PAK1 Promotes Intestinal Tumor Initiation

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Abstract

p21-activated kinase 1 (PAK1) is a serine/threonine kinase that is overexpressed in colorectal cancer. PAK1 is a target of mesalamine [5-aminosalicylic acid (5-ASA)], a common drug for the treatment of ulcerative colitis with prospective chemopreventive properties. Here, we investigated whether PAK1 deletion impedes tumorigenesis in murine intestinal cancer models. Ten-week-old APCmin or APCmin/PAK1−/− mice were monitored for 8 weeks, euthanized, and assessed for tumor number and size. Six- to 8-week-old PAK1−/− and wild-type (WT) mice received one 10 mg/kg intraperitoneal injection of azoxymethane (AOM) and four cycles of 1.7% dextran sodium sulfate (DSS) for 4 days followed by 14 days of regular water. Mice also received 5-ASA via diet. Tumor incidence and size was assessed via colonoscopy and pathology. Molecular targets of PAK1 and 5-ASA were evaluated via immunohistochemistry (IHC) in both models. PAK1 deletion reduced tumor multiplicity and tumor burden but did not alter average tumor size in APCmin mice. IHC revealed that PAK1 deletion reduced p-AKT, β-catenin, and c-Myc expression in APCmin adenomas. Colonoscopy and pathologic analysis revealed that PAK1 deletion reduced tumor multiplicity without affecting tumor size in AOM/DSS-treated mice. 5-ASA treatment and PAK1 deletion impeded tumor multiplicity and dysplastic lesions in AOM/DSS mice. IHC further revealed that 5-ASA blocked β-catenin signaling via inhibition of PAK1/p-AKT. These data indicate that PAK1 contributes to initiation of intestinal carcinogenesis. Cancer Prev Res; 8(11); 1093–101. ©2015 AACR.

Introduction

Inflammatory bowel disease (IBD), such as ulcerative colitis, increases the risk of developing colitis-associated cancer (CAC; ref. 1). Epidemiologic evidence suggests that treatment of ulcerative colitis with mesalamine (5-ASA; 5-aminosalicylic acid) reduces the risk of developing CAC (2). Mechanistically, 5-ASA activates the nuclear receptor PPARγ (3) and reduces oxidative stress through inhibition of PI3K activity (4, 5). 5-ASA not only induces S-phase arrest and blocks cell proliferation (6) but also reduces the activation of AKT/β-catenin (7) and NF-κB signaling (8). In addition, we recently reported that p21-activated kinase 1 (PAK1) is a 5-ASA target, and its inhibition by 5-ASA improved intestinal barrier function by increasing membranous E-cadherin and β-catenin (9, 10).

PAK1 is a serine/threonine kinase effector of the small Rho GTPases Rac1/Cdc42 with both kinase and scaffolding activities (11). Accumulating evidence suggests that PAK1 signaling actively participates in gut homeostasis (12). Within intestinal crypts, PAK1 facilitates cell proliferation through sustenance of Wnt/β-catenin signaling (13, 14). PAK1 also regulates cytoskeletal rearrangements as well as cell survival pathways, such as NF-κB, which may be implicated in cell shedding along the villus axis in response to inflammatory cytokines or bacterial effector proteins (15, 16).

We recently found that PAK1 is overexpressed in IBD and CAC and demonstrated that PAK1 overexpression in colonic epithelial cells promotes proliferation and survival via Wnt/β-catenin and AKT/mTOR pathways (17, 18). Previous reports established that PAK1 expression increases with colorectal cancer progression (19) and have shown that knockdown of PAK1 within xenograft models reduces tumor size (20). However, it is unclear whether PAK1 deletion interferes with intestinal tumorigenesis.

Here, we investigated (i) the role of PAK1 upon loss of APC using APCmin/PAK1−/− mice, (ii) the role of PAK1 in inflammation-driven colon carcinogenesis using a PAK1−/− azoxymethane (AOM)/dextran sodium sulfate (DSS) model, and (iii) modulation of PAK1 signaling by 5-ASA.

