Molecular Evaluation of Interleukin-8, Interleukin-27, and Vascular Endothelial Growth Factor Expression in Prostate Cancer Patients

Jutyar I. Saber[1], Hiwa R. Fatah[1] and Fikry A. Qadir[2†]

[1]Department of Biology, Faculty of Science and Health, Koya University, Koya 44023, Kurdistan Region – F.R. Iraq

[2]Department of Biology, College of Science, Salahaddin University-Erbil, Kurdistan Region – F.R. Iraq

***Abstract*—Globally, prostate cancer (PCa) is becoming more prevalent and lethal. The progression of PCa is linked to both inflammation and angiogenesis. Pro-inflammatory cytokines, interleukin (IL)-8) and vascular endothelial growth factor (VEGF), promote inflammation, angiogenesis, and PCa progression. On the other hand, IL-27 (IL-27) has antitumor effects, modulates immune system activity, and thus serves as a suppressor of tumor growth. In the Kurdistan Region of Iraq, there is limited evidence linking these cytokines and their gene polymorphisms to PCa. This case–control study included 50 PCa patients and 30 age-matched healthy controls. Serum levels of these three cytokines were evaluated by enzyme-linked immunosorbent assay, whereas PCR and Sanger sequencing were used to find polymorphisms in IL-8 (rs4073, rs2227306), IL-27 (rs153109), and VEGF (rs2010963) in formalin-fixed paraffin-embedded tissue DNA. GeneMANIA was used to assess gene-gene interaction networks. Statistical analyses were performed using Mann–Whitney U tests. Patients with PCa showed elevated IL-8 and VEGF levels and reduced IL-27 levels compared to controls ($p < 0.05$). Multiple single-nucleotide polymorphisms were found in all target genes, several of which were new to the GenBank. Gene-network analysis revealed that these three cytokines are involved in shared inflammatory, immunomodulatory, and angiogenic pathways. This study shows that altered serum levels and gene polymorphisms of these three cytokines may be biomarkers for PCa diagnosis and progression. It underlines the molecular interplay between inflammatory and angiogenic mediators and supports further cytokine-based diagnostic and therapeutic research.**

***Index Terms*—Angiogenesis, Interleukin-8, Interleukin-27, Prostate cancer, vascular endothelial growth factor.**

ARO-The Scientific Journal of Koya University
Vol. XIV, No. 1 (2026), Article ID: ARO.12718. 10 pages
DOI: 10.14500/aro.12718
Received: 03 November 2025; Accepted: 17 January 2026
Regular research paper; Published: 10 April 2026
†Corresponding author's e-mail: fikry.qadir@su.edu.krd

I. Introduction

In the United States, prostate cancer (PCa) is the most common cancer found among men, exceeding lung and colorectal cancers. In the United States, 2.04 million new cancer cases and 618 thousand cancer deaths are projected in 2025. These estimates highlight how significant PCa is within the framework of the cancer burden (Siegel et al., 2025). PCa was the 4th most prevalent cancer in Erbil in the Kurdistan Region of Iraq from 2013 to 2019, including approximately 10% of male cancer cases. In Duhok, it ranked 7th, accounting for 5% of cases among men (Karwan et al., 2022).

Risk factors for PCa that are established and unchangeable encompass getting older, ethnicity (notably Black race), specific gene mutations, insulin-like growth factors, and hereditary background of the disease. Modifiable factors, such as lifestyle choices, such as diet, cigarette and alcohol use, becoming overweight, and lack of physical activity, along with environmental factors, such as being exposed to chemicals or ionizing radiation, may increase the likelihood of developing metastatic PCa (Berenguer et al., 2023).

Interleukin (IL)-8 is a significant pro-inflammatory chemokine that functions through the G protein-coupled receptors CXCR1 and CXCR2. By activating these receptors, IL-8 triggers several intracellular signaling pathways that promote angiogenesis, cellular proliferation, and metastatic potential (McClelland et al., 2024).

IL-27 is a member of the IL-12 and IL-6 family whose immune-augmenting properties and unique structure make it a cytokine of dual functions. As a double-edged sword, IL-27 has been shown to perform dual functions in clinical models of a variety of solid and hematological tumors. Primarily, it exerts anti-tumor activity by inducing cytotoxic T cell and T helper cell one responses while simultaneously suppressing the growth, angiogenesis, proliferation, tumor cell invasion, metastasis, and survival. On the other hand, IL-27 may also have a pro-tumor effect in some cancers, facilitating tumor progression (Maleki et al., 2025).

Angiogenesis is one of the most important aspects of cancer, and for such processes, VEGF acts as a central regulator and is associated with the progression of cancer

(Furriol et al., 2024). Recent research demonstrates a significant association between PCa and angiogenesis (Sarkar et al., 2020). Growth and invasion of PCa tissues are linked with increased formation of neovascularization (Luo et al., 2022). These blood vessels not only supply oxygen and nutrients to the tumor cells but also help in immune evasion, which helps sustain the ongoing proliferation of PCa. The newly formed blood vessels also promote cancer metastasis, which aids in the development of extensive metastatic lesions and thus severely worsens the prognosis of PCa. Cells of PCa activate many angiogenesis-related signaling pathways, such as VEGF, fibroblast growth factor, and platelet-derived growth factor (Ene, Nicolae and Ene, 2023).

To the best of our knowledge, there is a lack of research investigating immune-related changes in patients with PCa in the Kurdistan region of Iraq, particularly concerning IL-8, IL-27, VEGF, and their genetic polymorphisms. To address this gap, this study examines these cytokines and variation in genes as potential biomarkers for the identification, monitoring, and treatment of PCa within this population.

II. Materials and Methods

A. Patient and Sample Collection

This study followed a case–control design, and clinical samples were collected from patients at the oncology departments of Par Hospital and Rizgary Hospital in the Kurdistan Region, Iraq. Ethical approval was granted by the Human Ethics Committee of the College of Science, Koya University (Approval No. 008 Bio) on November 14, 2024, in accordance with the Declaration of Helsinki. A total of 50 patients with confirmed PCa and 30 age-matched healthy controls were enrolled. The mean age of the control group was 69.43 ± 6.99 years, whereas the mean age of the patient group was 69.12 ± 6.61 years. Strict inclusion and exclusion criteria were applied. Individuals with acute seasonal respiratory infections (e.g., influenza or the common cold) were excluded from both groups. Venous blood samples and formalin-fixed paraffin-embedded (FFPE) tissue blocks were collected from all participants. Sample collection took place between October 2024 and February 2025.

