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Unleashing the therapeutic potential of tumor-draining lymph nodes: spotlight on bladder cancer

Xin Yang[1†], Xiangyu Wu[1†], Xuyang Hao[1], Tianhang Li[2,3*], Hongqian Guo[1*] and Rong Yang[1*]

Abstract

Tumor-draining lymph nodes (TDLNs) are often involved during the metastasis of bladder cancer (BC), which is associated with a poor prognosis. Recent studies have shown that TDLNs are a major source of host anti-tumor immunity, which can impede tumor progression and favor tumor immunotherapy. However, during tumor progression, various tumor-derived mediators modulate the TDLN microenvironment, impairing their protective function. Ultimately, TDLNs provide the soil for the proliferation and dissemination of tumor cells. Therefore, surgical removal of TDLNs is commonly recommended in various solid tumors to prevent metastasis, but this poses significant challenges for leveraging TDLNs in immunotherapy. Additionally, lymph node dissection (LND) has not shown survival benefits in some tumors. Hence, the decision to remove TDLNs in oncological treatment needs to be reconsidered. Herein, we spotlight the TDLNs of BC and introduce how BC cells modulate stromal cells and immune cells to shape an immunosuppressive TDLN microenvironment for BC progression. We summarize the existing therapeutic strategies to reinvigorate anti-tumor immunity in TDLNs. Furthermore, we discuss whether to preserve TDLNs and the role of LND during oncological treatment.

Keywords Tumor-draining lymph nodes, Bladder cancer, Immune evasion, Immunotherapy, Lymph node dissection

Introduction

Bladder cancer (BC), a global health issue, is the tenth most common malignancy worldwide, with nearly 600,000 new cases in 2020, while the number is expected to double by 2040 according to the World Health Organization [1]. Based on the extent of tumor invasion, approximately 75% of cases are non-muscle invasive bladder cancer (NMIBC), while 25% are muscle invasive bladder cancer (MIBC) [2]. Lymph node (LN) metastasis is the predominant metastatic mode in BC, contributing to an extremely poor prognosis, especially in MIBC [3].

Tumor-draining lymph nodes (TDLNs) are positioned along the lymphatic drainage route of the primary tumor and play a pivotal role in fueling anti-tumor immune responses. Sentinel lymph nodes (SLNs), the first TDLNs, acting as the initial barrier to prevent LN

†Xin Yang and Xiangyu Wu contributed equally to this work.

*Correspondence:
Tianhang Li
DG20350057@smail.nju.edu.cn
Hongqian Guo
dr.ghq@nju.edu.cn
Rong Yang
doctoryr@gmail.com
[1]Department of Urology, Nanjing Drum Tower Hospital, Affiliated Hospital of Medical School, Nanjing University, Nanjing, China
[2]Department of Urology, Zhongda Hospital, Southeast University, Nanjing, China
[3]Surgical Research Center, Institute of Urology, Southeast University Medical School, Nanjing, China

metastasis of the tumor, have been demonstrated to display a substantial anti-tumor response in BC [4, 5]. However, a growing body of evidence indicates that the malignant cells can hijack the TDLNs, subverting the immunity of TDLNs and leading to immune evasion [6–8]. Ultimately, the TDLNs serve as a hub for tumor metastasis [9, 10]. Therefore, TDLNs are situated at the intersection between immunity and metastasis [11, 12]. This dual role presents both opportunities and challenges for halting tumor progression by leveraging TDLNs for immunotherapy.

The role of TDLNs in tumor immunotherapy has long been overlooked, as they are often regarded as "transit stations" for tumor cell metastasis and are thus routinely removed. However, lymph node dissection (LND) does not improve overall survival (OS) in melanoma [13], thyroid cancer [14], and breast cancer [15]. Previous studies recommended extended LND in BC [16, 17], but recent findings indicated that such LND does not provide significant survival benefits, but instead leads to surgical complications and reduced quality of life [18, 19]. Preclinical models have also highlighted that TDLNs play a crucial role in immunotherapy [20–22], and surgical removal can substantially reduce therapeutic outcomes [23]. Therefore, the benefits of LND remain controversial. In the era of immuno-oncology, the question of whether to preserve TDLNs will provoke intense discussion, which could potentially transform the current landscape of tumor immunotherapy.

