Review

Current strategies to target p53 in cancer

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A R T I C L E   I N F O

A B S T R A C T

Tumor suppressor p53 is a transcription factor that guards the genome stability and normal cell growth. Stresses like DNA damage, oncogenic assault will turn on p53 function which leads to cell cycle arrest for DNA repair, senescence for permanent growth arrest or apoptosis for programmed cell death. At the late stage of cancer progression, p53 is hijacked in all forms of tumors either trapped in the negative regulator such as MDM2/viral proteins or directly mutated/deleted. Re-introduction of a functional p53 alone has been proven to induce tumor regression robustly. Also, an active p53 pathway is essential for effective chemo- or radio-therapy. The emerging cyclotherapy in which p53 acts as a chemoprotectant of normal tissues further expands the utility of p53 activators. Functionally, it is unquestionable that drugging p53 will render tumor-specific intervention. One direct method is to deliver the functional wild-type (wt) p53 to tumors via gene therapy. The small molecule strategies consist of activation of p53 family member such as p73, manipulating p53 posttranslational modulators to increase wt p53 protein levels, protein–protein interaction inhibitors to free wt p53 from MDM2 or viral protein, and restoring p53 function to mutant p53 by direct modulation of its conformation. Although most of the current pre-clinical leads are in μM range and need further optimization, the success in proving that small molecules can reactivate p53 marks the beginning of the clinical development of p53-based cancer therapy.

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1. Overview

The p53 tumor suppressor is a transcription factor which forms a homotetramer on the target gene response elements [1]. Its mRNA is ubiquitously expressed, but the expression levels vary depending on the development stages and tissue types. In normal cells under non-stressed condition, the biologically functional p53 protein has a short half-life and is hardly detectable via western analysis [2]. The protein level of p53 is under the tight control of its negative regulator murine/human double minute 2 and 4 (MDM2/HDM2) via ubiquitination. As MDM2 is also a target gene of p53, it forms a negative feedback loop [3]. Stress signals, such as oncogene assaults, chemo- or radiation-induced DNA damages, hypoxia or nucleotide depletion, can all induce the accumulation of p53 protein in normal cells which in turn triggers the transcription of different categories of p53 target genes, such as those regulating cell cycle arrest/DNA repairing for the cell survival or apoptosis for the cell death [1,4]. Survival or death is determined by the combination of the signal contents initiated by the activation of p53 under the specific cellular background. DNA damage signals will stabilize p53 which in turn regulates protein targets involved in cell cycle arrest/DNA repair, and apoptosis if damage is severe. So, activation of p53 via DNA damage may not absolutely lead to apoptosis. On the other hand, activation of p53 without DNA damage will likely avoid stimulation of the whole molecular machinery contributing to cell survival. Consequently, it may favor activation of p53-induced apoptotic pathway [5,6]. For example, non-genotoxic nutlin and RITA treatments induce massive apoptosis in tumor cell lines expressing wild-type p53 with or without cell cycle inhibition [7,8].

The fact that p53 signaling pathway is inactivated in all types of cancers has drawn great attention from the world-wide cancer researchers to target p53 pathway for the development of improved cancer therapies [9,10]. Specifically, ~50% of the patients contain various inactivating mutations in their p53 proteins, while the other ~50% of the patients possess defective components in posttranslational modification of p53 protein or abrogation of p53 signaling pathway [11]. It has also been well documented for years that a functional p53 plays a central role in mediating the tumor suppressive function derived from the chemotherapy or radiation treatment [9,12]. Recently, three seminal studies by Ventura et al., Xue et al. and Martins et al. demonstrated inactivation of p53 was indeed a necessary component in maintaining tumor phenotype as all three studies had showed that restoring p53 alone was sufficient to induce tumor regression in mice [13–15]. The transformed environment of tumor cells appears to be super-responsive to re-introduction of p53 activity which turns on the tumor suicidal path or senescence followed by immune system-assisted tumor clearance. The elegant studies have enhanced extra interest in developing approaches to restore p53 function specifically in human tumors so as to bypass the deleterious side effect resulting from most of the current cancer treatments based heavily on DNA damaging [1,5].

