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The Intratumoral Microbiota in Prostate Cancer: Mechanisms, Microbial–Androgen Crosstalk, and Urinary Exfoliative Signatures for Liquid Biopsy Development

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Abstract

Prostate cancer [PCa] is the second most prevalent malignancy diagnosed in men worldwide, constituting a considerable public health challenge due to the inadequacies of existing diagnostic methods focused on prostate-specific antigen [PSA] testing, multiparametric magnetic resonance imaging [MRI], and invasive biopsy. The recent paradigm shift towards viewing solid tumors as complex ecosystems has revealed the intratumoral microbiota, a community of bacteria, viruses, and fungi residing within the tumor microenvironment [TME] as a crucial regulator of carcinogenesis. The origin, spatial dynamics, and mechanistic functions of the prostate intratumoral microbiota are summarized in this thorough review. We present "The Prostate Microbial Triad," a novel conceptual framework that describes the co-evolutionary interaction of microbes, resident immune cells, and malignant epithelium. We explore particular mechanisms such as microbial reprogramming of tumor immunology and metabolism, the proposed "Microbial–Androgen Crosstalk Model" specific to PCa, and microbe-induced genomic instability. In order to create reliable liquid biopsies, we suggest a translational pipeline that connects tumor-resident microbes to urine signals through the "Microbial-Extracellular Vesicle [EV] Pathway," and we support multi-omics integration [metagenomics, metabolomics, and spatial transcriptomics] driven by machine learning. Decoding and utilizing the prostate intratumoral microbiome, according to this review, is a frontier with unmatched potential to transform PCa diagnostics by enabling accurate, non-invasive detection and risk stratification.

Introduction

In contemporary oncology, prostate cancer [PCa] poses a significant clinical challenge. It is a major cause of cancer-related morbidity and mortality in men, with an estimated 1.4 million new cases and over 375,000 deaths annually worldwide [1]. The clinical conundrum is caused by both its high incidence and its diverse behavior, which ranges from aggressive, metastatic variants to indolent tumors that might never pose a threat to life [2]. The crucial limitation of the diagnostic techniques used today is rooted in this heterogeneity. Although PSA screening is crucial in lowering mortality, its low specificity results in widespread overdiagnosis and overtreatment of clinically insignificant illness, which has negative effects on the body and mind [3,4]. Although subsequent diagnostic procedures, such as ultrasound-guided systematic or fusion biopsy and multiparametric MRI [mpMRI], have increased precision, they are still invasive, expensive, resource-intensive, and prone to sampling error [5]. This diagnostic impasse has fueled a decades-long quest for superior biomarkers. A transformative shift in cancer biology has recently emerged, framing malignancies not solely as genetic diseases but as complex "ecosystems" [6]. Malignant cells, stroma, vasculature, immune infiltrates, and a previously unrecognized element resident microbial communities are all included in this ecosystem. The identification of an intratumoral microbiota in several solid tumours, such as colorectal, pancreatic, breast, and prostate cancers, has significantly changed our knowledge

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of tumor biology [7]. There is strong evidence that these microbes actively affect tumour initiation, progression, metastatic dissemination, and response to therapy, making them more than just contaminants or passive bystanders [8,9]. A particularly interesting location to study tumor-microbe interactions is the prostate gland. Its unique microenvironment is produced by its ductal architecture, secretory functions [producing prostatic fluid rich in zinc, citrate, and antimicrobial peptides], and immunologically privileged status [10]. Advanced, contamination-controlled sequencing studies have verified the existence of a unique, low-biomass microbial community within both benign and malignant prostate tissue, which was previously thought to be sterile beyond the urethra [11,12]. These finding challenges long-held beliefs and raises important questions: Where did these microbes come from? How do they survive and arrange themselves spatially in the frequently hostile TME? Most importantly, do they contribute to or cause prostate cancer?

The intratumoral microbiota is a rich, unexplored source of molecular signals derived from tumors if it is functionally active. Capturing these microbial components or the host's reaction to them in an easily accessible biofluid presents a revolutionary diagnostic opportunity. Urine is the best medium for PCa. Exfoliated prostate cells, extracellular vehicles [EVs], cell-free nucleic acids, and metabolites that represent the physiological and pathological state of the gland are all present in this non-invasive sample that directly bathes the prostatic urethra. Moreover, EVs containing RNAs and proteins linked to infection provide possible biomarkers for infectious disease diagnosis. SEVs offer promising opportunities for the creation of novel cancer detection techniques. Endogenous, single-stranded, non-coding RNA molecules found in the majority of eukaryotic organisms, miRNAs have become useful tools for identifying abnormalities in regular cellular processes [13]. Although the idea of is well-established, the addition of biomarkers derived from microbes provides a potent, new biological information axis [14,15].