Materials and Methods

Animal experiments

C57Bl/6 PAK1−/− were obtained from the Mutant Mouse Regional Resource Center-University of North Carolina and bred with C57Bl/6 APCmin mice to generate APCmin/PAK1−/− mice. All mice were genotyped to confirm PAK1 knockout and APC heterozygote status as previously described (21, 22). Mice were housed at the Institute of Biomedical Research (Medical University Vienna, Vienna, Austria) and kept under 12-hour light/dark cycles. Chow and water were available ad libitum, and the animals were weighed weekly. All experiments were performed in accordance with the Austrian and European law, defined by the Good Scientific Practice guidelines of the Medical University Vienna (animal ethics approval number: BMWFV-66.009/0268-WF/V/3b/2014 and BMWFV-66.009/0294-11/3b/2012). Ten-week-old...
male and female APC\textsubscript{min} (n = 10) and APC\textsubscript{min}/PAK1\textsuperscript{1/-} (n = 18) were weighed weekly for 8 weeks and then euthanized by cervical dislocation. Intestines were excised, flushed with PBS, and Swiss roles were fixed and paraffin embedded as described previously (21). Tumor multiplicity and burden were analyzed microscopically on an Olympus BX51 microscope using Cellsens dimension life science imaging software (Olympus). Tumor burden was calculated by drawing a polygon around the tumor and recorded in (mm\textsuperscript{2}).

Six- to eight-week-old male and female C57BL/6 PAK1\textsuperscript{1/-} (n = 48) or C57BL/6 wild-type (WT; n = 54) mice were randomly separated into two groups each, which received either a control or 5-ASA supplemented diet (500 mg/kg chow) starting together with azoxymethane treatment after a 2-week adaption phase on regular chow. All mice received one intraperitoneal injection of 10 mg/kg AOM and four intermittent cycles of 1.7% DSS in drinking water for 4 days followed by 14 days of regular water. Before euthanasia, both tumor incidence and size was assessed via colonoscopy and scored into small (<25%), medium (25%–50%), or large tumors (>50% of circumference). The bowel preparation was carried out as follows: mice were fasted for 24 hours, receiving a rehydration solution (13.5 g glucose, 2.9 g trisodium citrate dihydrate, 2.6 g NaCl, 1.5 g KCl in 1 L) supplied with PEG (34.5 g/L PEG 35000) to clean the large bowel. After anesthesia with an intraperitoneal injection of ketamine and xylazine, the colonoscope (Karl Storz) connected to an airpump (Eheim) was inserted and the colon in situ stimulated. Independent of the colonicoscopy data, tumors were counted by a pathologist who was blinded to the group assignment and classified as dysplastic or invasive carcinoma microscopically. Colonic inflammation was evaluated by two separate investigators as previously described (23, 24). Briefly, inflammation was scored using 10 separate high-power (40×) fields of view on a scale from (0–4), grade 0, normal colonic mucosa; grade 1, slightly inflamed lamina propria and mild shortening of crypts; grade 2, moderate inflammation of the lamina propria and two thirds loss of crypts; grade 3, severe inflammation of lamina propria with intact surface epithelium and complete loss of crypts; and grade 4, severe inflammation of lamina propria and complete loss of the crypts and surface epithelium.

Immunohistochemical analysis

Immunohistochemistry (IHC) was performed on paraffin-embedded sections from mouse intestinal tissue as described previously (9). Sections were dried, dewaxed, and rehydrated followed by blocking of endogenous peroxidase using 15% H\textsubscript{2}O\textsubscript{2} in methanol for 10 minutes. Subsequent antigen retrieval was accomplished by boiling the sections in 10 mmol/L citrate buffer (pH 6). After blocking, the primary antibody was added in for overnight at 4°C in a humidified chamber (for a complete list of primary antibodies, see Supplementary Table S1). The biotinylated secondary antibody was applied for 30 minutes at room temperature. The avidin–biotin–HRP complex (Vectastain) was added (30 minutes), and 3,3′-diaminobenzidine (DAB; Fluka) was used to visualize the staining. Nuclear counterstaining was achieved with hematoxylin. Images were recorded on an Olympus BX51 microscope.

