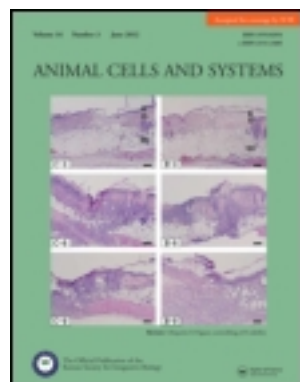


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Ubiquitin E3 ligases controlling p53 stability

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The p53 protein plays a pivotal role in tumor suppression. The cellular level of p53 is normally kept low by proteasome-mediated degradation, allowing cell cycle progression and cell proliferation. Under stress conditions, such as DNA damage, p53 is stabilized and activated through various post-translational modifications of itself as well as of its regulatory proteins for induction of the downstream genes responsible for cell cycle arrest, DNA repair, and apoptosis. Therefore, the level of p53 should be tightly regulated for normal cell growth and for prevention of the accumulation of mutations in DNA under stress conditions, which otherwise would lead to tumorigenesis. Since the discovery of Mdm2, a critical ubiquitin E3 ligase that destabilizes p53 in mammalian cells, nearly 20 different E3 ligases have been identified and shown to function in the control of stability, nuclear export, translocation to chromatin or nuclear foci, and oligomerization of p53. So far, a large number of excellent reviews have been published on the control of p53 function in various aspects. Therefore, this review will focus only on mammalian ubiquitin E3 ligases that mediate proteasome-dependent degradation of p53.

Keywords: p53; Mdm2; Pirh2; COP1; CARP1/CARP2; ARF-BP1; TOPORS; Synoviolin; CHIP; JFK; MKRN1; p300/CBP

Introduction

Nearly 50% of human cancers are attributed to the loss of p53 function. As to the importance of the tumor suppressive function of p53, a great deal of effort has been contributed to explore the mechanism for the control of p53 activity. One of the key mechanisms that control p53 activity is post-translational modification of the tumor suppressor protein. Nearly 100 proteins have been identified as the p53-binding proteins, which are involved in phosphorylation, methylation, acetylation, poly-ADP-ribosylation, glycosylation, ubiquitination, modification by ubiquitin-like proteins, such as SUMO and Nedd8, and their reversible processes. Of 393 amino acids that constitute the p53 protein, nearly 50 amino acids have been identified as the modification sites and perhaps many more of such sites are to be identified in the near future (Meek and Anderson 2009; Anderson and Appella 2010).

Of diverse post-translational modification processes, ubiquitination plays a fundamental role in the control of p53 function as it determines the cellular level of p53. The process of ubiquitination is catalyzed by three cascade enzyme system involving E1 activating enzymes, E2 conjugating enzymes, and E3 ligases (Hershko and Ciechanover 1998; Pickart 2001). Of these enzymes, E3 ligases determine the specificity of target proteins for ubiquitination. E6AP (human papilloma virus E6-associated cellular protein) is the first identified E3 ligase that targets p53 (Scheffner et al. 1993). The viral E6 protein promotes the human E6AP protein, also known as UBE3A (ubiquitin-

protein ligase E3A), to destabilize p53 for viral replication in the host cells. Mouse double minute 2 (Mdm2) was then discovered as a major E3 ligase for p53 ubiquitination and degradation (Haupt et al. 1997; Honda et al. 1997; Kubbutat et al. 1997; Li et al. 2003). Since then, numerous other E3 ligases were identified and characterized for their function (Brooks and Gu 2011). Here, we summarize the functions of the E3 ligases that interact with and ubiquitinate p53 in mammalian cells.

Mdm2

Mouse double minute 2 is a major ubiquitin E3 ligase that mediates really interesting new gene (RING)-dependent ubiquitination of p53 (Haupt et al. 1997; Honda et al. 1997; Kubbutat et al. 1997; Fang et al. 2000). Its human homolog is called as HDM2, whose gene is located in chromosome 12q14.3-q15 (Oliner et al. 1992). Mdm2 has an N-terminal p53-binding domain, acidic and zinc finger domains in the middle, and a C-terminal RING finger domain (Figure 1).

