

The interplay of extracellular matrix and microbiome in urothelial bladder cancer

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Abstract | Many pathological changes in solid tumours are caused by the accumulation of genetic mutations and epigenetic molecular alterations. In addition, tumour progression is profoundly influenced by the environment surrounding the transformed cells. The interplay between tumour cells and their microenvironment has been recognized as one of the key determinants of cancer development and is being extensively investigated. Data suggest that both the extracellular matrix and the microbiota represent microenvironments that contribute to the onset and progression of tumours. Through the introduction of omics technologies and pyrosequencing analyses, a detailed investigation of these two microenvironments is now possible. In urological research, assessment of their dysregulation has become increasingly important to provide diagnostic, prognostic and predictive biomarkers for urothelial bladder cancer. Understanding the roles of the extracellular matrix and microbiota, two key components of the urothelial mucosa, in the sequelae of pathogenic events that occur in the development and progression of urothelial carcinomas will be important to overcome the shortcomings in current bladder cancer treatment strategies.

Urothelial bladder cancer (UBC) is the most common malignancy of the urinary tract, causing 145,000 patient deaths per year¹. Based on tumour stage classification, UBC is grouped into non-muscle-invasive bladder cancer (NMIBC) and muscle-invasive bladder cancer (MIBC)^{2,3}. NMIBC includes carcinoma *in situ* (Cis or Tis), pTa and pT1 tumours, with cancer cells located in the mucosa and submucosa of the bladder, and MIBC includes pT2–pT4 tumour stages, indicating invasion of cancer cells into the muscle layer of the bladder and beyond^{2,3}. Most low-grade NMIBCs are prone to recurrence after treatment; flat Cis noninvasive lesions and papillary tumours are always characterized by high-grade cancer cells, with the risk of progression to MIBC and subsequent occurrence of metastases in 40–80% of patients, depending on the extent of the disease^{2,4,5}. Patients with MIBC are treated with radical cystectomy, lymph node dissection and urinary diversion, and these patients are at high risk of metastatic tumour progression and cancer-related death^{3,6}.

Modifications of the extracellular environments are mandatory for tumour progression, both at the primary site and in the metastatic niches^{7,8}. Data published in the past decade suggest that the tumour extracellular matrix (ECM) might have a principal rather than a supporting role in the onset of carcinoma. Indeed, the dysregulation

of the composition and stiffness of the ECM are associated with a lack of asymmetric division and differentiation of stem cells^{9,10}, as well as an epithelial–mesenchymal transition of cancer stem cells; thus, the ECM regulates tissue homeostasis and sustains the onset and progression of cancer, including bladder cancer^{7,9,11,12}.

The bacterial flora — collectively known as microbiota — is another extracellular microenvironment that is in contact with epithelium. Bacteria produce proteases, which can act intracellularly and/or extracellularly¹³. These enzymes function as extracellular virulence factors with important roles in host tissue degradation, as well as evasion and destruction of host physical barriers. Among these virulence factors, many bacterial enzymes that can degrade the ECM have been extensively characterized, including collagenase, elastase and hyaluronidase^{14,15}. In addition, the bacterial invasion of tissues induces inflammation, a reaction which further sustains ECM remodelling, as well as the generation of oxygen radicals, which induce DNA damage and mutations that drive cancer and cancer recurrence¹⁶.

Current methods of oncological outcome evaluation and imaging assessment still have a limited ability to provide reliable risk stratification and predict UBC aggressiveness, disease recurrence and survival probability.

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Key points

- The interplay between tumour cells and the extracellular microenvironment has been recognized as one of the key determinants of cancer development and progression
- Interaction between extracellular matrix components and bacterial products controls tissue homeostasis; its dysregulation might prepare protumorigenic environmental niches, which could also favour disease relapse
- Bladder colonization with specific bacterial genera throughout an individual's lifetime might influence the propensity for bladder pathology and partly explain the gender differences in the rate of urinary diseases
- Extracellular-matrix-based and microbiota-based biomarkers might be new prognostic factors in bladder cancer, exploitable for risk stratification, tumour staging and for predicting relapse and outcome
- Targeting the bladder extracellular matrix or the associated microbiota might bypass treatment resistance mechanisms of tumour cells

Hence, an urgent need exists to better understand processes underlying bladder carcinogenesis, progression and metastasis, and to identify new prognostic and predictive markers, as well as novel potential therapeutic targets^{17,18}. The ECM and microbiota represent two microenvironments that contribute to the onset and progression of tumours, which has been extensively studied in colorectal cancer¹⁹. In this Review, we provide an overview of the ECM and microbiota of the human epithelium, highlighting how dysregulation of either environment might influence the development and progression of UBC. An in-depth investigation of these two microenvironments and the role of their dysregulation in UBC might eventually provide novel prognostic, diagnostic and predictive biomarkers and new treatment strategies for this disease.

ECM

Molecular genetic evidence supports the existence of distinct pathogenetic pathways for the development of NMIBC and MIBC²⁰. Indeed, changes in the composition and biomechanical properties of the extracellular environment are required for disease progression, both at the primary site and in metastatic niches^{7,8}. Over the past years, it has become evident that tumour progression requires a microenvironment that is conducive to tumour growth, spread of tumour cells via the vasculature and lymphatic system and the formation of metastases in distant organs²¹. These steps in tumour progression are influenced by tumour cells as well as host factors²². Tumour-associated cells, such as fibroblast and macrophages, although they could be classified as nonmalignant based on the absence of specific genetic mutations, harbour epigenetic changes that modify their protein expression, resulting in modulation of the composition of the ECM itself and of ECM-embedded growth factors surrounding the neoplastic area^{22–24}.

The ECM is a complex environment made up of a network of proteins and proteoglycans that interact with each other. In addition, these components form supra-molecular structures in which their biological properties are modified. This system also incorporates cytokines and growth factors that are bound to glycosylated components of the ECM, thus, locating their activity to a specific compartment. Components of the ECM have been

categorized as structural molecules (the core matrisome, composed of 200 glycoproteins, 43 collagen subunits and 35 proteoglycans) and matrisome-associated molecules (176 ECM-affiliated proteins, 250 ECM regulators, 352 secreted factors bound to the ECM, such as matrix metalloproteinases (MMPs), mucins, MMP inhibitors and TGF- β)²⁵. In this context, not only ECM deposition but also its breakdown into smaller fragments and the crosslinking among ECM components contribute to the supramolecular assemblies found in the extracellular space. Intrinsic domains of the stromal proteins have growth-factor-like structures, which act as ligands for canonical growth factor receptors. Proteases and MMPs participate in ECM remodelling and turnover, thereby regulating cell–cell and cell–ECM adhesion, releasing ECM-bound cytokines and growth factors, as well as fragments of stromal components that function as growth factors, or proangiogenic or antiangiogenic molecules^{26–28}. Thus, the ECM has been described as a solid-phase organized assembly of ligands²⁹. Oxygen-dependent crosslinking has been reported to be mediated by lysyl oxidase^{30,31} and glycation end products³², which are increased in case of enhanced neovascularization.