Immunoreactivity score

An immunoreactivity score (IRS) was generated by two blinded investigators (K. Dammann, F. Harpain) as previously described (17). The intensity of staining included a score from 0 to 3, 0 (no staining), 1 (low), 2 (medium), and 3 (high). The percentage of positively stained epithelial cells was evaluated on a scale from 0 to 4, 0 (<1%), 1 (1%–10%), 2 (10%–50%), 3 (51%–80%), and 4 (>80%) in four separate fields of view. The mean intensity and percentage of positively stained epithelial cells was multiplied to generate the IRS, and the highest score attainable was 12. Data represent the mean of the 2 scorers’ findings.

Statistical analysis

Statistical analysis was performed using SPSS (version 21.0). Metric outcome variables were compared using univariate ANOVA and least significant difference (LSD) post hoc tests. P < 0.05 was considered significant (\(\ast\), \(P < 0.05\); \(\ast\ast\), \(P < 0.01\); \(\ast\ast\ast\), \(P < 0.001\)). All data are expressed as mean ± SD unless otherwise stated.

Results

The effect of PAK1 deletion on tumorogenesis in APC\textsubscript{min} mice

Intestinal tumorigenesis following loss of APC can be recapitulated using the APC\textsubscript{min} mouse model. We previously demonstrated that PAK1 is overexpressed in tumors of APC\textsubscript{min} mice and that 5-ASA reduced both PAK1 overexpression and small bowel tumor multiplicity in these mice (10). However, it was unclear whether inhibition of PAK1 by 5-ASA was required to carry out the effect of 5-ASA on reducing tumorogenesis. Here, we established APC\textsubscript{min}/PAK1\textsuperscript{1/-} mice to investigate the role of PAK1 on tumorogenesis upon loss of APC. Ten-week-old APC\textsuperscript{min} and APC\textsuperscript{min}/PAK1\textsuperscript{1/-} mice were housed for 8 weeks (Fig. 1A) and then sacrificed to analyze tumor multiplicity in the small bowel. PAK1 deletion reduced both small bowel tumor number (Fig. 1B), and tumor burden (Fig. 1C). However, average tumor size was not altered in APC\textsuperscript{min}/PAK1\textsuperscript{1/-} mice (Fig. 1D). These data indicated that following loss of APC, PAK1 deletion impedes tumor initiation but not tumor growth.

PAK1 deletion impairs Wnt signaling in APC\textsubscript{min} mice

Wnt/\beta-catenin signaling is a key regulator of crypt proliferation, and its aberrant activation upon loss of APC drives colorectal tumorigenesis (25). PAK1 phosphorylation of \(\beta\)-catenin at Ser-675 increases both \(\beta\)-catenin stability and transcriptional activation (13). IHC of \(\beta\)-catenin in untreated WT and PAK1\textsuperscript{1/-} mice, which were WT for APC, revealed that \(\beta\)-catenin expression was prominently reduced throughout the crypts in PAK1\textsuperscript{1/-} mice, in both the cytoplasm and nucleus (Supplementary Fig. S1A). This suggested that PAK1 was required for \(\beta\)-catenin stability within the normal mucosa. IHC staining of \(\beta\)-catenin in APC\textsubscript{min} and APC\textsubscript{min}/PAK1\textsuperscript{1/-} adenomas also revealed that in the absence of PAK1, \(\beta\)-catenin levels were significantly reduced in both the cytoplasm and the nucleus (Fig. 2A; Supplementary Fig. S2). We also stained sections with a \(\beta\)-catenin (Ser 675) antibody and observed that \(\beta\)-catenin levels were reduced upon PAK1 deletion in APC\textsubscript{min} mice (Fig. 2), and this effect was most profound in the nucleus (Supplementary Fig. S2).

