

The Urinary Microbiome: Implications in Bladder Cancer Pathogenesis and Therapeutics



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Recent investigation has proven that the bladder is not sterile. However, the implications of this finding in the pathophysiology and management of urothelial cell carcinoma have not been fully described. In this review, we summarize the literature relating to the urinary and gastrointestinal microbiomes in the context of urothelial cell carcinoma. The bladder microbiome may relate to urothelial cell carcinoma pathogenesis/progression, act as a noninvasive and modifiable urinary biomarker and have implications in treatment using immunotherapy agents such as intravesical bacillus Calmette-Guerin. Investigators should continue to optimize techniques to characterize this intriguing new area of human health. UROLOGY 126: 10–15, 2019. © 2019 Elsevier Inc.

While the relationship between schistosomiasis and squamous cell carcinoma is well accepted, the role of microbes in the pathophysiology and management of urothelial cell carcinoma (UCC) of the bladder has not been defined.¹ Internal surfaces, including gastrointestinal (GI) or vaginal epithelium, are widely known to possess commensal microbial communities. These microbiomes are believed to benefit human health, facilitating efficient removal of improperly functioning immune cells and protecting the host from pathogen infection.²⁻⁴ However, micro-organisms have also been implicated in the pathogenesis of numerous malignancies, including gastric cancer, colon cancer and cervical cancer.⁵ The bladder, another hollow muscular organ, has historically been considered “sterile” based on prevailing culture-dependent methods of bacterial detection. However, this “sterile bladder” paradigm was recently debunked with the discovery of resident bacteria.⁶⁻¹⁰

High throughput DNA sequencing and enhanced culture methods have revealed bacterial DNA and live bacteria, respectively, in standard culture-negative urine samples.¹⁻¹³ These results support the new dogma that the bladder possesses its own endogenous microbiome. Subsequent investigations support the notion that these microbes have protective capabilities and that disruption (dysbiosis) of the bladder microbiome can result in lower urinary tract (LUT) dysfunction.¹⁵⁻²⁰

For over 40 years, urologists have manipulated the bladder microbiome for treatment of UCC by instillation of

intravesical BCG (bacillus Calmette-Guerin immunotherapy, an attenuated form of the bacterium *Mycobacterium bovis*).¹¹ Based on this therapy's success, and on observations from other malignancies, the bladder microbiome may play a role in the pathophysiology of UCC, as a noninvasive biomarker, and/or in response to therapy (Fig. 1).

THE GUT MICROBIOME AND MALIGNANCY

Local microbiomes have been implicated in development of multiple malignancies, particularly in the GI tract, where the microbiome has been studied extensively. Animal studies have shown that the gut microbiome can activate or produce carcinogens, which act locally on the GI tract or remotely on other organs.¹² Other potential mechanisms for malignant transformation include induction of chronic inflammation, genotoxicity, and bacterial virulence.¹³

Perhaps the most well-known relationship between living microbe and malignancy is that of stomach cancer and *Helicobacter pylori*, which infects 50% of the world population and leads to gastric ulcers. The mechanism of carcinogenesis of *H. pylori* is most commonly via production of cytotoxin-associated gene A and vacuolating cytotoxin VacA. Among persons with *H. pylori* infection, strain-specific components, host immune responses, and environmental factors influence risk of adenocarcinoma of the stomach, although only a small proportion develop the malignancy.¹⁴

Similarly, associations have been identified between the gut microbiome and colorectal cancer. A dysbiotic gut microbiome is thought to contribute to the development of genetic mutations, epigenetic changes and aberrant immunological signaling pathways linked to disease development.¹⁵ More specifically, the virulence factor FadA of *Fusobacterium nucleatum* is reported to interact with E-cadherin, activate β -catenin signaling, and promote colorectal cancer.¹⁶

Attempts have also been made to associate the gut microbiome with urinary tract malignancy. Several small studies