There is an added advantage of directly targeting the mt p53 which is tumor specific. Mutant p53 is frequently overexpressed and posttranslationally modified in tumor cells. The cellular environment of tumor cells also favors the functional p53-induced apoptosis. Therefore, any small molecules or peptide chaperones that can stabilize mt p53 wt conformation will activate apoptosis pathway in tumor cells [5,16].

In addition, most chemotherapeutic agents and radiation therapy require a functional p53 pathway, therefore p53 small chemical activators can also increase the sensitivity of the chemotherapy and radiation therapy [12]. Further more, the emerging concept of cyclotherapy implies that p53-specific drugs will function as chemoprotectants on normal tissues when combined with anti-mitotic drug [17,18]. In a word, it cannot be disputed that p53 is the best target for cancer specific treatment development.

2. Challenges of p53 as a drug target

Although p53 is functionally an attractive target for cancer therapy development, the main question is whether it is a druggable protein? Based on the conventional drug discovery/development process, p53 is not an ideal drug target. It is not a receptor or an enzyme. To make matter worse, it is a homo-tetrameric nuclear transcription factor which plays a central role in keeping the genome stability and guarding normal cell growth and physiological functions [10,11]. Further more, the mode of p53 inactivation is diverse including deletion of p53, inhibition of p53 by MDM2 or viral proteins, and >2000 different types of mutations in p53 proteins depending on individual patient. Therefore, the development process of p53-based therapeutics not only faces the technical challenge, but also needs to discretely manipulate this target to achieve better therapeutic efficacy with reduced adverse effect. Nevertheless, the impressive advancement of the technology in drug development and the knowledge accumulated on p53 over the past 30 years after its discovery make it a realistic ambition to develop tumor-specific p53 restoration therapies. Progress has been made using virus-based gene therapy delivering a functional copy of p53 via adenoviral vector or oncolytic adenovirus ONYX-015 [19,20]. Small molecules inhibiting MDM2 and p53 interaction have also been identified [21]. Multiple approaches directly modulating p53 conformation and inducing its wt functions have been reported in recent years [11].

In this review, we would like to focus chronologically on strategies utilized in activating p53 pathway via p53 gene therapy, stimulating p63/p73, targeting p53 posttranslational modification proteins, disrupting wt p53 and MDM2 interaction and restoring mt p53 functions. Some details of each of the approach are also included so as to provide clues guiding future improvement on strategically targeting p53 for better cancer therapy development.

3. Adenovirus-based p53 gene therapy

As tumor cells cannot tolerate wt p53 or functional p53 [5], an obvious approach of cancer therapy is to deliver wt p53 gene

into tumor cells. Adenovirus-based p53 gene therapies, such as Advexin and Gendicine, are examples of this strategy [22,23]. Both therapies have been advanced to phases I, II and even III clinical trials. Results in general demonstrated that antitumor efficacy was associated with expression of the functional p53, and adverse effects were insignificant [1,22–24].

Instead of delivering a functional p53 into tumor cells and inducing apoptosis, ONYX-015 is an oncolytic adenoviral therapy. It selectively replicates in p53 dysfunctional tumor cells and results in the specific lysis of those cells. Phase I trial has showed its expected efficacy and non-significant side effects [25].

The encouraging data from adenovirus-based p53 gene therapy support further development of this clinical intervention so as to resolve the issues of viral clearance derived from immune response and broaden its utility beyond head and neck cancers.

4. Activation of wild-type p53 through drugging its modification proteins

Accumulated data have suggested that subtle variation in wt p53 protein levels can affect the threshold of the tumor on-set rate [32]. Therefore, compounds regulating p53 modification proteins will be expected to exert indirect impact on p53 protein levels and consequently its function. The following discovery proves that increasing wt p53 protein levels in cancer cells via manipulating p53 posttranslational modifiers contribute to enhanced p53 activity (Fig. 1).

