Targeting Tumor Suppressor p53 for Cancer Therapy: Strategies, Challenges and Opportunities

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Abstract: p53 is one of the most important tumor suppressor genes that is frequently mutated in human cancers. Generally, p53 functions as a transcription factor that is stabilized and activated by various genotoxic and cellular stress signals, such as DNA damage, hypoxia, oncogene activation and nutrient deprivation, consequently leading to cell cycle arrest, apoptosis, senescence and metabolic adaptation. p53 not only becomes functionally deficient in most cancers, but not infrequently mutant p53 also acquires dominant negative activity and oncogenic properties. p53 has remained an attractive target for cancer therapy. Strategies targeting p53 have been developed including gene therapy to restore p53 function, inhibition of p53-MDM2 interaction, restoration of mutant p53 to wild-type p53, targeting p53 family proteins, eliminating mutant p53, as well as p53-based vaccines. Some of these p53-targeted therapies have entered clinical trials. We discuss the therapeutic potential of p53, with particular focus on the therapeutic strategies to rescue p53 inactivation in human cancers. In addition, we discuss the challenges of p53-targeted therapy and new opportunities for the future.

Keywords: Tumor suppressor, p53, mutant p53, p53-targeted therapy, restoration of p53, MDM2, p53 family protein.

1. INTRODUCTION

Cancer is a genetic disease that can take decades to develop through the accumulation of genetic mutations that activate oncogenes and inactivate tumor suppressor genes. Tumor suppressors act as "cellular guardians", by inhibiting cell growth pathways and/or by inducing cellular apoptosis, consequently preventing cancer formation [1]. One of the most important tumor suppressors is p53, which functions as a sensor of DNA damage and other cellular and metabolic stresses including hypoxia, oncogenic activation and nutrient deprivation. In response to stress, p53 can induce cell cycle arrest and subsequent DNA repair, senescence or apoptosis, depending on the level of cellular compromise and cellular context. If p53 is dysfunctional, some DNA damage isn't repaired, and this can result in genomic instability, a hallmark of cancer. Moreover cells with deficient p53 are able to escape apoptosis pathways and proliferate indefinitely under the selection of these cellular stresses. Thus, p53 plays a key role in the prevention of carcinogenesis [2, 3]. Studies have indicated that p53 is mutated in more than 50% of human cancers. In the remaining cancers, components of p53's protein post-translational modifications, protein stability or p53's signaling function is deficient [4, 5]. There is a need to develop p53-targeted therapy as an approach to treat cancer. In this review, we discuss the therapeutic potential of p53, with particular focus on the therapeutic strategies to rescue p53 inactivation in human cancer.

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2. TUMOR SUPPRESSOR P53 AND CANCER

p53 functions as a transcription factor that is stabilized and activated by various genotoxic and cellular stress signals, such as DNA damage, hypoxia, oncogene activation and nutrient deprivation. A great variety of genes are now identified as p53 target genes, whose protein products cause multiple outcomes such as cell cycle arrest (e.g., p21 and GADD45), apoptosis (e.g., DR5, Bax, PUMA and Noxa), senescence (e.g., pai-1), autophagy (e.g., dram) and the adaptation of cellular metabolism (e.g. TIGAR) [6]. p53 and its downstream pathways play a critical role in preventing tumor formation. However, p53 deficiency is a common event occurring in patient tumors. More importantly, unlike other tumor suppressors that only lose their function in cancer, mutant p53 has dominant negative activity and oncogenic function that endow cancer cells with a growth advantage and resistance to anti-cancer therapy [7, 8]. Therefore, p53 inactivation represents a promising target and its functional restoration is an attractive strategy for human cancer therapy.

2.1. p53 Functional Loss in Cancers

More than 50% cancer patients harbor somatic mutations in p53 genes and about 80% of p53 mutations are missense mutations. Most of the p53 mutations are within the DNA binding domain, resulting in disruption of wild-type p53 conformation (e.g., R175H, G245S, G245D and R249S) or abolishment of its DNA contact (e.g., R248Q, R248W, R273H, R273C and R282W) [9, 10]. Germline p53 mutations cause a rare type of cancer predisposition disorder called Li-Fraumeni Syndrome (LFS). LFS patients often develop a variety of cancer types with initial occurrence at a relatively young age, including soft-tissue and bone sarcomas, breast cancer, adrenal cortical carcinoma, brain tumors

and leukemia. Approximately 70% of families with LFS have a mutation in the p53 gene [11]. Importantly, both somatic and germline p53 gene mutations are usually followed by loss of heterozygosity (LOH) during tumor progression, which results in the inactivation of the remaining wild-type allele of p53 [12]. Functionally, due to the deficiency of cell cycle control and apoptosis, p53-deficient cells are susceptible to malignant transformation. Beyond its classical functions to induce cel cycle arrest and/or apoptosis, p53 has been found to play an important role in DNA repair, cell migration and cellular metabolism. p53 deficiency in cancer is associated with increased genomic instability and metastasis, as well as metabolic adaptation under an unfavorable environment [13-15]. It is well known that p53 knock-out mice develop spontaneous tumors. Donehower *et al.* reported that 26 of 35 homozygote p53-deficient mice (74%) developed at least one obvious neoplasm by 6 month of age. By contrast, p53 wild-type mice did not develop any tumors by 9 month of age [16]. In clinical studies, p53 mutation is found to be associated with resistance to radiotherapy and chemotherapy, poor patient survival and tumor progression [17].

2.2. Dominant Negative Activity of Mutant p53

Unlike most tumor suppressor genes (e.g. RB, APC and BRCA1) which are typically deleted or truncated in cancer, the p53 gene is frequently inactivated by a single mono-allelic missense mutation, and the mutant p53 protein is usually overexpressed in the full-length form in human tumors [7]. Increasing evidence suggests that mutant p53 not only loses its tumor suppressive function, but also has dominant-negative activity on the remaining wild-type allele [8]. *In vitro* experiments have demonstrated that mutant p53 inhibits the activity of wild-type p53, as observed in the reduced ability of wild-type p53 in binding to p53-responsive elements, inducing endogenous target genes and growth suppression in the presence of mutant p53 [18]. Genetic knock-in mouse models further support the conclusions. p53$^{+/-}$ mice bearing A135V p53 mutant transgene vector exhibited accelerated tumor development as compared to p53$^{+/-}$ mice without the transgene [19]. Since p53 binds to DNA as a tetramer, a potential mechanism for the dominant negative activity involves wild-type p53 proteins forming hetero-oligomers with mutant p53 proteins, resulting in impaired DNA binding and transcriptional activity [20, 21].

