Am J Clin Exp Urol 2019;7(6):352-377
www.ajceu.us /ISSN:2330-1910/AJCEU0105685

Original Article

Genetic alterations of interleukin-17 and related genes in human prostate cancer

Ruoxin Lan[2], Kun Zhang[3], Tianhua Niu[4], Zongbing You[1,2,5,6,7,8]

[1]Southeast Louisiana Veterans Health Care System, New Orleans, LA, USA; [2]Department of Structural & Cellular Biology, Tulane University, New Orleans, LA, USA; [3]Department of Computer Science and Biostatistics Facility of RCMI Cancer Research Center, Xavier University of Louisiana, New Orleans, LA, USA; [4]Department of Biochemistry and Molecular Biology, Tulane University, New Orleans, LA, USA; [5]Department of Orthopaedic Surgery, Tulane University, New Orleans, LA, USA; [6]Tulane Cancer Center and Louisiana Cancer Research Consortium, Tulane University, New Orleans, LA, USA; [7]Tulane Center for Stem Cell Research and Regenerative Medicine, Tulane University, New Orleans, LA, USA; [8]Tulane Center for Aging, Tulane University, New Orleans, LA, USA

Received November 29, 2019; Accepted December 6, 2019; Epub December 15, 2019; Published December 30, 2019

Abstract: Interleukin-17 (*IL-17*) has been shown to promote development of hormone-naïve prostate cancer (HNPC) and castration-resistant prostate cancer (CRPC) as well as lymph node metastasis in mouse models. Gene alterations of *IL-17* family of cytokines and their downstream genes in human prostate cancer have not been investigated. We studied 7 datasets archived in cBioPortal and queried gene alterations in a total of 1303 cases of human prostate cancers. 35 genes were examined, including *IL-17* family of cytokines and receptors, *IL-17*-downstream genes, and genes related to *IL-17*-downstream genes. We found that 34/35 (97%) genes had significantly more alterations in metastatic prostate cancer (with alteration rates ranging from 3.42% to 13.01%) than primary prostate cancer (with alteration rates ranging from 0.40% to 2.96%). 15/35 (43%) genes had significantly more alterations in primary CRPC than primary HNPC. 34/35 (97%) genes had significantly more alterations in metastatic CRPC than primary HNPC. Only three genes (*S100A7*, *S100A8*, and *S100A9*) had significantly more alterations in metastatic CRPC than primary CRPC. The gene alterations were mostly gene amplifications (97%), while gene deep deletions, missense mutations, and truncating mutations were very rare. 7/35 (20%) genes had significantly more alterations in primary neuroendocrine prostate cancer (NEPC) than primary adenocarcinoma (AC). 23/35 (66%) genes had significantly more alterations in metastatic NEPC than metastatic AC. Only three genes (*S100A7*, *S100A8*, and *S100A9*) had significantly more alterations in metastatic NEPC than metastatic AC with neuroendocrine features. Most of the gene alterations in metastatic NEPC were gene amplifications (80%), while gene deep deletions, missense mutations, and truncating mutations were very rare. Our findings suggest that gene amplifications of *IL-17* and related genes are more frequently found in metastatic CRPC and NEPC than primary hormone-naïve prostate adenocarcinomas, implying that *IL-17* and related genes may play important roles in the progression from HNPC to CRPC and from primary location to metastasis as well as in development of metastatic NEPC.

Keywords: Prostate cancer, metastasis, CRPC, *IL-17*, gene amplification

Introduction

Prostate cancer is the most common malignancy and the second most common cause of cancer-related deaths in American men; the American Cancer Society estimates that there are approximately 174,650 new cases and 31,620 deaths due to prostate cancer in 2019 [1]. Distant metastases are common findings in autopsies of prostate cancer patients. Hematogenous metastases were present in 35% of 1,589 patients with prostate cancer, with the most frequent involvement being the bone (90%), lungs (46%), liver (25%), pleura (21%), and adrenals (13%) [2]. Metastatic prostate cancers are usually treated with androgen deprivation therapy (ADT) and they often respond to ADT as they are hormone-naïve prostate cancer (HNPC). Eventually, almost all of them become insensitive to ADT even when the blood levels of androgens are at castration levels, hence they are called castration-resistant prostate cancer (CRPC). A small portion (approximately 0.5%-2%) of prostate cancer, so called neuroendocrine prostate cancer (NEPC), is small cell carcinoma, instead of adenocarcinoma

(AC). NEPC does not express androgen receptor and is inherently CRPC [3]. Some adenocarcinomas (approximately 17% to 30%) may undergo focal neuroendocrine (NE) differentiation after ADT and present NE features [3]. The molecular and cellular mechanisms underlying the progression from primary prostate cancer to metastatic prostate cancer and from HNPC to CRPC have not been fully understood.

Interleukin-17 (*IL-17*) is a family of proinflammatory cytokines, including *IL-17A*, *IL-17B*, *IL-17C*, *IL-17D*, *IL-17E*, and *IL-17F* [4]. There are five receptors for *IL-17* cytokines, including *IL-17* receptor A (*IL-17RA*), *IL-17RB*, *IL-17RC*, *IL-17RD*, and *IL-17RE* [4]. IL-17RA acts as a shared subunit and forms heterodimers with other four receptors [5]. Homodimers of IL-17A or IL-17F and heterodimer of IL-17A/IL-17F bind to IL-17RA/IL-17RC receptor complex. Homodimers of IL-17B or IL-17E bind to IL-17RA/IL-17RB receptor complex. IL-17C homodimer binds to IL-17RA/IL-17RE receptor complex. Recently, it has been reported that IL-17A, but not IL-17F or IL-17A/F, also binds to IL-17RA/IL-17RD receptor complex [6]. IL-17A and IL-17F are produced by T helper 17 (Th17) cells, γδ T cells, natural killer cells, and other immune cells [7]. Binding of IL-17A or IL-17F to IL-17RA/IL-17RC receptor complex recruits nuclear factor-κB (NF-κB) activator 1 (Act1) through SEFIR (similar expression to fibroblast growth factor genes, IL-17 receptors and Toll-IL-1R) domains of IL-17RA, IL-17RC, and Act1. Act1 acts as an E3 ubiquitin ligase to ubiquitinate tumor necrosis factor receptor-associated factor 6 (TRAF6) through lysine-63-linked ubiquitination [8]. Then, TRAF6 activates transforming growth factor-β-activated kinase 1 (TAK1) and subsequently IκB kinase (IKK) complex, resulting in activation of NF-κB pathway that initiates transcription of a variety of cytokines, chemokines, matrix metalloproteinases (MMP), and growth factors, such as *IL-1α*, *IL-1β*, *IL-6,* tumor necrosis factor α *(TNFα),* colony stimulating factor (*CSF*), C-X-C motif ligand 1 (*CXCL1*), *CXCL2, CXCL5, CXCL8*, C-C motif ligand 7 (*CCL7*), S100 Calcium Binding Protein A7 (*S100A7*), *S100A8, S100A9*, CCAAT Enhancer Binding Protein Beta (*CEBPB*), Prostaglandin-Endoperoxide Synthase-2 (*PTGS2*), *MMP1*, *MMP2*, *MMP7*, *MMP9*, and *MMP13* [9-15]. IL-17 also induces expression of programmed cell death protein 1 (*PD-1*) ligand 1 (*PD-L1*), but not *PD-L2* in a human prostate cancer LNCaP cell line [16].

IL-17 has been shown to promote development of colon cancer [17-20], skin cancer [21, 22], breast cancer [23], prostate cancer [13, 24], lung cancer [25, 26], and pancreas cancer [27]. Using *Il-17rc*-null and phosphatase and tensin homolog (*Pten*)-null mouse models, we have demonstrated that *Il-17rc* knockout inhibits prostate cancer development [13]. IL-17 induces expression of MMP7 to cleave E-cadherin, thus activating β-catenin-mediated epithelial-to-mesenchymal transition, which subsequently enhances development of prostate cancer in *Pten*-null mice [14]. We have also shown that IL-17 promotes development of CRPC in *Pten*-null mice partially through increasing immunosuppressive M2 macrophages and myeloid-derived suppressor cells (MDSCs) in the tumor microenvironment [24]. Using an orthotopic allograft model in immunocompetent mice, we found that co-injection of recombinant IL-17A and mouse prostate cancer MPC3 cells slightly increased primary tumor growth, however, pelvic lymph node metastasis was significantly enhanced [28]. On the other hand, we found that Th17 inhibitor SR1001 and anti-mouse IL-17A antibody are able to partially inhibit formation of prostate cancer in *Pten*-null mice [29]. Recently, we have shown that Th17-polarized splenocytes promoted orthotopic allograft prostate tumor growth compared to the control splenocytes [30]. These studies suggest that Th17 cells and IL-17 cytokines secreted by Th17 cells may play important roles in development of primary prostate cancer, CRPC, and lymph node metastasis. The objective of this study was to analyze genetic alterations of *IL-17* family of cytokines and related genes aforementioned in primary and metastatic prostate cancers, using publicly archived datasets and bioinformatics tools.

Materials and methods

Data sources

All of the data were obtained through cBioPortal for Cancer Genomics (www.cbioportal.org) [31, 32]. cBioPortal has archived 20 datasets for gene alterations in human prostate cancers. We filtered through the datasets and excluded the datasets that potentially used overlapping original samples according to the linked publications. Seven datasets were included, which did not appear to have overlapping original samples (**Table 1**).

Table 1. Data sources

Datasets‡	Author	Year	Included cases (N=1303)	Excluded cases* (N=77)
MCTP	Grasso [33]	2012	59	NA (n=60)
SU2C/PCF Dream Team	Abida [34]	2019	427	Unknown (n=2)
Multi-Institute	Beltran [35]	2016	81	
Broad/Cornell	Barbieri [36]	2012	112	NA (n=11)
Fred Hutchinson CRC	Kumar [37]	2016	61	Not profiled (n=2)
SMMU	Ren [38]	2017	65	
TCGA	TCGA data		498	Not profiled (n=2)

‡MCTP, Michigan Center for Translational Pathology; SU2C/PCF Dream Team, Stand Up to Cancer/Prostate Cancer Foundation Dream Team; Fred Hutchinson CRC, Fred Hutchinson Cancer Research Center; SMMU, Second Military Medical University (Shanghai); TCGA, The Cancer Genome Atlas. *Cases were excluded from analysis if the sample types were "Not available (NA)", "Unknown", or "Not profiled"; the number of cases excluded is shown.

The data of Michigan Center for Translational Pathology (MCTP) were generated by Dr. Arul Chinnaiyan's and Dr. Scott Tomlins' labs at the University of Michigan [33]; the data of Stand Up to Cancer/Prostate Cancer Foundation Dream Team (SU2C/PCF Dream Team) were generated by researchers at Dana-Farber Cancer Institute, Karmanos Cancer Institute, Memorial Sloan Kettering Cancer Center, Royal Marsden, University of Michigan, University of Washington, and Weill Cornell Medicine [34]; the data of Multi-Institute were generated by researchers at Weill Cornell Medicine [35]; the data of Broad/Cornell were generated by Dr. Levi Garraway's lab at Broad Institute and Dr. Mark Rubin's lab at Weill Cornell Medicine [36]; the data of Fred Hutchinson Cancer Research Center were generated by researchers at the University of Washington and Fred Hutchinson Cancer Research Center [37]; the data of the Second Military Medical University (Shanghai) (SMMU) were generated by researchers at Shanghai Changhai Hospital and Fudan University Cancer Center [38]; the data of The Cancer Genome Atlas (TCGA) (https://www.cancer.gov/tcga) were a public databank managed by the National Cancer Institute, National Institutes of Health. A total of 1303 cases of prostate cancer samples (N=1303) were included, while 77 cases were excluded from analysis because the sample types were "Not available (NA)", "Unknown", or "Not profiled" (**Table 1**).

