REVIEW

Crosstalk between bladder cancer and the tumor microenvironment: Molecular mechanisms and targeted therapy

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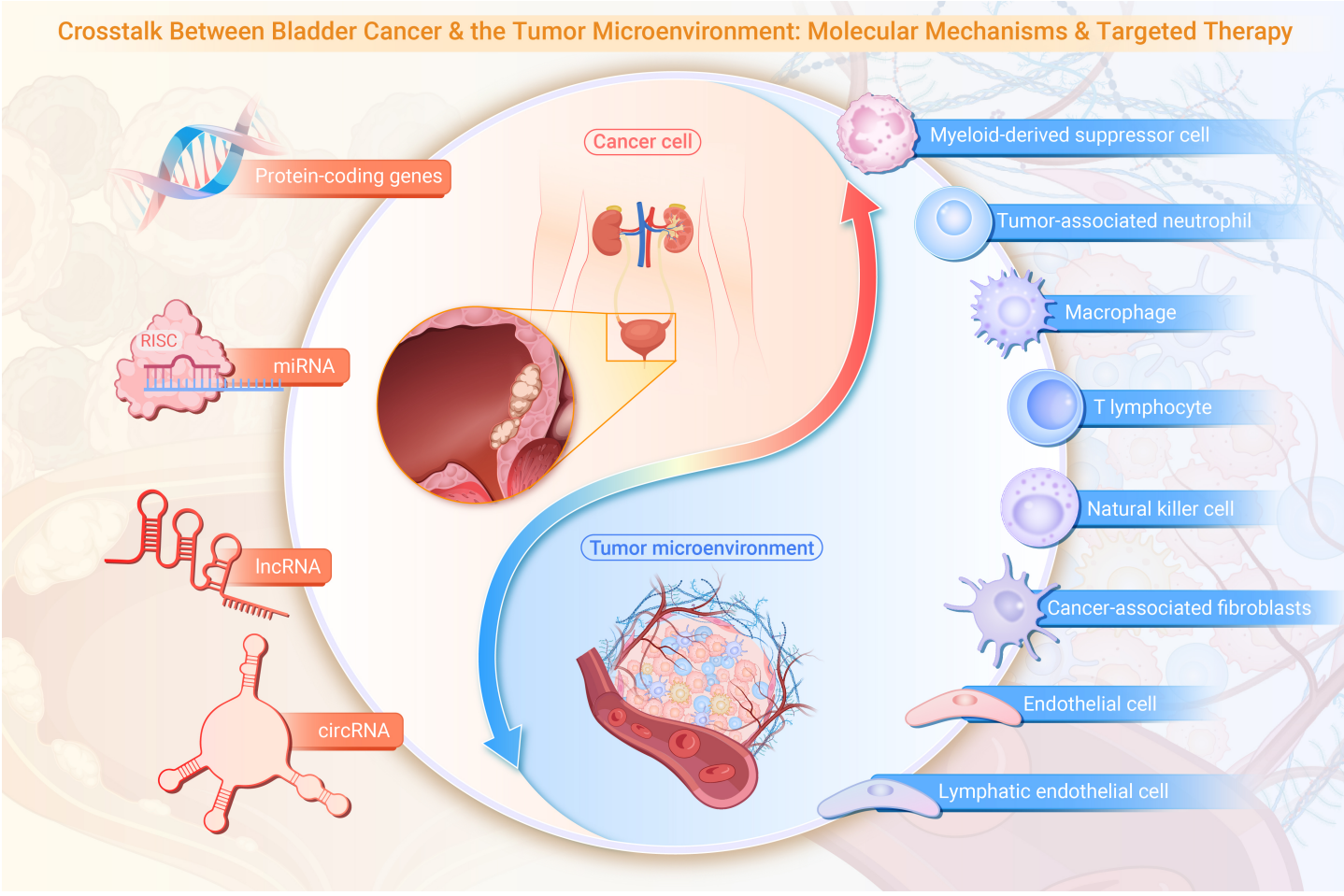
GRAPHICAL ABSTRACT



PUBLIC SUMMARY

- An overview of the multilevel crosstalk between bladder cancer and the tumor microenvironment (TME).
- The most advanced therapeutic approaches targeting the TME in BCa.
- Challenges and future perspectives associated with TME therapies.

REVIEW

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Bladder cancer (BCa) is the most common and lethal urological malignancy. Lymphatic metastasis is the main type of metastasis and a poor prognostic factor for bladder cancer patients. Although several treatments are approved for BCa patients, some patients are still resistant to current therapy. The tumor microenvironment (TME), which consists of diverse cellular components, is a crucial mediator of cancer progression and treatment resistance. However, the literature on the interactions between BCa and the TME lacks coherence and systematic analysis, while the impact of intratumoral heterogeneity (ITH) on the tumorigenesis and progression of BCa has not been fully summarized. Iterative insights into factors intrinsic to and extrinsic to BCa cells that regulate metastasis and treatment response are critically needed. Here, we provide an overview of the current knowledge of the multilevel crosstalk between BCa and the TME, including protein-coding genes and epigenetic factors in BCa cells, tumor-associated macrophages (TAMs), cancer-associated fibroblasts (CAFs), tumor-associated neutrophils (TANs), endothelial cells (ECs), lymphatic endothelial cells (LECs) and other cells that play crucial roles in tumorigenesis, progression, and the development of drug resistance. We also summarize the most advanced therapeutic approaches targeting the TME in BCa and discuss some of the challenges and future perspectives associated with TME therapies.

INTRODUCTION

Bladder cancer (BCa) is a prevalent urological malignancy, with an annual incidence of more than 550,000 newly diagnosed cases and resulting in approximately 200,000 fatalities worldwide.[1] Approximately 75% of cases are nonmuscle invasive,[2,3] with up to 20% of patients progressing to muscle-invasive bladder cancer (MIBC).[4] Few treatment options are available for MIBC patients,[5-11] whose five-year survival rate ranges from 5% to 36%. Notably, MIBC tends to progress and can spread from the bladder to the pelvic lymph nodes and subsequently to other organs. With recent advances in single-cell technologies such as single-cell RNA sequencing (scRNA-seq) and mass cytometry,[12,13] systematic interrogation of the TME is feasible and will provide insights into the functional diversities of tumor-infiltrating immune cells. Integrating multiomics data such as single-cell transcriptomics, proteomics, metabolomics, and spatial transcriptomics data will help to systematically depict the temporal and spatial atlas of tumors at different biological levels. Junpeng Fan et al. integrated multiomics data to depict three distinct tumor states, recapitulating different stages of squamous differentiation, and reported distinct tumor immune microenvironments (TMEs) and different prognoses.[14] This has spurred a recent surge of interest in the functioning of the TME, thereby enabling the development of novel treatments for patients with MIBC, either as standalone therapies or in combination with conventional chemotherapeutic regimens.

The complexity of the tumor microenvironment is identified as a key factor contributing to tumor heterogeneity and suboptimal responses to current anticancer therapies. The tumor microenvironment comprises tumor cells, immune and inflammatory cells, cancer-associated fibroblasts (CAFs), and endothelial cells, which establish intricate crosstalk among themselves.[15] Initially, these host cells were viewed as bystanders of tumorigenesis. However, recent advancements in single-cell sequencing and spatial transcriptomics technologies have shifted the focus of research on cell-to-cell communication (CCC) toward understanding the intricate interactions among diverse cellular entities rather than solely acknowledging their presence.[16-19] The composition, distribution, and functional attributes of individual cells within the TME undergo dynamic spatiotemporal changes that govern intratumoral heterogeneity (ITH).[20] The TME integrates various incoming signals from BCa and transmits new signals that together shape cancer prognosis. The presence of genetic alterations is necessary but not sufficient for the initiation and progression of BCa.[21] The current focus of BCa research, however, centers primarily on investigating the genetic and epigenetic alterations that accumulate within uroepithelial cells.[22] The literature on the interactions between BCa and the TME lacks coherence and systematic analysis, while the exploration of the impact of ITH on the tumorigenesis and progression of BCa has been insufficient.[23] Therefore, elucidating the intricate cellular interactions within the TME of BCa is crucial for identifying novel therapeutic targets and enhancing treatment efficacy for this malignancy.

The TME is a crucial mediator of cancer progression and therapeutic outcomes. Chen et al. classified the TME into immune-desert, immune-rejection, and immune-inflammatory subtypes on the basis of the degree of T-cell infiltration. Importantly, the first two subtypes correspond to cold tumors that exhibit limited responsiveness to immunotherapy.[24] Additionally, radiotherapy enhances the abundance of immunosuppressive cells in the TME, potentially conferring protection to tumor cells against treatment-induced cell death and facilitating tumor recurrence.[25-27] Therefore, the TME plays a crucial role in regulating tumor progression and influencing the therapeutic efficacy of conventional treatments. Targeted therapy for tumors, a therapeutic approach that selectively targets tumor cells while sparing other cells in the TME, has emerged as a promising and versatile method of cancer treatment because of its numerous advantages, such as precise targeting, mitigation of drug resistance, and broad applicability.[28] It specifically targets tumor-associated macrophages (TAMs) within the TME, T cells, endothelial cells (ECs) involved in promoting tumor angiogenesis, and cancer-associated fibroblasts (CAFs), which are responsible for extracellular matrix (ECM) remodelling.[29] BCa, in addition to melanoma, is a solid malignancy for which immunotherapy offers survival benefits. However, the population suitable for current targeted TME regimens remains uncertain, and predicting their efficacy on the basis of clinical trial data poses significant challenges.[30]

Here, we developed a comprehensive model to investigate the intricate interplay between BCa cells and the TME, aiming to comprehensively summarize the remarkable diversity of immune cells and ligands recruited and unravel the ITH in BCa across various levels of transcriptional regulation, epigenetic modifications, and TME dynamics as well as how the TME is tailored to suit the diversity of cancer behaviors. Furthermore, this review offers a comprehensive overview of recent advancements in therapeutic strategies targeting the TME in BCa. These strategies include immunotherapy, antiangiogenic agents and interventions aimed at CAFs and the ECM. This review provides an in-depth understanding of the current challenges associated with targeted TME approaches in BCa treatment and proposes future directions for research.