B. Collection of Blood Samples

Each participant provided blood samples, which were drawn with 5 mL syringes. For the complete blood count, 2 mL was put into EDTA blood tubes, and 3 mL was put into gel tubes for the determination of prostate-specific antigen (PSA) and cytokine measurements. The gel tubes were allowed to clot at room temperature and then centrifuged at 5000 rpm for 10 min to separate the sera. Then this serum was used for PSA analysis, and the remaining amount was transferred into 1.5 mL Eppendorf tubes, which were stored at −80°C for later cytokine measurement.

C. Determination of PSA and Complete Blood Counts

PSA levels were determined by using the Liaison XL system for chemiluminescence immunoassay (Diasorin, Italy) due to its sensitivity, automation capability, and ability to detect low quantities of PSA levels within serum samples. Complete blood counts were acquired utilizing the Sysmex XP-300 hematology analyzer (Sysmex Corporation, Japan). Each test was performed in accordance with the quality-control procedures to ensure the reliability of the findings and to minimize analytical variations across instruments.

D. Determinations of Cytokine Concentration

Specific enzyme-linked immunosorbent assay (ELISA) kits from ELK biotechnology (catalog ELK1159 for IL-8, ELK1527 for IL-27, and ELK1129 for VEGF) were used to determine the serum concentrations of each cytokine. The optical density values for each serum sample were obtained using an ELISA plate reader (BioTek Instruments, Inc.) at a wavelength of 450 nm, which is directly proportional to the serum cytokine concentration and can be quantified by a standard calibration curve.

E. DNA Extraction

Genomic DNA was isolated from histopathologically confirmed malignant FFPE PCa tissue samples. Extraction was performed using the PureLink Genomic DNA Mini Kit (Invitrogen, USA; Cat. No. 29650600), according to the manufacturer's protocol. The concentration and purity of the extracted DNA were assessed using a NanoDrop spectrophotometer (OneDrop TOUCH, Biometrics). Calibration was performed using the ATE elution buffer as the blank. The A260/A280 ratio was used as a standard measure to evaluate the quantity as well as the purity of extracted DNA.

F. Genotype Determinations

The study examined two polymorphisms within the *IL-8* gene and one polymorphism within the *IL-27* and the *VEGF* genes. The purified DNA samples were amplified by conventional PCR using a TC1000-G thermal cycler (DLAB Scientific, Beijing, China) equipped with the primer sequences shown in Table I. A commercially available master mix (Amplicon, Denmark; Cat. No. 5200300-1250) was used, containing Taq DNA polymerase, dNTPs, potassium chloride, and reaction buffer. The first step in PCR amplification was to denature the DNA at 95°C for 5 min. This was followed by 40 cycles, each of which included a 35s denaturation phase at 95°C. The annealing temperatures for each polymorphism were as follows: IL-8 rs4073 at 55°C, IL-8 rs2227306 at 56.5°C, VEGF at 55.7°C, and IL-27 at 62°C, each for 35 s. The elongation step was performed at 72°C for 1 min, followed by a final extension phase at the same temperature for 7 min. The PCR products of each gene were subjected to gel electrophoresis on a 2.0% agarose gel containing 12 μL of DNA Safe Stain (Lot No. EP5083, SinaClon Co., Iran) and 10 μL of a 100 bp plus 3 kb DNA Ladder (SMOBIO, Taiwan). Furthermore, 10 μL of the *PCR* gene product was incorporated and visualized with a ultraviolet transilluminator (BIO View).

TABLE I
List of Primers Used in the Study

Gene	Primer	Primer sequence 5′ - 3′	Product size (bp)
IL-8	Forward	5′-TCATCCATGATCTTGTTCTAA-3′	541
rs4073	Reverse	5′-GGAAAACGCTGTAGGTCAGA-3′	
IL-8	Forward	5′-ACCCTGATTATAGACCAGGCAT-3′	361
rs2227306	Reverse	5′-TCTGCCAGCTACTTCCTTTCTA-3′	
IL-27	Forward	5′-AACCCCATCCTCTCCCTGAA-3′	829
rs153109	Reverse	5′-TGGTTGATCCCAGAGTCCCA-3′	
VEGF	Forward	5′-ATTTATTTTTGCTTGCCA TT-3′	303
rs2010963	Reverse	5′-GTCTGTCTGTCTGTCCGT CA-3′	

IL: Interleukin, VEGF: Vascular endothelial growth factor

G. Database Analysis of Gene Interactions

The interactions among *IL-8*, *IL-27*, and *VEGF-A* genes were predicted using GeneMANIA (http://genemania.org). GeneMANIA is a user-friendly web-based tool that provides an accessible interface for generating and ranking hypotheses about gene functions within a gene list for functional assays and gene interaction networks, models, and predictions using gene and protein interaction data and phylogenetically localized genes, co-expressed genes, and shared biological pathways (Franz et al., 2018). The connection between these three genes illustrates the combined functions of these genes in inflammation, immune response, and angiogenesis.

H. Statistical Analysis

Before the statistical analysis, the distribution of the data was examined, and all the variables were non-normally distributed. For PCa and control participants, differences were analyzed using the Mann-Whitney U test. DNA sequencing data were processed with Mutation Surveyor and aligned to the reference sequences from GenBank. GraphPad Prism was used to perform the statistical analyses. Data were summarized as medians with interquartile ranges (IQR). A significance level of $p < 0.05$ was used for all the analyses.

III. Results

A. Total White Blood Cell (WBC) and Platelet Count

The total WBC count and the percentage of neutrophils were markedly increased in patients with PCa compared to the healthy controls with $p < 0.05$ and < 0.01, respectively. Patients with PCa and the controls showed no significant differences in the percentage of lymphocytes and the count of platelets. The results of this analysis are shown in Fig. 1, which illustrates the hematological features of the study participants.

B. PSA Levels in Patients and Control Individuals

As shown in Fig. 2, the level of PSA in patients with PCa was significantly higher than that of the control group (median 40.20 ng/mL; range, 12.38–102.3 vs. median 1.105 ng/mL; range, 0.6908–2.768) with $p < 0.0001$. These results confirm that PSA is a reliable marker for finding and monitoring PCa.

C. Concentrations of Serum IL-8, IL-27, and Vascular Endothelial Growth Factor (VEGF)

As illustrated in Fig. 3, patients exhibited higher serum concentrations of IL-8 and VEGF compared to the control group (IL-8 median 102.1 pg/mL; IQR, 81.36–123.1 vs. median 82.79 pg/mL; IQR, 64.04–91.18; VEGF median 172.5 pg/mL; IQR, 97.66–200.3 vs. median 131.3 pg/mL; IQR, 72.90–152.3). Conversely, serum IL-27 levels were significantly lower in patients with PCa compared to the control group (median 94.52 pg/mL; IQR, 87.62–107.8 vs. median 148.9 pg/mL; IQR, 124.6–162.8).