Bioinformatics analysis

We used cBioPortal query tools to find genetic alterations of 35 *IL-17* and related genes (**Figure 1**). These genes were chosen because they are *IL-17* family of cytokines and receptors, *IL-17*-downstream genes, and related to *IL-17*-regulated genes (e.g., *PD-1* and *PD-L2* are related to *PD-L1* that is regulated by *IL-17* [16]). The bioinformatics analysis procedures are briefly described here: first, we chose "Prostate" organ type on the main page of cBioPortal; second, we chose the dataset named "Metastatic Prostate Adenocarcinoma (MCTP, Nature 2012)" and clicked the round button on the right side; third, we typed in gene names (e.g., *IL17A*) in the query box on right top corner of the page; fourth, we clicked "Charts" below the query box and clicked "Deselect all"; fifth, we chose "Sample Type" and we could see a pie chart on this page where we chose the portion named "metastatic" and clicked "Query" on the right side of query box; sixth, we moved the mouse on the "2.1%" on the right side of "*IL17A*", which showed "altered/profiled 1/48". That means the number of overall *IL17A* gene alterations in metastatic prostate cancers was 1, and the number of total cases was 48. We used Object Query Language (OQL) to do queries of the 35 genes. The gene alterations were categorized into copy number alterations (amplifications and deep deletions) and mutations (missense mutations and truncating mutations) according to cBioPortal (**Figure 1**). Prostate cancer sample types were categorized into primary prostate cancer (including both HNPC and CRPC), metastatic prostate cancer (including both HNPC and CRPC), primary HNPC, primary CRPC, metastatic HNPC (not included in analysis because there was only one case), metastatic CRPC, primary adenocarcinoma (AC), primary NEPC, metastatic AC, metastatic NEPC, primary AC with NE feature (not included in analysis because there was only one case), and metastatic AC with NE feature. We identified and calculated the numbers and percentages of overall gene alterations and individual categories of gene alterations after pooling the query results from the 7 datasets.

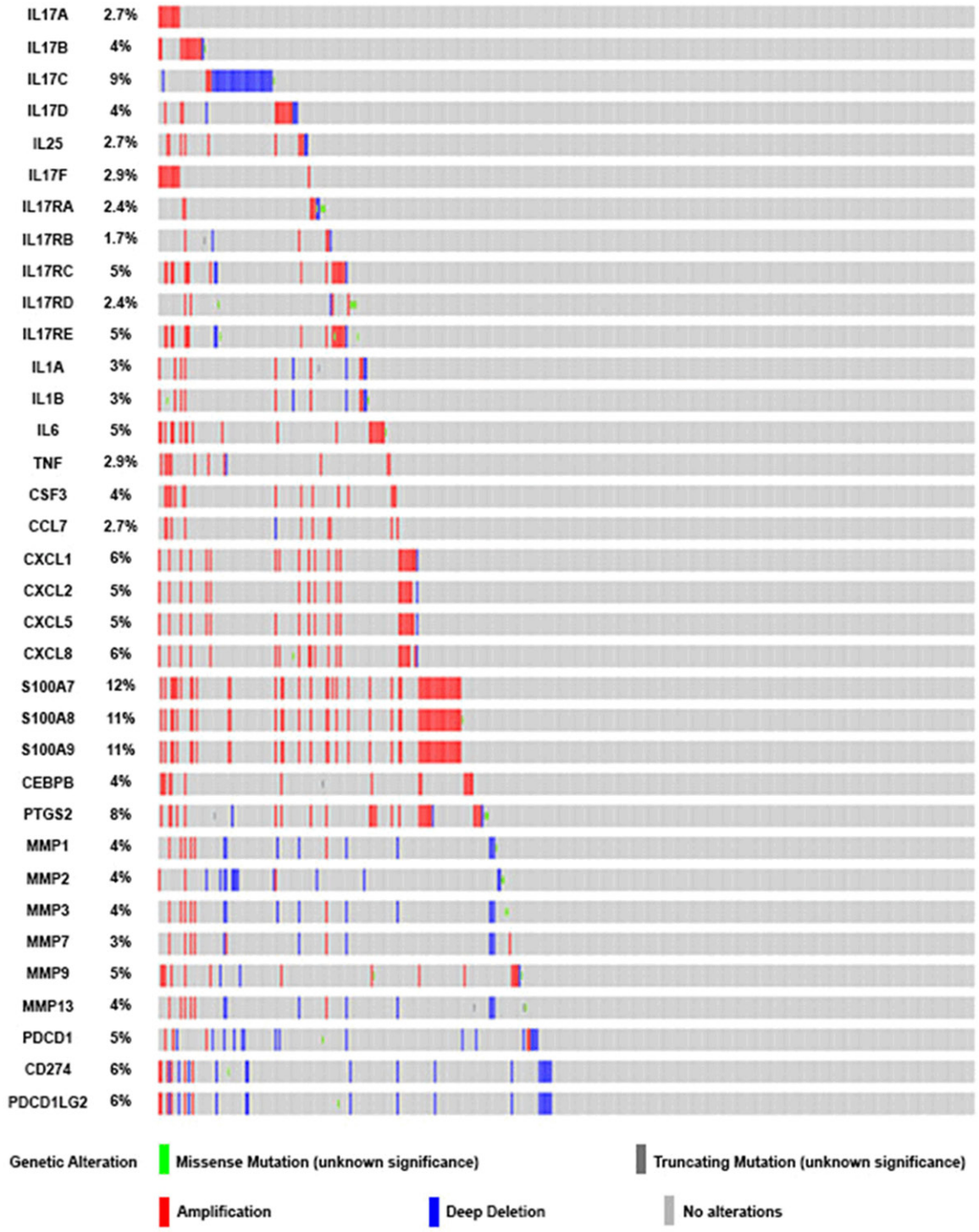

Figure 1. Representative illustration of cBioPortal query results. Metastatic prostate cancer samples from the SU2C/PCF Dream Team dataset were queried for overall gene alterations including missense mutations, truncating mutations, amplifications, and deep deletions (color bar-coded). 35 *IL-17* and related genes were analyzed using cBioPortal query tools and the percentages of overall gene alterations are shown.

Statistical analysis

R software package [R version 3.5.2 (2018-12-20), R Core Team (2018); R: A language and environment for statistical computing, R Foundation for Statistical Computing, Vienna, Austria. https://www.r-project.org/] was used to perform Fisher's exact test between two

sample types. $P<0.05$ was considered statistically significant.

Results

Human prostate cancer samples

Data from a total of 1303 human prostate cancer samples (N=1303) were included in the analysis, while 77 samples were excluded (N=77) (**Table 1**). The samples were categorized into primary prostate cancer (N=742), metastatic prostate cancer (N=584), primary HNPC (N=684), primary CRPC (N=56), metastatic HNPC (N=1), metastatic CRPC (N=583), primary AC (N=724), primary NEPC (N=16), metastatic AC (N=461), metastatic NEPC (N=45), primary AC with NE feature (N=1), and metastatic AC with NE feature (N=12).

Overall gene alterations

Thirty-four of the 35 *IL-17* and related genes studied had significantly higher rates of gene alterations in metastatic primary cancers (including both HNPC and CRPC) compared to primary prostate cancers (including both HNPC and CRPC) (**Table 2**). *IL17C* is the only gene that the gene alterations showed no significant difference. The alteration rate range was from 3.42% to 13.01%, with the top alterations in *S100A7* (13.01%), *S100A8* (12.50%), *S100A9* (12.33%), and *PTGS2* (10.27%) in metastatic prostate cancers (**Table 2**). Significantly higher rates of gene alterations were found in *S100A7*, *S100A8*, and *S100A9* genes, but not in other genes, in metastatic CRPC, compared to primary CRPC (**Table 3**). However, significantly higher rates of gene alterations were found in 15 of the 35 genes in primary CRPC, compared to primary HNPC (**Table 4**). Further, significantly higher rates of gene alterations were found in 34 of the 35 genes in metastatic CRPC, compared to primary HNPC (**Table 5**).

Individual categories of gene alterations

We further analyzed the gene alterations by dividing them into four categories, namely, amplifications, deep deletions, missense mutations, and truncating mutations according to the default classification of cBioPortal. We found that 34 of the 35 *IL-17* and related genes studied had significantly higher rates of gene amplifications in metastatic primary cancers (including both HNPC and CRPC) compared to primary prostate cancers (including both HNPC and CRPC) (**Table 6**). *IL17C* is the only gene that the gene amplifications showed no significant difference. The rate range was from 1.37% to 13.01%, with the top amplifications in *S100A7* (13.01%), *S100A8* (12.33%), *S100A9* (12.33%), and *PTGS2* (9.08%) in metastatic prostate cancers (**Table 6**). Gene deep deletions were significantly higher in *MMP1* (2.23%), *MMP3* (2.23%), *PDCD1* (3.60%), *CD274* (3.60%), and *PDCD1LG2* (3.60%) in metastatic prostate cancers, compared to primary prostate cancers (**Table 7**). In contrast, gene deep deletions were significantly higher in *CSF3* (0.94%) in primary prostate cancers, compared to metastatic prostate cancers (**Table 7**). Gene missense mutations were significantly higher in *IL17RD* (1.03%) and *IL17RE* (0.86%) in metastatic prostate cancers, compared to primary prostate cancers (**Table 8**). However, gene truncating mutations were very rare and there was no significant difference between metastatic and primary prostate cancers (**Table 9**).

