Epigenetics & Chromatin

METHODOLOGY

Open Access

Post-translational modifications of epigenetic modifier TIP60: their role in cellular functions and cancer

Himanshu Gupta[1] and Ashish Gupta[1*]

Abstract

TIP60 is a crucial lysine acetyltransferase protein that catalyzes the acetylation of histone and non-histone proteins. This enzyme plays a crucial role in maintaining genomic integrity, by participating in DNA damage repair, ensuring accurate chromosomal segregation, and regulating a myriad of cellular processes such as apoptosis, autophagy, and wound-induced cell migration. One of the primary mechanisms through which TIP60 executes these diverse cellular functions is via post-translational modifications (PTMs). Over the years, extensive studies have demonstrated the importance of PTMs in controlling protein functions. This review aims to summarize the findings on PTMs occurring on the TIP60 protein and their functional implications. We also discuss previously uncharacterized PTM sites identified on TIP60 and examine their relationship with cancer-associated mutations, with a particular focus on residues potentially modified by various PTMs, to understand the cause of deregulation of TIP60 in various cancers.

Keywords TIP60, Post-translational modifications, Phosphorylation, Acetylation, Cancer

Introduction

Organisms carry out a multitude of finely synchronized functions, which necessitates the cellular machinery to operate in a well-organized manner by precisely controlling the activity of proteins to ensure proper cellular function. This can be achieved through various mechanisms, including protein post-translational modifications (PTMs), which are covalent modifications that occur on the amino acid side chains of proteins after their translation. PTMs, most of these being reversible, add an extra layer of control over the biological activity of proteins by altering their structure, localization, stability, as well as modulate interactions with other molecules. Through these mechanisms, PTMs enable precise and dynamic regulation of protein functions, allowing cells to respond rapidly to environmental changes and maintain homeostasis. Although, scientists have understood the physiological relevance of PTMs for many decades, significant progress was only achieved in the early twenty-first century with the advent of high-resolution mass spectrometry techniques, that allow for the identification of less prevalent PTMs. As of now, more than 400 PTMs are known to exist, and the number continues to grow [1, 2].

Some of the key post-translational modifications include phosphorylation, acetylation, ubiquitination, SUMOylation, and methylation [2]. PTMs can influence various aspects of a protein's function, including localization, activity, stability, protein-protein interactions (PPIs), protein-DNA interactions, and phase separation [1–6]. For example, protein kinase A (PKA) phosphorylates the repression domain of the nuclear receptor

*Correspondence:
Ashish Gupta
ag315@snu.edu.in
[1]Epigenetics and Human Disease Laboratory, Centre of Excellence in Epigenetics, Department of Life Sciences, Shiv Nadar Institution of Eminence, deemed to be University, Delhi-NCR 201314, Uttar Pradesh, India

corepressor (N-CoR), which modulates several nuclear receptors, including progesterone, estrogen, and glucocorticoid receptors. This phosphorylation event results in increased nuclear translocation of N-CoR, thereby enhancing its repressive functions [7]. Thus, PTMs can lead to significant alterations in protein function. In the present work, we concentrated on the TIP60 protein, which serves as a protein modifier by acetylating both histone and non-histone proteins.

TIP60, a lysine acetyltransferase protein and a well-documented tumour suppressor, is a part of the MYST (*M*OF, *Y*bf2, *S*as3, & *T*IP60) family of acetyltransferases [8–10]. Initially discovered as a TAT (trans-activator of transcription) interacting protein of HIV-1, TIP60 was later identified as a nuclear histone acetyltransferase (HAT) capable of acetylating free histones (H2A, H3, and H4) [8, 11]. However, its acetylation activity significantly decreases when using nucleosome substrates, leading scientists to hypothesize that TIP60 exists within a multimeric protein complex [8]. Eventually, Ikura et al. demonstrated and confirmed that TIP60 forms a part of a 14-subunit complex (known as TIP60 complex), that is capable of acetylating nucleosomes [12]. Also, this complex possess ATPase and DNA binding activities that assists in DNA repair [12]. Recent cryo-EM studies by three independent groups elucidated the structure of the TIP60 complex, revealing it to consist of over 20 proteins, and providing insights into the configuration of several protein subunits [13–15]. All investigations highlighted EP400 as a key protein that interacts with all other modules, including TRRAP, ARP, TINTIN, HAT, and the base module, thus imparting flexibility to them. The TRRAP subunit was found to be essential for efficient and precise deposition of acetylation and H2AZ on chromatin by the TIP60 complex [14].

TIP60 is shown to be essential for survival in mammals as homozygous knockout of TIP60 results in embryonic lethality in mice [16]. Additionally, heterozygous TIP60 knockout mice exhibited a higher susceptibility to tumorigenesis [9]. TIP60 mediates the acetylation of both histone and non-histone proteins playing a pivotal role in various cellular functions, including transcriptional regulation, DNA damage repair, apoptosis, autophagy, cell cycle regulation, mitosis, and wound-induced cell migration [12, 17–22]. Under conditions of DNA damage, TIP60 acetylates ataxia telangiectasia mutated (ATM) kinase thereby activating it and facilitating ATM-dependent phosphorylation of p53, which is essential for DNA repair [17]. However, in case of extensive DNA damage, TIP60 shifts the cellular equilibrium towards apoptosis [23]. TIP60 interacts with and activates p53, resulting in the upregulation of p21 expression and consequent growth arrest. Conversely, in conditions, when DNA damage is beyond repair, TIP60 acetylates p53 at Lys 120, which triggers PUMA activation and leads to cellular apoptosis [23]. TIP60 also acetylates Aurora B kinase and Nup62, ensuring error-free chromosomal segregation during mitosis thereby helps in maintaining genomic integrity [24, 25].

Besides regulating the activity of various cellular factors, TIP60 controls the transcriptional regulation of several nuclear receptors by acting as their cofactor. Brady et al. have demonstrated that TIP60 functions as a ligand-dependent coactivator of the androgen receptor (AR), progesterone receptor (PR), and estrogen receptor (ER) [26, 27]. In one of our research work, we have demonstrated that TIP60 associates with and acetylates PXR (pregnane & xenobiotic receptor), a class II nuclear receptor [28]. Together, the TIP60-PXR complex promotes wound-induced cell migration by activating genes, such as *Cdc42*, *ROCK1*, *GADD45β*, and *IGFBP-1*, involved in actin reorganization and filopodia formation. This complex also acetylate histone H4 and H2B, leading to changes in the chromatin landscape that enhance the transcription of target genes [20, 21]. More lately, we have shown that TIP60 undergoes phase separation which is crucial for its interaction with its partners to perform its functions according to the cellular environment [29].

TIP60 is encoded by *KAT5* gene in humans and is predominantly present in three isoforms (isoform 1, 2, 3) due to alternative splicing [30–33]. Isoform 2 is 513 amino acid and is the most abundantly expressed [30]. TIP60 features various conserved domains, including a chromodomain that facilitates its loading onto chromatin, an intrinsically disordered region (IDR) that aids in its phase separation, a conserved MYST domain encompassing a catalytic HAT domain that regulates the KAT (lysine acetyl transferase) activity of TIP60 and a nuclear receptor box (NR box) which facilitates TIP60's interactions with different nuclear receptors (Fig. 1) [8, 20, 26, 27, 29, 30, 34–36].

Multiple studies have shown that TIP60 undergoes various post-translational modifications, which influence its functions. These modifications include phosphorylation, acetylation (including autoacetylation), SUMOylation, methylation, glycosylation, and ubiquitination (Fig. 2). In this review, we have summarized all the PTMs that occur on TIP60 and their implications in regulating TIP60-associated functions (Table 1). Additionally, we have discussed uncharacterized potential PTM sites on TIP60 and examined the relationship between cancer-associated mutations in TIP60 and its PTM sites. This aims to provide a deeper understanding of its deregulation in pathological conditions due to disruptions in its PTMs.

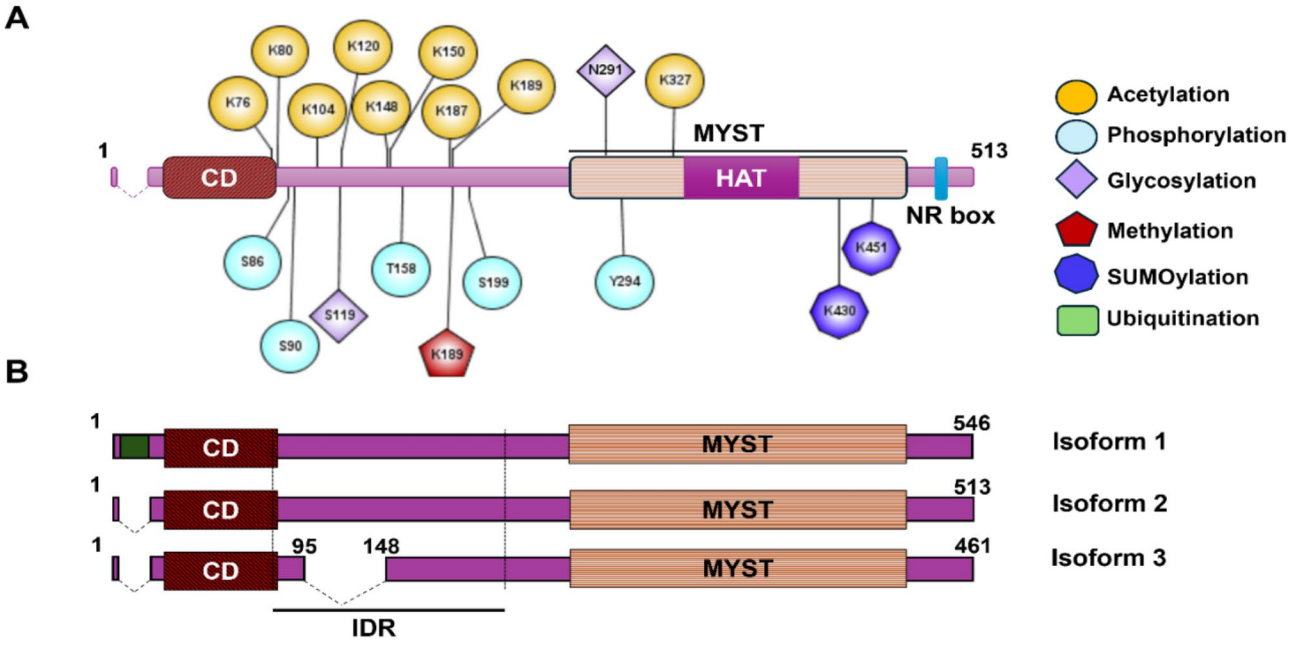

Fig. 1 Schematic diagram showing post-translational modifications in TIP60. (**A**) The domain map illustrates TIP60's various domains and the post-translational modifications associated with them (amino acid numbering is as per isoform 2). The different domains are CD (chromodomain), IDR (intrinsically disordered region), HAT domain (histone acetyltransferaese), and NR box (nuclear receptor) motif. Yellow color circle depicts acetylation, blue color circle represents phosphorylation, purple color quadrilateral shows glycosylation, pentagon represents methylation, dark blue octagon represents SUMOylation, and green rectangle represents ubiquitination modification. Note- Ubiquitination sites are yet not well charecterized. (**B**) The domain map of TIP60 isoforms 1, 2, and 3. Isoform 1 has an intron region of 33 amino acid, while isoform 2 and 3 lacks this intron region. Isoform 3 is also devoid of exon 5 (52 amino acid). Dash line depicts missing regions in the respective isoform

Phosphorylation

Phosphorylation is the most extensively studied reversible PTM, that occur on proteins within the nucleus or cytoplasm of cells, influencing protein activities in both prokaryotic and eukaryotic organisms [37]. This process involves the addition of phosphate group to serine (Ser), threonine (Thr), or tyrosine (Tyr) residues [38] facilitated by protein kinases that catalyze the transfer of a phosphate group from adenosine triphosphate (ATP) to the target protein. Conversely, dephosphorylation, the removal of the phosphate group, is carried out by phosphatases [39]. Protein phosphorylation plays a crucial role in regulating various cellular processes, including cell signalling, growth and division, cell cycle regulation, apoptosis, protein localization and stability [40–45].

