NF-κB: Regulation by Methylation

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Abstract

In normal cells exposed to stress, the central transcription factor NF-κB is activated only transiently, to modulate the activation of downstream immune responses. However, in most cancers, NF-κB is abnormally activated constitutively, contributing thus to oncogenesis and tumor progression. Therefore, downregulating NF-κB activity is an important goal of cancer treatment. In order to control NF-κB activity therapeutically, it is helpful to understand the molecular mechanisms that normally govern its activation and how dysregulated NF-κB activity may aid the development of disease. Recent evidence from our laboratories and others indicates that, in addition to various posttranslational modifications of NF-κB that have been observed previously, including phosphorylation, ubiquitination, and acetylation, NF-κB can be methylated reversibly on lysine or arginine residues by histone-modifying enzymes, including lysine and arginine methyl transferases and demethylases. Furthermore, these methylations are required to activate many downstream genes. Interestingly, amplifications and mutations of several such enzymes have been linked to cancer. We propose that some of these mutations may alter the methylation not only of histones but also of NF-κB, making them attractive therapeutic targets.

Overview of NF-κB Signaling

The transcription factor NF-κB plays a critical role in inflammation, oncogenesis, and tumor progression. Its family includes p65 (RelA), RelB, c-Rel, p50/p105 (NF-κB1), and p52/p100 (NF-κB2). All proteins of the family share a Rel homology domain (RHD) at their N-termini, which is required for dimerization, nuclear targeting, binding to DNA, and interaction with the inhibitory IkB proteins (IkB; refs. 1, 2). A subgroup of NF-κB family members, the Rel proteins, including p65, RelB, and c-Rel, also contain an additional carboxy-terminal transactivation domain (TAD). The prototype of NF-κB is the heterodimer of p65 and p50 subunits. In unstimulated normal cells, NF-κB exists as inactive heterodimers or homodimers of p65 that are bound to IkB (1). The NF-κB family uses canonical and noncanonical activation pathways (3). In the canonical pathway, signals from proinflammatory cytokines such as IL1 activate IkB kinase (IKK), which phosphorylates IkBα, leading to its ubiquitination and degradation by proteasomes (3). The released NF-κB (mostly p65/p50 heterodimers) translocates into the nucleus, binds to DNA to regulate the expression of many inflammatory and oncogenic genes (3). In the noncanonical pathway, phosphorylation of p100 on its C-terminus allows the processing of p100 to p52 (3). The freed NF-κB (mostly p52/RelB heterodimers) translocates into the nucleus and regulates the expression of genes whose products mostly regulate the development and maintenance of the secondary lymphoid organs (3).

Posttranslational Modifications of NF-κB

An important aspect of the complex regulation of NF-κB is multiple posttranslational modifications of the p65 subunit. These modifications include ubiquitination, phosphorylation, acetylation, sumoylation, and nitrosylation and, more recently, methylation (3–11). The nature and extent of these regulatory modifications can vary with different NF-κB stimulators and the same modifications may even facilitate quite different effects (11). In this review, we focus on the methylation of p65, the major functional subunit of NF-κB.

Methylation of NF-κB

In the past few years, accumulated evidence suggests that histone-modifying enzymes not only modify the histone proteins, but also play a role in the modification of nonhistone proteins, such as NF-κB. This interesting set of observations reminds us of the economy of nature, which has empowered histone-modifying enzymes with the dual ability to control both the histone proteins—which directly affect chromatin conformation and function, and the nonhistone proteins—which directly drive gene expression.

