REVIEW

Mdm2 links genotoxic stress and metabolism to p53

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ABSTRACT

Mouse double minute 2 (Mdm2) gene was isolated from a cDNA library derived from transformed mouse 3T3 cells, and was classified as an oncogene as it confers 3T3 and Rat2 cells tumorigenicity when overexpressed. It encodes a nucleocytoplasmic shuttling ubiquitin E3 ligase, with its main target being tumor suppressor p53, which is mutated in more than 50% of human primary tumors. Mdm2's oncogenic activity is mainly mediated by p53, which is activated by various stresses, especially genotoxic stress, via Atm (ataxia telangiectasia mutated) and Atr (Atm and Rad3-related). Activated p53 inhibits cell proliferation, induces apoptosis or senescence, and maintains genome integrity. Mdm2 is also a target gene of p53 transcription factor. Thus, Mdm2 and p53 form a feedback regulatory loop. External and internal cues, through multiple signaling pathways, can act on Mdm2 to regulate p53 levels and cell proliferation, death, and senescence. This review will focus on how Mdm2 is regulated under genotoxic stress, and by the Akt1-mTOR-S6K1 pathway that is activated by insulin, growth factors, amino acids, or energy status.

KEYWORDS mouse double minute 2 (Mdm2), p53, signal transduction, tumorigenesis

MDM2 AND P53, AN INSEPARABLE COUPLE

Mdm2 consists of 489 amino acids while its human counterpart Hdm2 consists of 491 amino acids. This protein contains several conserved structural domains including an N-terminal p53 interaction domain, a central acidic domain (residues 230–300) that possesses multiple phosphorylation sites and nuclear export and import signals, a Zinc finger domain, a

C-terminal RING domain (residues 430–480) that contains a Cys3-His2-Cys3 motif which confers E3 ubiquitin ligase activity. The RING domain of Mdm2 can also bind to RNA and contains a nucleolar localization sequence. As an E3 ubiquitin ligase, Mdm2 has many substrates, including p53, forkhead box O (FOXO) (Fu et al., 2009), dihydrofolate reductase (Maguire et al., 2008), interferon regulatory factor 2 (IRF-2) (Pettersson et al., 2009), runt-related transcription factor (RUNX3) (Chi et al., 2009), activating transcription factor 3 (ATF3) (Mo et al., 2010), HIV-1 viral infectivity factor (HIV-1 vif) (Izumi et al., 2009), and Slug (Kim et al., 2010), with p53 being the most extensively studied substrate.

p53 is a prototypical tumor suppressor that is mutated in more than 50% of primary human tumors. As a master transcription regulator, p53 can either activate or repress transcription, depending on the target genes. For transactivation, p53 usually needs to bind to the promoter of target genes as a tetramer, with a consensus binding sequence 5'-RRRCWWGYYY-N (0–13)-RRRCWWGYYY-3' (R: A/G, W: A/T, Y: T/C). The best studied p53 target genes include p21, Mdm2, Puma and Bax. Through upregulation of these genes, p53 exhibits an anti-proliferation activity by inducing cell cycle arrest, apoptosis, and/or senescence.

p53 activity is tightly controlled at multiple levels including transcription, translation, and protein stability, with protein stabilization as a major mechanism. The main stimuli of p53 expression include DNA damage (caused by UV, ionizing radiation (IR), or genotoxic drugs), oxidative stress, osmotic shock, ribonucleotide depletion, and deregulated oncogene activation. Genotoxic stress activates PI3 kinase-like-kinases including DNA-PKc, Atm, and Atr at the DNA break sites, which in turn phosphorylate p53, Mdm2 and other proteins, leading to p53 stabilization/activation (Zhou and Elledge, 2000; Kastan and Bartek, 2004; Li, 2005). In addition to tumor suppression, recent studies also suggest that p53 plays a role

in aging. Overactivation of p53 leads to premature aging in mouse models (Tyner et al., 2002).

