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Acetylation of TIP60 at K104 is essential for metabolic stress-induced apoptosis in cells of hepatocellular cancer

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Abstract

Tumor cells often encounter hypoglycemic microenvironment due to rapid cell expansion. It remains elusive how tumors reprogram the genome to survive the metabolic stress. The tumor suppressor TIP60 functions as the catalytic subunit of the human NuA4 histone acetyltransferase (HAT) multi-subunit complex and is involved in many different cellular processes including DNA damage response, cell growth and apoptosis. Attenuation of TIP60 expression has been detected in various tumor types. The function of TIP60 in tumor development has not been fully understood. Here we found that suppressing TIP60 inhibited p53 K120 acetylation and thus rescued apoptosis induced by glucose deprivation in hepatocellular cancer cells. Excitingly, Lys-104 (K104), a previously identified lysine acetylation site of TIP60 with unknown function, was observed to be indispensable for inducing p53-mediated apoptosis under low glucose condition. Mutation of Lys-104 to Arg (K104R) impeded the binding of TIP60 to human NuA4 complex, suppressed the acetyltransferase activity of TIP60, and inhibited the expression of pro-apoptotic genes including *NOXA* and *PUMA* upon glucose starvation. These findings demonstrate the critical regulation of TIP60/p53 pathway in apoptosis upon metabolic stress and provide a novel insight into the down-regulation of TIP60 in tumor cells.

Keywords

TIP60; p53; K104 Acetylation; Hypoglycemia; Metabolic Stress; Apoptosis

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Conflict of interest

None.

1. Introduction

Tumor cells usually show higher demand for glucose and other nutrients than normal cells in order to sustain high proliferation rates [1]. Due to the spatial and temporal heterogeneity of nutrients in the microenvironment of solid tumors, tumor cells undergo metabolic stress which might cause proliferation arrest and apoptosis. Therefore, preventing apoptosis induced by metabolic stress has been found to be essential for the survival and malignant progression of tumors [1–3]. Despite of extensive studies, it has not been fully understood how tumor cells adapt to the low-glucose environment.

As a major regulator in the process of metabolic stress, the tumor suppressor p53 plays a critical role in determining cell fate. Various post translational modifications (PTMs) have been identified on p53, which are indispensable for its function [4–7]. For example, acetylation at K120 is critical for the function of p53 in inducing apoptosis [8]. It has been shown that extended glucose starvation leads to elevated ROS (reactive oxygen species) and increased p53 acetylation at K120, which is required for the activation of p53 downstream apoptotic signaling in HepG2 cells [9]. Several acetyltransferases have been found to be able to acetylate p53 (PCAF, CBP/p300 and *etc.*) [10]. Among them, TIP60 (KAT5) is reported to acetylate p53 at K120, which promotes the expression of the pro-apoptotic p53 target gene *PUMA* and thus induces apoptosis upon DNA damage in human colon cancer cells [10]. Nevertheless, it remains elusive whether TIP60 activates p53 apoptotic signaling under metabolic stress in hepatocellular cells.

Attenuation of TIP60 expression has been observed in various human cancers and is a potential marker of tumor malignancy [11–17]. TIP60 carries out its biological functions both as a transcription factor and as an acetyltransferase [18]. On one hand, it can be recruited to the promoter of the pro-apoptotic gene *PUMA* and serves as a transcription coactivator upon DNA damage [10]. On the other hand, TIP60 functions as the catalytic subunit of the human NuA4 histone acetyltransferase (HAT) complex and participates in a wide range of biological processes including DNA damage response, cell cycle arrest and apoptosis [8, 10, 19–24]. The acetyltransferase activity of TIP60 is regulated by various PTMs, such as Y44-, S86-, T158-phosphorylation and K327-acetylation.[23–30]. Among all the PTMs mentioned above, S86-phosphorylation is most well-studied due to its central role in regulating TIP60-induced autophagy and apoptosis under various stress conditions [24, 26]. Interestingly, the level of S86-phosphorylation of TIP60 does not change under metabolic stress induced by glucose starvation, despite of the increased acetylation of p53 at K120 [9, 26]. This suggests that the activation of TIP60 might be regulated differently during the stress of glucose starvation. Of note, there are still a series of PTMs on TIP60 with unknown functions including K104, a newly identified acetylation site from a mass spectrometry study [28].

Here we report that the K104 acetylation of TIP60 plays an essential role in inducing apoptosis upon glucose starvation. Under low glucose condition, TIP60 is recruited to the human NuA4 complex which increased the acetyltransferase activity of TIP60. TIP60 activation leads to stimulated p53 pathway, enhanced TIP60 recruitment to *PUMA* promoter and elevated expression of the pro-apoptotic gene *PUMA*. K104R mutation markedly

interferes with the above processes and results in decreased apoptosis upon glucose starvation. Our results reveal that TIP60 mediates p53 apoptotic signaling under glucose starvation and that the K104 acetylation of TIP60 is required in the process, which suggests a possible mechanism how tumor cells adapt to the hypoglycemic microenvironment.

2. Materials and Methods

2.1. Cell culture and siRNA knockdown

HepG2 cells were grown in DMEM (Gibco) containing 10% FBS (Sigma). For glucose starvation assays, the cells were cultured in glucose-free DMEM (Sigma) supplemented with 10% FBS and 5 mM glucose (Sigma). To knockdown TIP60 by siRNA, siRNA duplexes against TIP60 (Sigma, predesigned siRNA pools) were transfected into HepG2 cells by RNAiMAX reagent (Invitrogen) following manufacturer's instructions.

