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# Resistance and gain-of-resistance phenotypes in cancers harboring wild-type p53

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## Abstract

Chemotherapy is the bedrock for the clinical management of cancer, and the tumor suppressor p53 has a central role in this therapeutic modality. This protein facilitates favorable antitumor drug response through a variety of key cellular functions, including cell cycle arrest, senescence, and apoptosis. These functions essentially cease once p53 becomes mutated, as occurs in ~50% of cancers, and some p53 mutants even exhibit gain-of-function effects, which lead to greater drug resistance. However, it is becoming increasingly evident that resistance is also seen in cancers harboring wild-type p53. In this review, we discuss how wild-type p53 is inactivated to render cells resistant to antitumor drugs. This may occur through various mechanisms, including an increase in proteasomal degradation, defects in post-translational modification, and downstream defects in p53 target genes. We also consider evidence that the resistance seen in wild-type p53 cancers can be substantially greater than that seen in mutant p53 cancers, and this poses a far greater challenge for efforts to design strategies that increase drug response in resistant cancers already primed with wild-type p53. Because the mechanisms contributing to this wild-type p53 "gain-of-resistance" phenotype are largely unknown, a concerted research effort is needed to identify the underlying basis for the occurrence of this phenotype and, in parallel, to explore the possibility that the phenotype may be a product of wild-type p53 gain-of-function effects. Such studies are essential to lay the foundation for a rational therapeutic approach in the treatment of resistant wild-type p53 cancers.

### Keywords

Tumor suppressor p53; Antitumor drug response; Drug resistance; Gain-of-function mutants; Gain-of-resistance phenotype; Post-translational modifications

## 1. Introduction

Chemotherapy is a critical modality in the fight against cancer. Indeed, antitumor drugs are commonly used for cancer therapy, and their effectiveness is a clear indication that such



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agents have positive therapeutic indices; that is, successful drugs must have greater activity against tumor cells than against normal cells. Conversely, when activity against tumor cells falls below that against normal cells because of drug resistance, the clinical utility of the antitumor drug is essentially lost. Resistance of tumor cells can be intrinsic, when tumor cells are predisposed at the outset to minimal or negligible response to therapy, or acquired, when tumor cells may initially have a drug-sensitive phenotype that is lost during the course of therapy.

Genetic alterations are generally the reason for greater drug sensitivity of tumor cells relative to normal cells, but which specific genes or sets of genes are involved to induce this differential response will continue to be a subject of concerted laboratory investigations. However, such investigations are more difficult than previously thought since tumor cells demonstrate abnormal expression of over several hundred genes [1,2] and because no two cancers, even from the same tissue type, exhibit identical patterns of the abnormally expressed genes [3,4]. Therefore, the greater drug sensitivity of tumor cells may depend on a unique gene signature profile, which lowers the threshold for sensing cellular damage by antitumor agents and allows efficient transduction of signals for anti-proliferative and/or pro-apoptotic effects.

The lack of an explicit understanding of which dysfunctional genes promote greater drug sensitivity of tumor cells relative to normal cells has not hindered the progress toward identifying critical normal genes that work in concert with the tumor's signaling network to enhance tumor sensitivity. One of these is the tumor suppressor *p53*, widely recognized as the "guardian of the genome," which in its wild-type state has an essential role in facilitating antitumor drug response. Indeed, tumor cells harboring wild-type *p53* are generally recognized as being sensitive to antitumor agents [5]. This premise is consistent with the understanding that activation of p53 is sufficient to induce cell death, even in the presence of strong survival signals from other deregulated genes that may be present [6]. Unfortunately, such tumor cells do eventually become resistant to therapy, and it is not surprising that inactivation of p53 function is an important resistance mechanism, both clinically and in tumor cell lines [7–11]. In this review, we discuss how wild-type p53 is inactivated to render cells resistant to antitumor drugs. We also consider evidence to support the notion of a gain-of-resistance phenotype, in which drug resistance in the presence of wild-type p53 can be greater than that anticipated from a simple loss of p53 function. Although this review uses reported examples of mechanisms in the context of resistance to specific agents, such as cisplatin, it should be noted that these mechanisms are also applicable to other antitumor drugs. This claim is supported by the observation that tumors resistant to cisplatin are generally cross-resistant to diverse, structurally unrelated cytotoxic agents [12].

## 2. Role of p53 in cancer and therapeutics

The p53 protein, identified as a tumor suppressor in 1989 [13], is encoded by the *TP53* gene located on chromosome 17p13.1 and is composed of several domains: (i) a DNA-binding domain, (ii) a transactivation domain, (iii) an oligomerization domain, (iv) a proline-rich domain and v) a C-terminal regulatory domain [14]. The p53 protein functions mainly as a transcriptional activator by binding to specific DNA sequences of target genes involved in a broad range of biological functions, including cell cycle arrest, DNA repair, senescence, apoptosis, and inhibition of angiogenesis [15].

Cellular levels of p53 are usually kept low because of its short half-life of ~20 minutes. Degradation of p53 occurs mainly by binding to Mdm2, an E3 ligase that catalyzes mono-ubiquitination of p53 in the C-terminal domain and thereby targets it for proteasomal degradation [13,16,17]. A close homolog, Mdm4, can also regulate p53 function by binding

to its transactivation domain, but unlike Mdm2 it cannot directly ubiquitinate p53 [18]. The E3 and E4 ubiquitin ligase p300 poly-ubiquitinates p53 in an Mdm2-dependent manner that also results in p53 degradation [16,19]. Several other molecules are involved in p53 regulation, including Pirh2, Parc, and $p14^{ARF}$, which can also affect p53-dependent therapeutic response. Pirh2 binds to the DNA-binding domain of p53 and promotes its degradation, whereas Parc fixes p53 in the cytoplasm, preventing it from entering the nucleus and thus suppressing its transcriptional activity [16]. From the perspective of therapy, the tumor suppressor $p14^{ARF}$ is of significant interest given that it positively regulates p53 by directly inhibiting Mdm2 [17,20].

For tumor suppressive function or to facilitate a therapeutic response to an antitumor agent, p53 needs to be not only stabilized but also activated. This is accomplished by post-translational modifications, including phosphorylation and dephosphorylation at ~23 distinct sites by a network of kinases and phosphatases [21,22]. Thus, under conditions of stress, as may ensue after DNA damage, specific kinases, such as ATM, ATR, Chk1, and Chk2, become activated and phosphorylate p53 target sites, such as Ser15, Thr18, and Ser20, and this phosphorylation forces dissociation of p53 from the Mdm2–p53 complex. The resulting stabilization of p53 and its translocation to the nucleus enable p53 to bind as a tetramer to specific DNA sequences and transactivate target genes [22,23]. This transactivation by p53, driven by upstream activating pathways, is critical for its cellular functions, which include arresting the cell cycle to permit DNA repair and, if the damage is too severe to be repaired, activating cell death pathways [24]. Although the p53 tumor suppressive signaling cascade paradoxically fails to prevent cancer from developing in the first place, it can still be activated in many cancer cells with DNA damaging drugs to induce antitumor response [25,26]. However, the eventual failure of p53 to become activated in tumor cells during the course of chemotherapy is the most significant mechanism of drug resistance. This failure results from the loss of three of the most significant cellular pathways involved in antitumor response during chemotherapy: (i) induction of programmed cell death (apoptosis), (ii) induction of checkpoint response and cell cycle arrest, and (iii) induction of permanent cell cycle arrest (senescence); these pathways normally contribute independently or collectively to final therapeutic outcomes in patients. A brief discussion of these processes is warranted to better appreciate p53-dependent resistance mechanisms.