D. Polymorphisms in the Genes IL-8, IL-27, and VEGF

The genetic polymorphisms detected in the three studied cytokine genes are shown in Fig. 4 and summarized in Table II. For the IL-8 rs4073 region, 78 substitution polymorphisms were identified, two of which resulted in amino acid changes, as shown in Table III, and two have previously been documented in external databases. For IL-8 rs2227306, 31 substitutions were identified, none of which caused an amino acid change, and one was previously recorded. The greatest variation was in the IL-27 rs153109 locus, which had 89 polymorphisms, most of which were substitutions, with 1 deletion, 1 duplication, and 1 insertion; none of these resulted in amino acid changes. In contrast, the VEGF rs2010963 locus had the fewest polymorphisms, totaling 25, which were mostly substitutions, along with 1 deletion and 1 duplication; only one of these variants resulted in an amino acid change.

E. Functional Interaction Network among IL-8, IL-27, and VEGF-A Genes

A gene-gene interaction network was constructed using GeneMANIA to analyze the molecular interactions and biological functions of IL-8, IL-27, and VEGF-A, as shown in Fig. 5. The network contained 23 genes and 355 interaction edges, including the three central genes and 20 associated genes linked through physical interactions, co-expression, co-localization, genetic associations, shared pathways, and domain homology, which formed a verified and physiologically realistic model. The hub genes with the most connections were CXCL8 (IL-8), IL-27, and VEGF-A. IL-8 was predominantly associated with CXCR1, CXCR2, CCL2, and CCL8, illustrating its chemokine role in tumor-related inflammation and neutrophil recruitment. IL-27, through IL27RA, EBI3, SHC2, NRP2,

http://dx.doi.org/10.14500/aro.12718

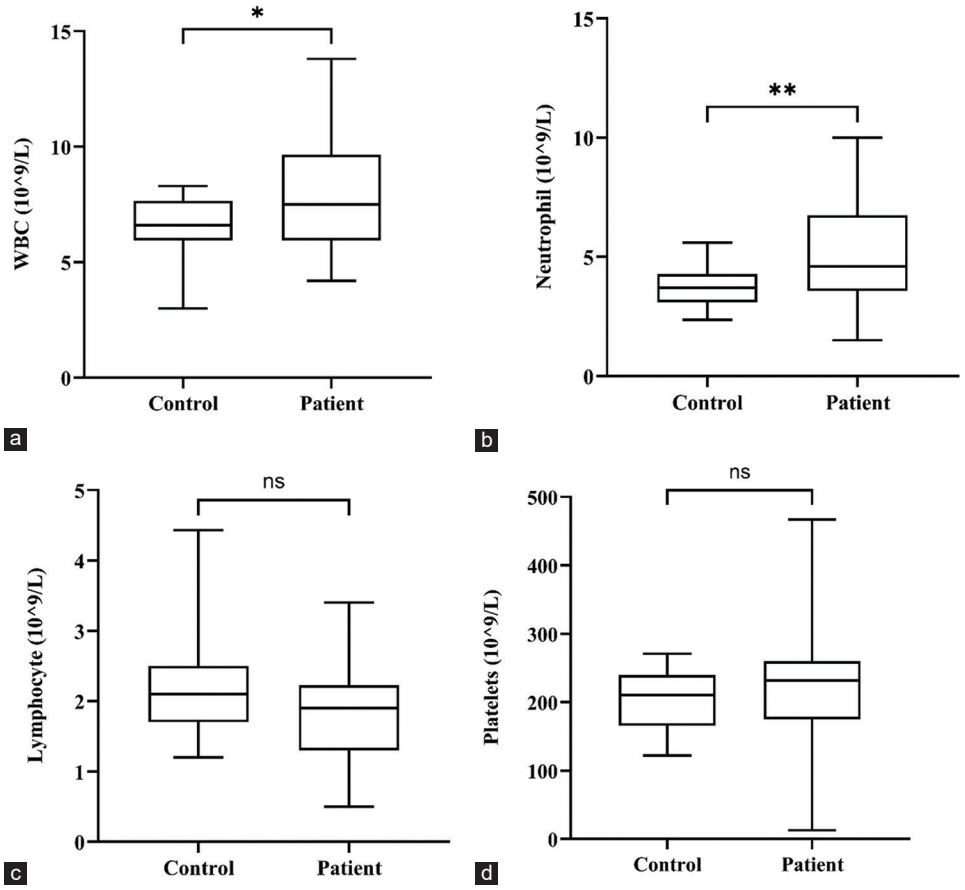

Fig. 1. Comparison of white blood cell (WBC) count, neutrophil percentage, lymphocyte percentage, and platelet count between prostate cancer patients and healthy controls: (a) Total WBC count, (b) Neutrophil percentage, (c) Lymphocyte percentage, (d) Total platelet count.

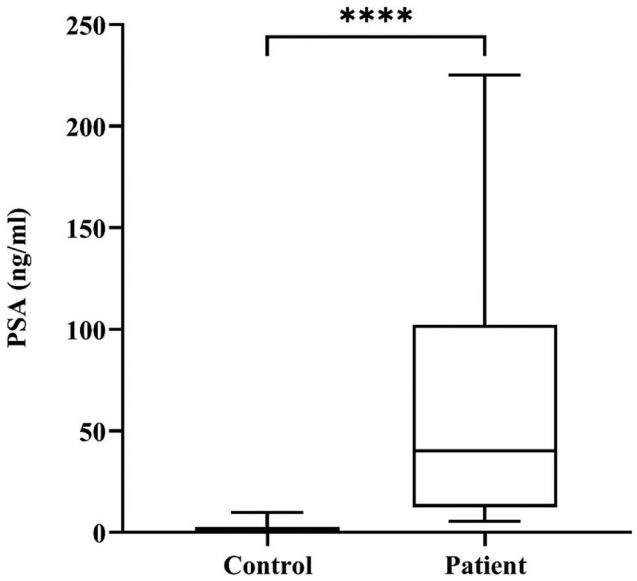

Fig. 2. Comparison of prostate-specific antigen concentrations (ng/mL) between prostate cancer patients and healthy individuals.

and various members of the signal transducer and activator of transcription (STAT) family, participated in Janus Kinase (JAK)-STAT–mediated anti-tumor and immunoregulatory pathways, which were largely IL-27 driven. VEGF-A interactions with KDR, FLT1, PGF, VEGF-B, NRP1, and NRP2 demonstrated its role in the formation of blood vessels, enabling endothelial migration and tumor vascularization. Collectively, these cytokine genes demonstrated an integrative control of inflammation, immune modulation, and angiogenesis in PCa, where IL-8 and VEGF enhance angiogenesis-associated inflammation and IL-27 targets immune checkpoints in the tumor microenvironment (TME).

