygen species; SAT1, spermidine/spermine N1-acetyltransferase 1; SLC7A11, solute carrier family 7 member 11; TFR1, transferrin receptor; TIGAR, TP53 induced glycolysis regulatory phosphatase; TP53INP1, tumor protein p53 inducible nuclear protein 1; XPC, xeroderma pigmentosum, complementation group C; YAP1, Yes1-associated transcriptional regulator.

mutants located in the cytoplasm were unable to inhibit autophagy (67).

A recent study by Wang *et al* (68) addressed the importance of mutant p53 GOF in neoplastic transformation by inactivating 12 TP53 mutations in a panel of 15 human cancer cell lines derived from breast cancer, colorectal cancer, lymphoma, hepatocellular carcinoma, leukaemia, osteosarcoma and lung cancer. They found that removing mutant TP53 using an inducible clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) platform neither reduced *in vitro* cell survival and proliferation, nor did it affect the killing of cancer cells in response to drug treatment. Furthermore, removing mutant p53 did not impact mitochondrial activity and intracellular reactive oxygen species (ROS) in the tested cell lines. In addition, knocking out the mutant TP53 did not affect the local growth of tumours derived from cancer cell lines or the number of lung metastasis in NOD scid gamma mice (68). Conversely, changing mutant TP53 to wild-type TP53 induced a reduction in tumour cell proliferation *in vitro*, suggesting that drugs capable of reshaping the structure of mutant p53 to that of wild-type p53 could be more effective as anticancer therapies.

The clinical outcome of p53 mutations has been extensively studied, yielding sometimes divergent results (53,69). Early studies in patients with Li-Fraumeni syndrome showed that the germline mutation p53 R248Q was associated with the development of cancer at a younger age compared with those with non-mutated TP53 (70). Conversely, somatic mutations in p53, commonly associated with a wide range of cancers, are more frequent in older populations (71). Overall, the majority of studies have demonstrated an association between TP53 mutations and more aggressive tumours, resistance to treatments and poor overall outcomes in several cancer types (17,72).

The chemoresistance of cancer cells to various drugs is strongly dependent on TP53 mutational status in numerous cancer types (50). Several studies have shown that mutant p53 causes an increased expression of the multidrug resistance 1 gene, an important regulator of the membrane pump, which induces chemoresistance by increasing the efflux of drugs out of cells (73). Conversely, depletion of mutant p53 reduces cell

