



REVIEW ARTICLE

The inflammatory microenvironment and the urinary microbiome in the initiation and progression of bladder cancer

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Abstract Accumulating evidence suggests that chronic inflammation may play a critical role in various malignancies, including bladder cancer. This hypothesis stems in part from inflammatory cells observed in the urethral microenvironment. Chronic inflammation may drive neoplastic transformation and the progression of bladder cancer by activating a series of inflammatory molecules and signals. Recently, it has been shown that the microbiome also plays an important role in the development and progression of bladder cancer, which can be

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mediated through the stimulation of chronic inflammation. In effect, the urinary microbiome can play a role in establishing the inflammatory urethral microenvironment that may facilitate the development and progression of bladder cancer. In other words, chronic inflammation caused by the urinary microbiome may promote the initiation and progression of bladder cancer. Here, we provide a detailed and comprehensive account of the link between chronic inflammation, the microbiome and bladder cancer. Finally, we highlight that targeting the urinary microbiome might enable the development of strategies for bladder cancer prevention and personalized treatment.

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Introduction

Bladder cancer (BC) is the 10th most common malignant disease worldwide, with an estimated 80,470 new cases and 17,670 deaths in 2019 in the United States alone.^{1,2} BC is divided into two clinically distinct types, muscle-invasive bladder cancer (MIBC) and non-muscle-invasive bladder cancer (NMIBC), and the latter accounts for 75% of BC.^{3,4} According to statistics, 90% of malignancies of the urinary tract are urothelial bladder cancer (UBC) when diagnosed, whereas the rest are mostly squamous cell carcinoma (SCC) and adenocarcinoma.⁵ The risk of developing bladder cancer increases with age, and men are more affected than women, with respective incidence and mortality rates of 9.6 and 3.2 per 100,000 in men, respectively, approximately 4 times those of women around the world.¹ Smoking is the most frequent risk factor for BC. In addition, heavy alcohol consumption, occupational exposure to polycyclic aromatic hydrocarbons or aromatic amines, and other environmental factors all contribute to the malignant transformation and progression of BC.^{6–8} Apart from the above, chronic inflammation has recently been recognized as another risk factor for BC.⁹

Chronic inflammation has been recognized as a hallmark of carcinoma. A common host response to any tumorigenic process is inflammation, an essential host defence mechanism for cell or organism injury in response to stresses, by which the immune system tries to neutralize or eliminate injurious stimuli and initiate regenerative or healing processes.¹⁰ Namely, the microenvironment in neoplastic tissues resembles the status of chronic inflammation.¹¹ It is now widely appreciated that excessive or persistent inflammation also contributes to carcinogenesis and tumour progression, even metastasis, by activating a series of inflammatory molecules and signals (Fig. 1).^{12,13}

The human microbial ecosystem plays an essential role in human health and disease and has gained strong research support for its promising perspectives in cancer research.¹⁴ The term “microbiome” means the whole collective genomes of commensal and pathogenic microorganisms that reside in an anatomical niche, including the products of the microbiota and the host environment, while the term “microbiota” describes the microorganisms themselves.^{15–17} Although terms microbiota and microbiome have different meanings, they are often used interchangeably. Historically, bladder epithelium and

urine have been considered sterile in healthy individuals based on microbiological urine cultures. However, evidence has accumulated during the last few years showing that the urinary tract harbours microorganisms.^{18–23}

This Review focuses on the potentially carcinogenic role of the inflammatory microenvironment and urinary microbiome in bladder cancer development. We discuss the relationships between chronic inflammation, urinary tract infections, the urinary microbiome and carcinogenesis, which have been uniquely difficult to define in bladder cancer, and summarize current findings that suggest that an inflammatory microenvironment including the urinary microbiome is involved in the progression of bladder cancer that drives cancer initiation. Furthermore, we highlight the possible involvement of the microbiome in the formation and development of bladder cancer. Finally, we propose future strategies for the study of the urinary tract microbiome and bladder cancer.

Urinary tract infections and BC

Urinary tract infections (UTIs) are among the most common urologic diseases. UTIs refer to the presence of microbial pathogens in the urinary tract. Risk factors for UTIs include catheterization, urinary tract obstruction, immune system suppression, oestrogen deficiency, genetic predisposition, and sexual intercourse and may differ between men and pre- and postmenopausal women.^{24–27} The majority of UTIs occur in women, and it is estimated that 40–50% of females undergo symptomatic UTI during their lifespan at least once, and half of them will experience a recurrence in a year.^{26,27} In the past, it was thought that approximately 80% of UTIs are caused by *E. coli* and manifest as cystitis.²⁸ However, this estimate is based on the use of the standard urine culture method designed to detect *E. coli* and other bacteria that grow rapidly under ambient atmospheric conditions.

Epidemiological studies have investigated the association between episodes of UTIs and BC. Most studies reported that UTIs increased the risk of BC, and UTIs tend to be related to worse outcomes.^{29–40} Controversially, the link between UTI frequency and duration and BC remains unclear. When the time lag between UTIs and BC is prolonged and the incidence of UTIs decreases, the positive relationship tends to weaken.^{32,34,38,41} In addition, Vermeulen et al analysed data from one of the largest bladder cancer

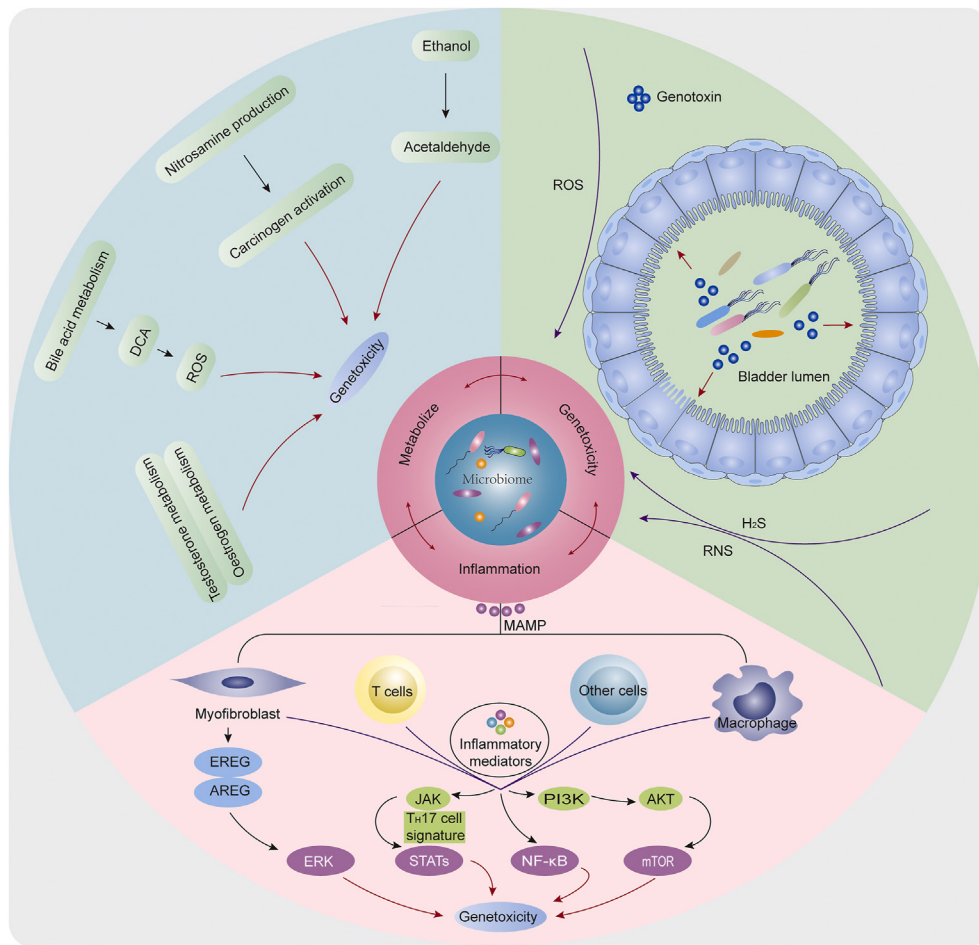


Figure 1 Possible mechanisms by which the bacterial microbiome modulates bladder carcinogenesis. Chronic inflammation is a significant event in bladder cancer. Bacterial translocation may be increased by changes in the microbiome and host defences, leading to increased inflammation, which is regulated by microorganism-associated molecular patterns (MAMPs) that activate Toll-like receptors (TLRs) in many cell types. Several signalling pathways are linked to bladder carcinogenesis during inflammation, including janus activated kinase (JAK)-STAT3, nuclear factor-kappaB (NF-κB), and phosphoinositide-3 kinase (PI3K)-Akt-mammalian target of rapamycin (mTOR). In addition, the microbiome can directly mediate genotoxic effects by releasing bacterial genotoxins. Reactive oxygen species (ROS) and reactive nitrogen species (RNS) released from inflammatory cells, as well as hydrogen sulphide (H₂S) from the microbiota, may also be genotoxic. In addition, microbiome metabolism may contribute to active changes in genotoxins such as acetaldehyde, dietary nitroamines and other carcinogens, hormone metabolism such as oestrogen and testosterone, bile acid metabolism and energy harvesting. Eventually, carcinogenesis coexists with genotoxicity and inflammation. Abbreviation: AREG, amphiregulin; DCA, deoxycholic acid; EREG, epi regulin; IL, interleukin; NF-κB, nuclear factor-κB; STATs, signal transducer and activator of transcriptions; T_H17, T helper 17; TNF, tumour necrosis factor.

case-control studies and concluded that a limited number of episodes of UTIs treated with antibiotics is associated with decreased urinary bladder cancer risk.⁴² There is a theory that planned colonization of the bladder with an avirulent strain of bacteria produces an asymptomatic bacteriuria state that restrains the ability of virulent strains to infect the bladder, which may reduce the incidence of bladder cancer in the high-risk population.⁴³

Overall, it is likely that UTIs play a role in bladder carcinogenesis. When considering the correlation of UTIs and BC, UTI onset age and duration, urinary tract location, and antibiotic usage should be emphasized in future research to allow for further unravelling of their separate effects.

Inflammation and BC

Emerging evidence indicates that inflammation is present at different stages of tumour development, including initiation, promotion, invasion, and metastasis.⁴⁴ Curiously, inflammation is "Janus-faced" in tumour biology; it possesses the capacity to elicit an antitumoural immune response that eliminates tumours, and simultaneously, prolonged inflammation can promote carcinogenesis.⁴⁵ Inflammation represents a host response resulting from various factors, including but not limited to proinflammatory mediators, environmental toxins, tissue injury and chronic infection.^{46–49} Studies have shown that proinflammatory cytokines are pivotal in some types of cancer

and that increasing inflammatory mediators could lead to cancer angiogenesis, development, and invasion.⁵⁰ To some extent, carcinoma acts as a wound that fails to heal.⁵¹ That is, malignancy may coexist with inflammation.

For BC, inflammatory events that induce tumorigenesis and angiogenesis have been observed.^{52,53} Secreted protein acidic and rich in cysteine (SPARC), a glycoprotein located in the extracellular matrix that is increasingly expressed during tissue remodelling, is involved in bladder carcinogenesis. Recent basic research indicates that SPARCs are produced both in cancer- and non-cancer-related compartments of bladder carcinomas, where they suppress bladder carcinogenesis and progression by modulating the inflammatory response to cancer cells.⁵⁴ In animal studies, chronic inflammation traces have also been reported in BC.^{55,56} Moreover, the fact that *Schistosoma haematobium* infections can induce BC through the induction of chronic inflammation has been demonstrated.⁵⁷ Nonsteroidal anti-inflammatory drugs (NSAIDs) are potent inhibitors of cyclooxygenase-2 (COX-2) and are able to induce apoptosis of bladder cancer cells. For example, aspirin, one of the NSAIDs that decreases the risk of BC, indirectly confirms the pivotal role of inflammation in BC.^{58,59} Therefore, inflammation may elicit bladder carcinogenesis.