IV. Discussion

Neutrophils are central to inflammation and the progression of PCa, and they are crucial to the TME (Venet and Monneret, 2018). When neutrophils are recruited to the tumor tissue, they are known to discharge proteases, reactive oxygen species, and neutrophil extracellular traps (NETs), thus driving chronic inflammation and breakdown of the extracellular matrix, which enables cancer cells to invade (Rizo-Téllez and Filep, 2024). Neutrophils associated with tumors (TANs) can shift in functionality, moving from an N1 (anti-tumor) to an N2 (pro-tumor) phenotype based on specific TME signals, and the N2 phenotype suppresses immune reactions, promotes blood-vessel formation, and enhances tissue remodeling and tumor growth (He et al., 2025). As tumors develop, neutrophils become increasingly

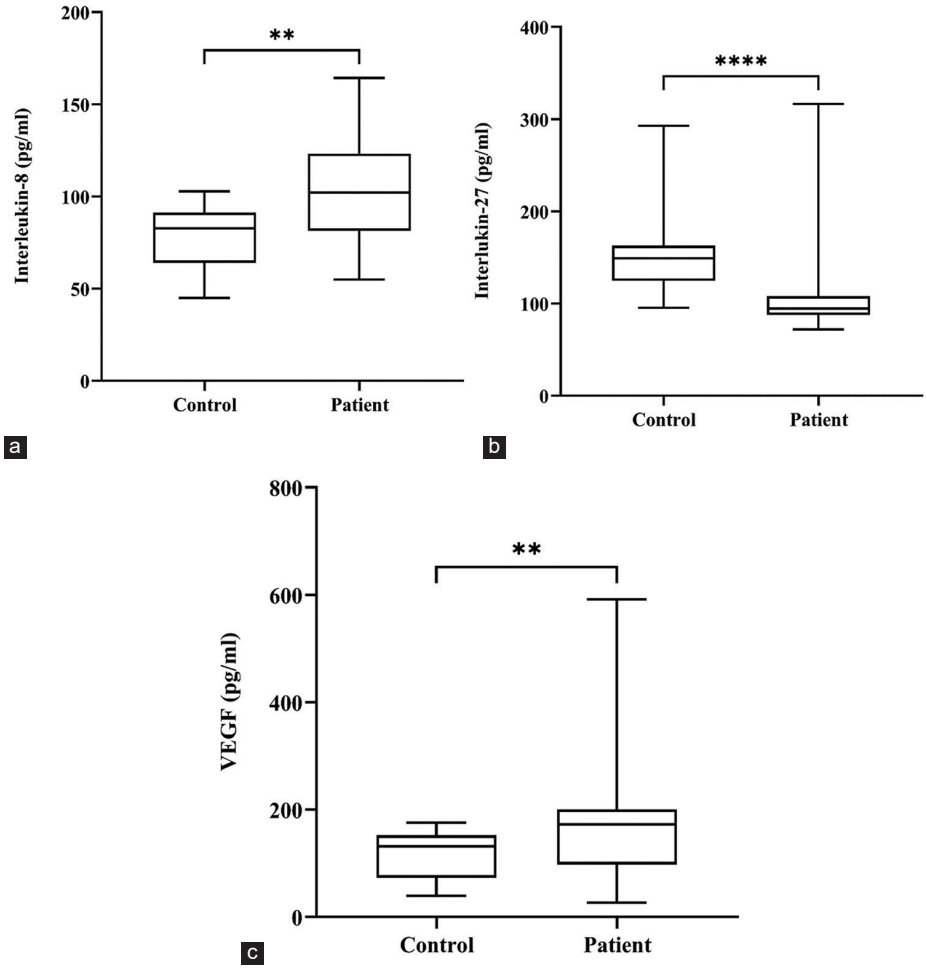

Fig. 3. Comparison of interleukin (IL)-8, IL-27, and vascular endothelial growth factor (VEGF) concentrations (pg/mL) between prostate cancer patients and the control group: (a) IL-8, (b) IL-27, (c) VEGF.

TABLE II
Summary of Genetic Variants via Mutation Surveyor

Gene	Mutation number	Type	Heterozygous/ homozygous	Unchanged amino acid	Changed Amino acid	External database
IL-8 *rs4073*	78	All Substitution	68 Heterozygous 10 Homozygous	76	2	2
IL-8 *rs2227306*	31	All Substitution	All Heterozygous	All of Them	None	1
IL-27	89	86 Substitution 1 Insertion 1 Deletion 1 Duplication	82 Heterozygous 7 Homozygous	All of Them	None	1
VEGF	25	23Substitution 1 Deletion 1 Duplication	24 Heterozygous 1 Homozygous	24	1	None

IL: Interleukin, VEGF: Vascular endothelial growth factor

TABLE III
The Mutation Surveyor found Changes in the *IL-8* and *VEGF* Genes that Affect Amino Acids in Prostate Cancer Patients

Gene	Chromosome position	Mutation	Genotype	Changed amino acid	Variants%	External database
IL-8 *rs4073*	4:74606401	Substitution	T>C: 9L>P	Leucine/Proline	100	Not found
IL-8 *rs4073*	4:74606402	Substitution	C>T: 9L>P	Leucine/Proline	100	Not found
VEGF	6:43738455	Substitution	A>AT: 4R>D	Arginine/Aspartic Acid	33	Not found