2.2. Plasmids

For Myc-TIP60, TIP60 cDNA was amplified by PCR and inserted into pCMV-Myc vector. K104R-mutation and HAT-dead mutant (HATm) of TIP60 were generated by overlapping PCR. To stably express wildtype and mutant HA-TIP60, corresponding cDNAs were first cloned into pENTR-EF1A vector and transferred into pHAGE-EF-Puro-Dest vector by LR recombination. For conditionally knockdown endogenous TIP60 by shRNA, a lentiviral shRNA-conditional-knockdown vector targeting the 3'UTR region of TIP60 was generated. Specifically, the shRNA sequence (ATCAGACCAACTCCAAGGTC) was cloned into pInducer10 vector. For conditional expressing HA-TIP60s by pInducer20 vector, wildtype and mutant TIP60 cDNAs were first cloned into pEntry vector and transferred into pInducer20 vector by LR recombination. For the construction of CRISPR plasmids, the CAS9 expression vector and LKO.1 guiding RNA vector are obtained from Dr. Jianping Jin's lab at University of Texas Health Science Center, Houston. To generate the LKO.1-sgTRRAP plasmid, the sgRNA oligo was designed on the website <http://www.genome-engineering.org/CRISPR>, synthesized and inserted into LKO.1 vector through recombination using Gibson recombinase (NEB, E5510). For Myc-HDM2, HDM2 cDNA was cloned into pCMV-Myc vector.

2.3. Virus package, infection and stable cell line generation

Virus packaging and infection was conducted as previously described [31]. In short, shRNA or TIP60-expressing vectors were co-transfected with virus packaging plasmids into 293T cells to generate the lentivirus. HepG2 cells were infected with respective lentivirus and selected according to the lentiviral vector information. Stable cell lines were generated and subjected to further analyses.

To generate the HepG2.PI10 cell line with the pInducer10 cassette for conditionally knockdown endogenous TIP60 after doxycycline (Dox) addition, HepG2 cells were infected with the lentivirus expressing the conditional-knockdown shRNA vector targeting the 3'UTR of TIP60, selected with puromycin and 0.02 µg/mL doxycycline were applied to cells in order to attenuate TIP60 expression in the experiments.

To generate the HepG2.PI10/20 cell line with both pInducer10 and pInducer20 cassettes for 1) conditionally knockdown endogenous TIP60 and 2) simultaneously overexpress HA-tagged wildtype (WT), K104R or HATm TIP60 in the presence of doxycycline, HepG2.PI10 cells were infected with the lentivirus conditionally overexpressing wildtype or mutant TIP60s and selected with G418. 0.02 µg/mL doxycycline were added to the cell culture medium to knockdown endogenous TIP60 and induce the expression of respective TIP60 proteins in subsequent analyses.

2.4. Co-IP

For Co-IP analysis, cells were lysed in NETN buffer with protease-inhibitor cocktail (Roche). Mouse anti-HA antibody (Milipore) was incubated with cell lysates for 3 hours at 4°C. Protein G agarose beads (GE Healthcare) were applied to precipitate the immune complexes. Resulting precipitants were washed four times with NETN buffer and boiled in 2×SDS loading buffer in preparation for Western blot analysis.

2.5. Western blot and antibodies

For Western blot analysis, cells were lysed in RIPA buffer containing protease-inhibitor mixture (Roche) and the Bradford method (Bio-rad) was applied to measure protein concentration. Cell lysates were boiled in 2×SDS loading buffer for 5 min and prepared for gel running. Antibodies used in Western blots were, anti-p53, anti-TUBULIN, anti-Myc, anti-GAPDH (Santa Cruz); anti-K120Ac-p53, anti-TIP60 (Abcam); anti-HA (Milipore); anti-cleaved-PARP, anti-TRRAP, anti-p400, anti-DMAP1 and anti-RUVBL1 (Cell Signaling) antibodies.

2.6. RNA extraction and quantitative RT-PCR analysis

Extraction of total RNA was performed with Trizol (Invitrogen) following manufacturer's instructions. A total of 2 µg RNA was used for first strand cDNA synthesis with M-MLV reverse transcriptase (Promega). Primers for qRT-PCR were generated as previously described [24].

2.7. In vitro HAT activity assay

The in vitro HAT activity assay was performed as previously described [32]. Briefly, TIP60 immunoprecipitates (prepared as above described) were washed twice in HAT assay buffer (50 mM Tris at pH 8.0, 0.1 mM EDTA, 1 mM DTT and 10% glycerol), and incubated in 100 µl HAT assay buffer containing 100 µM acetyl-CoA and 1 µg biotinylated histone H4 peptide for 30 min at 30°C. Reaction aliquots were immobilized onto 96-well plates coated with streptavidin, and H4-peptide acetylation was examined by HAT ELISA according to the manufacturer's instructions (Upstate Biotechnology). HAT activity was determined by the change in absorbance at 450 nm relative to the reference wavelength at 540 nm.

2.8. ChIP assay

The ChIP assay was conducted as earlier described on the "The Epigenome" website (http://www.epigenome-noe.net/researchtools/protocol.php_protid=10.html) with a little modification that the chromatin was fragmented by micrococcal nuclease (NEB M0247S).

