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SET7/9 mediated methylation of non-histone proteins in mammalian cells

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Abbreviations: DNMT1, DNA (cytosine-5) methyltransferase 1; H3K4, histone 3 lysine 4; LSD1, lysine-specific demethylase 1; 5-aza-CdR, 5-Aza-Deoxycytidine

Lysine methylation has emerged as a major posttranslational modification for histones in eukaryotes. Crosstalk between lysine methylation and other posttranslational modifications is crucial for transcriptional gene regulation and epigenetic inheritance. In addition to histones, several other cellular proteins including transcription factors, tumor suppressor and membrane-associated receptors are subject to lysine methylation. SET7/9 plays a prominent role in lysine methylation of histone and non-histone proteins. Recent reports have suggested a new mechanism of epigenetic gene regulation via SET7/9 modulated DNMT1 methylation. In this mechanism, SET7/9 may methylate DNMT1 leading to proteasome mediated protein degradation, and antagonist lysine specific demethylase (LSD), may prevent this degradation by removing the methyl mark. Thus a fine-tuning and balance between cellular SET7/9 and LSD interaction with DNMT1 may be means for epigenetic gene regulation.

Introduction

Post-translational modification (PTM) of histones, such as methylation, acetylation, phosphorylation, ADP-ribosylation and ubiquitination are shown to play important regulatory roles in defining the status of eukaryotic gene expression.[1] Several interrelated mechanisms facilitate the catalytic addition and removal of modification marks resulting in the transition between transcriptionally active and inactive chromatin. These modification marks potentiate recruitment of chromatin remodeling complexes, as demonstrated by increased retention of SWI/SNF on acetylated promoters[2] and deposition of HP1 complexes on the silenced chromatin.[3] Although the enzyme involved in histone modifications are sequence specific surrounding the target amino acid residue, it is widely believed that they might possess substrates outside the histone molecules. Indeed, several examples of lysine methylation of non-histone proteins have been discovered suggesting that this is a common post-translational modification for regulation of protein activity.[4-7] Other post-translational modifications of several non-histone proteins by different chromatin modifying activity, such as acetylation and arginine methylation have been described.[8-10]

Lysine methyltransferases contain an evolutionary conserved SET domain, initially identified in Drosophila PEV (position effect variegation) suppressor SU(VAR)39,[11] the polycomb group protein Enhancer of zeste[12] and the trithorax group protein Trithorax.[13] The major roles of these proteins were thought to be modulation of gene activity via histone methylation and alteration of chromatin structure.[14] SET7 contains a SET domain and it monomethylates histone 3 lys 4 (H3K4), predominately found in active chromatin. In the presence of H3K9 methylation, a general signature of repressed genes, SET7 is blocked from H3K4 methylation.[15] Currently many different substrates of SET7 have been discovered including tumor suppressor p53,[5] ribosomal protein Rpl42ab,[16] Rubisco LSMT,[17] ERα,[6] (nuclear hormone estrogen receptor alpha), and DNMT1 (mammalian major maintenance DNA cytosine-5 methyltransferase 1).[18,19] Among all the modifications, methylation was considered to be irreversible, but the recent discovery of histone lysine demethylases revealed a dynamic nature of histone methylation regulation on four of the main sites of methylation on histone H3 and H4 tails (H3K4, H3K9, H3K27, H3K36 and H4K20). Some of the histone lysine demethylases, particularly LSD1, may also contribute to demethylation of non-histone proteins, as discovered recently for methylated p53 and methylated DNMT1.[18,20]

Since the initial discovery of SET7, substantial structural and product specificity studies have been published.[21,22] The structural analysis of broader substrate specificity of SET domain containing histone methyltransferase, SET7, was established on TAF10, a component of general transcription factor TFIID complex.[23] SET7 co-crystal with TAF10 peptide bearing monomethyl Lys 192 and AdoHcy revealed similar structural organization as was observed previously in SET7 bound to histone H3 or p53. Further analysis between these complexes revealed a structurally conserved binding mode with short sequence of each substrate, K/R-S/T-K (where K being the target Lys residue), suggesting other potential target sites in the protein pool of the mammalian cells. Based on the consensus target site, Couture et al. have analyzed and identified several putative substrates of SET7.[23] The potential SET7 substrates are involved in cell cycle regulation, RNA polymerase dependent gene expression, proteins involved

