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Review

Surf the Post-translational Modification Network of p53 Regulation

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Abstract

Among the human genome, *p53* is one of the first tumor suppressor genes to be discovered. It has a wide range of functions covering cell cycle control, apoptosis, genome integrity maintenance, metabolism, fertility, cellular reprogramming and autophagy. Although different possible underlying mechanisms for p53 regulation have been proposed for decades, none of them is conclusive. While much literature focuses on the importance of individual post-translational modifications, further explorations indicate a new layer of p53 coordination through the interplay of the modifications, which builds up a complex 'network'. This review focuses on the necessity, characteristics and mechanisms of the crosstalk among post-translational modifications and its effects on the precise and selective behavior of p53.

Key words: p53; post-translational modification; crosstalk; protein-protein interaction; semiotic system.

Introduction

Since the discovery of p53 in 1979 [1-3], numerous studies have been conducted related to its functions and regulatory mechanisms. Previous research has confirmed that p53 is able to coordinate a regulatory network that supervises and responds to a variety of stress signals. These signals include: DNA damage, aberrant oncogenic activation, telomere erosion, ribosomal stress, loss of cell-cell or cell-matrix adhesion, and hypoxia [4]. Regulating a vast pool of external stimuli, p53 exerts irreplaceable anti-neoplastic functions at homeostasis and thus is considered to be 'the guardian of the genome' [5]. Mutations of *p53* or disruptions of p53 coordination, to a lesser extent, can disturb the normal physiological balance, and lead to cancer if genome disarrangement reaches a critical value.

Basic elements of the p53 coordination are its cellular localization, oligomerization [6] and concentration, which are tightly and exquisitely interrelated. Originally, p53 was thought to perform its functions in its tetrameric form in the nucleus by acting as a transcription factor or as a binding partner [7-10]. At homeostasis, the transcriptional activity of p53 is downregulated in three ways: 1. Ubiquitin-mediated proteasomal degradation of p53 in both cytoplasm and nucleus mainly through mouse double minute protein 2 (Mdm2) [11]; 2. Decrease in nuclear p53 levels through nuclear export by either the exposure of its nuclear export sequence (NES) [12, 13] or the NES of Mdm2 [14]; and 3. Transcriptional repression of chromatin-associated p53 by Mdm2-Mdmx-p53 complex formation [15-17].

Under stress, degradation and nuclear export of p53 are suppressed, and nuclear import of p53 is concomitantly enhanced, resulting in its nuclear accumulation. Recently proposed is another process involved in the activation of chromatin-bound p53 termed as 'anti-repression' [18]. Transcriptional levels

of p53 downstream target genes can be generally increased by p53 nuclear accumulation and the release of chromatin-bound p53 from repression state. On the other hand, selective functions of p53 can be fulfilled through enhancement of p53's transactivation of specific target genes [19]. Although p53 primarily acts as a transcription factor, a transcription-independent role of cytosolic p53 to trigger apoptosis and inhibit autophagy has also been discovered [20-22]. Researchers during the past decades have discovered that, in either the homeostatic maintenance or stress-induced activation of p53, covalent modifications play pivotal roles (summarized in Figure 1).

Although relatively unified findings related to the functions of post-translational modifications (PTMs) were obtained *in vitro*, the *in vivo* data are somehow contradictory, indicating a variable behavior of p53. This variability is characterized by cell type- and tissue-dependent [23, 24], genotype and stimuli-specific responses [25-27].