The goal of this review is to present a thorough summary of what is currently known about the intratumoral microbiota of prostate cancer. First, we will investigate its possible origins and the dynamic, spatially heterogeneous nature of its presence in the tumour. After that, we will analyse the potential mechanisms by which these microorganisms might interact with and alter important characteristics of prostate cancer, presenting new conceptual models to explain these interactions. The review's main focus will be on the translational opportunity, methodically analysing the justification, potential biomarkers, and difficult technical obstacles for identifying these intratumoral signals in urine.

Origin & Dynamics of the Prostate Intratumoral Microbiota

When microorganisms are found in prostate tissue, it is necessary to explain how they came to be in a glandular organ without being exposed to the outside world. Multifactorial colonisation routes and sophisticated adaptation that allows persistence within a distinct ecological niche are indicated by the evidence.

- **Sources of Microbial Colonization: Multiple Portals of Entry**

Clinical and molecular evidence suggests that microbial seeding of the prostate occurs through multiple non-exclusive pathways.

Ascending Urogenital Tract Colonization: This is the most straightforward path. A resident microbiome resides in the male urethra, and bacteria may ascend via retrograde flow, particularly in the event of urinary stasis, instrumentation, or microtrauma [16,17]. An increased long-term risk of PCa is linked to clinical histories of urethritis or prostatitis, indicating a connection between earlier microbial insult and subsequent carcinogenesis [18]. Prostate tissue studies often reveal bacterial species like Escherichia coli and Enterococcus faecalis that are frequently linked to UTIs and prostatitis [19].

The Gut–Prostate Axis: The intestinal microbiota and the prostate may communicate in both directions, similar to well-known systemic axes [20]. This could happen through: [a] Microbial metabolites, such as trimethylamine N-oxide and short-chain fatty acids, spread throughout the body and affect hormone metabolism and systemic inflammation [21,22]. [b] Bacterial translocation into the mesenteric lymphatics and systemic circulation through a compromised intestinal epithelial barrier ["leaky gut"], possibly seeding distant locations like the prostate [23,24]. [c] Immune modulation, in which T cell populations shaped by the gut microbiome travel and settle in peripheral tissues, influencing local immune surveillance [25].

Figure 1.0 Illustration of The Gut–Prostate Axis

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Hematogenous Dissemination: It is common for distant sites [oral, intestinal, or cutaneous] to cause transient bacteremia. The TME's distinct, frequently immunosuppressive, hyperpermeable vasculature may serve as a "sanctuary site," enabling circulating microbes to settle and endure [26]. Studies showing oral pathobionts like Fusobacterium nucleatum and Porphyromonas gingivalis in prostate tumors, which are similar to findings in colorectal cancer, lend credence to this [27,28]. A greater chance of PCa has been epidemiologically associated with periodontal disease [29].

Latent/Resident Pathobionts and Viral Co-factors: Early in life, certain microorganisms may develop latent, low-level residency, possibly in ductal systems or prostate stem cells. They may be reactivated by inflammatory triggers, immunological senescence, or hormonal changes [30]. Furthermore, oncogenic viruses like human papillomavirus [HPV] and cytomegalovirus [CMV] have been detected in some prostate tumours and may create a pro-inflammatory, pro-proliferative milieu that facilitates bacterial colonization or cooperatively drives oncogenic pathways [31].

- **Spatial Heterogeneity Within the Prostate Tumor Ecosystem**

The intratumoral microbiota has a complex spatial organization that is essential to its functional impact, but it is not uniformly distributed. Both macro and micro scales are affected by this heterogeneity.

Macro-scale: Tumor Core vs. Margin vs. Benign Tissue

The invasive front interacting with stroma and immune cells, the hypoxic, necrotic tumour core, and the histologically normal surrounding tissue can all have very different microbial biomass and composition. Fusobacterium nucleatum exhibits intra-tumoral regional variation and is more prevalent in colorectal cancer tumor tissue than in matched normal mucosa [32].

