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CCL11 Exacerbates Colitis and Inflammation-Associated Colon Tumorigenesis

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Abstract

CCL11, also known as eotaxin-1, is described as an eosinophil chemoattractant, which has been implicated in allergic and Th2 inflammatory diseases. We have reported that CCL11 is significantly increased in the serum of inflammatory bowel disease (IBD) patients, colonic eosinophils are increased and correlate with tissue CCL11 levels in ulcerative colitis patients, and CCL11 is increased in dextran sulfate sodium (DSS)-induced murine colitis. Here, we show that CCL11 is involved in the pathogenesis of DSS-induced colitis and in colon tumorigenesis in the azoxymethane (AOM)-DSS model of colitis-associated carcinogenesis (CAC). *Ccl11*$^{-/-}$ mice exposed to DSS then allowed to recover had significantly less body weight loss and a decrease in histologic injury versus wild-type (WT) mice. In the AOM-DSS model, *Ccl11*$^{-/-}$ mice exhibited decreased colonic tumor number and burden, histologic injury, and colonic eosinophil infiltration versus WT mice. *Ccl11* is expressed by both colonic epithelial and lamina propria immune cells. Studies in bone marrow chimera mice revealed that hematopoietic- and epithelial-cell derived CCL11 were both important for tumorigenesis in the AOM-DSS model. These findings indicate that CCL11 is important in the regulation of colitis and associated carcinogenesis and thus anti-CCL11 antibodies may be useful for treatment and cancer chemoprevention in IBD.

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CONTRIBUTIONS

Study concept and design: LAC, KTW. Data acquisition: DP, KS, MA, DPB, MMA, DMH, MBP, MKW, LAC. Analysis and interpretation of data: DP, KS, MMA, DMH, MBP, APG, KTW, LAC. Paper preparation: DP, LAC. Critical review: DP, MBP, APG, KTW, LAC.

CONFLICT OF INTEREST

The authors declare that no conflict of interest exists.

Keywords

CCL11; colon cancer; inflammation; tumorigenesis

Introduction

With more than 3 million Americans affected[1], inflammatory bowel disease (IBD), with its two main forms ulcerative colitis (UC) and Crohn's disease (CD), is characterized by chronic relapsing and remitting immune-mediated inflammation leading to the risk for eventual progression to colon cancer[2]. The mechanisms associated with the dysregulated immune response and chronic, persistent inflammation that characterize IBD continue to be investigated[3]. Increased secretion of proinflammatory cytokines has been implicated as exacerbating factors in the disease process[3]. A crucial issue is that the lifetime risk of carcinoma evolving from chronic colitis in UC has been estimated to be as high as 20%[2]. More recently it was found that, while UC patients continue to have increased risk of developing colorectal cancer and increased risk of mortality from this, the overall risk appears to be decreasing[4]. Importantly there is no proven chemopreventive strategy to reduce colitis-associated carcinogenesis (CAC).

We have previously shown that, out of 42 cytokines/chemokines assessed, CCL11 was one of only two targets that were increased in serum from 137 UC patients vs 38 control subjects, and the only one of these that was also increased in tissues from these patients at all levels of disease severity[5]. Increased CCL11 has also been shown in UC tissues in children[6]. In addition, we have demonstrated that CCL11 is significantly increased in the serum of 114 CD patients[7].

We now report that in an injury and recovery mouse model that mimics UC, loss of CCL11 is protective, with decreased body weight loss and histologic injury. Furthermore, *Ccl11* expression is present in both isolated epithelial cells and lamina propria (LP) immune cells, and loss of CCL11 leads to reduced development of CAC in the chronic azoxymethane (AOM)-dextran sulfate sodium (DSS) model. $Ccl11^{-/-}$ mice exhibit decreased tumor number and burden, decreased histologic injury and extent of dysplasia, along with alterations in the cytokine millue in the tumor microenvironment. Bone marrow chimera studies point to loss of both epithelial and myeloid cell-derived CCL11 as important for the protective phenotype. Thus, CCL11 plays a role in exacerbating colitis and the resultant risk of CAC.

RESULTS

Ccl11 deletion is protective in DSS-induced colitis

We have previously shown that CCL11 is increased in serum and colonic tissue from UC patients[5], in CD patients serum[7], and in murine colonic tissue after exposure to DSS[5]. When WT and $Ccl11^{-/-}$ mice were exposed to an injury and recovery model using 4% DSS as previously described[8], $Ccl11^{-/-}$ mice exhibited significantly less body weight loss beginning at Day 6 versus WT mice, and this protection continued through Day 10 (Fig.

1a). This was associated with decreased histological injury in *Ccl11*$^{-/-}$ mice versus WT mice with DSS colitis (Fig. 1b, c). As CCL11 is an eosinophil chemoattractant, we assessed colonic eosinophil infiltration via major basic protein (MBP) staining and found a significant increase in eosinophils in WT DSS colon tissue, which was markedly attenuated in *Ccl11*$^{-/-}$ mice (Fig. 1d, e). Interestingly, expression of *CCR3*, the highest affinity receptor for CCL11, has been shown to be significantly increased in UC patients[9]. Accordingly, *Ccr3* expression was significantly increased in the WT DSS group and was significantly decreased in the *Ccl11*$^{-/-}$ DSS group (Supplementary Fig. 1a).

