Interleukin-17 Promotes Formation and Growth of Prostate Adenocarcinoma in Mouse Models

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Abstract

The contributions of interleukin (IL)-17 to cancer remain unclear and somewhat controversial. We took a genetic approach to explore its role in prostate cancers by interbreeding IL-17 receptor C (IL-17RC)–deficient mice with mice that are conditionally mutant for PTEN, one established preclinical model for prostate cancer. Mice that were IL-17RC-deficient (IL-17RC–/) displayed prostates that were smaller than mice that maintained IL-17RC expression (IL-17RC+/+). In addition, IL-17RC–/– mice developed a reduced number of invasive prostate adenocarcinomas with lower rates of cellular proliferation and higher apoptosis than IL-17RC+/+ mice. Moreover, the fibromuscular stroma surrounding prostatic glands was relatively thicker in IL-17RC–/– mice and was associated with decreased matrix metalloproteinase (Mmp7) expression and increased Timp1, 2, and 4 expression, whereas administration of recombinant mouse IL-17 induced prostatic expression of Mmp7. Taken together, our results suggested that IL-17 promotes the formation and growth of prostate adenocarcinoma, and that an IL-17–MMP7 signaling axis is required for the transition of prostatic intraepithelial neoplasia to frank adenocarcinoma. Cancer Res; 72(10); 1–11. ©2012 AACR.

Introduction

Interleukin-17 (IL-17 or IL-17A) is the founder member of the IL-17 cytokine family, which shares 50% homology with IL-17F. IL-17 cytokines are produced by T-helper (TH)17 cells, γδ T cells, and other immune cells. IL-17A and IL-17F form homodimers and heterodimers as functional ligands (1, 2). IL-17 cytokines bind to 2 receptors, IL-17RA and IL-17RC (3). IL-17RC forms a receptor heterodimer with IL-17RA (4, 5). The cytokines bind to 2 receptors, IL-17RA and IL-17RC (3). IL-17RA and IL-17RC receptors are expressed in prostate cancer. However, the critical question of whether IL-17 has any role in prostate cancer has never been investigated in a mouse model of autochthonous prostate cancer. In the present study, we crossed Il17rc KO (Il17rc−/−) mice with Pten conditional KO mice (Ptenfl/fl/C0) mice. We found that in Pten-deficient context, Il17rc KO mice developed significantly smaller prostate tumors than in Il17rc WT mice. Our results suggest that IL-17 promotes formation and growth of prostate adenocarcinoma.

Materials and Methods

Mice

Animal protocol was approved by the Animal Care and Use Committee of Tulane University (New Orleans, LA). Ptenfl/fl/C0.
(Pten<sup>L/L</sup>) mice (ref. 18; strain name: C129S4-Pten<sup>tm1Hwu</sup>/J; genetic background: 129S4/SvJaeBALB/c) were obtained from the Jackson Laboratory. PB-Cre4 mice (ref. 19; strain name: B6.Cg-Tg(Psbn-cre)4Prb; genetic background: B6.Cg) were obtained from Mouse Models of Human Cancers Consortium (MMHCC) of the National Cancer Institute (Bethesda, MD). Il17rc<sup>−/−</sup> mice (a gift from Genentech; genetic background: B6.Cg) were generated by Lexicon Pharmaceuticals using standard homologous recombination (20). Il17rc<sup>−/−</sup> mice developed normally, but the mouse fibroblasts and colon tissues did not respond to either IL-17A or IL-17F stimulation (8, 20). The numbers of T cells, B cells, monocytes, neutrophils, and dendritic cells in the blood, lymph nodes, spleen, and bone marrow are comparable between Il17rc<sup>+/+</sup> and Il17rc<sup>−/−</sup> mice (8). The breeding strategy is shown in Fig. 1A. DNA was extracted from the tail biopsy for PCR genotyping as described (refs. 18–20; see primer sequences in Supplementary Table S1).