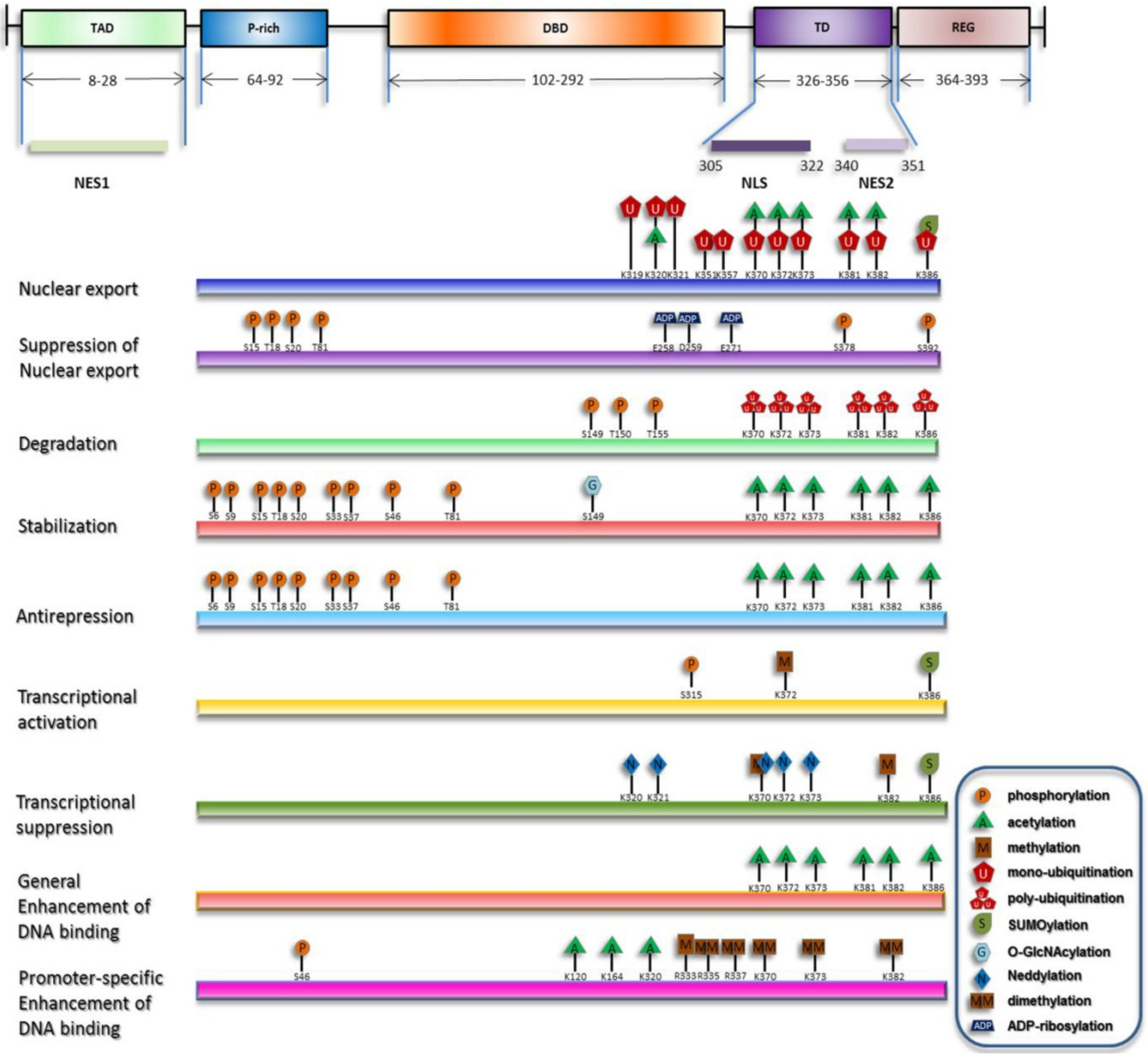

Figure 1. Overview of p53 posttranslational modifications. The major domains of p53 and their distributions are depicted and only the modifications directly responsible for the listed effects are plotted. The modification sites within p53 are primarily updated from W Gu [164, 165].

The stimuli-specific response has received intensive investigations, for it provides a potential model to study the discriminative behavior of p53 pathway. The prominent features of the stimuli-specific responses are distinct elevation manners and different gene expression profiles [28]. Because the concentration of p53 is tested indirectly by the antibody, Differences in the elevation manner of p53 can be partly explained by the occupation of p53 antibody binding epitopes by PTMs. Different gene expression profiles is confirmed to be a result of combinatorial expression of specific sets of p53 target gene. This can be accomplished either through the promoter selection by p53 or the dissimilar assembly manner of transcription complexes by chromatin-bound p53 [29]. Promoter selectivity is attributed to different binding affinities for different response elements (REs) [30], presumably due to PTM marks on p53 [31-33]. Likewise, different assembly manners of transcription complexes, such as chromatin remodelers, histone modifiers or RNA polymerase, can also be ascribed to p53 modifications [34, 35]. It is important to note that different types of stresses can result in different sets of modifications, which bolsters the relationship between covalent modifications and the variability of p53 response [36, 37].

Overall, PTM exerts both general and distinctive role in regulating p53 behavior. However, the contradictions between the results of the *in vitro* and *in vivo* experiments call for more in-depth studies and raise some open problems concerning the real regulatory network of PTMs.

The behavior of individual modifications

p53 harbors an array of amino acids subject to various kinds of PTMs, which are mainly concentrated in the tetramerization domain (TD) and C-terminal domain (CTD). The earliest-discovered behavior of individual modifications of p53 is the redundancy of many N-terminal and C-terminal modifications [18], which is characterized by either the flexible correspondence between the enzymes and modifications, or the subtle effects by the mutation of single site [38]. This can be explained by either the complementarity among the modifications, or an additive and synergistic performance of the modifications. Both mechanisms illustrate the significance of the crosstalk among the modifications.

There also exist switch-like behaviors of individual sites. The individual modifications involved in the transactivation by p53 reflect this behavior, of which the most scrutinized is acetylation. As an example, lysine 320 (K320) acetylation is necessary in antagonizing apoptotic activity of p53 [39]; in contrast, acetylation of K373 and K120 dominantly favors the activation of proapoptotic genes [31, 40]. Besides the acetylation, serine 46 (S46) phosphorylation is found to play critical roles in p53-mediated proapoptic gene induction but not in the induction of cell-cycle arrest [41-43]. Thus, it is possible that individual modifications with a predominant preference for specific physiological outcomes serve as 'binary switches' of different cell fates. Furthermore, with structural biological methods, threonine 18 (T18) has been found to exert 'on-off'-switch role controlling the binding of p53 with Mdm2 [44-46]. This suggests that development of test methods also influences the determination of the functions of modifications. However, switch-like behavior of the individual modifications is mostly identified by mutation assay. This method only proves the essentiality but not the sufficiency of these modifications in initiating specific effects. So it remains to be determined whether there is a simple correlation between an individual modification and a specific effect. In fact, another characteristic of the modifications is their multi-potency—that modification of one site exerts various effects in different contexts and under different stresses, even if the effects are seemingly conflicting. For example, our group found that $p21^{waf1/cip1}$, a canonical cell cycle regulating gene, is activated by K373/K382 acetylation after a specific histone deacetylase inhibitor (HDACi)—depsipeptide—is administered [47]. This finding contradicts previous results that K373 acetylation has a preference for proapoptotic genes [40]. Of note, depsipeptide also induces T18 and S37 phosphorylation. This specific combination pattern of phosphorylation and acetylation is a likely cause of the contrasting results. Similarly, it is shown that K120, with a widely accepted transcription-dependent apoptotic activity, has a transcription-independent proapoptotic function [48]. While a transcription-dependent activity requires prior nuclear accumulation, nuclear export is the prerequisite for transcription-independent apoptotic activity. S315 phosphorylation both increases p53 transactivation potential through nuclear retention, and promotes Mdm2-dependent proteolysis of p53 [49-51]. This raises the question of how a single modification is able to choose between two contrasting fates.