Under normal conditions, p53 activates the expression of Mdm2, which in turn ubiquitinates p53 for proteasome-mediated degradation, forming a negative feedback loop for keeping p53 in low levels (Haupt et al. 1997; Honda et al. 1997; Kubbutat et al. 1997). Under DNA damage or other stress conditions, however, p53 level is increased by disruption of p53-mediated Mdm2 expression as well as by post-translational modifications that inhibit Mdm2 function

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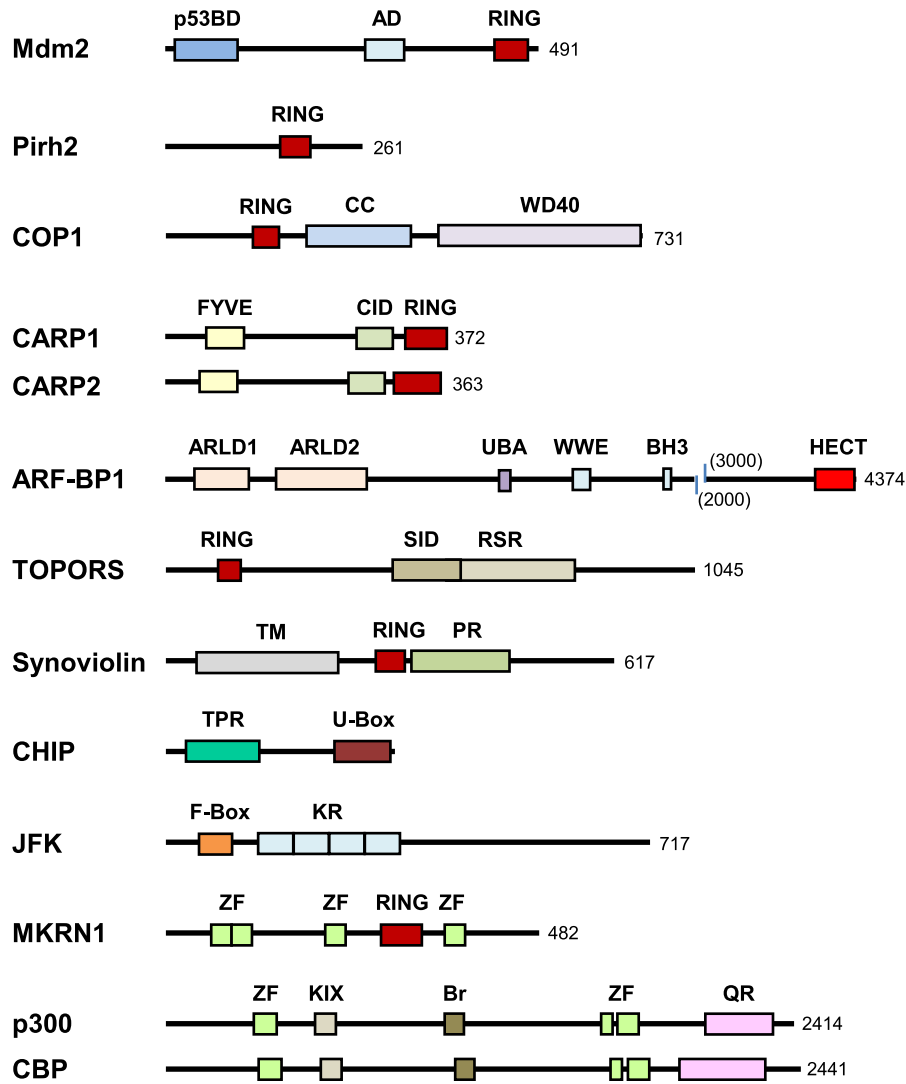


Figure 1. Domain structures of ubiquitin E3 ligases for p53 degradation. p53BD, p53-binding domain; AD, acidic domain; RING, really interesting new gene; CC, coiled coil; FYVE, Cys-rich zinc finger domain of Fab1, YOTB, Vac1, and EEA1; CID, caspase-interacting domain; ARLD, Armadillo repeat-like domain; UBA, ubiquitin-associated domain; BH3, Bcl-2 homology domain; HECT, homologous to the E6-AP carboxyl terminus; SID, SUMO-interacting domain; RSR, Arg/Ser-rich; TM, trans-membrane; PR, Pro-rich; TPR, tetratricopeptide repeat; KR, Kelch repeat; ZF, zinc finger; KIX, CREB and MYB interaction domain; Br, bromo domain; QR, Gln-rich domain. The numerals shown at the right ends indicate the number of amino acids.

