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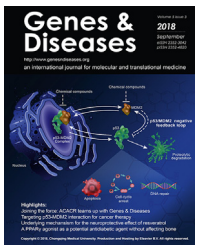

REVIEW ARTICLE

Natural products targeting the p53-MDM2 pathway and mutant p53: Recent advances and implications in cancer medicine

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Abstract The p53 tumor suppressor plays a major role in controlling the initiation and development of cancer by regulating cell cycle arrest, apoptosis, senescence, and DNA repair. The MDM2 oncogene is a major negative regulator of p53 that inhibits the activity of p53 and reduces its protein stability. MDM2, p53, and the p53-MDM2 pathway represent well-documented targets for preventing and/or treating cancer. Natural products, especially those from medicinal and food plants, are a rich source for the discovery and development of novel therapeutic and preventive agents against human cancers. Many natural product-derived MDM2 inhibitors have shown potent efficacy against various human cancers. In contrast to synthetic small-molecule MDM2 inhibitors, the majority of which have been designed to inhibit MDM2-p53 binding and activate p53, many natural product inhibitors directly decrease MDM2 expression and/or MDM2 stability, exerting their anticancer activity in both p53-dependent and p53-independent manners. More recently, several natural products have been reported to target mutant p53 in cancer. Therefore, identification of natural products targeting MDM2, mutant p53, and the p53-MDM2 pathway can provide a promising strategy for the development of novel cancer chemopreventive and chemotherapeutic agents. In this review, we focus our discussion on the recent advances in the discovery and development of anticancer natural products that target the p53-MDM2 pathway, emphasizing several emerging issues, such as the efficacy, mechanism of action, and specificity of these natural products.

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Introduction

Human cancer is associated with alterations in a number of oncogenes and tumor suppressor genes that occur at various stages, from carcinogenesis to tumor growth, progression and metastasis.[1,2] Proto-oncogenes normally control cell division and growth; molecular alterations, including gain-of-function mutations, amplification, and overexpression, can trigger the activation of oncogenes, leading to uncontrolled cell division, finally causing cancer.[3,4] In contrast, tumor suppressor genes are "good" genes that normally slow cell division and growth. When loss-of-function mutations and the deletion of tumor suppressor genes occur, tumor suppressors are inactivated. Consequently, cell division and growth become out of control, resulting in cancer.[5,6] Therefore, the balance between the oncogenes and tumor suppressor genes critically controls the initiation and progression of cancer. Inhibiting oncogenes and activating tumor suppressor genes are promising approaches for preventing or treating malignancies.

Natural products from edible and medicinal plants have shown potent cancer chemopreventive and chemotherapeutic activity in both preclinical and clinical studies.[7,8] Many natural products mediate their anticancer activities by targeting oncogenes and/or tumor suppressors (Fig. 1). In comparison to the synthetic anticancer compounds designed for a single molecular target, most anticancer natural products have a broader range of targets, including the MDM2 oncogene and p53 tumor suppressor gene.[7] The p53 tumor suppressor has a vital role in regulating cancer cell death, cell cycle arrest, apoptosis, senescence, and DNA repair.[9] p53 forms a negative feedback loop with MDM2, which directly interacts with p53 and inhibits its function and expression.[10] Many natural products have been reported to target the p53-MDM2 pathway, including chalcones, genistein, curcumin, sesquiterpenoids, ginsenosides, etc.; some of these have been summarized in a 2012 review paper published by our group.[11] In the present review, we focus on the newly-reported natural products that target the p53-MDM2 pathway, as well as the *in vitro* and *in vivo* activity and mechanisms of action of natural product-derived p53/MDM2 inhibitors. We also discuss the emerging issues, especially those related to the efficacy, bioavailability, and toxicity of these natural product inhibitors.

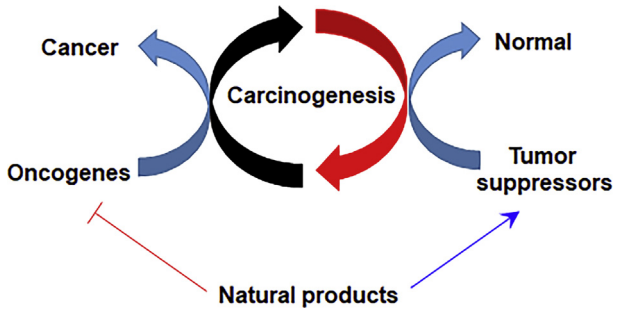

Figure 1 Natural products that target tumor suppressors and oncogenes in malignant cells. The tumor suppressor genes and oncogenes play critical roles in various stages of cancer development, from carcinogenesis to progression and metastasis. A number of natural products target tumor suppressor genes and oncogenes and have shown potential for cancer prevention and therapy.

The interplay between p53 and MDM2

The dysregulation of the p53-MDM2 pathway, including p53 mutations and deletions and/or MDM2 amplification and overexpression, is the most frequently observed molecular alteration in various human cancers.[12–15] Several recent reviews comprehensively discuss the roles of the p53-MDM2 pathway in the initiation, progression, and metastasis of human cancer.[10,16,17] In this section, we briefly introduce the p53-MDM2 interaction and the p53-independent functions of MDM2 in human cancer.

The p53 tumor suppressor and MDM2 interact with each other and participate in an autoregulatory feedback loop, which is critical for controlling their expression levels in both normal and cancer cells (Fig. 2). First, p53 binds to the *MDM2* P2 promoter and activates MDM2 expression at the transcriptional level.[18] Second, MDM2 binds to the transactivation domain of p53 and inhibits p53-mediated transcription of the downstream target genes, including MDM2 itself.[19] Third, MDM2 functions as an E3 ubiquitin ligase and promotes p53 ubiquitination.[20,21] The MDM2-mediated p53 ubiquitination facilitates the recognition and interaction of p53 by the proteasome, resulting in enhanced p53 degradation.[22] MDM2 is also responsible for p53 sumoylation and nuclear export through its interaction with a SUMO E3 ligase, PIASy.[23,24] In addition, many p53-MDM2 binding modulators are involved in regulating the functions, expression, and protein stability of MDM2 and p53, and have been comprehensively discussed in recent reviews.[10,11,25,26]

MDM2 also has various p53-independent functions, which have been discussed in several reviews.[27–29] Much attention has been paid to the p53-independent effects of MDM2 due to the frequent observations of MDM2 amplification and overexpression in human cancers harboring mutant p53.[26,30–32] Our lab have discovered that MDM2 plays an important role in regulating the expression of p21, Bax, pRb, ppRb, and E2F1 in p53 *null* PC3 cells.[33] Further studies have indicated that MDM2 binds to p21 and induces its degradation by causing a conformation change.[34–36] MDM2 inhibits the ubiquitination of E2F1 and enhances its protein

