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Review

Regulation of protein stability of DNA methyltransferase 1 by post-translational modifications

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DNA methylation is an important epigenetic mechanism that ensures correct gene expression and maintains genetic stability. DNA methyltransferase 1 (DNMT1) is the primary enzyme that maintains DNA methylation during replication. Dysregulation of DNMT1 is implicated in a variety of diseases. DNMT1 protein stability is regulated via various post-translational modifications, such as acetylation and ubiquitination, but also through protein–protein interactions. These mechanisms ensure DNMT1 is properly activated during the correct time of the cell cycle and at correct genomic loci, as well as in response to appropriate extracellular cues. Further understanding of these regulatory mechanisms may help to design novel therapeutic approaches for human diseases.

Keywords DNA (cytosine-5-)-methyltransferase; epigenetics; protein stability; post-translational modification; neoplasms

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Introduction

Epigenetic changes—inheritance of genetic traits that cannot be explained by changes in the DNA sequence—rely heavily on DNA methylation to propagate responses to environmental and/or developmental cues [1]. Enzymes that catalyze this modification are part of the DNA methyltransferase family. Among them, DNA (cytosine-5-)-methyltransferase 3 alpha and DNA (cytosine-5-)-methyltransferase 3 beta are thought to be *de novo* DNA methyltransferases, which methylate genes to regulate them in a developmental context. Conversely, DNA (cytosine-5-)-methyltransferase 1 (DNMT1) specifically methylates DNA during replication to maintain correct methylation patterns on the new DNA strand [2].

DNA methylation via DNMT1 ensures correct formation of heterochromatin [3,4] and promoter repression via histones [5]. Interactions between histone deacetylases and DNMT1 further underscore the relationship between chromatin and DNMT1 [6], but also belie a mechanism for DNMT1 regulation [7].

Correct regulation of DNMT1 and its relationship with histone modifications becomes especially important in the case of 'bivalent chromatin'—those promoters with both activating and repressive histone marks. The latter modifications could inadvertently recruit DNMT1 to aberrantly methylate promoters, permanently turning off a gene that should otherwise be temporarily silenced [8].

Dysregulation of DNMT1 activity causes human diseases, such as cancer [9] and various genetic disorders [10,11]. DNMT1 mutations are found in patients with hereditary sensory neuropathy [12,13] and in human cancers [14]. Cancer epigenetic landscapes are generally defined by global DNA hypomethylation with localized promoter hypermethylation at tumor suppressors [8]. These tumor suppressors include Cadherin 1, Type 1, E-Cadherin [15], adenomatous polyposis coli [16], Ras association domain family member 1 [17], p16 and TIMP metallopeptidase inhibitor 3 [18] among many others. As a corollary, both overexpression and developmental disruption of DNMT1 will lead to tumorigenesis in both experimental models and human cancer studies [19–24].

As such, given the complications of bivalent chromatin and the ramifications of incorrect DNMT1 protein levels, regulation of DNMT1 becomes especially vital and occurs through a variety of pathways [9,25] (summarized in **Table 1**). Although DNMT1 is also regulated at the transcriptional level [34–37], this review will focus on emerging mechanisms of post-translational regulation of DNMT1 protein stability.

Regulation of DNMT1 Protein Stability by Acetylation and Ubiquitination

DNMT1 protein abundance is tightly regulated during the cell cycle: it peaks at the early S phase, decreases after the S phase, and reaches lowest level at the G1 phase [26]. The primary

Table 1. List of modifications to DNMT1, the enzymes that catalyze them, and the resultant effects on DNMT1 stability

Modification	Enzyme	Effect on DNMT1	References
Acetylation	Tip60, p300, PCAF	Destabilizing	[26–28]
Deacetylation	HDAC1, Sirt1	Stabilizing	[26,28–30]
Methylation	Set7	Destabilizing	[31]
Demethylation	LSD1	Stabilizing	[32]
Phosphorylation	Akt1	Stabilizing	[33]