2.3. Oncogenic Properties of Mutant p53

In addition to dominant negative activity, mutant p53 acquires oncogenic properties that lead to "gain-of-function" [7]. Initially, p53 was identified as an oncogene, as early studies erroneously used mutant p53 sequence with oncogenic function [22]. *In vitro* studies have indicated that transfection of mutant p53 into p53-null cancer cells is able to suppress the promoter of p53 target genes and decrease p53 target genes expression [23]. Down-regulation of mutant p53 by siRNA increases the expression of p53 target proteins and suppresses cancer cell growth [24-26]. *In vivo* studies of p53 mutant mice (R172H and R270H) demonstrated that both p53$^{R172H/-}$ mice and p53$^{R270H/-}$ mice developed highly aggressive carcinoma, whereas p53$^{-/-}$ mice only developed lymphoma and sarcoma and never developed carcinoma. Forty-

three percent of p53$^{R270H/-}$ mice and 57% of p53$^{R172H/-}$ mice developed multiple tumors, whereas only 32% of p53$^{-/-}$ mice developed multiple tumors [27]. Clinical evidence indicates that p53 gene mutations are associated with poor clinical outcome in various cancer types [17]. Several mechanisms are involved in the oncogenic activity of mutant p53. First, mutant p53 can inhibit the function of the p53 family proteins p63 and p73 by protein-protein interaction. Increasing evidence has demonstrated that mutant p53 is able to bind to p63 and p73 and inhibit their function. Furthermore, it has been found that mutant p53 only inhibits p73 and p63 when mutant p53 is present in large excess to p63 and p73, which typically occurs in tumors [8, 28]. Second, regulation of gene transcription by mutant p53 is an important gain-of-function mechanism. Mutant p53 has the ability to activate the transcription of the multidrug resistance 1 (MDR1) gene, which causes drug resistance in mutant p53-expressing cancer cells [29]. Besides MDR1, mutant p53 has been implicated in the transcriptional regulation of several genes including PCNA [30], c-myc [31], FAS [32], bcl-xl [33] and VEGF [34]. The transcriptional regulation of these specific genes by mutant p53 may be modulated through preferential binding to structural DNA motifs such as non-B DNA structure [35], or through the interaction of mutant p53 with sequence-specific transcription factors, such as SP1 [36, 37], ETS [38, 39] and NF-Y [40]. Third, the inhibition of DNA repair pathways by mutant p53 is another mechanism for the gain-of-function. p53 mutants (R248W and R273H) can interact with the Mre11-Rad5-NBS1 (MRN) complex and impair its ability to recruit ATM kinase to DNA double-strand breaks, ultimately leading to genetic instability [41].

3. TARGETING P53 SIGNALING FOR CANCER THERAPY

Because of the critical role of p53 deficiency in tumorigenesis and therapy resistance, significant efforts have been devoted to developing p53-based cancer therapies. These approaches includes: (1), Gene therapy to restore p53 function; (2), Inhibition of p53-MDM2 interaction; (3), Restoration of mutant p53 to wild-type p53; (4), Targeting the p53 family proteins; (5), Eliminating mutant p53; (6), p53-based vaccines (Fig. **1** and Table. **1**).

3.1. Gene Therapy to Restore p53 Function

Delivery of wild-type p53 by adenovirus into cancer cells is a direct strategy to rescue p53 activity in cancer. *In vitro* and *In vivo* studies have demonstrated that adenoviral delivery of wild-type p53 (Ad-p53) triggers a dramatic apoptosis and tumor regression response in various cancer types including head and neck cancer [42], lung cancer [43], prostate cancer [44], cervical cancer [45], ovarian cancer [46] and glioma [47]. These viruses are replication defective because they lack certain early proteins for replication [48]. Remarkably, a previous study has shown that Ad-p53 does not have cytotoxic effect on normal fibroblast cells. Ad-p53 enhances the radiosensitivity of tumor cells, but not normal fibroblast cells [49]. Thus, adenovirus-delivered p53 gene therapy has shown a safety profile. The clinical application of Ad-p53 (Gendicine, Shenzhen Sibiono Genetech, China; ADVEXIN, Introgen Therapeutics Inc., USA) has been tested in phase I to IV clinical trials for the treatment of
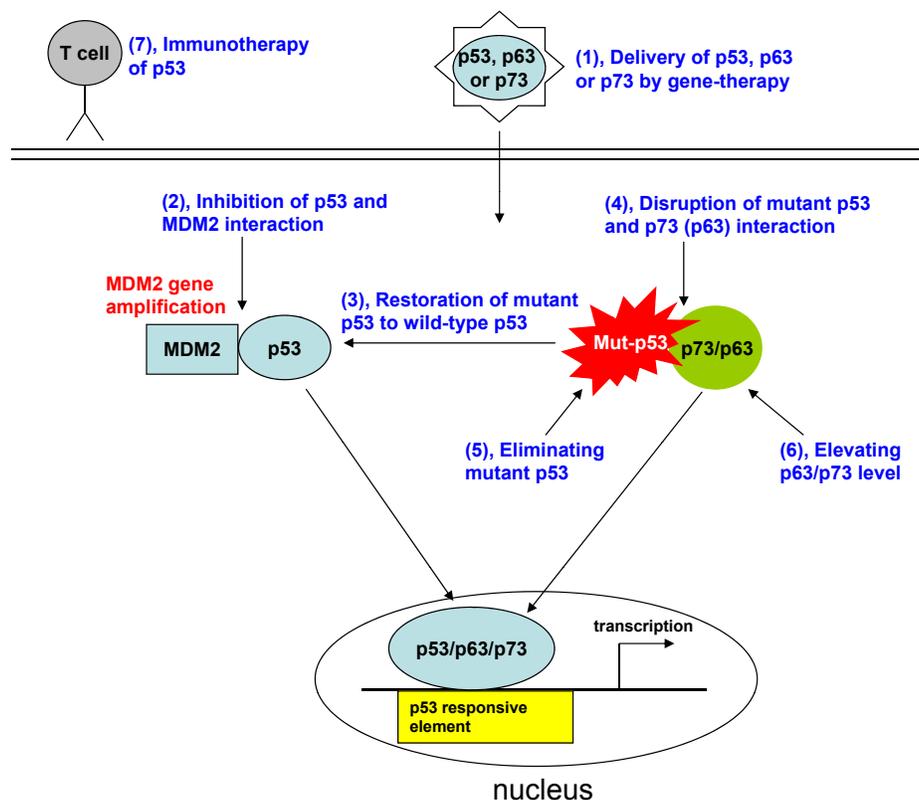
Fig. (1). p53 inactivation in cancer and strategies for targeting restoration of p53 function. p53 inactivation in cancer includes p53 muta-tion, MDM2 gene amplification, and the inhibition of transcriptional activity of p53 family proteins (p73 and p63) by interaction with mutant p53. Strategies for targeting restoration of p53 function include (1), delivery of p53, p63 and p73 by gene-therapy; (2), inhibition of p53 and MDM2 interaction; (3), restoration of mutant p53 to wild-type p53; (4), disruption of mutant p53 and p73 (p63) interaction; (5), eliminating mutant p53; (6), Elevating p63/p73 level; and (7), immunotherapy of p53.

patients with recurrent malignant gliomas [50], head and neck cancer [51], hepatocellular carcinoma [52], naso-pharyngeal carcinoma [53] and ovarian cancer [54]. The study in hepatocellular carcinoma demonstrated a significant increase in the response rate and survival rate when patients were treated by the combination of Ad-p53 (Gendicine) and radiotherapy as compared to radiotherapy alone [52]. The study in advanced nasopharyngeal carcinoma demonstrated that Ad-p53 was safe and improved the survival rate in pa-tients. Importantly, this study indicated that Ad-p53-expressed p53 mRNA was detected in biopsies from 16 of 17 patients after injection. Upregulation of p21 and Bax and downregulation of VEGF was observed in post-injection tumor biopsies. Complete response rate, 5-year overall sur-vival rate and 5-year disease-free survival rate were signifi-cantly increased in the group receiving Ad-p53 combined with radiotherapy as compared with the group receiving ra-diotherapy alone [53]. The study in ovarian cancer did not reveal any therapeutic effect of Ad-p53 [54]. Although these results in clinical trials are promising, the limitations of this approach are that virus is not able to infect every cancer cell due to the limitation of virus delivery, and that host antibod-ies reduce adenovirus infectivity.