To date, methylations of both lysine and arginine have been identified on the p65 subunit of NF-κB (4–10). The six methylated K sites, K37, 218, 221, 310, 314, and 315 (Fig. 1A), are modified by different histone-modifying enzymes (Supplementary Table S1; refs. 4–10). We used a novel genetic approach to identify previously unknown regulators (4–7), discovering that the nuclear receptor binding SET domain protein 1 (NSD1) and the demethylase F-box leucine-rich protein 11 (FBXL11) regulate...
Figure 1.
Methylation of NF-κB, genetic alteration of PRMT5, and hypothetical model. A, methylation of the p65 subunit of NF-κB. A schematic diagram of the principal structural motifs of p65. Rel homology domain (RHD, amino acids 19–301), transactivation domain (TAD, amino acids 428–551), and the linker region (amino acids 302–427). The mapped sites are the known methylation modifications on either K or R residues of the p65 subunit. (This figure is adapted from ref. 18.) B, cross-cancer genetic alteration summary for PRMT5 (89 studies). Green solid squares, mutations; blue solid squares, deletions; red solid squares, amplifications; gray solid squares, multiple alterations. Website: www.Cbioportal.org. C, a model for the time course of methylation of chromatin-bound NF-κB by histone-modifying lysine methyltransferases. I, NF-κB and the methyltransferases are free of DNA. II, NF-κB binds to a promoter. III, the methyltransferases are recruited. IV, the methyltransferases are activated and catalyze methylations of both histones and NF-κB. Alternatively, some methyltransferases may be preassociated with some promoters before NF-κB arrives. (This figure is adapted from refs. 19, 20.)
NF-κB through the reversible methylation of K218 and 221 of p65. Interestingly, another group (12) found that homeodomain finger protein 20 (PHF20, also called glioma-expressed antigen 2) promotes NF-κB transcriptional activity by interacting with methylated p65 at K218 and 221. The interaction between PHF20 and methylated p65 blocks the recruitment of phosphatase PP2A, thus maintaining the phosphorylation of serine 536 of the p65 subunit of NF-κB.

In addition to K218/221, Ea and colleagues (8) reported that, upon activation of NF-κB by TNFα, the histone-modifying enzyme SET9 monomethylates p65 at K37, and this epigenetic modification regulates the expression of a subgroup of target genes, including IκBα, IP-10, and TNFα. The induction of IP-10 and TNFα was greatly reduced in p65−/− MEF cells that express the K37Q mutant instead of wild-type p65 (8). Interestingly, the same SET9 enzyme is also able to modify other lysine residues of p65. Yang and colleagues reported that p65 is monomethylated by SET9 on K314 and 315 (9), leading to decreased NF-κB activity and target gene expression. This phenomenon further highlights the complex role that the histone-modifying enzymes play in the methylation of NF-κB. In addition to the results described above, Levy and colleagues identified that SETD6 monomethylates p65 on K310, leading to the induction of a repressed state of NF-κB target genes through the binding of C9a-like protein (10). Why does p65 need the methylation on multiple K sites? Recently, we compared the effects of methylation on the K37 and K218/221 sites of p65 (6), finding that mutations of K218/221 greatly reduced the expression of approximately 50% of NF-κB–inducible genes, whereas the K37Q mutation decreased the expression of approximately 25% of NF-κB–inducible genes. Analysis showed that the mutations K218/221Q greatly reduce the affinity of p65 for many promoters and that the K37Q mutation does not. Structural modeling revealed that the newly introduced methyl groups on K218/221 interact directly with DNA in some K8-specific binding sites to increase the affinity of p65. The difference between binding sites that do or do not interact with methylated K218/221 is not yet known. Thus, the K218/221 and K37 methylations have dramatically different effects on different genes by distinct mechanisms (6).

Distinct from the methylation of lysine residues, we recently discovered that the R30 of p65 is dimethylated by protein arginine methyltransferase 5 (PRMT5), leading to activation of NF-κB (7, 13). Microarray analysis revealed that approximately 85% of the NF-κB–inducible genes that are downregulated by the R30A mutation are similarly downregulated by the R30Q mutation does not. Structural modeling revealed that the newly introduced methyl group has not been identified. In vivo demethylases erase these methyl groups. To date, much less is known about how arginine is demethylated than about lysine demethylation. As shown in Supplementary Table S1, no example has been found as a “Reader” of NF-κB methylation. Readers are proteins that recognize methylated lysine or arginine. Because every key component in the cancer epigenome is potentially druggable, the current gap in our knowledge of “Readers” of NF-κB methylation will, hopefully, be filled in soon.

