Review

Deciphering the PTM codes of the tumor suppressor p53

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The genome guardian p53 functions as a transcription factor that senses numerous cellular stresses and orchestrates the corresponding transcriptional events involved in determining various cellular outcomes, including cell cycle arrest, apoptosis, senescence, DNA repair, and metabolic regulation. In response to diverse stresses, p53 undergoes multiple posttranslational modifications (PTMs) that coordinate with intimate interdependencies to precisely modulate its diverse properties in given biological contexts. Notably, PTMs can recruit ‘reader’ proteins that exclusively recognize specific modifications and facilitate the functional readout of p53. Targeting PTM–reader interplay has been developing into a promising cancer therapeutic strategy. In this review, we summarize the advances in deciphering the ‘PTM codes’ of p53, focusing particularly on the mechanisms by which the specific reader proteins functionally decipher the information harbored within these PTMs of p53. We also highlight the potential applications of intervention with p53 PTM–reader interactions in cancer therapy and discuss perspectives on the ‘PTMomic’ study of p53 and other proteins.

Keywords: p53, posttranslational modification, reader, PTM code, tumor therapy

Introduction

Although p53 was originally presumed to be an oncoprotein, subsequent studies have shown that wild-type p53 is a bona fide homeostasis maintainer and tumor suppressor that is extensively involved in cell cycle arrest, apoptosis, DNA repair, cellular senescence, metabolic processes, etc. (Kruiswijk et al., 2015; Kastenhuber and Lowe, 2017; Li et al., 2019; Liu and Gu, 2021). Among various regulatory modes, reversible posttranslational modification (PTM) is the most intricate and efficient pattern that dynamically modulates the flexible functional potential of p53 (Hafner et al., 2019; Liu et al., 2019). Analogous to the proposition of the ‘histone code’ hypothesis, PTM ‘writers’ (e.g. acetyltransferases) and ‘erasers’ (e.g. deacetylases) catalyze the addition/removal of chemical modifications to/from specific amino acid residues of p53 in a stress stimulus-dependent context (e.g. DNA damage, oncogenic stimuli, hypoxia, etc.). In addition to this ‘encoding’ process, a group of ‘reader’ proteins, which specifically recognize certain modifications, facilitate the ‘decoding’ and translation of an accurate cellular ‘readout’ by controlling the recruitment of the corresponding effectors. Relying on this highly organized modification system, a set of ‘PTM codes’, which correspond to methodical instructions to orchestrate the spatiotemporal regulatory functions of p53 via both individual modifications and combined modification (i.e. crosstalk) of multiple decorated residues, is generated. In this review, we outline the current knowledge on the PTM code of p53 and the readers that decode it, and we discuss the implications of targeting PTM–reader interplay as a potential clinical therapeutic strategy.

The PTM code and its functional readout for p53

Upon exposure to various cellular stresses, >36 distinct amino acid sites of p53 undergo biochemical modifications, including phosphorylation, acetylation, methylation, ubiquitination, and other modifications, which are dispersed widely throughout the transactivation domains (TADs), proline-rich domain, DNA-binding domain (DBD), tetramerization domain, and
C-terminal domain (CTD) (Figure 1). These equipped PTM codes recruit reader proteins containing specific modules to interpret the designated PTMs and coordinate intimately with p53 to control cellular outcomes (Figure 2).

Phosphorylation code

The results of extensive investigations imply that site-specific phosphorylation is strongly associated with the protein stability and protein–protein interactions of p53. Upon cellular stress signaling [including that initiated by DNA damaging agents, ultraviolet irradiation (UV), nutrient starvation, etc.], phosphorylation occurs at specific sites via a series of kinases, including ataxia telangiectasia-mutated serine/threonine kinase (ATM)/ATM and rad3-related serine/threonine kinase (ATR) and checkpoint kinase 1 (CHK1)/CHK2, either facilitating the release of p53 from degradation via murine double minute 2 (MDM2) [serine (S)6, S9, S15, threonine (T)18, S20, S37, and S106] or manipulating the transcriptional activity of p53 (S15, S33, S37, S46, T81, S215, and S392) (Shieh et al., 1997, 2000; Takekawa et al., 2000; Buschmann et al., 2001; Keller et al., 2001; D’Orazi et al., 2002; Saito et al., 2002; Li et al., 2006a; Teufel et al., 2009; Hsueh et al., 2013; Xu et al., 2016; Ishak Gabra et al., 2018). In contrast, phosphorylation at several other sites (S315, S362, and S366) assists ubiquitin ligase recruitment, which promotes p53 degradation (Katayama et al., 2004; Xia et al., 2009). In addition, p53 also undergoes dephosphorylation in response to genotoxic insults: phosphorylation of T55 of p53 is maintained by TATA box-binding protein-associated factor 1 (TAF1), which inactivates p53-mediated transcription, while upon DNA damage, T55 can be promptly dephosphorylated by protein phosphatase 2A (PP2A), which preserves p53 stability and induces cell cycle arrest (Li et al., 2007b; Wu et al., 2014). Similarly, S376 and S378 of p53 are constitutively phosphorylated in nonirradiated cells, whereas exposure to ionizing radiation (IR) leads to dephosphorylation at S376, which provides a docking site for 14-3-3 proteins and increases the binding affinity of p53 for its sequence-specific DNA elements (Waterman et al., 1998).