Micro-scale: Association with Specific Cellular and Structural Niches

Microscale colocalization is being revealed by sophisticated spatial profiling techniques. Microbes can be localized [33] in relation to particular cell types using imaging mass cytometry [IMC], multiplexed immunofluorescence [mIF], and in situ hybridization [e.g., RNAscope for bacterial rRNA] [33]. Do they accumulate in the extracellular stroma, within the intracellular compartments of cancer-associated fibroblasts or tumor-associated macrophages, or inside the lumens of tumor glands? There is evidence that bacteria can form biofilm-like aggregates inside dilated prostatic ducts, which confers immune clearance and antibiotic resistance [34].

- **Microbial Survivability in the Unique Prostate Tumor Microenvironment**

The prostate TME is a harsh environment that is frequently hypoxic, nutrient-deficient, acidic, and under immune siege. Certain adaptations are necessary for microbial persistence.

Metabolic Symbiosis and Competition

Through the Warburg effect, tumors frequently produce excess lactate due to metabolic reprogramming. Some Streptococcus and Veillonella species are among the bacteria that are adept at using lactate [35]. This leads to a possible symbiosis in which the tumor may benefit from bacterial metabolic byproducts while bacteria eat a waste product from the tumor, possibly reducing acidosis that prevents tumor growth. On the other hand, microbes may compete with immune cells and tumours for vital nutrients like amino acids [such as arginine and tryptophan], influencing the TME's metabolic limitations [36].

Exploiting Anaerobic and Immunosuppressive Niches

Facultative and obligatory anaerobes that are frequently found in prostate tissue, such as Fusobacterium spp. and Cutibacterium acnes, thrive in the hypoxic core of tumors [37]. Additionally, hypoxia provides a survival advantage by impairing neutrophil function and other oxygen-dependent phagocytic killing mechanisms [38]. Additionally, as explained in Section 3.3, microbes can actively suppress local immunity, changing their niche to make it more hospitable.

Formation of Biofilms and Intracellular Persistence

Physical and immunological protection is provided by living inside host cells [intracellular residency] or in dense, polymeric biofilm communities. It has been shown that the dominant prostate isolate Cutibacterium acnes, a known biofilm former, can invade and persist within prostate epithelial cell lines, possibly avoiding detection and destruction [39,40].

The Prostate Microbial Triad

According to this model, PCa progression is caused by a tripartite, dynamic crosstalk between tumor cells rather than by tumor cells alone [41]. The Intratumoral Microbiota: Actively shapes the immune response and modifies epithelial behavior [e.g., through genotoxins, metabolites].

This trio creates a coevolutionary ecosystem that reinforces itself. For example, microbes cause inflammation, which leads to epithelial DNA damage and proliferation; the ensuing tumor growth modifies immunity and local metabolism, further molding the microbial community, which in turn propels more aggressive tumor behavior [42]. This model provides a holistic framework for designing studies and therapies that target these interactions rather than individual components.

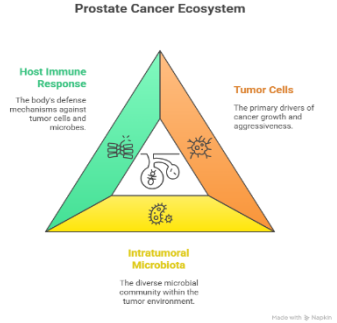

Figure 2.0 The Prostate Microbial Triad: A Coevolutionary Ecosystem

Intratumoral Microbiota–Tumor Interaction Mechanisms

The crucial question raised by the microbes' presence and spatial organization within the TME is whether or not they have functional implications for cancer biology. Emerging prostate-specific data and convergent evidence from multiple cancers point to an active, complex role [43].

- **Microbe-Induced DNA Damage and Genomic Instability**

Chronic inflammation is a recognized enabling characteristic of cancer, and persistent microbial presence is a potent inflammatory trigger.

Activation of the Inflammatory Pathway: Pattern-recognition receptors [PRRs] on epithelial and immune cells, such as Toll-like receptors [TLRs] and NOD-like receptors [NLRs], are activated by microbial components, such as LPS, lipoteichoic acid, flagellin, and bacterial DNA [44]. Reactive oxygen/nitrogen species [ROS/RNS], chemokines, and pro-inflammatory cytokines [IL-6, TNF-α, and IL-1β] are produced as a result of this engagement, which sets off downstream cascades, particularly NF-κB and STAT3 [45]. While long-term cytokine signaling encourages proliferative and anti-apoptotic programs in epithelial cells, ROS/RNS can directly cause DNA damage, including double-strand breaks, point mutations, and chromosomal instability [45].