A comprehensive mechanistic understanding of the biology of TDLNs in BC will not only help to elucidate the progression of BC but also provide effective information for clinical translation. In this review, we present the modulation of the immune microenvironment of TDLNs and summarize the current therapeutic advances targeting TDLNs in BC, particularly immunotherapy, and further discuss the current status of LND in tumor treatment, as well as whether to preserve TDLNs for immunotherapy.

The modulation of the TDLN microenvironment in BC

As the essential components of the immune system, TDLNs orchestrate the interactions among immune cells, playing an indispensable role in triggering anti-tumor immune response. The SLNs of BC display a robust ongoing immune response against tumors [4, 5]. However, as the tumor progresses, immune activation within the TDLNs transitions to immune suppression and this dynamic change facilitates tumor metastasis. The composition of the TDLN stroma in BC also undergoes significant changes. Extracellular vesicles (EVs) derived from BC can induce fibroblasts to secrete Tenascin-C via the NF-κB signaling pathway, promoting the formation of a pre-metastatic niche in LNs, which is a microenvironment conducive to the colonization and growth of tumor cells [24]. In addition, hyaluronic acid (HA) accumulates in TDLNs, associated with an increase in HA-producing fibroblast cells. HA supports the development of antigen-presenting cells (APCs) expressing PD-L1, ultimately contributing to immune tolerance [25]. Here, we focus on the advances in how BC-derived mediators domesticate stromal cells and immune cells in TDLNs to reprogram an immunosuppressive microenvironment that facilitates tumor cell colonization and metastasis (Fig. 1).

Lymphatic endothelial cells (LECs)

LECs are the principal structural cells of lymphatic vessels, possessing multifaced immunoregulatory functions [26]. The proliferation of LECs, a core event in lymphangiogenesis, has been observed in both intratumoral and peritumoral lymphatic vessels in muscle-invasive bladder transitional cell carcinoma, providing a channel for tumor cells to migrate to TDLNs [27, 28]. BC cells can regulate the proliferation of LECs through multiple mechanisms, especially epigenetic mechanisms. Long noncoding RNA (lncRNA) are transcripts longer than 200 nucleotides and are extensively involved in various pathophysiological processes [29]. A lncRNA derived from BC cells, lymph node metastasis-associated transcript 2 (LNMAT-2), is encapsulated in an exosome by interacting with the heterogeneous nuclear ribonucleoprotein A2B1 (hnRNPA2B1) [30]. The exosome can be internalized by LECs, epigenetically upregulating the expression of prospero homeobox 1 (PROX1) by increasing trimethylation of lysine 4 on histone H3 protein subunit (H3K4me3) level in the PROX1 promoter, ultimately leading to LEC proliferation. Another BC-derived exosomal lncRNA, BCYRN1, can upregulate WNT5A expression by inducing heterogeneous nuclear ribonucleoprotein A1 (hnRNPA1) associated with H3K4me3 in the WNT5A promoter, thereby activating the Wnt/β-catenin signaling pathway and promoting BC cells to secrete vascular endothelial growth factor-C (VEGF-C) [31]. Additionally, it can stabilize VEGF receptor 3 (VEGFR3) mRNA in LECs, forming a WNT5A/VEGF-C/VEGFR3 feed-forward loop to facilitate lymphangiogenesis and LN metastasis. EV-mediated LN-associated transcript 1 (ELNAT1) induces overexpression of ubiquitin carrier protein 9 (UBC9), thereby catalyzing small ubiquitin-like modifier binding (SUMOylation) of hnRNPA1 at lysine 113, promoting its transfer via EV to facilitate intercellular communication between BC cells and LECs [32]. It activates the transcription of SOX18 in LECs, inducing lymphangiogenesis. In addition, the interaction between LECs and cancer-associated fibroblasts (CAFs) involves in the LN metastasis. A PDGFRα$^+$ITGA11$^+$ CAF subtype was identified by single-cell multi-omics analysis,