TIP60 contains many serine, threonine and tyrosine residues that are conserved across various species and could serve as potential phosphorylation sites. Numerous studies have demonstrated the ability of TIP60 to undergo phosphorylation. According to available reports, five specific residues of TIP60 - Ser 86, Ser 90, Thr 158, Ser 199 and Tyr 294, are phosphorylated and play crucial role in regulating TIP60-mediated cellular activities, including DNA damage repair, autophagy, cell cycle progression, and apoptosis [24, 46, 47]. Lemercier and colleagues discovered TIP60 phosphorylation using an alkaline phosphatase assay (calf intestinal phosphatase or CIP) two decades ago [48]. They purified TIP60 and detected two bands on an SDS-PAGE gel. Interestingly, when they treated the protein with CIP and ran the gel they observed a single faster migrating band on the gel, suggesting phosphorylation of TIP60 [48]. Through mass spectrometric analysis, they identified two primary phosphorylation sites: Ser 86 and Ser 90. Mutations at these sites altered TIP60's migration pattern on the gel. Furthermore, they identified cyclin B/Cdc2 as the enzyme responsible for phosphorylating Ser 90, influencing TIP60's histone acetyltransferase activity. Given that Cdc2 is involved in mitosis, they noted increased TIP60 phosphorylation in the G2/M phase, suggesting a role for TIP60 phosphorylation in regulating the cell cycle [48]. Subsequent investigations by various groups explored the significance of these phosphorylation events and determined their involvement in multiple cellular processes including DNA damage repair, autophagy, apoptosis, and fatty acid synthesis. It was previously established that TIP60 plays a key role in apoptosis by acetylating p53 under DNA damage conditions, leading to either cell cycle arrest or apoptosis, depending on the extent of DNA damage [23]. Charvet and colleagues have demonstrated that TIP60 phosphorylation is crucial for this pathway selection. They demonstrated

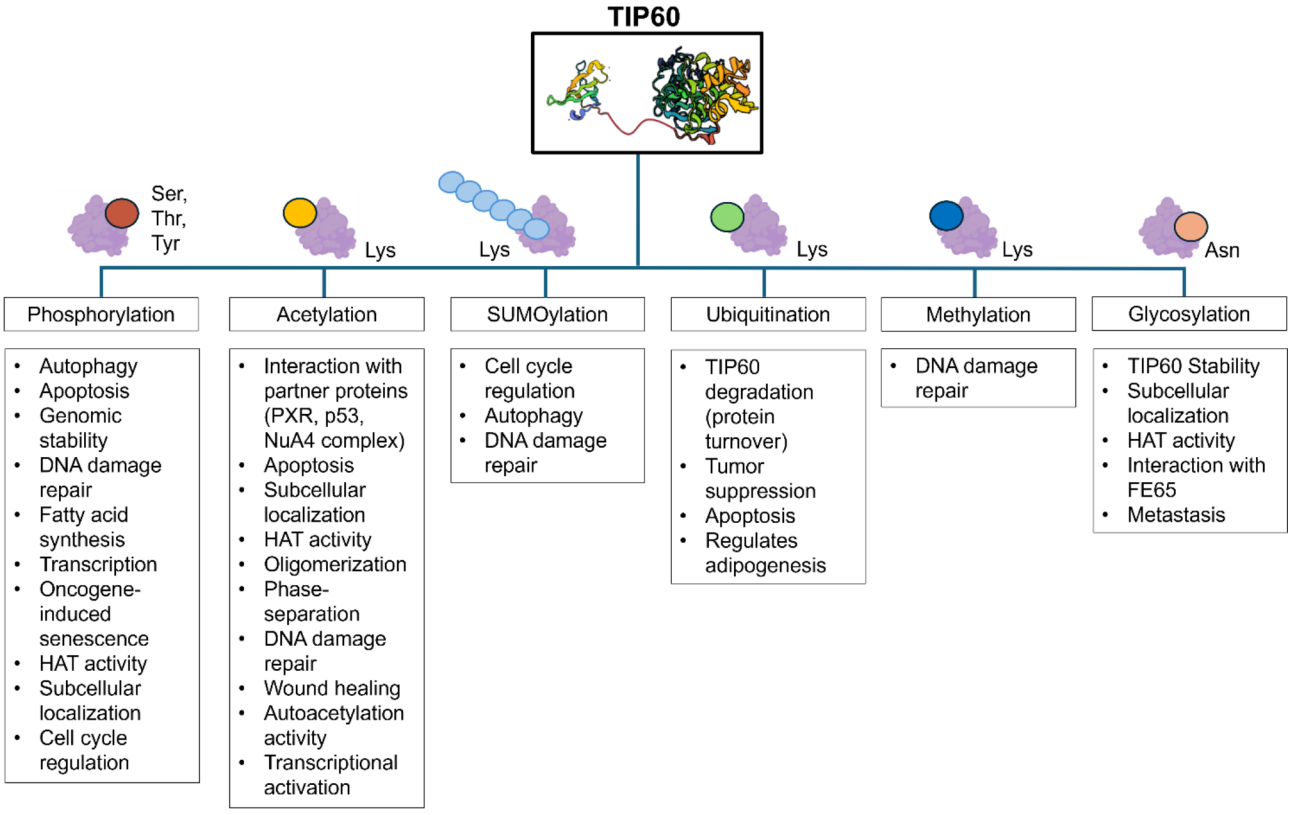

Fig. 2 The image depicts distinct types of TIP60's post-translational modifications as well as their effects on its functions. Modifications on the amino acid residues of TIP60 are indicated as Ser (Serine), Thr (Threonine), Tyr (Tyrosine), Lys (Lysine), and Asn (Asparagine) (Image is generated using Biorender.com and modified using Microsoft office)

that GSK-3 (glycogen synthase kinase 3) phosphorylates TIP60 at Serine 86 [49], and the mutant version of TIP60 at this site fails to acetylate p53 at the K120 position, which results in its inability to express PUMA, necessary for inducing apoptosis under DNA damage conditions. Additionally, the phosphorylation-defective TIP60 mutant fails to acetylate histone H4 at the PUMA promoter due to decreased HAT activity [49]. Notably, two other research groups have associated Serine 86 phosphorylation to the process of autophagy [19, 47]. In cells under serum deprivation conditions, the enzyme GSK-3 is activated, which phosphorylate TIP60 at the Ser 86 position, thereby influencing TIP60's activity as an acetyltransferase [47]. This phosphorylation enables TIP60 to bind to and acetylate ULK-1, a kinase involved in autophagy regulation. Moreover, it has been demonstrated that TIP60 depletion leads to compromised lipidation of LC3 (microtubule-associated protein 1), a marker for autophagosomes in cells [47]. When cells undergo endoplasmic reticulum (ER) stress induced by agents like tunicamycin (TM) and H_2O_2, GSK-3β becomes activated by a reduction in its Ser9 phosphorylation. The activated GSK-3β phosphorylates TIP60 at Ser86, leading to its activation. The activated TIP60 then acetylates ULK1, playing a role in apoptosis in response to ER stress. Furthermore, the GSK-3β-TIP60-ULK1 pathway not only regulates autophagy but also helps prevent ER stress-induced apoptosis by inhibiting CHOP, a transcription factor involved in the ER stress and unfolded protein response (UPR) pathways [19].

Research by Mo et al. and Mischa and colleagues has demonstrated the critical role of Ser 90 phosphorylation of TIP60 in maintaining genomic integrity [24, 50]. During mitosis, cells preserve their genomic stability through mechanisms like the DNA damage response (DDR) and the spindle assembly checkpoint (SAC) [51, 52]. Aurora B kinase (Ser/Thr kinase) a part of the chromosome passenger complex (CPC), is integral to SAC surveillance [53]. Mo et al. found that TIP60 acetylates Aurora B kinase, process that ensures precise chromosomal segregation during mitosis. The acetylation of Aurora B kinase by TIP60 is triggered by the phosphorylation of TIP60 at Ser 90 by the CDK1-cyclin B complex [24]. Furthermore, another study by Li et al. showed that phosphorylation of TIP60 at Ser 86 and Ser 90 is essential for balancing the localization and aggregation of 53BP1(p53-binding protein 1) and BRCA1 (breast cancer gene 1) at Double-Strand Breaks (DSBs) during the S and G2 phases of the cell cycle [50]. 53BP1 promotes non-homologous end joining (NHEJ), while BRCA1 facilitates homologous

Table 1 List of TIP60 PTM sites, catalyzing enzymes and their implications on TIP60-associated functions