(Ashcroft et al. 2000; Meek and Knippschild 2003; Michael and Oren 2003). For example, phosphorylation of Ser15 and Ser20 of p53 by stress-induced kinases (such as ATM/ATR, Chk1/Chk2, and DNA-PK) disrupts its interaction with Mdm2, leading to the stabilization of p53 (Shieh et al. 1997; Shieh et al., 1999; Tibbetts et al. 1999; Hirao et al. 2000). In addition, alternate open reading frame (ARF) directly binds and inhibits Mdm2, leading to the stabilization of p53 and the promotion of p53-mediated cell cycle arrest and apoptosis (Zhang et al. 1998). Remarkably, the relative amount of Mdm2 is important to decide the fate of ubiquitinated p53 between proteasome-

mediated destruction and translocation to the cytoplasm (Li et al. 2003). Low levels of Mdm2 activity induce mono-ubiquitination and nuclear export of p53, whereas high levels promote poly-ubiquitination and degradation of p53 in the nucleus.

MdmX, a structural homolog of Mdm2, which has a RING domain but lacks E3 ligase activity, ablates p53-mediated transcription by forming a ternary complex with Mdm2 and p53 (Shvarts et al. 1996; Stad et al. 2001). Knockout of MdmX is embryonic lethal. Knockout of Mdm2 is also embryonic lethal, and this phenotype can completely be rescued by knockout of both Mdm2 and p53 (Parant et al. 2001; Finch et al.

2002; Migliorini et al. 2002; Li et al. 2004). Elegant experiments involving the reintroduction of p53 to mice lacking p53 together with either Mdm2 or MdmX have shown that loss of Mdm2 promotes the activation of p53 target genes related to apoptosis, while loss of MdmX promotes the activation of genes mediating cell cycle arrest (Chavez-Reyes et al. 2003; Barboza et al. 2008). Human *MDM2* (*HDM2*) proto-oncogene is amplified in 30–40% of sarcomas and is overexpressed in leukemic cells (Oliner et al. 1992; Bueso-Ramos et al. 1993; Jones et al. 1995).

Other protein substrates that are ubiquitinated by Mdm2 for proteasome-mediated degradation include DYRK2 (dual-specificity tyrosine-regulated kinase 2), β -arrestin, IGF-1R (insulin-like growth factor-1 receptor), and androgen receptor (Shenoy et al. 2001; Lin et al. 2002; Girnita et al. 2003; Taira et al. 2010). Mdm2 also promotes proteasome-mediated degradation of Rb (retinoblastoma protein) and p21, but through an ubiquitin-independent pathway (Zhang et al. 2004; Sdek et al. 2005).

Pirh2

Pirh2 (p53-induced RING H2) was identified as an androgen receptor N-terminal interacting protein (ARNIP) and also known as Rchy 1 (RING finger and CHY zinc finger domain containing 1). Its gene is located in chromosome 4q21.1 (Beitel et al. 2002; Leng et al. 2003).

Like Mdm2, Pirh2 is transcriptionally activated by p53 and in turn negatively regulates p53 level by ubiquitination, forming a negative feedback loop (Leng et al. 2003; Feng et al. 2007; Sheng et al. 2008). However, unlike Mdm2, which promotes degradation of unmodified form of p53 under nonstressed conditions, Pirh2 preferentially ubiquitinates Ser15-phosphorylated p53, which can be generated under DNA damage conditions (e.g., IR or UV treatment), for proteasome-mediated degradation. Thus, Pirh2 negatively regulates the expression of p53 downstream genes for cell cycle arrest and apoptosis, because phosphorylation of Ser15 in p53 is critical for p53-dependent transactivation.

Pirh2 is mostly detected in the phosphorylated form in normal tissues, whereas it is present in unphosphorylated form in majority of tumors. Pirh2 is phosphorylated by calmodulin-dependent kinase II (CaMKII) in a cell cycle-dependent manner, and this phosphorylation promotes the localization of Pirh2 in the cytoplasm as well as self-ubiquitination, leading to p53 stabilization (Duan et al. 2007). Interestingly, the level of CaMKII is down-regulated in human tumors (Tombes et al. 1999). In addition, Pirh2 is overexpressed in a variety of human tumors, including

prostate, lung, head-and-neck, and breast cancers, and this overexpression is independent of p53, implicating its role as an oncogenic protein (Duan et al. 2006; Wang et al. 2011).