4.1. Tenovin-1 and Tenovin-6

Tenovin-1 was identified by screening ChemBridge DIVERSet small molecule collection utilizing a cell-based p53-dependent reporter assay. Compounds at 10 μM were screened against T22-RGC-ΔFos-lacZ murine cells expressing β-galactosidase under the control of a p53-dependent promoter. β-Galactosidase activity was measured 18 h post-treatment. Tenovin-6 is the more water-soluble analog of Tenovin-1. Both compounds activated p53 reporter robustly. Further profiling the compound via a yeast genetic screen revealed that Tenovin-1 inhibited the protein-deacetylating activities of SirT1 and SirT2 of the sirtuin family. Acetylation of p53 not only stabilized p53 but also interfered MDM2-mediated p53 degradation [33]. This result demonstrated that compounds targeting p53-modulating

Fig. 1. Strategies in wild-type p53 activation. (1) Tenovin-6 inhibits protein-deacetylase activity of SirT1 and SirT2. Acetylation results in the stabilization of p53 and interferes MDM2-mediated degradation. (2) NEI inhibits the nuclear export protein CRM1 which increases nuclear p53 level indirectly. (3) HLI98 blocks the ubiquitin ligase activity of HDM2. It prevents p53 degradation and elevates p53 level indirectly. (4) RITA binds to p53 and interferes with MDM2 and p53 interaction which activates p53 function. (5) Both Nutlins and MI219 interact with MDM2 and block p53 interaction. Consequently, they activate p53.

proteins could also achieve the goal of activating or increasing p53 activity.

4.2. Nuclear export inhibitors (NEIs)

New NEIs were synthesized based on leptomycin B (LMB) which is a specific inhibitor of the export protein CRM1. Blocking CRM1 actually shows therapeutic efficacy in inducing cancer cell apoptosis and inhibiting tumor growth in wt p53 cancer models. So increasing p53 protein level by blocking its export CRM1 can also achieve cancer therapeutic effect. The new improved NEIs exert better window for efficacy vs. adverse effect in comparison to the parent compound LMB. Their effect on normal lung fibroblasts is cell cycle arrest. The current study strongly supports targeting CRM1 as a novel strategy in cancer therapy [34].

4.3. HLI98 (HDM2 ubiquitin ligase inhibitor)

Wild-type p53 protein level is regulated by HDM2. HDM2 can not only interfere p53 interaction with other transcription factors, but also target p53 for degradation via its ubiquitin ligase (E3) function ubiquitylation [35,36]. Therefore, inhibition of E3 activity can stabilize wt p53 and elevate p53 target gene expression. As a result, rescuing of wt p53 via inhibition of E3 will cascade the apoptosis and tumor suppression function in cancer cells. Targeting HDM2 E3 activity presents another alternative approach in reactivating p53 in tumors, which bypasses the genotoxic damage associated with the current cancer therapeutic drugs. The identification of HLI98 validates HDM2 E3 as a target in restoring wt p53 activity in cancer cells. Further search of HDM2 E3 inhibitor with improved specificity and efficacy is warranted [37].

5. Inhibition of MDM2 and wild-type p53 protein–protein interaction

Technical advancement in developing small molecule inhibitor of protein–protein interactions (PPI) has opened a total new class of drug targets [38]. The successful identification of MDM2 and p53 PPI inhibitors proves the concept that it is feasible in tackling this new group of drug targets [39,40]. Biotech companies devoted to developing drug-like MDM2 and p53 PPI inhibitors have emerged with the advancement of technology [21]. The existing progress has also revealed that MDM2 inhibitors exhibit desirable safety profile with the advantage of selectively inducing cancer cell death while exerting cell cycle arrest in normal cells without initiating DNA damage [7] (Fig. 1).

