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Increased cytokine gene expression and cognition risk associated with androgen deprivation therapy

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Abstract

Background: Androgen deprivation therapy (ADT) is a standard treatment modality for locally advanced, high-risk, and metastatic hormone-sensitive prostate cancer. Long-term ADT treatment likely develops side-effects that include changes in cognition or onset of dementia. However, the molecular understanding of this effect remains elusive. We attempt to establish a link between ADT and changes in cognitive function using patient databases and bioinformatics analyses.

Methods: Gene expression profiling was performed using RNA sequencing data from Alzheimer patient cohort and compared with the data from advanced-stage prostate cancer patients receiving neoadjuvant antiandrogen therapy. Differentially expressed genes (DEGs) were analyzed using the Ingenuity knowledge database.

Results: A total of 1952 DEGs in the Alzheimer patient cohort and 101 DEGs were identified in ADT treated prostate cancer patients. Comparing both data sets provided a subset of 33 commonly expressed genes involving cytokine-cytokine signaling with an over representation of cytokine-cytokine receptor interaction, inflammatory cytokines, signaling by interleukins together with alterations in the circulating lymphocyte repertoire, adaptive immune responses, regulation of cytokine production, and changes in T-cell subsets. Additionally, lipopolysaccharide, tumor necrosis factor, and toll-like receptors were identified as upstream transcriptional regulators of these pathways. The most commonly expressed genes viz. *IL-17A, CCL2, IL-10, IL-6, IL-1RN, LIF/LIFR* were further validated by quantitative RT-PCR exhibited higher expression in antiandrogen treated neuronal, glial, and androgen-responsive prostate cancer cells, compared to no-androgen antagonist treatment.

Conclusions: Our findings suggest that changes in cytokine signaling under the influence of ADT in prostate cancer patients may be linked with cognitive impairment presenting new avenues for diagnostic and therapeutic development in combating brain deficits.

KEYWORDS
androgen deprivation therapy, antiandrogens, brain deficits, cognitive impairment, proinflammatory cytokines, prostate cancer

1 | INTRODUCTION

Androgen deprivation therapy (ADT) remains a primary approach in the palliative treatment of advanced-stage prostate cancer in men, and is increasingly used as neoadjuvant therapy for patients diagnosed with high-risk prostate cancer.[1,2] A majority of these patients will eventually receive ADT treatment, as part of their primary therapy, for local invasion, biochemical recurrence, or metastatic spread. ADT decreases testosterone levels, suppresses serum prostate-specific antigens to stabilize disease progression with potential increase in the survival of prostate cancer patients.[3] Despite these benefits, ADT has been associated with an increased risk of anemia, osteoporosis, gynecomastia, erectile dysfunction, and systemic disorders including diabetes and cardiovascular events decreasing the quality-of-life of these patients.[4–6]

A growing body of evidence supports a link between ADT and neurocognitive impairment.[7,8] In humans, behavioral and neurologic effects are recognized with an age-related decrease in circulating testosterone levels and cognitive function.[9] A causal relationship between lower testosterone levels and impaired cognitive function has been associated with diminished neuron growth and axonal regeneration.[10,11] A recent population-based study suggested an association between ADT and Alzheimer's disease[12]; together with an aberrant increase in the folded β-amyloid (Aβ) protein.[13,14] Both testosterone concentration and ADT increases the risk of cardio-metabolic diseases,[15] which are known risk factors for all-cause dementia.[16] Therefore, examination of the relationship of ADT with Alzheimer's disease and general dementia is critical to fully understand the molecular effects of this association.

In this study, we performed bioinformatics analyses on the transcriptomic data of prostate cancer patients who underwent pre- and post-ADT protocol and compared with RNA-sequencing data of postmortem putamen specimens diagnosed with Alzheimer disease and general symptoms of dementia. The outcome of the study was cross analyzed with the TCGA database on prostate cancer patients who received ADT with functional validation on brain and prostate-derived cell lines exposed to antiandrogens.

2 | MATERIALS AND METHODS

2.1 | Cell culture and treatment

Androgen-responsive human prostate cancer LNCaP and C4-2B cells, brain neuronal BT142 cells, and glial M059K cells were used for the experiment. Prostate cancer cells were grown in RPMI 1640 (Cat#SH30027.01; GE Healthcare), and brain cells were grown in Dulbecco's Modified Eagle's Medium and Ham's F12 medium (1:1) (Cat# 30-2006; ATCC) with an additional 0.9% glucose, 4 mM L-glutamine (Cat#30-2214; American Type Culture Collection), 25 µg/ml insulin, 100 µg/ml transferrin, 20 nM progesterone, 15 µM putrescine, and 30 nM selenite. Both cell lines were supplemented with 10% fetal bovine serum, 50 U/ml penicillin, and 50 µg/ml streptomycin in 100 mm tissue culture plates at 37°C in a humidified atmosphere (5% CO_2). All cell lines were treated with enzalutamide (20 µM) (Cat#A10562; Adooq

Bioscience) for 24 h along with their respective control. After the treatment, the cells were harvested and processed for the experiment.

2.2 | Data source

The Gene-Expression Omnibus (GEO) GSE150368 and GSE77668 data sets were downloaded from the National Center for Biotechnology Information (NCBI) database. The RNA sequencing data were downloaded using Version info: R 3.2.3, Biobase 2.30.0, GEOquery 2.40.0, limma 3.26.8. RNA sequencing analysis (GSE150368) was performed on paired pre-ADT and post-ADT prostate cancer specimens and paired benign and cancer tissue from patients receiving neoadjuvant ADT.[17] A total of 22 samples were subjected to RNA sequencing analysis. The GSE77668 data set consists of postmortem putamen samples of Alzheimer's disease patients.

In another analysis, RNA sequencing data of 107 patient's brain, including 377 specimens from different regions of the brain viz. cortical gray (parietal and temporal) and white matter (parietal) and hippocampus with dementia and Alzheimer's disease data were downloaded from the University of Washington, Washington Health Research Institute and the Allen Institute for Brain Science. From the above, 223 samples were considered for analysis and the female patient specimens were excluded from the study. The expression of cytokines, cytokines receptors, and growth factors were screened. The expression of each gene was represented in the form of heat map using a Z-score intensity scale.