Loss of CCL11 leads to decreased tumorigenesis

Using publicly-available bulk RNAseq datasets, we found that *CCL11* expression is increased in both UC and CAC human tissues versus control colon tissues (Supplementary Table 1). When WT and *Ccl11*$^{-/-}$ mice were subjected to the AOM-DSS protocol with repeated cycles of 4% DSS[10], *Ccl11*$^{-/-}$ mice exhibited significantly less tumors (Fig. 2a) with decreased tumor burden (Fig. 2b) and decreased mortality (Fig. 2c) versus WT mice. After exposure to 3 cycles of DSS alone, *Ccl11*$^{-/-}$ mice had decreased histologic injury versus WT mice (Fig. 2d), which paralleled the histologic protection seen in the injury and recovery model (Fig. 1b). Further, *Ccl11*$^{-/-}$ mice exposed to AOM-DSS exhibited decreased histologic injury in the non-tumor areas versus WT mice (Fig. 2d). There was decreased progression to high grade dysplasia in *Ccl11*$^{-/-}$ mice, with the majority (17/29) exhibiting low grade dysplasia, whereas WT mice were more likely to exhibit high grade dysplasia (20/27) as shown in Fig. 2e and f ($P = 0.0169$). When we assessed colonic eosinophil infiltration via MBP staining, we found a significant increase in eosinophils in the tumors of WT AOM-DSS colon tissues, which was markedly attenuated in *Ccl11*$^{-/-}$ mice (Fig. 2g, h). *Ccr3* expression was not increased in either the non-tumor or tumor areas in WT mice, but *Ccr3* was significantly decreased in the non-tumor areas of *Ccl11*$^{-/-}$ mice versus WT mice. (Supplementary Fig. 1b).

Ccl11$^{-/-}$ mice exhibit altered tumor chemokine and cytokine levels

The tumors in WT mice exhibited more advanced levels of dysplasia (Fig. 2e, f), and the tumors themselves exhibited significant inflammation including crypt abscesses, which were greatly diminished in the *Ccl11*$^{-/-}$ mice (Fig. 2e). Using a 24-plex Luminex Multiplex Array, we assessed the effects of CCL11 loss on the signaling milieu in the tumor microenvironment by comparing paired tumor and adjacent non-tumor colonic tissue pieces from AOM-DSS-treated mice. There were 18 cytokines/chemokines that were significantly increased in the tumors of either WT or *Ccl11*$^{-/-}$ mice compared to adjacent non-tumor areas (Fig. 3a–i, Supplementary Table 2). Though there was decreased histologic injury in *Ccl11*$^{-/-}$ mice exposed to chronic DSS, with or without AOM (Fig. 2d), the only significant differences in the cytokine levels between non-tumor areas of the WT versus *Ccl11*$^{-/-}$ mice were decreases in IL-13 and CCL11 (Supplementary Table 2).

Importantly, when tumors from WT or *Ccl11*$^{-/-}$ mice were compared to each other, there were further significant alterations found. In the tumors from *Ccl11*$^{-/-}$ mice, there were significant decreases (Fig. 3a, e) in the innate cytokine IL-1β and the chemokine CXCL2 (MIP2), with a modest reduction in CXCL1 (KC, GRO-α; Supplementary Table

2), the murine equivalent of CXCL8 (IL-8)[11]. CXCL1 and CXCL2, which are produced by macrophages[12] and epithelial cells[13, 14], are chemoattractants for innate immune cells. Multiple cytokines, including vascular endothelial growth factor (VEGF) A, and CXCL1, can contribute to angiogenesis, which is essential for tumor growth[15]. While both WT and *Ccl11*$^{-/-}$ tumors exhibited a similar increase in VEGF expression, CXCL1 was increased in both WT and *Ccl11*$^{-/-}$ tumors, but was modestly decreased in *Ccl11*$^{-/-}$ tumors (Supplementary Table 2). When directly comparing *Ccl11*$^{-/-}$ to WT tumors, CSF1, TNF-α, IFN-γ, IL-12p40, IL-15, and IL-17 were all decreased in the *Ccl11*$^{-/-}$ tumors (Fig. 3b, c, d, g, h, i), pointing to a dampening of pro-inflammatory cytokines in the tumor microenvironment. In addition, IL-4 was reduced in *Ccl11*$^{-/-}$ tumors (Fig. 3f); this cytokine has been implicated in the generation of tumor-associated macrophages[16], and this decrease may be contributing to the beneficial effect of *Ccl11* deletion. Taken together, tumor areas in *Ccl11*$^{-/-}$ mice exhibited an overall decrease in the inflammatory response versus WT mice, indicating that decreased inflammation contributes to the decreased tumorigenesis in *Ccl11*$^{-/-}$ mice.