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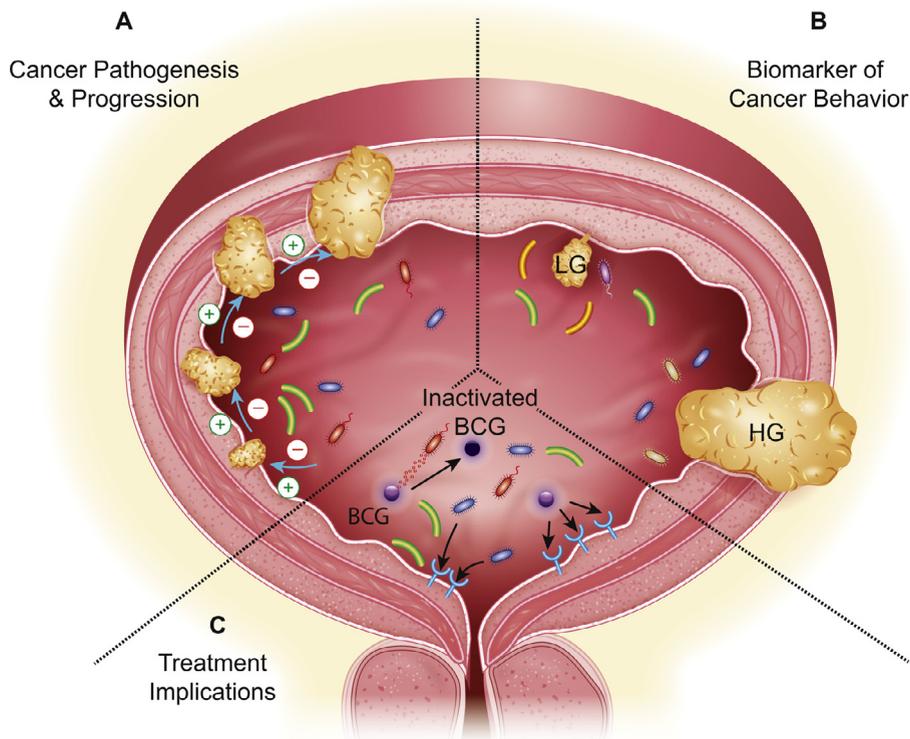


Figure 1. Possible relationships between the bladder microbiome and UCC. The bladder microbiome may promote or inhibit UCC pathogenesis and progression (A). Differing communities of microbiota may be associated with different types of tumors (B). Last, local microbiota may directly inactivate BCG in the bladder or modulate urothelial sensitivity to BCG through competitive binding of fibronectin (C). BCG, Bacillus Calmette-Guerin; UCC, urothelial cell carcinoma.

have explored the role of oral probiotics containing *Lactobacillus* in reducing recurrence of superficial UCC.^{17,18} One review suggested that certain commensal gut microbes may bind compounds linked to urinary tract malignancies—cadmium, other heavy metals and pesticides—and could have implications in reducing cancer rates.¹⁹ A specific pathophysiological link between gut microbiome and urinary tract malignancy has not been proposed.

THE DISCOVERY OF THE URINARY/BLADDER MICROBIOME

While investigation into the gut microbiome has been robust, the urinary microbiome has only recently been described. High throughput DNA sequencing and enhanced culture methods have been utilized to characterize female bladder bacteria in standard urine culture-negative samples.^{6-9,20-24} Since these studies were performed on urine obtained by transurethral catheterization, they have been able to reveal associations between the female bladder microbiome and postinstrumentation/postoperative UTIs, urgency urinary incontinence and response to overactive bladder treatment.^{7,8,10,20,22,25-27} Certain *Lactobacillus* species are even associated with lack of symptoms and protection against postinstrumentation urinary tract infection (UTI).²⁴ These results support the new dogma that the bladder possesses its own indigenous microbiome. They also support the notion that these microbes have protective

capabilities and that dysbiosis of this microbiome can result in LUT disorders.²⁸⁻³⁰

