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Critical role of ROCK2 activity in facilitating mucosal CD4$^+$ T cell activation in inflammatory bowel disease

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ABSTRACT

Rho-associated kinase (ROCK) has been found to be involved in the pathogenesis of a variety of autoimmune diseases, but the role of ROCK in inflammatory bowel disease (IBD) is still elusive. In this study, we demonstrated that the levels of ROCK2, but not ROCK1, activity were significantly upregulated in peripheral blood mononuclear cells (PBMC) and inflamed mucosa from IBD patients using a ROCK activity assay, and that ROCK2 activity in intestinal mucosa was positively correlated with disease severity. Stimulation with TNF markedly upregulated ROCK2 activity in IBD CD4$^+$ T cells through NF-κB signaling. Blockade of ROCK2 activity using Slx-2119 significantly suppressed proinflammatory cytokines in inflamed mucosa from IBD patients including IFX-unresponsive CD patients, and inhibited IBD CD4$^+$ T cells to differentiate into Th1 and Th17 cells through downregulating phosphorylated Stat1 and Stat3, but promoted Treg cell differentiation through upregulating phosphorylated Stat5. Furthermore, oral administration of Slx-2119 markedly ameliorated intestinal mucosal inflammation in TNBS-induced colitis in mice and decreased proinflammatory cytokines productions in inflamed colon. Our data indicate that ROCK2 plays a critical role in inducing mucosal T cell activation and inflammatory responses in IBD and that inhibition of ROCK2 activity might serve as a novel therapeutic approach in the management of IBD.

1. Introduction

Inflammatory bowel diseases (IBD), mainly including Crohn's disease (CD) and ulcerative colitis (UC), are chronic relapsing and remitting disorders affecting the gastrointestinal tract [1]. Although the etiology and pathology of IBD remain elusive, it is thought to be multifactorial and primarily involves an aberrant gut immune response to intestinal microbiota resulting from environmental triggers or genetic predisposition, characterized by an excessive amount of proinflammatory cytokines in intestinal mucosa [2–5]. As the crucial mediators in regulating immune responses, CD4$^+$ T cells have been implicated in the pathogenesis of IBD, mainly resulting from the imbalance of effector and regulatory CD4$^+$ T cells [2,6]. Among different subtypes of CD4$^+$ T cells, IFN-γ-producing Th1 cells and IL-4/IL-13-producing Th2 cells have been considered to mediate the immunopathology of CD and UC, respectively [7–9]. The critical roles of Th17 cells, which are characterized by producing IL-17A, IL-21, and IL-22, have been highlighted in the development of IBD [10–12]. Moreover, Treg cells are also reported to be essential for intestinal mucosal homeostasis by suppressing the proliferation and functions of effector cells [3,13]. However, the mechanisms by which the functions of CD4$^+$ T cells are regulated in IBD are still not completely understood.

Rho family of small GTPases, including RhoA, Rac, and Cdc42, have been found to be involved in a number of cellular processes, such as motility, cell proliferation, and differentiation [14]. Moreover, the Rho GTPase-mediated signaling pathway plays an important role in regulating T cell-mediated immune response, including development, activation, and differentiation of T cells [15]. Rho-associated kinase (ROCK), a serine/threonine kinase of about 160 kD, is composed of ROCK1 and ROCK2. It is originally identified as the key effector of RhoA, and regulates a wide range of physiological functions via phosphorylation of downstream targets

