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

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Running title: IL-17 promotes formation and growth of prostate cancer

Keywords: Prostate cancer, mouse model, IL-17, Pten knockout, IL-17RC Knockout

Financial support: This work was supported by NIH/NCRR COBRE grant (P20RR020152, now NIGMS P20GM103518), DoD grants (W81XWH-05-1-0567 and W81XWH-10-1-0937), Developmental Fund of Tulane Cancer Center, and Louisiana Cancer Research Consortium Fund (to Z.Y.).

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

**Other notes:** Word count (excluding references): 4993; total number of figures: 7
Abstract

The contributions of interleukin-17 (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+). Additionally, IL-17RC- mice developed a reduced number of invasive prostate adenocarcinomas with lower rates of cellular proliferation and higher apoptosis, compared to IL-17RC+ mice. Moreover, the fibromuscular stroma surrounding prostatic glands was relatively thicker in IL-17RC- mice and was associated with decreased Mmp7 expression and increased Timp1,2,4 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 (PIN) to frank adenocarcinoma.
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 $T_{H17}$ cells, $\gamma\delta$ 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 two receptors, IL-17RA and IL-17RC (3). IL-17RC forms a receptor heterodimer with IL-17RA (4, 5). The receptor heterodimer is preferred by IL-17 ligands (6), thus, either IL-17RA knockout (KO) or IL-17RC KO completely abolishes IL-17 signaling (7, 8). IL-17A and IL-17F are key proinflammatory cytokines involved in many inflammatory and autoimmune diseases (9).

Almost all surgical prostate specimens contain inflammation (10). Chronic inflammation invokes proliferative inflammatory atrophy (PIA) of prostate – a potential precursor lesion to prostatic intraepithelial neoplasia (PIN) and carcinoma (11). The cause of prostatic inflammation includes infection, urine reflux, diet, estrogen, and physical trauma (12). A high-fat diet increases intraprostatic inflammation and plasma levels of IL-17 in mice (13). IL-17A expression is increased in 58% of prostate cancer specimens (14) and both IL-17RA and IL-17RC receptors are expressed in prostate cancer (5, 14). It has been reported that $T_{H17}$ cell number is increased in prostate cancer (15). A higher percentage of $T_{H17}$ cells in blood is correlated to poorer outcome (16). When a mouse prostate cancer cell line TRAMP-C2 was implanted in IL-17RA KO mice, the tumor growth rate was slower than those tumors implanted in wild-type (WT) mice (17). Taken together, the current knowledge links IL-17 to 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 $\text{Il17rc}$ KO ($\text{Il17rc}^{-/-}$) mice with $\text{Pten}$ conditional KO mice ($\text{Pten}^{L/L;\text{Cre}^+}$). We found that, in $\text{Pten}$-deficient context,
I\textit{l17rc} KO mice developed significantly smaller prostate tumors compared to \textit{l17rc} WT mice. Our results suggest that IL-17 promotes formation and growth of prostate adenocarcinoma.

\textbf{Materials and Methods}

\textbf{Mice.} Animal protocol was approved by the Animal Care and Use Committee of Tulane University. \textit{Pten^{loxp/loxp}} (\textit{Pten^{L/L}}) mice (18) (strain name: C;129S4-\textit{Pten^{m1Hwu/J}}; genetic background: 129S4/SvJae*BALB/c) were obtained from the Jackson Laboratory, Bar Harbor, ME. PB-Cre4 mice (19) (strain name: B6.Cg-Tg(Pbsn-cre)4Prb; genetic background: B6.Cg) were obtained from Mouse Models of Human Cancers Consortium (MMHCC) of the National Cancer Institute. \textit{l17rc^{-/-}} mice (a gift from Genentech, South San Francisco, CA; genetic background: B6.Cg) were generated by Lexicon Pharmaceuticals using standard homologous recombination (20). \textit{l17rc^{-/-}} 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 \textit{l17rc^{+/+}} and \textit{l17rc^{-/-}} mice (8). The breeding strategy is shown in Figure 1A. DNA was extracted from the tail biopsy for PCR genotyping as described (18-20) (see primer sequences in Supplementary Table 1).

