Biochimica et Biophysica Acta 1853 (2015) 2349–2360

Contents lists available at ScienceDirect

Biochimica et Biophysica Acta

journal homepage: www.elsevier.com/locate/bbamcr

PAK1 modulates a PPARγ/NF-κB cascade in intestinal inflammation

Kyle Dammann [a], Vineeta Khare [a], Michaela Lang [a], Thierry Claudel [b], Felix Harpain [a], Nicolas Granofszky [a], Rayko Evstatiev [a], Jonathan M. Williams [c], D. Mark Pritchard [c], Alastair Watson [d], Christoph Gasche [a,*]

[a] Medical University of Vienna, Department of Internal Medicine III, Division of Gastroenterology and Hepatology, Christian Doppler Laboratory for Molecular Cancer Chemoprevention, Vienna, Austria
[b] Medical University of Vienna, Department of Internal Medicine III, Division of Gastroenterology and Hepatology, Hans Popper Laboratory for Molecular Hepatology, Vienna, Austria
[c] Department of Gastroenterology, University of Liverpool, Liverpool, United Kingdom
[d] Norwich Medical School, University of East Anglia, Norwich Research Park, Norwich, United Kingdom

ARTICLE INFO

Article history:
Received 24 March 2015
Received in revised form 13 May 2015
Accepted 28 May 2015
Available online 31 May 2015

Keywords:
PAK1
NF-κB
PPARγ
Inflammation
Ulcerative colitis
Colitis-associated cancer

ABSTRACT

P21-activated kinases (PAKs) are multifunctional effectors of Rho GTPases with both kinase and scaffolding activity. Here, we investigated the effects of inflammation on PAK1 signaling and its role in colitis-driven carcinogenesis. PAK1 and p-PAK1 (Thr423) were assessed by immunohistochemistry, immunofluorescence, and Western blot. C57BL6/J wildtype mice were treated with a single intraperitoneal TNFα injection. Small intestinal organoids from these mice and from PAK1-KO mice were cultured with TNFα. NF-κB and PPARγ were analyzed upon PAK1 overexpression and silencing for transcriptional/translational regulation. PAK1 expression and activation was increased on the luminal intestinal epithelial surface in inflammatory bowel disease and colitis-associated cancer. PAK1 was phosphorylated upon treatment with IFNγ, IL-1β, and TNFα. In vivo, mice administered with TNFα showed increased p-PAK1 in intestinal villi, which was associated with nuclear p65 and NF-κB activation. p65 nuclear translocation downstream of TNFα was strongly inhibited in PAK1-KO small intestinal organoids. PAK1 overexpression induced a PAK1–p65 interaction as visualized by co-immunoprecipitation, nuclear translocation, and increased NF-κB transactivation, all of which were impeded by kinase-dead PAK1. Moreover, PAK1 overexpression downregulated PPARγ and mesalamine recovered PPARγ through PAK1 inhibition. On the other hand PAK1 silencing inhibited NF-κB, which was recovered using BADGE, a PPARγ antagonist. Altogether these data demonstrate that PAK1 overexpression and activation in inflammation and colitis-associated cancer promote NF-κB activity via suppression of PPARγ in intestinal epithelial cells.

1. Introduction

Inflammatory bowel diseases (IBD) such as Crohn's disease (CD) and ulcerative colitis (UC) are associated with an increased risk of developing colorectal cancer (CRC). Mesalamine, 5-aminosalicyclic acid (5-ASA), is an anti-inflammatory drug used to treat UC, and epidemiological evidence suggests that it has chemopreventive effects [1]. We previously identified p-21 activated kinase-1 (PAK1) as a 5-ASA target [2]. PAK1 is a serine/threonine kinase effector of the small Rho GTPases Rac1/Cdc42 [3], which regulates cytoskeletal dynamics and epithelial cell (IEC) migration and homeostasis. Recently, we demonstrated that PAK1 is overexpressed in IBD and CAC and promotes cell survival pathways [4–6]. However, the exact cause and consequence of PAK1 overexpression in intestinal inflammation have yet to be defined.