### 2.1. Induction of apoptosis

Although p53 can mediate apoptosis in a transcription-dependent manner, it is also involved in transcription-independent apoptosis. In the transcription-dependent process, two distinct signaling pathways are involved: extrinsic and intrinsic pathways. In the extrinsic pathway, p53 induces the transcription of death receptors of the tumor necrosis factor receptor (TNF-R) family: Fas, APO-1, CD95, DR5, and PERP [27]. After a ligand binds to its specific receptor, the formation of the death-inducing signaling complex (DISC) is accomplished by recruitment of the Fas-associated death domain (FADD) and caspase-8 and -10, leading to the activation of the effector caspases (e.g., caspase-3 and -7) and resultant DNA fragmentation as a hallmark of apoptosis. In contrast, the intrinsic pathway is activated through a DNA damage mechanism that involves mitochondrial apoptotic events, which are regulated largely by the Bcl-2 family of proteins [27]. Thus, upon stabilization and activation, p53 translocates to the nucleus, where it transactivates the pro-apoptotic genes *Bax*, *Noxa*, *PUMA*, and *Bid* [28]. Of these, *Bax* is the most important pro-apoptotic gene to be induced by p53, but its translocation and functional multimerization depend on other pro-apoptotic family members [29]. Bax can homo-multimerize or it can hetero-multimerize with Bak in the outer mitochondrial membrane and thereby induce the release of cytochrome c, Smac/DIABLO, and apoptosis-inducing factor (AIF) from the mitochondrial intermembrane space to the cytosol. The apoptosome complex, formed by cytochrome c,

apoptotic protease-activating factor 1 (APAF-1), and pre-cleaved caspase-9, activates effector caspase-3, -6, and -7 to induce DNA fragmentation as the final stage of apoptosis [30]. Interestingly, the intrinsic and extrinsic pathways are connected via Bid, which upon cleavage to t-Bid by caspase-8 translocates to the mitochondria and activates the pro-apoptotic proteins Bax and Bak [28].

In the transcription-independent mechanism of apoptosis, some of the induced wild-type p53 translocates to the mitochondria and physically interacts with the anti-apoptotic proteins Bcl-$x_L$ and Bcl-2 to promote the pro-apoptotic homo- or hetero-multimerization between Bak and Bax [31,32]. This occurs rapidly and before p53 exhibits its transcriptional activity. In addition, p53 disrupts the inhibitory Bak–Mcl1 complex by binding to Bak directly [33]. Taken together, these actions of p53 permeabilize the outer mitochondrial membrane, allowing the release of pro-apoptotic factors into the cytoplasm. DNA damaging drugs may activate both membrane death receptors and the endogenous mitochondrial damage pathway, and because these apoptotic mechanisms are deregulated in cancers, proteins involved in these pathways are molecular targets of great interest in cancer therapy [34]. Indeed, most of the anticancer agents that act through DNA damage or stress-inducing mechanisms require wild-type p53 to exhibit the apoptotic phenotype [35].

### 2.2. Induction of checkpoint response and cell cycle arrest

The cell is endowed with elaborate checkpoint systems that, when activated by stressful stimuli such as DNA damage by antitumor agents, activate appropriate signal transduction pathways, which propagate information from the stress stimuli to the cell cycle machinery via sensor, transducer, and effector signaling proteins [36]. This integrated network of DNA damage signaling results in the inhibition of cyclin-dependent kinase (Cdk) activities, thereby impacting cell cycle kinetics. More specifically, inhibition of distinct Cdk/cyclin complexes that are present throughout the cell cycle prevents or slows $G_1$/S transition, S-phase progression, $G_2$/M transition, or all three [36,37]. Although many of the Cdks can be inhibited by members of the Cip/Kip family members of Cdk inhibitors (p21, p27, and p57), p21 as a critical target of p53 is the most significant for inhibiting $G_1$-phase Cdk4/cyclin D and Cdk2/cyclin E. This significance is reflected in the fact that p21 deletion alone can prevent $G_1$-phase arrest [37–39], whereas other mechanisms exist to inhibit S- and $G_2$-phase Cdk activities [38]. Indeed, DNA damaging agents can induce S- and $G_2$-phase arrest even if p53 function is not present [40].

Cell cycle arrest is considered a critical step to allow DNA repair and for cell survival, but if repair fails then apoptosis is induced. Whether persistent p21-dependent $G_1$ arrest is actually a trigger for cell death is unclear. However, tumors retaining $G_1$ checkpoint response appear to be more sensitive to therapeutic agents [5,41,42]. Indeed, a strong positive correlation has been demonstrated between the ability of tumor cells to arrest in $G_1$ and their sensitivity to platinum-based drugs in the National Cancer Institute (NCI) panel of 60 tumor cell lines [43]. This correlation is also consistent with the finding that mutant p53 tumor cells transfected with a p21 expression vector are sensitized to antitumor drugs [44,45]. In addition, small-molecule inhibitors of $G_1$-phase Cdks not only arrest cells but also induce apoptosis [46,47]. Moreover, cells from p21 knockout mice lack $G_1$ checkpoint response [48], and because such mice develop tumors [49], the tumor suppressive function of p53 may be mediated in part through the downstream effects of p21 in checkpoint response.

### 2.3. Induction of senescence

Cellular senescence is defined as a permanent cell cycle arrest that prevents cell immortalization and transformation to a genetically unstable phenotype [50]. Therefore, senescence is identified as an additional tumor suppressive mechanism in benign or

premalignant cancer lesions [51]. Senescent cells remain metabolically active but acquire distinct changes in morphology and physiology characterized by enlarged cell size, chromatin condensation, changes in gene expression, and high levels of senescence-associated β-galactosidase. The senescence phenotype occurs as a result of various forms of stress stimuli, such as telomerase shortening, oncogenic stimuli, ionizing radiation, and DNA damaging agents [52]. Not surprisingly, p53 is the pivotal player in regulating replicative as well as premature (stress-induced) senescence. In line with this evidence, tumor cells containing wild-type p53 are more likely to undergo senescence in response to chemotherapy [53].

Stress-induced senescence is driven via the ATM/ATR-Chk2/Chk1-p53 pathway, with the involvement of several p53-dependent downstream molecular markers, such as p21, PML, PAI-1, and DEC1 [53]. Of these, p21 is the seminal controller of the senescence program. In this respect, p21-dependent $G_1$ arrest in senescence is similar to that after checkpoint response, but the significant difference is that activation of the senescence program requires prolonged p53-dependent expression of p21, whereas the checkpoint response pathway requires a relatively transient transactivation of p21 by p53 [15]. Despite this difference, premature senescence, like apoptosis and $G_1$ checkpoint response, can significantly contribute to the antitumor effects mediated by p53 with a variety of anticancer agents [54–58]. Although apoptosis may have greater relevance in cancer chemotherapy, p53-dependent senescence may provide an important anti-proliferative option in cancer cells that have lost their ability to undergo p53-dependent apoptosis [54].

## 3. Mechanisms of resistance involving p53

Induction of cell death by antitumor drugs requires (i) the recognition of DNA damage, (ii) activation of specific kinases, (iii) stabilization and activation of p53, (iv) activation of downstream p53-dependent pathways, and finally (v) execution of cytotoxic programs (Fig. 1). The p53 protein is central to this process, and its critical roles in activating anti-proliferative/pro-apoptotic pathways and facilitating the effects of antitumor agents also make it a vulnerable target; tumor cells will readily select for attenuated p53 pathways that lead to drug resistance and cell survival. Interestingly, resistance in tumor cells harboring wild-type p53 is observed with a large variety of agents, ranging from ionizing radiation to several classes of cytotoxic drugs, such as doxorubicin, paclitaxel, cisplatin, etoposide, and vinblastine [59]. This resistance can occur directly, via factors that reduce or negate the functional activity of p53, or indirectly, by deregulation of pathways downstream of p53 (Fig. 1). Although the purpose of this review is to focus on mechanisms that directly affect p53 function to induce drug resistance, either through mutation in the p53 protein or through the failure of upstream pathways that stabilize and post-translationally activate wild-type p53, it is appropriate to first briefly discuss resistance arising from deregulated pathways that affect p53-mediated signaling.

### 3.1. Deregulation of pathways downstream of p53

The indirect pathways that affect p53 signaling can involve downstream failure either in the activity of target gene products, such as p21, Bax, miRNAs, and caspases, or in the expression or mutation of proteins interacting with p53. A case in point is where p53 is functional and transactivates p21, but this Cdk inhibitor becomes inactivated through cytoplasmic sequestration after Akt-mediated phosphorylation, leading to resistance of testicular and ovarian cancers to antitumor agents such as cisplatin [60,61]. In this case, a possible mechanism for the resistance is the binding of phosphorylated form of p21 in the cytoplasm to procaspase-3, preventing its conversion to caspase-3 and thereby blocking apoptosis [62]. Although the *p21* gene is rarely mutated, it can also influence therapeutic outcomes through other mechanisms. For instance, a recent report has demonstrated that a

single nucleotide polymorphism at codon 31 of p21 in ~10% of chronic lymphocytic leukemia (CLL) patients is sufficient to cause greater aggressiveness and therapeutic resistance of the cancer [63]. Similarly, reduced p21 expression through *p21* promoter hypermethylation in the corresponding acute lymphocytic leukemia (ALL) leads to a low survival rate of 6–8% in patients compared with a higher rate of ~60% when the promoter is hypomethylated and fully functional [64]. Bax expression is also known to be downregulated, but by mutation in the coding region, in ~50% of colorectal and gastric cancers, which then lose apoptotic activity and become drug-resistant, leading to poor patient survival [65–67]. Where Bax expression is normal, failure in its activation has also been reported as an alternative mechanism for Bax-related resistance in Hodgkin disease [68].

