REVIEW

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Molecularly targeted therapies for p53-mutant cancers

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Abstract The tumor suppressor p53 is lost or mutated in approximately half of human cancers. Mutant p53 not only loses its anti-tumor transcriptional activity, but also often acquires oncogenic functions to promote tumor proliferation, invasion, and drug resistance. Traditional strategies have been taken to directly target p53 mutants through identifying small molecular compounds to deplete mutant p53, or to restore its tumor suppressive function. Accumulating evidence suggest that cancer cells with mutated p53 often exhibit specific functional dependencies on secondary genes or pathways to survive, providing alternative targets to indirectly treat p53-mutant cancers. Targeting these genes or pathways, critical for survival in the presence of p53 mutations, holds great promise for cancer treatment. In addition, mutant p53 often exhibits novel gain-of-functions to promote tumor growth and metastasis. Here, we review and discuss strategies targeting mutant p53, with focus on targeting the mutant p53 protein directly, and on the progress of identifying genes and pathways required in p53-mutant cells.

Keywords p53 mutation · Targeted therapies ·
Survival pathway · Gain of function

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Introduction

The p53 protein is a tumor suppressor that serves as a genomic guardian to maintain a dynamic balance between cell growth and cell arrest in response to genomic stress [1–4]. As a transcription factor, the p53 gene contains a core DNA-binding domain, a transcriptional activation domain, and a tetramerization domain [5, 6]. p53 is normally expressed at low levels, and its expression is stabilized and increased upon various genotoxic and cellular stress signals, such as DNA damage, hypoxia, oncogene activation, and nutritional deprivation [7]. These stress signals stimulate the binding of p53 to a specific DNA sequence, activating or repressing a set of downstream targeting genes that can regulate cell cycle arrest, apoptosis, or DNA repair. Thus, dysfunction of p53 will disrupt the balance of cell growth and arrest, allowing abnormal cells to proliferate and develop into cancer.

In contrast to traditional tumor suppressors that are often reduced or deleted in human cancers, p53 is more commonly mutated in most human cancers [8, 9]. Genomic sequencing data across different human cancers reveal that p53 gene is the most frequently and commonly mutated tumor suppressor gene in human cancers, with over 50% of cancers harboring somatic p53 mutations [10–12]. The mutation rate is even higher in certain cancer subtypes, such as ovarian serous carcinomas, squamous lung cancer, and triple-negative breast cancer [13–15]. The presence of mutated p53 has been associated with poor prognosis in various tumor types [16, 17]. In general, over 75% of p53 mutations occur as missense mutations with a single amino acid change in the core DNA-binding domain, resulting in loss of its transcriptional activity and accumulation of dysfunction p53 protein [18]. Of these mutations, there are several hotspots that occur with a higher frequency across

cancer types. These hotspots include amino acids R175, Y220, R248, and R273. Additionally, two distinct types of p53 point mutations are frequently observed in cancers: conformational mutations, and DNA contact mutations. Conformational mutations of p53 disrupt the structure of p53 and abolish DNA-binding ability, while DNA contact mutants alter amino acids that directly bind DNA [19, 20]. Both mutations cause p53 to lose its transcription activity and gain dominate-negative (DN) activity over the remaining wildtype allele, through hetero-oligomerization with wildtype p53 [21]. Drugs have been developed to induce the degradation of mutant p53 or restore its wild-type function. Furthermore, increasing evidence suggests that mutant p53 also acquires new oncogenic functions (gain-of-function, GOF mutations) to increase tumor progression, metastasis, and drug resistance [22–26]. Therefore, p53 mutations cause loss of tumor suppressive activity, as well as gain of oncogenic activity to promote tumor development, posing an attractive druggable target for cancer therapy.

Compounds that directly target mutant p53

Given that p53 mutations are more frequent in cancers than normal cells, targeting mutant p53 through restoration of its wildtype tumor suppressive function (Table 1) or induction of its degradation (Table 2) has the potential to selectively kill cancer cells. Therefore, strategies have been taken to identify and develop small molecular compounds that

could directly and specifically target p53-mutant p53 for effective anticancer therapy (Fig. 1) [1].

Compounds that restore wildtype p53 function

CP-31398

CP-31398 is a styrylquinazoline identified from a high throughput screen for therapeutic agents that restore the wildtype epitope on the DNA-binding domain of the p53 protein [27, 28]. Studies found that CP-31398 not only restores p53 function in mutant p53-expressing cells but also significantly increases the protein expression of wildtype p53 through the stabilization of exogenous p53 in p53-mutant human cell lines. CP-31398 treatment increases the expression of p21 and cell cycle arrest. In addition, CP-31398 induces mitochondrial translocation of p53, leading to changes in mitochondrial membrane permeability pore transition and subsequent cytochrome c release and apoptosis in cancer cells [29]. Treatment of CP-31398 dramatically inhibited tumor growth of both melanoma and colon xenografts with naturally mutated p53 [28]. The compound appears to be safe at the dose of 200 mg/kg/day, as no mortality was observed for mice treated with CP-31398 for fourteen consecutive days. Tang et al. demonstrated that CP-31398 restored the tumor suppressive activity of UVB-induced mutant p53. Administration of CP-31398 not only inhibited the growth, but also prevented the development of UVB-induced skin cancers [29]. These findings suggest that CP-31398 has potential applications

Table 1 Compounds that restore wildtype function of p53

Compounds	Target mutants	Mechanisms of action	References
CP-31398	V173A, S241F, R249S, R273H	Stabilize the DNA-binding core domain and induce conformational change	[27, 29]
P53R3	R175H, R273H	Restore sequence-specific DNA-binding and p53 transcriptional activities	[30]
NSC319726	R175H, R172H	Restore wildtype p53 conformation and activity with MDM2-dependent degradation	[34]
PK7088	Y220C	Bind to a p53Y220C-specific surface cavity and stabilize p53Y220C with restored wildtype p53 conformation	[36]
Chetomin	R175H	Increase Hsp40 (DNAJB1) levels and Hsp40-p53R175H binding, restoring wildtype p53 conformation, activity, and MDM2- dependent degradation	[37, 38]
PEITC	R175H	Sensitize the p53(R175H) mutant to proteasome-mediated degradation and further restore p53 WT conformation and transactivation	[39, 40]
RITA	R175H, R248Q, R273H, R280K	Reactivate p53 in mutant p53 cancers by inhibiting the p53–HDM2 interaction	[41–43]
COTI-2	R175H, R273H	Restore wildtype p53 activity by targeting and binding to misfolded p53 mutant	[46, 47]
PRIMA-1 and PRIMA-1^{Met}	R175H, R273H	Bind to thiol groups in the core domain and restore wildtype conformation	[49, 50, 52, 54]