**Histopathology**

Mice were weighed at 4, 6, 9, 12, and 30 weeks of age. The genitourinary bloc consisting of the prostate lobes, seminal vesicles, ampullary glands, bladder, proximal ductus deferens, and proximal urethra was excised en bloc (21). The genitourinary blocs were photographed, weighed with an empty bladder, and fixed as described (21). Fifty-six consecutive 5-μm sections of each prostate were cut and 8 sections (from every seventh section) were hematoxylin and eosin (H&E)-stained for histopathologic assessment in a blinded fashion according to the Bar Harbor Classification (21). To measure the thickness of fibromuscular stroma, photomicrographs of the sections were captured with a Nikon DS-Fi1 camera at
× 200 magnifications; the length measurement function of computer software (NI-Elements Basic Research 3.0, Nikon Instruments Inc.) was used to measure the thickness at 6 different points of the stroma layer around each gland and the average of the 6 measurements represented the thickness of fibromuscular stroma of the gland. The number of inflammatory cells in the connective tissue space between the prostatic glands was counted in 5 high-power fields (×400 magnification) per lobe; the average number of inflammatory cells per high-power field in 7 to 9 mouse prostates per genotype was compared.

**Immunohistochemical and terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling staining**

Immunohistochemical staining and double immunofluorescent staining were conducted as described (18, 22). The antibodies used were rabbit anti-p-Akt (1:100) and mouse anti-Pten (26H9, 1:50; Cell Signaling Technology), rabbit anti-Ki-67 (1:100, Millipore), rabbit anti-IL-17RA (1:200; sc-30175), and anti-IL-17RC (1:200; sc-99936) from Santa Cruz Biotechnology; rabbit anti-laminin (1:100; Sigma-Aldrich), rabbit anti-α smooth muscle actin (1:200; Pierce Biotechnology), goat anti-MMP7 (1:200; R&D Systems), and Cy 3–conjugated anti-mouse IgG and DyLight 488–conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories). Terminal deoxynucleotidyl transferase-mediated dUTP nick end labeling (TUNEL) staining was conducted with TACS XL Blue Label In Situ Apoptosis Detection Kits (Trevenigen) according to the manufacturer’s instructions (23). To quantify Ki-67- and TUNEL-positive cells, 5 animals from each genotype group at 9, 12, and 30 weeks of age were randomly selected; 3 representative prostate sections from each animal were stained; approximately 300 cells per field of 3 high-power fields of each prostate lobe were counted; and the percentages of positive cells were calculated as the number of positive cells divided by the total number of cells.

**Western blot and quantitative reverse transcription PCR**

Prostates were pulverized for protein extracts. Western blot analysis of matrix metalloproteinase (MMP7) protein expression was conducted as described (22). For induction of MMP7 protein expression, prostates were minced into 2–3-mm pieces and cultured in serum-free medium; the prostate tissues were treated with or without 20 ng/mL of recombinant mouse IL-17 (rmIL-17; R&D Systems) for 24 hours and were analyzed for MMP7 expression. To quantify IL17rc gene expression and IL-17–induced gene expression, ex vivo cultured mouse prostate tissues were treated with or without 20 ng/mL of rmIL-17 for 2 hours and were analyzed by quantitative reverse transcription PCR (qRT-PCR) as described (22). To quantify mRNA levels of Mmps and tissue inhibitor of metalloproteinases (Timps) using qRT-PCR, 3 prostates were randomly selected from each genotype group at 9, 12, and 30 weeks (see PCR primer sequences in Supplementary Table S1).

**Statistical analysis**

Comparisons of the genitourinary bloc weights were analyzed with Student t test, followed by a 2-factor (age and genotype) ANOVA in conjunction with Tukey–Kramer method. The χ² test was used to compare the incidences of PIN and invasive adenocarcinoma. Student t test was used to analyze the remaining data.

**Results**

**IL-17RC⁻ mice developed smaller prostate tumors than IL-17RC⁺ mice**

The male pups were genotyped at 3 weeks of age (Fig. 1B–D). Double immunofluorescent staining confirmed loss of Pten and activation of p-Akt in the prostatic epithelium of Ptenfl/fl; Cre⁺ (Fig. 1E) as previously reported (18). IL17rc⁺/+ and IL17rc⁻/⁻ prostates expressed similar levels of IL17rc mRNA (Fig. 2A) and protein (Fig. 2B), whereas IL17rc⁻/⁻ prostates did not express any detectable levels of IL17rc mRNA (Fig. 2A) and protein (Fig. 2B). IL17rc⁺/+; IL17rc⁻/⁻, and IL17rc⁻/⁻ prostates expressed similar levels of IL-17RA (Fig. 2B). Similar levels of C-C motif ligand 2 (Cxcl2) and C-X-C motif ligand 1 (Ccx1l) mRNA expression were induced by rmIL-17 treatment in IL17rc⁺/+ and IL17rc⁻/⁻ prostates, but not in IL17rc⁻/⁻ prostates (Fig. 2C).