5.1. Nutlins

Nutlins were identified using the conventional in vitro biochemical screening method. This approach has the advantage of clear target engagement, but it lacks the ability to predict if the hits will exhibit cytotoxicity, poor permeability, and inferior stability in cells in the follow up assays. To carry out the screening, both HDM2 (residues 25–108) and human p53 (His6-residues 1–312) were expressed in E. coli. Screening assay was proceeded by incubating HDM2–p53 protein complex in the presence of the tested compounds. Compounds that inhibited the HDM2–p53 interaction as measured by Biacore S51 were identified as hits. Hits were further validated by various cell-based assays and in vivo tumor xenograft model. Nutlins fit in the binding pocket of p53 in MDM2 and inhibited p53 interaction. Consequently, these MDM2 antagonists reached the goal of tumor inhibition in nude mouse xenograft model [39]. One of the nutlins R7112 has advanced to the implementation of a phase I clinical trial to treat patients with hematologic neoplasms.
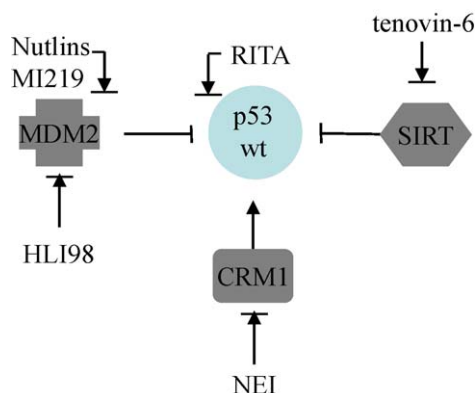
5.2. MI219

MI219 is a product of crystal structure guided designer molecule. With the emergence of more 3D X-ray or NMR structures on biomolecules and sophisticated molecular modeling/docking tools, structure-based drug design is gaining popularity. This approach has shown promise in accelerating drug discovery and development when coupled with various cell-based assays [41,42]. Yet, this method inherits the similar advantage and drawback of the in vitro biochemical screening. Based on the crystal structure of the MDM2–p53 complex [43], the research group from University of Michigan designed spiro-oxindoles as a new class of inhibitors of the MDM2–p53 interaction. Because of the poor pharmacokinetic (PK) profile of the lead compound MI63, MI-219 was developed by extensive modifications of this parent compound. MI-219 exhibits a high binding affinity to MDM2 with Ki value of 5 nM. It is ~10,000-fold selective for MDM2 over MDMX. Treatment of MI-219 causes cell cycle arrest in all cells with wt p53 and induces apoptosis in tumor cells. The discovery of MI219 demonstrated the utility of the crystal structure guided drug design [44].

5.3. RITA (reactivation of p53 and induction of tumor cell apoptosis)

Cell-based screening assays have been used extensively in recent drug development process because they eliminate the step of large quantity of purified protein preparation. Also, advancement in robot friendly cell-based assay format makes it possible to screen thousands of compounds in 1 or 2 weeks even in an academic lab. In addition, if the hits are scored based on up-regulation of signals, it also offers the advantage of obtaining lead compounds without issues of cytotoxicity, permeability or instability in cells. The main challenge of this approach is to prove the target engagement and mechanism of action as the final expected cellular phenotype can result from several different mechanisms [45].

RITA was discovered from the following cell-based assay. Cell lines of HCT116 containing wt p53 and HCT116TP53$^{-/-}$ lacking p53 gene were screened with compounds from NCI Diversity and Challenge sets at 25 μM. Cell viability was assessed using WST-1 assay 48 h post-treatment. One compound was identified that selectively and dose dependently repressed HCT116 cell growth but only slightly inhibited the growth of HCT116TP53$^{-/-}$. Further characterization of this compound which was designated as RITA demonstrated that it bound to p53 directly. The interaction of RITA with wt p53 prevented its interaction with HDM2 and resulted in accumulation of p53. Consequently, RITA induced p53 target gene expression and triggered massive apoptosis in various tumor cells expressing wt p53 [46].

So, MDM2 and P53 PPI inhibitors have been identified utilizing traditional in vitro biochemical screening, crystal structure guided design of drug-like molecule and cell-based screening assay.