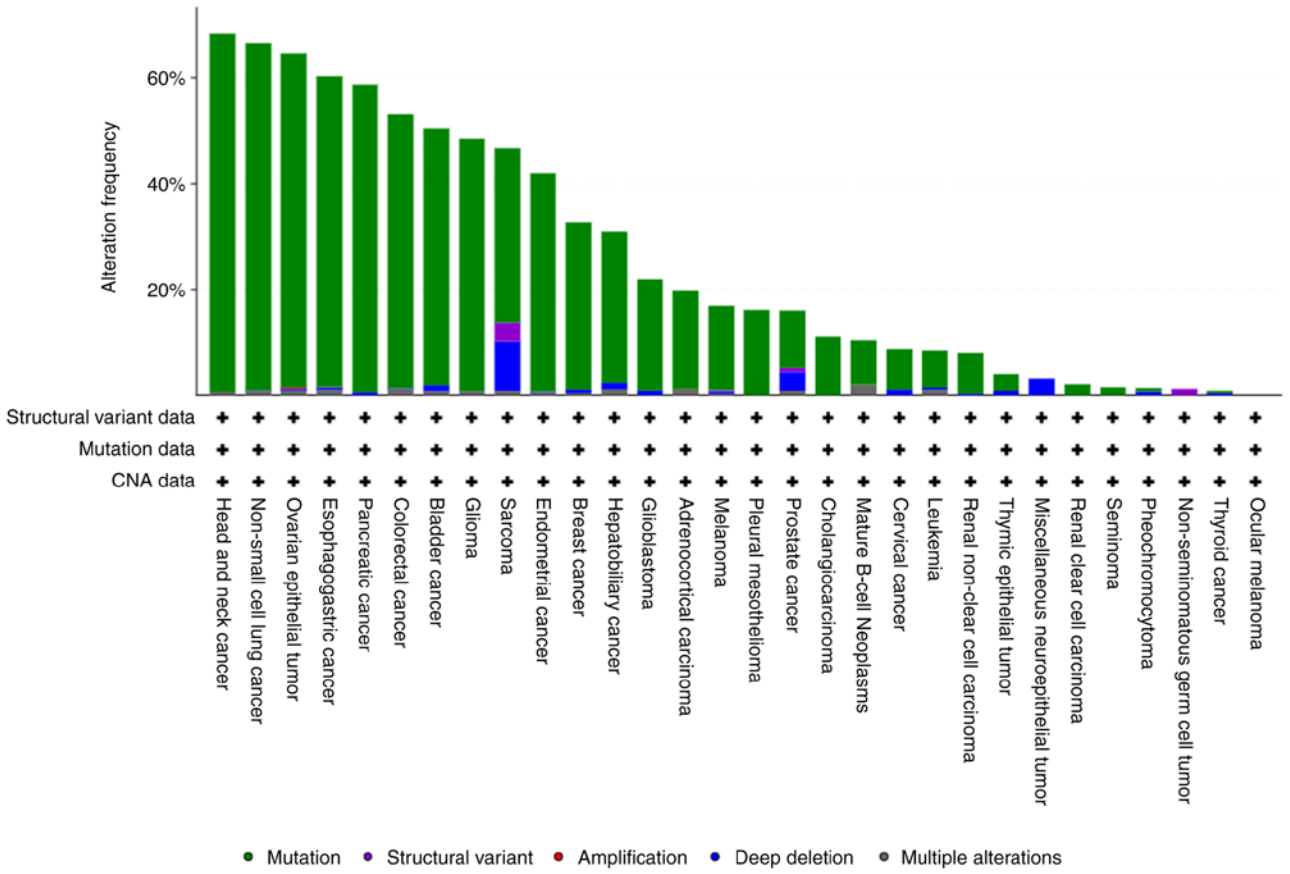

Figure 2. TP53 gene mutations in the curated set of non-redundant studies including TCGA and non-TCGA datasets (n=10,953 patients from 32 studies) that do not include overlapping samples (www.cbioportal.org/). TCGA, The Cancer Genome Atlas; CNA, copy number alterations.