Recently, wild-type p53 has been reported to regulate the expression of microRNAs [69]. The most significant effect involves the induction by p53 of microRNAs miR-34a, miR-34b, and miR-34bc, which are involved in apoptosis, cell cycle arrest, and senescence, and defective expression of these targets has been associated with resistance to p53-dependent therapeutic agents [70]. The mechanism for this reduced expression has been attributed to gene silencing by CpG hypermethylation of miR-34a, miR-34b, and/or miR-34c promoters in 60–100% of renal cell carcinoma (RCC), pancreatic, breast, colorectal, ovarian, and urothelial cancers [71]. Conversely, ectopic expression of miR-34a has been demonstrated to sensitize medulloblastoma and prostate cells to chemotherapeutic agents [72,73].

In apoptosis, the ultimate goal of antitumor agents is to activate caspases, which degrade DNA and complete the process of cell death. Therefore, inhibition of caspase activity will also result in tumor resistance. Indeed, inactivation or downregulation of caspase-3, -8, and -9 has been reported and directly correlated with drug resistance in neuroblastoma and cervical, head and neck, ovarian, and breast cancers [74–79]. Moreover, >100 proteins can bind with p53 to potentially regulate cytotoxic outcome, and a defect in this interaction through alterations in expression or mutation in the binding partner can have serious therapeutic consequences. For instance, the p53-dependent nuclear export of BRCA1 is important in enhancing DNA damage response, but overexpression of BRCA1 in wild-type p53 MCF-7 tumor cells induced resistance to cisplatin as a result of increased BRCA1-dependent DNA repair, most likely due to a resultant increase in levels of BRCA1 retained in the nucleus [80,81]. Although Mdm2 and Mdm4 are the most prominent of the proteins known to interact with p53, their impact is more direct and will be discussed later.

### 3.2. Mutations in p53

The high risk of patients with Li–Fraumeni syndrome to develop fibrosarcomas and breast cancer is linked directly to the loss of tumor suppressive function as a result of heterozygous germline mutations in *p53* [13,23,26,82]. Because *p53* is mutated in ~50% of human cancers, this loss of function has a widespread impact on tumor incidence in many tissue types. Loss of p53 function from mutation also results in poor response of tumors to a wide range of clinical treatment regimens, in stark contrast to excellent prognosis and treatment outcome in tumors harboring wild-type p53 [18,83]. This contrast is clearly and perhaps uniquely exemplified in pediatric patients with choroid plexus carcinomas treated with chemoradiation; essentially, the treatment induced universal response and long-term survival (>5 years) in all patients with tumors expressing wild-type p53, whereas most patients (~80%) with tumors expressing mutant p53 died within 6 months [84]. Although not as dramatic, the response of patients treated for advanced breast cancer with paclitaxel or the 5-fluorouracil (5-FU)/epirubicin/cyclophosphamide combination was 45–64% when wild-type was expressed, but responses were totally absent when mutant p53 was expressed [85,86]. As another example, the 5-year survival rate in CLL patients with tumors expressing wild-type p53 was three-fold greater than in patients with tumors expressing mutant p53 (59% vs.

20%) [87]. Similarly, the exquisite curative sensitivity of testicular cancers to platinum-based therapy is widely attributed to the rarity of p53 mutations in this disease [88]. Consequently, the negative therapeutic association with mutant p53 has made the mutant form a popular molecular target, and thus drugs that may restore wild-type p53 conformation and function have been aggressively pursued [83,89].

Cytotoxic responses based on p53 genotype have also been studied in tumor cell lines. The most extensive study conducted to date was from NCI using a broad tumor panel comprising 39 mutant p53 and 18 wild-type p53 cell lines across 9 tissue types and exposed to 123 agents from a variety of antitumor drug classes [5]. That study demonstrated that the median resistance of cell lines in the mutant p53 group to cisplatin, 5-FU, and bleomycin was 3–10 times that in the group of wild-type p53 cell lines. This drug-dependent variation in resistance is not unexpected, and it is important to note that due to cell context p53 mutation may not necessarily render tumor cells resistant to every antitumor agent. Thus, the p53-R273H mutant expressed in H1299 lung adenocarcinoma cells induced a low level of resistance to etoposide but a substantially greater resistance to cisplatin (see Table 1), whereas ectopic expression of p53-V143A did not induce resistance to several antitumor agents, including cisplatin, paclitaxel, vincristine, and teniposide [90,91].

Somatic p53 mutations are mostly missense in nature and are located predominantly within the DNA-binding domain, where a single mutation is sufficient to cause loss of normal p53 function [82,92]. Four positions in p53 are frequently mutated; these “hot spots” are located at amino acid positions 175, 248, 249, and 273 and collectively account for >25% of all missense mutations in human cancers. Specific mutations in p53 are grouped into “contact” and “structural” classes, but both types impede the pro-apoptotic p53 transactivation functions by preventing binding of p53 with target promoter sites on DNA. Thus, mutations at amino acid positions in p53 that normally make direct contact with DNA (e.g., R248W and R273H) will disrupt promoter binding without necessarily altering p53 conformation. In contrast, mutations in noncontact positions (e.g., R175H and R249S) induce local and/or global structural distortions and thereby prevent p53 from binding to the specific DNA sites [93,94].

**3.2.1. Mutations in p53 and the gain-of-function phenotype—**It is noteworthy that specific mutations in p53 not only may result in loss of normal p53 functions but also can induce novel functions, leading to the so-called p53 gain-of-function phenotype, which still confers drug resistance [95]. More specifically, the novel biological effects of p53 mutations, such as R175H, R175P, R248W, R249S, R273C, and R273L, involve transcriptional activation or repression of genes whose expression is not normally regulated by wild-type p53, for example *BFGF*, *EGFR*, *HSP70*, and *C-Myc* [94,96]. These genes when deregulated have been implicated in the promotion of tumor cell growth and survival and the development of resistance to several chemotherapeutic agents by inhibiting apoptosis in several cell lines and in patients [97–100].

Evidence suggests that p53 gain-of-function mutants may also mediate their effects through inhibitory interactions with other transcriptional factors that normally bind to gene promoters [96,101]. An important example is the interaction of such p53 mutants with the family members p63 and p73, which are then prevented from transactivating their gene targets [94]. Moreover, protein–protein interactions with these p53 mutants can also have profound cellular consequences in a promoter-independent manner. For instance, the hot spot mutant R248W or R273H interacts with the nuclease Mre11, thus interfering with the formation and recruitment of the Mre11/Rad50/NBS1 (MRN) complex at the site of DNA double-strand breaks (DSBs) and thereby abolishing the co-recruitment and activation of ATM for DNA damage response [94,96,101–103].

From the perspective of therapeutic response, it is clear that mutations in p53 will inhibit both anti-proliferative and pro-apoptotic pathways, and induce resistance as a result. For example, *p53* gene mutations may exist in only one allele, but if the mutant p53 molecule has a dominant-negative characteristic, its lone presence in the active tetrameric complex is sufficient to not only inhibit p53 function but also promote gain-of-function effects [104,105]. With p53 gain-of-function mutants, the resistance to DNA damaging agents arises both through downregulation of anti-proliferative and pro-apoptotic genes, such as *FAS* and *MST-1*, and through upregulation of pro-proliferative and anti-apoptotic genes, such as *BAG-1* and *NFKB2* [94,101]. This finding raises an important question of whether drug resistance from a combination of loss-of-function and gain-of-function effects will be greater than that from loss of p53 function alone. Investigations to address this question have used either transfection of p53-null cells (e.g., human lung adenocarcinoma H1299 and human osteosarcoma Saos-2 models) with p53 mutants or siRNA-dependent downregulation of p53 mutants. Interestingly, both approaches have demonstrated that tumor cells expressing p53 gain-of-function mutants, such as R175H, R179H, and R273H, exhibit significantly greater resistance to a number of antitumor drugs, including doxorubicin, cisplatin, etoposide, and 5-FU [90,94,95,103,106]. Examples of this effect are shown in Table 1. Note that increased resistance is not always observed but can be drug-dependent, as evidenced by the unexpectedly increased sensitivity to etoposide of H1299 tumor cells transfected with the p53-R273H mutant. With this mutant, the additional resistance to methotrexate or doxorubicin has been reported to arise through downregulation of procaspase-3 [107].

