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Inflammatory bowel disease induces inflammatory and pre-neoplastic changes in the prostate

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Abstract

Background: Inflammatory bowel disease (IBD) has been implicated as a risk factor for prostate cancer, however, the mechanism of how IBD leads to prostate tumorigenesis is not known. Here, we investigated whether chronic intestinal inflammation leads to pro-inflammatory changes associated with tumorigenesis in the prostate.

Methods: Using clinical samples of men with IBD who underwent prostatectomy, we analyzed whether prostate tumors had differences in lymphocyte infiltrate compared to non-IBD controls. In a mouse model of chemically-induced intestinal inflammation, we investigated whether chronic intestinal inflammation could be transferred to the wild-type mouse prostate. In addition, mouse prostates were evaluated for activation of pro-oncogenic signaling and genomic instability.

Results: A higher proportion of men with IBD had T and B lymphocyte infiltration within prostate tumors. Mice with chronic colitis showed significant increases in prostatic CD45+ leukocyte infiltration and elevation of three pro-inflammatory cytokines—TIMP-1, CCL5, and CXCL1 and activation of AKT and NF-kB signaling pathways. Lastly, mice with chronic colitis had greater prostatic oxidative stress/DNA damage, and prostate epithelial cells had undergone cell cycle arrest.

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Conflict of Interest

The authors have declared no conflict of interest exists.

Conclusions: These data suggest chronic intestinal inflammation is associated with an inflammatory-rich, pro-tumorigenic prostatic phenotype which may explain how gut inflammation fosters prostate cancer development in men with IBD.

Introduction

Prostate cancer (PCa) is the most common cancer in men in the United States, with an estimated 191,930 new cases in 2020 (1). Prostate-specific-antigen (PSA)-based screening for PCa in the general population can reduce mortality, but may lead to over-diagnosis and overtreatment of indolent cancers (2–4). Therefore, the United States Preventative Task Force (USPTF) has identified a critical need to study populations at increased risk of PC death that may benefit from focused screening (5). Our group has previously demonstrated men with inflammatory bowel disease (IBD) have a 4-fold increased risk of developing clinically significant (Gleason grade group ≥2) PCa (6), and recently this finding was validated within a largescale, population-based registry (7). We seek to elucidate the potential mechanism underlying the increased risk of PCa in men with IBD.

IBD is a biologically complex chronic inflammatory disease state of the gastrointestinal (GI) tract, affecting 1.2 million men in the United States alone (8). Patients with IBD are known to be at increased risk of colorectal carcinomas (CRC), primarily as the result of unmitigated chronic inflammation (9). Similarly, men with IBD may also have an increased risk of prostate tumorigenesis due to the effect of prolonged chronic gastrointestinal inflammation on the prostate. Here, we demonstrate that chronic gut inflammation is associated with significant increases in prostatic inflammatory infiltrate, upregulation of inflammation-associated pro-oncogenic signaling, and genomic instability—a characteristic phenotype preceding the development of inflammation-mediated cancer (10).

Results

IBD is associated with a lymphocyte-rich tumor microenvironment in human PCa

In an exploratory analysis, using a clinical cohort of men who underwent prostatectomy at our institution, we investigated whether PCa tissue in men with IBD had differences in lymphocyte infiltration compared to those without IBD. Despite similar baseline characteristics between groups (Fig. 1a), a higher proportion of men with IBD stained positively for CD4+ [4/10 (40%) vs. 3/22 (13.7%)], CD8+ [5/10 (50%) vs. 3/22 (13.7%)], and CD20+ [5/10 (50%) vs. 3/21 (14.3%)] lymphocytes within prostate tumors. Most men regardless of IBD status stained positively for CD4+ [8/10 (80%) vs. 14/22 (63.6%)], CD8+ [9/10 (90%) vs. 17/22 (77.3%] and CD20+ [9/10(90% vs. 16/22 (72.3%)] lymphocytes in benign glands (Fig. 1b and 1c). We also attempted to compare the inflammatory grade of each lymphocyte marker, however, there were too few patients to discern differences (Supplemental Table 1a and 1b). Overall, these data may suggest that prostate tumors in men with IBD are enriched with T and B lymphocytes.

Clinical samples were also evaluated for the prostatic presence of proliferative inflammatory atrophy (PIA), a background lesion containing chronic inflammation adjacent to atrophic glands. PIA potentially precedes prostate neoplasia in the setting of chronic inflammation

(11). In our cohort, there was no clear difference in the proportion of men with IBD who had prostatic PIA compared to controls [7/10 (70%) vs. 17/22 (77.2%)] (Figure 1b and Supplementary Figure 1).

