HDAC6 regulates DNA damage response via deacetylating MLH1*

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ABSTRACT

MLH1 is a key DNA mismatch repair (MMR) protein, which plays an important role in maintenance of genomic stability and the DNA damage response (DDR). Here, we report that MLH1 is a novel substrate of HDAC6. HDAC6 interacts with and deacetylates MLH1 both in vitro and in vivo. Interestingly, deacetylation of MLH1 blocks the assembly of the MutSα-MutLα complex. Moreover, we have identified four novel acetylation sites in MLH1 by mass spectrometry analysis. The deacetylation mimetic mutant, but not the wild-type and the acetylation mimetic mutant, of MLH1 confers resistance to 6-thioguanine. Overall, our findings suggest that the MutSα-MutLα complex serves as a sensor for DNA damage response and that HDAC6 disrupts the MutSα-MutLα complex by deacetylation of MLH1, leading to the tolerance of DNA damage.

Mismatch repair is a mutation avoidance mechanism that corrects DNA replication errors, including small insertions, deletions and mis-incorporated bases, as well as some forms of DNA damage (1-3). This process begins with mismatch recognition, which is mainly carried out by MSH2-MSH6 heterodimer (MutSα). Next, the DNA-bound MutSα recruits MLH1-PMS2 heterodimer
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(MutLa) to increase the footprint on the DNA. Eukaryotes have three different forms of heterodimers that include MLH1, designated as MLH1-PMS2 (MutLa), MLH1-PMS1 (MutLβ) and MLH1-MLH3 (MutLγ). Among these heterodimers, MutLa acts as the matchmaker and facilitator, coordinating events in mismatch repair (4,5). This step may serve as an entry point for the exonuclease activity that removes mismatched DNA in the presence of other required proteins, including MutSa and downstream protein PCNA. Due to its critical role in MMR, deletion or mutation of MLH1 causes genomic instability in hereditary nonpolyposis colorectal cancer (HNPCC) (6,7). More than 240 mutations have been described in HNPCC, and about 60% of these mutations occurred in hMLH1. From the analyses of HNPCC, more than 25% of gene alterations in hMLH1 were identified as minor variants such as amino acid replacement or small in-frame deletions. These mutations are scattered throughout the entire coding region, indicating the importance of every single domain of the MLH1 protein in its full function. To date, the UMD-MLH1 mutation database shows a total of 3,063 recorded mutations, and 568 different variants from 2,527 samples. Among all the mutations, 47 nonsense and 198 missense and synonymous variants in hMLH1 are reported (www.umd.be/mlh1/). Aside from cancer susceptibility, MLH1 also affects fertility, as reported in a knockout mice study. Both males and females exhibit normal mating behavior, however, both are sterile (8). In addition to mutations on MLH1 cDNA, hypermethylation of its promoter is another main cause of hMLH1 gene silencing involved in sporadic cancers (9).

Antimetabolites (e.g. 6-thioguanine (6-TG)), alkylating agents (e.g. N-methyl-N’-nitro-N-nitrosoguanidine (MNNG) and N-methyl-N-nitrosourea (MNU)) and platinum-containing drugs (e.g. cisplatin) can trigger cell cycle arrest and apoptosis in cells via MMR proteins (10). Cell lines, such as 2008/A and HCT116 which are deficient of hMLH1, became more resistant to MNNG and cisplatin, respectively (11), suggesting the involvement of MLH1 in DNA damage response. It has been reported that MLH1 plays a critical role in apoptosis in either p53-dependent or p53-independent mechanism. In the presence of p53, MLH1 mediates MNNG- and MNU-induced cell death by increasing phosphorylation of Ser15 of p53, leading to apoptosis (12). In the absence of p53, MLH1 is associated with the c-Abl-p73-apoptosis pathway in response to cisplatin-induced DNA damage (13). Overall, the above evidence has shown that MLH1 indeed plays an important role in DNA damage response. However, how MLH1 is regulated at the post-translational level is understudied.

Histone deacetylase 6 (HDAC6) was cloned from mouse and human as a mammalian homolog of yeast HDA1 (14,15). It contains two deacetylase domains, termed DAC1 and DAC2, as well as a ZnF-UBP domain in the C-terminus. Our previous studies have shown that DAC2 has full deacetylase activity, while DAC1 possesses an intrinsic E3 ligase activity both in vitro and in vivo (16). HDAC6 is now considered to be a master regulator of cellular response to cytotoxic assaults (17-19), and plays a role in genotoxic stress responses (16,20,21). Here, we have identified the MMR protein MLH1 as a factor for HDAC6-mediated DDR function. HDAC6 interacts with and deacetylates MLH1 both in vivo and in vitro. We also identified four new acetylation lysine sites in the MLH1 protein, and HDAC6 may regulate the acetylation status of these sites to disrupt the assembly of the MutSa-MutLa complex, leading to 6-TG tolerance. These findings suggest that as a downstream target of HDAC6, MLH plays an important role in HDAC6-mediated DNA damage response.
Results

MLH1 interacts with HDAC6

We have previously shown that HDAC6 forms a complex in the nucleus with several DNA mismatch repair proteins, including MSH2, MSH6 and MLH1, in HeLa cells (16). Although we have shown that HDAC6 sequentially deacetylates and ubiquitinates MSH2, it is completely unknown whether HDAC6 also regulates MLH1. To this end, we first verified that HDAC6 interacts with MLH1. We assessed the endogenous interaction between HDAC6 and MLH1 via co-immunoprecipitation. As shown in Figure 1A, lung cancer A549 cell extracts were immunoprecipitated with either anti-rabbit IgG or anti-HDAC6 antibody. MLH1 could only be detected in the immunoprecipitates from the anti-HDAC6 antibody, but not from the control anti-IgG. In the reciprocal experiment, anti-MLH1 antibody, but not the anti-rabbit IgG control, specifically pulled down HDAC6 protein (Figure 1B). Thus, HDAC6 and MLH1 are indeed associated with each other in vivo.