CROSSTALK BETWEEN BCA AND THE TME

Previously, the cellular components and ECM of the BCa TME were traditionally perceived as passive scaffolds that envelop neighboring tumor cells and function as barriers, hindering tumor invasion and metastasis. However, recent advancements in research have led to the widespread recognition that

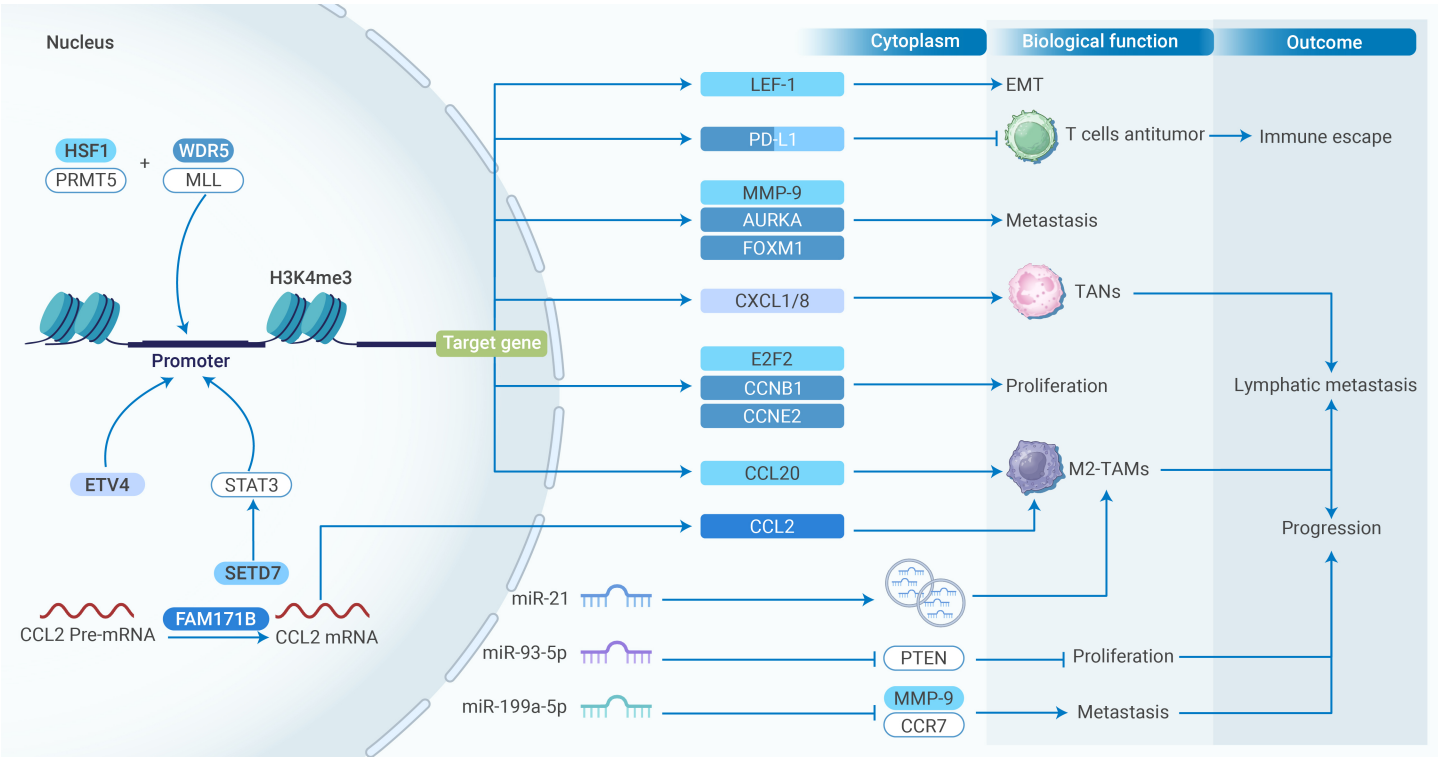

Figure 1. Transcriptional regulation and miRNAs through which tumor cells induce the transformation of the TME toward a protumorigenic phenotype Transcriptional heterogeneity in tumor cells is a crucial factor in tumor progression, metastasis, and therapeutic resistance. ETV4 enhances the secretion of CXCL1/8 by BCa cells, which recruits TANs and promotes lymphangiogenesis. FAM171B facilitates CCL2 mRNA splicing, recruiting TAMs and inducing M2 polarization. SETD7 enhances PD-L1 expression in BCa cells, suppressing CD8+ T-cell chemotaxis and cytotoxicity, resulting in immune evasion. Histone modifications such as H3K4me3 play a critical role in transcription regulation and are involved in determining biological processes. HSF1 interacts with PRMT5, leading to the recruitment of the WDR5/MLL complex and H3K4me3, thereby upregulating CCL20 expression, TAM recruitment, and M2 polarization. MiRNAs exert their regulatory function by binding to the 3' untranslated regions (3' UTRs) of mRNAs, thereby inhibiting gene expression. MiR-93-5p promotes BCa cell proliferation, invasion, and metastasis by downregulating PTEN expression.

tumorigenesis involves the coevolution of cellular components and the ECM within the TME, which act as nonautonomous drivers that contribute to ITH through interactions with tumor cells. This intricate interplay between the TME and tumor cells plays a crucial role in tumorigenesis, tumor progression, and the development of drug resistance.[31-33] Therefore, this review offers a comprehensive overview of the latest research advancements in BCa, with a focus on the complex interactions between tumor cells and the TME as well as their impact on tumorigenesis and progression.

Intrinsic molecular mechanisms of tumor cells: inducing the transformation of the TME toward a protumorigenic phenotype

A comprehensive analysis of single-cell RNA-Seq data across 15 cancer types revealed the intricate interplay between tumor cells and the TME, revealing a highly organized system that contributes to immune evasion, drug resistance, and metastasis.[34] During tumorigenesis, early tumor cells employ intrinsic molecular mechanisms to recruit and activate specific cells in the TME, which in turn influence tumor cells by activating relevant molecular pathways and remodelling their biological behavior.[16] Hence, tumor cells play a pivotal role in reshaping the surrounding TME.

Transcriptional heterogeneity in tumor cells is increasingly recognized as a crucial driver of tumor progression, metastasis, and therapeutic resistance.[15,35-37] The transcription factor ETV4 enhances the secretion of CXCL1/8 by BCa cells, recruiting tumor-associated neutrophils (TANs) and thereby increasing the release of VEGFA and MMP9 from TANs. Consequently, this cascade promotes lymphangiogenesis and facilitates lymph node metastasis.[38] FAM171B facilitates CCL2 mRNA splicing by interacting with heterogeneous nuclear ribonucleoprotein U (HNRNPU), thereby promoting the recruitment of tumor-associated macrophages (TAMs) and inducing M2 polarization.[39] SETD7, a lysine monomethyltransferase, enhances PD-L1 expression in BCa cells by binding to and promoting STAT3. This suppresses the chemotaxis and cytotoxicity of CD8+ T cells within the TME and induces immune evasion.[40] Histone modifications play pivotal roles in the regulation of transcription and are intricately involved in crucial biological processes, such as cell fate, development, and disease.[41,42] The trimethylation of histone H3 at the fourth lysine residue, known as H3K4me3, has attracted significant attention in scientific research because of its strong correlation with gene expression and cancer development.[43,44] HSF1 interacts with PRMT5, resulting in the induction of H3R2me1 and H3R2me2 modifications. The subsequent recruitment of the WDR5/MLL complex facilitates the deposition of H3K4me3, leading to the upregulation of CCL20 expression. This upregulation subsequently promotes TAM recruitment and enhances M2 polarization.[45] Furthermore, targeting WDR5 suppresses proliferation, metastasis and PD-L1-based immune evasion in BCa by blocking the WDR5-MLL complex (Figure 1).[46]

MicroRNAs

MicroRNAs (miRNAs) are a group of small noncoding RNAs approximately 22 nucleotides in length. They exert their regulatory function by binding to the 3' untranslated regions (3' UTRs) of mRNAs, thereby inhibiting gene expression.[47] PTEN inhibits tumor cell proliferation and invasion by negatively regulating the PI3K/Akt/mTOR signalling pathway.[48] MiR-93-5p promotes tumor cell proliferation, invasion, and metastasis in BCa by downregulating PTEN expression. Moreover, exosome-derived miR-93-5p secreted by tumor cells facilitates angiogenesis through its action on vascular endothelial cells.[49] Another study revealed that exosomal miR-21, which is secreted by BCa cells, influences macrophages, resulting in the downregulation of PTEN and subsequently enhancing the activity of the PI3K/Akt-induced STAT3 signalling pathway. This ultimately leads to increased recruitment of TAMs and promotes M2 polarization.[50] MiR-199a-5p targets the 3' UTR of CCR7 in BCa and inhibits the expression of CCR7, MMP9 and EMT-related proteins to suppress tumor metastasis (Figure 1).[51]

Long noncoding RNAs

Long noncoding RNAs (lncRNAs) are characterized as RNA molecules that exceed 200 nucleotides in length, and their regulatory influence on gene expression involves a diverse range of mechanisms.[52] At the transcriptional