2.3 | Data analysis

QIAGEN's Ingenuity® Pathway Analysis (IPA®, QIAGEN Redwood City, www.qiagen.com/ingenuity) was used as a comparison module tool to analyze the up- and downregulated differentially expressed genes (DEGs). The prostate cancer RNA sequence database GSE150368 was compared with normal putamen tissue and postmortem putamen samples of dementia with an additional neurological diagnosis of Alzheimer's disease (GSE77668).[18] The GSE77668 data were analyzed using the platform GPL21436, NanoString nCounter gene expression system for the association between prostate cancer receiving ADT and risk of aging-related disorders. The data sets were analyzed statistically with a score cutoff of −log (p value) <1.3, and Fisher's exact test p value was applied and adjusted for multiple testing using the Benjamini −Hochberg (B−H) method for false discovery rate (FDR correction.

2.4 | Cytokines gene expression in ADT treated patients

The cBioPortal (https://www.cbioportal.org; accessed on December 5, 2021) was used to explore prostate cancer patients who underwent ADT. A total of 198 patients' data were utilized for the analysis of which n = 92 patients who underwent ADT receiving second

generation antiandrogens (enzalutamide and abiraterone acetate) were compared with the expression value of $n = 106$ naïve patients (no ADT treatment). The average age of both patient cohorts who received ADT and no-ADT was 59.5 years. For the analysis, mRNA expression gene value fragments per kilobase of transcript per million (FPKM log2) was considered for analysis.

2.5 | Cytokines expression in prostate adenocarcinoma (PRAD) patients

To analyze the effect and the expression of cytokines in prostate cancer patients of different ages, UALCAN database (http://ualcan.path.uab.edu) was used. TCGA transcriptome and clinical patient data provides the cytokines expression level. A total of $n = 547$ patients sample were analyzed for cytokines gene expression consisting of normal subjects ($n = 52$), prostate cancer patients between 41 and 60 years ($n = 222$), and 61–80 years ($n = 273$). We analyzed the relative transcriptional expression of candidate genes in prostate cancer patients in a progressive age-specific manner with a cutoff value of $p < 0.05$.

2.6 | Cytokine and immune cell infiltration in PRAD

The potential association between cytokines and immune cell infiltration was analyzed using tumor immune estimation resource (TIMER) (https://cistrome.shinyapps.io/timer/). Cytokines such as IL-17A, IL-6, LIF, IL-10 LIFR, and IL-1RN were analyzed in the gene module-TIMER to explore the possible correlation between expression with the immune cell and infiltration abundance. These immune cells include T-cells, B-cells, monocytes, TAMs, macrophages, neutrophils, natural killer (NK) cells, dendritic cells (DCs), different T-helper cells, Tregs, and exhausted T-cells.

2.7 | quantitative RT-PCR (qRT-PCR)

Gene expression of *CCL2*, *IL-10*, *IL-6*, *IL-1RN*, *LIF*, and *LIFR* was validated by qRT-PCR in antiandrogen treated brain and androgen-responsive prostate cancer cells. The relative expression of these genes were compared with the expression of endogenous genes viz. GAPDH (NM_008084) and Actin (NM_007393) as internal controls in the reaction. Briefly, 5 ml of each 10 µg/ml sample were added for a total 25 µl volume with SYBR green (QuantaBio). The thermal cycler program was used at 48°C for 30 min to generate cDNA. The program then proceeded with 95°C for 15 min for initial denaturing, followed by 40 cycles of 95°C for 15 s, 60°C for 40 s, and 72°C for 35 s to collect cycle threshold (C_t) values. All reactions were performed in triplicate (three biological and three technical replicates) along with no-template controls. Primers for qRT-PCR were designed using PrimerExpress3.0 (Integrated DNA Technologies) targeting an amplicon size of 100 bp (Table 1). Primer specificity was tested by performing BLAST analysis (http://www.ncbi.nlm.nih.gov/).

2.8 | Statistical analysis

The canonical pathways were identified using B−H adjusted $p < 0.01$ for FDR correction. The qRT-PCR data were analyzed using REST@2009 (QIAGEN) and relative expression was calculated using formula ratio = (Etarget)ΔCPtarget (control-sample)/(Eref)ΔCPref (control-sample) and represented in log2 value, 2000 times the data were randomized

TABLE 1 List of primers used in the study

Name	Type	Strand	Sequence	Length	Start	End	Amplicon length
CCL2	Primer	+	AAGCAGAAGTGGGTTCAGG	19	300	318	90
	Primer	−	ATTCTTGGGTTGTGGAGTGAG	21	389	369	
IL-10	Primer	+	AATTCACCTTCCAGTGTCTCG	21	939	959	149
	Primer	−	AAGTGCTGGGATTACAGGC	19	1087	1069	
IL-6	Primer	+	AAAAGTCCTGATCCAGTTCCTG	22	435	456	135
	Primer	−	TGAGTTGTCATGTCCTGCAG	20	569	550	
IL-1RN	Primer	+	ACTCGTATATGTCTCAGGTCCC	22	1527	1548	116
	Primer	−	GAAGTCACAGGAAGTAGGGAAC	22	1642	1621	
LIF	Primer	+	GTTCTCTTCCCCGTGATCTTC	21	1199	1219	123
	Primer	−	GAACCTCTGCACCTGACTTAG	21	1321	1301	
LIFR	Primer	+	AGGGAAGTTGGAAGGAGATTG	21	6134	6154	147
	Primer	−	GGAGAAGCCACGAATCTAACTG	22	6280	6259	
IL-17A	Primer	+	CGCAATGAGGACCCTGAGAG	20	289	308	92
	Primer	−	TAGTCCACGTTCCCATCAGC	20	380	361	

(repetition of a function), and relative expression ratio was calculated. Prism-graph pad version 5 was used to plot the data.

3 | RESULTS

We performed ingenuity pathway analysis (IPA) of RNA-sequencing data to identify DEGs of prostate cancer patients under ADT protocol (GSE150368) and compared with the data set of patients diagnosed with Alzheimer/dementia (GSE77668). A total of 1952 DEGs in the Alzheimer/dementia patient cohort and 101 DEGs were identified in prostate cancer patients on ADT protocol (Figure 1A). A comparison between both data sets provided a subset of 33 commonly expressed genes; a majority of them belongs to the cytokine signaling network with the highest fold change in *IL-6* followed by *CSF3, CCL18, CCL20, MMP9, SERPIN1, CCL3, CXCL2, CCL24, CXCL8, CCL19, IL-1RN, OSM, LIF, CCL22, XCL1, CXCL1, CCL5, BMP2, BMP7, CXCL13, CCL2, IL-10, LTB, AKR1B1, IL-18, CDKN1A, HSPA4L, GADD45A, MCL1, FAS, CXCL16,* and *IL-16* (Figure 1B).