\textbf{Histopathology.} Mice were weighed at 4, 6, 9, 12, and 30 weeks of age. The genitourinary (GU) bloc consisting of the prostate lobes, seminal vesicles, ampullary glands, bladder, proximal ductus deferens, and proximal urethra was excised \textit{en bloc} (21). The GU blocs were photographed, weighed with an empty bladder, and fixed as described (21). Fifty-six consecutive 5-\textmu m sections of each prostate were cut and eight sections (from every 7\textsuperscript{th} section) were 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 200x magnifications; the length-measurement function of computer software (NIS-Elements Basic Research 3.0, Nikon Instruments Inc., Melville, NY) was used to measure the thicknesses at six different points of the stroma layer around each gland; and the average of the six 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 five high-power fields (x 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 TUNEL staining.** Immunohistochemical staining (IHC) and double immunofluorescent staining were performed as described (18, 22). The antibodies used were: rabbit anti-p-Akt (1:100) and mouse anti-PTEN (26H9, 1:50) (Cell Signaling Technology, Beverly, MA); rabbit anti-Ki-67 (1:100, Millipore, Temecula, CA); rabbit anti-IL-17RA (1:200; sc-30175) and anti-IL-17RC (1:200; sc-99936) from Santa Cruz Biotechnology, Santa Cruz, CA; rabbit anti-laminin (1:100; Sigma-Aldrich, St. Louis, MO); rabbit anti-α smooth muscle actin (1:200; Pierce Biotechnology, Rockford, IL), goat anti-MMP7 (1:200; R&D systems, Minneapolis, MN), and Cy™ 3-conjugated anti-mouse IgG and DyLight™ 488-conjugated anti-rabbit IgG (Jackson ImmunoResearch Laboratories, West Grove, PA). Terminal deoxynucleotidyl transferase dUTP nick end labeling (TUNEL) staining was performed using TACS.XL® Blue Label In Situ Apoptosis Detection Kits (Trevigen, Gaithersburg, MD) according to the manufacturer’s instructions (23). To quantify Ki-67-positive and TUNEL-positive cells, five animals from each genotype group at 12 and 30 weeks of age were randomly selected; three representative prostate sections from each animal were stained; approximately
300 cells per field of three 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 MMP7 protein expression was performed as described (22). For induction of MMP7 protein expression, prostates were minced into 2 to 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, Minneapolis, MN) 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 matrix metalloproteinases (*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 1).

**Statistical analysis.** Comparisons of the GU-bloc weights were analyzed using Student’s *t* test, followed by a two-factor (age and genotype) ANOVA in conjunction with Tukey-Kramer method. *χ²* test was used to compare the incidences of PIN and invasive adenocarcinoma. Student’s *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 $Pten^{L/L};\text{Cre}^+$ (Fig. 1E) as previously reported (18). $II17rc^{+/+}$ and $II17rc^{+/-}$ prostates expressed similar levels of $II17rc$ mRNA (Fig. 2A) and protein (Fig. 2B), whereas $II17rc^{-/-}$ prostates did not express any detectable levels of $II17rc$ mRNA (Fig. 2A) and protein (Fig. 2B). $II17rc^{+/-}, II17rc^{+/-}$ and $II17rc^{-/-}$ prostates expressed similar levels of IL-17RA (Fig. 2B). Similar levels of C-C motif ligand 2 ($Ccl2$) and C-X-C motif ligand 1 ($Cxcl1$) mRNA expression were induced by rmIL-17 treatment in $II17rc^{+/-}$ and $II17rc^{+/-}$ prostates, but not in $II17rc^{-/-}$ prostates (Fig. 2C).