3.2. Inhibition of p53-MDM2 Interaction

Mdm2, an E3 ubiquitin ligase is a critical feedback regu-lator of p53. Mdm2, which is upregulated by p53, interacts with p53 to promote polyubiquitination and subsequent pro-teasome-dependent degradation of p53 [55]. Mdm2 is fre-quently amplified in human cancer [56]. The transgenic mice containing increased copies of the MDM2 gene have an in-creased rate of tumor formation [57]. Due to the critical in-hibitory effect of MDM2 on p53, targeting the interaction of MDM2 with p53 is a potential cancer therapeutic strategy.

The experimental screening of chemical libraries has continued to identify an increasing number of MDM2-p53 interaction inhibitors including Nutlin 3 [58], RITA [59] and benzodiazepinedione [60]. Nutlin 3 is a potent and selective inhibitor of MDM2-p53 interaction. Protein crystal-structure studies demonstrate that nutlin 3 binds to MDM2 mimicking the crucial amino acid residues of p53 that are essential for MDM2 binding. Nutlin 3 has been shown to effectively block cells in G1 and G2 phases and induce apoptosis in a wild-type p53-dependent manner. Treatment by nutlin-3a shows effective tumor-growth inhibition and tumor shrink-age at non-toxic doses in human cancer xenograft mouse models [58, 61]. RITA is a small-molecule compound that is found to prevent the interaction of MDM2 with p53. RITA activates the p53 pathway and induces p53-dependent growth inhibition and apoptosis in tumor cells. In addition, RITA inhibits tumor growth in SCID mice with wild-type p53 HCT116 human tumor xenografts. In contrast to nutlin which binds to MDM2, RITA binds to p53 thereby prevent-ing MDM2-p53 interaction [59].

Table 1.   The Agents Targeting p53 under Investigation.

Therapeutics	Mechanism	Testing Stage	Refs.
1. Genetherapy to restore p53 function			
Gendicine	Delivery of wt p53 by adenovirus	Phase IV	[6]
Advexin	Delivery of wt p53 by adenovirus	Phase I/II	[6]
2. Targeting MDM2-p53 axis			
Nutlin	Inhibition of p53-MDM2 interaction	Preclinical	[58]
RITA	Inhibition of p53-MDM2 interaction	Preclinical	[59]
MI-219	Inhibition of p53-MDM2 interaction	Preclinical	[65]
RG7112	Inhibition of p53-MDM2 interaction	Phase I	[67]
3. Restoration of mutant p53 to wild-type p53			
PRIMA-1	Restores both DNA contact and structural p53 mutant to the wild-type conformation	Phase I/II	[70]
PhiKan083	Binds to a unique pocket in p53-mutant Y220C and stabilizes p53 mutant Y220C	Preclinical	[73]
NSC319726	Specifically restores wild-type p53 activity of p53 mutant R175H	Preclinical	[74]
CP31398	Stabilizes the core domain of mutant p53	Preclinical	[75]
SCH529074	Binds to DNA binding domain of mutant p53 and stabilizes mutant p53	Preclinical	[76]
Ellipticine	Restores the transcription activity of mutant p53	Preclinical	[77]
WR1065	Restores the wild-type conformation of the temperature-sensitive p53 mutant V272M	Preclinical	[80]
p53R3	Restores DNA binding of R175H and R273H p53 mutants	Preclinical	[78]
4. Targeting p53 family proteins			
RETRA	Increases the p73 levels and releases p73 from Mutant p53/p73 complex	Preclinical	[87]
37AA	Binds to iASPP, resulting in the release of p73 from iASSP/p73 complex	Preclinical	[88]
5. Eliminating mutant p53			
17AAG	Decreases mutant p53 by destroying the complex of mutant p53 and HSP90 to release mutant p53 for its degradation	Preclinical	[89]
SAHA	Degrades mutant p53 by inhibition of the HDAC6-Hsp90 chaperone axis	Preclinical	[90]
6. p53-based vaccines			
p53-SLP	A p53 synthetic long peptide vaccine	Phase I/II	[95]
INGN-225	A p53-modified adenovirus-transduced dendritic cell vaccine	Phase I/II	[96]

The crystal structure of the MDM2-p53 complex has been determined [62], which provides the possibility to screen and design inhibitors of MDM-p53 interaction using computer-based methodologies. Computational structure-based screening has identified NSC279287 [63] and NSC66811 [64], which disrupt the interaction between MDM2 and p53 proteins. Structure-based *de novo* design strategy has identified MI219. MI-219 is a second class of Mdm2 inhibitors. Similar to nutlin-3, MI-219 blocks the interaction of p53 with Mdm2 by mimicking critical residues of the p53-Mdm2 complex interface. Functionally, MI-219 activates the p53 pathway and triggers apoptosis in p53 wild-type cancer cells, but not in p53 mutant cancer cells. In a mouse model with wild-type p53 human cancer xenografts, MI-219 induces tumor regression with little toxicity to normal tissues [65, 66].

The first MDM2 inhibitor that was developed in clinical trial is RG7112 (Hoffmann-La Roche, USA). RG7112 is a member of the nutlin family with improved potency and pharmacological properties. RG7112 binds MDM2 with high affinity, blocking its interactions with p53. RG7112 activates the p53 pathway, leading to cell cycle arrest and apoptosis in wild-type p53 expressing cancer cells. RG7112 treatment shows the best apoptotic response observed in osteosarcoma cells with MDM2 gene amplification. Currently, phase I clinical trials have been conducted in patients with advanced solid tumors, hematologic neoplasms, or liposarcomas prior to debulking surgery. Preliminary clinical data indicate that RG7112 appears to be well tolerated in patients and provides initial evidence that clinical activity is consistent with targeting the MDM2-p53 interaction [67].

The key limitation of inhibitors of the p53-MDM2 interaction is that they are only effective in wild-type p53 expressing cancer cells, and have no effect in mutant p53-expressing cancer cells. In addition, it is important to consider the effect of p53 overexpression on wild-type p53-expressing normal cells. p53 over-expression in normal cells may be toxic. High level expression of wild-type p53 induces cell-cycle arrest and apoptosis in sensitive tissues, including the hematopoietic system, intestinal epithelium and other organs with a high proliferative index [68]. The lethality imposed by p53 expression in MDM2-null mice indicates the risk of triggering p53 in normal tissues, at least during development [69].

3.3. Restoration of Mutant p53 to Wild-Type p53

Mutant p53 is specifically overexpressed in tumor cells, representing a tumor-specific target. The restoration of the overexpressed mutant p53 to wild-type p53 could result in massive apoptosis of tumor cells. High through-put screening of chemical libraries and *in silico* screens with computational techniques has identified an increasing number of small molecules that could restore wild-type function of mutant p53, including PRIMA-1 [70], MIRA-3 [71], STIMA-1 [72], PhiKan083 [73], NSC319726 [74], CP31398 [75], SCH529074 [76], Ellipticine [77] and p53R3 [78]. The PRIMA-1 derivative, PRIMA-1^{MET} is the first compound to enter the clinical development.

PRIMA-1 is a promising compound and its optimized derivative PRIMA-1^{MET} (APR-246) has been tested in a phase 1 clinical trial. PRIMA-1 appears to specifically inhibit the growth of p53 mutant cancer cells. PRIMA-1 rescues both DNA contact and structural p53 mutants by restoring sequence-specific DNA binding and the wild-type p53 conformation *In vitro*. *In vivo* study has shown potent antitumor activity of PRIMA-1 alone or in combination with cisplatin with no apparent toxicity in lung and osteosarcoma xenografts [70]. Recently, the human phase I trial of PRIMA-1^{MET} (APR-246) was completed in patients with acute myeloid leukemia and prostate cancer. APR-246 was well tolerated with a favorable pharmacokinetic profile. Treatment of APR-246 in patients induced cell-cycle arrest, apoptosis and transcription of p53 target genes NOXA, PUMA, and BAX. One patient with acute myeloid leukemia who had a p53 core domain mutation showed a reduction of blast percentage from 46% to 26% in the bone marrow after APR-246 treatment [79].