14-3-3 family proteins specifically recognize phosphorylated serine/threonine residues in a context-specific manner and act as scaffolds or adapters to provide docking sites for protein binding.
and complex formation (Tzivion et al., 2001). For example, 14-3-3 specifically reads phosphorylated S378 in the CTD of p53 upon stress, an event that increases the sequence-specific DNA-binding affinity of p53 by enhancing its oligomerization (Rajagopalan et al., 2008). In particular, the r and s isoforms of 14-3-3 proteins display weaker binding affinity for the phosphorylated sites in p53 than do the e and c isoforms. However, all four isoforms preferentially bind to diphosphorylated sites over monophosphorylated sites. Functionally, the r and s isoforms stabilize the p53 level, while the e and c isoforms increase the DNA binding affinity of p53 (Figure 3A; Rajagopalan et al., 2008). The WW domain, another phosphorylation-associated protein domain named after its two signature tryptophan (W) residues, is the smallest natural domain, containing /32-40 amino acids. The WW domain contains a stable, triple-stranded b-sheet motif, which recognizes phosphorylated serine/threonine–proline sites (Salah et al., 2012). Peptidylprolyl cis/trans isomerase (Pin1) recognizes phosphorylated S33, T81, and S315 of p53 through its WW domain and is involved in p53-mediated checkpoint control after challenge with UV radiation or DNA-damaging agents (Figure 3B; Zacchi et al., 2002; Zheng et al., 2002). WW domain containing oxidoreductase (WOX1) recognizes p53 phosphorylation at S46 via its WW domain and plays a synergistic cell death-inducing role with p53 under tumor necrosis factor stimulation, UV light exposure, or staurosporine stimulation. Furthermore, T33-phosphorylated WOX1 may act as a protein chaperone that stabilizes S46-phosphorylated p53 via a direct physical interaction (Figure 3C; Chang et al., 2005). Furthermore, the forkhead-associated (FHA) domain, which contains 100–120 residues that fold into an 11-stranded b-sandwich, is strictly specific for phosphothreonine residues (Almawi et al., 2017). Phosphorylation of p53 at T18 creates a binding site for the FHA domain of Pellino1 (an E3 ubiquitin ligase), which increases their recruitment to the DNA damage site and activation of p21 transcription (Figure 3D; Dai et al., 2019).

**Acetylation code**

p53 was the first transcription factor identified to undergo acetylation (Gu and Roeder, 1997). The discovery of C-terminal acetylation [lysine (K)370, K372, K373, K381, K382, and K386], which is dynamically balanced by p300/CREB-binding
protein (CBP) and several deacetylases, e.g. histone deacetylase 1 (HDAC1) and sirtuin 1 (SIRT1), revealed a novel p53 transactivation pathway that dramatically enhances the sequence-specific DNA-binding activity of p53 in response to DNA damage both in vivo and in vitro (Gu and Roeder, 1997; Luo et al., 2000; Vaziri et al., 2001; Luo et al., 2004). Within the DBD of p53, different acetyltransferases, i.e. lysine acetyltransferase 8 (hMOF)/lysine acetyltransferase 5 (TIP60) or lysine acetyltransferase 6A (MOZ), catalyzing K120 acetylation result in differing transcriptional selectivity of p53 between mediators of cell cycle arrest and apoptosis after genotoxic stress (Sykes et al., 2006; Tang et al., 2006; Rokudai et al., 2013). Acetylation of K164, another critical acetyl-modified site within the DBD of p53, is responsible for p53-dependent growth arrest (Tang et al., 2008). Simultaneous mutation of K117, K161, and K162 of mouse p53 (corresponding to K120 and K164 in humans), which destroys acetyl modifications at these sites, completely abolishes p53-mediated cell cycle arrest, senescence, and apoptosis after treatment with IR or genotoxic agents but maintains the ability of p53 to regulate energy metabolism and reactive oxygen species (ROS) production (Li et al., 2012). More recently, we also identified that acetylation of K120 and K164 is critically involved in p53-mediated programmed cell death protein-1 transcription, suggesting a potential link between p53 acetylation and immune modulation in cancer (Cao et al., 2021). In addition, loss of K101 acetylation of p53 impairs solute carrier family 7 member 11 (SLC7A11)-dependent ferroportosis ( Wang et al., 2016b). Outside the DBD, acetylation of K320, which is induced by p300/CBP-associated factor, negatively regulates proapoptotic activities of p53 by repressing the transcription of apoptosis-inducing genes after DNA damage insult or antitumor drug treatment (Chao et al., 2006; Knights et al., 2006). Collectively, modulation of transcriptional profiles shows a major role of site-specific p53 acetylation, which may attribute to conformational alterations in p53 or the recruitment of specific cofactors by acetylated p53.

Correspondingly, the bromodomain was the first identified acetylated lysine residue reader; this domain consists of a conserved left-handed four-helix bundle that forms a hydrophobic cavity that selectively recognizes acetylated lysine residues (Marmorstein and Zhou, 2014). In the human proteome, 61 bromodomain modules are encoded within 42 proteins (especially nuclear histone acetyltransferases), which are involved in a wide range of functions, including chromatin remodeling, transcriptional modulation, or scaffolding for the recruitment of other transcriptional regulators (Fujisawa and Filippakopoulos, 2017). The bromodomain within CBP, a conserved histone acetyltransferase and transcriptional coactivator, specifically reads acetylated K382 of p53, which is indispensable for the activation of p53-induced cell cycle arrest after UV exposure (Figure 3E; Mujtaba et al., 2004). As the largest subunit of the transcription initiation factor TATA-box binding protein (TFIID), TAF1 recognizes acetyl groups on two lysine residues (K373 and K382) of p53 through its bromodomains after UV damage. Disruption of the acetyl–p53–bromodomain interaction abolishes TAF1 recruitment to both the distal p53-binding site and the core promoter of p21 (Figure 3F; Li et al., 2007a). Polybromo 1 (PBRM1) also recognizes and reads acetylated K382 of p53 via its bromodomain 4 (BRD4) after induction of DNA damage; however, mutations in key residues in BRD4 of PBRM1 compromise the transcriptional ability of p53 (Figure 3G; Cai et al., 2019).