Direct Genotoxicity: Toxins produced by some bacteria cause direct damage to DNA. Colibactin, a genotoxin encoded by the pks genomic island in particular strains of Escherichia coli, is the most well-studied. Adenine-to-cytosine substitutions in particular trinucleotide contexts are a characteristic mutational signature left by colibactin's interstrand crosslinks and double-strand breaks in DNA [46]. In colorectal cancer, this signature is noticeable. Although pks+ E. coli has been found in the prostate, research is currently being done to determine its genotoxic activity and mutational signature in this organ [47].

- **Modulation of Androgen Signaling: A PCa-Specific Axis**

The primary carcinogenic factor in all phases of prostate cancer is androgen receptor [AR] signaling. It's interesting to note that the microbiome may influence this crucial pathway both directly and indirectly, providing a special mechanistic connection to PCa biology.

Metabolite-Mediated Modulation: Compounds that mimic, oppose, or modify steroid synthesis and metabolism can be produced by microbial metabolism. Enzymes like β-glucuronidase and β-glucosidase, which deconjugate estrogen and phytoestrogen metabolites, reactivate them, and change the local hormonal milieu, are expressed by gut and possibly prostate bacteria [48]. A changed estrogen-to-androgen ratio can affect prostate growth, despite the complicated role that estrogens play in PCa. More specifically, although specific molecules in the prostate context are still unknown, microbial metabolites [such as particular SCFAs or secondary bile acids] may function as ligands or allosteric modulators of the AR [48].

Inflammation-Driven AR Activation: Microbe-induced inflammatory cytokines can trigger AR signaling. Through the MAPK and STAT3 signaling pathways, IL-6, a crucial cytokine in the PCa TME, can activate AR ligand-independently and work in concert with low androgen levels to enhance transcriptional activity [48]. In castration-resistant prostate cancer [CRPC], where tumor cells use different pathways to sustain AR signaling, this mechanism is extremely important.

Impact on Intratumoral Androgen Synthesis: Through "intracrine" pathways, advanced prostate cancers can produce androgens from cholesterol precursors. By influencing the expression of important enzymes [like AKR1C3] through inflammatory signals or by directly adding to or consuming the metabolic pool of precursors, the microbiome may have an impact on this intratumoral steroidogenesis [49].

The Microbial–Androgen Crosstalk Model

This model demonstrates a self-reinforcing, vicious cycle:

- The prostate TME develops a chronic inflammatory state due to intratumoral or gut-derived dysbiotic microbes.
- ROS and cytokines [IL-6, TNF-α] are produced continuously as a result of inflammation.
- These inflammatory mediators encourage epithelial survival and proliferation by directly or indirectly activating AR signaling.
- AR-active, proliferating epithelial cells change the local TME by releasing more pro-inflammatory damage-associated molecular patterns [DAMPs], increasing hypoxia, and changing nutrient availability.
- This modified TME reinforces the cycle by selectively enriching for microorganisms that flourish in these environments [such as anaerobes and inflammation-tolerant pathobionts] and/or worsening inflammation.

This model suggests that microbiome modulation could be a novel adjunct to androgen deprivation therapy [ADT] and offers a testable hypothesis for how microbes could contribute to both the critical transition to castration resistance and the initiation of cancer [50,51].

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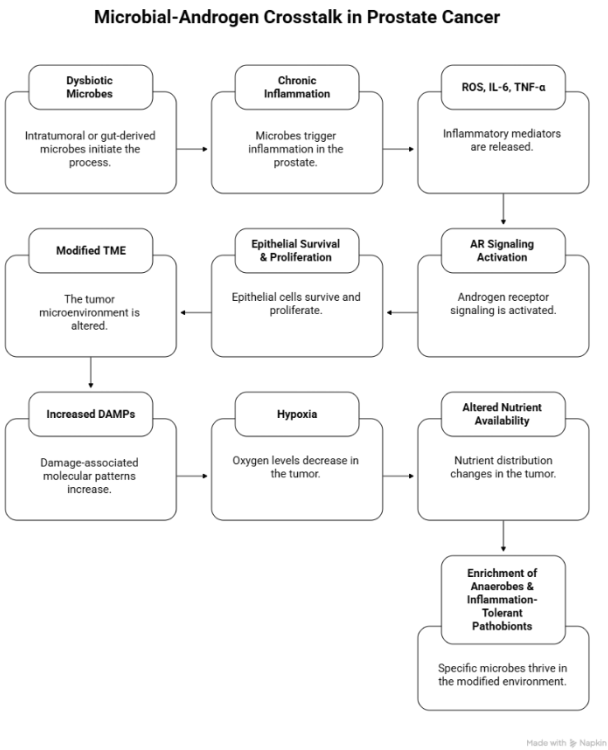