In addition to the biochemical signals provided to cells by ECM proteins, the stiffness, dimensionality and the geometry of the extracellular space also modulate the cell behaviour, cell phenotype and function of any organ^{33–35}. As a result, the extracellular environment of different tissues and organs also regulates recruitment of cells from the bloodstream and the fate of these cells, with stem cells or monocytes differentiating into a variety of cellular populations^{33–35}.

Bladder ECM

In the past few years, the function of the ECM in the bladder has been examined and a number of genes regulating ECM remodelling and composition have been found to be associated with UBC progression and poor prognosis. The ECM composition of the bladder mucosa, in particular the basal membrane (BOX 1) and the submucosa (BOXES 2,3), have been assessed for healthy tissue and tumours of epithelial origin.

Urothelium. The urothelium is covered by glycosaminoglycans (such as hyaluronic acid, heparan sulfate, heparin, chondroitin sulfate, dermatan sulfate and keratan sulfate), which form a gel-like barrier against urine and bacteria^{36,37}. Other proteoglycans (such as decorin, nidogen-1, biglycan, fibulin-1 and tenascins) are also present in the urothelium, but their role in the modulation of tissue functionality has not been clarified³⁸.

Hyaluronic acid is the main ECM-related glycosaminoglycan and it regulates biomechanical activity of tissues and cell functionality. Hyaluronic acid does not induce cell transformation, but it supports many important aspects of the malignant cell phenotype, such as proliferation, migration, resistance to apoptosis and epithelial–mesenchymal transition^{39,40}. Formation of supramolecular hyaluronic acid structures (termed hyaluronic acid cables) can even promote inflammation through the binding of monocytes and lymphocytes⁴¹.

Box 1 | Composition of bladder basal membrane extracellular matrix

Collagen IV

- One of the main components of the basal membrane
- Composed of six distinct α chains

Laminins

- Some of the main components of the basal membrane
- A large family of heterotrimeric glycoproteins, consisting of α , β and γ chains, which are encoded by several different genes
- Involved in cell differentiation, migration and adhesion
- Support attachment of epithelial cells by participating in the formation of hemidesmosomes²⁰³
- In particular, laminin-5 is the major component of the anchoring filament that attaches hemidesmosomes to the basal membrane
- Cells interact with laminins through several cell surface receptors, such as integrins, membrane-bound proteoglycans and glycoproteins

Nidogen-1 (REF. 204) and nidogen-2 (REF. 205)

- Also known as entactin-1 and entactin-2
- Glycoproteins mainly expressed by mesenchymal cells and deposited into the epithelial and endothelial basal membranes during development²⁰⁶
- Structures and abilities to bind to extracellular matrix proteins are similar for both proteins
- Bind and form a ternary complex with collagen IV and laminins, connecting the two networks and stabilizing the 3D structure of the basal membrane²⁰⁷
- Can compensate for each other, owing to their similar structures and binding affinities^{208,209}, but have different tissue distributions^{210–212}, interact with different receptors^{212–214} and have diverse functions^{215,216}
- Highly sensitive to proteolytic cleavage, although the binding of nidogen-1 to laminin- γ 1 decreases susceptibility to proteolysis²¹⁷, protecting laminins from proteolysis and contributing to basal membrane stability
- Removal of nidogens contributes to basal membrane disintegration, favouring epithelial–mesenchymal transition and metastasis²⁰⁶

Perlecan

- A ubiquitous proteoglycan supporting the basal membrane structure
- Binds to a variety of growth factors, such as FGF, VEGF, PDGF and TGF- β , through both a domain of the core protein and the carbohydrate chain^{218–220}, highlighting a function in cell differentiation, cell proliferation and, in particular, in angiogenesis²¹⁸

Lamina propria and muscularis propria. Collagen is the main structural protein of the ECM of various connective, fibrous and muscle tissues. Upon interaction with other ECM components, collagen provides the proper structure and function to tissues and organs. In the lamina propria and muscularis propria, the ECM is composed of collagens, and studies in female Sprague-Dawley rats and in women have revealed that a specific ratio among different collagens (mainly collagen I, III, IV, VI and XII)³⁶ is required for adequate bladder functioning^{42–45}. Collagenases (MMP-1, MMP-2, MMP-9 and MMP-13) break peptide bonds in collagen, enabling tissue ontogenesis and turnover, and participate in the normal immune response (that is, wound healing). Collagenases generate a variety of collagen-derived fragments with anti-angiogenic activity, such as endostatin⁴⁶, tumstatin⁴⁷, arresten⁴⁸, canstatin⁴⁹ and restin⁵⁰. In the bladder, MMPs (such as MMP-1, MMP-2, MMP-7, MMP-9, MMP-11, MMP-14 and MMP-28), serine proteases (plasmin and kallikreins) and urokinase plasminogen activator have been identified^{36,51–56}.

Osteopontin and fibronectin are also expressed in both the lamina propria and the muscularis propria of the bladder³⁶. Fibronectin is present in the ECM in its insoluble form and is critical to the assembly of collagen fibrils in the extracellular space during development *in vivo*⁵⁷. In the bladder, several fibronectin variants generated by alternative splicing are present^{58,59}. Many studies have used rats or *in vitro* models to assess the role of plasma-purified fibronectin and fibronectin variants in tissue homeostasis and remodelling throughout disease processes^{60–63}; however, a study focusing on the human bladder has not yet been reported.

Laminins are also present in the lamina propria and, together with elastic fibres, contribute to bladder functionality⁶⁴. Elastin is also an ECM protein, which, along with collagen, determines the structural and mechanical properties of connective tissues. Elastic fibres (microfibrils) are formed by elastin covalently linking to fibrillin, fibulin and microfibril-associated glycoprotein^{53,65–67}, by the action of lysyl oxidase⁶⁵. Increased levels of lysyl oxidase have been reported in a variety of solid tumours as a responsible factor for the increased stiffness of neoplasia compared with normal tissues and they have been associated with poor prognosis^{68–71}. In a murine model, lysyl oxidase was shown to regulate bladder tissue elasticity, and a deficiency in that enzyme has been associated with pelvic prolapse⁷². Indeed, elastic fibres are present in all bladder layers and are long-lasting molecules with only 1% turnover per year⁶⁶, suggesting that any damage in the microfibrils is also long-lasting. Chymotrypsin, cathepsin G, neutrophil elastase and macrophage metalloelastase are enzymes that can cleave elastin⁷³.