Loss of *Ccl11* does not alter macrophage activation pattern

As many of the most significantly altered cytokines and chemokines are produced by macrophages or are related to macrophage polarization, we assessed the response of naïve bone marrow-derived macrophages (BMmacs) to classical stimuli. Exposing naïve WT BMmacs to either classical M1 (LPS + IFN-γ) or M2 (IL-4 + IL-10) stimuli led to significant increases in *Ccl11* mRNA levels (Supplementary Fig. 2a), while this response was not present in *Ccl11*$^{-/-}$ BMmacs. When markers of M1 and M2 macrophage activation status were assessed, WT and *Ccl11*$^{-/-}$ BMmacs exhibited similar polarization patterns in response to M1 or M2 stimuli as assessed by the M1 markers *Nos2* and *Tnfa*, and the M2 markers *Arg1, Chil3*, and *Retnla* (Supplementary Fig. 2b, c). Consistent with the *Nos2* findings, nitric oxide (NO) production (measured as NO_2^-) was increased with M1 stimulation in WT cells but was similarly increased in *Ccl11*$^{-/-}$ BMmacs (Supplementary Fig. 2d). Interestingly, the M1 marker *IL1b* was significantly decreased in *Ccl11*$^{-/-}$ BMmacs (Supplementary Fig. 2b) similar to the pattern seen when IL-1β was assessed in *Ccl11*$^{-/-}$ tumors (Fig. 3e). The polarization data indicate that there is no apparent overall predisposition toward M1 or M2 macrophage polarization in BMmacs in the setting of CCL11 loss, but IL-1β may be an important signal as inflammasome dysregulation has been linked to tumorigenesis in CAC[17].

Loss of both hematopoietic and epithelial CCL11 is required for protection from tumorigenesis

While eosinophil chemotaxis is the most well-known function of CCL11, working through the CCR3 receptor[18], there are many cell types that produce CCL11 and more recently, CCL11 has been found to also interact with CCR2 and CCR5[18]. Upregulation of CCR3 by CCL11 is associated with wound repair in respiratory epithelial cells[19]. In addition, CCR2 has been shown to be expressed by both LP immune cells and epithelial cells[20], and our data indicate *Ccl11* expression is altered in the AOM-DSS model. Specifically, *Ccl11* mRNA expression was increased in both isolated colonic epithelial cells (CECs) and LP immune cells (Fig. 4a, b).

Thus, the alterations in intra-tumoral cell populations and signaling observed in the setting of *Ccl11* deletion could be due to its loss from either the epithelial or immune cell compartments leading to the decreased tumorigenesis observed in *Ccl11*$^{-/-}$ mice. We exposed WT and *Ccl11*$^{-/-}$ mice to the AOM-DSS model 8 weeks following bone marrow transplantation to test whether the decreased tumorigenesis was due to a CCL11 hematopoietic cell-autonomous effect. Bone marrow transplantation efficiency was confirmed using genotyping (Fig. 4c). WT mice receiving *Ccl11*$^{-/-}$ bone marrow did not show protection from tumorigenesis. *Ccl11*$^{-/-}$ mice receiving WT bone marrow showed a modest decrease in tumorigenesis versus WT mice receiving WT bone marrow, but the additional loss of hematopoietic cell-derived CCL11 was required to fully reproduce the protection phenotype seen in *Ccl11*$^{-/-}$ mice (Fig. 4d, e). *Ccl11*$^{-/-}$ mice receiving *Ccl11*$^{-/-}$ marrow exhibited decreased tumor number and tumor burden (Fig. 4d, e) versus WT mice receiving WT bone marrow, mimicking the protection phenotype observed in non-transplanted mice. These data demonstrate that loss of CCL11 in both the epithelial and hematopoietic cell compartments is required for the protection phenotype in the AOM-DSS model caused by *Ccl11* deletion, consistent with the mixed CCL11 localization observed in the AOM-DSS model.

Discussion

We have previously shown that CCL11 expression is increased in UC patients' serum and colonic tissues[5], increased in CD patients' serum[7], and now show that *CCL11* is increased in UC and CAC tissues. Given this, we performed studies in mice lacking CCL11 to determine the role of CCL11 in colitis and CAC. We have previously shown *Ccl11* expression is increased in DSS-induced colitis[5]. Prior studies have shown increased CCL11 expression in macrophages after acute DSS exposure, CCL11 expression in both macrophages and epithelial cells in pediatric UC patients[6], and *Ccl11*$^{-/-}$ mice have been shown to be protected in an 8 day-DSS model, with decreased colonic eosinophil infiltration, but not a complete lack of eosinophils[21]. Furthermore, treatment with an anti-CCL11 antibody has been shown to improve clinical disease indices and colon weight to length ratio in a 7-day DSS model[22]. Based on the protection phenotype seen in acute DSS[21] and in the current injury and repair DSS model, we hypothesized that CCL11 may modulate the inflammatory response that is thought to drive the colon tumorigenesis observed in the AOM-DSS model[10, 23, 24]. The development of colon tumorigenesis is important as patients with colonic inflammation associated with IBD are at increased risk for colonic dysplasia and carcinoma[25].