PhiKan083, a carbazole derivative, was identified by *in silico* analysis of the crystal structure of p53 Y220C mutant protein using virtual screening and rational drug design. PhiKan083 can selectively bind to a unique pocket in p53 Y220C mutant protein, and stabilize the p53 Y220C mutant. PhiKan083 significantly increases Y220C mutant melting temperature and slows down its rate of denaturation. The detailed biological activity of the compound is still to be assessed [73].

NSC319726 was identified by *in silico* analysis with the NCI60 cell-line panel. The screening was designed to identify compounds with increased sensitivity in a panel of cell lines carrying p53 mutations, relative to wild-type p53 tumor cells. NSC319726 was found to restore wild-type p53 activity in R175H-mutant cancer cell lines. NSC31397 displays anti-tumor activity in specific p53 R172H (equivalent to human R175H) mutant genetically engineered mice, and selectively inhibits xenograft tumor growth of R175H-mutant p53 cancer cells [75].

Additional compounds targeting mutant p53 include (a) CP31398, which stabilizes the core domain of mutant p53 protein, increases DNA binding and transcriptional activity, and shows anti-tumor efficacy in colon cancer and melanoma mice models [75]; (b) SCH529074, which binds to the DNA binding domain of mutant p53, stabilizes mutant p53 and induces p53-dependent apoptosis [76]; (c) Ellipticine, which restores the transcriptional activity of mutant p53 [77]; (d) WR1065, the active metabolite of amifostine, which restores the wild-type conformation of the temperature-sensitive V272M p53 mutant, resulting in enhanced transcriptional induction of p21, GADD45 and MDM2, and leading to G1 cell cycle arrest [80]; and (e) p53R3, which restores DNA binding of R175H and R273H p53 mutants, strongly induces DR5 expression, and sensitizes cancer cells to TRAIL-induced apoptosis [78].

3.4. Targeting p53 Family Proteins

p53 family proteins p63 and p73 share a high degree of sequence homology with p53 and regulate the expression of similar genes by binding to p53 responsive elements within gene promoters and perform similar functions to p53. p63 and p73 can functionally replace p53 [81, 82]. Like the gene therapy using adenovirus delivered wild-type p53, the same approach has been extended to p73 and p63. Studies have indicated that the adenovirus mediated delivery of p63 and p73 into tumor cells is an efficient gene therapy approach, even better than p53-delivered gene therapy. Ad-p73 activates p21 and induces significant cell cycle arrest and apoptosis in multiple cancer cell lines. Ad-p73 sensitizes p53 mutant cancer cells to adriamycin with a higher efficiency than Ad-p53. Importantly, Ad-p73 infection does not induce apoptosis in human normal cells [83]. Ad-p63 induces apoptosis in osteosarcoma cells that are resistant to Ad-p53 mediated apoptosis. The apoptosis-inducing effects of Ad-p63γ are also found to be greater than Ad-p53 in osteosarcoma cells with Mdm2 amplification. Intratumoral injection of Ad-p63 significantly suppresses tumor growth in human osteosarcoma xenografts. p63 sensitizes osteosarcoma cells to the chemotherapeutic agents doxorubicin and cisplatin [84].

p73 function has been found to be inhibited by the interaction with mutant p53 [85]. Thus, although p73 expression is up-regulated, it is likely that the increased p73 is inhibited by interaction with the overexpressed mutant p53 in cancer cells. Short-interfering mutant p53 (SIMP) peptides are designed based on mutant p53/p73 binding regions. Indeed, SIMP effectively disrupts the interaction of mutant p53 with p73 and restores p73-mediated transcriptional activity. SIMP sensitizes mutant p53 cancer cells to adriamycin and cisplatin. Of note, the effects of SIMP are mutant p53-specific and SIMP has no effect on wild-type p53 and p53-null cancer cells [86]. Small molecule RETRA was identified in a cell-based screening for compounds that reactivate the transcriptional activity of p53 in mutant p53 cancer cells. RETRA activates a number of p53 target genes and selectively inhibits the growth of p53 mutant cancer cells both *In vitro* and *In vivo*. Mechanistically, RETRA increases p73 levels and releases p73 from the complex of mutant p53/p73 [87]. Taken together, these studies have demonstrated that disruption of the interaction of mutant p53 and p73 is a promising approach for effective cancer therapy.

37AA is a p53-derived peptide. The 37AA induces cell death through binding to iASPP, a common negative regulator of p53 family members, resulting in the release of TA-p73 from iASSP/TA-p73 complex. Nanoparticle delivery of a vector expressing this peptide causes tumor regression *In vivo* via p73, and tumors with p73 knockdown are resistant to the peptide [88].

3.5. Eliminating Mutant p53

Mutant p53 acquires dominant negative activity and oncogenic function, and is highly expressed in the majority of human tumors. Thus, targeting mutant p53 for degradation can be explored as a therapeutic strategy to manage tumors that depend on mutant p53 for survival. For example, therapeutic administration of p53 siRNA to cancer patients is a promising direction. *In vitro* experiments have shown that down-regulation of mutant p53 by siRNA inhibits cancer cell growth. Small molecules that degrade mutant p53 are also promising for cancer therapy. The molecular chaperone, HSP90 can interact mutant p53, consequently stabilizing the mutant p53 conformation. The inhibitor of HSP90, 17AAG can decrease mutant p53 by destroying the complex of mutant p53 and HSP90 to release mutant p53 for its degradation [89]. Histone deacetylase, HDAC6 is a positive regulator of HSP90 chaperone activity by regulating HSP90 deacetylation. Histone deacetylase (HDAC) inhibitor SAHA exhibits preferential cytotoxicity for mutant p53 human cancer cells, due to its ability to degrade mutant p53 by inhibition of the HDAC6-HSP90 chaperone axis [90]. Arsenic trioxide, a drug for the treatment of acute promyelocytic leukemia, and Gambogic acid, a Chinese medicine, are both found to degrade mutant p53 [91, 92].

3.6. p53-Based Vaccines

Besides the functional restoration of p53 activity, a totally different strategy of targeting p53 mutant tumors, p53 vaccine has entered phase I/II clinical trials. Tumor-specific and high expression of mutant p53 in human cancers, make p53 a promising target antigen. Wild-type p53 protein has a

short half-life and is therefore present in very low levels in normal cells. This differential level of p53 expression between normal and cancer cells could provide a basis for p53 immunotherapy [93]. The antibodies against p53 have been found in patients with various types of cancer, which indicates that the human immune system can recognize and respond to tumor-associated p53 [94]. Speetjens *et al.* reported a phase I/II clinical trial of a p53 synthetic long peptide (p53-SLP) vaccine for the treatment of metastatic colorectal cancer. The p53-SLP vaccine was non-toxic and p53-specific T-cell responses were induced in 9 of 10 colorectal cancer patients [95]. In a phase I/II clinical trial of INGN-225, a p53-modified adenovirus-transduced dendritic cell vaccine for the treatment of small cell lung cancer was reported. INGN-225 was well tolerated and a specific anti-p53 immune response was observed in 18 out of 43 (41.8%) patients. INGN-225 appeared to sensitize SCLC to subsequent chemotherapy [96].