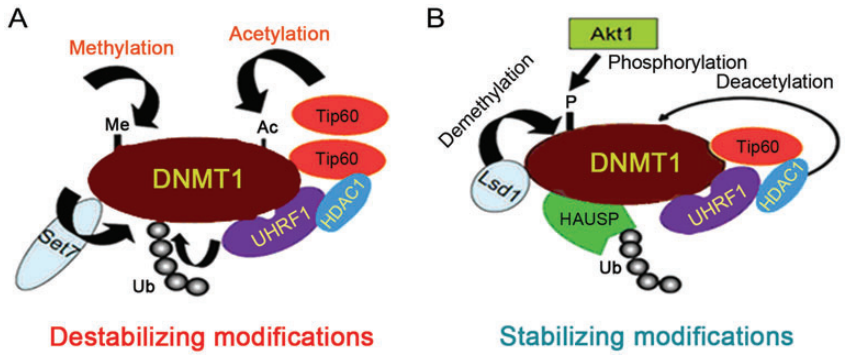

Figure 1. Post-translational modifications regulate DNMT1 protein stability (A) Destabilizing modifications. Methylation catalyzed by Set7 and Tip60 acetylation activity result in UHRF1 ubiquitination of DNMT1. (B) Stabilizing modifications. Demethylation by Lsd1 and/or Akt1 phosphorylation counteracts Set7 methylation. Deacetylation via HDAC1 stabilizes DNMT1, and HAUSP/USP7 deubiquitinates DNMT1.

function of DNMT1 is to methylate newly synthesized DNA during the S phase [1]. A crucial component of the replication machinery, proliferating cell nuclear antigen (PCNA), interacts with DNMT1 to ensure its correct positioning at the replication fork and at the appropriate time during replication [38,39]. The chromatin-associated ubiquitinase ubiquitin-like with PHD and ring finger domains 1 (UHRF1) [40,41] binds to hemi-methylated loci that are formed during DNA replication, helping DNMT1 co-localize with the replication fork [42] as well as chromatin [43]. A recent study using an Xenopus inter-phase egg extract system suggests an alternative mechanism that DNMT1 is recruited to the replication forks through binding to ubiquitinated histone H3, whereas the ubiquitination of histone is catalyzed by UHRF1 [44].

In addition to localizing DNMT1 correctly, UHRF1 also causes its degradation, as it ubiquitinates acetylated DNMT1. The acetyltransferase Tip60 protein levels are elevated in the late S phase, which, in turn, leads to DNMT1 acetylation. Acetylation of DNMT1 enhances its binding affinity with UHRF1 and thus triggers DNMT1 ubiquitination and degradation (**Fig. 1**) [26]. Conversely, DNMT1 is deacetylated by histone deacetylase 1 (HDAC1) and deubiquitnated by ubiquitin specific peptidase 7 (HAUSP), thereby stabilizing DNMT1 (**Fig. 1**) [26,29,30]. This model bears out *in vivo*, as DNMT1 levels correlate with HAUSP levels in human tumors [26]. Furthermore, the role of Tip60 and UHRF1 in DNMT1 stability connects with the DNA damage response via ataxia telangiectasia mutated (ATM). When phosphorylated ATM interacts with DNMT1, it coordinates its acetylation and subsequent ubiquitination via Tip60 and UHRF1 [45], consistent with previous reports [26]; accordingly, Rb antagonizes ATM and helps stabilize DNMT1 [45]. Tip60 acetylation can be regulated via extracellular cues, as the autocrine/paracrine signaling molecule gastrokine 1 decreases DNMT1 levels via upregulation of Tip60 and concomitant DNMT1 acetylation [27]. Other enzymes that modulate DNMT1 acetylation include K (lysine) acetyltransferase 2B for acetylation or sirtuin 1 for deacetylation [28]. Sirt1 deacetylation is especially notable for modulating DNMT1's methyltransferase activity as well [28].

Regulation of DNMT1 Protein Stability by Phosphorylation and Methylation

DNMT1 stability is also affected by lysine methylation. Specifically, DNMT1 binds with the histone methyltransferase Set7 and becomes methylated, especially during the S/G2 phase of the cell cycle. This mark catalyzes DNMT1 ubiquitination and subsequent degradation [31]. Conversely, the histone demethylase lysine (K)-specific demethylase 1A (LSD1) interacts with and demethylates DNMT1; when LSD1 is knocked out, cells experience global DNA hypomethylation and decreased DNMT1 levels (**Fig. 1**) [32].

Moreover, DNMT1 methylation at K142 occurs in opposition to its phosphorylation. While phosphorylation of DNMT1 had been shown to affect its methyltransferase activity [46] and its interaction with PCNA and UHRF1 [47], it also plays a role in DNMT1 stability [33]. When v-akt murine thymoma viral oncogene homolog 1 phosphorylates DNMT1 at S143, Set7 becomes unable to methylate DNMT1 at K142 and DNMT1 is stabilized. Much like ubiquitination, DNMT1 phosphorylation changes during the cell cycle: another example of the relationship between DNMT1 stability and appropriate timing of DNA methylation.