The GU-bloc weight is proportional to the prostate weight, thus it has often been used to represent the prostate tumor burden (24, 25). Representative GU blocs of mice at 9, 12, and 30 weeks of age are presented in Figure 2D, showing that the GU blocs of $II17rc^{+/-};Pten^{L/L};\text{Cre}^+$ and $II17rc^{+/-};Pten^{L/L};\text{Cre}^+$ mice were clearly larger than those of $II17rc^{-/-};Pten^{L/L};\text{Cre}^+$ mice at 30 weeks of age. Because $II17rc^{+/-}$ and $II17rc^{+/-}$ mouse prostates expressed similar levels of $II17rc$ (Fig. 2A and 2B) and responded similarly to IL-17 stimuli (Fig. 2C), and because there were no significant differences in the GU-bloc weights and histopathology (see description below) between $II17rc^{+/-};Pten^{L/L};\text{Cre}^+$ and $II17rc^{+/-};Pten^{L/L};\text{Cre}^+$ mice, we put $II17rc^{+/-};Pten^{L/L};\text{Cre}^+$ and $II17rc^{+/-};Pten^{L/L};\text{Cre}^+$ mice into one group (named IL-17RC+, or RC+ in abbreviation). Likewise, $II17rc^{-/-};Pten^{L/L};\text{Cre}^+$ mice were named as IL-17RC- (or RC- in abbreviation). As shown in Figure 2E, there were no significant differences in the GU-bloc weights between IL-17RC+ and IL-17RC- mice at 4, 6, and 9 weeks of age ($P > 0.05$). However, the GU-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’s $t$ test). We did not find any significant differences in body weights between the two groups. We normalized the GU-bloc
weight of each mouse by their corresponding body weight and found that the differences were still the same between the two groups (Fig. 2F).

In addition, we assessed the GU-bloc weights using a two-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 GU-bloc weights through the first 12 weeks ($P$ values varied from 0.2517 to 0.9635). However, the trajectories of the GU-bloc weights diverged soon after week 12 (see Fig. 2E) and there were highly significant differences at 30 weeks ($P < 0.0001$).

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

It was reported that $Pten^{L/L};\text{Cre}^+$ mice (with $Il17rc^{+/-}$ genotype) developed epithelial hyperplasia at 4 weeks, PIN at 6 weeks, and invasive adenocarcinoma at 9 weeks of age with 100% penetrance (18). We found epithelial hyperplasia in all of the IL-17RC+ and IL-17RC- mouse prostates at 4 weeks (Fig. 3A-C). At 6 weeks, mouse PIN was observed in all of the IL-17RC+ and IL-17RC- mouse prostates and the PIN-involved gland was bounded by an intact layer of fibromuscular stroma (Fig. 3D-F). At 9 weeks, IL-17RC+ prostates developed invasive adenocarcinoma (Fig. 3G-H). 87% of prostate lobes had invasive adenocarcinoma and other 13% had PIN in IL-17RC+ mice. In contrast, only 25% of prostate lobes had invasive adenocarcinoma, while other 75% had PIN (Fig. 3I) in IL-17RC- mice. At 12 weeks, IL-17RC+ mice presented with invasive adenocarcinoma in 85% of prostate lobes (Fig. 3J-K) and with PIN in other 15%. On the other hand, IL-17RC- mice presented with invasive adenocarcinoma in 44% of prostate lobes, while other 56% had PIN (Fig. 3L). At 30 weeks, 100% of prostate lobes...
presented as invasive adenocarcinoma in IL-17RC+ mice (Fig. 3M-N). In contrast, only 70% of prostate lobes showed invasive adenocarcinoma (Fig. 3O) in IL-17RC- mice, with the remaining 30% still presented as PIN. Invasion of the basement membrane and fibromuscular layer was confirmed by α smooth muscle actin staining (Fig. 3P). The percentages of PIN and invasive adenocarcinomas were significantly different between IL-17RC+ and IL-17RC- mice at 9, 12, and 30 weeks ($P < 0.001$, $P < 0.001$, and $P < 0.005$, respectively) (Fig. 3Q; see data from individual prostatic lobes in Supplementary Figure 1).