Murine chronic colitis model

To determine whether intestinal inflammation leads to changes in the intra-prostatic inflammatory milieu, we induced chronic colitis by administering wild-type, C57BL/6 mice with 3 cycles of 3% dextran-sodium sulfate DSS (12). Mice were sacrificed for immunophenotyping following the third cycle of DSS (Fig. 2a). After administration, colitis (DSS-treated) mice exhibited progressively bloody diarrhea and weight loss. Histologic analysis of the colon invariably demonstrated epithelial erosion and infiltration of granulocytes into the submucosa (Fig. 2c–d). To rule out the possibility of direct toxicity of DSS on prostate, we checked the presence of DSS in prostate by Toluidine blue staining (13). We observed positive Toluidine blue staining indicating DSS in the colons of colitis mice but there was no change in prostates of either control or DSS-treated colitis mice (Fig. 2e), supporting the notion DSS has no direct effect on the prostate.

Chronic colitis is associated with intra-prostatic immune cell infiltration

Given we observed increased immune cell infiltration in prostate tumors of men with IBD, we investigated how chronic colitis changes the murine prostatic inflammatory infiltrate. By H&E staining, colitis mice had greater prostatic inflammation compared to controls (Fig. 3a). In addition, colitis mice had significantly elevated prostatic CD45+ leukocyte and relatively more T (CD8+) lymphocyte infiltration (Fig. 3b and 3c). In contrast to the findings from our clinical prostatectomy cohort, we observed a relative absence of B (CD45-B220+) lymphocyte infiltration in both groups (Fig. 3c). Overall, these results validate the findings from our patient cohort that chronic intestinal inflammation is associated with prostatic immune cell infiltration.

Chronic colitis upregulates intra-prostatic expression of pro-inflammatory cytokines

Within inflamed tissue, immune cells contribute to imbalances of cytokines which can drive several stages of cancer development (14). In an exploratory analysis, we analyzed prostatic levels of 40 cytokines and chemokines (n=3/group) after colitis induction. We observed relative trends in differences of several pro and anti-inflammatory factors (Fig. 4a). The greatest increase was observed in CXCL1 in all three colitis replicates compared to controls (Fig. 4b and 4c). Additionally, we observed >2-fold increases in CCL5 (RANTES) and TIMP-1 in two of three replicates compared to controls (Fig. 4b and 4c) and validated the increases by immunostaining (Fig. 4d). Notably, prostatic levels of TNF-α, IL-6, and the IL-1β—important cytokines in IBD pathogenesis in the intestines—were not different between groups (Fig. 4a and Supplementary Fig. 2). These results indicate chronic colitis is associated with elevated prostatic expression of pro-inflammatory cytokines, further supporting our hypothesis gut inflammation leads changes in the immune profile of the prostate.

Chronic colitis is associated with prostatic pro-oncogenic AKT and NF-kB signaling, DNA damage, oxidative stress, and cell cycle arrest.

Previous studies have shown TIMP1, CCL5 and CXCL3 regulate AKT and NF-kB pathways(15–17). We tested the hypothesis chronic colitis-associated changes to the prostate immune microenvironment leads to activation of prostatic AKT and NF-kB signaling. We found significantly increased phosphorylation of AKT at S473 (p-AKT), a trend toward elevated total AKT level in colitis mice (Fig. 5a and 5b), and also an increased p-AKT expression using immunofluorescence (Fig. 5c). Furthermore, phosphorylated NF-kB at S-536 (p-NF-kB) was significantly increased in colitis mice (Fig. 5a and 5b). Aberrant AKT and NF-kB signaling pathways play important roles in inflammation and also implicated in the regulation of several prostate cancer oncogenes including *c-MYC* (18, 19). We found prostatic levels of *c-MYC* significantly increased in colitis mice (Fig. 5a and 5b). Collectively, our results indicate chronic colitis is associated with activation of prostatic AKT and NF-kB signaling and upregulation of *c-MYC.*

One of the critical components of inflammation associated tumorigenesis includes the development of immune-mediated DNA damage that results in acquired molecular alterations (20). Therefore, we analyzed levels of phosphorylated-ɣ-H.2AX at Ser 139 (p-ɣ-H.2AX)— DNA damage marker—after induction of chronic colitis. We observed an increase in prostatic expression (Fig. 5d) and protein levels (Fig. 5e) of p-ɣ-H.2AX in colitis mice. Given the central role of oxidative stress in inducing DNA damage in inflamed tissues,(20) we analyzed levels of 4-Hydroxy-2-Nonenal (4-HNE), a marker for oxidative stress.(21) We found colitis mice had an upward trend toward greater murine prostatic 4-HNE expression (Fig. 5f) and protein levels (Fig. 5g).