Therefore, an individual modification of p53 is far from discriminating p53 isoforms in deciding biological effects. Instead, certain combinations of modifications can expand the functional scope of individual modifications and explain the results from the functional studies. In this respect, combined with

other modifications, an individual modification can exert various functions, which interprets the multi-potency of the individual modifications.

Sequential crosstalk among modifications

While individual modifications show little significance in coordinating the vast pool of upstream stresses and the downstream repertoire of the target genes, crosstalk among different modifications may provide a way to guarantee the complexity of the p53 network. In normal cells during cell cycle progression, a modification cascade of p53 exists. Phosphorylation of S9, 15, 20 and 372 peaks during G1, whereas S37 and S392 phosphorylation peak during G2/M. S37 is the only site to be phosphorylated during S phase and acetylation is mostly abundant at G0 [52]. This demonstrates that at homeostasis, p53 modification is a dynamic and transient event which may be predicted under controlled conditions.

Under stress, p53 is modified more extensively. At the center of the p53 activation are acetylation and phosphorylation. Phosphorylation of the N terminus serves as the initial wave of response to stress, which shows strong inter-dependence between one another. For example, T18 is phosphorylated *in vitro* and *in vivo* subject to the prior phosphorylation of S15, which is a prerequisite of S20 phosphorylation [36, 53]. Thus, N-terminal phosphorylations can be classified into several clusters. For each cluster, one site is directly modified by the kinases, i.e. nucleating sites, whereas others are modified followed by the nucleating sites. Not only does sequential inter-site dependence exist among the N-terminal phosphorylation sites, C-terminal phosphorylation sites are also involved [54]. Thus, for phosphorylation, inter-site dependence and activation cascades set a new level for more comprehensive and precise coordination of different types of stress.

Phosphorylation is also influenced by other upstream modifications, such as the addition of O-linked β-N-acetylglucosamine (O-GlcNAcylation) and poly (ADP-ribosylation) [55-57]. poly (ADP-rybosylation) of different nuclear acceptors by poly (ADP-ribose) polymerase 1 (PARP-1) is believed to be a damage sensing modification, and thus may bridge the gap between the DNA damage sensing and p53 stabilization. Considering the scarcity of the sites subject to either O-GlcNAcylation or poly (ADP-rybosylation), they may merely exert subtle effects or perform switch-like roles in p53 activation.

Specific phosphorylation patterns induce the acetylation of the C terminus and initiate a phosphorylation-acetylation cascade [58]. Consistently, with the use of specific DNA damage agents, we and other groups testified this hypothesis [59-61]. This suggests that this cascade is generally implicated in all circumstances. It is now confirmed that Mdm2, CREB binding protein (CBP)/p300 (specific enzymes for the acetylation of p53) and p53 form a ternary complex in unstressed cell, and phosphorylation of S15, T18 and S20 increase p53's affinity for CBP/p300 [62]. Phosphorylation of the C terminus also differentially influences the acetylation status. For example, phosphorylation of S378 and T377 reduces the acetylation of K373, K382 and K320, and phosphorylation of S366 and T387 enhances the C-terminal acetylation [54, 58]. Since S378 is constitutively phosphorylated in unstressed cells, T377 and S378 phosphorylation may suppress p53 activation through inhibition of acetylation. Moreover, C-terminal phosphorylation also mediates ubiquitination by Mdm2 [51, 63]. Therefore, p53 degradation may be accomplished through a series of interlocking processes, which is initiated by C-terminal phosphorylation [51, 63], relayed by inhibition of C-terminal acetylation and ended up with the ubiquitination (Figure 2). In addition to acetylation, other modifications including mono-ubiquitination, poly-ubiquitination [64, 65] and ubuiquitin-like modifications [66], are also included in various cascades.

Acetylation is the hub of p53 transactivation and is contained within a network of various upstream and downstream modifications. Loss of Set7/9 in mouse embryonic fibroblasts cells (MEFs) prevents acetylation at K117, K317, K370 and K379 (human homologous sites are K120, K320, K373 and k382), suggesting a general effect of K372 methylation on the acetylation status of p53 [67]. Consequently, acetylated p53 can recruit coactivators to the binding promoters and mediate the acetylation of histone H4 [68, 69]. In addition, SUMOylation also influences the acetylation of the C terminus and illustrates a unidirectional cascade. K386 SUMOylaiton of at least one subunit of p53 tetramer inhibits the consecutive acetylation by p300/CBP, whereas prior acetylation by p300 remains permissive for the SUMOylation machinery [70]. This demonstrates distinct behaviors of a pathway cascading in opposite directions and a coordination mechanism of a level higher than the primary structure of p53.

Therefore, the modification of p53 is a dynamic process that rapidly relays the sequential signals to the final target during the course of p53 activation. (Summarized in Figure 2).