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

To understand why IL-17RC+ mice developed bigger prostate tumors than IL-17RC- mice at 12 and 30 weeks, we assessed cellular proliferation and apoptosis by performing 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+ prostates than in IL-17RC- prostates (Fig. 4A-B). In addition, there were significantly fewer apoptotic cells in IL-17RC+ prostates than in IL-17RC- prostates (Fig. 4C-D).

**IL-17RC knockout 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 histopathological features of the mouse prostates. Normal mouse prostate has a thin rim of fibromuscular stroma surrounding individual glands (Fig. 5A) (21). The layer of fibromuscular stroma in IL-17RC- mice was dramatically thicker compared to age-matched normal prostates (Fig. 5B). In contrast, the layer of fibromuscular stroma in IL-17RC+ mice (Fig. 5C) was obviously thinner than that of IL-17RC- mice. Laminin staining confirmed what were observed with H&E staining (Fig. 5D-I, see whole prostate...
sections in Supplementary Figure 2A-B). The thickness of the fibromuscular stroma was measured using computer software (Fig. 5J). As shown in Figure 5K, the thickness of the fibromuscular stroma was significantly thicker in IL-17RC- mice compared to 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 Figure 3A).

**IL-17RC knockout decreased $Mmp7$ expression**

To investigate the molecular mechanisms underlying the different thicknesses of fibromuscular stroma, we screened seven $Mmps$ and four $Timps$ 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 over 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 IHC, 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 6J), whereas only a few neoplastic epithelial cells in IL-17RC- mice stained positive (Fig. 6K and 6L). We did not see positive MMP7 staining in the stromal cells (Fig. 6C to 6L). Similar results were obtained in other prostatic lobes (see data of dorsal lobes in Supplementary Figure 3B). To confirm the differences of MMP7 protein expression, we performed 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).

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

**IL-17RC knockout 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-D).

**Discussion**
The role of IL-17 in cancer growth has been debated for over a decade (27, 28). One opinion is that IL-17 is pro-tumorigenic (29, 30), whereas another notion is that IL-17 is anti-tumorigenic (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, two 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 IL-17’s action 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, in order to properly evaluate IL-17’s role 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 GU-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’s $t$ test and Tukey-Kramer method. At 12 weeks, the GU-bloc weight was 14% heavier in IL-17RC+ mice than in IL-17RC- mice, which was statistically significant in Student’s $t$ test ($P = 0.005$) but...
insignificant in Tukey-Kramer method ($P = 0.2517$). This is because Student’s $t$ test only compared two groups at the designated time point, whereas Tukey-Kramer method took multiple comparisons at the five time points into consideration. Of note, because accumulation of fluid in the anterior lobes may not be the same in all animals, the GU-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 prior to 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 to IL-17RC+ mice. IL-17 does not directly stimulate cancer cellular proliferation in in vitro studies (29, 31); however, it may indirectly stimulate cellular proliferation in vivo via induction of other target genes such as $Ccl2$. We found that IL-17 induced $Ccl2$ expression in IL-17RC+ mouse prostates and CCL2 has been shown to enhance prostate cancer cellular proliferation (38). We previously demonstrated that IL-17RC over-expression inhibited apoptosis in LNCaP cells via blocking activation of caspase-3 (22). It is possible that $Ii17rc$ 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 compared to 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 $Il17rc$ 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. Since 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 4C), 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 to 9 weeks. More importantly, Mmp7 mRNA and protein expression was significantly reduced in IL-17RC- mice compared to 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 compared to IL-17RC+ mouse prostates. This finding is consistent with IL-17’s proinflammatory function. 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 knockout 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, vascular endothelial growth factor, 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 pathological 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 et al. 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). IL-17’s pro-tumorigenic role has also been demonstrated in several recent studies. Xiao et al (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.

Acknowledgments

We thank Dr. Prescott L. Deininger (Tulane Cancer Center) for his mentoring and support. This study used several Tulane Cancer Center Core Facilities. We thank Dr. Wenjun Ouyang and Genentech for providing the Il17rc−/− mice and NCI MMHCC for providing the PB-Cre4 mice.
References:


Figure legends

Figure 1. Strategy of animal breeding and genotyping. A, strategy of animal breeding. B to D, representative gel images of PCR genotyping. E, Double immunofluorescent staining of PTEN (in red) and p-Akt (in green) in the dorsal prostatic lobes of 9-week-old mice.