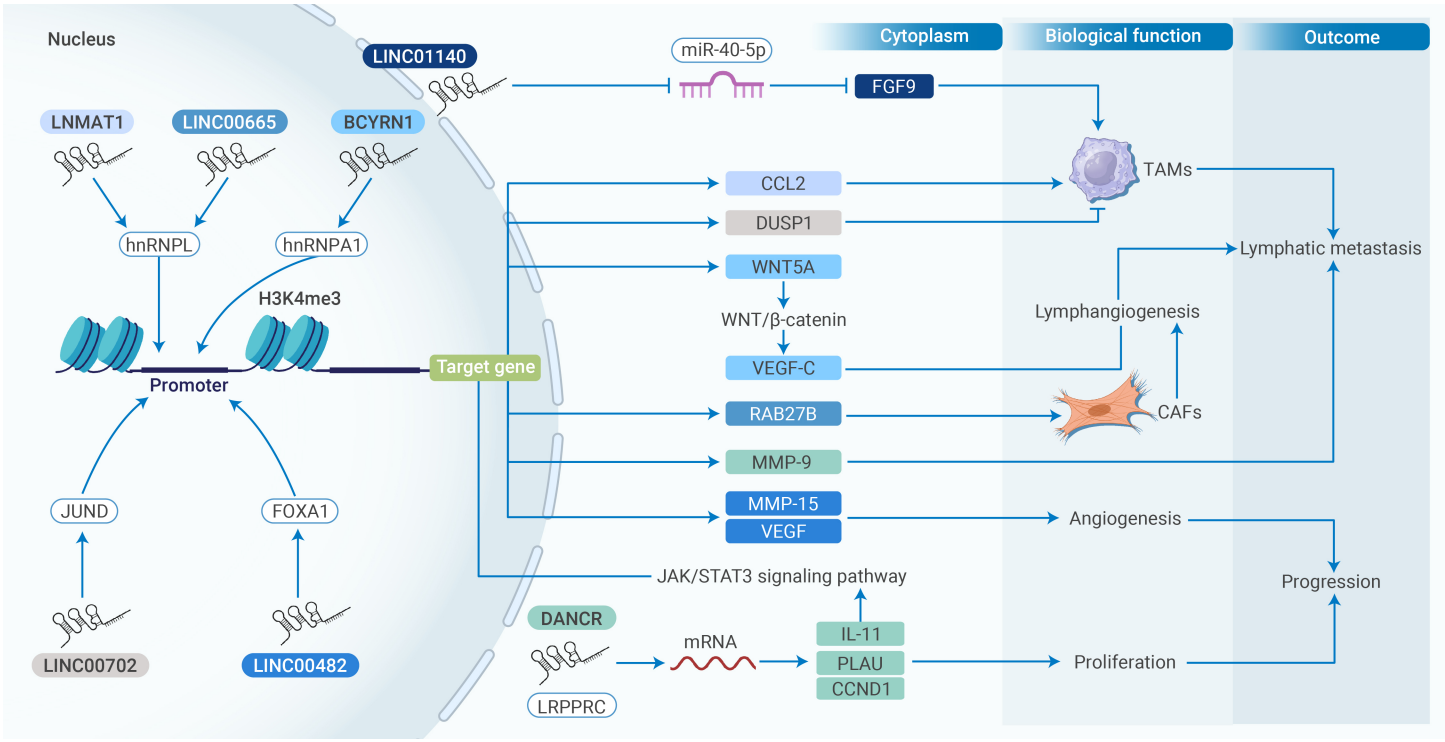

Figure 2. Mechanisms of lncRNAs in Regulating BCa Progression LncRNAs are RNA molecules over 200 nucleotides in length that regulate gene expression through various mechanisms. They directly participate in transcription by interacting with transcription complexes or DNA elements, such as promoters involved in gene expression. BCYRN1 activates the WNT/β-catenin signalling pathway, enhancing VEGF-C secretion and lymphangiogenesis. LINC00702 recruits transcription factors to bind to the DUSP1 promoter, inhibiting BCa cell proliferation and suppressing IL-4, IL-10, and TNF-α secretion. LINC01140 targets miR-140-5p in BCa, upregulates FGF9 expression, and induces macrophage M2 polarization, thereby promoting tumor cell invasion. LncRNAs also interact with heterogeneous nuclear ribonucleoproteins (hnRNPs), enhancing H3K4me3-mediated transcription. DANCR, a lncRNA involved in posttranscriptional regulation, stabilizes mRNA, increases CCND1, PLAU, and IL-11 expression, activates the JAK/STAT3 signalling pathway, enhances MMP9 transcription, and promotes BCa cell proliferation and metastasis.

level, lncRNAs directly participate in transcription by interacting with transcription complexes or DNA elements, such as promoters involved in gene expression.[53] The lncRNA BCYRN1 activates the WNT/β-catenin signalling pathway by upregulating the expression of WNT5A, thereby increasing VEGF-C secretion from BCa cells and leading to lymphangiogenesis and lymphatic metastasis through its interaction with the VEGFR3 receptor on lymphatic endothelial cell (LEC) membranes. Notably, the tumor cell-derived exosome BCYRN1 exerts an early effect on LECs by stabilizing VEGFR3 mRNA to increase its expression, thereby establishing a feed-forward loop.[54] LINC00702 recruits the transcription factor JUND to bind to the DUSP1 promoter, thereby promoting its transcription and subsequently inhibiting BCa cell proliferation while suppressing IL-4, IL-10, and TNF-α secretion by M2-TAMs.[55] In addition, LINC01140, which targets miR-140-5p in BCa, upregulates FGF9 expression to induce macrophage M2 polarization, thereby promoting tumor cell invasion.[56] LINC00482 binding to FOXA1 upregulates MMP15 expression, which induces angiogenesis in BCa.[57] Recent studies have shown that lncRNAs interact with heterogeneous nuclear ribonucleoproteins (hnRNPs). LNMAT1 recruits hnRNPL to bind to the CCL2 promoter, thereby increasing H3K4me3-mediated transcription and increasing CCL2 expression in BCa. Additionally, LNMAT1 augments the secretion of CCL2 by tumor cells, thereby promoting the recruitment of TAMs that facilitate lymphatic metastasis through VEGF-C secretion.[58] By recruiting hnRNPL to the promoter of RAB27B, LINC00665 can enhance the transcriptional upregulation of RAB27B expression. The secretion of extracellular vesicles (EVs) by this entity additionally induces a phenotypic transformation of fibroblasts into CAFs, thereby facilitating lymphatic metastasis in BCa.[59] By acting as a scaffold and binding to both enhancers EZH2 and hnRNPK, lnc-LBCS can construct a complex that inhibits SOX2 transcription by inducing H3K27me3, which in turn suppresses stemness and increases chemosensitivity.[60] The posttranscriptional regulation of lncRNAs primarily encompasses the modulation of mRNA stability, alternative splicing, and modifications. By stabilizing mRNA through binding to LRPPRC, DANCR increases the expression of CCND1, PLAU, and IL-11. This mechanism enhances MMP9 transcription and activates the IL-11/JAK/STAT3 signalling pathway through autocrine secretion, thereby promoting BCa cell proliferation and metastasis (Figure 2).[61]

Circular RNAs

Circular RNAs (circRNAs) are a distinct subclass of noncoding RNAs characterized by their unique covalent single-stranded closed-loop structure, which makes them more resistant to degradation by RNase A than linear RNAs.[62] CircRNAs act as sponge adsorbents for miRNAs, thereby exerting regulatory effects on gene transcription, alternative splicing, and protein translation. They also form complexes with RNA-binding proteins, resulting in synergistic effects. circEHBP1 exerts an upregulatory effect on TGFBR1 expression by counteracting the inhibitory influence of miR-130a-3p on TGFBR1, thereby activating the TGF-β/SMAD3 signalling pathway and augmenting VEGF-D secretion, ultimately facilitating lymphangiogenesis and promoting lymphatic metastasis in BCa.[63] Additionally, CircDHTKD1 in BCa enhances the expression of CXCL5 by sequestering miR-149-5p, thereby facilitating the recruitment and activation of TANs. This process promotes lymphangiogenesis through VEGF-C release.[64] circTRPS1 captures miR-141-3p to increase GLS1 expression, activate the mTOR signalling pathway, and mitigate ROS levels through GLS1-mediated reprogramming of glutamine metabolism. This process sustains the malignant phenotype of BCa cells while also upregulating PD-L1 expression for immune evasion. Moreover, CD8+ T cells are activated by CircTRPS1, an exosome generated from BCa cells, to upregulate the expression of PD-1 and Tim-3, which results in T-cell dysfunction.[65] During tumorigenesis and development, circRNAs play dual roles in both promoting and inhibiting tumor progression. Additionally, the nuclear localization of CircNCOR1 facilitates the recruitment of hnRNPL to the SMAD7 promoter, thereby promoting the increased transcription of H3K9ac. This subsequently inhibits the TGF-β/SMAD signalling pathway and effectively suppresses lymphatic metastasis in BCa.[66] In BCa cells, circFAM13B competitively binds to IGF2BP1, thereby attenuating its interaction with PKM2 and subsequently reducing the stability of PKM2 mRNA. This