Investigators have also explored the role of the male urinary microbiome in urologic disease.³¹⁻³⁶ Most of these studies assessed only voided urine or urethral swabs; thus, their conclusions cannot be extended to the bladder and must be referred to as the urinary microbiome. However, like the bacteria that comprise the female bladder microbiome, those that form the male urinary microbiome are distinct from bacteria that cause overt clinical UTI. As in females, the urinary microbiome of healthy men differs from that of males experiencing LUT symptoms.^{34,35} One study of the effect of aging on the male urinary microbiome reported an intriguing trend: while the total number of bacteria in the male urinary microbiome substantially decreased with age, the number of genera increased.³⁶ Thus, the male urinary microbiome may diminish in size and increase in diversity with age. A recent study of men showed that severity of LUT symptoms was associated with detection of bacteria in urine obtained by transurethral catheterization (ie, the male bladder microbiome).³⁴

METHODS FOR URINARY MICROBIOME CHARACTERIZATION

To characterize the bladder/urinary microbiome, investigators primarily have utilized 2 assays: high throughput 16S ribosomal RNA gene sequencing and expanded quantitative urine culture.

16S ribosomal RNA sequencing is the most common technique to classify bacteria within a population because it is highly conserved among bacteria. Much human microbiome research has relied on 16S sequencing to obtain a first glimpse of bacteria present in different niches, at different times, and between different disease states.

The use of 16S sequencing, a very sensitive test, provided early evidence that bacterial DNA was present in the female bladder; however, it could not determine whether bacteria were alive or dead. For that purpose, clinical urine cultures were needed. While the standard clinical microbiology urine culture was established and refined to detect *Escherichia coli* and a few other well-established uropathogens,³⁷ this test does not detect most of the microbes of the bladder microbiome, which often require special growth conditions. Simple refinements to the standard urine culture protocol (increased volume, various growth conditions, increased duration of incubation) allow a more complete description of the microbes present. Expanded quantitative urine culture and other enhanced culture methods have shown definitively that the bacteria detected by 16S sequencing are indeed alive.^{9,21-23}

THE URINARY MICROBIOME AND BLADDER CANCER

While these techniques have been utilized extensively in benign urologic disease states, few studies to date have explored the role of the urinary microbiome in urologic malignancies. A study by Xu, et al, compared the voided urine of 6 controls to 8 UCC patients using 16S sequencing, and reported the genus *Streptococcus* was more commonly identified in patients with UCC.³⁸ Another study by Popovic, et al, compared the voided urine of 12 UCC patients to 11 controls using 16S sequencing, showing no significant differences with respect to microbial diversity or overall microbiome composition. The authors did note, however, that *Fusobacterium*, a genus associated with colorectal cancer, was enriched in the UCC group.³⁹ Finally, Wu, et al, compared 31 male UCC patients to 18 healthy controls using 16S sequencing of midstream voided urine. UCC was associated with enrichment of the genera *Acinetobacter*, *Anaerococcus*, and *Sphingobacterium*. Furthermore, enrichment of *Herbaspirillum*, *Porphyrrobacter*, and *Bacteroides* were observed in UCC patients with high risk of recurrence and progression, indicating these may serve as potential biomarkers for risk stratification.⁴⁰

Unfortunately, these studies have all been limited by their use of voided urine. An early urinary microbiome study of women indicated that voided urine is often not representative of the bladder microbiome, as the bacterial DNA detected in midstream voided urine diverged substantially from the DNA detected in urine obtained by transurethral catheter. The authors concluded that catheterization sampled bladder urine because the DNA detected in the catheterized urine resembled the DNA profile of urine obtained by suprapubic aspirate, which bypasses external contamination.⁶ A more recent study compared voided and catheterized urine obtained from men and found that the paired samples often did not match, providing evidence that voided urine does not

adequately characterize the male bladder microbiome.³⁴ Thus, future studies of the role that the bladder microbiome plays in UCC should ideally utilize catheterized urine, which is in direct contact with the urothelium.