Our recent study revealed a novel class of acetylation-associated reader proteins: acidic domain (AD)-containing proteins that conversely recognize the unacetylated CTD of p53, e.g. SET nuclear proto-oncogene (SET), DDB1 and CUL4-associated factor 1 (VPRBP), death domain associated protein (DAXX), and proline, glutamate and leucine rich protein 1 (PELP1) (Wang et al., 2016a). Specifically, SET directly binds to the unacetylated CTD of p53 via its acidic domain and acts as a transcriptional repressor of p53 in unstimulated cells, while upon stress-induced acetylation of the p53 CTD, the SET–p53 interaction and SET-mediated p53 repression are completely abolished. Mechanistically, the positive charge of the p53 CTD attracts the negative charge of the acidic domain, which provides docking sites for acidic domain-containing regulators. Upon acetylation, the positive charge of the lysine side chains is neutralized, which subsequently disrupts the docking site for acidic domain-containing regulators (Figure 3H). Notably, this acetylation-dependent association/dissociation mode occurs ubiquitously in acidic domain-containing cofactors of p53, which contributes to the fine-tuned regulation of p53 under different stress conditions. Intriguingly, the same recognition of unacetylated p53 by the acidic domain reader has been further demonstrated during the virus infection process. Latency-associated nuclear antigen, which is encoded by Kaposi’s sarcoma (KS)-associated herpesvirus (KSHV) and contains two tandem acidic domains, selectively interacts with unacetylated p53 and facilitates KSHV latency establishment (Juillard et al., 2020).

**Methylation code**

Combinatorial methylation of p53 is believed to create modification marks that facilitate downstream effector recognition and binding. Two nearby lysine sites in the C-terminus of p53 function exclusively upon methylation: SET domain containing 7 (SET7/9)-mediated K372 methylation positively affects p53 stability, whereas SET and MYND domain containing 2 (SMYD2)-induced monomethylation of K370 represses the transcriptional activation of p53 (Chuikov et al., 2004; Huang et al., 2006). K370 can also be dimethylated by an unknown methyltransferase, and this event is reversed by the demethylase lysine demethylase 1A (LSD1). Dimethylation of K370 (K370me2) differentially manipulates p53 activity by promoting its association with the coactivator p53-binding protein 1 (53BP1) (Huang et al., 2007). An additional study reported that p53 dimethylation at K373 by euchromatic histone lysine methyltransferase 2 (G9A) and euchromatic histone...
lysine methyltransferase 1 (GLP) negatively regulates p53 activity (Huang et al., 2010). At the distal end of the CTD, lysine methyltransferase 5A (SET8/PR)-SET7 monomethylates p53 at K382, which robustly suppresses the transactivation of p53 (Shi et al., 2007). In addition to the lysine sites, three arginine (R) residues in p53—R333, R335, and R337—are also subject to methylation by protein arginine methyltransferase 5 (PRMT5) during the DNA damage response, which affects the gene-specific transcription of p53 (Jansson et al., 2008). The ‘Royal Family’ reader domains that specifically recognize lysine methylation marks are chromodomains, Tudor domains, Pro-Trp-Trp-Pro motif (PWWP) domains, and malignant brain tumor (MBT) repeat domains, which share homologous core regions originating from a common ancestor (Maurer-Stroh et al., 2003). In the absence of DNA damage, monomethylated K382 of p53 is read by the MBT repeat domain of the chromatin compaction factor L3MBTL histone methyl-lysine binding protein 1 (L3MBTL1). Functionally, L3MBTL1 interacts with

Figure 3 Functional regulation of p53 by PTM–reader recognition. Upon activation, various PTMs recruit corresponding reader domain-containing proteins that facilitate the promotion of p53-mediated cellular responses. (A) Phosphorylation of p53 recruits 14-3-3, which facilitates p53 stabilization and transactivation. (B) Three phosphorylation sites in p53 provide a binding site for the WW domain of Pin1, which facilitates p53 stabilization and transactivation. (C) WOX1 recognizes phosphorylated p53 via the WW domain to promote transcriptional activation of p53 and DNA repair. (E) One or more bromodomains of CPB bind to acetylated p53 to activate p53-mediated transcription. (F) Two acetylated sites in p53 create a binding site for the bromodomain of TAF1 to activate p53-mediated transcription. (G) One or more bromodomains in PBRM1 recognize acetylated p53 to activate p53-mediated transcription. (H) The acid domain of SET specifically recognizes the unacetylated CTD of p53 to repress its transactivation. Upon stress-induced acetylation of CTD, the interaction of SET and p53 is abolished, which induces transactivational activity of p53. (I) L3MBTL1 reads methylated p53 via its MBT repeat domain and functions as a repressor of p53. (J) 53BP1 recognizes methylated p53 via its Tudor domain to facilitate DNA repair. (K) Two methylation sites in p53 recruit the Tudor domain of PHF20 to facilitate p53 stabilization and transcriptional activation. (M) The UIM of S5a recognizes ubiquitinated p53 to accelerate its degradation. (N) The ubiquitin of hHR23 binds to ubiquitinated p53 to accelerate its degradation. (O) USP7 modulates the ubiquitination of p53 to maintain its stabilisation. USP7 also regulates the ubiquitination of MDM2, which indirectly affects p53 stabilization. (P) USP10 modulates the ubiquitination of p53 to impact its stabilisation and cellular location. A, acetylation; M, methylation; P, phosphorylation; U, ubiquitination; Un, unmodified.
monomethylated K382 and functions as a negative regulator of p53 by promoting the compacted chromatin state to repress target gene expression; in contrast, upon DNA damage, the monoubiquitination level of K382 is decreased, which abolishes the p53–L3MBTL1 interaction and dissociates L3MBTL1 from the promoters of p53 target genes (Figure 3I; West et al., 2010). Upon DNA damage, the DNA repair factor 53BP1 recognizes dimethylated K382 of p53 via its tandem Tudor domain, which facilitates the accumulation of p53 at DNA damage sites to repair the process (Figure 3I). Structurally, dimethylated K382 of p53 can insert into the aromatic-lined pocket of the tandem Tudor domain of 53BP1 (Kachiriskaia et al., 2008; Roy et al., 2010). At nearby sites, the Tudor domain in PHD finger protein 20 (PHF20) recognizes dimethylated K370 or K382 in p53, which promotes the stabilization and activation of p53 by disrupting its MDM2-mediated degradation (Figure 3K).