Table 2 Compounds that induce degradation of mutant p53

Compounds	Target mutants	Mechanisms of action	References
Hsp90 inhibitors	R175H, L194F, R248Q, R273H, R280K	Reverse the Hsp90's function to inactivate MDM2 and CHIP	[56, 57]
Statins	V157F, R172H, R175H, Y220C, R248W, R273H, R280K	Induce CHIP-dependent degradation of p53 with conformational or misfolded changes	[61]
HDAC inhibitors	R175H, R280K	Inhibit HDAC6 and disrupt the HDAC6/Hsp90/mutant p53 complex	[56–59]
Gambogic Acid	R175H, G266E, R273H, R280K	Inhibit the mutant p53-Hsp90 complex and induce CHIP-dependent degradation or induce autophagy	[64, 65]
YK-3-237	V157F, M237I, R249S, R273H, R280K	Decrease mutant p53 levels through deacetylation at lysine 382 by activating SIRT1	[66]
Capsaicin	R175H, R273H	Unknown	[67]

Fig. 1 Strategies to target mutant p53 in cancers. Two approaches have been used to target mutant p53 for cancer treatment. The first approach is to using small molecular compounds to directly target mutant p53 by induction of its degradation or reactivation of its tumor suppressive transcriptional activity. Several well-studied small molecular compounds are listed in the left part of this figure. The alternative approach is to targeting pathways that are critical for the survival and growth of p53-mutant cancers. Specific molecular targets, including G2/M regulators, kinases and are listed in the right part of this figure
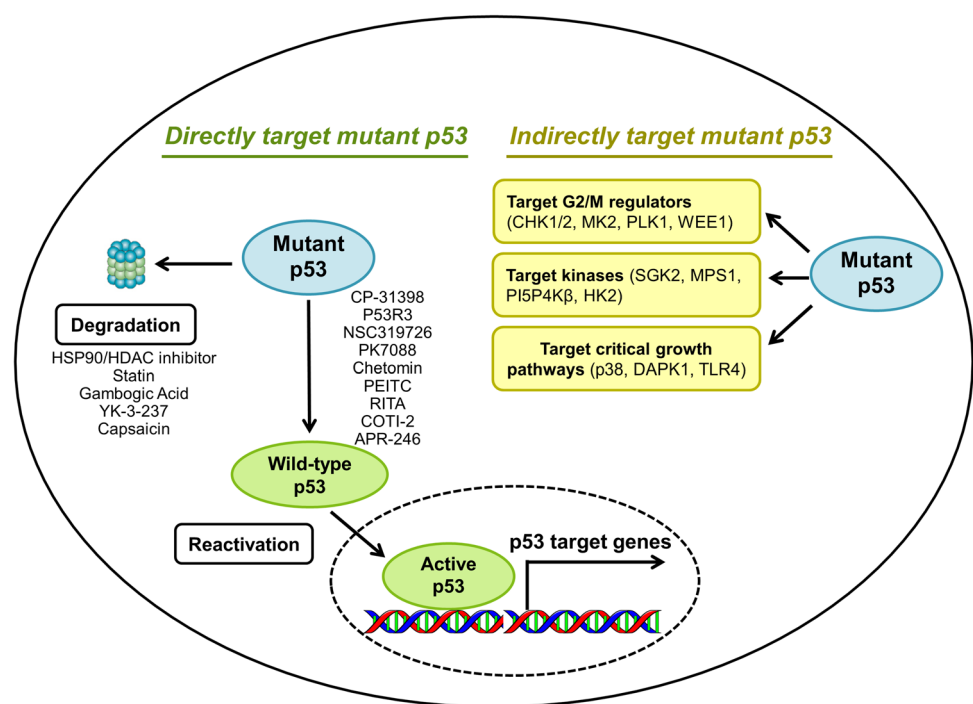
for the treatment as well as chemoprevention for mutant p53 cancers.

P53R3

Another novel p53 "restorer", named P53R3, was identified from a small library of compounds using an in vitro DNA-binding assay [30]. It restores sequence-specific DNA binding of endogenously expressed p53^{R175H} and p53^{R273H} mutants in glioma cell lines. P53R3 treatment inhibits cell proliferation by inducing the expression of p53 target genes, including MDM2, p21, PUMA and BAX. The P53R3-mediated increase of p53 target genes seems to be relatively more specific to mutant p53 cells, as little effects

were observed in wildtype p53 cells. Furthermore, P53R3 strongly enhances expression of death receptor 5 (DR5) at the cell surface and sensitizes the cell to Apo2L/TRAIL-induced cell death [30]. This new p53 rescue compound opens up novel opportunities for the treatment of p53-mutant cancers.

NSC319726

It is known that p53 binds a single zinc ion near its DNA-binding interface, which is critical for proper folding, site-specific DNA binding, and transcriptional activation [31]. Insufficient zinc causes misfolding and functional loss of p53 [32]. Treating tumor-bearing mice with zinc has been

shown to restore DNA-binding activity of mutant p53, leading to tumor inhibition [33]. Using the National Cancer Institute's anticancer drug screen data, Yu et al. identified a compound named NSC319726 from the thiosemicarbazone family that exhibited selective growth inhibitory activity against mutant p53^{R175H}, but not wildtype p53 cells [34]. NSC319726 treatment restores wildtype structure and function of the p53^{R175H} mutant and upregulates p53 target genes such as p21, MDM2 and PUMA [34]. NSC319726 may also increase the degradation of p53^{R175H}. Although high dose (10 mg/kg/day) of NSC319726 shows strong toxicity to both p53^{WT} and p53^{R175H} mice, a lower dose (5 mg/kg/day) induces xenograft inhibition with extensive apoptosis only in p53^{R172H}, but not in p53^{WT} mice [34]. Thus, NSC319725 functions as an effective activator of p53^{R175H} mutant and could be used for the treatment of p53^{R175H} expressing cancers.

PK7088

The p53-mutant Y220C has a unique surface crevice that can be targeted by small molecular stabilizers [35]. PK7088Was identified from a compound library screen, and was found to specifically bind and stabilize the p53^{Y220C} mutant. It restores wildtype p53 conformation and increases the expression of p21 and the proapoptotic protein NOXA [36]. Consequently, treatment of PK7088 induces p53^{Y220C}-dependent G2/M cell-cycle arrest, apoptosis and growth inhibition in cancer cells [36]. In addition, PK7088Works synergistically with Nutlin-3 to further upregulate expression of p21 and NOXA [36].

Chetomin

Chetomin was identified as a specific mutant p53^{R175H} activator from cell-based luciferase-reporter screen [37]. It restores wildtype p53 transactivation and upregulates MDM2, p21 and PUMA expression. In mouse xenograft models, chetomin selectively inhibits the growth of tumor cells harboring p53^{R175H}, but not p53^{R273H} [37]. Chetomin binds and increases the interaction of Hsp40 with p53^{R175H}, leading to a conformational change of p53^{R175H} and restoration of wildtype p53 function [37]. However, further studies found that chetomin also suppresses tumor growth of colon cancer expressing wildtype p53, suggesting that chetomin may exert anti-cancer effects independently of mutant p53 [38].