Site	Modifying Enzyme	Eraser Enzyme	Function	Reference
Ser 86 Phosphorylation	GSK3 β		HAT activity of TIP60, cell cycle regulation & progression, chromosomal segregation, autophagy, apoptosis, DSB repair, and TAG (triacyl glycerol) synthesis	[19, 47, 48, 49, 55]
Ser 90 Phosphorylation	Cyclin B/ cdc2, CDK9		HAT activity of TIP60, cell cycle regulation & progression, chromosomal segregation, association with H3 and RNA pol II.	[24, 48, 57]
Thr 158 phosphorylation	p38α, VRK1		Apoptosis, DNA damage response, TIP60 autoacetylation, oncogene-induced senescence, chromatin loading	[59, 60, 62]
Ser 199 phosphorylation	DNA-PK		TIP60 autoacetylation, DNA damage response	[60]
Tyr 294 phosphorylation	Ab1		HAT activity, protein localization, cell cycle regulation, interaction with Fe65	[63]
Lys 104 acetylation	TIP60 (auto-acetylation)		Apoptosis, NuA4 complex partner interaction	[84]
Lys 104, Lys 120, Lys 148, Lys 150, Lys 187, and Lys 189 acetylation	TIP60 (auto-acetylation)	HDAC3, SIRT1	Apoptosis	[83]
Lys 187 acetylation	TIP60 (auto-acetylation)		Nuclear localization, phase separation, HAT activity, DNA damage repair, and wound healing	[29]
Lys 327 acetylation	TIP60 (auto-acetylation), p300		HAT & autoacetylation activity, FOXP3 transcriptional activity	[82, 85]
Lys 268 and Lys 282 acetylation	p300/CBP		Function not known	[86]
Lys 189 Methylation	SET7	LSD1	DNA double strand break repair, Colon cancer cell proliferation	[99]
Lys 430 SUMOylation	PIASγ, Ubc9	SENP3	DNA damage repair, Autophagy	[92, 93, 94, 95]
Lys 451 SUMOylation	PIASγ, Ubc9		DNA damage repair, Autophagy	[92, 93]
Ser 119 O-GlcNAc	(OGT) O-linked N-acetylglucosaminyltransferase		TIP60 stabilization, upregulation of MMP9 & 14 (c-myc mediated), Metastasis (TWIST1 mediated)	[104]
Asn 291 N-glycosylation			TIP60 stability, subcellular localization, HAT activity, interaction with Fe65	[103]
Ubiquitination (sites not known)	MDM2		TIP60 degradation, protein turnover	[111]
Ubiquitination (sites not known)	UHRF1		TIP60 stability, tumor suppression	[112]
Ubiquitination (sites not known)		USP7	Apoptosis	[113]
Lys35, Lys150, Lys243, Lys359, Lys296, and Lys404 Ubiquitination		USP7	Regulates adipogenesis	[114]

recombination repair (HRR), both critical for DSB repair [54]. Additionally, the phosphorylation of Ser 90 is a key component in TIP60-mediated histone acetylation during DNA damage, as Ser 90 mutants exhibit lower levels of H4K16 and H2AZ acetylation compared to wild-type TIP60 [50]. This evidence underscores the pivotal role of Ser 90 phosphorylation in maintaining genomic integrity, particularly through its involvement in chromosomal segregation, DSB repair, and histone acetylation during DNA damage.

Recently the significance of TIP60 Serine 86 phosphorylation has been highlighted in the synthesis of triacylglycerols (TAGs) [55], which serve as the body's primary energy storage molecules. During periods of starvation, TAGs are hydrolyzed to meet the body's energy demands [56]. This phosphorylation enables TIP60 to interact with acetylate lipin1(phosphatidic acid phosphatase), which is necessary for lipin1's translocation to the endoplasmic reticulum. This translocation is a pre-requisite for the conversion of phosphatidic acid to diacylglycerol, a vital step in TAG synthesis [55]. Under dietary stress conditions like a high-fat diet, TIP60 S86A knock-in mice, which have a mutation leading to reduced acetyltransferase activity, exhibited lower body mass and reduced liver TAG levels compared to their wild-type counterparts. This highlights the importance of TIP60's acetyltransferase activity in maintaining normal TAG synthesis and storage and suggests that disruptions to TIP60 function might lead to metabolic alterations and affect how the body handles high-fat diets. In another study, Prisca and colleagues have demonstrated the importance of Ser 90 phosphorylation catalyzed by CDK9, in regulating the association of TIP60 with the transcription machinery [57]. They revealed that a mutation in Ser 90 disrupts TIP60's ability to bind chromatin and decreases its interaction with histone H3 and RNA polymerase II. Additionally, they found that phosphorylation at TIP60 Ser 86 and Ser 90 is essential for preserving TIP60's KAT activity [57]. In a separate study, it was observed that the use of Ser 86 and Ser 90 mutants along with the CDK inhibitor roscovitine led to decreased activation of TIP60 by APP/Fe65, resulting in its enhanced nuclear localization [58].

Similarly, TIP60's Thr 158 residue is of significant importance. Phosphorylation of TIP60 at the Thr 158 residue by p38α (mitogen-activated protein kinase) and VRK1 (vaccinia-related kinase 1) has multiple important biological roles [59, 60]. Hui Zheng's research highlighted the pivotal role of Thr 158 phosphorylation in oncogene-induced senescence, a key defense mechanism that inhibits tumor growth [59]. The proto-oncogene Ras activates p38α [61], which subsequently phosphorylates TIP60 at Thr 158. This, in turn, leads to TIP60 binding and TIP60-mediated acetylation of PRAK (p38-regulated/activated protein kinase) at the K364 position, a crucial step for PRAK's activation and its role in oncogene-induced senescence [59]. In addition to its role in senescence, Thr 158 phosphorylation is also implicated in apoptosis [62]. When cells are treated with doxorubicin, a DNA-damaging agent, p38α phosphorylates TIP60 at Thr 158. This phosphorylated TIP60 aids in the apoptosis process through the p38α-TIP60-p53 axis [62]. Furthermore, Thr 158 phosphorylation also facilitates TIP60's role in DNA damage repair. When cells are exposed to doxorubicin, a DNA-damaging agent, VRK1 kinase phosphorylates TIP60, which subsequently acetylates H4K16, a marker of open chromatin. This acetylation promotes chromatin relaxation, facilitating the interaction of DDR (DNA damage repair) proteins with the damaged DNA [46]. Besides, more recent studies have shown that phosphorylation at Thr 158 mediates TIP60's loading onto chromatin [60]. Additionally, DNA-PK (DNA-dependent protein kinase) phosphorylates chromatin-bound TIP60 at Ser 199, activating TIP60. The activated TIP60 further acetylates H4K16 and ATM (ataxia telangiectasia mutated), thereby regulating the DDR response [60].

Shin and Kang demonstrated the importance of TIP60 tyrosine 294 phosphorylation, in maintaining TIP60's protein interactions, HAT activity and localization [63]. They found that TIP60 needs to be phosphorylated at tyrosine 294 (reported as tyrosine 327) by Ab1 kinase to bind with FE65. Mutating tyrosine 294 to phenylalanine disrupts this interaction. Furthermore, the phosphorylation mutant form shows reduced TIP60 autoacetylation but increased HAT activity, indicating the critical nature of this specific phosphorylation. The phosphorylation at tyrosine 294 also influences TIP60's nuclear localization and facilitates cell cycle progression from the G0 to G1 phase [63]. From these research work, it becomes evident that phosphorylation of TIP60 is vital for mediating crucial cellular functions, such as apoptosis, autophagy, DNA damage response (DDR), cell cycle regulation, and TAG synthesis.

Acetylation

Acetylation is a common PTM in proteins that typically occurs on the lysine residues of a polypeptide chain. This process involves the transfer of an acetyl group from acetyl coenzyme-A to the ε-amino group of a lysine residue. The enzymes responsible for catalyzing these reactions are known as lysine acetyltransferases (KATs). Conversely, the removal of the acetyl group from lysine residues is carried out by enzymes known as lysine deacetylases (KDACs). Acetylation can occur on both histone and non-histone proteins. When it takes place on histone tails, acetylation neutralizes the charge interaction between DNA and histone proteins, leading to the loosening of chromatin [64].

Starting with the discovery of histone acetylation by Allfrey and colleagues in the 1960s, where they proposed its potential role in regulating gene expression [65], the field expanded to include the discovery of acetylation in other important proteins such as HMG (chromatin-binding protein) [66], tubulin, and p53 [67, 68]. In 1995, Kleff and colleagues discovered that HAT1 in yeast has HAT activity and can acetylate histone H4 at Lys12 [69]. A year later, Allis and coworkers identified Gcn5 as a HAT protein and its role in transcription [70]. These discoveries marked the beginning of extensive research into protein acetylation and the enzymes responsible for these reactions. Subsequently the discovery of various histone acetyltransferases (HATs) like CBP, EP300, and MYST further emphasized the importance of acetylation in cellular functions [8, 71–75]. Apart from exploring the role of acetylation in regulating essential processes like gene transcription, DNA damage repair, cell signalling, protein folding, subcellular localization, phase separation, and protein catalytic activity [29, 76–80], the phenomenon of autoacetylation, where proteins can acetylate themselves, was identified.

TIP60 is one such enzyme that can autoacetylate. Martina and colleagues were pioneers in demonstrating that TIP60 undergoes autoacetylation, independently of its interaction with the HIV-1 Tat protein [35]. Later in 2010, Wang and Chen established that TIP60 acetylates itself in response to UV radiation, which augments its interaction with p53. They identified four autoacetylation sites in TIP60 (Lys 76, 80, 189, and 327) using mass spectrometry [81]. Later another study by Yang et al. expanded on this finding by identifying 7 lysine residues in TIP60 that can be auto-acetylated (Lys 76, 80, 104, 150, 187, 327 and 383) [82]. Among these, Lys 327, located near the catalytic pocket, was found to be conserved within all the MYST family members of HATs and plays a crucial role in maintaining the enzyme's HAT and autoacetylation activity [82]. Jingjie and co-workers reported 6 putative autoacetylation sites (Lys 104, 120, 148, 150, 187, and 189) in TIP60 through a unique approach involving the purification of different TIP60 domains followed by an in-vitro autoacetylation assay, which allowed them to discover previously unknown autoacetylation sites [83]. Mutating these sites resulted in no detectable autoacetylation levels in TIP60 autoacetylation mutants *in-cellulo*, compared to the wild-type protein, indicating the importance of these sites for TIP60's autoacetylation [83]. Notably, 4KR mutant of TIP60 (K120R, K148R, K187R, and K189R) failed to acetylate p53 at K120, crucial for p53 activation. Additionally, their research indicated that HDAC3, alongside SIRT1, plays a role in TIP60 deacetylation, leading to an increase in its half-life and ubiquitination. They observed diminished apoptosis in cells after DNA damage when TIP60 was deacetylated by HDAC3 and SIRT1, emphasizing the importance of TIP60's autoacetylation in p53 activation and the cellular apoptosis process [83].

Xiao and colleagues underscored the significance of TIP60's autoacetylation at the Lys104 residue in p53-mediated apoptosis [84]. They observed that under metabolic stress conditions, like glucose deprivation, cells are more inclined to undergo apoptosis, and this process necessitates TIP60-mediated acetylation of p53 at the K120 site. The study further revealed that mutating the Lys104 residue diminishes cellular apoptosis compared to wild-type TIP60, as the mutant fails to acetylate p53 at the K120 site, consequently leading to reduced PUMA expression. Additionally, they showed that K104 is essential for TIP60's interaction with its complex partners [84]. In a separate study, Fang et al. discovered that the autoacetylation of TIP60 at the conserved lysine residue K327 functions as a molecular switch for modulating its binding preference with partner proteins like FOXP3 (a transcription factor that regulates the immune system) [85]. p300-mediated autoacetylation of TIP60 at K327 promotes TIP60's interaction with FOXP3, thereby facilitating FOXP3's acetylation by TIP60 which is necessary for its efficient transcriptional activity [85]. Recently our lab findings highlight the critical role of TIP60's autoacetylation at K187 in its nuclear import, oligomerization, and phase separation [29]. Moreover, autoacetylation at K187 is essential for maintaining TIP60's catalytic activity. When this site is mutated (replacing lysine with arginine), TIP60 loses its ability to autoacetylate and transfer acetyl groups to histones. Besides, this mutant version of TIP60 shows a deficiency in oligomerization and phase separation capabilities. Furthermore, we also demonstrated that this mutant form of TIP60 is functionally compromised, unable to protect cells from DNA damage, and ineffective in promoting wound-induced cell migration in conjunction with the nuclear receptor PXR [29]. In essence, K187 autoacetylation is vital for TIP60's structure, activity, and its role in cellular protection and repair mechanisms.