Apart from the crosslinking enzyme lysyl oxidase, advanced glycation end products, such as carboxymethyllysine and pentosidine, have been observed in connective tissues between muscle bundles and in the connective tissue between muscle fibres in non-neoplastic areas of human bladder specimens obtained from patients who underwent radical cystectomy for cancer³².

ECM and UBCs

Expression of extracellular matrix metalloproteinase inducer (EMMPRIN, also known as CD147) on malignant cells has been associated with an invasive phenotype, as well as advanced stage and grade of both transitional cell carcinoma and squamous cell carcinoma. Its expression has also been associated with disease progression⁷⁴, because of the induced expression of MMPs⁷⁵. Indeed, increased expression levels of MMP-10 in the bladder have been associated with an invasive phenotype of UBC⁷⁶, increased expression levels of MMP-7 (both in tissue and serum samples) have been associated with the occurrence of metastatic disease in patients with UBC⁷⁷, and MMP-14 expression levels correlated with tumour stage and grade, as well as poor prognosis^{78,79}. MMP-2 and MMP-9 have also been extensively evaluated in UBC, owing to their collagenolytic activity against collagen IV, which is distributed in the basal membrane, but the results were conflicting⁸⁰. In agreement with the increased expression levels of MMP-7 in UBC, MMP-7-mediated shedding of the transmembrane proteoglycan syndecan-1

Box 2 | Composition of bladder submucosa extracellular matrix

Collagen

- Main structural protein of the extracellular matrix (ECM) of various connective, fibrous and muscle tissues
- 28 forms have been identified
- Interaction with other ECM components provides the structure and function of tissues and organs
- Together with elastin, forms elastic fibres (microfibrils) through covalent links with fibrillin, fibulin and microfibril-associated glycoprotein^{53,65–67}, determining structural and mechanical properties of connective tissues

Proteoglycans

- Consist of a core protein with one or more covalently attached glycosaminoglycan chains
- Divided into small proteoglycans (decorin, biglycan, fibromodulin, lumican and testican) and large proteoglycans (versican, perlecan, neurocan and aggrecan)
- The extracellular proteoglycan decorin acts as an important regulator of collagen fibrillogenesis and inhibitor of cellular proliferation via sequestration of TGF- β and other growth factors^{221,222}
- The decorin-related proteoglycan biglycan does not participate in fibrotic processes²²³, but mainly sustains pro-inflammatory signalling via the binding of TLR-2 and TLR-4 (REFS 224,225)

Fibromodulin

- Controls collagen assembly and the maintenance of the matrix structure in tendons and ligaments^{226–228}
- Has also been detected in the stroma of a variety of solid malignancies, such as lung, breast and prostate carcinomas^{229–231}

Hyaluronic acid

- Main glycosaminoglycan of the ECM, regulating biomechanical activity of tissues and cell functionality
- Does not induce cell transformation but supports aspects of the malignant cell phenotype, such as proliferation, migration, resistance to apoptosis and epithelial–mesenchymal transition^{39,40}
- Can promote inflammation by forming supramolecular structures (hyaluronic acid cables) that bind monocytes and lymphocytes⁴¹

was found to be independently associated with UBC progression and poor survival⁸¹, and the same process has been reported to be involved in chemotherapy resistance of colorectal cancer⁸².

Clearly, MMPs have a fundamental role in tumorigenesis and disease progression, but their application as diagnostic and prognostic markers or as therapeutic targets was not very successful⁸⁰, probably because MMPs represent only few of the many extracellular factors (both stromal and microbial) contributing to UBC pathogenesis. Mapping the upstream regulatory pathways or establishing combination therapies that include MMP-targeted agents might offer new approaches for more effective treatments against UBC progression and relapse in comparison with agents targeting MMPs alone.

Regarding matrix-associated enzymes that degrade and remodel the ECM, the tissue level of hyaluronidase-1 has been reported as a potential prognostic marker predicting progression to muscle invasion and tumour recurrence⁸³. The turnover of hyaluronic acid is profoundly altered during UBC progression, owing to increased expression of hyaluronan synthase 1 (REF. 84) and hyaluronidase-1 (REF. 83), resulting in increased levels of hyaluronic acid fragments, and hyaluronic

acid receptors (CD44 and the receptor for hyaluronan-mediated motility, also known as RHAMM)⁸⁴. These factors contribute to the formation of a protumoural environment. Hyaluronic acid fragments boost the inflammatory response by releasing hyaluronic-acid-bound proinflammatory mediators⁸⁵, and by inhibiting type 2 immune responses, thus, favouring angiogenesis and cell motility⁸⁶, which are likely to contribute to the tumour-supporting effect of hyaluronic acid^{87,88}. In addition, RHAMM expression in the urothelium has been associated with MIBC progression⁸⁴, holding promise for further evaluation as a prognostic marker or therapeutic target in UBC treatment. In 2014, the determination of urinary levels of hyaluronic acid and hyaluronidase was reported to be a highly accurate and noninvasive method for detecting bladder transitional cell carcinoma regardless of tumour grade^{89,90}.

The small leucine-rich proteoglycan (SLRP) decorin has been reported to inhibit cell motility and its loss has been associated with tumour aggressiveness and unfavourable prognosis in both NMIBC and MIBC^{91–93}. The SLRP biglycan has been reported to inhibit UBC cell proliferation, with an increased tissue level being associated with a favourable prognosis⁹¹. However, in colorectal, gastric and pancreatic adenocarcinoma, biglycan expression has been associated with disease progression^{94–96}.

Matrix-associated galectin-3 has been identified in the healthy urothelium⁹⁷. Several authors have reported overexpression of galectin-3 in UBCs, but contrasting findings were reported when comparing different tumour grades of MIBC and NMIBC^{96–98}. Tissue overexpression and increased urinary levels of galectin-3 have been suggested as potential biomarkers for UBC diagnosis, staging and outcome prognosis⁹⁸. Considering all discussed ECM components, the contribution of galectin-3 expression to tumour outcome seems to be tumour-type-specific and, correspondingly, microenvironment-specific: a positive correlation between the expression of galectin-3 and tumour progression has been observed in both UBC and colon cancer^{98,99}, but not in breast and gastric cancers^{100,101}.

Taken together, these findings highlight the important role of the ECM in UBC outcome, while outlining the relevance of the tissue-specific composition and complexity of the different 3D microenvironments. Indeed, ECM derived from healthy small-intestine submucosa, but not ECM from mouse sarcoma (Matrigel®, Corning, New York, USA) or composed of collagen I, suppressed the malignant phenotype of highly invasive J82 bladder cancer cells¹⁰². In agreement, the ECM from healthy human colon allowed binding but not invasion of metastatic LoVo cells, whereas the same cells were able to infiltrate peritumoural ECM and ECM derived from colorectal carcinoma¹⁰³. In 2015, IL-1 α from UBC cell lines was shown to induce the expression of ECM-associated chemokine MCP-1 (encoded by *CCL2*) from fibroblasts, and the level of MCP-1 expressed from normal fibroblasts was lower than that of tumour-associated fibroblasts¹⁰⁴.