Important transcriptional targets of \(\beta\)-catenin include cyclin D1 and c-Myc. Cyclin D1 levels were not altered upon PAK1 deletion in APC\textsubscript{min} adenomas, although total and nuclear c-Myc levels were significantly reduced in APC\textsubscript{min}/PAK1\textsuperscript{1/-} tumors (Fig. 2; Supplementary Fig. S2). Previous work demonstrated that 5-ASA reduces nuclear accumulation of \(\beta\)-catenin via inhibition of p-AKT.
Considering that 5-ASA inhibits PAK1 and complete activation of AKT requires PAK1 (26), we also investigated whether PAK1 deletion impaired p-AKT (Thr-308) in APCmin adenomas. PAK1 deletion reduced total p-AKT levels and this effect was prominent within the nuclei of APCmin/PAK1/C0/C0 tumors (Fig. 2; Supplementary Fig. S2). These data suggest that PAK1 deletion reduces b-catenin, c-Myc, as well as the nuclear p-AKT.

The effect of PAK1 deletion on tumorigenesis in an AOM/DSS model of CAC
PAK1 is overexpressed in both IBD and CAC (17). However, it is unclear whether PAK1 overexpression promotes CAC and whether inhibition of PAK1 by 5-ASA is sufficient to curtail tumorigenesis in vivo. WT and PAK1/C0/C0 male and female mice received either a control or 5-ASA diet. After one intraperitoneal injection of AOM, mice received four cycles of DSS, which induced a chronic colitis that resulted in weight loss and bloody stool, followed by weight gain in the absence of DSS. Male and female mice were analyzed separately, as the PAK1/C0/C0 phenotype and 5-ASA effects were more pronounced in male mice. PAK1 deletion did not alter the severity of colitis (as measured by weight loss) in male (Fig. 3A) and female mice (Supplementary Fig. S3A). However, following the second cycle of DSS, 5-ASA-treated male WT mice weighed 26 g (24–28) and displayed a median difference of 3 g in comparison to untreated WT mice weighing 23 g (22–24; P = 0.004, ANOVA, LSD). This effect of 5-ASA was not present in PAK1/C0/C0 mice that weighed 24 g (23–25) and displayed a median difference of 1 g in comparison to 5-ASA-treated PAK1/C0/C0 mice (25g (24–26; P = 0.25, ANOVA, LSD; Fig. 3A) implicating that 5-ASA uses PAK1 to carry out certain protective effects. Such weight differences were not observed in female mice (Supplementary Fig. S3A).

Histologic inflammation scoring revealed that WT and PAK1/C0/C0 mice presented with a moderate to severe colitis, such as a loss of two thirds of the crypts and severe infiltration of inflammatory cells in the lamina propria (Supplementary Fig. S3B). 5-ASA restored crypt architecture and reduced inflammation in the lamina propria in WT and PAK1/C0/C0 mice (Supplementary Fig. S3B and S3C).

Tumor multiplicity and size were evaluated via mouse colonoscopy 2 weeks after the fourth DSS cycle just before euthanization and by pathology (Fig. 3B). Colonoscopic scores revealed a median of 4 (2–10) tumors with no effect of PAK1 deletion on tumor multiplicity or size (for male and female subpopulations, see Fig. 3C; Supplementary Fig. S3D). Histologic analysis, however, identified far more lesions—a median of 14 (11–18)—per mouse (P < 0.0001 compared with colonoscopy; Fig. 3D; Supplementary Fig. S3E). Both PAK1
deletion and 5-ASA treatment reduced tumor multiplicity in male mice only (Fig. 3D; Supplementary Fig. S3E). 5-ASA treatment in PAK1/C0/C0 mice did not further impede total tumor number. Both PAK1 deletion and 5-ASA similarly reduced the number of dysplastic lesions but not the number of carcinoma in situ (Fig. 3D). These data suggest that (1) PAK1 deletion inhibits tumor initiation independent of intestinal inflammation, and (2) 5-ASA likely uses PAK1 to impede tumor initiation independent of its anti-inflammatory properties.