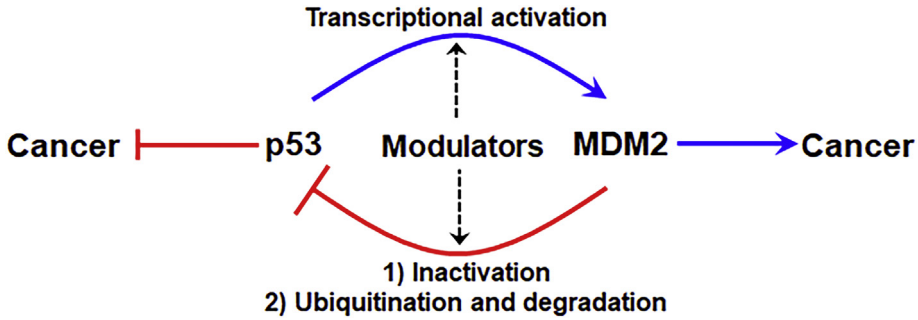

Figure 2 The p53-MDM2 pathway and natural products inhibitors. The p53 tumor suppressor and MDM2 oncogene form an autoregulatory feedback loop. The p53 protein binds to the *MDM2* P2 promoter and increases MDM2 expression. MDM2, in turn, binds to the transactivation domain of p53 and inhibits its ability to activate the transcription of its target genes. MDM2 also acts as an E3 ligase and promotes p53 ubiquitination and degradation. A number of natural products have been reported to exert their anticancer activity by inhibiting MDM2 and/or activating p53.

stability and activity.[37,38] MDM2 also destabilizes the Rb, FOXO3a, FOXO4, and E-cadherin proteins, which are important for the p53-independent activity of MDM2 in regulating cancer development and metastasis.[39–43] Therefore, direct inhibition of MDM2 may have preventive and therapeutic potential because it can inhibit both the p53-dependent and p53-independent functions of MDM2.

Natural products targeting the p53-MDM2 pathway

Natural products that target the p53-MDM2 pathway typically fall into three categories: 1) natural products that directly inhibit MDM2 expression and/or protein stability, 2) natural products that inhibit p53-MDM2 binding and activate wild-type p53, and 3) natural products that inhibit MDM2's E3 ligase activity and stabilize p53. Numerous natural product MDM2 inhibitors (Table 1) have previously been discussed in our 2012 review paper.[11] There are many newly-discovered natural product MDM2 inhibitors (Fig. 3), which mainly fall into categories 1 and 2, that have shown potent cancer preventive and therapeutic activities *in vitro* and *in vivo*. In this section, we focus our discussion on these new natural product MDM2 inhibitors, as well as their efficacy and mechanisms of action (Table 1).

Natural products that inhibit MDM2 expression and/or decrease its protein stability

Nature-derived flavonoids and isoflavonoids, including genistein,[44] apigenin,[45] and oroxylin A,[46] have shown excellent anticancer activity *in vitro* and *in vivo*, and these effects are at least partly mediated by inhibiting MDM2 expression, as discussed previously.[11] Recent studies have discovered a new flavonoid MDM2 inhibitor, flavopiridol (**1**) (Fig. 3 and Table 1), which has displayed broad-spectrum cytotoxicity against glioma, leukemia, lung, prostate, and colon cancer cell lines *in vitro* (IC_{50} values in the range of 50–500 nM).[47,48] Flavopiridol arrests cells at the G2/M phase and induces cell apoptosis, independent of p53. Mechanistically, flavopiridol decreases the *MDM2* mRNA level without affecting the half-life of the MDM2 protein, regardless of the p53 status of the cells.[44] However, the anticancer activity of flavopiridol and its inhibitory effects on MDM2 have not been examined in any *in vivo* models yet, which is critical for the further development of this natural product.

We have identified a novel class of ginsenosides, including 25-OCH_3-PPD and 25-OH-PPD, which inhibit cancer cell growth *in vitro* and *in vivo* by decreasing the MDM2 protein levels.[49–52] Our recent studies have further demonstrated that 25-OCH_3-PPD prevents breast cancer cell migration *in vitro* and inhibits tumor metastasis *in vivo* by inhibiting *MDM2* transcription and promoting MDM2 ubiquitination and degradation.[53] Another ginsenoside MDM2 inhibitor, 20(S)-Ginsenoside Rg3 (**2**) (Fig. 3 and Table 1), has been shown to inhibit gallbladder cancer cell growth and colony formation, arrest the cell cycle at the G1 phase, and promote senescence and apoptosis by decreasing the MDM2 protein levels.[54] An anabolic steroid, nandrolone (**3**) (Fig. 3 and Table 1) has also been found to reduce MDM2 expression.[55] However, all of these studies were performed in *in vitro* cell models, and further *in vivo* evaluation is needed for both 20(S)-Ginsenoside Rg3 and nandrolone. Studies of the molecular mechanisms underlying the inhibitory effects of these agents on MDM2 are still required.

More recently, platycodin D (**4**) (Fig. 3 and Table 1), a triterpenoid saponin, has been shown to inhibit triple negative breast cancer (MDA-MB-231) cell growth *in vitro* and xenograft tumor growth *in vivo*, which are attributed to platycodin D's inhibitory effects on MDM2 and MDMX.[56] MDMX is also an important regulator of p53 and has been demonstrated as a potential drug target for treating and preventing human cancer.[17] Interestingly, platycodin D also decreases the expression level of mutant p53 in MDA-MB-231 cells, although the molecular mechanism(s) is unclear.

Natural alkaloids represent an important class of natural products that target the p53-MDM2 pathway in human cancer. It has been known that the alkaloid berberine induces MDM2 self-ubiquitination and degradation by inhibiting MDM2-DAXX-HAUSP interactions.[57] A recent study has further indicated that berberine down-regulates MDM2 expression, resulting in a reduction in XIAP expression and leukemia cell apoptosis, independent of p53.[58] Our lab has identified a class of tricyclic pyrroloquinone alkaloid

Table 1 Natural products as preventive and therapeutic agents that target the p53-MDM2 pathway.