Overall, these findings indicate that features of the healthy ECM suppress malignancy. Understanding

Box 3 | Glycoproteins of bladder submucosa extracellular matrix

Tenascin

- Polymorphic with a high molecular mass, mainly expressed during embryonic development
- In adults, normally absent or expressed at greatly reduced levels, but again expressed in association with cell migration, for example during inflammation, wound healing and in tumours²³²
- Structure and size vary owing to alternative splicing, with some forms appearing to be expressed in a tumour-specific manner²³³

Fibronectin

- Multifunctional and adhesive glycoprotein widely distributed in connective tissues and subendothelial matrices, as well as in many cell types
- Present in a soluble form in body fluids and in an insoluble form in the extracellular matrix where it interacts with many other matrix components, such as collagen, fibrin, several integrins and syndecans²³⁴
- Originates from a primary transcript, which can be alternatively spliced generating at least 20 different variants

Osteopontin

- Multifunctional glycoprophosphoprotein highly expressed in bone, but also by various other cell types^{235,236}
- Participates in the regulation of both physiological and pathological mineralization²³⁷, but also in acute and chronic inflammation in which it can be expressed by resident epithelial, endothelial and smooth muscle cells, and infiltrating macrophages and T cells
- Might have both proinflammatory and anti-inflammatory effects, depending on the biological scenario²³⁸

the ECM features that account for the suppression mechanism might help identify new therapeutic targets to control UBC outcomes. Numerous findings support the notion that oncogenesis recapitulates embryogenesis^{105–109}, as cells in both processes are plastic, hypoxia-dependent and perform epithelial–mesenchymal transitions (in the primary tumour) and mesenchymal–epithelial transitions (in metastases)⁹. Thus, pathways associated with embryogenesis are now being investigated as therapeutic targets in cancer¹¹⁰.

Recapitulation of fetal gene expression also occurs during wound healing and organ regeneration, with generation of differentiated cells from stem cells and deposition of ECM components. Studies in murine models have shown activation and deactivation of ECM-related genes during bladder development. Of particular interest might be genes that are switched off after birth, such as those coding for tenascin-C¹¹¹, decorin¹¹², elastin and collagen IV¹¹³. In the normal human bladder, tenascin-C is located in the basal membrane; however, in UBC, increased levels of tenascin-C and diffuse tenascin-C staining in the stroma of invasive tumours have been associated with significantly worse prognosis¹¹⁴, which had also been observed in other solid tumours^{115,116}. Loss of basal membrane components, such as laminin-5, collagen IV and collagen VII, has also been investigated in UBC, without univocal findings¹¹⁴. Conversely, in pancreatic cancer, an increase in collagen IV expression has been reported¹¹⁷.

Microbiota

A vast number of ecological niches exist in the human body, and the continuous process of tissue morphogenesis

and structural regeneration — during both physiological and pathological conditions — can be directly or indirectly influenced by the resident normal microbiota or by pathogenic microorganisms. The organisms involved in this process can include bacteria, viruses and fungi. For instance, in physiological conditions, the mucosal barriers (such as the intestinal or bladder mucosa together with the gut mucus or the glycosaminoglycans secreted by the urothelium, respectively)³⁷ might limit the direct interaction of the majority of bacteria that are present in the intestinal or in the bladder lumen with the ECM environment. However, as observed in the gut¹¹⁸, continuous transitory bacterial translocations or tissue invasions usually occur by members of the microbiota or by pathogenic bacteria, which can adhere to the mucosal surfaces. Moreover, bacteria present in the bladder can form biofilms that enable a continuous direct and prolonged contact with the urothelium. These events can bring bacteria to a location where they can directly alter the composition and structure of ECMs.

Bacterial proteases and the ECM

A number of bacteria produce proteases (BOX 4), which can act in an intracellular and/or extracellular manner¹³. These enzymes serve as extracellular virulence factors, as they have an important role in host tissue degradation and immune system evasion and/or destruction of host physical barriers. Among them, alkaline protease, elastase and phospholipase C have been extensively characterized^{14,15}. In addition, most Gram-positive bacteria produce hyaluronidase as a means of using hyaluronic acid as a carbon source, and probably to facilitate the spread of pathogens through the mucosa of the host organism and the onset of productive infection^{119,120}.

Many bacteria also produce and release collagenases that promote bacterial spread^{121–123}. Collagenases are endopeptidases that digest native collagen in its triple helix region^{124,125}. Bacterial collagenases exhibit broader substrate specificity than vertebrate collagenases and, thus, digest collagens regardless of their type or size^{126,127}; they can nonspecifically bind to and degrade various types of fibrils and sheets formed by collagens^{128,129}. Interestingly, abnormal collagen degradation can be observed in many human diseases in which a role for microorganisms has been hypothesized, such as cancer, arthritis and atherosclerosis^{130,131}. Collagenases enable tissue degradation, the acquisition of nutrients for growth and proliferation, colonization, evasion of host defences and the dissemination of biofilm-forming bacteria. In particular, these extracellular proteases are essential in hydrolysis of proteins in cell-free environments, enabling bacteria to absorb the hydrolytic products¹³².

Exoprotease production by bacteria is also usually regulated by the environment and by the bacterial growth modality, for example in biofilm formation or gaining access to specific environments, such as the suburothelial space and the bladder ECM. For example, the proteases secreted by *Pseudomonas aeruginosa* via the type II or general secretory pathway differ under aerobic and anaerobic growth conditions: under aerobic growth, this microorganism primarily secretes elastase, whereas

Box 4 | **Bacterial enzymes acting on the ECM and affecting immune responses**

Alkaline protease

- Has optimal enzymatic activity at neutral and alkaline pH
- Degrades extracellular matrix (ECM) components and interferes with lymphocyte proliferation through degradation and inactivation of IFN- γ
- Expressed by *Staphylococcus aureus*, *Pseudomonas aeruginosa*, *Serratia marcescens*, *Listeria monocytogenes*, *Bacillus subtilis*, *Clostridium perfringens* and others^{13,14,146,239}

Elastase

- Breaks down elastin and other molecules
- Disrupts tight junctions, damages tissue, degrades cytokines and α protease inhibitors
- Cleavage of IgA, IgG, complement factor C3b and complement receptor type 1 decreases phagocytosis by neutrophils
- Expressed by *S. aureus*, *P. aeruginosa*, *S. marcescens*, *L. monocytogenes*, *B. subtilis*, *C. perfringens* and others^{13,14,146,239}

