

Role of the tumor microenvironment in promoting treatment resistance in urothelial carcinoma (Review)

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Abstract. Urothelial carcinoma (UC) presents considerable treatment challenges, primarily due to the high rates of therapeutic resistance observed in affected patients. Currently, therapeutic strategies often fail to yield satisfactory outcomes; therefore, there is a need for innovative treatment approaches. The tumor microenvironment (TME) serves a key role in driving treatment resistance in UC, revealing that components such as cancer-associated fibroblasts, tumor-associated macrophages and the extracellular matrix contribute to tumor survival and evasion of therapy. Understanding the mechanisms through which the TME influences treatment resistance is key for developing effective countermeasures. Targeting the TME may be an effective therapeutic strategy, as this approach may increase the efficacy of existing treatments and overcome barriers to response. The present review aims to summarize current knowledge regarding the role of the TME in UC treatment resistance, discuss potential strategies for targeting the TME and highlight future research directions. The translation of insights from studies on the TME into therapeutic strategies may improve clinical outcomes for patients suffering from this challenging malignancy.

Contents

1. Introduction
2. Composition of the TME in UC
3. Mechanisms by which TME promotes treatment resistance
4. Advances in research related to TME and UC treatment
5. Discussion
6. Conclusion

1. Introduction

Urothelial carcinoma (UC), previously known as transitional cell carcinoma, is the most common malignancy of the urinary tract; it predominantly affects the bladder but also frequently occurs in the renal pelvis and ureters (1). The incidence of bladder cancer in the United States is ~19 cases per 100,000 individuals, but its incidence in other regions varies markedly. For instance, Southern Europe has the highest age-standardized incidence rates (ASIRs) globally, at 26.5 cases per 100,000 individuals in men and 5.8 cases per 100,000 individuals in women (2). By contrast, Central Africa has the lowest ASIR in men (2.2 cases per 100,000 individuals), which is likely associated with reduced tobacco exposure and limited access to diagnostic resources (2). Northern Africa has disproportionately high mortality rates (9.2 cases per 100,000 individuals in men), reflecting advanced disease presentation and suboptimal health care infrastructure (2). Among Asian countries, India exhibits a moderate incidence rate (1.6 cases per 100,000 of all individuals) but an increasing mortality rate (0.87 deaths per 100,000 of all individuals), which is attributed to underdiagnosis and the limited availability of tumor microenvironment (TME)-targeted therapies (2). China and Japan have ASIRs of 3.4 and 7.0 cases per 100,000 of all individuals, respectively, with corresponding age-standardized mortality rates of 1.4 and 2.4 per 100,000 individuals; these rates, particularly in men, are influenced by smoking and occupational exposures (2). UC exhibits an increased prevalence in men compared with women and is associated with risk factors such as smoking, chronic irritation and exposure to certain chemicals. The disease is classified into non-muscle invasive and muscle-invasive forms, with ~70% of patients presenting with non-muscle invasive disease at diagnosis. However, ~30% of these patients may progress to muscle-invasive disease, highlighting the need for effective therapeutic strategies (3).

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Abbreviations: UC, urothelial carcinoma; BCG, Bacillus Calmette-Guerin; ICI, immune checkpoint inhibitor; TME, tumor microenvironment; ECM, extracellular matrix; CAF, cancer-associated fibroblast; FGFR3, fibroblast growth factor receptor 3; TAM, tumor-associated macrophage; PD-1, programmed death 1; CTLA-4, cytotoxic T lymphocyte-associated protein 4; NK, natural killer cell; VEGF, vascular endothelial growth factor; PD-L1, programmed death-ligand 1; MIBC, muscle-invasive bladder cancer; ASIR, age-standardized incidence rate; CCL, chemokine (C-C motif) ligand

Key words: TME, UC, treatment resistance, CAF, TAM, ECM

Current therapeutic options for UC vary based on the stage and grade of the tumor. Non-muscle invasive disease is often treated with transurethral resection of the bladder tumor followed by intravesical therapies, such as Bacillus Calmette-Guerin (BCG) immunotherapy or chemotherapeutic agents, such as mitomycin C (4). For muscle-invasive disease, radical cystectomy, chemotherapy and emerging approaches, such as immunotherapy, including immune checkpoint inhibitors (ICIs) such as pembrolizumab and atezolizumab, are utilized (5). Despite these advancements, treatment resistance remains a key challenge, ultimately contributing to a poor prognosis and high recurrence rates (6).

The TME comprises a complex and dynamic network of various cellular and acellular components, including cancer cells, immune cells, fibroblasts, endothelial cells and the extracellular matrix (ECM) (7). In UC, the TME serves a key role in tumor biology and markedly influences disease progression and therapeutic outcomes (8). The complexity and heterogeneity of the TME in UC can manifest as differences in cellular compositions, pathological signaling pathway enrichment profiles and interactions that contribute to cancer development and treatment resistance. Previous studies have revealed that the TME can modulate the aggressive behavior of UC cells, facilitating tumor growth and metastasis (9-12). For example, the presence of immune suppressive cells, such as regulatory T cells and myeloid-derived suppressor cells, has been associated with a detrimental effect on antitumor immunity (10). As activated fibroblasts, cancer-associated fibroblasts (CAFs), secrete factors such as TGF- β to induce epithelial-mesenchymal transition in UC cells, increasing their invasiveness while suppressing immune cell functions and remodeling the ECM to create a pro-tumorigenic niche (11). Tumor-associated macrophages (TAMs), predominantly M2-polarized in UC, secrete anti-inflammatory cytokines (such as IL-10) and proangiogenic factors [such as vascular endothelial growth factor (VEGF)], facilitating tumor immune evasion, angiogenesis and tumor cell survival/proliferation through direct interactions or paracrine signaling (12).