PEITC

The natural compound PEITC (phenethyl isothiocyanate), derived from cruciferous vegetables, was recently demonstrated to reactivate the wildtype function of p53 mutant in cancer cells [39]. Aggarwal et al. discovered that PEITC exhibits growth inhibitory activity in cancer cells expressing p53^{R175H} [40]. Mechanistically, PEITC restores the wildtype conformation and transactivation of the p53^{R175H} mutant. It also sensitizes the p53^{R175H} mutant to proteasome-mediated degradation [40]. Accordingly, PEITC treatment in p53^{R175H} mutant cells induces apoptosis and a delay in S and G2/M phase, through the activation of canonical wildtype p53 targets. Further, dietary supplementation of PEITC in xenograft mouse model significantly inhibited tumor growth in vivo [40]. No difference in body weights was observed between control and PEITC-treated groups, suggesting the safety of this natural compound. These findings provide the first example of mutant p53 reactivation by a dietary compound, and have important implications for the treatment of p53^{R175H} mutant cancers.

RITA

Reactivation of p53 and induction of tumor cell apoptosis (RITA) is another compound that can reactivate p53 function [41]. It was originally identified as a molecule that inhibited growth of p53-wildtype HCT 116 cells, but not HCT 116 p53$^{-/-}$ cells, by inhibiting the p53–HDM2 interaction and inducing p53-target genes, such as p21 and PUMA [41]. Subsequent studies have demonstrated that RITA can also reactivate p53 in mutant p53 cancers, but not p53-null cell lines, by inducing apoptotic genes such as NOXA, p21, and GADD45, in addition to suppressing oncogenic genes such as N-Myc and Bcl-2 [42, 43]. However, another study in ovarian cancer showed that RITA-induced cell death occurred independently of p53, as RITA treatment induces caspase-dependent apoptosis in p53-null cells, as well as cells harboring wildtype p53 and mutant p53 [44]. RITA enhances cisplatin-induced cytotoxicity in p53-wildtype head and neck cancers [45], and additional studies showed that reactivation of p53 by RITA synergistically enhances gemcitabine-induced apoptosis in p53 mutant pancreatic cancer [45].

COTI-2

COTI-2 was discovered by a pharmaceutical company named Critical Outcome Technologies as an orally available third generation thiosemicarbazone with ability to restore wildtype p53 from misfolded p53 mutants (R175H, R273H) (http://criticaloutcome.com/coti-2-and-pipeline/coti-2/). COTI-2 also acts as a negative regulator of the PI3K/AKT/mTOR pathway [46]. Salim et al. recently demonstrated that COTI-2 treatment reduces cell proliferation and inhibits xenograft growth of multiple human cancer cell lines expressing either mutant p53 or wildtype

p53 [47]. This suggests that COTI-2 may affect mutant p53 in human tumors, but likely has additional effects on other targets in the PI3K/AKT/mTOR pathway in wildtype p53 tumors. Despite its potent anti-tumor efficacy, COTI-2 was well tolerated and did not affect mice weights in vivo [47]. Although the anti-tumor mechanisms of COTI-2 are still under investigation, it is currently undergoing a Phase I clinical trial at MD Anderson Cancer Center for the treatment of advanced or recurrent gynecologic malignancies (www.clinicaltrials.gov, NCT02433626).

PRIMA-1 and PRIMA-1^{Met} (APR-246)

PRIMA-1 was identified as a small molecule with the ability to suppress growth of p53^{R175H} and p53^{R273H} cells through the reactivation of p53 target genes and induction of apoptosis by binding to the core DNA-binding domain [34, 48, 49]. This occurs by forming the compound methylene quinuclidinone, which covalently binds to thiol groups in mutant p53, refolding p53 into a wildtype conformation, thus restoring its anti-tumor transcriptional activity. Further testing of PRIMA-1 demonstrated that its methylated analogue, PRIMA-1^{Met} (APR-246), is more potent and less toxic than the parental PRIMA-1 [50]. In SCLC (small cell lung cancer) lines expressing mutant p53, APR-246 treatment induces apoptosis through activation of caspase-3 and upregulation of pro-apoptotic proteins such as Bax and Noxa. Moreover, APR-246 significantly inhibits in vivo tumor growth of p53-mutant SCLC models without apparent toxicity [51]. Since many chemotherapy drugs depend on wildtype p53 for induction of tumor cell apoptosis, the restoration of wildtype p53 by APR-246 has the potential to sensitize p53-mutant cancers to these drugs. Indeed, APR-246 has showed strong synergy with traditional chemotherapy drugs such as cisplatin, 5-fluorouracil and doxorubicin in multiple p53-mutant expressing ovarian cancers [52]. Furthermore, mutant p53 targeting with APR-246 effectively eliminates resistance to the proteasome inhibitor carfilzomib [53]. In a triple-negative breast cancer xenograft model, APR-246 combined with carfilzomib not only synergistically reduced primary tumor growth, but also efficiently eradicated lymph node and lung metastasis [53].

APR-246 is another p53-reactivating drug that has undergone clinical trials. The phase I/IIa clinical trial of APR-246, conducted in individuals with hematological malignancies and prostate cancer, showed both effectiveness and safety in patients with mutation of p53 [54]. Combined APR-246 with carboplatin is currently being tested in phase Ib/II clinical trials for patients with recurrent high-grade serous ovarian cancer, 95% of which carry p53 mutations (http://www.clinicaltrials.gov, NCT02098343) [55].

Compounds that induce mutant p53 degradation

HSP90 and HDAC inhibitors

In human cancers, mutant p53 is more stable than wildtype p53. This is mainly due to the interaction of mutant p53 with the HDAC6/Hsp90 chaperone complex [56]. This complex stabilizes mutant p53 through preventing its degradation mediated by chaperone-associated E3 ubiquitin ligase. Therefore, disruption of HDAC6/HSP90 complex by either HDAC or Hsp90 inhibitors will induce the degradation of mutant p53 [57] (Fig. 1). Heat shock protein 90 inhibitors (17-AAG and the more potent ganetespib) and HDAC inhibitors (such as SAHA or vorinostat) have been shown to promote proteasome-dependent degradation of mutant p53 [56, 58]. These drugs are currently being tested in clinical trials for treatment of p53-mutant cancers. The Hsp90 inhibitor ganetespib entered phase III trials for the treatment of non-small cell lung cancer, but the trial was terminated following the first interim analysis because of futility [59]. However, ganetespib is still in phase I and II trials for other cancers with mutant p53, including acute myeloid leukemia, ovarian, and breast cancers [59].