IL: Interleukin, VEGF: Vascular endothelial growth factor

http://dx.doi.org/10.14500/aro.12718

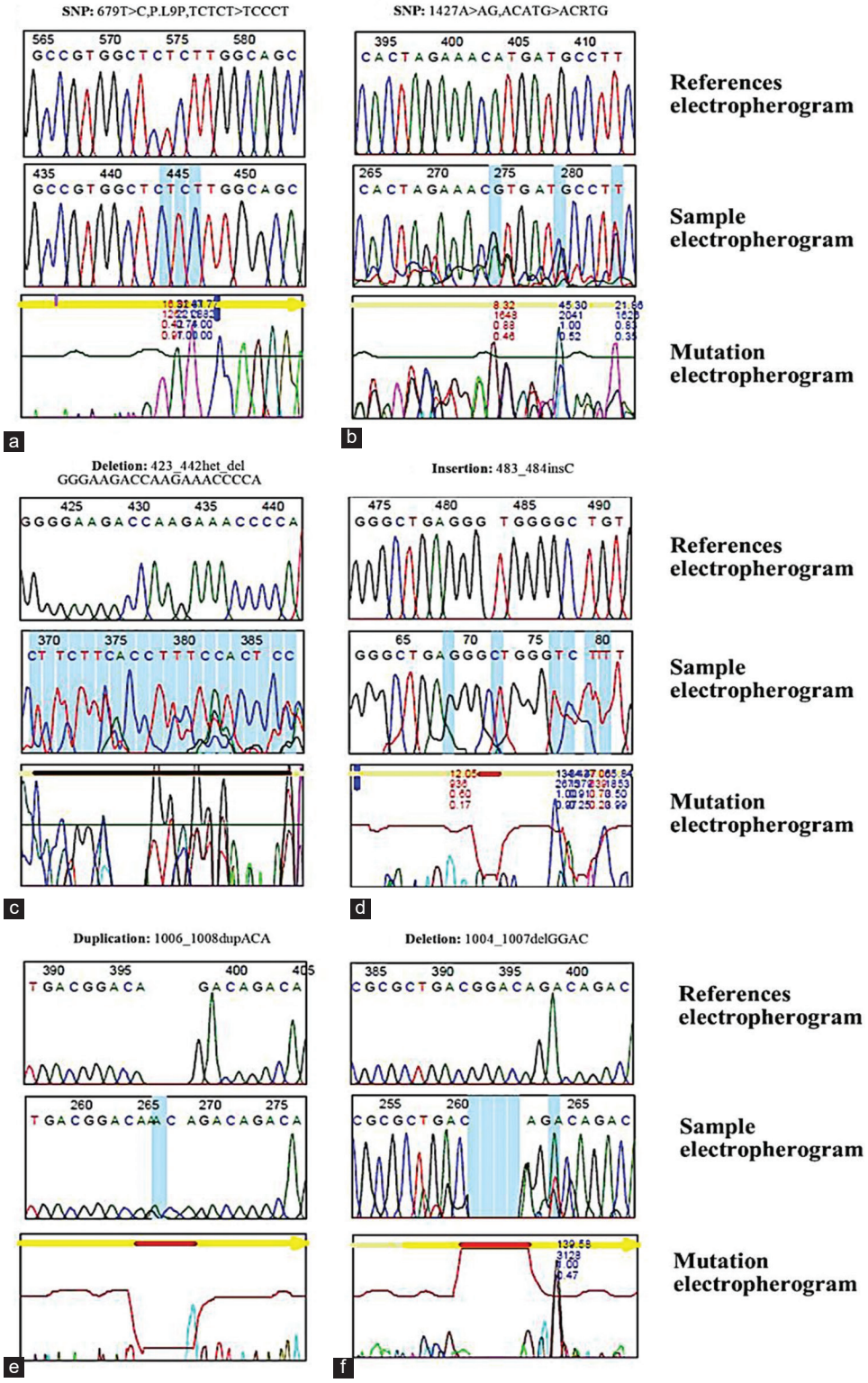

Fig. 4. DNA electropherogram profiles display nucleotide variations in the *interleukin* (*IL*)-8, *IL*-27, and vascular endothelial growth factor (*VEGF*) genes. (a) IL-8 rs4074 Homozygous substitution (b) IL-8 rs2227306 Heterozygous substitution (c and d) IL-27 rs153109 Heterozygous deletion and homozygous insertion (e and f) VEGF rs2010963 heterozygous duplication and deletion.

less able to display cytotoxic abilities, thereby allowing the immune evasion of the PCa cells. TANs have also been shown to contribute to bone metastasis, and in this case, the increase in neutrophils and NET formation may restrict metastatic dissemination but promote TME remodeling (Alsamraae et al., 2023). In our study of patients, neutrophil and WBCs counts were high, whereas lymphocytes and platelets were stable. These findings resulted in a higher neutrophil-to-lymphocyte ratio, mechanistically a shift to a pro-tumor inflammatory milieu skewed toward neutrophils

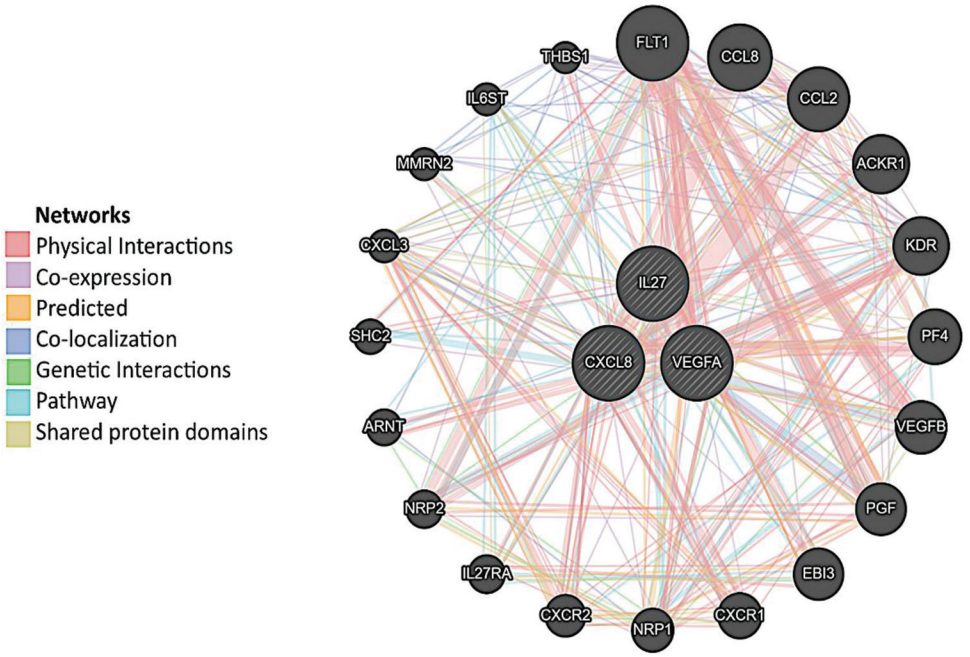

Fig. 5. The functional relationship network between IL-8, IL-27, and VEGF-A based on the GeneMANIA database. IL: Interleukin, VEGF: Vascular endothelial growth factor.

and dominated by neutrophil activity (Watts et al., 2020). In contrast, the platelet-to-lymphocyte ratio did not change, suggesting that this cohort did not have significant tumor-promoting inflammation driven by platelets.