Natural product	Cancer type	*In vitro* activity	*In vivo* efficacy	Mechanism(s) of action	Reference(s)
1. Natural products that inhibit MDM2 expression and/or decrease its protein stability					
Flavonoids and isoflavonoids					
Genistein	Prostate, colon, and breast cancer	Inhibits cell proliferation, arrests cells at G2/M phase, and induces cell apoptosis, regardless of p53 status	Inhibits tumor growth in PC3 xenograft model and sensitizes tumors to gemcitabine	Inhibits NFAT1-mediated *MDM2* transcription and promotes MDM2 autoubiquitination and degradation	44
Apigenin	Ovarian cancer	Inhibits tube formation	Not reported	Inhibits MDM2 phosphorylation and decreases MDM2 protein level	45
Oroxylin A	Liver, cervical, breast, ovarian, and colon cancer, leukemia	Inhibits the growth of cancer cells (at 10—200 μM) and induces cell apoptosis	Not reported	Decreases MDM2 protein expression level	46
Flavopiridol (**1**)	Glioma	Inhibits the growth of glioma cells (200—500 nM), arrests cells at G2/M phase, and induces cell apoptosis	Not reported	Inhibits MDM2 expression at mRNA level	47
	Lung, prostate, and colon cancer, leukemia	Inhibits the growth of glioma cells (at 50—500 nM), arrests cells at G2/M phase, and induces cell apoptosis	Not reported	Decreases MDM2 protein expression level	48
Ginsenosides and saponins					
25-OCH_3-PPD	Prostate, pancreatic and lung cancer	Inhibits cell growth (IC_{50} = 4.9—19.1 μM) and proliferation, induces cell cycle arrest at G1 phase and apoptosis, regardless of p53	Inhibits tumor growth in PC3, Panc-1 and A549 xenograft models and enhances the antitumor effects of taxotere, gemcitabine and radiation.	Decreases MDM2 protein level	49—51
	Breast cancer	Inhibits cell migration	Inhibits tumor growth in MCF7 and MDA-MB-469 xenograft models and inhibits lung metastasis in MDA-MB-231 metastatic model	Inhibits *MDM2* transcription and promotes MDM2 ubiquitination and degradation	53
25-OH-PPD	Prostate and pancreatic cancer	Inhibits cell growth (IC_{50} = 21—60 μM) and proliferation, induces cell cycle arrest at G1 phase and apoptosis, regardless of p53	Inhibits tumor growth in PC3 and Panc-1 xenograft models and enhances the antitumor effects of taxotere, gemcitabine and radiation.	Decreases MDM2 protein level	50,52
20(S)-Ginsenoside Rg3 (**2**)	Gallbladder cancer	Inhibits cell growth (IC_{50} = ~100 μM) and colony formation, and induces cell cycle arrest at G1 phase, apoptosis, and senescence	Not reported	Decreases MDM2 protein level	54

(*continued on next page*)

Table 1 (*continued*)

Natural product	Cancer type	*In vitro* activity	*In vivo* efficacy	Mechanism(s) of action	Reference(s)
Nandrolone (**3**)	Not reported	Not reported	Not reported	Decreases MDM2 protein level	55
Platycodin D (**4**)	Breast cancer	Inhibits MDA-MB-231 cell growth (IC_{50} = 7.8 μM) and proliferation and induces cell cycle arrest at G0/G1 phase	Inhibits tumor growth in MDA-MB-231 xenograft model	Decreases the protein levels of MDM2, MDMX, and mutant p53	56
Alkaloids					
Berberine	Acute lymphoblastic leukemia	Induces cell death and apoptosis	Not reported	Increases MDM2 self-ubiquitination by disrupting MDM2—DAXX—HAUSP interactions	57
	Leukemia	Induces cell death and apoptosis	Not reported	Decreases MDM2 protein level	58
FBA-TPQ	Breast, prostate, ovarian, and pancreatic cancer	Inhibits cell growth (IC_{50} = 0.1—1.8 μM) and proliferation, induces cell cycle arrest and apoptosis, regardless of p53	Inhibits tumor growth in MCF7, OVCAR-3, and Panc-1 xenograft models.	Decreases MDM2 protein level	88—91
PEA-TPQ	Breast cancer	Inhibits cell growth (IC_{50} = 0.1—2.5 μM) and proliferation, induces cell cycle arrest and apoptosis, regardless of p53	Not reported	Decreases MDM2 protein level	88
MPA-TPQ	Breast cancer	Inhibits cell growth (IC_{50} = 0.6—4.9 μM) and proliferation, induces cell cycle arrest and apoptosis, regardless of p53	Not reported	Decreases MDM2 protein level	88
DPA-TPQ	Breast cancer	Inhibits cell growth (IC_{50} = 0.3—24.4 μM) and proliferation, induces cell cycle arrest and apoptosis, regardless of p53	Not reported	Decreases MDM2 protein level	88
BA-TPQ	Breast cancer	Inhibits cell growth (IC_{50} = 0.1—0.4 μM) and induces cell cycle arrest and apoptosis, regardless of p53	Inhibits tumor growth in MCF7 and MDA-MB-468 xenograft models	Decreases MDM2 protein level	92
TCBA-TPQ	Lung cancer	Inhibits cell growth (IC_{50} = 0.39—1.41 μM) and induces cell cycle arrest and apoptosis, regardless of p53	Not reported	Decreases MDM2 protein level	93
Matrine (**5**)	Liver cancer	Induces cell apoptosis, independent of p53	Not reported	Decreases *MDM2* mRNA synthesis	60
Melatonin (**6**)	Breast cancer	Not reported	Not reported	Inhibits *MDM2* transcription, decreases MDM2p (Ser166) level, and enhances p53 acetylation	61

Xanthones, naphthoquinones, and polyphenols					
Gambogic acid	Breast and non-small cell lung cancer	Inhibits the growth of MCF7 (IC_{50} = 3.5 μM) and H1299 (IC_{50} = 3.5 μM) cells, arrests cells at G2/M phase, and induces cell apoptosis, regardless of p53 status	Inhibits tumor growth in H1299 xenograft model	Inhibits *MDM2* transcription and promotes MDM2 ubiquitination and degradation	62
Plumbagin (**7**)	Osteosarcoma	Inhibits the growth of U2OS (IC_{50} = 2.5 μM) cells, arrests cells at S phase, and induces cell apoptosis	Not reported	Decreases MDM2 protein expression level	63
Gossypol (**8**)	Breast cancer	Induces cell death and apoptosis in MCF7 and MDA-MB-468 cells	Suppresses the tumor growth in MCF7 and MDA-MB-468 xenograft models	Inhibits the binding of MDM2 to *VEGF* mRNA and induces MDM2 self-ubiquitination and protein degradation	64
Terpenoids					
Triptolide	Acute lymphoblastic leukemia	Inhibits cell growth (IC_{50} = 47 −73 nM) and induces cell apoptosis	Not reported	Inhibits MDM2 at the transcriptional level by suppressing its mRNA synthesis	65
	Gastric cancer	Induces cell apoptosis	Not reported	Decreases MDM2 protein level	66
Parthenolide	Colon cancer	Inhibits the growth of HCT116 (IC_{50} = 6 μM) and HCT116 p53 $^{-/-}$ (IC_{50} = 10 μM) cells and induces cell apoptosis	Not reported	Promotes MDM2 ubiquitination and degradation	67
Japonicone A (**9**)	Breast cancer	Inhibits cell growth (IC_{50} = 0.5 −2 μM), proliferation, and colony formation and induces cell cycle arrest at G2/M phase and apoptosis, regardless of p53	Inhibits tumor growth in MCF7 and MDA-MB-231 xenograft models	Inhibits NFAT1-mediated *MDM2* transcription and promotes MDM2 ubiquitination and degradation	68,69
Inulanolide A (**10**)	Breast cancer	Inhibits cell growth (IC_{50} = 0.9 −4.1 μM), proliferation, and colony formation, induces cell cycle arrest at G2/M phase and apoptosis, and prevents cell migration and invasion, regardless of p53	Inhibits tumor growth in MDA-MB-231 orthotopic model	Inhibits NFAT1-mediated *MDM2* transcription and promotes MDM2 ubiquitination and degradation	70
	Prostate cancer	Inhibits cell growth (IC_{50} = 1.3 −4 μM), proliferation, and colony formation and prevents cell migration and invasion, regardless of p53	Not reported	Inhibits NFAT1-mediated *MDM2* transcription, disrupts MDM2-MDMX binding, and promotes ubiquitination and degradation of both MDM2 and MDMX	71

