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Review Article

Nonhistone Lysine Methylation as a Protein Degradation Signal

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Protein degradation is a fundamental feature of cellular life, and malfunction of this process is implicated in human disease. Ubiquitin tagging is the best characterized mechanism of targeting a protein for degradation; however, there are a growing number of distinct mechanisms which have also been identified that carry out this essential function. For example, covalent tagging of proteins with sequestosome-1 targets them for selective autophagy. Degradation signals are not exclusively polypeptides such as ubiquitin, NEDD8, and sequestosome-1. Phosphorylation, acetylation, and methylation are small covalent additions that can also direct protein degradation. The diversity of substrate sequences and overlap with other pleotrophic functions for these smaller signaling moieties has made their characterization more challenging. However, these small signals might be responsible for orchestrating a large portion of the protein degradation activity in the cell. As such, there has been increasing interest in lysine methylation and associated lysine methyltransferases (KMTs), beyond canonical histone protein modification, in mediating protein degradation in a variety of contexts. This review focuses on the current evidence for lysine methylation as a protein degradation signal with a detailed discussion of the class of enzymes responsible for this phenomenon.

1. Introduction

Protein degradation is a critical function for regulating signaling and removal of dysfunctional or misfolded proteins. There are two canonical methods by which this occurs. The ubiquitin/proteasome system (UPS) is the most well-described while the second is autophagy, which can be further distinguished as macroautophagy, microautophagy, or chaperone-mediated autophagy. The importance of these processes is illustrated by protein aggregation and other maladies that occur subsequent to the failure of protein turnover machinery. Many diseases feature protein aggregation as their hallmark, and it is reasonable that this could result from aberrant protein degradation as these are often shown to be linked [1, 2].

Similarly, protein degradation during cellular differentiation is the key to the changing of cellular identity. This process, though, requires that the cell target large numbers of proteins for degradation as global proteome turnover occurs. This dictates a few key requirements that would be necessary for an effective global degradation signal including that it be small and energetically inexpensive, unrestricted by sequence, and paired with an enzyme whose dysfunction is linked to disorders that include aberrant cell differentiation. One potential solution to the problem is lysine methylation.

Nonhistone lysine methylation is a post-translational modification that has garnered increased attention over recent years. While histone methylation has been well-studied since its discovery in 1964, nonhistone substrates for lysine methylation have begun to be identified of late [3, 4]. The enzymes responsible for the methylation of lysine residues are termed lysine methyltransferases (KMTs), and those that remove them are termed lysine demethylases (KDMs). The amine group that is characteristic of lysine residues can accept up to three methyl groups (Figure 1). This is an interesting alteration to the chemical state of lysine in that the positively charged side chain accepts between one and three hydrophobic groups that reside in close proximity to the positive charge. This creates a diametric epitope that could be readily discriminated by the cell. In fact, a variety of