Inflammatory microenvironment in BC

It has been documented that the tumour microenvironment is similar to the chronic inflammation status.¹¹ The inflammatory response to tumours tends to persist because of the persistence of the initiating factors or mechanisms required for resolving the inflammatory response disorder. Tumour cells can attract inflammatory cells and produce various cytokines and chemokines. During the development of neoplasms, the inflammatory component includes a diverse leukocyte population — for example, macrophages, dendritic cells, neutrophils, eosinophils, and lymphocytes — all of which are capable of producing an assorted array of cytokines; cytotoxic mediators including reactive oxygen species, serine and cysteine proteases, matrix metalloproteinase (MMPs) and membrane-perforating agents; and soluble mediators of cell killing, such as tumour necrosis factor (TNF- α), interleukins and interferons (IFNs).^{60,61} As a result, inflammation generates reactive oxygen and nitrogen species and tissue, proteins, lipids and DNA damage.^{46,62}

Cytokines are a family of proteins that are an important component of the immune system, acting as mediators between cells to regulate the human immune response.⁶³ The word cytokine is derived from the Greek root words “cyto” for cell and “kinos” for movement. Released by cells, cytokines regulate the growth, maturation, and responsiveness of certain cell populations through receptors. As biomolecules, cytokines play a vital role in infections, haematopoiesis, and homeostasis, controlling the response against infectious diseases and even carcinogenesis by controlling tissue renewal, cellular sprouting, and growth. Previous studies have implicated the unique pattern of inflammatory cytokines in the serum of patients with particular types of cancer. The involvement of

inflammatory cytokines in the formation of BC has also been highlighted.⁶⁴ We discuss inflammatory cells and cytokines in the following sections.

Macrophages

Tumour-associated macrophages (TAMs) are the main component of the infiltrate of most tumours.⁶⁵ TAMs originate in the circulation and are recruited to the tumour site by chemokines and specific tumour-derived chemo-attractant cytokines. Different from macrophages in inflammation, TAMs proliferate at the tumour site. Macrophages are capable of mediating tumour cytotoxicity and stimulating antitumour lymphocytes; however, tumour cells can not only block the host's defence programme but also benefit from abnormally activated TAMs. This dual potential of TAMs has been interpreted in the “macrophage balance” hypothesis.⁶⁵ Hence, TAMs can stimulate tumour cell proliferation, promote angiogenesis, and favour invasion and metastasis.⁶⁶

The dual role of TAMs in BC depends on the different polarization states classified as M1 and M2.⁶⁷ In the process of tumour development, M1 macrophages play a role in the inflammatory response and antitumour immunity. In contrast, M2 macrophages have anti-inflammatory and tumour-promoting properties. M1 macrophages can be driven by IFN- γ and LPS and produce interleukin-6 (IL-6), interleukin-12 (IL-12), interleukin-23 (IL-23), and TNF- α (Table 1).⁶⁸ M2 macrophages with high levels of scavenger receptor, mannose receptor, interleukin-1 (IL-1) receptor antagonist, and IL-1 decoy receptor produce interleukin-4 (IL-4), interleukin-10 (IL-10), interleukin-13 (IL-13) or transforming growth factor- β (TGF- β) to promote cancer cell proliferation, migration, invasion, metastasis and suppression of antitumour immune responses (Table 1).^{68–70} In addition, direct evidence for the role of TAMs in the carcinogenesis and progression of BC has been reported. BAY 11–7082 treatment suppressed both oncogenic and metastatic potential while preventing M2 polarization of TAMs in bladder cancer cells.⁷¹ The predominance of M2-polarized macrophages in the stroma of low-hypoxic BC was related to *Bacillus Calmette-Guérin* (BCG) immunotherapy failure, possibly owing to the immunosuppressive function of M2.⁷⁰ OK-432, a *Streptococcus*-derived anticancer immunotherapeutic agent, suppresses cell proliferation and metastasis by inducing M1 to secrete cytokines in BC.⁷² ATP-binding cassette transporter G1 (ABCG1) impeded BC development through a phenotypic shift from a tumour-promoting M2 to a tumour-fighting M1.⁷³ Moreover, studies suggested that TAM phenotypes provide prognostic information and testified that MAC387, alone and in combination with CD68, was associated with poorer survival in univariate analyses, particularly in bladder cancers undergoing transurethral resection.⁷⁴ The count of TAMs infiltrating the tumour area is useful for predicting the response of bladder carcinoma *in situ* to intravesical bacillus Calmette-Guérin instillation before treatment commissioning.⁷⁵ Collectively, TAMs are important in initiating tumorigenesis and facilitating the malignant progression of the bladder, but there is room to explore.

Table 1 Inflammatory microenvironment and urinary microbiome of bladder cancer.

Class	Mechanism of action	Effect	Refs
Inflammatory cells			
TAMs	M1 phenotype: driven by IFN- γ and LPS, and produce IL-6, IL-12, IL-23, TNF- α	Inhibition	66
	M2 phenotype: with high levels of scavenger receptor, mannose receptor, IL-1 receptor antagonist, and IL-1 decoy receptor, and produce IL-4, IL-10, IL-13 or TGF- β	Promotion	66,67,68
MDSCs	Acting on the CXCL2/MIF-CXCR2 axis; attracting IL-8 and CCL22; decrease of T cells and NK cells	Promotion	83,84,85
Tregs	Active JAK/STAT3 signal	Promotion	88
DCs	Immune evasion and immune tolerance	Promotion	97
MCs	Modulate ER β /CCL2/CCR2 EMT/MMP9 signals	Promotion	98
Inflammatory cytokines			
TNF- α	Angiogenesis; stimulating secret MMP9 in the tumour microenvironment	Promotion	107,111,112
IL-1	ER β /IL-1/c-MET signalling modulation	Promotion	120
	AKR1C1 increase	Promotion	121
IL-4	IL-4 rs2243250 genetic variants	Promotion	123
IL-6	upregulating N-myc downstream gene 1, KAI1 proteins, and the mammary serine protease inhibitor; inhibition on epithelial–mesenchymal transitions	Inhibition	126
IL-10	Inhibit cell–immune reaction	Promotion	116
Microbiome studies			
<i>Streptococcus</i>	Enrichment	Promotion	14
<i>Acinetobacter</i>	Enrichment	Promotion	169
<i>Anaerococcus</i>			
<i>Sphingobacterium</i>			
<i>Serratia</i>	Decrease	Promotion	169
<i>Proteus</i> <i>Roseomonas</i>			
<i>Herbaspirillum</i> <i>Porphyrobacter</i>	Enrichment	Promotion	169
<i>Bacteroides</i>			
<i>Fusobacterium</i>	Enrichment	Promotion	170
<i>Firmicutes</i>	Male and female	/	194
<i>Actinomycetes</i>	Female only	/	194
<i>Lactobacillus</i> <i>Gardnerella</i>	The most represented in men	/	196
<i>Corynebacterium</i>	Predominant in women	/	155
<i>Staphylococcus</i> <i>Streptococcus</i>			
<i>Lactobacillus</i>	The most common in women	/	197
<i>Gardnerella</i>	More common in younger women	/	197
<i>Escherichia</i>	More common in older women	/	197

Abbreviation: TAMs, tumour-associated macrophages; MDSCs, myeloid-derived suppressor cells; Tregs, regulatory T cells; DCs, dendritic cells; MCs, mast cells; ER β , estrogen receptor- β ; CCL2, chemokine(C–C motif) ligand 2; EMT, epithelial–mesenchymal transition; MMP9, matrix metalloproteinases 9; TNF- α , tumour necrosis factor- α ; TGF- β , transforming growth factor- β ; IL, Interleukins; AKR1C1, aldo-keto reductase 1C1.

Myeloid-derived suppressor cells

The first observations of suppressive myeloid cells were noted more than 30 years ago in patients with cancer.^{76–78} The name myeloid-derived suppressor cells (MDSCs) was introduced to the scientific literature in 2007.⁷⁹ MDSCs stem from the bone marrow or peripheral lymphoid organs and play a role in disease progression and reduced survival in many types of malignancy.^{80,81} Tumour cells have been demonstrated to induce MDSC expansion by secreting tumour-derived factors (TDFs), which comprise a variety of biologically active compounds, including growth factors, cytokines and chemokines.⁸² High levels of arginase or tryptophan activity as well as nitric oxide (NO), reactive oxygen species (ROS) and prostaglandin E(2) (PGE(2)) induction contributed to MDSC suppression of antitumour immunity.^{83,84}

MDSCs are significant mediators of BC cell-associated immune suppression. The CXCL2/MIF-CXCR2 axis is an important mediator in MDSC recruitment and is viewed as a predictor and potential therapeutic target in BC patients (Table 1).⁸⁵ Another study showed that bladder cancer tissues spontaneously produce MDSCs attracting CXCL8 (interleukin-8, IL-8) and CCL22, which are correlated with poor prognosis (Table 1).⁸⁶ In addition, the increased tumour infiltration of MDSCs with a concomitant decrease in T cells and NK cells was documented in a tyrosine kinase Rip2-deficient mouse model of BC, resulting in enhanced metastases (Table 1).⁸⁷ Together, MDSCs may be a potential target for the progression and treatment of BC, and monitoring MDSC proliferation is of great clinical importance.

Regulatory T cells (Tregs)

Regulatory T cells (Tregs) have been implicated in the pathogenesis of inflammation and a variety of autoimmune diseases, especially cancer. Tregs have been considered unmitigated suppressors of antitumour immunity because antitumour T-cell responses represent a favourable prognostic factor. Tregs can promote cancer progression by suppressing antitumour immune responses or expressing inflammatory cytokines.^{88,89} Furthermore, they represent a major barrier in antitumour immunity and immunotherapy.

Tregs have long been viewed as one-sided suppressors of antitumour immune responses with poor patient outcome in cancer. S1PR1 signalling in T cells can drive Treg accumulation in tumours by means of JAK/STAT3 activation, leading to the promotion of BC growth (Table 1).⁹⁰ Moreover, there is a relative enrichment of Tregs in peripheral blood compared with patients with BC and healthy controls.^{91,92} Treg suppression contributes to an antitumour effect in an orthotopic BC model that received AdCD40L gene therapy.⁹³ In contrast, evidence of a paradoxical positive prognostic effect of Tregs on BC is mounting. Researchers found a considerable survival benefit of Tregs at the invasive front, supporting the notion that Tregs may positively influence prognosis on survival in BC.⁹⁴ Thus, the true efficacy of targeting Tregs in carcinomas has yet to be determined, and the gaps that remain are promising for future studies.

Dendritic cells

Dendritic cells (DCs) are a handful of cell populations of distinct subtypes derived from the bone marrow that are interspersed among antigen-exposed tissues (e.g., skin, lung and intestine) and their draining lymph nodes (LNs). Functionally, DCs are one of the major antigen-presenting cells (APCs) of the innate immune system, inducing antigen-specific immunity, and DC-based vaccines are readily adapting to the shift in cancer immunotherapy.⁹⁵

DCs have been reported in BC. In the MB49 bladder cancer model, the intravesical Ty21a vaccine promotes dendritic cells and T-cell-mediated tumour regression.⁹⁶ The impairment of myeloid DC (mDC) counts and monocyte-derived DC (MoDC) function are closely related to proliferation and recurrence of superficial transitional cell carcinoma of the bladder (STCCB).⁹⁷ A high level of CD83(+) mature tumour-infiltrating dendritic cells (TIDCs) increases the risk of muscle-invasive BC.⁹⁸ Conversely, TIDCs were inversely correlated with the degree of malignancy and prognosis of bladder transitional cell carcinoma (BTCC), and a decreased number of TIDCs could have a notable relation to tumour immune evasion and immune tolerance, as Xiang et al reported (Table 1).⁹⁹ Altogether, DCs, specifically TIDCs, may be risk factors for BC.