Next, we performed the IPA analysis to identify various differentially regulated canonical pathways between prostate cancer patients on ADT and Alzheimer's disease. The IPA analysis identified differentially regulated canonical pathways which were ranked and categorized using Z score values (Z-score indicates the predicted activation state of the canonical pathway). Among the top 21 canonical pathways, IL-17 signaling was identified as the highest-ranked pathway (Z score 3.46), followed by B-cell signaling pathway (Z score 2.8), differential regulation of cytokines production in macrophage and T-helper cells by IL-17A and IL-17F (Z score 2.6), TREM1 signaling, HMGB1 signaling, differential regulation of cytokines production in intestinal epithelial cells, neuroinflammation signaling pathway (Z score 2.33), IL-6 signaling, tumor microenvironment pathway (Z score 2.2), HIF1α signaling (Z score 2), crosstalk between dendritic cell and NK cells (Z score 2) as major signaling pathways (Table 2). Besides these top 21 canonical pathways, other signaling pathways associated with cytokine gene expression and their regulation are listed in Suppporting Information: Table S1.

To validate the expression of cytokine dysregulation after ADT exposure obtained from the RNA sequencing data set, we used androgen-responsive human prostate cancer LNCaP and C4-2B cells, brain neuronal BT142 cells, and glial M059K cells subjected to antiandrogen exposure (enzalutamide). We compared the expression of seven cytokines with a log2 fold change with their respective vehicle
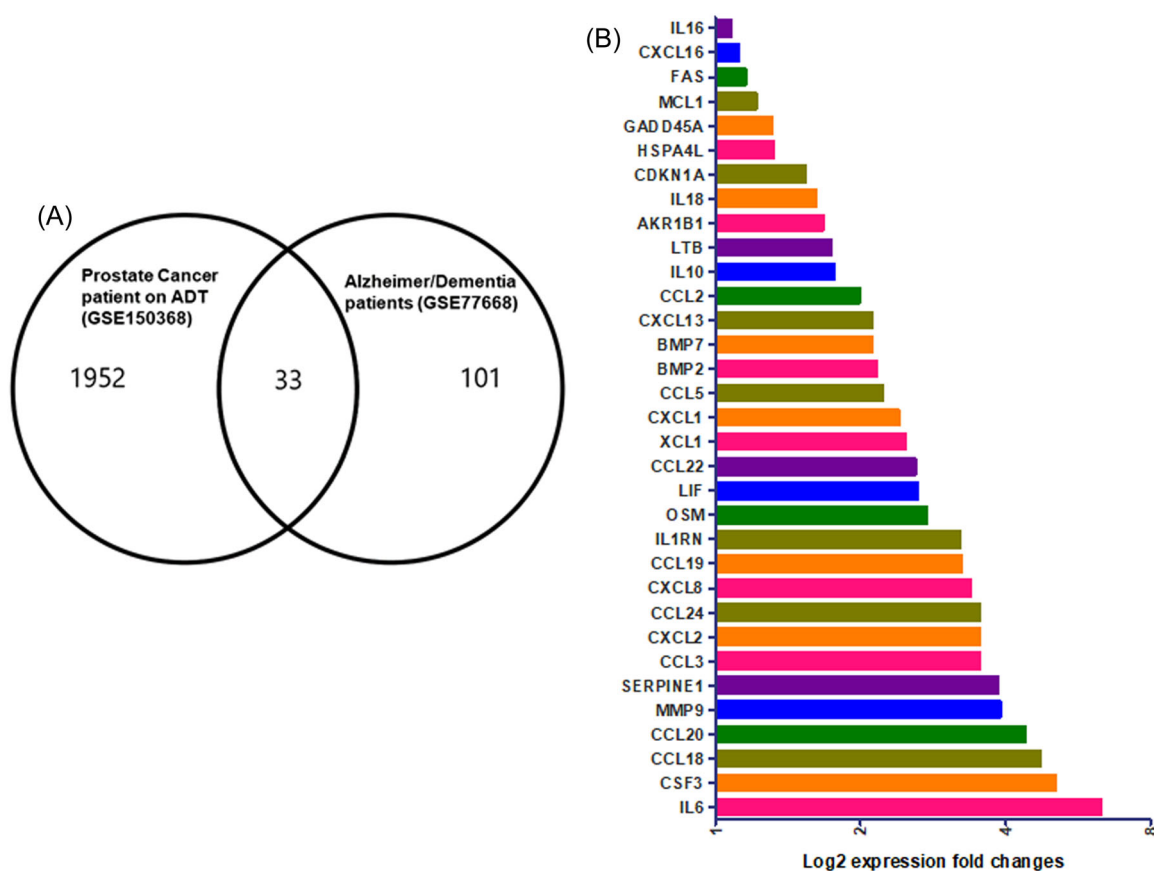

FIGURE 1 Comparative analysis of RNA sequencing data. (A) IPA comparative module was explored to analyze the RNA sequencing data of differentially expressed genes of prostate cancer patients under ADT overlaid with Alzheimer/dementia patient database. (B) The identified 33 genes belong to the cytokines gene family along with growth factors genes differentially expressed and shared in both data sets. Data represented in log2 expression fold change. ADT, androgen deprivation therapy; IPA, ingenuity pathway analysis. [Color figure can be viewed at wileyonlinelibrary.com]

TABLE 2 List of top 22 canonical signaling pathways their respective ratio, Z score, and –log (BH p values) and associated cytokines molecules