Phospholipase C

- Highly heterogeneous class of enzymes
- Cleaves various bonds in phospholipids
- Lipase activity releases the secondary messengers inositol triphosphate and diacylglycerol in host cells, ultimately resulting in degradation of host cell membranes
- Expressed by *S. aureus*, *P. aeruginosa*, *S. marcescens*, *L. monocytogenes*, *B. subtilis*, *C. perfringens* and others^{13,14,146,239}

Hyaluronidases

- Degrade hyaluronic acid, resulting in a carbon source and destabilization of eukaryotic cells
- Expressed by most Gram-positive bacteria^{119,120}

Collagenases

- Endopeptidases that digest collagen in its triple helix region
- Bacterial collagenases have broader substrate specificity than vertebrate collagenases and can digest collagen regardless of type or size
- Expressed by *S. aureus*, *Streptococcus agalactiae*, *P. aeruginosa*, *Aeromonas hydrophila*, *Streptococcus bovis*, *Bacteroides fragilis*, *S. marcescens* and *C. perfringens*^{121–125,132,133}

Serine proteases

- Ubiquitously present in nature, classified into >50 families
- Differently distributed in bacteria, owing to environmental adaptation
- Cleave peptide bonds in which serine serves as the nucleophilic amino acid at the enzyme's active site
- Expressed by *S. aureus*, *S. agalactiae*, *P. aeruginosa*, *A. hydrophila*, *S. bovis*, *B. fragilis*, *S. marcescens* and *C. perfringens*^{121–125,132,133}

Metalloproteinases

- Metal-containing proteases classified into nine families on the basis of differing primary sequences and structural characteristics
- Degrade environmental proteins and peptides for bacterial heterotrophic nutrition
- Expressed by *S. aureus*, *S. agalactiae*, *P. aeruginosa*, *A. hydrophila*, *S. bovis*, *B. fragilis*, *S. marcescens* and *C. perfringens*^{121–125,132,133}

Streptococcal pyrogenic exotoxin B cysteine protease

- Secreted protease
- Degrades host serum proteins, such as immunoglobulins, ECM and complement components
- Cleaves transmembrane proteins associated with the epithelial barrier, enabling bacterial penetration and direct access to the ECM
- Expressed by Group A *Streptococcus pyogenes*¹³³

These enzymes were characterized in the gut microbiota.

in anaerobic conditions *in vitro* (and, hence, probably within tumour tissues *in vivo*) alkaline protease represents the predominantly secreted protease¹³³. Importantly, during biofilm formation, bacteria such as *Aeromonas hydrophila* and *P. aeruginosa* can increase the production of extracellular proteases, such as serine proteases, metalloproteinases and elastases¹³⁴. Some pathogens, for example *Streptococcus pyogenes*, can cause invasive diseases through the degradation of intercellular junctions together with the host cysteine protease calpain¹³⁵. During infection of human tissues, *S. pyogenes* produces numerous secreted and cell-associated proteins, including a number of known proteases. Among the secreted cysteine proteases, streptococcal pyrogenic exotoxin B (SpeB) effectively cleaves transmembrane proteins associated with the epithelial barrier to enable bacterial penetration and direct access to the ECM¹³⁵. Moreover, if a lesion of the mucocutaneous barrier (such as a wound) occurs, many bacteria can directly and specifically bind to ECM components, such as collagen fibrils, and start the formation of microcolonies or biofilms¹³⁶.

Post-translational modifications of host proteins can also be induced by bacterial enzymes, enabling diversification of activities of the host protein¹³⁷. Regarding ECM proteins, bacterial peptidyl-arginine deiminase expressed from *Porphyromonas gingivalis* has been suggested to citrullinate collagen I, thus, affecting interaction of fibroblasts expressing integrin $\alpha 1 \beta 1$ with collagen I, and has been associated with the development and progression of destructive arthritis^{138,139}. However, whether bacterially induced post-translational modifications of ECM proteins have a role in the onset and progression of bladder cancer, or other solid tumours, has not yet been clarified. Future studies should evaluate the relevance of this hypothesis.

Bacterial proteases and immune responses

Bacterial proteases are able to degrade growth factors and their receptors and can perturb host cytokine networks^{140,141}. Bacteria also release elastases that cleave and release matrix-associated components¹²¹, such as cytokines and protease inhibitors, factors of the complement system and complement receptors from neutrophils, thus, reducing immune responses (that is, proinflammatory activity and phagocytosis) against the invading bacteria. Furthermore, bacterial elastase has been shown to disrupt epithelial tight junctions^{142–144}, causing proteolytic damage to tissues. In this context, bacterial elastases have been shown to be involved in the formation of leg ulcers¹²¹, as well as in a variety of chronic diseases, such as cystic fibrosis and chronic wounds^{136,145}.

As shown *in vitro*, both alkaline proteases and elastases from *P. aeruginosa* are able to modulate the local host immune response and the machinery that produces and degrades the ECM by inhibiting IL-2-induced proliferation of lymphocytes and through degradation and inactivation of IFN- γ ¹⁴⁶. Proteases expressed by several microorganisms can cleave the IL-6 receptor from human monocytes¹³. Bacterial proteases have been shown to mimic endogenous host-membrane-bound metalloproteinases and enable the shedding of various

host signalling factors, including the IL-6 receptor, the FAS ligand, as well as TNF and its receptors^{147,148}. Hence, these mechanisms enable bacterial proteases to exert not only direct enzymatic activity on the ECM but also an indirect activation or increased production of host MMPs by inflammatory cells, which in turn can enhance the perturbation of the physiological process of ECM regeneration, thus, promoting an altered and potentially cancer-promoting extracellular environment.

Many members of this vast array of proteases, which is produced by both Gram-positive and Gram-negative bacteria and fungi (viruses do not produce metalloproteinases but usually endopeptidases), can directly digest ECM components and are also becoming commercially important, particularly in terms of protein degradation in various pharmaceutical, clinical or industrial applications^{149,150}.

Although many mechanisms involve direct enzymatic action on ECM components, other immune-system-related processes can influence the ECM indirectly. Endotoxins, for example lipopolysaccharide, can recruit and activate inflammatory cells, such as neutrophils, macrophages and even T cells and B cells, owing to their superantigenic nature. The release of pathogen-associated molecular patterns occurs during both physiological and pathological bacterial replication and induces the migration and activation of inflammatory cells. This continuous stimulation has been shown to be essential in the development of a mature and effective immune system, although perturbations of this equilibrium are often observed during pathogenic processes.