4. CHALLENGES OF P53 TARGETING

From a conventional point of view, p53 is a challenging target for drug discovery. p53 does not offer the accessibility of a receptor-ligand interaction or an enzyme active site. Instead, it is a tetrameric transcription factor with complicated protein-protein interactions. There have been many obstacles in the development of p53 targeted therapy. These are some of the critical challenges:

4.1. Complexity of the p53 Pathway

p53 signaling is a complex network involving p53, the p53 family of proteins (p63 and p73), their various isoforms, p53 regulators (MDM2) and p53 downstream target genes, together with numerous posttranslational modification of p53. In human cancer, thousands of different p53 mutations have been detected although there are common hotspots for mutation within its DNA-binding domain. Different missense mutations in p53 could confer unique structures and activities, and thereby produce different mutant p53 proteins. A p53-targeted drug may only target a particular p53 mutant, and therefore the drug may only be effective in cancer patients with a particular p53 mutant in the tumor. For example, NSC319726 selectively restores the activity of the R175H-mutant of p53, so NSC319726 may only be effective for the treatment of cancer patients whose tumor harbors the R175H-mutant of p53. Clearly, it is important to know the mechanism by which the p53 pathway is deficient in cancer patients, to determine patient stratification for p53-targeted therapy and to choose the right p53-targeted drug for treatment. Taken together, therapeutic targeting of the p53 pathway has various levels of complexity.

4.2. Lack of an Apoptotic Response to p53- Targeted Therapy

p53 reactivation in tumors may only induce cell cycle arrest rather than apoptosis, which may lead to recurrence of the tumor once the drug is withdrawn. Endogenous activation of p53 triggers apoptosis in some tumor types, with lymphomas showing the most drastic apoptotic response. However, in some solid tumors with a low apoptotic index, cell cycle arrest and senescence are the most prominent re-

sponses to p53 restoration [97]. In addition, not all tumor cells are likely to possess sufficient stress signals to activate a restored p53 protein. The restored p53 protein may not be activated in a significant number of tumor cells with low cellular stress thereby providing a mechanism of escape from the therapy. In lung cancer mouse models, studies have shown that p53 restoration selectively targets high-grade tumor cells with high oncogenic stress [98, 99]. These studies have implied that, although p53 restoration will kill high-grade malignant tumor cells, low-grade tumor cells driven by low-level oncogenic signals would presumably survive to lead to relapse of the tumor.

4.3. Acquired Resistance to p53-Targeted Therapy

p53-targeted therapy may select for emergence of p53 therapy-resistant tumors. Martins *et al.* found that tumors treated with p53 restoration therapy exhibited resistance to p53 function. Inactivation of p19^{ARF} is a frequent mechanism by which tumors acquire resistance to the therapeutic impact of p53 functional restoration [100]. In addition, p53-targeted therapy can lead to the acquisition of somatic mutations in the p53 gene. Aziz *et al.* have found that p53 wild-type cancer cells acquired mutations in the p53-DNA-binding domain when these cells were grown in repeated nutlin treatments. The individual clones with acquired mutations of p53 were resistant to both apoptosis and growth arrest induced by nutlin [101].

4.4. Metabolic Adaptation to p53-Targeted Therapy

In vitro or *In vivo* modeling of p53-targeted therapy evaluated p53 responses including cell cycle arrest and apoptosis. p53 function impacts highly diverse biological processes such as metabolism, angiogenesis, metastasis, stem cell homeostasis and age. Recent evidence has shown that regulation of metabolism by p53 may not only prevent tumor formation, but also increase tumor survival. p53-mediated metabolic adaptation may prevent the apoptotic response, promote the long-term survival of cancer cells and increase the risk of relapse. Thus, tumor cells could benefit from wild-type p53 function. It was recently suggested that the presence of wild-type p53 in primary glioblastoma could protect tumor cells from glucose deprivation and the detrimental effects of hypoxia by restraining glycolysis [102]. Thus, p53-mediated metabolic function further complicates therapeutic targeting of the p53 pathway.

5. NEW OPPORTUNITIES

Despite a number of challenges, some promising advances have been reported in the field. These advances could provide new opportunities to improve p53-targeted therapy. p53 restoration compounds, such as PRIMA-1^{MET}, have started to emerge in clinical trials and have the potential to impact on therapy of cancer patients.

5.1. Personalized Selection of Potentially Efficacious p53-Targeted Therapy

Due to the complexity of the p53 pathway, cancer patients could have a different p53 status and expression. The precise therapeutic strategy in any given patient will need to be tailored to the mechanism by which the p53 pathway has been disrupted. The development of a tumor p53 biomarker profile could provide guidance for the most appropriate application of these strategies in cancer patients. For example, if the tumor p53 biomarker profile indicates that the patient has a tumor expressing mutant p53, treatment with a drug that restores mutant p53 to wild-type p53 would be more appropriate than another approach to p53 activation. In a study of p53 gene therapy in recurrent squamous cell carcinoma of the head and neck (SCCHN), novel biomarkers predictive of p53 gene therapy efficacy that may guide individualized patient treatment were described. The tumor p53 biomarker profile classified the patients into two groups: one group included patients with wild-type p53 or low mutant p53 expression predicted to be favorable for p53 gene therapy and the other group included patients with high mutant p53 expression predicted to be unfavorable for p53 gene therapy. The study showed that patients with an unfavorable tumor p53 profile had significantly reduced tumor response and survival as compared with patients with a favorable tumor p53 profile. Thus, a p53 biomarker profile permits personalized selection of potentially efficacious p53-targeted therapy for the treatment of cancer [51].

5.2. Combination Therapy

Targeting multiple signaling pathways to achieve increased cancer cell killing is a widely accepted concept in cancer therapy. A new opportunity to be explored is the combination of p53-targeted therapy with conventional chemotherapeutic drugs or other molecularly targeted therapies. Many of the chemotherapeutic drugs currently used induce cell cycle arrest or apoptosis through activation of p53, and therefore mutant p53 reactivation may increase the sensitivity of chemo-resistant tumors to conventional chemotherapeutic drugs. Nutlin has been shown to potentiate the p53-dependent apoptosis induced by chemotherapeutic drugs such as doxorubicin, chlorambucil, or fludarabine [103]. The p53-targeted therapy can also produce synergistic antitumor killing when combined with other molecularly targeted therapy. Sullivan *et al.* have established a genome-wide short hairpin RNA screen for genes that are lethal in combination with p53 activation by Nutlin-3. The data have demonstrated that inhibition of the ATM or the MET signaling pathways could synergize with nutlin in inducing p53-dependent apoptosis [104]. Cheok *et al.* have shown that CDK inhibitors roscovitine and DRB synergize with nutlin-3a in inducing p53 activity and promoting p53-dependent apoptosis [105]. Clearly, the combination of conventional chemotherapeutic drugs or other molecularly targeted therapy with p53-targeted drugs may result in a synergistic therapeutic effect.

5.3. Optimization of p53 Restoration Therapy

Restoration of p53 function may select for the emergence of tumor cell populations that are resistant to p53 function by abrogating p53-activating signals, i.e., loss of p19^{ARF} or amplification of MDM2 or other mechanisms. Shchors *et al.* found that sustained p53 restoration drives rapid emergence of resistant tumor cells, however the repeated transient imposition of p53 restoration does not lead to development of such resistance, as observed by their sensitivity to undergo

apoptosis subsequently in response to additional rounds of transient p53 restoration. Thus, intermittent dosing regimes of drugs that restore wild-type p53 function may be more efficacious than traditional chronic dosing by reducing adaptive resistance. The data make intermittent therapy worthy of consideration in future clinical trial designs for p53-targeted agents [106]. However, intermittent p53 restoration typically delays, rather than stops, cancer progression. The application of combination therapy with other anticancer strategies will be more beneficial for patients than p53 restoration alone. Optimization of p53 restoration therapy provides an opportunity to achieve maximal therapeutic efficacy and decrease tumor resistance.