Ingenuity canonical pathways	–Log (B–H p value)	Ratio	Z-score	Molecules
IL-17 signaling	14.9	0.0642	3.464	CCL2, CCL20, CCL22, CSF3, CXCL1, CXCL8, IL-18, IL-6, LIF, LTB, MMP9, OSM
B-cell signaling pathway	7.4	0.0291	2.828	CXCL8, IL-10, IL-18, IL-6, LIF, LTB, MCL1, OSM
Differential regulation of cytokine production in macrophages and T-helper cells by IL-17A and IL-17F	14.0	0.389	2.646	CCL2, CCL3, CCL5, CSF3, CXCL1, IL-10, IL-6
Hepatic fibrosis signaling pathway	5.3	0.0185	2.646	CCL2, CCL3, CCL5, CXCL8, IL-18, IL-1RN, SERPINE1
TREM1 signaling	8.0	0.08	2.449	CCL2, CCL3, CXCL8, IL-10, IL-18, IL-6
HMGB1 signaling	8.9	0.0485	2.449	CCL2, CXCL8, IL-18, IL-6, LIF, LTB, OSM, SERPINE1
Differential regulation of cytokine production by IL-17A and IL-17F	10.8	0.261	2.449	CCL2, CCL3, CCL5, CSF3, CXCL1, IL-10
Neuroinflammation signaling pathway	8.5	0.03	2.333	CCL2, CCL3, CCL5, CXCL8, FAS, IL-10, IL-18, IL-6, MMP9
Acute phase response signaling	4.6	0.0278	2.236	IL18, IL-1RN, IL-6, OSM, SERPINE1
IL-6 signaling	5.3	0.0397	2.236	CXCL8, IL-18, IL-1RN, IL-6, MCL1
Cardiac hypertrophy signaling (enhanced)	3.5	0.0121	2.236	CXCL8, IL-18, IL-6, LIF, LTB, OSM
Senescence pathway	3.7	0.0182	2.236	CDKN1A, CXCL8, GADD45A, IL-6, SERPINE1
Tumor microenvironment pathway	8.8	0.0455	2.121	CCL2, CSF3, CXCL8, FAS, IL-10, IL-6, MMP9, OSM
HIF1α signaling	3.1	0.0195	2	CDKN1A, IL-6, MMP9, SERPINE1
Crosstalk between dendritic cells and natural killer cells	4.4	0.0449	2	FAS, IL-18, IL-6, LTB
Role of IL-17F in inflammatory diseases	5.5	0.093	2	CCL2, CXCL1, CXCL8, IL-6
T-cell signaling pathway	2.4	0.012	2	FAS, GADD45A, IL-10, IL-6
Role of MAPK signaling	4.7	0.0533	2	CCL2, CCL5, CXCL8, IL-6
Dendritic cell maturation	4.5	0.0272	1.342	IL-10, IL-18, IL-1RN, IL-6, LTB
LXR/RXR activation	5.3	0.1413	−2.236	CCL2, IL-18, IL-1RN, IL-6, MMP9
Erythropoietin signaling pathway	5.9	0.0347	−2.449	CXCL8, IL-18, IL-6, LIF, LTB, OSM

Abbreviation: B–H, Benjamini–Hochberg.

controls by performing qRT-PCR. The enzalutamide treated C4-2B cells demonstrated higher levels of cytokine gene expression, in particular *IL-6* (6.5-fold; $p < 0.025$), *IL-17A* (5.33-fold; $p < 0.001$), *LIFR* (4.26-fold; $p < 0.001$), *CCL2* (5.15-fold; $p < 0.001$), *IL-10* (4.0-fold; $p < 0.009$), *LIF* (3.0-fold; $p < 0.001$), and *IL-1RN* (2.7-fold; $p < 0.001$) (Figure 2A). Similar trend of increased cytokine expression was observed in LNCaP cells post-enzalutamide exposure including *CCL2* (6.6-fold; $p < 0.001$), *IL-6* (6.08-fold; $p < 0.001$), *IL-17A* (5.33-fold; $p < 0.025$), *IL-10* (3.48-fold; $p < 0.001$), *LIF* (3.0-fold; $p < 0.009$), *IL-1RN* (2.4-fold; $p < 0.001$), and *LIFR* (2.3-fold; $p < 0.001$. However, expression of IL-1RN in LNCaP cells exposed to enzalutamide was statistically insignificant ($p < 0.18$) (Figure 2B).

Exposure of brain glial M059K cells to enzalutamide resulted in higher cytokine gene expression viz. *LIF* (11.5-fold; $p < 0.001$), *IL-17A* (3.7-fold; $p < 0.001$), *LIFR* (2.89-fold; $p < 0.001$), *IL-1RN* (2.4-fold;

$p < 0.001$), *IL-6* (2.36-fold; $p < 0.001$), and *IL-10* (2.2-fold; $p < 0.001$) (Figure 2C). The neural BT142 cells exposed to enzalutamide also exhibited similar trend in expressing higher levels of cytokine genes, *IL-17A* (4.29-fold; $p < 0.001$), *LIFR* (2.8-fold; $p < 0.001$), *IL-6* (2.65-fold; $p < 0.043$), *CCL2* (2.2-fold; $p < 0.002$), *LIF* (1.9-fold; $p < 0.002$), and *IL-1RN* (1.6-fold; $p < 0.35$) (Figure 2D). Moreover, the expression of *CCL2* in glial cells and *IL-10* in neural cells did not exhibit any expression and were statistically insignificant ($p < 0.3$) (Figure 2C,D).

