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Acetylation of MKP-1 and the Control of Inflammation

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Abstract

Innate immune responses mediated by Toll-like receptors (TLRs), a class of pattern-recognition receptors, play a critical role in the defense against microbial pathogens. However, excessive TLR-mediated responses result in sepsis, autoimmunity, and chronic inflammation. To prevent deleterious activation of TLRs, cells have evolved multiple mechanisms that inhibit innate immune reactions. Stimulation of TLRs induces the expression of the gene encoding the mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1), a nuclear-localized dual-specificity phosphatase that preferentially dephosphorylates p38 MAPK and c-Jun N-terminal kinase (JNK), resulting in the attenuation of TLR-triggered production of proinflammatory cytokines. MKP-1 is posttranslationally modified by multiple mechanisms, including phosphorylation. A study now demonstrates that MKP-1 is also acetylated on a key lysine residue following stimulation of TLRs. Acetylation of MKP-1 promotes the interaction of MKP-1 with its substrate p38 MAPK, which results in dephosphorylation of p38 MAPK and the inhibition of innate immunity.

Chromatin remodeling by acetylation of histone molecules plays an essential role in the activation of gene expression. By decreasing the acetylation of lysine residues in histone tails, histone deacetylases (HDACs) condense chromatin structure and repress gene transcription. HDAC inhibitors induce the expression of a subset of genes involved in cell growth arrest, differentiation, and apoptosis, which accounts for the antitumor effects of these compounds (1). Paradoxically, HDAC inhibitors also possess potent anti-inflammatory effects by shutting down the expression of genes encoding proinflammatory cytokines and molecules. Findings by Cao *et al.* have provided insight into the molecular mechanisms involved in this process by identifying that HDAC inhibitors promote acetylation of a new target—mitogen-activated protein kinase (MAPK) phosphatase-1 (MKP-1)—a phosphatase in the MAPK pathway that is a key inhibitor of inflammation. Acetylation of MKP-1 promotes its association with its substrate p38 MAPK, which leads to the inhibition of p38 MAPK activity and the expression of genes that encode proinflammatory proteins (2). Here, we discuss the role of MKP-1 in the control of inflammation and how both the abundance and function of MKP-1 are regulated at the molecular level.

MKP-1 is a Key Inhibitor of Innate Immune Responses

Upon pathogen infection, the immune system rapidly mounts defense mechanisms, which are characterized by massive production of cytokines, interferon, and other immune factors. This response, known as the innate immune response, is mediated by receptors found on macrophages and dendritic cells that detect conserved structures present in a broad range of

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pathogens. The best-studied innate recognition receptors are the Toll-like receptors (TLRs), a class of membrane receptors that sense microbes either in the extracellular space or in intracellular compartments such as endosomes (3). Although pathogen recognition begins at the level of the receptor, it is the signaling components downstream of each receptor and the ways in which they interact that ultimately determine the specific transcriptional response and immunological outcome (3). Among the central pathways activated by innate signals are those involving the MAPKs. The MAPK family, which includes extracellular signal-regulated kinase (ERK), c-Jun N-terminal kinase (JNK), and p38 MAPK, is a family of serine/threonine kinases that play prominent roles in the immune system (4,5). Activation of MAPK is mediated by a core kinase module consisting of MAPK kinase kinase (MAPKKK, also known as MAP3K), MAPK kinase (MAPKK, also known as MAP2K), and MAPK through sequential protein phosphorylations. Activated MAPKs, in turn, phosphorylate activating protein 1 (AP-1) transcription factors and other targets to stimulate gene transcription and immune responses (4,5). Inhibition of MAPK activity is effected primarily by MAPK phosphatases (MKPs), a group of more than 10 dual-specificity phosphatases that dephosphorylate the regulatory threonine and tyrosine residues of their target MAPK (6,7).