Figure 2. IL-17RC knockout decreased growth of prostate tumors. A, qRT-PCR analysis of Il17rc mRNA levels in the whole prostates from 9-week-old Il17rc+/+, Il17rc+/−, and Il17rc−/− mice. B, IHC of IL-17RC and IL-17RA in 9-week-old Pten−/− dorsal prostatic lobes; original magnifications, 400x. C, ex vivo cultured prostate tissues from 9-week-old Il17rc+/+, Il17rc+/−, and Il17rc−/− mice were treated with or without 20 ng/ml of rmIL-17 for 2 hours, followed by qRT-PCR analysis. D, representative photographs of the GU blocs. E, the GU-bloc weights over the time course; the number of animals from the RC+ and RC- groups and P values (Student’s t test) are shown under the abscissa. F, the ratios of GU-bloc weights normalized by body weights over the time course.

Figure 3. IL-17RC knockout did not affect formation of PIN, but decreased formation of invasive prostate adenocarcinoma. A to O, representatives of H&E stained lateral prostatic lobes. Original magnifications, 200x for photomicrographs and 400x for inserts. P, anti-α smooth muscle actin staining; the tissue sections were consecutive sections corresponding to each of the panels G to L; arrows indicate invasion where continuity of staining is broken. Q, percentages of PIN and invasive adenocarcinomas in dorsal, lateral, and ventral prostatic lobes.

Figure 4. IL-17RC knockout decreased cellular proliferation and increased apoptosis in the prostate lesions. A, Ki-67 staining; arrows indicate the positive cells. B, percentages of Ki-67-positive cells in dorsal (DP), lateral (LP), and ventral (VP) prostate lobes; *P < 0.05 and **P <
0.01. *C*, TUNEL staining; arrows indicate the positive cells. *D*, percentages of apoptotic cells in prostate lobes; **P < 0.05** and **P < 0.01**. Original magnifications, 400x.

**Figure 5.** IL-17RC knockout increased the thickness of fibromuscular stroma surrounding each prostatic gland. *A* to *C*, representatives of H&E stained prostate sections from 9-week-old mouse dorsal lobes; arrows in the inserts indicate the fibromuscular stroma; original magnifications, 100x for photomicrographs and 400x for inserts. *D* to *F*, representatives of laminin staining in 9-week-old mouse dorsal prostatic lobes; original magnifications, 100x. *G* to *I*, 400x magnification of the selected regions in *D* to *F*; arrows indicate the fibromuscular stroma. *J*, representative of computer-aided measurements of the thicknesses of fibromuscular stroma at six points (arrows) of each prostatic gland; scale bar, 50 μm. *K*, comparisons of the thicknesses of fibromuscular stroma between age-matched mouse dorsal, lateral, and ventral prostatic lobes; **P < 0.001**.

**Figure 6.** IL-17RC knockout decreased *Mmp7* expression in the prostate lesions. *A*, qRT-PCR analysis of mRNA levels of *Mmps* and *Timps* in 9-week-old mouse whole prostates; **P < 0.05** and **P < 0.01**. *B*, qRT-PCR analysis of *Mmp7* mRNA levels in mouse whole prostates; **P < 0.01**. *C* to *L*, IHC of MMP7 in lateral prostatic lobes; original magnifications, 200x for photomicrographs and 400x for inserts. *M*, Western blot of MMP7 protein expression in 9-week-old mouse whole prostates. *N*, qRT-PCR analysis of *Mmp7* mRNA levels in *ex vivo* cultured mouse prostates treated with or without 20 ng/ml of rmIL-17 for 2 hours; **P < 0.01**. *O*, Western blot of MMP7 protein expression in *ex vivo* cultured mouse prostates treated with or without 20 ng/ml of rmIL-17 for 24 hours.