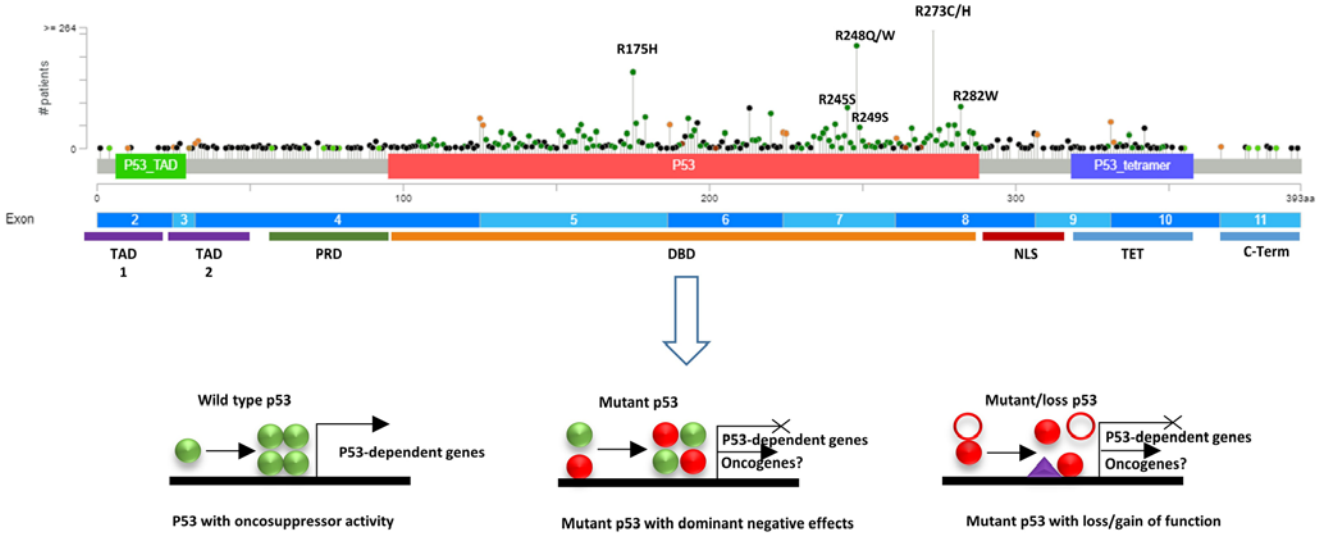

Figure 3. Lollipop plot produced by the MutationMapper tool of cBioPortal shows the frequency and position of TP53 mutations in 10953 tumours from 32 studies. Domain organization of p53 is also described, followed by the C-terminus containing the nuclear export signal. TAD, transactivation domain; PRD, proline rich domain; DBD, DNA binding domain; NLS, nuclear localization signal; NLS, nuclear localization signal; TET, tetramerisation domain.

resistance to cisplatin, adriamycin and etoposide in multiple cancer cell lines (74). Mutations in the TP53 gene identified in colorectal cancer have been associated with resistance to insulin-like growth factor-1 receptor inhibitors (75). In osteosarcoma, the p53 R273H mutant is associated with reduced expression of procaspase-3 and failure of methotrexate and doxorubicin to induce apoptosis (76). In colon cancer, mutant p53 that fails to activate the PUMA promoter causes evasion from apoptosis and reduces the sensitivity of tumour cells to 5-fluorouracil (77). Considering all these findings, it is highly plausible that mutant p53 plays a critical role in the chemoresistance of several tumour types.

The response to radiation therapy is also influenced by the p53 status. A study of 60 different cancer cell lines carrying mutant p53 revealed an inability to induce key regulatory genes such as CDKN1A, MDM2 and growth arrest and DNA damage inducible alpha (GADD45), leading to a failure in G1 phase arrest after gamma-irradiation (78). This lack of response resulted in increased radioresistance. Similarly, ovarian cancer, head and neck cancer, hepatocellular carcinoma, cervical cancer and endometrial cancer cells carrying mutant p53 are more resistant to radiation therapy compared to cells carrying wild-type p53 (79). Therefore, additional research is necessary to fully characterise the relationship between mutant p53 and response to radiotherapy, which is crucial for improving patient treatment.

3. Therapeutic strategies to target mutant p53

The primary effect of p53 mutations in cancer is the loss of tumour suppressor function, making therapeutic reactivation of p53 a priority. Several therapeutic approaches include refining earlier strategies with improved understanding and delivery methods or utilising novel drug design techniques. New strategies encompass gene therapy to restore normal p53 function, inhibition of MDM2-p53 interaction, p53-based vaccines and the use of small molecules capable of reinstating a wild-type-like status of mutant p53. Several excellent reviews have reported state-of-the-art therapeutic strategies to enhance wild-type p53 activity as well as p53-based immunotherapy (80,81). The present review reassesses the progress in promising therapeutic strategies targeting mutant p53 for degradation or reinstating its wild-type-like conformation to restore its oncosuppressor activities.

Degradation of mutant p53 has emerged as a promising antitumour strategy. Various compounds, including gambogic acid (GA), capsaicin, MCB-613 and NSC59984, have demonstrated the ability to effectively degrade mutant p53 (82). GA was reported to reduce the expression of MDM2, thereby increasing the levels of wild-type p53 and inhibiting tumour growth (83). Later studies revealed that GA also reduces mutant p53 levels by hindering the formation of heat shock protein (Hsp)90/mutant p53 complexes, leading to the ubiquitin/proteasome-mediated degradation of mutant p53 (84). However, GA was observed to drive other mechanisms critical for malignant cell death, such as the activation of the intrinsic apoptotic pathway in TP53-null prostate cancer cells (85). Thus, GA inhibits tumour growth by activating several cell growth inhibitory pathways, with mutant p53 degradation being one possible contributing mechanism.