Figure 3.0 Model Diagram: Microbial-Androgen Crosstalk in Prostate Cancer

❖ **Effects on Tumor Immunology: Sculpting the Immune Landscape**

- The immune system can both eradicate and promote cancer, which makes its role in the disease paradoxical. One powerful regulator of this equilibrium is the intratumoral microbiota. Immunosuppressive cell populations can be recruited, expanded, and activated by bacterial signals. Myeloid progenitor cells that receive TLR signalling can differentiate into myeloid-derived suppressor cells [MDSCs], which effectively inhibit T cell and NK cell function by producing arginase-1, iNOS, and ROS [52]. Similarly, the growth and recruitment of regulatory T cells [Tregs] can be facilitated by microbial antigens and metabolites. For instance, it is known that SCFA butyrate promotes Treg differentiation and function through epigenetic mechanisms [HDAC inhibition]. These cells collectively create an "immunosuppressive shield" that protects both the microbe and the tumour from immune attack [53].
- Immune checkpoint molecule expression can be affected by microbial presence. Certain gut microbes are linked to increased PD-L1 expression on tumour and antigen-presenting cells in lung and colorectal cancers, which exacerbates T cell fatigue. Prostate intratumoral microbes may have comparable effects, which could account for PCa's generally poor response to immune checkpoint inhibitors [ICIs] and imply that microbiome modification could increase ICI efficacy [54].

❖ **Microbial Metabolic Reprogramming of the TME**

A significant metabolic reprogramming occurs in cancer cells. This altered metabolism is facilitated by and exploited by intratumoral microbes. Large volumes of lactate are secreted by tumors that rely on aerobic glycolysis, which contributes to extracellular acidosis and compromises immune cell function. Lactate can be the main carbon source for some bacteria, such as some Streptococcus species and Veillonella species. Bacteria may reduce tumor acidosis by consuming lactate, which would indirectly promote tumor growth and lessen the environment's hostility to immune cells like T cells, which are also susceptible to low pH. A possible metabolic symbiosis is represented by this [55].

SCFAs such as butyrate, propionate, and acetate are produced by microbial fermentation. These play intricate, concentration-dependent, and context-dependent roles. Butyrate, an HDAC inhibitor, may have immunosuppressive [pro-Treg] and possibly pro-tumor effects systemically or within the TME by promoting epigenetic changes in cancer cells. However, at high concentrations in the colon lumen, it promotes healthy colonocyte differentiation and apoptosis. Almost nothing is known about the function of SCFAs generated in the prostate TME [56].

Urinary Exfoliative Markers: A New Frontier in Liquid Biopsy

Urine is a readily available biological fluid that contains a number of different substances, some of which are filtered out of the bloodstream. These include tiny proteins secreted by different cell types and a variety of metabolic waste products. Additionally, it includes bigger proteins and cells that come from the urinary tract after glomerular filtration. Urine's liquid and solid components can be separated by low-speed centrifugation [57]. Cells, casts, and debris are typically found in the pellet, whereas soluble elements like proteins, exosomes, and cell-free nucleic acids that can be separated and assessed are kept in the supernatant. Every malignant lesion excretes cancerous cells. PCa can be detected in urine that has been voided via prostatic pathways. Genomic VPAC receptors are highly expressed on the surface of MCs [57,58]. Numerous prostatic biomarkers, including both cell-associated and cell-free indicators, are typically found in urine. Urine is a better option for treating prostate cancer than serum because of the possibility of contamination from other body tissue types and the enriched cell population as the urine travels through the prostatic urethra [59]. Even highly skilled cytopathologists find it challenging to identify prostate cancer cells based solely on morphology and immunohistochemistry due to the scarcity of cells and overlap of cytologic findings with urothelial cell carcinoma, even though cytologic identification and descriptions of shed prostate cancer cells in urine samples have been reviewed and reported [59,60].

❖ **Biology of Urinary Exfoliation: Capturing the Tumoral Snapshot**

Malignant prostate epithelial cells are constantly shed into the ductal network of the gland, which empties into the prostatic

urethra. A molecular cargo representative of the tissue of origin is carried by this physiological exfoliation. Tumor architecture and grade may have an impact on the type and rate of shedding. Tumors that are poorly differentiated and have problems with cell-cell adhesion, such as a loss of E-cadherin, may shed more easily. Microbially active tumors are characterized by inflammation, which increases exfoliation and epithelial turnover. Urine samples taken after a digital rectal examination [DRE] are frequently used to boost the yield of material derived from the prostate [43,61].