Beyond TIP60's autoacetylation, research on its acetylation by other acetyltransferases is limited. So far, only two studies have suggested TIP60's acetylation by CBP/p300 [85, 86]. Among them, only one report indicated that CBP/p300 acetylates TIP60 at the K268 and K282 residues [86]. Additionally, it has been demonstrated that p300/CBP enhances the polyubiquitination of TIP60 by HIV-1 Tat [86]. Another study revealed that p300 stimulates TIP60 autoacetylation at the K327 position [85]. However, there is still a lack of information on whether other acetyltransferases are directly acetylating TIP60 or influencing its autoacetylation. In summary, while autoacetylation is a key process for TIP60, more research is needed to fully understand the potential contributions of other acetyltransferases to its regulation and function.

SUMOylation

In 1995, researchers identified SUMOylation through a yeast genetic screen as a modifiable post-translational modification [87–89]. SUMO (small ubiquitin-like modifier) proteins, approximately 10 kDa in size, are encoded by distinct SUMO genes. Humans have four SUMO isoforms: SUMO-1, SUMO-2, SUMO-3, and SUMO-4. While SUMO-1 to SUMO-3 are ubiquitously expressed, SUMO-4 is predominantly expressed in the kidney and spleen [90]. The process of SUMO conjugation to target proteins involves several steps, that involves maturation, activation, conjugation, ligation, and demodification. Initially, a SUMO-specific protease cleaves the immature pro-form of SUMO proteins to activate them and allowing them to attach to the target. The E1 activating enzyme, in an ATP-dependent reaction, then activates the matured SUMO. This activated SUMO is subsequently transferred to the SUMO E2 conjugating enzyme. Finally, the SUMO protein is attached to the lysine residue of the substrate through a reaction catalyzed by the SUMO E3 ligase. Being a reversible post-translational modification, deSUMOylation or the removal of SUMO is mediated by SENP proteins [91]. SUMOylation is crucial for regulating various molecular and cellular processes, such as DNA damage repair, cell cycle progression, and the development of cancer [89].

SUMOylation of TIP60 was initially identified by Cheng et al. in 2008 [92]. They found that the enzyme Ubc9 catalyzes the SUMOylation of TIP60 in response to UV irradiation and identified lysine residues 430 and 451 as the SUMOylation sites under these conditions. Moreover, their research illustrated that SUMOylated TIP60 contributes to the stabilization of the p53 protein via the ATR/chk1 pathway, which in turn increases p21 levels to regulate the cell cycle during UV-induced DNA damage [92]. Subsequent studies revealed that lysine residues 430 and 451 of TIP60 are SUMOylated by the SUMO E3 ligase PIASγ, which triggers p53 acetylation at K120, thereby stimulating autophagy [93]. Further research by Gao et al. indicated that the SUMOylation of TIP60 at lysine 430 is essential for HR-mediated DNA damage repair, showing that this modification reduces the interaction between TIP60 and DNA-PKcs (DNA-dependent protein kinase catalytic subunit) during the S-phase of the cell cycle [94]. PIASγ catalyzes the addition of SUMO2 to TIP60 at K430. TIP60's SUMOylation reduces the phosphorylation of DNA-PKcs at serine 2056, promoting homologous recombination (HR) pathway-dependent repair during the S-phase of the DNA damage response (DDR) [94]. In a follow-up study by the same group it was revealed that under normal conditions, TIP60 is normally highly SUMOylated, however, when cells are exposed to irradiation-induced DNA damage, TIP60 undergoes deSUMOylation at K430, facilitated by SENP3 [95]. This deSUMOylation enables TIP60 to interact with DNA-PKcs, leading to its acetylation and autophosphorylation, which promotes non-homologous end joining (NHEJ)-mediated DNA damage repair [95]. Together, these findings demonstrate that SUMOylation state of TIP60 plays a crucial role in generating appropriate cellular response to DNA damage and stress, particularly safeguarding cells under conditions of UV irradiation and is crucial for maintaining genomic integrity by regulating DNA repair mechanisms.

Methylation

Methylation is a reversible post-translational protein modification that occurs at lysine or arginine residues. This process is catalyzed by methyltransferases (writers) using S-adenosylmethionine (SAM) as a methyl group donor. Distinct methyltransferases are responsible for methylating lysine and arginine. Lysine can undergo mono-, di-, or tri-methylation, primarily facilitated by lysine-specific methyltransferases (PKMTs). In contrast, arginine can be mono- or di-methylated by arginine methyltransferases (PRMTs), with the latter occurring symmetrically or asymmetrically [96, 97]. Methylation can take place at both histone and non-histone proteins, influencing processes such as chromatin remodelling, DNA damage repair, gene transcription, and protein synthesis [98]. To date, only one study has examined the methylation of TIP60. In this study, Kim et al. reported that the SET7 methyltransferase mediates the methylation of TIP60 at lysine 189 (reported as lysine 137 in isoform 3) under DNA damage conditions induced by hydroxyurea (HU) [99]. This modification enhances homologous recombination (HR)-directed double-strand break (DSB) repair. Additionally, it was shown that this methylation of TIP60 at lysine 189 enhances the proliferation of colon cancer cells. This highlights the importance of methylation in regulating TIP60-mediated cellular processes and its potential implications in cancer research.

Glycosylation

Glycosylation is a prevalent and reversible post-translational modification (PTMs) where a glycan (a polysaccharide) is enzymatically added to a protein, either during or after translation [100, 101]. The process is tightly regulated and involves various enzymes. Glycans can be N-linked (N-acetylglucosamine added to asparagine) or O-linked (N-acetylgalactosamine added to serine or threonine). N-linked glycosylation involves the addition of N-acetylglucosamine to the amide group of asparagine, while O-linked glycosylation entails the addition of N-acetylgalactosamine to the hydroxyl groups of serine or threonine [102]. Glycosylation has a profound impact on many critical protein functions, such

as solubility, protein-protein interactions, localization, protein folding, and activity [100, 101]. Lee et al. were the first to report the possible N-glycosylation of TIP60 at asparagine 291 (reported as 324 in isoform 1) [103]. Their findings showed that TIP60's binding to concanavalin A, which is reliant on its glycosylation at the Asn 291 site, is essential under endoplasmic reticulum (ER) stress. This glycosylation influences TIP60's retention in the ER, its stability, localization, histone acetyltransferase (HAT) activity, and interaction with FE65. Nearly a decade later, Liu et al. discovered that OGT (O-GlcNAc transferase) catalyzes the O-GlcNAcylation of TIP60 at serine 119 [104]. Their data revealed that the downregulation of the enzyme PCK-1 (phosphoenolpyruvate carboxykinase-1), which plays a role in hepatic gluconeogenesis, promotes the O-GlcNAcylation of TIP60, which in turn stabilizes TIP60 by inhibiting its ubiquitination and degradation. Moreover, they linked O-GlcNAcylation of TIP60, to the development and metastasis of hepatocellularcarcinoma by showing TIP60's dual role in transcriptional activation of *TWIST1* and c-Myc acetylation [104].

Ubiquitination

About fifty years ago, in the 1970s, scientists discovered a protein called ubiquitin, whose function was initially unknown [105] and it was thought to be ubiquitous (hence the name) because it was found in all living cells [105]. Later research discovered that APF1 (ATP-dependent proteolysis factor-1) is actually ubiquitin, which binds to protein substrates and acts as signal for downstream protease action [106]. Ubiquitination is a reversible post-translational modification involving the addition of ubiquitin moieties (around 8.6 kDa) to proteins, typically marking them for degradation via the proteasome pathway and the process is known as ubiquitin-mediated proteolysis. This process is mediated by action of three enzymes: E1 (ubiquitin-activating enzyme), E2 (ubiquitin-conjugating enzyme), and E3 (ubiquitin-ligating enzyme) [105, 107, 108]. Ubiquitin first binds to E1 using ATP as an energy source, then is transferred to E2, and finally to E3, which attaches ubiquitin to the target protein. Deubiquitinases (DUBs) remove ubiquitin moieties from proteins, regulating protein turnover through ubiquitination-deubiquitination cycles [109, 110].

Legube and colleagues were the first to demonstrate the ubiquitination of TIP60 [111]. They found that Mdm2 (an E3 ubiquitin ligase) binds to TIP60 (within the 258–364 amino acid region) leading to its ubiquitination and subsequent degradation via the proteasomal pathway. Additionally, they observed that TIP60 stabilizes upon UV radiation exposure due to decreased Mdm2 levels [111]. Conversely, in another study, TIP60 was shown to be ubiquitinated by UHRF1, another E3 ligase, which does not result in TIP60 degradation [112], but rather interferes TIP60's interaction with p53, thereby inhibiting processes like growth arrest and apoptosis mediated by p21 and PUMA, respectively. These findings indicated that Ubiquitination plays a significant role in regulating the stability and function of the TIP60 protein. Different E3 ubiquitin ligases, such as Mdm2 and UHRF1, can ubiquitinate TIP60, leading to different outcomes. USP-7 (Ubiquitin-specific protease 7) has been identified as deubiquitinases (DUBs) for TIP60 [113, 114]. USP7-mediated deubiquitination of TIP60, stabilizes TIP60 levels in cells. This stabilization plays crucial roles in different cellular processes, such as apoptosis under conditions of genotoxic stress and early adipogenesis [113, 114]. Through, mass spectrometric analysis, authors identified six lysine residues at position, K35, K150, K243, K359, K296, and K404, as ubiquitination sites on TIP60. Mutating all six lysine residues to arginine did not affect the ubiquitination levels of TIP60. This implies that the ubiquitination of TIP60 might not be confined to specific lysine residues [114]. Interestingly, no study has yet successfully pinpointed the specific ubiquitination sites of TIP60, suggesting that TIP60's ubiquitination may be nonspecific implying at a broader and more flexible mechanism of regulation through ubiquitination.