Statins

Statins are a class of cholesterol-lowering compounds used to treat and prevent cardiovascular disease [60]. By conducting a chemical library screen to identify compounds that can degrade mutant p53, Parrales and colleagues recently discovered statins (lovastatin, atorvastatin and mevastatin) as biologically active compounds that preferentially induce degradation of p53 with conformational or misfolded mutation changes (V157F, R172H, R175H, Y220C, R248W, R273H, and R280K) [61]. Mechanistic studies revealed that lovastatin treatment inhibits the mevalonate-5-phosphate pathway and subsequently induces CHIP (carboxyl terminus of Hsp70-interacting protein) ubiquitin ligase-mediated nuclear export, ubiquitylation, and degradation of mutant p53 by inhibiting the interaction of mutant p53 with DNAJA1 (DnaJ Heat Shock Protein Family (Hsp40) Member A1) [61]. The effects of statins-mediated HMG-CoA inhibition and p53 degradation were highly specific to cancer cells expressing mutant p53 as minimal effects were observed in cancer cells expressing wildtype p53. Accordingly, lovastatin treatment reduces in vitro and in vivo tumor growth only in p53 mutant, but not in p53-wildtype cancers [61]. Furthermore, statins synergistically enhanced the anti-tumor effects of chemotherapeutic drugs such as doxorubicin exclusively in p53 mutant cancers [62]. Thus, inhibition of the

mevalonate pathway with statins may represent a novel and effective strategy to treat p53 mutant cancers.

Gambogic acid

A natural product derived from the *Garcinia hanburyi* tree, gambogic acid, upregulates protein expression of p53 and induces apoptosis of wildtype p53 cells [63]. It also down-regulates mutant p53 at post-transcription level by targeting the nuclear export of various p53 mutants for ubiquitination and subsequent protein degradation, mediated by the CHIP ubiquitin ligase [64]. Furthermore, gambogic acid induces mutant p53 degradation through autophagy in cancer cells expressing the $p53^{R280K}$ and the $p53^{S241F}$ proteins, as the inhibition of autophagy with bafilomycin A1 or chloroquine counteracted mutant p53 degradation by gambogic acid [65].

YK-3-237

The small molecule compound YK-3-237 was discovered to induce degradation of mutant p53 in triple-negative breast cancers (TNBCs) [66]. It reduces the acetylation of various p53 mutants (V157F, M237I R249S, R273H, and R280K) through activation of protein deacetylase SIRT1 (NAD-dependent deacetylase sirtuin-1) [66]. Deacetylation of mutant p53 destabilizes its protein level and upregulates the expression of p53-target genes including PUMA and NOXA [66]. YK-3-237 preferentially inhibits cell proliferation through induction of cell cycle arrest and PARP-dependent apoptotic cell death in TNBC harboring mutant p53. However, it remains to be determined whether YK-3-237 inhibits xenograft tumor growth of p53 mutant TNBCs.

Capsaicin

To explore natural compounds that induce degradation of mutant p53, Garufi et al. recently discovered that capsaicin, the constituent of peppers responsible for their pungency, could induce the protein degradation of mutant p53 (R175H, R273H) in both glioblastoma and breast cancer cell lines [67]. Abrogation of mutant p53 by capsaicin treatment restored wildtype p53 activities, such as upregulation of PUMA and Bax, and induction of cancer cell death. Interestingly, capsaicin also decreased the expression of MDR1 (multidrug resistance gene) and therefore sensitized tumors to chemotherapy drugs such as cisplatin [67]. However, the mechanism by which capsaicin induces degradation of mutant p53 remains unknown.

Targeting pathways that are critical for survival of p53 mutant cancers

The concept of synthetic lethality originates from studies in drosophila model systems, in which a combination of mutations in two or more separate genes leads to cell death [68]. In cancers, synthetic lethality can also occur by inhibiting a gene product in combination with another single-gene mutation [69]. The relevance of synthetic lethal screens is supported by multiple observations that oncogenic mutations, or tumor suppressor defects, may lead to the development of secondary dependencies in cancer cells [70]. Therefore, investigators are working to identify drugs targeting these critical survival pathways that specifically induce the death of cancer cells harboring oncogenic mutations [71, 72]. In light of its high mutational rate across different tumors, p53 provides an attractive target for identifying candidate genes or pathways that are synthetically lethal with p53 mutations [73]. The compounds that target these genes and pathways may selectively kill cancer cells expressing mutant p53 without affecting normal cells expressing wildtype p53 (Fig. 1).

Multiple approaches, including RNA interference and chemical screens, have been used in search for synthetic lethality to p53 mutations [11, 74]. Several different genes have shown synthetic lethality with p53 mutations, and these genes function through regulating cell cycle and critical kinase pathways. Targeted inhibition of these genes, or a block in relevant pathways, provides a unique therapeutic window to treat aggressive p53-mutant tumors.

Targeting the G2/M checkpoint

When p53 is mutated, the G1 arrest function of p53 is lost, causing the cell to rely solely on the G2/M checkpoint for DNA repair [4, 8]. Therefore, inhibition of cell cycle regulating genes or pathways that control the G2/M checkpoint can potentially induce synthetic lethality in p53-mutant cancers [75, 76]. Several G2/M checkpoint regulators, including CHK1/2, MK2, PLK1 and WEE1, have been identified as druggable targets in p53-mutant cancers (Fig. 1).

CHK1/2

The ATR/CHK1 signaling pathway is activated to regulate cell cycle arrest in response to explicative and genomic stress, such as DNA damage [77, 78]. ATR/CHK1 activation prevents collapse of DNA-single strand breaks and stalled replication forks, and inhibits cell-cycle progression through the G2/M checkpoint [79, 80].

Suppression of the G2/M checkpoint by inhibition of ATR/CHK1 in p53-mutant cells has been shown to induce loss of G1 and G2/M cell cycle checkpoints, resulting in cell death [81, 82]. UCN-01 was the first CHK1 inhibitor that exhibited preclinical synergistic effects in combination with several DNA-damaging agents including irinotecan, a topoisomerase I inhibitor [83–85]. Other potent and more selective CHK1 inhibitors, such as PF477736, A-690002, and SCH900776, have since been developed. These drugs potentiate the cytotoxicity of topoisomerase inhibitors and ionizing radiation in p53 mutant, but not p53-wildtype cancer cells [86, 87]. In a recent study, Ma et al. employed elegant human-in-mouse models of triple-negative breast cancer and showed that combination treatment of AZD7762, a highly selective CHK1 inhibitor, with irinotecan, significantly inhibited tumor growth and prolonged survival in mice bearing p53-mutant or -deficient tumors [88]. Furthermore, this selective CHK1/2 inhibitor can overcome the cisplatin resistance of head and neck p53-mutant cancer cells, reconfirming the feasibility of treating p53-mutant cancers with CHK inhibitors [89].

