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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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Progression;
Urinary microbiome

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.

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

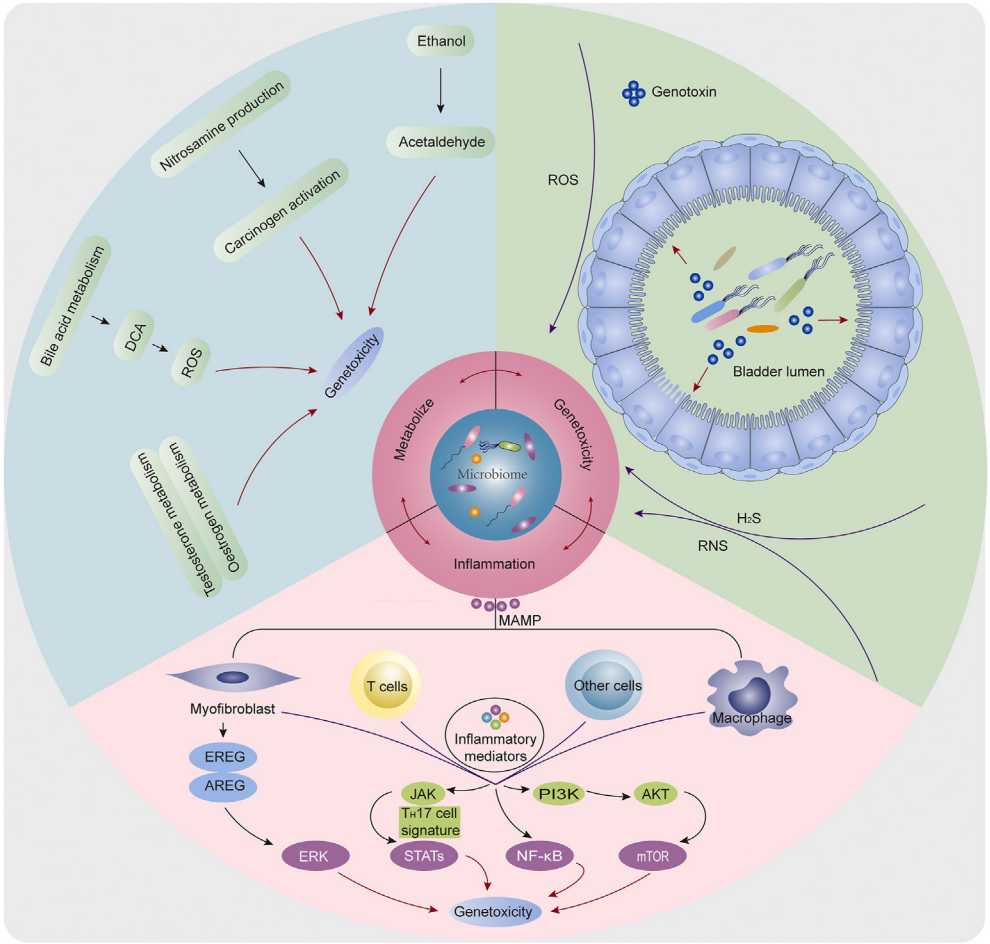

Figure 1 Possible mechanisms by which the bacterial microbiome modulates bladder carcinogenesis. Chronic inflammation is a significant event in bladder cancer. Bacterial translocation may be increased by changes in the microbiome and host defences, leading to increased inflammation, which is regulated 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 (H_2S) 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 chemoattractant 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-Guerin 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 ERβ/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	ERβ/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			
Streptococcus	Enrichment	Promotion	14
Acinetobacter *Anaerococcus* *Sphingobacterium*	Enrichment	Promotion	169
Serratia *Proteus Roseomonas*	Decrease	Promotion	169
Herbaspirillum Porphyrobacter *Bacteroides*	Enrichment	Promotion	169
Fusobacterium	Enrichment	Promotion	170
Firmicutes	Male and female	/	194
Actinomycetes	Female only	/	194
Lactobacillus Gardnerella	The most represented in men	/	196
Corynebacterium *Staphylococcus Streptococcus*	Predominant in women	/	155
Lactobacillus	The most common in women	/	197
Gardnerella	More common in younger women	/	197
Escherichia	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; ERβ, 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-κB-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-α308 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 H_2O_2.[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 (LTβR) 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-1α and IL-1β. Seddighzadeh et al concluded that IL-1α 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-1α 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 ERβ/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-4Rα) is correlated with the pathological grade and stage of bladder tumours; thus, IL-4Rα 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 IFNc-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 ≥ 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 Leeewenhoek 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]