2.9. Statistical analysis

Statistical analysis was performed by Student's-t test. Data were presented as mean $\pm$ standard deviation (SD). $p < 0.05$ was considered as statistically significant.

3. Results

3.1. TIP60 is required for apoptosis induced by glucose starvation

Glucose starvation induces metabolic stress and leads to elevated ROS, increased p53 acetylation at K120 and apoptosis in HepG2 cells [9]. However, it remains elusive how p53 is activated during the process. Since TIP60 is the known acetyltransferase of p53 that regulates its function in apoptosis in human colon cancer cells upon DNA damage [10], we investigated whether TIP60 played any role under metabolic stress. To that end, we depleted TIP60 in HepG2 cells by siRNA (Fig. 1A) and subjected the cells to low-glucose (5 mM) condition. As shown by growth curve analysis, repressing TIP60 expression significantly rescued cell growth after glucose starvation (Fig. 1B). Consistent with this observation, qRT-PCR analyses demonstrated that knockdown of TIP60 markedly suppressed mRNA expression of the pro-apoptotic gene *PUMA* (Fig. 1C). Further, we analyzed cleaved-PARP (poly ADP-ribose polymerase) as an indicator of apoptosis. After been kept in low glucose medium for 48 h, TIP60 knockdown cells showed reduced apoptosis in comparison with control cells (Fig. 1D), suggesting that TIP60 knockdown attenuated p53-mediated apoptotic signaling upon glucose deprivation. Above conclusion was further supported by cell cycle analysis using flow cytometry. Significantly decreased ratio of sub-G1 was detected in TIP60 knockdown cells comparing to control cells after 48 h of glucose starvation (Fig. 1E and 1F). Moreover, we generated a TIP60-stable knockdown cell line by lentiviral expression of an shRNA targeting 3'UTR of TIP60 (HepG2. PI10 cells), in which endogenous TIP60 can be conditionally knocked down in the presence of doxycycline (Fig. 1G). Similarly, decreased apoptosis was found in the TIP60-knockdown cells (Fig. 1H). Together, these results demonstrate that TIP60 plays an essential role in glucose-starvation-induced apoptosis.

3.2. Human NuA4 complex regulates the acetyltransferase activity of TIP60 and apoptosis induced by glucose starvation

As reported before, TIP60 is the catalytic subunit of human NuA4 complex [33, 34]. Earlier studies have demonstrated that the components of NuA4 complex, such as TRRAP (transformation/transcription domain associated protein), p400 (E1A binding protein p400), RUVBL1 (RuvB like AAA ATPase 1) and DMAP1 (DNA methyltransferase 1-associated protein 1) participate in DNA damage response via modulating the function of TIP60, the acetyltransferase activity of which is shown to be indispensable for DNA repair and apoptosis [19, 22, 35–37]. To better understand how TIP60 regulates apoptosis induced by glucose deprivation, we first examined whether the acetyltransferase activity of TIP60 is affected by other components of the human NuA4 complex under metabolic stress. The HepG2 cell line was first infected with lentivirus to ectopically express HA-TIP60 and then TRRAP was depleted by CRISPR/Cas9-mediated gene editing in the cells. The acetyltransferase activity of HA-TIP60 isolated from these cells was evaluated. As expected, depletion of TRRAP substantially decreased TIP60 HAT activity under both normal and

low-glucose conditions (Fig. 2A). We further investigated whether human NuA4 complex proteins are required for glucose starvation-induced apoptosis. Indeed, silencing TRRAP, p400 and RUVBL1 by siRNA all suppressed apoptosis (Fig. 2B–D). CRISPR-mediated depletion of TRRAP showed similar results (Fig. 2E). Taken together, these data demonstrate that the acetyltransferase activity of TIP60 resides in human NuA4 complex and it is the human NuA4 complex that regulates apoptosis under glucose-starvation-induced metabolic stress.

3.3. The acetylation of TIP60 at K104 is critical for its apoptosis-inducing function

It is known that posttranslational modifications such as acetylation mediate protein function [38]. The activity of TIP60 is regulated by autoacetylation and SIRT1-directed deacetylation [29]. A number of lysine residues in TIP60 have been found to be acetylated. In particular, acetylation at K327 which is located around the catalytic site contributes to the acetyltransferase activity of TIP60 [28]. The functions of acetylation at other lysine residues are unclear. To begin elucidating the function of other acetylation sites, we focused on lysine 104 (K104) [28] first by mutating lysine 104 to arginine (K104R) which cannot be acetylated but retains the positive charge of lysine. We created K104R TIP60 as well as HAT-deleted TIP60 (HATm) which served as a positive control. The TIP60 mutants were expressed in HepG2 cells in which the endogenous TIP60 could be depleted inducibly (Fig. 3A). When these cells were treated with glucose starvation, apoptosis (cleaved PARP) as well as K120-acetylation of p53 was markedly attenuated in K104R and HATm TIP60-expressing cells comparing to wildtype TIP60-expressing cells (Fig. 3B and 3C). qRT-PCR analysis of the expression of two pro-apoptotic target genes of p53 (*NOXA* and *PUMA*) [9, 10] indicated similarly that K104R TIP60 was defective in activating apoptosis (Fig. 3D and 3E). These results suggest that K104 acetylation of TIP60 is critical for its function in stress-induced p53 activation and apoptosis.