6. Activation of p53 pathway via family member p63/p73

The p53 protein family consists of p53, p63 and p73. As transcription factors, they share similar protein structure and biological function. In addition, there exist various isoforms of each gene and they can heterodimerize with each other. These add extra complexity in the p53 signaling [26]. Worth noting here is that p53 but not p63 or p73 is frequently mutated in human cancer cells [26,27]. Therefore, p63 or p73 could step up their tumor suppressive function in p53 defective cancers. Treatments that can increase p63 or p73 protein levels will deliver p53-like function [26]. The discovery of NSC176327 is a good example (Fig. 2).
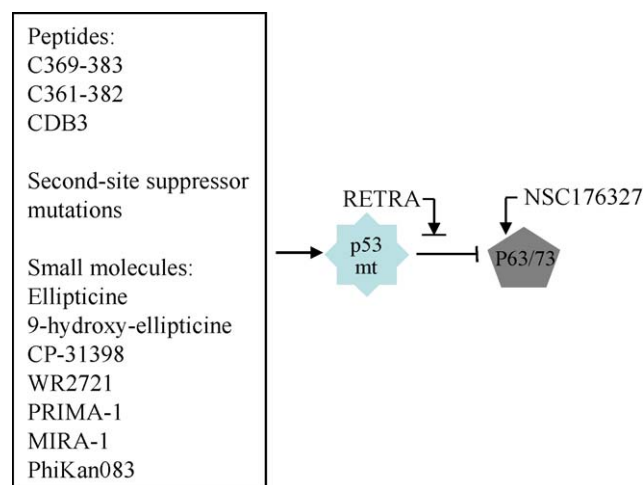

Peptides:
C369-383
C361-382
CDB3

Second-site suppressor mutations

Small molecules:
Ellipticine
9-hydroxy-ellipticine
CP-31398
WR2721
PRIMA-1
MIRA-1
PhiKan083

RETRA ⊣ p53 mt ⊣ NSC176327 → P63/73

Fig. 2. Strategies in reactivation of mutant p53. (1) NSC176327 induces p73 expression and activates p53-like activity in a p53 independent manner. This approach is especially useful in tumors with p53 deletion. (2) RETRA relieves the negative effect of mt p53 on p73 and induces p73 expression. Treatment of RETRA exhibits p53-like activity. (3) All molecules/approaches in the box rescue mt p53 function by assisting mt p53 refolding or driving the protein folding equilibrium from denatured/mt conformation more toward the native functional/wt conformation. Restoration of native conformation to mt p53 endows its wild-type activity.

6.1. NSC176327 (a close derivative of ellipticine)

Human colon adenocarcinoma cell line SW480 bearing mt p53 (R273H, P309S) was stably integrated with a p53 reporter containing the firefly luciferase gene under the control of 13 copies of p53 response elements. This p53 defective cell line was screened against compounds from NCI diversity set at 10 and 50 μM. The transcriptional activity of p53 reporter was measured using IVIS imaging system for a time period ranging from 12 to 72 h. A number of small molecules up-regulating p53 target genes had been identified through this imaging-based cell assay. Further characterization of the compounds revealed that some of the hits actually activated p53-like activity independent of p53 status via elevating p73 expression [28]. NSC176327, an ellipticine derivative, was one of the compounds identified in this screening process. Treatment of NSC176327 increased p53 target genes DR5 and p21 expression. NSC176327 was less effective in inhibiting cell growth when p73 was knockdown in HCT116 p53$^{-/-}$ cells indicating p73 played a critical role in NSC176327-induced p53-like activity [29].

Besides inducing the expression of p53 family members, compounds releasing inhibition of mt p53 on p73 has also been reported to exert p53-like function. Mutations in p53 result in loss of its tumor suppressive function. Some of the p53 mutants even gain dominant-negative activity and inhibit transcriptional function of its family member p73 through protein–protein interaction [30]. Compounds that block mt p53 and p73 interaction can also activate p53 target genes and rescue p53 function.