Mast cells

Mast cells (MCs) have been viewed, for the most part, as effectors of allergy, particularly in the early and acute phases of allergic reactions, since 1878. Recently, studies have indicated that MCs play an important role in a variety of inflammation-associated diseases related to cancer.

Studies have demonstrated that MCs can influence the neoplasia and progression of BC. Recruited mast cells in the tumour microenvironment enhance bladder cancer metastasis via modulation of ERβ/CCL2/CCR2 EMT/MMP9 signals (Table 1).¹⁰⁰ Tumour stroma-infiltrating mast cells predict prognosis and adjuvant chemotherapeutic benefits in patients with muscle-invasive bladder cancer.¹⁰¹ c-Kit-positive MCs may contribute to tumour angiogenesis in tumour invasion of the urinary bladder.¹⁰² However, stem cell marker-positive MCs are decreased in the stroma of benign-appearing mucosa of BC patients, indicating that MCs could suppress carcinogenesis.¹⁰³ Overall, the mechanisms of how mast cells influence the formation and progression of BC could be an object of intensive study.

TNF-α

As a pivotal mediator of inflammation, TNF is a potential molecular link between chronic inflammation and cancer.⁶⁴ TNF-α is a key factor in the onset of infectious disease and malignancy and is secreted by many cells, such as monocytes/macrophages, T-cells, and fibroblasts.¹⁰⁴ There are two types of TNF-receptors, TNFRI and TNFRII. TNFRI is expressed on the membrane of all cell types (except erythrocytes), and it can activate both apoptosis by the Fas-associated death domain and the proinflammatory pathway through TNF-α receptor-associated factor 2; however, TNFRII exists in haematopoietic cells and can only

activate the proinflammatory pathway.^{104,105} TNF- α can protect chemotherapeutic agents depending on NF- κ B-mediated antagonism of apoptosis signaling.⁹² In addition, TNF- α also contributes to the production of angiogenic factors (such as vascular endothelial growth factor, VEGF) and proteases, which can pave the way for cancer invasion and metastasis.¹⁰⁶

Compared with healthy urothelium, tumorous tissue with high TNF- α expression, observed by Feng et al.¹⁰⁷ Moreover, they found that the expression of TNF- α was related to angiogenesis in BC (Table 1).¹⁰⁷ In addition, a correlation between a single-nucleotide polymorphism of the TNF- α gene promoter (308 A/G) and muscle invasion BC has been previously reported.^{64,108} A meta-analysis showed that the TNF- α 308 G/A polymorphism was associated with the risk of urogenital cancer, particularly in the Caucasian population.¹⁰⁹ A case-control study has also demonstrated a significant relationship between the TNF- α –308 A/A genotype (the high-risk genotypes) and the odds ratio for urothelial carcinoma.¹¹⁰ The serum level of TNF- α is clearly increased in bladder cancer patients with or without schistosomiasis infection; in addition, higher levels of TNF- α have been seen in patients with progressive stages of BC (T2–T4) compared with patients with less advanced stages (Ta–T1), indicating that the TNF- α level might predict the progression of BC.¹⁰⁸ Furthermore, TNF- α is implicated in promoting the invasion and migration of BC cells by stimulating secret matrix metalloproteinases-9 (MMP-9) in the tumour microenvironment, and MMP9 expression could be suppressed by cordycepin (Table 1).^{111,112} TNF- α release in inflammation is also associated with the transformation of BC due to the induction of H₂O₂.¹¹³ In contrast, studies have documented that some TNF-related factors affect cancer cell apoptosis and that regulating TNF- α expression could change cancer development. In this regard, it has been shown that lymphotoxin β receptor (LT β R) activation could promote inflammation-induced carcinogenesis via the upregulation of TNF- α expression.¹¹⁴ It has also been implied that recombinant TNF-related apoptosis-inducing ligand inhibited proliferation and development of BC cells, leading to their own apoptosis.¹¹⁵ Moreover, it has been suggested that the effectiveness of doxorubicin chemotherapy in BC is partly mediated by the low regulation of TNF- α .¹¹⁶ As a proinflammatory cytokine, TNF- α could surely contribute to the formation and development of BC. However, it also plays a potential role in the tumour microenvironment, which has yet to be undetermined. Thus, the exact role of this cytokine in BC development or inhibition requires further molecular studies.

Interleukins

Interleukins (ILs) were first discovered in the 1970s and belong to the superfamily of cytokines secreted by immune system cells, which possess complex immunological functions. To date, dozens of ILs have been identified, binding to their own receptor separately, holding a specific origin, structure, and properties. The major objective of ILs is to mediate intercellular communication in the immune system, including cell migration, proliferation, maturation, and adhesion, which, as mentioned, are vital in the inflammatory

response.¹¹⁷ ILs partake in both acute and chronic inflammatory responses. They respond to the stimulation of specific receptors expressed on the cell surface and then activate a particular signalling pathway to exert both inflammatory and anti-inflammatory actions. Over the past decades, ILs have been shown to play a critical role in cancer initiation, migration, and progression.

IL-1

As a key proinflammatory cytokine, IL-1 represents a family of two agonistic proteins, IL-1 α and IL-1 β . Seddighzadeh et al concluded that IL-1 α was significant in bladder cancer biology and that measurements of this cytokine might contribute to pretreatment characterization of BC.¹¹⁸ Further study demonstrated that low levels of IL-1 α mRNA expression were related to an expanded risk for BC-specific death.¹¹⁹ Recent studies, both *in vitro* and *in vivo*, have shown that BC cells could recruit more infiltrated T cells, which can stimulate proliferation of the cancer cells through modulation of the ER β /IL-1/c-MET signalling pathways (Table 1).¹²⁰ Moreover, it has been investigated whether IL-1 could give rise to metastasis and drug resistance by increasing aldo-keto reductase 1C1 (AKR1C1) in BC cell lines (Table 1).¹²¹

IL-4

IL-4, a T helper 2 (Th2)-related cytokine, promotes the production of antibodies and induces B-cell activation/differentiation and macrophage inhibition. Signal transducers and activators of transcription (STATs) induce the IL-4 signalling pathway and regulate the transcription of related genes. It has been suggested that the IL-4 gene intron-3 polymorphism is associated with transitional cell carcinoma of the urinary bladder and introduces IL-4 gene variants as an appropriate genetic marker for BC.¹²² Similarly, the IL-4 rs2243250 genetic variants could contribute to the risk of multiple bladder tumours (Table 1).¹²³ In addition, overexpression of IL-4 receptor- α (IL-4R α) is correlated with the pathological grade and stage of bladder tumours; thus, IL-4R α has been recognized as a prognostic indicator and therapeutic target for BC.¹²⁴

IL-6

IL-6, a multifunctional cytokine mainly produced by monocytes/macrophages in acute and chronic inflammation, triggers the signal transducers and activators of transcription 3 (STAT3) signalling pathway, regulating tumour growth and metastasis. It has been reported that excessive IL-6 expression is closely associated with tumour progression, especially in BC.¹²⁵ According to a study, IL-6 could reduce the proliferation, migration, and invasion of BC cells by upregulating N-myc downstream gene 1, KAI1 proteins, and the mammary serine protease inhibitor; moreover, IL-6 inhibited epithelial–mesenchymal transitions (EMT) via modulation of vimentin and N-cadherin proteins and promotion of E-cadherin expression (Table 1).¹²⁶ Okamoto et al discovered that the growth of bladder carcinoma cells was markedly inhibited by anti-IL-6

neutralizing antibody or the antisense oligonucleotide, concluding that IL-6 functioned as an autocrine growth factor for bladder carcinoma cells but not for normal urothelial cells.¹²⁷ Furthermore, studies revealed that IL-6 (−174 C>G) genotypes are significantly related to an increased risk of bladder cancer.¹²⁸ IL-6 causes hepatocytes to release serum C-reactive protein (CRP), which can activate the complement system. Moreover, serum CRP was an independent risk factor for cancer-specific survival after radical cystectomy.¹²⁹

IL-10

IL-10 has pleiotropic effects in immunoregulation and inflammation. This cytokine is involved in the regulation of the JAK-STAT signalling pathway. Doxorubicin removed the inhibition of regulatory T cells by decreasing the expression of IL-10 and consequently resulted in the effective treatment of BC (Table 1).¹¹⁶ It has also been reported that the serum and urinary concentration of IL-10 rises greatly in recurrent BC patients, and its levels are correlated with poor recurrence-free survival.¹³⁰ In summary, IL-10 may be a possible marker of BC initiation and progression.

Other interleukins

IL-8, namely, CXCL8, overproduction is an important factor in monomethylarsonous acid [MMA(III)]-induced malignant transformation of urothelial cells and subsequently promotes angiogenesis, which can pave the way for tumorigenesis and metastasis.¹³¹ IL-17 is one of the newly highlighted cytokines that can promote tumour growth through the IL-6-STAT3 signalling pathway, whereas a low level of IL-17 in peripheral blood could be used as an indicator for worse prognosis of BC patients.^{132,133} IL-18, also called IFN γ -inducing factor, belongs to the IL-1 family, an antitumour factor that modulates tumorigenesis, angiogenesis, and stimulation of apoptosis.^{134,135} Increased serum levels of IL-18 in patients with BC may prevent cancer progression; moreover, an association between the IL-18 polymorphism and the risk of BC has been documented.^{136,137}

Although the exact mechanism of interleukins in the development or prevention of BC has not yet been clearly illustrated, preliminary studies emphasize their important role in cancer. Further studies are needed to handle the roles of interleukins in BC and to use them as biomarkers or therapeutic targets.

TGF- β

TGF- β regulates cell growth and differentiation, apoptosis, cell motility, extracellular matrix production, angiogenesis, and cellular immune responses.^{138–140} Essentially, TGF- β has a paradoxical role in cancer, as it inhibits cellular transformation and prevents cancer progression in the early stages; however, in later stages, it plays a key role in promoting tumour progression.¹⁴¹ A positive correlation was reported between the TFG- β level and grade of bladder carcinoma ($G \geq 2$) in a study, and in another study, it was demonstrated that TGF- β could induce the migration/invasion of BC cells through the mammalian target of

rapamycin complex 2 and by stimulating T24 cells. Overall, TGF- β may be involved in the pathogenesis of BC and its progression and be considered a potential prognosis and novel therapeutic, and further studies are necessary to explore this topic in this field.

Microbiome and BC

Microbiome: a “forgotten organ”

Since van Leeuwenhoek first described protozoa in his stool as well as in saliva and dental plaque in 1676, human-associated microbiota has been an area of attention.¹⁴² The microbiome of humans contains trillions of microbiota that make a host out of the human body that are particularly distributed around the body, genital tract and other mucosal surfaces.¹⁴³ Developing at birth, with influence from both the maternal microbiota and the environment, the human microbiome varies between individuals by virtue of host factors and environmental exposures.^{144,145} The explosion of next-generation sequencing studies has enabled identification and relative quantitation of the species present in each of the human-associated microbial communities.

Dysbiosis or disruption of normal human microbiotas has an effect on human health and disease. These microbial constellations have been viewed as forgotten organs that exist throughout the human body. It has been confirmed that the microbiome generates a metagenome that is 100 times larger than the whole host genome.^{15,146,147} There is a specific environment between each microbiome and its host, which makes it ideal for maintaining symbiotic relationships. The host and its microbiome together form a supraorganism.