One potential downstream implication of DNA damage is in order to repair altered genetic material, cells may undergo senescence, a state of irreversible cell cycle arrest (22). The relative absence of Ki67, a proliferative marker, in the setting of elevated p-ɣ-H.2AX, implies cell cycle arrest and may be useful for the determination of cellular senescence (23). We found a significant reduction in Ki67 expression in colitis mice (Fig. 5h and 5i). Taken together, our results suggest chronic colitis is associated with prostatic DNA damage and oxidative stress as well as an associated state of cell cycle arrest.

Discussion

In this study, we showed chronic intestinal inflammation has a pro-inflammatory effect on the prostate that is associated with intra-prostatic activation of pro-oncogenic signaling pathways and genomic instability. Clinically, these findings have implications for PCa screening and may suggest subgroups of men with IBD with severe chronic intestinal inflammation should undergo more focused PCa screening.

Chronic inflammation in one organ leading to inflammation-mediated tumorigenesis in a distinct organ has not been previously described. In determining the impact of chronic intestinal inflammation on prostate tumorigenesis, a critical question is whether the primary inflammatory signal is transferred from the gut to the prostate. Our results may suggest a greater proportion of men with IBD have tumor-associated prostatic T and B lymphocyte

infiltration compared to men without IBD. Similarly, in our murine model we found chronic colitis was associated with greater prostatic immune cell infiltration. In contrast to findings from our clinical cohort, chronic colitis in mice was not associated with prostatic B lymphocyte infiltration, which may be explained by distinctions in B-cell function between mice and humans (24) and differences in the inflammatory response between DSS-colitis and human IBD (25). Notably, the role of lymphocytes in cancer development is complex and likely explained by the activation status of individual cell types (26).

Immune cells can exacerbate inflammation by secreting pro-inflammatory factors that drive pro-oncogenic signaling (14). Our results indicate chronic intestinal inflammation is associated with greater prostatic immune cell infiltration, both within human prostate tumors and chronic colitis mice and enhanced prostatic expression of pro-inflammatory cytokines TIMP1, RANTES, and CXCL1. These pro-inflammatory factors have broad roles in cancer initiation and progression (15, 27, 28). Specifically, elevated levels of these factors have all been shown to lead to AKT and NF-kB pathway activation (15–17), which are central events in the pathogenesis of inflammation-associated cancer (29, 30). Notably, prostatic levels of important cytokines in IBD associated CRC pathogenesis—TNF-α, IL-6, and IL-1β—were not elevated in chronic colitis. We found chronic colitis was associated with activation of both the prostatic AKT and NF-kB pathways. These pathways play numerous important roles in cancer development and can regulate important PCa-related oncogenes including *c-MYC* (18, 19). We found chronic colitis was associated with greater prostatic levels of *c-MYC.*

Inflammation can also drive tumorigenesis by promoting genomic instability, which can result in the development of acquired genetic alterations in key oncogenes (20). We observed chronic colitis was associated with features of genomic instability in the prostate, including elevated DNA damage, oxidative stress, and prostate epithelial cells had undergone cell cycle arrest. Irreversible cell cycle arrest is known as cellular senescence, where cells can engender a unique local microenvironment by secreting pro-inflammatory factors, which may in turn lead to more DNA damage, driving cancer initiation (31). In human IBD, Risques et. al. postulated DNA damage and associated cell cycle senescence are important features of pre-neoplastic colon tissue in IBD related CRC (22).

This study has several limitations. First, there is heterogeneity of clinical factors within our human RP cohort with respect to PCa and IBD disease factors, which we are unable to control for within this small group of patients. Second, we harvested benign prostate tissues from mice at a relatively early time-point after colitis induction and further investigation is needed to determine whether the effects of chronic intestinal inflammation on the prostate are sustained and eventually lead to PCa. Third, we are unable to determine within our model whether the inflammatory signal from the colon is transmitted to the prostate by local translocation—given the proximity of rectum to the prostate—or by systemic elevation of circulating cytokines. Other organs were not evaluated at the time of our analysis.