3.4. K104R mutation abolishes TIP60 interaction with human NuA4 complex components and its acetyltransferase activity

Next we examined whether the interaction of TIP60 with other components of human NuA4 complex is affected by K104 acetylation. HA-TIP60 was immunoprecipitated from the cells (Fig. 4A). The interactions of wildtype TIP60 with TRRAP, RUVBL1 and DMAP1 were all increased respectively upon glucose starvation as shown by western blot (Fig. 4A), indicating the induction of NuA4 complex formation by stress. However, K104R as well as the HATm TIP60 failed to do so. Interestingly, unlike HATm, K104R TIP60 interacted normally with SIRT1 (Fig. 4A), which functions as the TIP60 deacetylase [39]. The failure to form human NuA4 complex by K104R TIP60 suggests that the mutant would have attenuated acetyltransferase activity. However, unlike HAT-mutant of TIP60, which completely lost the acetyltransferase activity, K104R mutant maintained basal level of HAT activity as the wildtype but the acetyltransferase activity failed to increase in response to hypoglycemic stress (Fig. 4B). These results suggest that the acetylation at K104 is required for the recruitment of TIP60 to human NuA4 complex proteins and the induction of TIP60 acetyltransferase activity upon metabolic stress.

3.5. K104 acetylation is required for the recruitment of TIP60 to PUMA promoter

Upon DNA damage, TIP60 is recruited to PUMA promoter by p53 and functions as a transcription coactivator to induce PUMA expression [10]. To further understand the regulatory role of K104 acetylation on stress-induced apoptosis, we investigated whether the recruitment of TIP60 to PUMA promoter region depends on this modification. Through chromatin immunoprecipitation analysis, we detected a 3-fold increase of TIP60 occupying the PUMA promoter region upon glucose starvation, whereas such increase was abolished by the K104R or HAT mutation of TIP60 (Fig. 5), which is consistent with the expression of PUMA at mRNA levels (Fig. 3E).

3.6. K104R TIP60 binds normally to HDM2 for proteasome-mediated-degradation

Acetylation at internal lysine residues is a common type of post-translational modification on proteins and causes multiple changes in protein features, including protein stability [40]. To better understand the regulation of TIP60 function by K104 acetylation, we examined whether the stability of TIP60 was changed by K104R mutation. As reported before, TIP60 is degraded by the ubiquitin-proteasome pathway [40]. To determine whether degradation of K104R-TIP60 was regulated by the proteasome pathway, we treated HepG2 cells which stably expressed wildtype and K104R-TIP60 with cycloheximide (CHX) in the presence and absence of the proteasome inhibitor MG132. Based on Western blot results, treatment of MG132 stabilized both wildtype and mutant TIP60s after CHX treatment (Fig. 6A). Degradation of a protein in the ubiquitin-proteasome pathway is regulated by the E3 ligase of the protein and HDM2 was reported to be the E3 ligase of TIP60 [9]. The interaction between TIP60 and HDM2 was examined to determine whether K104R mutation interferes with the binding of TIP60 to its E3 ligase. Myc-HDM2 was transiently transfected into HepG2 cells stably expressing wildtype or mutant TIP60s. Cells were collected 48 h after transfection and prepared for Co-IP analysis. K104R mutation did not show changed strength of interaction to HDM2 compared to wildtype TIP60 (Fig. 6B). These together suggest that K104 acetylation status does not affect the binding of TIP60 to HDM2 for proteasome-mediated degradation. Further, HepG2 cells which stably expressed wildtype or K104R-TIP60 were treated with CHX and collected at different time points for Western blot analysis. No significant difference in stability was observed between wildtype and K104R-TIP60 (Fig. 6C). Together, these results demonstrate that K104-acetylation of TIP60 does not change the stability of TIP60 protein.

4. Discussion

Being the catalytic subunit of human NuA4 complex, TIP60 is down-regulated in various human cancers and the down-regulation predicts poor clinic outcomes [11–17]. TIP60 functions as a tumor suppressor and is activated in response to genotoxic stress to acetylate ATM (ataxia telangiectasia mutated) kinase and thus promote DNA repair [32]. Aberrant TIP60 signaling in DDR (DNA damage response) might drive tumor formation, especially when p53 is synergistically mutated [17, 32]. In this study we demonstrated that repressing TIP60 expression improved the survival of tumor cells under metabolic stress induced by glucose starvation. Given the heterogeneity of the microenvironment in glucose and other nutrients supply for solid tumors [1], our observations suggest that repressed TIP60

expression in cells favors tumorigenesis due to not only attenuated DDR, but also enhanced adaptability to metabolic stress. In support of this notion, deficient expression of other NuA4 complex proteins has been reported in multiple human tumors [41–43].

As been reported before, TIP60 modulates the choice between apoptosis and cell cycle arrest by regulating p53 acetylation at K120 upon stress [10]. Short-term treatment of low glucose (< 36 h) leads to p53-dependent p21 induction and cell cycle arrest, while prolonged glucose starvation results in p53 K120-acetylation and apoptosis in HepG2 cells [9]. In this study, we found that TIP60 regulates p53 K120-acetylation and thus apoptosis at extended periods of glucose starvation. Although p21 is not significantly induced after long-term of glucose deprivation [9], it would be interesting to know whether TIP60 regulates p21 expression and cell cycle progression at early time of low glucose treatment (< 36 h). Of note, although depleting TIP60 expression repressed apoptosis after glucose starvation (Fig. 1C–F), the growth potential is not markedly rescued in the starved cells. Two possible explanations for this are 1) the knockdown efficiency of TIP60 by RNAi is limited and 2) rather than restore the growth potential, TIP60 knockdown might only delay the cell death caused by glucose deprivation, since glucose supply is indispensable for cell growth. Further studies on TIP60 knockout mice and other cell lines will help improve the understanding of TIP60-mediated cell survival under stress.