Furthermore, the ECM components, such as fibronectin and hyaluronic acid, can alter cellular signaling, promoting a pro-tumorigenic environment (13). Such interactions not only enhance tumor cell survival but also promote an escape from immune surveillance, complicating treatment efforts. The TME markedly contributes to the treatment resistance observed in UC. Studies have revealed that the presence of hypoxic regions within tumors can activate hypoxia-inducible factors, leading to increased expression of genes associated with aggressive behavior and treatment resistance (10,14). Additionally, the dense ECM within the TME creates physical barriers that impede drug delivery and limit immune cell infiltration, further supporting tumor evasion of therapeutic effects (15). Furthermore, molecular interactions within the TME can trigger the activation of survival pathways that enable cancer cells to withstand cytotoxic treatments. This interaction often results in the upregulation of drug efflux transporters and anti-apoptotic factors, which complicate treatment responses (16). Research suggests that targeting the TME, in combination with conventional therapies, could mitigate resistance and enhance therapeutic efficacy (17).

A comprehensive understanding of the complex interactions that occur within the TME is essential for developing innovative therapeutic strategies aimed at overcoming these barriers. Future research should focus on the specific mechanisms by which the TME influences treatment outcomes, ultimately contributing to the improved management of UC and enhanced patient survival rates. The present review will delve into the various aspects of the role of the TME in facilitating treatment resistance, outlining potential pathways for therapeutic intervention and optimization.

2. Composition of the TME in UC

The TME of UC is composed of a diverse array of cellular and acellular components that intricately interact to promote tumor growth, progression and treatment resistance (18). Recognizing the roles of CAFs, various vascular elements and the ECM highlights potential therapeutic targets for overcoming treatment challenges in UC (Fig. 1) (19,20). Future research should continue to unravel the complex relationships within the TME, aiming to develop strategies that modify the microenvironment to improve patient outcomes. Moreover, integrating therapies that target both cancer cells and their microenvironment hold promise for enhanced therapeutic efficacy in UC.

Cellular components

CAFs. CAFs are key players in the TME of UC (16). Emerging evidence reveals that CAFs enhance tumor proliferation and invasive capabilities through various mechanisms (21-23). CAFs are activated by factors secreted by tumor cells and serve a key role in remodeling the ECM, thereby providing structural support for tumor growth and invasion (22). CAFs release a variety of cytokines, chemokines and growth factors, such as TGF- β , fibroblast growth factor and hepatocyte growth factor, all of which stimulate cancer cell proliferation and migration (23).

In UC, CAFs are associated with the promotion of various signaling pathways, including the Hedgehog, Wnt/ β -catenin and NF- κ B pathways, all of which have been implicated in cancer cell growth and metastasis (23). Studies have demonstrated that the depletion or inhibition of CAFs can slow tumor growth and reduce invasiveness, suggesting that targeting CAFs may be a potential therapeutic strategy to improve outcomes for patients with UC (19,22).

Immune cells. TAMs are a prominent component of the immune cell population within the TME of UC (24). TAMs can either adopt a pro-inflammatory (M1) or anti-inflammatory (M2) phenotype, with M2-like TAMs generally being associated with tumor progression and a poor prognosis (25). M2 TAMs secrete immunosuppressive factors and promote angiogenesis, ECM remodeling and tumor cell proliferation (26). The recruitment of TAMs to the tumor site is mediated by various chemokines, such as chemokine (C-C motif) ligand (CCL) 2 and CCL5, which are produced by both tumor cells and CAFs (27). Increased levels of TAM infiltration have been associated with advanced disease stages and an increased likelihood of metastasis in patients with UC (28).

T cells are key mediators of antitumor immunity. However, in the context of UC, T-cell function can be severely

