Resuscatiting Wild-Type p53 Expression by Disrupting Ceramide Glycosylation: A Novel Approach to Target Mutant p53 Tumors

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Abstract

Mutant p53 is frequently detected in cancers in which p53 has lost its ability in tumor suppression and gained function in promoting tumor progression. Restoration of p53 functions by replacement of wild-type p53 and inhibition of its degradation or increment of its transcriptional activity has been applied to the prevention and treatment of cancers. Recent evidence indicates that disrupting ceramide glycosylation can resuscitate wild-type p53 expression and p53-dependent apoptosis in mutant p53 tumors. A posttranscriptional process that can turn on wild-type p53 expression and abrogate mutant p53 may provide a new strategy to eradicate mutant p53 cancers. Cancer Res; 71(20); 1–5. ©2011 AACR.

Introduction

p53 protein, encoded by the human gene TP53, is a key tumor suppressor in preventing tumorigenesis and cancer progression. As an essential transcriptional factor, p53 activates the expression of p21Waf1/Cip1, Bax, Puma, FAS, and other p53-responsive genes, and consequently promotes cell-growth arrest, apoptosis, DNA repair, and cell differentiation. These cell processes remove damaged or transformed cells from normal tissues (1). The transcriptional activity of p53 on p53-responsive genes is sequence specific and relies mainly on its DNA-binding domain (DBD, residues 102–292) encoded by the sequence from exon 4 to exon 8 (1, 2). In normal cells, p53 is tightly controlled and kept at a low level. A wide variety of signals involved in DNA damage, oncogenic stress, hypoxia, and cellular distress activate p53 primarily through posttranslational modifications that result in an augmented level of p53 protein and its transactivation activity. Ubiquitin ligase MDM2, which interacts with and recruits p53 to the ubiquitin-proteasome, regulates p53 degradation (1, 3).

p53 function is compromised in most tumors as a result of somatic TP53 mutations, followed by loss of heterozygosity during the course of carcinogenesis (1, 4). The frequencies of p53 mutations vary considerably among cancer types, ranging from ~10% in hematopoietic malignancies to 50% to 70% in ovarian, colorectal, and head and neck cancers (1, 4). The majority of p53 mutants in human cancers abrogate sequence-specific DNA binding to the promoter element of the p53-responsive genes. Moreover, p53 mutants confer a dominant-negative activity over the remaining wild-type allele by functionally inactive hetero-oligomers of the mutants with the wild-type protein (1, 3). Increasing evidence indicates that many p53 mutants also gain new oncogenic properties that are independent of wild-type p53 (1, 3). p53 mutants that promote tumor progression and resistance to therapies have become the most common prognostic indicator of both tumor recurrence and cancer death (1, 4, 5). Restoration of p53 function has been shown to achieve regression of tumors and thus represents a potential approach to treat cancers (1, 6). This review highlights resuscitation of wild-type p53 expression by targeting ceramide glycosylation, a novel approach for eradicating mutant p53 cancers.

Reactivation of the p53 Pathway in Tumor Suppression

Most malignant tumors that disrupt p53 signaling pathways remain addicted to p53 mutants. Various strategies have been successfully developed to reconstitute p53 functions to abrogate tumor progression (1, 3, 6–8). On the basis of the action sites, these strategies can be divided into 3 groups: (i) replacing wild-type p53 by gene therapy, (ii) augmenting wild-type p53 by inhibiting MDM2-mediated degradation, and (iii) reactivating mutant p53 by altering the protein conformation (Fig. 1A).

It has been shown that restoring p53 function by introducing the wild-type p53 gene alone is sufficient to cause regression of several different types of tumors in mice (1, 6). Gene therapy for p53 replacement delivered by adenoviral vectors to human tumors has shown very promising results in a number of clinical trials (9, 10). p53 gene therapy [Gendicine (Shenzhen SiBiono GeneTech) and Advexin (Introgen)] administered locally has shown at least partial clinical responses as monotherapy, and it has increased the effectiveness of radiation therapy and chemotherapy.
Expression of the p53 transgene occurs at high levels and is associated with the activation of other genes in the p53 pathway after treatment (9). Although these studies provide a proof-of-principle for p53 replacement, it is also noted that adenoviral p53 gene therapy by intraperitoneal injection could not significantly improve standard chemotherapy in ovarian cancers harboring p53 mutants (10). Several factors, including inefficient systemic delivery, nonspecific immune responses, and p53 mutants in cancers, may limit the efficacy and application of p53 gene therapy (refs. 1 and 10; Fig. 1A).

