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Mkp-1 cross-talks with Nrf2/Ho-1 pathway protecting against intestinal inflammation

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ABSTRACT

Inflammatory bowel disease (IBD) is associated with intense oxidative stress, contributes to colonic damage and tumorigenesis. Mitogen-activated protein kinase phosphatase 1 (Mkp-1) is an essential negative regulator of the innate immune response. However, its role in colitis, and its association with the nuclear factor-erythroid 2 related factor 2 (Nrf2), a master regulator of cytoprotection program against oxidative stress, in inflammatory response, is elusive. In this study, we found that increased expression of Mkp-1, Nrf2, and heme oxygenase 1 (Ho-1) was correlated in colonic tissues in patients with ulcerative colitis and Crohn's disease, as well as wild-type mice with colitis induced by dextran sodium sulfate (DSS). *Mkp-1*$^{-/-}$ mice were more susceptible to DSS-induced colitis with more severe crypt injury and inflammation. Mechanistically, directly interacting with the DIDLID motif of Nrf2, Mkp-1 increased Nrf2 stability and positively regulated the constitutive and lipopolysaccharide (LPS)-inducible Nrf2/Ho-1 expression. Conversely, upon exposure to LPS, Nrf2 activated Mkp-1 transcription through the antioxidant response elements in the promoter of *Mkp-1*. Our results revealed a novel link between Mkp-1 and Nrf2 signaling pathways in protecting against colonic inflammation. Mkp-1 might be a therapeutic target for IBD.

1. Introduction

Inflammatory bowel diseases (IBDs) are prevalent and serious gastrointestinal diseases in Western countries and are associated with the development of cancer [1–3]. With the popular adoption of a western lifestyle, IBDs are becoming increasingly common worldwide [1]. They are forms of chronic, recurrent colitis, most commonly Crohn's disease (CD) and ulcerative colitis (UC). Current concepts attribute IBDs to inappropriate chronic inflammatory responses to commensal microbes in genetically susceptible patients [4]. Conventional treatment of colitis can reduce periods of active disease and help to maintain remission, but they often achieve marginal results, patients become refractory, and there are side-effects. As a result, there is an unmet need for alternative biological therapies.

Abbreviations: ARE, antioxidant response element; BHA, butylated hydroxyanisole; CD, Crohn's disease; ChIP, Chromatin immunoprecipitation; CHX, cycloheximide; COX-2, cyclooxygenase-2; DMSO, dimethyl sulfoxide; DSS, dextran sulfate sodium; Ho-1, heme oxygenase 1; IBD, inflammatory bowel disease; IHC, immunohistochemistry; IL-6, interleukin 6; KEAP1, Kelch-like ECH-associated protein 1; LPS, lipopolysaccharide; MAPK, mitogen-activated protein kinase; Mkp-1, mitogen-activated protein kinase phosphatase 1; Nrf2, nuclear factor-erythroid 2 related factor 2; RT-qPCR, real-time quantitative PCR; ROS, reactive oxygen species; RSV, resveratrol; tBHQ, *tert*-butylhydroquinone; TNF-alpha, tumor necrosis factor alpha; TUNEL, terminal deoxynucleotidyl transferase dUTP nick end labeling; UC, ulcerative colitis; WT, wild-type

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Considerable evidence points to a role for mitogen-activated protein kinases (MAPKs) in the pathogenesis of IBDs [5]. MAPKs are regulated by phosphorylation and dephosphorylation. The latter is carried out by a large group of dual-specificity phosphatases, which dephosphorylate tyrosine and threonine residues in their target proteins [6,7]. MAPK phosphatase-1 (Mkp-1) is a nuclear phosphatase, which plays essential regulatory roles in both innate and adaptive immunity [8–10]. The phosphatase dephosphorylates all three major MAPK families (p38, c-Jun N-terminal kinases (JNK), and extracellular-signal-related kinase (ERK)). Mkp-1 reduces oxidative stress-induced JNK and p38 activation, which is involved in mitochondrial integrity, reactive oxygen species (ROS) production, and cell viability [6,7]. Mkp-1 attenuates the synthesis of pro-inflammatory cytokines and limits the inflammatory response *in vivo* [7]. *Mkp-1*$^{-/-}$ mice undergo enhanced inflammation and organ damage in response to both localized and systemic bacterial ligands [7]. These findings suggest the existence of an unexplored role of Mkp-1 in the pathogenesis of colitis.