To probe the association between age and cytokine expression in patients with prostate cancer we used the UALCAN transcriptome database. Patient data revealed that the *LIFR, IL-1RN, IL-6, IL-10, CCL2, LIF,* and *IL-17A* genes exhibited a low level of expression with no statistical significance at a $p < 0.05$, compared to normal control subjects (Figure 3).
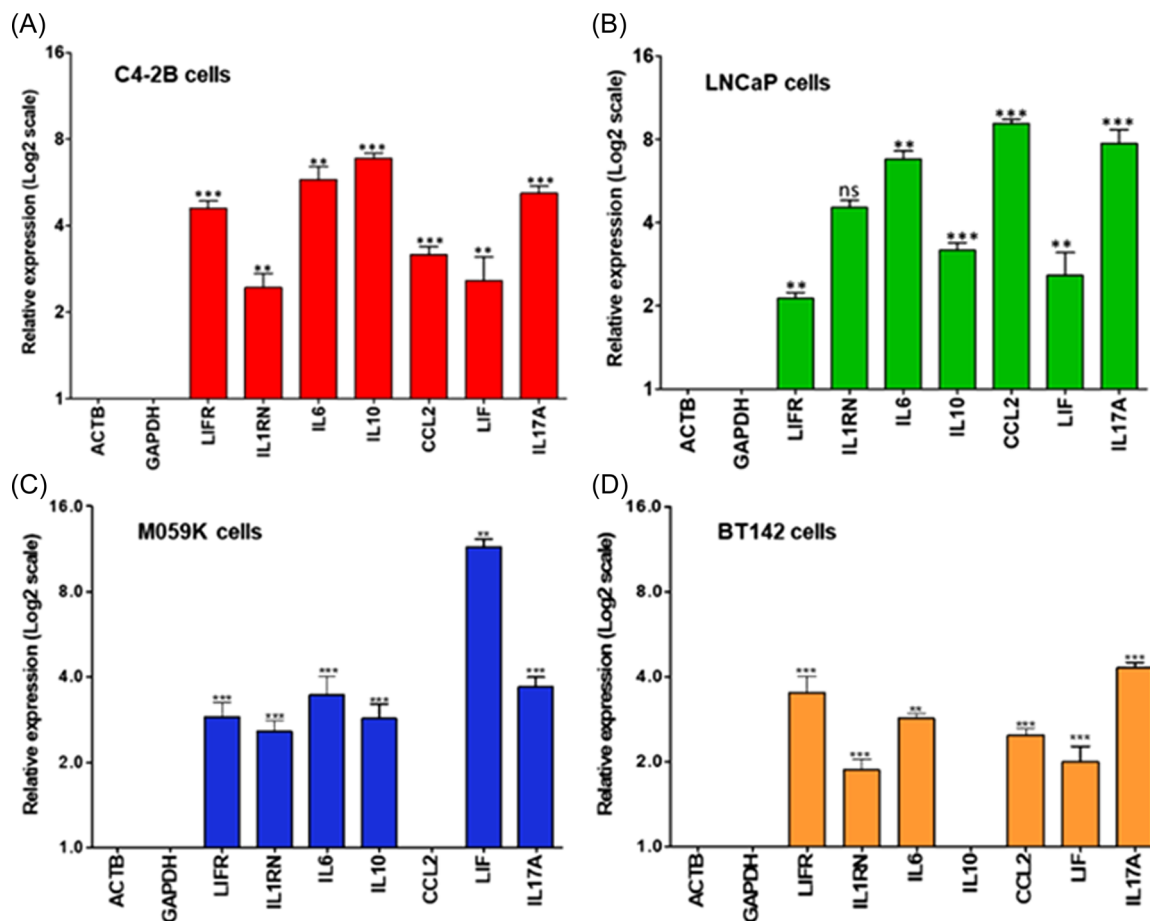
FIGURE 2 Quantitative real time-PCR (qRT-PCR) analysis of cytokines. qRT-PCR was performed in cytokines which include LIFR, IL-1RN, IL-6, IL-10, CCL2, LIF, and IL-17A. (A) Prostate cancer cell lines C4-2B and (B) LNCaP cells, and (C) brain glial M059K cells and (D) brain neural BT142 cells were treated with 20 µM enzalutamide for 24 h. X-axis of the graph denotes relative expression values (log2) and Y-axis denotes the cytokine's levels. Experiments were performed using three biological and three technical replicates. The expression of GAPDH and ACTB was used as internal control in the experiment. The error bars in the graph show standard deviation (+SD). ***$p < 0.001$, and **$p < 0.05$; ns, nonsignificant. [Color figure can be viewed at wileyonlinelibrary.com]

Next, the levels of *IL-17A, IL-17RA, IL-6, CCL2, LIFR, IL-10,* and *IL-1RN* gene expression were analyzed in prostate cancer patients exposed to ADT compared to patients who did not receive ADT. Analysis was conducted on 23 studies that contain 9043 patients and among them 203 patients who received ADT, out of which, 197 patients were considered for analysis, compared with naïve prostate cancer patients. The mRNA expression of cytokines in these patients were provided in log2 value of fragments per kilobase of transcript per million (FPKM) mapped fragments. Patients exposed to ADT treatment ($n = 197$) resulted in an increased expression of *LIFR, IL-1RN, IL-10, CCL2,* and *IL-17RA* whereas *IL-6, LIF,* and *IL-17A* exhibited inconsistent expression and were insignificant, compared to naïve prostate cancer patients ($n = 210$). (Figure 4).

We next investigated the association between cytokine gene expressions in prostate cancer patients who received ADT with neuropathological changes in the brain tissue. The transcriptome data of aged neuropathological patients ($n = 223$) diagnosed with Alzheimer/dementia were explored. The analysis revealed a high level of gene expression of proinflammatory cytokines and others that include

IL-17RA, IL-10, CSF3R, CXCL1, CXCL2, LIFR, IL-6, CCL19, IL-16, IL-18, CCL22, CXCL16, CCL5, CCL3, LIF, and *OSM* (shown in red color), in the frontal white matter and hypothalamus. However, a low level of gene expression of cytokines including *XCL1, CXCL20, LTB, IL-17A, CCL24,* and *CCL18* (low intensity depicted by blue or white color) was observed in the temporal cortex and parietal cortex of the brain (Figure 5).