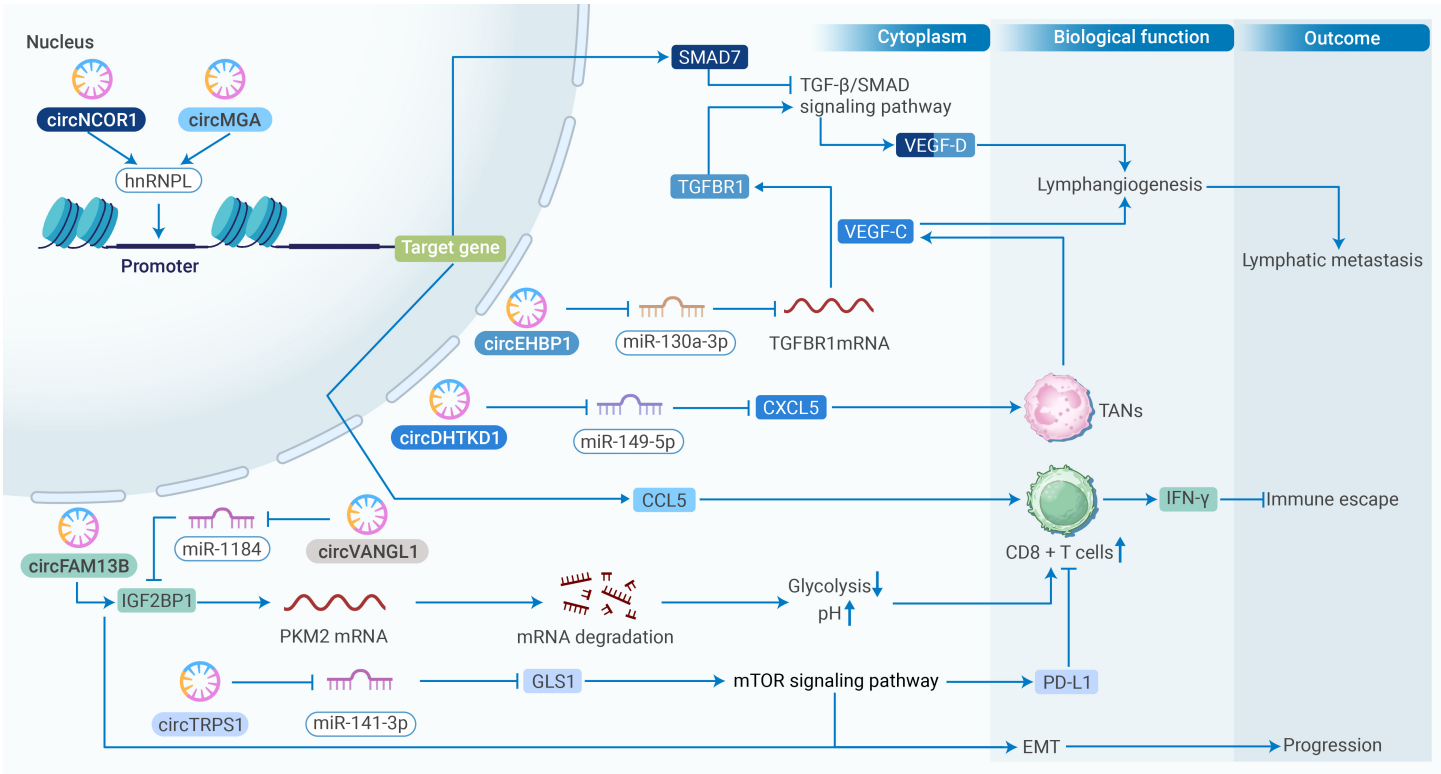

Figure 3. Mechanisms by which circRNAs regulate the progression of BCa CircRNAs are noncoding RNAs with a unique closed-loop structure, making them resistant to degradation by RNase A. These RNAs act as sponge adsorbents for miRNAs, regulating gene transcription, alternative splicing, and protein translation. CircEHBP1 upregulates TGFBR1 expression, activating the TGF-β/SMAD3 signalling pathway and VEGF-D secretion and promoting lymphangiogenesis and lymphatic metastasis. circDHTKD1 enhances CXCL5 expression, recruits TANs, and promotes lymphangiogenesis. CircTRPS1 sequesters miR-141-3p, enhancing GLS1 expression, activating the mTOR signalling pathway, and upregulating PD-1 and Tim-3 expression, resulting in T-cell dysfunction. CircRNAs play dual roles in tumorigenesis and development. CircNCOR1 recruits hnRNPL to the SMAD7 promoter, hindering TGF-β/SMAD signalling and suppressing lymphatic metastasis. CircFAM13B binds to IGF2BP1, attenuating its interaction with PKM2, reducing its stability, and enhancing its immunotherapeutic sensitivity. circMGA interacts with hnRNPL, promoting CCL5 expression and secretion and augmenting the effectiveness of PD-1 immune checkpoint inhibitor therapy.

leads to decreased glycolysis, reversal of the acidic TME, prevention of immune evasion, and enhancement of immunotherapeutic sensitivity.[67] Additionally, circMGA interacts with hnRNPL, resulting in increased secretion and expression of CCL5, promoting intratumoral infiltration of CD8+ T cells, and increasing the effectiveness of PD-1 immune checkpoint inhibitor therapy (Figure 3; Table 1).[68]

TAMs: Diverse functions and impact on tumor progression

TAMs, which include proliferating in situ-resident macrophages as well as recruited complementary circulating monocyte-derived macrophages, are the main infiltrating inflammatory cells in the TME and exhibit phenotypic and functional diversity in response to different signalling stimuli in the TME.[69,70] Lineage tracing experiments have shown that resident macrophages can self-renew in most tissues of adult mice and are not dependent on bone marrow-derived macrophages (BMDMs).[71-73] The proliferation and survival of resident macrophages rely primarily on stromal cell-secreted CSF-1, which functions by binding to CSF1R expressed on macrophages.[74,75] Additionally, BCa tumor cells engage in interactions with macrophage membranes through the secretion of CCL2, CXCL1, and BMP4, thereby engaging CCR2, CXCR2, and BMPR2-BMPR1 receptors.[39,58,76-78] This enables them to facilitate the recruitment and differentiation of monocyte-derived macrophages into M2-type TAMs. Furthermore, TAMs themselves secrete the circulating chemokines CCL2 and CXCL1.[77] The morphological and functional characteristics of resident macrophages differ significantly from those of circulating monocyte-derived macrophages.[79] Monocyte-derived macrophages exert multifaceted control over tumor progression, including facilitating tumorigenesis through inflammation, promoting tumor invasion and metastasis by inducing epithelial–mesenchymal transition (EMT) in neoplastic cells, stimulating angiogenesis, and exerting immunosuppressive effects. Tumor cells in BCa secrete TNF-α along with TNFR1 from TAMs, which stimulates VEGFR3 signalling on LECs through the secretion of VEGF-C. This process facilitates lymphangiogenesis and promotes lymphatic metastasis in BCa.[80] TAMs also secrete TGF-β, which binds to TGFBR on tumor cells and facilitates immune evasion by upregulating the expression of METTL3, thereby increasing the level of m6A modification on PD-L1 mRNA in BCa tumor cells. Moreover, activation of the TGF-β/Smad3 signalling pathway induces EMT and enhances glycolysis in BCa cells.[81] TAMs also induce EMT and promote tumor invasion by secreting CXCL1, which binds to CXCR2 in BCa tumor cells (Figure 4).[77]

CAFs: Insights into heterogeneity and diverse functions in tumor progression

CAFs have multiple origins, including the activation of in situ resident fibroblasts;[82] the differentiation of bone marrow-derived mesenchymal stem cells;[83] the recruitment and activation of bone marrow-derived fibroblasts; the transdifferentiation of pericytes, smooth muscle cells, adipocytes; epithelial EMT; and endothelial EndMT,[84] most of which are derived from in situ resident fibroblasts.[85] In BCa, tumor cells secrete IFN-I, TGF-β1, IL-1α, and CXCL1 to interact with IFNAR, TGFBR1, IL-1R, and CXCR2 on normal fibroblasts, respectively. This interaction induces the activation of normal fibroblasts into CAFs.[77,86-88] Initially, the majority of CAFs were believed to be αSMA+ CAFs; however, an increasing number of studies have revealed a much wider range of CAF heterogeneity than previously anticipated.[89,90] The molecular markers of CAFs are heterogeneous among different subtypes. For example, myofibroblast CAFs (myCAFs) exhibit elevated expression levels of αSMA, TGF-β, COL1A1, and IGFBP3, which primarily participate in extracellular matrix remodelling and facilitate tumor growth and invasion. Inflammatory CAFs (iCAFs) exhibit increased expression of IL-6/8/11, CCL2, and HGF, which are predominantly associated with inflammation, the recruitment of immune cells, and the establishment of an immunosuppressive microenvironment. Vascular CAFs (vCAFs) display increased expression levels of VEGF, FGF, and MMPs, among other factors, to promote tumor angiogenesis.

Table 1. Molecular mechanisms, including protein-coding genes and epigenetic factors (microRNAs, long noncoding RNAs, circular RNAs, DNA and histone modifications), that regulate BCa cell functions and the immunosuppressive tumor microenvironment.

Molecular mechanisms	Expression in tumour	Patient survival	Regulatory mechanism	Related pathway	Biological Function	Outcome	Ref.
Protein-coding genes							
ETV4	upregulated	poor	BCa cells-derived CXCL1/8 to recruit TANs↑, leading to VEGFA and MMP9 from TANs↑	ETV4/CXCL1/8/CXCR2/VEGFA, MMP9	Lymphangiogenesis↑ Recruits TANs	Lymphatic metastasis	38
FAM171B	upregulated	poor	vimentin stabilization↑ by its ubiquitination↓ CCL2 mRNA splicing↑ by interacting with HNRNPU	vimentin stabilization, HNRNPU/CCL2	TAMs and M2 polarization↑	Progression	39
SETD7	upregulated	poor	PD-L1 expression↑ by binding and promoting STAT3	SETD7/STAT3/PD-L1	Antitumour activities of T cells↓	Immune escape	40
HSF1	upregulated	poor	PRMT5-WDR5-mediated H3K4me3	PRMT5/WDR5/LEF1, MMP9, E2F2, CCL20	EMT↑(initiate metastasis) Proliferation↑ Macrophages infiltration and M2 polarization↑	Lymphatic metastasis	45
WDR5	upregulated	poor	WDR5-MLL complex-mediated H3K4me3	BIRC5, XRCC2, CCNB1, CCNE2, AURKA, FOXM1, PD-L1	Apoptosis↓ Proliferation↑ Cisplatin chemosensitivity↓ Antitumour activities of T cells↓	Treatment resistance	46
Sig1R	upregulated	poor	forms a complex with β-integrin	Sig1R/β-integrin/CLIC4	Proliferation↑ VEGF-A secretion↑ and angiogenesis↑	Progression	133
noncoding RNAs							
microRNA							
miR-93-5p	upregulated	poor	PTEN↓	miR-93-5p/PTEN	Proliferation↑, Migration↑, Invasion↑ Angiogenesis↑	Progression	49
miR-21	NA	NA	PTEN↓ and PI3K/AKT↑, STAT3↑ in macrophages	miR-21/PTEN, PI3K/AKT, STAT3	TAMs and M2 polarization↑	Progression	50
miR-199a-5p	downregulated	good	binding 3' UTR of CCR7 and CCR7↓, MMP-9↓ and EMT-related proteins↓	miR-199a-5p/CCR7, MMP-9	Migration↓, Invasion↓	Progression-free	51
lncRNA							
BCYRN1	upregulated	poor	WNT5A expression↑ to WNT/β-catenin↑ and VEGF-C↑	WNT5A/VEGF-C/VEGFR3	Lymphangiogenesis↑	Lymphatic metastasis	54
LINC00702	downregulated	good	recruit JUND to DUSP1 promoter to facilitate its transcription	LINC00702/JUND/DUSP1	Proliferation↓ IL-4, IL-10 and TNF-α secretion by M2-TAMs↓	Progression-free	55
LINC01140	upregulated	poor	targets miR-140-5p	LINC01140/miR-140-5p/FGF9	Proliferation↑, Migration↑, Invasion↑ M2 macrophage polarization↑	Lymphatic metastasis	56