6.2. RETRA (reactivation of transcriptional reporter activity)

Human epidermal carcinoma cell line A431 bearing R273H p53 mutant was engineered to contain a β-galactosidase p53 reporter (A431/LC5). A control cell line with the reporter structure lacking p53 response element (A431/LC0) was used to determine the background level reporter activity. A431/LC5 cells were treated with compounds at 5 μM for 14 h before the β-galactosidase activity was measured. RETRA was identified to enhance p53 reporter activity in a mt p53-dependent manner. RETRA treatment increased p73 expression and prevented p53 inhibition of p73,

which produced a p53-like tumor suppression effect. The exact mechanism of how RETRA interfering mt p53 and p73 interaction is unclear currently [31].

7. Reactivation of mutant p53—war against the remaining 50% of the cancers

As ~50% tumors possess primarily missense mutations, alternative strategies are needed to tackle cancers in this category. Mutations in p53 are a late-occurring event during cancer progression. Mutations not only disable p53 tumor suppressive function but also exhibit cancer-promoting activity by gaining oncogenic properties or inactivating remaining wt p53 and/or p73 via dominant-negative (DN) function [10,30]. More than 2000 different types of mutations have been identified in p53 in various cancers [31]. About 95% of the mutations occur in the core DNA binding domain, which emphasizes that p53 tumor repressor functions mainly as a transcription factor. Tumor-associated mt p53 proteins are mainly full length with a single amino acid change. These mutations can be roughly categorized into two classes: structural and DNA contact. Structural mutations can disrupt local or global protein folding, while DNA contact mutations predominantly interfere with amino acid residues interaction with DNA response element exhibiting little effect on the protein folding [11]. While wt p53 has short half-life and low abundance, mt p53 proteins are quite stable, abundant and posttranslationally modified in tumor cells [16]. Mere synthesis of wt p53 in normal cells is not enough to activate p53 signaling, while restoring p53 wt function in cancer cells can turn on p53 signaling pathway. This demonstrates that the cellular environment of cancer cells has some kind of built-up potential which is ready to activate expressed wt p53 [47]. Based on the above facts, it is logical to postulate that tumor cells will be highly sensitive to mt p53 reactivation. Therefore, drugging mt p53 can achieve great specificity and efficacy in treating cancers while avoiding deleterious adverse effects associated with many of the current cancer therapies.

Consequently, restoration of wide-type function to mt p53 via direct modulation of mt p53 conformation has been attempted extensively by many research groups [48]. Even though the mutations are diverse in tumors, there is a common feature among most of the mt p53 conformation changes in the DNA binding core domain resulting in loss of DNA binding activity [9,49]. Based on the intrinsic property of a peptide, the p53 protein is in a constant equilibrium between native functional/wt and denatured dysfunctional/mt conformations. Mutations in p53 drive the equilibrium more towards the denatured conformation. It is reasonable to postulate that there exist small molecules termed molecular chaperones to force the equilibrium towards the native functional/wt conformation for mt p53 [50]. Current progress has demonstrated the feasibility of this approach (Fig.2).

7.1. Allosteric activation of p53 via small peptides

C-terminal negative regulatory domain locks the unphosphorylated p53 tetramer in an inactive state. Activation of p53 via phosphorylation of the C-terminus or mAB directed to this domain does not require increase in protein level. Therefore, small peptides derived from the C-terminal negative regulatory domain were tested and peptide C1 (C369–383) was identified to activate p53 specifically at 100 μM. The results not only demonstrated the allosteric mechanism of negative regulation derived from the C-terminus, but also provided the proof of concept that p53 could be activated via low molecular mass modulator [51]. Selivanova et al. further revealed that peptide corresponding to residues 361–382 of p53 could also restore the transcriptional activity of some mt

p53 in living cells in addition to activating wt p53. The above observation further pushes the hypothesis of small molecule chemicals can allosterically stabilize mt p53 conformation, restore its biological function and selectively eliminating tumor cells [52].