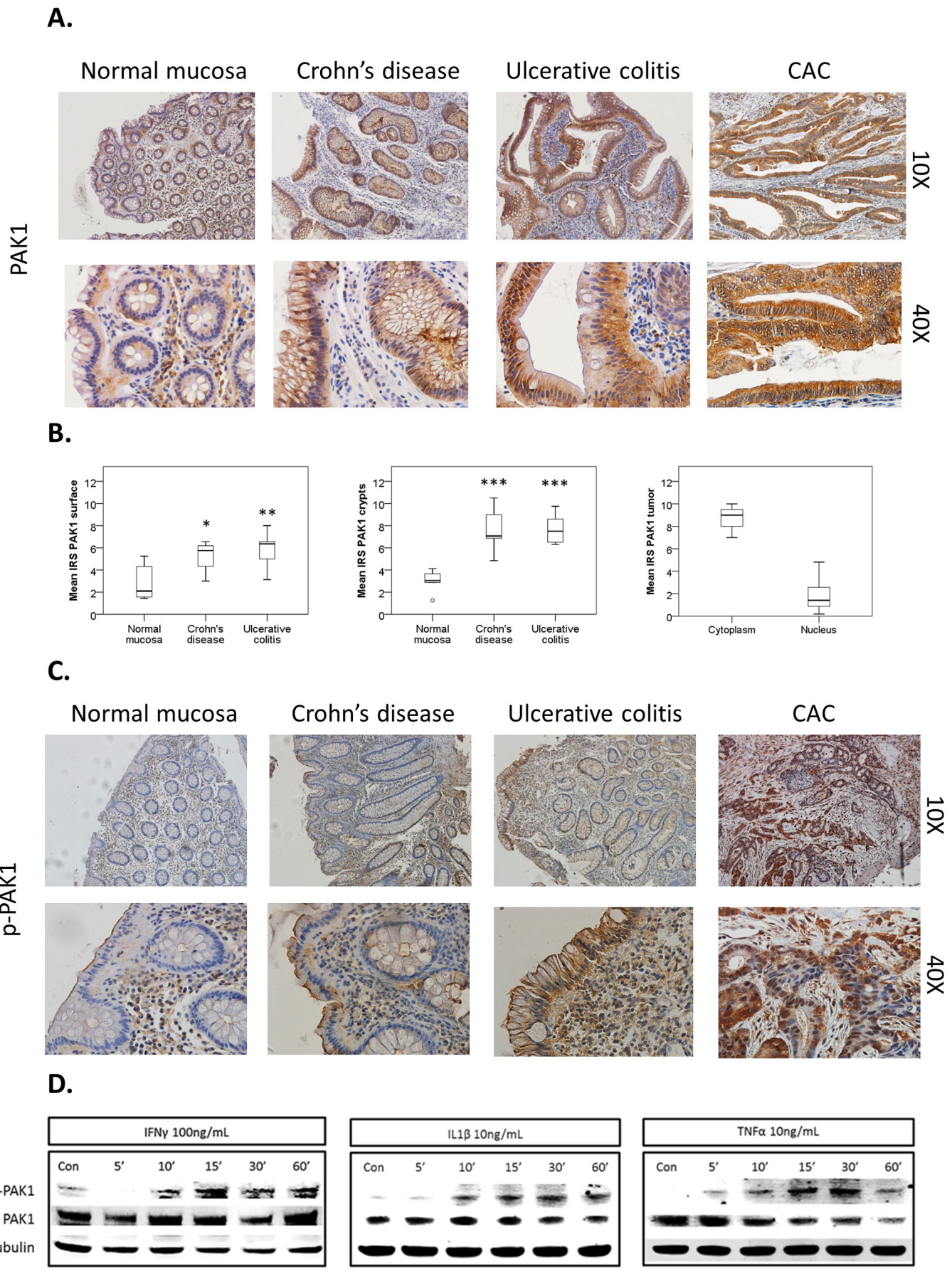
Several studies support the notion that canonical NF-κB activation promotes intestinal tumorigenesis through the upregulation of pro-inflammatory cytokines, proliferation, and cell survival [7–10]. NF-κB activation is regulated by the transcription factor RelA (p65). At basal levels, p65 is sequestered in the cytoplasm by its inhibitors IKKα/β and IκB. Upon pathway activation, IκB is degraded, and p65 translocates into the nucleus [11]. NF-κB signaling in immune cells drives the expression of pro-inflammatory cytokines such as TNFα or IL-1β, which subsequently activate NF-κB in IECs thereby promoting cell survival [12]. In support of this, TNFα-administered NF-κB1$^{-/-}$ mice show increased IEC apoptosis [12]. PAK1 was previously reported to stimulate NF-κB [13], albeit its exact mechanism within the canonical pathway is unknown.

Here, we have investigated the effect of PAK1 activation in IECs upon inflammation and its relevance for NF-κB signaling. We observed that

Abbreviations: PAK1, p-21 activated kinase 1; PPARγ, peroxisome proliferator associated receptor gamma; IBD, inflammatory bowel disease; NF-κB, nuclear factor-kappa B; CRC, colorectal cancer; UC, ulcerative colitis; CD, Crohn's disease; CAC, colitis-associated cancer; EV, empty vector; WT, wild type; KD, kinase dead; KO, knock out; SIO, small intestinal organoids; SB, small bowel; LB, large bowel; IEC, intestinal epithelial cells; Rosi, Rosiglitazone

* Corresponding author at: Division of Gastroenterology and Hepatology, Medical University of Vienna, Währinger Gürtel 18-20, 1090 Vienna, Austria.
E-mail address: christoph.gasche@meduniwien.ac.at (C. Gasche).

http://dx.doi.org/10.1016/j.bbamcr.2015.05.031


A.
Normal mucosa
Crohn's disease
Ulcerative colitis
CAC
PAK1
10X
40X
B.
Mean IRS PAK1 surface
Mean IRS PAK1 crypts
Mean IRS PAK1 tumor
Normal mucosa
Crohn's disease
Ulcerative colitis
Cytoplasm
Nucleus
C.
Normal mucosa
Crohn's disease
Ulcerative colitis
CAC
p-PAK1
10X
40X
D.
IFNγ 100ng/mL
IL1β 10ng/mL
TNFα 10ng/mL
Con
5′
10′
15′
30′
60′
p-PAK1
PAK1
α-tubulin