Histone-modifying enzymes that regulate NF-κB methylation are frequently amplified or mutated in different cancers. A comprehensive review summarizes the roles of histone methyltransferases in cancer (15). As shown in Supplementary Table S1, the histone methyltransferases SET19, SETD6, and NSD1 are “Writers” that have frequent genetic alterations in cancers. For example, NSD1 has been linked to tumorigenesis in prostate cancer and childhood acute myeloid leukemia (15). Our discovery that NSD1 is capable of activating NF-κB by methylating K218 and K221 of p65 (4, 5) provides a potential mechanism for how NSD1 might contribute to tumor formation, as constitutive activation of NF-κB is a hallmark of most cancers.

In addition to lysine methylase, the arginine methyltransferase PRMT5 also shows many genetic alterations in a spectrum of cancers. As illustrated in Fig. 1B, http://www.cbioportal.org data (16, 17) suggest that PRMT5 is either amplified or mutated in 89 cases of cancer studied. In addition, Oncomine and GeneNote data also show that PRMT5 is frequently overexpressed—often to a striking degree—in many types of cancer, such as colon, ovary, kidney, lung, bladder, liver, pancreas, breast, prostate, cervix, and skin (7). We suggest that high levels of this enzyme may promote tumorigenesis, at least in part, by facilitating NF-κB–induced gene expression (7).

Perspectives

Although several methylation sites of NF-κB have been identified, the details of what happens at responsive promoters are not yet completely clear. Examples from three studies of NF-κB indicate that the methylation of NF-κB by histone-modifying enzymes might take place on promoters in the context of chromatin. Our study of NF-κB methylation showed that the p65 subunit is not associated with histone-modifying enzymes until it is activated (4, 5), suggesting that this event happens only after NF-κB is released from IκB. Furthermore, Yang and colleagues (9) provided important evidence that the methylation of NF-κB occurs only when it is in the nucleus and can bind to DNA, as a DNA binding–deficient mutant of p65 was no longer a substrate for methylation by SET7/9. Levy and colleagues (10) also showed that the methylation of p65 by SETD6 occurs on the chromatin-associated protein. In most of the studies cited above, the levels of the methylases and demethylases have been increased or decreased exogenously, with effects on the in vivo functions of NF-κB, results that are consistent with the effects of the methylases. The endogenous levels of some methylases and demethylases are also subject to change, for example, the gene encoding the demethylase FBXL11 is activated by NF-κB (4, 5).

Taken together, these observations lead to an interrelated set of hypotheses (Fig. 1C: refs. 4, 5). First, methylation of NF-κB may...
occur, in concert with histone modifications, only when NF-κB is bound to specific promoters, where the local chromatin remodelling machinery is active (Fig. 1C–IV). Second, methylation profoundly affects the functions of NF-κB at these promoters, altering their stability, transactivation potency, and affinity for DNA, and thus affecting the strength and duration of inducible gene expression. Third, methylation is gene specific, leading to differential effects on individual genes that give plasticity to the dependent biologic responses. As a likely example, the well-described differential methylation of K37 and K218/221 on NF-κB (6) may constitute "bar codes" that direct differential activation of individual promoters. Although a variety of mechanisms are surely required to achieve cell-type specificity of gene activation, the ability to modify NF-κB differently at specific promoters in different types of cells may contribute importantly.

As described above, there are a variety of methylations of NF-κB. Different methylations determine the activity of NF-κB and regulate its gene expression. The most recent discovery regarding NF-κB methylation, the identification of PRMT5 as the enzyme that methylates R30 of p65, is particularly important in shedding light on this previously understudied area. Future studies of the methylation of NF-κB will not only provide further insight into the basic mechanisms of its regulation but also, given the role of NF-κB in human health and disease, may well provide additional drug targets and biomarkers to aid in the diagnosis and prognosis of many pathologic conditions.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

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