In this study, we have not investigated other mechanisms that may explain the association between IBD and PCa. The urinary microbiome may influence PCa development (32) and it is unknown how it may be altered in the context of IBD. Patients with IBD are

on immunosuppressive medications, and further study is necessary to determine whether these medications rescue the effects of chronic gut inflammation on the prostate, or rather, exacerbate carcinogenesis by reducing anti-cancer immune surveillance (33). Lastly, there is possibly genetic overlap between IBD and PC—which includes a candidate gene FOLH1/PSMA and several susceptibility SNPs—that may contribute to carcinogenesis either through an effect on inflammation or on other pathways (6).

In summary, our data suggest chronic intestinal inflammation is associated with greater prostatic inflammation, upregulation of pro-oncogenic signaling pathways, and genomic instability. These characteristics may cooperate to enable the growth and survival of cell populations within inflamed tissue (Supplementary Fig. 3), are hallmark features of the pre-neoplastic phenotype in inflammation-mediated human malignancies, and potentially helps to explain the association between IBD and PCa.

Methods

A detailed description of materials and methods is provided in Supplementary methods. In short, RP specimens of patients who had IBD and patients without a history of IBD who have undergone RP at our institution (Northwestern Medicine) for localized prostate adenocarcinoma.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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A

	Control (n=22)	Inflammatory Bowel Disease (n=10)
Age [years, medial (IQR)]	63.8 (52.9 - 81.6)	62.4 (53.4 - 73.9)
Black Race [number(%)]	5 (22.7%)	1 (10%)
PSA [median (range)]	5.82 (1.38 - 15.37)	5.4 (2.3 - 9.4)
Gleason Grade Group		
1	1 (4.5%)	4 (40%)
2	20 (90.9%)	4 (40%)
3	1 (4.5%)	2 (20%)
Prostate Volume [mL, median (range)]	63.3 (32.6 - 155)	64.8 (18.5 - 190)
IBD subtype		
Ulcerative Colitis	-	2 (20%)
Crohn's Disease	-	7 (70%)
Unspecified	-	1 (10%)
IBD treatment		
Medication	-	9 (90%)
Surgery	-	4 (40%)
Unknown	-	1

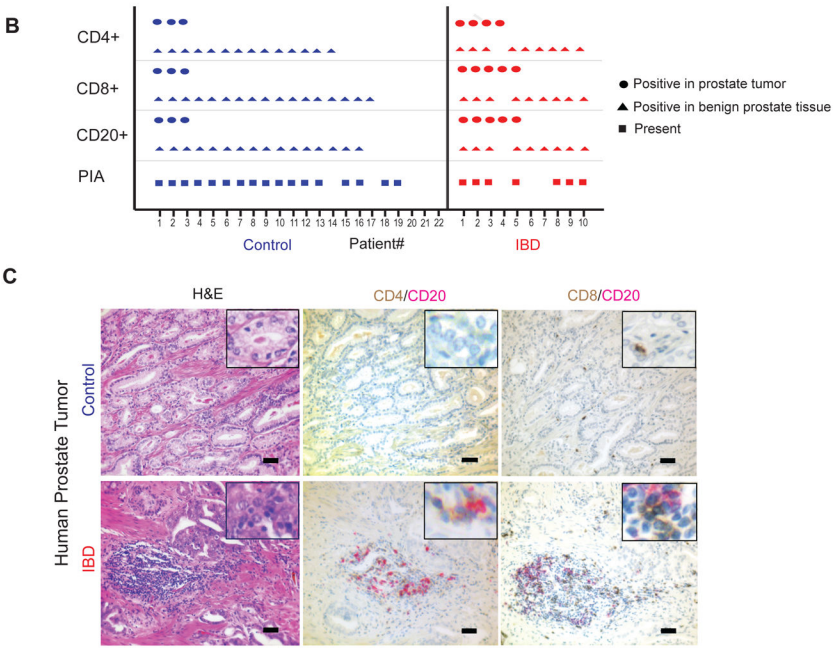

Figure 1. IBD is associated with a lymphocyte-rich tumor microenvironment in human prostate cancer.

a, Baseline demographic and clinical characteristics of human RP cohort. b, Immunohistochemistry (IHC) was performed to analyze the presence of CD4+, CD8+, and CD20+ lymphocytes, and proliferative inflammatory atrophy in human RP specimens of men with IBD (n=10) and non-IBD controls (n=22). c, Representative H&E and accompanying IHC staining of prostatectomy specimens in regions of prostate tumor. Sections were dual stained for CD4/CD20 and CD8/CD20. Images are visualized at 20x and scale bar represents 50 μm.