Table 1. *(Continued)*

Molecular mechanisms	Expression in tumour	Patient survival	Regulatory mechanism	Related pathway	Biological Function	Outcome	Ref.
LINC00482	upregulated	poor	MMP15↑ and VEGF↑ by interacting with FOXA1	LINC00482/FOXA1/MMP15, VEGF	Tumor-associated inflammation↑ Angiogenesis↑	Progression	57
LNMAT1	upregulated	poor	CCL2 transcription↑ by promoting H3K4me3 at the CCL2 promoter	LNMAT1/hnRNPL/CCL2/VEGF-C	CCL2-dependent TAMs recruitment↑	Lymphatic metastasis	58
LINC00665	upregulated	poor	RAB27B↑ by recruiting hnRNPL to induce H3K4me3 modification on RAB27B promoter	LINC00665/hnRNPL/RAB27B/HGF/c-Myc	CAF↑ Lymphangiogenesis↑	Lymphatic metastasis	59
lnc-LBCS	downregulated	good	binds to hnRNPK and EZH2 to repress SOX2 transcription by mediating H3K27me3	lnc-LBCS/hnRNPK/EZH2/SOX2	BCa stem cells self-renewal↓	Sensitive to chemotherapy	60
DANCR	upregulated	poor	cyclin D1↑ and PLAU↑ via LRPPRC-mediated mRNA stabilization	IL-11-mediated activation of JAK-STAT3 signalling	Proliferation↑, Migration↑, Invasion↑	Lymphatic metastasis	61
circRNA							
circEHBP1	upregulated	poor	miRNA sponge	circEHBP1/miR-130a-3p/TGFBR1/VEGF-D	Lymphangiogenesis↑	Lymphatic metastasis	63
circDHTKD1	upregulated	poor	miRNA sponge	circDHTKD1/miR-149-5p/CXCL5/VEGF-C	Lymphangiogenesis↑ Recruits and activates TANs	Lymphatic metastasis	64
circTRPS1	upregulated	poor	miRNA sponge	circTRPS1/miR141-3p/GLS1/mTOR	Proliferation↑, Invasion↑ Antitumour activities of T cells↓	Immune escape	65
circNCOR1	downregulated	good	protein binding role	circNCOR1/HNRNPL/SMAD7	Lymphangiogenesis↓	Progression-free	66
circFAM13B	downregulated	good	protein binding role	circFAM13B/IGF2BP1/PKM2	glycolysis↓ and reverses the acidic TME IFN-γ and granzyme B secretion by CD8+ T cells↑	Sensitive to immunotherapy	67
circMGA	downregulated	good	protein binding role	circMGA/HNRNPL/CCL5	Intratumoral infiltration of CD8+ T cells↑	Sensitive to immunotherapy	68
circVANGL1	upregulated	poor	miRNA sponge	circVANGL1/miR-1184/IGF2BP1	Proliferation↑, Migration↑, Invasion↑	Progression	134

Finally, antigen-presenting CAFs (apCAFs) are characterized by high expression levels of MHC II and CD74.[91] In BCa, CAFs secrete IL-6, IL-1β, TGF-β1, CXCL1, MFAP5, and VCAN, which interact with IL-6R, IL-1R, TGFBR1, CXCR2, and NOTCH2 on tumor cells, respectively, thereby inducing tumor cells to undergo EMT, which promotes tumor growth and invasion.[77,87,92-95] CAFs can secrete HGF, which interacts with c-MET receptors on tumor cells, activating the c-Myc signalling pathway. This process facilitates lymphangiogenesis and promotes lymphatic metastasis in BCa.[59] Additionally, PDGFRα+ITGA11+ CAFs are identified through the combination of scRNA-seq and spatial transcriptomics, which can promote lymphangiogenesis by recognizing the ITGA11 surface receptor SELE on LECs to activate the SRC-p-VEGFR3-MAPK pathway, fostering early-stage BCa lymphovascular invasion and LN metastasis, as confirmed by a BBN-induced BCa model and a PDX model.[96] By combining scRNA-seq analysis results with clinical data from BCa patients, SLC14A1+ CAFs were shown to secrete WNT5A, which interacts with FZD6 receptors on BCa cells and activates the WNT5A/β-catenin signalling pathway. This interaction confers stemness and drug resistance properties to BCa cells.[86] CAFs also secrete IGF-1 to interact with the tumor cell line IGF-1R, which promotes antiapoptotic effects and cisplatin resistance in BCa cells by activating the IGF-1/ERβ/Bcl-2 signalling pathway.[97] In addition, an integrated analysis of scRNA - seq data obtained from both primary and recurrent BC samples revealed that ICAM1+ iCAFs secrete FGF2 to interact with FGFR in cancer stem-like cells (CSCs) and maintain the stemness of BCa cells.[98] ICAM1+ iCAFs also secrete CCL2 to interact with the myeloid-derived suppressor cell (MDSC) CCR2, which activates the JAK/STAT3 signalling pathway and recruits MDSCs to hypoxic tumor areas, thereby creating an immunosuppressive microenvironment that promotes tumor progression.[98] Through integrated single-cell and bulk RNA sequencing analyses, LOXL2+

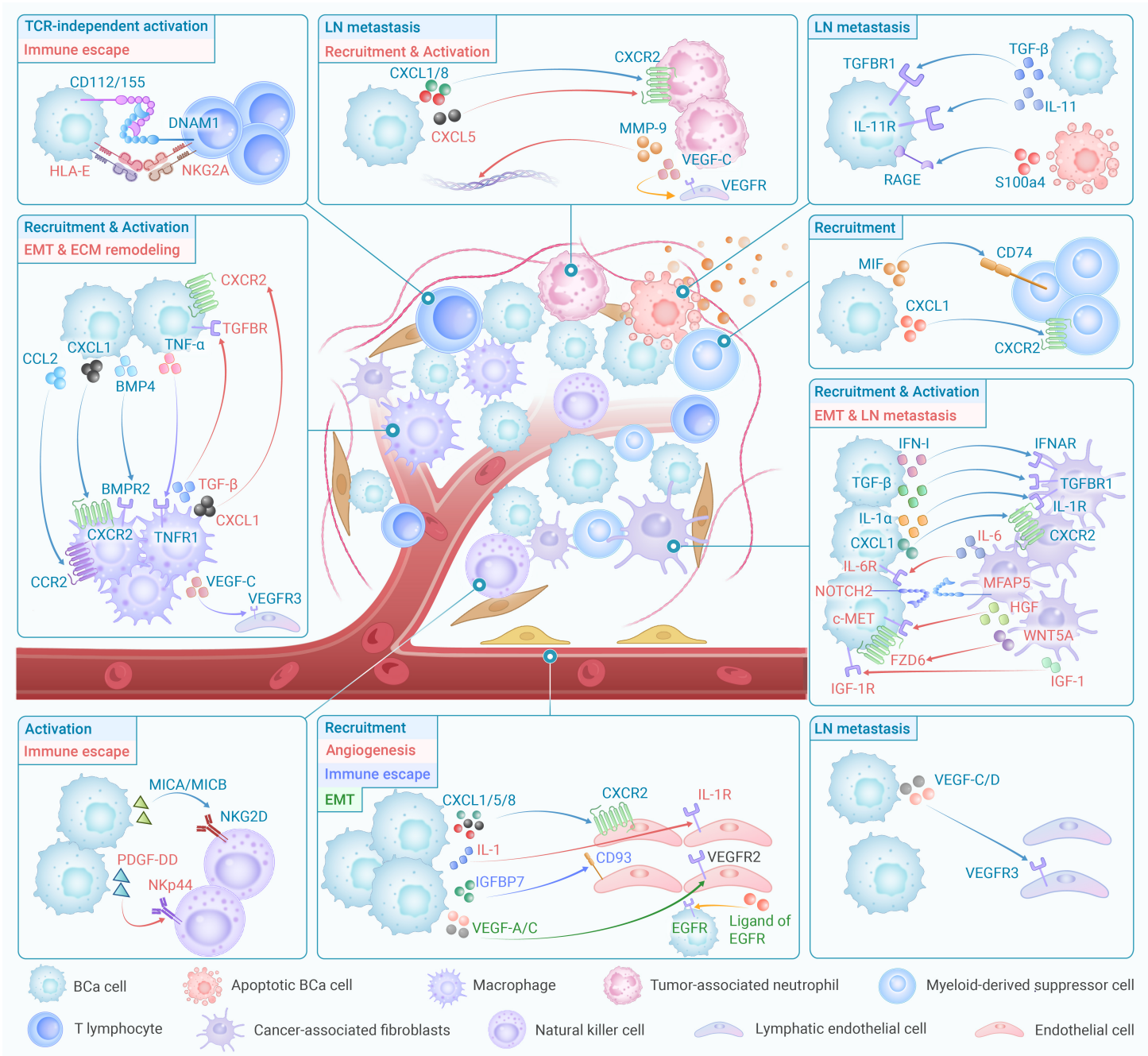

Figure 4. Crosstalk between BCa and the tumor microenvironment drives BCa progression through ligand–receptor interactions The figure illustrates the intricate and complex interactions between BCa cells and the surrounding TME, emphasizing the crucial role of ligand–receptor interactions in driving cancer progression. The figure depicts various cellular components, such as immune cells, fibroblasts, and ECs/LECs, that tightly surround BCa cells. Specific pathways, including immune evasion, angiogenesis, lymphangiogenesis, EMT, and ECM remodelling, play significant roles under the influence of these interactions. The arrows indicate the direction of impact or activation. The arrows of different colors in each diagram have the same effect as their corresponding colors.

iCAFs were shown to promote BCa cell proliferation and invasion by secreting IL-32 and inducing macrophage M2 polarization, resulting in an immunosuppressive TME (Figure 4).[99] Additionally, integrated single-cell and spatial transcriptomic profiling revealed a marked increase in activity between CAFs and malignant cells, as well as other immune cells, in recurrent tumors.[100]