CONFLICT OF INTEREST

The authors confirm that this article content has no conflicts of interest.

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LIST OF ABBREVIATIONS

MDM2/HDM2	=	Murine / human double minute 2
LFS	=	Li-Fraumeni Syndrome
LOH	=	Loss of heterozygosity
MDR1	=	Multidrug resistance 1
MRN	=	Mre11-Rad5-NBS1
Ad-p53	=	Adenoviral delivery of wild-type p53
SIMP	=	Short-interfering mutant p53 peptides
HDAC	=	Histone deacetylase
p53-SLP	=	p53 synthetic long peptide
SCLC	=	Small cell lung cancer
SCCHN	=	Squamous cell carcinoma of the head and neck

REFERENCES

[1] Vogelstein B, Kinzler KW. Cancer genes and the pathways they control. Nat Med 2004; 10: 789-99.

[2] Prabhu VV, Allen JE, Hong B, *et al.* Therapeutic targeting of the p53 pathway in cancer stem cells. Expert Opin Ther Targets 2012; 16: 1161-74.

[3] Chen F, Wang W, El-Deiry WS. Current strategies to target p53 in cancer. Biochem Pharmacol 2010; 80: 724-30.

[4] Wang W, El-Deiry WS. Restoration of p53 to limit tumor growth. Curr Opin Oncol 2008; 20: 90-6.

[5] Lu C, El-Deiry WS. Targeting p53 for enhanced radio- and chemosensitivity. Apoptosis 2009; 14: 597-606.

[6] Stegh AH. Targeting the p53 signaling pathway in cancer therapy - the promises, challenges and perils. Expert Opin Ther Targets 2012; 16: 67-83.

[7] Brosh R, Rotter V. When mutants gain new powers: news from the mutant p53 field. Nat Rev Cancer 2009; 9: 701-13.

[8] Goh AM, Coffill CR, Lane DP. The role of mutant p53 in human cancer. J Pathol 2011; 223: 116-26.

[9] Hollstein M, Rice K, Greenblatt MS, *et al.* Database of p53 gene somatic mutations in human tumors and cell lines. Nucleic Acids Res 1994; 22: 3551-5.

[10] Freed-Pastor WA, Prives C. Mutant p53: one name, many proteins. Genes Dev 2012; 26: 1268-86.

[11] Mai PL, Malkin D, Garber JE, *et al.* Li-Fraumeni syndrome: report of a clinical research workshop and creation of a research consortium. Cancer Genet 2012; 205: 479-87.

[12] Rivlin N, Brosh R, Oren M, Rotter V. Mutations in the p53 Tumor Suppressor Gene: Important Milestones at the Various Steps of Tumorigenesis. Genes Cancer 2011; 2: 466-74.

[13] Hanel W, Moll UM. Links between mutant p53 and genomic instability. J Cell Biochem 2012; 113: 433-9.

[14] Vousden KH, Ryan KM. p53 and metabolism. Nat Rev Cancer 2009; 9: 691-700.

[15] Muller PA, Vousden KH, Norman JC. p53 and its mutants in tumor cell migration and invasion. J Cell Biol 2011; 192: 209-18.

[16] Donehower LA, Harvey M, Slagle BL, *et al.* Mice deficient for p53 are developmentally normal but susceptible to spontaneous tumours. Nature 1992; 356: 215-21.

[17] Robles AI, Harris CC. Clinical outcomes and correlates of TP53 mutations and cancer. Cold Spring Harb Perspect Biol 2010; 2: a001016.

[18] Willis A, Jung EJ, Wakefield T, Chen X. Mutant p53 exerts a dominant negative effect by preventing wild-type p53 from binding to the promoter of its target genes. Oncogene 2004; 23: 2330-8.

[19] Harvey M, Vogel H, Morris D, *et al.* A mutant p53 transgene accelerates tumour development in heterozygous but not nullizygous p53-deficient mice. Nat Genet 1995; 9: 305-11.

[20] Milner J, Medcalf EA. Cotranslation of activated mutant p53 with wild type drives the wild-type p53 protein into the mutant conformation. Cell 1991; 65: 765-74.

[21] Kern SE, Pietenpol JA, Thiagalingam S, *et al.* Oncogenic forms of p53 inhibit p53-regulated gene expression. Science 1992; 256: 827-30.

[22] Dittmer D, Pati S, Zambetti G, *et al.* Gain of function mutations in p53. Nat Genet 1993; 4: 42-6.

[23] Vikhanskaya F, Lee MK, Mazzoletti M, Broggini M, Sabapathy K. Cancer-derived p53 mutants suppress p53-target gene expression--potential mechanism for gain of function of mutant p53. Nucleic Acids Res 2007; 35: 2093-104.

[24] Zhu HB, Yang K, Xie YQ, *et al.* Silencing of mutant p53 by siRNA induces cell cycle arrest and apoptosis in human bladder cancer cells. World J Surg Oncol 2013; 11: 22.

[25] Yan W, Liu G, Scoumanne A, Chen X. Suppression of inhibitor of differentiation 2, a target of mutant p53, is required for gain-of-function mutations. Cancer Res 2008; 68: 6789-96.

[26] Bossi G, Lapi E, Strano S, *et al.* Mutant p53 gain of function: reduction of tumor malignancy of human cancer cell lines through abrogation of mutant p53 expression. Oncogene 2006; 25: 304-9.

[27] Olive KP, Tuveson DA, Ruhe ZC, *et al.* Mutant p53 gain of function in two mouse models of Li-Fraumeni syndrome. Cell 2004; 119: 847-60.

[28] Jung MS, Yun J, Chae HD, *et al.* p53 and its homologues, p63 and p73, induce a replicative senescence through inactivation of NF-Y transcription factor. Oncogene 2001; 20: 5818-25.

[29] Lin J, Teresky AK, Levine AJ. Two critical hydrophobic amino acids in the N-terminal domain of the p53 protein are required for the gain of function phenotypes of human p53 mutants. Oncogene 1995; 10: 2387-90.

[30] Deb S, Jackson CT, Subler MA, Martin DW. Modulation of cellular and viral promoters by mutant human p53 proteins found in tumor cells. J Virol 1992; 66: 6164-70.

[31] Frazier MW, He X, Wang J, *et al.* Activation of c-myc gene expression by tumor-derived p53 mutants requires a discrete C-terminal domain. Mol Cell Biol 1998; 18: 3735-43.

[32] Zalcenstein A, Stambolsky P, Weisz L, *et al.* Mutant p53 gain of function: repression of CD95(Fas/APO-1) gene expression by tumor-associated p53 mutants. Oncogene 2003; 22: 5667-76.

[33] Bossi G, Marampon F, Maor-Aloni R, *et al.* Conditional RNA interference *In vivo* to study mutant p53 oncogenic gain of function on tumor malignancy. Cell Cycle 2008; 7: 1870-9.

[34] Khromova NV, Kopnin PB, Stepanova EV, Agapova LS, Kopnin BP. p53 hot-spot mutants increase tumor vascularization via ROS-mediated activation of the HIF1/VEGF-A pathway. Cancer Lett 2009; 276: 143-51.

[35] Gohler T, Jager S, Warnecke G, *et al.* Mutant p53 proteins bind DNA in a DNA structure-selective mode. Nucleic Acids Res 2005; 33: 1087-100.

[36]   Bargonetti J, Chicas A, White D, Prives C. p53 represses Sp1 DNA binding and HIV-LTR directed transcription. Cell Mol Biol (Noisy-le-grand) 1997; 43: 935-49.

[37]   Chicas A, Molina P, Bargonetti J. Mutant p53 forms a complex with Sp1 on HIV-LTR DNA. Biochem Biophys Res Commun 2000; 279: 383-90.