Capsaicin, the main bioactive component of chili peppers, has been shown to induce autophagy and mutant p53 protein degradation in cells carrying mutant p53 and in H1299 (p53 null) overexpressing p53 R175H and p53 R273H mutant proteins (86). The small molecule MCB-613 has been demonstrated to cause fast and selective degradation of p53 R175H protein in ovarian cancer cells by inhibiting ubiquitin carboxyl-terminal hydrolase 15, enabling selective depletion of oncogenic p53 R175H levels (87). In addition, the small molecule NSC59984 has been shown to specifically restore the p53 pathway through p73 and deplete mutant p53 proteins in colorectal cancer cells (88).

Several drugs effective in degrading mutant p53, such as ganetespib, statins and suberoylanilide hydroxamic acid (SAHA), are currently under evaluation in clinical trials. Ganetespib is a potent Hsp90 inhibitor that has shown remarkable effectiveness in degrading mutant p53 and killing cancer cells. It has exhibited strong cytotoxic effects across various haematological and solid tumours and has been successful in inhibiting tumour growth and extending survival in mouse models with specific mutant p53 expressions (89). Combining ganetespib with chemotherapy agents such as cyclophosphamide has been shown to further enhance its tumour-suppressing effects (90).

It has been shown that statins, which target the rate-limiting enzyme in cholesterol biosynthesis, inhibit the growth of cancer cells expressing mutant TP53 and increase their sensitivity to chemotherapeutics (91,92). Statins induce degradation of certain forms of mutant p53 through a mechanism involving the inhibition of mutant p53 interaction with DnaJ homolog subfamily A member 1, leading to E3 ubiquitin-protein ligase CHIP-mediated degradation while sparing wild-type p53 and other mutants (93). However, there are conflicting results regarding the ability of statins to selectively kill tumour cells depending on the TP53 mutation status (94). Hence, the mechanisms by which statins kill tumour cells require further investigation.

Similarly to statins, SAHA targets mutant p53 by disrupting its association with Hsp90 via histone deacetylase 6 (HDAC6) inhibition, resulting in the reactivation of degradation pathways and exhibiting selective toxicity towards cancer cells harbouring mutant p53 (95). These findings underscore the potential of mutant p53 degradation as an effective therapeutic approach against cancer. However, clinical trials are necessary to fully establish the safety and efficacy of these treatments for widespread clinical use.

Restoration of wild-type activity by using small molecules capable of restoring a wild-type-like structure and the ability to promote the transcription of p53-dependent genes or to inhibit the oncogenic function of GOF mutant p53 is gaining attention (96,97). Several compounds have been developed to reinstate the wild-type-like activity of mutant p53 (Table I). These molecules can target mutant p53 and activate the transcription of p53-dependent genes by restoring the structural conformation of wild-type p53. Such compounds exert antitumour activity, inhibiting cell proliferation and tumour growth (98).

Among these, p53 reactivation with induction of massive apoptosis 1 (PRIMA-1) is a low-molecular-weight compound that was identified in 2002 through chemical screening and was observed to restore the wild-type p53 conformation to several p53 mutants (99). PRIMA-1 and its methylated form, namely APR-246, were found to delay tumour growth and increase apoptosis in tumour cells with mutant TP53 but not in those with wild-type TP53 via reactivation of p53-dependent target genes and induction of the pro-apoptotic protein NOXA (100-102). APR-246, alone or in combination with other drugs, is currently being tested in phase I-III clinical trials, including TP53-mutant myeloid malignancies, high-grade serous ovarian cancer, oesophageal cancer and melanoma (103-105). Two phase I/II trials showed significant effects when APR-246 was combined

Table I. Therapeutic compounds changing mutant p53 conformation to wild-type-like structure that are under evaluation in clinical trials.