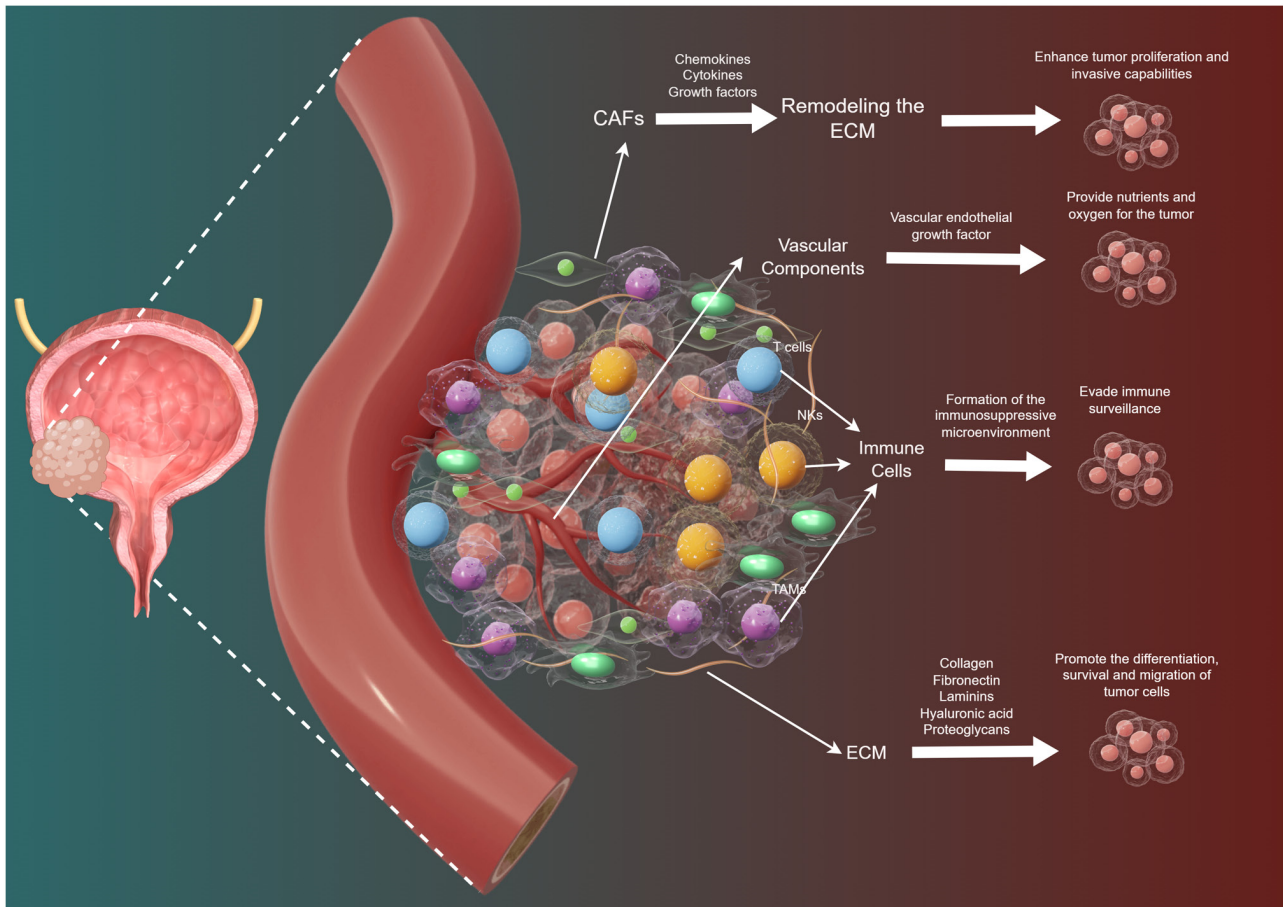


Figure 1. Composition of the tumor microenvironment in urothelial carcinoma. This image was drawn using Figdraw (www.figdraw.com; ID: UORAS20309). CAFs, cancer-associated fibroblasts; ECM, extracellular matrix.

compromised, leading to a state known as ‘functional exhaustion’ (29). The exhaustion is characterized by the upregulation of inhibitory receptors, such as programmed death 1 (PD-1) and cytotoxic T lymphocyte-associated protein 4 (CTLA-4), and a corresponding decrease in the production of effector cytokines (29). The TME actively promotes T-cell exhaustion through multiple mechanisms, including the production of immunosuppressive cytokines, such as IL-10 and TGF- β , by tumor and stromal cells (30,31). The infiltration of CD8⁺ T cells within the TME is often an indicator of a favorable prognosis (32). Nevertheless, the presence of exhausted T cells has been associated with resistance to immunotherapies, such as ICIs (29). Strategies aimed at revitalizing exhausted T cells or reversing T-cell dysfunction are currently under investigation as potential treatments for UC (29).

Natural killer cells (NKs) are a key component of the innate immune response and serve a fundamental role in targeting tumor cells (33). In UC, NKs are often present but can be functionally impaired within the TME due to the influence of immunosuppressive factors (34). The presence of TGF- β , IL-10 and TGF- α can inhibit NK cytotoxicity and proliferation (34). Furthermore, the crosstalk between NKs and other immune cells or stromal components can impact their effectiveness in targeting tumor cells, thus contributing to therapeutic resistance (35,36). Efforts to enhance NK functionality, either through adoptive transfer or combination

therapies with ICIs, are being explored to improve responses in patients with UC (37,38).

Vascular components. Endothelial cells line the blood vessels and serve a key role in angiogenesis, the process of forming new blood vessels from existing ones. In UC, angiogenesis is often stimulated by hypoxic conditions and is mediated by factors such as VEGF (39). The requirement of the tumor for nutrients and oxygen drives the formation of new blood vessels, with increased VEGF expression being associated with histological high grade and a poor prognosis (40). Angiogenesis not only supports tumor growth but also contributes to metastasis by promoting tumor cell intravasation and dissemination into the circulation. Inhibition of angiogenesis has been explored as a therapeutic strategy in UC, with anti-VEGF therapies demonstrating efficacy in certain cases, particularly in patients with high levels of VEGF expression (41).

Lymphatic vessels facilitate the transport of immune cells and the drainage of interstitial fluid. In the context of UC, the presence of lymphatic vessels is key in the metastatic spread of cancer cells (42). Tumors induce lymphangiogenesis, an increase in the formation of lymphatic vessels, through factors such as VEGF-C and VEGF-D, allowing cancer cells to spread to the regional lymph nodes and beyond (43). The interaction between cancer cells and lymphatically-associated fibroblasts can modify the behavior of lymphatic vessels, enhancing their