7.2. Ellipticine

Ellipticine (EPC) is a natural compound derived from Aspidosperma williansii (Apocynaceae), and the purified form of EPC was obtained in 1959. Its anticancer and cytotoxic activities on various types of tumors are well-known. The toxic side effects have limited EPC to be developed into a cancer therapeutic agent despite of its attractive structural nature that can serve as the basis for various modes of mechanism of action. However, extensive attempts have been made to systematically optimize EPC structure [53,54]. One such compound 9-hydroxy-ellipticine (9HE) has been shown to induce G1 arrest and trigger G1 phase-restricted apoptosis in a mt p53-dependent manner in several tumor cells [55]. Exactly how 9HE restores mt p53 wild-type p53 function remains to be elucidated.

7.3. CP-31398

CP-31398 is the first small synthetic prototype molecule that rescues mt p53 tumor suppressor function. A screening assay was established utilizing DNA binding domain (DBD) of p53, monoclonal antibody (mAB) mAb1620 which recognizes p53 DBD active conformation and mAb240 for DBD with disrupted conformation. Small molecule CP-31398 had been identified that could restore active conformation in mt p53 DBD, up-regulate p53 target gene expression and repress tumor growth in mice [56]. This work has provided the proof of concept that it is possible to identify small synthetic compounds that can reactivate even mt p53. Further characterization of CP-31398 demonstrated that restoration of DNA binding activity is p53-specific without effect on its family member p63 or p73 [57].

7.4. CDB3—molecular chaperone

The work of C-terminal peptides and CP-31398 have paved the road for further identification of small molecules that can rescue mt p53 function. Through rational search, a nine-residue peptide, CDB3, derived from a p53 binding protein, was identified. CDB3 binds at the edge of the p53 DNA binding site and stabilizes it in vitro. This binding can be competed by gadd45 DNA. Binding of CDB3 to wt or mt p53 (R249S) core domain has raised the apparent melting temperatures. The molecular chaperone model has been proposed that CDB3 binds to the native form of p53 and drives the equilibrium from denatured/mutant conformation more towards the native functional/wild-type conformation. It is proposed that CP-31398 stabilizes the p53 core domain via the similar mechanism, and more small molecules of this type are predicted to be discovered in the coming years [50].

7.5. Second-site suppressor mutations

Through extensive genetic approaches and p53 assays in yeast and mammalian cells, Baroni et al. reported the identification of second-site mutations involving residues 235, 239 and 240 that endowed wt p53 function back to 16 out of 30 of the most common p53 mutants tested. The study demonstrates that it is possible to activate p53 mutants by modifying a small region of the core domain [58]. Even though it is not a method for therapeutic agents discovery, it further proves that the global p53 conformation can be re-shaped by manipulating locally with small molecules. By now, the concept that small synthetic compounds that can achieve the goal of restoring mt p53 tumor suppressor function has been solidified.

7.6. WR2721 (amifostine)

Amifostine (ethyol) is a radio- and chemoprotector which has been reported to activate wt p53 in cultured mammalian cells. WR2721 is dephosphorylated in vivo by alkaline phosphatase and converted to WR1065, the active metabolite. Further investigation of WR2721 using a yeast functional assay revealed that WR2721 could also restore completely or partially the transcriptional activity of p53 mutants. However, similar to other cell-based assays, this yeast functional assay cannot depict the exact mechanism on how the compounds modulate p53 conformation [59].

7.7. PRIMA-1

PRIMA-1 was identified via cell-based screening assay. Saos-2 cell line was engineered to contain His-273 mt p53 with expression under the regulation of tetracycline (Tet-Off). Saos-2–His-273 cells were grown in the presence or absence of doxycyline and treated with compounds from NCI diversity set at 25 μM in 96-well plates. Cell viability was assessed using WST-1 assay 48 h post-treatment. One compound was identified that inhibited Saos-2 cell growth in a mt p53-dependent manner. It was designated as PRIMA-1 short for p53 reactivation and induction of massive apoptosis. PRIMA-1 was also shown to inhibit the cell growth of other cell lines containing Tet-Off regulated mt p53 expression including SKOV–His-175, SKOV–His-273 and H1299–His-175. PRIMA-1 can rescue p53 activity of mutants with DNA contact or global structural mutation. Inhibition of human xenograft tumor growth can be achieved via systemic administration of PRIMA-1 or its methylated version PRIMA-1^{MET} [60]. Extensive characterization of PRIMA-1 reveals that it forms adducts with thiols in mt p53 core domain. This covalent modification reactivates mt p53 and induces apoptosis in tumor cells [61].