The urinary microbiome and BC

Urinary microbiome overview

The urinary tract hosts its own microbiome, probably since urine passing through the urethra borders the external environment (the openings of the gastrointestinal tract, vaginal mucosae and the skin), which harbour distinct commensal microorganisms. A large number of ecological niches present in the human body and the continuous process of tissue morphogenesis and structural regeneration—regardless of physiological or pathological conditions—can be influenced by the resident normal microbiota or by pathogenic microorganisms directly or indirectly.¹⁴⁸ The urinary tract microbiota may act similarly to that of bacterial communities at other mucosal sites. It is assumed that microbiota benefit from the host's nutrient supply and other necessary survival factors, indicating a reciprocal relationship; however, it is not clear what advantage the urinary microbiota provides to the host.

The bladder, a hollow muscular organ, was traditionally thought to be a completely sterile environment. Namely, the urine had been considered sterile before reaching the urethra in healthy individuals. However, this “sterile bladder” paradigm no longer persists. In recent years, the sterile bladder has been debunked with the discovery of an

indigenous microbiome in the absence of clinical urinary tract infection; in other words, the bladder has its own diverse microbiome (Fig. 2A).^{149–153} High-throughput DNA sequencing and enhanced culture-dependent methods have been applied to token female bladder bacteria in standard urine culture-negative samples.^{154–158} In other words, standard urinary culture methods do not detect slower-growing bacteria such as *Lactobacillus* and *Corynebacterium*, but these species can be detected using current sequencing technologies.^{149,159}

Of note, studies in the field of benign urology have supported the notion that certain microbial members of the bladder microbiome may be protective and that disruption, or dysbiosis, of this community may induce lower urinary tract (LUT) dysfunction.^{155,160–162} Evidence has accumulated that the urinary microbiome may change in disease conditions, such as overactive bladder, urinary incontinence, interstitial cystitis, neuropathic bladder, sexually transmitted infections, chronic prostatitis or chronic pelvic pain syndrome.^{150,163–167}

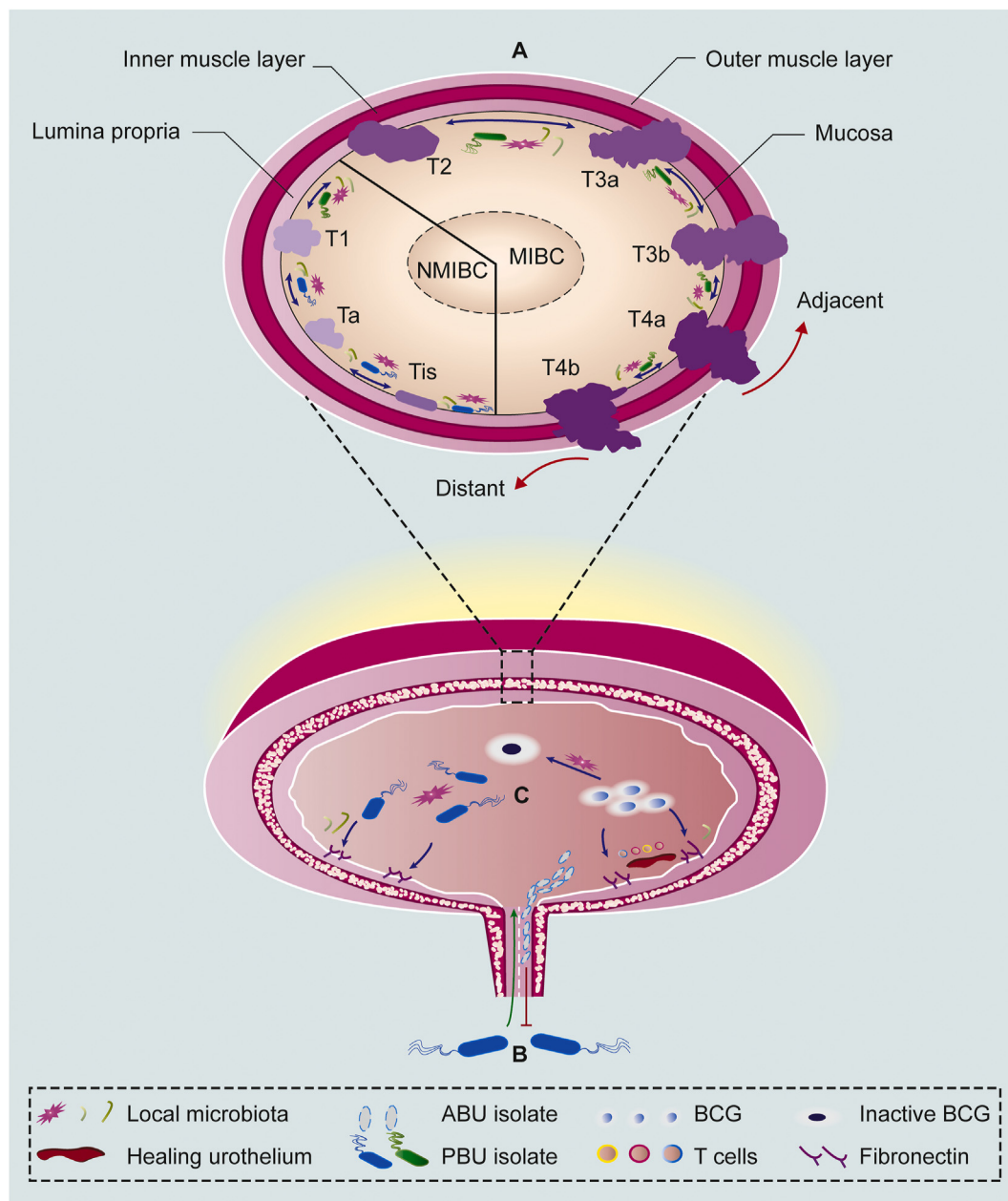


Figure 2 Possible relationships between the urinary microbiome and BC during progression. **(A)** The bladder has its own diverse microbiome, which may involve BC pathogenesis and progression, and differing microbiota isolates may be linked to different types of BC. **(B)** Intentional colonization of the bladder with an avirulent strain of bacteria may inhibit the ability of virulent strains to infect the bladder. **(C)** The immune response is induced by intravesical BCG, but symbiotic microbiota may inactivate BCG in the bladder or regulate urothelial sensitivity to BCG. Abbreviation: BCG, *Bacillus Calmette-Guerin*; ABU, asymptomatic bacteriuria; PBC, pathogenic bacteriuria.

Bladder microbiome research

Wu et al performed a study to characterize the potential urinary microbial community possibly associated with bladder cancer. They compared 31 male bladder cancer patients to 18 healthy controls based on 16S sequencing of midstream voided urine. Bladder cancer was associated with enrichment of certain genera (*Acinetobacter*, *Anaerococcus*, and *Sphingobacterium*) and a decrease in other genera (*Serratia*, *Proteus*, and *Roseomonas*). Furthermore, enrichment of *Herbaspirillum*, *Porphyrobacter*, and *Bacteroides* was detected in the patient group with a high risk of recurrence and progression, indicating that these genera may be potential biomarkers for risk stratification (Table 1).¹⁶⁸ Another study by Xu et al compared the voided urine of 8 bladder cancer patients to 6 controls using 16S sequencing and discovered that the genus *Streptococcus* was significantly elevated in 5 of the 8 cancer samples, suggesting that urothelial carcinoma may be related to altered microbiota of the urinary tract (Table 1).¹⁴ Then, to characterize and compare the urinary microbiome of bladder cancer patients, Popovic et al examined the urinary microbiome in bladder cancer using 16S sequencing, finding an OTU belonging to the genus *Fusobacterium*, a possible putumorigenic pathogen in urine (Table 1).¹⁶⁹

One thing all of these studies have in common is that the samples are from voided urine. Voided, midstream urine samples had been historically considered representative of the bladder in both men and women for minimizing distal urethral contamination.¹⁷⁰ However, voided urine is often not representative of the bladder microbiome because bacterial DNA detected in midstream urine diverges substantially from that obtained by a transurethral catheter.^{149,171,172} A recent study compared microbiome metrics resulting from 16S rRNA gene sequencing between urine obtained from voided midstream urine and cystoscopy among a population of individuals regularly undergoing surveillance cystoscopy with a prior history of superficial bladder cancer, and the results conclude that microbiomes in the urine of the two collection methods are not equivalent to each other, at least in males.¹⁷³

There were differences in the urine microbiome metrics of different urine collection methods. It is critical that bladder microbiome studies remove direct contact with the urothelium as a way of completely and accurately represent the bladder microbiome.

Bladder microbiome and BCG

A live attenuated strain of the bacterium *M. bovis*, the BCG vaccine, was first instilled into the human bladder to treat UCC by Alvaro Morales in 1972, and the FDA approved the use of intravesical BCG in patients with superficial bladder tumours in 1990.¹⁷⁴ According to the recommendation of the European Association of Urology and the American Urological Association, BCG is the most effective immunotherapy for patients with intermediate- and high-risk urothelial NMIBC and carcinoma *in situ*.^{175–177} The mechanism by which BCG immunotherapy mediates body immunity remains unclear. Based on recent evidence, triggering both a local and systemic immune response is the most accepted mechanism. BCG may interact with the bladder microbiome

and recruit inflammatory cells through the secretion of cytokines and chemokines due to the activation of antigen-presenting cells.

Immunological studies have shown that some resident commensal and probiotic bacterial strains have the capability to attenuate mucosal inflammation by down-regulating the NF- κ B pathway, IL-6 and IL-8.¹⁷⁸ Commensal microbial communities in urine are thought to have a positive impact on human health by eliminating inadequately working immune cells and protecting hosts from pathogens (Fig. 2B).¹⁷⁹ Dysbiosis of these microbes with protective abilities may cause lower urinary tract dysfunction.¹⁵⁷ It has been assumed that the bladder microbiome may influence the possible response to BCG therapy through the destruction or inactivation of BCG in bladder lumens or by the regulation of urothelial sensitivity to BCG activity by attachment to fibronectin (Fig. 2C),¹⁸⁰ which suggests that the members of the local microbiota may competitively bind fibronectin in the presence of BCG. A related study found that *Lactobacillus* may be superior to other species at binding fibronectin.¹⁸¹ Furthermore, *Lactobacillus* species have also been researched as an alternative to BCG for the treatment of bladder cancer.¹⁸²

With our growing understanding of BCG immunotherapy, the bladder microbiome has incredible potential for further research. The potential impact of using microbiota to treat bladder cancer is immense. Future research can start from more aspects, such as technology development, and focus on developing techniques to regulate the bladder microbiome to optimize responses to BCG and other therapies. Urine transplantation may be a possible immunotherapy.

The interweaving of the urinary inflammatory microenvironment and the microbiome

UTIs cause inflammatory microenvironmental changes in the local region, so a history of UTIs may be a risk factor for the progression of certain urinary malignancies. However, the existence of microbiota is not equal to infection. The chronic inflammatory response is triggered by many infectious agents after resolution of the acute infection. A long inflammatory response drives carcinogenesis (Fig. 3). For example, SCC can develop after schistosomiasis. The mechanisms involved can be explained by the fact that schistosomiasis induces chronic irritation and inflammation in the urinary bladder. Over time, this state promotes changes in at least two phases of tumour progression: first, premalignant lesions are initiated, and second, these lesions transform into cancerous lesions by the persistent inflammatory response.⁵⁷ Surprisingly, certain commensal strains of bacteria in the urinary microbiome may regulate pathogenic outgrowth of bacteria in the genitourinary tract, similar to the positive effect on controlling vaginal infections of vaginal *Lactobacillus*, a major species in female urine.¹⁸³ Additionally, *Lactobacillus* can also be found in sexually active adolescent men but not in their non-experienced counterparts, which denotes a potential interaction between the female and male urinary microbiome.¹⁸⁴

Therefore, further study of the urinary microbiome is necessary to fully elucidate its role in regulating pathogenic infections and mediating the development of BC.