Regulation of DNMT1 Protein Stability by Associated Proteins

DNMT1 interacts with a multitude of proteins that have varying functions within the cell [48]. These proteins variously affect DNMT1 stability, activity, and localization. As an example, we recently identified an interaction between DNMT1 protein and the wingless-type mouse mammary tumor virus integration site family (Wnt) pathway effector, β-catenin. DNMT1 levels increase upon stimulation with Wnt3a in a β-catenin-dependent manner; moreover, the interaction between β-catenin and DNMT1 is mutually stabilizing (J. Song, Z. Wang and R. Ewing, unpublished data). Given the crucial role of Wnt signaling in development, these findings suggest a mechanism by which developmental cues can have an impact on the epigenetic landscape of a developing organism.

Therapeutic Implications

Several USA Food and Drug Administration approved epigenetic drugs have been shown to target DNMT1 protein stability. DNMT1 inhibitors (DNMTi), azacitidine and decitabine, are used to treat myelodysplastic syndromes. Canonically, they act via targeting DNMT1 [49], covalently binding with DNA methyltransferases to inactivate them [50–52]. However, recent research demonstrates that DNMTi also induce the proteosomal degradation of DNMT1 [53–55]. Interestingly, HDAC inhibitors (HDACi), another kind of epigenetic drug, also cause proteasome-mediated DNMT1 degradation [7,26]. HDACi, such as vorinostat, are currently on the market for treating cutaneous T-cell lymphoma [56]. However, neither HDACi nor DNMTi as single agent has an effect on solid tumors. Several ongoing clinical trials indicate that at least a subset of lung cancer patients is responsive to combination treatment of HDACi and DNMTi [57].

The HDAC1-HAUSP axis of DNMT1 regulation provides rationale for novel combinatorial therapy options. Knockout of HAUSP in colon cancer cells potentiates HDACi-induced DNMT1 degradation and thus enhances the tumor inhibitory effect of HDACi in a xenograft tumor model. [26]. These observations suggest that combination of HDACi with a HAUSP inhibitor (HAUSPi) may be an effective approach to treat solid tumors. In fact, several distinct HAUSPi have been developed. Some, such as HBX 19,818 [58,59] and P5091 [60,61] were derived from high-throughput compound screens. Another group rationally derived a peptide based on the interaction between HAUSP and a viral protein that inhibits its deubiquitinase activity [62]. Moreover, a compound isolated from sponges also has anti-HAUSP activity [63]. Many of these demonstrate cytotoxicity *in vitro* and in animal models of disease, particularly through potentiating p53-mediated apoptosis. As predicted by the interaction between HDAC1 and HAUSP, two different HAUSPis demonstrate synergistic effects with HDACi in cell culture [61, and P. Zhang and Z. Wang, unpublished data].

Perspectives and Future Directions

As increasing emphasis is placed on epigenetic mechanisms of gene expression, understanding DNMT1 stability and regulation becomes paramount. Like many other proteins, post-translational modifications of DNMT1 play a crucial role in how and when it is activated. When this protein becomes dysregulated, aberrant methylation patterns and subsequent tumor initiation can occur. Anti-cancer therapies increasingly emphasize the release of tumor suppressor genes from epigenetic mechanisms of repression [64,65]. One important way to effect this goal is to choose compounds that can affect DNMT1 stability.

An important outstanding question in the field of DNMT1 and cancer development is the mechanism by which specific loci become silenced, especially in the context of global demethylation. Compounds that target specific loci for demethylation rather than causing global hypomethylation have an advantage from a therapeutic perspective, preventing chromosomal instability while restoring tumor suppressor expression; one such compound is the DNMT1 inhibitor RG108 [66]. Long non-coding RNAs (lincRNAs) are proposed as a way for gene silencing machinery to target-specific gene loci [9,67,68]. It has been shown that the lincRNA, KCNQ1 opposite strand/antisense transcript 1, interacts with DNMT1 and mediates imprinting at imprinted loci. A recent study demonstrated that a nuclear RNA transcribed from the CCAAT/enhancer binding protein alpha (CEBPA) locus interacts with DNMT1, blocks it from that locus and thus inhibits methylation around CEBPA's promoter [69]. It is possible that other DNMT1-associated RNAs target DNMT1 around other gene loci or specific chromatin regions. In addition, the DNMT1 binding partner UHRF1 contains domains that bind specific H3 tail modifications and therefore can target DNMT1 to specific loci via the histone code [70]. Protein–protein interactions are also

implicated, as the DNMT1-binding partner nibrin helps DNMT1 localize and repress survivin during DNA damage [71]. Other evidence suggests that the N-terminal domains of DNMT1, while not necessary for catalytic methyltransferase activity, are still important to target the protein to correct genomic locations [72]. Extending these models to tumor suppressor loci will be useful in expanding the role of DNMT1 in tumorigenesis and may belie alternate mechanisms for treatment.

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