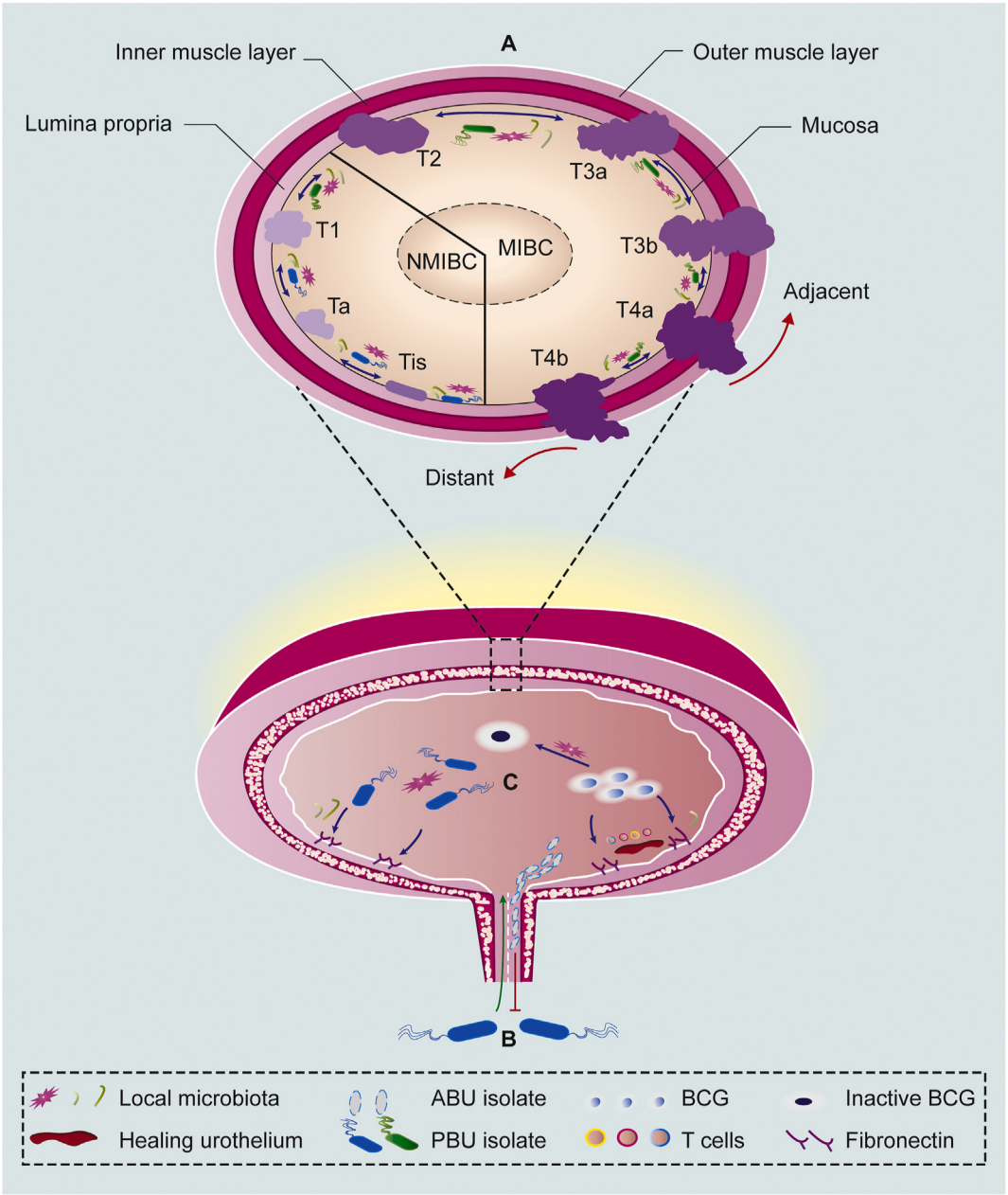

Figure 2 Possible relationships between the urinary microbiome and BC during progression. **(A)** The bladder has its own diverse microbiome, which may involve BC pathogenesis and progression, and differing microbiota isolates may be linked to different types of BC. **(B)** Intentional colonization of 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 protumorigenic 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-κB 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.

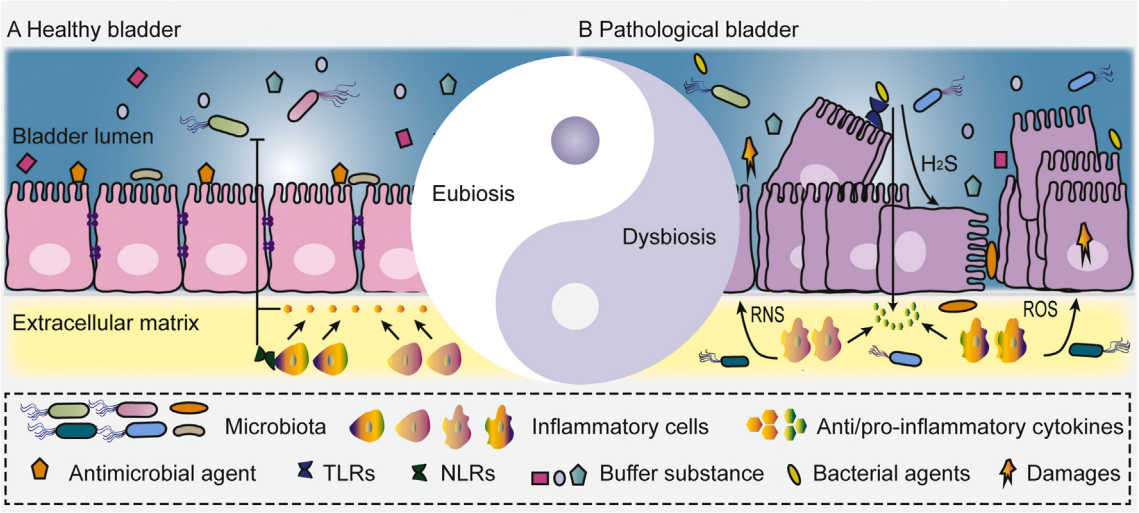

Figure 3 Assumption of the mutual effect between microbial organisms and inflammation. In a healthy bladder (eubiosis), metabolic production of commensal organisms can stimulate the host to produce anti-inflammatory cytokines to maintain homeostasis. Additionally, NLRs protect against microbial invasion. 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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