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REVIEW AND PERSPECTIVES

Chronic inflammation in urothelial bladder cancer

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Abstract The association between inflammation and cancer has been pointed out in epidemiological and clinical studies, revealing how chronic inflammation may contribute to carcinogenesis in various malignancies. However, the molecular events leading to malignant transformation in a chronically inflamed environment are not fully understood. In urothelial carcinoma of the urinary bladder, inflammation plays a dual role. On the one hand, chronic inflammation is a well-established risk factor for the development of bladder cancer (BC), as seen in *Schistosoma haematobium* infection. On the other, intravesical therapy by bacillus Calmette-Guérin (BCG), which induces inflammation, offers protection against cancer recurrence. The large variety of pro-inflammatory mediators expressed by BC and immune cells binds to specific receptors which control signalling pathways. These activate transcription of a plethora of downstream factors. This review summarizes recent data regarding inflammation and urothelial carcinoma, with special emphasis on the role the inflammatory response plays in BC recurrence risk and progression.

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Introduction

Inflammation is a self-limiting host defence mechanism against biological, chemical and physical agents. However, its persistence can lead to cellular damage causing various diseases, including cancer [1].

As long ago as 1863, Virchow hypothesized that inflammation might induce cancer due to stimulation of cell proliferation by tissue injury and the local host response it induces [2]. Cell proliferation per se does not cause cancer, but a microenvironment rich in inflammatory cells, growth factors, enhanced angiogenesis and DNA-damaging agents may superimpose tumour development onto proliferative activity [1, 3]. In approximately 20 % of cancers, oncogenesis is associated with chronic infection and inflammation. Etiological factors eliciting chronic inflammation related to cancer development include microbial (such as *Helicobacter pylori* gastritis for gastric cancer and MALT lymphoma) and viral (such as hepatitis B or C for hepatocellular carcinoma) infections as well as autoimmune diseases (such as inflammatory bowel disease for colon cancer) [4–5].

Bladder cancer (BC) ranks ninth among the most common cancers worldwide, comprising about 3 % of all malignancies [6]. Males are affected more often than females. BC is rare before the age of 50 but its incidence increases sharply after the age of 65 [7]. When diagnosed, 75–85 % of bladder carcinomas are superficial tumours. Following transurethral resection (TUR), 70 % of patients experience relapses while in 15–25 % the disease eventually progresses to muscle-invasive BC (MIBC) [8]. Prognosis mainly relies on grade and stage of

the tumour at presentation, as well as patient age and gender [9].

In addition to well-known causes such as smoking and occupational exposure to aromatic amines, chronic inflammation is recognized as a risk factor for BC [1] (Fig. 1). The association between squamous cell carcinoma of the urinary bladder and urogenital schistosomiasis has been known for decades [10].

BC is a highly immunogenic malignancy. However, neoplastic urothelial cells may inhibit cytotoxic functions of immunocompetent cells and stimulate secretion of growth promoting factors. Conversely, intravesical instillation of bacillus Calmette-Guerin (BCG) offers long-term recurrence-free survival in about two thirds of patients [11, 12]. Although the mechanisms responsible for the anti-proliferative effects of BCG on BC cells are poorly known, the clinical benefits suggest that the immune system itself contributes to halting BC progression [12].

This review provides an update regarding the role of inflammation in development and progression of BC.

Causes of bladder inflammation

Schistosomiasis

According to the World Health Organization, schistosomiasis affects 200 million people worldwide, in endemic areas of sub-Saharan Africa, Sudan, Egypt and Yemen, where BC is the most common type of cancer in men and the second in women after breast cancer [13]. Squamous cell carcinomas account for 60–90 % of all schistosomiasis-associated BC, adenocarcinomas for 5–15 % while the remainder are urothelial carcinomas [14] (Fig. 2).

It has been suggested that the inflammatory response to *Schistosoma haematobium* eggs may give rise to genotoxic factors causing genomic instability in host cells. This not only induces a proliferative response to repair tissue damage caused by the inflammation but also might activate oncogenes or inactivate tumour suppressor genes [15–16].