(*continued on next page*)

Table 1 (*continued*)

Natural product	Cancer type	*In vitro* activity	*In vivo* efficacy	Mechanism(s) of action	Reference(s)
Linearііfolianoid A (**11**)	Breast cancer	Inhibits cell growth (IC_{50} = 4.4 −9.1 μM), proliferation, and colony formation, induces cell cycle arrest at G2/M phase and apoptosis, and prevents cell migration and invasion, regardless of p53	Not reported	Inhibits NFAT1-mediated *MDM2* transcription and promotes MDM2 ubiquitination and degradation	72
Curcumin and derivatives					
Curcumin	Prostate, lung, and breast cancer	Inhibits cell proliferation and colony formation and induces cell apoptosis, regardless of p53 status	Inhibits tumor growth in PC3 xenograft model and enhances the antitumor effects of gemcitabine and irradiation.	Inhibits *MDM2* transcription through the PI3K/mTOR/ETS2 pathway	73
Curcumin derivative 1	Neuroblastoma	Inhibits SH-SY5Y cell growth (IC_{50} = 8 μM), arrests cells at S phase, and induces cell apoptosis	Not reported	Decreases the MDM2 protein level	74
Hispolon (**12**)	Liver and breast cancer	Induces autophagy	Not reported	Enhances the binding of MDM2 with HSP90, HSP70, HSC70, and LAMP2A and decreases the MDM2 protein level	75
Chalcone and derivatives					
Chalcone N9 (**13**)	Glioma	Inhibits U87-MG cell growth (IC_{50} = 0.72 μg/mL) and colony formation, arrests cells at G1 phase, and induces cell apoptosis	Inhibits tumor growth in U87-MG xenograft model.	Decreases MDM2 protein level	76
2. Natural products that inhibit MDM2-p53 binding					
Chalcone and derivatives					
Chalcone derivative A	Not reported	Not reported	Not reported	Inhibits MDM2-p53 binding (K_i = 206 μM)	77
Chalcone derivative B	Not reported	Not reported	Not reported	Inhibits MDM2-p53 binding (K_i = 49 μM)	77
Chalcone derivative B-1	Not reported	Not reported	Not reported	Inhibits MDM2-p53 binding (K_i = 117 μM)	77
Chalcone derivative C	Not reported	Not reported	Not reported	Inhibits MDM2-p53 binding (K_i = 250 μM)	77
Hexylitaconic acid					
Hexylitaconic acid	Not reported	Not reported	Not reported	Inhibits MDM2-p53 binding (K_i = 50 μg/mL)	78
Natural peptide chlorofusin and derivatives					
Chlorofusin	Liver cancer	No cytotoxicity against HepG2 cells at 4 μM	Not reported	Inhibits MDM2-p53 binding (K_i = 4.6 μM)	79

Chlorofusin derivative 17 (**14**)	Osteosarcoma	Inhibits SJSA-1 cell growth (IC_{50} = 33.1 μM)	Not reported	Inhibits MDM2-p53 binding (K_i = 3.1 μM)	81
Chlorofusin derivative 18 (**15**)	Osteosarcoma	Inhibits the growth of SJSA-1 (IC_{50} = 31.2 μM) and A375 (IC_{50} = 49.3 μM) cells	Not reported	Inhibits MDM2-p53 binding (K_i = 7.0 μM)	81
Hoiamide D	Non-small cell lung cancer	Inhibits H460 cell growth (IC_{50} = 40 μM)	Not reported	Inhibits MDM2-p53 binding (K_i = 4.5 μM)	80
Flavonoids					
Tricetin (**16**)	Breast cancer	Inhibits MCF7 cell growth (IC_{50} = 32.2 μM) and colony formation, and induces cell cycle arrest at G2/M phase and apoptosis.	Not reported	Inhibits MDM2-p53 binding and induces p53 phosphorylation at Ser15 and Ser392	82
Alkaloids					
Indole-3-carbinol (**17**)	Breast	Induces MCF10A cell cycle arrest at G1 phase	Not reported	Inhibits MDM2-p53 binding and induces p53 phosphorylation at Ser15	83
Fluspirilene (**18**)	Colon cancer	Inhibits HCT116 cell growth at 10 μM	Not reported	Inhibits MDM2-p53 binding	84
Lithocholic acid, Isokotomolide A, and Leptomycin B					
Lithocholic acid (**19**)	Colon cancer	Induces HCT116 cell apoptosis at 300 μM	Not reported	Dually inhibits MDM2-p53 (K_i = 66.0 μM) and MDMX-p53 (K_i = 15.4 μM) binding	85
Isokotomolide A (**20**)	Lung cancer	Inhibits A549 cell growth (IC_{50} = 4.4 μM) and colony formation, and induces cell cycle arrest at G0/G1 phase and apoptosis	Not reported	Inhibits MDM2-p53 binding	86
Leptomycin B (**21**)	Osteosarcoma and lung cancer	Not reported	Not reported	Protects p53 from MDM2-mediated degradation	87
Marine compounds					
Sempervirine	Osteosarcoma	Induces U2OS cell apoptosis	Not reported	Inhibits MDM2 E3 ligase activity (IC_{50} = 8 μg/mL)	94
Isolissoclinotoxin B	Not reported	Not reported	Not reported	Inhibits MDM2 E3 ligase activity (IC_{50} = 58.6 μM)	95
Varacin	Not reported	Not reported	Not reported	Inhibits MDM2 E3 ligase activity (IC_{50} > 295 μM)	95
N,N-dimethyl-5-methylvaracin	Not reported	Not reported	Not reported	Inhibits MDM2 E3 ligase activity (IC_{50} = 120.8 μM)	95
Diplamine B	Not reported	Not reported	Not reported	Inhibits MDM2 E3 ligase activity (IC_{50} = 101.3 μM)	95
Lissoclinidine B	Osteosarcoma	Induces U2OS cell death	Not reported	Inhibits MDM2 E3 ligase activity (IC_{50} = 98.1 μM)	95