7.8. MIRA-1

MIRA-1 was identified from the same screen that discovered PRIMA-1. It exhibited similar biological activity to PRIMA-1 except that it was slightly more potent in inducing cell death than that of PRIMA-1. In addition, it is structurally different from PRIMA-1 or CP-31398, representing a novel class of mt p53-reactivating molecules. MIRA-1 and its analogs lead the rescue of mt p53 by shifting the equilibrium between the native and denatured conformation of p53 toward the native/active conformation. The molecular mechanism of how MIRA-1 interacts with mt p53 and drives the equilibrium toward native functional conformation is unclearly. It is hypothesized that the maleimide group in MIRA compounds may react with thiol and amino groups in proteins, which may likely stabilize the native fold of p53 [62].

7.9. PhiKan083

Y220C is a common mutation reducing p53 stability with about 75,000 new patients of this category per year. Through in silico screening targeting the surface cavity resulted from Y220C mutation, a carbazole derivative (PhilKan083) was identified that bound to the cavity with a Kd ~ 150 μM and increased the melting temperature of the mutant. The current progress provides a foundation for further lead optimization in targeting Y220C mutant [63].

8. Summary

Major strategies employed in reactivating p53 pathway have been updated in the current review. For small molecules obtained through target engagement-based method, such as in vitro biochemical screening, crystal structure guided designer molecule, or in silico screening, their mechanism of activation is clear. Lead compounds usually can be further optimized via comprehensive medicinal chemistry, a process known as structure–activity relationship (SAR) study. On the other hand, cell-based assay has the advantage of drugging any biological outcome, but the challenge is to delineate the mechanism of activation so as to move to the SAR process [11]. As exemplified in the review, compounds derived from cell-based screening can fall in all the categories of mechanism of activation, which are activating p53 family members, modulating p53 posttranslational modifiers, MDM2 and p53 PPI inhibitor and mt p53 refolding chaperone. Still, the mechanism of activation remains to be determined for compounds like 9HE, WR1065 or MIRA-1. One common feature of all cell-based assays in this review is that all screens had been performed against the chemical libraries at >5 μM, and the treatment effects were evaluated >12 h post-treatment. This procedure could produce false negative on potent compounds when assays examine up-regulation of the p53 reporter. This is because the endogenous p53 activity is also awakened when p53 reporter shows increased activity. Activation of p53 signaling can trigger cell death which may result in low p53 reporter activity 12 h post-treatment especially for potent p53 activators. Also, except M-219, all lead compounds stimulate p53-like activity in the μM range. Therefore, the identification of potent p53 activators with favorable pharmacokinetic properties should drive the future assay improvement. Nevertheless, achievements in reactivating p53 from different angles warrant further investment in targeting p53 for cancer selective therapy development.

9. Future perspectives

It is no doubt that reactivating p53 provides a general strategy in conquering cancer specifically. As the cause of p53 malfunction varies among patients, developing a magic bullet to be effective for most of the patients is still far reaching currently. Personalized medicine has to be implemented in treating each cancer patient. Therefore, genetic diagnosis will have to come hand in hand with the development of p53-based cancer therapies. In addition, resistance to the treatments derived from new p53 mutation or pathway abrogation will haunt the field resembling the drug resistance situation in the treatment of virus, bacteria or yeast infections. So new molecules directed towards the newly occurred mutations will be developed on a continuing base. After 30 years of p53 and over 100 years of cancer research, we are never this much ready to declare a long-term war against cancer.

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