Agent	Target	Class	Mechanism	Phases of clinical trials	Clinical trial nos.
APR-246	p53 R175H p53 R273H	Small molecule	Wild-type-like conformation of p53 by binding to thiol groups	I-III	NCT03072043, NCT03588078, NCT03745716, NCT04383938, NCT04419389, NCT03931291, NCT04214860
PEITC	R175H P223L	Phytochemical	Wild-type-like conformation of p53, oxidative stress	I-II	NCT01790204, NCT03034603
PC14586	Y220C	Small molecule	Selectively restores wild-type p53 conformation	I	NCT04585750
ATO	R175H V272M R282W E285K Y234C	Small molecule	Restores wild-type p53 conformation	I-II	NCT03855371, NCT04489706, NCT04695223, NCT04869475
Sodium stibogluconate	65 mutants	Pentavalent antimony compound	Restores wild-type p53 conformation	II	NCT04906031
COTI2	R175H R273H R273C R282W	Zn^{2+} chelator	Inhibition of mutant p53 misfolding	I	NCT02433626

with azacitidine in patients with myelodysplastic syndrome (MDS) or acute myeloid leukaemia (AML) carrying p53 mutations. Azacitidine is the first Food and Drug Administration (FDA)-approved drug for treating MDS and the combination with APR-246 demonstrated substantial therapeutic potential in these patients (104,105). Despite their initial specificity for mutant TP53, both PRIMA-1 and APR-246 could also induce autophagy in certain cancer cells independently of their TP53 status (106). This highlights the complexity of the molecular mechanisms implicated in APR-246 activity, which remain to be fully elucidated.

Phenethyl isothiocyanate (PEITC), a natural compound present at high levels in cruciferous vegetables, has been shown to possess chemotherapeutic activity by targeting mutant TP53. Unlike APR-246, which can restore wild-type p53 function in several mutant p53 proteins, PEITC specifically targets the mutant R175 p53, restoring wild-type p53 conformation and transactivation functions (107). In addition, PEITC exerts significant anti-cancer activity in cells with structural TP53 mutants, such as P223L, but not in those with contact TP53 mutants (108). Importantly, PEITC is able to reduce the quantity of mutant p53 protein post-transcriptionally, with minimal impact on wild-type p53 levels. This dual action suggests that PEITC reactivates wild-type p53 functions while decreasing mutant p53 protein levels (107). However, further research is required to validate the efficacy of PEITC and clarify the critical mechanisms for killing cancer cells.

The small molecule PC14586 specifically targets the Y220C mutant. This orally bioavailable drug has achieved clinical responses in patients with various tumour types, including ovarian, breast, prostate, lung and endometrial cancers. In patients with solid tumours carrying the p53 Y220C mutation and wild-type KRAS, the overall response rate was 38% (6 of 16 evaluable patients) (109). Due to these promising results, the FDA granted fast-track designation of PC14586 for treating locally advanced or metastatic solid tumours with the p53 Y220C mutation. However, considering that the Y220C mutation is relatively rare, applying similar structure-based approaches to other p53 mutants may prove significantly challenging for several tumour types.

Arsenic trioxide (ATO) is a small molecule that can restore wild-type p53 function in tumour cells expressing structural p53 mutants by binding to their DNA-binding domain. This binding induces transcriptional activities characteristic of wild-type p53, suppressing tumour growth both *in vitro* and *in vivo* (110). Although ATO shows promise as a therapy for cancers expressing mutant p53, further studies are required to validate its specificity and efficiency.

Sodium stibogluconate (SSG), originally an antiparasitic drug, has recently been identified as a potent reactivator of temperature-sensitive p53 mutants in a high-throughput screen.