In experimental schistosomiasis, the activity of carcinogen-metabolizing enzymes increases soon after infection but

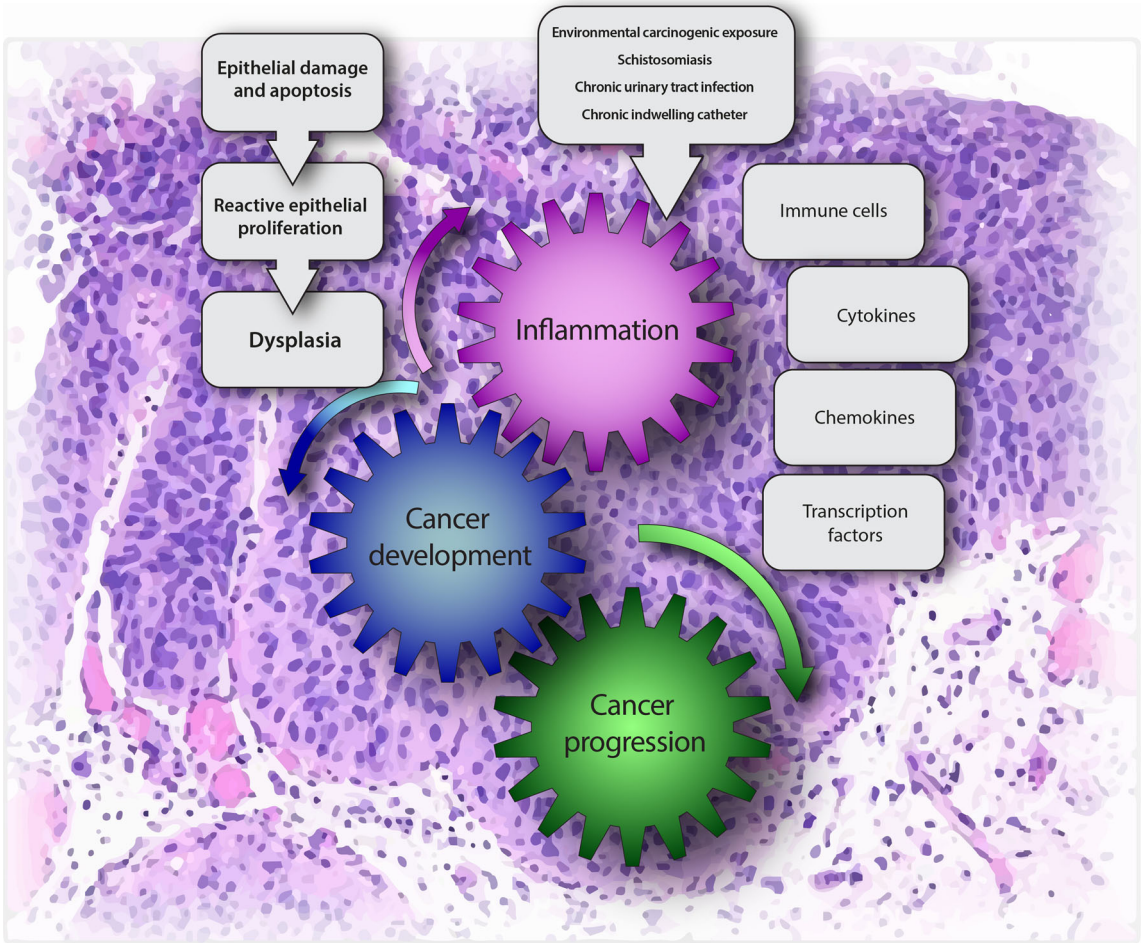

Fig. 1 Chronic inflammation in BC development and progression. Tumour microenvironment is largely conditioned by inflammatory cells and signalling molecules promoting cancer cell growth, invasion and metastasis. Inflammation may be triggered by numerous environmental factors, infectious agents and mechanical injuries