MK2

MAPKAP kinase 2 (MK2) is another important regulator of the G2/M checkpoint. In normal cells with wildtype p53, MK2 is a critical regulator of the cell cycle by sustaining the G2/M checkpoint in response to genotoxic stress or UV irradiation [90]. Synthetic lethality between MK2 and p53-deficiency following genotoxic stress was identified using RNA interference (RNAi)-mediated depletion of MK2 in p53-proficient and p53-deficient settings [91]. Knockdown of MK2 dramatically sensitized p53-deficient murine embryonic fibroblasts (MEFs), and H-RasV12-driven p53-deficient allografts, to the cytotoxic effects of cisplatin and the topoisomerase II inhibitor doxorubicin. In contrast, loss of MK2 in p53-proficient cells did not increase cell death or sensitivity to chemotherapy. Further mechanistic investigation revealed that MK2-depletion dramatically reduced phosphorylation of Cdc25A and B [91]. Subsequent studies by Morandell et al. were conducted with isogenic MK2-proficient and deficient non-small-cell lung cancer tumors, which were oncogenically driven by K-Ras and a lack of p53. Using this model, they demonstrated that $MK2^{-/-}$ tumors are more sensitive to cisplatin treatment than $MK2^{+/+}$ tumors, suggesting that MK2 is responsible for resistance of p53-deficient tumors to cisplatin [92]. Therefore, targeting inhibition of MK2 in p53-deficient or mutant cancers may sensitize chemotherapy agents such as cisplatin.

PLK1

Polo-like kinase 1 (PLK1) is an enzyme that controls G2/M checkpoint, and its inhibition has been shown to induce synthetic lethality in cells with p53 mutations [93, 94]. Transcriptome analysis revealed a consistent upregulation of PLK1 in P53-mutant ($p53^{R248W}$ and $p53^{S241F}$), but not wildtype p53 colorectal cancer lines. Inhibition of PLK1 by small molecular inhibitor BI-2536 significantly enhanced cytotoxic effects of ionizing radiation in p53-mutant, but not p53-wildtype cancers cells. Furthermore, BI-2536 treatment dramatically reduced the side effects of Nutlin-3 (MDM2 inhibitor) by protecting neutrophil depletion in nude mice bearing HCT116 $p53^{-/-}$ xenografts. Although clinical trials using PLK1 inhibitor as monotherapy have been terminated, the second generation of PLK1 inhibitor, GSK461364, has shown greater sensitivity in p53-mutated cancer compared with that of p53-wildtype cancer cells [95, 96]. Anti-mitotic agents such as PLK1 inhibitors may synergistically enhance the therapeutic efficacy of chemotherapy agents in p53-mutant tumors.

WEE1

WEE1 is another checkpoint kinase that mediates G2/M cell cycle arrest through phosphorylation of CHK1, which inhibits mitotic transition [97, 98]. Functional analysis identified WEE1 as an important survival factor in p53-mutant head and neck squamous cell carcinomas [99, 100]. The synthetic lethality between WEE1 and mutant p53 was established by studies showing that MK-1775, a selective WEE1 inhibitor, sensitizes p53-mutant cancer cells to DNA-damaging agents such as cisplatin [100]. Clinical trials utilizing a combination of MK-1775, carboplatin, and paclitaxel have been used in patients with p53-mutant ovarian cancer [101].

Targeting kinase pathways

SGK2/PAK3

SGK2 and PAK3 were identified as two novel kinases that when inhibited cause synthetic lethality with p53 dysfunction in cervical cancer cell lines in which p53 was inactivated by HPV infection [102]. While loss of p53, SGK2 or PAK3 alone did not significantly affect cell viability, loss of p53 in combination with depletion of either SGK2 or PAK2 led to cell death in primary human epithelial cells derived from tissues of cervical carcinoma. Two different mechanisms of synthetic lethality in p53-deficient cervical carcinoma were proposed: SGK2 depletion induces autophagy, and PAK3 depletion increases apoptosis. However, it remains to be determined whether

kinase activity of SGK2 or PAK3 is required for their synthetic interactions with p53 loss.

MPS1

Monopolar Spindle 1 (MPS1) kinase is a dual-specificity protein kinase that represents an essential component of the spindle assembly checkpoint [103, 104]. It functions in a number of mitosis steps, including regulation of activities at the kinetochore in both chromosome attachment and spindle checkpoint [105]. Using video microscopy and fluorescent TP53$^{+/+}$ and TP53$^{-/-}$ human colon carcinoma cells, Jemaa et al. discovered that SP600125, a serine–threonine kinase inhibitor which acts on MPS1, kills more p53-deficient cells but not p53-proficient cells [106]. This preferential cytotoxicity of MSP1 inhibition is dependent on p53. TP53$^{-/-}$ cells treated with SP600125 failed to undergo cell cycle arrest and became polyploid upon mitotic abortion, resulting in apoptosis. The gene that encodes MPS1 is also significantly correlated with p53 mutation in several breast cancer datasets [107]. Similarly, the inhibition of MSP1 by SP600125 reduced cell viability and increased cell death selectively in p53-mutant breast cancers [107]. In addition, MSP1 inhibition sensitized breast cancers to conventional chemotherapy treatments [107]. Therefore, MPS1 is a potential therapeutic target for TP53 mutated colon and breast cancers.

PI5P4Kβ

It has been shown that p53 supports cell survival by maintaining metabolic homeostasis by regulating mitochondrial respiration and limiting reactive oxygen species (ROS) [12, 108]. Thus, p53-mutant cells lack the ability to cope with metabolic changes in conditions of low ROS [109]. PI5P4Kβ (type 2 phosphatidylinositol-5-phosphate 4-kinase beta) was recently identified as a novel gene that, when inhibited, reduces the growth of p53-mutant breast cancers by inhibiting glucose metabolism and increasing levels of ROS [110, 111]. PI5P4Kβ is found frequently amplified in breast cancers, often in co-occurrence with a p53 mutation. Knockdown of PI5P4Kβ specifically impaired tumor growth in p53-deficient breast cancers. These novel findings suggest that inhibitors of PI5P4Kβ could be effective in treating cancers with p53 mutations.

HK2

Wan et al. recently identified an interaction between p53 and hexokinase-2 (HK2), a metabolic-related kinase highly expressed in many cancer cells [112]. HK2 catalyzes the phosphorylation of glucose in glycolysis, and increases glucose metabolism that is required for tumorigenesis

[113, 114]. The expression of HK2 was selectively upregulated by the combined loss of PTEN and p53 in prostate cancer. Genetic deletion of HK2 demonstrates that HK2-mediated aerobic glycolysis, known as the Warburg effect, is required for PTEN- and p53-deficient tumor growth in xenograft mouse models of prostate cancer [112]. Thus, targeting inhibition of HK2 might be effective for the treatment of prostate tumors with PTEN loss and a p53 mutation.