Inhibition of PAK1 impairs p-AKT, β-catenin signaling in chronic inflammation

PAK1 deletion and 5-ASA treatment similarly reduced tumor multiplicity and early dysplastic lesions. This suggested that PAK1
is required for tumor initiation; however, it was unclear whether 5-ASA actually inhibited PAK1 in AOM/DSS tumors. IHC detection of PAK1 in control and 5-ASA–treated WT mice confirmed that PAK1 was overexpressed in tumors but not in the corresponding normal mucosa (Fig. 4A; Supplementary Fig. S4A). Importantly, 5-ASA treatment reduced PAK1 expression in tumors (Fig. 4A).

To elucidate pathways interfered by PAK1, we stained multiple 5-ASA targets (27), including p65, PPARγ, p-ERK, β-catenin, p-AKT, and p-mTOR (17) via IHC. Sequestration of p65 in the cytoplasm maintains NF-κB signaling in an inactive state, although its nuclear translocation is required for transcriptional activation. Staining of p65 revealed its expression was low, cytoplasmic, and largely absent throughout the majority of nuclei in tumors (Supplementary Fig. S4B). PAK1 deletion did not change p65 levels in the cytoplasm, nor in the nucleus. However, 5-ASA had a modest effect in reducing cytoplasmic p65 levels in WT (P = 0.281, ANOVA, LSD), but not PAK1–/– mice (Supplementary Fig. S4B). Next we asked whether the anti-inflammatory effect of 5-ASA required activation of the nuclear receptor PPARγ.

5-ASA was previously reported to increase the nuclear expression of PPARγ (3). Although PPARγ was expressed in tumors of WT mice, its nuclear expression was not increased upon PAK1 deletion or 5-ASA treatment (Supplementary Fig. S4B). We examined p-ERK1/2 levels to assess MAPK activation. p-ERK was highly expressed in both the cytoplasm and nucleus of WT tumors. PAK1 deletion but not 5-ASA treatment resulted in a modest reduction of p-ERK expression (Supplementary Fig. S4B).

Considering that PAK1 deletion reduced β-catenin expression in APCmin mice, we investigated the expression of total and p-β-catenin (Ser-675) in AOM/DSS tumors. Both PAK1 deletion and 5-ASA treatment reduced total and p-β-catenin levels in the cytoplasm, and p-β-catenin inhibition was more profound within the nucleus (Supplementary Fig. S4C). Importantly, PAK1 deletion and 5-ASA treatment together did not synergistically impede total or p-β-catenin expression. The downstream β-catenin target c-Myc was strongly downregulated upon PAK1 deletion and 5-ASA treatment (Fig. 5A; Supplementary Fig. S4D).
We also investigated whether PAK1 deletion impaired either p-AKT (Thr-308) in tumors of AOM/DSS mice. Both PAK1 deletion and 5-ASA treatment in WT mice reduced expression of p-AKT (308; Fig. 5A; Supplementary Fig. S4D). However, 5-ASA did not block p-AKT (308) expression in PAK1−/− mice indicating that 5-ASA uses PAK1 inhibition to block AKT. These data indicate that PAK1 may initiate tumorigenesis through activation of a p-AKT (Thr 308)–β-catenin cascade.

mTOR) signaling is downstream of p-AKT (308) and has been implicated in tumor growth and survival. Activation of mTOR implicated in tumorigenesis was also examined in these samples, as PAK1 contributes to mTOR signaling and 5-ASA inhibits mTOR (17, 28). IHC staining of p-mTOR revealed its activation in tumors of WT and PAK1−/− mice, although this effect was more profound in WT mice. Importantly, 5-ASA treatment reduced p-mTOR in WT mice and this effect was diminished upon PAK1 deletion (Supplementary Fig. S4E). These data suggest that 5-ASA uses PAK1 inhibition to impede mTOR signaling.