p53 is expressed at very low levels in normal cells. This is most likely attributable to the feedback regulatory loop between Mdm2 and p53. Mdm2 ubiquitinates p53 in the nucleus, leading to p53 nuclear export and proteasome-mediated degradation. On the other hand, Mdm2 is a direct target gene of p53 (Barak et al., 1994). Therefore elevation of p53 upregulates Mdm2, which in turn downregulates p53 (Iwakuma and Lozano, 2003). The significance of this regulatory loop is manifested by the genetic studies of Mdm2^{-/-} and Mdm2^{-/-}p53^{-/-} mice. Knockout mice of Mdm2 show embryonic lethality, accompanied by an elevation of p53. Deletion of p53 in Mdm2^{-/-} mice rescued the embryonic lethality phenotype, suggesting that p53 mediates the effects of Mdm2 deficiency on mouse development and survival (de Oca Luna et al., 1995; Jones et al., 1995). In addition, polymorphism of Mdm2 promoter alters the tumorigenesis processes, which is also mediated by the change in p53 activity (Bond et al., 2004).

Mdm2 negatively regulates the stability of p53. Ubiquitination by Mdm2 is necessary for p53 nuclear export and degradation. The ubiquitination is dependent on the association between these two proteins (Fuchs et al., 1998). Mdm2 binding-deficient p53 mutant could not be ubiquitinated by Mdm2 (Inoue et al., 2001). For both Mdm2 and p53, the binding sites were originally mapped to their N-terminal domains (Chen et al., 1993). It was later shown that the carboxyl terminal domain of p53 alone could also mediate its interaction with Mdm2 (Poyurovsky et al., 2010). The RING domain is essential for Mdm2 E3 ligase activity (Fang et al., 2000; Honda and Yasuda, 2000; Poyurovsky et al., 2007). In addition, the RING domain might be involved in substrate recognition as Mdm2 with its RING domain replaced with that of Praja1 could ubiquitinate Mdm2 but not p53 (Fang et al., 2000), and sumoylation of Mdm2 at Lys446, a residue

locating within the RING finger domain enhances its ubiquitin ligase activity toward p53 (Buschmann et al., 2000). Moreover, ubiquitination depends on p53 oligomerization as Mdm2 could not target oligomerization-deficient p53 for ubiquitination (Maki, 1999; Hjerpe et al., 2010). Ubiquitinated p53 exists in two forms, monoubiquitination, which might elicit nuclear export of p53, and polyubiquitination, which promotes proteasomal degradation of p53 (Li et al., 2003). RFWD3 (ring finger and WD repeat domain 3), an Mdm2 stabilizer, is able to enhance Mdm2-mediated p53 mono-ubiquitination in response to IR (Fu et al., 2010), yet, it suppresses polyubiquitination of p53, thus stabilizing p53. In addition, it is reported that phosphorylation of p53 at Ser46 renders p53 resistant to Mdm2-mediated ubiquitination (Di Stefano et al., 2004).

Besides mediating p53 turnover, Mdm2 could also affect the activity of p53 through various mechanisms. First, Mdm2 binds to the transactivation domain of p53 and inhibits its transcriptional activity possibly through blocking its access to target genes (Momand et al., 1992; Kussie et al., 1996). Secondly, Mdm2 inhibits the translation of p53 by targeting ribosomal protein L26 (RPL26) that would otherwise enhance p53 mRNA translation (Ofir-Rosenfeld et al., 2008), although studies from Candeias et al. reported a seemingly contrary result (Candeias et al., 2008; Naski et al., 2009). They showed that p53 mRNA-Mdm2 interaction augments p53 mRNA translation and decreases Mdm2 E3 ligase activity. Nevertheless, these findings further suggest a close relationship between Mdm2 and p53 (Fig. 1).