Targeting pathways that are critical for growth of p53-mutant cancers

P38

p38 kinases are members of the mitogen-activated protein kinase (MAPK) family, which transduce signals from environmental stresses, growth factors, inflammatory cytokines to regulate cell growth, differentiation and apoptosis [115]. During the onset of malignant transformation, p38 has been shown to exert tumor suppressive activity by phosphorylation and activation of p53, leading to cell cycle inhibition and apoptosis [116, 117]. However, increased p38 expression and activation has been correlated with poor survival in patients with breast cancer or liver cancer, suggesting the potential oncogenic role of p38 [118, 119]. Indeed, inhibition of p38 preferentially suppressed tumor growth of breast cancers expressing mutant, but not wildtype p53 [120]. Another study showed that p38 inhibition also sensitizes breast cancer cells to cisplatin-induced apoptosis [121]. Thus, targeting p38 by small molecular inhibitors may be clinically effective for the treatment of highly aggressive triple-negative breast cancers that harbor p53 mutations.

DAPK1

Death associated protein kinase 1 (DAPK1) is a calcium/calmodulin (CaM)-regulated protein kinase that activates death signaling in response to interferon-gamma (INF-γ), tumor necrosis factor-alpha (TNF-α), and transforming growth factor-beta (TGF-β) [122–124]. It has been shown to be highly expressed in ER negative breast cancers when compared to ER positive breast cancers [125]. Recent studies suggest that DAPK1 is essential for growth of p53-mutant cancers, which account for over 80% of TNBCs. DAPK1 expression is elevated in p53-mutant cancers compared to p53-wildtype cells. The depletion or inhibition of DAPK1 suppressed growth of p53-mutant, but not p53-wildtype breast cancer cells, suggesting that targeting DAPK1 may possess a therapeutic strategy for p53-mutant cancers.

TLR

The Toll-like receptors (TLR) play important role in activating early innate immunity in response to different pathogens and orchestrating late adaptive immune responses [126, 127]. The functions of at least two TLRs including TLR4 and TLR3 have been linked to mutant p53 cancers. Toll-like receptor 4 (TLR4) is expressed on the cell surface of immune cells to activate innate immune response to gram-negative bacterial lipopolysaccharide (LPS) [126, 127]. It is also expressed in breast epithelial tumors, and has oncogenic functions to promote tumor growth and drug resistance [128]. Therefore, TLR4 appears to be a promising target for immune-based therapeutic options and is the focus of many drugs currently in development. However, a recent study demonstrated that TLR4 activation promotes cell growth in p53-mutant breast cancers, but inhibits cell growth in p53-wildtype breast cancers [129]. The differential effects appear to be mediated by tumor cell cytokine secretions upon TLR4 activation. In p53-wildtype breast cancer cells, TLR4 activation increases type I IFN (IFN-γ), resulting in p21 expression and tumor inhibition. However, in p53-mutant breast cancer cells, TLR4 activation promotes tumor growth by inducing the secretion of CXCL1 and CD154. These findings suggest the need to determine p53 status for anti-TLR4 therapy, as the therapy may only be beneficial for p53-mutant tumors.

Recently studies suggest that wildtype p53 can amplify immune response against cancer cells by regulating the expression of immune-related TLRs including TLR2, TLR3 and TLR5 [130]. Menendez and colleagues transfected several specific p53 mutants (R138 V, P151H, R175H, H178Y) into HCT 116 p53$^{-/-}$ cells and found that those with p53 mutants decreased the expression of TLR3 and inhibited TLR3-mediated cytokine secretion and apoptotic response after stimulation by the cognate ligand ploy [131]. Furthermore, treatment with the p53 reactivating agent RITA rescued TLR3 expression and enhanced DNA damage-induced apoptosis via TLR3 signaling in a p53 mutant lymphoma cell line, Raji, that harbors loss-of-function alleles (R213Q and Y234H) [131]. Therefore, targeting mutant p53 to increase TLR3 expression may enhance anti-tumor immune responses in cancer cells harboring specific p53 mutations.

Targeting p53 "gain-of-function" pathways

The fact that most tumors contain p53 missense mutations, rather than p53 deletions, raises the possibility that tumor cells harboring p53 mutations may acquire advantages over cells lacking p53 [22]. Although mutations can

occur throughout p53 protein, the most common mutations occur in the DNA-binding domain, of which the majority are substitution mutations in six codons which contain CpG dinucleotide sequences (5 missense: R175, G245, R248, R273, R282; 1 nonsense: R213*) [132]. Accumulating evidence has shown that the mutations of p53 not only cause loss of transcriptional activity, but also allow the acquisition of novel oncogenic functions, which contribute to tumor development and progression. p53 mutants that localize in the cytoplasm have been shown to favor cancer cell survival by activating mTOR signaling. p53 mutants can also activate cell migratory pathways (e.g. PDGFRβ signaling, RhoA/ROCK, EGFR/integrin recycling, Myo10, Pla2g16) to promote cancer invasion and metastasis. Studies have also demonstrated that introducing p53 mutants into p53-null cancer cells resulted in an increase in tumor growth and enhanced tumor invasion through metabolic alterations, epigenetic modifications, and increased drug resistance, as detailed in Fig. 2 [26]. Therefore, targeted inhibition of the pathways that contribute to mutant p53′s GOF activities offers an alternative strategy for effectively treating p53-mutant cancers.
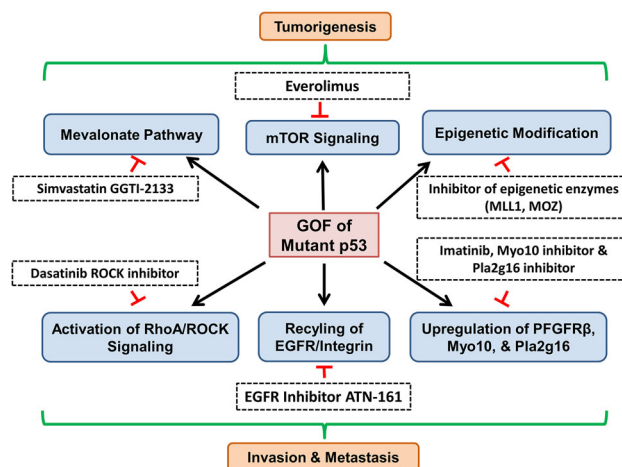