Intriguingly, the homodimeric form of the Tudor domain can bind to two dimethylated sites of p53 simultaneously with increased binding affinity (Cui et al., 2012). Additionally, a group of proteins containing a module called the plant homeodomain (PHD) finger, which constitutes zinc finger-like domain with a Cys4–His–Cys3 motif, selectively reads dimethylation and trimethylation marks (Patel, 2016). The PHD finger domain exists in diverse eukaryotic proteins involved in transcriptional regulation and chromatin dynamics. The direct ‘reading’ characteristics of the PHD finger domain on methylation marks in p53 have not yet been elucidated.

**Ubiquitination and ubiquitination-like codes**

Ubiquitination tightly modulates p53 protein stability. As the primary negative regulator of p53, MDM2 ubiquitinates p53 at K370, K372, K373, K381, K382, and K386 within its CTD, which drives its proteasomal degradation (Rodriguez et al., 2000). Remarkably, MDM2 can catalyze both mono- and polyubiquitination of p53 depending on its level: a low level of MDM2 induces monoubiquitination, which triggers cytoplasmic translocation of p53, whereas a high level of MDM2 leads to degradation of polyubiquitinated p53 (Li et al., 2003). In addition, a plethora of E3 ligases, such as ring finger and C HvA zinc finger domain containing 1 (PHR2), COP1 E3 ubiquitin ligase (COP1), STIP1 homology and U-box containing protein 1 (CHIP), and tripartite motif (TRIM) proteins, and deubiquitinases (DUBs), such as ubiquitin-specific protease 7 (USP7/HAUSP) and ubiquitin-specific peptidase 10 (USP10), act cooperatively to maintain the appropriate abundance of p53 in a ubiquitin-dependent manner (Kwon et al., 2017; Bang et al., 2020).

Ascribed to the flexible assembly of ubiquitin moieties, the three-dimensional polyubiquitin code recruits >200 readers containing ubiquitin-binding domains from 20 families, which drive ubiquitin-dependent recognition by the proteasome (Dikic et al., 2009). The intrinsic 55a subunit of the 26S proteasome has been proven to recognize ubiquitinated p53 with two ubiquitin-interacting motifs (UIMs) for proteasomal degradation (Figure 3I; Sparks et al., 2014). Nucleotide excision repair protein (hHR23), with a C-terminal ubiquitin-associated domain (Uba) that binds to ubiquitin chains, directly recognizes ubiquitin moieties on p53 and delivers p53 to the 26S proteasome for degradation (Figure 3M; Glockzin et al., 2003). Moreover, many DUBs, belonging to the family of Cys proteases that specifically recognize ubiquitin on lysine residues, have been demonstrated to be related to p53 modulation in human cancer. These DUBs, including ubiquitin-specific peptidase 2 (USP2), USP7, USP10, and OTU deubiquitinase (OTUB1), extensively engage in regulating the stability or cellular localization of p53 (Figure 3N and O; Deng et al., 2020).

Furthermore, several ubiquitination-like PTMs of p53 have been identified. Conjugation of the most homologous ubiquitin-like protein, neural precursor cell expressed developmentally downregulated protein 8 (NDE08), termed as NEDDylation, has been found at K320, K321, K370, K372, and K373, which suppresses the transcriptional activity of p53 (Xirodimas et al., 2004; Abida et al., 2007). Upon DNA damage and oxidative stress, the small ubiquitin-like modifier (SUMO) protein is conjugated to p53 (SUMOylation) at K386, which impacts both p53-dependent transcription and nuclear export of p53 (Kwek et al., 2001; Li et al., 2006b; Wu and Chiang, 2009; Santiago et al., 2013). Meantime, the SUMO protease SUMO-specific peptidase 1 (SENP1) has been found to directly deSUMOylate p53 in cells (Chauhan et al., 2021). In addition, p53 has been proven to be a substrate for conjugation of UFM (UFMylation), a recently identified ubiquitin-like modification (Liu et al., 2020). UFMylation occurs at K351, K357, K370, or K373 of p53 and maintains the stability of p53 by interfering with its ubiquitination-mediated proteasomal degradation. Since these ubiquitin and ubiquitin-like modifications compete for the same lysine sites in p53, their mutual interplay determines the functional outcome of p53. Coordinated NEDDylation and ubiquitination control the subcellular localization of p53 (Liu and Xirodimas, 2010). Moreover, monoubiquitination promotes SUMOylation of p53, which further promotes its nuclear export (Carter et al., 2007).

Notably, in addition to DNA damage, different stress signaling pathways usually activate and stabilize p53 by interfering with its ubiquitination modification. aberrant oncogenic stimuli, such as RAS or c-MYC, activate the tumor suppressor cyclin-dependent kinase inhibitor 2A (ARF), which sequesters MDM2 to block the ubiquitination of p53 (Zhang and Xiong, 2001; Sherr, 2006). Impaired ribosome biogenesis (nucleolar stress) results in the interaction of ribosomal proteins (RPs) and nucleolar factors with the central domain of MDM2, thereby releasing p53 from ubiquitination-mediated degradation to block cell cycle progression and prevent incomplete cell division (Zhang and Lu, 2009; Turi et al., 2019). Equally importantly, p53 is involved in metabolic homeostasis upon nutrient and oxygen stress (Humpton and Vousden, 2016). Nutrient starvation inhibits AKT signaling, which elicits MDM2 suppression and subsequent p53 activation. ROS stress activates the ROS sensor thioredoxin-interacting protein (TXNIP) in hematopoietic stem cells, which
abolishes the MDM2–p53 interaction to initiate p53-mediated antioxidant programs (Jung et al., 2013). Under hypoxic conditions, hypoxia-inducible factor 1 alpha stabilizes and activates p53 by directly binding to MDM2, thus suppressing the ubiquitination of p53 and protecting it from degradation (Chen et al., 2003). Additionally, severe hypoxia induces p53 phosphorylation on S15, which results in cell growth arrest and apoptosis (Hammond et al., 2002; Lee et al., 2007). Broader investigations are required to characterize the spectrum of stress specifically induced p53 modifications, which help to potentiate the stress response mechanism of p53.