The genitourinary bloc weight is proportional to the prostate weight, thus it has often been used to represent the prostate tumor burden (24, 25). Representative genitourinary blocks of mice at 9, 12, and 30 weeks of age are presented in Fig. 2D, showing that the genitourinary blocs of IL17rc⁺/+; Ptenfl/fl; Cre⁺ and IL17rc⁻/⁻; Ptenfl/fl; Cre⁺ mice were clearly larger than those of IL17rc⁻/⁻; Ptenfl/fl; Cre⁻ mouse at 30 weeks of age. Because IL17rc⁺/+ and IL17rc⁻/⁻ mouse prostates expressed similar levels of IL17rc (Fig. 2A and B) and responded similarly to IL-17 stimuli (Fig. 2C) and because there were no significant differences in the genitourinary bloc weights and histopathology (see description below) between IL17rc⁺/+; Ptenfl/fl; Cre⁺ and IL17rc⁻/⁻; Ptenfl/fl; Cre⁺ mice, we put IL17rc⁺/+; Ptenfl/fl; Cre⁺ and IL17rc⁻/⁻; Ptenfl/fl; Cre⁺ mice into one group (named IL-17RC⁺ or RC⁺ in abbreviation). Likewise, IL17rc⁻/⁻; Ptenfl/fl; Cre⁻ mice were named as IL-17RC⁻ (or RC⁻ in abbreviation). As shown in Fig. 2E, there were no significant differences in the genitourinary bloc weights between IL-17RC⁺ and IL-17RC⁻ mice at 4, 6, and 9 weeks of age (P > 0.05). However, the genitourinary bloc weight of IL-17RC⁻ mice was 14% and 47% heavier than that of IL-17RC⁻ mice at 12 and 30 weeks of age, respectively (P = 0.005 and P = 0.004, respectively, using Student t test). We did not find any significant differences in body weights between the 2 groups. We normalized the genitourinary bloc weight of each mouse by their corresponding body weight and found that the differences were still the same between the 2 groups (Fig. 2F).

In addition, we assessed the genitourinary bloc weights by a 2-factor ANOVA and found that there was a significant age and genotype interaction (P = 0.0012). Using Tukey–Kramer adjustment for multiple comparisons, there were no significant differences in the genitourinary bloc weights through the first 12 weeks (P values varied from 0.2517 to 0.9635).
However, the trajectories of the genitourinary bloc weights diverged soon after week 12 (see Fig. 2E) and there were highly significant differences at 30 weeks (P < 0.0001).

**IL-17RC KO did not affect formation of PIN but decreased formation of invasive adenocarcinoma**

It was reported that Pten<sup>−/−</sup>Cre<sup>+</sup> mice (with Il17rc<sup>+/+</sup> genotype) developed epithelial hyperplasia at 4 weeks, PIN at 6 weeks, and invasive adenocarcinoma at 9 weeks of age with 100% penetration (18). We found epithelial hyperplasia in all of the IL-17RC<sup>+</sup> and IL-17RC<sup>/−</sup> mouse prostates at 4 weeks (Fig. 3A–C). At 6 weeks, mouse PIN was observed in all of the IL-17RC<sup>+</sup> and IL-17RC<sup>/−</sup> mouse prostates, and the PIN-involved gland was bounded by an intact layer of fibromuscular stroma (Fig. 3D–F). At 9 weeks, IL-17RC<sup>+</sup> prostates developed invasive adenocarcinoma (Fig. 3G and H). Eighty-seven percent of prostate lobes had invasive adenocarcinoma and other 13% had PIN in IL-17RC<sup>+</sup> mice. In contrast, only 25% of prostate lobes had invasive adenocarcinoma whereas other 75% had PIN (Fig. 3I). At 12 weeks, IL-17RC<sup>+</sup> mice presented with invasive adenocarcinoma in 85% of prostate lobes (Fig. 3J and K) and with PIN in other 15%. On the other hand, IL-17RC<sup>/−</sup> mice presented with invasive adenocarcinoma in 44% of prostate lobes whereas other 56% had PIN (Fig. 3L). At 30 weeks, 100% of prostate lobes presented as invasive adenocarcinoma in IL-17RC<sup>+</sup> mice (Fig. 3M and N). In contrast, only 70% of prostate lobes showed invasive adenocarcinoma (Fig. 3O) in IL-17RC<sup>/−</sup> mice, with the remaining 30% still presented as PIN. Invasion of the basement membrane and fibromuscular layer was confirmed by smooth muscle actin staining (Fig. 3P). The percentages of PIN and invasive adenocarcinomas were significantly different between IL-17RC<sup>+</sup> and IL-17RC<sup>/−</sup> mice at 9, 12, and 30 weeks (P < 0.001, P < 0.001, and P < 0.005, respectively; Fig. 3Q; see data from individual prostate lobes in Supplementary Fig. S1).