Fig. 2 Strategies to target pathways induced by p53 GOF mutants. Mutant p53 gains new functions to promote tumorigenesis by activation of the metabolic melanovate pathway or upregulation of epigenetic enzymes. Drugs that target the melanovate pathway (simvastatin, GGTI-2133) or epigenetic enzymes (MLL1, MOZ inhibitors) show promise for the treatment of p53-mutant cancers. Cytoplasmic mutant p53 also promote tumor growth through activation of mTOR signaling, providing an opportunity to using mTOR inhibitors to treat cancers with cytoplasmic mutant p53. In addition, mutant p53 often gains novel function(s) to promote tumor invasion and metastasis through activation of cell invasion pathways (PDGFRβ signaling, RhoA/ROCK, EGFR/integrin recycling, Myo10, Pla2g16). Small molecular inhibitors (ROCK inhibitor, EGFR inhibitor, Myo10 inhibitor and Pla2g16 inhibitor) have been used to target each pathway to inhibit the metastatic ability of p53-mutant cancers

Targeting mTOR pathway induced by gain-of-function p53 mutants

Autophagy is an intracellular process by which damaged organelles and macromolecules are targeted by lysosomes for degradation via autophagic vesicles [133]. Under normal conditions, autophagy functions to maintain cellular homeostasis by monitoring the integrity of long-lived proteins and organelles. Under cellular stress, such as nutrient starvation and oxidase stress, autophagy is crucial for maintaining primary biological activities to recycle intracellular contents as an alternative energy for cell survival [133]. Accumulating data indicate that autophagy plays tumour suppressive role during tumorigenesis [134]. Previous studies showed that genomic stress can induce autophagy in a p53-dependent fashion and wildtype p53 can activate autophagy by upregulating autophagy-related gene DRAM1 (damage-regulated autophagy modulator 1) [135]. However, several line of recent studies demonstrated that specific p53 mutants (R175H, R273H, R273L) that localize in the cytoplasmic can gain new function to inhibit autophagy either through blockade of AMPK signaling or activation of Akt/mTOR signaling [136, 137]. In contrast, other p53 mutants (P151H, R282W) that localize in the nucleus failed to inhibit autophagy [138]. Those cytoplasmic p53 mutants counteract the formation of autophagic vesicles and fusion with lysosomes through the repression of several key autophagy-related proteins and enzymes such as BECN1, DRAM1 and ATG12 [137]. While AMPK activation triggers autophagy by inducing degradation of macromolecules, mTOR signaling inhibits autophagy by stimulating anabolic biosynthesis for cancer cell growth [139]. Consequently, the inhibition of autophagy by cytoplasmic p53 mutants increases the proliferation and survival of cancer cells. In addition, the Akt/mTOR activation by GOF p53 mutants sensitizes cancer cells to the treatment with mTOR inhibitor everolimus [137]. Therefore, targeting inhibition of mTOR signaling to induce autophagy has potential therapeutic applications to treat human cancers harboring cytoplasmic p53 mutants.

Targeting p53-activated invasion pathways

RhoA/ROCK pathway

It is known that aerobic glycolysis is primarily utilized by tumor cells for energy production, a phenomenon known as the Warburg effect [140]. A novel mutant p53 GOF was shown to be a driving of the Warburg effect and promotion of tumorigenesis in a murine knock-in p53^{R172H} model [141]. Tumor formation was promoted by the translocation of the glucose transporter GLUT1 to the plasma membrane, which was induced by increased RhoA/ROCK signaling in p53^{R172H} knock-in mice. Similar studies using FLIM-FRET imaging to track RhoA activity in pancreatic cancer driven by the p53^{R172H} mutant found that this mutant p53 increased RhoA activity in vivo [142]. Dasatanib, a clinically used kinase inhibitor agent, inhibited the activity of RhoA and invasive ability of p53^{R172H} cells in vivo [141]. These studies therefore suggest that targeted inhibition of RhoA/ROCK signaling in p53-mutant cancers may inhibit tumorigenesis, and possibly metastasis.

EGFR/integrin recycling

Muller and coworkers showed that cells containing mutant p53 gain novel functions to promote cell migration and tumor metastasis [143]. They demonstrated that p53^{R175H} and p53^{R273H} mutants promote recycling of EGFR and integrin alpha-5 beta-1 (α5β1) to the cell surface, thereby increasing invasive and metastatic potential [143]. Recycling of integrin/EGFR by mutant p53 is linked to the transcriptional inhibition of p63. Consequently, the loss of p53 and p63 can phenocopy a p53-mutant cancer. Although the molecular details of how mutant p53 inhibits p63 remain to be determined, these findings suggest that inhibiting integrin α5β1integrin or blocking EGFR signaling (Cetuximab) may have therapeutic benefits in cancers with GOF p53 mutations.

PDGFRβ

Platelet-derived growth factor receptor beta (PDGFRβ) is mainly expressed by stromal cells, where PFGFRβ signaling contributes to tumor-associated invasion and metastasis [144, 145]. A recent study demonstrated that PDGFRβ signaling enhanced the expression of a GOF mutant p53 induced pancreatic cancer metastasis in PKC mice harboring one oncogenic allele of Kras^{G12D} and one allele of p53^{R172H} [146]. Inhibition of PDGFRβ using imatinib effectively prevented cell invasion and metastasis of pancreatic cancer with p53 mutations [146]. Given than over 90% of pancreatic cancers contain p53 mutations, these studies highlight the potential targeted therapy of blocking PDGFRβ signaling to treat p53-mutant pancreatic cancers.

AdPLA

Adipocyte phospholipase A2 (AdPLA), a transcript from the *PLA2G16* gene, was recently identified in expression array studies comparing primary osteosarcomas from metastatic p53$^{R172H/+}$ mice and non-metastatic p53$^{+/-}$ mice [147]. AdPLA is a phospholipase that catalyzes phosphatidic acid into lysophosphatidic acid and free fatty acid, both of which are implicated in metastasis. Xiong

et al. found that murine mutant p53^{R172H} increased *PLA2G16* expression in mouse osteosarcoma cells, suggesting that *PLA2G16* is a p53-regulated gene [147]. Indeed, ChIP analysis revealed that transcription factor EST2 recruited p53-mutant protein to bind the *PLA2G16* promoter at the E26 transformation specific binding motif. Functionally, AdPLA knockdown inhibited migration and invasion in mutant p53-expressing cells. These studies identify *PLA2G16* as a transcriptional target of mutant p53 and suggest that targeting AdPLA will inhibit invasion and metastasis of p53-mutant tumors.

Targeting cellular and nucleotide metabolism induced by gain-of-function p53 mutants

Several studies demonstrated that gain-of-function (GOF) p53 mutants regulate cellular and nucleotide metabolism [148–150]. Using a three-dimensional (3D) culture model, Freed-Pastor et al. discovered that breast cancer cell lines expressing either p53^{R273H} or p53^{R280K} disrupted acinar morphology through upregulation of the mevalonate pathway, which is responsible for cholesterol synthesis [148]. Treatment with simvastatin, a clinically approved statin that inhibits cholesterol synthesis, induced cell death in p53^{R273H} cells and reduced the invasive morphology of the p53^{R280K} cells. Geranylgeranyl transferase was identified to be the critical enzyme in this mevalonate pathway, as its inhibitor (GGTI-2133) significantly reduced the growth and 3D invasive morphology of p53^{R280K} cells. Mechanistic studies revealed that SREBPs (sterol regulatory element-binding protein) recruited mutant p53 to gene promoters that encode mevalonate pathway enzymes, which increased protein geranylgeranylation and altered acinar morphogenesis and promoted tumorigenesis [148]. These findings provide strong evidence for targeting the mevalonate pathway for the treatment of p53-mutant breast cancers.