Previous studies have shown that acetylation of p53 at K120 is essential for inducing apoptosis after glucose starvation [9]. However, the acetyltransferase responsible for the K120 acetylation of p53 and its regulation under low glucose condition remain elusive. In this study, we report that TIP60 functions to mediate the acetylation of p53 at K120 and thus to initiate the downstream apoptotic signaling upon glucose starvation, and that the K104 acetylation of TIP60 is indispensable in this process (Fig. 7). Abolishing the acetylation with K104R mutation resulted in the attenuation of apoptosis induced by glucose deprivation to a similar degree as the HAT mutant. However, unlike the HAT mutant, K104R TIP60 possessed comparable basal acetyltransferase activity with wildtype TIP60 (Fig. 4B). The mutant just failed to up-regulate its acetyltransferase activity as the wildtype protein does in response to glucose starvation. Since the mutant fails to be incorporated into the human NuA4 complex, which is also critical in stress-induced apoptosis, it is very likely that under stress conditions, TIP60 (acetylated, at least at K104) forms human NuA4 complex and it is the NuA4 complex that possesses higher acetyltransferase activity than TIP60 alone, acetylates p53, and induces apoptosis. It is unclear at the moment how stress induces human NuA4 complex formation.

As K104 acetylation is constitutive [28], acetylation at this site is unlikely the regulatory mechanism responsible for human NuA4 complex formation. Since TIP60 is activated under various stresses to regulate p53 activity [18, 19], it is possible that metabolic stress induces a previously undefined PTM of TIP60 and promotes the formation of NuA4 complex to cause p53-dependent apoptosis (Fig. 7). A mass spectrometry study comparing the PTM sites of TIP60 under normal and low glucose condition will be performed to gain a better knowledge of the above process. Moreover, TRRAP, one key component of the NuA4 complex was detected to regulate TIP60 acetyltransferase activity as well as its recruitment to PUMA promoter (Figure 2A and S1). Given the fact that TRRAP also serves as a common subunit

of three mammalian equivalents of yeast SAGA complex (STAGA, PCAF and TFTC) [34, 44], TIP60-induced apoptotic signaling is at least indirectly modulated by the three coactivator complexes. Further investigations are needed to fully dissect the regulation of TIP60 pathway upon metabolic stress.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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Abbreviations

HAT	histone acetyltransferase
PTM	post translational modification
ROS	reactive oxygen species
PARP	poly ADP-ribose polymerase
Dox	doxycycline
TRRAP	transformation/transcription domain associated protein
p400	E1A binding protein p400
RUVBL1	RuvB like AAA ATPase 1
DMAP1	DNA methyltransferase 1-associated protein 1
CHX	cycloheximide
DDR	DNA damage response

Reference

- [1]. Cairns RA, Harris IS, Mak TW, Regulation of cancer cell metabolism, Nat Rev Cancer 11 (2011) 85–95. [PubMed: 21258394]
- [2]. Warburg O, On the origin of cancer cells, Science 123 (1956) 309–314. [PubMed: 13298683]
- [3]. Smith B, Schafer XL, Ambeskovic A, Spencer CM, Land H, Munger J, Addiction to Coupling of the Warburg Effect with Glutamine Catabolism in Cancer Cells, Cell Rep 17 (2016) 821–836. [PubMed: 27732857]