SSG works by releasing antimony ions that bind to a specific pocket in the mutant p53 protein, increasing its stability and restoring its tumour-suppressing functions (111). It has shown effectiveness in rescuing 65 different p53 mutations, though none were hotspot mutations. Given its existing approval as an antiparasitic, repurposing SSG for cancer treatment is a cost-effective strategy. A phase II clinical trial is underway for patients with specific p53 mutations in MDS or AML cells.

A thiosemicarbazone, namely COTI-2, shows preferential activity against p53-mutated cancer cells but also affects p53-wild-type cells to a certain extent. In head and neck squamous cell carcinoma, COTI-2 demonstrated both p53-dependent and p53-independent effects, inducing cell death by causing DNA damage, replication stress and activating p53 target genes through the p53 family member p63 (112). COTI-2 has progressed to phase I clinical trials, while other related thiosemicarbazones targeting p53 R175H have not yet entered clinical trials despite promising preclinical results.

Numerous other compounds targeting mutant p53 are showing promising results as anticancer agents. However, they have yet to be evaluated in clinical trials. These include the small molecule reactivation of TP53 and induction of tumour cell apoptosis (RITA), which was identified by chemical screening and initially reported to inhibit the p53-MDM2 interaction, thereby activating the anti-tumour effects of wild-type p53 (113). A subsequent study revealed that RITA could also suppress proliferation and induce apoptosis in tumour cells expressing mutant TP53 by restoring wild-type p53 transcriptional activities in several hotspot p53 mutants (114). This was demonstrated by the expression of wild-type p53 target genes such as GADD45, BCL2 binding component 3, BAX and CDKN1A in these cells. Furthermore, RITA was also shown to induce apoptosis in tumour cells with wild-type p53 and also in TP53-deficient cancer cells, suggesting that its anticancer effects may not be specifically dependent on mutant p53 (115).

Similarly to the above-described small molecules, CP-31398 has been shown to restore functional activity to mutant p53 proteins, enabling them to exert wild-type p53 transcriptional activity (116). This synthetic compound induces cell death in a p53-dependent manner and is effective only in tumour cells expressing wild-type or mutant p53 but not in p53-deficient cells (117). CP-31398 increases levels of wild-type p53 by preventing its ubiquitination and proteasomal degradation, thus promoting cell cycle arrest and apoptosis (118). In addition, CP-31398 has been shown to restore wild-type p53 function in various p53 mutants, delaying growth in hepatocellular and colorectal cancer cells expressing different p53 mutations both *in vitro* and *in vivo* (119,120). The inhibitory effects on cell growth are consistent across different cancer types carrying diverse p53 mutations. Furthermore, CP-31398 has been shown to increase ROS production, triggering apoptosis in multiple myeloma cells regardless of p53 status (121). Further research is needed to fully understand the mechanisms behind the restoration of p53 activity.

Several compounds have been identified that target uncommon p53 mutants or distinct groups of mutants. Among these, PK7088 is a small molecule that specifically binds the unique surface crevice created by the amino acid change Y220C in mutant p53, converting the mutant structure to the wild-type conformation (122). PK7088 restores p53-dependent cell cycle arrest and apoptosis by activating p21 and NOXA expression, respectively.

Other mutants of p53 targeted by specific compounds include the amino acid change R175H, which causes structural modification of the p53 DNA-binding domain (123). The p53 R175H is specifically targeted by the ZMC1 (NSC319726) molecule, which is a metal ion chelator promoting p53-dependent apoptosis *in vitro* and inducing tumour regression *in vivo* (124). NSC319726 selectively kills cells carrying the p53 R175H mutation without impacting non-transformed cells or those with wild-type p53. Related compounds like ZMC2 and ZMC3 from the thiosemicarbazone family also induced a wild-type-like conformation of p53 R175H *in vitro* (125). Mutant p53 can interact with various oncogenic factors, forming complexes that promote cancer cell growth. Compounds that disrupt such interactions can inhibit cancer cell proliferation and induce apoptosis. For instance, molecules that inhibit the interaction between mutant p53 and its chaperone HSP90 cause enhanced ubiquitination and proteasomal degradation of mutant p53 while promoting apoptotic pathways and the death of cancer cells. The HDAC inhibitor SAHA has been reported to synergise with the HSP90 inhibitor 17AAG to degrade mutant TP53, inducing apoptosis and decreasing tumour growth in xenografts (126). Another HDAC inhibitor, FK228, also inhibits growth and induces apoptosis in tumour cells. Unlike SAHA, which specifically kills tumours carrying mutant TP53, FK228 induces cell death in both mutant and wild-type TP53-expressing tumour cells (127-129).