Cancer-associated mutations and post-translational modifications of TIP60

Generally, TIP60 helps maintain genome stability and regulate gene expression, functioning as a tumor suppressor however atypical expression and deregulated activity of TIP60 have been detected in various cancers [115–123]. In some cancers, such as certain epithelial tumors, in colorectal cancer, down-regulation of TIP60 is associated with larger tumor size, poor differentiation, peritoneal dissemination, distant metastasis, and higher TNM stage [118]. Conversely, in certain types of epithelial tumors, such as those involving overexpression of ornithine decarboxylase (ODC), TIP60 levels are found to be elevated [124]. This overexpression can contribute to abnormal histone acetyltransferase (HAT) activity, potentially promoting tumour development and progression. This dual role of TIP60 underscores its complex involvement in cancer, highlighting its importance in maintaining cellular integrity while also having potential tumor-promoting effects depending on the specific context. To probe into the less-explored aspects of TIP60 in cancer, particularly how cancer-associated mutations might influence TIP60 functions through post-translational modifications, we conducted a comparative analysis of TIP60 residues mutated in different cancers, and compared them against both characterized PTM sites and those reported as PTM sites in various databases but not yet fully characterized (Table 2). We identified seven

Table 2 List of post-translational modification sites on TIP60 identified through various databases

Modification (Phosphorylation/Acetylation/SUMOylation/Ubiquitination)	Databases		
	Phosphosite Plus https://www.phosphosite.org	dbPTM http://dbptm.mbc.nctu.edu.tw	BioGRID https://orcs.thebiogrid.org
Phosphorylation			
S33	-	✓	-
Y44	✓	-	-
T77	-	✓	-
T79	-	✓	-
S86	✓	✓	-
S90	✓	✓	-
S98	-	✓	-
S102	-	✓	-
S155	✓	✓	-
T158	✓	✓	✓
S162	-	✓	-
T164	-	✓	-
S168	-	✓	-
S190	✓	✓	-
T195	✓	✓	-
S199	✓	✓	-
S202	✓	✓	✓
S203	✓	✓	-
S208	-	✓	-
T227	✓	-	-
T281	✓	-	-
Y294	✓	-	-
S431	✓	-	-
Y472	✓	✓	-
Acetylation			
K52	✓	✓	-
K76	-	✓	-
K80	-	✓	-
K104	✓	✓	-
K120	✓	✓	-
K148	✓	✓	-
K150	✓	✓	-
K187	✓	✓	-
K189	✓	✓	-
K268	✓	✓	-
K282	✓	✓	-
K327	✓	✓	-
K383	-	✓	-
K404	-	✓	-
SUMOylation			
K430	✓	✓	-
K451	✓	✓	-
Ubiquitination			
K35	✓	✓	✓
K230	✓	-	✓
K274	✓	-	-

Table 2 (continued)

Modification (Phosphorylation/Acetylation/SUMOylation/Ubiquitination)	Databases		
	Phosphosite Plus https://www.phosphosite.org	dbPTM http://dbptm.mbc.nctu.edu.tw	BioGRID https://orcs.thebiogrid.org
K282	✓	✓	✓
K296	✓	-	✓
K310	✓	-	✓
K404	✓	-	-
K451	-	-	✓
K498	✓	-	-
K505	✓	-	-

Table 3 List of PTM sites on TIP60 reported as mutations in various cancer

S. No.	Mutations	Modification	Cancer	Reference (https://www.cbioportal.org/)
1	K35R	Ubiquitination	Head and neck squamous cell carcinoma	[125, 126]
2	T77A	Phosphorylation	Colorectal adenocarcinoma	125, 126]
3	S155L	Phosphorylation	Cervical squamous cell carcinoma & Bladder urothelial carcinoma	[125, 126]
4	S190L	Phosphorylation	Pancreatic adenocarcinoma	[125, 126]
5	K274N	Ubiquitination	Liver hepatocellular carcinoma	[125, 126]
6	K310N	Ubiquitination	Ovarian serous cystadenocarcinoma & Skin cutaneous melanoma	[125, 126]
7	S431L	Phosphorylation	Uterine corpus endometrial carcinoma	[125, 126]

specific sites in TIP60 associated with cancer mutations [125, 126] that could potentially serve as PTM sites (Table 3). Two of these sites, K35 and T77, are located in the chromodomain, with K35 being a ubiquitination site and T77 a phosphorylation site. Two other sites, S155 and S190 are found in the linker region between the chromodomain and the MYST domain. Both S155 and S190 are phosphorylation sites located in the identified IDR region. The remaining three sites, K274, K310 and S431, are found in the MYST domain, with K274 and K310 being a ubiquitination target and S431 a phosphorylation modification site. Cancer-associated mutations at these PTM sites, can affect TIP60's catalytic activity, substrate preferences, intracellular dynamics, or interactions with binding partners, potentially leading to different cellular outcomes. By disrupting TIP60's normal function, these mutations and the resulting alterations in PTM sites may lead to genomic instability and can contribute to the initiation and progression of cancer. However, all these speculations need to be experimentally verified.

Domain-specific and isoform specific post-translational modifications in TIP60

Functional domains are specific regions of a protein that have distinct structural and functional properties. Since these domains often have unique amino acid sequences and structural characteristics, they can be targeted by different modifying enzymes, leading to various PTMs. PTMs occurring in structurally significant regions such as active sites, binding interfaces, surface exposed residues or regulatory motifs, are more likely to be functionally important, controlling protein function. Understanding the PTMs of specific domains of TIP60 can provide valuable insights into the protein's overall regulation and the roles it plays in different cellular processes. TIP60 has many characteristic domains and while domain structures of the chromodomain [127] and MYST domain (PDB ID: 2OU2) are available, the presence of an intrinsically disordered region (IDR) between these domains complicates crystallization, due to its high flexibility. Cryo-EM studies by different groups have determined the TIP60 complex, yet the TIP60 protein structure itself remains unresolved [13–15]. In the absence of a full-length crystal structure for TIP60, it is difficult to comment on how PTMs and mutations might impact TIP60 protein's structure and functions. However, we know a lot about the different functional domains of TIP60, which are very well characterized both structurally and functionally. On the basis of available information, we can predict the functional outcomes of PTMs occurring within these domains. The chromodomain, also known as the tudor-knot domain, plays a critical role as a reader of the epigenetic code, enabling TIP60 to load onto chromatin [128, 129]. TIP60 specifically anchors to histone H3 tri-methylated at lysine 9 (H3K9me3) during DNA damage, and mutations in the chromodomain

can prevent this anchoring. Research also highlights the importance of the chromodomain for cell survival as deletion or point mutations in this domain can lead to reduced global H4 acetylation and decreased cell numbers [130]. A study by Dubey et al. showed that mutations in the chromodomain can significantly reduce TIP60's chromatin binding, autoacetylation, and HAT activity, impacting TIP60-mediated activation of genes related to wound healing [21]. Therefore, it is plausible that post-translational modifications and cancer-associated mutations in the chromodomain may impact TIP60's interaction with the histone code, which is essential for its chromatin loading.

Another very important domain of TIP60 is the MYST domain which encompasses the catalytic HAT domain, is crucial for its catalytic functions, and interactions with other proteins [17, 20, 29, 85, 131]. PTMs in the MYST domain have a significant impact on its functions. For instance, autoacetylation of TIP60 at lysine 327, promoted by interaction with p300, shifts its interaction towards FOXP3, which is vital for the survival and function of Treg cells [85]. These findings suggest that PTMs in TIP60, as well as variations in its PTMs influenced by interactions with other proteins or mutations, impact the protein's structure, which in turn affects its catalytic activity and overall function.

Elevated frequencies of PTMs within the intrinsically disordered region (IDR) of TIP60 have been noted, including region that contain isoform-specific sites absent in isoform 3 (Fig. 1A). Recent studies indicate that TIP60's IDR is integral to its phase separation [29]. Although IDRs lack stable structures, they are known for weak yet specific interactions with partner proteins [132–135]. Consequently, PTMs associated with TIP60's IDR might enable it to interact with a wide array of partners under varying cellular conditions by providing local stability to its disordered nature, acting as a scaffold for the binding of other proteins.

TIP60 is encoded by 14 exons, producing three main isoforms through alternative mRNA splicing [30, 31]. The canonical isoform, isoform 2, consists of 513 amino acids. Isoform 1 includes all 14 exons plus an intron, while isoforms 2 and 3 lack the intron region [31–33, 73]. Notably, isoform 3 specifically lacks exon 5, which is a proline-rich region (Fig. 1B). While various functions of TIP60 have been identified, there remains a gap in understanding its cell-type and tissue-specific roles, particularly in relation to its isoforms and their post-translational modifications, given they all share conserved domains. By analyzing the tissue-specific gene expression data for TIP60 and its isoforms from the GTEx portal, *[data source: GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2)]*, we observed distinct differences in TIP60 isoform expression patterns across different tissues (Fig. 3). While most tissues primarily express isoform 2, isoforms 1 and 3 show variations in tissue-specific manner. Isoforms are variations of a protein and can have different amino acid sequences and structural features, which can influence the sites as well as types of PTMs they receive. These differences in PTMs can lead to variations in the protein's function, stability, localization, and interactions with other molecules. For instance, a study suggested that TIP60 isoform 2 is localized in the nucleus, whereas isoform 3 can be found in both the nucleus and cytoplasm [31]. K104, K120, and S119 residues which are validated PTM sites in TIP60 are present in isoform 1 and 2 but are absent in isoform 3, may possibly be playing a role in influencing isoform localization and expression levels. Additionally, TIP60 is known to form a complex (TIP60 complex); therefore, isoform 2, which remains in the nucleus, might serve as a complex partner, whereas isoform 3, present in both the nucleus and cytoplasm, could interact with cytoplasmic proteins and function independently. Understanding the cell- and tissue specific variations in different isoforms of TIP60, would be crucial for comprehending the complexity of TIP60 protein regulation and the diverse roles that proteins can play in different cellular contexts.

Present gaps and future directions

Despite current technological advancements, which have significantly enhanced the process of PTM identification, our comprehension of the precise conditions and mechanisms under which TIP60 undergoes specific modifications are not fully understood. The influence of different environmental or cellular contexts on TIP60's PTMs needs further exploration. Comprehensive mapping of all potential PTMs on TIP60 across various tissues and conditions is lacking, and the functional consequences of these modifications on TIP60's activity, interactions, and stability are not fully elucidated. Additionally, the interplay between different PTMs on TIP60 is not well understood, necessitating studies on how one modification may influence another. There is also limited knowledge on the differential regulation of TIP60 isoforms by PTMs and their functional impacts. Most studies have been conducted in vitro, highlighting the need for in vivo validation. The specific role of TIP60's PTMs in disease pathogenesis is not fully explored, and the potential for therapeutic targeting of these modifications remains underexplored. Addressing these gaps is crucial for a comprehensive understanding of TIP60's regulation and its implications for cellular function and various diseases.