Normal, benignly enlarged, and even malignant prostate cells all secrete PSA (Denmeade et al., 2003). It is primarily produced by columnar epithelial cells within the prostate and is classically known as a serine protease (Schalken, 2004). PSA, as a prostate enzyme, functions as a biocatalyst in the cleavage of gel-forming proteins of semen, thus aiding sperm motility. The gene responsible for its production is KLK3, which is a member of the kallikrein-related peptidase family (Lilja, Ulmert and Vickers, 2008). It has been indicated that although malignant cells synthesize less PSA (Occhipinti et al., 2021), the destruction of the prostatic epithelium during the invasive phase of a tumor allows more PSA to enter the blood circulation system (Lilja, Ulmert and Vickers, 2008). This explanation may account for the significant differences in serum PSA levels observed between patients and controls during the study.

The involvement of inflammation in the early stages of development and progression of a tumor is well documented, and PCa is notable for its high level of inflammatory activity. In the context of inflammatory cytokines, IL-8 is particularly important, as it acts directly on tumor cells and indirectly within the TME to enhance both proliferation and disease progression (Chen et al., 2021). Our study demonstrated that IL-8 concentrations were much higher in patients with PCa than in the control group. This result was supported by both *in vitro* and *in vivo* investigations, with higher levels seen in malignant cells and the sera of patients and with significant correlations to disease stage and tumor progression (Ferrer et al., 1998; Veltri et al., 1999). Sanger sequencing of the two *IL-8* gene areas in PCa samples revealed numerous single-nucleotide polymorphisms (SNPs), all of which were nucleotide substitutions. Of note, two rs4073 substitutions gave rise to changes of amino acids, which may suggest profound changes at the protein level. A 2020 meta-analysis supported our findings and revealed a substantial association between the IL-8 rs4073 polymorphism and heightened PCa risk across various genetic models (Chen et al., 2020). Mechanistically, IL-8 via the CXCR2 signaling axis modulates immune-cell infiltration, angiogenic-factor production, and inflammatory signaling, thereby influencing both TME and tumor cell behavior (McClelland et al., 2024). Processes driven by CXCR2 recruit neutrophils and tumor-associated macrophages (TAMs) while stimulating angiogenesis, supporting IL-8's pro-tumor and pro-angiogenic effects within the TME (Dahal et al., 2023). At the cellular level, IL-8 promotes proliferation and invasion and inhibits apoptosis (Guo et al., 2017). The secretion levels for many of these factors are strongly associated with tumor aggressiveness (Archer et al., 2020) and may also relate to polymorphisms of the *IL-8* gene (Ghazy and Alenzi, 2021).

IL-27 exhibits multiple antitumor functions, including the ability to activate immune effector cells, control angiogenesis, inhibit tumor-cell proliferation, promote apoptosis, and upregulate TLR3 expression, both *in vitro* and *in vivo* in PCa (Kourko et al., 2019; Di Carlo et al., 2013). In this study, however, the concentration of IL-27 in PCa patients was significantly lower than in healthy controls, reflecting diminished immunostimulatory and tumor-suppressive activity within the TME. Reduced IL-27 levels may impair antitumor activity by decreasing the influence of IL-27 on the STAT1/STAT4 pathway and activating $CD8^+$ T and NK cells (granzyme, perforin, IFN-γ) (Schneider et al., 2011), as well

as by diminishing the anti-proliferative tumor-cell signaling of IL-27 (Zhang et al., 2016). A previous study supported our findings, reporting decreased expression of IL-27 in PCa patients relative to healthy controls, associated with disease progression (Zolochevska et al., 2013). Furthermore, a recent study has reported the dualistic nature of IL-27 in tumor immunity, in which it generally promotes antitumor responses but in certain situations may function as a pro-tumor cytokine by inducing immune suppression and aiding in tumor growth (Mirlekar and Pylayeva-Gupta, 2021). Thus, in our patients, reduced expression may shift from an immunostimulatory to an uncontrolled, tumor-promoting state. The Sanger sequencing revealed that IL-27 showed the highest number of mutations in comparison to the *IL-8* and *VEGF* genes, totaling 89 alterations comprising 86 nucleotide substitutions and one each of insertion, deletion, and duplication. Such polymorphisms might change the expression of the *IL-27* gene or alter the stability of its protein, which in turn diminishes its functional activity and antitumor immune response. Taken together, these reasons demonstrate why IL-27 cannot perform its primary function as an antitumor and further serve as a factor for promoting angiogenesis and disease progression.

The critical functions of VEGF in microvascular remodeling and metastasis during the advancement of PCa have been documented and highlighted (Wu et al., 2007). Not only does VEGF serve as the strongest activator of endothelial cells associated with normal and pathological angiogenesis (Elaimy and Mercurio, 2018), but it also serves as the primary and most aggressive promoter of PCa in all of its stages (Kluetz, Figg and Dahut, 2010). Of the multiple protein isoforms that VEGF possesses, VEGF-A is the most studied form and is especially known for its role in angiogenesis in PCa (Wong et al., 2005). VEGF exerts its action by binding with the relevant cell surface receptors, which are the tyrosine kinase receptors, and stimulates signaling processes, such as the Ras–MAPK and PI3K/Akt pathways that enhance angiogenesis (Catena et al., 2010). The results of our study demonstrated that PCa patients had higher VEGF levels in comparison to the healthy population; this result agrees with a previous study showing that increased levels of VEGF are indicative of disease prognosis (Leach et al., 2025). VEGF continues to modify the TME by controlling the T-lymphocytes and the TAMs, which in turn modify the immune cell's dynamics, enabling immune evasion (Basagiannis et al., 2016). Their combined action supports the proliferation, invasion, and metastasis of tumor cells in the microenvironment of PCa (Larsson et al., 2020). For the *VEGF* gene, 25 SNPs were recorded, which comprise 23 substitutions, one deletion, and one duplication, of which only one caused an amino acid change. Out of the 25 recorded variants, none had been reported earlier in GenBank. This range of gene polymorphisms observed in the *VEGF* gene indicates its potential role in PCa metastasis and angiogenesis.

The connection networks between IL-8, IL-27, and VEGF-A, along with other genes, were constructed and presented in a model by GeneMANIA. The developed network indicates that these genes operate in cooperation and not in isolation, integrating the inflammatory, immunomodulatory, and angiogenic elements that characterize the TME. Such profile interactions correlate with our observations that IL-8 enhances inflammation and neutrophil responses (McClelland et al., 2024b); IL-27, which was reduced in our patient groups, indicates a reduction of immune-stimulating signals and an immune-dysregulated state (Mirlekar and Pylayeva-Gupta, 2021); and VEGF-A stimulates angiogenesis and tissue remodeling (Wu et al., 2007). These mechanisms together could promote alterations in these genes, indicating dominant signaling pathways that sustain inflammation, angiogenesis, and tumor proliferation. Thus, the network provides supportive evidence of a unified mechanistic model in which IL-8, IL-27, and VEGF-A functionally interact in PCa pathology.