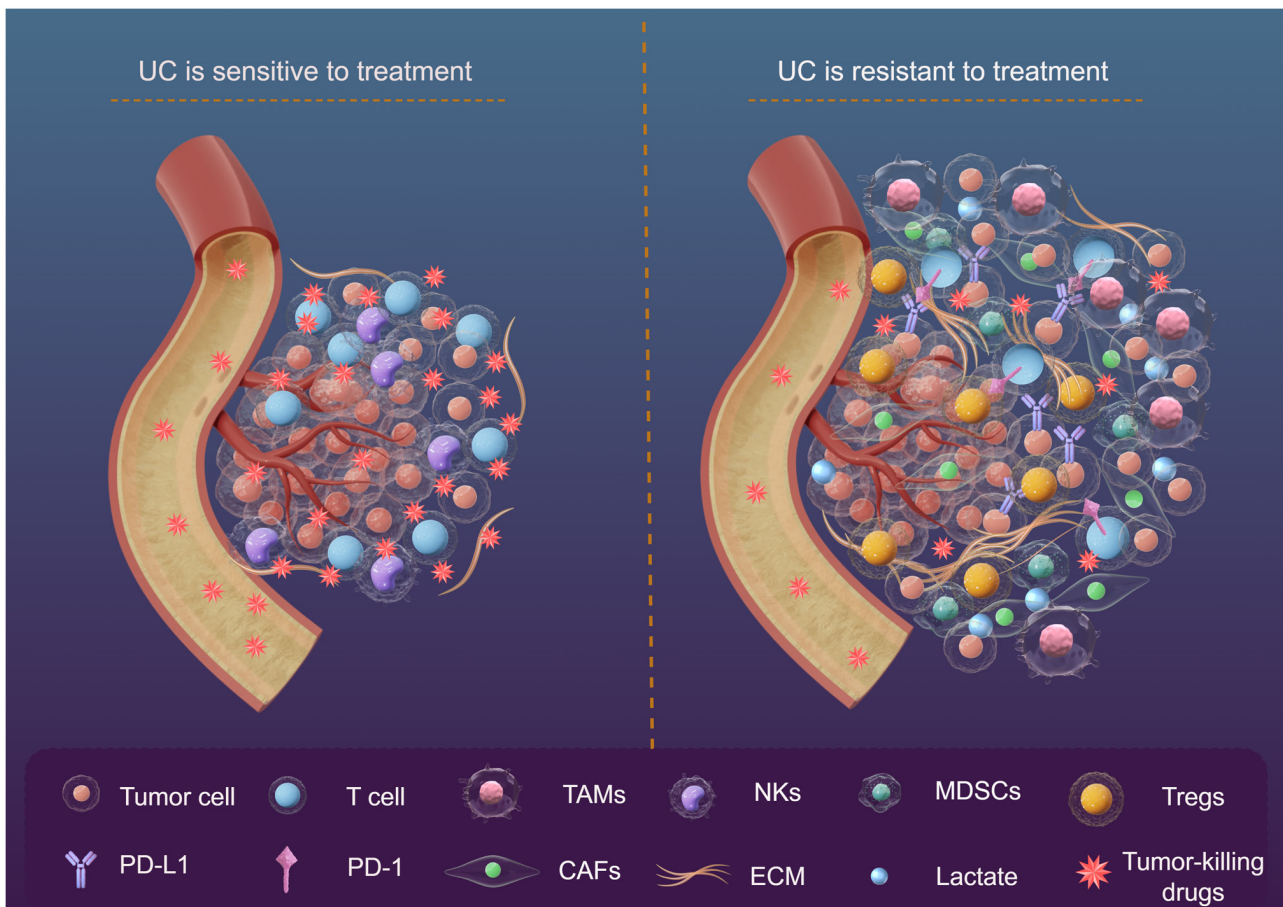


Figure 2. Diagram presenting the role of the tumor microenvironment of UC on therapeutic resistance. This image was drawn using Figdraw (www.figdraw.com; ID: UIAIT9e999). UC, urothelial carcinoma; TAMs, tumor-associated macrophages; NKs, natural killers; MDSCs, myeloid-derived suppressor cells; Treg, regulatory T cell; PD-L1, programmed death-ligand 1; PD-1, programmed death 1; CAFs, cancer-associated fibroblasts; ECM, extracellular matrix.

capacity to transport tumor cells (42). Understanding lymphangiogenesis and lymphatic metastasis may provide insights into novel therapeutic targets, as inhibiting these processes may impede metastasis and improve patient outcomes.

ECM. The ECM is a key acellular component of the TME that provides structural support and biochemical signaling to cells (44). The ECM in UC comprises various proteins, including collagen, fibronectin, laminins, hyaluronic acid and proteoglycans (44). The composition and organization of the ECM can considerably influence tumor behavior, affecting cell adhesion, migration and proliferation. In UC, the ECM undergoes profound remodeling driven by enzymes such as MMPs produced by CAFs and tumor cells (45). This remodeling can lead to increased stiffness and altered architecture, which can promote invasive capabilities and facilitate metastatic spread (46). Changes in ECM composition can also affect the recruitment and activation of immune cells, contributing to an immunosuppressive microenvironment.

Tumor cells interact dynamically with the ECM through integrins and other receptors, which relay signals that can modulate cell behavior. These interactions influence cellular processes such as differentiation, survival and migration (47). In UC, ECM components such as fibronectin and hyaluronic acid can exert pro-tumorigenic effects, enhancing growth and invasiveness (48). Research indicates that the degree of ECM

rigidity can impact tumor cell behavior; stiffer ECM environments tend to promote a more aggressive phenotype in cancer cells (49). Additionally, the ECM can serve as a reservoir for growth factors and cytokines, facilitating sustained tumor growth and evasion of therapeutics.

3. Mechanisms by which TME promotes treatment resistance

The TME serves a key role in the development of treatment resistance in UC, a malignancy known for its high recurrence rates and propensity to develop resistance to various therapeutic modalities (50,51). This section explores several key mechanisms through which the TME promotes treatment resistance, including its impact on drug delivery and distribution, metabolic adaptations, immune evasion, induction of cellular senescence and quiescence, and intratumoral heterogeneity (Fig. 2). Understanding these mechanisms is key for developing innovative therapeutic strategies aimed at overcoming resistance and improving patient outcomes. Future research should focus on integrating TME-targeted approaches with conventional and novel therapies to enhance the effectiveness of treatment strategies in UC.

Impact on drug delivery and distribution. ECM in the TME serves not only as a structural scaffold but also as a modulator

of drug delivery (52). In UC, the ECM often becomes dysregulated and excessively remodeled, leading to increased stiffness and density. The TME in UC drives treatment resistance via ECM remodeling, characterized by increased collagen I/III, fibronectin, laminin and hyaluronic acid, which enhance matrix stiffness and activate pro-tumor signaling (such as integrin/CD44 pathways). This remodeling, mediated by MMPs, creates physical/biochemical barriers to drug delivery and immune cell infiltration. Targeting ECM constituents (such as, hyaluronidase and MMP inhibitors) may help to overcome resistance and enhance therapeutic efficacy (53). This remodeling is primarily driven by components released from CAFs and other stromal cells (54). The resulting physical barriers impede the penetration of therapeutic agents, making it increasingly difficult for drugs to reach their target sites effectively.