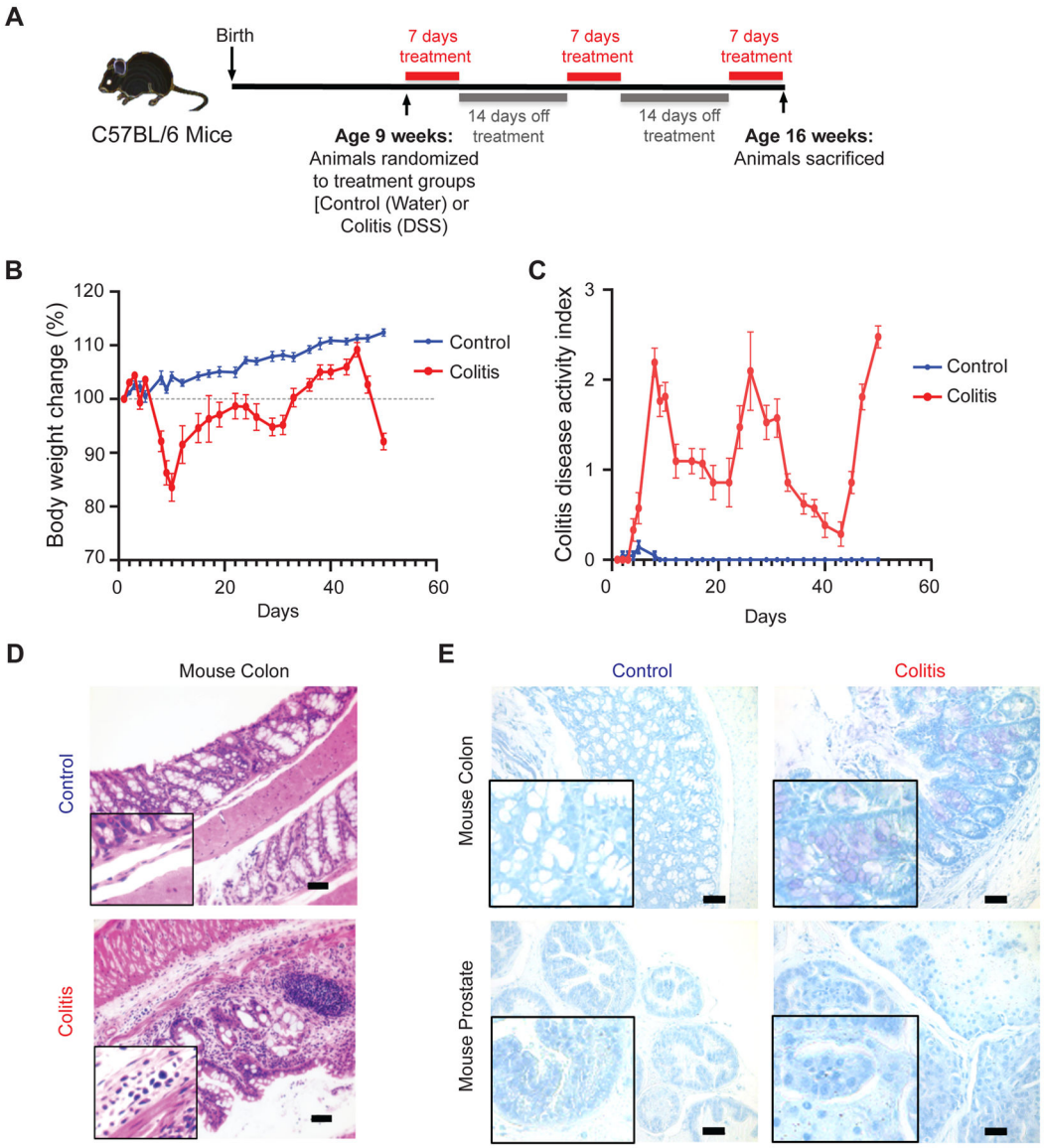

Figure 2. Dextran-sodium-sulfate (DSS) administration led to induction of chronic colitis in mice, mimicking human IBD.

a, Schematic of chronic colitis induction with DSS. **b,** Mouse body weight relative to baseline during DSS treatment (n=7/group). Day 0 refers to the day of treatment initiation. **c,** Colitis severity was measured using the disease activity index (DAI) scoring system composed of the average change in body weight, stool consistency, and diarrhea and ranges from 0 to 4. **d,** Representative H&E of mouse colon sections in control and colitis group and **e**, Toluidine blue staining of mouse colon and mouse prostate in control and colitis groups. Images are visualized at 20x and scale bar represents 50 μm.