This study certainly adds to the understanding of PCa at the molecular level by analyzing the expression and mutations of IL-8, IL-27, and VEGF; however, the study is limited to a single region and is constrained by a moderate sample size. Future work, especially that involving more complex functional assays on larger and more heterogeneous populations, should further examine cytokine gene polymorphisms and how they relate to the inflammation, immune response, and angiogenesis in the development and progression of PCa.

V. Conclusion

This study showed that PCa patients had altered serum levels of IL-8, IL-27, and VEGF and distinct gene polymorphisms. Elevated IL-8 and VEGF can be interpreted as enhanced inflammation, angiogenesis, and tumor progression, whereas decreased IL-27 suggests weakened antitumor immune signaling, possibly influenced by genetic alterations. The identified SNPs may alter pro-inflammatory and anti-inflammatory cytokine expression, thus contributing to the pathogenesis of PCa. GeneMANIA demonstrated functional interconnections among these cytokines to export inflammation, immune regulation, and angiogenesis, showing why those processes are necessary for the progression of PCa. Targeting these cytokines and their pathways may assist in finding novel PCa treatments and requires further study.

References

Alsamraae, M., Costanzo-Garvey, D., Teply, B.A., Boyle, S., Sommerville, G., Herbert, Z.T., Morrissey, C., Dafferner, A.J., Abdalla, M.Y., Fallet, R.W., Kielian, T., Jensen-Smith, H., DeOliveira, E.I., Chen, K., Bettencourt, I.A., Wang, J.M., MeVicar, D.W., Keeley, T., Yu, F., and Cook, L.M., 2023. Androgen receptor inhibition suppresses anti-tumor neutrophil response against bone metastatic prostate cancer via regulation of TβRI expression. *Cancer Letters,* 579, p.216468.

Archer, M., Dogra, N., and Kyprianou, N., 2020. Inflammation as a driver of prostate cancer metastasis and therapeutic resistance. *Cancers (Basel),* 12, p.2984.

Basagiannis, D., Zografou, S., Murphy, C., Fotsis, T., Morbidelli, L., Ziche, M., Bleck, C., Mercer, J., and Christoforidis, S., 2016. VEGF induces signalling and

angiogenesis by directing VEGFR2 internalisation through macropinocytosis. *Journal of Cell Science,* 129, pp.4091-4104.

Berenguer, C.V., Pereira, F., Câmara, J.S., and Pereira, J.A., 2023. Underlying features of prostate cancer-statistics, risk factors, and emerging methods for its diagnosis. *Current Oncology,* 30, pp.2300-2321.

Catena, R., Larzabal, L., Larrayoz, M., Molina, E., Hermida, J., Agorreta, J., Montes, R., Pio, R., Montuenga, L.M., and Calvo, A., 2010. $VEGF_{121}b$ and $VEGF_{165}b$ are weakly angiogenic isoforms of VEGF-A. *Molecular Cancer,* 9, p.320.

Chen, C.H., Ho, C.H., Hu, S.W., Tzou, K.Y., Wang, Y.H, and Wu, C.C., 2020. Association between interleukin-8 rs4073 polymorphism and prostate cancer: A meta-analysis. *Journal of the Formosan Medical Association,* 119, pp.1201-1210.

Chen, K., Jiang, K., Tang, L., Chen, X., Hu, J., and Sun, F., 2021. Analysis of clinical trials on therapies for prostate cancer in mainland China and globally from 2010 to 2020. *Frontiers in Oncology,* 11, p.647110.

Dahal, S., Chaudhary, P., Jung, Y.S., and Kim, J.A., 2023. Megakaryocyte-Derived IL-8 acts as a paracrine factor for prostate cancer aggressiveness through CXCR2 activation and antagonistic AR downregulation. *Biomolecules and Therapeutics,* 31, pp.210-218.

Denmeade, S.R., Litvinov, I., Sokoll, L.J., Lilja, H., and Isaacs, J.T., 2003. Prostate-specific antigen (PSA) protein does not affect growth of prostate cancer cells *in vitro* or prostate cancer xenografts *in vivo*. *The Prostate,* 56, pp.45-53.

Di Carlo, E., Sorrentino, C., Zorzoli, A., Di Meo, S., Tupone, M.G., Ognio, E., Mincione, G., and Airoldi, I., 2013. The antitumor potential of Interleukin-27 in prostate cancer. *Oncotarget,* 5, pp.10332-10341.

Elaimy, A.L., and Mercurio, A.M., 2018. Convergence of VEGF and YAP/TAZ signaling: Implications for angiogenesis and cancer biology. *Science Signaling,* 11, p.eaau1165.

Ene, C., Nicolae, I., and Ene, C.D., 2023. Angiogenic systemic response to the hypoxic microenvironment in prostate tumorigenesis: A pilot study. *Experimental and Therapeutic Medicine,* 26, p.483.

Ferrer, F.A., Miller, L.J., Andrawis, R.I., Kurtzman, S.H., Albertsen, P.C., Laudone, V.P., and Kreutzer, D.L., 1998. Angiogenesis and prostate cancer: *In vivo* and *in vitro* expression of angiogenesis factors by prostate cancer cells. *Urology,* 51, pp.161-167.

Franz, M., Rodriguez, H., Lopes, C., Zuberi, K., Montojo, J., Bader, G.D., and Morris, Q., 2018. GeneMANIA update 2018. *Nucleic Acids Research,* 46, pp.W60-W64.

Furriol, J., Wik, E., Aziz, S., Askeland, C., Knutsvik, G., and Akslen, L.A., 2024. *VEGFA* gene variants are associated with breast cancer progression. *The Journal of Pathology: Clinical Research,* 10, p.e12393.

Ghazy, A.A., and Alenzi, M.J., 2021. Relevance of interleukins 6 and 8 single nucleotide polymorphisms in prostate cancer: A multicenter study. *Prostate Cancer,* 2021, p.3825525.

Guo, Y., Zang, Y., Lv, L., Cai, F., Qian, T., Zhang, G., and Feng, Q., 2017. IL8 promotes proliferation and inhibition of apoptosis via STAT3/AKT/NFκB pathway in prostate cancer. *Molecular Medicine Reports,* 16, pp.9035-9042.

He, W., Yan, L., Hu, D., Hao, J., Liou, Y.C., and Luo, G., 2025. Neutrophil heterogeneity and plasticity: Unveiling the multifaceted roles in health and disease. *Medicine Communications,* 6, p.e70063.