To identify the correlation between cytokine expression and tumor-infiltrating immune cells in PRAD data sets, we used the TIMER. The correlation value of cytokine expression with tumor purity and the abundance of immune cells were recovered. Tumor purity is an important factor that influences the analysis of immune infiltration in clinical samples by genomic approaches. The analysis revealed a positive association between the expression of IL-17A, CCL2, IL-10, IL-6, IL-1RN, LIF, and LIFR with the infiltration of B-cells, CD8+ T-cells, CD4+ T-cells, macrophages, neutrophils, and DCs - (Figure 6). The expression levels of the above-mentioned cytokines positively correlate with the infiltration of neutrophil and DCs ($p < 0.05$). The correlation score along with p value of cytokines infiltration is listed in Supporting Information: Table S2.
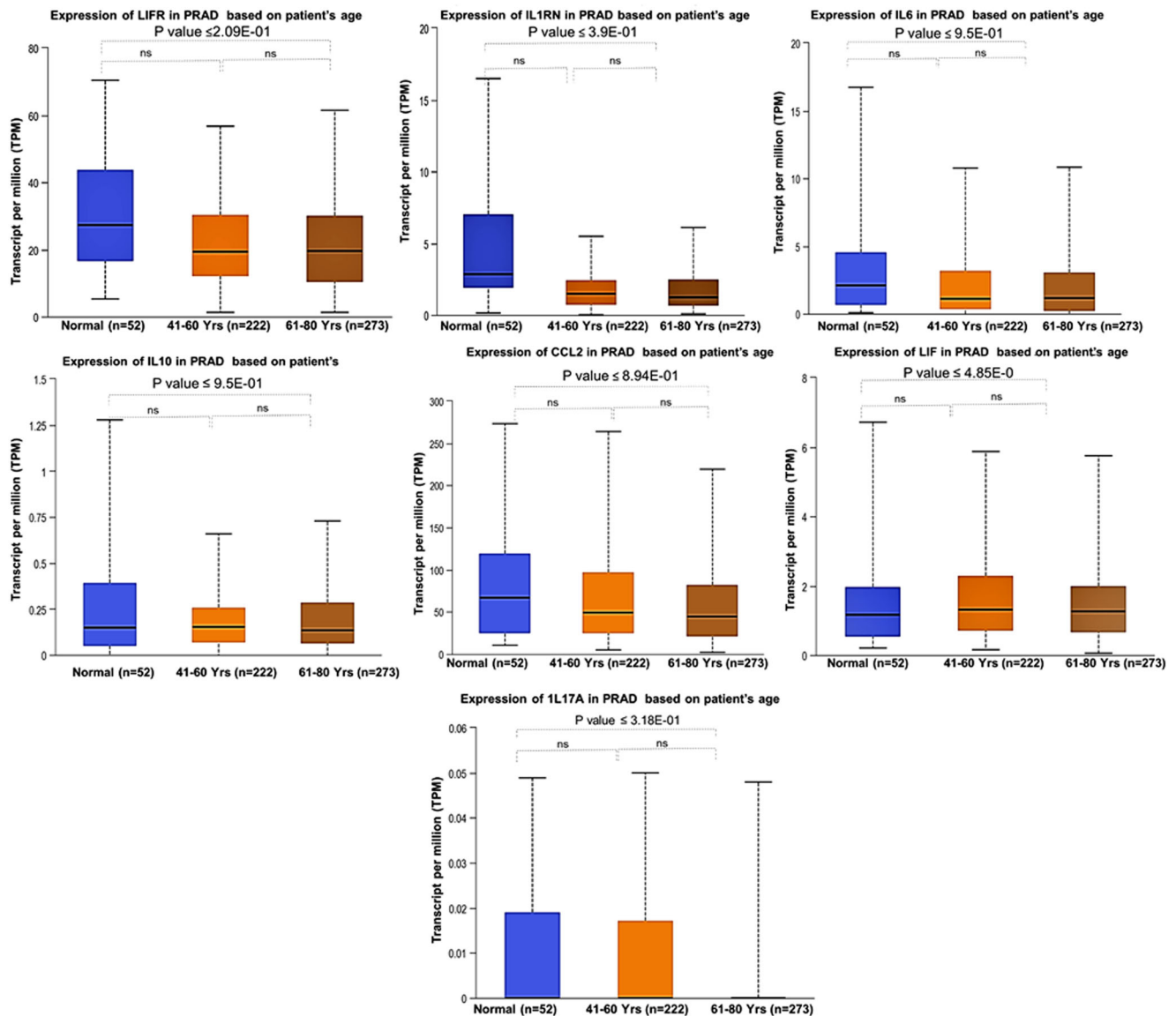
FIGURE 3 Cytokine gene expression in PRAD. Expression of cytokines LIFR, IL-1RN, IL-6, IL-10, CCL2, LIF, and IL-17A in aged (41–80 years) prostate cancer patients using UALCAN. Box-whisker plots showing the expression of cytokines in prostate cancer patients (PRAD) in age-wise manner. The expression of normal patients was compared to prostate cancer aged patients and analyzed statistically. The X-axis represents patients' age and Y-axis denoted gene expression value in transcript per million (TPM). ns, nonsignificant; PRAD, prostate adenocarcinoma. [Color figure can be viewed at wileyonlinelibrary.com]

4 | DISCUSSION

In the present study, we demonstrate an association between prostate cancer patients undergoing ADT and increased cytokine signaling, suggesting that proinflammatory molecules can trigger the progression of brain deficits. To recognize this positive association, using IPA, we compared the RNA sequencing data of prostate cancer patients who underwent ADT with postmortem putamen samples of the degenerated brain (dementia/Alzheimer's disease). The transcriptome gene expression data of ADT patients compared with Alzheimer's disease demonstrated higher expression of 33 proinflammatory cytokines. Our analysis further revealed IL-17 as the top

listed cytokine expressed by T-helper cells, and other immune cells that include macrophages, fibroblasts, endothelial cells, communication between innate and adaptive immune response, and the tumor microenvironment. Although the pathogenesis of ADT-mediated brain cognition is not fully understood, it appears that increased production of proinflammatory cytokines is the most critical component initiating cognitive impairment.

Subsets of immune and inflammatory cells that interact through interleukins and interferons play an important role in facilitating crosstalk between cells of the innate and adaptive systems.[19] Cytokines such as IL-8, IL-1, IL-6, and IL-10 are shown to be frequently associated with prostate cancer patients under ADT

treatment[20] and Alzheimer disease.[21] These cytokines are typically produced by macrophages that lead to the activation of neutrophils, mast cells, basophils, and T-helper cells.[22] DCs and macrophages also produce a host of cytokines including IL-1, IL-15, IL-4, TNF, and IFN which activate NK cells, T-cells, and also lead to the maturation of
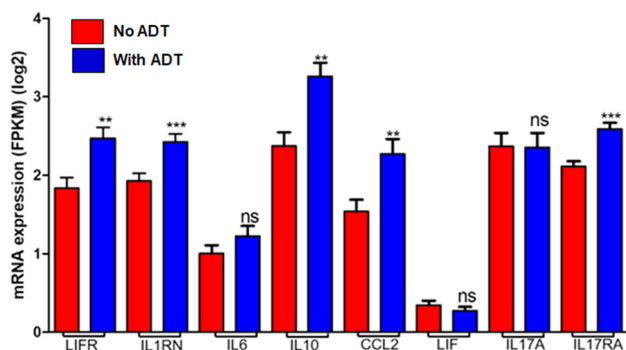

FIGURE 4 The cBioPortal prostate cancer database. Cytokine expression in prostate cancer patients exposed to ADT. TCGA data—expression in log2 value of fragments per kilobase of exon per million (FPKM) mapped fragments of cytokines IL-6, IL-17A, IL-17RA, CCL2, LIFR, IL-10, IL-1RN, and LIF in prostate cancer patient on ADT treatment. ADT, androgen deprivation therapy. [Color figure can be viewed at wileyonlinelibrary.com]

DCs. T-helper cells then lead to the differentiation of T helper 17 (Th17) cells and secrete IL-17A and IL-17F.[23] The IL-17 cytokines mediate their biological functions through surface receptors on target cells by binding to IL-17 receptor A (IL-17RA), which stimulates the production of several proinflammatory cytokines including IL-6 and IL-10, CCL2, and others.[24] The function of IL-17 signaling is also important to a subset of CD4+ T-cells referred as Th17 cells.[25] Consequently, IL-17 is linked to transmit inflammatory signaling via the recruitment of Th17 cells either by autocrine or paracrine manner to other organs of the body including brain. In our study, gene expression of proinflammatory cytokines and their receptors viz. *ILFR*, *IL1-RN*, *IL-6*, *IL-10*, *LIF*, *CCL2*, and *IL-17A* were increased in brain-derived neural and glial cells and malignant prostate cancer cells treated with antiandrogen, compared to no-androgen antagonist treatment.