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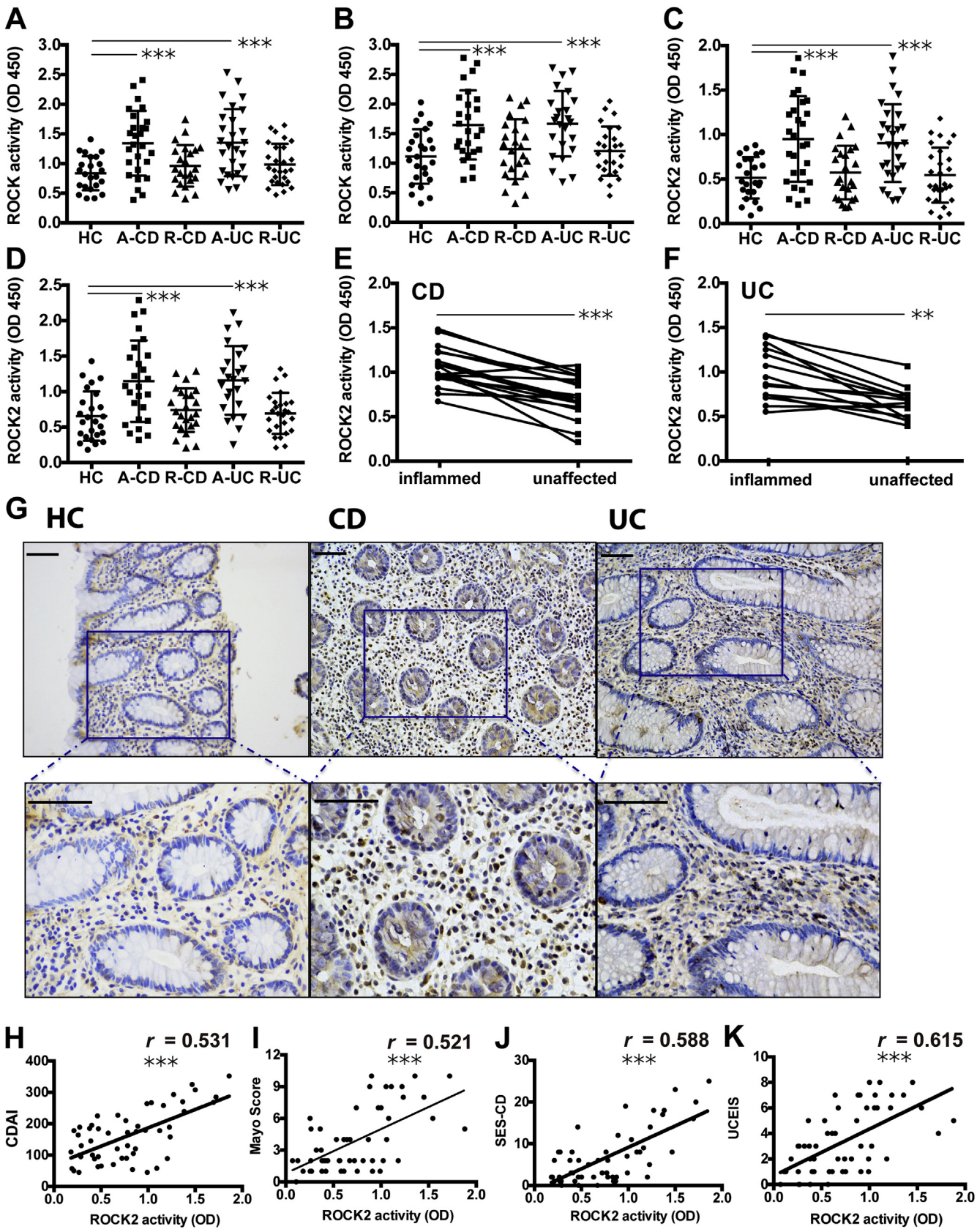

Fig. 1. ROCK2 activity is significantly increased in inflamed mucosa and PBMC from IBD patients. (A) Lysates of inflamed mucosa (50 μg) were isolated from colonic mucosa of healthy control (HC, n = 24), active CD patients (A-CD, n = 28), CD patients with remission (R-CD, n = 24), active UC patients (A-UC, n = 28), and UC patients with remission (R-UC, n = 28). The level of ROCK activity was assessed using an ELISA–based ROCK activity assay. ***p < .001 versus HC. (B) Lysates of PBMC (50 μg) were isolated from PBMC of HC (n = 25), patients with A-CD (n = 26), R-CD (n = 24), A-UC (n = 24), and R-UC (n = 26). The levels of ROCK activity were determined with an ELISA–based ROCK activity assay. ***p < .001 versus HC. (C) ROCK2 was pulled down by immunoprecipitation from the lysates (50 μg) isolated from colonic biopsies of HC (n = 24), patients with A-CD (n = 28), R-CD

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