Extracellular Vesicles EVs are heterogeneous, membrane-bound nanoparticles [exosomes, microvesicles] released by all cells that contain a substantial amount of urine molecular information [62]. EVs transport metabolites, proteins, lipids, and nucleic acids [DNA, RNA, and miRNA] from their parent cell. Importantly, new data suggests that EVs can carry microbiological components. Urinary nucleases and proteases cannot break down bacterial DNA fragments, proteins, or metabolites that are encapsulated in tumor-derived EVs [63].

❖ A Taxonomy of Urinary Microbiome-Derived Biomarkers

Direct Microbial Signatures

RNA or cell-free microbial DNA [cfmDNA] released by living or dead bacteria inside the tumour. Urine shotgun metagenomic sequencing [64] can identify the existence, relative abundance, and potential functions of particular bacterial taxa. Targeted identification of pathobionts frequently linked to PCa tissue [such as Enterococcus faecalis, Fusobacterium nucleatum, and Cutibacterium acnes] in urine, particularly when enriched in comparison to controls, may function as a diagnostic signal [65,66]. Microbe-Associated Molecular Patterns [MAMPs]: Immunoassays can identify these conserved microbial structures. Lipoteichoic acid [LTA], lipopolysaccharide [LPS], and bacterial flagellin are a few examples. An active immune response to a specific microbial community may be indicated by elevated levels of particular MAMPs or host antibodies against them in urine [67].

Microbial Metabolic Byproducts

Microbial Volatile Organic Compounds [VOCs]: Typical VOCs are produced by bacteria. These tiny, carbon-based substances can enter the bloodstream and be eliminated through breath and urine. Research on other cancers, such as ovarian and bladder, has demonstrated that VOC profiles can differentiate patients from healthy controls. Electronic nose devices or gas chromatography-mass spectrometry [GC-MS] analysis of urine headspace may identify a PCa-specific "microbial metabolic fingerprint [68]. Non-volatile Metabolites: Certain microbial metabolites in urine, such as distinct SCFAs, polyamines, or genotoxin activity products [like colibactin-DNA adducts if excreted], may function as extremely specific biomarkers [69].

Host Response Signatures Induced by Microbes

Epigenetic Changes: Certain DNA methylation changes may result from long-term inflammation. A urine test that finds methylation of genes involved in microbial sensing [like TLR pathway genes] or genes known to be hypermethylated in PCa [like GSTP1, RASSF1, and APC] may serve as a stand-in for a microbially active TME [70].

Profiles of microRNA [miRNA]: Cellular stress and inflammation change the expression of miRNA. miRNAs are abundant in urinary EVs. Signatures of particular bacterial responses or miRNAs linked to TLR/NF-κB pathway activation [e.g., miR-21, miR-155] could be created [71].

Cytokine/Chemokine Panels: Inflammatory cytokines [IL-8, IL-6, CXCL1, and CXCL2] are frequently elevated in PCa and may be further amplified in the presence of an inflammatory intratumoral microbiome [72].

Linking Intratumoral Microbiota with Urinary Exfoliative Profiles

Urinary microbial biomarkers must ultimately be validated by proving a direct connection between the signals in the urine of the same person and the microbes in the tumor.

❖ Evidence for Shared Microbial Signatures

Men with positive and negative prostate biopsies have different urinary microbiome profiles, according to several studies. For example, it has been noted that PCa patients' urine has an enrichment of genera such as Propionibacterium [now Cutibacterium], Staphylococcus, and Anaerococcus [73]. Studies that sequence both compartments from the same patient provide the strongest evidence. According to preliminary reports, certain taxa identified in the patient's urine and the tumor are consistent, but not in the urine of controls. Cutibacterium acnes, Fusobacterium spp., Enterococcus spp., and Streptococcus spp. are frequently reported overlapping taxa in PCa [74]. For instance, one study discovered that matched urine samples contained Fusobacterium from prostate tumor tissue. To identify reliable, shared signatures, larger, carefully monitored cohort studies using deep metagenomic sequencing are required [75].