Another interesting aspect of post-translational modifications is that identical residues can undergo multiple modifications that might interact, conflict, or exhibit context-dependent effects based on their chemistry

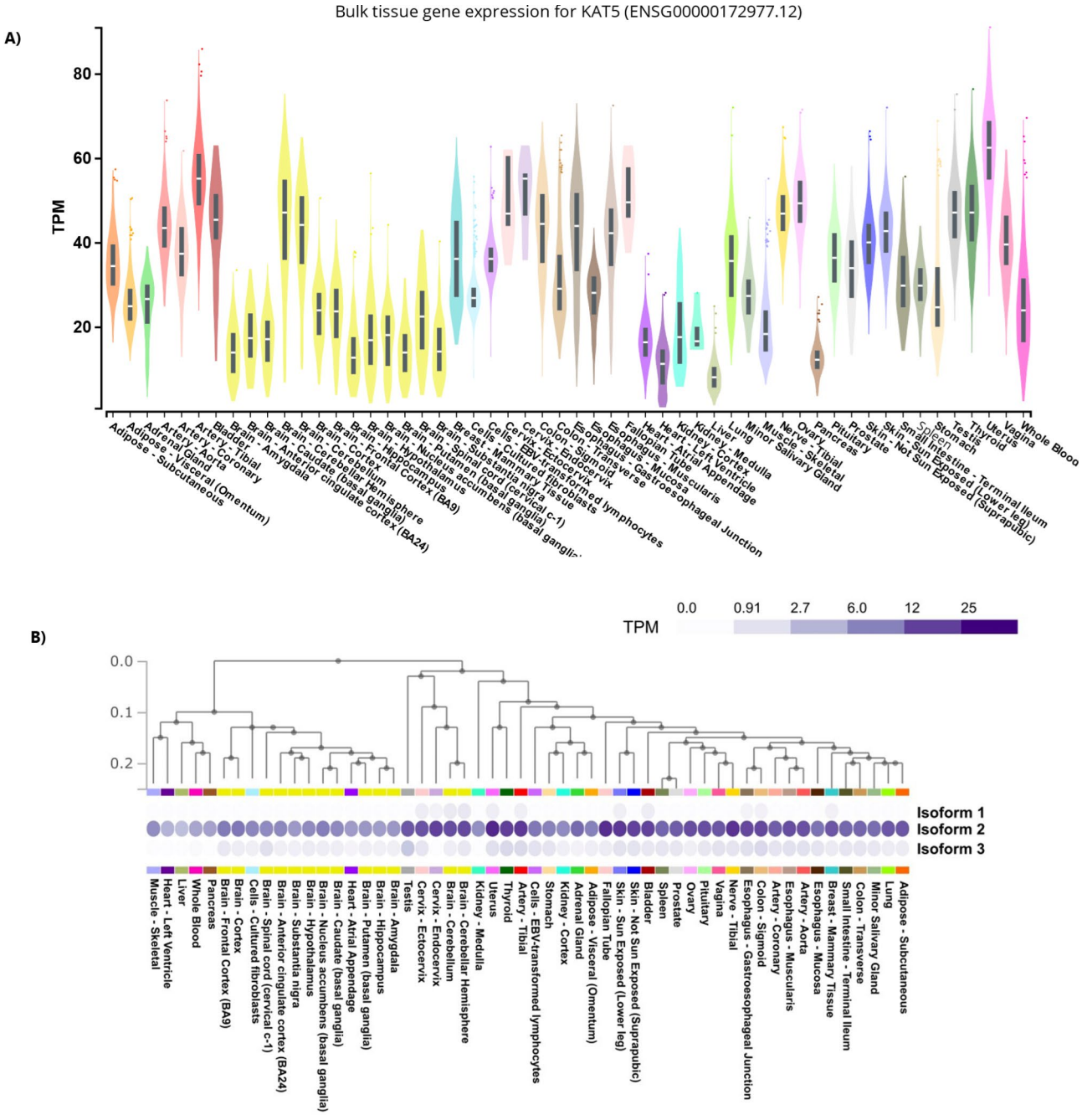

Fig. 3 TIP60 expression profile. (**A**) Violin plot depicts tissue-wise expression of TIP60 (data source: GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2)). Expression values are shown in transcripts per million (TPM). The data of violin plots are displayed for the median, 25th percentile, and 75th percentile. Points that fall above or below 1.5 times the interquartile range are identified as outliers. (**B**) Heat map depicts the TIP60 isoform-specific expression among different tissue samples. The expression values are displayed as transcripts per million (TPM) (data source: GTEx Analysis Release V8 (dbGaP Accession phs000424.v8.p2)). Figures are modified using INKSCPAE 1.2

and biological conditions [136, 137]. For instance, the K189 residue in TIP60 can be both acetylated or methylated. Specifically, acetylation of TIP60 at K189 has been observed in HEK293 (human embryonic kidney) cells, whereas methylation at the same residue has been reported in HCT116 (human colorectal cancer) cells [83, 99]. Due to the use of different cell lines in these studies, it remains inconclusive whether these distinct PTMs on the same residue are cell-specific or could occur simultaneously or sequentially in a context-dependent manner within the same cell. Further studies are needed to determine whether these modifications are mutually exclusive or can coexist in a context-dependent manner in the same cellular environment. Recent research has revealed

the presence of acetyl-methyllysine (Kacme), a newly identified post-translational modification, challenging the typical exclusivity of acetylation and methylation [138]. This novel PTM has been specifically observed on histone H4, with evidence suggesting that the information encoded by Kacme is distinct from the information encoded by either methylation or acetylation alone. This emergent aspect of PTMs warrants further investigation, especially in relation to the regulation of TIP60. Moreover, it is crucial to explore the interplay between acetylation and ubiquitination. While acetylation enhances TIP60's histone acetyltransferase activity, ubiquitination reduces its activity and promotes its degradation [112]. Both modifications target lysine residues, raising intriguing questions about whether they compete for the same sites. Additionally, it is essential to determine the factors that lead to a functional trade-off between activation and degradation when the same TIP60 molecule undergoes both acetylation and ubiquitination. This balance and its regulatory cues present compelling areas for future research. Recently, we have demonstrated that TIP60 undergoes oligomerization [29], this will be intriguing to explore if various subunits of the oligomer could experience different post-translational modifications either sequentially or concurrently. We postulate that TIP60's susceptibility to PTMs might be affected by the specific tissue where it is expressed, as well as the predominant isoform in that tissue. For instance, Isoform 3, can localize both in the nucleus and cytoplasm, and intracellular localization of SET7, the enzyme responsible for catalyzing TIP60 methylation at K189 also varies depending on the tissue of expression [139]. The tissue-specific differential localization of SET7 could lead to differential methylation of TIP60 isoforms in various tissues, adding another layer of complexity to the regulation of TIP60's PTMs.

Many residues of TIP60 could potentially serve as sites for PTMs, but remain uncharacterized. Researchers from different labs have utilized mass spectrometric analysis, combining curated data with mathematical modelling, to create online databases storing details of known PTMs for all proteins. In this review, using three different databases: Phosphosite Plus, dbPTM, and BioGRID: https://www.phosphosite.org, http://dbptm.mbc.nctu.edu.tw, and https://orcs.thebiogrid.org, we have identified PTMs on TIP60 including the uncharacterized sites that may potentially undergo post-translational modifications (Table 2). Understanding these post-translational modifications could shed light on the previously undiscovered functions of the TIP60 protein.

Conclusions

Selective PTM of targeted residues may result from the combined effects of structural accessibility, enzyme specificity, functional importance, competition with other modifications, cellular conditions, and regulatory networks. To ensure that modification occurs precisely where and when it is needed for proper protein regulation. Aberrant PTMs are often linked to diseases like cancer, neurodegenerative disorders, and diabetes. For example, hyper phosphorylation of tau protein is associated with Alzheimer's disease [140]. By systematically studying PTMs within defined domains, altered modifications in disease states can be identified, leading to identification of potential biomarkers or therapeutic targets. Overall, this review sheds light on TIP60's PTMs and their implications in its diverse cellular functions expanding our comprehension of about how these PTM can alter TIP60's functions under different cellular context. It also introduces some new potential PTMs/sites on TIP60 and provides a comparative analysis revealing how certain cancer-associated mutations might impact PTM sites on TIP60, consequently affecting its functionality. Despite these insights, our knowledge of the full significance of these PTMs is still limited, and the enzymes responsible for these modifications often remain unidentified, possibly due to their condition-specific or tissue-specific expression patterns. Future research should focus on developing innovative methods to detect transient post-translational changes in this vital multifunctional protein, to examine the impact of these PTMs on TIP60 itself, as well as on the structure and function of the TIP60 complex and its associated subunits.

Abbreviations

TIP60	Tat interactive protein of 60kDa
PTM	Post-translational modification
NR	Nuclear receptor
CD	Chromodomain
HAT	Histone acetyltransferase
AR	Androgen receptor
PXR	Pregnane & xenobiotic receptor
MDM2	Mouse double minute 2 homolog
DNA	Deoxy-ribonucleic acid
UHRF1	Ubiquitin-like with PHD and RING finger domains1

Acknowledgements

AG and HG thank SNIoE for all the necessary infrastructure and resources. HG acknowledges ICMR for Senior Research Fellowship while AG acknowledge SERB TARE fellowship.

Author contributions

H.G.- data collection, analysis, writing original draft and editing, prepared figures and tables, preparation of final draft. A.G.- conceptualization and design of the study, data analysis, writing original draft and editing, preparation of final draft.

Funding

No funding is available for this study.

Data availability

No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Received: 13 October 2024 / Accepted: 27 January 2025
Published online: 04 April 2025