Microenvironmental factors, including the dysregulation of interstitial fluid pressure, can further complicate drug distribution (55). Elevated interstitial fluid pressure, often associated with increased ECM deposition, creates a physical barrier that restricts the diffusion of therapeutic agents into tumor regions (55). Consequently, drugs that are effective *in vitro* may fail to show similar efficacy *in vivo* due to inadequate delivery.

Additionally, hypoxia within the TME can affect drug metabolism and efficacy. Hypoxic tumor regions may exhibit altered expression of drug transporters and metabolic enzymes, limiting the activation of prodrugs or the effectiveness of conventional chemotherapeutics (56). Together, these barriers contribute markedly to the therapeutic resistance observed in UC.

Metabolic adaptations. UC cells often exhibit altered metabolism, characterized by the Warburg effect, where cancer cells preferentially utilize glycolysis for energy production even in the presence of adequate oxygen (aerobic glycolysis) (57). This metabolic reprogramming supports rapid tumor growth and proliferation, allowing tumor cells to thrive under the nutritionally and energetically challenging conditions often found in the TME. The Warburg effect provides several advantages to UC cells, including the generation of metabolic intermediates required for biosynthesis and increased lactate production, which can create an immunosuppressive microenvironment (58). Increased lactate levels can inhibit the activation of T cells and promote differentiation of immunosuppressive cells such as regulatory T cells (59).

Moreover, metabolic adaptations can induce resistance to therapies targeting the cell cycle. Cancer cells that exhibit altered metabolic pathways can survive cytotoxic treatments by upregulating survival pathways and evading apoptosis (60). Consequently, metabolic reprogramming driven by the TME can notably impact how tumor cells respond to conventional therapies.

Immune evasion. The TME is often enriched with immunosuppressive factors that facilitate the evasion of antitumor immunity. UC cells can produce cytokines such as TGF- β , IL-10, C-X-C motif chemokine ligand 12 and VEGF, which foster an immunosuppressive milieu by promoting regulatory immune cell populations and inhibiting antitumor effector

functions (61,62). Immune checkpoint molecules, such as programmed death-ligand 1 (PD-L1) and CTLA-4, are frequently upregulated in UC (62,63). The interaction between PD-L1 expressed on tumor cells and PD-1 on T cells leads to T-cell exhaustion, characterized by reduced cytokine production and impaired cytotoxic activity. This immune checkpoint pathway prohibits the host immune system from effectively targeting and destroying tumor cells, thus facilitating the development of resistance to immunotherapeutic strategies (62).

Additionally, the presence of TAMs and myeloid-derived suppressor cells in the TME can further exacerbate immune evasion by secreting anti-inflammatory cytokines, promoting immune tolerance and supporting tumor growth (64,65). The accumulation of these immunosuppressive cells highlights the key role of the TME in shaping the immune landscape and facilitating escape from immune-mediated destruction (66).

Induction of senescence and quiescence. Cellular senescence and quiescence are mechanisms through which tumor cells can evade treatment-induced cell death (67). In the context of the TME, factors such as cytokines, oxidative stress and ECM components can induce a senescent state in tumor cells, characterized by permanent cell cycle arrest (68). While senescent cells are typically resistant to apoptosis, they can still contribute to tumor progression through the senescence-associated secretory phenotype, which involves the release of pro-inflammatory cytokines, growth factors and ECM remodeling enzymes (69).

Furthermore, quiescent tumor cells can survive unfavorable conditions, such as cytotoxic therapy, by remaining in a non-proliferative state (70). This dormancy allows tumor cells to evade detection by therapies that target actively dividing cells. When conditions improve, these dormant cells can re-enter the cell cycle, leading to tumor recurrence and metastasis (70). The interplay between the TME and cancer cell states of senescence and quiescence underscores the complexity of treatment resistance in UC. Strategies that target the pathways involved in cellular senescence and promote re-sensitization to therapies are being actively investigated as potential approaches to improve treatment outcomes.

Heterogeneity in tumor response. Tumor heterogeneity is a hallmark of UC, manifesting as genetic, epigenetic and phenotypic variations among subclones within the same tumor (71). This heterogeneity allows for diverse responses to treatment, whereby some subclones may be sensitive to specific therapies while others remain resistant. This adaptive behavior contributes to treatment failure and disease progression (72). The TME influences tumor heterogeneity through interactions between cancer cells and stromal cells, as well as via environmental factors such as oxygen tension and nutrient availability. Heterogeneous expression of drug transporters, anti-apoptotic proteins and growth factor receptors among tumor subclones can lead to differential responses to chemotherapeutics and targeted therapies (73). Consequently, the presence of resistant subclones can facilitate treatment escape, even after initial responses to therapy.

Intratumoral heterogeneity poses notable challenges for effective treatment in UC. Novel therapeutic strategies that target multiple pathways or pathways essential for maintaining

Table I. Exploratory studies of targeting TME strategies in the treatment of UC.

First author/s, year	Cell type	Target/intervention/ observational index	Study types	Results	(Refs.)
Chen <i>et al</i> , 2022	iCAFs	LOXL2	Bioinformatics analysis and <i>in vitro</i> study	The associated features of iCAFs can predict the prognosis and response to immunotherapy in UC. In addition, the central gene LOXL2 may be a promising target for bladder cancer therapy	(74)
Qin <i>et al</i> , 2023	CAF subtypes	-	Single-cell RNA-seq and cohort study	The CAF subtypes have the potential to predict the prognosis and immunotherapy response of patients with BLCA	(75)
Cui <i>et al</i> , 2024	CAFs	TSLP	<i>In vitro</i> and <i>In vivo</i> study	JNK signaling serves an immunosuppressive role in the TME by promoting TSLP expression levels in CAFs, suggesting that inhibiting JNK signaling may be a promising immunotherapy strategy for UC	(76)
Shan <i>et al</i> , 2021	CAFs	microRNA-148b-3p	<i>In vitro</i> and <i>In vivo</i> study	Downregulated exosomal microRNA-148b-3p in CAFs enhances the chemosensitivity of UC cells by downregulating the Wnt/ β -catenin pathway and upregulating PTEN	(77)
Sun <i>et al</i> , 2022	TAMs	The infiltration level and polarization status of TAMs	Observational study	The infiltration and polarization status of TAMs shapes distinct immune microenvironments with predictive ability for survival outcome, ACT benefit and PD-L1 blockade therapy sensitivity in UC	(78)
Hu <i>et al</i> , 2020	TAMs	DC-SIGN	<i>In vitro</i> study	DC-SIGN ⁺ TAM infiltration is associated with a protumor immune microenvironment and may serve as a promising therapeutic target in the immunotherapy of UC	(79)
An <i>et al</i> , 2023	TAMs	CD206	<i>In vitro</i> and <i>In vivo</i> study	A bsGP reduces the matrix microenvironment barrier, increasing the spatially redirected CD8 ⁺ T cells to tumor cells	(80)