Whereas activation of TLRs is essential for provoking the acute inflammatory response necessary for the effective clearance of pathogens, excessive responses, such as occur in sepsis, a condition caused by uncontrolled inflammation and innate responses, are dangerous to the host. To prevent deleterious inflammation, a number of signaling mechanisms are evoked, including the down-regulation of the surface abundance of TLRs and the transcriptional induction of genes encoding negative regulatory proteins (8,9). Chief among these feedback mechanisms is the rapid induction of the expression of *MKP-1* to restrain excessive MAPK activation. *MKP-1*$^{-/-}$ macrophages produce excessive amounts of cytokines, including tumor necrosis factor α (TNF-α), interleukin-6 (IL-6), and IL-10. In addition, mice deficient in *MKP-1* show greatly elevated susceptibility to endotoxic shock (10–14). Two other MKP genes, *MKP-5* and *DUSP2*, also play important roles in innate immunity, and a number of other MKP genes are expressed by innate immune cells (15–19). However, the striking and unique phenotypes of *MKP-1*$^{-/-}$ mice in innate immune responses highlight MKP-1 as a critical regulator of innate immunity.

Transcriptional Regulation of *MKP-1* Expression

Given the pivotal role of MKP-1 in innate immunity, there is growing interest in exploring how its gene expression and biochemical activity are regulated. Expression of *MKP-1* is induced by a number of growth factors and stresses in multiple cell types. In macrophages responding to TLR stimulation, there is a strong and rapid induction of *MKP-1* mRNA and increase in MKP-1 protein abundance, peaking at 1 hour after stimulation (10,13,20–22). The induction of *MKP-1* correlates with a decline in the activities of JNK and p38 MAPK, which is consistent with a role for MKP-1 in the inactivation of these MAPKs as a feedback mechanism to restrain excessive inflammation. The signals involved in TLR-induced expression of *MKP-1* are beginning to be defined. Stimulation of TLRs activates two distinct pathways that are mediated by the adaptor proteins myeloid differentiation marker 88 (MyD88) and TRIF [Toll-IL-1 receptor (TIR) domain-containing adapter inducing interferon-β (IFN-β)], respectively (3). TLR-induced expression of *MKP-1* is reduced in mice lacking either MyD88 or TRIF compared to that in wild-type mice, suggesting that *MKP-1* is induced through both MyD88- and TRIF-dependent pathways in response to activation of TLRs (10). In addition, a signaling pathway consisting of the MAP3K Raf-1 and protein kinase C ε (PKC ε) is required for TLR-induced expression of *MKP-1* in macrophages (23,24). Furthermore, ERK, JNK, and p38 MAPK have all been suggested to facilitate TLR-induced expression of *MKP-1* (20,22,25).

MKP-1 is also induced by multiple immunosuppressive agents, including glucocorticoids and anti-inflammatory cytokines, and this induction partially mediates the inhibitory effects of these agents on MAPK activation and inflammation (Fig. 1A). Notably, glucocorticoid-dependent inhibition of JNK and p38 MAPK activation and proinflammatory gene expression is impaired in *MKP-1*$^{-/-}$ macrophages after stimulation of TLRs (26). Moreover, in vivo immunosuppressive effects of glucocorticoids on zymosan-induced inflammation are lost in *MKP-1*$^{-/-}$ mice. Therefore, induction of *MKP-1* by glucocorticoids is required for the suppressive effects of glucocorticoids on proinflammatory signaling pathways (26). Induction of *MKP-1* by transforming growth factor β (TGF-β) and IL-10 also contributes to the suppressive effects of these potent anti-inflammatory cytokines on the expression of genes encoding proinflammatory mediators (27,28). Conversely, proinflammatory stimuli such as interferon γ (IFN-γ) attenuate *MKP-1* expression to facilitate their positive effects on MAPK activation (13). Therefore, it appears that the induction of *MKP-1* represents a common mechanism by which immunomodulatory agents “fine tune” innate immune responses.

Posttranslational Modifications of MKP-1 Stability and Activity

Posttranslational modifications of MKP-1 also play an important role in regulating its activity (Fig. 1B). For example, glucocorticoids increase the expression of *MKP-1* as well as attenuating proteasomal degradation of MKP-1. Both of these mechanisms are necessary for glucocorticoid-mediated inhibition of ERK activation in mast cells (29). How is proteasomal degradation of MKP-1 regulated at the biochemical level? Interestingly, MKP-1 is phosphorylated by ERK in two distinct regions, which have opposing effects on its stability. Transient activation of ERK phosphorylates MKP-1 at the two extreme C-terminal Ser^{359} and Ser^{364}, which enhances MKP-1 stabilization without altering its phosphatase activity (30). This serves as a negative feedback mechanism to inhibit ERK activation. In contrast, sustained activation of ERK results in phosphorylation of MKP-1 at Ser^{296} and Ser^{323}. This facilitates the interaction of MKP-1 with the Skp-cullin-F-box (SCF^{Skp2}) ubiquitin ligase, which targets MKP-1 for proteasomal degradation (31,32). Therefore, it appears that signals transmitted by transient or sustained ERK activation play distinct roles in controlling the stability of MKP-1.