References

1. Choudhary C, Mann M. Decoding signalling networks by mass spectrometry-based proteomics. Nat Rev Mol Cell Biol. 2010;11:427–39.
2. Ramazi S, Zahiri J. Posttranslational modifications in proteins: resources, tools and prediction methods. Database (Oxford). 2021, (2021).
3. Audagnotto M, Dal Peraro M. Protein post-translational modifications: in silico prediction tools and molecular modeling. Comput Struct Biotechnol J. 2017;15:307–19.
4. Ryšlavá H, Doubnerová V, Kavan D, Vaněk O. Effect of posttranslational modifications on enzyme function and assembly. J Proteom. 2013;92:80–109.
5. Luo Y-Y, Wu J-J, Li Y-M. Regulation of liquid–liquid phase separation with focus on post-translational modifications. Chem Commun. 2021;57:13275–87.
6. Ohtsubo K, Marth JD. Glycosylation in cellular mechanisms of health and disease. Cell. 2006;126:855–67.
7. Choi H-K, et al. Protein kinase A phosphorylates NCoR to enhance its nuclear translocation and repressive function in human prostate cancer cells. J Cell Physiol. 2013;228:1159–65.
8. Yamamoto T, Horikoshi M. Novel substrate specificity of the histone acetyltransferase activity of HIV-1-Tat interactive protein Tip60. J Biol Chem. 1997;272:30595–8.
9. Gorrini C, et al. Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response. Nature. 2007;448:1063–7.
10. Yang X-J. The diverse superfamily of lysine acetyltransferases and their roles in leukemia and other diseases. Nucleic Acids Res. 2004;32:959–76.
11. Kamine J, Elangovan B, Subramanian T, Coleman D, Chinnadurai G. Identification of a Cellular protein that specifically interacts with the essential Cysteine Region of the HIV-1 Tat Transactivator. Virology. 1996;216:357–66.
12. Ikura T, et al. Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis. Cell. 2000;102:463–73.
13. Chen K, et al. Structure of the human TIP60 complex. Nat Commun. 2024;15:7092.
14. Yang Z, et al. Structural insights into the human NuA4/TIP60 acetyltransferase and chromatin remodeling complex. Science. 2024;385:eadl5816.
15. Li C, et al. Structure of human TIP60-C histone exchange and acetyltransferase complex. Nature. 2024. https://doi.org/10.1038/s41586-024-08011-w.
16. Hu Y, et al. Homozygous disruption of the Tip60 gene causes early embryonic lethality. Dev Dyn off Publ Am Assoc Anat. 2009;238:2912–21.
17. Sun Y, Jiang X, Chen S, Fernandes N, Price BD. A role for the Tip60 histone acetyltransferase in the acetylation and activation of ATM. Proc Natl Acad Sci. 2005;102:13182–7.
18. Wu W et al. Tip60 phosphorylation at ser 99 is essential for Autophagy induction in Bombyx mori. Int J Mol Sci 21, (2020).
19. Nie T, et al. Regulation of ER stress-induced autophagy by GSK3β-TIP60-ULK1 pathway. Cell Death Dis. 2016;7:e2563.
20. Bakshi K, et al. Novel complex of HAT protein TIP60 and nuclear receptor PXR promotes cell migration and adhesion. Sci Rep. 2017;7:3635.
21. Dubey S, Jaiswal B, Gupta A. TIP60 acts as a regulator of genes involved in filopodia formation and cell migration during wound healing. J Biol Chem. 2022;298:102015.
22. Song X, et al. Dynamic crotonylation of EB1 by TIP60 ensures accurate spindle positioning in mitosis. Nat Chem Biol. 2021;17:1314–23.
23. Tang Y, Luo J, Zhang W, Gu W. Tip60-Dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis. Mol Cell. 2006;24:827–39.
24. Mo F, et al. Acetylation of Aurora B by TIP60 ensures accurate chromosomal segregation. Nat Chem Biol. 2016;12:226–32.
25. Akbar H, et al. Acetylation of Nup62 by TIP60 ensures accurate chromosome segregation in mitosis. J Mol Cell Biol. 2022;14:mjac056.
26. Brady ME, et al. Tip60 is a nuclear hormone receptor Coactivator. J Biol Chem. 1999;274:17599–604.
27. Gaughan L, Brady ME, Cook S, Neal DE, Robson CN. Tip60 is a Co-activator Specific for Class I Nuclear hormone Receptors*. J Biol Chem. 2001;276:46841–8.
28. Ihunnah CA, Jiang M, Xie W. Nuclear receptor PXR, transcriptional circuits and metabolic relevance. Biochim Biophys Acta - Mol Basis Dis. 2011;1812:956–63.
29. Dubey S, Gupta H, Gupta A. Autoacetylation-mediated phase separation of TIP60 is critical for its functions. https://doi.org/10.7554/eLife.93418.1
30. Sapountzi V, Logan IR, Robson CN. Cellular functions of TIP60. Int J Biochem Cell Biol. 2006;38:1496–509.
31. Ran Q, Pereira-Smith OM. Identification of an alternatively spliced form of the Tat Interactive protein (Tip60), Tip60(β). Gene. 2000;258:141–6.
32. Legube G, Trouche D. Identification of a larger form of the histone acetyl transferase Tip60. Gene. 2003;310:161–8.
33. Sheridan AM, et al. PLIP, a novel splice variant of Tip60, interacts with group IV cytosolic phospholipase A(2), induces apoptosis, and potentiates prostaglandin production. Mol Cell Biol. 2001;21:4470–81.
34. Sun Y, Jiang X, Price BD. Tip60: connecting chromatin to DNA damage signaling. Cell Cycle. 2010;9:930–6.
35. Creaven M, et al. Control of the histone-acetyltransferase activity of Tip60 by the HIV-1 transactivator protein, Tat. Biochemistry. 1999;289:8826–30.
36. Jaiswal B, Gupta A. Modulation of nuclear receptor function by chromatin modifying factor TIP60. Endocrinology. 2018;159:2199–215.
37. Duan G, Walther D. The roles of post-translational modifications in the context of protein interaction networks. PLoS Comput Biol. 2015;11:e1004049.
38. Panni S. Phospho-peptide binding domains in S. Cerevisiae model organism. Biochimie. 2019;163:117–27.
39. Skamnaki VT, et al. Catalytic mechanism of phosphorylase kinase probed by mutational studies. Biochemistry. 1999;38:14718–30.
40. Devaiah BN, et al. MYC protein stability is negatively regulated by BRD4. Proc Natl Acad Sci U S A. 2020;117:13457–67.
41. Gjertsen BT, Døskeland SO. Protein phosphorylation in apoptosis. Biochim Biophys Acta. 1995;1269:187–99.
42. Kwon YG, Lee SY, Choi Y, Greengard P, Nairn AC. Cell cycle-dependent phosphorylation of mammalian protein phosphatase 1 by cdc2 kinase. Proc. Natl. Acad. Sci. U. S. A. 1997;94:2168–2173.
43. Moutal A, et al. CRMP2 phosphorylation drives Glioblastoma Cell Proliferation. Mol Neurobiol. 2018;55:4403–16.
44. Olsen JV, et al. Global, in vivo, and site-specific phosphorylation dynamics in signaling networks. Cell. 2006;127:635–48.
45. Simke WC et al. Phosphorylation of RGS regulates MAP kinase localization and promotes completion of cytokinesis. Life Sci Alliance 5, (2022).
46. García-González R, Morejón-García P, Campillo-Marcos I, Salzano M, Lazo PA. VRK1 phosphorylates Tip60/KAT5 and is required for H4K16 acetylation in response to DNA damage. Cancers (Basel). 12, (2020).
47. Lin S-Y, et al. GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy. Science. 2012;336:477–81.
48. Lemercier C, et al. Tip60 acetyltransferase activity is controlled by phosphorylation. J Biol Chem. 2003;278:4713–8.
49. Charvet C, et al. Phosphorylation of Tip60 by GSK-3 determines the induction of PUMA and apoptosis by p53. Mol Cell. 2011;42:584–96.
50. Li ML et al. Phosphorylation of TIP60 suppresses 53BP1 localization at DNA damage sites. Mol Cell Biol 39, (2019).
51. De Marco Zompit M, Stucki M. Mechanisms of genome stability maintenance during cell division. DNA Repair (Amst). 2021;108:103215.
52. Luna-Maldonado F, Andonegui-Elguera MA, Díaz-Chávez J, Herrera LA. Mitotic and DNA damage response proteins: maintaining the Genome Stability and Working for the Common Good. Front Cell Dev Biol 9, (2021).
53. Krenn V, Musacchio A, The Aurora B. Kinase in chromosome bi-orientation and spindle checkpoint signaling. Front Oncol 5, (2015).
54. Georgieva D et al. BRCA1 and 53BP1 regulate reprogramming efficiency by mediating DNA repair pathway choice at replication-associated double-strand breaks. Cell Rep 43, (2024).
55. Li TY, et al. Tip60-mediated lipin 1 acetylation and ER translocation determine triacylglycerol synthesis rate. Nat Commun. 2018;9:1916.
56. Coleman RA, Mashek DG. Mammalian triacylglycerol metabolism: synthesis, lipolysis, and signaling. Chem Rev. 2011;111:6359–86.

