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Small molecule inhibitors of histone acetyltransferases and deacetylases are potential drugs for inflammatory diseases☆

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Lysine acetylation is a reversible post-translational modification (PTM) of cellular proteins and represents an important regulatory switch in signal transduction. Lysine acetylation, in combination with other PTMs, directs the outcomes as well as the activation levels of important signal transduction pathways such as the nuclear factor (NF)-κB pathway. Small molecule modulators of the 'writers' (HATs) and 'erasers' (HDACs) can regulate the NF-κB pathway in a specific manner. This review focuses on the effects of frequently used HAT and HDAC inhibitors on the NF-κB signal transduction pathway and inflammatory responses, and their potential as novel therapeutics.

Introduction

Lysine acetylation is a reversible post-translational modification (PTM) of cellular proteins and represents an important regulatory switch in signal transduction cascades [1,2]. An increasing number of studies highlight the importance of lysine acetylation as a key PTM, directing the outcomes as well as the activation levels of important signal transduction pathways such as the nuclear factor (NF)-κB pathway. For example, acetylation of NF-κB transcription factors p65 and p50 plays an important part in their nuclear localization and transcriptional activity [3]. Similar phenomena have been observed for other pathways [4]. Next to this, acetylation of histones connected to specific genes has an important role in gene-specific transcription in the NF-κB pathway [3]. Furthermore, an increasing number of reports describe significant levels of crosstalk between lysine acetylation and other PTMs, such as ubiquitinylation, methylation and phosphorylation, in the NF-κB pathway. For example, competition between acetylation and ubiquitinylation on the same lysine residues is observed for transcription factor p65 [5]. This highlights the fact that acetylation is not a sole determining factor but, rather, is a regulator working in concert with other PTMs at multiple levels in signaling cascades.

Lysine acetylations are generally regulated by 'writers' and 'erasers', which are denoted as histone acetyltransferases (HATs) and histone deacetylases (HDACs), respectively, owing to their original discovery as histone-modifying enzymes. An important future challenge is to identify and quantify distinct HAT and HDAC activities in distinct signaling pathways such as the NF-κB pathway, as well as their aberrations in disease (models). Considering the importance of lysine acetylation in the NF-κB pathway (Fig. 1), small molecule modulators of HATs and HDACs have great potential to regulate this signaling cascade specifically, which is an important aim in drug discovery.

Focusing on the NF-κB pathway, here we summarize the effects of lysine acetylation of the p65 transcription factor as well as histones. In addition, we highlight the role of crosstalk between lysine acetylation and other PTMs such as methylation and phosphorylation. Furthermore, we discuss the effects of frequently used small molecule HAT and HDAC inhibitors on the NF-κB signal transduction pathway and inflammatory responses *in vitro* and *in vivo*.

Lysine acetylation as a regulator of the NF-κB pathway

In 2001, it was discovered that acetylation of p65 inhibits binding to the inhibitory complex IκBα, and thus stimulates gene transcription; whereas deacetylation promotes IκBα binding and nuclear export [6]. This study triggered intense interest in lysine

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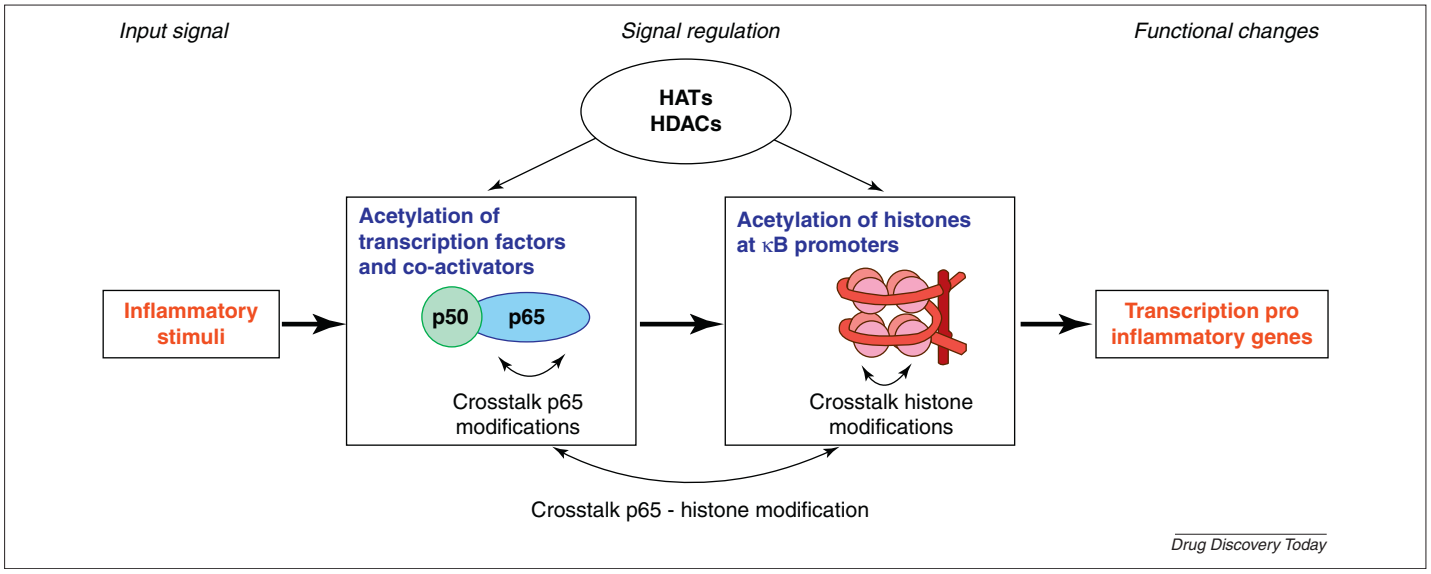