**IL-17RC KO decreased cellular proliferation and increased apoptosis**

To understand why IL-17RC<sup>+</sup> mice developed bigger prostate tumors than IL-17RC<sup>/−</sup> mice at 12 and 30 weeks, we assessed cellular proliferation and apoptosis by conducting Ki-67 staining and TUNEL assays on each prostate lobe. We found that there were significantly more Ki-67–positive epithelial cells in IL-17RC<sup>+</sup> prostates than in IL-17RC<sup>/−</sup> prostates (Fig. 4A and B). In addition, there were significantly fewer apoptotic cells in IL-17RC<sup>+</sup> prostates than in IL-17RC<sup>/−</sup> prostates (Fig. 4C and D).
**IL-17RC KO increased the thickness of fibromuscular stroma**

To understand why IL-17RC−/− mice developed fewer invasive adenocarcinomas than IL-17RC+/+ mice at 9 to 30 weeks, we examined the histopathologic features of the mouse prostates. Normal mouse prostate has a thin rim of fibromuscular stroma surrounding individual glands (Fig. 5A; ref. 21). The layer of fibromuscular stroma in IL-17RC−/− mice was dramatically thicker than in age-matched normal prostates (Fig. 5B). In contrast, the layer of fibromuscular stroma in IL-17RC+/+ mice (Fig. 5C) was obviously thinner than that in IL-17RC−/− mice. Laminin staining confirmed what we observed with H&E staining (Fig. 5D–I, see whole prostate sections in Supplementary Fig. S2A and S2B). The thickness of the fibromuscular stroma was measured using computer software (Fig. 5J). As shown in Fig. 5K, the thickness of the fibromuscular stroma was significantly thicker in IL-17RC−/− mice than in either IL-17RC+/+ mice or normal mice at 9, 12, and 30 weeks ($P < 0.001$ for all comparisons; see data from individual prostatic lobes in Supplementary Fig. S3A).
IL-17RC KO decreased Mmp7 expression

To investigate the molecular mechanisms underlying the different thickness of fibromuscular stroma, we screened 7 Mmp genes and 4 Timp genes that are associated with prostate cancer (26). We found that in the mouse prostates, the most abundant Mmp mRNA was Mmp7, the level of which was more than 15,000 times higher than the levels of Mmp2, 8, 9, 10, and 13. The second most abundant Mmp mRNA was Mmp3, the level of which was approximately 3% of Mmp7 (Fig. 6A). More importantly, Mmp7 mRNA level was significantly higher in IL-17RC+ mice than in IL-17RC− mice at 9 weeks (P < 0.01; Fig. 6A). Mmp7 mRNA expression increased at 12 and 30 weeks in both IL-17RC+ and IL-17RC− mice, but the levels of Mmp7 mRNA were still significantly higher in IL-17RC+ mice than in IL-17RC− mice (P < 0.01; Fig. 6B). In addition, we found that the mRNA levels of Timp1, 2, and 4 were significantly higher in IL-17RC+ mice than in IL-17RC− mice (P < 0.05 or P < 0.01; Fig. 6A). Using immunohistochemistry, we found that MMP7 protein was not detectable in either IL-17RC+ (Fig. 6C) or IL-17RC− (Fig. 6D) mouse prostatic lateral lobes at 4 weeks. At 6 weeks, only a few cells of the PIN lesions in IL-17RC+ mice stained positive for MMP7 (Fig. 6E), whereas the PIN cells in IL-17RC− mice stained negative (Fig. 6F). At 9 weeks, almost all neoplastic epithelial cells including the cells at the front edge of tumor invasion stained positive for MMP7 in IL-17RC+ mice (Fig. 6G). In contrast, only a few cells of the PIN lesions in IL-17RC− mice stained positive (Fig. 6H). At 12 and 30 weeks, the invasive adenocarcinoma cells in IL-17RC+ mice stained strongly positive for MMP7 (Fig. 6I and J), whereas only a few neoplastic epithelial cells in IL-17RC− mice stained positive (Fig. 6K and L). We did not see positive MMP7 staining in the stromal cells (Fig. 6C–L). Similar results were obtained in other prostatic lobes (see data of dorsal lobes in Supplementary Fig. S3B). To confirm the differences of MMP7 protein expression, we conducted Western blot analysis using protein lysates from 9-week-old mouse prostates. We found that indeed MMP7 protein level was higher in IL-17RC+ mice than in IL-17RC− mice and was not detectable in normal mouse prostates at 9 weeks of age (Fig. 6M).