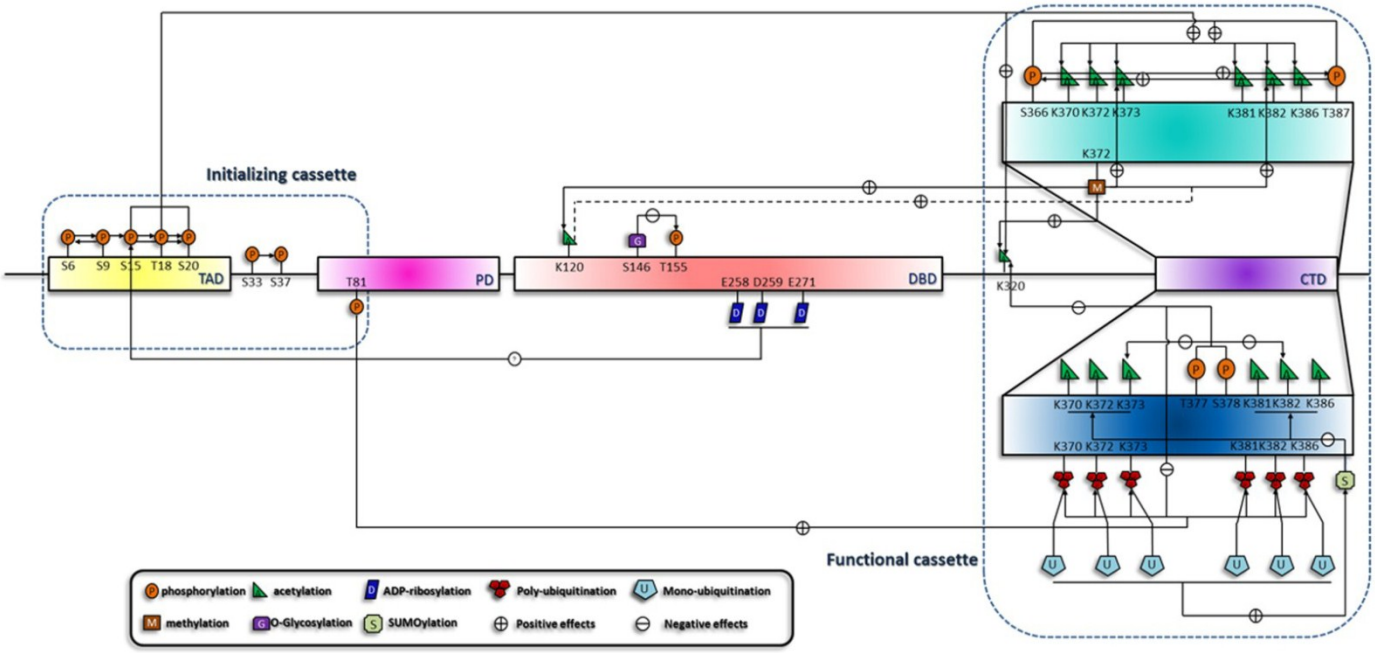

Figure 2. Summary of the sequential interplay of modifications of p53. This figure shows the modification cascades which can be classified into short-range and long-range influences, which is reminiscent of the model raised by X-J Yang [127]. In addition, effects on the downstream modification sites can be either negative (indicated by arrows with a '-' in a circle) or positive (indicated by arrows with a '+' in a circle). According to the sequential order of the modifications, they are crudely classified into two cassettes. Modifications in the initializing cassette are responsible for the sensing and distinguishing of the stresses and can transmit the signals to the functional cassette. Modification combinations in the functional cassette can induce specific biological outcome.

Spatial crosstalk among the modifications

In addition to sequential crosstalk, there is also spatial crosstalk among the PTMs, which is characterized by combinations of multisite modifications to trigger p53 response cooperatively (i.e. combinatorial behaviors). Series of combinatorial behaviors of different modifications on other proteins have already been characterized, including Forkhead Box protein O (FoxO) family [71-74], tubulin and the C-terminal domain of RNA polymerase II [75, 76]. Analogous with the interplay of histone modifications and the none-histone protein modifications [77, 78], p53 modifications also demonstrate spatial crosstalk. The simplest behavior is the competition of the same site by different kinds of modifications. Mostly influenced are lysines located in the CTD of p53, especially the 6 lysines acetylated by p300/CBP and K320 acetylated by P300/CBP-associated factor (PCAF). All the acetylation sites are ubiquitination targets [79], with some of them competing with methylation, SUMOylation and neddylation, suggesting mutual exclusivity of these modifications. Moreover, functions of the modifications of the same sites vary according to the number of moieties added. This is exemplified by methylation of K370, K373 and K382 [80-82] and the competitive mono- and polyubiquitination of several lysines in the C terminus [83]. Besides the competition for the same amino acid, crosstalk among adjacent sites in the primary amino acid sequence or higher order structure of protein also exists. The spatial crosstalk has both antagonistic and synergistic effects. Antagonism is exemplified by the interplay of modifications on the CTD, such that methylation of K370 and K372 and phosphorylation of S315 and S392 occur in a mutually exclusive way [84, 85]. Synergy is characterized by the sites functioning or modified simultaneously. As shown in Figure 3, the key problem resolved by this hypothesis is the multisite phosphorylation of the N terminus. The multiplicity may indicate the need for a critical amount of phosphorylation sites to reach an activation threshold, forming a multisite switch, which is reminiscent of the regulation of Ste5 by cyclin-cyclin dependent kinase complex (Cln/CDK) [86]. In addition, simultaneous phosphorylation and acetylation of several sites is essential for the interaction of p53 with Pin1 and TAF1 respectively [87, 88]. Apart from addition of covalent moieties, deletion is also indispensable. For example, simultaneous phosphorylation of S378, S366 and T387 and dephosphorylation of S376 results in p53 tetramerization and transcriptional activation [89-91].