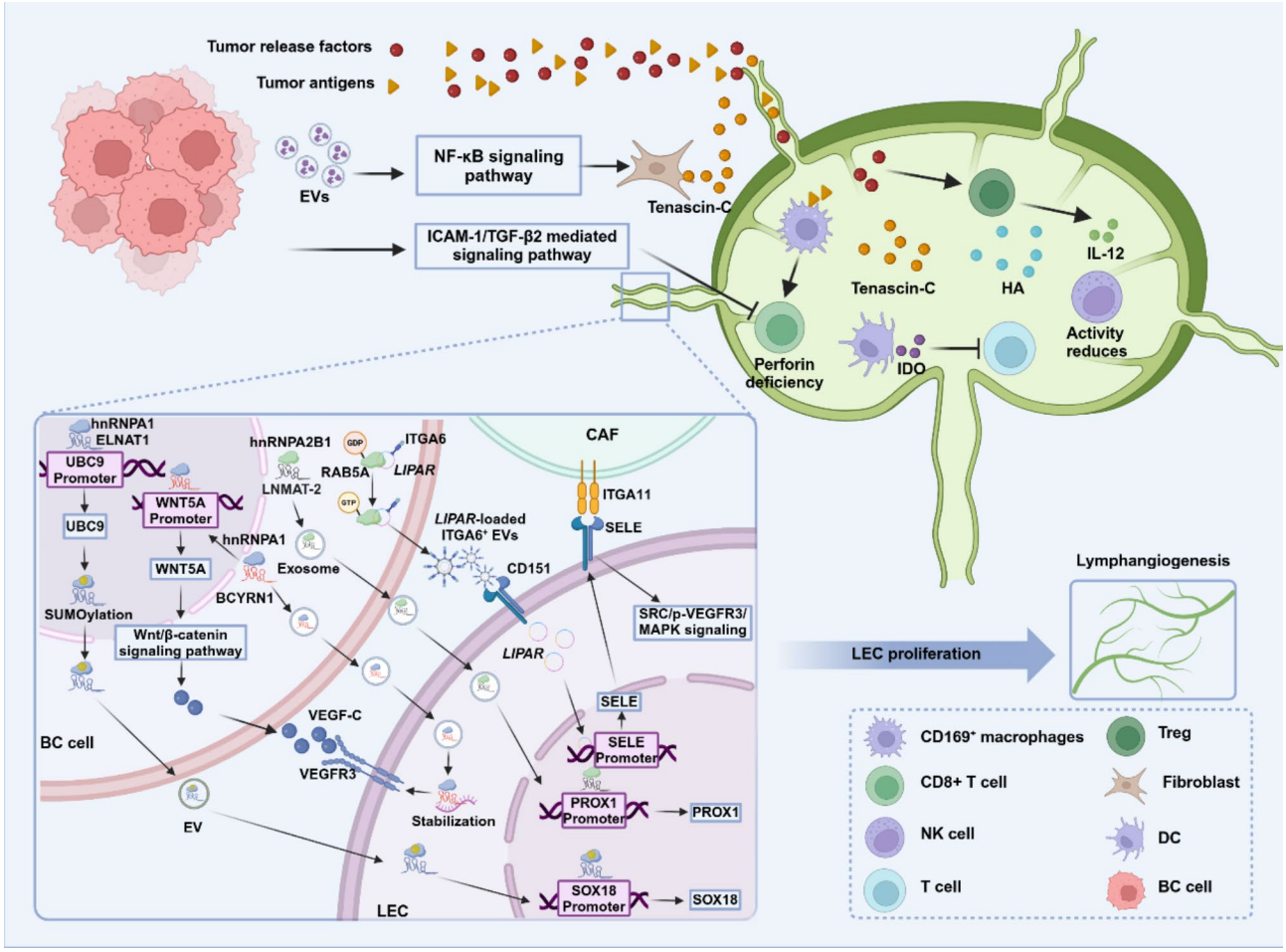

Fig. 1 The modulation of the TDLN microenvironment in BC. In the TDLN microenvironment, LECs proliferate primarily under the epigenetic regulation by BC cells, promoting lymphangiogenesis and ultimately facilitating LN metastasis. Besides, the function of various antitumor immune cells, such as $CD8^+$ T cells, NK cells, and DCs, is impaired, while the immunosuppressive capacity of Tregs is enhanced. In addition, the alteration of TDLN stroma also favors the metastasis of BC cells