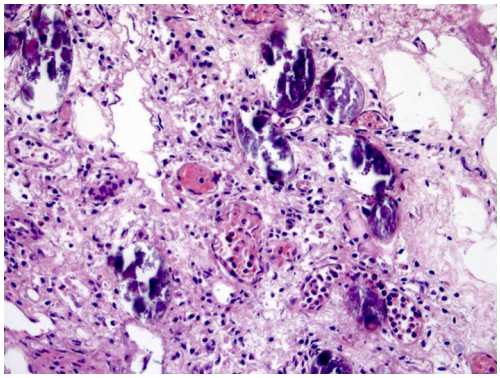

Fig. 2 Schistosomiasis. Numerous calcified eggs of *Schistosoma haematobium* in inflamed bladder mucosa

decreases in the later chronic stages of the disease, which prolongs exposure of urothelium to activated N-nitrosamines. Activated inflammatory cells induce endogenous synthesis of N-nitrosamines as well as production of oxygen radicals. Levels of DNA damage in host cells are high and correlate with intensity of the inflammatory reaction [17]. Intravesical instillation of *S. haematobium* antigens induced inflammation as well as urothelial dysplasia in CD-1 mice [18]. It has been suggested that *S. haematobium* may be oncogenic by inducing *K-RAS* mutations [19].

Bacterial infections

Kantor et al. published a case-control study showing that the risk of BC was higher in patients with a history of urinary tract infections (UTI) and more significantly in individuals reporting three or more infection episodes (RR = 2.0) [20]. Recently, Vermeulen et al. confirmed that BC and regular cystitis are positively associated, while infrequent occurrences of UTI treated with antibiotics were associated with decreased BC risk [21].

Over the last decade, several studies have questioned whether uropathogenic *Escherichia coli* (UPEC) strains are strictly extracellular pathogens. UPEC can invade host epithelial cells in the urinary tract by means of filamentous adhesive organelles and may persist for long periods, unaffected by host defence mechanisms and antibiotics [22]. The host cell response to intracellular bacteria reservoirs triggers innate immunity, apoptosis, increased proliferation and differentiation. Activation of cell surface receptors, such as Toll-like receptors, induces pro-inflammatory cytokine transcription, ultimately recruiting neutrophils to the site of infection. Recruitment of neutrophils and increased urothelial cell apoptosis lead to exfoliation of infected urothelial cells into the urine, which decreases the bacterial burden and reduces risk of reinfection [23–24]. Conversely, these mechanisms can drive genetic alterations, including promoter hypermethylation, resulting in downregulation of the expression of *MLH1* and *MGMT* and deficient DNA repair systems [25].

In contrast, Jiang et al. reported that patients with recurrent UTI have a significantly reduced risk of BC [26]. Possible mechanisms involved in this paradox might be the anti-cancer effect of antibiotic treatment for bladder infections, higher exposure to non-steroidal anti-inflammatory drugs (NSAIDs) of patients with a history of bladder infections and the immune response triggered by bladder infection [26].

Human papilloma virus

A large volume of evidence indicates that high-risk human papillomavirus (HPV) infection plays a role in the development of malignancies other than cervical cancer (i.e. oral and laryngeal carcinoma, penile and anal carcinoma) [27]. A possible role of HPV in the pathogenesis of BC has not been fully clarified. Some studies suggested that HPV might be a causative agent in BC [28–30] while others failed to confirm this [31, 32].

The HPV genome contains genes encoding for E6 and E7 oncoproteins [33]. In an HPV-infected cell, E7 inactivates the retinoblastoma (Rb) tumour suppressor gene that normally inhibits p16. As a result, in HPV-related cancers, a high level of p16 accumulates, which can be detected immunohistochemically and used as a surrogate marker for HPV infection. However, in urothelial carcinoma and squamous cell carcinoma of the urinary bladder, there is no use for p16 expression as a screening test for evidence of HPV infection [34–35].

Mechanical injury

Metaplastic changes of the urothelium tend to occur in response to local injury, resulting in a variety of benign lesions, including *cystitis cystica et glandularis*, intestinal metaplasia and nephrogenic adenoma. Conversely, keratinizing squamous metaplasia is associated with the development of cancer and therefore requires complete resection and diligent follow-up [36].

While it has been suggested that chronic indwelling urinary catheters and spinal cord injury are risk factors for BC, data from the literature are non-conclusive. In patients with spinal cord injury, a risk of BC has been reported at 16–28 times higher than that in the general population [37]. Along this line, Kalisvaart et al. demonstrated that the neurogenic bladder rather than the indwelling catheter is a significant risk factor for BC [38]. However, in a case series of over 33,000 patients with spinal cord injury, squamous cell carcinoma was more common in patients with indwelling catheter (42 %) than in those using clean intermittent catheterization, condom catheterization or spontaneous voiding (19 %) [39]. In contrast, a

recent study showed no significant difference in the risk of BC between spinal cord injury patients and control groups [40]. However, this study has some limitations since it provides no information on relevant clinical variables including the presence of an indwelling catheter.