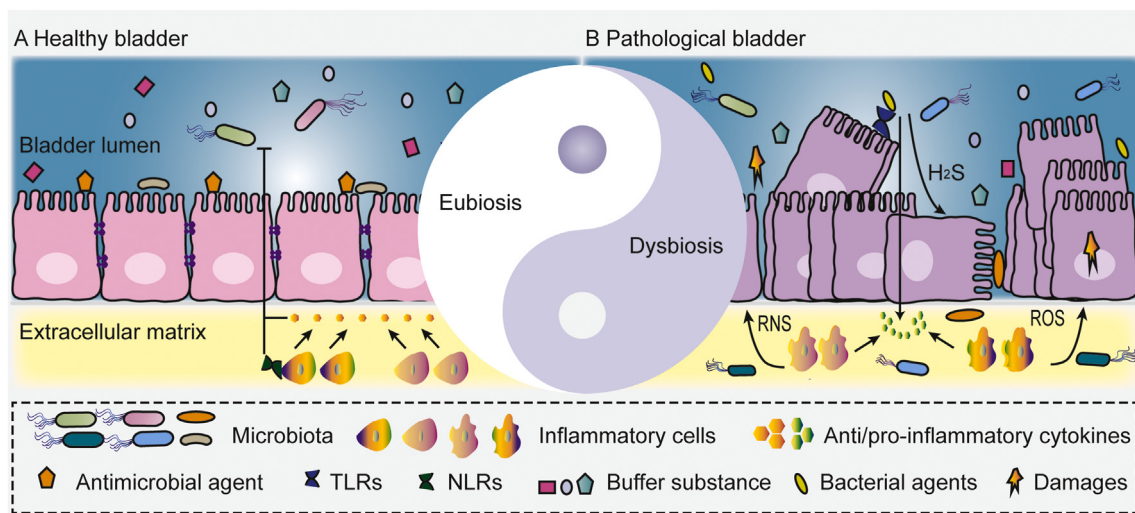


Figure 3 Assumption of the mutual effect between microbial organisms and inflammation. In a healthy bladder (eubiosis), metabolic production of commensal organisms can stimulate the host to produce anti-inflammatory cytokines to maintain homeostasis. Additionally, NLRs protect against microbial invasion. Under pathological conditions (dysbiosis), pathological microbiota outcompete the resident microbiota and stimulate inflammation. Chronic inflammation damages epithelial cells through the production of reactive oxygen species (ROS) and reactive nitrogen species (RNS), leading to cell death and further destruction of the epithelial barrier. A long repeated inflammatory response drives carcinogenesis.

Dysbiosis signatures of the microbial profile in bladder cancer patients

Malignant tissue itself presents with an altered microbiome; however, it is well known that many cancers seem to develop in previously inflamed tissues, increasing the likelihood that microorganisms may have a “field effect” that promotes tumour processes.¹⁸⁵ In addition, tissue adjacent to tumours becomes a special existence, between normal and abnormal. Compared to true “normal” tissue, tumour-adjacent “normal” tissue is probably altered as well. This is mainly due to changes in the extracellular matrix, such as tumour-associated inflammation, immune cell infiltrate and fibrosis. A study performed by Allali et al examining colorectal cancer and adjacent tissue from patients found that tumour and adjacent tissues had very close bacteria, with lower diversity in tumour tissues.¹⁸⁶ Remarkably, tumour and adjacent normal tissue were found to have similar microbiota in other types of cancers, including breast cancer, laryngeal cancer and oral cancers.^{187–189} Regrettably, the associated results have not been found in bladder cancer so far. Thus, a similar study is warranted to be carried out in bladder cancer. Moreover, the microflora characteristics of infiltrating bladder cancer tissue and non-infiltrating bladder cancer tissue are also worth exploring.

However, to examine the microbial profiles in parenchyma tissues in bladder cancer, Fei Liu et al described and analysed the dysbiosis motifs of urinary microbiota in tissue samples of cancerous bladder mucosa (22 carcinoma tissues and 12 adjacent normal tissues) via 16S rRNA gene sequencing.¹⁹⁰ Their results indicated that the predominant phylum in both tissues was Proteobacteria. In addition, lower species richness and diversity were exhibited in cancerous tissues, with beta diversity obviously differing between the cancerous and normal tissues. Similarly,

Popovic et al had previously examined the microbiome of bladder cancer tissue, and *F. nucleatum* 16S rRNA was found in 11 of 42 (26%) bladder tumour tissue samples.¹⁶⁹ There are few studies on the characteristics of urinary tract flora in patients with bladder cancer at the tissue level. As research continues, however, bladder microbiome alteration may be considered a biomarker for BC in the future.

Sex differences in the urinary microbiome

BC is diagnosed more often in men than women, but it may be two to five times more common in women,¹⁹¹ which leads us to reconsider the prevention and risk factors of bladder cancers. It is worth noting that most of the urine contained microbiota, which is different in men and women.¹⁹² An intriguing trend was reported by a study on the effect of ageing on the male urinary microbiome: the whole number of bacteria in the male urinary microbiome substantially decreased with age, but the number of genera increased; *Firmicutes* were found among both, yet *Actinomycetes* were found only among women (Table 1).¹⁹³ A study in 2017 demonstrated that the male urinary microbiome is overall more diverse between samples than the female urinary microbiome, with higher Shannon diversity (but less within sample species richness and therefore lower alpha diversity).¹⁹⁴ Additionally, *Lactobacillus* and *Gardnerella* are the most represented genera in the female microbiota, whereas *Corynebacterium*, *Staphylococcus*, and *Streptococcus* are predominant in the male microbiota (Table 1).^{155,195} To characterize the bladder microbiota of adult women, Price et al performed a cross-sectional study of catheterized urine samples and found that the most common urotype was *Lactobacillus* (19%), while the *Gardnerella* ($P < 0.001$) and *Escherichia* ($P = 0.005$) urotypes were more common in younger and older women, respectively (Table 1).¹⁹⁶

The mechanism of the differences in the incidence of bladder cancer between men and women is unclear and complex and needs further study. For the time being, it may be postulated that the sex difference in the commensal urinary flora might contribute to the well-known sex differences in bladder cancer incidence.

Conclusions

The worldwide burden of bladder cancer has aroused considerable concern worldwide. The involvement of inflammation in tumour formation is becoming increasingly clear. On the one hand, it is increasingly crucial that inflammation is involved in the pathogenesis of bladder cancer. Interference with the inflammatory microenvironment has been shown to inhibit antitumour activity. On the other hand, current evidence supports that the urinary tract is inhabited by a variety of microorganisms that were previously thought not to be present. Furthermore, treatment by modulating the inflammatory microenvironment, especially the regulation of urinary tract microorganisms, shows promise in patients with bladder cancer. Therefore, characterization of the link between the urinary microbiome and chronic inflammation in the bladder might be critical to enable the development of strategies for bladder cancer prevention and treatment. However, research on the pathogenesis of urinary tract microbes in bladder cancer and its prevention and treatment is still in its infancy. To date, there has been no prospective, comparative, large-scale clinical trial that combines regulation of the inflammatory microenvironment as well as the urinary microbiome with conventional treatment of bladder cancer. We recommend progress on trials and preclinical studies to develop innovative protocols to help improve survival and cure rates for bladder cancer in the future.

Conflict of Interests

The authors declare no conflict of interest. None of the contents of this manuscript has been previously published or is under consideration elsewhere. All the authors read and approved the final version of the manuscript prior to submission.

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References

- Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. *CA Cancer J Clin*. 2018;68(6):394–424.
- Siegel RL, Miller KD, Jemal A. Cancer statistics, 2019. *CA Cancer J Clin*. 2019;69(1):7–34.
- Knowles MA, Hurst CD. Molecular biology of bladder cancer: new insights into pathogenesis and clinical diversity. *Nat Rev Cancer*. 2015;15(1):25–41.
- Sanli O, Dobruch J, Knowles MA, et al. Bladder cancer. *Nat Rev Dis Primers*. 2017;3, e17022.
- Prasad SM, Decastro GJ, Steinberg GD, Medscape. Urothelial carcinoma of the bladder: definition, treatment and future efforts. *Nat Rev Urol*. 2011;8(11):631–642.
- Zeegers MP, Tan FE, Dorant E, van Den Brandt PA. The impact of characteristics of cigarette smoking on urinary tract cancer risk: a meta-analysis of epidemiologic studies. *Cancer*. 2000;89(3):630–639.
- Burger M, Catto JWF, Dalbagni G, et al. Epidemiology and risk factors of urothelial bladder cancer. *Eur Urol*. 2013;63(2):234–241.
- Latifovic L, Villeneuve PJ, Parent MÉ, Johnson KC, Kachuri L, Harris SA. Bladder cancer and occupational exposure to diesel and gasoline engine emissions among Canadian men. *Cancer Med*. 2015;4(12):1948–1962.
- Gakis G. The role of inflammation in bladder cancer. *Adv Exp Med Biol*. 2014;816:183–196.
- Trikha M, Corringham R, Klein B, Rossi JF. Targeted anti-interleukin-6 monoclonal antibody therapy for cancer: a review of the rationale and clinical evidence. *Clin Cancer Res*. 2003;9(13):4653–4665.
- Balkwill F, Mantovani A. Inflammation and cancer: back to Virchow? *Lancet*. 2001;357(9255):539–545.
- Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. *Nature*. 2008;454(7203):436–444.
- Ma C, Kesarwala AH, Eggert T, et al. NAFLD causes selective CD4(+) T lymphocyte loss and promotes hepatocarcinogenesis. *Nature*. 2016;531(7593):253–257.
- Xu W, Yang L, Lee P, et al. Mini-review: perspective of the microbiome in the pathogenesis of urothelial carcinoma. *Am J Clin Exp Urol*. 2014;2(1):57–61.
- Turnbaugh PJ, Ley RE, Hamady M, Fraser-Liggett CM, Knight R, Gordon JI. The human microbiome project. *Nature*. 2007;449(7164):804–810.
- Wolfe AJ, Brubaker L. "Sterile urine" and the presence of bacteria. *Eur Urol*. 2015;68(2):173–174.
- Thomas-White K, Brady M, Wolfe AJ, Mueller ER. The bladder is not sterile: history and current discoveries on the urinary microbiome. *Curr Bladder Dysfunct Rep*. 2016;11(1):18–24.
- Whiteside SA, Razvi H, Dave S, Reid G, Burton JP. The microbiome of the urinary tract—a role beyond infection. *Nat Rev Urol*. 2015;12(2):81–90.
- Tang J. Microbiome in the urinary system—a review. *AIMS Microbiol*. 2017;3(2):143–154.
- Price TK, Hilt EE, Dune TJ, Mueller ER, Wolfe AJ, Brubaker L. Urine trouble: should we think differently about UTI? *Int Urogynecol J*. 2018;29(2):205–210.
- Mueller ER, Wolfe AJ, Brubaker L. Female urinary microbiota. *Curr Opin Urol*. 2017;27(3):282–286.
- Brubaker L, Wolfe AJ. The female urinary microbiota, urinary health and common urinary disorders. *Ann Transl Med*. 2017;5(2), e34.
- Aragón IM, Herrera-Imbroda B, Queipo-Ortuño MI, et al. The urinary tract microbiome in health and disease. *Eur Urol Focus*. 2018;4(1):128–138.
- Foxman B, Gillespie B, Koopman J, et al. Risk factors for second urinary tract infection among college women. *Am J Epidemiol*. 2000;151(12):1194–1205.
- Nicolle LE. Urinary tract infection in geriatric and institutionalized patients. *Curr Opin Urol*. 2002;12(1):51–55.