UCC AND BCG THERAPY

UCC causes significant morbidity and mortality worldwide and is categorized as nonmuscle invasive (NMI; 70%) or muscle invasive (30%). Twenty percent of non-invasive tumors will progress to invasive cancers and 50% with invasive disease will develop metastases. Despite systemic therapy, metastatic UCC confers a high mortality rate with a median survival of 12-15 months and a 5% 5-year survival rate.⁴¹

Investigation into the relationship between UCC and the bladder microbiome may elucidate a pathophysiological relationship between the 2 (Fig. 1A). The bladder microbiome may also act as a noninvasive biomarker for tumor behavior (Fig. 1B). While these associations have not yet been fully investigated, urologists have been manipulating the bladder microbiome for treatment of UCC for more than 40 years, treating high grade (HG) NMI UCC with intravesical BCG immunotherapy.

The BCG vaccine, a live attenuated strain of the bacterium *M. bovis*, was first instilled into human bladders to treat UCC by Alvaro Morales in 1972. A course of 6 weekly treatments was selected based on the 6-vial packaging of the BCG strain available at the time (Institute Armand Frappier, Montreal, CA) and it was presumed this schedule would be adequate for a patient to mount an immune response.¹¹ The work of Dr. Morales and others led to the eventual FDA approval for use of intravesical BCG in patients with superficial bladder tumors in 1990 and it has since endured as the recommended standard treatment of HG NMI UCC, with 6 weekly treatments remaining the most common protocol for induction therapy. While the dose, frequency, and duration of treatment (maintenance therapy) have been subject to debate, maximizing effectiveness while minimizing side effects (eg, urinary frequency, urgency, dysuria, hematuria, and more rare side effects [eg, BCG sepsis]) has remained a challenge. Unfortunately, rates of recurrence and progression remain exceedingly high.^{11,42}

In 1 contemporary mechanistic model of BCG activity, the bacterium *M. bovis* first attaches to the urothelium via fibronectin and the integrin $\alpha 5 \beta 1$.⁴³ UCC cells (which overexpress $\alpha 5 \beta 1$) then internalize BCG via macropinocytosis. Following internalization, UCC cells upregulate expression of MHC class II and ICAM-1, and secrete cytokines (IL-6, IL-8, GM-CSF, TNF- α) that, along with dendritic cells, recruit immune cells (granulocytes, CD4+ & CD8+ lymphocytes, NK cells, macrophages) to the site, resulting in release of IL-1, IL-2, IL-5, IL-6, IL-8, IL-12, IL-18, TNF- α , IFN- γ , and GM-CSF. This results in cytotoxicity to UCC cells, proceeding through various immune mechanisms including NK cells, CD8+ cytotoxic T cells, macrophages, and granulocytes (via tumor necrosis factor-related apoptosis-inducing ligand, or TRAIL).⁴³

THE BLADDER MICROBIOME AND BCG

Numerous biomarkers for predicting response to BCG have been investigated. Of these, only clinicopathologic features and urinary cytokine profiles have been moderately successful although none are integrated into widespread clinical practice.⁴⁴ Although studies have shown promise in improving our ability to predict BCG response during therapy, a biomarker detectable prior to initiation of BCG therapy is still needed. It is plausible that the bladder microbiome affects the likelihood of response to BCG therapy by way of various mechanisms. These mechanisms include destruction or inactivation of BCG in the bladder lumen or modulation of urothelial sensitivity to BCG activity by attachment to fibronectin (Fig. 1C). Immunological studies have indicated that numerous indigenous commensal and probiotic bacterial strains exhibit the ability to attenuate mucosal inflammation via inhibition of the NF- κ B pathway, as well as IL-6 and IL-8.⁴⁵ Other studies have shown that certain organisms, such as *Lactobacillus iners*, may be superior at binding fibronectin compared to other species.⁴⁶ This may indicate that the local microbiome may competitively bind fibronectin in the presence of BCG. *Lactobacillus* species have also been investigated as an alternative to BCG for the treatment of UCC.⁴⁷

ADVANCED BLADDER CANCER AND THE MICROBIOME

The bladder microbiome also may have implications in advanced and metastatic UCC. The use of immunotherapy

in the treatment of advanced malignancies has increased in recent years, with numerous new agents utilizing the PD-1/PD-L1 axis. Recent studies have investigated the association between the human gut microbiome and the efficacy of anti-PD-1 therapy.⁴⁸⁻⁵⁰ These studies have associated commensal microbial composition and clinical response. Specifically, response to therapy for metastatic melanoma has been associated with the presence of the species *Bifidobacterium longum*, *Collinsella aerofaciens*, and *Enterococcus faecium*.⁴⁸ It is plausible that response to treatment of metastatic urothelial carcinoma with anti-PD-1/PD-L1 agents may be associated with the presence of certain organisms in the gut or bladder microbiome, although this has not yet been investigated.