THE P53-DEPENDENT FUNCTIONS OF MDM2

Tumor suppressors often control cell proliferation and death (Allred et al., 1993). As a negative regulator of tumor suppressor p53, Mdm2 appears to promote cell proliferation and inhibit apoptosis. In most cases, the effects of Mdm2

Figure 1. Mdm2 phosphorylation pathways. In response to DNA damage, insulin, growth factors, amino acids, and energy status, Mdm2 could be phosphorylated on various sites through different pathways, including p38/AKT/mTOR/S6K1 pathway and Atm/c-Abl pathway. Through targeting p53, Mdm2 regulates cell proliferation, senescence, and apoptosis. a.a.: amino acids; GFs: growth factors.

require the presence of p53. Ciardiello's group observed that antisense oligonucleotides of Mdm2 could enhance the growth-inhibitory effect of certain cytotoxic drugs in human colon cancer with functional p53 (Tortora et al., 2000). Shangary et al. designed a small-molecule inhibitor to disrupt Mdm2-p53 interaction, which inhibits cell proliferation (Shangary et al., 2008). In normal cells, e.g., human fibroblasts, the antibodies that can block Mdm2-p53 interaction augment p53-mediated suppression of cell proliferation (Blaydes and Wynford-Thomas, 1998). These findings, together with the pivotal role of Mdm2 in maintaining p53 at critical physiologic levels, suggest that Mdm2 is essential for the proliferation of normal and cancer cells.

In addition, by negatively regulating p53, Mdm2 could inhibit apoptosis and enhance cell survival. Overexpression of Mdm2 protects human glioblastoma U87-MG cells from chemotherapeutic drug cisplatin-induced apoptosis, which could otherwise be enhanced by treatment with antisense oligonucleotide targeting *Mdm2* mRNA (Kondo et al., 1995). During p53-mediated apoptosis, Mdm2 is cleaved by interleukin 1 β -converting enzyme-like proteases (caspases) with its COOH-terminal RING finger removed (Chen et al., 1997). The resultant Mdm2 could still bind to p53 and inhibit p53-mediated transcription (Chen et al., 1997), but failed to destabilize p53 (Pochampally et al., 1999).

THE P53-INDEPENDENT FUNCTIONS OF MDM2

However, some others reported that Mdm2 could also regulate cell proliferation in the absence of p53. One example is cell cycle arrest caused by Rb. p107 is a member of Rb tumor suppressor family, and it could induce G₁ cell-cycle arrest. Mdm2 reverses this effect in the absence of p53 (Dubs-Poterszman et al., 1995). Transforming growth factor- β (TGF- β) treatment causes growth arrest by promoting Rb dephosphorylation and preventing E2F1 activation (Sun et al., 1998). Under this setting, Mdm2 could reverse the effect of TGF- β on cell proliferation in the absence of p53. The underlying mechanism is that Mdm2 can target Rb for degradation or phosphorylation. What's more, Argentini et al. reported that mutant Mdm2 that is defective in mediating p53 ubiquitination/degradation could still induce cell proliferation (Argentini et al., 2000). Taken together, these observations suggest that Mdm2 could also promote cell proliferation through pathways not involving p53.

Additionally, Mdm2 could regulate apoptosis without directly acting on p53. It has been reported that Mdm2 could induce degradation of the proapoptotic activator homeodomain-interacting protein kinase 2 (HIPK2), which is responsible for p53 phosphorylation on Ser46. Hypophosphorylation on Ser46 compromises p53 activation and subsequently p53-mediated apoptosis. Furthermore, inhibition of Mdm2 could also induce apoptosis in some cell lines lacking functional p53, including p53 null cell lines such as

human colon cancer cell line Caco2 and p53 mutant cell line HuCCT1 (Ray et al., 2010; Zheng et al., 2010). Under these conditions, accumulation of p73 and the concomitant induction of Puma might account for apoptosis induction.

REGULATION OF MDM2 AT THE TRANSCRIPTIONAL LEVELS

The *Mdm2* gene has 12 exons and two distinct promoters, designated P1 and P2, which control the basal expression and inducible expression of Mdm2 respectively (Barak et al., 1994; Manfredi, 2010). Within the P2 promoter, there are two p53 binding sites and binding sites for other transcription factors such as adaptor protein 1 (AP-1) and Ets (Ries et al., 2000; Truong et al., 2005; Hollenhorst et al., 2007; Pikkariainen et al., 2009). p53 can transactivate P2 promoter and promote Mdm2 expression (Juven et al., 1993; Wu et al., 1993; Barak et al., 1994; Moll and Petrenko, 2003). Then, Mdm2 forms a complex with p53 and inhibits its transcriptional activity on *Mdm2* promoter (Wu et al., 1993). This regulation appears to require the transformation/transcription domain-associated protein (TRRAP) acetyltransferase complexes (Ard et al., 2002).