The potential of knocking down p53 gain-of-function mutants as a therapeutic option to sensitize tumor cells to antitumor agents is apparent from the above discussion and has been proposed independently by others [108]. However, it should be noted that this sensitization will be partial because p53-knockdown tumor cells will still retain resistance due to persistent loss of p53 functions. Therefore, approaches that convert the p53 gain-of-function mutant configuration to wild-type p53 are likely to be more successful. Indeed, substantial effort has been devoted by the research community toward the ultimate goal of identifying a viable therapeutic option for cancers expressing mutant p53. This concerted effort has led to the identification of several agents with clinical potential, such as the small molecules CP-31398, MIRA-1, STIMA-1, and PRIMA-1, and the PRIMA-1 analog APR-246 [109,110], which has recently entered phase I/II clinical trials [111].

### 3.3. Resistance in tumor cells harboring wild-type p53

The ~50% mutation rate of p53 in cancer and the associated failure in antitumor drug response has been pivotal in maintaining much interest in mutant p53 with the goal of developing more effective therapeutic solutions [112]. However, antitumor drug resistance is not limited to tumors expressing mutant or null p53. Indeed, in direct contradiction of the normal role of p53 in promoting apoptotic response with antitumor agents, as discussed here, it has become increasingly clear from the clinical literature that resistance is also observed in cancers harboring wild-type p53. The major evidence supporting this conclusion can be gleaned from the clinical reports summarized in Table 2. In the examples shown, the incidence of wild-type p53 is in the range from 40% to almost 100%. As mentioned earlier, cancers of the choroid plexus and germ cell tumors expressing wild-type p53 are exquisitely sensitive to therapeutic regimens, which are highly curative, as indicated by the 5-year survival rate of 90–100% [84,88,113]. In contrast, the presence of wild-type p53 in other advanced cancers, such as bladder, breast, CLL, mesothelioma, non-small cell lung cancer (NSCLC), ovarian, and RCC, results in significantly lower response and/or curative rates, ranging from 0% to ~60% [85,87,114–126]. A low p53 mutation rate is not predictive of greater therapeutic responses or long-term survival rates. This is best illustrated by

comparing CLL, germ cell tumors, mesothelioma, and RCC. All of these cancers have a wild-type p53 incidence of ≥90%, but the response rate was best in germ cell tumors (~90%), intermediate in CLL (59–83%), and worst in mesothelioma and RCC (0–33%) [87,88,113,119–122,126,127].

The clinical data presented here clearly support the notion that the presence of wild-type p53 is a poor predictor of response and/or cure; many cancers harboring wild-type p53, as with mutant p53, are resistant to a variety of antitumor agents, including cisplatin, paclitaxel, and 5-FU. To explain this paradox requires a discussion of possible mechanisms that may downregulate wild-type p53 function. The mechanisms can involve deregulation of any component of the DNA damage pathway (see Fig. 1), but here we focus on mechanisms that directly impinge on p53: upregulation of pathways that promote p53 degradation and downregulation of pathways involved in post-translational modification.

**3.3.1. Mechanisms promoting degradation of wild-type p53—**Mechanisms that enhance degradation of wild-type p53 are the simplest way for tumor cells to select for attenuated p53 functions and demonstrate resistance to therapeutic agents. Indeed, accelerated proteasomal degradation of p53 and induction of resistance to anticancer therapy have been demonstrated through upregulation of specific p53-binding proteins, such as human papillomavirus type 16 (HPV-16) E6, Mdm2, and Mdm4 [22]. HPV-16 is found in several cancers, the most common of which is cervical cancer. HPV-16 expresses the E6 viral oncoprotein, which promotes ubiquitin-dependent proteasomal degradation of p53 and leads to low levels of this tumor suppressor in infected tumor cells [128]. As a result, HPV-positive cervical cancer cells are resistant to chemotherapeutic agents such as camptothecin and cisplatin [129].

As discussed here, p53 activation and stability are negatively regulated in a controlled manner via proteasomal degradation by Mdm2. However, Mdm2 gene expression can become deregulated, leading to overproduction of this oncoprotein in human and mouse tumors, as well as tumor cell lines, by one of three different mechanisms: gene amplification, increased transcription, or enhanced translation [130,131]. The overall frequency of Mdm2 amplification in human tumors is ~7–10%, but tissues such as osteosarcomas (16%), soft tissue tumors (31%), hepatocellular carcinoma (44%), and Hodgkin disease (67%) have some of the highest frequencies [22]. The overexpression of Mdm2 in cells substantially alters the stoichiometry with wild-type p53 and likely promotes cancer development [132]. Indeed, a 2-fold increase in Mdm2 has been reported as sufficient to attenuate p53 activation and thereby increase the risk of breast cancer [133]. In parallel, overexpression of Mdm2 leads to a reduction in therapeutic response. This is again well demonstrated with breast cancer, where in an extensive analysis of >2000 patients, those with tumors overexpressing Mdm2 had a significantly lower 10-year survival rate (58–61% vs. 73%) [134]. Such negative roles of Mdm2 in antitumor response have stimulated the search for small-molecule antagonists of p53–Mdm2 interactions to enhance the apoptotic activity of wild-type p53 [135]. Several molecules of interest have been identified, but probably the best known is Nutlin-3a, which displays p53-dependent activity against tumors overexpressing Mdm2, including those with upregulated Mdm2 as a result of ATM mutation [22,136–138]. Nutlin-3a can effectively stabilize wild-type p53 and induce antitumor activity not only as a single agent but also as a potent binary combination with other antitumor agents such as etoposide and cisplatin [139].

Alternative mechanisms involving protein–hprotein interactions can also modulate Mdm2 levels and contribute to the failure of antitumor agents to activate wild-type p53 and induce apoptosis. Mdm4 is one such protein that stabilizes Mdm2 by preventing its proteasomal degradation. Interestingly, 10–20% of cancers overexpress or amplify the *Mdm4* gene,

which results in a failure to disrupt the p53–Mdm2 complex and culminates in drug resistance [22,140]. This is clearly demonstrated by the ability of Mdm4 to attenuate p53-dependent transactivation of Bax and induction of apoptosis [141]. In contrast to Mdm4, 14-3-3σ normally negatively regulates Mdm2, but downregulation of 14-3-3σ in several cancers, such as ovarian, lung, breast, and prostate cancer, results in significant increases in Mdm2 levels and inhibition of antitumor drug response [142]. Similarly, deregulation of the tumor suppressor $p14^{ARF}$ by deletion, mutation, or epigenetic silencing in human tumors prevents its sequestration of Mdm2 in the nucleolus and thereby increases Mdm2 interactions with wild-type p53 to inhibit p53 functions and induce drug resistance [143–146]. In agreement with this finding, ectopic expression of $p14^{ARF}$ in tumor cells substantially increases p53-dependent $G_1$-phase arrest, apoptosis, and sensitivity to antitumor drugs such as cisplatin [147].

**3.3.2. Mechanisms downregulating post-translational activation of wild-type p53—**To facilitate antitumor response to cytotoxic drugs, wild-type p53 must be transcriptionally active [148]. However, in drug resistance, this function is lost, and the two genes critically impeded are the checkpoint response gene *p21* and the pro-apoptotic gene *Bax*. Defects in Bax expression have indeed been correlated with adverse drug responses in many human cancers, including pancreatic, colorectal, and breast tumors, irrespective of p53 status [65–67,149]. Similarly, loss of p21 transactivation after exposure of resistant wild-type p53 tumor cells to antitumor agents, such as those based on platinum, has also been reported [150–152]. This finding is consistent with the role of p21 as a tumor suppressor [49,153,154] and with correlations of p21 expression with superior therapeutic drug effects in patients with ovarian cancer and NSCLC [155,156] and in experimental tumor models [44,45,157–159].