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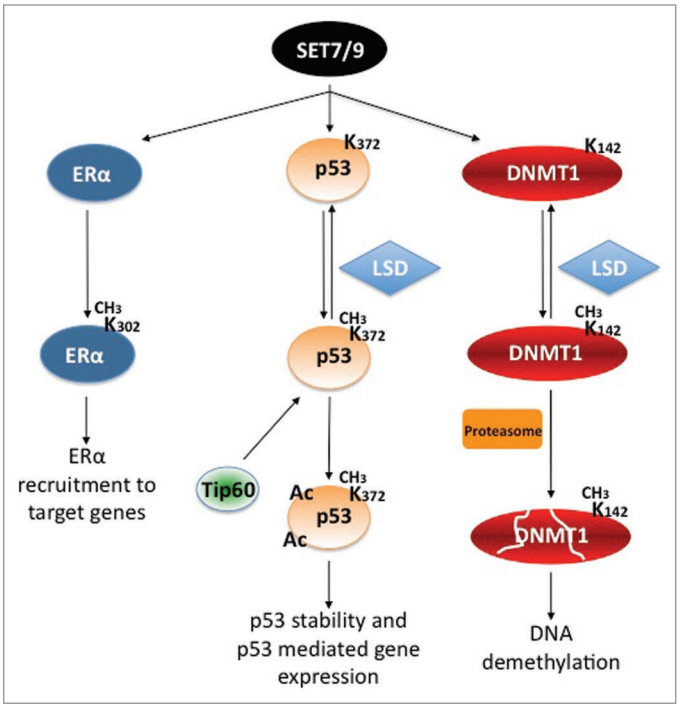

Figure 1. SET7/9 methylate lysine on non-histone proteins such as ERα, p53 and DNMT1. This methylation can be reversed by lysine specific demethylases (LSD). Methylation of lysine may affect protein stability and facilitates gene expression of ERα or p53 responsive genes. In contrast, methylated DNMT1 gets degraded.

in maintenance of chromatin architecture and calmodulin binding. Furthermore, SET7 mediated methylation of different cellular proteins have resulted in various cellular response including altered gene expression, protein stability and loss of DNA methylation. Here we have discussed the functional consequence of SET7 methylation on various target proteins and their impact on cellular function.

SET7 is a Lysine Methyltransferase

Comparison of the structure of SET7 methyltransferase bound to TAF10, p53 and histone H3 revealed plasticity of its amino acid recognition adjacent to the target lysine. Kinetic parameters of full-length SET7 with peptide substrates representing the above proteins demonstrated similar catalytic efficiency. Recently, we have also observed similar catalytic efficiency between peptides representing histone H3 tail and DNMT1 in a SET7 methylation reaction.[19] Mass-spectroscopic analysis of the reaction products on DNMT1, TAF10, H3, TAF7 and p53 revealed an addition of a single methyl group on the target lysine. Using saturated enzyme concentration and overnight incubation conditions, the enzyme did not add a second methyl group. This observation suggests relaxed sequence specificity with a stronger product specificity of the enzyme. Indeed the substrate specificity between DIM5, a lysine trimethylase and SET7 may be attributed to the residues in the lysine binding channel of SET7, that may sterically exclude a mono-methyl lysine. The above hypothesis was proven further by point mutation of critical amino acids (Y305F) in the SET7 pocket resulting in a change of substrate specificity to a di/tri-lysine methyltransferase.[24] A similar change in specificity was also observed for DIM5 (F281Y) from trimethylation to predominantly mono and dimethylation. Quantum mechanical/molecular mechanical (QM/MM) studies on SET7 product specificity indicated that the methylation state specificity is mainly controlled by the methyl-transfer reaction step itself, thus confirming that SET7/9 is a mono-methyltransferase. Furthermore, the binding of the methylated lysine substrate in the active site of SET7/9 opens up the cofactor AdoMet binding channel allowing the solvent water molecules to get access to the active site. This event disrupts the catalytic machinery of SET7/9 for the di-methylation reaction, leading to a higher activation barrier.[25]

Biological Response of SET7 Methylation

As described above, SET7 mediated methylation occurs in both histone and non-histone proteins. SET7 mediated mono methylation of H3K4 makes a platform for both H3K4 di and trimethylation by cellular enzymatic apparatus. There are few H3K4 methyl (H3K4me) binding proteins such as BPTF, CHD1 and ING2 that can tether to the modified histone and direct enzymatic activities onto chromatin. For example, BPTF, a component of the NURF chromatin modification complex is responsible for activation of HOXC8 genes by SNF2L ATPase activity.[26] Similarly, ING2 can recruit the mSin3A-HDAC1 complex to proliferation specific genes following exposure of cells to DNA damaging agents and there by repressing gene expression.[27,28] Some enzymes with methylated H3K4 binding activity, such as histone demethylase JMJD2A with double tudor domain, and histone ATPase CHD with chromodomain, can exert the specific cellular response including gene activation.[29,30]