FUTURE DIRECTIONS

The recent discovery of the bladder microbiome mandates a re-evaluation of the pathophysiology of various urologic disease states. Numerous studies have been performed in the realm of benign urology, but there has been only limited investigation into associations between the urinary microbiome and UCC, and none of these studies have assessed catheterized urine (Table 1). As a leading cause of cancer-related mortality in the urologic population, the diagnosis and treatment of UCC may be enhanced by investigating its relationship with the bladder microbiome. Furthermore, the possibility of using the bladder microbiome as a modifiable, noninvasive biomarker is highly intriguing. Future investigation may aid in the development of techniques to modify the bladder microbiome,

Table 1. Urinary microbiome studies arranged by subject. No studies to date have related catheterized urine to bladder cancer

	Year	n	Sex	Catheterized Urine	Voided Urine
Characterization of the Urinary Microbiome					
Dong Q, et al ³²	2011	32	Male	No	Yes
Wolfe AJ, et al ⁶	2012	23	Female	Yes	Yes
Nelson DE, et al ³³	2012	18	Male	No	Yes
Lewis DA, et al ³⁶	2013	15	Both	No	Yes
Hilt EE, et al ⁹	2014	65	Female	Yes	No
Price TK, et al ²³	2016	150	Female	Yes	No
Urinary Microbiome and Benign Urologic Conditions					
Nelson DE, et al ³¹	2010	19	Male	No	Yes
Khasriya R, et al ²¹	2013	81	Female	Yes	Yes
Fok CS, et al ²⁵	2013	284	Female	Yes	No
Nienhouse V, et al ⁷	2014	54	Female	Yes	No
Pearce MM, et al ⁸	2014	118	Female	Yes	No
Brubaker L, et al ¹⁰	2014	155	Female	Yes	No
Pearce MM, et al ²²	2015	182	Female	Yes	No
Nickel JC, et al ³⁵	2015	110	Male	No	Yes
Karstens L, et al ²⁰	2016	20	Female	Yes	No
Thomas-White KJ, et al ²⁴	2016	134	Female	Yes	No
Thomas-White KJ, et al ²⁶	2018	104	Female	Yes	No
Bajic P, et al ³⁴	2018	49	Male	Yes	Yes
Urinary Microbiome and Bladder Cancer					
Xu W, et al ³⁸	2014	8	Unknown	No	Yes
Bucevic Popovic V, et al ³⁹	2017	23	Male	No	Yes
Peng W, et al ⁴⁰	2018	49	Male	No	Yes

either through oral probiotics or intravesical instillations, for optimization of response to BCG and other therapies.

As investigation into the bladder microbiome continues, the importance of using catheterized urine cannot be understated. Voided urine introduces contamination from the highly colonized distal urethra. Frequent surveillance cystoscopy in this population may provide an avenue for obtaining “catheterized” urine with minimal added discomfort. Unfortunately, frequent use of antibiotics and urinary tract instrumentation likely leads to changes in the bladder microbiome. The dynamics of these changes have not yet been described, making it difficult to define a recruitment cutoff for antibiotic use and instrumentation.

With these factors being considered, the bladder microbiome has incredible potential for further investigation. Through characterization of the bladder microbiome in UCC patients, mechanistic relationships between UCC and the microbiome may be established, and new diagnostic biomarkers may be discovered. The potential impact of using the microbiome to improve UCC diagnosis and treatment is immense. Investigators should continue to work diligently to improve methods for studying this intriguing area of human health.

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