Because Mmp7 expression was reduced in IL-17RC− prostates compared with IL-17RC+ prostates, we investigated whether Mmp7 expression was induced by IL-17 in the mouse prostates. We cultured mouse prostate tissues ex vivo from Il17rc−/− and Il17rc+/+ mice. After 2 hours of treatment with rmIL-17, Mmp7 mRNA expression was increased approximately 5-fold in Il17rc+/+ mice but was not increased in Il17rc−/− mice (Fig. 6N). Furthermore, we found that rmIL-17 treatment increased MMP7 protein expression in Il17rc+/+ but not in Il17rc−/− mouse prostate tissues (Fig. 6O).

IL-17RC KO decreased inflammatory cell infiltration in the prostate

We found that IL-17RC+ mouse prostates had many inflammatory cells in the connective tissue space between the prostatic glands, particularly at 30 weeks of age; however, the number of inflammatory cells was significantly reduced in IL-17RC− mouse prostates (Fig. 7A–E). The inflammatory cell population was mainly composed of macrophages and lymphocytes, with few neutrophils (Fig. 7C and D).

Discussion

The role of IL-17 in cancer growth has been debated for more than a decade (27, 28). One opinion is that IL-17 is protumorigenic (29, 30), whereas another notion is that IL-17 is antitumorigenic (31, 32). The discrepancies may be caused...
by many factors, such as using nude mice, overexpression of IL-17, and grafting of different tumor types. It must be pointed out that IL-17 exerts its proinflammatory effects by targeting immune cells and body tissues with cytokines, chemokines, and MMPs (9). Therefore, an intact immune system is critical for the proper functioning of IL-17, and the findings from the allograft studies in immunocompetent mice are presumably more convincing than the ones from nude mice. However, 2 groups of investigators have found opposite functions of IL-17 in tumor growth using the same IL-17 KO mouse strain but different murine tumor allografts (33, 34). One interpretation for the conflicting results is that the action of IL-17 may be tumor-specific. Yet, the differences in strengths of the antigenicity of tumor allografts may also cause different responses from the animal hosts, as IL-17 promotes organ allograft rejection (35). Taken together, to properly evaluate the role of IL-17 in cancer, it would be better to use immunocompetent animal models of autochthonous cancer, rather than tumor xenograft or allograft models.

In this study, we used the Pten conditional KO mouse model of autochthonous prostate adenocarcinoma (18, 36), which among the currently available animal models of prostate cancer, most closely imitates formation and growth of the human prostate adenocarcinomas (37). Two significant by many factors, such as using nude mice, overexpression of IL-17, and grafting of different tumor types. It must be pointed out that IL-17 exerts its proinflammatory effects by targeting immune cells and body tissues with cytokines, chemokines, and MMPs (9). Therefore, an intact immune system is critical for the proper functioning of IL-17, and the findings from the allograft studies in immunocompetent mice are presumably more convincing than the ones from nude mice. However, 2 groups of investigators have found opposite functions of IL-17 in tumor growth using the same IL-17 KO mouse strain but different murine tumor allografts (33, 34). One interpretation for the conflicting results is that the action of IL-17 may be tumor-specific. Yet, the differences in strengths of the antigenicity of tumor allografts may also cause different responses from the animal hosts, as IL-17 promotes organ allograft rejection (35). Taken together, to properly evaluate the role of IL-17 in cancer, it would be better to use immunocompetent animal models of autochthonous cancer, rather than tumor xenograft or allograft models.