Among the non-chromatin proteins, SET7 mediated methylation of p53 is well studied. SET7 is shown to methylate K372 resulting in its stability and retention in the mammalian nucleus, thus influencing on the regulation of p53 mediated gene expression (**Fig. 1**).[5] The molecular mechanism of p53K372me stability is shown to be associated with enhanced acetylation of the protein. Indeed, p53 modification events can be observed on the promoter of the p21 gene, a known transcriptional target of p53, resulting in its transcriptional activation via acetylation leading to subsequent cell cycle arrest.[31] Further investigation has identified Tip60 as the p53 specific acetyltransferase and demonstrated mechanistic link between methylation and acetylation of p53.[4] Similarly, methylation TAF10 at K189 potentiates transcription of a set of RNA Pol II dependent genes. TAF10K189me has more affinity for RNA Pol II, perhaps resulting in preinitiation complex formation.[7] This observation suggests that TAF10 and histone H3 methylation may be interdependent, sequential event for SET7 mediated gene expression. Such a functional synergy may be observed in activation of beta cell specific genes, particularly of those encoding preproinsulin. Experimental knockdown of SET7 resulted in repression of Ins1/2, Glut2 and MafA correlated with loss of H3K4 methylation and RNA Pol II on their respective promoter.[32] Another example of SET7 mediated

transcriptional activation is the nuclear factor kappaB (NFκB), where chromatin immunoprecipitation revealed that SET7/9 small interfering RNA could reduce TNFalpha-induced recruitment of NFkappaB p65 to inflammatory gene promoters.[33] In both the above examples, the roles of SET7/9 mediated methylation on non-histone protein methylation are not fully established yet. Another case of SET7/9 mediated gene expression is exemplified by estrogen receptor alpha (ERα) methylation at K302. ERαK302 methylation stabilizes it and is necessary for the efficient recruitment of ERα to its target genes and for their transactivation[6] (**Fig. 1**). Therefore, Lys methylation plays an important role in signal transduction and transactivation of genes like other post-translational modifications such as phosphorylation and acetylation.

Lysine Specific Demethylase Acts as an Antagonist to SET7 Methylation

Post-translational modifications are reversible, thus regulating dynamic gene expression in mammalian cells. For example, acetylation, phosphorylation and methylation are reversed by deacetylases, phosphatases and demethylases. The enzymatic addition of covalent modification marks and its rapid removal makes the cell more dynamic to respond to various stimuli in order to control and establish regulated signaling pathways. The first lysine specific demethylase (LSD1) demethylates mono methyl lysine 4 of histone H3 the exact reaction product of SET7/9. The degree of H3K4 methylation in the genome is potentially associated with different outcome for the cell. In a recent report, Wang et al. have demonstrated that LSD1 can demethylate a methylated non-histone protein, murine DNMT1 at K1096 (mDNMT1K1096). The enzyme DNMT1 is well established as the major maintenance DNA (Cytosine-5) methyltransferase 1. Its major role is to maintain DNA methylation in mammalian cells during DNA replication. DNMT1 is essential for mammalian development, since DNMT1 null mutation is embryonic lethal in mice.[34] Based on experimental observations by Wang et al. mDNMT1 gets immuno-precipitated with LSD1 and SET7/9 is capable of methylation at K1096 of murine DNMT1. Pargyline (N-Methyl-N propargylbenzylamine, C11H13N), an LSD1 inhibitor prevented the enzymatic demethylation in vitro. Furthermore, LSD1 deficiency in ES cells resulted in severe reduction in 5meC content. The loss of methylation was significant in the IAP repetitive DNA, C-type retroviral DNA and imprinted genes H19, Igf2 and Xist. Reduction in methylated cytosine was attributed to severe decrease in mDNMT1 protein level, since the mDNMT3a and mDNMT3b level remained unchanged.[18] Therefore, the authors concluded that lysine methylated mDNMT1 is prone to degradation, and fine-tuning of the methylation status of the enzyme may be involved in regulating gene expression.