which was able to interact with LECs via ITGA11-SELE and activate SRC/p-VEGFR3/MAPK signaling in LECs, thereby promoting lymphangiogenesis [33]. Recently, a study focused on the formation of pre-metastasis niche in TDLNs, deciphering a mechanism in which *LIPAR* links ITGA6 to RAB5A to form a ternary complex that sustains RAB5A GTP-bound activated state, thus triggering sustained production of *LIPAR*-loaded $ITGA6^+$ EVs [34]. These EVs target lymphatic vessels via the ITGA6-CD151 interaction between BC cells and LECs, delivering *LIPAR* to activate SELE transcription. The transcriptional activation of SELE may create a vicious cycle, which would enhance the interaction between $PDGFR\alpha^+ITGA11^+$ CAFs and LECs, further promoting LN metastasis. Therefore, mutual crosstalk between tumor cells, LECs, and CAFs reshapes the structure of lymphatic vessels, providing new insights into the diagnosis and treatment of LN metastasis in BC.

In addition to LEC proliferation, other stromal changes, including the dilation and dedifferentiation of high endothelial venules and the remodeling of fibroblastic reticular cells, collectively create a supportive niche for tumor cell metastasis [11]. However, research on these stromal changes has not yet been conducted in BC, offering a new direction for future studies.

T cells

While stromal cells remodel the TDLN structure, immune cells undergo functional and quantitative shifts (Table 1). $CD8^+$ T cells are a key component of adaptive immunity, playing a significant role in defending against tumors. Whereas, the reduced cytotoxicity of $CD8^+$ T cells in SLNs is attributed to the deficiency of perforin on their surface, caused by BC cells via the ICAM-1/TGF-β2 mediated signaling pathway [35]. However, the expression of perforin can be restored through the provision of a type 1 $CD8^+$ T cells (Tc1)-promoting condition,

Table 1 The roles of the immune cells in the TDLN microenvironment of BC

Cell type	Role	Mechanism	Reference
$CD8^+$ T cell	The cytotoxicity is suppressed, contributing to tumor immune evasion.	BC cells may downregulate perforin expression in $CD8^+$ T cells through an ICAM-1/TGF-β2 mediated signaling pathway.	[35]
Treg	The cells promote tumor immune evasion.	BC-releasing factors activate caspase-3, thereby increasing IL-16 expression, which reinforces the immunosuppressive capacity of Tregs.	[39]
GM-CSF-producing lymphocyte	The cells decrease with tumor infiltrating TDLNs.	GM-CSF inhibits lymphangiogenesis and recruitment of M2 macrophage.	[40]
Memory $CD8^+$ T cell	The cells are increasing with BC progression.	The immune system is exposed to continuous stimulation by tumor antigens to create a memory T cell reservoir, but this may be suppressed by tumor-releasing factors.	[42]
$CD4^+$ Tregs	The cells are negatively correlated with antitumor immunity.	/	[43]
IFN-γ/IL-17-prodcing $CD8^+$ T cell	The cells are negatively correlated with antitumor immunity.	/	[44]
$CD27^+$ $CD11b^{+/-}$ NK cell	The cells increase and display a regulatory phenotype with BC progression.	/	[47]
$CD169^+$ macrophage	The cells are correlated with a good prognosis.	The cells can present tumor antigens to $CD8^+$ T cells and activate them, which infiltrate into BC and inhibit BC cell proliferation.	[49]
CD163-expressing malignant cell	The cells are associated with EMT and enhanced metastatic activity.	TAMs induce the malignant cells in TDLNs to express CD169 in an IL-6/IL-10 independent manner.	[51]
IDO-expressing DC	The cells suppress anti-tumor T cell immunity.	DCs with high IDO expression can lead to T cell anergy.	[52]

offering a potential approach to rescue the cytotoxicity of $CD8^+$ T cells. Mounting evidence has also demonstrated that the major histocompatibility complex class I (MHC-I) expression on the surface of tumor cells is downregulated prior to their arrival at TDLNs, which will further impair the immune recognition and response of $CD8^+$ T cells [36–38]. In addition, regulatory T cells (Tregs), serving a central role in tumor immune evasion, can be activated by factors released from BC to activate caspase-3, thereby increasing IL-16 expression, which reinforces their immunosuppressive capacity in SLNs [39]. Thus, the antitumor immunity of $CD8^+$ T cells is compromised, while the immunosuppressive activity of Tregs is enhanced, facilitating tumor cell metastasis.