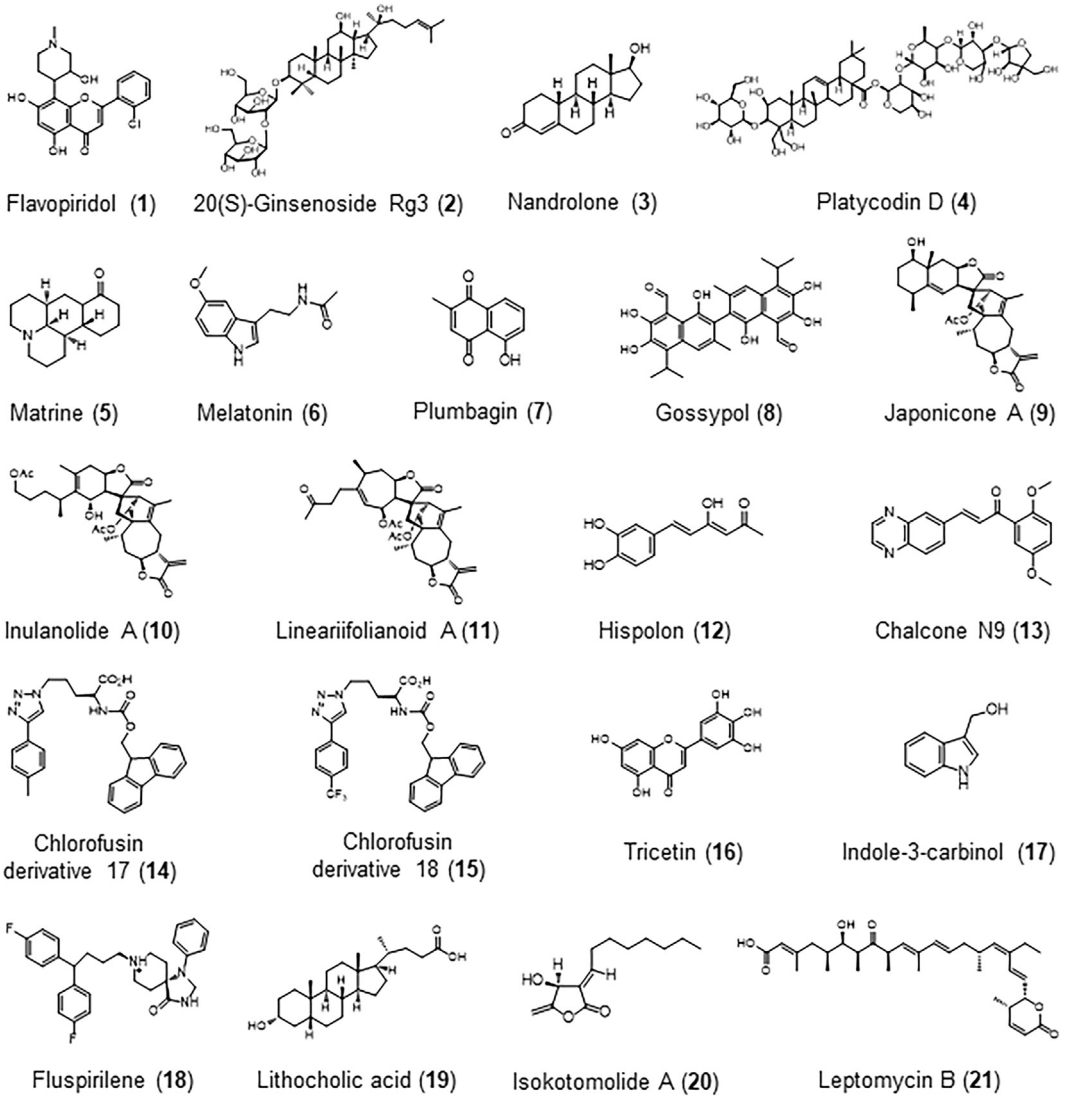

Figure 3 The structures of newly-discovered natural product MDM2 inhibitors.

analogs as novel MDM2 inhibitors, including FBA-TPQ, PEA-TPQ, MPA-TPQ, DPA-TPQ, BA-TPQ, and TCBA-TPQ (Table 1). All of these compounds have shown excellent *in vitro* and *in vivo* activities in various cancer models with different p53 status (wild-type, mutant, and *null*), as were discussed in a recent review.[59] Matrine (**5**), an alkaloid derived from the traditional Chinese medical herb *Sophora flavescens* Ait, has been shown to inhibit MDM2 expression by decreasing *MDM2* mRNA synthesis in liver cancer cells (Fig. 3 and Table 1).[60] Matrine also sensitizes MDM2-overexpressing liver cancers to etoposide-induced apoptosis, independent of p53. Melatonin (**6**), a monoamine alkaloid, inhibits MDM2 at the transcriptional level in MCF7 breast cancer cells (Fig. 3 and Table 1). Melatonin also inhibits MDM2 phosphorylation and enhances p53 acetylation, resulting in the disruption of p53-MDM2 binding and p53 stabilization.[61] The anticancer activity of matrine and melatonin, and their inhibitory effects on MDM2, need to be further investigated in *in vivo* cancer models.

Gambogic acid, a natural xanthone, has been reported to inhibit MDM2 at both the transcriptional and post-translational levels and exerts anticancer activity *in vitro* and *in vivo*, regardless of the p53 status of the cells/tumors.[62] Several natural products with similar structural features have been identified to inhibit MDM2 and exert cancer preventive and therapeutic effects. For example, plumbagin (**7**), a natural naphthoquinone derivative, has been identified as a new MDM2 inhibitor that decreases MDM2 protein expression levels, independent of p53 (Fig. 3 and Table 1).[63] Plumbagin also inhibits the growth of osteosarcoma U2OS cells (IC_{50} = 2.5 μM), arrests cells at the S phase, and induces cell apoptosis *in vitro*, regardless of the p53 status of the cells. The *in vivo* efficacy and safety profiles of plumbagin have not yet been reported. Gossypol (**8**) is a natural polyphenol that has been identified as an inhibitor of MDM2-*VEGF* mRNA binding via a high-throughput screening assay (Fig. 3 and Table 1).[64] Gossypol induces MDM2 self-ubiquitination and protein degradation and inhibits VEGF translation in breast cancer cell lines harboring

wild-type p53 and mutant p53. Consequently, gossypol treatment causes cancer cell death and apoptosis *in vitro* and suppresses the xenograft tumor growth *in vivo* in a p53-independent manner.