In this study, we demonstrate that consistent with our findings of decreased histologic damage in the injury and repair model, *Ccl11*$^{-/-}$ mice exposed to chronic DSS alone exhibited decreased histologic damage versus WT mice. Furthermore, after exposure to the AOM-DSS model, *Ccl11*$^{-/-}$ mice exhibit decreased mortality, tumor number and burden, and likelihood of developing high-grade dysplasia versus WT mice. This protection phenotype in *Ccl11*$^{-/-}$ mice was associated with decreased infiltration of eosinophils into the tumors. Previous studies in eosinophil-deficient mice have shown mixed phenotypes in acute colitis studies[26, 27], but have shown improved survival and decreased tumor burden in both CAC and *Apc*$^{min/+}$ models[28]. The protection phenotype in *Ccl11*$^{-/-}$ mice was not associated with increased immune surveillance. In fact, the downregulation of multiple

cytokines/chemokines in the tumors from *Ccl11*$^{-/-}$ mice points to a dampening of immune responses including the M1, Th1, Th2, and Th17 response[29, 30]. While expression of the CCL11 receptor, *Ccr3*, was increased in the WT DSS group and significantly attenuated in the *Ccl11*$^{-/-}$ DSS group, a recent study found that *Ccr3*-deficient mice exhibit exacerbated DSS-induced colitis[31]. Given that CCL11 can interact with multiple receptors[18, 32], and that studies in eosinophil-deficient mice have shown mixed results[26, 27], the protection from CAC may not be an eosinophil-dependent effect.

Furthermore, we found in WT mice that *Ccl11* mRNA expression is increased in CECs in the AOM-DSS group versus both control and DSS groups. While *Ccl11* mRNA expression is increased in LP cells from both the DSS and AOM-DSS groups, the apparent difference between these treatment groups is not statistically significant. This alteration is most likely due to fewer LP immune cells isolated from the AOM-DSS group given the decreased histologic injury seen in the WT AOM-DSS group and the WT DSS group. Subsequent bone marrow transplant studies indicated that the decreased tumorigenesis observed requires loss of both epithelial and hematopoietic cell-derived *Ccl11* expression and is not an immune cell-autonomous effect. Thus, it may be that host surveillance is altered in the presence of CCL11 leading to a permissive tumor microenvironment that supports aberrant epithelial cell growth. While our data do not suggest that CCL11 contributes directly to macrophage polarization, epithelial responses to CCL11 in the AOM-DSS model may be a key regulator.

We have shown increased serum and tissue CCL11 expression in IBD patients[5, 7]. We now show that *CCL11* is increased in CAC, though, we have not been able to assess CCL11 in human CAC tissues by immunohistochemistry despite testing 5 commercially available antibodies from 4 different companies. However, there are studies showing increased serum CCL11 expression in patients with sporadic colon cancer[32, 33] as well as other solid tumors such as breast, lung, and pancreatic cancer[32]. In another study, colon cancer tissue CCL11 levels were elevated versus paired adjacent normal tissue, though serum levels in this cohort were decreased in the colon cancer patients versus healthy controls[34]. CCL11 has also been assessed as a potential serum marker to help differentiate between benign prostatic hypertrophy and prostate cancer[35, 36]. While further studies assessing the role of CCL11 in IBD patients who are at risk for progression to CAC are needed, it appears that CCL11 is acting to modulate colitis via more than simply the classical definition of eosinophil chemotaxis. Anti-CCL11 therapies may present a potential therapeutic strategy in patients with IBD to reduce colitis and therefore the risk for development of CAC.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