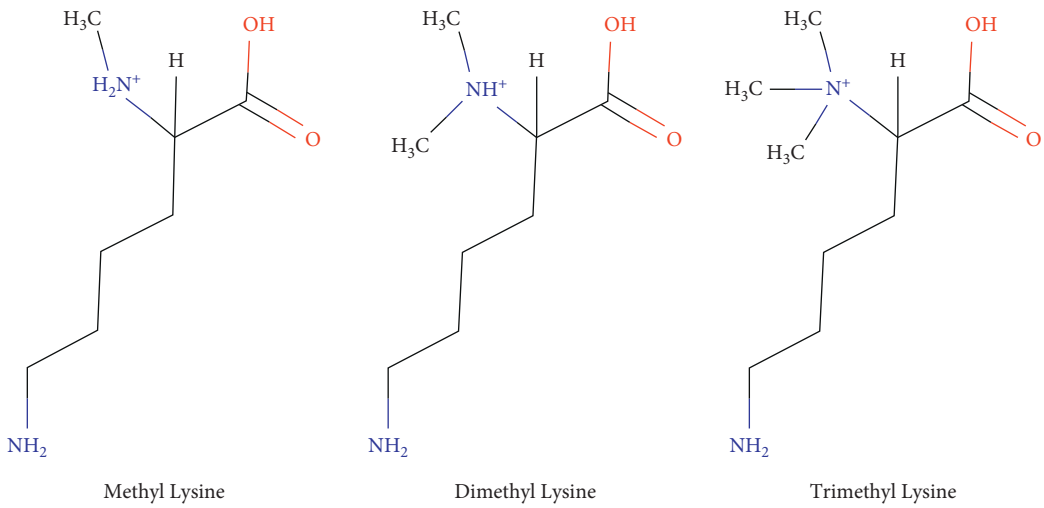

Figure 1: Potential methylation states of lysine. Generation of these three species is catalyzed by lysine methyltransferases (KMTs) and lysine demethylases (KDMs) through the addition and removal of methyl groups, respectively.

proteins that can recognize methylated lysines have been identified [5–7]. These methyl-lysine-binding proteins are specific to the number and position of methyl groups added to a lysine residue, making each its own distinct signal. The addition of methyl groups to lysine residues has been shown to impact protein-protein interactions, protein-DNA interactions, and protein stability [8–13]. A number of enzymes exist that have been shown to perform mono-, di-, and trimethylations of lysine residues (Table 1). While most of these enzymes are canonically histone methylators, many of them have overlapping function as nonhistone methylators as well [48].

2. Methylation of Nonhistone Proteins

Investigation of proteome methylation has generally been centered on histone modification. This is likely due to the challenges associated with the identification of methylation events using common methods (e.g., western blotting). Methylation results in small changes to mass and no alteration of charge making them difficult to observe. This feature also reduces the numbers of antibody reagents that can specifically recognize this post-translational modification (PTM). When considering nonhistone proteins, the relative low abundance of methylations when compared to histone methylation also presents a challenge. Enrichment techniques have allowed for the application of a few analytical methods for identifying methylated proteins. Mass spectrometry is the most widely used method because when paired with enrichment steps, it can identify not only the presence of methylation but also the nature of methylation (mono-, di-, or tri-) [49, 50]. Chemical enrichment has also enabled the application of affinity tagging for labeling lysine methyltransferase substrates (e.g., bioorthogonal profiling and click labeling) [51–53]. These techniques have led to the identification of a number of nonhistone lysine methyltransferase targets.

Nonhistone lysine methylation is a relatively new field, but it is fast becoming increasingly apparent that this post-translational modification plays a role in cell signaling, particularly within the context of cancer [29, 54]. A central example of this is p53. The tumor suppressor has recently been identified as a substrate for SETD7-mediated methylation [55]. It is important to note that p53 methylation by SETD7, specifically at K372, prevents the addition of other PTMs that inhibit p53 activity [56–59]. Methylation of p53 is site- and context-specific with regard to protein function and stability. Both methylation by SETD7 [55] and demethylation by KDM4A [60] alter p53 stability.

Another protein exhibiting regulation by regulatory methylation is β-catenin. β-catenin is involved in a variety of cellular activities including proliferation, while its dysfunction has been well characterized in tumorigenesis. Methylation of β-catenin has effects on stability, translocation, and activity as a transcriptional regulator [61–63]. Other nuclear targets of nonhistone lysine methylation include RB transcriptional corepressor 1, E2F1, HIF1, reptin, FOXO3, NFKB complex components, and ARTD1 [64–70]. In addition, there have been several other nuclear proteins (nonhistone) identified as targets that play a wide variety of roles in within the cell [71]. Adding complexity is the fact that lysine methylation occurs across the three states: mono-, di-, and trimethylation. Therefore, understanding these individual PTMs in the context of specific signaling events as they relate to protein degradation could inform novel therapeutic approaches.

3. Lysine Methyltransferases

There is a wide spectrum of enzymes capable of catalyzing the addition of methyl groups to lysine residues. As previously discussed, the majority of them perform their function on histones, but many have nonhistone substrates. In line with this notion, the vast majority of these proteins are localized to the nucleus. A select few, though, are localized to, and active in the cytosol.