Natural terpenoids, especially diterpenoids and sesquiterpenoids, represent a promising source of cancer preventive and therapeutic agents; some of which have entered clinical trials.[8] Triptolide, a diterpene triepoxide, has been found to inhibit *MDM2* mRNA synthesis in leukemia and gastric cancer cells.[65,66] Triptolide inhibits cancer cell growth and induces apoptosis at nanomolar concentrations *in vitro*, and these effects are dependent on its inhibiting MDM2. Parthenolide, a sesquiterpene lactone, has been demonstrated to induce MDM2 ubiquitination and protein degradation and to inhibit cell growth and induce apoptosis in colon cancer cells in a p53-independent manner.[67] However, the inhibitory effects of triptolide and parthenolide on MDM2 have not been reported in any *in vivo* tumor models yet.

We have recently discovered a novel class of dimeric sesquiterpene lactones, including Japonicone A (JapA, **9**),[68,69] Inulanolide A (InuA, **10**),[70,71] and Linearifolianoid A (LinA, **11**)[72] as MDM2 inhibitors (Fig. 3 and Table 1), which have been reviewed in recent papers.[8,59] JapA, InuA, and LinA have been demonstrated to directly bind to the MDM2 protein and promote MDM2 ubiquitination and degradation. These natural products also bind to the transcription factor NFAT1 and inhibit NFAT1-mediated *MDM2* transcription. More recently, we have discovered that InuA binds to the RING domain of MDMX and induces MDMX ubiquitination and degradation, which is important for InuA-induced MDM2 degradation.[71]

Curcumin, a dietary polyphenol, has shown promising cancer chemopreventive and therapeutic efficacy in preclinical and clinical studies.[73] We have demonstrated that curcumin inhibits *MDM2* transcription through the PI3K/mTOR/ETS2 pathway, which is important for its anti-prostate cancer activity *in vitro* and *in vivo*.[73] Many curcumin derivatives have been developed and shown inhibitory effects on MDM2 in various cancer models. A curcumin derivative has been reported to induce cell cycle arrest at the S phase and apoptosis in neuroblastoma cells by decreasing MDM2 protein expression and increasing p53 expression.[74] Hispolon (**12**), a natural phenol derivative, induces autophagy in liver and breast cancer cells by decreasing the MDM2 protein levels (Fig. 3 and Table 1).[75] Further studies have indicated that hispolon enhances the binding of MDM2 with HSP90, HSP70, HSC70, and LAMP2A. The mechanisms underlying the binding to these various targets and the *in vivo* efficacy of hispolon have not yet been determined. A chalcone derivative, N9 (**13**), has been reported to inhibit glioma cell growth and colony formation, arrest cells at the G1 phase, and induce cell apoptosis *in vitro*, and also inhibits xenograft tumor growth *in vivo* by decreasing the MDM2 expression levels (Fig. 3 and Table 1).[76] Further studies are needed to determine the mechanism(s) underlying N9's inhibitory effects on MDM2.

Natural products that inhibit MDM2-p53 binding

The major focus of the discovery and development of synthetic small molecule MDM2 inhibitors is still inhibiting MDM2-p53 binding to activate p53 and protect p53 from MDM2-mediated p53 ubiquitination and degradation. There are a number of natural products that have shown significant inhibitory effects on p53-MDM2 binding, including chalcone derivatives,[77] hexylitaconic acid and its derivatives,[78] natural peptide chlorofusin,[79] and hoiamide D (Table 1).[80] Recently, two new chlorofusin derivatives (**14**, **15**) have been reported to inhibit osteosarcoma SJSA-1 cell growth by inhibiting p53-MDM2 binding (Fig. 3 and Table 1), although the *in vivo* efficacy is still being investigated.[81]

Tricetin (**16**), a dietary flavonoid, has recently been reported to inhibit cell growth and colony formation, induce cell cycle arrest at the G2/M phase, and lead to apoptosis in breast cancer MCF7 cells (p53 wild-type) *in vitro*.[82] Tricetin directly inhibits the p53-MDM2 interaction and stabilizes p53, which is critical for the anticancer activity of this natural product (Fig. 3 and Table 1). The dietary phytochemical, indole-3-carbinol (**17**), has been considered a promising cancer preventive natural product. A recent study demonstrated that indole-3-carbinol induces p53 phosphorylation at Ser15, resulting in the inhibition of p53-MDM2 binding and p53 stabilization and cell cycle arrest at the G1 phase.[83] A virtual screening of p53-MDM2 binding inhibitors has recently been performed, and fluspirilene (**18**) was identified as a new natural product MDM2 inhibitor.[84] Similar to other inhibitors that inhibit MDM2-p53 binding, fluspirilene significantly inhibited the growth of HCT116 $p53^{+/+}$ colon cancer cells, but not HCT116 $p53^{-/-}$ cells *in vitro*.[84] In another *in silico* screening for dual inhibitors of MDM2-p53 and MDMX-p53 binding, lithocholic acid (**19**) was identified and showed inhibitory effects at micromolar concentrations.[85] Lithocholic acid has also been shown to induce apoptosis in p53 wild-type HCT116 cells *in vitro*.

Isokotomolide A (**20**) (Fig. 3 and Table 1), a natural butanolide, has been reported to inhibit cancer cell growth and colony formation and induce cell cycle arrest at the G0/G1 phase and apoptosis, in lung cancer A549 (p53 wild-type) cells by activating p53 and p21.[86] Further studies have indicated that isokotomolide A directly inhibits the binding of MDM2 to p53 and prohibits MDM2-mediated p53 degradation. Leptomycin B (**21**), a natural product that inhibits nuclear export, protects p53 from MDM2-mediated degradation by inducing a modification at the amino-terminal half of the full-length MDM2 protein.[87] Interestingly, this MDM2 modification also protects the amino 32 kDa fragment of MDM2 from complete degradation. However, all of these studies have only been done in *in vitro* cancer models, and the further *in vivo* evaluation of these natural p53-MDM2 binding inhibitors is needed to confirm these effects.

Natural products targeting mutant p53

In addition to targeting the p53-MDM2 pathway, targeting mutant p53 with natural products represents another promising approach to treating human cancers. Mutations of p53 are common in human cancers, although the mutation rate varies widely among different types of cancers.[96] These mutations often result in the loss of p53 function,

i.e., the inability of the protein to bind to p53-binding sites on DNA and to exert its functions in signaling checkpoint arrest and apoptosis. Moreover, mutant p53 (*e.g.*, p53R273H) may exert oncogenic functions through trans-dominant repression of wild-type p53 activities.[97] Mutant p53 proteins have been reported to regulate cancer cell survival, proliferation, migration, invasion, chemo-resistance, inflammation, *etc.*, which have been discussed in several reviews.[98,99] Therefore, mutant p53 has been proposed as a preventive and therapeutic target in cancer. Many molecules have been developed to reactivate or reinstate the wild-type functions of mutant p53 or inhibit its oncogenic functions.[9] Considerable effort has also been expended to identify natural products that target mutant p53 in cancer. In this section, we focus our discussion on the natural products targeting mutant p53 and their anticancer activities and molecular mechanisms (Fig. 4 and Table 2).