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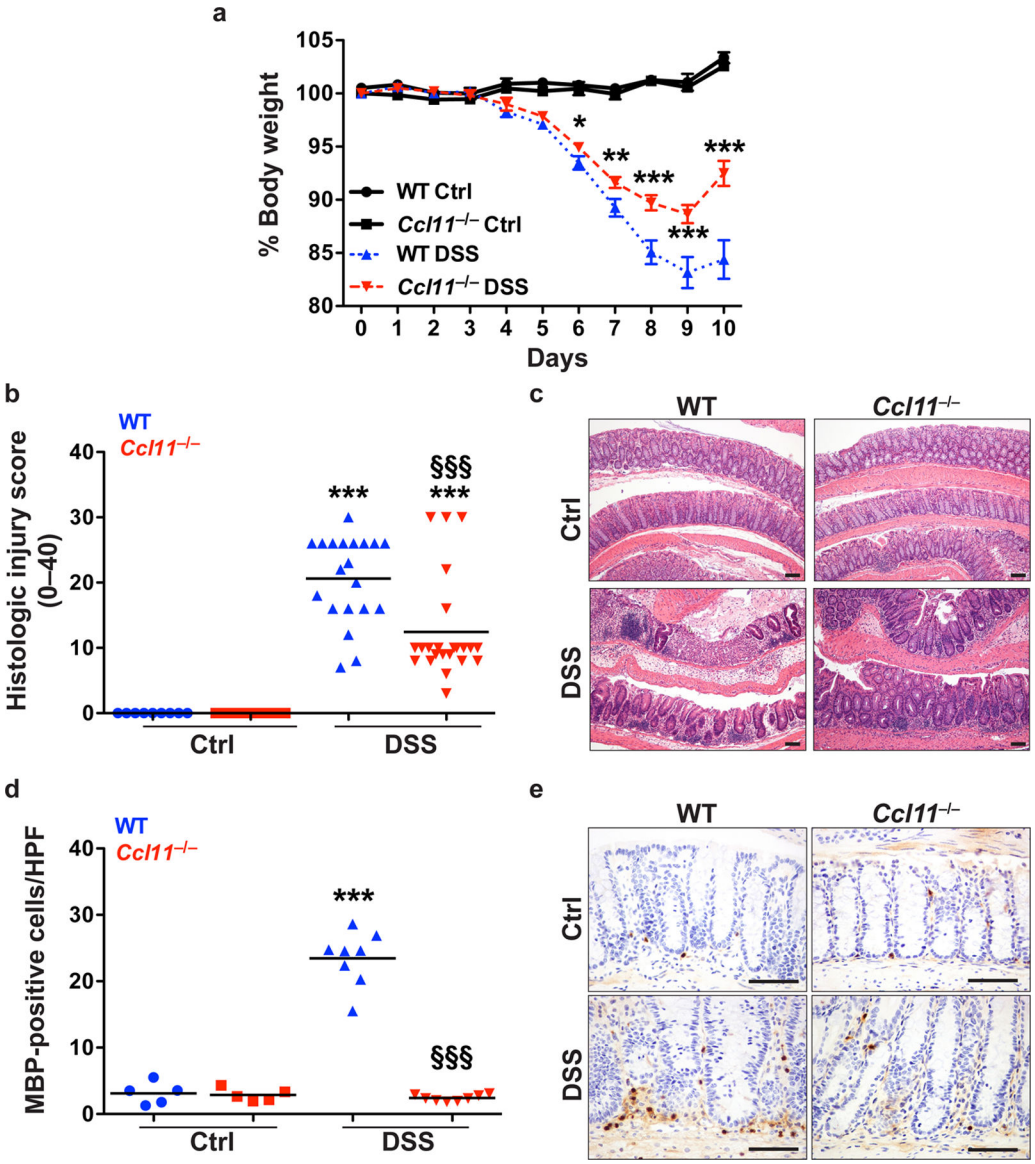

Fig. 1. *Ccl11* deletion is protective in DSS-induced colitis.

WT and *Ccl11*$^{-/-}$ mice were exposed to 4% DSS for 5 days, followed by 5 more days of regular drinking water. (a) Body weights were assessed daily and are presented as percentage of initial body weight. **P* < 0.05, ***P* < 0.01, ****P* < 0.001 versus WT DSS. (b) Histologic injury score. ****P* < 0.001 compared to WT control; §§§*P* < 0.001 versus WT DSS mice. One-way ANOVA with Student-Newman-Keuls posthoc multiple comparisons test. *n* = 9 control mice each in the WT and *Ccl11*$^{-/-}$ groups, *n* = 20 WT DSS-treated mice, and *n* = 23 *Ccl11*$^{-/-}$ DSS-treated mice. (c) Representative H&E-stained images from Control (top row) and DSS (bottom row) mice. Scale bar = 100 μm. (d) Colon sections were stained for the eosinophil granule, major basic protein (MBP) by immunohistochemistry. Number of MBP-positive cells per HPF (400x) assessed by a GI pathologist (M.B.P.) in a blinded manner. ****P* < 0.001 vs WT control; §§§P < 0.001 vs. WT DSS by ANOVA with

Student-Newman-Keuls test. (e) Representative images from Control (top row) and DSS (bottom row) mice. Scale bar = 100 μm.