Gene alterations in NEPC

Since NEPC is inherently CRPC, we singled out this prostate cancer type and compared it against AC. We found that 7 of the 35 genes (namely, *IL17B*, *IL25*, *IL17RB*, *TNF*, *CXCL8*, *CEBPB*, and *MMP9*) had significantly more overall gene alterations (12.50%) in primary NEPC, compared to primary AC (**Table 10**). More dramatically, 23 of the 35 genes had significantly more overall gene alterations (ranging from 11.11% to 33.33%) in metastatic NEPC, compared to metastatic AC (ranging from 2.17% to 11.93%) (**Table 11**). *S100A7* (28.89%), *S100A8* (28.89%), *S100A9* (28.89%), *CEBPB* (28.89%), *PTGS2* (28.89%), and *MMP9* (33.33%) were the genes with top alteration rates in metastatic NEPC. *S100A7* (28.89%), *S100A8* (28.89%) and *S100A9* (28.89%) were also the genes with higher rates of gene alterations in metastatic NEPC, compared to metastatic AC with NE feature (**Table 12**). When comparing between metastatic AC with NE feature and metastatic AC, there were some genes with more or less gene alterations, but the differences were not statistically significant (**Table 13**). Further analysis of each individual categories of gene alterations showed that 28 of the 35 genes had significantly higher rates of gene

Table 2. Overall gene alterations in metastatic vs primary prostate cancer

Genes*	Metastatic (N=584)		Primary (N=742)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	23	3.94%	6	0.81%	1.86E-04
IL17B	36	6.16%	11	1.48%	7.10E-06
IL17C	48	8.22%	56	7.55%	6.81E-01
IL17D	31	5.31%	20	2.70%	2.05E-02
IL25	26	4.45%	5	0.67%	8.63E-06
IL17F	23	3.94%	3	0.40%	2.88E-06
IL17RA	24	4.11%	8	1.08%	4.46E-04
IL17RB	26	4.45%	11	1.48%	1.27E-03
IL17RC	42	7.19%	22	2.96%	4.35E-04
IL17RD	32	5.48%	9	1.21%	8.58E-06
IL17RE	43	7.36%	19	2.56%	6.13E-05
IL1A	20	3.42%	9	1.21%	7.58E-03
IL1B	22	3.77%	8	1.08%	1.30E-03
IL6	49	8.39%	13	1.75%	1.11E-08
TNF	28	4.79%	9	1.21%	1.38E-04
CSF3	31	5.31%	15	2.02%	1.36E-03
CCL7	30	5.14%	8	1.08%	1.60E-05
CXCL1	37	6.34%	6	0.81%	1.41E-08
CXCL2	31	5.31%	6	0.81%	6.97E-07
CXCL5	34	5.82%	7	0.94%	4.26E-07
CXCL8	35	5.99%	11	1.48%	1.22E-05
S100A7	76	13.01%	9	1.21%	<2.2e-16
S100A8	73	12.50%	11	1.48%	<2.2e-16
S100A9	72	12.33%	9	1.21%	<2.2e-16
CEBPB	36	6.16%	9	1.21%	7.87E-07
PTGS2	60	10.27%	12	1.62%	3.08E-12
MMP1	38	6.51%	9	1.21%	2.34E-07
MMP2	25	4.28%	14	1.89%	1.33E-02
MMP3	38	6.51%	9	1.21%	2.34E-07
MMP7	34	5.82%	11	1.48%	2.07E-05
MMP9	42	7.19%	14	1.89%	2.85E-06
MMP13	38	6.51%	10	1.35%	7.77E-07
PDCD1	38	6.51%	15	2.02%	5.18E-05
CD274	36	6.16%	11	1.48%	7.10E-06
PDCD1LG2	36	6.16%	12	1.62%	1.13E-05

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene alterations in metastatic than primary prostate cancer.

Table 3. Overall gene alterations in metastatic CRPC vs primary CRPC

Genes*	Metastatic CRPC (N=583)		Primary CPRC (N=56)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	23	3.95%	3	5.36%	4.90E-01
IL17B	36	6.17%	5	8.93%	3.92E-01
IL17C	48	8.23%	1	1.79%	1.11E-01
IL17D	31	5.32%	4	7.14%	5.36E-01
IL25	26	4.46%	4	7.14%	3.24E-01
IL17F	23	3.95%	2	3.57%	1.00E+00
IL17RA	24	4.12%	1	1.79%	7.16E-01
IL17RB	26	4.46%	5	8.93%	1.80E-01
IL17RC	42	7.20%	4	7.14%	1.00E+00
IL17RD	32	5.49%	5	8.93%	3.61E-01
IL17RE	43	7.38%	3	5.36%	7.88E-01
IL1A	20	3.43%	2	3.57%	1.00E+00
IL1B	22	3.77%	2	3.57%	1.00E+00
IL6	49	8.40%	2	3.57%	3.00E-01
TNF	28	4.80%	4	7.14%	5.14E-01
CSF3	31	5.32%	3	5.36%	1.00E+00
CCL7	30	5.15%	3	5.36%	1.00E+00
CXCL1	37	6.35%	3	5.36%	1.00E+00
CXCL2	31	5.32%	3	5.36%	1.00E+00
CXCL5	34	5.83%	3	5.36%	1.00E+00
CXCL8	35	6.00%	5	8.93%	3.83E-01
S100A7	76	13.04%	2	3.57%	3.35E-02
S100A8	73	12.52%	2	3.57%	4.92E-02
S100A9	72	12.35%	2	3.57%	4.92E-02
CEBPB	36	6.17%	4	7.14%	7.71E-01
PTGS2	60	10.29%	4	7.14%	6.41E-01
MMP1	38	6.52%	2	3.57%	5.66E-01
MMP2	25	4.29%	0	0.00%	1.55E-01
MMP3	38	6.52%	2	3.57%	5.66E-01
MMP7	34	5.83%	2	3.57%	7.61E-01
MMP9	42	7.20%	4	7.14%	1.00E+00
MMP13	38	6.52%	2	3.57%	5.66E-01
PDCD1	38	6.52%	1	1.79%	2.40E-01
CD274	36	6.17%	2	3.57%	7.65E-01
PDCD1LG2	36	6.17%	2	3.57%	7.65E-01

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene alterations in metastatic CRPC than primary CRPC.

Table 4. Overall gene alterations in primary CRPC vs primary HNPC

Genes*	Primary CRPC (N=56)		Primary HNPC (N=684)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	3	5.36%	3	0.44%	6.98E-03
IL17B	5	8.93%	6	0.88%	6.77E-04
IL17C	1	1.79%	55	8.04%	1.13E-01
IL17D	4	7.14%	16	2.34%	5.73E-02
IL25	4	7.14%	1	0.15%	1.40E-04
IL17F	2	3.57%	1	0.15%	1.61E-02
IL17RA	1	1.79%	7	1.02%	4.69E-01
IL17RB	5	8.93%	6	0.88%	6.77E-04
IL17RC	4	7.14%	18	2.63%	7.72E-02
IL17RD	5	8.93%	4	0.58%	2.08E-04
IL17RE	3	5.36%	16	2.34%	1.68E-01
IL1A	2	3.57%	7	1.02%	1.44E-01
IL1B	2	3.57%	6	0.88%	1.17E-01
IL6	2	3.57%	11	1.61%	2.58E-01
TNF	4	7.14%	5	0.73%	2.79E-03
CSF3	3	5.36%	12	1.75%	9.75E-02
CCL7	3	5.36%	5	0.73%	1.75E-02
CXCL1	3	5.36%	3	0.44%	6.98E-03
CXCL2	3	5.36%	3	0.44%	6.98E-03
CXCL5	3	5.36%	4	0.58%	1.16E-02
CXCL8	5	8.93%	6	0.88%	6.77E-04
S100A7	2	3.57%	7	1.02%	1.44E-01
S100A8	2	3.57%	9	1.32%	2.00E-01
S100A9	2	3.57%	7	1.02%	1.44E-01
CEBPB	4	7.14%	5	0.73%	2.79E-03
PTGS2	4	7.14%	8	1.17%	9.23E-03
MMP1	2	3.57%	7	1.02%	1.44E-01
MMP2	0	0.00%	14	2.05%	6.16E-01
MMP3	2	3.57%	7	1.02%	1.44E-01
MMP7	2	3.57%	9	1.32%	2.00E-01
MMP9	4	7.14%	10	1.46%	1.67E-02
MMP13	2	3.57%	8	1.17%	1.71E-01
PDCD1	1	1.79%	14	2.05%	1.00E+00
CD274	2	3.57%	9	1.32%	2.00E-01
PDCD1LG2	2	3.57%	10	1.46%	2.28E-01

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene alterations in primary CRPC than primary HNPC.

Table 5. Overall gene alterations in metastatic CRPC vs primary HNPC

Genes*	Metastatic CRPC (N=583)		Primary HNPC (N=684)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	23	3.95%	3	0.44%	8.75E-06
IL17B	36	6.17%	6	0.88%	9.91E-08
IL17C	48	8.23%	55	8.04%	9.18E-01
IL17D	31	5.32%	16	2.34%	6.74E-03
IL25	26	4.46%	1	0.15%	1.94E-08
IL17F	23	3.95%	1	0.15%	1.90E-07
IL17RA	24	4.12%	7	1.02%	4.02E-04
IL17RB	26	4.46%	6	0.88%	4.61E-05
IL17RC	42	7.20%	18	2.63%	1.62E-04
IL17RD	32	5.49%	4	0.58%	6.71E-08
IL17RE	43	7.38%	16	2.34%	2.39E-05
IL1A	20	3.43%	7	1.02%	3.28E-03
IL1B	22	3.77%	6	0.88%	7.68E-04
IL6	49	8.40%	11	1.61%	1.32E-08
TNF	28	4.80%	5	0.73%	4.02E-06
CSF3	31	5.32%	12	1.75%	5.23E-04
CCL7	30	5.15%	5	0.73%	1.12E-06
CXCL1	37	6.35%	3	0.44%	3.33E-10
CXCL2	31	5.32%	3	0.44%	4.18E-08
CXCL5	34	5.83%	4	0.58%	1.69E-08
CXCL8	35	6.00%	6	0.88%	1.86E-07
S100A7	76	13.04%	7	1.02%	<2.2e-16
S100A8	73	12.52%	9	1.32%	<2.2e-16
S100A9	72	12.35%	7	1.02%	<2.2e-16
CEBPB	36	6.17%	5	0.73%	2.19E-08
PTGS2	60	10.29%	8	1.17%	1.75E-13
MMP1	38	6.52%	7	1.02%	1.19E-07
MMP2	25	4.29%	14	2.05%	2.28E-02
MMP3	38	6.52%	7	1.02%	1.19E-07
MMP7	34	5.83%	9	1.32%	1.34E-05
MMP9	42	7.20%	10	1.46%	2.33E-07
MMP13	38	6.52%	8	1.17%	2.51E-07
PDCD1	38	6.52%	14	2.05%	8.80E-05
CD274	36	6.17%	9	1.32%	2.55E-06
PDCD1LG2	36	6.17%	10	1.46%	7.09E-06

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene alterations in metastatic CRPC than primary HNPC.