- [4]. Kruiswijk F, Labuschagne CF, Vousden KH, p53 in survival, death and metabolic health: a lifeguard with a licence to kill, *Nat Rev Mol Cell Biol* 16 (2015) 393–405. [PubMed: 26122615]
- [5]. Vousden KH, Ryan KM, p53 and metabolism, *Nat Rev Cancer* 9 (2009) 691–700. [PubMed: 19759539]
- [6]. Tang Y, Zhao W, Chen Y, Zhao Y, Gu W, Acetylation is indispensable for p53 activation, *Cell* 133 (2008) 612–626. [PubMed: 18485870]
- [7]. Brooks CL, Gu W, Ubiquitination, phosphorylation and acetylation: the molecular basis for p53 regulation, *Curr Opin Cell Biol* 15 (2003) 164–171. [PubMed: 12648672]
- [8]. Sykes SM, Mellert HS, Holbert MA, Li K, Marmorstein R, Lane WS, McMahon SB, Acetylation of the p53 DNA-binding domain regulates apoptosis induction, *Mol Cell* 24 (2006) 841–851. [PubMed: 17189187]
- [9]. Sen N, Satija YK, Das S, PGC-1 α , a key modulator of p53, promotes cell survival upon metabolic stress, *Mol Cell* 44 (2011) 621–634. [PubMed: 22099309]
- [10]. Tang Y, Luo J, Zhang W, Gu W, Tip60-dependent acetylation of p53 modulates the decision between cell-cycle arrest and apoptosis, *Mol Cell* 24 (2006) 827–839. [PubMed: 17189186]
- [11]. Pandey AK, Zhang Y, Zhang S, Li Y, Tucker-Kellogg G, Yang H, Jha S, TIP60-miR-22 axis as a prognostic marker of breast cancer progression, *Oncotarget* 6 (2015) 41290–41306. [PubMed: 26512777]
- [12]. Sakuraba K, Yokomizo K, Shirahata A, Goto T, Saito M, Ishibashi K, Kigawa G, Nemoto H, Hibi K, TIP60 as a potential marker for the malignancy of gastric cancer, *Anticancer Res* 31 (2011) 77–79. [PubMed: 21273583]
- [13]. Sakuraba K, Yasuda T, Sakata M, Kitamura YH, Shirahata A, Goto T, Mizukami H, Saito M, Ishibashi K, Kigawa G, Nemoto H, Sanada Y, Hibi K, Down-regulation of Tip60 gene as a potential marker for the malignancy of colorectal cancer, *Anticancer Res* 29 (2009) 3953–3955. [PubMed: 19846935]
- [14]. Zhang Y, Subbiah VK, Rajagopalan D, Tham CY, Abdullah LN, Toh TB, Gong M, Tan TZ, Jadhav SP, Pandey AK, Karnani N, Chow EK, Thiery JP, Jha S, TIP60 inhibits metastasis by ablating DNMT1-SNAIL2-driven epithelial-mesenchymal transition program, *J Mol Cell Biol* (2016).
- [15]. Bassi C, Li YT, Khu K, Mateo F, Baniasadi PS, Elia A, Mason J, Stambolic V, Pujana MA, Mak TW, Gorrini C, The acetyltransferase Tip60 contributes to mammary tumorigenesis by modulating DNA repair, *Cell Death Differ* 23 (2016) 1198–1208. [PubMed: 26915295]
- [16]. 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* 132 (2012) 2632–2641. [PubMed: 22673729]
- [17]. Gorrini C, Squatrito M, Luise C, Syed N, Perna D, Wark L, Martinato F, Sardella D, Verrecchia A, Bennett S, Confalonieri S, Cesaroni M, Marchesi F, Gasco M, Scanziani E, Capra M, Mai S, Nuciforo P, Crook T, Lough J, Amati B, Tip60 is a haplo-insufficient tumour suppressor required for an oncogene-induced DNA damage response, *Nature* 448 (2007) 1063–1067. [PubMed: 17728759]
- [18]. Squatrito M, Gorrini C, Amati B, Tip60 in DNA damage response and growth control: many tricks in one HAT, *Trends Cell Biol* 16 (2006) 433–442. [PubMed: 16904321]
- [19]. Ikura T, Ogryzko VV, Grigoriev M, Groisman R, Wang J, Horikoshi M, Scully R, Qin J, Nakatani Y, Involvement of the TIP60 histone acetylase complex in DNA repair and apoptosis, *Cell* 102 (2000) 463–473. [PubMed: 10966108]
- [20]. Kusch T, Florens L, Macdonald WH, Swanson SK, Glaser RL, Yates JR, 3rd, Abmayr SM, Washburn MP, Workman JL, Acetylation by Tip60 is required for selective histone variant exchange at DNA lesions, *Science* 306 (2004) 2084–2087. [PubMed: 15528408]
- [21]. 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 USA* 102 (2005) 13182–13187. [PubMed: 16141325]
- [22]. Murr R, Loizou JI, Yang YG, Cuenin C, Li H, Wang ZQ, Herceg Z, Histone acetylation by Trapp-Tip60 modulates loading of repair proteins and repair of DNA double-strand breaks, *Nat Cell Biol* 8 (2006) 91–99. [PubMed: 16341205]