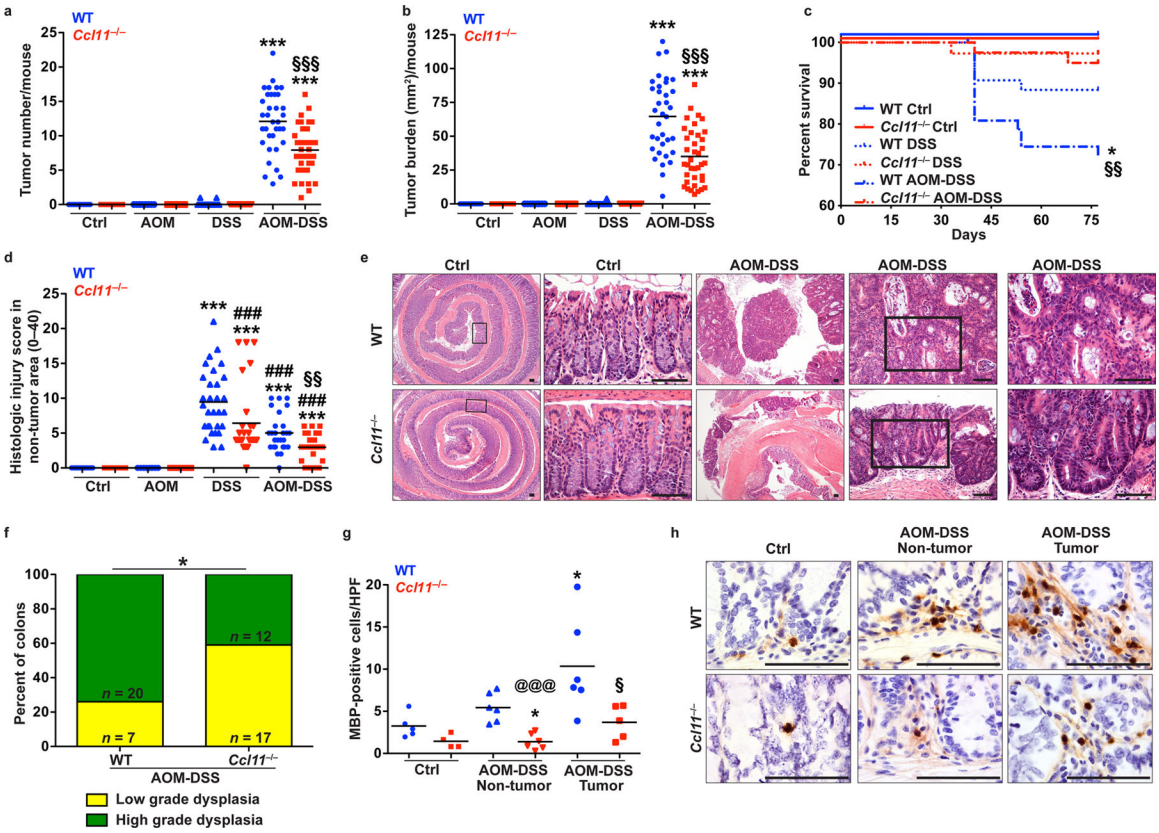

Fig. 2. *Ccl11*$^{-/-}$ mice exhibit decreased tumorigenesis after exposure to the AOM-DSS model of CAC.

WT and *Ccl11*$^{-/-}$ mice treated or not with AOM were exposed to 3 cycles of 4% DSS for 5 days, alternating with regular drinking water for a total of 77 days. (a) Tumor number was assessed by gross visual inspection, utilizing a dissecting microscope. (b) Tumor burden was determined by the addition of the calculated area of each identified tumor, as assessed with an electronic caliper for both length and width. (c) Survival curve assessed by log-rank (Mantel-Cox) test. *$P < 0.05$ versus WT Ctrl; §§$P < 0.01$ versus *Ccl11*$^{-/-}$ AOM-DSS. (d) Histologic colitis score in non-tumor area. In (a), (b), and (d), ***$P < 0.001$ versus WT Ctrl; ###$P < 0.001$ vs WT DSS; §§$P < 0.01$ and §§§$P < 0.001$ versus WT AOM-DSS by one-way ANOVA with Student-Newman-Keuls test. In (a) and (b), $n = 18$ control mice each in the WT and *Ccl11*$^{-/-}$ groups, $n = 10$ WT and 8 *Ccl11*$^{-/-}$ AOM-treated mice, $n = 38$ WT and 36 *Ccl11*$^{-/-}$ DSS-treated mice, and $n = 35$ WT and 38 *Ccl11*$^{-/-}$ AOM-DSS-treated mice. In (c), $n = 18$ control mice each in the WT and *Ccl11*$^{-/-}$ groups. In the following groups, the starting number of mice were $n = 43$ WT and 37 *Ccl11*$^{-/-}$ DSS-treated mice, and $n = 48$ WT and 40 *Ccl11*$^{-/-}$ AOM-DSS-treated mice. In (d), $n = 13$ control mice each in the WT and *Ccl11*$^{-/-}$ groups, $n = 10$ WT and 8 *Ccl11*$^{-/-}$ AOM-treated mice, $n = 30$ WT and 28 *Ccl11*$^{-/-}$ DSS-treated mice, and $n = 27$ WT and 29 *Ccl11*$^{-/-}$ AOM-DSS-treated mice. The mouse numbers in (d) reflect that some of the colons were used for cell isolation for other studies and were not examined for histologic injury. (e) Representative H&E-stained images from WT (top row) and *Ccl11*$^{-/-}$ mice illustrate that WT AOM-DSS-treated mice exhibited more and larger tumors with crypt abcesses and progression to high grade dysplasia, while

Ccl11$^{-/-}$ AOM-DSS-treated mice had fewer and smaler tumors that were more likely to exhibit low grade dysplasia. Scale bar = 100 μm. (f) Percentage of cases with either low grade dysplasia or high grade dysplasia determined by a GI pathologist (M.K.W.) in a blinded manner. *P = 0.0169 by Fisher's exact test. The number of mice with each diagnosis is shown on the graph. (g) Colon sections were stained for MBP by immunohistochemistry. Number of MBP positive cells per HPF (400x) assessed by a GI pathologist (M.B.P.) in a blinded manner. *P < 0.05 vs WT control; @@@P < 0.001 vs. WT AOM-DSS non-tumor; §P < 0.05 vs WT AOM-DSS tumor by ANOVA with Student-Newman-Keuls test. The tumor and non-tumor areas were assessed in the same cases. (h) Representative images from WT (top) and *Ccl11*$^{-/-}$ mice. Scale bar = 100 μm.