To determine whether HDAC6 directly binds to MLH1 or through other associated proteins, we performed in vitro GST pull-down assays with bacterially-purified GST-HDAC6 and His-MLH1. As shown in Figure 1C, GST-HDAC6, but not GST or glutathione agarose, efficiently pulled down His-MLH1. This result indicates a direct binding between HDAC6 and MLH1. We next attempted to map which region of HDAC6 interacts with MLH1. HDAC6 has two deacetylation domains in the N-terminus, termed DAC1 and DAC2, and a ZnF-UBP domain in the C-terminus. As shown in Figure 1D and 1E, both DAC1 (1-503 aa) and DAC2 (488-839 aa), but not the C-terminus (835-1215 aa), bind to MLH1. We then examined which region of MLH1 binds to HDAC6. MLH1 has an ATPase domain (1-147 aa) in the N-terminus. The middle region (147-320 aa) is for MutSα binding, and the very C-terminus domain is for PMS2/MLH3/PSM1 binding (491-756 aa). We found that both N- and C-terminal domains can bind to HDAC6, except a portion of the middle region (320-491 aa) that binds to the MutSα complex (Figure 1F & 1G). We previously showed that HDAC6 may interact with MLH1 in HeLa nuclear extracts (16). To confirm whether HDAC6 binds to MLH1 in the nucleus, we performed the co-IP experiments using anti-IgG or anti-HDAC6 to pull down MLH1 in T29 nuclear extracts. The method of preparing nuclear extracts was described in Zhang et al. (16) As shown in Figure 2A, endogenous HDAC6 indeed binds to MLH1 in the nucleus. Next, we performed immunofluorescence staining of MLH1 and HDAC6. As shown in Figure 2B and 2C, HDAC6 is co-localized with MLH1 in H1299 cells, an effect that is enhanced upon etoposide treatment. The result suggests that HDAC6 may be translocated into the nucleus upon DNA damage.

To examine the portion of HDAC6 in the nucleus, we performed nuclear and cytoplasmic fractionation of four non-small cell lung cancer cell lines, A549, H460, H292 and H125. PARP1 and Hsp60 were used as nuclear- and cytoplasmic-specific marker, respectively. As shown in Figure 2D, it appears that HDAC6 is almost equally distributed in nuclear and cytoplasmic fractions in all four cell lines except H460, in which there is at least twice as much HDAC6 protein in cytoplasm compared to the nucleus. Because equal amounts of protein were loaded in each lane, and our prior experience has indicated that there is 3-4-fold more protein in the cytosol than in the nucleus, we therefore estimated that approximately 20% of HDAC6 is localized in the nucleus of A549, H292 and H125 cell lines and 10% of HDAC6 is localized in the nucleus of H460 cell line.
**HDAC6 deacetylates MLH1**

Given the fact that HDAC6 is a deacetylase, we hypothesized that MLH1 could be a substrate of HDAC6. To this end, we tested whether MLH1 is an acetylated protein. We treated 293T cells with Trichostatin A (TSA), a class I, II and IV HDAC inhibitor, and immunoprecipitated MLH1 with an anti-MLH1 antibody. As shown in Figure 3A, the level of acetylated MLH1 is increased upon TSA treatment, suggesting that HDACs regulate MLH1 acetylation. To determine the involvement of HDAC6 in MLH1 deacetylation, we treated 293T cells with Tubastatin A, an HDAC6-specific inhibitor. As shown in Figure 3B, the level of acetylated MLH1 is increased upon Tubastatin A treatment, suggesting that HDAC6 deacetylates MLH1. Next, we tested whether histone acetyltransferases (HATs) could elevate MLH1 acetylation. As shown in Figure 3C, p300, a HAT, enhances the acetylation of MLH1 in 293T cells. To determine whether MLH1 is a substrate of HDAC6, we then performed a deacetylation assay. As shown in Figure 3D, acetylation of MLH1 was reduced in the cells overexpressing HDAC6 compared to the control cells, indicating that HDAC6 deacetylates MLH1 in vivo. To exclude the possibility that HDAC6 deacetylates MLH1 through its associated proteins, we performed an in vitro deacetylation assay. HDAC6 was purified from 293T cells and incubated with acetylated MLH1. As shown in Figure 3E, the acetylation level of MLH1 was significantly decreased in the reaction with purified HDAC6. Thus, HDAC6 can deacetylate MLH1 both in vivo and in vitro.

**Lysines 33, 241, 361 and 377 of MLH1 are targeted for acetylation**

To detect the acetylation sites present in MLH1, we overexpressed Flag-MLH1 in 293T cells followed by treatment with TSA and subjected the samples to mass spectrometry analyses. As shown in Figure 4, K33, K241, K361 and K377 were identified as acetylation sites, which spread across the ATPase and MutSα interaction domains. Lysine 33 is located in the ATPase domain and conserved among mammals, Zebrafish (D. rerio), clawed frog (X. tropicalis), fruit fly (D. melanogaster), Caenorhabditis (C. elegans), and even in plant (A. thaliana), yeast (S. cerevisiae and S. pombe) and E.coli (Figure 5A), indicating that lysine 33 plays an important role in MLH1 function. Lysine 241 is located in the MutS homologs interaction domain and conserved among mammals, zebrafish and clawed frog, indicating their potential role in MMR complex formation between Mutsα and MutLα (Figure 5B). Lysine 361 is also located in the MutS homologs interaction domain and is conserved from mammals to clawed frog, with the exception of mouse (Figure 5B). The K377 site shows a less conserved pattern compared with the other three sites, indicating a distinct regulatory role during evolutionary processes (Figure 5B). The domain structure of MLH1 and the positions of these four lysine sites are shown in Figure 5C.

**Deacetylation of MLH1 by HDAC6 blocks the MutLa-MutSa complex formation**

To further confirm whether the identified sites could be acetylated in vivo, we mutated each of the four lysine residues to arginine. However, single site mutation of MLH1 did not show a significant difference in acetylation level when compared to wild-type MLH1 (data not shown). Thus, we mutated all four lysine sites of MLH1 either to arginine (4KR) or glutamine (4KQ) to mimic the non-acetylated and acetylated form, respectively. As shown in Figure 6A, acetylation of the 4KR and 4KQ mutants was abolished, indicating that these four lysine sites are indeed the major acetylation sites in vivo. With a longer exposure of Western blots...
for the anti-total acetylated lysine antibody, 4KR and 4KQ showed weak signals (data not shown), suggesting that there are additional minor unidentified acetylation sites in MLH1. To determine the biological role of MLH1 acetylation, we first tested whether acetylation/deacetylation of MLH1 affects MLH1-PMS2 association. As shown in Figure 6B, no observable difference existed between wild-type MLH1 and its mutants in terms of their binding to PMS2 (panel 4). We next examined whether the acetylation status of MLH1 affects the MutLα-MutSα complex formation. As shown in Figure 6B, the MLH1-4KR mutant loses its binding affinity to both MSH6 and MSH2, while MLH1-4KQ displays the same binding affinity as wild-type MLH1 (panels 1 and 2). This result suggests that deacetylation of MLH1 diminishes its binding to the MutSα complex. To determine whether binding deficiency of MLH1-4KR is due to the change in acetylation levels of all four lysines or a site specific effect, we examined the single lysine to arginine mutants, and found that none of the single sites affected the binding to both MSH2 and MSH6 (Figure 6C). We next established HDAC6 tet-on inducible knockdown U2OS and H292 cell lines to examine the role of HDAC6 in the MutSα-MutLα complex formation. As shown in Figure 6D and 6E, knockdown of HDAC6 increases the binding of MutSα to MLH1 in both U2OS and H292 cell lines, indicating that HDAC6 prevents the formation of the MutSα-MutLα complex.