The transcription factor nuclear factor-erythroid 2 related factor 2 (Nrf2) is a master regulator of the cytoprotective program against oxidative stress [11]. Under basal conditions, Nrf2 is localized in the cytosol *via* a physical interaction with Kelch-like ECH-associated protein 1 (KEAP1), which promotes proteasome-dependent degradation of the protein. When exposed to environmental or intracellular stresses such as ROS and reactive nitrogen species, Nrf2 is translocated into the nucleus and activates the expression of cytoprotective enzymes through binding to the antioxidant response element (ARE) in their promoters [12,13]. However, a growing body of evidence has shown that Nrf2 also plays a critical role in regulating inflammation [14]. Nrf2 has been shown to regulate the innate immune response in various experimental models of disease [14]. The protective role of Nrf2 in colitis has been demonstrated using Nrf2-knockout mice [15]. *Nrf2*$^{-/-}$ mice have an increased susceptibility to colitis-associated colorectal cancer [16]. Among the enzymes regulated by Nrf2, heme oxygenase 1 (Ho-1) has pronounced anti-inflammatory as well as anti-oxidative properties. Ho-1 is prominently up-regulated in the inflamed colon of the dextran sulfate sodium (DSS)-induced model of experimental colitis [17–19] and is significantly higher in the colonic mucosa of patients with active UC than in normal mucosa. These studies suggest that the Nrf2/Ho-1 pathway is implicated in limiting the tissue damage induced by excessive inflammation and inhibiting colitis-associated tumorigenesis.

Through activating Nrf2 signaling, several functional food components and other chemicals such as curcumin, epigallocatechin gallate, resveratrol (RSV), and butylated hydroxyanisole (BHA) have been reported to inhibit colitis and suppress colon carcinogenesis in several animal models [20–25]. In a recent study, we found that Nrf2 signaling pathway is impaired in *Mkp-1*$^{-/-}$ mice, and that Mkp-1 protects mice against toxin-induced liver damage by upregulating Nrf2 cytoprotective system [26]. However, it is not known whether Mkp-1 could cross-talk with Nrf2 signaling pathway in inflammatory response. The present study was therefore carried out to elucidate the potential role of Mkp-1 in the context of colitis.

2. Results

2.1. *Mkp-1*$^{-/-}$ mice are more susceptible to DSS-induced colitis and epithelial damage

To investigate the function of Mkp-1 and its regulation in colitis, we used an experimental acute colitis model induced by 3% DSS given to wild-type (WT), *Mkp-1*$^{-/-}$, and *Nrf2*$^{-/-}$ mice for 7 days. We found that, under normal physiological conditions, the colon from *Nrf2*$^{-/-}$ mice was significantly shorter than that from WT mice. Interestingly, *Mkp-1*$^{-/-}$ mice also had a shorter colon (Fig. 1**A, a–b**), indicating the involvement of Nrf2 and Mkp-1 in colon development. In response to DSS, although all the genotypes had a shortened colon, the colonic epithelium suffered a loss of crypt architecture, mucosal inflammation primarily in mid-colon, and extensive crypt injury and inflammation (Figs. 1**B and C**), and the *Nrf2*$^{-/-}$ mice showed the worst colitis phenotype. Compared to the WT mice, *Mkp-1*$^{-/-}$ mice had increased inflammatory infiltrate, and a higher percentage of colon involved in inflammation following the injury (Fig. 1**B, a–f** and Fig. 1**C**). RT-qPCR revealed that, in untreated animals, the mRNA levels of cyclooxygenase-2 (COX-2), interleukin 6 (IL-6), tumor necrosis factor alpha (TNF-alpha), and iNOS were comparable among the three genotypes. However, following DSS treatment, while only a 6-fold induction of COX-2 mRNA was detected in the colon of WT mice, 9- and 32-fold was induced in the colon of the *Mkp-1*$^{-/-}$ and *Nrf2*$^{-/-}$ mice, respectively (SFig. 1). Similarly, much higher mRNA levels of IL-6, TNF-alpha, and iNOS were induced in the colon of *Mkp-1*$^{-/-}$ and *Nrf2*$^{-/-}$ mice than in WT mice (SFig. 1). IHC for nitrotyrosine, a marker of oxidative and nitroactive stress, demonstrated stronger staining in the colonic crypts and epithelial cells of DSS-treated *Mkp-1*$^{-/-}$ mice than in the WT (SFig. 2**A,** d and e). TUNEL assay revealed that DSS exposure induced significantly greater apoptosis in the colon of *Mkp-1*$^{-/-}$ mice following DSS treatment than in WT mice (SFig. 2**B**). Moreover, following DSS treatment, fewer goblet cells were observed in the colon of *Mkp-1*$^{-/-}$ mice than in WT mice (SFig. 2**C**, d and e). Furthermore, the mucous layer became significantly thinner in treated *Mkp-1*$^{-/-}$ mice than in the WT (SFig. 2**D**, d and e), suggesting the involvement of Mkp-1 in the function of goblet cells. These data indicated that, similar to Nrf2, Mkp-1 protects the colon against the mucosal inflammatory response induced by DSS.