Table 6. Gene amplifications in metastatic vs primary prostate cancer

Genes*	Metastatic (N=584)		Primary (N=742)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	22	3.77%	2	0.27%	1.87E-06
IL17B	34	5.82%	9	1.21%	2.62E-06
IL17C	8	1.37%	6	0.81%	4.19E-01
IL17D	25	4.28%	8	1.08%	2.59E-04
IL25	24	4.11%	4	0.54%	6.93E-06
IL17F	23	3.94%	2	0.27%	9.36E-07
IL17RA	13	2.23%	1	0.13%	1.82E-04
IL17RB	20	3.42%	3	0.40%	2.41E-05
IL17RC	37	6.34%	6	0.81%	1.41E-08
IL17RD	21	3.60%	5	0.67%	1.80E-04
IL17RE	36	6.16%	5	0.67%	5.20E-09
IL1A	14	2.40%	2	0.27%	4.88E-04
IL1B	14	2.40%	2	0.27%	4.88E-04
IL6	47	8.05%	10	1.35%	1.68E-09
TNF	26	4.45%	6	0.81%	1.71E-05
CSF3	31	5.31%	8	1.08%	9.07E-06
CCL7	27	4.62%	3	0.40%	1.62E-07
CXCL1	36	6.16%	4	0.54%	9.29E-10
CXCL2	30	5.14%	4	0.54%	1.29E-07
CXCL5	32	5.48%	5	0.67%	8.43E-08
CXCL8	33	5.65%	8	1.08%	1.59E-06
S100A7	76	13.01%	9	1.21%	<2.2e-16
S100A8	72	12.33%	8	1.08%	<2.2e-16
S100A9	72	12.33%	9	1.21%	<2.2e-16
CEBPB	35	5.99%	8	1.08%	4.51E-07
PTGS2	53	9.08%	6	0.81%	8.75E-14
MMP1	23	3.94%	3	0.40%	2.88E-06
MMP2	8	1.37%	1	0.13%	1.29E-02
MMP3	23	3.94%	3	0.40%	2.88E-06
MMP7	24	4.11%	4	0.54%	6.93E-06
MMP9	33	5.65%	8	1.08%	1.59E-06
MMP13	23	3.94%	3	0.40%	2.88E-06
PDCD1	16	2.74%	8	1.08%	3.59E-02
CD274	14	2.40%	1	0.13%	8.44E-05
PDCD1LG2	14	2.40%	1	0.13%	8.44E-05

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene amplifications in metastatic than primary prostate cancer.

Table 7. Gene deep deletions in metastatic vs primary prostate cancer

Genes*	Metastatic (N=584)		Primary (N=742)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	0	0.00%	1	0.13%	1.00E+00
IL17B	1	0.17%	1	0.13%	1.00E+00
IL17C	39	6.68%	50	6.74%	1.00E+00
IL17D	6	1.03%	12	1.62%	4.75E-01
IL25	2	0.34%	1	0.13%	5.86E-01
IL17F	0	0.00%	1	0.13%	1.00E+00
IL17RA	7	1.20%	3	0.40%	1.17E-01
IL17RB	5	0.86%	5	0.67%	7.57E-01
IL17RC	4	0.68%	14	1.89%	9.17E-02
IL17RD	5	0.86%	3	0.40%	3.11E-01
IL17RE	4	0.68%	14	1.89%	9.17E-02
IL1A	5	0.86%	6	0.81%	1.00E+00
IL1B	5	0.86%	6	0.81%	1.00E+00
IL6	1	0.17%	2	0.27%	1.00E+00
TNF	1	0.17%	2	0.27%	1.00E+00
CSF3	0	0.00%	7	0.94%	2.01E-02
CCL7	3	0.51%	4	0.54%	1.00E+00
CXCL1	1	0.17%	2	0.27%	1.00E+00
CXCL2	1	0.17%	2	0.27%	1.00E+00
CXCL5	1	0.17%	2	0.27%	1.00E+00
CXCL8	1	0.17%	2	0.27%	1.00E+00
S100A7	0	0.00%	0	0.00%	1.00E+00
S100A8	0	0.00%	1	0.13%	1.00E+00
S100A9	0	0.00%	0	0.00%	1.00E+00
CEBPB	0	0.00%	0	0.00%	1.00E+00
PTGS2	3	0.51%	2	0.27%	6.60E-01
MMP1	13	2.23%	5	0.67%	1.74E-02
MMP2	15	2.57%	12	1.62%	2.44E-01
MMP3	13	2.23%	5	0.67%	1.74E-02
MMP7	10	1.71%	6	0.81%	2.04E-01
MMP9	3	0.51%	2	0.27%	6.60E-01
MMP13	12	2.05%	6	0.81%	5.83E-02
PDCD1	21	3.60%	6	0.81%	5.65E-04
CD274	21	3.60%	10	1.35%	9.50E-03
PDCD1LG2	21	3.60%	10	1.35%	9.50E-03

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene deep deletions in metastatic than primary prostate cancer and green indicates significantly more gene deep deletions in primary than metastatic prostate cancer.

Table 8. Gene missense mutations in metastatic vs primary prostate cancer

Genes*	Metastatic (N=584)		Primary (N=742)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	1	0.17%	3	0.40%	6.35E-01
IL17B	1	0.17%	1	0.13%	1.00E+00
IL17C	2	0.34%	0	0.00%	1.94E-01
IL17D	0	0.00%	0	0.00%	1.00E+00
IL25	0	0.00%	1	0.13%	1.00E+00
IL17F	0	0.00%	0	0.00%	1.00E+00
IL17RA	5	0.86%	3	0.40%	3.11E-01
IL17RB	0	0.00%	2	0.27%	5.07E-01
IL17RC	1	0.17%	2	0.27%	1.00E+00
IL17RD	6	1.03%	1	0.13%	4.84E-02
IL17RE	5	0.86%	0	0.00%	1.64E-02
IL1A	0	0.00%	1	0.13%	1.00E+00
IL1B	3	0.51%	0	0.00%	8.52E-02
IL6	1	0.17%	1	0.13%	1.00E+00
TNF	1	0.17%	1	0.13%	1.00E+00
CSF3	0	0.00%	0	0.00%	1.00E+00
CCL7	0	0.00%	1	0.13%	1.00E+00
CXCL1	0	0.00%	0	0.00%	1.00E+00
CXCL2	0	0.00%	0	0.00%	1.00E+00
CXCL5	1	0.17%	0	0.00%	4.40E-01
CXCL8	1	0.17%	1	0.13%	1.00E+00
S100A7	0	0.00%	0	0.00%	1.00E+00
S100A8	1	0.17%	2	0.27%	1.00E+00
S100A9	0	0.00%	0	0.00%	1.00E+00
CEBPB	0	0.00%	0	0.00%	1.00E+00
PTGS2	2	0.34%	4	0.54%	7.00E-01
MMP1	2	0.34%	1	0.13%	5.86E-01
MMP2	2	0.34%	1	0.13%	5.86E-01
MMP3	2	0.34%	1	0.13%	5.86E-01
MMP7	0	0.00%	1	0.13%	1.00E+00
MMP9	5	0.86%	4	0.54%	5.18E-01
MMP13	1	0.17%	1	0.13%	1.00E+00
PDCD1	1	0.17%	1	0.13%	1.00E+00
CD274	1	0.17%	0	0.00%	4.40E-01
PDCD1LG2	1	0.17%	1	0.13%	1.00E+00

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene missense mutations in metastatic than primary prostate cancer.

Table 9. Gene truncating mutations in metastatic vs primary prostate cancer

Genes*	Metastatic (N=584)		Primary (N=742)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	0	0.00%	0	0.00%	1.00E+00
IL17B	0	0.00%	0	0.00%	1.00E+00
IL17C	0	0.00%	0	0.00%	1.00E+00
IL17D	0	0.00%	0	0.00%	1.00E+00
IL25	0	0.00%	0	0.00%	1.00E+00
IL17F	0	0.00%	0	0.00%	1.00E+00
IL17RA	0	0.00%	1	0.13%	1.00E+00
IL17RB	1	0.17%	1	0.13%	1.00E+00
IL17RC	0	0.00%	0	0.00%	1.00E+00
IL17RD	0	0.00%	0	0.00%	1.00E+00
IL17RE	0	0.00%	0	0.00%	1.00E+00
IL1A	1	0.17%	0	0.00%	4.40E-01
IL1B	0	0.00%	0	0.00%	1.00E+00
IL6	0	0.00%	0	0.00%	1.00E+00
TNF	0	0.00%	0	0.00%	1.00E+00
CSF3	0	0.00%	0	0.00%	1.00E+00
CCL7	0	0.00%	0	0.00%	1.00E+00
CXCL1	0	0.00%	0	0.00%	1.00E+00
CXCL2	0	0.00%	0	0.00%	1.00E+00
CXCL5	0	0.00%	0	0.00%	1.00E+00
CXCL8	0	0.00%	0	0.00%	1.00E+00
S100A7	0	0.00%	0	0.00%	1.00E+00
S100A8	0	0.00%	0	0.00%	1.00E+00
S100A9	0	0.00%	0	0.00%	1.00E+00
CEBPB	1	0.17%	0	0.00%	4.40E-01
PTGS2	2	0.34%	0	0.00%	1.94E-01
MMP1	0	0.00%	0	0.00%	1.00E+00
MMP2	0	0.00%	0	0.00%	1.00E+00
MMP3	0	0.00%	0	0.00%	1.00E+00
MMP7	0	0.00%	0	0.00%	1.00E+00
MMP9	1	0.17%	0	0.00%	4.40E-01
MMP13	2	0.34%	0	0.00%	1.94E-01
PDCD1	0	0.00%	0	0.00%	1.00E+00
CD274	0	0.00%	0	0.00%	1.00E+00
PDCD1LG2	0	0.00%	0	0.00%	1.00E+00

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*.

Table 10. Overall gene alterations in primary NEPC vs primary AC

Genes*	Primary NEPC (N=16)		Primary AC (N=724)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	1	6.25%	5	0.69%	1.23E-01
IL17B	2	12.50%	8	1.10%	1.78E-02
IL17C	0	0.00%	56	7.73%	6.25E-01
IL17D	0	0.00%	20	2.76%	1.00E+00
IL25	2	12.50%	3	0.41%	4.22E-03
IL17F	1	6.25%	2	0.28%	6.36E-02
IL17RA	0	0.00%	8	1.10%	1.00E+00
IL17RB	2	12.50%	9	1.24%	2.15E-02
IL17RC	1	6.25%	20	2.76%	3.72E-01
IL17RD	1	6.25%	8	1.10%	1.80E-01
IL17RE	1	6.25%	18	2.49%	3.43E-01
IL1A	1	6.25%	8	1.10%	1.80E-01
IL1B	1	6.25%	7	0.97%	1.61E-01
IL6	0	0.00%	13	1.80%	1.00E+00
TNF	2	12.50%	7	0.97%	1.45E-02
CSF3	1	6.25%	14	1.93%	2.82E-01
CCL7	1	6.25%	7	0.97%	1.61E-01
CXCL1	0	0.00%	6	0.83%	1.00E+00
CXCL2	0	0.00%	6	0.83%	1.00E+00
CXCL5	0	0.00%	7	0.97%	1.00E+00
CXCL8	2	12.50%	9	1.24%	2.15E-02
S100A7	0	0.00%	9	1.24%	1.00E+00
S100A8	0	0.00%	11	1.52%	1.00E+00
S100A9	0	0.00%	9	1.24%	1.00E+00
CEBPB	2	12.50%	7	0.97%	1.45E-02
PTGS2	0	0.00%	11	1.52%	1.00E+00
MMP1	1	6.25%	8	1.10%	1.80E-01
MMP2	0	0.00%	14	1.93%	1.00E+00
MMP3	1	6.25%	8	1.10%	1.80E-01
MMP7	1	6.25%	10	1.38%	2.15E-01
MMP9	2	12.50%	12	1.66%	3.43E-02
MMP13	1	6.25%	9	1.24%	1.97E-01
PDCD1	1	6.25%	14	1.93%	2.82E-01
CD274	0	0.00%	11	1.52%	1.00E+00
PDCD1LG2	0	0.00%	12	1.66%	1.00E+00

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene alterations in primary NEPC than primary AC.