Other proteins that are ubiquitinated by Pirh2 for proteasome-mediated degradation include DNA polymerase η , HDAC1, p27, and ϵ -COP (Logan et al. 2006; Hattori et al. 2007; Maruyama et al. 2008; Jung et al. 2010). Significantly, Pirh2 also ubiquitinates p73, a member of p53 family, which shows tumor suppressive functions by activating downstream target genes for cell cycle arrest and apoptosis (Jung et al. 2011; Wu et al. 2011).

COP1

COP1 (constitutive photomorphogenesis protein 1 homolog) was originally identified as a central regulator of photomorphogenic development in plant (Deng et al. 1991). Its gene is located in the human chromosome 1q25.1-q25.2. COP1 is also called as RFW2 (RING finger and WD repeat domain 2) and RNF200 (RING finger protein 200). It contains an N-terminal RING finger domain, a coiled-coiled domain in the middle, and a C-terminal WD40 repeat domain. COP1 has a central bipartite nuclear localization signal (NLS), and is localized in both the nucleus and the cytoplasm (Yi et al. 2002; Bianchi et al. 2003).

Like Mdm2, COP1 is transcriptionally activated by p53 and in turn negatively regulates p53 level by ubiquitination in mammalian cells, forming a negative feedback loop. Under normal conditions, COP1 and Mdm2 may synergistically down-regulate p53 by ubiquitination, thus allowing cell growth and proliferation (Dornan et al. 2004b). In response to DNA damage, such as ionizing radiation (IR), however, ATM phosphorylates COP1 on Ser387, triggers its export from the nucleus to the cytoplasm, and stimulates its self-ubiquitination, resulting in the stabilization of p53 for cell cycle arrest or apoptosis (Dornan et al. 2006). COP1 is overexpressed in more than 80% of breast and nearly half of ovarian adenocarcinomas, implicating its role as an oncogenic protein (Dornan et al. 2004a).

However, recent studies have shown that COP1 acts as a tumor suppressor by promoting ubiquitination and degradation of the c-Jun oncoprotein in cooperation with DET1 (de-etiolated 1), DDB1 (DNA damage binding protein 1), cullin-4A, and Roc1 (Wertz et al. 2004). The tumor suppressive role of COP1 was further supported by the finding that *Cop1*-hypomorphic mice, which express only 10% of Cop1 with increased c-Jun accumulation, develop spontaneous malignancy and are more prone to radiation-induced lymphomagenesis (Migliorini et al. 2011; Wei and Kaelin 2011). COP1 also down-regulates ETV1, ETV4, and ETV5, which

are oncogenic transcription factors and most frequently rearranged and overexpressed in prostate cancers, providing an additional evidence for the role of COP1 as a tumor suppressor (Vitari et al. 2011).

Other COP1 substrates identified so far include ACC1 (acetyl-coenzyme A carboxylase alpha), MTA1 (metastasis-associated protein 1), FOXO1 (forkhead box protein O1), TORC2 (transducer of regulated CREB activity 2), and PEA3 (polyomavirus enhancer activator-3) (Qi et al. 2006; Kato et al. 2008; Li et al. 2009; Baert et al. 2010; Wei and Kaelin 2011).

CARP1 and CARP2

CARPs (caspase-8- and caspase-10-associated RING proteins) were identified as a family of apoptotic inhibitors that bind to and down-regulate the death effector domain (DED)-containing caspases (McDonald and El-Deiry 2004). Their genes are located in chromosomes 12q24.31 and 17q12 for CARP1 and CARP2, respectively. CARP1 is also called as RFI (RING finger homologous to inhibitor of apoptosis protein [IAP]) and RNF34, while CARP2, a splicing variant of CARP1, is also called as RNFL1 (RING finger and FYVE-like domain containing 1), RNF189, and RNF34L (RNF34-like). CARPs contain an N-terminal FYVE-type zinc finger domain, two CIDs (caspase-interacting domains) in the middle, and a C-terminal RING finger domain (Sasaki et al. 2002; Coumailleau et al. 2004).