FIGURE 1

Schematic representation of the diverse roles of lysine acetylation in the activation of the nuclear factor (NF)-κB pathway. Lysine acetylations of the transcription factors as well as their co-activators play an important part in the duration of the response and the signaling output. Lysine acetylation status of the histones works in concert with acetylation status of the transcription factors to enable or disable transcription of specific genes. Crosstalk of acetylation with other PTMs is an important component in the NF-κB pathway. Abbreviations: HATs, histone acetyltransferases; HDACs, histone deacetylases.

acetylations of the seven lysine residues (122, 123, 218, 221, 310, 314, 315) of p65 that are subject to this PTM. These acetylations have specific roles in activation of the NF-κB pathway and have been previously reviewed [7,8]. Importantly: acetylation of lysines 122 and 123 decreases DNA binding [9]; acetylation at lysines 218 and 221 increases binding to κB enhancers; and acetylation at lysine 310 is essential for full transcriptional activity [10]. In addition, acetylations of specific lysine residues in histone H3 and H4 play an important part in NF-κB-mediated gene transcription as reviewed [3].

Lysine acetylation does not act alone: crosstalk between lysine acetylation and other PTMs

The acetylation of lysine residues in the NF-κB transcription factor and in histones (and numerous other cellular targets) can be dramatically affected by the PTM state of other constituent amino acids. These so-called 'crosstalk' mechanisms act, presumably, *via* increasing or decreasing the affinity of the substrate protein for the respective HAT or HDAC complexes involved in their acetylation. A recent review nicely illustrates the importance of crosstalk between PTMs on the NF-κB transcription factor [8]. In addition, previous reviews illustrate the importance of crosstalk between lysine acetylation and other PTMs in the histones [11–14]. Here, we highlight some specific examples that demonstrate the crucial involvement of crosstalk in NF-κB activation as well as in histones implicated in inflammation. The examples described below are limited to known cases of crosstalk within the same protein (*cis* crosstalk). In addition, a growing number of examples make it clear that similar mechanisms also operate in modulating protein–protein interactions including those between the peptides tails of different histones (*trans* crosstalk).

A specific example of crosstalk in the NF-κB pathway involves the phosphorylations of p65 at serines 276 and 536, which serves to enhance the p300-mediated acetylation of lysine 310. This, in turn, leads to an overall transcriptional activation of the NF-κB pathway (Fig. 2a) [15]. In addition, it has been found that phosphorylation of serine 276 is required for binding of p65 to the coactivator CREB-binding protein (CBP), which promotes proinflammatory gene transcription.

Phosphorylation also has a major role in the crosstalk observed within histone proteins. One of the earliest reported and best-studied examples of crosstalk in histones involves the phosphorylation of serine 10 in histone 3 (H3S10) and its effect on lysine acetylation (Fig. 2b). Several kinases are known to phosphorylate H3S10. These include AuroraB and other members of the Aurora/Ipl 1 kinase family, as well as kinases implicated in transcriptional regulation such as the yeast non-specific serine/threonine protein kinase 1 (Snf1) and mammalian Proto-oncogene serine/threonine protein kinase 1 (Pim1), Ribosomal s6 kinase (Rsk), Mitogen and stress activated kinase 1 (Msk1), and Mitogen and stress activated kinase 2 (Msk2) kinases [16–18]. Phosphorylation of H3S10 leads to the stimulation of acetylation at lysine 14 (H3K14) with the prototypical histone acetyltransferase general control nonderepressible (Gcn)5 displaying an up to tenfold preference for acetylation of H3K14 when H3S10 is phosphorylated [19,20]. In contrast to the enhancement of acetylation at H3K14, phosphorylation of H3S10 completely blocks acetylation at the directly adjacent lysine 9 (H3K9) residue [21], clearly illustrating the varying crosstalk effects that the same PTM can impart.

In addition to serine phosphorylation, the methylation of lysine and arginine side chains is another common PTM known to impact lysine acetylation *via* crosstalk. The side chain of lysine can be mono-, di- or tri-methylated, often with varying crosstalk effects (Fig. 2c). For example, lysine methylation at lysine 4 in histone H3 (H3K4) leads to increased acetylation of H3K14 and other lysine residues in histone H3 by p300 and other acetyltransferases [22]. Further evidence suggests that the degree to which H3K4 is methylated directly influences the associated extent of

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