Table 11. Overall gene alterations in metastatic NEPC vs metastatic AC

Genes*	Metastatic NEPC (N=45)		Metastatic AC (N=461)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	10	22.22%	11	2.39%	1.95E-06
IL17B	9	20.00%	24	5.21%	1.19E-03
IL17C	2	4.44%	33	7.16%	7.58E-01
IL17D	8	17.78%	22	4.77%	2.75E-03
IL25	9	20.00%	15	3.25%	7.50E-05
IL17F	10	22.22%	10	2.17%	1.09E-06
IL17RA	3	6.67%	16	3.47%	2.34E-01
IL17RB	6	13.33%	17	3.69%	1.12E-02
IL17RC	8	17.78%	31	6.72%	1.54E-02
IL17RD	7	15.56%	20	4.34%	6.24E-03
IL17RE	10	22.22%	30	6.51%	1.29E-03
IL1A	5	11.11%	11	2.39%	9.25E-03
IL1B	6	13.33%	13	2.82%	3.97E-03
IL6	9	20.00%	39	8.46%	2.73E-02
TNF	12	26.67%	14	3.04%	2.19E-07
CSF3	9	20.00%	22	4.77%	7.15E-04
CCL7	10	22.22%	19	4.12%	6.45E-05
CXCL1	5	11.11%	30	6.51%	2.24E-01
CXCL2	5	11.11%	25	5.42%	1.73E-01
CXCL5	5	11.11%	27	5.86%	1.90E-01
CXCL8	5	11.11%	28	6.07%	2.00E-01
S100A7	13	28.89%	55	11.93%	4.39E-03
S100A8	13	28.89%	52	11.28%	3.63E-03
S100A9	13	28.89%	52	11.28%	3.63E-03
CEBPB	13	28.89%	22	4.77%	1.37E-06
PTGS2	13	28.89%	42	9.11%	3.64E-04
MMP1	6	13.33%	30	6.51%	1.19E-01
MMP2	1	2.22%	19	4.12%	1.00E+00
MMP3	6	13.33%	30	6.51%	1.19E-01
MMP7	6	13.33%	26	5.64%	5.40E-02
MMP9	15	33.33%	26	5.64%	2.02E-07
MMP13	6	13.33%	29	6.29%	1.13E-01
PDCD1	5	11.11%	26	5.64%	1.81E-01
CD274	9	20.00%	22	4.77%	7.15E-04
PDCD1LG2	9	20.00%	23	4.99%	9.28E-04

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene alterations in metastatic NEPC than metastatic AC.

Table 12. Overall gene alterations in metastatic NEPC vs metastatic AC with NE feature

Genes*	Metastatic NEPC (N=45)		Metastatic AC with NE feature (N=12)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	10	22.22%	0	0.00%	9.97E-02
IL17B	9	20.00%	1	8.33%	6.71E-01
IL17C	2	4.44%	1	8.33%	5.15E-01
IL17D	8	17.78%	1	8.33%	6.67E-01
IL25	9	20.00%	1	8.33%	6.71E-01
IL17F	10	22.22%	0	0.00%	9.97E-02
IL17RA	3	6.67%	1	8.33%	1.00E+00
IL17RB	6	13.33%	1	8.33%	1.00E+00
IL17RC	8	17.78%	2	16.67%	1.00E+00
IL17RD	7	15.56%	2	16.67%	1.00E+00
IL17RE	10	22.22%	2	16.67%	1.00E+00
IL1A	5	11.11%	1	8.33%	1.00E+00
IL1B	6	13.33%	1	8.33%	1.00E+00
IL6	9	20.00%	1	8.33%	6.71E-01
TNF	12	26.67%	0	0.00%	5.28E-02
CSF3	9	20.00%	1	8.33%	6.71E-01
CCL7	10	22.22%	1	8.33%	4.26E-01
CXCL1	5	11.11%	0	0.00%	5.73E-01
CXCL2	5	11.11%	0	0.00%	5.73E-01
CXCL5	5	11.11%	0	0.00%	5.73E-01
CXCL8	5	11.11%	0	0.00%	5.73E-01
S100A7	13	28.89%	0	0.00%	4.98E-02
S100A8	13	28.89%	0	0.00%	4.98E-02
S100A9	13	28.89%	0	0.00%	4.98E-02
CEBPB	13	28.89%	1	8.33%	2.58E-01
PTGS2	13	28.89%	3	25.00%	1.00E+00
MMP1	6	13.33%	1	8.33%	1.00E+00
MMP2	1	2.22%	2	16.67%	1.09E-01
MMP3	6	13.33%	1	8.33%	1.00E+00
MMP7	6	13.33%	1	8.33%	1.00E+00
MMP9	15	33.33%	1	8.33%	1.48E-01
MMP13	6	13.33%	1	8.33%	1.00E+00
PDCD1	5	11.11%	1	8.33%	1.00E+00
CD274	9	20.00%	2	16.67%	1.00E+00
PDCD1LG2	9	20.00%	2	16.67%	1.00E+00

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene alterations in metastatic NEPC than metastatic AC with NE feature.

Table 13. Overall gene alterations in metastatic AC with NE feature vs metastatic AC

Genes*	Metastatic AC with NE feature (N=12)		Metastatic AC (N=461)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	0	0.00%	11	2.39%	1.00E+00
IL17B	1	8.33%	24	5.21%	4.83E-01
IL17C	1	8.33%	33	7.16%	5.96E-01
IL17D	1	8.33%	22	4.77%	4.54E-01
IL25	1	8.33%	15	3.25%	3.42E-01
IL17F	0	0.00%	10	2.17%	1.00E+00
IL17RA	1	8.33%	16	3.47%	3.59E-01
IL17RB	1	8.33%	17	3.69%	3.76E-01
IL17RC	2	16.67%	31	6.72%	2.01E-01
IL17RD	2	16.67%	20	4.34%	1.03E-01
IL17RE	2	16.67%	30	6.51%	1.92E-01
IL1A	1	8.33%	11	2.39%	2.68E-01
IL1B	1	8.33%	13	2.82%	3.06E-01
IL6	1	8.33%	39	8.46%	1.00E+00
TNF	0	0.00%	14	3.04%	1.00E+00
CSF3	1	8.33%	22	4.77%	4.54E-01
CCL7	1	8.33%	19	4.12%	4.08E-01
CXCL1	0	0.00%	30	6.51%	1.00E+00
CXCL2	0	0.00%	25	5.42%	1.00E+00
CXCL5	0	0.00%	27	5.86%	1.00E+00
CXCL8	0	0.00%	28	6.07%	1.00E+00
S100A7	0	0.00%	55	11.93%	3.76E-01
S100A8	0	0.00%	52	11.28%	3.78E-01
S100A9	0	0.00%	52	11.28%	3.78E-01
CEBPB	1	8.33%	22	4.77%	4.54E-01
PTGS2	3	25.00%	42	9.11%	9.62E-02
MMP1	1	8.33%	30	6.51%	5.61E-01
MMP2	2	16.67%	19	4.12%	9.48E-02
MMP3	1	8.33%	30	6.51%	5.61E-01
MMP7	1	8.33%	26	5.64%	5.10E-01
MMP9	1	8.33%	26	5.64%	5.10E-01
MMP13	1	8.33%	29	6.29%	5.49E-01
PDCD1	1	8.33%	26	5.64%	5.10E-01
CD274	2	16.67%	22	4.77%	1.19E-01
PDCD1LG2	2	16.67%	23	4.99%	1.28E-01

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Purple indicates more gene alterations in metastatic AC with NE feature than metastatic AC and blue indicates more gene alterations in metastatic AC than metastatic AC with NE feature, but without statistical significance.

amplifications in metastatic NEPC, compared to metastatic AC (**Table 14**). Gene deep deletions (**Table 15**), gene missense mutations (**Table 16**), and gene truncating mutations (**Table 17**) were very rare and there were no significant differences between metastatic NEPC and metastatic AC, except that *MMP9* had significantly higher rate of gene missense mutations in metastatic NEPC than metastatic AC (**Table 16**).

Discussion

In the present study, we included 1303 cases of human prostate cancers in 7 datasets archived in cBioPortal and queried gene alterations in 35 *IL-17* and related genes. We found that 34/35 (97%) genes had significantly more alterations in metastatic prostate cancer (with alteration rates ranging from 3.42% to 13.01%) than primary prostate cancer (with alteration rates ranging from 0.40% to 2.96%). 15/35 (43%) genes had significantly more alterations in primary CRPC than primary HNPC. 34/35 (97%) genes had significantly more alterations in metastatic CRPC than primary HNPC. Only three genes (*S100A7*, *S100A8*, and *S100A9*) had significantly more alterations in metastatic CRPC than primary CRPC. The gene alterations were mostly gene amplifications (97%), while gene deep deletions, missense mutations, and truncating mutations were very rare. 7/35 (20%) genes had significantly more alterations in primary NEPC than primary AC. 23/35 (66%) genes had significantly more alterations in metastatic NEPC (ranging from 11.11% to 33.33%) than metastatic AC (ranging from 2.17% to 11.93%). Only three genes (*S100A7*, *S100A8*, and *S100A9*) had significantly more alterations in metastatic NEPC than metastatic AC with neuroendocrine features. Most of the gene alterations in metastatic NEPC were gene amplifications (80%), while gene deep deletions, missense mutations, and truncating mutations were very rare. A prominent trend is that gene amplifications are more frequently found in metastatic CRPC and NEPC than primary hormone-naïve prostate adenocarcinomas (**Figure 2**).

Three *S100* family members (*S100A7*, *S100A8*, and *S100A9*) are among the genes with the highest gene amplifications (12-13% in metastatic prostate cancers and 28.89% in metastatic NEPC, respectively). S100 proteins comprise a family of 23 different members characterized by high homology, low-molecular weight, and two calcium-binding EF-hands, as well as tissue-specific expression [39]. S100A7/8/9 are induced by IL-17 to recruit neutrophils that protect against infections [40] or exacerbate psoriasis [41]. S100A7 protein expression was found to be increased in prostate cancer compared to normal prostate, and S100A7 promotes survival and invasiveness of prostate cancer cells [42]. S100A8 and S100A9 were found to be up-regulated in prostatic intraepithelial neoplasia and preferentially in high-grade adenocarcinomas, and S100A9 serum levels were significantly elevated in prostate cancer patients compared with patients with benign prostatic hypertrophy or healthy individuals [43]. Gene amplifications of *S100A7/8/9* genes in metastatic CRPC/NEPC may lead to overexpression of them at mRNA and protein levels, which remains to be determined. It is not known if S100A7/8/9 proteins play any roles in the progression from HNPC to CRPC/NEPC and from the primary tumor to metastatic tumor, which warrants future studies.

One potential caveat of the present study is that we cannot completely rule out the possibility of redundant samples among different datasets. For example, we are not sure if any of the sample data from the five U.S. datasets were also deposited into the TCGA dataset. We checked the patient identification (ID) numbers and sample IDs and could not find any overlapping numbers, however, we did not know if the IDs were separately assigned in different datasets. We had to use different datasets because TCGA dataset contains only primary adenocarcinomas. The five U.S. datasets might also share some samples, but we did not find any identical patient IDs. Since more gene amplifications are found in metastatic CRPC/NEPC that mostly (N=415) came from SU2C/PCF Dream Team, we think that the gene alteration rates might not be dramatically affected even if there were a few overlapping cases. Another caveat is that we did not include all *IL-17*-downstream genes, which may miss some genes that may be more important than the ones studied. The choice of these genes is arbitrarily based on our previous research work and we include only the well-known *IL-17*-downstream genes.