The alterations in the quantity of T cells in the TDLN microenvironment of BC also constitute a significant factor contributing to immune evasion. When the tumor infiltrates TDLNs, the level of granulocyte-macrophage-colony-stimulating factor (GM-CSF)-producing lymphocytes in TDLNs is significantly reduced [40], increasing the risk of metastasis, as GM-CSF has been demonstrated a protective role by inhibiting lymphangiogenesis and recruitment of M2 macrophage in BC [41]. Interestingly, the frequency of memory $CD8^+$ T cells is increasing with BC progression, which seems paradoxical, suggesting that the immune system is exposed to continuous stimulation by tumor antigens to create a memory T cell reservoir, but this may be suppressed by tumor-releasing factors [42]. In addition, immunosuppressive $CD4^+$ Tregs and IFN-γ/IL-17-prodcing $CD8^+$ T cells are negatively correlated with antitumor immunity and may promote the progression and metastasis of BC, with their proportion increasing in TDLNs [43, 44]. Therefore, these cells collectively induce an immunosuppressive microenvironment in TDLNs, while further investigations are warranted to elucidate the underlying mechanisms.

Natural killer (NK) cells

NK cells are the first responders in tumor immunity, capable of non-specifically directly killing tumor cells. Cytotoxic $CD56^{dim}$ NK cells are predominant among NK cell subsets. However, it has been observed that the percentage of NK cells in LN mononuclear cells is significantly lower compared with peripheral blood mononuclear cells in BC patients [45]. And their activity is reduced when LN metastasis occurs [46]. Furthermore, with tumor growth, there is an increase in $CD27^+$ $CD11b^{+/-}$ NK cells with a regulatory phenotype as well as a decrease in $CD56^{dim}$ NK cells with a cytotoxic phenotype in TDLNs of BC, and there may be a conversion between these two phenotypes [47]. Thus, the subtypes of NK cells undergo dynamic changes during tumor progression, suggesting that tumor cells may regulate the variations in different NK cell subtypes through certain mechanisms.

APCs

Macrophages are multifunctional and heterogeneous innate immune cells that undertake a central role in balancing the immune response to maintain organic homeostasis [48]. A subset of macrophages in TDLNs, $CD169^+$ macrophages, can present tumor antigens to $CD8^+$ T cells and activate them, which infiltrate into BC

and inhibit BC cell proliferation [49]. Therefore, $CD169^+$ macrophages in TDLNs are correlated with a good prognosis. However, tumor-associated macrophages (TAMs) also express CD169. Such $CD169^+$ macrophages exhibit an opposite role when infiltrating into BC, as they may be associated with lymphovascular invasion, which is a crucial step in LN metastasis [50]. Hence, more in-depth research to comprehensively elucidate the role of $CD169^+$ macrophages in immune regulation in BC is necessary. In addition, Maniecki et al. found that TAMs can induce malignant cells in TDLNs to express the macrophage-specific receptor CD163 in an IL-6/IL-10 independent manner [51]. These CD163-expressing malignant cells may be related to epithelial-mesenchymal transition (EMT) and enhanced metastatic activity in BC.

Moreover, the most potent APCs, dendritic cells (DCs), which act as shepherds of T cell immunity, overexpress an immune-negative regulatory molecule——indoleamine 2,3-dioxygenase (IDO) in TDLNs [52]. Conversely, DCs with high IDO expression can lead to T cell anergy, thereby suppressing anti-tumor T cell immunity.

To conclude, the TDLNs play a complex paradoxical role in BC, acting as orchestrators in the initiation and maintenance of anti-tumor immune responses, while also serving as key sites and sources for the induction of immune tolerance. Reversal of local immunosuppression and remodeling of the anti-tumor immune microenvironment in TDLNs hold substantial potential for the development of novel therapies for BC. However, research on the TDLN microenvironment in BC is still in preliminary stages, with further in-depth research needed to unveil the complex immune landscape of BC and propose potential therapeutic interventions.

Current strategies to unleash the therapeutic potential of TDLNs in BC

Regarding the immunological role of TDLNs, a variety of therapies have been developed to reinvigorate the anti-tumor immune response in TDLNs, substantially transforming the current treatment landscape for BC (Fig. 2).