Direct evidence is still lacking for bladder diseases, but, according to what has been observed in inflammatory bowel diseases, extensive alterations in the gut mucosal structure are always associated with modifications in the microbiota composition¹⁵¹. Indeed, in the gut, the microbiota has been shown to induce the epithelial expression of genes involved in ECM formation¹⁵², and microbiota-derived proteases degrade collagens of the intestinal mucosa ECM¹⁵³. Hence, these phenomena might also have an important role in the bladder, which is constantly colonized by microorganisms that are predominantly of gastrointestinal origin. The microbiota could affect both the integrity of the bladder urothelial barrier and ECM formation in physiological as well as in disease conditions¹⁵⁴.

Indeed, in several animal models, microorganisms either initiate or perpetuate organ inflammation and fibrosis^{155,156}. Although almost all available data come from observations made in the gut, they clearly demonstrate that microbial products can be directly profibrogenic. For example, progressive fibrosis was observed in the intestine of rats 17–26 days after injection of a bacterial cell wall component into the rat bowel^{155,156}. Similarly, the same consequences were observed 7 days after autologous injection of faecal material or anaerobic bacteria into rats¹⁵⁷. In both experiments, fibrogenesis was associated with an increase in classical profibrotic mediators, such as TGF- β in the mucosa, and could be inhibited by antibiotic treatment. These findings suggest that if microbial components can cross through

urothelial barriers, similar to intestinal ones, they could also trigger inflammation, as well as fibrosis and bladder stiffness at the same sites. Moreover, underscoring the complexity of this interaction, the microorganisms found in a patient with established fibrosis might not be the same as the organisms that triggered the initial events. Indeed, as observed in intestinal fibrosis in mice, once fibrogenesis is initiated, it might be self-perpetuating¹⁵⁸. In addition, ECM accumulation leads to increased tissue stiffness that can in itself drive fibrogenesis through an integrin-mediated fibroblast activation¹⁵⁹.

The many interactions of bacteria with the host ECM metabolism have also evolved in a way that, in physiological conditions, resulted in the microbiota having a key role in the regeneration of a disrupted host epithelial layer¹⁶⁰. The microbiota has the capacity to contribute to host mucosal homeostasis and is likely to participate in the pathogenesis of a variety of diseases including carcinoma. Recent observations have shown that in patients with colorectal carcinoma, the tumour-associated microbiome differs in composition compared with the microbiome of healthy individuals, with either increased or reduced representation of specific bacterial genera or species^{161,162}. Unfortunately, no data exist for the microbiome associated with bladder cancer.

Urinary and bladder-associated microbiota

Urinary microbiota

Historically, the bladder and urine — until reaching the urethra — have been considered sterile in healthy individuals. Conventional microbiological methods could neither isolate nor characterize the full spectrum of urinary bacterial species, which can now be identified by 16S ribosomal RNA sequencing and have been shown to be present in the urinary tract¹⁶³. Ultra-deep pyrosequencing revealed the (relatively) most abundant bacterial taxa in the urine of healthy individuals: *Lactobacillus*, *Corynebacterium*, *Staphylococcus*, *Prevotella*, *Gardnerella* and *Streptococcus*, with a preponderance of *Lactobacillus*, *Prevotella* and *Gardnerella* in women and *Corynebacterium* in men^{163,164}. The urinary tract has its own microbiome because urine passing through the urethra is contiguous to the external environment and is exposed to the skin and the openings of the gastrointestinal tract and vaginal mucosae, which host their own microbiota.

Similar to the intestinal microbiota, urinary microbiota is age-dependent¹⁶⁵, with significant differences among age groups. For instance, *Jonquetella*, *Parvimonas*, *Proteiniphilum* and *Saccharofermentans* mostly occur in adults over the age of 70.¹⁶⁵ Data from investigations of midstream urine (used as a proxy of the bladder microbiome) showed that a more heterogeneous mix of bacterial genera is present in samples from women (6–36 genera) than in samples from men (1–8 genera, but also one sample with 51 genera)¹⁶⁵. Moreover, regardless of sex, in 75% of samples, more than 50% of bacteria belonged to the phylum Firmicutes. Samples from women also had members of the phyla Actinobacteria and Bacteroidetes, which were generally absent from the samples collected from men. In addition, midstream urinary analysis confirmed

the existence of a core microbiome — also in the bladder — but with variability in the amount of the core bacteria, along with a variable prevalence of other bacteria, across age groups. This observation was even more pronounced in the urinary tract than in the gut microbiome^{166,167}, supporting the hypothesis that bladder colonization with specific genera over the course of a lifetime might ultimately influence the propensity for bladder pathology in later life. These findings might also explain the difference in the frequency of urinary diseases observed in men and women.

Bladder-associated microbiota

Obtaining bladder biopsies or suprapubic aspirates in healthy individuals, which would provide the best samples to characterize the bladder microbiome without sample contamination with microorganisms present in the urethra, is unethical. Indeed, this was one of the reasons why the bladder microbiome was not originally included in the *Human Microbiome Project*. However, sampling of the midstream bacterial population only might not enable detection of bacterial communities in biofilms in the bladder that adhere to the mucosa for long periods of time in direct interaction with the urothelium. For example, a different composition of mucosa-associated and luminal microbiota has been reported in the process of characterizing the intestinal microbiota^{168–171}. Thus, similar to the intestinal microbiota, further studies are needed to help understand the modulation of the composition and variety of bladder-associated bacterial strains, which are likely to be dependent on age, gender, breast feeding, dietary and socioeconomic status.

Indeed, epidemiological studies have revealed that UBC incidence is age-dependent, with men having a higher risk than women with a rate ratio of at least 3:1 (REFS 2,3). The association between bladder-associated microbiota and the incidence of cancer in men and women has not yet been comprehensively assessed. For example, whether the preponderant bacterial strain *Lactobacillus* in the bladder of women might provide protection from UBC is not known, although many reports have shown that *Lactobacillus* might reduce chronic inflammation and potentiate a number of immune responses^{172–176}. A multicentre, double-blind, placebo-controlled, randomized trial in 138 patients with primary bladder tumours reported that daily oral administration of freeze-dried *Lactobacillus casei* sp. Shirota for 1 year prevented the recurrence of UBC after transurethral resection of the tumours¹⁷⁷. Another multicentre, prospective, randomized, controlled trial that enrolled 207 patients demonstrated that patients treated orally for 1 year with *L. casei* sp. Shirota in addition to transurethral epirubicin (for 3 months) had a significantly lower UBC recurrence rate at 3 years compared with the epirubicin only group, although the overall survival did not differ between the groups¹⁷⁸. In addition, a case-control study in 180 patients and 445 population-based controls showed that regular (1–2 times per week) probiotic intake reduced UBC risk in the healthy population¹⁷⁹. Taken together, these results strongly support the protective role of *L. casei* Shirota against bladder cancer.