In summary, our findings suggest that gene amplifications of *IL-17* and related genes are more frequently found in metastatic CRPC and

Table 14. Gene amplifications in metastatic NEPC vs metastatic AC

Genes*	Metastatic NEPC (N=45)		Metastatic AC (N=461)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	10	22.22%	10	2.17%	1.09E-06
IL17B	9	20.00%	22	4.77%	7.15E-04
IL17C	2	4.44%	6	1.30%	1.54E-01
IL17D	7	15.56%	18	3.90%	3.88E-03
IL25	9	20.00%	15	3.25%	7.50E-05
IL17F	10	22.22%	10	2.17%	1.09E-06
IL17RA	3	6.67%	8	1.74%	6.54E-02
IL17RB	6	13.33%	13	2.82%	3.97E-03
IL17RC	8	17.78%	26	5.64%	6.45E-03
IL17RD	6	13.33%	13	2.82%	3.97E-03
IL17RE	8	17.78%	25	5.42%	5.29E-03
IL1A	5	11.11%	7	1.52%	2.21E-03
IL1B	5	11.11%	7	1.52%	2.21E-03
IL6	9	20.00%	37	8.03%	1.38E-02
TNF	12	26.67%	12	2.60%	6.98E-08
CSF3	9	20.00%	22	4.77%	7.15E-04
CCL7	10	22.22%	16	3.47%	2.09E-05
CXCL1	5	11.11%	29	6.29%	2.12E-01
CXCL2	5	11.11%	24	5.21%	1.66E-01
CXCL5	5	11.11%	25	5.42%	1.73E-01
CXCL8	5	11.11%	27	5.86%	1.90E-01
S100A7	13	28.89%	55	11.93%	4.39E-03
S100A8	13	28.89%	52	11.28%	3.63E-03
S100A9	13	28.89%	52	11.28%	3.63E-03
CEBPB	13	28.89%	21	4.56%	9.18E-07
PTGS2	12	26.67%	37	8.03%	4.58E-04
MMP1	6	13.33%	16	3.47%	8.87E-03
MMP2	1	2.22%	6	1.30%	4.81E-01
MMP3	6	13.33%	16	3.47%	8.87E-03
MMP7	6	13.33%	17	3.69%	1.12E-02
MMP9	13	28.89%	20	4.34%	6.04E-07
MMP13	6	13.33%	16	3.47%	8.87E-03
PDCD1	4	8.89%	12	2.60%	4.50E-02
CD274	8	17.78%	5	1.08%	1.95E-06
PDCD1LG2	8	17.78%	5	1.08%	1.95E-06

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene amplifications in metastatic NEPC than metastatic AC.

Table 15. Gene deep deletions in metastatic NEPC vs metastatic AC

Genes*	Metastatic NEPC (N=45)		Metastatic AC (N=461)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	0	0.00%	0	0.00%	1.00E+00
IL17B	0	0.00%	1	0.22%	1.00E+00
IL17C	0	0.00%	26	5.64%	1.54E-01
IL17D	1	2.22%	4	0.87%	3.74E-01
IL25	0	0.00%	0	0.00%	1.00E+00
IL17F	0	0.00%	0	0.00%	1.00E+00
IL17RA	0	0.00%	6	1.30%	1.00E+00
IL17RB	0	0.00%	3	0.65%	1.00E+00
IL17RC	0	0.00%	4	0.87%	1.00E+00
IL17RD	0	0.00%	4	0.87%	1.00E+00
IL17RE	0	0.00%	4	0.87%	1.00E+00
IL1A	0	0.00%	4	0.87%	1.00E+00
IL1B	0	0.00%	4	0.87%	1.00E+00
IL6	0	0.00%	1	0.22%	1.00E+00
TNF	0	0.00%	1	0.22%	1.00E+00
CSF3	0	0.00%	0	0.00%	1.00E+00
CCL7	0	0.00%	3	0.65%	1.00E+00
CXCL1	0	0.00%	1	0.22%	1.00E+00
CXCL2	0	0.00%	1	0.22%	1.00E+00
CXCL5	0	0.00%	1	0.22%	1.00E+00
CXCL8	0	0.00%	1	0.22%	1.00E+00
S100A7	0	0.00%	0	0.00%	1.00E+00
S100A8	0	0.00%	0	0.00%	1.00E+00
S100A9	0	0.00%	0	0.00%	1.00E+00
CEBPB	0	0.00%	0	0.00%	1.00E+00
PTGS2	1	2.22%	1	0.22%	1.70E-01
MMP1	0	0.00%	12	2.60%	6.13E-01
MMP2	0	0.00%	11	2.39%	6.10E-01
MMP3	0	0.00%	12	2.60%	6.13E-01
MMP7	0	0.00%	9	1.95%	1.00E+00
MMP9	0	0.00%	3	0.65%	1.00E+00
MMP13	0	0.00%	11	2.39%	6.10E-01
PDCD1	1	2.22%	13	2.82%	1.00E+00
CD274	1	2.22%	17	3.69%	1.00E+00
PDCD1LG2	1	2.22%	17	3.69%	1.00E+00

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*.

Table 16. Gene missense mutations in metastatic NEPC vs metastatic AC

Genes*	Metastatic NEPC (N=45)		Metastatic AC (N=461)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	0	0.00%	1	0.22%	1.00E+00
IL17B	0	0.00%	1	0.22%	1.00E+00
IL17C	1	2.22%	1	0.22%	1.70E-01
IL17D	0	0.00%	0	0.00%	1.00E+00
IL25	0	0.00%	0	0.00%	1.00E+00
IL17F	0	0.00%	0	0.00%	1.00E+00
IL17RA	0	0.00%	2	0.43%	1.00E+00
IL17RB	0	0.00%	0	0.00%	1.00E+00
IL17RC	0	0.00%	1	0.22%	1.00E+00
IL17RD	1	2.22%	3	0.65%	3.12E-01
IL17RE	2	4.44%	3	0.65%	6.51E-02
IL1A	0	0.00%	0	0.00%	1.00E+00
IL1B	1	2.22%	2	0.43%	2.44E-01
IL6	0	0.00%	1	0.22%	1.00E+00
TNF	0	0.00%	1	0.22%	1.00E+00
CSF3	0	0.00%	0	0.00%	1.00E+00
CCL7	0	0.00%	0	0.00%	1.00E+00
CXCL1	0	0.00%	0	0.00%	1.00E+00
CXCL2	0	0.00%	0	0.00%	1.00E+00
CXCL5	0	0.00%	1	0.22%	1.00E+00
CXCL8	0	0.00%	0	0.00%	1.00E+00
S100A7	0	0.00%	0	0.00%	1.00E+00
S100A8	0	0.00%	0	0.00%	1.00E+00
S100A9	0	0.00%	0	0.00%	1.00E+00
CEBPB	0	0.00%	0	0.00%	1.00E+00
PTGS2	0	0.00%	2	0.43%	1.00E+00
MMP1	0	0.00%	2	0.43%	1.00E+00
MMP2	0	0.00%	2	0.43%	1.00E+00
MMP3	0	0.00%	2	0.43%	1.00E+00
MMP7	0	0.00%	0	0.00%	1.00E+00
MMP9	2	4.44%	2	0.43%	4.14E-02
MMP13	0	0.00%	1	0.22%	1.00E+00
PDCD1	0	0.00%	1	0.22%	1.00E+00
CD274	0	0.00%	0	0.00%	1.00E+00
PDCD1LG2	0	0.00%	1	0.22%	1.00E+00

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*. Red indicates significantly more gene missense mutations in metastatic NEPC than metastatic AC.

Table 17. Gene truncating mutations in metastatic NEPC vs metastatic AC

Genes*	Metastatic NEPC (N=45)		Metastatic AC (N=461)		*P*-value (Fisher's exact)
	Number	Percent	Number	Percent	
IL17A	0	0.00%	0	0.00%	1.00E+00
IL17B	0	0.00%	0	0.00%	1.00E+00
IL17C	0	0.00%	0	0.00%	1.00E+00
IL17D	0	0.00%	0	0.00%	1.00E+00
IL25	0	0.00%	0	0.00%	1.00E+00
IL17F	0	0.00%	0	0.00%	1.00E+00
IL17RA	0	0.00%	0	0.00%	1.00E+00
IL17RB	0	0.00%	1	0.22%	1.00E+00
IL17RC	0	0.00%	0	0.00%	1.00E+00
IL17RD	0	0.00%	0	0.00%	1.00E+00
IL17RE	0	0.00%	0	0.00%	1.00E+00
IL1A	0	0.00%	0	0.00%	1.00E+00
IL1B	0	0.00%	0	0.00%	1.00E+00
IL6	0	0.00%	0	0.00%	1.00E+00
TNF	0	0.00%	0	0.00%	1.00E+00
CSF3	0	0.00%	0	0.00%	1.00E+00
CCL7	0	0.00%	0	0.00%	1.00E+00
CXCL1	0	0.00%	0	0.00%	1.00E+00
CXCL2	0	0.00%	0	0.00%	1.00E+00
CXCL5	0	0.00%	0	0.00%	1.00E+00
CXCL8	0	0.00%	0	0.00%	1.00E+00
S100A7	0	0.00%	0	0.00%	1.00E+00
S100A8	0	0.00%	0	0.00%	1.00E+00
S100A9	0	0.00%	0	0.00%	1.00E+00
CEBPB	0	0.00%	1	0.22%	1.00E+00
PTGS2	0	0.00%	2	0.43%	1.00E+00
MMP1	0	0.00%	0	0.00%	1.00E+00
MMP2	0	0.00%	0	0.00%	1.00E+00
MMP3	0	0.00%	0	0.00%	1.00E+00
MMP7	0	0.00%	0	0.00%	1.00E+00
MMP9	0	0.00%	1	0.22%	1.00E+00
MMP13	0	0.00%	1	0.22%	1.00E+00
PDCD1	0	0.00%	0	0.00%	1.00E+00
CD274	0	0.00%	0	0.00%	1.00E+00
PDCD1LG2	0	0.00%	0	0.00%	1.00E+00