Investigators have applied a group of small molecules to target the p53-MDM2 protein interaction to enhance wild-type p53 protein (7, 11–13). Wild-type p53 usually presents at a low level because of its short half-life (15–30 min), which is maintained primarily by ubiquitin-mediated degradation. As a crucial negative regulator, MDM2 works together with MDMX to ubiquitinate p53 for proteasome-mediated degradation. Nutlin and MI-219, which accumulate wild-type p53 protein by binding to MDM2 and blocking p53-MDM2 interaction, have shown activity against human xenografts in preclinical models (11, 12). RITA (reactivation of p53 and induction of tumor apoptosis) accumulates wild-type p53 by binding to p53 protein and preventing p53 interaction with MDM2 (7, 13). Consequently, these compounds induce the expression of p53-responsive genes and trigger apoptosis in various tumor cells expressing wild-type p53 (11–13). However, the dominant-negative effects and gain-of-function of p53 mutants that present in almost half of all cancer cases may severely compromise the effectiveness of compounds that target the p53-MDM2 interaction (refs. 1, 5, and 10; Fig. 1A).

Restoration of wild-type function to mutant p53 tumors has been attempted extensively in modulation of the protein conformation (3, 8). Binding of wild-type p53 to the DNA to ubiquitinate p53 for proteasome-mediated degradation. Nutlin and MI-219, which accumulate wild-type p53 protein by binding to MDM2 and blocking p53-MDM2 interaction, have shown activity against human xenografts in preclinical models (11, 12). RITA (reactivation of p53 and induction of tumor apoptosis) accumulates wild-type p53 by binding to p53 protein and preventing p53 interaction with MDM2 (7, 13). Consequently, these compounds induce the expression of p53-responsive genes and trigger apoptosis in various tumor cells expressing wild-type p53 (11–13). However, the dominant-negative effects and gain-of-function of p53 mutants that present in almost half of all cancer cases may severely compromise the effectiveness of compounds that target the p53-MDM2 interaction (refs. 1, 5, and 10; Fig. 1A).

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element primarily depends on its protein structure, which is divided into a β-sandwich scaffold and a DNA-binding surface that includes a loop-sheet-helix motif and 2 loops (L2 and L3; ref. 3). Compound PhiLan083 binds with high affinity to a crevice created by Cys220 of the p53 mutant and stabilizes the β-sandwich, which serves as a basic scaffold for the DNA-binding surface (7, 8, 14). On the other hand, another compound, P53Rx, restores the sequence-specific DNA binding of both His273 and His275 of mutant p53 in the DNA-binding surface (15). CP-31998 prevents unfolding of wild-type or mutant p53 and stabilizes p53 through reduced ubiquitination. In these ways, CP-31998 induces the expression of p53-responsive genes, such as p21, but also induces p53-independent cell death (3, 7, 16). Ellipticine enhances the sequence-specific DNA binding and transcriptional activity of mutant p53 and consequently induces mutant p53-dependent cell death (1, 3, 7). PRIMA-1 (p53 reactivation and induction of massive apoptosis) restores the wild-type conformation to mutant p53 protein by covalently binding to and modifying the thiol groups of His273 and His275 in the core domain (8). The PRIMA-1 analog, APR-246, which inhibits human tumor growth and is able to synergize with chemotherapeutic drugs, has shown great potential and is currently being tested in a clinical trial (8, 17). Additionally, synthetic peptides C369-383 and C361-382, corresponding to the C-terminal residues of p53, can alter p53 protein conformation by an allosteric mechanism and restore p53 transactivation in cancer cells, because the C-terminal negative-regulatory domain of p53 locks the unphosphorylated p53 tetramer in an inactive state (7). The compounds in this category directly target particular types of p53 mutants and can be developed as specific therapeutic agents against cancers harboring these mutants. Because p53 binds to promoters of the responsive genes in tetramers, the heterotetramer of wild-type with mutants or homotetramer of mutants may interfere with the effects of these compounds on p53 transactivation. Whether these compounds are able to mediate the formation of p53 tetramer remains to be studied in p53 mutant cells (Fig. 1A).