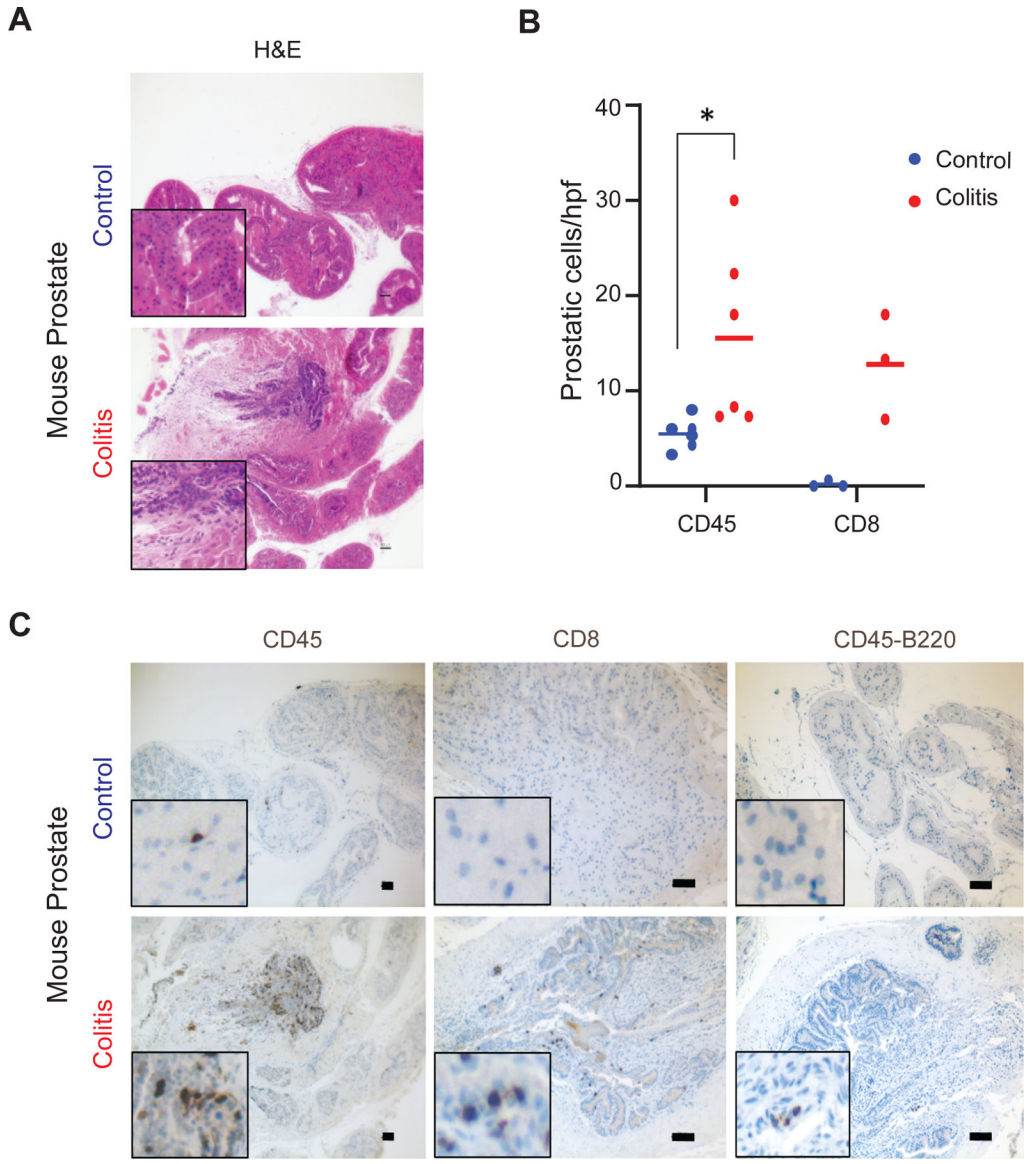

Figure 3. Chronic colitis is associated with prostatic immune cell infiltration.

a, H&E staining of murine prostate sections of control and colitis mice. **b,** Immunohistochemical analysis of prostatic immune cells on control and colitis mice and quantification of CD45 (n=6 biological replicates/group, 3 regions per replicate) and CD8 (n=3 biological replicates/group, 3 regions per replicate) expression. Dots represents an individual biological replicates and the line denotes the mean. *P < 0.05, **P < 0.01. hpf = high power field. **c,** IHC was performed on murine prostate sections for immune cell markers (CD45, CD8, CD45B-220) and representative images are shown. H&E and CD45 images are visualized at 10x, CD8 and CD45-B220 at 20x. Scale bar represents 50 μm.