**Other modification codes**

Moreover, novel modifications of p53 have been successively verified. Crotonylation occurs at S46 of p53 in response to crotonic acid, which induces glycolytic activity and promotes cancer cell growth (Liao et al., 2020). Upon β-hydroxybutyrate (BHB) treatment, p300/CBP-mediated β-hydroxybutyrylation (Kbb) occurs at K120, K319, and K370 of p53, which impairs the transcriptional activity of p53 due to the attenuated acetylation level (Liu et al., 2019a). Hydroxylation of p53 occurs on two distinct residues: Jumonji domain-containing 6 (JMJD6)-catalyzed hydroxylation at K382 negatively affects p53 transcriptional activity, whereas PHD3-mediated hydroxylation at proline (P)359 enhances p53 protein stability (Wang et al., 2014; Rodriguez et al., 2018).

**Combinatorial readout of cooperative PTMs**

In addition to modifications at individual sites, the alternative coordination and combination of different PTMs constitutes an advanced regulatory mechanism of p53. Phosphorylation in the TAD of p53 often initiates a response to cellular stimuli and converts signaling to acetyl modifications in the CTD, which collectively control the transcriptional activation of p53 (Lee et al., 2010). Similarly, site-specific methylation is a prerequisite for the subsequent acetylation that leads to p53 stabilization and transcriptional activation (Kurash et al., 2008). Notably, PTMs are mutually exclusive at the same residue, thus setting the stage for crosstalk among various PTMs. At the competitive overlapping lysine sites in the CTD, acetylation and ubiquitination antagonize each other to control the transactivation and stability of p53 (Li et al., 2002).

Similarly, few of the reader domains function independently, yet they frequently occur in multiple copies or in tandem with other reader domains within a single protein. Studies have proven the multivalent readout by the tandem Tudor–PHD finger domain combination (Marmorstein and Zhou, 2014; Patel, 2016). PHF20, which is involved in acetyl group transfer and transcriptional regulation, recognizes dimethylated K4 of histone H3 (H3K4me2) through its PHD finger domain as a native reader. In addition, PHF20 selectively binds to dimethylated p53 through another methyllysine reader domain, Tudor2 (Klein et al., 2016). This finding implies that PHF20 may link the histone acetylation, transcriptional activation, and activity of p53 through the combination of its PHD and Tudor2 domains. However, readers of p53 PTMs and their functional interplay are not completely determined. In particular, extensive studies are needed to focus on deciphering the combinatorial readout of multiple domain-containing readers and highlighting the functional crosstalk among different PTMs in the readout of p53.

**Targeting reader proteins as a therapeutic strategy**

Recently, epigenetic therapy has become a promising therapeutic strategy for cancers (Bates, 2020). Although writers and erasers have been proven to be potential targets for drug development, a new generation of anticancer agents targeting the reader domains has established a novel therapeutic approach with increased selectivity and context-specificity (Cipriano et al., 2020).

Several highly potent small molecules targeting bromodomain and extracellular (BET) family reader proteins with superior specificity for a subset of targets are currently in clinical trials. The BET bromodomain-specific inhibitor JQ1 has been demonstrated to bind competitively to acetyllysine binding sites with high potency and specificity (Filippakopoulos et al., 2010). Competitive binding by JQ1 displaces BRD4 from chromatin, which has been shown to induce differentiation and antiproliferative effects in BRD4-dependent cell lines and xenograft models. In acute myeloid leukemia and neuroblastoma, JQ1 treatment induces cell death via the p53-mediated apoptotic pathway, suggesting that bromodomain inhibition might enhance the current efficacy of chemotherapy (Stewart et al., 2013; Mazar et al., 2020). The CBP bromodomain is another reader target that has attracted attention. Studies have reported small-molecule inhibitors and cyclopeptides that can selectively target the bromodomain of CBP, which negatively affects the ability of CBP to bind to acetylated K382 of p53 and consequently blocks p53-mediated transcription and promotes p53 degradation (Sachchidanand et al., 2006; Gerona-Navarro et al., 2011).

In addition, numerous DUB inhibitors that target MDM2 to activate p53 exhibit unique value as antitumor drugs. Several such compounds (FT671, FT827, and XL188) have been shown to target USP7 with high affinity, leading to reactivation of p53 and subsequent induction of the tumor suppressor p21, which has been shown to inhibit tumor growth in mice (Turnbull et al., 2017; Deng et al., 2020). High-throughput screening also identified the small-molecule compounds HBX 19818 and HBX 28258, which specifically inhibit USP7 by binding to its catalytic cysteine. Mechanistically, these compounds destabilize MDM2 to induce p53-dependent proliferation inhibition and apoptosis in cancer cells (Reverdy et al., 2012).