2.2. *Mkp-1 up-regulates the Nrf2/Ho-1 pathway in inflammatory response*

We next examined the expression of Mkp-1, Nrf2, and Ho-1 in the colon of mice with colitis. Western immunoblotting revealed that the expression of Nrf2 (Fig. 2A, lane 2) and Ho-1 (Fig. 2B, lane 2) was increased concomitantly with that of Mkp-1 (Fig. 2A, lane 2). Strikingly, basal and inducible Nrf2 (Fig. 2A, lanes 3 and 4) and Ho-1 (Fig. 2B, lanes 3 and 4) expression was markedly reduced in *Mkp-1*$^{-/-}$ mice, indicating that the activation of Nrf2/Ho-1 in DSS-induced colitis is regulated by Mkp-1. To further assess the relationship between Nrf2 and Mkp-1 in the inflammatory response, peritoneal macrophages were prepared from WT, *Mkp-1*$^{-/-}$, and *Nrf2*$^{-/-}$ mice. RT-PCR analysis showed that Mkp-1 deficiency reduced the basal Ho-1 mRNA level by 27% (Fig. 3**A, a**). Following stimulation with lipopolysaccharide (LPS) (30 ng/ml) for 16 h, the inflammatory response marker genes for the cytokines IL-6 and COX-2 were induced ~ 20-fold in WT macrophages. Strikingly, the IL-6 mRNA level was induced 108-fold in *Mkp-1*$^{-/-}$ macrophages, and 204-fold in *Nrf2*$^{-/-}$ macrophages (Fig. 3**A, b**). Similarly, the COX-2 mRNA level was induced > 40-fold in the knockout macrophages (Fig. 3**A, b**). RT-PCR analysis revealed that the Ho-1 mRNA level was induced 5-fold in WT macrophages (Fig. 3**A, a**). As expected, < 2-fold induction of Ho-1 was detected in *Nrf2*$^{-/-}$ macrophages. Interestingly, the induction of Ho-1 was also compromised as a result of Mkp-1 deficiency. A < 4-fold induction of Ho-1 mRNA level was detected in *Mkp-1*$^{-/-}$ macrophages (Fig. 3**A**, **a**). Next, we examined the connection between Mkp-1 and Nrf2 in colon carcinoma epithelial LS174T cells using two independent Mkp-1 siRNAs to knock down Mkp-1 and minimize any off-target effects. In both cases, Mkp-1-knockdown was concomitant with reduction of the basal and LPS-inducible levels of Nrf2 (Fig. 3**B, ab,** lanes 3 and 4). The basal and LPS-inducible expression of the mRNA of Ho-1 was also repressed at the same time (Fig. 3**C**). Furthermore, when LS174T cells were exposed to H_2O_2, increased cytotoxicity was detected for the cells transfected with Mkp-1 siRNA. At 0.2 mM H_2O_2, the cell viability was 42% for the transfection with scrambled siRNA, while only 26% for that with Mkp-1 siRNA (Fig. 3**D**), indicating that Mkp-1 is required for the cytoprotection system against oxidative stress. Taken together, our results demonstrated that Mkp-1 positively regulates the constitutive and LPS-induced expression of Nrf2 and Ho-1 in both macrophages and colonic epithelial cells.