A novel gain-of-function of mutant p53 in promoting cancer cell metabolism was reported in head and neck squamous cell carcinoma [23, 151]. Under energy stress conditions, mutant p53, but not wildtype p53, inhibits the activation of adenosine monophosphate-activated protein kinase (AMPK) by binding with its α subunit. Inhibition of AMPK by mutant p53 impaired metabolic checkpoint and increased aerobic tumor growth and progression [23]. Given that wildtype p53 could activate AMPK activity through transcriptional activation of the gene encoding β subunit of AMPK [152], these results strongly support a transcription-independent mechanism by which mutant p53 promotes tumor progression by activating cancer cell metabolism. Regulation of nucleotide metabolism by mutant p53 was recently found to be functionally important for GOF activities [150]. CHIP-seq analysis of mutant p53 knockdown breast cancer cells demonstrated that loss of mutant p53 reduced many nucleotide metabolism genes (NMGs) and substantially depleted nucleotide pools, which attenuated GTP-dependent protein activity and cell invasion [150]. The reduction of invasion by mutant p53 knockdown was rescued by addition of exogenous GTP, suggesting that mutant p53 promotes invasion by increasing GTP. Mutant p53 appears to depend on deoxycytidine kinase, a nucleoside salvage pathway enzyme, to maintain a proper balance of the dNTP pools required for cell proliferation [150]. These data reveal the mechanism underlying the lethal genetic interaction between mutant p53 and deoxycytidine kinase.

Targeting epigenetic changes induced by gain-of-function p53 mutants

A novel GOF mutant p53 activity was discovered by Zhu and colleagues, who demonstrated that chromatin regulation by mutant p53 can drive aggressive cancer growth. This group showed that mutant p53 regulates gene expression through induction of histone modifying proteins [153]. Using genome-wide measurements of p53 chromatin occupancy in a panel of breast cancer cell lines, Zhu et al. demonstrated that mutant p53, but not wildtype p53, is recruited to chromatin via interaction with transcription factor ETS2. This interaction led to binding and upregulation of genes encoding epigenetic enzymes, including the methyltransferases KMT2A (MLL1) and KMT2D (MLL2), and acetyltransferase KAT6A (MOZ), resulting in genome-wide increases of histone methylation and acetylation [153]. Chromatin regulatory genes, especially MLL1, appear to be responsible for cell proliferation driven by mutant p53, as genetic or pharmacological inhibition of MLL1 significantly attenuated cancer cell proliferation. In support of these observations, analysis of The Cancer Genome Atlas shows specific upregulation of epigenetic regulatory genes including MLL1, MLL2, and MOZ in GOF p53-mutant patient-derived tumors, but not in p53-wildtype or p53 null tumors [154]. GOF p53 mutants were also recently shown to act with the SWI/SNF chromatin-remodeling complex to upregulate VEGFR2, which contributed to tumorigenesis [155]. Taken together, the links between p53 mutation, chromatin regulation, and gene expression, could explain why so many genes are affected by the presence of mutant p53. In addition, these findings also highlight new therapeutic opportunities for designing combinatorial chromatin-based therapies to treat cancers harboring p53 mutations.

Targeting drug resistance induced by gain-of-function p53 mutants

An accumulating body of evidence suggests that GOF p53 mutants also mediate drug resistance through multiple

mechanisms, including inhibiting apoptotic proteins and gene regulations [10, 156]. Early studies have shown that p53 mutations correlated with resistance to chemotherapy drugs such as cisplatin, doxorubicin and paclitaxel [157, 158]. The first line evidence comes from studies demonstrating that knockdown of mutant p53 in human squamous cell carcinoma A431 cells harboring p53^{R273H} increases procaspase-3 and sensitizes cells to doxorubicin-induced apoptosis [159]. Conversely, transfection of p53^{R273H} into p53-null human osteosarcoma Saos-2 cells down-regulated procaspase-3 level and induced resistance to doxorubicin-induced apoptosis [159]. Further studies found that expressing p53^{R248Q} in p53-null liver cancer cells conferred cross-resistances to doxorubicin and paclitaxel through upregulation of P-glycoprotein, a known multidrug resistance protein that is responsible for decreased drug accumulation in multidrug-resistant cells [158]. Therefore, combination of P-glycoprotein inhibitors with doxorubicin may overcome drug resistance in p53-mutant cancers. Subsequently studies investigating mutant p53 binding sites in Li-Fraumeni cell line MDAH087 harboring p53^{R248W} revealed that mutant p53 regulates gene expression through binding to the promoters of ETS-binding motif and that ETS2 mediates the interaction with this motif [160]. Do et al. identified TDP2 (tyrosyl-DNA phosphodiesterase 2), a DNA phosphodiesterase involved in the repair of DNA damage caused by chemotherapy drug etoposide, as a transcriptional target of mutant p53. Consequently, inhibition of TDP2 sensitizes cells to the treatment of etoposide, a chemotherapy drug for lung cancer. Since mutant p53 and TDP2 are frequently overexpressed in lung cancer, TDP2 may serve as a "druggable" target to increase chemotherapy sensitivity for p53-mutant lung cancer. In pancreatic cancers, Fiorini and colleagues recently demonstrated that the expression of mutant p53 confers chemoresistance to gemcitabine [161]. Gemcitabine treatment induces phosphorylation and nuclear stabilization of mutant p53 which further upregulate Cdk1 and CCNB1, leading to increased cell proliferation. Restoration of wildtype p53 function by p53-reactivating agent (RITA and CP-31398) induced apoptosis, resulting in synergistic anti-tumor effects with gemcitabine [161].

Concluding remarks and future directions

The fact that most cancers have mutation of p53 makes this molecule an attractive therapeutic target. Several approaches, including inactivation of mutant p53, degradation of mutant p53, and restoration of the wildtype function of p53, have been studied. However, these strategies often work only for specific p53 mutations and not for others. An alternative strategy, targeting signaling pathways essential in cells p53 mutations, has been effective in many types of cancers. RNA interference and chemical genetic screens have enabled investigators to identify molecules critical for survival or growth of cells with p53 mutations that can be targeted for the selective treatment of p53-mutant cancers. Ongoing studies continue to identify such critical mutant p53-specific survival and growth regulatory pathways. Thus, novel drugs that target mutant p53 or the critical pathways activated by p53 mutation are highly promising for effective treatment of many cancers.

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