Roles of TANs in lymphatic metastasis and immune modulation

The presence and significance of TANs in the TME have long been overlooked. Current research in the field of tumor immunology focuses on investigating the associations between peripheral blood neutrophil counts and patient overall survival (OS). However, the role of TANs in tumorigenesis and development has not yet been fully investigated.[101] BCa cells secrete CXCL1/5/8 to interact with TANs via CXCR2, thereby recruiting and activating neutrophils and promoting their secretion of VEGF-A and MMP9, ultimately leading to lymphatic metastasis.[38,64] Moreover, TANs secrete VEGF-C to reciprocally interact with VEGFR3 on LECs, thereby facilitating the process of lymphangiogenesis and promoting lymphatic metastasis in BCa.[64] Pathologically activated neutrophils (PMNs), known as myeloid-derived suppressor cells (PMN-MDSCs), predominantly exert immunosuppressive effects.[102] Rina Kim et al. reported that PMN-MDSCs can induce the release of oxidized lipids, thereby impeding T-cell activity through spontaneous iron-mediated cell death.[103] This explains why iron-based cytotoxic agents, despite their efficacy against tumor cells in vitro, exhibit limited success in immunocompetent animal models. Moreover, BCa cells secrete CXCL2 and MIF to interact with PMN-MDSCs through the receptors CXCR2 and CD74, respectively, thereby activating the MyD88-dependent MAPK and NF-κB signalling path-

ways. Consequently, this process promotes the recruitment of PMN-MDSCs while concurrently inhibiting T-cell infiltration (Figure 4).[104]

ECs and LECs: Roles in angiogenesis, lymphangiogenesis, and immune modulation

Angiogenesis serves as the fundamental basis for tumor progression, and once a tumor exceeds 1–2 mm in size, neovascularization becomes imperative to facilitate the acquisition of oxygen and nutrients.[21] In BCa, tumor cells secrete CXCL1/5/8 to interact with ECs via CXCR2 and facilitate the recruitment of ECs.[105] BCa cells secrete VEGF-A/C, which interacts with VEGFR2 in ECs to activate the NF-κB signalling pathway and facilitate angiogenesis. Additionally, this secretion promotes the release of EGFR ligands by ECs, thereby stimulating tumor cell infiltration and invasion in BCa.[105] Tumor cells secrete IL-1 to interact with IL-1R in ECs, activating the NF-κB signalling pathway and promoting immune cell recruitment and angiogenesis.[106] Moreover, a study combining exosome multiomics, proteomics, and metabolomics revealed that EVs derived from BCa cells induce angiogenesis in ECs by promoting O-GlcNAc glycosylation of SerRS through GFAT1, thereby leading to metabolic reprogramming of glucose metabolism in ECs.[107] Furthermore, under conditions of tumor starvation, tumor cells can secrete EVs containing CTSB that are internalized by ECs. This process enhances TPX2-mediated phosphorylation of the AURKA-PI3K-AKT axis and facilitates angiogenesis in BCa.[108] BCa cells also secrete IGFBP7, which acts on CD93 on ECs to increase the recruitment of tumor-infiltrating immune cells (TIICs) while concurrently suppressing T-cell recognition and cytotoxicity against tumor cells.[109] Lymphatic metastasis is the main mode of metastasis of BCa and is a multistep and complex process involving tumor invasion, lymphangiogenesis, dissemination to lymphatic vessels, transport, dissemination, and proliferation within lymph nodes.[110] Tumor cells can promote lymphangiogenesis and lymphatic metastasis in BCa by secreting VEGF-C/D and interacting with VEGFR3 in LECs (Figure 4).[54,63]

Other cells

Currently, research on the interaction between tumor cells and T cells has focused primarily on the release of certain cytokines by tumor cells through their influence on intermediary cells (such as TAMs, CAFs, etc.), which subsequently affects the cytotoxicity of T cells. Studies investigating the direct interactions between tumor cells and T cells have focused mainly on immune checkpoints. Tumor cells can engage with immune checkpoints, including PD-1, CTLA-4, TIM-3, LAG-3, and TIGIT, expressed on the surface of CD8+ T cells to facilitate immune evasion. Moreover, certain nonclassical HLA molecules, such as HLA-E molecules expressed on the surface of BCa tumor cells, can interact with NKG2A receptors present on CD8+ T-cell membranes, thereby impeding the recognition and cytotoxicity of T cells toward tumor cells.[111] The DNAM-1 receptor on the membranes of CD8+ T cells interacts with tumor cell CD112/CD155 ligands to recognize tumor cells exhibiting deficiencies in HLA class I molecules.[111] In BCa, tumor cells can induce the development of a protumorigenic NK cell phenotype by secreting PDGF-DD, which interacts with NK cells through NKp44.[112] Furthermore, tumor cells secrete MICA/MICB to interact with the NK cell receptor NKG2D and activate the antitumour functionality of NK cells.[112] Tumor cells also interact with IgG1+ plasma cells, CD44, and SDC1 through LAMB3 and ANGPTL4. These interactions facilitate tumor cell progression and confer resistance to immunotherapy in BCa (Figure 4; Table 2).[113]

THERAPEUTIC APPROACHES TARGETING THE TME

In recent years, therapeutic strategies targeting the TME have emerged as promising approaches for treating tumors because the TME plays a critical role in regulating tumor progression and modulating the response to conventional therapies. Therefore, this review provides a comprehensive overview of current immunotherapies, antiangiogenic agents, and therapies targeting CAFs and the ECM in BCa, aiming to enhance the understanding of the existing challenges in targeting the TME and to propose future trends in BCa treatment.

Targeting T cells

Currently, the majority of antitumour therapies aimed at T cells focus on increasing the antitumour potential of effector T cells through immune checkpoint inhibition, which predominantly targets the PD-1 and CTLA4 receptors. Pembrolizumab and nivolumab, antagonistic antibodies targeting PD-1, were first approved by the FDA in 2014 for the treatment of patients with melanoma and subsequently approved for the treatment of patients with uroepithelial carcinoma expressing PD-1.[113,114] Currently, several phase I and phase II clinical trials are underway to evaluate the effectiveness of pembrolizumab and nivolumab in patients with locally progressive or metastatic BCa. Patients with advanced metastatic uroepithelial cancer treated with the adjuvant pembrolizumab after first-line chemotherapy had a median RFS time of 5.4 months and a median OS time of 22 months.[115] PD-L1, a ligand for PD-1, is normally expressed on antigen-presenting cells (APCs). The infiltration of MDSCs and DCs within the TME often facilitates tumor growth and invasion by upregulating PD-L1 expression, thereby inducing T-cell depletion and fostering an immunosuppressive TME that promotes further tumor progression.[116,117] Atezolizumab, avelumab, and other neutralizing antibodies targeting PD-L1 are in clinical trials for the treatment of patients with locally progressive or metastatic BCa. Avelumab was tested in a phase III clinical trial, which revealed that patients had a median RFS time of 5.5 months and a median OS time of 21.4 months. In addition, antagonistic antibodies such as tremelimumab and ipilimumab, which target CTLA4, are in phase I and phase II clinical trials for the treatment of patients with progressive or metastatic BCa. Some emerging and attractive immune checkpoint molecules, such as the TIM3, LAG3, and TIGIT proteins, have also been recently identified. LAG3, for example, is a negative regulator of T-cell activation, proliferation, and exertion.[118] It is structurally similar to the CD4 receptor and binds to MHC-II-like molecules with higher affinity.[119] INCAGN02385 and INCAGN02390, antagonistic antibodies targeting LAG3 that inhibit the interaction between LAG3 and MHC-II, are currently in phase II clinical trials. Both antibodies are used in combination with retifanlimab for neoadjuvant therapy as an alternative option for patients with MIBC who are ineligible for or refuse cisplatin therapy. Currently, targeted PD-1 and CTLA-4 therapies have significant individual variations, and many patients fail to respond effectively to these therapies. In addition, considering the functional complementarity of CTLA-4 and PD-1, the combination of anti-CTLA-4 and PD-1 therapies has been acknowledged as a viable strategy for increasing response rates. However, strategies that combine anti-CTLA-4 and PD-1 therapies exhibit increased toxic side effects.[120] Therefore, further extensive research is imperative to investigate combination regimens of immune checkpoint inhibitors that can effectively maintain clinical efficacy while minimizing potential toxic side effects (Table 3).

Targeting TAMs

Various therapeutic strategies have been developed to target TAMs, including the inhibition of monocyte recruitment and infiltration, the suppression of M2 polarization in TAMs, and the reduction in pro-inflammatory cytokine release. Notably, therapeutic interventions targeting TAMs not only impede the direct protumorigenic effects exerted by TAMs but also enhance their interactions with CD8+ T cells, thereby enhancing the antitumour efficacy of the latter.[121] Several drugs targeting TAMs have entered clinical trials, including inhibitors of the CSF1 receptor (CSF1R) and CCL2/CCR2 to inhibit monocyte recruitment and infiltration, antagonists of the CD47/SIRPα complex to increase TAM-mediated phagocytosis of tumor cells, inhibitors of the PI3Kγ and TREM2 proteins to reprogram TAMs toward antitumour M1 polarization, and CD40 agonists to enhance T-cell-mediated antitumour effects.[28]

Several ongoing clinical trials are investigating the efficacy of CD40 agonists in treating BCa. The results of experiments with AdCD40L, an adenoviral vector expressing a CD40 ligand, in patients with invasive BCa scheduled for radical cystectomy revealed that CD40L interacts with the CD40 receptor expressed by intratumoral dendritic cells (DCs), which in turn promotes the maturation of DCs, thus increasing intratumoral effector T-cell infiltration.[122] CDX-1140, a CD40 agonistic antibody, promotes the polarization of TAMs toward an antitumour phenotype and induces a macrophage-dependent antitumour response. It is currently being tested in a phase I clinical trial in patients with advanced BCa. In addition, another drug that targets TAMs is in clinical trials in the field of BCa treatment. Evorpacept (ALX148), a CD47 antagonistic antibody, is being tested in a phase I clinical trial in

Table 2. Crosstalk between BCa cells and the Tumor Microenvironment: Regulating Cancer Cell Functions and the Immunosuppressive Tumor Microenvironment through Ligand–Receptor Interactions