In this study, we used the Pten conditional KO mouse model of autochthonous prostate adenocarcinoma (18, 36), which among the currently available animal models of prostate cancer, most closely imitates formation and growth of the human prostate adenocarcinomas (37). Two significant
phenotypical differences were found between the IL-17RC\(^+\) and IL-17RC\(^/-\) groups. The first is that the genitourinary bloc weight was 47% heavier in IL-17RC\(^+\) mice than in IL-17RC\(^/-\) mice at 30 weeks. The difference was statistically significant in both Student t test and Tukey–Kramer method. At 12 weeks, the genitourinary bloc weight was 14% heavier in IL-17RC\(^+\) mice than in IL-17RC\(^/-\) mice, which was statistically significant in Student t test (\(P = 0.005\)) but insignificant in Tukey–Kramer method (\(P = 0.32517\)). This is because Student t test only compared 2 groups at the designated time point, whereas Tukey–Kramer method took multiple comparisons at the 5 time points into consideration. Of note, because accumulation of fluid in the anterior lobes may not be the same in all animals, the genitourinary bloc weight may not be an accurate indicator of the disease development in this model. This limitation may be overcome by cutting open the anterior lobes and draining off the fluid before weighing. Our explanation for the differences in prostate sizes is that there was a reduction in cellular proliferation and an increase in apoptosis in IL-17RC\(^-\) mice compared with IL-17RC\(^+\) mice. IL-17 does not directly stimulate cancer cellular proliferation in \textit{in vitro} studies (29, 31); however, it may indirectly stimulate cellular proliferation \textit{in vivo} via induction of other target genes such as Ccl2. We found that IL-17 induced Ccl2 expression in IL-17RC\(^+\) mouse prostate and Ccl2 has been shown to enhance prostate cancer cellular proliferation (38). We previously showed that \textit{Il17rc} KO inhibited apoptosis in LNCaP cells via blocking activation of caspase-3 (22). It is possible that \textit{Il17rc} KO removed the survival mechanism provided by IL-17RC, thus rendering the neoplastic cells more sensitive to apoptotic stimuli.

The second significant phenotype is that IL-17RC\(^-\) mice developed significantly fewer invasive adenocarcinomas than in IL-17RC\(^+\) mice. It is worth pointing out that both IL-17RC\(^-\) and IL-17RC\(^+\) mice developed epithelial hyperplasia at 4 weeks and PIN at 6 weeks, suggesting that \textit{Il17rc} KO did not affect the...
initiation of prostate cancer. It appears that Il17rc KO affects the transition from PIN to invasive adenocarcinoma. It is well recognized that high-grade PIN may proceed to become invasive adenocarcinoma (21). However, it is not clear what the precise mechanisms are in driving PIN-to-adenocarcinoma transition. The distinction between PIN and adenocarcinoma is whether the neoplastic cells penetrate through the basement membrane of the PIN-involved glands and invade into the surrounding stroma (21). Thus, degradation of the basement membrane and invasion of the stroma are critical steps in PIN-to-adenocarcinoma transition. Interestingly, we found that the layer of fibromuscular stroma surrounding the glands was significantly thicker in IL-17RC− mice than in IL-17RC+ mice, which suggests that there is either an increase of stroma synthesis or a decrease in stroma degradation, or both in IL-17RC− mice. Because we did not find any differences in cellular proliferation or apoptosis of the stromal cells between IL-17RC+ and IL-17RC− mice (Fig. 4A and C), we focused on stroma degradation. Matrix-degrading enzymes are critical for the PIN-to-adenocarcinoma transition as they are needed in dissolution of basement membrane components. These enzymes include MMPs, aspartyl proteases (e.g., cathepsin D), and serine proteases (e.g., urokinase-like plasminogen activator and prostate-specific antigen). MMP2, 3, 7, and 9 as well as their inhibitors TIMP1 and TIMP2 have been associated with malignant progression of prostate cancer (26). We found that Mmp7 mRNA was the most abundant Mmp in the mouse prostates. MMP7 protein levels were not readily detectable at 4 and 6 weeks but dramatically increased at 9 weeks in IL-17RC+ mouse prostates. The timing of MMP7 protein expression matches the critical period of PIN-to-adenocarcinoma transition between 6 and 9 weeks. More importantly, Mmp7 mRNA and protein expression was significantly reduced in IL-17RC− mice compared with IL-17RC+ mice at 9, 12, and 30 weeks, which paralleled the increase in stroma thickness and the decrease in forming invasive adenocarcinomas in IL-17RC− mice. Furthermore, we found that rmIL-17 treatment induced Mmp7 mRNA and protein expression in the ex vivo cultured IL-17RC+ prostates but not in IL-17RC− prostates, which suggests that Mmp7 is a new downstream
target gene of IL-17 signaling. It is known that IL-17 induces expression of MMP1, 2, 3, 9, and 13 in synoviocytes and chondrocytes (9). Thus, we conclude that Il17rc KO leads to decreased Mmp7 expression and increased expression of Timp1, 2, and 4, resulting in reduced stroma degradation and inhibition of invasive adenocarcinoma formation.