Both CARP1 and CARP2 physically interact with and ubiquitinate p53 in the absence of Mdm2 for proteasome-mediated degradation. Interestingly, CARPs are also capable of down-regulating Ser20-phosphorylated p53. Under DNA damage conditions, such as treatment with adriamycin, phosphorylation of Ser20 of p53 as well as of Ser395 of Mdm2 occurs and disrupts their interaction, leading to p53 stabilization. Thus, the ability of CARPs, with that of Pirh2, in down-regulating phosphorylated-p53 may provide oncogenic potential capable of eliminating chemotherapy-induced p53 protein. Consistently, CARPs are frequently overexpressed in human tumors, such as esophageal cancers (Yang et al. 2007).

ARF-BP1

ARF-BP1 (ARF-binding protein 1) is the largest ubiquitin E3 ligase (~ 500 kDa) that harbors a HECT (homologous to E6AP carboxyl terminus) domain. It is also called as HectH9 (HECT homologous protein 9), UREB1 (upstream regulatory element-binding protein 1), LASU1 (large structure of UREB1), Mule (MCL-1 ubiquitin ligase E3), and HUWE1 (HECT, UBA, and WWE domain-containing

protein 1). Its gene is located in chromosome Xp11.22 (Adhikary et al. 2005; Chen et al. 2005; Liu et al. 2005; Zhong et al. 2005).

Like Mdm2, ARF-BP1 directly binds and ubiquitinates p53, and this activity is strongly inhibited by ARF. Depletion of ARF-BP1 by RNA interference stabilizes p53 and induces apoptosis (Chen et al. 2005). However, ARF expression results in cell growth inhibition even in p53/Mdm2-double knockout MEF cells, indicating that ARF exerts its tumor-suppressive function in p53- and Mdm2-independent manner (Weber et al. 2000; Kuo et al. 2003). Moreover, ARF-BP1 depletion induces cell growth arrest in p53 null MEF cells. Thus, it appears that ARF-BP1 plays a role not only as an ubiquitin E3 ligase of p53 but also as a key target of ARF, which in turn exerts its tumor suppressive function in a p53-independent manner. However, target substrate(s) of ARF-BP1, which is stabilized by ARF and thereby induces cell growth inhibition, remains unknown (Chen et al. 2005, 2006). ARF-BP1 is overexpressed in a variety of primary tumor samples, including breast cancer cell lines (Adhikary et al. 2005; Yoon et al. 2005). Significantly, defects in ARF-BP1 are a causative of mental retardation syndromic X-linked Turner type (MRXST), also known as mental retardation and macrocephaly syndrome (Froyen et al. 2008).

Other proteins that are ubiquitinated by ARF-BP1 for proteasome-mediated degradation include MCL1 (myeloid cell leukemia 1), DNA polymerase β , Cdc6, and MyoD (Zhong et al. 2005; Hall et al. 2007; Parsons et al. 2009; Noy et al. 2012). Interestingly, ARF-BP1 promotes the degradation of MyoD by ubiquitinating its N-terminus (Noy et al. 2012). ARF-BP1 also ubiquitinates Myc through Lys63-linked isopeptide bonds, leading to the stabilization of Myc rather than its degradation (Adhikary et al. 2005).

TOPORS

TOPORS was identified as topoisomerase I-binding protein containing RING finger and arginine/serine (R/S)-rich sequences. It is also called as p53BP3 because it binds to p53 and as LUN because it is highly expressed in the lung. Its gene is located in chromosome 9p21. TOPORS has an N-terminal RING domain, an R/S-rich domain and a bipartite NLS in the middle, and five PEST sequences (three in the N-terminus and two in the C-terminus) (Haluska et al. 1999; Chu et al. 2001). It dynamically associates with PML nuclear bodies in the nucleus (Rasheed et al. 2002).

TOPORS was shown to ubiquitinate p53, leading to proteasome-mediated degradation, albeit less efficiently than Mdm2 (Rajendra et al. 2004). By contrast,

it was also shown that TOPORS overexpression stabilizes p53 and in turn induces p53 downstream genes, such as p21 and Bax, for cell cycle arrest and apoptosis. Furthermore, in response to DNA damage, TOPORS accumulates and facilitates p53-mediated apoptosis in tumor and primary cells, implicating the role of TOPORS as a tumor suppressor (Lin et al. 2005). Interestingly, the expression of TOPORS is down-regulated during the development and metastasis of lung cancer, again suggesting the role of TOPORS in the suppression of tumorigenesis. However, it remains unclear how the ubiquitin ligase activity of TOPORS is related with its tumor suppressive function (Oyanagi et al. 2004; Saleem et al. 2004).