**Deacetylation mimetic mutant of MLH1 confers 6-TG resistance**

We previously showed that low levels of the MutSα complex confers 6-TG resistance (16). However, it is not clear whether the MutLα complex is involved in a 6-TG-mediated DNA damage response. To this end, we utilized the human embryonic kidney (HEK) 293T cell line to test whether MutLα plays a role in 6-TG sensitivity. HEK293T is considered to be an MMR-deficient cell line because of promoter hypermethylation on MLH1 and extremely low expression of PMS2 (22). We stably transfected vector or MutLα (MLH1-PMS2) in 293T cells. As shown in Figure 7A, compared to the vector control, 293T cells harboring wild-type MutLα (MLH1-PMS2) showed a damage-susceptible phenotype in response to 6-TG, with cleaved PARP-1 protein being used as a surrogate for apoptosis, indicating that ectopically expressed MutLα indeed functions as a sensor in 6-TG-induced cell death signaling. We next tested the difference between wild-type MLH1 and its mutants in response to 6-TG treatment. We stably transfected MLH1(4KR)-PMS2 and MLH1(4KQ)-PMS2 into 293T cells. As shown in Figure 7B, both wild-type MLH1 and acetylation mimetic mutant 4KQ, but not deacetylation mimetic mutant 4KR, showed an increase in apoptosis upon 6-TG treatment. As we have shown in Figure 6B, 4KR cannot form the complex with MutSα efficiently, and thus we conclude that the assembly of MutSα-MutLα is necessary for mediating 6-TG-induced apoptosis. The expression levels of exogenous MLH1 and PMS2 are shown in Figure 7E.

To further confirm the effect of the decetylation mimetic mutant 4KR, MLH-PMS2-, MLH1(4KR)-PMS2- and MLH1(4KQ)-PMS2-rescued 293T pools were used for MTT assays to determine whether expression of these mutants affects cell viability in response to 6-TG. As shown in Figure 7C, MLH1(4KQ)-PMS2 exhibited a similar cell viability in response to 6-TG compared to MLH1-WT, while MLH1-4KR exhibited a 2-fold increase of cell survival compared with MLH1-WT, suggesting that MLH1(4KR)-PMS2 confers resistance to 6-TG. To test the long-term cell survival, we also conducted the colony formation assays.
As shown in Figure 7D, MLH1(4KR)-PMS2-rescued 293T cells were more resistant to 6-TG compared with MLH1-PMS2- and MLH1(4KQ)-PMS2-rescued 293T cells, suggesting that deacetylating MLH1 is associated with a cellular tolerance to DNA damage.

**Acetylation/deacetylation of K33 and K241 in MLH1 may be important for MSH2 binding**

Finally, we explored the mechanism by which the four acetylated lysine residues in MLH1 regulate the assembly of the MutS\(\alpha\)-MutL\(\alpha\) complex. Of the four residues, Lys361 and Lys377 are outside the structured area, and K361 is not conserved in mouse (Figure 5). We then focused on Lys33 and Lys241. As shown in Figure 8, both Lys33 and Lys241 are located in a region enriched with acidic residues. Lys33 is located near Glu34 and Glu37. Lys241 is located close to Glu234 and Glu297. These acidic residues form a continuous negatively charged surface patch that covers the majority of the surface of an elongated groove. If this groove is a potential site for MSH2 binding, the interaction regions in MSH2 are likely positively charged. Without acetylation, Lys33 and Lys241 may interfere with MSH2 binding via electrostatic repulsion. Contrarily, the interaction with MSH2 may be enhanced because of lysine neutralization by acetylation.

**Discussion**

In this study, we have shown that a key DNA mismatch repair protein, MLH1, is a novel substrate of HDAC6. Acetylation/deacetylation of MLH1 can be regulated by p300 and HDAC6, respectively. Deacetylation of MLH1 leads to disassociation of the MutS\(\alpha\)-MutL\(\alpha\) complex, a core component of DNA mismatch repair machinery, and induces 6-TG tolerance (Figure 9).

Much attention has been focused on how germline or somatic mutations, as well as promoter methylation, modulate the MLH1 gene, leading to the accumulation of DNA replication errors (7,23). These errors eventually manifest in a high frequency of microsatellite instability (MSI-H). However, post-translational modifications of MLH1 protein, and how these modifications regulate MLH1-mediated DNA damage response, are largely unknown. Our study, for the first time, has revealed that deacetylation of MLH1 by HDAC6 confers 6-TG resistance via disruption of the MutS\(\alpha\)-MutL\(\alpha\) complex. Because both TSA and nicotinamide treatment significantly increase the acetylation of MLH1 (Figure 3A and data not shown), and nicotinamide is a Sirtuin inhibitor, we speculate that our finding—HDAC6 deacetylates MLH1—may be the tip of the iceberg. Other HDACs and Situins could also modulate the status of MLH1 acetylation. Conversely, we suspect that in addition to p300, other HATs may acetylate MLH1. Therefore, multiple HDACs, Situins, and HATs could govern MLH1 functionality.

A report has shown that N-terminus of MLH1 (1-505aa) is essential for its binding to the MutS\(\alpha\) complex, while the C-terminus of MLH1 is important for the MLH1-PMS2 heterodimer formation (24). The results of our study are consistent with this published report. All four acetylated lysines (K33, K241, K361 and K377) are located in the N-terminus of MLH1. The deacetylation mimetic mutant of MLH1 exhibits a significantly reduced binding affinity to the MutS\(\alpha\) complex compared to the wild-type and acetylation mimetic mutant of MLH1. However, the acetylation mimetic mutant of MLH1 displays a similar binding affinity to the MutS\(\alpha\) complex compared to the wild-type MLH1. Previously, we have identified four acetylated lysine sites (K845, K847, K871 and K892) in the C-terminus of MSH2, where MSH2 binds to the MutL\(\alpha\).
complex (16). An interesting continuation of this work would be to study whether acetylation/deacetylation of these sites would affect MSH2’s binding to MLH1 in the future.