**Resuscitating Wild-Type p53 Expression by Disrupting Ceramide Glycosylation**

The *TP53* mutation is usually heterozygous in both germline and somatic cells (1, 4). This may provide opportunities to modulate the transcriptional and posttranscriptional processes to restore wild-type p53 expression in mutant p53 cancer cells. Little is known about whether p53 expression can be restored in p53 mutant tumors (1, 7, 8); however, it was recently reported that suppression of glucosylceramide synthase restores wild-type p53 expression and p53-dependent apoptosis in mutant cancer cells (18). This intriguing finding provides insight into the p53 mutation in transcriptional and posttranscriptional processes, as well as the role of sphingolipid in regulating expression, and it provides a novel approach for targeting cancers that harbor p53 mutations.

It has been speculated that p53 mutants can be corrected in gene expression level as well as posttranslational modification, and the latter has been broadly demonstrated (1, 3, 8). Heterozygous *TP53* that is able to express either wild-type or mutant heterogeneous nuclear RNA (hnRNA) has been detected in most (but not all) mutant p53 tumors (1, 4, 5). More likely, mutant p53 cancer cells generate heterogeneous hnRNA from the transcription using both wild-type and mutant DNA; however, these cells produce mutant protein after translation. NCI/ADR-RES and OVCAR-8 cancer cells are mutant p53 cell lines that dominantly express the p53 mutants with a deletion of 7 and 6 amino acids (encoded by exon 5), respectively, within the DBD (18, 19). These deleted mutants lack p53 transactivation activity and render these cells resistant to apoptosis induced by DNA damage (18, 19). Analyses of hnRNA and mRNA revealed that both NCI/ADR-RES and OVCAR-8 cell lines expressed the same p53 hnRNA as in wild-type cells, even though these p53 mutant cells could not generate wild-type p53 mRNA when they were exposed to doxorubicin (18). Of interest, wild-type p53 mRNA and the phosphorylated p53 protein (at Ser15) presented in the p53 mutant cells after treatment with mixed backbone oligonucleotide against human glucosylceramide synthase (MBO-asGCS) and doxorubicin exposure. Consequently, the functional p53 activated the expression of p53-responsive genes and induced apoptosis in the p53 mutant cells (18). As a proof of concept, this study indicates that dysfunctional regulation in transcriptional and posttranscriptional processes is an important cause of p53 mutants in cancer cells (Fig. 1B). This finding raises many questions, and further investigations should determine which types and locations of p53 mutations result from dysfunctional regulation of the transcriptional/posttranscriptional processes, as well as the molecular mechanisms that underlie these processes. On the basis of these findings, we should be able to develop therapeutic approaches to correct or resuscitate wild-type p53 expression in cancers harboring p53 mutants.