**IL17A,* also named interleukin-17A, *IL17*; *IL17B,* also named interleukin-17B, *IL-20*; *IL17C,* also named interleukin-17C, *CX2*; *IL17D,* also named interleukin-17D, *IL-27*; *IL25,* also named interleukin-25, *IL17E*; *IL17F,* also named interleukin-17F, *IL-17F*; *IL17RA,* also named interleukin-17 Receptor A, *IL-17RA*; *IL17RB,* also named interleukin-17 Receptor B, *IL-17RB*; *IL17RC,* also named interleukin-17 Receptor C, *IL-17RL*; *IL17RD,* also named interleukin-17 Receptor D, *IL-17RD*; *IL17RE,* also named interleukin-17 Receptor E, *IL-17RE*; *IL1A,* also named interleukin-1 Alpha, *IL1F1*; *IL1B,* also named interleukin-1 Beta, *IL-1 Beta*; *IL6,* also named interleukin-6, *IL-6*; *TNF,* also named Tumor Necrosis Factor, *TNFA*; *CSF3,* also named Colony Stimulating Factor-3, *GCSF*; *CCL7,* also named C-C Motif Chemokine Ligand-7, *SCYA6*; *CXCL1,* also named C-X-C Motif Chemokine Ligand-1, *NAP-3*; *CXCL2,* also named C-X-C Motif Chemokine Ligand-2, *MIP2A*; *CXCL5,* also named C-X-C Motif Chemokine Ligand-5, *SCYB5*; *CXCL8,* also named C-X-C Motif Chemokine Ligand-8, *GCP-1*; *S100A7,* also named S100 Calcium Binding Protein A7, *PSOR1*; *S100A8,* also named S100 Calcium Binding Protein A8, *CAGA*; *S100A9,* also named S100 Calcium Binding Protein A9, *MRP-14*; *CEBPB,* also named CCAAT Enhancer Binding Protein Beta, *TCF5*; *PTGS2,* also named Prostaglandin-Endoperoxide Synthase-2, *PGHS-2*; *MMP1,* also named Matrix Metallopeptidase-1, *CLG*; *MMP2,* also named Matrix Metallopeptidase-2, *CLG4A*; *MMP3,* also named Matrix Metallopeptidase-3, *STMY1*; *MMP7,* also named Matrix Metallopeptidase-7, *MMP-7*; *MMP9,* also named Matrix Metallopeptidase-9, *CLG4B*; *MMP13,* also named Matrix Metallopeptidase-13, *MMP-13*; *PDCD1,* also named Programmed Cell Death 1, *PD1*; *CD274,* also named CD274 Molecule, *PD-L1*; *PDCD1LG2,* also named Programmed Cell Death 1 Ligand 2, *PD-L2*.

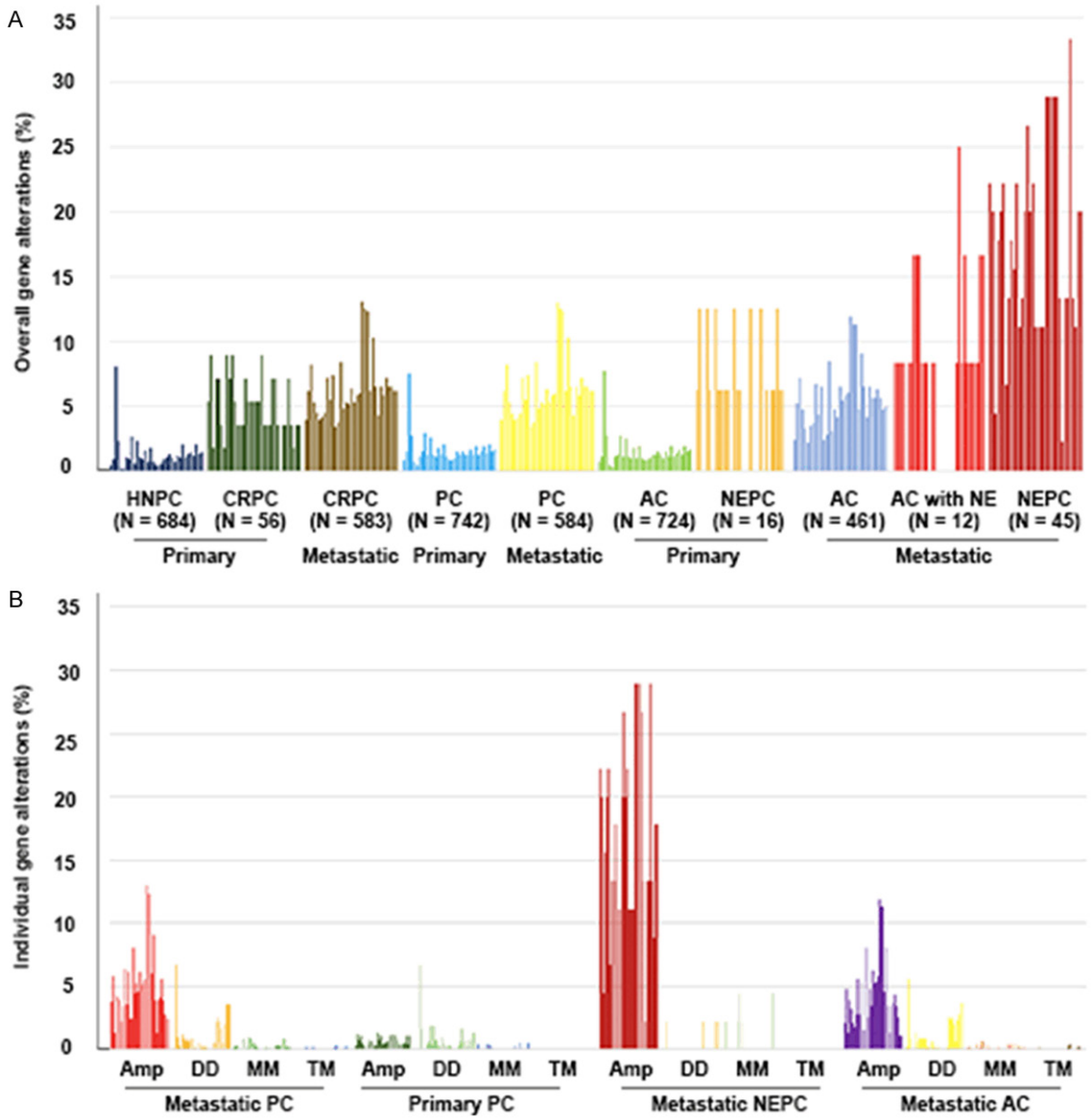

Figure 2. Trend of gene alterations in prostate cancers. A. Overall gene alterations. B. Individual gene alterations including amplifications (Amp), deep deletions (DD), missense mutations (MM), and truncating mutations (TM). Each bar represents a gene; gene names (from left to right) within each color-coded block are the same as shown in **Figure 1** or **Table 2** (from top to bottom). HNPC, hormone-naïve prostate cancer; CRPC, castration-resistant prostate cancer; PC, prostate cancer including HNPC and CRPC; AC, adenocarcinoma; NEPC, neuroendocrine prostate cancer; NE, neuroendocrine feature.

NEPC than primary hormone-naïve prostate adenocarcinomas, implying that *IL-17* and related genes may play important roles in the progression from HNPC to CRPC and from primary location to metastasis as well as in development of metastatic NEPC. Future studies shall determine if expression at mRNA and protein levels of these genes is increased in metastatic CRPC and NEPC and if the genes function to promote prostate cancer progression.

Acknowledgements

This work is part of Mr. Ruoxin Lan's thesis required to obtain a Master's Degree. All of the research activities were conducted at VA and Tulane University and all intellectual property rights belong to the U.S. institutions. The results shown here are in whole or part based upon data generated by the TCGA Research Network: https://www.cancer.gov/tcga. Dr.

Zongbing You was partially supported by a Merit Review Award (I01BX004158) from the Department of Veterans Affairs, Veterans Health Administration, Office of Research and Development, Biomedical Laboratory Research & Development Service. Dr. Zongbing You is a Research Physiologist employed by the Research Service, Southeast Louisiana Veterans Health Care System, New Orleans, LA-629. Dr. Kun Zhang was partially supported by Research Centers in Minority Institutions (RCMI) funded by National Institutes of Health (2U54-MD007595). The content of this article is solely the responsibility of the authors and does not necessarily represent the official views or policies of the Department of Veterans Affairs, National Institutes of Health, or the United States government.

Disclosure of conflict of interest

None.

Address correspondence to: Dr. Zongbing You, Department of Structural & Cellular Biology, Tulane University School of Medicine, 1430 Tulane Avenue Mailbox 8649, New Orleans, Louisiana 70112, USA; Southeast Louisiana Veterans Health Care System, 2400 Canal St, New Orleans, LA 70119, USA. Tel: +1-504-988-0467; +1-504-507-2000 Ext. 67364; Fax: +1-504-988-1687; E-mail: zyou@tulane.edu; Zongbing.You@va.gov

References

[1] Siegel RL, Miller KD and Jemal A. Cancer statistics, 2019. CA Cancer J Clin 2019; 69: 7-34.

[2] Bubendorf L, Schopfer A, Wagner U, Sauter G, Moch H, Willi N, Gasser TC and Mihatsch MJ. Metastatic patterns of prostate cancer: an autopsy study of 1,589 patients. Hum Pathol 2000; 31: 578-583.

[3] Patel GK, Chugh N and Tripathi M. Neuroendocrine differentiation of prostate cancer-an intriguing example of tumor evolution at play. Cancers (Basel) 2019; 11.

[4] Moseley TA, Haudenschild DR, Rose L and Reddi AH. Interleukin-17 family and IL-17 receptors. Cytokine Growth Factor Rev 2003; 14: 155-174.

[5] McGeachy MJ, Cua DJ and Gaffen SL. The IL-17 family of cytokines in health and disease. Immunity 2019; 50: 892-906.

[6] Su Y, Huang J, Zhao X, Lu H, Wang W, Yang XO, Shi Y, Wang X, Lai Y and Dong C. Interleukin-17 receptor D constitutes an alternative receptor for interleukin-17A important in psoriasis-like skin inflammation. Sci Immunol 2019; 4.

[7] McAleer JP and Kolls JK. Directing traffic: IL-17 and IL-22 coordinate pulmonary immune defense. Immunol Rev 2014; 260: 129-144.

[8] Liu C, Qian W, Qian Y, Giltiay NV, Lu Y, Swaidani S, Misra S, Deng L, Chen ZJ and Li X. Act1, a U-box E3 ubiquitin ligase for IL-17 signaling. Sci Signal 2009; 2: ra63.

[9] Onishi RM and Gaffen SL. Interleukin-17 and its target genes: mechanisms of interleukin-17 function in disease. Immunology 2010; 129: 311-321.

[10] Shen F, Ruddy MJ, Plamondon P and Gaffen SL. Cytokines link osteoblasts and inflammation: microarray analysis of interleukin-17- and TNF-alpha-induced genes in bone cells. J Leukoc Biol 2005; 77: 388-399.

[11] Li Q, Liu L, Zhang Q, Liu S, Ge D and You Z. Interleukin-17 indirectly promotes M2 macrophage differentiation through stimulation of COX-2/PGE2 pathway in the cancer cells. Cancer Res Treat 2014; 46: 297-306.

[12] You Z, Ge D, Liu S, Zhang Q, Borowsky AD and Melamed J. Interleukin-17 induces expression of chemokines and cytokines in prostatic epithelial cells but does not stimulate cell growth in vitro. Int J Med Biol Front 2012; 18: 629-644.

[13] Zhang Q, Liu S, Ge D, Xue Y, Xiong Z, Abdel-Mageed AB, Myers L, Hill SM, Rowan BG, Sartor O, Melamed J, Chen Z and You Z. Interleukin-17 promotes formation and growth of prostate adenocarcinoma in mouse models. Cancer Res 2012; 72: 2589-2599.