Other attractive therapeutic approaches include gene therapy to repair or replace the mutant TP53 gene, for example using the CRISPR/Cas9 technique, as well as RNA interference to silence critical genes, including the mutant TP53 gene that is required for tumour cell proliferation (130,131). Immunotherapeutic strategies, such as vaccines targeting mutant p53 neoantigens, the use of bispecific antibodies and adoptive T-cell therapies using engineered T-cells that recognise mutant p53-expressing cells also show promise for the treatment of p53-mutant tumours (58,80,132,133).

Small molecules that interfere with the binding of p53 to its negative regulator MDM2 may be effective in increasing the levels of p53 in tumours carrying the wild-type TP53 gene (80,134). Despite the promising results of certain clinical trials of MDM2 inhibitors and mutant p53-restoring compounds, none of these agents have been approved by the FDA.

In conclusion, targeting mutant p53 in cancer therapy is an advancing field with substantial therapeutic potential. Understanding the diverse roles of mutant p53 in tumour progression and utilising emerging strategies to counteract its oncogenic functions are essential for developing effective cancer treatments.

4. Conclusions

Mutations in the TP53 gene or functional inactivation of the p53 protein play a crucial role in cell transformation and the development of cancers. Missense nucleotide changes in the TP53 gene are the most frequent mutations observed in human cancers, making mutant p53 a key target for cancer research and development of therapies. Most of these genetic alterations

are specific to cancer histotypes (135). Targeting mutant p53 to restore the activity of wild-type p53, by using specific drugs and immunotherapies, may be effective in all these cancers presenting a high frequency of mutations in the TP53 gene.

The screening of chemical libraries has allowed the identification of small molecules capable of specifically binding to mutant p53, guiding its folding to a wild-type-like p53 and restoring tumour suppressor activity. Such therapeutic strategies are likely to have a significant clinical impact in the treatment of numerous types of human cancers characterised by these mutations.

Nonetheless, important considerations remain to be resolved. These include questions related to the functional inactivation of the p53 protein mediated by viral oncoproteins that can bind to and degrade wild-type p53 in tumours caused by infectious agents (4). The BamHI Z fragment leftward open reading frame 1 encoded by Epstein Barr virus, the Early 6 protein of high-risk human papillomavirus and the non-structural protein 5 of hepatitis C virus have been shown to directly bind to and degrade p53 (136-138). Other viruses have been shown to inhibit the activity of wild-type p53 through the modulation of p300/ CREB-binding protein nuclear factors, causing a decrease in its levels in infected cells. No drugs have been developed to disrupt the complex formed by viral oncoproteins and p53.

In conclusion, therapies targeting mutant p53 offer several potential advantages, such as specificity for cancer cells, thereby minimizing damage to normal cells, wide applicability given the high prevalence of p53 mutations in numerous tumour types, and the potential to reverse cancer progression, as these drugs can reinstate the normal tumour-suppressive function of p53. However, only a small number of these drugs have reached late-stage clinical trials, commonly due to off-target effects and nonspecific toxicity, which hinder the evaluation of their efficacy in clinical trials. Therefore, further efforts are needed to achieve the goal of targeting all forms of p53 dysfunction with specific molecules or drugs to address them effectively.

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Author's contributions

Conceptualization, MLT; data curation, MLT; writing, review and editing, MLT. The author has read and approved the final manuscript. Data authentication is not applicable.

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Not applicable.

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Competing interests

The author declares that she has no competing interests.

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