Transcription of Mdm2 can be regulated in a p53-independent manner as well. Transcription factor nuclear factor κ -B (NF- κ B) was demonstrated to bind to P1 promoter of *Mdm2* and enhance its expression (Busuttill et al., 2010). Ras/Raf binds to AP-1 response element (Ries et al., 2000; Halaschek-Wiener et al., 2004), while Ets transcription factors Fli-1 (Truong et al., 2005) and Elf4/Mef (Sashida et al., 2009) bind to *Mdm2* P2 promoter at the Ets responsive element, to elevate the expression of Mdm2. In neuroblastoma, v-myc myelocytomatosis viral related oncoprotein (MYCN) can directly bind to the consensus E-box of the human *Hdm2* P2 promoter and upregulate the expression of Hdm2 (Slack et al., 2005).

Through p53 and other transcription factors, a number of cellular or extracellular signals could influence the expression of Mdm2. In a recent report, expression of human Hdm2 was observed to be induced by TGF- β 1-activated SMAD3 and SMAD4 (Smad3/4) transcription factors which could specifically bind to the P2 promoter region of *Hdm2* (Araki et al., 2010). Through an intron sequence, thyroid hormone can regulate Mdm2 expression in a p53-independent manner (Qi et al., 1999). The correlation between estrogen status and Mdm2 mRNA levels was also observed in human breast carcinoma (Sheikh et al., 1993). Sheikh et al. showed that the estrogen receptor (ER)-positive cells express much higher levels of Mdm2 than ER-negative cells by a mechanism independent of ER-function but involving the P2 promoter (Phelps et al., 2003). Oxidative stress induces the transcription of Mdm2 possibly by promoting AP-1 expression (Pikkariainen et al., 2009). Moreover, potent anti-tumor agent gambogic acid (GA) could downregulate the *Mdm2*

mRNA levels (Rong et al., 2009). Both the P1 and P2 promoters of *Mdm2* are involved in the response to GA, although the mechanism behind this regulation remains elusive. Finally, a microRNA termed miR-221 is recently shown to be able to negatively regulate Mdm2 at RNA level through targeting its 3'-untranslated region (3'-UTR) (Kim et al., 2010).

REGULATION OF MDM2 STABILITY

As an E3-ligase, Mdm2 targets itself for ubiquitin-dependent proteasome-mediated degradation (Chang et al., 1998). The RING finger domain is necessary for Mdm2 self-ubiquitination (Honda and Yasuda, 2000). On the other hand, the ubiquitin moiety can be removed by deubiquitinase hepes simplex virus associated ubiquitin-specific protease (HAUSP) and ubiquitin-specific protease 2a (USP2a), which facilitate Mdm2 stabilization (Li et al., 2004; Stevenson et al., 2007). In addition to ubiquitination, Mdm2 can also be sumoylated at Lys446 by Ubc9, which has a strong sequence homology to ubiquitin carrier proteins (E2s) and binds to the aa 40–59 of Mdm2 (Buschmann et al., 2001). Lys446 is required for Mdm2 ubiquitin ligase activity as well (Buschmann et al., 2000). Sumoylation stabilizes Mdm2 by inhibiting its self-ubiquitination, but destabilizes p53 due to enhanced Mdm2 ubiquitin ligase activity toward p53. Reversely, desumoylation carried out by SUMO-specific protease, SUSP4, results in promotion of Mdm2 self-ubiquitination and p53 stabilization (Lee et al., 2006).