Owing to the multifaceted modulatory effects of PTMs on p53 behaviors, targeting PTMs to restore the appropriate tumor-suppressive effect of wild-type p53 has become a prospective strategy for cancer treatment (Figure 4). Notably, the
Figure 4 (Re)activation of p53 by targeting specific PTM–reader interplay events is a promising cancer therapeutic strategy. Several small-molecule inhibitors have been verified to block the recognition of specific PTMs in p53 by their corresponding readers (summarized in the syringe icons at the top). These inhibitors usually function by impacting the stability of p53 and transcription of its targets, which further impedes cancer cell growth (summarized in the ring at the bottom). A, acetylation; BRD, bromodomain; U, ubiquitination; USP7, ubiquitin-specific protease 7.
application of pharmaceutical inhibitors targeting readers may predominate over those targeting writers or erasers, for the following reasons. (i) Most available small molecules that target PTMs are pan-inhibitors that act nonspecifically on a plethora of proteins with identical modifications, including chromatin histones, thus probably leading to unexpected side effects. Comparatively, targeting specific reader interactions relatively limits such effects. (ii) To add more complexity, p53 undergoes multiple layers of PTMs upon activation. For example, the same lysine residue may accept diverse types (acetylation, methylation, and ubiquitination) and even degrees (monomethylation, dimethylation, and trimethylation) of modification upon activation. Hence, fine-tuned regulation of p53 PTMs requires strict intervention, which could be achieved through inhibition/activation of specific readers.

Perspectives

Increasing progress has advanced the knowledge of p53 PTMs; however, the biological significance of these regulatory modifications remains enigmatic. p53 is one of the most commonly mutated genes in human cancers, and clinical information of patients with cancer has revealed that genetic alterations in p53 are prevalent in the DBD (amino acid residues 100–300); these alterations result in either disruption of the DNA binding ability or alterations in the conformational structure, accompanied by impaired transcriptional activation of p53 (Bouaoun et al., 2016). However, cancer-derived mutations are rarely located at the sites of PTMs in p53, and in vivo substitutions of single codon or a few codons where PTMs occur generally induce subtle functional attenuation of p53 (Bruins et al., 2004; Krummel et al., 2005). The PTM network thus seems to constitute a critical but redundant regulatory mechanism of p53. However, the elaborate crosstalk and coordination among multiple modifications (especially sites in the CTD harboring diverse types of modifications) greatly augment the regulatory role of p53 in the hub of the stress response. First, considering individual modifications or a small portion of modifications as the on–off switch of p53 is an oversimplification. Due to the pivotal role of p53 as the genome guardian, multiple layers of regulatory mechanisms, including overlapping PTM-mediated modulation of certain functional controls (e.g. cell cycle arrest vs. apoptosis), are prerequisite to maintain it in a steady state. However, simultaneous multisite mutations are rare in tumors; thus, we infrequently find mutations in the regions of p53 subject to PTMs in human cancer databases. Second, different modifications that occur at the same residues (e.g. acetylation vs. ubiquitination) may result in opposite effects on p53, probably as a means to achieve the optimal and most balanced outcome in response to intricate stimuli. Therefore, entire mutation or complete deletion of these sites may counteract or neutralize the mutual effects in response to stress, leading to moderate phenotypes in vivo. Finally, before we can determine the value of these modifications, the overall landscape of PTMs in p53 must be explored. Extensive studies on the newly identified chemically modified groups are necessary. Hence, further investigations are needed to delineate the context-specific permutation and combination of PTMs, along with their translating readers, in order to acquire a comprehensive understanding of the complex properties of p53 and to shed light on potential anticancer strategies.

Along with the expanding interest and breakthroughs in PTM research, the scope of basic and clinical studies has deepened exceed our understanding of the transcriptome and proteome. Notably, 21% of disease-associated amino acid substitutions are located in the PTM sites in proteins (Krassowski et al., 2018). Although a substantial number of PTM sites in p53 have been identified, their concrete functional annotations are just beginning to emerge. With improvements in state-of-the-art high-throughput mass spectrometry (MS) technology, optimized human sample preparation, and bioinformatic analysis approaches, it has become feasible to map the diseasespecific alterations in p53 PTMs, which may also further facilitate depiction of the disease-related interactome of p53. Hence, systematic qualitative and quantitative investigations of the disease-derived ‘PTMomic code’ landscape of p53 and other proteins may become trends in pathophysiological research, and this approach will greatly benefit clinical biomarker detection and pharmacological target augmentation.

From a technological perspective, prior to MS analysis, appropriate methods that capture and enrich the modified protein are vital for identifying and quantifying PTMs. Currently applied enrichment strategies rely largely on immunoprecipitation with modification-specific antibodies, but these methods have several limitations, including (i) the high cost of antibody generation, (ii) the variability of antibodies during production, and (iii) the compromise of binding affinity by neighboring modifications. However, native reader domain sequences could be alternatively complemented as a powerful tool to identify the specific PTMs of a given target (e.g. p53) or a wide array of modified proteins during PTMomic analysis. This method has distinct advantages in (i) its high selectivity and affinity, (ii) the easy and reproducible recombining engineering techniques involved, and (iii) the independence of neighboring modifications (Moore et al., 2013; Kungulovski et al., 2014). Furthermore, these dedicated interactions between the PTMs and the reader domains widen the avenues of innovation for PTMomic studies. For instance, recombinational fusion of double (or multiple) domain sequences with dual (or more extensive) specificity can potentially be used to investigate the coexistence and dependency of combined modifications within the same protein; this strategy is conducive to resolving multiple readouts and biological functions beyond those resulting from individual modification.

Overall, as a dynamic ‘switch’, the PTM cryptosystem of p53 remains obscure but a fascinating topic for exploration. A comprehensive manual covering the PTM codes and PTM-associated partners of p53 could be deemed a worthy prognostic guide and therapeutic target reference in cancer and other diseases (Figure 5).
Figure 5 Dynamic regulation of p53 readouts by the PTM codes and their readers. In response to cellular stimuli (pulling the lever), alternative arrangements and combinations of PTMs (colored circles) plus the corresponding PTM readers (colored triangles) generate distinct regulatory functions of p53 (coins cascaded at the bottom). Ac, acetylation; BRD, bromodomain; Me, methylation; Pho, phosphorylation; Tudor, Tudor domain.