Because MLH1’s mutation rate is high in HNPCC also called Lynch syndrome, we examined whether the four acetylation sites of MLH1 (K33, K241, K361, and K377) are mutated in HNPCC. By searching the online database (https://preview.ncbi.nlm.nih.gov/clinvar), we found that three out of four sites (K33, K241, K361 and K377) are mutated in HNPCC patients. These mutations are K33E, K241E, K241R, and K361E. Although the phenotypes of these mutations have yet to be reported, we predict that the patients harboring these mutations may exhibit MMR deficiency. Of these sites, K33 is highly conserved among species and located within the ATPase domain. We conducted the ATPase assays using the mutant of MLH1 containing K33R and found that the mutant displays the same ATPase activity as the wild-type (data not shown), suggesting that HDAC6 doesn’t regulate the ATPase activity of MLH1. Our structural analysis suggests that acetylation of the K33 and K241 sites favors MLH1’s binding to MutSα. Because both K361 and K377 sites are located in the unstructured region, we couldn’t perform molecular surface analysis. We assume that acetylation of both sites may assist MLH1’s binding to MutSα.

Our lab and others have shown that depletion of HDAC6 sensitizes cells to different drugs, including cisplatin, doxorubicin, MNN and 6-TG (16,20,21). We revealed that HDAC6 regulates DNA damage response and mismatch repair activities via regulation of MutSα homeostasis through sequential deacetylation and ubiquitylation of MSH2 (16). In this study we have, for the first time, shown that HDAC6 also regulates the DNA damage response by disrupting the assembly of the MutSα-MutLα complex via deacetylation of MLH1. Interestingly, both acetylation sites K33 and K377 of MLH1 can also be ubiquitinated (25). Expanding the data presented in this study may include testing whether HDAC6 ubiquitinates MLH1. Overall, our past and present studies have demonstrated that HDAC6 modulates the DNA damage response by regulating at least two critically important mismatch repair proteins, MSH2 and MLH1.

**Experimental procedures**

**Cell culture and transfection**— All cell lines were grown in Dulbecco’s Modified Eagle’s Medium (DMEM) with 10% fetal bovine serum or Tet-free serum for doxycycline inducible cell lines, penicillin (100 U/ml), and streptomycin (100 U/ml), except H292 inducible cells which were grown in RPMI 1640 medium with 10% Tet-free serum. Cells were incubated at 37 °C with 5% CO₂. The plasmids were transiently or stably transfected into cells using Lipofectamine 2000 (Invitrogen).

**Plasmids, antibodies, and chemicals**— The full-length GST-HDAC6 was constructed by inserting the cDNA into the pGEX-4T1 vector. Briefly, HDAC6 full-length cDNA was amplified by PCR with HA-HDAC6-F (16) as a template and primers GST-HDAC6-F and GST-HDAC6-R. The full-length GST-HDAC6 was constructed by inserting the cDNA into SalI and NotI sites into the pCMV-3Tag-1a vector between HindIII and SalI sites. The Flag-MLH1 and its deletion mutants were constructed by inserting cDNA into p3XFlag-CMV10 vector (Sigma) between EcoR1 and BamH1 sites. The single site mutants (K33R, K241R, K361R, and K337R) of MLH1 were generated using this plasmid as the template. Myc-MLH1 was constructed...
by inserting cDNA into pCMV-3Tag-2a (Agilent Technologies) between EcoRI and XhoI sites. Flag-MLH1 and PMS2 were tandemly cloned to LentiORF pLEX-MCS vector (Thermo Fisher Scientific Open Biosystems) with a T2A sequence between two cDNAs to generate Flag-MLH1/4KR/4KQ-T2A-PMS2 constructs (Please see supplemental materials and Table S1 for detailed information). The bacterial expressed His-MLH1 was constructed by inserting cDNA sequences of full-length MLH1 into the pET-28a vector between EcoRI and XhoI. HA-PMS2 plasmid was constructed by inserting PMS2 cDNA between the BamH1 and XhoI sites in pcDNA3.1-C-HA vector. Briefly, PMS2 cDNA was amplified by PCR using pBluescript PMS2 (Addgene Plasmid #16457) as a template and primers PMS2F-BamHI, and PMS2R-Sall. HA-PMS2 was obtained by inserting PMS2 cDNA between BamH1 and XhoI sites in pcDNA3.1-HA (with a c-terminal HA tag). Please note that SalI and XhoI generate the same cohesive end after cutting. The Inducible HDAC6 shRNAs were purchased from Dharmacoyn Inc, and the targeted sequences are: V3THS_330047, TTCGCTTTGAAGTGACACT and V2THS_71188, TTCTGTGGACATAGCGGG. Please see Table S1 for primers used in generating the above constructs. The anti-HDAC6 (H-300), anti-MLH1 (C-20, N-20 and B-12), anti-PMS2 (C-20), anti-HA (Y-11), anti-Myc (8E10) antibodies were purchased from Santa Cruz, and the anti-MSH2 antibody was purchased from Calbiochem and the anti-MSH6 antibody from BD Biosciences. The anti-PARP1 antibody was purchased from Cell Signaling Technology. The anti-Flag-M2 antibody and agarose beads, anti-β-actin antibody, 6-TG, doxycycline, etoposide, tubastatin A, MTT, and protease inhibitor cocktail were purchased from Sigma. Ni-NTA resin was purchased from Clontech.

6-TG treatment— 6-TG was dissolved in the medium directly from a stock of 1.5 mM. Cells were washed with PBS and incubated in fresh medium with 6-TG at 37 °C for different time periods, as indicated in the figures. For longer treatments, 6-TG was replenished every 2 days.