❖ Mechanistic Pathways of Microbe Shedding into Urine

As cells exfoliate, bacteria that live in the ductal lumens or stick to the epithelial surface may be passively transported by fluid flow. Bacteria or bacterial components internalized by immune or cancerous prostate cells can be packaged into multivesicular bodies and released as exosomes [76]. As an alternative, bacteria have the ability to produce their own outer membrane vesicles [OMVs]. The ductal lumen is subsequently filled with these microbe-filled EVs. In addition to shielding their cargo from deterioration, EV membranes may exhibit surface markers that could be utilized for urine

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immunocapture, enriching for signals derived from the prostate [77].

When neutrophils or macrophages that have phagocytosed bacteria within the tumor migrate into the ductal system, they can undergo NETosis, apoptosis, or release their contents into the prostatic fluid, including partially digested microbial fragments [such as peptidoglycan and bacterial DNA] [78].

❖ **The "Microbial Fingerprint" Diagnostic Pipeline**

Utilizing post-DRE urine from a well-phenotyped cohort and carefully collected matched tumor tissue [from radical prostatectomy] for deep shotgun metagenomic and metatranscriptomic sequencing. Finding microbial taxa, genes, and pathways that are consistently found in both compartments in men with PCa [especially high-grade] but not in controls [such as men with benign prostatic hyperplasia or normal prostates] is the aim [79]. Finding the minimal set of microbial and host-response characteristics that best distinguish PCa from non-cancer and, importantly, from indolent PCa by applying machine learning techniques [such as random forest and LASSO regression] to the multi-omics data. transforming the genomic signature into a useful, affordable diagnostic test. This could be a targeted metagenomic sequencing panel, a nanopore-based rapid sequencing assay, or a multiplexed qPCR or digital PCR panel for 10–30 important bacterial taxa and host genes. rigors testing in sizable prospective multicenter cohorts of men undergoing MRI abnormalities or elevated PSA biopsies. Key metrics include diagnostic accuracy [sensitivity, specificity, AUC, PPV, NPV] for identifying any PCa and, more crucially, for identifying clinically significant PCa [Gleason Grade Group ≥2], in comparison to and in conjunction with current biomarker tests [e.g., 4Kscore, SelectMDx], MRI PI-RADS score, and PSA density [79].

Multi-Omics Integration for Next-Generation Liquid Biopsy Development

❖ **Metagenomics & Metatranscriptomics**

In contrast to 16S sequencing, shotgun metagenomics provides functional information about microbial genes [virulence factors, antibiotic resistance, metabolic pathways like colibactin synthesis] and profiles bacteria, viruses, fungi, and eukaryotic microbes by sequencing all of the DNA in a sample. This makes it possible to identify functionally significant but low-abundance taxa and genes [80]. Metatranscriptomics, by sequencing all RNA, it is possible to determine which host and microbial genes are actively expressed, giving a dynamic picture of host response and microbial activity rather than just microbial presence. This could reveal pathways that were actively influencing the TME during the sampling period [81].

❖ **Metabolomics**

Mass Spectrometry-Based Profiling: Hundreds to thousands of small molecules in urine can be measured using high-throughput liquid chromatography-mass spectrometry [LC-MS] or GC-MS. PCa-specific metabolic profiles can be found through unsupervised analysis, such as principal component analysis. By comparing to databases of microbial metabolites, such as ECMDB, supervised analysis can then determine which metabolites are most likely microbial in origin or are host metabolites whose levels are correlated with particular microbial characteristics [82].

❖ **Spatial Multi-Omics within Tissue**

Gene or protein expression can be measured in precise, microscopically defined areas of a tissue section thanks to technologies like 10x Genomics Visium, NanoString GeoMx DSP, and CODEX. Mechanistic questions such as "What is the immune gene expression signature in a region rich in Fusobacterium compared to a region without it?" can be directly addressed by applying this to prostate tumours with defined microbial areas [via simultaneous in situ hybridization]. This can detect host response signatures [such as a particular cytokine profile] for downstream targeting in liquid biopsies and offers causal insights [83].