Whereas phosphorylation of MKP-1 regulates its stability, it does not alter the intrinsic phosphatase activity of MKP-1, which is affected by another mechanism of posttranslational modification (33). Exposure of cells to TNF-α triggers cell proliferation or cell death, depending on the signaling pathways that it initiates. In the presence of the transcription factor nuclear factor-κB (NF-κB), TNF-α induces a rapid and transient activation of JNK. In contrast, in NF-κB–deficient cells, TNF-α induces sustained JNK activation, which is a major determinant of cytotoxicity. Reactive oxygen species (ROS) mediate sustained JNK activation in NF-κB–deficient cells by inactivating MKP-1 and several other MKP molecules. This is achieved by ROS-induced oxidation of the catalytic Cys residue in the MKP molecules, resulting in the loss of phosphatase activity. Consequently, JNK activation becomes unconstrained, eventually leading to cellular apoptosis (33).

Acetylation of MKP-1 Enhances its Association with p38 MAPK

Cao *et al.* have now identified another mode of posttranslational modification of MKP-1 that does not affect protein stability or intrinsic phosphatase activity (2). The authors initiated their studies by trying to identify the molecular mechanisms responsible for the suppressive effects of HDAC inhibitors on the TLR-induced expression of genes encoding proinflammatory molecules. Specifically, they found that HDAC inhibitors down-regulated a subset of inflammation-associated genes, including *nitric oxide synthase 2* (*NOS2*), *TNFα*, and *IL-6*. Given the central roles of the NF-κB and MAPK pathways in inflammation, the authors examined whether HDAC inhibitors affected these signaling pathways. They found that HDAC

inhibitors reduced the activation of p38 MAPK and ERK, but not JNK or the NF-κB pathway. In particular, HDAC inhibitors decreased phosphorylation of p38 MAPK and its downstream target Elk-1 but not the upstream MAP2Ks MKK3 or MKK6, suggesting that these inhibitors act upon the MAPK pathway at the level of p38, possibly upon a kinase or phosphatase that modifies p38 MAPK. To identify the acetylated component of the p38 MAPK pathway, Cao *et al*. immunoprecipitated the histone acetylase p300 and showed that it was associated with MKP-1. Upon stimulation of TLRs, MKP-1 was acetylated at Lys^{57}, and mutation of this residue abrogated the acetylation of MKP-1 by p300 in vitro and in TLR-stimulated cells, suggesting that Lys^{57} is the crucial residue required for the acetylation of MKP-1.

What is the biochemical and physiological importance of the acetylation of MKP-1? Through a number of biochemical approaches, the authors demonstrated that acetylation of MKP-1 potentiated the interaction between MKP-1 and p38 and, consequently, increased the dephosphorylation of p38 by MKP-1. However, acetylation of Lys^{57} did not affect the intrinsic phosphatase activity of MKP-1. Instead, acetylation of MKP-1 indirectly increased its activity by increasing its affinity for p38 MAPK. To investigate the physiological importance of MKP-1 acetylation on TLR signaling, the authors performed experiments with MKP-1 knockdown and knockout cells and found that HDAC inhibitors were no longer able to suppress proinflammatory gene expression in the absence of MKP-1. Moreover, ectopic expression of wild-type MKP-1, but not a mutant form of MKP-1 that lacked Lys^{57}, restored the sensitivity of the cells to HDAC inhibitors, highlighting the specific effect of acetylation of MKP-1 Lys^{57} on MAPK signaling. Finally, deletion of *MKP-1* also substantially reduced the immunosuppressive effects of HDAC inhibitors in septic shock, a mouse model of acute inflammation.