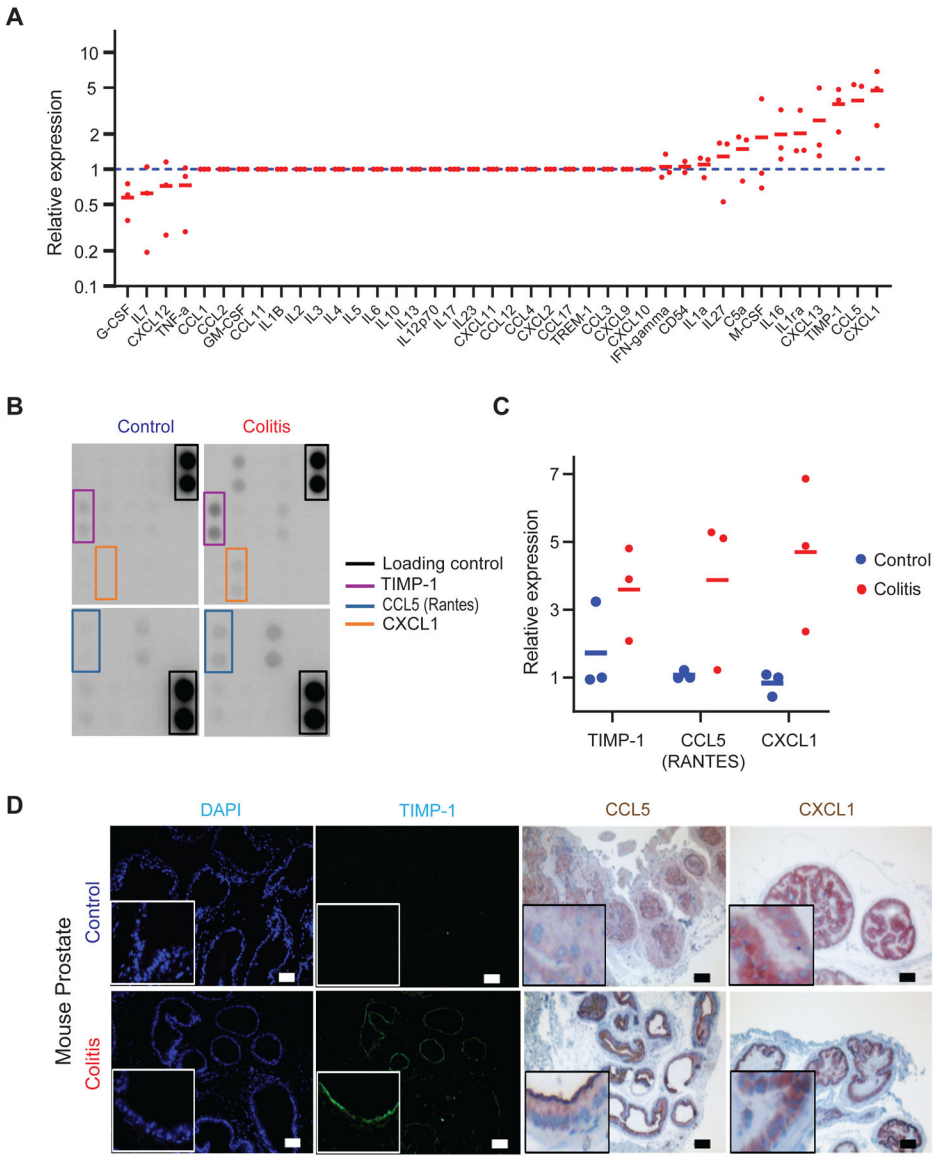

Figure 4. Chronic colitis is associated intra-prostatic expression of pro-inflammatory cytokines.

a, Cytokine profiling was performed with multiplex ELISA on prostatic tissue lysates (n=3 biological replicates/group). The scatter plot depicts the fold change of each cytokine in colitis mice relative to controls. **b, and c,** Representative Cytokine antibody array probe with murine prostatic tissue lysate and quantification for TIMP1, CCL5, and CXCL1. **d,** Immunostaining of TIMP1, CCL5, and CXCL1 in murine prostate sections and representative images from 3 biological replicates.