**Figure 7.** IL-17RC knockout decreased inflammatory cell infiltration in the mouse prostate tumors. *A* and *B*, representatives of H&E stained sections from 30-week-old mouse ventral
prostatic lobes; original magnifications, 100x. \textit{C and D}, 400x magnification of the selected regions in \textit{A} to \textit{B}; arrows indicate macrophages; open arrows indicate lymphocytes; arrowhead indicates a neutrophil. \textit{E}, comparison of the numbers of inflammatory cells per high-power field. \textit{F}, a summary of the time course of pathologic changes in the mouse prostate with \textit{Pten} conditional deletion in the prostatic epithelium and the effects of IL-17 signaling on the pathologic changes.
Figure 1

A. Schematic diagram showing the breeding strategies and genetic composition of the experimental groups. The crossbreeding of 

**II**17rc+/−;PtenL/L;Cre+ with different genotypes resulted in the following genotypes: 

- **II**17rc+/−;PtenL/L;Cre+ 
- **II**17rc+/−;PtenL/L;Cre+ 
- **II**17rc+/−;PtenL/L;Cre+ 

The breeding strategy includes: 

- **Male** PB-Cre4 x **Female** II17rc−/− 
- **Male** II17rc+/−;PtenL/L;Cre+ x **Female** II17rc+/−;PtenL/L;Cre+ 
- **Male** II17rc+/−;PtenL/L;Cre+ x **Female** II17rc+/−;PtenL/L;Cre+ 

Animal age: Birth, 3 wk, 4 wk, 6 wk, 9 wk, 12 wk, 30 wk. 

Physiological milestones: Birth, Weaning, Hyperplasia, Sexual maturity, PIN, Invasive cancer.

B. Gel electrophoresis showing the amplification of II17rc with different genotypes: 

- II17rc+/+, II17rc−/−, II17rc+/−, II17rc−/−;PtenL/L, II17rc−/−;PtenL/L, II17rc+/−;PtenL/L, II17rc+/−;PtenL/L

The bands are 534 bp and 404 bp.

C. Gel electrophoresis showing the amplification of Pten with different genotypes: 

- PtenL/L, PtenL/L, PtenL/L, PtenL/L, PtenL/L

The bands are 650 bp and 500 bp.

D. Gel electrophoresis showing the amplification of Cre with different genotypes: 

- Cre+, Cre−, Cre+, Cre+

The bands are 324 bp and 199 bp.

E. Immunofluorescence images showing PTEN/p-Akt in different genotypes: 

- WT (normal), II17rc−/−;PtenL/L;Cre+, II17rc+/−;PtenL/L;Cre+

The images are captured at 50 μm.
Figure 5

A Normal
B Il17rc^-/-;Pten^-/-
C Il17rc^+/+;Pten^-/-

Laminin staining

D
E
F

G
H
I

J

K

Thickness of stroma (μm)

<table>
<thead>
<tr>
<th></th>
<th>Normal</th>
<th>RC-</th>
<th>RC+</th>
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<tbody>
<tr>
<td>9 wk</td>
<td></td>
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<tr>
<td>12 wk</td>
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<tr>
<td>30 wk</td>
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* indicates significant difference.
Figure 6

A

Relative mRNA levels (x1000 fold):

RC- □ RC+

Mmps

7 8 9 10 13

Timps

2 3

**

**

B

Relative mRNA levels (x1000 fold):

RC- □ RC+

Age (wk)

9 12 30

**

**

C

4 wk

E

6 wk

G

9 wk

I

12 wk

J

30 wk

D

RC-

M

MMP7 GAPDH

II17rc-/- II17rc+/+

L

N

Mmp7 mRNA (fold):

II17rc-/- II17rc+/+

rmIL-17 - +

**

O

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Figure 7
Interleukin-17 Promotes Formation and Growth of Prostate Adenocarcinoma in Mouse Models

Qiuyang Zhang, Sen Liu, Dongxia Ge, et al.

Cancer Res  Published OnlineFirst March 28, 2012.

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