Immunoprecipitation and immunoblotting— For immunoprecipitations, cells were lysed in LS buffer (PBS, pH 7.5, 10% glycerol, 0.1 % Nonidet P-40, protease inhibitor cocktail), BC100 buffer (20 mM Tris, pH 7.9, 100 mM NaCl, 10% glycerol, 0.2 mM EDTA, 0.2% Triton X-100 and protease inhibitor cocktail), or cell lysis buffer (10 mM Tris, pH 8.0, 85 mM KCl, 5 mM EDTA, 0.5% NP-40, 0.25% Triton X-100 and fresh protease inhibitor cocktail). Lysates were incubated for 2 hours with protein A- or protein G- agarose beads for pre-clearing. Primary antibodies were added to the supernatants for 12 hours at 4 °C, followed by the addition of protein A- or G-agarose beads for an extra 2 hours. Immune complexes were collected, washed 3 times in lysis buffer, and resolved on SDS-PAGE. For immunoblotting, samples were transferred to nitrocellulose membranes then probed with indicated antibodies. Bound antibodies were detected using a Chemiluminescent Detection Kit (Pierce).

Establishment of stable clones— For construction of the stable cell lines, F-MLH1-PMS2, F-MLH1-4KR-PMS2, F-MLH1-4KQ-PMS2, and empty vector were used to transfext 293T cells. One day after transfection, cells were split. After 24 hrs, 2 μg/ml puromycin was added into the medium.
to select positive cells. Ten days later, stable cell lines were subcloned into 60 mm dishes and 1 μg/ml puromycin was added to the medium to maintain the stable clones in the subsequent culture. The well-isolated single clones were transferred into 24-well plates. The ectopic protein expression was verified by Western blotting analyses using anti-Flag antibodies. Wild-type MLH1 and its mutant clones with comparable protein expression were isolated for further experiments.

Establishment of HDAC6 inducible knockdown stable clones— The TRIPZ inducible lentiviral vectors containing shHDAC6-047 and 088 were transfected into U2OS and H292 cells. One day after transfection, 1 μg/ml puromycin was added into the medium to select positive cells. Two weeks later, stable cell lines were subcloned to 60-mm dishes. Then cells were cultured for another 4 weeks with puromycin for further selection. Positive cells were confirmed by the manufacturer’s protocol. For induction of HDAC6 knockdown, vehicle or doxycycline (0.5 μg/ml) was added to each positive clone for four days. Anti-HDAC6 Western blotting analysis was used to verify HDAC6 knockdown.

Colony formation assay—Flag-MLH1/4KR/4KQ-PMS2 or empty vector was stably transected, 293T cells were seeded in triplicates (500 cells per 60-mm dish), and the cells were incubated overnight at 37 °C to allow for adherence to the dishes. Cells were then treated with a vehicle control or 6-TG at 0.5 μM for 10 days. Afterwards, cells were directly fixed and stained with crystal violet (0.05% w/v, 1% formaldehyde, 1% methanol in PBS) for 20 minutes. Colonies on each plate were scanned and counted using OpenCFU software.

MTT (3-(4, 5-Dimethylthiazol-2-yl)-2, 5-diphenyltetrazolium bromide) assay—2,000 cells were seeded in 96-well plates and incubated at 37 °C overnight. 6-TG was added into the medium for 2 days at the indicated concentrations. Afterwards, 15 μl MTT was added into medium for 4 hours before being replaced and dissolved with DMSO. The absorbance was quantified by spectrophotometer at 570 nm wavelength.

Identification of acetylation sites of MLH1 by LC-MS/MS— The gel band containing acetylated MLH1 was excised, and treated with Tris (2-carboxy-ethyl) phosphine hydrochloride (TCEP) and iodoacetamide. Trypsin in-gel digestion was carried out at 37 °C overnight. The extracted peptides were analyzed by LCMS/MS. A nanoflow liquid chromatograph (U3000, Dionex, Sunnyvale, CA) coupled to an electrospray ion trap mass spectrometer (LTQ-Orbitrap, Thermo, San Jose, CA) was used for tandem mass spectrometry peptide sequencing experiments. The sample was first loaded onto a pre-column (5 mM x 300 μM ID packed with C18 reversed-phase resin, 5 μm, 100Å) and washed for 8 minutes with aqueous 2% acetonitrile and 0.04% trifluoroacetic acid. The trapped peptides were eluted onto the analytical column, (C18, 75 μm ID x 15 cm, Pepmap 100, Dionex, Sunnyvale, CA). The 120-minute gradient was programmed as follows: 95% solvent A (2% acetonitrile + 0.1% formic acid) for 8 min, solvent B (90% acetonitrile + 0.1% formic acid) from 5% to 50% for 35 minutes, then solvent B from 50% to 90% for 2 min and holding at 90% for 5 minutes, followed by solvent B from 90% to 5% for 1 min and re-equilibration for 10 min. The flow rate on the analytical column was 300 nl/min. Tandem mass spectra were collected in a data-dependent manner following each survey scan. The MS scans were performed in Orbitrap to obtain accurate peptide mass measurement and the MS/MS scans were performed in a linear ion trap using 60 sec exclusion for previously sampled peptide peaks. Sequest and Mascot searches were performed against the Swiss-Prot human
database downloaded on February 10th, 2009. Two trypsin-missed cleavages were allowed, and the precursor mass tolerance was 1.08 Da. MS/MS mass tolerance was 0.8 Da. Both MASCOT and SEQUEST search results were summarized in Scaffold 2.0.

**GST Pulldown**— GST fusion proteins were purified as previous described (16). For *in vitro* binding assays, His tagged MLH1 protein was incubated with 10 μl glutathione agarose beads or beads with 1 μg of the GST protein or GST-HDAC6 protein in BC100 at 4°C overnight with gentle rotation. The GST beads were washed 4 times with BC100 buffer and eluted with 40 μl BC100 plus 20 mM reduced glutathione for 2 hours with gentle rotation. Half of the elution was resolved on a SDS-PAGE gel and detected by Western blot.