iCAFs, inflammatory cancer-associated fibroblasts; LOXL2, lysyl oxidase homolog 2; TSLP, thymic stromal lymphopoietin; TME, tumor microenvironment; TAMs, tumor-associated macrophages; ACT, adjuvant chemotherapy; PD-L1, programmed cell death 1 ligand 1; DC-SIGN, dendritic cell-specific c-type lectin; UC, urothelial carcinoma; RNA-seq, RNA-sequencing; BLCA, bladder urothelial carcinoma; bsGP, bispecific glycopeptide.

tumor cell diversity, such as combination therapies or personalized medicine approaches, may be required to overcome the barriers posed by heterogeneous tumor populations.

4. Advances in research related to the TME and UC treatment

Targeting the TME presents a promising strategy for overcoming treatment resistance in UC. Current research highlights the potential of approaches targeting CAFs and TAMs to enhance therapeutic efficacy through a variety of mechanisms (19,26). Innovations in immunotherapy that incorporate TME modulation pave the way for novel therapeutic combinations that may further improve patient outcomes. Future efforts to target ECM components and pursue personalized medicine approaches based on TME

analysis hold great promise for advancing therapeutic strategies in the management of UC. Building upon these findings will be key for developing more effective cancer therapies that address the multifaceted nature of the TME.

Exploratory studies on targeting the TME. Targeting the TME is a promising strategy to overcome treatment resistance in UC. Studies have identified various components of the TME, such as CAFs and TAMs, as key players influencing tumor progression and treatment response (Table I). Further exploratory studies in this direction could lead to considerable advancements in the management of this malignancy.

CAFs are pivotal in constructing the TME and are known to influence immunotherapeutic responses in bladder cancer. Chen *et al* (74) identified inflammation-associated CAFs subtypes that can effectively predict the prognosis and

response to immunotherapy in patients with bladder cancer. Furthermore, Qin *et al* (75) provided a CAF-subtype-based signature that aids in evaluating patient responses to immunotherapy, indicating the clinical implication of CAF heterogeneity in therapeutic contexts. These findings suggest that targeting specific CAF populations could potentially enhance immunotherapy outcomes.

Inhibition of signaling pathways within CAFs is another promising approach. Cui *et al* (76) demonstrated that the blockade of JNK signaling in CAFs can alleviate tumor-induced immunosuppression, augmenting the effectiveness of immunotherapy in bladder cancer. This indicates that not only does the subtype of CAFs matter, but also the intra-cellular signaling pathways that govern their immunological environment. Moreover, the regulatory role of exosomal microRNAs within CAFs has notable therapeutic implications. A study by Shan *et al* (77) revealed that down-regulated exosomal microRNA-148b-3p in CAFs can enhance the chemosensitivity of bladder cancer cells by modulating the Wnt/ β -catenin pathway. This highlights the potential of using exosomal microRNAs as therapeutic agents to manipulate the TME and improve chemosensitivity in bladder cancer.

TAM infiltration and polarization are key components of the TME influencing therapeutic efficacy. Sun *et al* (78) observed that the profile of TAMs could predict prognosis and therapeutic benefits in muscle-invasive bladder cancer. Specifically, TAMs expressing dendritic cell-specific C-type lectin has demonstrated promise in reactivating antitumor immunity; Hu *et al* (79) revealed that blockade of these macrophages enhances the efficacy of immunotherapy in muscle-invasive bladder cancer (MIBC). These insights suggest that TAMs are key targets to shift the balance towards a more favorable immune response. Innovations such as bispecific glycopeptides offer new strategies for modulating the TME. Recent research by An *et al* (80) demonstrated that these agents can spatially and temporally regulate the TME to prevent bladder cancer recurrence. This innovative approach underscores the potential for fine-tuning the TME to enhance therapeutic efficacy and combat resistance.

Studies have emphasized the potential of combination therapies that integrate TME modulation with conventional treatments. For instance, the co-administration of anti-angiogenic agents (such as bevacizumab) and chemotherapy has revealed synergistic effects in UC by normalizing the tumor vasculature, thereby improving drug delivery and reducing hypoxia-driven resistance (81-83). A phase II trial demonstrated that combining atezolizumab (anti-PD-L1) with fibroblast growth factor receptor 3 (FGFR3) inhibitors in patients with UC with FGFR3 alterations resulted in a 45% objective response rate, compared with 20% when using monotherapy, underscoring the importance of targeting both tumor cells and their microenvironment (82). Additionally, CAF-directed therapies are being explored in combination with ICIs. Preclinical models revealed that focal adhesion kinase inhibitors, which disrupt CAF-ECM interactions, enhance the efficacy of anti-PD-1 therapy by reducing stromal barriers and promoting T-cell infiltration (83,84). Clinical trials such as NCT04616248 are currently evaluating this combination in metastatic UC, with preliminary data revealing prolonged progression-free survival times.