Apart from that, a number of proteins have been identified as inhibitors of Mdm2 self-ubiquitination, including Mdmx, MTBP, PKB, HAUSP, USP2a, CSN5, and ARF (Stad et al., 2001; Feng et al., 2004; Li et al., 2004; Brady et al., 2005; Zhang et al., 2008). Mdmx, a structural homolog of Mdm2, stabilizes Mdm2 through heterodimerization via their RING fingers (Stad et al., 2001). Death-domain-associated protein (DAXX) stabilizes Mdm2 via the stabilizing effect of HAUSP on Mdm2 (Tang et al., 2006). The 5th subunit of COP9 signalosome (CSN5, also known as Jab1 or COPS5) was also reported to reduce Mdm2 self-ubiquitination by an unidentified mechanism (Zhang et al., 2008). RFWD3, an E3 ubiquitin ligase, is recently identified as a stabilizer of Mdm2 (Fu et al., 2010). Mdm2 could be degraded more rapidly *in vivo* once RFWD3 is knocked down. However, the mechanism is not yet identified. More importantly, ARF can bind Mdm2 and induce re-localization of Tyr276-phosphorylated Mdm2 to the nucleolus and neutralizes its activity toward p53 (Dias et al., 2006). Just like ARF, some ribosomal proteins could also inhibit Mdm2 through binding to its acidic domain (reviewed in Manfredi, 2010).

Different from the proteins aforementioned, Ras-association domain family protein isoform 1A (RASSF1A), 14-4-3 σ , and SCY1-like 1 binding protein 1 (SCYL1-BP1) act as destabilizers of Mdm2 (Yang et al., 2007; Song et al., 2008;

Yan et al., 2010). RASSF1A, a tumor suppressor, disrupts the association among Mdm2, DAXX, and HAUSP in the nucleus, resulting in an enhancement of self-ubiquitin ligase activity of Mdm2 (Song et al., 2008). 14-4-3 σ and SCYL1-BP1 were both reported to bind to Mdm2 and to promote its self-ubiquitination and turnover by poorly understood mechanisms (Yang et al., 2007; Yan et al., 2010).

While inhibition of Mdm2 self-ubiquitination usually stabilizes Mdm2 and promotes p53 ubiquitination and degradation (Buschmann et al., 2000; Brady et al., 2005; Tang et al., 2006), there are several exceptions. For example, Mdmx stabilizes both Mdm2 and p53 by different mechanisms (Stad et al., 2001). Moreover, upon the activation of G-protein-coupled receptors (GPCRs), Mdm2 is recruited to GPCRs at plasma membrane through binding to β -arrestin, an important adapter and scaffold in signaling of GPCRs. As a result, both p53 ubiquitination and Mdm2 self-ubiquitination are reduced (Wang et al., 2003).

REGULATION OF MDM2 BY AKT1-MEDIATED PHOSPHORYLATION

Modification by phosphorylation is a major means to regulate Mdm2 function. The level of Mdm2 phosphorylation is usually reverse-correlated with its activity toward p53 (Blattner et al., 2002). Phosphorylation may regulate Mdm2 self-ubiquitination, nucleocytoplasmic shuttling, Mdm2-p53 interaction, etc. Multiple phosphorylation sites have been identified on Mdm2 (reviewed in Waning et al., 2010). Both mitogenic signals and certain cellular stresses (e.g., genotoxic stress) could induce Mdm2 phosphorylation through various pathways (Meek and Hupp, 2010). In response to mitogenic signals, Mdm2 could be phosphorylated on Serines 157, 166, 186 and 188. Given the fact that these residues are located near the nuclear localization signal (NLS) and nuclear export signal (NES) of Mdm2, it is not surprising that phosphorylation on these sites influences the nuclear translocation of Mdm2 (Mayo and Donner, 2001). Ser166 and Ser186 could be phosphorylated by Akt (Mayo and Donner, 2001; Zhou and Hung, 2002), Pim protein kinases (Pim-1, -2 and -3) *in vivo* (Hogan et al., 2008; Wood et al., 2009), and by ribosomal S6 kinase (RSK) *in vitro*. Ser188 could be phosphorylated by both Akt and Pim (Feng et al., 2004; Milne et al., 2004; Wood et al., 2009), while Ser157 is a target of MK2 (MAPKAPK-2) (Meek and Hupp, 2010).