**Immunofluorescence staining**—H1299 cells were plated in a 12-well plate containing a circular glass coverslip in 1ml RPMI media. 24 hours post-plating, etoposide was added for a final concentration of 10 μM. At the indicated time, glass coverslips were removed from the wells and fixed in 4% paraformaldehyde in a 24-well plate at room temperature (RT) with shaking for 10 min, and stored in PBS at 4°C until all timepoints were collected. Then, coverslips were washed with PBS, then washed with 0.3% Triton-X in PBS for 10 min two times, then washed with 2% BSA 0.3% Triton-X in PBS for 15 minutes. Then, primary antibodies, a 1:800 dilution of rabbit anti-HDAC6 (Sigma) and mouse anti-MLH1 (Abcam G168-15, ab14206), were added in a mixture of 150 μl of 2% BSA and 0.1% Triton-X in PBS which was added to the coverslips. The plate was sealed with paraffin wrap and incubated on the shaker in the cold room (4 °C) for 48 hours. After 48 hours, the plate was removed from the cold room and placed on the RT shaker for 20 minutes. Primary antibody was removed, and coverslips were washed with PBS for 15 minutes three times. Then the secondary antibodies, a 1:800 dilution of AlexaFluor 488-tagged anti-rabbit secondary and AlexaFluor 594-tagged anti-mouse secondary (Thermo Fisher Scientific), were added to a solution of 2% BSA and 0.1% Triton-X in PBS. 150 μl of this mix was added to each coverslip well, and the plate was incubated at RT in the dark for 4 hours. Orientation of the plate was reversed halfway through the incubation. Then, coverslips were washed with 0.3% Triton-X in PBS for 15 minutes three times, and mounted on glass slides with a drop of Vectashield® antifade mounting medium with DAPI. Coverslips were sealed to the slide with clear nail polish. Images were taken using a Zeiss LSM 780 Confocal Microscope paired with Carl Zeiss Zen 2012 SP1 (black edition) (64 bit) software. Acquisition settings were the same for all images. Dimensions: 1024x1024, 3 channels, 8-bit; Objective: EC Plan-neofluar 40x/1.30 oil Ph3 M27; Pixel dwell: 1.58μS; Average: line 8.

**Histone Deacetylation Assay** —The ac-Myc-MLH1 and the F-HDAC6 full-length proteins were purified from 293T cells. Briefly, Myc-MLH1 was transfected into 293T cell for 2 days and treated with TSA overnight prior to harvest. Purified ac-Myc-MLH1 on the Myc-beads was incubated with either F-HDAC6 or buffer control in 50 μl of ice-cold HD buffer (20 mM Tris [pH 8.0], 150 mM NaCl, and 10% glycerol) at 37°C for 2hr. The reaction was terminated by adding the SDS-PAGE loading buffer.

**Nuclear/cytoplasmic fraction preparation**—The adherent cells in 60 mm plates were washed with cold PBS and cells were scraped in PBS. Then cells were suspended in 0.4 ml lysis buffer (10 mM Hapes, pH7.9, 10 mM KCl, 0.1 mM EDTA and 0.1 mM EGTA with protease inhibitors)
and left on ice for 15 min. Then, 12.5 µl of
10% NP-40 was added and the mixture was
vortexed for 10 seconds. The mixture was
spun down for 1 min at 14,000 rpm at 4°C.
The supernatant was kept, which is the
cytoplasmic fraction. Then 40 µl extraction
buffer (20 mM Hepes, pH7.9, 0.4 M NaCl, 1
mM EDTA, 1 mM EGTA with protease
inhibitors) was added. The sample was left on
ice for 30 min, and it was vortexed for 3-5
seconds every 5 min. Then it was spun for 5
min at full speed (14,000 rpm) at 4°C. The
resultant supernatant was the nuclear fraction.
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HDAC6 deacetylates MLH1

FOOTNOTES

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The abbreviations used are: PTM, post-translational modification, HDAC6, histone deacetylase 6, MLH1, MutL homolog1, MMR. DNA mismatch repair, DDR, DNA damage response, HNPCC, hereditary nonpolyposis colorectal cancer, PCNA, proliferating cell nuclear antigen, EXO1, Exonuclease 1
HDAC6 deacetylates MLH1

Zhang et al., Figure 1

A

IP
Input
Anti-IgG
Anti-HDAC6

IB anti-MLH1
75

IB anti-HDAC6
150

IB anti-MLH1

IB anti-HDAC6

B

IP
Input
Anti-IgG
Anti-MLH1

IB anti-HDAC6
150

IB anti-MLH1
75

IB anti-HDAC6

IB anti-MLH1

D

HDAC6
1-1215 aa
1-503 aa
488-839 aa
835-1215 aa

D1

D2

ZnF

C

His-MLH1

GST pull-down
IB anti-MLH1
75

IB anti-HDAC6

Coomassie
Staining

GST

GST-HDAC6

E

Myc-MLH1

Interaction with MLH1

F

PMS2/MLH3/MLH1

binding domain

Interaction with HDAC6

MLH1

ATPase
MutS binding domain

1-756 aa
1-147 aa
147-320 aa
320-491 aa

G

HA-HDAC6

F-HD6

F-HD6(835-1215)

F-HD6(1-503)

F-HD6 (488-839)

Input
IB anti-Myc

IB anti-Flag

IB anti-Flag

IB anti-Flag

IB anti-Flag

IB anti-HA

F-HD6

Myc-MLH1

Myc-MLH1

Myc-MLH1

Myc-MLH1

F-MLH1

F-MLH1 (1-147)

F-MLH1 (491-756)

F-MLH1 (147-320)

F-MLH1 (229-491)

F-MLH1(1-147)
Figure 1. **HDAC6 interacts with MLH1.** A and B, Endogenous interaction of HDAC6 and MLH1 in A549 cells. A, Immunoprecipitation (IP) was performed with either anti-IgG or anti-HDAC6 antibody in A549 cells, followed by anti-MLH1 and anti-HDAC6 Western blotting analyses as shown in the upper and lower panel, respectively. The input of MLH1 and HDAC6 are also shown. B, The reciprocal IP of A was performed. C, Physical interaction between HDAC6 and MLH1. GST pull-down assays were performed with the bacterially-expressed His-MLH1 and GST-HDAC6 proteins, followed by anti-His Western blotting analysis (upper panel). Purified GST and GST-HDAC6 proteins were analyzed by coomassie blue staining (lower panel). Glutathione agarose was used for pull-down as a negative control. D, Schematic representation of HDAC6 full-length protein and its deletion mutants. The strength of interaction between HDAC6 and MLH1 was shown as ++, very strong binding: +, strong binding, and -, no binding. E, MLH1 binds to HDAC6 deacetylase domain 1 (DAC1) and 2 (DAC2). Empty vector, Flag-tagged full-length HDAC6 (1-1215), DAC1 (1-503), DAC2 (488-834) or ZnF-UBP (835-1215) was co-transfected with Myc-MLH1 in 293T cells. Anti-Flag immunoprecipitation was performed, followed by Western blotting using anti-Myc and anti-Flag antibodies (upper and middle panels). The input of Myc-MLH1 is shown in the anti-Myc Western blotting analysis in the lower panel. F, Schematic representation of MLH1 full-length protein and its deletion mutants. The strength of interaction between MLH1 and HDAC6 was shown as +, strong binding, +/-, weak binding, and -, no binding. G, HDAC6 binds to MLH1’s N-terminal (1-320) and C-terminal (491-756) regions. Empty vector, Flag-tagged full-length MLH1(1-756), MLH1(1-147), MLH1(147-320), MLH1(320-491) or MLH1(491-756) was co-transfected with HA-HDAC6 in 293T cells. Anti-Flag immunoprecipitation was performed and followed by Western blots using anti-HA and anti-Flag antibodies (upper and middle panels). The input of HA-HDAC6 is shown in the anti-HA Western blot (lower panel).
HDAC6 deacetylates MLH1