Inflammatory cells in the tumours

Tumour-associated macrophages

Monocytes, immature precursors of macrophages, are released from the bone marrow into the blood stream. From there, they migrate to various tissues where they differentiate in response to specific microenvironmental cues [41–42]. The association between a high density of tumour-associated macrophages (TAMs) and poor prognosis in several types of human tumour, e.g. lymphoma, cervix, breast and lung cancers, suggests that they play a role in promoting tumour growth [41]. However, other studies have proven the contrary with a high density of macrophages correlated to longer survival [43–46].

Dufresne et al. demonstrated that pro-inflammatory macrophages trigger cellular invasion and activate the phosphoinositide 3-kinase (PI3-K)/Akt signalling pathway in BC T24 cells [47]. Using an orthotopic urinary BC model, Yang et al. reported massive TAM infiltration in primary tumours and lymph node metastases. TAMs clustered towards lymphatic vessels and expressed high VEGFC/D levels. As VEGFR-3 was expressed in lymphatic vascular endothelial cells, TAMs might promote lymphangiogenesis in bladder tumours through paracrine action. Moreover, depletion of TAMs with clodronate liposomes considerably inhibited both lymphangiogenesis and lymphatic metastases [48].

Hanada et al. found that in patients with high TAM count, cystectomy and distant spreading were significantly more frequent and 5-year survival rates lower [49]. Following BCG therapy, a high density of CD68+ TAM was significantly correlated with worse response to treatment and shorter recurrence-free survival [50–51]. The use of CD6+ cell density as biomarker of an inflammatory response to tumour needs further validation, because in the published reports, patient characteristics, tissue location and quantification methods varied strongly [52].

Tumour-associated myeloid-derived suppressor cells

Myeloid-derived suppressor cells (MDSCs) originate from progenitors in the bone marrow. They tend to accumulate in the blood and the spleen, but some proceed directly to tumour sites [4]. Their involvement in pathological conditions, including neoplasia, and their ability to suppress an immune response were first observed in the late 1970s. More recent studies on their role in tumour progression and their adverse effect on response to therapy have shown that cytokines, chemokines and transcription factors regulate recruitment and function of MDSC [53].

In xenograft models of human BC, Eruslanov et al. studied prostaglandin E (PGE) metabolism in relation to tumour-infiltrating myeloid cell subsets. Human SW780 BC cells were found to secrete substantial amounts of PGE while heterogeneous CD11b myeloid cell subsets, including TAMs and MDSCs, infiltrated the xenografts. These findings suggest that cancer-associated inflammation and deregulated PGE metabolism induce an immunosuppressive pro-tumour myeloid cell phenotype in BC [54].

In animals with malignant mesothelioma and three lung carcinoma, viral immunogene therapy after three weekly administrations of cisplatin/gemcitabine reduced the density of immunosuppressive cells including MDSCs, which markedly enhanced anti-tumour efficacy [55]. Since cisplatin/gemcitabine is the standard of care in patients affected by locally advanced or metastatic BC, MDSCs may be a potential target to improve therapy efficacy in BC.

Tumour-associated T cells

T cells (tumour-infiltrating lymphocytes, TILs) are frequently found in the microenvironment in BC and elicit both tumour-suppressive and tumour-promoting effects [56] (Fig. 3). In