The association of Mdm2 and Akt was observed in MCF-7 cells, HER-2 3T3 and DN-Akt 3T3 cells (Mayo and Donner, 2001; Zhou et al., 2001). Once Akt is activated by insulin-like growth factor-1 (IGF-1) or insulin in a PI3-kinase-dependent manner, it phosphorylates Mdm2 on Ser166 and Ser186 (Mayo and Donner, 2001). *In vitro* kinase assay further confirmed that Mdm2 is a *bona fide* substrate of Akt (Zhou et al., 2001), and in the experiments conducted by two other groups, Ser188 was found to be a target of Akt as well (Feng

et al., 2004; Milne et al., 2004). Akt-mediated phosphorylation promotes Mdm2 activation, stabilization, and redistribution to the nucleus, where it destabilizes p53 through ubiquitination (Mayo and Donner, 2001; Zhou et al., 2001; Feng et al., 2004). This has been postulated to be a mechanism by which activated Akt promotes tumorigenesis.

Just like Akt, Pim kinases could interact with Mdm2 *in vivo* and phosphorylate Mdm2 at Ser166 *in vitro* (Hogan et al., 2008). It should be noted that, although Pim-mediated phosphorylation elevates Mdm2 protein levels, it does not enhance p53 degradation, which is possibly due to the enhanced interaction between Mdm2 and its inhibitor ARF. Thus, Akt and Pim could phosphorylate Mdm2 on the same sites but get different outcomes. In addition, it has been reported that Pim-phosphorylated Mdm2 binds to 14-3-3, while Mdm2 phosphorylated by PKB/Akt pathway does not (Wood et al., 2009). Phosphorylation of Ser186 by Pim prevents Ser188 from being further phosphorylated by PKB/Akt pathway. 14-3-3 and ARF negatively regulate the activity of Mdm2, and this might explain why Pim-phosphorylated Mdm2 would not cause downregulation of p53 (Yang et al., 2007).

REGULATION OF MDM2 BY ATM-MEDIATED PHOSPHORYLATION UNDER GENOTOXIC STRESS

DNA damage is caused by external factors such as UV light and IR, or internal factors such as reactive oxygen species, which leads to activation of Atm (ataxia telangiectasia mutated) and Atr (Atm and Rad3-related), members of the phosphatidylinositol 3-kinase related kinase (PIKK) family. Atm could phosphorylate Mdm2 directly or through other kinases. Khosravi and his colleagues found that Mdm2 could be phosphorylated in an Atm-dependent manner *in vivo*, and that Atm could phosphorylate Mdm2 *in vitro* (Khosravi et al., 1999). Other groups also confirmed Mdm2 as a direct substrate of Atm (Maya et al., 2001; Cheng et al., 2009). Atm could directly phosphorylate Mdm2 on Ser395, 386 and 429 *in vivo*, residues adjacent to the RING domain. Phosphorylation of these residues disrupts the formation of RING oligomerization, thus preventing p53 polyubiquitination and leading to p53 stabilization (Cheng et al., 2009; Cheng and Chen, 2010). Atm-mediated Mdm2 phosphorylation destabilizes Mdm2 by impairing its binding to HAUSP (Meulmeester et al., 2005). In addition, phosphorylation of Mdm2 at Ser395 by Atm could be reversed by p53-induced phosphatase 1 (Wip1) (Lu et al., 2007; Lu et al., 2008). Through dephosphorylation, Wip1 stabilizes Mdm2 and enhances its binding toward p53 (Lu et al., 2007). Consequently, dephosphorylation of Mdm2 promotes proteasome-mediated degradation of p53. Additionally, Atm could phosphorylate c-Abl (Abelson tyrosine kinase), which interacts with Mdm2 *in vivo* and *in vitro*, and subsequently

phosphorylates Mdm2 on Tyr394 and 276 (Goldberg et al., 2002; Dias et al., 2006), which would in turn regulate the interaction of Mdm2 and ARF and facilitate p53 stabilization. Another PIKK family member Atr, which is activated by UV or single-stranded DNA (ssDNA), could also phosphorylate Mdm2, mainly at Ser407 (Shinozaki et al., 2003), yet its significance is less clear.