[38]   Sampath J, Sun D, Kidd VJ, *et al.* Mutant p53 cooperates with ETS and selectively up-regulates human MDR1 not MRP1. J Biol Chem 2001; 276: 39359-67.

[39]   Do PM, Varanasi L, Fan S, *et al.* Mutant p53 cooperates with ETS2 to promote etoposide resistance. Genes Dev 2012; 26: 830-45.

[40]   Di Agostino S, Strano S, Emiliozzi V, *et al.* Gain of function of mutant p53: the mutant p53/NF-Y protein complex reveals an aberrant transcriptional mechanism of cell cycle regulation. Cancer Cell 2006; 10: 191-202.

[41]   Song H, Hollstein M, Xu Y. p53 gain-of-function cancer mutants induce genetic instability by inactivating ATM. Nat Cell Biol 2007; 9: 573-80.

[42]   Clayman GL, el-Naggar AK, Roth JA, *et al. In vivo* molecular therapy with p53 adenovirus for microscopic residual head and neck squamous carcinoma. Cancer Res 1995; 55: 1-6.

[43]   Zhang WW, Fang X, Mazur W, *et al.* High-efficiency gene transfer and high-level expression of wild-type p53 in human lung cancer cells mediated by recombinant adenovirus. Cancer Gene Ther 1994; 1: 5-13.

[44]   Yang C, Cirielli C, Capogrossi MC, Passaniti A. Adenovirus-mediated wild-type p53 expression induces apoptosis and suppresses tumorigenesis of prostatic tumor cells. Cancer Res 1995; 55: 4210-3.

[45]   Hamada K, Zhang WW, Alemany R, *et al.* Growth inhibition of human cervical cancer cells with the recombinant adenovirus p53 *In vitro*. Gynecol Oncol 1996; 60: 373-9.

[46]   Kim J, Hwang ES, Kim JS, *et al.* Intraperitoneal gene therapy with adenoviral-mediated p53 tumor suppressor gene for ovarian cancer model in nude mouse. Cancer Gene Ther 1999; 6: 172-8.

[47]   Kock H, Harris MP, Anderson SC, *et al.* Adenovirus-mediated p53 gene transfer suppresses growth of human glioblastoma cells *In vitro* and *In vivo*. Int J Cancer 1996; 67: 808-15.

[48]   Lane DP, Cheok CF, Lain S. p53-based cancer therapy. Cold Spring Harb Perspect Biol 2010; 2: a001222.

[49]   Kawabe S, Munshi A, Zumstein LA, *et al.* Adenovirus-mediated wild-type p53 gene expression radiosensitizes non-small cell lung cancer cells but not normal lung fibroblasts. Int J Radiat Biol 2001; 77: 185-94.

[50]   Zhu JX, Li ZM, Geng FY, *et al.* [Treatment of recurrent malignant gliomas by surgery combined with recombinant adenovirus-p53 injection]. Zhonghua Zhong Liu Za Zhi 2010; 32: 709-12.

[51]   Nemunaitis J, Clayman G, Agarwala SS, *et al.* Biomarkers Predict p53 Gene Therapy Efficacy in Recurrent Squamous Cell Carcinoma of the Head and Neck. Clin Cancer Res 2009; 15: 7719-25.

[52]   Yang ZX, Wang D, Wang G, *et al.* Clinical study of recombinant adenovirus-p53 combined with fractionated stereotactic radiotherapy for hepatocellular carcinoma. J Cancer Res Clin Oncol 2010; 136: 625-30.

[53]   Pan JJ, Zhang SW, Chen CB, *et al.* Effect of recombinant adenovirus-p53 combined with radiotherapy on long-term prognosis of advanced nasopharyngeal carcinoma. J Clin Oncol 2009; 27: 799-804.

[54]   Zeimet AG, Marth C. Why did p53 gene therapy fail in ovarian cancer? Lancet Oncol 2003; 4: 415-22.

[55]   Freedman DA, Wu L, Levine AJ. Functions of the MDM2 oncoprotein. Cell Mol Life Sci 1999; 55: 96-107.

[56]   Momand J, Jung D, Wilczynski S, Niland J. The MDM2 gene amplification database. Nucleic Acids Res 1998; 26: 3453-9.

[57]   Jones SN, Hancock AR, Vogel H, Donehower LA, Bradley A. Overexpression of Mdm2 in mice reveals a p53-independent role for Mdm2 in tumorigenesis. Proc Natl Acad Sci USA 1998; 95: 15608-12.

[58]   Vassilev LT, Vu BT, Graves B, *et al. In vivo* activation of the p53 pathway by small-molecule antagonists of MDM2. Science 2004; 303: 844-8.

[59]   Issaeva N, Bozko P, Enge M, *et al.* Small molecule RITA binds to p53, blocks p53-HDM-2 interaction and activates p53 function in tumors. Nat Med 2004; 10: 1321-8.

[60]   Grasberger BL, Lu T, Schubert C, *et al.* Discovery and cocrystal structure of benzodiazepinedione HDM2 antagonists that activate p53 in cells. J Med Chem 2005; 48: 909-12.

[61]   Tovar C, Rosinski J, Filipovic Z, *et al.* Small-molecule MDM2 antagonists reveal aberrant p53 signaling in cancer: implications for therapy. Proc Natl Acad Sci USA 2006; 103: 1888-93.

[62]   Kussie PH, Gorina S, Marechal V, *et al.* Structure of the MDM2 oncoprotein bound to the p53 tumor suppressor transactivation domain. Science 1996; 274: 948-53.

[63]   Galatin PS, Abraham DJ. A nonpeptidic sulfonamide inhibits the p53-mdm2 interaction and activates p53-dependent transcription in mdm2-overexpressing cells. J Med Chem 2004; 47: 4163-5.

[64]   Lu Y, Nikolovska-Coleska Z, Fang X, *et al.* Discovery of a nanomolar inhibitor of the human murine double minute 2 (MDM2)-p53 interaction through an integrated, virtual database screening strategy. J Med Chem 2006; 49: 3759-62.

[65]   Shangary S, Qin D, McEachern D, *et al.* Temporal activation of p53 by a specific MDM2 inhibitor is selectively toxic to tumors and leads to complete tumor growth inhibition. Proc Natl Acad Sci USA 2008; 105: 3933-8.

[66]   Yu S, Qin D, Shangary S, *et al.* Potent and orally active small-molecule inhibitors of the MDM2-p53 interaction. J Med Chem 2009; 52: 7970-3.

[67]   Saha MN, Qiu L, Chang H. Targeting p53 by small molecules in hematological malignancies. J Hematol Oncol 2013; 6: 23.

[68]   Gudkov AV, Komarova EA. The role of p53 in determining sensitivity to radiotherapy. Nat Rev Cancer 2003; 3: 117-29.

[69]   Ringshausen I, O'Shea CC, Finch AJ, Swigart LB, Evan GI. Mdm2 is critically and continuously required to suppress lethal p53 activity *In vivo*. Cancer Cell 2006; 10: 501-14.

[70]   Bykov VJ, Issaeva N, Shilov A, *et al.* Restoration of the tumor suppressor function to mutant p53 by a low-molecular-weight compound. Nat Med 2002; 8: 282-8.

[71]   Bykov VJ, Issaeva N, Zache N, *et al.* Reactivation of mutant p53 and induction of apoptosis in human tumor cells by maleimide analogs. J Biol Chem 2005; 280: 30384-91.

[72]   Zache N, Lambert JM, Rokaeus N, *et al.* Mutant p53 targeting by the low molecular weight compound STIMA-1. Mol Oncol 2008; 2: 70-80.