Although loss of transactivation function of wild-type p53 can be explained by deregulated expression of Mdm2, Mdm4, 14-3-3σ, or $p14^{ARF}$, this is not always the case. Thus, in breast cancer, the 45% incidence rate of resistant tumors harboring wild-type p53 (see Table 2) is not consistent with the lower (7–14%) rate of occurrence of Mdm2 overexpression or downregulation of $p14^{ARF}$ [160]. Similarly, the differential half-life of wild-type p53 in sensitive and resistant ovarian tumor cells after DNA damage with cisplatin does not correlate with Mdm2 levels [161]. Moreover, in RCC, in which p53 mutations are rare, wild-type p53 transactivation functions were still reported as defective when tumor cells were exposed to 5-FU, camptothecin, or doxorubicin, but interestingly no changes in Mdm2, Mdm4, or $p14^{ARF}$ were detected to explain these observations [162]. Therefore, it is clear that other mechanisms attenuating wild-type p53 function are also involved, and defects in post-translational modification are a primary mechanism to consider.

**3.3.2.1. Significance of phosphorylation in post-translational modifications of p53:** Stabilization and activation of p53 is governed by covalent post-translational modifications, such as phosphorylation, ubiquitination, acetylation, methylation, SUMOylation, neddylation, glycosylation, and ribosylation [163,164]. A total of ~50 sites on p53 are now known to be subject to modifications after a stress stimulus [165]. The N-terminus is modified primarily by phosphorylation, whereas the C-terminus can be the target of a variety of modifications, including phosphorylation [163]. Although an optimal combination of post-translational modifications is required for maximal p53 function, the antitumor effects of DNA damaging agents are dominantly governed by phosphorylation events [166,167]. There are ~23 possible sites on p53 that can be phosphorylated, and each site can be targeted by multiple kinases, suggesting possible redundancy [22]. However, it is likely that a specific antitumor agent only activates an individual kinase for each of the several sites targeted for phosphorylation. As a result, a DNA damaging agent produces a unique signature of p53-phosphorylated sites that induces p53 to adopt a specific structural

conformation and thereby transactivate a select set of downstream target genes specific for that agent. It is highly likely that deregulation of any critical kinase will alter the unique p53 phosphorylation signature, impede gene transactivation, and attenuate antitumor response.

Of the multiple p53 phosphorylation sites that have been associated with its anti-proliferative and apoptotic functions, Ser15, Thr18, and Ser20 in the DNA-binding domain at the N-terminus are recognized as perhaps the most critical and are therefore the most extensively studied [22,168]. Indeed, these three sites are highly conserved between urochordates, where evolution of the p53–Mdm2 axis appeared, and humans [168]. Studies demonstrate that phosphorylations at these sites promote not only dissociation of p53 from Mdm2, as a prerequisite for p53 stabilization, but also its transactivation function, by inducing concomitant binding of the freed p53 with transcription co-activators, such as p300 and cAMP-response element-binding protein-binding protein (CBP) [165,169–171]. Moreover, several studies suggest that the effects of phosphorylation at these sites may be cooperative, particularly because Thr18 and Ser20 phosphorylations are strongly dependent on the priming phosphorylation at Ser15 [172–174]. Indeed, dual phosphorylation at Ser20 with either Ser15 or Thr18 is sufficient to induce apoptosis in glioma cells harboring wild-type p53 [175,176]. Further support is provided by studies in transgenic mice where mutations induced by replacing both Ser15 and Ser20 with alanine produced a more severe phenotype than a single mutation at either site, as evidenced by loss in apoptotic capacity, defects in replicative senescence, and latency in tumor appearance [163].

**3.3.2.2. Downregulation of kinases involved in post-translational modifications of p53:** Because of the seminal role of phosphorylation in regulating p53 stabilization and function, loss of this post-translational event at any one of the critical sites is likely to modulate cytotoxic response to antitumor agents. A case in point is with ATM, a transducer of DNA damage signals that upon exposure of tumor cells to antitumor agents phosphorylates p53 primarily at Ser15; this, in turn, stimulates phosphorylation at Thr18 and Ser20 by other kinases, such as Chk2. ATM also phosphorylates and activates homeodomain-interacting protein kinase 2 (HIPK2), a kinase that, in turn, modifies p53 at Ser46 [165]. Therefore, it is not surprising that mutation of ATM in ataxia telangiectasia leads to defects in p53 regulation and checkpoint response after DNA damage with ionizing radiation [177]. Interestingly, defective ATM function has been observed in ~20% of patients with B-cell CLL, which predominantly harbors wild-type p53, and ATM dysfunction correlates with resistance to fludarabine and with poor prognosis in patients [137,178]. In addition, deregulation of Ser376 phosphorylation of p53 has been observed in radioresistant melanoma, with loss of wild-type p53 function attributed to a loss in ATM-dependent signaling [179]. Because ATM can also affect the function of HIPK2 and, thereby, p53-dependent apoptosis, it would be expected that direct loss of HIPK2 would similarly induce drug resistance. Indeed, knockdown of HIPK2 by siRNA in wild-type p53 colorectal tumor cells resulted in resistance to doxorubicin and, more importantly, patients with colorectal cancers expressing low levels of HIPK2 had a significantly lower survival rate [180].

The DNA damage checkpoint kinase Chk2 is also a downstream target of ATM and has been identified as a tumor suppressor, the loss of which leads to multi-organ susceptibility to cancer [181]. Not surprisingly, suppression of either ATM or Chk2 in the presence of wild-type p53 decreased sensitivity of mouse embryonic fibroblasts (MEFs) or tumor cells to doxorubicin [182]. As a member of the calcium calmodulin kinase superfamily, Chk2 has the intrinsic ability to phosphorylate both the Thr18 and Ser20 sites, as well as several other sites in the C-terminus of p53 [183–185]. Interestingly, Chk2 can also be activated independently of ATM, and in the case of cisplatin, the homolog ATR appears to be involved in not only upregulating Chk2 to mediate the DNA damage signal to p53 but also phosphorylating p53 directly at Ser15 [186].

The importance of Chk2 in DNA damage response can be readily appreciated from reports that Chk2-null cells from genetically engineered mice are remarkably resistant to some DNA damaging agents [181,187,188]. The significance of these observations becomes clear when it is noted that Chk2 downregulation has been correlated with resistance to cisplatin in lung cancer cell lines [189]. Moreover, defects in Chk2 through epigenetic mechanisms have been observed clinically in 83% of tumor specimens from patients with NSCLC [189], a highly refractory cancer where cisplatin-based therapy is heavily used, but with a poor survival rate (see Table 2). In addition, 23% of clinical ovarian cancers are reported to be negative for Chk2 [190], a rate that is similar to that of cisplatin-resistant ovarian cancers harboring wild-type p53 (20–22%) [116,191]. It should also be noted as a caution that the results of independent studies with DNA damaging agents in knockout cell lines or *in vivo* murine models have been at odds with the roles of Chk2 and site-specific phosphorylation of the transactivation domain in p53 stabilization and stimulation and in cell death [22,192]. It has also been reported that defects in Chk2 are rare and, therefore, not relevant [193]. Nevertheless, other clinical assessments strongly indicate that a Chk2 defect is a significant occurrence [189]. In strong support of this is the recent finding that defects in p53 and Chk2 are mutually exclusive, as determined from an extensive analysis of epithelial malignancies, which revealed that 37% of tumors (124 of 335 cases) had either a p53 or a Chk2 defect but that a defect in both within the same tumor was rare (2/335) [182]. A similar mutual exclusivity between defects in either p53 or ATM was also demonstrated in that study. Thus, defects in ATM and Chk2 could account for drug resistance in tumor cells harboring wild-type p53.

A second critical member of the calcium calmodulin kinase superfamily involved in checkpoint response is Chk1, which is also activated by cisplatin in an ATR-dependent manner and targets p53 sites similar to those targeted by Chk2 [22,184]. However, the importance of the ATR/Chk1 pathway in p53 regulation and apoptosis has been difficult to demonstrate because ATR or Chk1 deficiency is embryonically lethal [194,195]. Other approaches using dominant-negative ATR mutants or the Chk1 kinase inhibitor UCN-01 have been possible, but the data have surprisingly demonstrated increased tumor cell sensitivity to DNA damaging agents [195–198]. It is notable that ATR and Chk1 mutations have been observed in 10–20% of gastric cancers [199], but studies to ascertain resultant effects on therapeutic outcomes and p53 functions are needed to determine whether defects in ATR and Chk1 can affect drug response in this disease.