In a screen to identify compounds that selectively inhibit the growth of mutant p53-expressing cells, furcreastatin (**22**), a steroidal saponin, has been identified and shown selective cytotoxicity against oral squamous cell carcinoma and breast cancer cell lines with mutant p53.[100] However, the molecular mechanisms underlying furcreastatin's selective cytotoxicity against mutant-p53 cancer cells and its *in vivo* efficacy are still unclear. Green tea and caffeine have been reported to prevent UVB irradiation-induced tumor formation in female SKH-1 hairless mice by inhibiting the formation of mutant p53-positive cellular patches.[101,102] Mechanistic studies have indicated that oral exposure to green tea and caffeine changes the mutation profile of p53 in early mutant p53-positive epidermal patches *in vivo*.[101,102] The direct effects of green tea and caffeine on mutant p53 need to be further investigated.

Triptolide (**23**), a previously-reported MDM2 inhibitor,[65,66] has recently been reported to decrease the protein expression level of mutant p53 in MDA-MB-231 breast cancer cells. The decrease in mutant p53 was partially responsible for triptolide's inhibitory effects on cell growth and cell cycle progression.[103] An *in silico* screen of the NCI natural product database has been performed to identify compounds and plant extracts that inhibit the growth of mutant p53-expressing cancer cells. An extract from the terrestrial plant *Brachylaena ramiflora*, named N37063, has been found to inhibit cell growth and induce apoptosis in osteosarcoma, lung, and colon cancer cells in a mutant p53-dependent manner.[104] Further studies have shown that N37063 restores wild-type p53 function to His175 and His273 mutant p53 proteins and induces the expression of p53 target genes. Eupatilin (**24**), a dietary flavonoid, has been found to induce G2/M cell cycle arrest in Hec1A (mutant p53) endometrial cancer cells by activating p21.[105] Further studies have shown that eupatilin decreases the expression level of mutant p53, resulting in the upregulation of p21. However, neither N37063 nor eupatilin has been examined in any animal models containing specific mutant p53.

A class of new triterpene derivatives has recently been identified to target mutant p53 N236S, and yunnanterpene D (**25**) has shown great selective cytotoxicity against p53 N236S-expressing cells.[106] However, the specific effects of yunnanterpene D on the mutant p53 N236S protein have not been examined yet. *Origanum majorana* ethanolic extract (OME) has been shown significant effects against MDA-MB-231 breast cancer cells by inhibiting cell growth and colony formation and inducing G2/M cell cycle phase arrest and apoptosis.[107] In studies of its mechanism of action, OME has been found to inhibit the expression of mutant p53, resulting in the activation of p21, which is mainly responsible for the OME-induced G2/M phase arrest. Six steroidal glycoalkaloids, including SM (**26**), have been shown to inhibit the growth of MGC-803 gastric cancer cells and

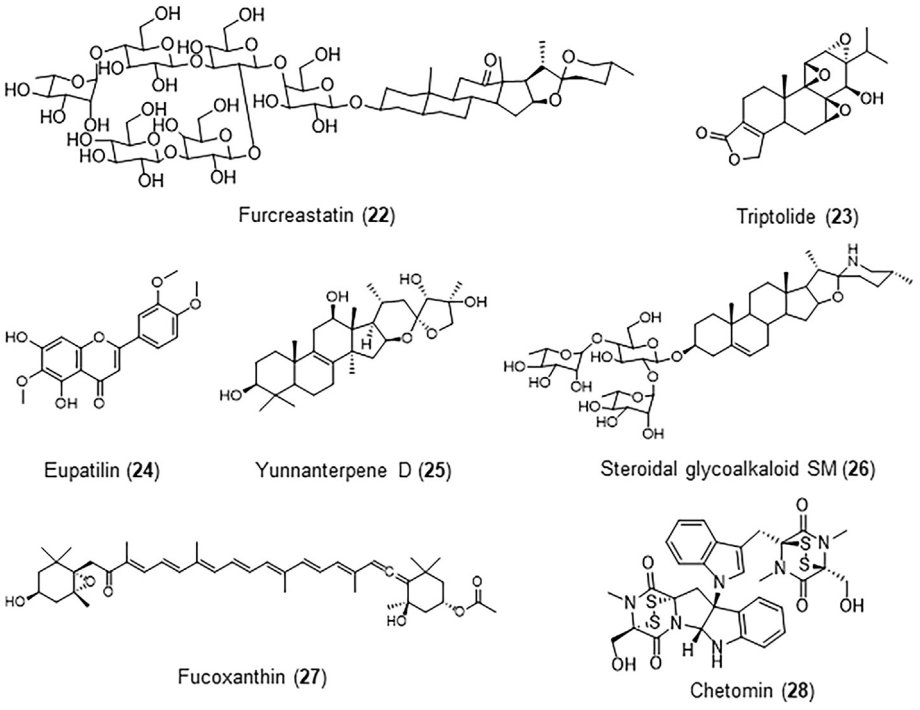

Figure 4 The structures of natural products targeting mutant p53 in cancer.

Table 2 Natural products as preventive and therapeutic agents that target mutant p53.