**Discussion**

PAK1 is overexpressed in both sporadic and colitis-associated colorectal cancer (17, 19). Prolonged use of 5-ASA may impede colorectal tumorigenesis (2, 29), although the underlying mechanisms are unknown. We previously identified PAK1 as a 5-ASA target and here we investigated whether PAK1 deletion impaired tumorigenesis in mouse models of sporadic and CAC. We used APCmin/PAK1−/− mice to investigate the role of PAK1 upon loss of APC and found that PAK1 deletion impeded tumorigenesis without affecting tumor size. Furthermore, Wnt signaling was impaired via downregulation of β-catenin and c-Myc. We previously reported that PAK1 is overexpressed in IBD (17), and it is known that chronic inflammation in IBD increases the risk of CAC (30). We used an AOM/DSS mouse model in which WT and PAK1−/− mice received a 5-ASA–enriched diet and found that PAK1 deletion did not affect the severity of colitis but impaired tumor initiation without affecting tumor size. Mechanistically, PAK1 deletion diminished the effect of 5-ASA on reducing AKT–β-catenin signaling, resulting in downregulation of c-Myc, and reducing tumor initiation.

Mutation in the tumor suppressor APC is an initiating step in familial adenomatous polyposis and sporadic colorectal tumorigenesis (31) and results in aberrant β-catenin activation, which drives cell transformation within the crypt axis (32). The APCmin mouse model quickly recapitulates this process exhibiting a robust tumor load following loss of APC within 12 weeks (33). We previously observed that PAK1 was overexpressed in tumors of APCmin mice and that 5-ASA not only impeded tumor multiplicity...
but also reduced expression of PAK1, nuclear β-catenin, and its downstream target c-Myc. It was however unclear whether this effect of 5-ASA required PAK1 inhibition. Here, we bred APC<sup>min</sup> and PAK1<sup>−/−</sup> mice to assess whether PAK1 deletion reduces tumorigenesis similar to 5-ASA. Indeed, PAK1 deletion reduced tumor multiplicity and the expression and phosphorylation of β-catenin and its target c-Myc. The transcription factor c-Myc regulates cell transformation and tumorigenesis and its deletion in APC<sup>min</sup> mice reduced tumor multiplicity, which was also in line with our findings upon PAK1 deletion (34). This suggested that PAK1 drives tumorigenesis via β-catenin–c-Myc signaling.

In our model of CAC, PAK1 deletion impeded tumorigenesis specifically in male mice. Although we did not investigate this further, PAK1 mediates the effects of both testosterone and estrogen (35, 36). Therefore it is not surprising that PAK1 may play a gender-specific role in colorectal tumorigenesis. PAK1 deletion blocked tumor multiplicity and dysplastic lesions similar to the effect of 5-ASA in WT mice. Importantly, 5-ASA inhibited PAK1 expression in AOM/DSS tumors and did not further reduce tumor multiplicity or dysplastic lesions in PAK1<sup>−/−</sup> mice. This supported that 5-ASA uses PAK1 inhibition to impede tumor initiation, but not progression, as neither PAK1 deletion nor 5-ASA reduced tumor size or the number of in situ carcinoma.

5-ASA treatment, and not PAK1 deletion, reduced inflammation in this AOM/DSS model, implicating a PAK1-independent effect of 5-ASA on intestinal inflammation. He and colleagues reported that PAK1 knockdown reduced tumor growth using a xenograft model (20). We, however, did not observe this phenotype upon PAK1 deletion in either of our APC<sup>min</sup> or AOM/DSS models. In the AOM/DSS model, the presence of chronic inflammation likely drives tumor growth even in the absence of PAK1. 5-ASA activation of PPARγ was required to reduce neoplasia in experimental models of colitis (3, 37). However, 5-ASA did not increase PPARγ expression in our AOM/DSS model, indicating that 5-ASA blocked inflammation and tumor size through an alternative pathway. Baan and colleagues reported that 5-ASA...
block proliferation though inhibition of mTOR signaling (28), and we recently demonstrated that 5-ASA inhibits an AKT–mTOR cascade (17), although an in vivo proof of concept was lacking. Here we found that 5-ASA blocked p-mTOR in AOM/DSS tumors, and this effect was reduced upon PAK1 deletion. These data led us to conclude that in addition to its anti-inflammatory effect, 5-ASA may reduce tumor size through inhibition of mTOR signaling.