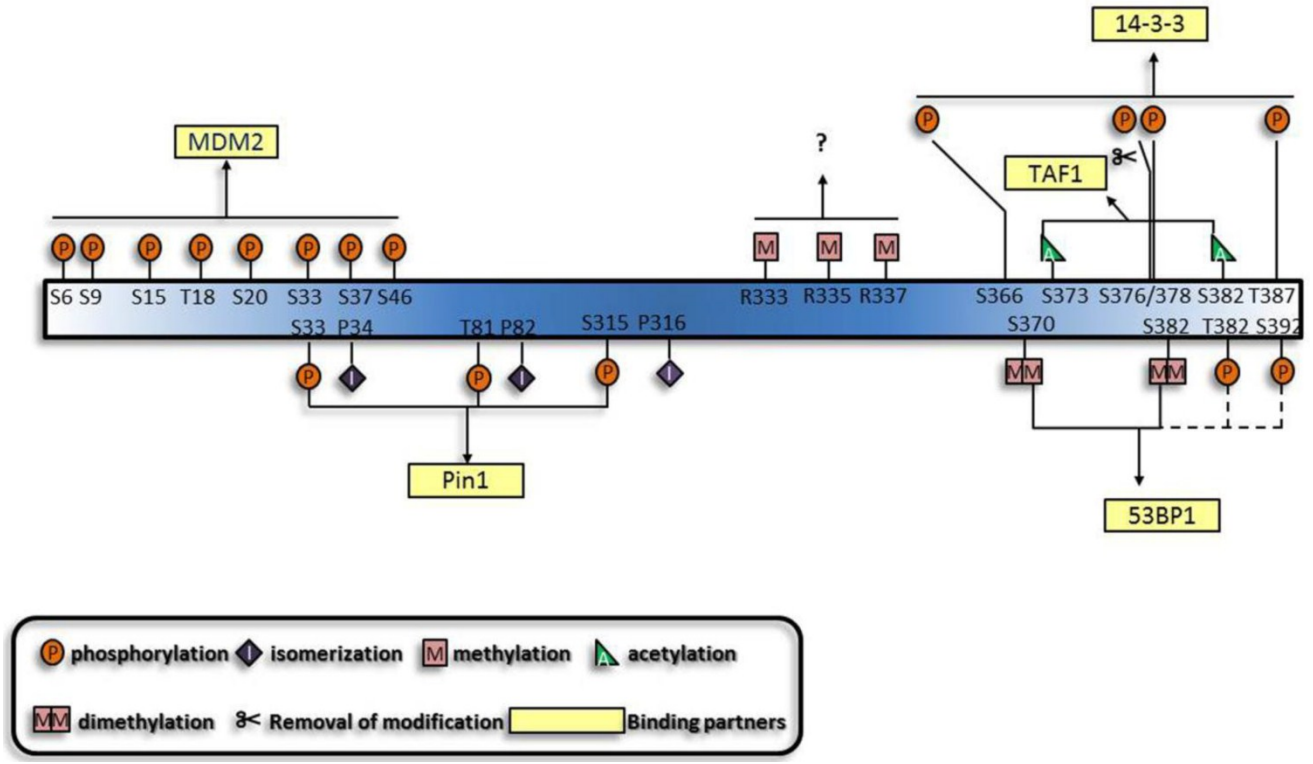

Figure 3. Generalization of the synergistic manner of different modifications and their binding partners. The functions of the p53 modifications are mutually dependent. A given combination of modifications can exert their specific functions simultaneously, and a specific binding partner, usually protein, mediates the function of modifications. Dashed lines and questions marks highlight that these modifications are likely to function synergistically.

Based on the established cooperative manners of modifications, lots of other modifications are predicted to perform synergistically. Multi-site-monoubiquitination that was previously thought to function redundantly, now seems to strengthen the binding affinity between proteins [92-94]. Likewise, methylation of three arginines in the C terminus may also perform simultaneously [95]. Furthermore, the dual modification pattern in the interaction between 14-3-3 and histone through phospho-acetylation [96] is indicative of the interaction between 14-3-3 and p53. Apart from the established role of K370 and K382 di-methylation [80, 81] in the interaction between p53 and p53 binding protein 1 (53BP1), phosphorylation shows great potential to be involved [97-100]. Furthermore, motifs which can associate with several modifications and proteins with multiple modification-recognizing motifs have also been characterized [101]. On the whole, protein-protein interactions appear to underlie the majority of cooperative regulations by modifications.

Both sequential and spatial crosstalk represents combinatorial performance of covalent modifications. Due to this combinatoriality, the selectivity and variability of p53 functions are yielded.

Regulation mechanisms by PTMs

PTM cascade of p53 is always accompanied by a binding partner cascade, indicating a role of the modifications to mediate the interaction between p53 and its partners. This has raised tantalizing questions of how the interactions are regulated. In fact, there are various underlying mechanisms for the interaction which should be discussed.

Conformational changes

Conformation is the most important feature of protein structure, which dramatically influences the function of the protein. Since PTMs can elicit significant effect on protein function through conformational changes [102-104], different combinations of PTMs may yield distinct protein conformations, resulting in the ensuing specific interaction [76].