[14] Zhang Q, Liu S, Parajuli KR, Zhang W, Zhang K, Mo Z, Liu J, Chen Z, Yang S, Wang AR, Myers L and You Z. Interleukin-17 promotes prostate cancer via MMP7-induced epithelial-to-mesenchymal transition. Oncogene 2017; 36: 687-699.

[15] Li X, Bechara R, Zhao J, McGeachy MJ and Gaffen SL. IL-17 receptor-based signaling and implications for disease. Nat Immunol 2019; 20: 1594-1602.

[16] Wang X, Yang L, Huang F, Zhang Q, Liu S, Ma L and You Z. Inflammatory cytokines IL-17 and TNF-alpha up-regulate PD-L1 expression in human prostate and colon cancer cells. Immunol Lett 2017; 184: 7-14.

[17] Wu S, Rhee KJ, Albesiano E, Rabizadeh S, Wu X, Yen HR, Huso DL, Brancati FL, Wick E, McAllister F, Housseau F, Pardoll DM and Sears CL. A human colonic commensal promotes colon tumorigenesis via activation of T helper type 17 T cell responses. Nat Med 2009; 15: 1016-1022.

[18] Chae WJ and Bothwell AL. IL-17F deficiency inhibits small intestinal tumorigenesis in Apc-Min/+ mice. Biochem Biophys Res Commun 2011; 414: 31-36.

[19] Chae WJ, Gibson TF, Zelterman D, Hao L, Henegariu O and Bothwell AL. Ablation of IL-17A abrogates progression of spontaneous intestinal tumorigenesis. Proc Natl Acad Sci U S A 2010; 107: 5540-5544.
[20] Hyun YS, Han DS, Lee AR, Eun CS, Youn JH and Kim HY. Role of IL-17A in the development of colitis-associated cancer. Carcinogenesis 2012; 33: 931-6.
[21] Xiao M, Wang C, Zhang J, Li Z, Zhao X and Qin Z. IFNgamma promotes papilloma development by up-regulating Th17-associated inflammation. Cancer Res 2009; 69: 2010-2017.
[22] Wang L, Yi T, Zhang W, Pardoll DM and Yu H. IL-17 enhances tumor development in carcinogen-induced skin cancer. Cancer Res 2010; 70: 10112-10120.
[23] Novitskiy SV, Pickup MW, Gorska AE, Owens P, Chytil A, Aakre M, Wu H, Shyr Y and Moses HL. TGF-beta receptor II loss promotes mammary carcinoma progression by Th17 dependent mechanisms. Cancer Discov 2011; 1: 430-441.
[24] Zhang Q, Liu S, Xiong Z, Wang AR, Myers L, Melamed J, Tang WW and You Z. Interleukin-17 promotes development of castration-resistant prostate cancer potentially through creating an immunotolerant and pro-angiogenic tumor microenvironment. Prostate 2014; 74: 869-879.
[25] Chang SH, Mirabolfathinejad SG, Katta H, Cumpian AM, Gong L, Caetano MS, Moghaddam SJ and Dong C. T helper 17 cells play a critical pathogenic role in lung cancer. Proc Natl Acad Sci U S A 2014; 111: 5664-5669.
[26] Xu B, Guenther JF, Pociask DA, Wang Y, Kolls JK, You Z, Chandrasekar B, Shan B, Sullivan DE and Morris GF. Promotion of lung tumor growth by interleukin-17. Am J Physiol Lung Cell Mol Physiol 2014; 307: L497-508.
[27] McAllister F, Bailey JM, Alsina J, Nirschl CJ, Sharma R, Fan H, Rattigan Y, Roeser JC, Lankapalli RH, Zhang H, Jaffee EM, Drake CG, Housseau F, Maitra A, Kolls JK, Sears CL, Pardoll DM and Leach SD. Oncogenic Kras activates a hematopoietic-to-epithelial IL-17 signaling axis in preinvasive pancreatic neoplasia. Cancer Cell 2014; 25: 621-637.
[28] Cunningham D, Zhang Q, Liu S, Parajuli KR, Nie Q, Ma L, Zhang A, Chen Z and You Z. Interleukin-17 promotes metastasis in an immunocompetent orthotopic mouse model of prostate cancer. Am J Clin Exp Urol 2018; 6: 114-122.
[29] Zhang Q, Liu S, Ge D, Cunningham DM, Huang F, Ma L, Burris TP and You Z. Targeting Th17-IL-17 pathway in prevention of micro-invasive prostate cancer in a mouse model. Prostate 2017; 77: 888-899.
[30] Duan Z, Miller HD, Fu X, Ge D, Jin B, Moustafa AA, Lan R, Zhang K, Chen Z and You Z. Th17 cells promote tumor growth in an immunocompetent orthotopic mouse model of prostate cancer. Am J Clin Exp Urol 2019; 7: 249-261.
[31] Cerami E, Gao J, Dogrusoz U, Gross BE, Sumer SO, Aksoy BA, Jacobsen A, Byrne CJ, Heuer ML, Larsson E, Antipin Y, Reva B, Goldberg AP, Sander C and Schultz N. The cBio cancer genomics portal: an open platform for exploring multidimensional cancer genomics data. Cancer Discov 2012; 2: 401-404.
[32] Gao J, Aksoy BA, Dogrusoz U, Dresdner G, Gross B, Sumer SO, Sun Y, Jacobsen A, Sinha R, Larsson E, Cerami E, Sander C and Schultz N. Integrative analysis of complex cancer genomics and clinical profiles using the cBioPortal. Sci Signal 2013; 6: pl1.
[33] Grasso CS, Wu YM, Robinson DR, Cao X, Dhanasekaran SM, Khan AP, Quist MJ, Jing X, Lonigro RJ, Brenner JC, Asangani IA, Ateeq B, Chun SY, Siddiqui J, Sam L, Anstett M, Mehra R, Prensner JR, Palanisamy N, Ryslik GA, Vandin F, Raphael BJ, Kunju LP, Rhodes DR, Pienta KJ, Chinnaiyan AM and Tomlins SA. The mutational landscape of lethal castration-resistant prostate cancer. Nature 2012; 487: 239-243.
[34] Abida W, Cyrta J, Heller G, Prandi D, Armenia J, Coleman I, Cieslik M, Benelli M, Robinson D, Van Allen EM, Sboner A, Fedrizzi T, Mosquera JM, Robinson BD, De Sarkar N, Kunju LP, Tomlins S, Wu YM, Nava Rodrigues D, Loda M, Gopalan A, Reuter VE, Pritchard CC, Mateo J, Bianchini D, Miranda S, Carreira S, Rescigno P, Filipenko J, Vinson J, Montgomery RB, Beltran H, Heath EI, Scher HI, Kantoff PW, Taplin ME, Schultz N, deBono JS, Demichelis F, Nelson PS, Rubin MA, Chinnaiyan AM and Sawyers CL. Genomic correlates of clinical outcome in advanced prostate cancer. Proc Natl Acad Sci U S A 2019; 116: 11428-11436.
[35] Beltran H, Prandi D, Mosquera JM, Benelli M, Puca L, Cyrta J, Marotz C, Giannopoulou E, Chakravarthi BV, Varambally S, Tomlins SA, Nanus DM, Tagawa ST, Van Allen EM, Elemento O, Sboner A, Garraway LA, Rubin MA and Demichelis F. Divergent clonal evolution of castration-resistant neuroendocrine prostate cancer. Nat Med 2016; 22: 298-305.
[36] Barbieri CE, Baca SC, Lawrence MS, Demichelis F, Blattner M, Theurillat JP, White TA, Stojanov P, Van Allen E, Stransky N, Nickerson E, Chae SS, Boysen G, Auclair D, Onofrio RC, Park K, Kitabayashi N, MacDonald TY, Sheikh K, Vuong T, Guiducci C, Cibulskis K, Sivachenko A, Carter SL, Saksena G, Voet D, Hussain WM, Ramos AH, Winckler W, Redman MC, Ardlie K, Tewari AK, Mosquera JM, Rupp N, Wild PJ, Moch H, Morrissey C, Nelson PS, Kantoff PW, Gabriel SB, Golub TR, Meyerson M, Lander ES, Getz G, Rubin MA and Garraway LA. Exome sequencing identifies recurrent SPOP, FOXA1 and

MED12 mutations in prostate cancer. Nat Genet 2012; 44: 685-689.

[37] Kumar A, Coleman I, Morrissey C, Zhang X, True LD, Gulati R, Etzioni R, Bolouri H, Montgomery B, White T, Lucas JM, Brown LG, Dumpit RF, DeSarkar N, Higano C, Yu EY, Coleman R, Schultz N, Fang M, Lange PH, Shendure J, Vessella RL and Nelson PS. Substantial interindividual and limited intraindividual genomic diversity among tumors from men with metastatic prostate cancer. Nat Med 2016; 22: 369-378.

[38] Ren S, Wei GH, Liu D, Wang L, Hou Y, Zhu S, Peng L, Zhang Q, Cheng Y, Su H, Zhou X, Zhang J, Li F, Zheng H, Zhao Z, Yin C, He Z, Gao X, Zhau HE, Chu CY, Wu JB, Collins C, Volik SV, Bell R, Huang J, Wu K, Xu D, Ye D, Yu Y, Zhu L, Qiao M, Lee HM, Yang Y, Zhu Y, Shi X, Chen R, Wang Y, Xu W, Cheng Y, Xu C, Gao X, Zhou T, Yang B, Hou J, Liu L, Zhang Z, Zhu Y, Qin C, Shao P, Pang J, Chung LWK, Xu J, Wu CL, Zhong W, Xu X, Li Y, Zhang X, Wang J, Yang H, Wang J, Huang H and Sun Y. Whole-genome and transcriptome sequencing of prostate cancer identify new genetic alterations driving disease progression. Eur Urol 2017; [Epub ahead of print].

[39] Gebhardt C, Nemeth J, Angel P and Hess J. S100A8 and S100A9 in inflammation and cancer. Biochem Pharmacol 2006; 72: 1622-1631.

[40] Naik S, Bouladoux N, Linehan JL, Han SJ, Harrison OJ, Wilhelm C, Conlan S, Himmelfarb S, Byrd AL, Deming C, Quinones M, Brenchley JM, Kong HH, Tussiwand R, Murphy KM, Merad M, Segre JA and Belkaid Y. Commensal-dendritic-cell interaction specifies a unique protective skin immune signature. Nature 2015; 520: 104-108.

[41] Bertelsen T, Iversen L and Johansen C. The human IL-17A/F heterodimer regulates psoriasis-associated genes through IkappaBzeta. Exp Dermatol 2018; 27: 1048-1052.

[42] Ye L, Sun PH, Martin TA, Sanders AJ, Mason MD and Jiang WG. Psoriasin (S100A7) is a positive regulator of survival and invasion of prostate cancer cells. Urol Oncol 2013; 31: 1576-1583.

[43] Hermani A, Hess J, De Servi B, Medunjanin S, Grobholz R, Trojan L, Angel P and Mayer D. Calcium-binding proteins S100A8 and S100A9 as novel diagnostic markers in human prostate cancer. Clin Cancer Res 2005; 11: 5146-5152.