Releaser	Ligand	Recipient	Receptor	Biological Function	Outcome	Ref.
BCa cells	IL-11	BCa cells	IL-11R	IL-11/JAK/STAT3↑ Proliferation↑, Migration↑, Invasion↑	Lymphatic metastasis	61
BCa cells	TGF-β	BCa cells	TGFBR1	TGF-β/SMAD3/VEGF-D/VEGFR3↑ Lymphangiogenesis↑	Lymphatic metastasis	63
apoptotic BCa cells	S100a4	Neighboring live BCa cells	RAGE	Erk↑ metastatic outgrowth of neighboring live BCa cells↑	Lymphatic metastasis	135
BCa cells	CCL2	Macrophages	CCR2	Recruitment and M2 polarization↑	Progression Lymphatic metastasis	39,58,76
BCa cells	CXCL1	Macrophages	CXCR2	Recruitment and M2 polarization↑	Repeated intravesical recurrence Progression Drug resistance	77
BCa cells	BMP4	Macrophages	BMPR2-BMPR1	SMAD1/5/8 phosphorylation↑ Recruitment and M2 polarization↑	Progression	78
BCa cells	TNF-α	TAMs	TNFR1	VEGF-C↑ Lymphangiogenesis↑	Lymphatic metastasis	80
TAMs	VEGF-C	LECs	VEGFR3	Lymphangiogenesis↑	Lymphatic metastasis	58,80
TAMs	TGF-β	BCa cells	TGFBR	Glycolysis↑through the TGF-β/Smad2/3↑ PD-L1 mRNA m6A levels↑ by METLL3↑ expression CSCs↑ and EMT↑	Immunotherapy resistance Progression	81
TAMs	CXCL1	BCa cells	CXCR2	Invasion↑, EMT↑, and ECM remodelling↑	Repeated intravesical recurrence Progression Drug resistance	77
BCa cells	IFN-I	Fibroblasts	IFNAR	CAF↑	Chemotherapy resistance	86
BCa cells	TGF-β1	Fibroblasts	TGFBR1	FAP↑, EMT↑ CAF↑	Progression	87
BCa cells	IL-1α	CAFs	IL-1R	IL-8, GM-CSF, CCL2, MMP-2, and HGF↑ Invasion↑, Migration↑	Progression	88
BCa cells	CXCL1	CAFs	CXCR2	Recruitment and infiltration	Progression	77
CAFs	IL-6	BCa cells	IL-6R	JAK/STAT3↑ EMT↑	Progression	92
CAFs	IL-1β	BCa cells	IL-1R	IL-1β/Wnt↑ Proliferation↑, Invasion↑	Progression	93
CAFs	TGF-β1	BCa cells	TGFBR1	TGF-β1/Smad2↑ EMT↑	Progression	94
CAFs	VCAN	BCa cells		EMT↑	Progression	87
CAFs	MFAP5	BCa cells	NOTCH2	NOTCH2/HEY1↑ EMT↑, proliferation↑ and metastasis↑	Lymphatic metastasis	95
CAFs	HGF	BCa cells	c-MET	c-Myc↑ Lymphangiogenesis↑	Lymphatic metastasis	59
CAFs	CXCL1	BCa cells	CXCR2	Proliferation↑, Growth↑	Repeated intravesical recurrence Progression Drug resistance	77
CAFs	WNT5A	BCa cells	FZD6	WNT5A/β-catenin↑ Confers stemness and drug resistance to BCa cells	Neoadjuvant chemotherapy or immunotherapy resistance	86
CAFs	IGF-1	BCa cells	IGF-1R	IGF-1/IGF-1R/AKT/c-Jun/ERβ/Bcl-2↑ BCa cells anti-apoptosis↑ and cisplatin resistance↑	Chemotherapy resistance	97

Table 2. *(Continued)*

Releaser	Ligand	Recipient	Receptor	Biological Function	Outcome	Ref.
CAFs	ITGA11	LECs	SELE	SRC-p-VEGFR3-MAPK↑ Early-stage BCa lymphovascular invasion↑	Lymphatic metastasis	96
CAFs	FGF2	CSCs	FGFR	Maintains BCa stemness and EMT↑	Repeated intravesical recurrence	98
CAFs	CCL2	MDSCs	CCR2	JAK/STAT3↑ MDSCs↑ to hypoxic tumor areas, creating an immune-suppressive microenvironment	Progression	76,98
BCa cells	CXCL1/8	TANs	CXCR2	VEGF-A↑ and MMP9↑ Lymphangiogenesis↑	Lymphatic metastasis	38
BCa cells	CXCL5	TANs	CXCR2	Recruitment and activation of neutrophils	Lymphatic metastasis	64
TANs	VEGF-C	LECs	VEGFR3	Lymphangiogenesis↑	Lymphatic metastasis	64
BCa cells	CXCL2	MDSCs	CXCR2	MyD88-dependent MAPK and NF-κB↑ MDSCs recruitment↑ and T-cell proliferation↓	Progression	104
BCa cells	MIF	MDSCs	CD74	MyD88-dependent MAPK and NF-κB↑ MDSCs recruitment↑ and T-cell proliferation↓	Progression	104
BCa cells	LAMB3	IgG1 PCs	CD44		Progression Immunotherapy resistance	113
BCa cells	ANGPTL4	IgG1 PCs	SDC1		Progression Immunotherapy resistance	113
BCa cells	CD112/ CD155	CD8+ T cells	DNAM-1	DNAM-1-mediated TCR-independent activation↑ Recognition of HLA class I-deficient tumors through TCR-independent innate-like mechanisms↑	Sensitive to immunotherapy	111
BCa cells	HLA-E	CD8+ T cells	NKG2A	The function of CD8+ T cells↓	Immunotherapy resistance	111
BCa cells	MICA/MICB	NK cell	KLRK1（NKG2D）	NK cell antitumour functions↑	Sensitive to immunotherapy	112
BCa cells	PDGF-DD	NK cell	NKp44	Protumorigenic NK cell↑	Immunotherapy resistance	112
BCa cells	VEGF-C/D	LECs	VEGFR3	Lymphangiogenesis↑	Lymphatic metastasis	54,63
BCa cells	VEGF-A/C	ECs	VEGFR2	VEGFR2/NF-κB↑ EGFR ligand↑ Proliferation↑, Migration↑, Invasion↑	Progression	105
BCa cells	CXCL1/5/8	ECs	CXCR2	Recruitment	Progression	105
BCa cells	IL-1	ECs	IL-1R	NF-κB↑ Pro-inflammatory, pro-coagulatory, and cell-adhesive ECs phenotype↑ Leukocyte recruitment↑ and platelet adhesion↑ Angiogenesis↑	Dissemination	106
BCa cells	IGFBP7	ECs	CD93	Tumor-infiltrating immune cells↑, but the activities of T-cell recognition and killing↓	Immunotherapy resistance	109
ECs	Ligand of EGFR	BCa cells	EGFR	EGFR↑ and induces the phosphorylation of AKT and NF-κB↑ Proliferation↑, Migration↑, Invasion↑	Progression	105

patients with advanced unresectable, locally progressive or metastatic BCa. The results of studies targeting TAM-related drugs for the treatment of patients with BCa have not yet been published. The screening of relevant drug-sensitive populations and potential synergistic effects when combined with other therapeutic approaches (e.g., ICIs, antiangiogenic drugs) still needs to be further confirmed. However, it is undeniable that targeting TAM-related drugs can provide another potentially effective treatment option for patients with advanced BCa (Table 3).

Table 3. Current therapeutic approaches targeting the tumor microenvironment in bladder cancer

Target	Drug name	Drug type	Mechanism	Current Status	ClinicalTrials.gov
Targeting T cells	Pembrolizumab Nivolumab	antagonistic antibodies targeting PD-1	Binds to the PD-1 receptor and blocks its interaction with PD-L1 and PD-L2, releasing PD-1 pathway–mediated inhibition of antitumour responses	Phase I/II	NCT02619253 NCT02500121 NCT02897765 NCT03633110 NCT02500121
	Atezolizumab；Avelumab	neutralizing antibodies targeting PD-L1	Binds to PD-L1 to stop the interaction between PD-1 and PD-L1 in order to restore antitumour T-cell functions	Phase II/III	NCT02951767 NCT01772004 NCT02603432
	Tremelimumab Ipilimumab	Antagonistic antibodies targeting CTLA4	Blocking of the inhibitory signal CTLA4, allowing CTLs to destroy tumor cells	Phase I/II	NCT03601455 NCT02812420 NCT02496208 NCT04940299
	INCAGN02385 INCAGN02390	antagonistic antibodies targeting LAG3	Blocking MHC-II–LAG3 interaction	Phase II	NCT04586244
Targeting ECM and CAFs-FGFR	Infigratinib	FGFR1-3 targeted small-molecule inhibitors	Prevents CAF activation	Phase III	NCT04197986
	ICP-192; TYRA300; LOXO-435; Derazantinib; Rogaratinib	Small molecule inhibitors of the FGFR signalling	Prevents CAF activation	Phase I/II	NCT04492293 NCT05544552 NCT05614739 NCT04045613 NCT03473756
	Denosumab	RANKL (NF-κB receptor activator ligand) inhibitor	Prevents CAF activation	Phase II	NCT03520231
	PF-03446962	Monoclonal antibody targeting the TGF-β receptor ALK1	Prevents CAF activation	Phase II	NCT01620970
Targeting Angiogenesis	Ziv-aflibercept	Genetically engineered soluble receptor that targets VEGF	Anti-angiogenic therapy	Phase II	NCT00407485
	Pazopanib hydrochloride	Small molecule inhibitor targeting the VEGF/VEGFR signalling pathway	Anti-angiogenic therapy	Phase II	NCT00471536
	Bevacizumab; Ramucirumab	Antagonistic antibody targeting the VEGF/VEGFR signalling pathway	Anti-angiogenic therapy	Phase I-III	NCT00942331 NCT02443324
	Sitravatinib; Cabozantinib	Small-molecule inhibitors targeting the VEGF/VEGFR signalling pathway	Anti-angiogenic therapy	Phase Ib/II	NCT03170960 NCT03606174

Targeting CAFs and the ECM

Since the activation and function of CAFs are driven by signalling pathways such as the Hedgehog, NF-κB, FGFR, TGF-β, and CXCR4 pathways, current antitumour therapies targeting CAFs and the ECM in BCa are focused mainly on specific inhibitors of relevant pathways,[89] especially those targeting the FGFR signalling pathway. Infigratinib, an oral FGFR1-3-targeted inhibitor, is currently in phase III clinical trials for adjuvant treatment after radical cystectomy in patients with MIBC. In addition, small molecule inhibitors of the FGFR signalling pathway, such as ICP-192, TYRA300, LOXO-435, derazantinib, and rogaratinib, are currently in phase I and phase II clinical trials for the treatment of patients with advanced unresectable, locally progressive, or metastatic FGFR-mutated BCa. Denosumab, the first NF-κB receptor activator ligand (RANKL) inhibitor, is currently in a phase II clinical trial in combination with first-line platinum-based chemotherapy for the treatment of patients with advanced metastatic BCa. PF-03446962, a monoclonal antibody targeting the TGF-β receptor ALK1 for the treatment of patients with recurrent or refractory uroepithelial cancer. However, the results of a phase II clinical trial revealed that PF-03446962 did not provide a significant clinical benefit when used as a single agent, with a median RFS time of 1.8 months and a median OS time of 8 months. This phenomenon may be attributed to the dualistic nature of CAFs, as they exhibit both antitumour and protumour effects. For example, depletion of SMA+ CAFs in a murine model of pancreatic cancer led to immunosuppression, thereby adversely impacting OS.[123] Therefore, current therapeutic strategies aimed at targeting CAFs have progressively shifted from depleting CAFs to reprogramming or normalizing their phenotypes. Mara H Sherman et al. reported that the use of vitamin D analogues induced the reversal of CAFs to stellate cells and promoted antitumour efficacy.[124] Currently, paricalcitol, a vitamin D analogue, is in phase I and phase II clinical trials for the treatment of patients with advanced metastatic pancreatic and breast cancer (NCT03520790, NCT00637897). However, similar related studies have not been conducted in BCa. CAFs exhibit significant heterogeneity and can transition between distinct states, with each subpopulation of CAFs exhibiting unique functional characteristics. Consequently, a comprehensive understanding of diverse CAF subtypes is imperative for elucidating their precise contributions to tumor progression, facilitating the development of targeted therapeutic strategies against CAFs (Table 3).