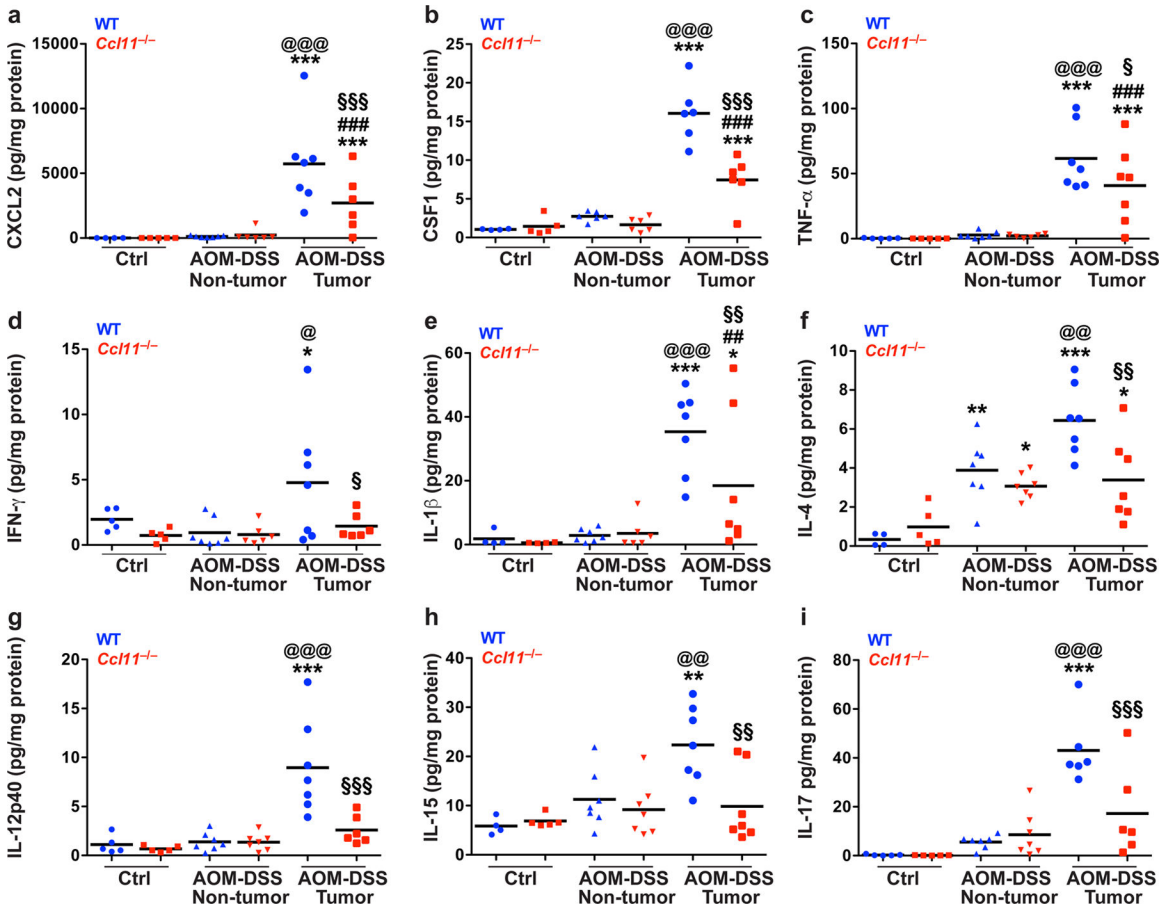

Fig. 3. *Ccl11*$^{-/-}$ mice exhibit altered tumor chemokine and cytokine levels.

Protein levels were assessed by Luminex Multiplex Array from colonic tissues from the 77-day AOM-DSS model. (a) CXCL2. (b) CSF1. (c) TNF-α. (d) IFN-γ. (e) IL-1β. (f) IL-4. (g) IL-12p40. (h) IL-15. (i) IL-17. In all panels, *$P < 0.05$, **$P < 0.01$, ***$P < 0.001$ vs WT control; @$P < 0.05$, @@$P < 0.01$, @@@$P < 0.001$ vs WT AOM-DSS non-tumor; ##$P < 0.01$, ###$P < 0.001$ vs *Ccl11*$^{-/-}$ AOM-DSS non-tumor; §$P < 0.05$, §§$P < 0.01$, §§§$P < 0.001$ vs WT AOM-DSS tumor by ANOVA with Student-Newman-Keuls test. $n = 5$ control and 7 AOM-DSS-tumors with paired non-tumor area per genotype.