## 4. Wild-type p53 gain-of-resistance phenotype

Given the seminal antitumor and apoptotic properties of p53, tumor cells resistant to antitumor agents in the presence of wild-type p53 represent somewhat of a contradiction; more importantly, this contradiction poses a major challenge in using p53 status as a prognostic factor in personalized medicine. However, the challenge may be far greater than envisaged so far, as it becomes apparent from sporadic reports in the literature that drug resistance in the context of a retained wild-type p53 genotype is greater than expected. Clinical evidence supports this notion, which is endorsed by data garnered from preclinical studies. For the purpose of this review, we term this observation a “gain-of-resistance” phenotype and consider whether it may be a direct result of a gain-of-function effect associated with wild-type p53.

### 4.1. Clinical data

Several clinical studies have examined the role of *p53* gene status in tumor response, and have concluded that the prognostic value of this tumor suppressor is low. This conclusion stems from clinical observation of therapeutic resistance in both mutant and wild-type p53 cancers, as discussed here. Similarly, tumor responses can also be observed independent of

p53 status. For instance, in a clinical study of advanced ovarian cancer, cisplatin treatment induced a response in 46% of the patients in the wild-type p53 group and in a similar percentage (37%) of patients in the mutant p53 group [191]. In direct contrast, other studies have demonstrated that responses in the mutant p53 group are in fact significantly greater, as has been seen in advanced ovarian cancer with the platinum/paclitaxel combination (mutant, 86%; wild-type, 47%) [116], in NSCLC with paclitaxel (mutant, 75%; wild-type, 47%) [114], in metastatic bladder cancer with platinum-based combinations (mutant, 50%; wild-type, 27%) [200], and in advanced breast cancer with paclitaxel (mutant, 83%; wild-type, 38%) [86]. In non-inflammatory breast cancer, the contrast is even more striking. In this disease, >50% of the patients harboring mutant p53 responded to a dose-intense epirubicin–cyclophosphamide regimen, whereas none of the >100 patients with tumors harboring wild-type p53 responded to this regimen [14]. Interestingly, in a rare study involving serial sampling of ovarian cancer ascites and assessment *in vitro* of drug response, the intrinsic resistance of tumor cells to cisplatin increased 1.4-fold during therapy while cells retained wild-type p53 status; however, this resistance decreased below the intrinsic level after p53 became mutated as therapy progressed [201]. Moreover, because clinical responses are limited by the maximum tolerated dose, one may predict that responses would increase if higher doses were permissible. This appears to be the case with high-dose cisplatin or carboplatin therapy, where a 30% response rate has been observed in ovarian tumors already resistant to platinum therapy [202]. However, this response rate was concluded not to increase further with increasing dose on the basis of a critical analysis of carboplatin dose–response data from clinical investigations [203]. Thus, there appears to be a fraction of refractory ovarian tumors that are supra-resistant, and, on the basis of the discussion so far and from the preclinical data presented in Figure 2 (see below), these tumors are predicted to harbor wild-type p53.

Collectively, the clinical studies discussed here have been instrumental in justifying the use of appropriate therapeutic strategies involving selective antitumor agents (e.g., paclitaxel) against mutant p53 tumors in specific disease types. However, the underlying basis for the relatively poorer responses when wild-type p53 is present has been overlooked, even when it is clear that the activity of the agent (e.g., paclitaxel) is also dependent on the presence of wild-type p53 [204]. Nevertheless, these reports provide evidence for the existence of wild-type p53 cancers that are more resistant to therapy than mutant p53 cancers are; that is, the wild-type p53 cancers are displaying the gain-of-resistance phenotype.

### 4.2. Preclinical data

As in clinical studies, the effect of p53 status on response to antitumor agents has also been determined in tumor panels of various disease types. The cytotoxic data from head and neck squamous cell carcinoma (HNSCC), RCC, and ovarian cancer after exposure to cisplatin or paclitaxel have been reported in subgroups of mutant/null p53 or wild-type p53 models [40,205–207] and are listed in Table 3A. In these examples, the cytotoxicity is relatively lower (that is, the drug concentration that inhibits cell proliferation by 50% [$IC_{50}$] is higher, or the resistance is higher) when p53 is wild-type. Interestingly, the degree of increased resistance with wild-type p53 was significant, and the ratio of $IC_{50}$ between wild-type p53 and mutant/null p53 ranged from 2 to 77. More importantly, these data demonstrate the presence of additional drug resistance in wild-type p53 tumor models over and above that displayed in mutant p53 cancers.

Additional data to support the wild-type p53 gain-of-resistance phenotype have been garnered from studies in isogenic systems after disruption of p53 with dominant-negative mutant p53, p53 knockout, or p53 knockdown by siRNA. Thus, if wild-type p53 indeed contributes to gain of resistance, then disruption of p53 should lower resistance. This is analogous to the studies with the p53 gain-of-function mutant, where the higher level of

resistance to antitumor agents, such as cisplatin, was reduced by p53 siRNA [108]. Indeed, cell lines derived from gliomas, colon cancers, or breast cancers that were exposed to DNA damaging agents from three different structural classes displayed reduced $IC_{50}$ (decreased resistance) by 2-fold to >400-fold after disruption of p53 by any of the three established procedures (Table 3B) [208–211]. The cell lines MCF-7 and HCT-116 have generally been considered to have a drug-sensitive phenotype, but reports indicate that they harbor a defect in apoptosis [210,211] and therefore may carry some intrinsic resistance that is reduced when wild-type p53 is disrupted. Sensitization of tumor cells to cisplatin by knockdown of wild-type p53 with siRNA has also been observed in Bcr/Abl-transformed murine hematopoietic cells [212]. In an alternative approach, these cells were transfected with temperature-sensitive p53, and resistance to cisplatin was found to be higher in cells expressing wild-type p53 at the permissive temperature of 32°C than in cells expressing mutant p53 at 37°C. A limitation of these data is the difficulty in ascertaining the level of resistance in mutant/null p53 versus wild-type p53 cell lines relative to a standard sensitive model. This limitation can be partially addressed using our previous findings in an ovarian tumor panel [40,213], which are shown in Figure 2. Compared with established cisplatin-sensitive A2780 cells, which harbor wild-type p53 and have intact checkpoint responses, all other models shown in the figure were resistant to cisplatin (had higher $IC_{50}$ values), irrespective of their p53 status. However, it is notable that models harboring wild-type p53 had greater resistance than those harboring mutant p53.

The underlying basis of chemotherapy is that normal cells with wild-type p53 must have relatively greater resistance to DNA damaging drugs than tumor cells have. This is amply demonstrated by the report that the resistance of normal human foreskin fibroblasts to cisplatin, carboplatin, and paclitaxel is reduced 6- to 12-fold when wild-type p53 is disrupted by ectopic expression of the HPV-16 *E6* oncogene [214]. Taken together, these findings are consistent with the premise that wild-type p53 tumor cells can indeed demonstrate supra-resistance, not unlike that in normal cells, and that wild-type p53 contributes to the greater resistance.

### 4.3. Underlying basis for wild-type p53 gain-of-resistance phenotype

Although the data presented here support the concept of a wild-type p53 gain-of-resistance phenotype in specific tumor systems, the underlying mechanisms are not known. However, the mechanism requires the presence of wild-type p53 because its knockdown reduces drug resistance in these systems. Thus, mechanisms that promote degradation may be eliminated from consideration. Indeed, such gain-of-resistance model systems do not necessarily demonstrate increased expression of Mdm2, Mdm4, or $p14^{ARF}$. From an analysis of the available data, it is likely that defects in post-translational mechanisms play a role. This possibility is based in part on data from RCC, where wild-type p53 transactivation functions failed when tumor cells were exposed to antitumor agents even though levels of Mdm2, Mdm4, and $p14^{ARF}$ remained normal [162]. Further support comes from the demonstration that resistance to structurally distinct platinum drugs cisplatin and the trinuclear analog BBR3464 is associated with the inability of DNA damage to induce p21 expression in wild-type p53 models [151,215]. However, normal p21 expression could be restored if cells resistant to cisplatin were exposed to *1R,2R*-diaminocyclohexane(*trans*-diacetato)(dichloro)platinum(IV) (DAP) or if cells resistant to BBR3464 were exposed to cisplatin; that is, the signal transduction pathways induced by BBR3464 and DAP are distinct from those induced by cisplatin. This is well demonstrated with DAP, which fails to phosphorylate p53 at Ser392; in contrast, cisplatin is very effective in modifying this site [215]. Moreover, in the ovarian panel shown in Figure 2, DAP was demonstrated to be highly effective against the wild-type p53 supra-resistant models but was not effective against the mutant/null models [40,213]. Therefore, it can be concluded that, at least for

platinum-based drugs, there is a minimum of two parallel upstream pathways that independently converge on wild-type p53 to activate the tumor suppressor, and when one becomes defective and induces supra-resistance, the alternative intact pathway can be activated to bypass the defect and restore p53-dependent drug sensitivity. Furthermore, the transactivation of p21, the anti-proliferative activity of wild-type p53, and the cytotoxicity of antitumor drugs appear to be more dependent on phosphorylation of p53 at Ser15, Thr18, and Ser20 [175,216–218]. This finding supports post-translational defects as a likely cause of a failure to activate p53 and a likely mechanism of the greater resistance observed in tumor cells harboring the wild-type p53 genotype.