Remarkably, TOPORS also serves as a SUMO E3 ligase for p53 as well as other proteins, such as IKK ϵ (Weger et al. 2005; Renner et al. 2010). Although the physiological relevance for SUMO modification of p53 remains unclear, it has recently shown that in response to DNA damage TOPORS-mediated SUMO modification of IKK ϵ is induced and triggers phosphorylation of NF- κ B p65, contributing to the anti-apoptotic function of NF- κ B (Renner et al. 2010).

Synoviolin

Synoviolin (synovial apoptosis inhibitor 1) is an endoplasmic reticulum (ER) stress-inducible ER membrane protein that acts as an ubiquitin E3 ligase with a RING finger motif, originally identified in yeast. It is also called as HRD1 (hydroxymethyl glutaryl CoA reductase degradation 1), named after its substrate. Synoviolin contains an N-terminal transmembrane domain, a RING finger domain in the middle, and a C-terminal cytoplasmic domain. Its gene is located in chromosome 11q13 (Kaneko et al. 2002; Amano et al. 2003; Nadav et al. 2003).

Synoviolin sequesters p53 in the cytoplasm and ubiquitinates it for proteasome-mediated degradation (Yamasaki et al. 2007). Synoviolin has been regarded as a causative factor of arthropathy, as it is highly expressed in the rheumatoid synovium and triggers the outgrowth of synovial cells due to its anti-apoptotic effects, and mice overexpressing this enzyme spontaneously develop arthropathy (Amano et al. 2003). Moreover, mice lacking p53 have severe collagen-induced arthritis, although they do not develop spontaneous arthropathy, suggesting the operation of the synoviolin-p53 pathway at least in controlling the severity of arthritis (Simelyte et al. 2005).

CHIP

CHIP (carboxyl terminus of Hsc70-interacting protein) is a cofactor that modulates the functions of cytosolic

chaperones, such as Hsc/Hsp70. It contains an N-terminal TPR (tetratricopeptide repeat) domain and a C-terminal U-box domain, which forms a structure similar to a RING finger. Its gene is located in chromosome 16p13.3 (Min et al. 2008).

CHIP ubiquitinates p53 in cooperation with the chaperones Hsc70 and Hsp90 for degradation by proteasome, thereby negatively regulating p53-mediated transcription (Esser et al. 2005). Many transforming mutants of p53 display structural defects and associate with Hsc70 (Davidoff et al. 1992; Blagosklonny et al. 1995; Whitesell et al. 1998). For example, Arg175 mutations destabilize loop regions of the DNA-binding domain, leading to partial unfolding and more stable association of p53 mutants with Hsc70 (Gannon et al. 1990; Hinds et al. 1990; Bargonetti et al. 1993). CHIP promotes the degradation of both wild-type and mutant forms of p53. Interestingly, however, geldanamycin, an inhibitor of Hsp90, accelerates the degradation of oncogenic mutant forms but not that of wild-type p53, providing a relevance for the use of Hsp90 inhibitors as chemotherapeutics (Esser et al. 2005).

Other chaperone-assisted protein substrates for CHIP-mediated ubiquitination include glucocorticoid receptor (Connell et al. 2001; Demand et al. 2001), oncogenic receptor tyrosine kinase ErbB (Xu et al. 2002), and cystic fibrosis transmembrane conductance regulator (Meacham et al. 2001), and hyperphosphorylated Tau (Petrucelli et al. 2004; Shimura et al. 2004).

SCF^{JFK}

Among the total of 68 F-box proteins in human, JFK (just one F-box- and Kelch-domain contain protein) is the only F-box protein that also contains Kelch repeats. JFK is also called as FBXO42 (F-box protein 42). Its gene is located in 1p36.23-p36.11 (Sun et al. 2009).