- [23]. Kaidi A, Jackson SP, KAT5 tyrosine phosphorylation couples chromatin sensing to ATM signalling, *Nature* 498 (2013) 70–74. [PubMed: 23708966]
- [24]. Charvet C, Wissler M, Brauns-Schubert P, Wang SJ, Tang Y, Sigloch FC, Mellert H, Brandenburg M, Lindner SE, Breit B, Green DR, McMahon SB, Borner C, Gu W, Maurer U, Phosphorylation of Tip60 by GSK-3 determines the induction of PUMA and apoptosis by p53, *Mol Cell* 42 (2011) 584–596. [PubMed: 21658600]
- [25]. Zheng H, Seit-Nebi A, Han X, Aslanian A, Tat J, Liao R, Yates JR, 3rd, Sun P, A posttranslational modification cascade involving p38, Tip60, and PRAK mediates oncogene-induced senescence, *Mol Cell* 50 (2013) 699–710. [PubMed: 23685072]
- [26]. Lin SY, Li TY, Liu Q, Zhang C, Li X, Chen Y, Zhang SM, Lian G, Ruan K, Wang Z, Zhang CS, Chien KY, Wu J, Li Q, Han J, Lin SC, GSK3-TIP60-ULK1 signaling pathway links growth factor deprivation to autophagy, *Science* 336 (2012) 477–481. [PubMed: 22539723]
- [27]. Creaven M, Hans F, Mutskov V, Col E, Caron C, Dimitrov S, Khochbin S, Control of the histone-acetyltransferase activity of Tip60 by the HIV-1 transactivator protein, Tat, *Biochemistry* 38 (1999) 8826–8830. [PubMed: 10393559]
- [28]. Yang C, Wu J, Zheng YG, Function of the active site lysine autoacetylation in Tip60 catalysis, *PLoS One* 7 (2012) e32886. [PubMed: 22470428]
- [29]. Wang J, Chen J, SIRT1 regulates autoacetylation and histone acetyltransferase activity of TIP60, *J Biol Chem* 285 (2010) 11458–11464. [PubMed: 20100829]
- [30]. Yi J, Huang X, Yang Y, Zhu WG, Gu W, Luo J, Regulation of Histone Acetyltransferase TIP60 Function by Histone Deacetylase 3, *J Biol Chem* 289 (2014) 33878–33886. [PubMed: 25301942]
- [31]. Li M, Fang X, Baker DJ, Guo L, Gao X, Wei Z, Han S, van Deursen JM, Zhang P, The ATM-p53 pathway suppresses aneuploidy-induced tumorigenesis, *Proc Natl Acad Sci USA* 107 (2010) 14188–14193. [PubMed: 20663956]
- [32]. Sun Y, Jiang X, Xu Y, Ayrapetov MK, Moreau LA, Whetstine JR, Price BD, Histone H3 methylation links DNA damage detection to activation of the tumour suppressor Tip60, *Nat Cell Biol* 11 (2009) 1376–1382. [PubMed: 19783983]
- [33]. Doyon Y, Selleck W, Lane WS, Tan S, Cote J, Structural and functional conservation of the NuA4 histone acetyltransferase complex from yeast to humans, *Mol Cell Biol* 24 (2004) 1884–1896. [PubMed: 14966270]
- [34]. Knutson BA, Hahn S, Domains of Tra1 important for activator recruitment and transcription coactivator functions of SAGA and NuA4 complexes, *Mol Cell Biol* 31 (2011) 818–831. [PubMed: 21149579]
- [35]. Tyteca S, Vandromme M, Legube G, Chevillard-Briet M, Trouche D, Tip60 and p400 are both required for UV-induced apoptosis but play antagonistic roles in cell cycle progression, *EMBO J* 25 (2006) 1680–1689. [PubMed: 16601686]
- [36]. Jha S, Shibata E, Dutta A, Human Rvb1/Tip49 is required for the histone acetyltransferase activity of Tip60/NuA4 and for the downregulation of phosphorylation on H2AX after DNA damage, *Mol Cell Biol* 28 (2008) 2690–2700. [PubMed: 18285460]
- [37]. Penicud K, Behrens A, DMAP1 is an essential regulator of ATM activity and function, *Oncogene* 33 (2014) 525–531. [PubMed: 23318425]
- [38]. Yang XJ, Seto E, Lysine acetylation: codified crosstalk with other posttranslational modifications, *Mol Cell* 31 (2008) 449–461. [PubMed: 18722172]
- [39]. Peng L, Ling H, Yuan Z, Fang B, Bloom G, Fukasawa K, Koomen J, Chen J, Lane WS, Seto E, SIRT1 negatively regulates the activities, functions, and protein levels of hMOF and TIP60, *Mol Cell Biol* 32 (2012) 2823–2836. [PubMed: 22586264]
- [40]. Legube G, Linares LK, Lemercier C, Scheffner M, Khochbin S, Trouche D, Tip60 is targeted to proteasome-mediated degradation by Mdm2 and accumulates after UV irradiation, *EMBO J* 21 (2002) 1704–1712. [PubMed: 11927554]
- [41]. Wang J, Shan M, Liu T, Shi Q, Zhong Z, Wei W, Pang D, Analysis of TRRAP as a Potential Molecular Marker and Therapeutic Target for Breast Cancer, *J Breast Cancer* 19 (2016) 61–67. [PubMed: 27066097]

- [42]. Macher-Goeppinger S, Bermejo JL, Schirmacher P, Pahernik S, Hohenfellner M, Roth W, Senescence-associated protein p400 is a prognostic marker in renal cell carcinoma, *Oncol Rep* 30 (2013) 2245–2253. [PubMed: 23982490]
- [43]. Yamaguchi Y, Takenobu H, Ohira M, Nakazawa A, Yoshida S, Akita N, Shimozato O, Iwama A, Nakagawara A, Kamijo T, Novel 1p tumour suppressor Dnmt1-associated protein 1 regulates MYCN/ataxia telangiectasia mutated/p53 pathway, *Eur J Cancer* 50 (2014) 1555–1565. [PubMed: 24559687]
- [44]. Spedale G, Timmers HT, Pijnappel WW, ATAC-king the complexity of SAGA during evolution, *Genes Dev* 26 (2012) 527–541. [PubMed: 22426530]

Highlights

- TIP60 acetylates p53 at K120 and induces apoptosis in glucose-starved HepG2 cells.
- Binding of TIP60 to human NuA4 complex up-regulates TIP60 acetyltransferase activity.
- K104-acetylation of TIP60 is essential for TIP60 function upon glucose deprivation.
- TIP60 suppresses tumor by sensitizing tumor cells to hypoglycemic microenvironment.

Figure 1. TIP60 is required for glucose-starvation-induced apoptosis.