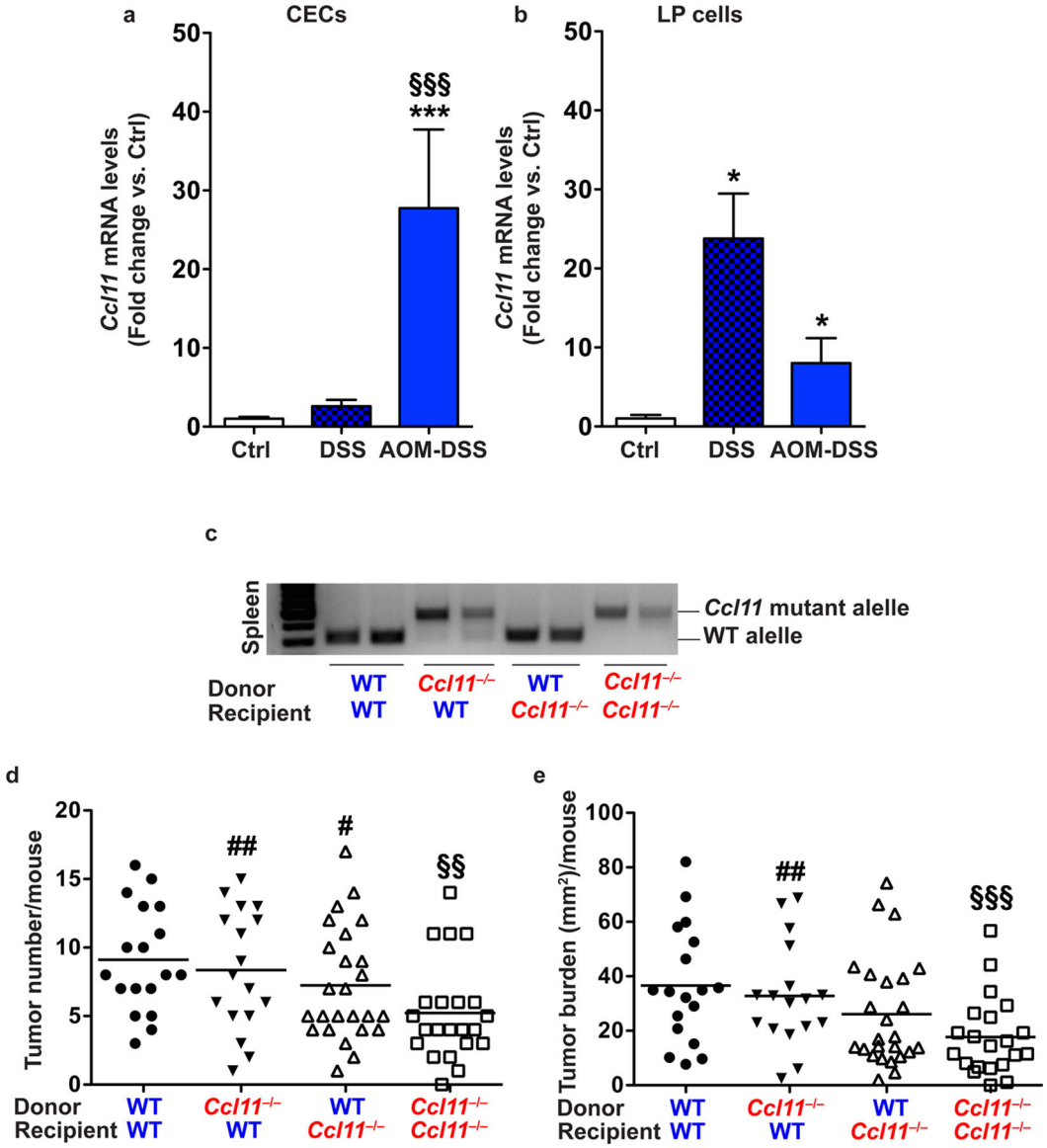

Fig. 4. Loss of both hematopoietic and epithelial CCL11 is required for protection from tumorigenesis.

Whole colon epithelial (CECs) and lamina propria (LP) cells were isolated from colonic tissues from the 77-day AOM-DSS model, mRNA extracted, and assessed by real-time PCR. (a) CECs. (b) LP cells. *$P < 0.05$, ***$P < 0.001$ vs control; §§§$P < 0.001$ vs DSS by ANOVA with Student-Newman-Keuls test. In (a) and (b), $n = 4$ control mice, $n = 8$ DSS-treated mice, and $n = 4$ AOM-DSS-treated mice. Irradiated animals were given bone marrow-derived hematopoietic cells and were treated with AOM-DSS. (c) After bone marrow transplantation, DNA from the spleen of recipient animals was analyzed by PCR for the wildtype and mutant *Ccl11* alleles. Representative PCR gel of two animals in each condition. The predominant band is that of donor mice. (d) Tumor number was assessed by gross visual inspection, utilizing a dissecting microscope. (e) Tumor burden was determined by the addition of the calculated area of each identified tumor, as assessed with an electronic caliper for both length and width. In (d) and (e), §§$P < 0.01$, §§§$P < 0.001$ vs WT to WT

AOM-DSS; #$P < 0.05$, ##$P < 0.01$ vs *Ccl11*$^{-/-}$ to *Ccl11*$^{-/-}$ AOM-DSS by ANOVA with Student-Newman-Keuls test. In (d) and (e), $n = 18$ WT to WT AOM-DSS-treated mice, $n = 17$ *Ccl11*$^{-/-}$ to WT AOM-DSS-treated mice, $n = 25$ WT to *Ccl11*$^{-/-}$ AOM-DSS-treated mice, and $n = 22$ *Ccl11*$^{-/-}$ to *Ccl11*$^{-/-}$ AOM-DSS-treated mice.