In a parallel publication by Estève et al. in human cell lines, the authors observed a similar phenomenon. After detailed biochemical studies the authors have concluded that human DNMT1 (hDNMT1) undergoes lysine methylation at K142. The hDNMT1K142me remained very unstable in cells. The half-life of hDNMT1K142me was ~6 hrs in contrast to over 18 hrs for DNMT1K142A mutant enzyme. In addition, overexpression of SET7 led to decreased DNMT1 levels, while siRNA-mediated knockdown of SET7 stabilizes DNMT1. Application of proteasome inhibitors in the cultured cells resulted in stability of the hDNMT1K142me. In these experiments, ubiquitin mediated degradation of hDNMT1 seems to be the plausible cause for the shorter half-life for the modified enzyme. Based on reports from both Wang et al. and Estève et al. it seems likely that lysine methylation is a signal for protein degradation via proteasome pathways. Similarly, proteasome mediated degradation of DNMT1 has been observed in cells treated with 5-aza-CdR or 5-azacytidine.[35] However, both murine and human DNMT1 have two different amino acids residues (hDNMT1:K142 and mDNMT1:K1096) identified as the substrate, and signal inducer for possible enzymatic degradation. Indeed, the amino terminal target lysine in both hDNMT1 and mDNMT1 possess the same flanking

A

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Np-hDNMT1:   RTPRRSKSDGEA
Np-mDnmt1:   RGPRRSKSDGEA
Cp-hDNMT1:  EAYNAKSKSFED
Cp-mDnmt1:   LEAYNSKTKNEF
Histone H3:    CARTKQTARKSTGG
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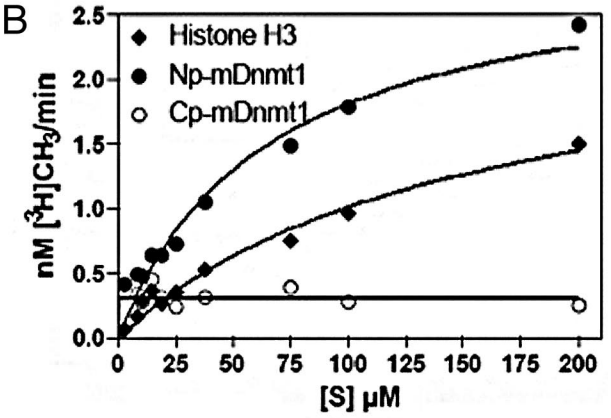
B



C

Peptide	k_{cat} h^{-1}	K_m μM	k_{cat}/K_m $10^6 xM^{-1}xh^{-1}$
Np-mDnmt1:	2984	72	41
Cp-mDnmt1:	~300	ND	ND
Histone H3:	2654	149	17.8

Figure 2. An amino terminus lysine is the preferred substrate for SET7/9 mediated lysine methylation in both human and mouse DNA cytosine-5 methyltransferases. (A) Peptides representing the putative target lysine are shown. The SET7/9 methylated lysine residue is underlined. Peptides are shown as N-terminal peptide (Np) or C-terminal peptide (Cp) along with N-terminal peptide of histone H3. Experimentally established major lysine residue in each peptide is shown in gray shade. (B) Steady state kinetic assay to determine the catalytic turnover values for mouse Dnmt1 peptides by SET7/9. (C) Table representing the kinetic constants using different peptides representing portion of mDnmt1 as compared with histone H3 tail.

sequence "SKS" (**Fig. 2A**). To validate the substrate specificity, we used highly purified SET7/9 and corresponding peptides in a steady state kinetic assay. The amino terminus peptides encompassing K142 for human DNMT1 displayed a similar catalytic turnover as compared to histone H3K4 amino terminus peptide.[19] In the same set of experiments, the peptide encompassing the K1094 was a poor substrate for SET7 methylation, thus, suggesting only one major SET7 methylation site on human DNMT1 (data not shown). Since the amino acid sequence of mDNMT1 and hDNMT1 have a high degree of identity, we performed a similar steady state kinetic assay with peptides corresponding to mDNMT1 K139 and K1096. We found that only the amino terminus peptide of mDNMT1 (Np-mDNMT1) displayed a similar turnover number comparable to the hDNMT1K142 peptide (**Fig. 2B and C**; ref. 19). Therefore, we conclude that the amino terminus lysine (K142) is the preferred substrate for SET7 mediated methylation.

Conclusion and Future Direction

Methylation of non-histone protein will likely receive increased attention in the near future. Identification of broad substrate specificity for lysine methyltransferases will establish new networks of signal transduction events that can be written and erased by enzymes. Specifically, how non-histone proteins get regulated by lysine methylation, and how lysine methylation related with other posttranslational modifications such as acetylation, phosphorylation and ubiquitinylation will more clearly be defined. If additional protein molecules that are methylated by SET7 prior to protein degradation, a generalized mechanism of methyl-lysine mediated protein degradation will emerge. These processes will obviously draw more attention to uncovering the mechanism of the proteasome machinery in recognizing the methylated substrate and directing its degradation.

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