The relationship between inflammatory cytokines and altered brain cognition in prostate cancer patients under ADT treatment has not been fully investigated. We explored prostate cancer database comprising of 23 studies containing 9043 patients and among them 203 patients received ADT treatment. Interestingly, the cytokines LIFR, IL-10, IL-17A, and CCL2 showed higher levels except IL-6, IL1-RN, LIF, and LIFR in patients exposed to ADT. This further support our rationale that cytokines secreted from the tumor microenvironment may exert their effects through paracrine mechanisms,
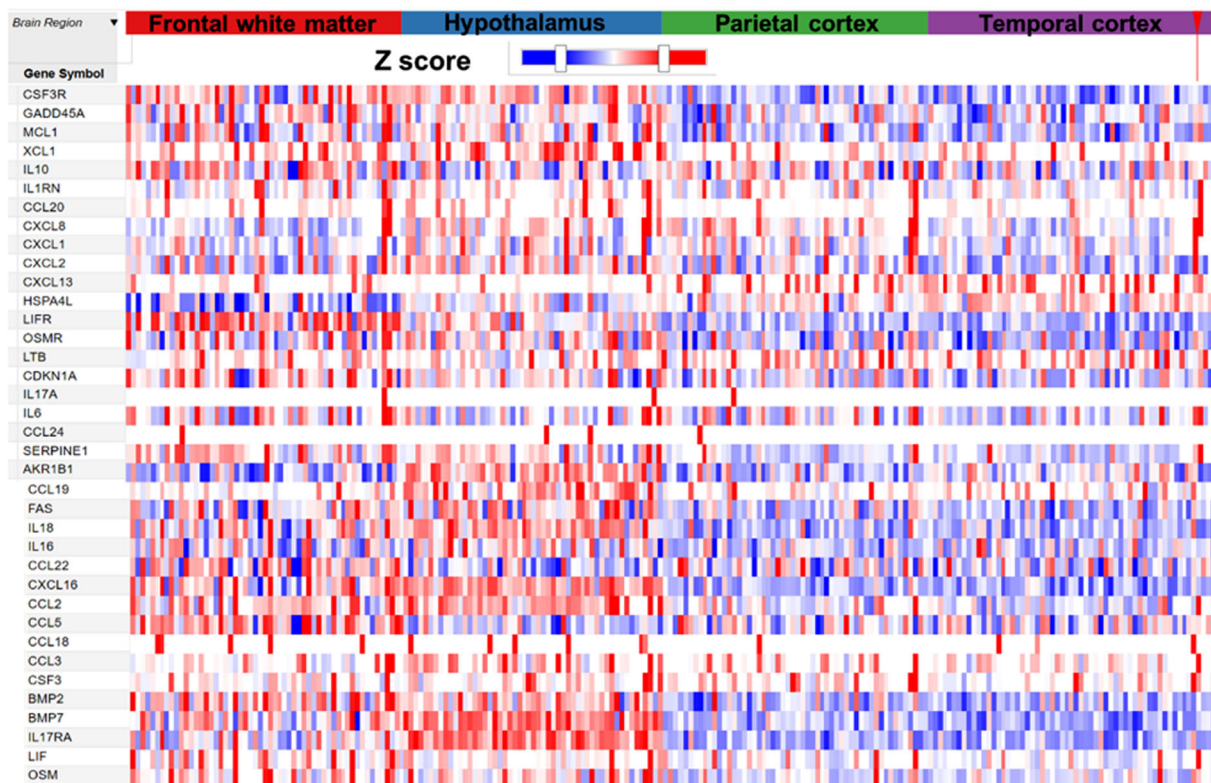

FIGURE 5 Heat map representation of cytokine gene expression in Alzheimer, dementia, and aging patients in different regions of brain which include frontal white matter and hypothalamus, temporal cortex, and parietal cortex. Z-score was calculated and shown in colors, red color denotes high expression, blue color represents low level of expression, and white represent nil or zero expression. [Color figure can be viewed at wileyonlinelibrary.com]
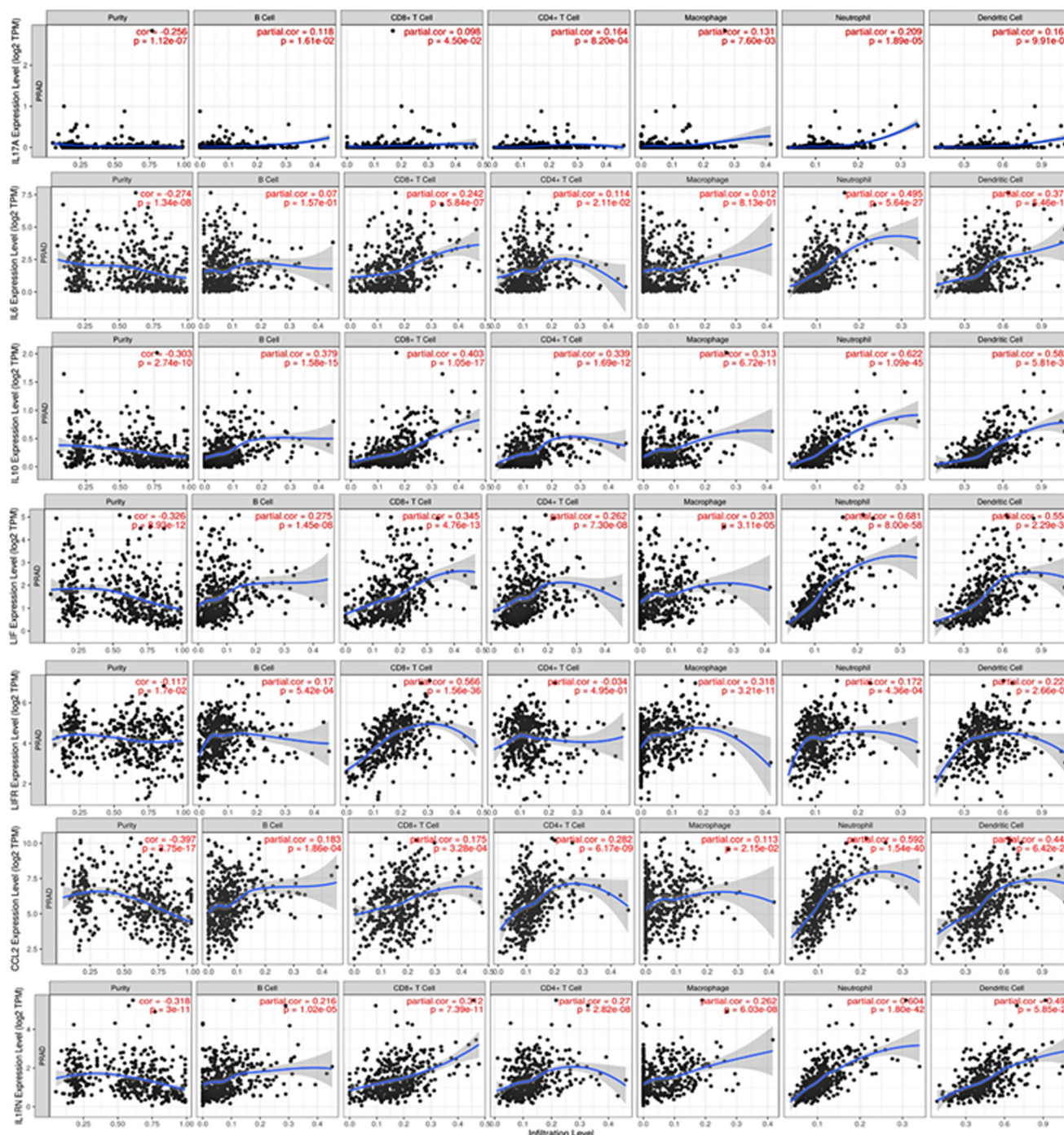
FIGURE 6 Cytokine and immune cell infiltration in PRAD (prostate adenocarcinoma); scatter plots were generated using with the tumor immune estimation resource gene module tool to identify the immune cell profiles associated with cytokines expression in PRAD. For each gene and respective immune cells correlation score was calculated along with p values. [Color figure can be viewed at wileyonlinelibrary.com]