(A) TIP60 was knocked down in HepG2 cells by siRNA and the knockdown efficiency was detected by Western blot analysis. (B) Growth curve analysis of control and TIP60 knockdown cells. Control and TIP60 knockdown cells by siRNA were plated into 6 cm plate (3×10^5 cells/well), collected at indicated time points and the cell number was counted in each group. (C, D) Control and TIP60-knockdown HepG2 cells were glucose starved for 48 h. qRT-PCR analysis on *PUMA* mRNA expression (C), Western blot on apoptosis (D) and flow cytometry analysis on sub-G1 ratio (E) were performed in these cells. (F) Statistical analysis of the results in E. (G) The HepG2.PI10 cell line was generated by infecting HepG2 cells with the lentivirus harboring the conditionally-expressed shRNA cassette targeting 3'UTR of TIP60. Endogenous TIP60 was knocked down after addition of 0.02 $\mu\text{g/mL}$ Dox and the knockdown efficiency was examined by Western blot analysis. (H) HepG2.PI10 cells

were glucose starved for 48 h with or without Dox treatment and apoptosis was analyzed by immunoblotting. *, $p<0.05$; **, $p<0.01$.

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Figure 2. Human NuA4 complex regulates TIP60 acetyltransferase activity and apoptosis in glucose-starved cells.

(A) HepG2 cells were first infected by lentivirus to conditionally overexpress HA-TIP60. TRRAP was then stably knocked down in these cells by CRISPR/Cas9-mediated gene editing via a second round of lentivirus infection. HA-TIP60 was pulled down by HA antibody after glucose starvation for in vitro HAT assay. (B-D) TRRAP (B), p400 (C) or RUVBL1 (D) was knocked down in HepG2 cells by siRNA and Western blot analysis was applied to monitor apoptosis in these cells after low glucose treatment for 48 h. (E) TRRAP was knocked down in HepG2 cells through CRISPR/Cas9 gene editing and the cells were glucose starved for indicated time points. Western blot was performed to evaluate apoptosis in the cells. **, $p < 0.01$.

Figure 3. K104 acetylation mediates the function of TIP60 in inducing apoptosis after low-glucose treatment.

(A) HepG2.PI10/20 cells were generated by infecting the HepG2.PI10 cells with the lentivirus conditionally expressing HA-tagged wildtype (WT), K104R or HATm TIP60. Dox was added to the culture medium at gradient concentrations to knockdown endogenous TIP60 and simultaneously overexpress wildtype or mutant TIP60. Western blot was performed to detect TIP60 status in the cells and the Dox concentration (0.02 $\mu\text{g/mL}$) at which cellular TIP60 level was most approaching physiological conditions was chosen for the following experiments. (B) HepG2.PI10/20 cells were treated with Dox at 0.02 $\mu\text{g/mL}$ to induce the expression of HA-TIP60s. After glucose starvation for 48 h, apoptosis and p53 status in the cells were monitored by immunoblotting. (C) Measurement of signal intensity

of the result in B. (D, E) HepG2.PI10/20 cells were treated with Dox at 0.02 $\mu\text{g/mL}$ to knockdown endogenous TIP60 and simultaneously overexpress HA-empty vector or HA-TIP60s. After glucose starvation for 48 h, qRT-PCR analysis was performed to detect mRNA expression of *NOXA* (D) and *PUMA* (E) in the cells. *, $p < 0.05$; **, $p < 0.01$.

Figure 4. K104R mutation attenuates TIP60 interaction with TIP60 HAT complex and its acetyltransferase activity upon glucose starvation.

(A) HepG2.PI10/20 cells were treated with Dox, glucose starved for 48 h and collected for Co-IP analysis. HA-TIP60 was pulled down by anti-HA antibody and human NuA4 complex components in the immune complex were detected by Western blot with indicated antibodies. (B) HepG2.PI10/20 cells were treated at 0.02 $\mu\text{g/mL}$ and collected as described in A. HA-TIP60s in the cell lysis were immunoprecipitated with anti-HA antibody and in vitro HAT assay was performed to evaluate the HAT activity of wildtype and mutant TIP60s. **, $p < 0.01$.

Figure 5. TIP60 K104 acetylation modulates its recruitment to PUMA promoter.

HepG2, PI10/20 cells were treated as described in Fig. 3B. ChIP analysis was performed to examine the binding of wildtype or mutant TIP60 to PUMA promoter region by pulling down HA-TIP60s with anti-HA antibody. *, $p < 0.05$; **, $p < 0.01$.

Figure 6. TIP60 stability is not affected by K104 acetylation.

(A) HepG2 cells stably expressing HA-tagged wildtype and K104R-TIP60 were treated with CHX at 100 ng/ml in the presence and absence of 10 μ M MG132. Cells were collected at indicated time and prepared for Western blot analysis. (B) Co-IP analysis on the interaction between TIP60 and HDM2. Myc-HDM2 was transfected into control and HepG2 cells, which stably expressed HA-tagged wildtype and K104R-Tip60. 48h after transfection, cells were collected for Co-IP analysis. (C) Western blot on TIP60 stability in wildtype and K104R-TIP60 stable-expressing HepG2 cells. Cells were treated with CHX at 100 ng/ml for indicated time and collected for Western blot analysis.

Figure 7. A model for the regulation of TIP60 under metabolic stress.

K104-acetylation is a constitutive PTM of TIP60, which is a prerequisite for TIP60 to fully carry out its apoptosis-inducing function. Metabolic stress leads to the activation of TIP60 (possibly by a novel PTM which has not been identified yet) and the activated TIP60 is recruited to NuA4 complex proteins, which increases the acetyltransferase activity of TIP60 and initiates downstream p53 apoptotic signaling.