JFK forms an SCF complex with Skp1, cullin-1, and Rbx1, and promotes ubiquitination of p53 for proteasome-mediated degradation. Depletion of JFK by RNA interference stabilizes p53, leading to promotion of cell cycle arrest and apoptosis. As SCF ligases mainly participate in the control of cell cycle, particularly on G1/S transition, and p53 plays a critical role in regulating the G1/S cell cycle checkpoint, JFK may contribute to the maintenance of p53 levels in unstressed cells (Sun et al. 2009).

MKRNI

MKRNI (Makorin RING finger protein 1) was first identified as an intron-containing source gene for MKRN gene family, majority of which are intronless

and imprinted. MKRN1 is also called as RNF61, and its gene is located in chromosome 7q34.

MKRN1, like Mdm2, ubiquitinates and destabilizes p53 under normal conditions, but by targeting distinct Lys residues (i.e., K291 and K293). Under DNA damage conditions, however, MKRN1 ubiquitinates p21, but not p53, for proteasome-mediated degradation and thereby stimulates p53-mediated apoptosis (Lee et al. 2009). MKRN1 also ubiquitinates hTERT, the catalytic subunit of telomerase, for proteasome-mediated degradation, leading to a decrease in telomere length (Kim et al. 2005).

p300/CBP

Both p300 and CBP (CREB-binding protein) are transcriptional coactivators that have histone acetyltransferase (HAT) activity. Although both proteins are mainly localized in the nucleus, their substantial amounts also reside in the cytoplasm. Both contain three conserved Zn^{2+} -binding Cys/His-rich domains, one of which (C/H1-TAZ1) responsible for E3 and E4 ligase activity lies in the N-terminal region (Grossman et al. 2003). The genes for p300 and CBP are located in chromosome 22q13.2 and 16p13.3, respectively.

Under unstressed conditions, both p300 and CBP catalyze poly-ubiquitination of p53 that had been mono-ubiquitinated by Mdm2 in the cytoplasm, leading to destabilization of p53. Under stress conditions, however, both proteins catalyze acetylation of p53 for its stabilization and activation in the nucleus (Grossman et al. 2003; Shi et al. 2009). Thus, p300 and CBP appear to engage in compartmentalized control of p53 by ubiquitination in the cytoplasm and acetylation in the nucleus under normal and stress conditions, respectively.

Others

The E3 ligases described here ubiquitinate and destabilize p53 by proteasome-mediated degradation. In addition to these enzymes, mammalian cells contain a number of E3 ligases that catalyze ubiquitination of p53, but do not promote its proteasome-mediated degradation. Ubc13 that commonly acts as an E2 enzyme for the synthesis Lys63-linked poly-ubiquitin chains can directly ubiquitinate p53 without intervention of a partner E3 ligase, leading to prevention of p53 tetramerization (Laine et al. 2006). MSL2 (male-specific lethal 2) and WWP1 (WW domain-containing E3 ubiquitin protein ligase 1) ubiquitinate p53 and induce its nuclear export (Laine and Ronai 2007; Kruse and Gu 2009). On the other hand, E4F1 (E4F transcription factor 1) promotes translocation of p53 to chromatin by ubiquitination (Le Cam et al. 2006).

Significantly, E4F1 promotes the ubiquitination of Lys residues in the hinge region of p53, which are acetylated by PCAF but distinct from those targeted by Mdm2, and this modification stimulates transcriptional activation of p53, specific for cell cycle arrest.

So many E3s for p53

Nearly 20 different E3 ligases for p53 have been identified in mammalian cells so far. Why the cells have so many E3 ligases for p53 ubiquitination? Based on their primary functions, they can at least be divided into two groups: one for the degradation of p53 by proteasome and the other for the proteasome-independent control of p53 activity, such as by subcellular localization and oligomerization. The E3 ligases that mediate proteasome-dependent p53 degradation can also be roughly divided into two groups: one, such as Mdm2, for the degradation of unmodified p53 for keeping p53 level low for cells to proliferate normally and the other, such as Pirh2 and CARP1/2, for the degradation of stress-induced, phosphorylated p53 to overcome p53-mediated cell cycle arrest and apoptosis. In addition, whole-mount *in situ* expression analysis has recently shown that E3 ligases for p53 display distinct expression pattern during mouse embryonic development, suggesting that the ligases may acquire specific functions at different stage of development (Jain and Barton 2010). Nonetheless, it remains largely unknown how different E3 ligase cooperates and cross talks with each other for the control of p53 function in tumor suppression.

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