The bladder epithelium can act as a persistent reservoir for viable but nonculturable uropathogenic bacterial strains, which can ultimately lead to bladder or kidney infection^{163,180,181}. In these cases, *Escherichia coli*, *Klebsiella pneumoniae* and *Staphylococcus saprophyticus* strains are the most often isolated species, but many other bacteria can also be found^{163,180–182}, suggesting that bladder commensal populations are polymicrobial and variable^{162,164,175–177}.

In a murine model, researchers found that 2 weeks after acute *E. coli* infection the bladder can tolerate the colonization of bacterial strains that are in a resting state, which, thus, do not induce an immune response and are unresponsive to many antibiotics^{183,184}. Whether a chronic state of bacterial colonization is associated with a chronic state of low-grade bladder inflammation still requires investigation. In healthy individuals, intentional instillation into the bladder of *E. coli* sp. 83972 (the prototype strain of asymptomatic bacteriuria)¹⁸⁵ resulted in a normal acute neutrophil response but only a modest inflammatory response¹⁸⁶. This finding suggests that, indeed, chronic low-grade bladder inflammation can be present even in the absence of symptomatic infection and that this inflammation can be associated with low pathogenic or nonpathogenic bacterial strains that colonize the urothelium. The association between chronic inflammation, mucosa-associated microbiota and the development and outcome of solid tumours has been validated for a variety of neoplasia, in particular colorectal cancer^{187,188}. In bladder cancer, a preliminary study found an association between urinary dysbiosis (specifically, an altered ratio among *Pseudomonas* and *Anaerococcus* versus *Streptococcus*) and urothelial carcinoma¹⁸⁹.

Overall, the urinary microbiota differs between men and women and urinary dysbiosis might be associated with UBC. Indeed, the urinary microbiome might be different from the bacteria strictly associated with the urothelium, and a clear association between mucosa-associated microbiota and the incidence and outcome of UBC is lacking. Identification of bacterial strains associated with UBC and clarification of their interaction with the ECM might lead to new therapeutic options for patients with NMIBC experiencing tumour recurrence after BCG treatment^{190,191}, and those with MIBC for whom immunotherapy is not indicated^{2,3,192}.

Conclusions

In UBC, a patient's most important prognostic factors are still based on morphology, including tumour size, multiplicity, associated Cis, grade and stage^{2,3}. Further thorough studies are needed to establish the detailed ECM composition of all layers of the human bladder and UBC. Despite many authors reporting expression and localization of a single or some ECM components in healthy and neoplastic bladders, a thorough examination of composition, 3D structure and biomechanical properties of the ECMs of the human bladder and human bladder carcinoma has not yet been undertaken. Assessing the entire complexity of ECM composition, for example via a proteomic approach, and biomechanical features

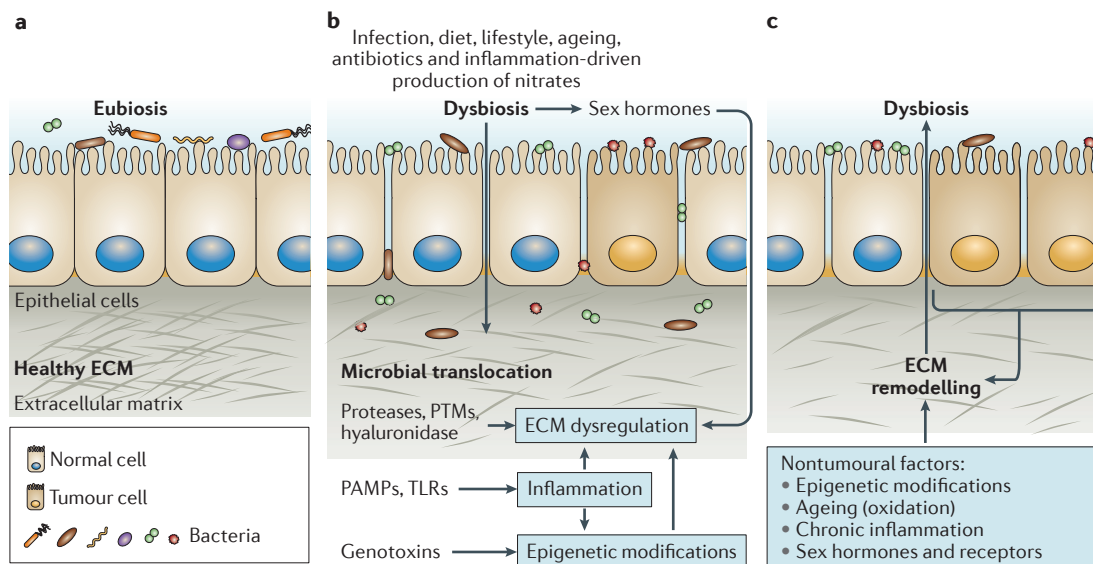


Figure 1 | Dysbiosis and ECM modifications. **a** | In many tissues, homeostasis depends on ecological community with microbial organisms (eubiosis). Disruption of this balance (dysbiosis) and extracellular matrix (ECM) remodelling are associated with a variety of diseases, for example, colorectal carcinoma. Future studies are likely to also establish associations between the functions of the microbiota and the urothelium, connecting dysbiosis with the onset, progression and relapse of urothelial bladder cancer. **b** | These associations have been mainly viewed as dysbiosis inducing ECM remodelling, for example, through the release of bacterial enzymes that degrade ECM components or introduce post-translational modifications (PTMs)¹³⁷, activation of inflammatory pathways and epigenetic modifications of fibroblasts. Microbial translocation can occur during this process and exacerbate ECM dysregulation. Interaction between the microbiome and sex hormones can also regulate ECM features^{240,241}. **c** | However, dysbiosis can also follow ECM remodelling at the onset of disease; for example, a solid tumour can establish a new niche for the growth of bacterial strains by creating hypoxic and/or acidic conditions or changing the ECM composition^{242,243}. Dysregulation of the ECM composition can also follow nontumoural conditions, for example, through epigenetic modifications of fibroblasts induced by toxins, irradiation and chronic inflammatory responses, an imbalance between sex hormones and/or their receptors, and ageing. Changes in the ECM composition provide conditions for altered binding of bacterial strains, thus, favouring dysbiosis and microbial translocation. PAMPs, pathogen-associated molecular patterns; TLRs, Toll-like receptors.

(that is, ultrastructure and stiffness) will reveal new or dysregulated pathways that are associated with UBCs.