Collectively, these studies indicate that acetylation of MKP-1 inhibits TLR signaling by increasing the association of MKP-1 with p38 and thus inhibiting the MAPK signaling cascade. They have also raised interesting questions. First, are there additional anti-inflammatory targets of HDAC inhibitors? This is probably the case because these inhibitors only partially decrease the production of proinflammatory cytokines in macrophages and mice deficient in *MKP-1*. Second, does acetylation of MKP-1 play a key role in controlling inflammation in vivo? Answering this question will require the generation of a knockin allele of *MKP-1* by mutating the Lys^{57} residue in vivo.

Conclusion

Since its identification in the early 1990s, MKP-1 has been extensively studied in the context of regulating various physiological processes, including cell proliferation, apoptosis, and inflammation. Recent studies have identified this molecule as one of the key negative regulators of innate immune responses. Molecular pathways that regulate MKP-1 expression and function are also beginning to be identified. The findings of Cao *et al.* have advanced our knowledge in this area by identifying acetylation of MKP-1 as an important mechanism by which TLR signaling is inhibited. This finding should open the way to gaining deeper mechanistic insights into the molecular and cellular mechanisms that control innate immunity. Ultimately, acetylation of target proteins in inflammatory signaling pathways may provide novel therapeutic targets in patients with sepsis or autoimmune diseases.

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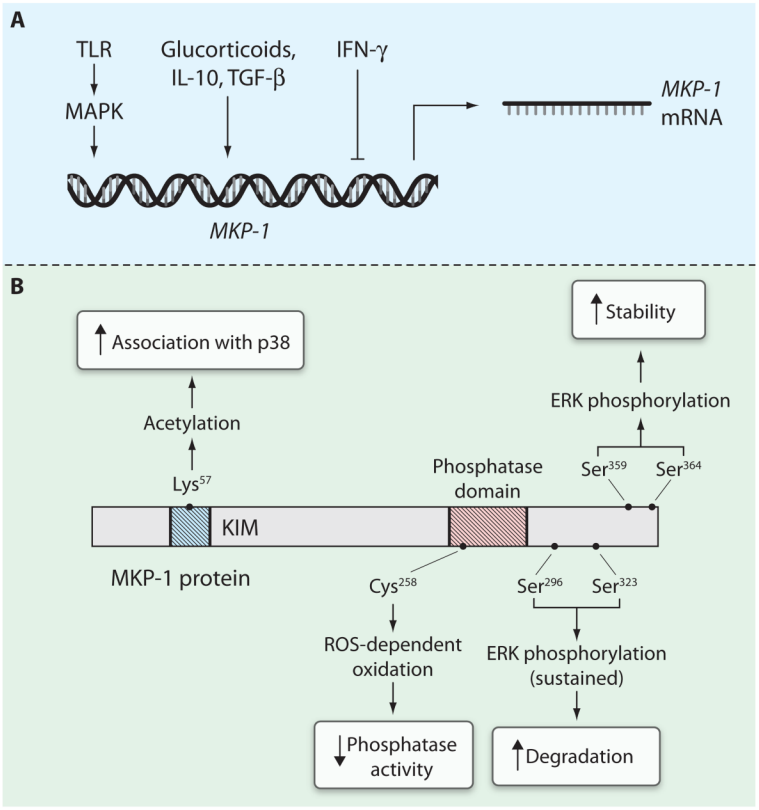

Fig. 1.

Multiple layers of regulation of MKP-1. (**A**) At the transcriptional level, TLR signaling induces *MKP-1* expression as a feedback mechanism to switch off MAPK activation, and immunosuppressive agents, such as glucocorticoids and IL-10, induce the expression of *MKP-1* to mediate their inhibitory effects on the activation of MAPKs. Conversely, immunostimulatory agents, such as IFN-γ, suppress *MKP-1* expression to promote MAPK activation. (**B**) At the posttranslational level, acetylation of Lys^{57} of the MKP-1 protein promotes its association with p38 MAPK, and phosphorylation of Ser^{359} and Ser^{364} in the C terminus of MKP-1 by ERK enhances its stability. ROS-dependent oxidation of the catalytic Cys^{258} of MKP-1 inactivates its phosphatase activity, whereas phosphorylation of Ser^{296} and Ser^{323} by sustained ERK activation leads to proteasomal degradation of MKP-1. KIM, kinase-interaction motif.