As discussed here, part of the evidence supporting the gain-of-resistance phenotype comes from p53 knockdown studies. This phenotype is analogous to the increase in resistance that results from p53 gain-of-function mutants, where sensitivity to antitumor agents is increased after knockdown of the p53 mutant [108]. The supra-resistance associated with wild-type p53 is strongly suggestive of additional functions acquired by p53, although direct evidence for this conjecture is lacking at this stage. Hypothetically, if post-translational defects are indeed involved, as may be envisaged in the absence of ATM, Chk2, or HIPK2 activity, then wild-type p53 may assume a conformation not unlike that of p53 gain-of-function mutants. Whether the specific mechanism involved in the gain-of-resistance phenotype is indeed a result of gain-of-function effects is a question that must await rigorous studies, which are desperately needed not only for the rational design of novel drugs, but also for the clinical application of traditional antitumor agents.

## 5. Conclusions and future directions

From the limited available data presented in this review, it is evident that drug resistance can be significant, particularly when resistant tumor cells harbor wild-type p53. This indeed represents a paradox as it deviates from the established role of this genotype in facile antitumor drug response. Moreover, the resistance in tumor cells expressing wild-type p53 can be much greater than that in tumor cells expressing mutant p53, leading to the recognition of a wild-type p53 gain-of-resistance phenotype, and it is tempting to speculate that this resistance may be a result of gain-of-function effects associated with wild-type p53. It could be argued that the clinical and preclinical data discussed here represent an increase in the sensitivity of mutant/null p53 cancers to a specific drug through the phenomenon of synthetic lethality, rather than an increase in the resistance of wild-type p53 tumors. A concerted effort by the research community will be required to unravel this question and to define the underlying mechanism for the supra-resistance of wild-type p53 cancers. Such data will be of paramount importance to change the treatment paradigm and to increase responses and cure rates in wild-type p53 gain-of-resistance cancers. Effective prototypical small-molecule drugs (e.g., DAP) already exist and provide evidence that activity against such cancers is possible, but recognition of this gain-of-resistance phenotype in cancer is the first step toward defining the mechanisms involved and thus laying the foundation for rational-based therapies. Such a progression is vital to ultimately achieve success at the clinical level against resistant cancers harboring wild-type p53.

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## Abbreviations

| | |
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| **ALL** | acute lymphocytic leukemia |
| **APAF-1** | apoptotic protease-activating factor 1 |
| **AIF** | apoptosis-inducing factor |
| **ATM** | ataxia telangiectasia mutated |
| **ATR** | ataxia telangiectasia and Rad3-related |
| **CBP** | cAMP-response element-binding protein-binding protein |
| **Cdk** | cyclin-dependent kinase |
| **CLL** | chronic lymphocytic leukemia |
| **DAP** | *1R,2R*-diaminocyclohexane(*trans*-diacetato)(dichloro)platinum(IV) |
| **DISC** | death-inducing signaling complex |
| **DSBs** | DNA double-strand breaks |
| **FADD** | Fas-associated death domain |
| **5-FU** | 5-fluorouracil |
| **HIPK2** | homeodomain-interacting protein kinase 2 |
| **HNSCC** | head and neck squamous cell carcinoma |
| **HPV-16** | human papillomavirus type 16 |
| **$IC_{50}$** | drug concentration that inhibits cell proliferation by 50% |
| **MEF** | mouse embryonic fibroblasts |
| **MRN** | Mre11/Rad50/NBS1 complex |
| **NSCLC** | non-small cell lung cancer |
| **RCC** | renal cell carcinoma |
| **TNF-R** | tumor necrosis factor receptor |

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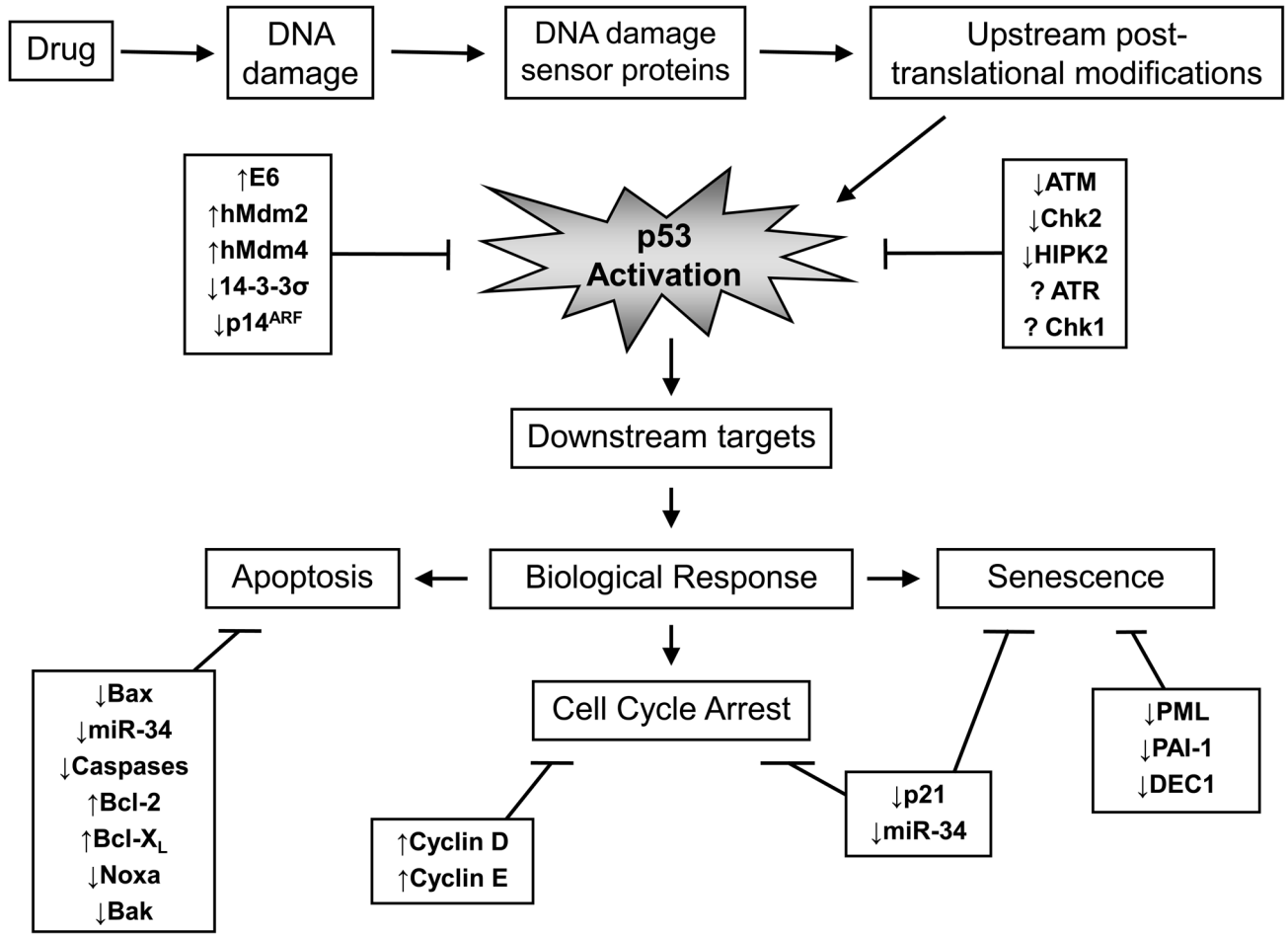