IL-17 signaling plays an important proinflammatory role in many diseases (9). We found that IL-17RC- mouse prostates had significantly less inflammatory cell infiltration than IL-17RC+ mouse prostates. This finding is consistent with the proinflammatory function of IL-17. It has been reported that IL-17 can recruit neutrophils into the rat airways (39). The main cell types in the mouse prostate appear to be macrophages and lymphocytes. It is not clear whether these inflammatory cells are attracted directly by IL-17 or indirectly by IL-17–induced cytokines/chemokines in IL-17RC+ mice, as IL-17RC KO may abate both the direct and indirect effects to reduce inflammatory cell infiltration in IL-17RC+ mice. The function of the inflammatory cells in mouse prostate tumor growth awaits further research. It has been reported that macrophages in TRAMP mouse prostates are mainly M2 macrophages (40). We have recently reported that 70% of macrophages in human lung tumors are M2 macrophages (41). It is believed that M2 macrophages promote tumor growth and metastasis by secretion of growth factors, VEGF, and immunosuppressive cytokines/chemokines (42). Thus, we speculate that a reduction in inflammatory cell infiltration partially contributes to the observed tumor growth inhibition in IL-17RC+ mice.

In summary, the present study provides evidence that IL-17 promotes formation and growth of prostate adenocarcinoma in a mouse model. In this model, Pten deletion is the driving force of a series of pathologic changes (Fig. 7F). However, IL-17 cytokines act through IL-17RA:IL-17RC heterodimer receptors to induce Mmp7 expression, which plays an important role in the PIN-to-adenocarcinoma transition (Fig. 7F). Recently, Fukuda and colleagues reported that Mmp7 KO did not affect formation of ductal metaplasia and intraepithelial neoplasia but reduced development of pancreatic ductal adenocarcinoma and metastasis (43). This finding is consistent with our result that IL-17–induced Mmp7 expression did not affect PIN formation but affected PIN-to-adenocarcinoma transition. Similarly, Mmp7 is required for intestinal adenocarcinoma formation (44, 45) and development of early focal lesions in mammary tumorigenesis (46). These findings including ours suggest that MMP7 may play a critical role in formation of a variety of adenocarcinomas. In addition, IL-17 signaling may increase cellular proliferation, inhibit apoptosis, and enhance inflammation, thus promoting tumor growth (Fig. 7F). The protumorigenic role of IL-17 has also been shown in several recent studies. Xiao and colleagues (47) found that anti-IL-17 antibodies decreased inflammation and delayed papilloma formation in mouse skin, which was confirmed by a study using IL-17 KO mice (48). Neutralization of IL-17 in mice also inhibited colon cancer formation (49), which was confirmed independently by another study using IL-17 KO mice (50). Taken together, these recent findings support the opinion that IL-17 promotes tumor formation and growth, at least in skin cancer, intestinal adenocarcinoma, and prostate adenocarcinoma.

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

Authors’ Contributions
Conception and design: Qiuyang Zhang, A.B. Abdel-Mageed, S.M. Hill, B.G. Rowan, O. Sartor, Z. You
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Acknowledgments
The authors thank Dr. Prescott L. Deininger (Tulane Cancer Center) for his mentoring and support. This study used several Tulane Cancer Center Core Facilities. The authors also thank Dr. Wenjun Ouyang and Genentech for providing the Il17rc−/− mice and NCI-MMHCC for providing the PB-Cre4 mice.

Grant Support
This work was supported by NIH/NCRR COBRE grant (P20RR020152, now NGMS P20GM103518), DoD grants (W81XWH-05-1-0567 and W81XWH-10-1-0937), Developmental Fund of Tulane Cancer Center, and Louisiana Cancer Research Consortium Fund (Z. You).

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Received November 17, 2011; revised March 7, 2012; accepted March 19, 2012; published OnlineFirst March 28, 2012.

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Cancer Res  Published OnlineFirst March 28, 2012.

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doi:10.1158/0008-5472.CAN-11-3795

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