57. Brauns-Schubert P, et al. CDK9-mediated phosphorylation controls the interaction of TIP60 with the transcriptional machinery. EMBO Rep. 2018;19:244–56.
58. Hass MR, Yankner BA. A {gamma}-secretase-independent mechanism of signal transduction by the amyloid precursor protein. J Biol Chem. 2005;280:36895–904.
59. Zheng H, et al. A posttranslational modification cascade involving p38, Tip60, and PRAK mediates oncogene-induced senescence. Mol Cell. 2013;50:699–710.
60. García-González R, Monte-Serrano E, Morejón-García P, Navarro-Carrasco E, Lazo PA. The VRK1 chromatin kinase regulates the acetyltransferase activity of Tip60/KAT5 by sequential phosphorylations in response to DNA damage. Biochim Biophys Acta Gene Regul Mech. 2022;1865:194887.
61. Chen G, Hitomi M, Han J, Stacey DW. The p38 pathway provides negative feedback for ras proliferative Signaling*. J Biol Chem. 2000;275:38973–80.
62. Xu Y, Liao R, Li N, Xiang R, Sun P. Phosphorylation of Tip60 by p38α regulates p53-mediated PUMA induction and apoptosis in response to DNA damage. Oncotarget. 2014;5:12555–72.
63. Shin SH, Kang SS. Phosphorylation of Tip60 tyrosine 327 by Abl Kinase Inhibits HAT activity through Association with FE65. Open Biochem J. 2013;7:66–72.
64. Bannister AJ, Kouzarides T. Regulation of chromatin by histone modifications. Cell Res. 2011;21:381–95.
65. Allfrey VG, Faulkner R, Mirsky AE, Acetylation and methylation of histones, and their possible role in the regulation of rna synthesis. Proc. Natl. Acad. Sci. U. S. A. 1964;51:786–794.
66. Sterner R, Vidali G, Allfrey VG. Studies of acetylation and deacetylation in high mobility group proteins. Identification of the sites of acetylation in HMG-1. J Biol Chem. 1979;254:11577–83.
67. L'Hernault SW, Rosenbaum JL. Chlamydomonas alpha-tubulin is post-translationally modified in the flagella during flagellar assembly. J Cell Biol. 1983;97:258–63.
68. Gu W, Roeder RG. Activation of p53 sequence-specific DNA binding by acetylation of the p53 C-terminal domain. Cell. 1997;90:595–606.
69. Kleff S, Andrulis ED, Anderson CW, Sternglanz R. Identification of a gene encoding a yeast histone H4 acetyltransferase. J Biol Chem. 1995;270:24674–7.
70. Brownell JE, et al. Tetrahymena histone acetyltransferase A: a homolog to yeast Gcn5p linking histone acetylation to gene activation. Cell. 1996;84:843–51.
71. Borrow J, et al. The translocation t(8;16)(p11;p13) of acute myeloid leukaemia fuses a putative acetyltransferase to the CREB-binding protein. Nat Genet. 1996;14:33–41.
72. Reifsnyder C, Lowell J, Clarke A, Pillus L. Yeast SAS silencing genes and human genes associated with AML and HIV-1 Tat interactions are homologous with acetyltransferases. Nat Genet. 1996;14:42–9.
73. Hilfiker A, Hilfiker-Kleiner D, Pannuti A, Lucchesi JC. Mof, a putative acetyl transferase gene related to the Tip60 and MOZ human genes and to the SAS genes of yeast, is required for dosage compensation in Drosophila. EMBO J. 1997;16:2054–60.
74. Mizzen CA, et al. The TAF(II)250 subunit of TFIID has histone acetyltransferase activity. Cell. 1996;87:1261–70.
75. Ogryzko VV, Schiltz RL, Russanova V, Howard BH, Nakatani Y. The transcriptional coactivators p300 and CBP are histone acetyltransferases. Cell. 1996;87:953–9.
76. Reed SM, Quelle DE. p53 acetylation: regulation and consequences. Cancers (Basel). 2014;7:30–69.
77. Kawasumi R, et al. ESCO1/2's roles in chromosome structure and interphase chromatin organization. Genes Dev. 2017;31:2136–50.
78. Jacquet K, et al. The TIP60 complex regulates bivalent chromatin recognition by 53BP1 through direct H4K20me binding and H2AK15 acetylation. Mol Cell. 2016;62:409–21.
79. Okumura K, et al. PCAF modulates PTEN activity. J Biol Chem. 2006;281:26562–8.
80. Bock AS, et al. N-terminal acetylation modestly enhances phase separation and reduces aggregation of the low-complexity domain of RNA-binding protein fused in sarcoma. Mol Cell. 2000;30:601–7.
81. Wang J, Chen J. SIRT1 regulates autoacetylation and histone acetyltransferase activity of TIP60. J Biol Chem. 2010;285:11458–64.
82. Yang C, Wu J, Zheng YG. Function of the active site lysine autoacetylation in Tip60 catalysis. PLoS ONE. 2012;7:e32886.
83. Yi J, et al. Regulation of histone acetyltransferase TIP60 function by histone deacetylase 3. J Biol Chem. 2014;289:33878–86.
84. Fang X, et al. Acetylation of TIP60 at K104 is essential for metabolic stress-induced apoptosis in cells of hepatocellular cancer. Exp Cell Res. 2018;362:279–86.
85. Xiao Y, et al. Dynamic interactions between TIP60 and p300 regulate FOXP3 function through a Structural switch defined by a single lysine on TIP60. Cell Rep. 2014;7:1471–80.
86. Col E, et al. HIV-1 Tat targets Tip60 to impair the apoptotic cell response to genotoxic stresses. EMBO J. 2005;24:2634–45.
87. Meluh PB, Koshland D. Evidence that the MIF2 gene of Saccharomyces cerevisiae encodes a centromere protein with homology to the mammalian centromere protein CENP-C. Mol Biol Cell. 1995;6:793–807.
88. Okura T, et al. Protection against Fas/APO-1- and tumor necrosis factor-mediated cell death by a novel protein, sentrin. J Immunol. 1996;157:4277–81.
89. Celen AB, Sahin U. Sumoylation on its 25th anniversary: mechanisms, pathology, and emerging concepts. FEBS J. 2020;287:3110–40.
90. Acuña ML, et al. Alternative splicing of the SUMO1/2/3 transcripts affects cellular SUMOylation and produces functionally distinct SUMO protein isoforms. Sci Rep. 2023;13:2309.
91. Kunz K, Piller T, Müller S. SUMO-specific proteases and isopeptidases of the SENP family at a glance. J Cell Sci. 2018;131:jcs211904.
92. Cheng Z, et al. Functional characterization of TIP60 sumoylation in UV-irradiated DNA damage response. Oncogene. 2008;27:931–41.
93. Naidu SR, Lakhter AJ, Androphy EJ. PIASy-mediated Tip60 sumoylation regulates p53-induced autophagy. Cell Cycle. 2012;11:2717–28.
94. Gao S-S, et al. TIP60 K430 SUMOylation attenuates its interaction with DNA-PKcs in S-phase cells: facilitating homologous recombination and emerging target for cancer therapy. Sci Adv. 2020;6:eaba7822.
95. Han Y et al. SENP3-mediated TIP60 deSUMOylation is required for DNA-PKcs activity and DNA damage repair. MedComm 2022;3:e123.
96. Lee DY, Teyssier C, Strahl BD, Stallcup MR. Role of protein methylation in regulation of transcription. Endocr Rev. 2005;26:147–70.
97. Lyko F. The DNA methyltransferase family: a versatile toolkit for epigenetic regulation. Nat Rev Genet. 2018;19:81–92.
98. Biggar KK, Li SS-C. Non-histone protein methylation as a regulator of cellular signalling and function. Nat Rev Mol Cell Biol. 2015;16:5–17.
99. Kim SH, et al. SET7-mediated TIP60 methylation is essential for DNA double-strand break repair. BMB Rep. 2022;55:541–6.
100. Dwek RA. Biological importance of glycosylation. Dev Biol Stand. 1998;96:43–7.
101. Wandall HH, Nielsen MAI, King-Smith S, de Haan N, Bagdonaite I. Global functions of O-glycosylation: promises and challenges in O-glycobiology. FEBS J. 2021;288:7183–212.
102. Abou-Abbass H, et al. Glycosylation and other PTMs alterations in neurodegenerative diseases: current status and future role in neurotrauma. Electrophoresis. 2016;37:1549–61.
103. Lee EJ, Shin SH, Hyun S, Chun J, Kang SS. Endoplasmic reticulum (ER) stress enhances Tip60 (a histone acetyltransferase) binding to the Concanavalin A. 2012;60:1–10.
104. Liu R, et al. O-GlcNAc modified-TIP60/KAT5 is required for PCK1 deficiency-induced HCC metastasis. Oncogene. 2021;40:6707–19.
105. Ciechanover A. The unravelling of the ubiquitin system. Nat Rev Mol Cell Biol. 2015;16:322–4.
106. Ciechanover A, Elias S, Heller H, Ferber S, Hershko A. Characterization of the heat-stable polypeptide of the ATP-dependent proteolytic system from reticulocytes. J Biol Chem. 1980;255:7525–8.
107. Ciehanover A, Hod Y, Hershko A. A heat-stable polypeptide component of an ATP-dependent proteolytic system from reticulocytes. Biochem Biophys Res Commun. 1978;81:1100–5.
108. Ciechanover A. Proteolysis: from the lysosome to ubiquitin and the proteasome. Nat Rev Mol Cell Biol. 2005;6:79–87.
109. Damgaard RB. The ubiquitin system: from cell signalling to disease biology and new therapeutic opportunities. Cell Death Differ. 2021;28:423–6.
110. Gao M, Karin M. Regulating the regulators: Control of Protein Ubiquitination and ubiquitin-like modifications by Extracellular Stimuli. Mol Cell. 2005;19:581–93.
111. Legube G, et al. Tip60 is targeted to proteasome-mediated degradation by Mdm2 and accumulates after UV irradiation. EMBO J. 2002;21:1704–12.
112. Dai C, Shi D, Gu W. Negative regulation of the acetyltransferase TIP60-p53 interplay by UHRF1 (ubiquitin-like with PHD and RING finger domains 1)*. J Biol Chem. 2013;288:19581–92.

113. Dar A, Shibata E, Dutta A. Deubiquitination of Tip60 by USP7 determines the activity of the p53-dependent apoptotic pathway. Mol Cell Biol. 2013;33:3309–20.
114. Gao Y, et al. Early adipogenesis is regulated through USP7-mediated deubiquitination of the histone acetyltransferase TIP60. Nat Commun. 2013;4:2656.
115. Zhang Y, et al. TIP60 inhibits metastasis by ablating DNMT1– SNAIL2-driven epithelial-mesenchymal transition program. J Mol Cell Biol. 2016;8:384–99.
116. McGuire A, et al. Quantifying Tip60 (Kat5) stratifies breast cancer. Sci Rep. 2019;9:3819.
117. Mattera L, et al. The p400/Tip60 ratio is critical for colorectal cancer cell proliferation through DNA damage response pathways. Oncogene. 2009;28:1506–17.
118. Sakuraba K, et al. Down-regulation of Tip60 gene as a potential marker for the malignancy of Colorectal Cancer. Anticancer Res. 2009;29:3953–5.
119. Chen G, Cheng Y, Tang Y, Martinka M, Li G. Role of Tip60 in Human Melanoma Cell Migration, Metastasis, and patient survival. J Invest Dermatol. 2012;132:2632–41.
120. Zhang Y, et al. Tip60 suppresses Cholangiocarcinoma Proliferation and Metastasis via PI3k-AKT. Cell Physiol Biochem. 2018;50:612–28.
121. Bassi C, et al. The acetyltransferase Tip60 contributes to mammary tumorigenesis by modulating DNA repair. Cell Death Differ. 2016;23:1198–208.
122. Jha S, et al. Destabilization of TIP60 by human papillomavirus E6 results in attenuation of TIP60-Dependent transcriptional regulation and apoptotic pathway. Mol Cell. 2010;38:700–11.
123. Sakuraba K, et al. TIP60 as a potential marker for the malignancy of gastric cancer. Anticancer Res. 2011;31:77–9.
124. Hobbs CA, et al. Tip60 protein isoforms and altered function in skin and tumors that overexpress ornithine decarboxylase. Cancer Res. 2006;66:8116–22.
125. Cerami E, et al. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov. 2012;2:401–4.
126. Gao J et al. Integrative Analysis of Complex Cancer Genomics and Clinical profiles using the cBioPortal. Sci Signal 6, (2013).
127. Zhang Y, et al. Structural and histone binding studies of the chromo barrel domain of TIP60. FEBS Lett. 2018;592:1221–32.
128. Kim C-H, et al. The chromodomain-containing histone acetyltransferase TIP60 acts as a code reader, recognizing the epigenetic codes for initiating transcription. Biosci Biotechnol Biochem. 2015;79:532–8.
129. Sun Y, et al. Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60. Nat Cell Biol. 2009;11:1376–82.
130. Xuan F, et al. The tudor-knot domain of KAT5 regulates nucleosomal substrate acetylation. J Mol Biol. 2024;436:168414.
131. Zhao H, Jin S, Gewirtz AM. The histone acetyltransferase TIP60 interacts with c-Myb and inactivates its transcriptional activity in human leukemia. J Biol Chem. 2012;287:925–34.
132. Trivedi R, Nagarajaram HA. Intrinsically disordered proteins: an overview. Int J Mol Sci 23, (2022).
133. Cermakova K, Hodges HC. Interaction modules that impart specificity to disordered protein. Trends Biochem Sci. 2023;48:477–90.
134. Protter DSW, et al. Intrinsically disordered regions can contribute promiscuous interactions to RNP Granule Assembly. Cell Rep. 2018;22:1401–12.
135. Macossay-Castillo M, et al. The Balancing Act of intrinsically disordered proteins: enabling functional diversity while minimizing Promiscuity. J Mol Biol. 2019;431:1650–70.
136. Javaid N, Choi S. Acetylation- and Methylation-Related Epigenetic Proteins in the context of their targets. Genes (Basel). 8, (2017).
137. Liu Y, Tavana O, Gu W. p53 modifications: exquisite decorations of the powerful guardian. J Mol Cell Biol. 2019;11:564–77.
138. Lu-Culligan WJ, et al. Acetyl-methyllysine marks chromatin at active transcription start sites. Nature. 2023;622:173–9.
139. Daks A, Vasileva E, Fedorova O, Shuvalov O, Barlev NA. The role of lysine methyltransferase SET7/9 in proliferation and cell stress response. Life (Basel Switz) 12, (2022).
140. Wang J-Z, Xia Y-Y, Grundke-Iqbal I, Iqbal K. Abnormal hyperphosphorylation of tau: sites, regulation, and molecular mechanism of neurofibrillary degeneration. J Alzheimers Dis. 2013;33(Suppl 1):S123–39.

Publisher's note