As for p53, modification confirmed to influence its conformation is the phosphorylation-dependent isomerization by Pin1 [105], which is supported by the fact that p53 needs to form a complex with Pin1 to exert its functions [87, 105, 106]. This indicates that

other modifications of p53 may also show great potential in converting PTMs into conformational changes.

p53 CTD can either positively or negatively regulate p53's transactivation ability, and its acetylation can potentiate p53 sequence-specific binding *in vitro* [19, 107, 108]. The underlying mechanism for sequence-specific binding was suggested to be allosteric activation [19, 58, 109], which either exposes the DNA binding domain (DBD) of p53, or influences the interaction between other proteins. DBD of p53 mainly mediates its direct binding with its consensus sequence and is heavily influenced by conformation. This is consolidated by the structural study revealing that p53 sequence-specific binding involves a conformational switch in its DBD [110]. Unlike DBD of FoxO family whose binding affinity is substantially influenced by its phosphorylation and acetylation [111], DBD of p53 shows poor access for covalent modifications. However, since the mechanism for K164 and K120 acetylation of p53 remains unclear, a conformational change similar to FoxO may cause this effect. Likewise, ubiquitination, especially mono-conjugated, shows great potential to regulate p53 conformation, possibly owing to its function as a chaperone to promote folding of nascent proteins [112].

Conformation is closely related to the energy of the molecule and serves as a major regulator of backbone structure. By virtue of the conformational change, the otherwise buried docking sites or catalytic sites can be exposed and thus induce the interaction between proteins, DNA and chromatin. Since these modifications can induce significant structural changes, they may serve as simple 'on/off' switches to regulate the qualitative responses of p53. However, as have been suggested, conformational changes cannot be incorporated into specific kind of protein during evolution, which limits the generality of this mechanism [101].

Combinations of docking motifs

Histone tails are heavily modified and can be read by effector proteins through direct binding. It was hypothesized that this effect is mediated by the covalent modifications embedded in specific motifs [113]. In support of this hypothesis, protein modules specific for recognizing modifications on histone tails are identified. These include bromodomain [114, 115], chromodomain [116, 117] and the more recent plant homeodomain (PHD) [118-121], Tudor and MBT [122] domains. Furthermore, it is confirmed that a protein can contain more than two modification recognition domains [123-125], and some protein complexes can embrace subunits with distinct modification recognition motifs. This provides a novel way to recognize cooperatively the modification signals [126].

The functions of non-histone protein modifications can be extrapolated from the histone modifications. Consistently, protein modules that specifically recognize modifications on non-histone proteins are characterized [127]. Together with the flanking sequences, modifications can mediate specific binding of p53 with the modification recognizing modules (summarized in Table 1). Therefore, docking motifs appear to be a precise commander enabling dynamic and specific binding of p53 with other partners.

Table 1. Modifications as docking motifs and their binding proteins.

Modification	Sites	Binding protein	domain	Reference
phosphorylation	S46, S33, T81, S127, T150, S315	Pin1	WW	Ref 105
dephosphorylation	S376	14-3-3		Ref 95
acetylation	K382	P300/CBP	bromo	Ref 159
di-acetylation	K373, K382	TAF1	Tadem bromo	Ref 88
SUMOylation	K386		SIM	Ref 160, 161
di-methylation	K382, K370	53BP1	Tudor	Ref 80, 81
Ubiquitination			UBD	Ref 83, 162
monomethylation	K382	L3MBTL1	MBT	Ref 163
	K372	Tip60	Chromo	Ref 67

Bulk electrostatics

Intrinsically disordered regions of proteins which are quite frequent in nature perform important functions in cells [128]. These regions always serve as the linkers between different domains of the proteins. There are three unstructured regions in p53: the linker between N-terminal transactivation domain (TAD) and DBD, the linker between DBD and TD, and CTD [129, 130].

Many kinds of modifications, including phosphorylation, acetylation and ADP-ribosylation, can change the overall charge of the amino acid residues, and in turn contribute to the electrostatic force-mediated interactions [131]. The first to be mentioned is the multisite phosphorylation of the TAD of p53 which mediates the binding with p300/CBP. Rather than a switch-like behavior, an additive manner of phosphorylations of S15, S20, T18, S46, S33, S37 and T55 was demonstrated [45]. In this scenario, the electrostatic forces generated by negative charges of phosphate and the positive charge of CBP contribute to the interaction. In addition, p53 transcriptional activity can exhibit both on/off switch and graded response after genotoxic stress [132]. An extension of this fact is that the seeming redundancy of the N-terminal phosphorylation can contribute to the graded response. The redundancy may also play critical roles in sensing the nature and the severity of cellular stresses, whereby prolonged or severe genotoxic stress leads to phosphorylation of additional sites and gradual increase in the affinity for CBP/p300. Apart from the TAD, modifications of lysines on CTD, especially the acetylation of the six lysines in proximity, may also neutralize the positive charge on CTD [19]. Other models of multisite acetylation functioning as charged patches have already been established, including histone acetylation and p300 autoacetylation [133, 134]. These models further increase the possibility of the acetylation on p53's CTD to function as electrostatic regulator.

The bulk electrostatic mechanism explains the graded response of p53 and confers a quantitative feature on p53 response. However, since various modifications share the same electric property, this mechanism is not precise enough. Hence, structure of different kinds of moieties is necessary in distinguishing different modifications.