26. Foxman B. The epidemiology of urinary tract infection. *Nat Rev Urol.* 2010;7(12):653–660.
27. Salvatore S, Salvatore S, Cattoni E, et al. Urinary tract infections in women. *Eur J Obstet Gynecol Reprod Biol.* 2011;156(2):131–136.
28. Stamm WE. Scientific and clinical challenges in the management of urinary tract infections. *Am J Med.* 2002;113(Suppl 1A):1S–4S.
29. Wynder EL, Onderdonk J, Mantel N. An epidemiological investigation of cancer of the bladder. *Cancer.* 1963;16:1388–1407.
30. Dunham LJ, Rabson AS, Stewart HL, Frank AS, Young JL. Rates, interview, and pathology study of cancer of the urinary bladder in New Orleans, Louisiana. *J Natl Cancer Inst.* 1968;41(3):683–709.
31. Howe GR, Burch JD, Miller AB, et al. Tobacco use, occupation, coffee, various nutrients, and bladder cancer. *J Natl Cancer Inst.* 1980;64(4):701–713.
32. Kantor AF, Hartge P, Hoover RN, Narayana AS, Sullivan JW, Fraumeni Jr JF. Urinary tract infection and risk of bladder cancer. *Am J Epidemiol.* 1984;119(4):510–515.
33. Claude J, Kunze E, Frentzel-Beyme R, Paczkowski K, Schneider J, Schubert H. Life-style and occupational risk factors in cancer of the lower urinary tract. *Am J Epidemiol.* 1986;124(4):578–589.
34. La Vecchia C, Negri E, D'Avanzo B, Savoldelli R, Franceschi S. Genital and urinary tract diseases and bladder cancer. *Cancer Res.* 1991;51(2):629–631.
35. Kunze E, Chang-Claude J, Frentzel-Beyme R. Life style and occupational risk factors for bladder cancer in Germany. A case-control study. *Cancer.* 1992;69(7):1776–1790.
36. Sturgeon SR, Hartge P, Silverman DT, et al. Associations between bladder cancer risk factors and tumor stage and grade at diagnosis. *Epidemiology.* 1994;5(2):218–225.
37. Jhamb M, Lin J, Ballow R, Kamat AM, Grossman HB, Wu X. Urinary tract diseases and bladder cancer risk: a case-control study. *Cancer Causes Control.* 2007;18(8):839–845.
38. Sun LM, Lin CL, Liang JA, et al. Urinary tract infection increases subsequent urinary tract cancer risk: a population-based cohort study. *Cancer Sci.* 2013;104(5):619–623.
39. Stone L. Urinary tract infection increases risk. *Nat Rev Urol.* 2015;12(1), e4.
40. Richards KA, Ham S, Cohn JA, Steinberg GD. Urinary tract infection-like symptom is associated with worse bladder cancer outcomes in the Medicare population: implications for sex disparities. *Int J Urol.* 2016;23(1):42–47.
41. González CA, Errezola M, Izarzugaza I, et al. Urinary infection, renal lithiasis and bladder cancer in Spain. *Eur J Cancer.* 1991;27(4):498–500.
42. Vermeulen SH, Hanum N, Grotenhuis AJ, et al. Recurrent urinary tract infection and risk of bladder cancer in the Nijmegen bladder cancer study. *Br J Cancer.* 2015;112(3):594–600.
43. Ingersoll MA, Albert ML. From infection to immunotherapy: host immune responses to bacteria at the bladder mucosa. *Mucosal Immunol.* 2013;6(6):1041–1053.
44. Grivennikov SI, Greten FR, Karin M. Immunity, inflammation, and cancer. *Cell.* 2010;140(6):883–899.
45. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011;144(5):646–674.
46. Coussens LM, Werb Z. Inflammation and cancer. *Nature.* 2002;420(6917):860–867.
47. Shacter E, Weitzman SA. Chronic inflammation and cancer. *Oncology (Williston Park).* 2002;16(2):217–226.
48. Ohshima H, Tatemichi M, Sawa T. Chemical basis of inflammation-induced carcinogenesis. *Arch Biochem Biophys.* 2003;417(1):3–11.
49. Rubin DC, Shaker A, Levin MS. Chronic intestinal inflammation: inflammatory bowel disease and colitis-associated colon cancer. *Front Immunol.* 2012;3, e107.
50. Abdel-Latif MMM, Duggan S, Reynolds JV, Kelleher D. Inflammation and esophageal carcinogenesis. *Curr Opin Pharmacol.* 2009;9(4):396–404.
51. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. *N Engl J Med.* 1986;315(26):1650–1659.
52. Swana HS, Smith SD, Perrotta PL, Saito N, Wheeler MA, Weiss RM. Inducible nitric oxide synthase with transitional cell carcinoma of the bladder. *J Urol.* 1999;161(2):630–634.
53. Xu W, Liu LZ, Loizidou M, Ahmed M, Charles IG. The role of nitric oxide in cancer. *Cell Res.* 2002;12(5–6):311–320.
54. Said N, Frierson HF, Sanchez-Carbayo M, Brekken RA, Theodorescu D. Loss of SPARC in bladder cancer enhances carcinogenesis and progression. *J Clin Invest.* 2013;123(2):751–766.
55. Yamamoto M, Wu HH, Momose H, Rademaker A, Oyasu R. Marked enhancement of rat urinary bladder carcinogenesis by heat-killed *Escherichia coli*. *Cancer Res.* 1992;52(19):5329–5333.
56. Okamoto M, Kawamata H, Kawai K, Oyasu R. Enhancement of transformation in vitro of a nontumorigenic rat urothelial cell line by interleukin 6. *Cancer Res.* 1995;55(20):4581–4585.
57. Mostafa MH, Sheweita SA, O'Connor PJ. Relationship between schistosomiasis and bladder cancer. *Clin Microbiol Rev.* 1999;12(1):97–111.
58. Klän R, Knispel HH, Meier T. Acetylsalicylic acid inhibition of n-butyl-(4-hydroxybutyl)nitrosamine-induced bladder carcinogenesis in rats. *J Cancer Res Clin Oncol.* 1993;119(8):482–485.
59. Cohen SM, Hasegawa R, Sakata T, Johansson SL. Effect of aspirin on urinary bladder carcinogenesis initiated with N-[4-(5-nitro-2-furyl)-2-thiazolyl]formamide in rats. *Cancer Res.* 1989;49(2):372–377.
60. Wahl LM, Kleinman HK. Tumor-associated macrophages as targets for cancer therapy. *J Natl Cancer Inst.* 1998;90(21):1583–1584.
61. Kuper H, Adami HO, Trichopoulos D. Infections as a major preventable cause of human cancer. *J Intern Med.* 2000;248(3):171–183.
62. Hussain SP, Hofseth LJ, Harris CC. Radical causes of cancer. *Nat Rev Cancer.* 2003;3(4):276–285.
63. Mantovani A, Bottazzi B, Colotta F, Sozzani S, Ruco L. The origin and function of tumor-associated macrophages. *Immunol Today.* 1992;13(7):265–270.
64. Mantovani A, Bussolino F, Dejana E. Cytokine regulation of endothelial cell function. *FASEB J.* 1992;6(8):2591–2599.
65. Biswas SK, Mantovani A. Macrophage plasticity and interaction with lymphocyte subsets: cancer as a paradigm. *Nat Immunol.* 2010;11(10):889–896.
66. Chanmee T, Ontong P, Konno K, Itano N. Tumor-associated macrophages as major players in the tumor microenvironment. *Cancer (Basel).* 2014;6(3):1670–1690.
67. Maniecki MB, Etzerodt A, Ulhøi BP, et al. Tumor-promoting macrophages induce the expression of the macrophage-specific receptor CD163 in malignant cells. *Int J Cancer.* 2012;131(10):2320–2331.
68. Lima L, Oliveira D, Tavares A, et al. The predominance of M2-polarized macrophages in the stroma of low-hypoxic bladder tumors is associated with BCG immunotherapy failure. *Urol Oncol.* 2014;32(4):449–457.
69. Zhang Q, Mao Z, Sun J. NF- κ B inhibitor, BAY11-7082, suppresses M2 tumor-associated macrophage induced EMT potential via miR-30a/NF- κ B/Snail signaling in bladder cancer cells. *Gene.* 2019;710:91–97.
70. Tian Y-F, Tang K, Guan W, et al. OK-432 suppresses proliferation and metastasis by tumor associated macrophages in bladder cancer. *Asian Pac J Cancer Prev.* 2015;16(11):4537–4542.