Suppression of GCS has resuscitated p53-dependent apoptosis in p53 mutant cells, indicating that active sphingolipids play a role in turning the expression of mutant to wild-type p53 (ref. 18; Fig. 1B). MBO-asGCS that silenced GCS expression significantly increased the levels of phosphorylated p53 (at Ser15 in DBD) and p53-responsive genes, including *p21Waf1/Cip1*, *Bax*, and *Puma*, in a dose-dependent manner in p53 mutant cells (18). Restoration of p53-dependent apoptosis by MBO-asGCS dramatically sensitized mutant p53 cancer to doxorubicin-induced apoptosis in cell and animal studies (18). Resuscitation of p53 expression with GCS suppression was confirmed in NCI/ADR-RES cells treated with *d*-threo-1-phenyl-2-decanoylamino-3-morpholino-1-propanol, a GCS inhibitor. Cellular immunofluorescent staining revealed that MBO-asGCS treatment dramatically decreased GCS protein in the Golgi apparatus, where ceramide glycosylation occurs, and consequently increased nuclear phosphorylated p53 in NCI/ADR-RES cells exposed to doxorubicin (ref. 18; Fig. 1B). Because GCS catalyzes ceramide glycosylation, converting ceramide to glucosylceramide, disrupting ceramide glycosylation by silencing of GCS can increase ceramide and decrease glucosylceramide and other glycosphingolipids (20), as shown in Fig. 1B. Ceramide is an active sphingolipid that plays critical roles in processing of apoptosis and other cellular functions.
(21–24). It was found that in NCI/ADR-RES cells, MBO-asGCS treatments significantly increased endogenous ceramide in a dose-dependent fashion and were directly associated with p53 resuscitation. Phosphorylated wild-type p53 accumulated in the nucleus, and endogenous ceramide appeared in the cytoplasm of NCI/ADR-RES cells after disruption of ceramide glycosylation. Fumonisin B1 treatments that inhibited ceramide synthase in the de novo pathway eliminated the effects of MBO-asGCS on restoration of p53, and exogenous C<sub>e</sub>-ceramide (but not C<sub>e</sub>-dihydroceramide) presented the resuscitation of p53 pathways as MBO-asGCS in p53 mutant cells (18). It is not clear how ceramide modulates p53 resuscitation, but several studies have suggested that ceramide plays a role in mediating posttranscriptional processing because it alters the isoform expression of caspase-9 and bcl-x in cancer cells (25, 26).

Targeting ceramide glycosylation is an alternative approach to restore p53 function and improve cancer treatment. Overexpression of GCS, which confers cell resistance to apoptosis, is a potential marker for predicting tumor response to chemotherapy and clinical progression (27–29). Inhibition of GCS by gene silencing or tamoxifen leads p53 mutant cancer cells to apoptosis (20, 28, 30). It has been reported that p53 mutants upregulate the expression of MDR1, HERT, bFGF, and HSP70 in cancer cells, enhancing cell growth and resistance to therapies (21, 27). On the other hand, inhibition of glycosphingolipid synthesis or increased endogenous ceramide represses the expression of MDR1, HERT, bFGF, and HSP70 in cancer cells, enhancing cell growth and resistance to therapies (21, 27). On the other hand, inhibition of glycosphingolipid synthesis or increased endogenous ceramide represses the expression of MDR1, HERT, bFGF, and HSP70 in cancer cells, enhancing cell growth and resistance to therapies (21, 27). On the other hand, inhibition of glycosphingolipid synthesis or increased endogenous ceramide represses the expression of MDR1, HERT, bFGF, and HSP70, and other genes, and downregulate the expression of FAS, PTEN, and others, gaining the oncogenic functions to promote tumor progression (1, 3, 7). Coincidently, glycosphingolipids (globo- and ganglio-series) produced after ceramide glycosylation upregulate the expression of MDR1, HERT, bFGF, and HSP70 in cancer cells, enhancing cell growth and resistance to therapies (21, 27). On the other hand, inhibition of glycosphingolipid synthesis or increased endogenous ceramide represses the expression of MDR1, HERT, bFGF, and HSP70, and other genes, and downregulate the expression of FAS, PTEN, and others, gaining the oncogenic functions to promote tumor progression (1, 3, 7). Coincidently, glycosphingolipids (globo- and ganglio-series) produced after ceramide glycosylation upregulate the expression of MDR1, HERT, bFGF, and HSP70, and other genes, and downregulate the expression of FAS, PTEN, and others, gaining the oncogenic functions to promote tumor progression (1, 3, 7). Coincidently, glycosphingolipids (globo- and ganglio-series) produced after ceramide glycosylation upregulate the expression of MDR1, HERT, bFGF, and HSP70, and other genes, and downregulate the expression of FAS, PTEN, and others, gaining the oncogenic functions to promote tumor progression (1, 3, 7). Coincidently, glycosphingolipids (globo- and ganglio-series) produced after ceramide glycosylation upregulate the expression of MDR1, HERT, bFGF, and HSP70.

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