The three mechanisms presented above function cooperatively. The conformational switch regulates the rigid backbone of p53's globular regions and exposes the docking motifs for interaction proteins. Docking motifs, in turn, directly coordinate the binding of p53 with its partners through interacting with specific modification recognizing modules. Electrostatic forces increase the binding efficiency by specifically utilizing the flexibility of the unstructured regions. Henceforth, modifications inducing conformational changes behave as 'on/off' switches toggling between different subsets of p53 events through exposure of different groups of docking motifs. Specific docking motifs serve as combinatorial signals to recruit binding partner. Modifications regulating the electrostatic attraction yield a rheostat behavior of p53 to quantitatively coordinate the events like a sensor or blocker. In consequence, these mechanisms form a complex and precise coordination network for p53.

Deciphering the 'p53 code'

Spatial and sequential interplay of p53 modifications provides it with vast indexing potential and expands its functional spectrum. A semiotic view, such as 'code', 'barcode' or 'cassette', is adopted to define the complex network among the modifications of both histones and non-histone proteins [40, 135]. In this semiotic system, several elements, including code, regulator and the 'meaning' of the code are indispensable. Regulators can be classified into 'reader', 'writer' and 'eraser' (see Figure 4). 'Reader' refers to binding partner like chromatin, non-histone protein and DNA that interacts specifically with the modification marks and initiates specific effects. These include conformational changes, catalytically activation and transcriptional activation. Protein adding chemical groups to specific sites is defined as 'writer'; by contrast, 'eraser' is responsible for the removal of chemical group from specific sites. However, there are no strict divisions between the definitions of these regulators; a protein can have multiple properties such that the process of reading and writing can be completed by the same protein. These regulators in the semiotic system are coordinated either at protein level or, more precisely, at the posttranslational level. Specific modification at particular sites can distinguish between different forms of a protein and guarantee the functional specificity of the regulators. Basic to p53 degradation is Mdm2, of which the most relevant modifications are autoubiquitination [136, 137], phosphorylation [138-142], SUMOylation [143, 144] and acetylation [145]. Similar to Mdm2, other ubiquitin ligases including Pirh2 [146] and constitutively photomorphogenic 1 (COP1) are also subject to the regulation by PTMs [147].