**Fig. 1.**
Deregulation of pathways attenuating p53-dependent drug response.

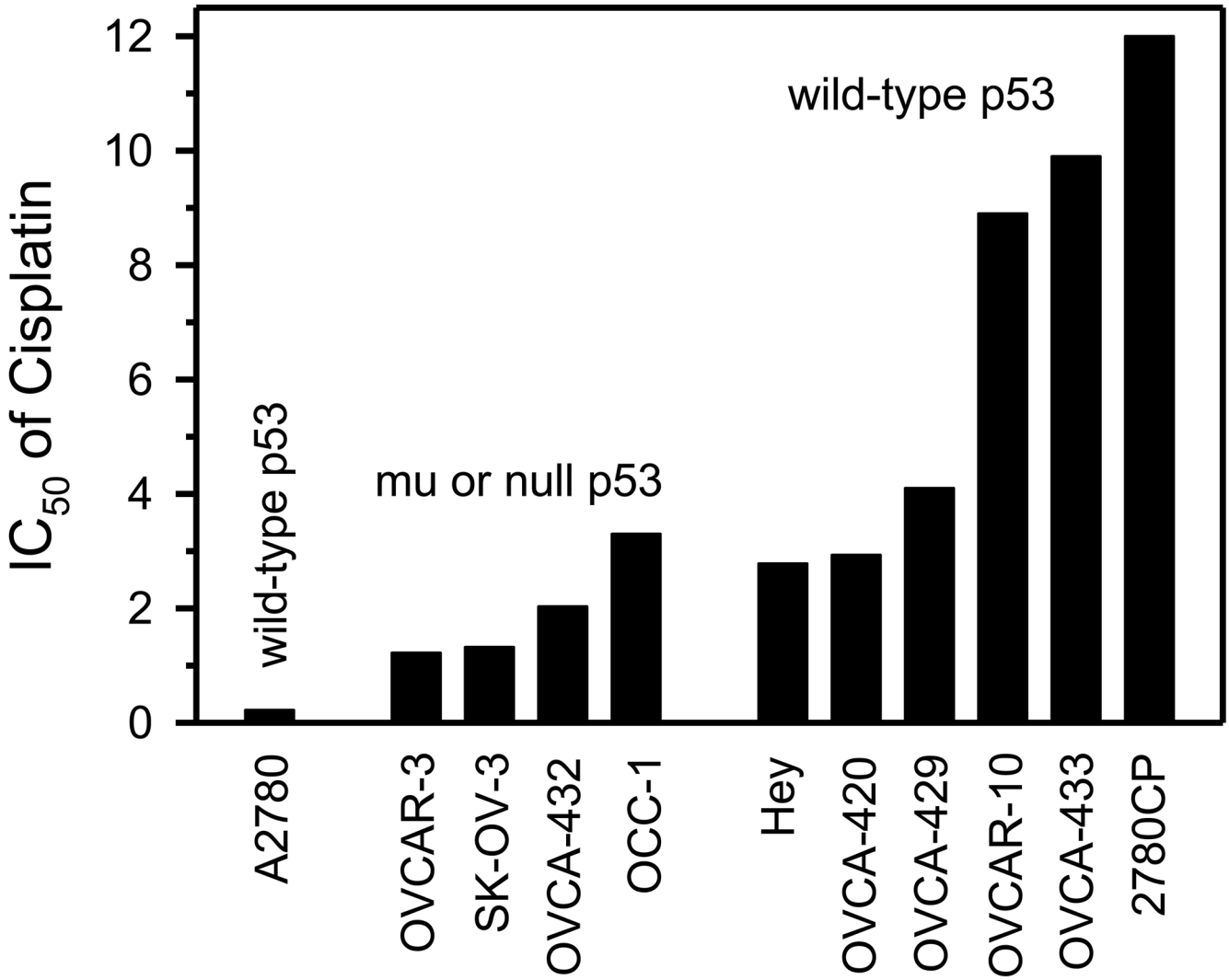


**Fig. 2.**

Cisplatin resistance in an ovarian tumor panel according to p53 status. The A2780 cell line is the sensitive tumor model. The resistant models are grouped into mutant (mu)/null p53 (*N* = 4) and wild-type p53 (*N* = 6); the 2780CP cell line was developed in tissue culture from A2780 cells, whereas all others were established directly from patients' biopsies. The $IC_{50}$ value is the concentration of cisplatin that inhibits cell proliferation by 50% and is expressed as μM. Adapted from Hagopian et al. [40] and Siddik et al. [213].

## Table 1

Cytotoxic drug response of tumor cell lines transfected with p53 gain-of-function mutant.

| | | | $IC_{50}$ (μM) | |
|---|---|---|---|---|
| **p53-Null tumor model [Ref.]** | **p53 Mutation** | **Treatment** | **Control** | **Mutant p53** |
| H1299 [90] | R175H | Etoposide | <10 | >10 |
| | R175H | Cisplatin | <8 | >8 |
| | R273H | Etoposide | >10 | <10 |
| | R273H | Cisplatin | <8 | >8 |
| Saos-2 [107] | R273H | Methotrexate | 0.012 | 0.025 |
| | R273H | Doxorubicin | 0.03 | >0.2 |

*Abbreviation:* $IC_{50}$, drug concentration that inhibits cell proliferation by 50%.

## Table 2

Clinical response of cancers harboring wild-type p53 to chemotherapy.

| Cancer type [Ref.] | Treatment | Incidence (%) | Response rate (%) | 5-Year survival rate (%) |
|---|---|---|---|---|
| Bladder [123] | Various | 49 | Not given | 45 |
| Breast [85] | Paclitaxel | 67 | 45 | Not given |
| Choroid plexus [84] | Various | 50 | Not given | 100 |
| CLL [87] | Chl/flu/cyc | 92 | 83 | 59 |
| Germ cell [88,113] | Platinum | ~100 | ~90 | ~90 |
| Mesothelioma [117–121] | Various | ~90 | 5–33 | 0–14 |
| NSCLC [114,115,125] | Various | 68 | 6–43 | 0–13 |
| Ovarian [116,124] | Paclitaxel/platinum | 40 | 47 | 43 |
| RCC [122,126,127] | Various | ~90 | Not given | ~30 |

*Abbreviations:* Chl/flu/cyc, chlorambucil/fludarabine/cyclophosphamide; CLL, chronic lymphocytic leukemia; NSCLC, non-small cell lung cancer; RCC, renal cell carcinoma.

**Table 3**

Greater drug resistance in tumor panels and cell lines expressing wild-type p53.

**A. Studies in tumor panels**

| Tumor panel [Ref.] | Panel size (*N*) | Treatment (unit of $IC_{50}$) | Mean $IC_{50}$ (*N*) | | Fold[a] |
|---|---|---|---|---|---|
| | | | wt p53 | mu/null p53 | |
| HNSCC [205] | 23 | Cisplatin (μM) | 13.7 (10) | 6.8 (13) | 2 |
| Ovarian [40] | 9 | Cisplatin (μM) | 5.7 (5) | 2.0 (4) | 2.9 |
| Ovarian [206] | 15 | Paclitaxel (μM) | 0.077 (6) | 0.015 (9) | 5 |
| RCC [207] | 8 | Paclitaxel (μM) | 1.54 (6) | 0.02 (2) | 77 |

**B. Studies modulating wild-type p53 in cell lines by molecular approaches**

| Tumor type [Ref.] | Cell line | Treatment (unit of $IC_{50}$) | Mean $IC_{50}$ | | Fold[a] |
|---|---|---|---|---|---|
| | | | wt p53 | dn/ko/kd p53 | |
| Glioma [208] | U87MG | Carmustine (μg/mL) | 7 | 3 | 2.3 |
| Colon [209] | HCT-116 | Trabectedin (nM) | 14.6 | 4.4 | 3.3 |
| Colon [210] | HCT-116 | Cisplatin (μM) | 3.9 | 0.9 | 4.3 |
| Breast [209] | MCF-7 | Trabectedin (nM) | >100 | 0.23 | >400 |
| Breast [211] | MCF-7 | Cisplatin (μM) | 3.38 | 1.69 | 2 |

*Abbreviations:* dn, dominant-negative; HNSCC, head and neck squamous cell carcinoma; $IC_{50}$, drug concentration (in μg/ml, nM, or μM) that inhibits cell proliferation by 50%; kd, knockdown; ko, knockout; *N*, number of cell lines in group; RCC, renal cell carcinoma; wt, wild-type.

[a]Fold, ratio of $IC_{50}$ between wt p53 and mu/null p53 or dn/ko/kd p53 groups.