Wnt–β-catenin signaling is a key driver of tumorigenesis, and PAK1 regulates stability and transcriptional activity of β-catenin (13). Without inflammation, we observed that PAK1 deletion reduced total β-catenin expression throughout the crypt axis and in tumors of APCmin mice. However, PAK1 deletion was not sufficient to fully block its nuclear accumulation in chronic inflammation. It is likely that other PAKs, such as PAK4, which also phosphorylate β-catenin, may be triggered upon DSS, thereby bypassing the effect of PAK1 deletion and promoting tumor growth (38). Brown and colleagues demonstrated that 5-ASA blocks β-catenin via AKT inhibition in IL10−/− mice (7). In addition, we also observed that PAK1 is required for AKT–β-catenin activation in both APCmin and AOM/DSS tumors, and its deletion reduced c-Myc expression. Taken together, these data indicate that inhibition of PAK1 by 5-ASA blocks AKT–β-catenin signaling thereby reducing tumorigenesis.

Population-based studies suggest a chemopreventive role of nonsteroidal anti-inflammatory drugs (NSAID) in colorectal cancer; however, their efficacy and safety are not yet established. Anti-inflammatory effects of NSAIDs are through inhibition of COX1 and COX2 activity in the prostaglandin synthesis pathway. In contrast to COX1, COX2 is inducible by pro-inflammatory cytokines and oncogenic signaling. Whether PAK1 interferes with COX2-mediated tumorigenesis warrants further investigations. In a rat intestinal cell line IEC-18, angiotensin-II-dependent activation of COX2 promoter was shown to be mediated by a PAK1/MKK6/p38beta/CREB signaling cascade (39). Another study showed that in respiratory papilloma, COX2 expression was mediated by a Rac1/PAK/NF-kB pathway (ref. 40). A role of COX1 signaling was demonstrated in T-cell activation that was mediated by a Fyn/Vav/PAK1 cascade and NSAIDs inhibited this pathway, including PAK1 activation (41). Altogether, these data demonstrate that inhibition of PAK1 might modulate COX signaling. Although, efficacy of NSAIDs is not examined upon PAK1 deletion, we expect downregulation of COX activity in PAK1−/− mice. PAK1 has a role in regulating both immune functions and epithelial homeostasis; therefore, pharmacologic inhibition of PAK1 should be carefully considered to target its overexpression. PAK1−/− mice were reported with no distinct phenotype except for an impairment of the pathways, such as MAPK, AKT, and Wnt–β-catenin, which require PAK1 for full activation and also contribute to oncogenesis. PAK1 deletion however was associated with defects in the immune functions such as mast cell degranulation (42). In our study, germline deletion of PAK1 modulated tumorigenesis in both APCmin mice and in chemically induced AOM/DSS model; however, inflammation was not affected in the latter, indicating its role in the stromal compartment.

This is the first study to demonstrate that PAK1 modulates tumorigenesis in an APCmin model as well as in an AOM/DSS model of chronic inflammation. PAK1 deletion and 5-ASA treatment impeded tumorigenesis via impairment of a PAK1–AKT–β-catenin axis and the downstream target c-Myc (Figure 5B). Our results suggest that PAK1 may be a plausible target for chemoprevention, although further investigations are warranted to understand its specific role in epithelial and stromal compartments.

Disclosure of Potential Conflicts of Interest
No potential conflicts of interest were disclosed.

Authors’ Contributions
Conception and design: K. Dammann, V. Khare, F. Harpain, C. Gasche
Development of methodology: K. Dammann
Acquisition of data (provided animals, acquired and managed patients, provided facilities, etc.): K. Dammann, F. Harpain
Analysis and interpretation of data (e.g., statistical analysis, biostatistics, computational analysis): K. Dammann, V. Khare, F. Harpain, M. Lang, A. Kurtovic, I. Mesteri, R. Evstatiev, C. Gasche
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