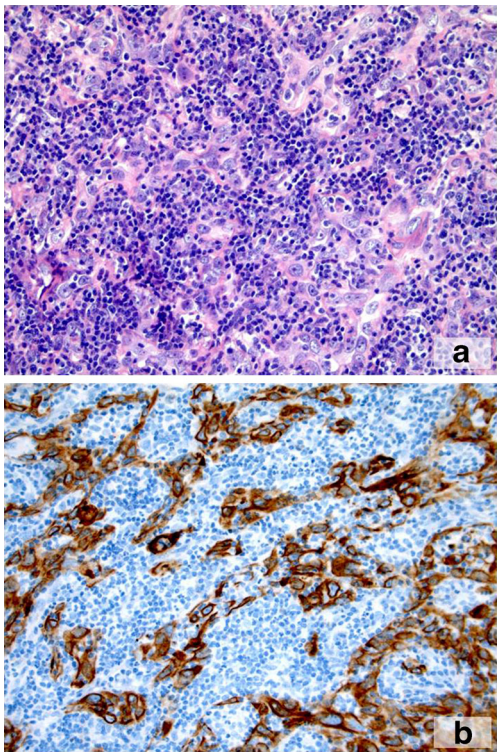

Fig. 3 Inflammatory cells and bladder cancer. Lymphocyte-rich stroma with invasive high-grade urothelial carcinoma (haematoxylin-eosin (**a**); cytokeratin 7 (**b**))

patients with pT1-4 BC subjected to radical cystectomy, Winerdal et al. showed that a high number of CD3+ lymphocytes is associated with better overall survival [57]. In patients with non-muscle-invasive bladder cancer (NMIBC) treated with TUR or radical cystectomy, Sharma et al. found no correlation between CD8+ TILs and disease-free and overall survival, while in MIBC patients CD8+ TILs were associated with better survival [58]. Krpina et al. showed in patients with a single NMIBC that the recurrence rate is higher in those with a high number of TILs at the time of initial TUR. They also noted significantly higher levels of CD3+ and CD8+ (but not CD4+) TILs in patients with tumour recurrence [59].

A CD4+ T helper cell subset (Th17) is crucial in the pathogenesis of many inflammatory and autoimmune diseases, but its function in human tumour immunity remains largely unknown. In patients with BC, the proportion of Th17 cells in the tumour was found to be higher than in patient and control peripheral blood. In contrast, the proportion of regulatory T (Treg) cells in peripheral blood was higher in BC patients than in healthy controls. Chi et al. suggested that the Th17/Treg ratio might be associated with development and progression of urothelial carcinoma and that targeting this might be considered in developing novel therapeutic approaches for invasive disease [60].

Molecular links between inflammation and BC

Cytokines

Cytokines are expressed by neoplastic and immune cells, bind to specific receptors and activate a multitude of downstream factors through signalling pathways [1]. Tumour necrosis factor (TNF) was first identified in view of its capacity to induce tumour necrosis but was later found to also exert tumour-promoting effects. In human BC cells (5637), TNF-alpha stimulates secretion of matrix metalloproteinase-9 (MMP-9) which contributes to tumour invasion and metastasis [61]. Feng et al. observed overexpression of TNF-alpha in recurrent large bladder carcinomas in association with angiogenesis [62].

The multifunctional cytokine interleukin-6 (IL-6) is a major activator of the signal transducers and activators of transcription 3 (STAT3) signalling pathway, involved in regulating tumour growth and metastatic spread. Its role in BC is still a matter of debate. In vitro and in vivo studies have demonstrated that IL-6 can reduce cell proliferation, migration and invasion [63]. In contrast, in a tissue-based study, Chen et al. showed that BC expresses IL-6 at a higher level than adjacent normal mucosa. Furthermore, high IL-6 was found to correlate with higher clinical stage (T2–T4 vs. T1 and CIS), recurrence rate after treatment and lower survival rate [64].

IL-6 induces hepatocytes to release serum C-reactive protein (CRP). In a recent screening study on healthy individuals, Trichopoulos et al. demonstrated that a high serum CRP level is associated with increased risk of developing BC [65]. Serum CRP was also found to be an independent risk factor for cancer-specific survival after radical cystectomy in patients with invasive BC. Gakis et al. developed the TNR-C (tumour stage, node density, resection margin status, C-reactive protein level) score, which increases predictive accuracy of well-established pathological prognosticators through addition of serum CRP [66].

Polymorphisms of cytokine interleukin-8 (IL-8) (i.e. IL-8 -251 T/T variant) have been correlated with the risk of urothelial BC in a case-control study [67]. In BC, the expression of IL-8 was significantly higher than in normal bladder tissue [68] and urinary IL-8 levels were higher in BC patients than in patients with a history of successfully treated urothelial carcinoma [69]. Inoue et al. observed that IL-8 boosts angiogenic activity by inducing MMP-9 expression which regulates tumorigenicity and metastasis [70]. In high-grade BC, levels of MMP-9 and IL-8 were significantly higher than in low-grade BC [71].