Targeting Angiogenesis

Tumor angiogenesis is a complex process orchestrated by multiple growth factors, including VEGF, PDGF, EGF, and FGF2. Among these factors, targeting the VEGFA/VEGFR2 signalling pathway has emerged as the predominant antiangiogenic therapeutic strategy. Ziv-aflibercept, also known as the VEGF trap, is a genetically engineered soluble receptor that targets VEGF. It is currently undergoing phase II clinical trials for the treatment of patients with recurrent or metastatic uroepithelial carcinoma and has a median patient RFS time of 2.79 months. Pazopanib hydrochloride, a small molecule inhibitor targeting the VEGF/VEGFR signalling pathway, was used for the treatment of patients with metastatic uroepithelial carcinoma, with a median RFS time of 1.85 months and a median OS time of 5.83 months. Currently, the majority of ongoing clinical trials of antiangiogenic therapy in BCa are focused on exploring the efficacy of anti-VEGF/VEGFR therapy in combination with chemotherapeutic agents or immunotherapeutic agents. The use of bevacizumab, an antagonistic antibody targeting the VEGF/VEGFR signalling pathway, in combination with gemcitabine and cisplatin for the treatment of patients with advanced unresectable, locally progressive, or metastatic BCa is currently in phase III clinical trials. Patients had a median RFS time of 8.0 months and a median OS time of 14.5 months. Currently, the predominant therapeutic strategies for BCa focus on inhibiting tumor angiogenesis rather than promoting vascular normalization to enhance drug delivery. However, recent findings have demonstrated that the administration of high doses of antiangiogenic drugs induces a hypoxic microenvironment within the tumor, thereby facilitating tumor cell invasion and metastasis.[125] Therefore, meticulous adjustment of the dosage of antiangiogenic drugs and subsequent normalization of the tumor vasculature frequently result in enhanced clinical benefits for patients; however, no relevant clinical trials have been conducted in BCa patients. Despite decades of research on tumor angiogenesis, the heterogeneity of tumor vascular components has been significantly underestimated. Currently, a recent single-cell analytical study on mouse lung cancer identified previously unidentified tumor ECs.[126] Therefore, future advancements require a deeper and more comprehensive understanding of the intricate biological processes underlying tumor angiogenesis to facilitate the development of targeted angiogenic therapies with enhanced efficacy (Table 3).

CONCLUSION

Previously, the genetic and epigenetic alterations occurring in the uroepithelium emerged as a primary focus of research in the field of BCa.[22] With recent advances in single-cell technologies such as scRNA-seq and mass cytometry, systematic interrogation of the TME has become feasible, which has spurred a recent surge of interest in the functioning of the TME. Fei Li et al. utilized single-cell RNA sequencing to identify a distinct subset of BCa cells that exhibit marked resistance to cisplatin in association with glycolytic metabolism.[127] Integrated multiomics analysis of a Chinese urothelial carcinoma patient cohort elucidated the immune subtyping characteristics of bladder urothelial carcinoma.[128] There has been gradual recognition of the heterogeneity of the TME and oversimplification of the early understanding of the TME. Delving into the microenvironment of BCa reveals a labyrinthine network system characterized by complexity and multilevel interactions. Currently, there is a lack of information regarding the relationship between BCa and the TME, and the role of ITH in the development and progression of BCa is not fully understood.[23] Furthermore, it is important to consider the significance of histological subtypes and molecular backgrounds when interpreting results. Therefore, it is crucial to elucidate the complex cellular interactions within the ITH and TME of BCa in the context of histological subtypes and molecular backgrounds to identify novel therapeutic targets for enhancing the effectiveness of BCa treatment.

Targeting the nonneoplastic constituents of the TME, owing to their greater genomic stability and lower susceptibility to mutation, has long been considered a promising therapeutic strategy. Currently, numerous ongoing clinical trials are investigating a wide range of targeted TME treatment strategies for BCa that aim to modulate T cells, TAMs, and CAFs, which are responsible for ECM remodelling, and ECs, which are involved in tumor angiogenesis. However, monotherapy alone is insufficient to achieve sustainable cancer control, and enrolling an adequate number of patients for clinical studies focused on combination therapy poses significant challenges. The small size of these cohorts is a major limiting factor for the development of targeted TME therapy drugs. In addition, inaccurate trial designs have crucially hampered the optimization of therapeutic strategies among subgroups. ICI therapy, which has demonstrated remarkable efficacy in recent years, still faces several challenges, including low and unpredictable response rates as well as significant toxicity when treated with two ICIs. The urgent task at hand is to develop a rational and highly effective approach that integrates diverse strategies for targeting the TME while reducing toxicity and side effects. Consequently, there is currently a significant focus on investigating therapeutic approaches that combine ICIs or cell-based immunotherapy with targeted therapies for the TME. For example, Bempegaldesleukin (BEMPEG), a CD122-preferential interleukin-2 pathway agonist, combined with nivolumab has shown promising results in BCa patients, with a higher response rate and longer progression-free survival. BEMPEG selectively activates CD8+ T and natural killer cells while limiting regulatory T-cell expansion without increasing immune-related adverse events when used with nivolumab.[129] These findings demonstrate that the era of targeted combination immunotherapy is imminent. Moreover, increasing attention has been given to the synergistic effects achieved by combining antiangiogenic inhibitors with chemotherapy or immunotherapy agents.

The TME is a complex and dynamic network of interactions between mesenchymal tissue cells and tumor cells, forming an intricate 'tumor ecosystem'. To comprehensively understand the role of the TME in tumorigenesis and development, it is crucial to adopt an ecological perspective rather than focusing solely on one cell type within the TME. In addition, histological subtypes and molecular backgrounds are critical features to consider in the interpretation of results. Collaborative efforts are needed to study and advance the understanding of rare subtypes that have been understudied, such as bladder squamous cell carcinoma, to provide the best chance for meaningful progress in the treatment of BCa. Furthermore, comprehensive analysis of patients should be conducted to identify specific populations that are responsive to targeted TME therapies. Additionally, the impact of systemic factors such as the gut flora, metabolism, diet or exercise, as well as underlying conditions such as aging, inflammation and malignant diseases, on the TME should be investigated.[130] There is significant heterogeneity among different patients; for example, high-fat diet-induced obesity can impair the function of CD8+ T cells and promote tumor growth.[131] Different tumors present diverse tumor microenvironment subtypes, and these subtypes are closely associated with prognosis and immune therapy response.[132] Therefore, screening specific populations for targeted TME therapeutic strategies is crucial.

Despite preclinical data continue supporting the use of targeted TME therapeutic strategies, targeted TME therapeutic strategies have not yet been firmly established in the therapeutic landscape of BCa. However, with novel cutting-edge technologies such as single-cell sequencing and spatial molecular profiling, gaining a better understanding of spatial and temporal interactions is expected to offer the most effective combination strategies. It is essential to incorporate translational endpoints into clinical trial designs to develop personalized and efficient targeted therapeutic strategies for patients with BCa. We are convinced that targeting the TME could offer more effective treatment options for BCa and benefit a larger number of patients.

Abbreviation	Explanation
apCAF	antigen-presenting CAFs
ADC	antibody drug conjugates
APCs	antigen-presenting cells
BCa	bladder cancer
BCG	Bacillus Calmette-Guérin
BMDMS	bone marrow-derived macrophages
CAFs	cancer-associated fibroblasts
CCC	cell-to-cell communication

circRNAs	circular RNAs
CSCs	cancer stem-like cells
DCs	dendritic cells
ECM	extracellular matrix
ECs	endothelial cells
EMT	epithelial–mesenchymal transition
EVs	extracellular vesicles
HNRNPU	heterogeneous nuclear ribonucleoprotein U
HSF1	heat shock factor 1
iCAFs	inflammatory CAFs
ICIs	immune checkpoint inhibitors
ITH	intratumoral heterogeneity
LECs	lymphatic endothelial cells
lncRNAs	long noncoding RNAs
MDSCs	myeloid-derived suppressor cells
MIBC	muscle invasive bladder cancer
miRNAs	microRNAs
myCAFs	myofibroblast CAFs
NMIBC	nonmuscle invasive bladder cancer
OS	overall survival
RFS	recurrence-free survival
scRNA-seq	single-cell RNA sequence
TAMs	tumor-associated macrophages
TANs	tumor-associated neutrophils
TIICs	tumor-infiltrating immune cells
TME	tumor microenvironment
TURBT	transurethral resection of bladder tumor
vCAFs	vascular CAFs
3′ UTRs	3′ untranslated regions

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AUTHOR CONTRIBUTIONS

Xiaole Lu and Liang Cheng drafted the manuscript. Chenwei Yang contributed to the acquisition and interpretation of the data. Jian Huang and Xu Chen contributed to the structural design and edited the manuscript. All the authors read and approved the final manuscript.

DECLARATION OF INTERESTS

The authors declare no competing interests.

DATA AND CODE AVAILABILITY

Not applicable.

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