Zhang et al., Figure 2

A

T29 Nuclear Extract

IP

Mr (Kd)

IB anti-MLH1 75

IB anti-HDAC6 150

1 2 3

MLH1

BD

C

Etoposide

B

Vehicle

Merged

MLH1

HDAC6

DAPI

C

Etosopside

Merged

MLH1

HDAC6

DAPI

D

| Mr (Kd)    | A549 C | H460 C | H292 C | H125 C |
|------------|--------|--------|--------|--------|
| IB anti-HDAC6 |        |        |        |        |
| IB Anti-PARP1 |        |        |        |        |
| IB Anti-Hsp60 |        |        |        |        |
Figure 2. **HDAC6 interacts with MLH1 in the nucleus.** *A*, HDAC6 interacts with MLH1 in the T29 nuclear extracts. Immunoprecipitation (IP) was performed with either anti-IgG or anti-HDAC6 antibody in T29 nuclear extracts, followed by anti-MLH1 and anti-HDAC6 Western blotting analyses as shown in the upper and lower panel, respectively. The input of MLH1 and HDAC6 are also shown. *B and C*, HDAC6 is co-localized with MLH1 in H1299 cells upon etoposide treatment. Representative images of immunofluorescence staining of HDAC6, MLH1 and DAPI as well as merged images in vehicle-treated H1299 cells (*B*) and 10 μM etoposide-treated H1299 cells. *D*, Nuclear (N)/cytoplasmic (C) expression of HDAC6 in four non-small cell lung cancer cell lines. The nuclear and cytoplasmic fractions were prepared as described in the Experimental Procedures. The Western blot analyses were performed with indicated antibodies.
Figure 3. **HDAC6 is an MLH1 deacetylase.** *A*, The level of acetylated MLH1 is increased upon TSA treatment. 293T cells were treated with vehicle or 200 μM of TSA for 12 hours. Thirty-six hours after transfection, cells were lysed and anti-MLH1 agarose beads were used to immunoprecipitate MLH1. The resulting immuno-complexes were resolved on SDS-PAGE, followed by anti-acetylated-lysine (anti-AcK) Western blotting analysis (upper panel). The membrane was stripped and reprobed with the anti-MLH1 antibody (lower panel). *B*, The level of acetylated MLH1 is increased upon Tubastatin A treatment. 293T cells were treated with vehicle or 10 μM of Tubastatin A for 12 hours. Thirty-six hours after transfection, cells were lysed and anti-MLH1 agarose beads were used to immunoprecipitate MLH1. The resulting immuno-complexes were resolved on SDS-PAGE, followed by anti-acetylated-lysine (anti-AcK) Western blotting analysis (upper panel). The membrane was stripped and reprobed with the anti-MLH1 antibody (lower panel). *C*, p300 acetylates MLH1. 293T cells were transfected with an empty vector, F-MLH1, or F-MLH1 with HA-p300. Cell lysates were immunoprecipitated with anti-Flag-M2 agarose beads, and an anti-Ac-K antibody was used to detect the Ac-MLH1 levels by Western blotting analysis (upper panel). The membrane was then stripped and reprobed with the anti-Flag antibody (lower panel). *D*, HDAC6 deacetylates MLH1 in 293T cells. Myc-MLH1 was transfected with either empty vector or Flag-HDAC6 in 293T cells. The cell lysates were immunoprecipitated with anti-Myc agarose beads, followed by anti-AcK Western blotting analysis (upper panel). The membrane was stripped and reprobed with anti-Myc antibody (middle panel). The expression of Flag-HDAC6 was detected by anti-Flag Western blotting analysis (lower panel). *E*, HDAC6 deacetylates MLH1 *in vitro*. Flag-HDAC6 was purified from 293T cells. To obtain acetylated Myc-MLH1, 293T cells were transiently transfected with Myc-MLH1, followed by TSA treatment. Acetylated Myc-MLH1 was then purified and incubated with either buffer or purified Flag-HDAC6. Reactions were stopped by adding SDS-PAGE loading buffer directly, and were then subjected to Western blots with anti-AcK, and anti-Myc antibodies. The lower panel shows purified Flag-HDAC6 by coomassie blue staining. For all the panels, the acetylated MLH1 bands were quantified and the fold-changes were shown below the those bands.
Figure 4. Lysines 33, 241, 361 and 377 are acetylated in MLH1. A, Lysine 33 is acetylated in MLH1. The peptide was detected with a mass-to-charge ratio of 625.3074, which represents an error of 6.1 ppm. The tandem mass spectrum matched the following sequence, PANAIKEMIENCLDAK, indicating that the first lysine was acetylated. B, Lysine 241 is acetylated in MLH1. The peptide was detected with a mass-to-charge ratio of 1040.0105, which represents an error of 4.2 ppm. The tandem mass spectrum matched the following sequence, TLAFKMNGYISNANYSVK, indicating that the first lysine was acetylated. C, Lysine 361 is acetylated in MLH1. The peptide was detected with a mass-to-charge ratio of 1237.5928, which represents an error of 1.4 ppm. The tandem mass spectrum matched the following sequence, MYFTQTLPLLPLGSPGEMVKSTTSSTSSSTSGSSDK, indicating that the first lysine was acetylated. D, Lysine 377 is acetylated in MLH1. The peptide was detected with a mass-to-charge ratio of 858.7390, which represents an error of 4.3 ppm. The tandem mass spectrum matched the following sequence, STTSLTSSSTSGSSDKVYAHQMVR, indicating that the first lysine was acetylated.
**Figure 5. Conservation of four acetylated lysines in MLH1.**