Serum levels of the anti-inflammatory cytokine interleukin-18 (IL-18) were higher in BC patients than in controls. In this study, IL-18 levels in patients were not significantly different according to stage or grade [72].

Chemokines and chemokine receptors

Chemokines are released by neoplastic as well as other cells in the tumour microenvironment (e.g. fibroblasts, endothelial cells, TAMs and MDSCs) [73].

It has been suggested that chemokine (C-X-C motif) ligand 1 (CXCL1) regulates tumour epithelial-stromal interactions facilitating tumour growth and invasion. Its expression confers a more aggressive phenotype in several tumours including urothelial carcinoma. Miyake et al. reported that overexpression of CXCL1 is associated with high grade and stage in BC as well as reduced disease-specific survival and overall survival [74].

The CXC chemokine receptor 4 (CXCR4) is upregulated in some malignant cells, and its expression correlates with lymph node metastasis in oesophageal, colorectal and breast cancer [75–77]. CXCR4 expression in BC is significantly higher than in normal bladder tissue [68, 78]. Furthermore, high expression of CXC chemokine ligand-12 (CXCL12) and CXCR4 protein has been associated with high tumour grade and advanced pT stage in both primary and recurrent BC [79].

Using high-density tissue microarrays, Hao et al. found that CXCR7 is involved in the development of urothelial carcinoma, in regulating the expression of pro-angiogenic factors such as IL-8 or VEGF [80]. In addition, they found that in BC cells CXCR7 activates AKT and ERK pathways,

responsible for in vitro and in vivo epithelial-mesenchymal transition in BC.

Cyclooxygenase-2

Cyclooxygenase-2 (COX-2) converts arachidonic acid into pro-inflammatory prostanoids, and its aberrant induction is involved in the pathogenesis of various malignancies [4]. COX-2 is commonly expressed in BC cells but not in normal urothelium [81]. In spite of numerous studies undertaken to evaluate how COX-2 promotes bladder carcinogenesis, its precise role remains controversial. Margulis et al. found COX-2 protein expression to be associated with higher pathological stage, vascular invasion and nodal metastasis [81]. Loss of immunoreactivity to COX-2 in recurrent NMI bladder carcinomas was demonstrated by Tadin et al., which suggests than an inverse correlation exists between the expression of this enzyme and recurrence and progression of BC [82]. Discordant results on record may depend on multiple factors, including diversity of the used scoring systems and differences between patient series as regards histotype and tumour stage.

It has been suggested that cancer stem cells are responsible for BC recurrence and progression [83]. Immunohistochemical investigation has revealed that the expression of markers of stemness such as Oct3/4, CD44V6 and COX-2 is significantly higher in cystitis and cancer patients than in healthy controls. Interestingly, nuclear localization of COX-2 correlated with upregulation of Oct3/4 and CD44v6 expression in BC, regardless of the degree of inflammation. As a consequence, COX-2 activation might contribute to stem cell proliferation during inflammation-induced bladder carcinogenesis [84].

Prostaglandin E2

Prostaglandins are involved in a variety of physiological functions. In humans, the most abundant prostanoid is prostaglandin E2 (PGE2), of which synthesis is driven by COX enzymes [85]. It has been suggested that prostaglandin synthesis may be boosted by FGFR1 activation in urothelial BC and may contribute to epithelial-mesenchymal transition, through APK/PLCγ/COX-2-mediated mechanisms [86]. Data obtained from animal models have proven that PGE2 levels are higher in BC than in normal urothelium and bladder papilloma, implying that increased PGE2 levels may contribute to bladder carcinogenesis [87].

Reports concerning PGE2 in human BC are scanty. Wheeler et al. demonstrated that urinary PGE2 levels in patients with UTI and BC are higher than those in age-matched controls [88]. Patients with successfully treated UTI and those with BC in remission exhibit decreased urinary PGE2 levels. Immunohistochemical expression of prostaglandin receptor (EP1-4) has recently been investigated in BC patients [89]. EP staining pattern correlated with tumour stage and grade in NMIBC. In multivariate analysis, nuclear EP1 expression remained as independent predictive marker for cancer recurrence [89].