71. Sag D, Cekic C, Wu R, Linden J, Hedrick CC. The cholesterol transporter ABCG1 links cholesterol homeostasis and tumour immunity. *Nat Commun.* 2015;6, e6354.
72. Bostrom MM, Irjala H, Mirtti T, et al. Tumor-associated macrophages provide significant prognostic information in urothelial bladder cancer. *PLoS One.* 2015;10(7), e0133552.
73. Ajili F, Kourda N, Darouiche A, Chebil M, Boubaker S. Prognostic value of tumor-associated macrophages count in human non-muscle-invasive bladder cancer treated by BCG immunotherapy. *Ultrastruct Pathol.* 2013;37(1):56–61.
74. Buessow SC, Paul RD, Lopez DM. Influence of mammary tumor progression on phenotype and function of spleen and in situ lymphocytes in mice. *J Natl Cancer Inst.* 1984;73(1):249–255.
75. Young MR, Newby M, Wepsic HT. Hematopoiesis and suppressor bone marrow cells in mice bearing large metastatic Lewis lung carcinoma tumors. *Cancer Res.* 1987;47(1):100–105.
76. Seung LP, Rowley DA, Dubey P, Schreiber H. Synergy between T-cell immunity and inhibition of paracrine stimulation causes tumor rejection. *Proc Natl Acad Sci U S A.* 1995;92(14):6254–6258.
77. Gabrilovich DI, Bronte V, Chen SH, et al. The terminology issue for myeloid-derived suppressor cells. *Cancer Res.* 2007;67(1), e425.
78. Brandau S, Trellakis S, Bruderek K, et al. Myeloid-derived suppressor cells in the peripheral blood of cancer patients contain a subset of immature neutrophils with impaired migratory properties. *J Leukoc Biol.* 2011;89(2):311–317.
79. Damuzzo V, Pinton L, Desantis G, et al. Complexity and challenges in defining myeloid-derived suppressor cells. *Cytometry B Clin Cytom.* 2015;88(2):77–91.
80. Solito S, Pinton L, Damuzzo V, Mandruzzato S. Highlights on molecular mechanisms of MDSC-mediated immune suppression: paving the way for new working hypotheses. *Immunol Invest.* 2012;41(6–7):722–737.
81. Gabrilovich DI, Nagaraj S. Myeloid-derived suppressor cells as regulators of the immune system. *Nat Rev Immunol.* 2009;9(3):162–174.
82. Obermajer N, Wong JL, Edwards RP, Odunsi K, Moysich K, Kalinski P. PGE(2)-driven induction and maintenance of cancer-associated myeloid-derived suppressor cells. *Immunol Invest.* 2012;41(6–7):635–657.
83. Zhang H, Ye YL, Li MX, et al. CXCL2/MIF-CXCR2 signaling promotes the recruitment of myeloid-derived suppressor cells and is correlated with prognosis in bladder cancer. *Oncogene.* 2017;36(15):2095–2104.
84. Muthuswamy R, Wang L, Pitteroff J, Gingrich JR, Kalinski P. Combination of IFN α and poly-I:C reprograms bladder cancer microenvironment for enhanced CTL attraction. *J Immunother Cancer.* 2015;3, e6.
85. Zhang H, Chin AI. Role of Rip2 in development of tumor-infiltrating MDSCs and bladder cancer metastasis. *PLoS One.* 2014;9(4), e94793.
86. Ménétrier-Caux C, Curiel T, Faget J, Manuel M, Caux C, Zou W. Targeting regulatory T cells. *Target Oncol.* 2012;7(1):15–28.
87. Chen X, Du Y, Lin X, Qian Y, Zhou T, Huang Z. CD4+CD25+ regulatory T cells in tumor immunity. *Int Immunopharmacol.* 2016;34:244–249.
88. Priceman SJ, Shen S, Wang L, et al. S1PR1 is crucial for accumulation of regulatory T cells in tumors via STAT3. *Cell Rep.* 2014;6(6):992–999.
89. Loskog A, Ninalga C, Paul-Wetterberg G, de la Torre M, Malmström PU, Tötterman TH. Human bladder carcinoma is dominated by T-regulatory cells and Th1 inhibitory cytokines. *J Urol.* 2007;177(1):353–358.
90. Chi LJ, Lu HT, Li GL, et al. Involvement of T helper type 17 and regulatory T cell activity in tumour immunology of bladder carcinoma. *Clin Exp Immunol.* 2010;161(3):480–489.
91. Loskog AS, Fransson ME, Totterman TT. AdCD40L gene therapy counteracts T regulatory cells and cures aggressive tumors in an orthotopic bladder cancer model. *Clin Cancer Res.* 2005;11(24 Pt 1):8816–8821.
92. Winerdal ME, Krantz D, Hartana CA, et al. Urinary bladder cancer Tregs suppress MMP2 and potentially regulate invasiveness. *Cancer Immunol Res.* 2018;6(5):528–538.
93. Hansen M, Andersen MH. The role of dendritic cells in cancer. *Semin Immunopathol.* 2017;39(3):307–316.
94. Domingos-Pereira S, Sathiyandan K, La Rosa S, et al. Intravesical Ty21a vaccine promotes dendritic cells and T cell-mediated tumor regression in the MB49 bladder cancer model. *Cancer Immunol Res.* 2019;7(4):621–629.
95. Lang F, Linlin M, Ye T, Yuhai Z. Alterations of dendritic cell subsets and TH1/TH2 cytokines in the peripheral circulation of patients with superficial transitional cell carcinoma of the bladder. *J Clin Lab Anal.* 2012;26(5):365–371.
96. Ayari C, LaRue H, Hovington H, et al. High level of mature tumor-infiltrating dendritic cells predicts progression to muscle invasion in bladder cancer. *Hum Pathol.* 2013;44(8):1630–1637.
97. Xiang St, Zhou SW, Guan W, Liu JH, Ye ZQ. [Expression of MUC1 and distribution of tumor-infiltrating dendritic cells in human bladder transitional cell carcinoma]. *Di Yi Jun Yi Da Xue Xue Bao.* 2005;25(9):1114–1118.
98. Rao Q, Chen Y, Yeh CR, et al. Recruited mast cells in the tumor microenvironment enhance bladder cancer metastasis via modulation of ER β /CCL2/CCR2 EMT/MMP9 signals. *Oncotarget.* 2016;7(7):7842–7855.
99. Liu Z, Zhu Y, Xu L, et al. Tumor stroma-infiltrating mast cells predict prognosis and adjuvant chemotherapeutic benefits in patients with muscle invasive bladder cancer. *Oncoimmunology.* 2018;7(9), e1474317.
100. Sari A, Calli A, Cakalagaoglu F, Altinboga AA, Bal K. Association of mast cells with microvessel density in urothelial carcinomas of the urinary bladder. *Ann Diagn Pathol.* 2012;16(1):1–6.
101. Isfoss BL, Busch C, Hermelin H, et al. Stem cell marker-positive stellate cells and mast cells are reduced in benign-appearing bladder tissue in patients with urothelial carcinoma. *Virchows Arch.* 2014;464(4):473–488.
102. Tayal V, Kalra BS. Cytokines and anti-cytokines as therapeutics—an update. *Eur J Pharmacol.* 2008;579(1–3):1–12.
103. Zhu Z, Shen Z, Xu C. Inflammatory pathways as promising targets to increase chemotherapy response in bladder cancer. *Mediators Inflamm.* 2012;2012, e528690.
104. Aggarwal BB. Signalling pathways of the TNF superfamily: a double-edged sword. *Nat Rev Immunol.* 2003;3(9):745–756.
105. Balkwill F. Tumour necrosis factor and cancer. *Nat Rev Cancer.* 2009;9(5):361–371.
106. Keane MP, Strieter RM. The role of CXC chemokines in the regulation of angiogenesis. *Chem Immunol.* 1999;72:86–101.
107. Feng CC, Wang PH, Ding Q, et al. Expression of pigment epithelium-derived factor and tumor necrosis factor- α is correlated in bladder tumor and is related to tumor angiogenesis. *Urol Oncol.* 2013;31(2):241–246.
108. Raziuddin S, Masihuzzaman M, Shetty S, Ibrahim A. Tumor necrosis factor alpha production in schistosomiasis with carcinoma of urinary bladder. *J Clin Immunol.* 1993;13(1):23–29.
109. Cai J, Yang MY, Hou N, Li X. Association of tumor necrosis factor- α 308G/A polymorphism with urogenital cancer risk: a systematic review and meta-analysis. *Genet Mol Res.* 2015;14(4):16102–16112.
110. Wu CC, Huang YK, Huang CY, et al. Polymorphisms of TNF- α -308 G/A and IL-8 -251 T/A genes associated with urothelial carcinoma: a case-control study. *Biomed Res Int.* 2018;2018, e3148137.
111. Lee SJ, Park SS, Cho YH, et al. Activation of matrix metalloproteinase-9 by TNF- α in human urinary bladder

- cancer HT1376 cells: the role of MAP kinase signaling pathways. *Oncol Rep.* 2008;19(4):1007–1013.
112. Lee EJ, Kim WJ, Moon SK. Cordycepin suppresses TNF- α -induced invasion, migration and matrix metalloproteinase-9 expression in human bladder cancer cells. *Phytother Res.* 2010;24(12):1755–1761.
 113. Okamoto M, Oyasu R. Transformation in vitro of a non-tumorigenic rat urothelial cell line by tumor necrosis factor- α . *Lab Invest.* 1997;77(2):139–144.
 114. Shen M, Zhou L, Zhou P, Zhou W, Lin X. Lymphotoxin β receptor activation promotes mRNA expression of RelA and pro-inflammatory cytokines TNF α and IL-1 β in bladder cancer cells. *Mol Med Rep.* 2017;16(1):937–942.
 115. Hao L, Zhao Y, Li ZG, et al. Tumor necrosis factor-related apoptosis-inducing ligand inhibits proliferation and induces apoptosis of prostate and bladder cancer cells. *Oncol Lett.* 2017;13(5):3638–3640.
 116. Zirakzadeh AA, Kinn J, Krantz D, et al. Doxorubicin enhances the capacity of B cells to activate T cells in urothelial urinary bladder cancer. *Clin Immunol.* 2017;176:63–70.
 117. Dmitrieva OS, Shilovskiy IP, Khaitov MR, Grivennikov SI. Interleukins 1 and 6 as main mediators of inflammation and cancer. *Biochemistry (Mosc).* 2016;81(2):80–90.
 118. Seddighzadeh M, Steineck G, Jansson O, et al. Low interleukin-1 α messenger RNA levels predict decreased overall survival time of patients with urinary bladder carcinoma. *Br J cancer.* 2001;84(3):329–334.
 119. Seddighzadeh M, Larsson P, Ulfgrén AC, et al. Low IL-1 α expression in bladder cancer tissue and survival. *Eur Urol.* 2003;43(4):362–368.
 120. Tao L, Qiu J, Slavin S, et al. Recruited T cells promote the bladder cancer metastasis via up-regulation of the estrogen receptor β /IL-1/c-MET signals. *Cancer Lett.* 2018;430:215–223.
 121. Matsumoto R, Tsuda M, Yoshida K, et al. Aldo-keto reductase 1C1 induced by interleukin-1 β mediates the invasive potential and drug resistance of metastatic bladder cancer cells. *Sci Rep-UK.* 2016;6, e34625.
 122. Tsai FJ, Chang CH, Chen CC, Hsia TC, Chen HY, Chen WC. Interleukin-4 gene intron-3 polymorphism is associated with transitional cell carcinoma of the urinary bladder. *BJU Int.* 2005;95(3):432–435.
 123. Luo Y, Ye Z, Li K, Chen R, Li S, Pang J. Associations between polymorphisms in the IL-4 and IL-4 receptor genes and urinary carcinomas: a meta-analysis. *Int J Clin Exp Med.* 2015;8(1):1227–1233.
 124. Joshi BH, Leland P, Lababidi S, Varrichio F, Puri RK. Interleukin-4 receptor alpha overexpression in human bladder cancer correlates with the pathological grade and stage of the disease. *Cancer Med.* 2014;3(6):1615–1628.
 125. Vinocha A, Grover RK, Deepak R. Clinical significance of interleukin-6 in diagnosis of lung, oral, esophageal, and gall bladder carcinomas. *J Cancer Res Ther.* 2018;14(Supplement):S758–S760.
 126. Tsui KH, Wang SW, Chung LC, et al. Mechanisms by which interleukin-6 attenuates cell invasion and tumorigenesis in human bladder carcinoma cells. *BioMed Res Int.* 2013;2013, e791212.
 127. Okamoto M, Hattori K, Oyasu R. Interleukin-6 functions as an autocrine growth factor in human bladder carcinoma cell lines in vitro. *Int J Cancer.* 1997;72(1):149–154.
 128. Ebadi N, Jahed M, Mivehchi M, Majidzadeh T, Asgary M, Hosseini SA. Interleukin-12 and interleukin-6 gene polymorphisms and risk of bladder cancer in the Iranian population. *Asian Pac J Cancer Prev.* 2014;15(18):7869–7873.
 129. Gakis G, Todenhöfer T, Renninger M, et al. Development of a new outcome prediction model in carcinoma invading the bladder based on preoperative serum C-reactive protein and standard pathological risk factors: the TNR-C score. *BJU Int.* 2011;108(11):1800–1805.
 130. Kumari N, Agrawal U, Mishra AK, et al. Predictive role of serum and urinary cytokines in invasion and recurrence of bladder cancer. *Tumour Biol.* 2017;39(4), e1010428317697552.
 131. Inoue K, Slaton JW, Kim SJ, et al. Interleukin 8 expression regulates tumorigenicity and metastasis in human bladder cancer. *Cancer Res.* 2000;60(8):2290–2299.
 132. Wang L, Yi T, Kortylewski M, Pardoll DM, Zeng D, Yu H. IL-17 can promote tumor growth through an IL-6-Stat3 signaling pathway. *J Exp Med.* 2009;206(7):1457–1464.
 133. Baharlou R, Ahmadi Vasmehjani A, Dehghani A, Ghobadifar MA, Khoubyari M. Reduced interleukin-17 and transforming growth factor Beta levels in peripheral blood as indicators for following the course of bladder cancer. *Immune Netw.* 2014;14(3):156–163.
 134. Ohtsuki T, Micallef MJ, Kohno K, Tanimoto T, Ikeda M, Kurimoto M. Interleukin 18 enhances Fas ligand expression and induces apoptosis in Fas-expressing human myelomonocytic KG-1 cells. *Anticancer Res.* 1997;17(5A):3253–3258.
 135. Park CC, Morel JC, Amin MA, Connors MA, Harlow LA, Koch AE. Evidence of IL-18 as a novel angiogenic mediator. *J Immunol.* 2001;167(3):1644–1653.
 136. Bukan N, Sözen S, Coskun U, et al. Serum interleukin-18 and nitric oxide activity in bladder carcinoma. *Eur Cytokine Netw.* 2003;14(3):163–167.
 137. Jaiswal PK, Singh V, Srivastava P, Mittal RD. Association of IL-12, IL-18 variants and serum IL-18 with bladder cancer susceptibility in North Indian population. *Gene.* 2013;519(1):128–134.
 138. Massagué J. TGF- β signal transduction. *Annu Rev Biochem.* 1998;67:753–791.
 139. Derynck R, Akhurst RJ, Balmain A. TGF- β signaling in tumor suppression and cancer progression. *Nat Genet.* 2001;29(2):117–129.
 140. Dumont N, Arteaga CL. Targeting the TGF β signaling network in human neoplasia. *Cancer Cell.* 2003;3(6):531–536.
 141. Wojtowicz-Praga S. Reversal of tumor-induced immunosuppression by TGF- β inhibitors. *Invest New Drugs.* 2003;21(1):21–32.
 142. Dobell C. The discovery of the intestinal Protozoa of man. *Proc R Soc Med.* 1920;13 (Sect Hist Med):1–15.
 143. Grieneisen LE, Blekhman R. Crowdsourcing our national gut. *mSystems.* 2018;3(3), e00060–18.
 144. Palmer C, Bik EM, DiGiulio DB, Relman DA, Brown PO. Development of the human infant intestinal microbiota. *PLoS Biol.* 2007;5(7), e177.
 145. Dominguez-Bello MG, Costello EK, Contreras M, et al. Delivery mode shapes the acquisition and structure of the initial microbiota across multiple body habitats in newborns. *Proc Natl Acad Sci U S A.* 2010;107(26):11971–11975.
 146. Gill SR, Pop M, Deboy RT, et al. Metagenomic analysis of the human distal gut microbiome. *Science.* 2006;312(5778):1355–1359.
 147. Schwabe RF, Jobin C. The microbiome and cancer. *Nat Rev Cancer.* 2013;13(11):800–812.
 148. Alfano M, Canducci F, Nebuloni M, Clementi M, Montorsi F, Salonia A. The interplay of extracellular matrix and microbiome in urothelial bladder cancer. *Nat Rev Urol.* 2016;13(2):77–90.
 149. Wolfe AJ, Toh E, Shibata N, et al. Evidence of uncultivated bacteria in the adult female bladder. *J Clin Microbiol.* 2012;50(4):1376–1383.
 150. Fouts DE, Pieper R, Szpakowski S, et al. Integrated next-generation sequencing of 16S rDNA and metaproteomics differentiate the healthy urine microbiome from asymptomatic bacteriuria in neuropathic bladder associated with spinal cord injury. *J Transl Med.* 2012;10, e174.