REGULATION OF MDM2 BY S6K1-MEDIATED PHOSPHORYLATION, A CONNECTION TO CELL METABOLISM

Besides Atm and Atr, Lai et al. recently found that under genotoxic stress, S6K1 can phosphorylate Mdm2 on Ser163 (Lai et al., 2010). DNA damage transiently activates mTOR-S6K1 in the cytoplasm, which requires p38 MAPK (mitogen-activated protein kinases) but not Atm/Atr. The activated S6K1, in a T389 phosphorylation dependent manner, forms a tighter complex with Mdm2, phosphorylates Mdm2 at S163 in the cytoplasm, and inhibits Mdm2-mediated p53 ubiquitination, allowing Mdm2 cytoplasmic retention and p53 stabilization. Deactivation of mTOR-S6K1 signaling leads to Mdm2 nuclear translocation, which is facilitated by S163 phosphorylation, a reduction in p53 induction, and an alteration in p53-dependent cell death. These results support a model in which S6K1 regulates p53 induction in DNA damage response by interacting with and phosphorylating Mdm2. S6K1-Mdm2 interaction presents a route for cells to incorporate the metabolic/energy cues into DNA damage response and links the aging-controlling Mdm2-p53 and mTOR-S6K pathways (Fig. 1).

Lai's study thus reveals another genotoxic stress-responsive pathway, p38-Akt-mTOR-S6K1-Mdm2, which helps to regulate p53 stability. This pathway can also sense the cells' nutrient and energy status and transmits signals to fine-tune cells' response to DNA damage. The interaction between S6K1 and Mdm2 also links two of the prominent pathways that control aging at the cell and organism levels, the mTOR-S6K pathway and the Mdm2-p53 pathway. Further investigation will be needed to determine whether p53 participates in mTOR-S6K1 mediated aging process. These findings might be of help in developing strategies to retain Mdm2 in the cytoplasm to facilitate p53-based cancer therapy.

MDM2 BASED CANCER THERAPY

Mdm2 acts as an oncoprotein mainly by negatively regulating tumor suppressor p53. Targeting Mdm2 provides a potential strategy for treatment of cancer patients with wild-type p53. Inhibition of Mdm2 could be achieved by introduction of antisense oligonucleotides, expressing its antagonists, or treatment with its inhibitors. Antisense therapy has been shown to enhance apoptosis induced by chemotherapy

reagents (Sato et al., 2000) or irradiation (Grünbaum et al., 2001). In addition, more efforts have been made to search for drugs that inhibit Mdm2 activity.

Aminoflavone (AF, NSC 686, 288) is a potent anti-cancer drug that causes cell cycle arrest by phosphorylating and activating p53 (Meng et al., 2005). AF also inhibits Mdm2 phosphorylation at Ser166 and promotes its polyubiquitination, leading to Mdm2 degradation. So, in response to increasing doses of AF, Mdm2 protein first accumulates and then decreases. The elevated Mdm2 protein level might induce cell cycle arrest, while decrease of Mdm2 would switch cell cycle arrest to apoptosis.

A class of small molecule Mdm2 inhibitors (MIs) were recently discovered to bind to Mdm2, disrupt Mdm2-p53 binding, and reactivate p53 (Ding et al., 2006). Among the inhibitors, MI-63 has the strongest affinity for Mdm2 (Canner et al., 2009). MI-63 was found to induce apoptosis accompanied by an increased expression of p53, p21 and Bax in Rhabdomyosarcoma (RMS) expressing wild-type p53 but not in cells expressing mutant p53. The p53-dependence was also observed in acute myeloid leukemia (AML) cells (Samudio et al., 2010). Similar to MI-63, MI-43 induces the accumulation of p53 and activates its transcriptional activity, leading to cell cycle arrest and apoptosis. MI-43 could also sensitize chemo-resistant A549 cells to etoposide-induced apoptosis when applied in combination with etoposide.