Innovations in immunotherapy. TME serves a key role in influencing the efficacy of immune therapy in UC. Immune therapy strategies targeting the TME in UC demonstrated substantial promise. Studies have explored various immunotherapeutic strategies that target the TME to enhance treatment efficacy and overcome drug resistance (Table II). Further investigations are key to elucidating optimal approaches in harnessing the TME for therapeutic benefit in UC.

The use of ICIs has revealed promising results in managing advanced and metastatic UC, particularly in patients who exhibit resistance to conventional therapies. In a phase 1 study assessing chemoradiotherapy combined with nivolumab $\pm$ ipilimumab for MIBC, de Ruiter *et al* (85) reported improved safety and tolerability, along with promising objective response rates, suggesting that this combination may potentiate anti-tumor immune responses while modifying the TME to favor immune infiltration and activity. Balar *et al* (86) conducted a phase 2 trial showcasing the efficacy of pembrolizumab monotherapy in high-risk non-MIBC unresponsive to BCG. This research illustrates how ICIs can reprogram the TME, potentially reversing immune evasion mechanisms associated with treatment resistance.

The application of immunotherapy in the neoadjuvant setting is being investigated as a means to alter the TME prior to definitive surgery. Grivas *et al* (87) evaluated the feasibility of neoadjuvant nivolumab, with or without lirilumab, in patients with MIBC who were ineligible for or refused cisplatin-based chemotherapy. The results revealed that initiating treatment with ICIs can modify the TME to favor an immune-active state, enhancing the chances of surgical success. Powles *et al* (88) compared pembrolizumab alone or in combination with chemotherapy against chemotherapy alone in advanced UC. This phase 3 trial indicated that the combination not only improved patient outcomes but also highlighted the capacity of immunotherapy to reshape the TME by promoting immune cell activation and increasing intratumoral T-cell levels.

Understanding and targeting the tumor stroma is key for influencing the TME. Stroma-modulating agents are being integrated into immunotherapeutic regimens. A multicohort phase 2 study by Qu *et al* (89) demonstrated the efficacy of combining camrelizumab with famitinib in advanced UC after platinum-based therapy. This combination targets both immune checkpoints and the vascular component of the TME, which can contribute to enhancing therapeutic effectiveness and addressing resistance associated with hypoxic stroma.

The integration of radiation therapy with immunotherapy is another promising strategy to potentiate the antitumor immune response by modifying the TME. The phase 1/2 trial conducted by Sundahl *et al* (90) assessed the safety and response of fixed-dose stereotactic body radiotherapy with sequential or concurrent pembrolizumab in metastatic UC. This approach leverages radiation-induced tumor antigen release and immune activation, thereby reshaping the TME to enhance the impacts of immunotherapy. Emerging approaches include tailoring immunotherapy based on individual TME characteristics (91). The study by Hsu *et al* (92), focusing on outcomes associated with first-line PD-1/PD-L1 inhibition, underscores the potential for personalized medicine whereby treatment regimens may be adjusted based on specific TME

Table II. Studies of tumor microenvironment-related immunotherapy strategies in the treatment of UC.

First author/s, year	Intervention	Phase	Target	Results	(Refs.)
de Ruiter <i>et al.</i> , 2022	Nivolumab ± ipilimumab	Phase 1	PD-1	Nivolumab monotherapy and nivolumab with ipilimumab are safe and can be used in phase 3 trials	(85)
Balar <i>et al.</i> , 2021	Pembrolizumab	Phase 2	PD-1	Pembrolizumab monotherapy was tolerated in patients with non-muscle-aggressive bladder cancer who did not respond to BCG and revealed promising antitumor activity	(86)
Grivas <i>et al.</i> , 2024	Nivolumab	Phase 1b	PD-1	Neoadjuvant nivolumab-based immunotherapy is safe, feasible and well tolerated in cisplatin-ineligible patients with MIBC	(87)
Powles <i>et al.</i> , 2021	Pembrolizumab ± Chemotherapy vs. Chemotherapy	Phase 3	PD-1	Combination improved outcomes by reshaping TME (↑T-cell infiltration, immunosuppression), especially in advanced UC	(88)
Qu <i>et al.</i> , 2022	Camrelizumab + Famitinib	Phase 2	PD-1/ VEGFR	Synergistic efficacy in advanced UC post-platinum therapy; targets hypoxic TME and vascular components to overcome resistance	(89)
Sundahl <i>et al.</i> , 2022	Pembrolizumab + SBRT	Phase 1/2	PD-1	Radiotherapy releases tumor antigens and remodels TME, enhancing pembrolizumab response in metastatic UC	(90)
Hsu <i>et al.</i> , 2020	Atezolizumab or pembrolizumab	A single institution experience	PD-L1/ PD-1	The use of ICIs did not improve outcomes in patients with advanced UC	(92)

PD-1, programmed death 1; BCG, Bacillus Calmette-Guerin; UC, urothelial carcinoma; ICIs, immune checkpoint inhibitors; PD-L1, programmed death-ligand 1; MIBC, muscle-invasive bladder cancer; SBRT, stereotactic body radiotherapy; VEGFR, vascular endothelial growth factor receptor; CTLA-4, cytotoxic T lymphocyte-associated protein 4; KIR, killer-cell immunoglobulin-like receptor.

profiles and immune microenvironment assessments. The promising results from various trials exploring immunotherapeutic strategies that target the TME indicate the importance of the microenvironment in the development of treatment resistance in UC. As the understanding of TME dynamics deepens, the future of UC therapies will likely involve a multi-faceted approach, combining immunotherapy with other modalities to effectively counteract resistance and improve clinical outcomes.

Future directions. As the understanding of the TME continues to evolve, future research may increasingly focus on targeting specific components of the ECM, which serve key roles in modulating cancer cell behavior and therapeutic responses (93). Pro-fibrotic signaling pathways, such as the TGF- β , NF- κ B and Notch pathways, present potential therapeutic targets for modifying the TME (47,94).