Clinical Translation: Toward a Microbiome-Driven Diagnostic Tool

❖ **Prototype Development for a Microbial Liquid Biopsy Kit**

The structure of a pragmatic first-generation test could look like this:

- **Standardized Collection Kit**: A urine collection vial containing a preservative [such as Zymo's DNA/RNA Shield or Norgen's Urine Preservation Buffer] to instantly stabilize nucleic acids, stop the growth of contaminating bacteria, and enable transport at room temperature.
- **Automated Nucleic Acid Extraction**: A platform designed for low-biomass samples that includes internal spike-in controls [like synthetic DNA sequences] to track extraction efficiency and measure absolute abundance, as well as steps to remove human DNA [like sialidase or selective lysis] to enrich for microbial signals.
- **Detection Module**: Multiplexed qPCR/ddPCR Panel: A 96-well plate format that tests for a few host response genes/miRNAs and 20–30 predetermined bacterial targets [specific to a species or strain]. Quick, affordable, and appropriate for clinical labs with high throughput. For low-abundance targets, digital PCR [ddPCR] provides better sensitivity and absolute quantification.
- **Next-Generation Sequencing [NGS] Panel**: This hybridization-based targeted capture panel for microbial genomic regions and host response markers yields more detailed information, but it is more expensive and takes longer to complete.
- **Point-of-Care Long-term vision**: a disposable cartridge that uses electrochemical/optical biosensors [e.g., functionalized gold nanoparticles]

or CRISPR-based detection [e.g., SHERLOCK, DETECTR] to identify a critical microbial DNA sequence or metabolite directly in urine, offering a quick "yes/no" or risk score in a clinical setting.

Knowledge Gaps & Future Directions

Even though the field is developing quickly, there are still significant gaps that need to be filled in order to go from correlation to causation and application.

Conclusive Causation Research: The majority of the evidence is correlative. Functional in vivo research is required: Can certain human PCa-associated bacterial isolates [e.g., pks+ E. coli, Fusobacterium nucleatum] colonize germ-free or antibiotic-treated mouse models of prostate neoplasia [e.g., TRAMP, Hi-Myc] and change tumorigenesis? Can the use of targeted antibiotics or bacteriophages to eradicate particular microbes change the course of tumors or the response to therapy in models?

Longitudinal Cohort Studies: There aren't many studies that follow the prostate and urinary microbiome over time, from benign tissue to localized cancer to metastatic CRPC to prostatic intraepithelial neoplasia [PIN]. Microbial drivers of initiation and progression may be found through such investigations. Potential resources are provided by biobanks containing serial serum/urine samples [such as the PLCO and ERSPC cohorts].

Analytical frameworks and standardized sampling: There is an urgent need for agreement on the best way to process low-biomass urine samples and sample the prostate tissue microbiome [avoiding transrectal needle contamination via transperineal approach?]. It is necessary to create reference materials for urine microbiome analysis.

Mechanistic Depth in the Context of PCa: The microbial–androgen crosstalk requires a more thorough molecular understanding. Which particular microbial metabolites affect intracrine synthesis or interact with the AR? Which specific signalling pathways in prostate epithelial cells connect particular MAMPs to AR activation?

Conclusion

The invasiveness of biopsy and the imperfect sensitivity-specificity trade-offs of PSA limit the treatment of prostate cancer. More than just a microbiological curiosity, the discovery of an intratumoral microbiota within prostate tumors represents a fundamental extension of our knowledge of the disease as a complex ecosystem controlled by the Prostate Microbial Triad. Within this trio, microbes actively alter the immunological and metabolic landscape of the tumor, influencing its progression and response to treatment, through complex Microbial–Androgen Crosstalk.

This biological realization opens up a revolutionary diagnostic possibility. These microorganisms and the host reactions they cause leave a detectable fingerprint in urine by functioning as a permanent "molecular broadcast" inside the tumor. Innovative frameworks like the Microbial-EV Pathway of Biomarker Release and the integration of multi-omics with machine learning offer a clear, practical roadmap despite the significant technical challenges associated with contamination and low biomass.

One concrete and pressing objective is the creation of a microbiome-driven, clinically validated urine liquid biopsy. Such a tool could significantly increase the specificity of PCa detection, lessen the financial, psychological, and physical burden of needless procedures, and improve risk stratification to tailor care. In the end, it might prevent overtreatment of men with indolent disease while more successfully identifying those with deadly cancer at a stage that can be cured. A persistent, multidisciplinary effort involving urologists, oncologists, microbiologists, bioinformaticians, diagnostic engineers, and regulatory scientists is required to realize this potential. Deciphering this new aspect of cancer biology and fulfilling the promise of precise, non-invasive medicine for the millions of men afflicted by prostate cancer worldwide are imperative.

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Declarations:

Authors' Contribution:

- **All authors** Conceptualization, data collection, interpretation, drafting of the manuscript, Intellectual revisions
- The authors agree to take responsibility for every facet of the work, making sure that any concerns about its integrity or veracity are thoroughly examined and addressed

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