Many attempts to develop bladder scaffolds or substitutes have failed because of mechanical, structural or functional problems¹⁹³. Given the critical role of the ECM in determining cell behaviour, a thorough examination of the features of the ECM of the healthy bladder will pave the way for bladder regenerative medicine in patients needing bladder augmentation (that is, patients with congenital bladder exstrophy, posterior urethral valves and benign prostatic hyperplasia)^{193,194} or undergoing nonradical surgery. As an example, ECM stiffness modulates cellular functions as diverse as migration, proliferation, differentiation and apoptosis, and is crucial for organ development and homeostasis¹⁰. Establishing the stiffness of bladder ECM and the correct ratio of ECM components, in particular the total amount of collagens and elastin, will probably contribute to the design of scaffolds resembling native features of the bladder. Moreover, elastin content in the bladder is continuously decreasing after birth, suggesting that features of bladder scaffolds should differ according to the decade of life.

The human body coexists with a variety of commensal microorganisms, referred to as the normal microbiota. The variety of bacterial strains has been described for most but not all human tissues¹⁹⁵, and the accurate

amount and diversity of microbial species of any healthy tissue, sometimes termed eubiosis, was associated with the function of the organ (FIG. 1a). The microbial translocation of commensal bacteria through the epithelium with altered permeability, dysbiosis and infection by pathogenic bacteria contribute to a variety of diseases. Tissue invasion by resident bacteria, biofilm formation in the mucosa or colonization by pathogenic bacteria might support the onset, progression and relapse of carcinoma. Malignant tumours often develop at sites of chronic injury, with chronic inflammation being the most important risk factor, as it increases the risk that a mutated cell accumulates additional deleterious mutations¹⁶.

Some bacterial strains release exoproteases that degrade the ECM to enable tissue colonization and the evasion of host defences, acquisition of nutrients for growth and proliferation and dissemination of biofilm-forming bacteria to different areas of the organism. Among these bacterial virulence factors, enzymes degrading ECM components have been extensively characterized and include bacterial hyaluronidase, collagenase and elastase^{14,15} (BOX 4). In terms of cancer outcome, different mechanisms have been proposed, such as the release of antiapoptotic and proliferative cytokines by TLR⁺ cells and the induction of mutations in epithelial cells (both induction of neoplastic mutations and accumulation in

addition to pre-existing mutations). These mutations can be caused by oxygen species and reactive nitrogen species released from inflammatory cells, genotoxins or cancerogenic metabolites occurring during bacterial metabolism¹⁹⁶ (FIG. 1b).

Indeed, in order to spread, bacteria invading tissues need to break the epithelial barrier and create an ecological niche within the lamina propria. Bacteria release a variety of enzymes that degrade ECM components, such as collagenase, hyaluronidase and elastase, which degrade the tight junctions between epithelial cells and ECM components in the lamina propria¹⁹⁷. Thus, bacterial enzymes that degrade ECM components might create an environmental niche that supports the productive seeding of transformed cells. Abnormal ECM composition and structure affect cancer progression by either promoting cellular transformation and metastasis or deregulating behaviour of stromal cells, eventually facilitating tumour-associated angiogenesis and inflammation⁹. Furthermore, an inflammatory response against bacterial antigens and as a consequence of the presence of bacterial elastase would contribute to the acquisition of new mutations.

In addition, bacteria-induced post-translational modifications of ECM components are likely to represent a new field of research. As post-translational modifications of host proteins have a fundamental role in the diversification of protein function, it is not surprising that bacteria evolved strategies to interfere with the functionality of host proteins to create their own niche and to evade the immune system. Bacteria-induced posttranslational modifications of host proteins might also alter functions that are regulated by ECM components, such as cell localization, proliferation and adhesion. Identification of pathogenic bacteria associated with specific diseases will open the way for searching post-translational modifications associated with the onset and progression of these diseases. Post-translational modifications of ECM components will probably also be discovered for solid tumours and other solid diseases. Finally, ECM modifications that occur in ageing tissues, induced by chronic immune responses or fibrosis, as well as by tumour-associated microenvironments (for example, acidosis), probably selectively promote the growth of specific bacterial species (FIG. 1c).

New prognostic factors, such as ECM-based and microbiota-based biomarkers, might be exploitable for risk stratification, tumour staging and to predict relapse

and outcome of UBCs. Similarly, ECM-based biomarkers might also be highlighting targets for antineoplastic drugs and the design of new therapeutic strategies. In addition, once ECM modifications and the diversity of the bacterial flora associated with UBC have been established, urine samples should be investigated for the same biomarkers, as urine samples ultimately represent the most useful specimen to be assessed in the real-life clinical setting. Extending the search to other biomarkers in addition to hyaluronic acid and hyaluronidase will improve the chance to identify rapid, cheap, noninvasive and easily repeatable assays that combine multiple parameters for diagnosis, as well as prognostic indicators for UBC relapse. Integration of biomarkers indicating ECM modifications induced by the bacterial flora associated with UBC into current diagnostic and prognostic assays is likely to strengthen their power.

Since the 1950s, faecal microbiota transplantation has been used to ‘re-establish the balance of nature’ within the intestinal environment, by correcting the dysbiosis caused by antibiotic treatment¹⁹⁸. Now, the modulation of gut microbiota composition is regarded as an emerging treatment for several gastrointestinal and metabolic disorders, such as refractory *Clostridium difficile* infection and ulcerative colitis^{199,200}. Animal models have been used to establish evidence (for example, lower incidence of certain cancers in germ-free mice compared to conventionally raised animals) and mechanisms underlying the observation that bacterial microbiota promote colorectal, gastric, liver, lung and breast cancer¹⁹⁶, but such studies have not been performed for UBC. Indeed, strategies to restore normal bladder-associated microbiota might be a potential option to reduce UBC incidence or relapse. Future studies assessing efficacy of intravesical instillation of prebiotics and probiotics are likely to prove the beneficial effects of targeting the bladder-associated microbiome in patients with UBC.

Finally, targeting the bladder ECM or the associated microbiota might bypass treatment resistance mechanisms of tumour cells. Bladder-associated microbiota should also be investigated in UBC responses to anti-neoplastic therapy, as has previously been reported for colorectal carcinoma^{201,202}. Strategies to improve UBC responses to antineoplastic therapies might come from studies assessing the difference of bladder-associated microbiota between men and women according to different decades of patient age, ultimately leading to a more patient-tailored approach.

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Author contributions

M.A., F.C. and A.S. researched data for the article, wrote and reviewed and/or edited the manuscript before submission. All authors substantially contributed to discussion of the article content.

Competing interests statement

The authors declare no competing interests.

Review criteria

A systematic literature search for English-language original and review articles was performed using Google and PubMed. Key words used were “bladder”, “urine”, “extracellular matrix”, “bacteria”, “microbioma”, “microbiota”. All available full-text original articles and reviews published since 1970 were used. Original articles referenced in the identified reviews were also searched and discussed.

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