Nitric oxide

Nitric oxide (NO), generated by nitric oxide synthases (NOS), performs several regulatory functions, including angiogenesis, in both benign and malignant tissues of the human bladder [90]. Data from a cross-sectional study showed that NO levels are significantly higher in BC patients than in controls, although NO and tumour grade were not correlated [91]. The relationship between the distribution of the polymorphisms of eNOS4a/b correlated with clinical features of NMIBC in a study published by Amasyali et al. Notably, the aa plus ab genotype was significantly more common in patients with a high-grade tumour and in those progressing to muscle-invasive disease [92].

Immunoelectron microscopy revealed that inducible NOS (iNOS) is localized in the mitochondria of urothelial cells in several bladder diseases, including NMIBC [93]. An inverse correlation was found between iNOS staining and urothelial cell differentiation, which suggests that pathological conditions of the urinary system may alter urothelial cell differentiation, in terms of induction of iNOS expression in partially differentiated cells [93].

Nuclear factor-kappaB

The nuclear factor-kappaB (NF-kB), a heterodimeric protein ubiquitous to all cell types, was first described in the kappa chain of immunoglobulin and in the nucleus of B cells. Recent evidence suggests it might be a molecular link between inflammation and cancer, since NF-kB is constitutively active in most tumours as well as in chronic inflammatory diseases [94]. It propels the expression of cytokines, adhesion molecules and angiogenic factors in immune and neoplastic cells. By activating genes encoding proteins essential to the cell cycle (e.g. cyclin D1, c-Myc) and apoptosis (e.g. BCL-2, c-FLIP), NF-kB also fosters cancer cell survival and proliferation [95–96]. NF-kB activity is, in turn, regulated by other transcription factors, e.g. Notch-1, STAT3, beta-catenin and p53 [97].

Nuclear expression of NF-kB has been correlated with histological grade and T category in urothelial BC [98–99]. The NFKB1-94ins/del promoter polymorphism has been proposed as a biomarker for identifying NMIBC patients with a high risk of recurrence. However, a recent meta-analysis showed that this polymorphism is not associated with the risk of BC [100, 101].

Signal transducers and activators of transcription 3

The transcription factor STAT3 is activated in several human cancer cells by a variety of factors, e.g. IL-6 and IL-17 [102, 103] in a NF-kB/IL-6/STAT3 cascade. If STAT3 is persistently activated, it will maintain constitutive NF-kB activity in cancer cells and tumour-associated leukocytes, which points at a relationship between oncogenic signalling pathways within the inflammatory microenvironment [104]. *STAT3* silencing via small interfering RNA (siRNA) vectors inhibits proliferation of T24 BC cells in vitro and in vivo [105]. In a cohort study conducted on patients with urothelial BC, NF-kB, STAT3 and some of their target genes (i.e. cyclin D1, VEGFA and TGFβ1) appeared to be activated in tumour tissue relative to healthy bladder tissue. This suggests that chronic inflammatory disorders promote the development of this tumour type [106].

Ho et al. showed in STAT3-transgenic mice that invasive BC arises directly from CIS and is mainly composed of cytokeratin 14 (CK14+) stem cells. In addition, human invasive bladder carcinomas rich in CK14+ stem cells showed diffuse nuclear expression of STAT3. Together, these findings suggest that activation of STAT3 may contribute to BC progression via the CIS pathway [107].

BCG immunotherapy

BCG, an attenuated strain of *Mycobacterium bovis*, was produced in 1921 as a vaccine for tuberculosis. Instillation of BCG in superficial bladder tumours was firstly reported in the 1930s [108] and is now the treatment of choice in NMIBC after TUR, producing a 60–70 % response rate [1, 109]. Despite its wide clinical use, the mechanism of action of intravesical BCG is still not fully understood. However, activation of the immune system and induction of inflammation are crucial to achieve a therapeutic response [110]. A further factor that directly influences BC response is the viability of BCG [111]. After vesical instillation, BCG attach to the urothelium via the extracellular protein fibronectin (FN). FN-BCG complexes, which are internalized by urothelial and inflammatory cells, elicit cytokine release and immune cell recruitment [109]. The first cells to respond are neutrophils and macrophages, followed by CD4+ T cells. A wide range of cytokines, including IL-2, IL-6, IL-8, IL-10, IL-12, IL-18, TNF-alpha, IFN-gamma and granulocyte/macrophage colony stimulating factor (GM-CSF), as well as the macrophage-derived chemokine (MDC) are involved. Tumour destruction is associated with cellular immunity as reflected in a high proportion of T helper (Th) cells and to some degree to the direct action of BCG itself (Fig. 4) [110].