Karwan, M.A., Abdullah, O.S., Amin, A.M., Mohamed, Z.A., Hasan, B., Shekha, M., Najmuldeen, H.H., Rahman, F.M., Housein, Z., Salih, A.M., Mohammed A.S., Sulaiman, L.R., Barzingi, B.T., Mahmood, D., Othman, H.E.,… & Salihi, A., 2022. Cancer incidence in the Kurdistan region of Iraq: Results of a seven-year cancer registration in Erbil and Duhok Governorates. *Asian Pacific Journal of Cancer Prevention,* 23, pp.601-615.

Kluetz, P.G., Figg, W.D., and Dahut, W.L., 2010. Angiogenesis inhibitors in the treatment of prostate cancer. *Expert Opinion on Pharmacotherapy,* 11, pp.233-247.

Kourko, O., Smyth, R., Cino, D., Seaver, K., Petes, C., Eo, S.Y., Basta, S., and Gee, K., 2019. Poly(I: C)-mediated death of human prostate cancer cell lines is induced by interleukin-27 treatment. *Journal of Interferon and Cytokine Research,* 39, pp.483-494.

Larsson, P., Syed Khaja, A.S., Semenas, J., Wang, T., Sarwar, M., Dizeyi, N., Simoulis, A., Hedblom, A., Wai, S.N., Ødum, N., Persson, J.L., 2020. The functional interlink between AR and MMP9/VEGF signaling axis is mediated through PIP5K1α/pAKT in prostate cancer. *International Journal of Cancer,* 146, pp.1686-1699.

Leach, D.A., Chatterjee, N., Spahr, K., De Almeida, G.S., Varela-Carver, A., Shah, T.T., Winkler, M., Ahmed, H.U., and Bevan, C.L., 2025. Simultaneous inhibition of TRIM24 and TRIM28 sensitises prostate cancer cells to antiandrogen therapy, decreasing VEGF signalling and angiogenesis. *Molecular Oncology*. 19, pp.2797-2821.

Lilja, H., Ulmert, D., and Vickers, A.J., 2008. Prostate-specific antigen and prostate cancer: Prediction, detection and monitoring. *Nature Reviews Cancer,* 8, pp.268-278.

Luo, Y., Yang, Z., Yu, Y., and Zhang, P., 2022. HIF1α lactylation enhances KIAA1199 transcription to promote angiogenesis and vasculogenic mimicry in prostate cancer. *International Journal of Biological Macromolecules,* 222, pp.2225-2243.

Maleki, A.H., Rajabivahid, M., Khosh, E., Khanali, Z., Tahmasebi, S., and Ghorbi, M.D., 2025. Harnessing IL-27: Challenges and potential in cancer immunotherapy. *Clinical and Experimental Medicine,* 25, p.34.

McClelland, S., Maxwell, P.J., Branco, C., Barry, S.T., Eberlein, C., and Labonte, M.J., 2024a. Targeting IL-8 and its receptors in prostate cancer: Inflammation, stress response, and treatment resistance. *Cancers (Basel),* 16, p.2797.

McClelland, S., Maxwell, P.J., Branco, C., Barry, S.T., Eberlein, C., and Labonte, M.J., 2024b. Targeting IL-8 and its receptors in prostate cancer: Inflammation, stress response, and treatment resistance. *Cancers (Basel),* 16, p.2797.

Mirlekar, B., and Pylayeva-Gupta, Y., 2021. IL-12 family cytokines in cancer and immunotherapy. *Cancers (Basel),* 13, p.167.

Occhipinti, S., Mengozzi, G., Oderda, M., Zitella, A., Molinaro, L., Novelli, F., Giovarelli, M., and Gontero, P., 2021. Low levels of urinary PSA better identify prostate cancer patients. *Cancers (Basel),* 13, p.3570.

Rizo-Téllez, S.A., and Filep, J.G., 2024. Beyond host defense and tissue injury: The emerging role of neutrophils in tissue repair. *American Journal of Physiology-Cell Physiology*, 326, pp.C661-C683.

Sarkar, C., Goswami, S., Basu, S., and Chakroborty, D., 2020. Angiogenesis inhibition in prostate cancer: An update. *Cancers (Basel),* 12, p.2382.

Schalken, J.A., 2004. Molecular and cellular prostate biology: Origin of prostate-specific antigen expression and implications for benign prostatic hyperplasia. *BJU International,* 93, pp.5-9.

Schneider, R., Yaneva, T., Beauseigle, D., El-Khoury, L., and Arbour, N., 2011. IL-27 increases the proliferation and effector functions of human naive CD8+ T lymphocytes and promotes their development into $T_{c1\ cells}$. *European Journal of Immunology,* 41, pp.47-59.

Siegel, R.L., Kratzer, T.B., Giaquinto, A.N., Sung, H., and Jemal, A., 2025. Cancer statistics, 2025. *CA: A Cancer Journal for Clinicians,* 75, pp.10-45.

Veltri, R.W., Miller, M.C., Zhao, G., Ng, A., Marley, G.M., Wright G.L. Jr., Vessella, R.L., and Ralph, D., 1999. Interleukin-8 serum levels in patients with benign prostatic hyperplasia and prostate cancer. *Urology,* 53, pp.139-147.

Venet, F., and Monneret, G., 2018. Advances in the understanding and treatment of sepsis-induced immunosuppression. *Nature Reviews Nephrology,* 14, pp.121-137.

Watts, E.L., Perez-Cornago, A., Kothari, J., Allen, N.E., Travis, R.C., and Key, T.J., 2020. Haematological markers and prostate cancer risk: A prospective

analysis in UK Biobank. *Cancer Epidemiology, Biomarkers and Prevention,* 29, pp.1615-1626.

Wong, S.Y., Haack, H., Crowley, D., Barry, M., Bronson, R.T., and Hynes, R.O., 2005. Tumor-secreted vascular endothelial growth factor-C is necessary for prostate cancer lymphangiogenesis, but lymphangiogenesis is unnecessary for lymph node metastasis. *Cancer Research,* 65, pp.9789-9798.

Wu, T.T.L., Wang, J.S., Jiann, B.P., Yu, C.C., Tsai, J.Y., Lin, J.T., and Huang, J.K., 2007. Expression of vascular endothelial growth factor in Taiwanese benign and malignant prostate tissues. *Journal of the Chinese Medical Association,* 70, pp.380-384.

Zhang, Z., Zhou, B., Zhang, K., Song, Y., Zhang, L., and Xi, M., 2016. IL-27 suppresses SKOV3 cells proliferation by enhancing STAT3 and inhibiting the Akt signal pathway. *Molecular Immunology,* 78, pp.155-163.

Zolochevska, O., Diaz-Quiñones, A.O., Ellis, J., and Figueiredo, M.L., 2013. Interleukin-27 expression modifies prostate cancer cell crosstalk with bone and immune cells *in vitro*. *Journal of Cellular Physiology,* 228, pp.1127-1136.