providing the link between changes in the brain. The data further tempted us to examine the expression of cytokines in different brain regions during degenerative diseases like Alzheimer and dementia. We explored the longitudinal population-based prospective cohort study of brain aging and incident dementia. The data revealed that proinflammatory cytokines exhibited higher level of expression in the frontal white matter and hypothalamus. Moreover, the secreted

cytokines influence the tumor microenvironment and causes chemotaxis of immune cells. To understand this further we correlated the immune cell infiltration with inflammatory cytokines such as IL-17A, IL-6, CCL2, IL-10, LIF, and LIFR in prostate adenocarcinoma (TCGA-PRAD) from the TCGA data set. The results indicate that among the above-mentioned cytokines IL-6 showed a higher rate of infiltration to DCs compared to B-cells, CD8+ T-cells, CD4+ T-cells, macrophages,

and neutrophils. This observation illustrates the requirement of IL-6 by DCs for priming the pathogenic Th17 cells. Interestingly, prostate tumors also induce adaptive immune cells; produce cytokines that stimulate DCs priming CD4+ T-cells to a proinflammatory phenotype leading to the differentiation of naive CD4+ T-cells into Th17 effector cells and secretes IL-17A and IL-17F, which act as heterodimers in the IL-17RA and IL-17RC receptors on the cell surface. Our analysis demonstrates the model that ADT treatment in prostate cancer is associated with higher circulating IL-17A which promotes the secretion of proinflammatory cytokine(s) through the paracrine loop delivered to the brain and contributes to neurodegeneration and cognitive impairment. Along these lines, a recent study has demonstrated that neutralization of IL-17 rescues Aβ-induced neuroinflammation and memory impairment in mouse brain.[26]

Long term ADT treatment in prostate cancer patients suppress the androgen receptor (AR), and in turn influences the tumor microenvironment in generating a proinflammatory condition. Previous studies demonstrate distribution of AR in the brain, with high expression in the hippocampus and cortex and areas associated with memory, emotions and others, and blocking of androgen signaling axis can impair object recognition memory particularly in males.[27,28] The rationale of this study is built on previous patient's cohorts based studies which showed positive correlation between prostate cancer patients who received ADT, linked to a higher likelihood of being diagnosed with Alzheimer's disease and dementia compared to patients who do not receive ADT therapy.[12] To support the above findings, we investigated the level of inflammatory cytokines and their receptor expression in healthy males, prostate cancer patients, and patients undergoing ADT. The expression of LIFR, IL-1RN, IL-6, IL-10, CCL2, LIF, and IL-17A were significantly lower in aged prostate cancer patients, compared to healthy individuals; whereas ADT treatment markedly increases the levels of these cytokines.

The present study has some limitations. First, the association between ADT and brain cognition is correlative and derived from data set analysis as there is a lack of brain specimens of prostate cancer patients undergoing ADT treatment for validation. Second, there is also lack of appropriate in vivo cognitive model(s) of ADT, which limits our studies to cell culture system. Finally, the partial data on ADT exposure to prostate cancer patients and the expression of various cytokines without cognition data limits the analysis to identify direct association between cognition and inflammatory cytokines and did not allow us to rule out the possibility that these associations may be due to noncausal relationships.

5 | CONCLUSIONS

The results of our study provide first-hand evidence linking brain deficits to a proinflammatory state and paracrine autocrine loop of cytokine expression in prostate cancer patients undergoing ADT. As such, studies specifically assessing for cognition in prostate cancer patients undergoing ADT are lacking, and timely recognition of these effects can lead to treatment regimens that could significantly impact quality-of-life of patients and their caring families.

AUTHOR CONTRIBUTIONS

Conceptualization: Shiv Verma, Lee E. Ponsky, and Sanjay Gupta. *Methodology*: Shiv Verma, Eswar Shankar, and Prem Prakash Kushwaha. *Software*: Shiv Verma and Sanjay Gupta. *Validation*: Shiv Verma and Sanjay Gupta. *Formal analysis*: Shiv Verma and Sanjay Gupta. *Investigation*: Shiv Verma, Eswar Shankar, and Prem Prakash Kushwaha. *Resources*: Sanjay Gupta. *Data curation*: Shiv Verma. *Writing—original draft preparation*: Shiv Verma and Sanjay Gupta. *Writing—review and editing*: Shiv Verma, Lee E. Ponsky, and Sanjay Gupta. *Visualization*: Shiv Verma and Sanjay Gupta. *Supervision*: Sanjay Gupta. Project administration: Sanjay Gupta. *Funding acquisition*: Sanjay Gupta. All authors have read and agreed to the published version of the manuscript.

CONFLICT OF INTEREST

The authors declare no conflict of interest.

DATA AVAILABILITY STATEMENT

The RNA-sequencing data are available in NCBI-GEO database.

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SUPPORTING INFORMATION

Additional supporting information can be found online in the Supporting Information section at the end of this article.