A. A stretch of MLH1 amino acids shows the conservation of lysine 33 among different species. B. Stretches of MLH1 amino acids show the conservation of lysines 241, 361 and 377 among different species. Consensus amino acids are indicated as *. C. A diagram of MLH1’s domain structure showing the locations of four acetylated lysines.
Figure 6. Acetylation/deacetylation of MLH1 regulates MLH1’s binding to MutSα. A, The K33, K241, K361 and K377 sites are the major acetylation sites in MLH1. MLH1-deficient SKOV3 cells were stably transfected with Flag-tagged control vector, MLH1-PMS2, MLH1(4KR)-PMS2 or MLH1(4KQ)-PMS2. The cell lysates were immunoprecipitated with anti-Flag-M2 agarose beads followed by Western blotting analysis with anti-AcK. The membrane was stripped and rebotted with the anti-Flag antibody. The input is shown by anti-Flag, anti-PMS2, and anti-β-actin antibodies. B, The MLH1-4KR mutant exhibits reduced binding affinity to MSH2 and MSH6. 293T cells were stably transfected with Flag-tagged control vector, MLH1-PMS2, MLH1(4KR)-PMS2 or and MLH1(4KQ)-PMS2. The cell lysates were immunoprecipitated with anti-Flag-M2 agarose beads followed by Western blots with indicated antibodies. The expression of F-MLH1, PMS2, MSH2 and MSH6 in transfected HEK293T cells was shown by indicated Western blots. C, All MLH1 mutants (K33R, K241R, K361R and K377R) display the same affinity of binding to MSH2 and MSH6 as wild-type MLH1. F-MLH1, F-MLH1-K33R, F-MLH1-K241R, F-MLH1-K361R or F-MLH1-K377R was transiently co-transfected with HA-PMS2 into HEK293T cells. The cell lysates were immunoprecipitated with anti-Flag-M2 agarose beads followed by Western blotting analysis with the indicated antibodies. The immunoprecipitated F-MLH1 (WT and mutants) and HA-PMS2 are indicated by Ponceau staining. The expression of MSH2, MSH6, input of F-MLH1 (WT and mutants) and HA-PMS2 is shown by anti-MSH2, anti-MSH6, anti-Flag, and anti-PMS2 Western blotting analyses. D and E, Knockdown of HDAC6 enhances the formation of the MutLα-MutSα complex in U2OS and H292 cells. U2OS (D) or H292 (E) Tet-on inducible HDAC6 knockdown cells were cultured with the Tet-free medium (lane 1) or doxycycline-containing medium (0.5 μg/ml) (lane 2). The cell lysates were immunoprecipitated with an anti-MLH1 antibody, followed by Western blotting with the indicated antibodies. The inputs of D and E were shown by direct Western blots with indicated antibodies.
Zhang et al., Figure 7

A

Vector | F-MLH1-PMS2
---|---
6-TG | 0 | 1 | 2 | 3 | 0 | 1 | 2 | 3 | days
Mr (Kd)
IB anti-PARP1
IB anti-β-actin

B

F-MLH1-PMS2 | F-MLH1(4KR)-PMS2 | F-MLH1(4KO)-PMS2
---|---|---
6-TG | 0 | 2 | 3 | 0 | 2 | 3 | 0 | 2 | 3 | days
Mr (Kd)
IB anti-PARP1
IB anti-β-actin

C

6-TG | 0 μM | 2.5 μM
---|---|---
Cell viability (% of control)
Vec | MLH1-PMS2 | 4KR-PMS2 | 4KQ-PMS2

D

6-TG | 0 μM | 0.5 μM
---|---|---
Colony Formation (% of Control)
Vec | MLH1-PMS2 | 4KR-PMS2 | 4KQ-PMS2

E

IB anti-Flag
IB anti-PMS2
IB anti-β-actin

Mr (Kd)
76 - | 100 - | 37 -
Figure 7. **The deacetylation mimetic mutant of MLH1 confers 6-TG tolerance in HEK293T cells.** A, Reintroduction of wild-type MLH1 and PMS2 in HEK293T cells sensitizes cells to 6-TG. F-MLH1-PMS2 stably transfected 293T cells were treated with 50 μM 6-TG for the indicated time intervals. Anti-PARP1 and anti-β-actin Western blotting analyses were performed. B, The deacetylation mimetic mutant of MLH1 cannot sensitize cells to 6-TG as does wild-type MLH1. The Flag-tagged MLH1-PMS2, 4KR-PMS2 or 4KQ-PMS2 was stably transfected into 293T cells, followed by 50 μM 6-TG treatment for indicated time intervals. Anti-PARP1 and anti-β-actin Western blotting analyses were performed. C and D, The deacetylation mimetic mutant of MLH1 confers 6-TG tolerance in 293T cells, as assessed via MTT and colony formation assays. The Flag-tagged empty vector, MLH1-PMS2, 4KR-PMS2 or 4KQ-PMS2 was stably transfected into 293T cells. MTT assays were performed with indicated 6-TG concentrations for 3 days (C). Colony formation assays were performed with vehicle or 0.5 μM 6-TG for 10 days. Cells were stained with crystal violet and survival colonies were counted, as shown in D. For C and D, Students’ t test was performed with *, p<0.05; ns, not significant. Error bars, S.D. E, The expression of F-MLH1-WT, F-MLH1-4KR, F-MLH1-4KQ and PMS2 in 293T stable cell lines. Lysates from the aforementioned cell lines were subjected to anti-Flag, anti-PMS2, and anti-β-actin Western blotting analyses.
Figure 8. MLH1 structure represented by molecular surface. Surface coloring is according to the electrostatic potential: red, white, and blue corresponding to negative, neutral, and positive potential, respectively. Two acetylated lysines, K33 and K241, are indicated. The potential MSH2 binding site is indicated in the region encircled by the dotted line. The structures of the sites of K33 and K241 are also enlarged.
Figure 9. A working model of how HDAC6 regulates MutSα’s recruiting MutLα via deacetylation of MLH1. Upon 6-TG treatment, 6-thioguanine (sG) and S⁶-methylthioguanine (S⁶mG) can be inserted into the DNA, so that sG:T and S⁶mG:T mispairs can be formed. These mispairs would be recognized by the MSH2-MSH6 heterodimer (MutSα), and MutSα would then recruit the MLH1-PMS2 heterodimer (MutLα) and additional components to initiate futile mismatch repair, leading to apoptosis. However, HDAC6 can deacetylate MLH1 and prevent MutLα from being recruited to MutSα, leading to 6-TG tolerance.
HDAC6 regulates DNA damage response via deacetylating MLH1
Mu Zhang, Chen Hu, Niko Moses, Joshua Haakenson, Shengyan Xiang, Daniel Quan, Bin Fang, Zhe Yang, Wenlong Bai, Gerold Bepler, Guo-Min Li and Xiaohong Mary Zhang

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