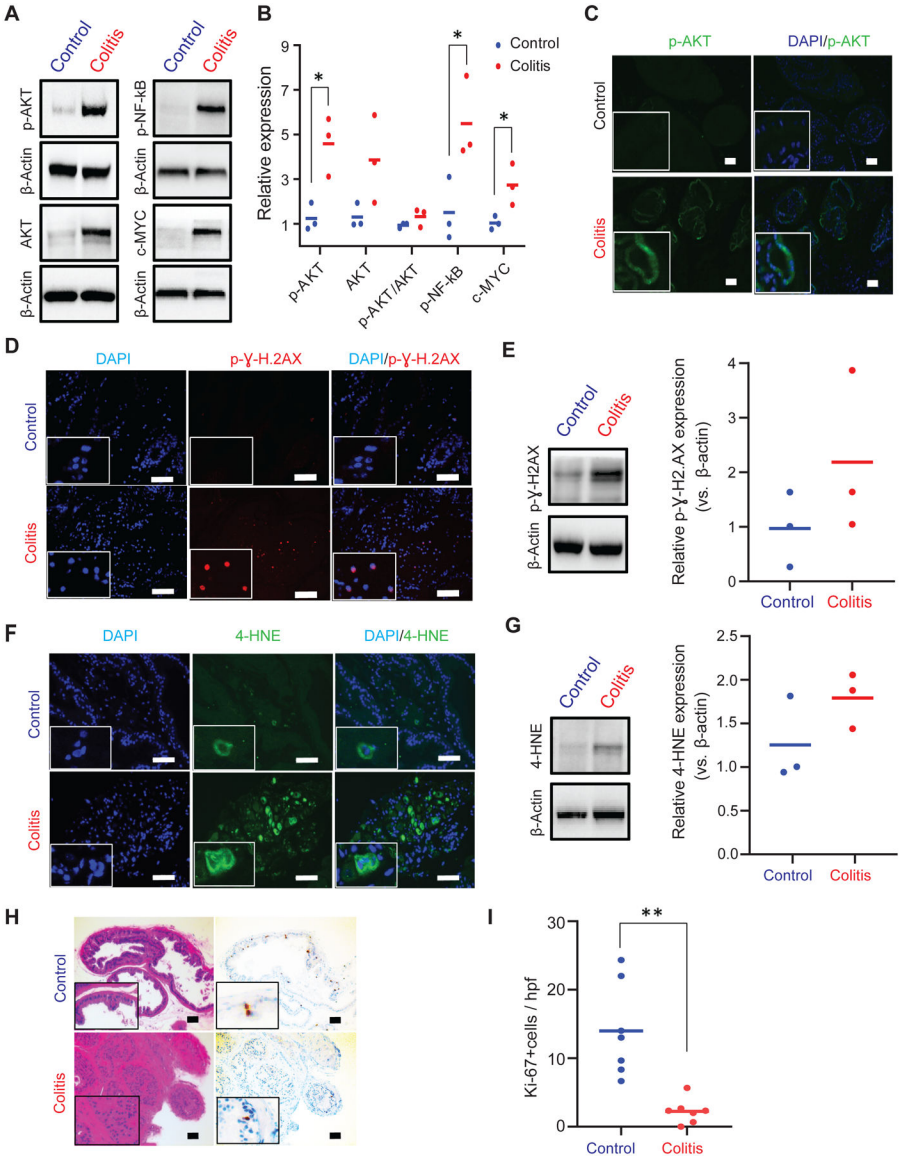

Figure 5. Chronic colitis is associated with prostatic pro-oncogenic AKT and NF-kB signaling, DNA damage, oxidative stress, and cell cycle arrest.

a and b Western analysis with indicated antibodies on extract from murine prostate tissue. **c,** p-AKT expression was analyzed by immunofluorescence (IF) in murine prostate sections (n=3/group). Images are visualized at 20x. **d,** Phosphorylated-ɣ-H.2AX at Ser139 (p-ɣ-H.2AX) expression analyzed by IF and visualized at 40x (n=3/group). **e,** Representative western blot and scatter plot depicting quantification for p-ɣ-H.2AX (n=3/group). **f,** 4-Hydroxynonenal (4-HNE) expression analyzed by IF and visualized at 40x (n=3/group). **g,** Representative western blot and quantification of 4-HNE (n=3/group). **h** and **i,** Cell cycle arrest (ki67) was assessed by IHC. Representative IHC images are shown at 20x and quantification (n=7 biological replicates/group, mean of 3 regions per biological replicate). For all scatter plots, the line denotes the mean. Scale bar represents 50 μm.