As a high percentage of patients do not respond to BCG, it is imperative to identify early markers predictive of response

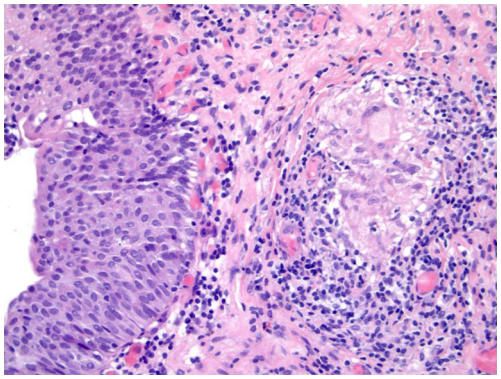

Fig. 4 BCG-related cystitis. Non-caseating granulomas containing epithelioid histiocytes and multinucleated giant cells in superficial lamina propria

to BCG. In addition to clinicopathological parameters, several inflammatory, intracellular and genetic markers have been investigated. IL-2 appears to be one of the best predictors of BCG response [112]. However, a single marker is rarely sufficient and a combination of inflammatory markers has been proposed for clinical use (e.g. IL-6/IL-10 ratio) [113].

Inflammation as a therapy target in bladder cancer

Although most patients with NMIBC respond to post-TUR BCG immunotherapy or intravesical chemotherapy, tumour recurrence and progression remains a significant problem. Better understanding of molecular oncogenesis of BC and innovative approaches with anti-inflammatory drugs have identified new targets for therapy of BC [114], but these need to be confirmed in clinical trials.

In a prospective pilot study conducted on patients elected to undergo cystectomy for invasive BC, Dhawan et al. reported that short-term pre-surgical administration of celecoxib, a selective COX-2 inhibitor, was associated with increased apoptosis in the tumour [115]. However, in a randomized phase IIb/III trial in NMIBC patients, Sabichi et al. reported that celecoxib only marginally reduced metachronous recurrence compared with a placebo [116].

An innovative example of adjuvant treatment is BCG combined with ALT-803. The IL-15 super-agonist ALT-803 consists of two molecules of IL-15 N-to-D substituted at position 72, bound to two molecules of the "sushi" domain of the IL-15 alpha receptor and a single Fc fragment of IgG1. A phase I trial of intravesical BCG in combination with ALT-803 in BCG-naïve patients is currently ongoing [117]. In addition to the classical endpoints (e.g. maximum tolerated dose, safety), the study assesses the efficacy and will characterize the molecular, immunogenic and pharmacokinetic effects of BCG plus ALT-803.

ALT-801 is a bifunctional fusion protein comprising the cytokine IL-2 linked to a soluble, single-chain T cell receptor

domain that recognizes the aa264-272 peptide epitope of the tumour suppressor p53 antigen in the context of HLA-A*0201 (p53+/HLA-A*0201). The tumour cell-localized IL-2 moiety stimulates natural killer (NK) cells and T cell cytotoxic immune responses against p53-expressing tumour cells. Preliminary data from a phase I trial of ALT-801 combined with gemcitabine in BCG-resistant NMIBC patients have recently revealed immune responses and potential durable clinical activity [118].

Concluding remarks

Infectious organisms as well as mechanical injury can trigger chronic inflammation linked to the development of BC, one of the most common urological malignancies worldwide. Once chronic inflammation sets in, it can mediate BC pathogenesis by stimulating cancer cell growth, invasion and metastasis through the recruitment of inflammatory cells and signalling molecules. However, inflammation plays a key role following intravesical BCG therapy in NMIBC. Cytokines, chemokines, signal transducers and transcription activators have all been proposed as targets in BC treatment, prompting innovative strategies which might be particularly relevant for patients unlikely to benefit from BCG immunotherapy. Nevertheless, further preclinical studies and controlled clinical trials are mandatory if we are to unravel the potential of such approaches.

Conflict of interest The authors declare that they have no competing interests.

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