151. Hilt EE, McKinley K, Pearce MM, et al. Urine is not sterile: use of enhanced urine culture techniques to detect resident bacterial flora in the adult female bladder. *J Clin Microbiol.* 2014;52(3):871–876.
152. Nienhouse V, Gao X, Dong Q, et al. Interplay between bladder microbiota and urinary antimicrobial peptides: mechanisms for human urinary tract infection risk and symptom severity. *PLoS One.* 2014;9(12), e114185.
153. Pearce MM, Hilt EE, Rosenfeld AB, et al. The female urinary microbiome: a comparison of women with and without urgency urinary incontinence. *mBio.* 2014;5(4), e01283–14.
154. Khasriya R, Sathiananthamoorthy S, Ismail S, et al. Spectrum of bacterial colonization associated with urothelial cells from patients with chronic lower urinary tract symptoms. *J Clin Microbiol.* 2013;51(7):2054–2062.
155. Pearce MM, Zilliox MJ, Rosenfeld AB, et al. The female urinary microbiome in urgency urinary incontinence. *Am J Obstet Gynecol.* 2015;213(3), e347.
156. Brecher SM. Complicated urinary tract infections: what's a lab to do? *J Clin Microbiol.* 2016;54(5):1189–1190.
157. Karstens L, Asquith M, Davin S, et al. Does the urinary microbiome play a role in urgency urinary incontinence and its severity? *Front Cell Infect Microbiol.* 2016;6, e78.
158. Thomas-White KJ, Hilt EE, Fok C, et al. Incontinence medication response relates to the female urinary microbiota. *Int Urogynecol J.* 2016;27(5):723–733.
159. Nazzi F, Pennacchio F. A framework for human microbiome research. *Nature.* 2012;486(7402):215–221.
160. Brubaker L, Nager CW, Richter HE, et al. Urinary bacteria in adult women with urgency urinary incontinence. *Int Urogynecol J.* 2014;25(9):1179–1184.
161. Brubaker L, Wolfe A. The urinary microbiota: a paradigm shift for bladder disorders? *Curr Opin Obstet Gynecol.* 2016;28(5):407–412.
162. Thomas-White KJ, Gao X, Lin H, et al. Urinary microbes and postoperative urinary tract infection risk in urogynecologic surgical patients. *Int Urogynecol J.* 2018;29(12):1797–1805.
163. Nelson DE, Van Der Pol B, Dong Q, et al. Characteristic male urine microbiomes associate with asymptomatic sexually transmitted infection. *PLoS One.* 2010;5(11), e14116.
164. Siddiqui H, Lagesen K, Nederbragt AJ, Jeansson SL, Jakobsen KS. Alterations of microbiota in urine from women with interstitial cystitis. *BMC Microbiol.* 2012;12, e205.
165. Shoskes DA, Altemus J, Polackwich AS, Tucky B, Wang H, Eng C. The urinary microbiome differs significantly between patients with chronic prostatitis/chronic pelvic pain syndrome and controls as well as between patients with different clinical phenotypes. *Urology.* 2016;92:26–32.
166. Brubaker L, Wolfe AJ. Microbiota in 2016: associating infection and incontinence with the female urinary microbiota. *Nat Rev Urol.* 2017;14(2):72–74.
167. Thomas-White KJ, Kliethermes S, Rickey L, et al. Evaluation of the urinary microbiota of women with uncomplicated stress urinary incontinence. *Am J Obstet Gynecol.* 2017;216(1), e55.
168. Wu P, Zhang G, Zhao J, et al. Profiling the urinary microbiota in male patients with bladder cancer in China. *Front Cell Infect Microbiol.* 2018;8, e167.
169. Bučević Popović V, Šitum M, Chow CT, Chan LS, Roje B, Terzić J. The urinary microbiome associated with bladder cancer. *Sci Rep.* 2018;8(1), e12157.
170. Manoni F, Gessoni G, Alessio MG, et al. Mid-stream vs. first-voided urine collection by using automated analyzers for particle examination in healthy subjects: an Italian multicenter study. *Clin Chem Lab Med.* 2011;50(4):679–684.
171. Bajic P, Van Kuiken ME, Burge BK, et al. Male bladder microbiome relates to lower urinary tract symptoms. *Eur Urol Focus.* 2020;6(2):376–382.
172. Chen YB, Hochstedler B, Pham TT, Acevedo-Alvarez M, Mueller ER, Wolfe AJ. The urethral microbiota – a missing link in the female urinary microbiota. *J Urol.* 2020;204(2):303–309.
173. Hourigan SK, Zhu W, Wong WSW, et al. Studying the urine microbiome in superficial bladder cancer: samples obtained by midstream voiding versus cystoscopy. *BMC Urol.* 2020;20(1), e5.
174. Bajic P, Wolfe AJ, Gupta GN. The urinary microbiome: implications in bladder cancer pathogenesis and therapeutics. *Urology.* 2019;126:10–15.
175. Morales A, Eiding D, Bruce AW. Intracavitary Bacillus Calmette-Guerin in the treatment of superficial bladder tumors. *J Urol.* 1976;116(2):180–183.
176. Chang SS, Boorjian SA, Chou R, et al. Diagnosis and treatment of non-muscle invasive bladder cancer: AUA/SUO guideline. *J Urol.* 2016;196(4):1021–1029.
177. Babjuk M, Burger M, Compérat EM, et al. European association of urology guidelines on non-muscle-invasive bladder cancer (TaT1 and carcinoma in situ) - 2019 update. *Eur Urol.* 2019;76(5):639–657.
178. Cosseau C, Devine DA, Dullaghan E, et al. The commensal *Streptococcus salivarius* K12 downregulates the innate immune responses of human epithelial cells and promotes host-microbe homeostasis. *Infect Immun.* 2008;76(9):4163–4175.
179. Frank DN, Zhu W, Sartor RB, Li E. Investigating the biological and clinical significance of human dysbioses. *Trends Microbiol.* 2011;19(9):427–434.
180. Larsen ES, Joensen UN, Poulsen AM, Goletti D, Johansen IS. Bacillus Calmette-Guerin immunotherapy for bladder cancer: a review of immunological aspects, clinical effects and BCG infections. *APMIS.* 2020;128(2):92–103.
181. McMillan A, Macklaim JM, Burton JP, Reid G. Adhesion of *Lactobacillus iners* AB-1 to human fibronectin: a key mediator for persistence in the vagina? *Reprod Sci.* 2013;20(7):791–796.
182. Seow SW, Rahmat JN, Bay BH, Lee YK, Mahendran R. Expression of chemokine/cytokine genes and immune cell recruitment following the instillation of *Mycobacterium bovis*, bacillus Calmette-Guérin or *Lactobacillus rhamnosus* strain GG in the healthy murine bladder. *Immunology.* 2008;124(3):419–427.
183. Spiegel CA. Bacterial vaginosis. *Clin Microbiol Rev.* 1991;4(4):485–502.
184. Nelson DE, Dong Q, Van der Pol B, et al. Bacterial communities of the coronal sulcus and distal urethra of adolescent males. *PLoS One.* 2012;7(5), e36298.
185. Picardo SL, Coburn B, Hansen AR. The microbiome and cancer for clinicians. *Crit Rev Oncol Hematol.* 2019;141:1–12.
186. Allali I, Delgado S, Marron PI, et al. Gut microbiome compositional and functional differences between tumor and non-tumor adjacent tissues from cohorts from the US and Spain. *Gut Microbes.* 2015;6(3):161–172.
187. Thompson KJ, Ingle JN, Tang X, et al. A comprehensive analysis of breast cancer microbiota and host gene expression. *PLoS One.* 2017;12(11), e0188873.
188. Gong H, Shi Y, Xiao X, et al. Alterations of microbiota structure in the larynx relevant to laryngeal carcinoma. *Sci Rep.* 2017;7(1), e5507.
189. Mukherjee PK, Wang H, Retuerto M, et al. Bacteriome and mycobiome associations in oral tongue cancer. *Oncotarget.* 2017;8(57):97273–97289.
190. Liu F, Liu A, Lu X, et al. Dysbiosis signatures of the microbial profile in tissue from bladder cancer. *Cancer Med.* 2019;8(16):6904–6914.
191. Gram IT, Sandin S, Braaten T, Lund E, Weiderpass E. The hazards of death by smoking in middle-aged women. *Eur J Epidemiol.* 2013;28(10):799–806.

192. Whiteside SA, Razvi H, Dave S, Reid G, Burton JP. The microbiome of the urinary tract—a role beyond infection. *Nat Rev Urol*. 2015;12(2):81–90.
193. Lewis DA, Brown R, Williams J, et al. The human urinary microbiome; bacterial DNA in voided urine of asymptomatic adults. *Front Cell Infect Microbiol*. 2013;3, e41.
194. Gottschick C, Deng ZL, Vital M, et al. The urinary microbiota of men and women and its changes in women during bacterial vaginosis and antibiotic treatment. *Microbiome*. 2017;5(1), e99.
195. Shrestha E, White JR, Yu SH, et al. Profiling the urinary microbiome in men with positive versus negative biopsies for prostate cancer. *J Urol*. 2018;199(1):161–171.
196. Price TK, Hilt EE, Thomas-White K, et al. The urobiome of continent adult women: a cross-sectional study. *BJOG*. 2020; 127(2):193–201.