Research on the composition and organization of the ECM in UC could inform the development of therapies aimed at normalizing ECM architecture, thereby enhancing drug delivery and efficacy. Altering ECM properties might improve the penetration of chemotherapeutic agents, thereby overcoming one of the key barriers to effective cancer treatment (95). The TME markedly drives treatment resistance in UC, with the ECM and its interacting molecules, such as integrins, serving roles (96). While integrins mediate tumor

cell-ECM adhesion and signaling, clinical trials targeting integrin-mediated pathways (such as $\alpha 5 \beta 1$ and $\alpha v \beta 3$) have revealed limited monotherapeutic efficacy in UC, primarily due to compensatory activation of alternative adhesion molecules and stromal heterogeneity (97). However, emerging strategies focus on combinatorial approaches that pair integrin inhibitors with ECM-degrading enzymes or ICIs, leveraging synergistic effects to disrupt tumor-stromal crosstalk (98).

The emerging field of personalized medicine emphasizes the importance of tailoring treatment strategies based on individual patient characteristics, including TME profiling (99). Advances in genomic, transcriptomic and proteomic technologies enable the comprehensive characterization of the TME in clinical samples, facilitating the identification of specific alterations associated with treatment resistance (100). For instance, stratifying patients based on the expression levels of CAF or TAM markers could guide the selection of targeted therapies aimed at these specific components of the TME (64,101). Moreover, integrating TME analysis into clinical trial design could aid in identifying suitable patient cohorts who are more likely to benefit from specific combination therapies. Bioinformatics approaches that analyze the spatial distribution of immune cells and ECM components within tumor samples can provide insights into tumor heterogeneity and therapeutic targets (100). Implementing these personalized medicine

approaches in the clinical setting may enhance treatment effectiveness, minimize adverse effects and ultimately improve patient outcomes in UC.

5. Discussion

UC poses a considerable challenge in the field of oncology, particularly due to its propensity to develop treatment resistance (102). Given the limitations of current therapies and the high rates of recurrence and mortality, there is an urgent need to develop innovative treatment strategies (102). Advances in understanding the TME have shed light on the complex interactions that drive therapeutic resistance, offering new avenues for intervention.

Research has increasingly indicated that the TME serves a key role in the treatment resistance observed in UC. Components of the TME, including CAFs, TAMs and ECM proteins, actively contribute to the survival and evasion of cancer cells in response to therapeutic agents (103-107). These elements not only provide structural support but also secrete a variety of cytokines and growth factors that promote tumor growth and resist pharmacological interventions (18). Studies have demonstrated that the interactions between tumor cells and their microenvironment can lead to metabolic changes, impaired immune responses and the promotion of prosurvival pathways, all of which facilitate treatment resistance (102,108,109).

Understanding the mechanisms by which the TME influences treatment resistance in UC is of paramount importance for overcoming these challenges (18). Insights into specific molecular and cellular pathways affected by the TME may guide the development of targeted therapeutic approaches (110). For example, elucidating the role of TGF- β in fibroblast activation or the impact of hypoxia on immune cell infiltration within the TME can yield information that may guide effective treatment strategies (111). Furthermore, identifying biomarkers associated with TME characteristics may facilitate patient stratification so that treatment regimens can be optimized according to individual TME profiles (18).

The therapeutic potential of targeting the TME in UC is increasingly recognized. Strategies that involve the modulation of CAFs and TAMs, as well as the targeting of ECM components, have shown supporting results in preclinical studies (75,76,78,79). For example, disrupting the protumorigenic signaling pathways driven by these components can resensitize tumors to chemotherapeutic agents and enhance the efficacy of immunotherapy. Additionally, combination therapies that integrate TME modulation along with existing treatment paradigms may improve the outcomes of patients with UC (85,87). By reshaping the immunosuppressive TME, it is possible to enhance antitumor immunity and augment the effectiveness of checkpoint inhibitors, leading to improved responses in patients who are traditionally less responsive to standard therapies.

However, despite the promising outlook for targeting the TME, several challenges need to be addressed. One major obstacle is the heterogeneity of the TME, which can lead to variable therapeutic responses among different patients or even within the same tumor. This complexity requires a more

refined understanding of the diverse cellular interactions and signaling pathways in the TME (110). Additionally, the potential for non-specific effects and the risk of adverse events arising from TME-targeted therapies pose notable concerns, underscoring the need for precision medicine approaches (81). Identifying which components of the TME to target and developing selective agents that minimize off-target effects will be crucial for the successful translation of these strategies into clinical practice.

It is important to acknowledge that the present review has several limitations. Firstly, the studies included in the present review may have varying experimental designs and methodologies, which can affect the comparability and generalizability of the findings. Secondly, the majority of the evidence presented is derived from preclinical models, which may not fully recapitulate the complexity of human UC and its TME. Thirdly, the dynamic nature of the TME and its interactions with cancer cells can be challenging to capture in a static analysis, potentially leading to an oversimplification of the mechanisms involved. Lastly, there may be publication bias, with studies reporting positive outcomes being more likely to be published, which could skew the overall understanding of TME-targeted therapies. Future research should aim to address these limitations by employing more standardized and rigorous experimental approaches, validating findings in diverse patient cohorts and conducting long-term follow-up studies to assess the true impact of TME-targeted interventions on clinical outcomes.

6. Conclusion

In conclusion, the TME, including CAFs, TAMs and ECM, drives treatment resistance in UC. Targeting TME components via strategies such as TGF- β inhibition, TAM repolarization or ECM modulation alongside chemotherapy/immunotherapy could revolutionize UC treatment by enhancing drug delivery, reversing immunosuppression and overcoming resistance. Integrating such approaches holds promise for improving patient outcomes through multitargeted, TME-focused interventions.

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LY and XS made significant contributions to the conception of the manuscript. LY and XS wrote the main manuscript text, and PS prepared the figures. LY, XS and PS reviewed the manuscript. Data authentication is not applicable. All authors have read and approved the final manuscript.

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Competing interests

The authors declare that they have no competing interests.

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