Natural product	Cancer type	*In vitro* activity	*In vivo* efficacy	Mechanisms of action	References
Furcreastatin (**22**)	Oral squamous cell carcinoma and breast cancer	Selectively inhibits the growth of p53-mutant cancer cells (IC_{50} = 2.6—6.3 μg/mL)	Not reported	Not reported	100
Green tea or caffeine	Skin cancer	Not reported	Prevents UVB irradiation-induced tumor formation in female SKH-1 hairless mice	Changes the mutation profile of p53 in early mutant p53-positive epidermal patches	101,102
Triptolide (**23**)	Breast cancer	Inhibits cell growth and induces S phase cell cycle arrest	Not reported	Decreases the protein expression level of mutant p53	103
N37063	Osteosarcoma, lung and colon cancer	Inhibits cancer cell growth and induces apoptosis in a mutant p53-dependent manner	Not reported	Restores wild-type p53 function to His175 and His273 mutant p53 proteins and induces the expression of p53 target genes	104
Eupatilin (**24**)	Endometrial cancer	Inhibits cell growth and induces cell cycle arrest at the G2/M phase	Not reported	Decreases the protein expression level of mutant p53	105
Yunnanterpene D (**25**)	Various cancer types	Selectively inhibits the growth of cancer cells (IC_{50} = 5.5 μM)	Not reported	Decreases the protein expression level of mutant p53	106
Origanum majorana extract	Breast cancer	Inhibits cell growth and colony formation in MDA-MB-231 cells and induces cell cycle arrest at the G2/M phase and apoptosis	Not reported	Decreases the protein expression level of mutant p53	107
Steroidal glycoalkaloid SM (**26**)	Gastric cancer	Inhibits the growth of MGC-803 cells and induces cell cycle arrest at the S phase and apoptosis	Not reported	Decreases the protein expression level of mutant p53	108
Fucoxanthin (**27**)	Bladder cancer	Inhibits T24 cell growth and colony formation and induces cell cycle arrest at the G0/G1 phase and apoptosis	Not reported	Inhibits the mortalin-p53 complex and reactivates mutant p53	109
Turmeric and curcumin	Epidermoid cancer	Induces apoptosis and autophagy in A431 cells	Not reported	Induces the degradation of mutant p53	110
Chetomin (**28**)	Pancreatic, colon, ovarian, lung, prostate, breast, epidermoid, bile duct and tongue cancer, renal cell carcinoma	Selectively inhibits the growth of cancer cells with p53 R175H	Specifically inhibits tumor growth in TOV-112D (p53 R175H) and CAL-33 (p53 R175H) xenograft models without significant effects on A431 (R273H) and H1299 (p53 *null*) xenograft tumor growth	Reactivates mutant p53 R175H by increasing the binding capacity of Hsp40 to mutant p53 R175H and causing a potential conformational change to a wild-type-like p53	111

induce S phase arrest and apoptosis.[108] SM has been also found to decrease the expression level of mutant p53, which is important for its anticancer activity.[108]

Fucoxanthin (**27**), a natural carotenoid, inhibits cell growth and colony formation, induces cell cycle arrest at the G0/G1 phase, and leads to apoptosis in T24 bladder cancer cells.[109] Fucoxanthin inhibits the mortalin-p53 complex and reactivates mutant p53 in these cells, resulting in the upregulation of p21. The crude extract of turmeric (*Curcuma longa*) and its bioactive component, curcumin, have been found to induce apoptosis and autophagy in A431 epidermoid cancer cells, which express mutant p53 R273H.[110] Both turmeric and curcumin induce macroautophagy, resulting in the degradation of mutant p53. In a cell-based, high-throughput small-molecule screening study, chetomin (**28**) has been identified as a mutant p53 R175H reactivator.[111] Chetomin has been further tested in several human cancer cell models with different p53 status, and the compound only selectively inhibited the growth of cancer cells with p53 R175H. Chetomin has also been shown to specifically inhibit the tumor growth in TOV-112D (p53 R175H) and CAL-33 (p53 R175H) xenograft models without affecting the growth of A431 (R273H) and H1299 (p53 *null*) xenograft tumors. Further mechanistic studies have shown that chetomin increases the binding capacity of Hsp40 to mutant p53 R175H and causes a potential conformational change to a wild-type-like p53, resulting in reactivation of the mutant p53 R175H.

Future research directions

The p53-MDM2 pathway is commonly dysregulated and involved in cancer initiation, progression, and metastasis.[10,17] The tumor suppressor role of p53 and the oncogenic functions of MDM2 and mutant p53 are well characterized in various cancers. Considerable efforts have been made to discover and develop inhibitors of the p53-MDM2 pathway for cancer prevention and treatment.[11,112,113] Several synthetic small molecules that specifically inhibit p53-MDM2 binding or MDM2 E3 ligase activity have entered clinical trials as cancer chemotherapeutic drugs.[114] However, all of these inhibitors exert their anticancer activity in a p53-dependent manner, and wild-type p53 is critical for their effects. More importantly, concerns regarding the drug resistance and side effects of these synthetic inhibitors have been raised.[114] Natural products targeting the p53-MDM2 pathway have recently gained momentum in their development as cancer chemopreventive and chemotherapeutic agents due to their lower toxicity compared to synthetic compounds. As reviewed above, a number of natural products (*e.g.*, flavonoids, isoflavonoids, ginsenosides, terpenoids, alkaloids, curcumin, and peptides) have been identified to target MDM2, p53 or the p53-MDM2 pathway, and many of these are under development as cancer chemopreventive and chemotherapeutic agents.

Although these natural products have shown significant anticancer activity in various *in vitro* models, many of them have not been evaluated for *in vivo* efficacy yet. Further studies must be performed to examine the efficacy of these natural products in clinically-relevant cancer models, *e.g.*, orthotopic models, metastasis models, transgenic mouse models, and patient-derived cancer models. These natural products often have poor bioavailability due to their high molecular weight, poor water solubility, and low dissolution rate, making it difficult to test them in preclinical and clinical studies. Nanotechnology holds promise in improving the bioavailability of these natural products through novel nano-delivery methods. Structural modification of these natural products is another way to enhance their bioavailability and increase their efficacy.

In addition, while most of these natural product inhibitors have been observed to decrease the expression levels of MDM2 and/or mutant p53, or to restore normal p53 activity, the detailed molecular mechanisms are still unclear. Having a thorough understanding of these natural products, especially their *in vivo* efficacy, physicochemical properties, bioavailability, and mechanisms of action, will lead to better translation of their cancer preventive and therapeutic activity to the preclinical and clinical settings.

Conclusion

Targeting the p53-MDM2 pathway can be a promising approach to develop compounds for cancer treatment and prevention. A number of natural products have been developed to target the p53-MDM2 pathway by 1) inhibiting MDM2 expression or protein stability, 2) inhibiting the p53-MDM2 interaction, 3) inhibiting the E3 ligase activity of MDM2, 4) reactivating or reinstating the wild-type functions of mutant p53, and/or 5) inhibiting the expression or protein stability of mutant p53. These natural products have shown potent chemopreventive and chemotherapeutic activity in various preclinical cancer models. Based on our experience, the natural products falling into category 1 may prove to be most potent, because they directly target MDM2 and exert activities against cancers regardless of the p53 status (wild-type, mutant, or *null*). Natural product MDM2 inhibitors in categories 2 and 3 selectively target cancers harboring wild-type p53, while the natural products in categories 4 and 5 specifically inhibit cancers containing mutant p53.

Overall, there has been progress in the development of natural products targeting the p53-MDM2 pathway for cancer therapy and prevention, but there are various issues that still need to be addressed. In particular, more detailed investigations of the *in vivo* efficacy, toxicity, bioavailability, and mechanisms of action are needed to hasten the development of effective and safe cancer preventive and therapeutic drugs for clinical use.

Conflicts of interest

These authors have no conflicts of interest to declare.

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apologize for not being able to cite all of the recent publications due to the limited space allotted for this review.

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