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References
Abida, W.M., Nikolaev, A., Zhao, W., et al. (2007). FBXO11 promotes the Neddylation of p53 and inhibits its transcriptional activity. J. Biol. Chem. 282, 1797–1804.
Almawi, A.W., Matthews, L.A., and Guarne, A. (2017). FHA domains: phosphopeptide binding and beyond. Prog. Biophys. Mol. Biol. 127, 105–110.
Bang, S., Kaur, S., and Kurokawa, M. (2020). Regulation of the p53 family proteins by the ubiquitin proteasomal pathway. Int. J. Mol. Sci. 21, 261.
Bates, S.E. (2020). Epigenetic therapies for cancer. N. Engl. J. Med. 383, 650–663.
Bouaoun, L., Sonkin, D., Ardin, M., et al. (2016). TP53 variations in human cancers: new lessons from the IARC TP53 database and genomics data. Hum. Mutat. 37, 865–876.
Bruins, W., Zwart, E., Attardi, L.D., et al. (2004). Increased sensitivity to UV radiation in mice with a p53 point mutation at Ser389. Mol. Cell. Biol. 24, 8884–8894.
Bushmann, T., Potapova, O., Bar-Shira, A., et al. (2001). Jun NH2-terminal kinase phosphorylation of p53 on Thr-81 is important for p53 stabilization and transcriptional activities in response to stress. Mol. Cell. Biol. 21, 2743–2754.
Jansson, M., Durant, S.T., Cho, E.C., et al. (2008). Arginine methylation regulates the p53 response. Nat. Cell Biol. 10, 1431–1439.
Juillard, F., de Miranda, M.P., Li, S., et al. (2020). KSHV LANA acetylation-selective acidic domain reader sequence mediates virus persistence. Proc. Natl Acad. Sci. USA 117, 22443–22451.
Jung, H., Kim, M.J., Kim, D.O., et al. (2013). TXNIP maintains the hemotopoietic cell pool by switching the function of p53 under oxidative stress. Cell Metab. 18, 75–85.
Kachirskaia, I., Shi, X., Yamaguchi, H., et al. (2008). Role for 53BP1 Tudor domain recognition of p53 dimethylation at lysine 382 in DNA damage signalling. J. Biol. Chem. 283, 34660–34666.
Kastenhuber, E.R., and Lowe, S.W. (2004). Fine-tune P300 activity. Biochem. Biophys. Res. Commun. 324, 730–735.
Luo, G., and Xirodimas, D.P. (2010). NUB1 promotes cytoplasmic localization of p53 through cooperation of the NEDD8 and ubiquitin pathways. Oncogene 29, 2252–2261.
Liu, J., Guan, D., Dong, M., et al. (2020). UFMylation maintains tumour suppressor p53 stability by antagonizing its ubiquitination. Nat. Cell Biol. 22, 1056–1063.
Li, K., Li, F., Sun, Q., et al. (2019a). p53 β-hydroxybutyrylation attenuates p53 activity. Cell Death Dis. 10, 263.
Liu, Y., and Gu, W. (2021). The complexity of p53-mediated metabolic regulation in tumor suppression. Semin. Cancer Biol. doi: 10.1016/j.semcancer.2021.03.010.
Luo, J., Li, M., Tang, Y., et al. (2004). Acetylation of p53 augments its site-specific DNA binding both in vitro and in vivo. Proc. Natl Acad. Sci. USA 101, 2259–2264.
Maurer-Stroh, S., Dickens, N.J., Hughes-Davies, L., et al. (2003). The Tudor domain ‘Royal Family’: Tudor, plant Agenet, Chromo, PWWP and MBT domains. Trends Biochem. Sci. 28, 69–74.
Mujtaba, S., He, Y., Zeng, L., et al. (2006). Distinct p53–UVMylation sites of proteins. Nucleic Acids Res. 34, 392–398.
Murrin, J.K., Liu, G., and Xirodimas, D.P. (2010). Discovery of specific inhibitors of the coactivator CBP in p53 transcriptional activation. Mol. Cell 33, 251–263.
Patel, D.J. (2016). A structural perspective on readout of epigenetic histone and DNA methylation marks. Cold Spring Harb. Perspect. Biol. 6, a018762.
Rajagopalan, S., Jaulent, A.M., Wells, M., et al. (2008). 14-3-3 activation of DNA binding of p53 by enhancing its association into tetramers. Nucleic Acids Res. 36, 5983–5991.
Rajagopalan, S., Sade, R.S., Townsley, F.M., et al. (2010). Mechanistic differences in the transcriptional activation of p53 by 14-3-3 isoforms. Nucleic Acids Res. 38, 893–906.
Reverdy, C., Conrath, S., Lopez, R., et al. (2012). Discovery of specific inhibitors of human USP7/HAUSP deubiquitinating enzyme. Chem. Biol. 19, 467–477.
Rodriguez, J., Herrero, A., Li, S., et al. (2018). P HD3 regulates p53 protein stability by hydroxylating proline 359. Cell Rep. 24, 1316–1329.
Rodriguez, M.S., Desterro, J.M., Lain, S., et al. (2000). Multiple C-terminal lysine residues target p53 for ubiquitin–proteasome-mediated degradation. Mol. Cell Biol. 20, 8458–8467.
Rokudai, S., Laptenko, O., Amal, S.M., et al. (2013). MOZ increases p53 acetylation and premature senescence through its complex formation with PML. Proc. Natl Acad. Sci. USA 110, 3893–3900.
Roy, S., Musselman, C.A., Kachirskaia, I., et al. (2010). Structural insight into p53 recognition by the 53BP1 tandem Tudor domain. J. Mol. Biol. 398, 489–496.
Sachchidanand, Resnick-Silverman, L., Yan, S., et al. (2006). Target structure-based discovery of small molecules that block human p53 and CREB binding protein association. Chem. Biol. 13, 81–90.
Tang, Y., Goodarzi, A.A., Hashimoto, Y., et al. (2002). ATM mediates phosphorylation at multiple p53 sites, including Ser46, in response to ionizing radiation. J. Biol. Chem. 277, 12491–12494.