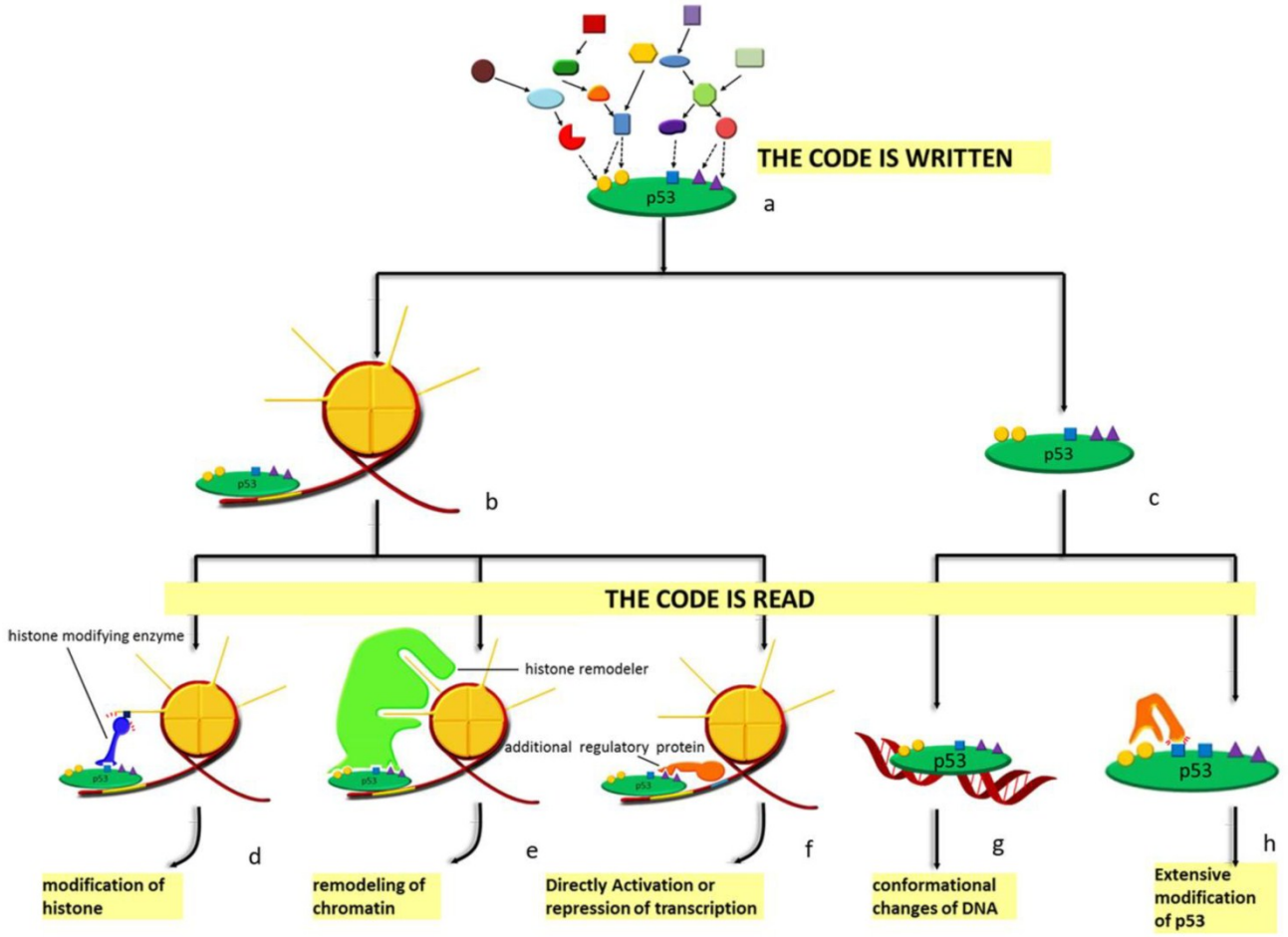

Figure 4. Diagram of the p53 'code system'. At homeostasis, p53 is mainly presented in two forms: chromatin-bound (**b**) form and unbound form (**c**). Under stress, p53 is modified combinatorially by various enzymes. Thus, the 'code' is written (**a**). Specific 'code' on chromatin-bound p53 can recruit either histone modifying enzymes (**d**), histone remodelers (**e**) or other regulatory proteins (**f**) to the vicinity of the response element p53 is bound to. As for the unbound form, DNA with p53-binding sites (**g**) and other enzymes (**h**) recognize the code. Different 'readers' lead to distinct outcomes. Modifications of histones, remodeling of chromatin, directly activation or repression of transcription, conformational changes as well as extensive modifications of p53 are the effects of histone modifying enzymes, histone remodelers, additional regulatory proteins, specific DNA sequences and other enzymes, respectively.

histone acetyltransferase (HAT) and histone deacetylase (HDAC) regulate acetylation of both histones and transcription factors, yielding a connection between chromatin accessibility and transcription activity. p300 and CBP are coactivators for a variety of transcription factors, whose activation is mainly regulated by autoacetylation [134, 148]. Similarly, we and other groups also found that class III HDACs sirtuins, especially SIRT1 and SIRT7 participating the deacetylation of p53, are phosphorylated and methylated [149-151]. Notably, different proteins modified by the same enzyme can function either synergistically to enhance the overall effects, or antagonistically to create a delicate balance [152]. This well explains the inconsistencies in the *in vitro* and in vivo experimental results: the *in vitro* methods used may always disturb the stoichiometry between different targets subject to same modifications.

Another critical element within this semiotic system of p53 is the interpretation of the code. 'PTM code' of non-histone proteins [153] is originally extrapolated from the 'histone code', [77, 78] which is interpreted as 'transcription starts or stops at a specific time and place' [154]. Although there are still arguments against the code's generality considering the context-dependent meaning and the weak predictability of modifications themselves [113], extensive combinations of modifications strengthen the specificity and more clearly define the concepts of 'code'. The meaning of the 'code' of p53 modification can be interpreted as the functions of interaction partners—including non-histone proteins, histones as

well as DNA—encoded combinatorially by the modifications (Figure 4). PTMs on the regulators can be translated into anti-code matching with specific code on p53. In this way, only the properly modified regulators can recognize a specific form of p53. More recently, as revealed by the interdependence between p53 modifications and histone H3 modifications [155, 156], a 'p53-histone' code-to-code model connecting the histone and non-histone modifications has been raised.

With respect to this semiotic system, a stress-specific performance of p53 can be explained by the differences of modification marks induced by distinct stresses [157]. Likewise, cell- and tissue-type dependency of p53 behavior can be attributed to specific combinations of p53 modifications introduced by intrinsically distinct regulator pools in different types of cells or tissues [158]. Furthermore, a modification cascade can serve as a driving force for p53 pathway to progress spontaneously, which indicates a more general regulating rationale for other signaling pathways.

Future perspectives

Promising as the 'code' model of p53 PTM is, the following questions remain open such as: what is the real basis for the redundancy of the individual modifications *in vivo*? What is the real mechanism that regulates the context-dependent behavior of p53? How exactly is p53 involved in the regulation of one specific biological effect? How general is the mechanism for the regulation of PTM and in what way do they really cooperate? With the emergence of the novel functions regulated by p53, such as metabolism and nutrient stress responses, is there a possibility to revise the demarcations between different phenotypic outcomes to a more subtle one? In order to tackle these problems, numerous further investigations are required: (i) more precise and subtle distinction of the effects of the 'code' in molecular level instead of phenotypic level; (ii) discrimination between the direct and indirect effects of specific modifications; (iii) identification of the combinatorial behaviors of the modifications using high-throughput testing method; (iv) *in situ* observation of the dynamic changes of the modifications marks using more reliable and direct time-resolved method. Although there is still a long way to go, it is believed that the final decipherment of the p53 code will arrive in the near future.

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Abbreviations

53BP1: p53 binding protein 1; CBP: CREB binding protein; Cln/CDK: cyclin-cyclin dependent kinase complex; COP1: constitutively photomorphogenic 1; CTD: C-terminal domain; DBD: DNA binding domain; FoxO: forkhead box protein O; HAT: histone acetyltransferase; HDAC: histone deacetylase; HDACi: histone deacetylase inhibitor; MEF: embryonic fibroblasts cells; Mdm2: mouse double minute 2; NES1: N-terminal nuclear export sequence; NES2: C-terminal nuclear export sequence; NLS: nuclear localization sequence; PARP-1: Poly (ADP-ribose) polymerase 1; PTM: post-translational modification; P-rich: proline rich domain; RE: response element; REG: C-terminal regulatory domain; TAD: transactivation domain; TD: tetramerization domain.

Competing Interests

The authors have declared that no competing interest exists.

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