Lysine methyltransferases exist in one of two superfamilies: the SET-domain containing superfamily and the

Table 1: Enzymes with lysine-methylating activity. The enzymes in the SET-domain family and the 7β-strand family that are active in lysine methylation. Also listed are the known methylation states each enzyme is capable of producing. Many lysine-methylating enzymes have disputed function, and the 7β-strand family is still being investigated in eukaryotes.

Gene name	Superfamily	Lysine methylation	Reference
EZH1/KMT6B	SET-domain	Mono, di, tri	[14, 15]
EZH2/KMT6A	SET-domain	Mono, di, tri	[16]
EHMT1/KMT1D	SET-domain	Mono, di	[17]
EHMT2/KMT1C	SET-domain	Mono, di, tri	[18]
NSD1/KMT3B	SET-domain	Mono, di	[19]
NSD2	SET-domain	Mono, di	[20]
NSD3	SET-domain	Mono, di	[20]
SETD1A	SET-domain	Mono, di, tri	[21]
SETD1B	SET-domain	Mono, di, tri	[22]
SETD4	SET-domain	Mono, di	[23]
SETD6	SET-domain	Mono	[24]
SETD7	SET-domain	Mono, di	[25]
SETD9	SET-domain	Mono, di	[25]
KMT5A	SET-domain	Unknown	[26]
SMYD2	SET-domain	Mono, di	[27, 28]
SMYD3	SET-domain	Mono, di, tri	[29]
SMYD5	SET-domain	Unknown	[30]
SETMAR	SET-domain	Mono, di, tri	[30]
ASH1L/KMT2H	SET-domain	Di, tri	[28]
DOT1L	SET-domain	Mono, di, tri	[31]
MLL	SET-domain	Mono, di, tri	[32]
MLL2	SET-domain	Mono, di, tri	[33]
MLL3	SET-domain	Mono, di, tri	[31]
MLL4	SET-domain	Mono, di, tri	[34]
MLL5	SET-domain	Unknown	[35]
SUV39H1	SET-domain	Di, tri	[36]
SUV39H2	SET-domain	Di, tri	[37]
SUV420H1	SET-domain	Di, tri	[38]
SUV420H2	SET-domain	Di, tri	[38]
PRDM2-17	SET-domain	Disputed or unknown	[39]
CAMKMT	7β-strand	Tri	[40]
VCPKMT	7β-strand	Tri	[41]
EEF1-AKMT1	7β-strand	Tri	[42]
EEF2KMT	7β-strand	Tri	[43]
METTL10	7β-strand	Mono, di, tri	[44]
METTL20	7β-strand	Tri	[45]
METTL21A	7β-strand	Tri	[46]
METTL22	7β-strand	Unknown	[47]

7β-strand enzymes. The SET-domain superfamily (55 human enzymes) functions primarily on histones with some members acting on both histone and nonhistone proteins. However, only a markedly small portion of function is on nonhistone proteins. Near half of the SET-domain members are orphans (substrates unknown). The 7β-strand superfamily contains 125 enzymes and is not lysine-specific nor is protein-specific. This group as a whole can methylate several different amino acids as well as nucleic acids [72].

SET-domain KMTs contain a four-motif conserved domain that facilitates catalysis. These domains are twisted into knot-like β-sheets, and it is thought that these "pseudoknots" are key to determining substrate specificity [73]. SET-domain lysine methyltransferases that are able to perform nonhistone methylations are EZH1, EZH2, EHMT1, EHMT2, NSD1, SETD3, SETD4, SETD6, SETD7, SETD8, SMYD2, SMYD3, SMYD5, and SETMAR. It is worthwhile to point out that a few of these (SMYD3, SETD3, and SETD4) not only have nuclear nonhistone targets but also nonhistone targets in the cytosol [74–76]. This is notable due to the fact that KMTs are largely associated with the nuclear localization and activity. This also supports a widespread role of lysine methylation as a degradation signal since most of the cellular protein content is housed in the cytoplasm, making non-nuclear KMTs needed for targeting large swaths of the proteome.