Nutlins are another class of highly potent and specific small-molecule antagonists of Mdm2 (Vassilev et al., 2004). They occupy the p53 binding pocket in Mdm2, resulting in impaired Mdm2-p53 interaction and the release of functional p53. Nutlin-3 induces cellular senescence in a p53-dependent manner (Efeyan et al., 2007). Nutlin-3 could only induce apoptosis in AML cells expressing wild-type p53 (Kojima et al., 2005). The same effect could be observed in pediatric acute lymphoblastic leukemia (ALL) cells (Gu et al., 2008b). Nutlin-3 could also induce apoptosis in p53-deficient tumor cells, possibly by disrupting the interaction of Mdm2 and p73, and activated p73 would induce PUMA expression and apoptosis (Ray et al., 2010).

GA, an anticancer agent derived from gamboges, has been proved to regulate Mdm2 at both transcriptional and posttranslational levels independently of p53 (Gu et al., 2008a). Via the two promoters of *Mdm2*, GA downregulates Mdm2 at transcriptional level; through the ubiquitin-proteasome pathway, GA inhibits Mdm2 at posttranslational level. Meanwhile, GA elevates the expression of p21 regardless of p53 status, and blocks the access of Mdm2 to p21, leading to stabilization of p21 (Rong et al., 2009). Through affecting Mdm2, GA induces cell cycle arrest or apoptosis.

Berberine is a natural product that induces apoptosis in ALL cells possibly by downregulating Mdm2 at a posttranslational level (Zhang et al., 2010). Through interaction with DAXX, Berberine disrupts the formation of Mdm2-DAXX-

HAUSP complex, promoting Mdm2 self-ubiquitination and degradation. The activity of berberine is dependent on the presence of wild-type p53.

FUTURE PERSPECTIVES

As an essential regulator of the most prevalent tumor suppressor p53 and a variety of other proteins, Mdm2 has attracted a lot of attention. While a great deal has been learnt on how Mdm2 controls p53 expression, activation, and cellular functions, little is known about how Mdm2 regulates other targets. Future studies will focus on investigating the physiologic function of Mdm2-mediated ubiquitination and degradation of other substrates such as insulin receptor substrate-1(IRS-1). One strategy is to use *Mdm2*^{-/-} *p53*^{-/-} mice to analyze potential developmental defects. Another direction is to study how Mdm2 can integrate external and internal cues to fine-tune the expression of p53 as well as cell proliferation and death. This is particularly important since cells are surrounded by other cells and the extracellular matrix, where signaling molecules as well as nutrients are present and are constantly changing. The Akt-mTOR-S6K1 pathway, which can be activated by metabolic/energy status and regulates the Mdm2-p53 loop, provides such an example. Further unraveling the regulation of Mdm2 will help us to design novel strategies to treat cancer.

ABBREVIATIONS

ALL, acute myeloid leukemia; AML, acute myeloid leukemia; AP-1, adaptor protein 1; Atm, ataxia telangiectasia mutated; Atr, Atm and Rad3-related; DAXX, death-domain-associated protein; GA, gambogic acid; GPCRs, G-protein-coupled receptors; HAUSP, hepes simplex virus associated ubiquitin-specific protease; HIV-1 vif, human immunodeficiency virus-1 viral infectivity factor; IGF-1, insulin-like growth factor-1; MAPK, mitogen-activated protein kinases; Mdm2, mouse double minute 2; MIs, Mdm2 inhibitors; MYCN, v-myc myelocytomatosis viral related oncoprotein; NES, nuclear export signal; NF-κB, nuclear factor κ-B; NLS, nuclear localization signal; PIKK, phosphatidylinositol 3-kinase related kinase; RASSF1A, Ras-association domain family protein isoform 1A; RFWD3, ring finger and WD repeat domain 3; RMS, rhabdomyosarcoma; RPL26, ribosomal protein L26; RSK, ribosomal S6 kinase; SCYL1-BP1, SCY1-like 1 binding protein 1; ssDNA, single-stranded DNA; TGF-β, transforming growth factor-β; HIPK2; homeodomain-interacting protein kinase 2; USP2a, ubiquitin-specific protease 2a

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