[73]   Boeckler FM, Joerger AC, Jaggi G, *et al.* Targeted rescue of a destabilized mutant of p53 by an in silico screened drug. Proc Natl Acad Sci USA 2008; 105: 10360-5.

[74]   Yu X, Vazquez A, Levine AJ, Carpizo DR. Allele-specific p53 mutant reactivation. Cancer Cell 2012; 21: 614-25.

[75]   Foster BA, Coffey HA, Morin MJ, Rastinejad F. Pharmacological rescue of mutant p53 conformation and function. Science 1999; 286: 2507-10.

[76]   Demma M, Maxwell E, Ramos R, *et al.* SCH529074, a small molecule activator of mutant p53, which binds p53 DNA binding domain (DBD), restores growth-suppressive function to mutant p53 and interrupts HDM2-mediated ubiquitination of wild type p53. J Biol Chem 2010; 285: 10198-212.

[77]   Peng Y, Li C, Chen L, Sebti S, Chen J. Rescue of mutant p53 transcription function by ellipticine. Oncogene 2003; 22: 4478-87.

[78]   Weinmann L, Wischhusen J, Demma MJ, *et al.* A novel p53 rescue compound induces p53-dependent growth arrest and sensitises glioma cells to Apo2L/TRAIL-induced apoptosis. Cell Death Differ 2008; 15: 718-29.

[79]   Lehmann S, Bykov VJ, Ali D, *et al.* Targeting p53 *In vivo*: a first-in-human study with p53-targeting compound APR-246 in refractory hematologic malignancies and prostate cancer. J Clin Oncol 2012; 30: 3633-9.

[80]   North S, Pluquet O, Maurici D, El-Ghissassi F, Hainaut P. Restoration of wild-type conformation and activity of a temperature-sensitive mutant of p53 (p53(V272M)) by the cytoprotective aminothiol WR1065 in the esophageal cancer cell line TE-1. Mol Carcinog 2002; 33: 181-8.

[81]   Jost CA, Marin MC, Kaelin WG, Jr. p73 is a simian [correction of human] p53-related protein that can induce apoptosis. Nature 1997; 389: 191-4.

[82]   Bisso A, Collavin L, Del Sal G. p73 as a pharmaceutical target for cancer therapy. Curr Pharm Des 2011; 17: 578-90.

[83]   Das S, Nama S, Antony S, Somasundaram K. p73 beta-expressing recombinant adenovirus: a potential anticancer agent. Cancer Gene Ther 2005; 12: 417-26.

[84]   Oshima Y, Sasaki Y, Negishi H, *et al.* Antitumor effect of adenovirus-mediated p53 family gene transfer on osteosarcoma cell lines. Cancer Biol Ther 2007; 6: 1058-66.

[85]   Di Como CJ, Gaiddon C, Prives C. p73 function is inhibited by tumor-derived p53 mutants in mammalian cells. Mol Cell Biol 1999; 19: 1438-49.

[86]   Di Agostino S, Cortese G, Monti O, *et al.* The disruption of the protein complex mutantp53/p73 increases selectively the response of tumor cells to anticancer drugs. Cell Cycle 2008; 7: 3440-7.

[87]   Kravchenko JE, Ilyinskaya GV, Komarov PG, *et al.* Small-molecule RETRA suppresses mutant p53-bearing cancer cells through a p73-dependent salvage pathway. Proc Natl Acad Sci USA 2008; 105: 6302-7.

[88]   Bell HS, Dufes C, O'Prey J, *et al.* A p53-derived apoptotic peptide derepresses p73 to cause tumor regression *In vivo*. J Clin Invest 2007; 117: 1008-18.

[89]   Li D, Marchenko ND, Schulz R, *et al.* Functional inactivation of endogenous MDM2 and CHIP by HSP90 causes aberrant stabilization of mutant p53 in human cancer cells. Mol Cancer Res 2011; 9: 577-88.

[90]   Li D, Marchenko ND, Moll UM. SAHA shows preferential cytotoxicity in mutant p53 cancer cells by destabilizing mutant p53 through inhibition of the HDAC6-Hsp90 chaperone axis. Cell Death Differ 2011; 18: 1904-13.

[91]   Yan W, Zhang Y, Zhang J, *et al.* Mutant p53 protein is targeted by arsenic for degradation and plays a role in arsenic-mediated growth suppression. J Biol Chem 2011; 286: 17478-86.

[92]   Wang J, Zhao Q, Qi Q, *et al.* Gambogic acid-induced degradation of mutant p53 is mediated by proteasome and related to CHIP. J Cell Biochem 2011; 112: 509-19.

[93]   DeLeo AB. p53-based immunotherapy of cancer. Crit Rev Immunol 1998; 18: 29-35.

[94]   Chada S, Mhashilkar A, Roth JA, Gabrilovich D. Development of vaccines against self-antigens: the p53 paradigm. Curr Opin Drug Discov Devel 2003; 6: 169-73.

[95]   Speetjens FM, Kuppen PJ, Welters MJ, *et al.* Induction of p53-specific immunity by a p53 synthetic long peptide vaccine in patients treated for metastatic colorectal cancer. Clin Cancer Res 2009; 15: 1086-95.

[96]   Chiappori AA, Soliman H, Janssen WE, Antonia SJ, Gabrilovich DI. INGN-225: a dendritic cell-based p53 vaccine (Ad.p53-DC) in small cell lung cancer: observed association between immune response and enhanced chemotherapy effect. Expert Opin Biol Ther 2010; 10: 983-91.

[97]   Ventura A, Kirsch DG, McLaughlin ME, *et al.* Restoration of p53 function leads to tumour regression *In vivo*. Nature 2007; 445: 661-5.

[98]   Feldser DM, Kostova KK, Winslow MM, *et al.* Stage-specific sensitivity to p53 restoration during lung cancer progression. Nature 2010; 468: 572-5.

[99]   Junttila MR, Karnezis AN, Garcia D, *et al.* Selective activation of p53-mediated tumour suppression in high-grade tumours. Nature 2010; 468: 567-71.

[100]   Martins CP, Brown-Swigart L, Evan GI. Modeling the therapeutic efficacy of p53 restoration in tumors. Cell 2006; 127: 1323-34.

[101]   Aziz MH, Shen H, Maki CG. Acquisition of p53 mutations in response to the non-genotoxic p53 activator Nutlin-3. Oncogene 2011; 30: 4678-86.

[102]   Wanka C, Brucker DP, Bahr O, *et al.* Synthesis of cytochrome C oxidase 2: a p53-dependent metabolic regulator that promotes respiratory function and protects glioma and colon cancer cells from hypoxia-induced cell death. Oncogene 2011; 31: 3764-76.

[103]   Coll-Mulet L, Iglesias-Serret D, Santidrian AF, *et al.* MDM2 antagonists activate p53 and synergize with genotoxic drugs in B-cell chronic lymphocytic leukemia cells. Blood 2006; 107: 4109-14.

[104]   Sullivan KD, Padilla-Just N, Henry RE, *et al.* ATM and MET kinases are synthetic lethal with nongenotoxic activation of p53. Nat Chem Biol 2012; 8: 646-54.

[105]   Cheok CF, Dey A, Lane DP. Cyclin-dependent kinase inhibitors sensitize tumor cells to nutlin-induced apoptosis: a potent drug combination. Mol Cancer Res 2007; 5: 1133-45.

[106]   Shchors K, Persson AI, Rostker F, *et al.* Using a preclinical mouse model of high-grade astrocytoma to optimize p53 restoration therapy. Proc Natl Acad Sci USA 2013; 110: E1480-9.