SET-domain KMTs have been shown to be clinically relevant. For example, ASH1L is linked to fascioscapulohumeral muscular dystrophy [77]. MLL (or KMT2a), SUV420H1 (KMT5b), and MLL5 (KMT2e) have all been linked to autism [78–80]. Numerous families including the PRDMs, SETDs, and SMYDs contain members with associations to a wide variety of cancers. The clinical significance of SET-domain KMTs along with their ability to methylate both histone and nonhistone targets in the nucleus and the cytosol make them an important line of future inquiry as well as potential therapeutic targets.

4. Evidence for Lysine Methylation as a Degradation Signal

Lysine methylation is capable of inducing numerous effects depending on the site and the protein. We have discussed changes to protein-protein interactions as well as changes to protein-DNA interactions. Perhaps the most interesting downstream effect of lysine methylation is destabilization of the protein target. There are instances where methylations at different sites can either induce degradation or even inhibit ubiquitination and, thereby, increase stability [81]. Both the positive and negative regulation has been demonstrated to occur, as well as the three different methylation states having differential effects.

DNMT1, a major DNA methyltransferase, exhibits both positive and negative regulation of stability by lysine methylation. If the K142 residue of DNMT1 is methylated, the recognition complex determines the outcome. PHF20L1 contains an MBT domain that can bind to this methylation side and prevent ubiquitin ligases from acting, leading to inhibition of the degradation process [82]. Conversely, if L3MBTL3 binds the same methylation site, ubiquitin ligases are recruited and DMNT1 is degraded [83]. This careful interplay of lysine methylation and recognition proteins determines the fate of DNMT1 and may represent broader paradigm for the investigation of other proteins that may have similar duality.

Many other proteins have been reported to be degraded upon lysine methylation. FOXO1 is a well-studied example. It has been shown that methylation of FOXO1 by G9a (EHMT2) leads to increased interaction with the E3 ubiquitin ligase SKP2 while also decreasing the protein's stability [65]. Similarly, lysine methylation has shown to have negative effects on the stability of proteins such as p53 and AR [65, 84]. The NFKB complex component Rel A is degraded in response to methylation by Set9 [70]. Another protein whose degradation is mediated by lysine methylation is the transcription factor E2F1. Multiple post-translational modifications act in tandem on E2F1 to stimulate degradation. When K185 is methylated, it prevents the acetylation and phosphorylation at a few positions far from K185 while inducing ubiquitination and, in turn, degradation [65]. These changes in stability are widely accepted to originate from one of a few sources including disruption of charge-stabilized regions of the protein leading to misfolding or unfolding, recruitment of unfolding chaperones, or direct proteasome targeting.

5. Summary and Future Perspectives

As our knowledge base regarding KMTs and lysine methylation expands, so does the breadth of roles this PTM plays within the cell. It has become clear the lysine methylation is not limited to histones, nor is it limited to nuclear roles. This is evidence that lysine methylation can instigate a multitude of cellular effects beyond the assembly of heterochromatin. In particular, target destabilization is an important function with growing interest. In considering the potential for lysine methylation to constitute a degradation signal, the current understanding of the PTM's ability to induce misfolding or direct proteasome targeting becomes increasingly notable. Lysine methylation satisfies the qualifications of being both unbound by amino acid sequence, unlike pathways relying upon consensus sequences, and relatively small and energetically inexpensive to deliver, unlike ubiquitination. It is possible that protein destabilization and/or recruitment of E3 ligases is the mechanism by which lysine methylation acts as a panproteomic degradation signal. While this line of thinking is interesting, it has yet to be reported. As discussed in this review, there have been major advances in our understanding of nonhistone lysine methylation. Still, questions remain as to whether alterations to KMTs associated with nonhistone methylation lead to aberrant protein aggregation on a broader scale or with direct human disease relevance. Such evidence would directly signify the physiological importance of protein degradation through lysine methylation.

Conflicts of Interest

The authors have no conflicts of interest to declare.

Authors' Contributions

NAL and BEM wrote and edited this manuscript.

Acknowledgments

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