Salah, Z., Afar, A., and Aqil, R.I. (2012). WW domain-containing proteins: retrospectives and the future. Front. Biosci. 17, 331–348.

Santiago, A., Li, D., Zhao, L.Y., et al. (2013). p53 SUMOylation promotes its nuclear export by facilitating its release from the nuclear export receptor CRM1. Mol. Biol. Cell 24, 2739–2752.

Sherr, C.J. (2006). Dividing ARF and p53: an unsettled case. Nat. Rev. Cancer 6, 663–673.

Shi, X., Kachirskaya, I., Yamaguchi, H., et al. (2007). Modulation of p53 function by SET8-mediated methylation at lysine 382. Mol. Cell 27, 636–646.

Shieh, S.Y., Ahn, J., Tamai, K., et al. (2000). The human homologs of checkpoint kinases Chk1 and Cds1 (Chk2) phosphorylate p53 at multiple DNA damage-inducible sites. Genes Dev. 14, 289–300.

Shieh, S.Y., Ikeda, M., Taya, Y., et al. (1997). DNA damage-induced phosphorylation of p53 alleviates inhibition by MDM2. Cell 91, 325–334.

Sparks, A., Dayal, S., Das, J., et al. (2014). The degradation of p53 and its major E3 ligase Mdm2 is differentially dependent on the proteasomal ubiquitin receptor S5a. Oncogene 33, 4685–4696.

Stewart, H.J., Home, G.A., Bastow, S., et al. (2013). BRD4 associates with p53 in DNMT3A-mutated leukemia cells and is implicated in apoptosis by the bromodomain inhibitor JQ1. Cancer Med. 2, 826–835.

Sykes, S.M., Mellert, H.S., Holbert, M.A., et al. (2006). Acetylation of the p53 DNA-binding domain regulates apoptosis induction. Mol. Cell 24, 841–851.

Takekawa, M., Adachi, M., Nakahata, A., et al. (2000). p53-inducible wip1 phosphatase mediates a negative feedback regulation of p38 MAPK–p53 signaling in response to UV radiation. EMBO J. 19, 6517–6526.

Tang, Y., Luo, J., Zhang, W., et al. (2006). Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. Mol. Cell 24, 827–839.

Tang, Y., Zhao, W., Chen, Y., et al. (2008). Acetylation is indispensable for p53 activation. Cell 133, 612–626.

Teufel, D.P., Bycroft, M., and Fersht, A.R. (2009). Regulation by phosphorylation of the relative affinities of the N-terminal transactivation domains of p53 for p300 domains and Mdm2. Oncogene 28, 2112–2118.

Turi, Z., Lacey, M., Mistrik, M., et al. (2019). Impaired ribosome biogenesis: mechanisms and relevance to cancer and aging. Aging 11, 2512–2540.

Turnbull, A.P., Ioannidis, S., Krajewski, W.W., et al. (2017). Molecular basis of USP7 inhibition by selective small-molecule inhibitors. Nature 550, 481–486.

Tzivion, G., Shen, Y.H., and Zhu, J. (2001). 14-3-3 proteins; bringing new definitions to scaffolding. Oncogene 20, 6331–6338.

Vaziri, H., Dessain, S.K., Ng Eaton, E., et al. (2001). HAT inhibitors function as an NAD-dependent p53 deacetylase. Cell 107, 149–159.

Wang, D., Kon, N., Lasso, G., et al. (2016a). Acetylation-regulated interaction between p53 and SET reveals a widespread regulatory mode. Nature 538, 118–122.

Wang, F., He, L., Kuangyang, P., et al. (2014). JMJD6 promotes colon carcinogenesis through negative regulation of p53 by hydroxylation. PLoS Biol. 12, e1001819.

Wang, S.J., Li, D., Ou, Y., et al. (2016b). Acetylation is crucial for p53-mediated ferroptosis and tumor suppression. Cell Rep. 17, 366–373.

Waterman, M.J., Stavridi, E.S., Waterman, J.L., et al. (1998). ATM-dependent activation of p53 involves dephosphorylation and association with 14-3-3 proteins. Nat. Genet. 19, 175–178.

West, L.E., Roy, S., Lachmi-Weiner, K., et al. (2010). The MBT repeats of L3MBTL1 link SET8-mediated p53 methylation at lysine 382 to target gene repression. J. Biol. Chem. 285, 37725–37732.

Wu, S.Y., and Chiang, C.M. (2009). Crosstalk between sumoylation and acetylation regulates p53-dependent chromatin transcription and DNA binding. EMBO J. 28, 1246–1259.

Wu, Y., Lin, J.C., Plisso, L.G., et al. (2014). Phosphorylation of p53 by TAF1 inactivates p53-dependent transcription in the DNA damage response. Mol. Cell 53, 63–74.

Xia, Y., Padre, R.C., De Mendoza, T.H., et al. (2009). Phosphorylation of p53 by IkB kinase 2 promotes its degradation by β-TrCP. Proc. Natl Acad. Sci. USA 106, 2629–2634.

Xirodimas, D.P., Saville, M.K., Bourdon, J.C., et al. (2004). Mdm2-mediated NEDD8 conjugation of p53 inhibits its transcriptional activity. Cell 118, 83–97.

Xu, H.T., Lai, W.L., Liu, H.F., et al. (2016). PAK4 phosphorylates p53 at serine 215 to promote liver cancer metastasis. Cancer Res. 76, 5732–5742.

Zacchi, P., Gostissa, M., Uchida, T., et al. (2001). Impaired ribosome biogenesis: mechanisms and relevance to cancer and aging. Aging 11, 2512–2540.

Zheng, H., You, H., Zhou, X.Z., et al. (2002). The prolyl isomerase Pin1 is a regulator of p53 in genotoxic response. Nature 419, 849–853.