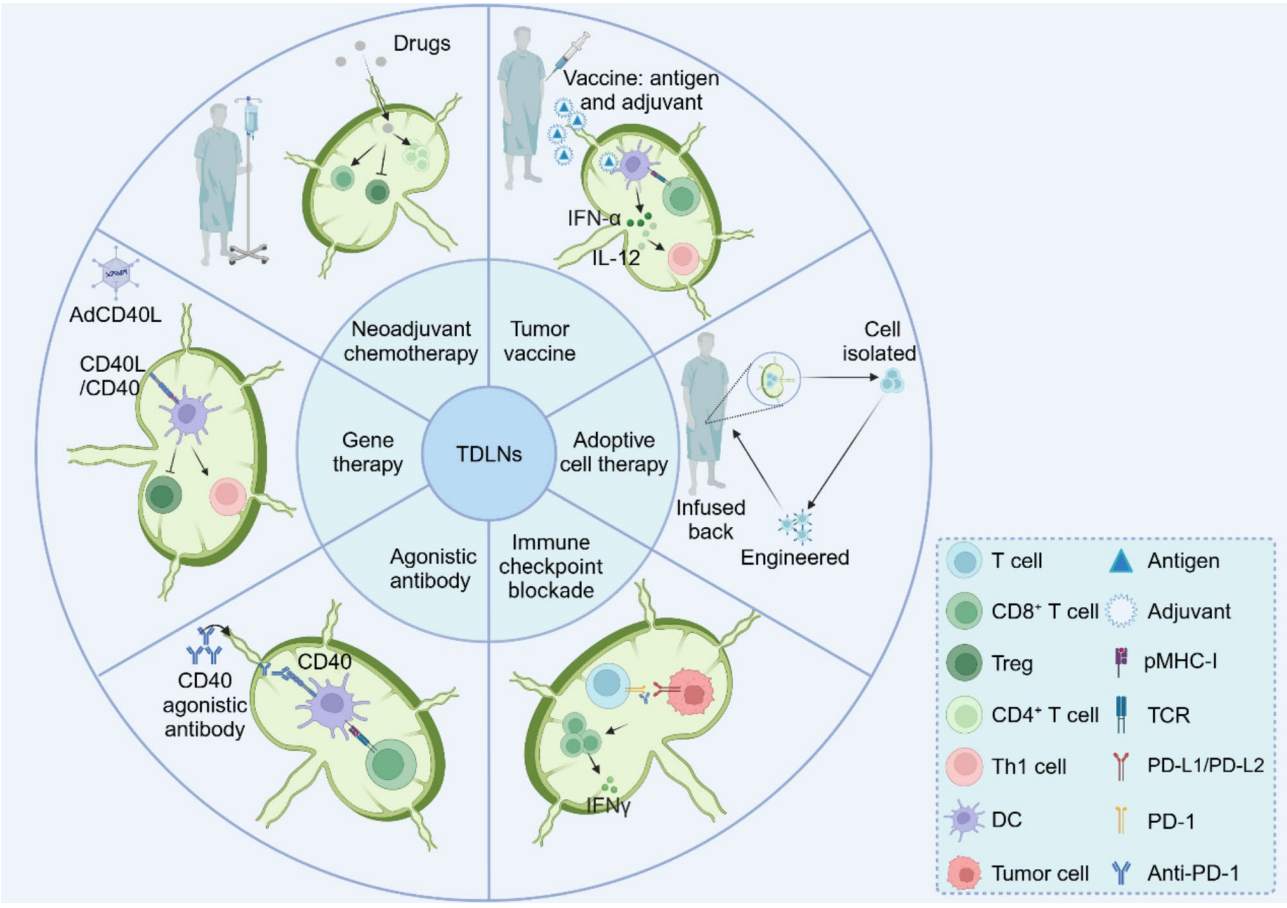

Fig. 2 Current strategies to unleash the therapeutic potential of TDLNs in BC. Various immunotherapies and NAC can lift immunosuppression and reactivate antitumor immunity within TDLNs to exert therapeutic effects. ACT, which involves isolating and engineering immune cells from TDLNs and reinfusing them, has been demonstrated to be both safe and feasible in BC

Tumor vaccines

Tumor vaccines, aiming to prevent or treat tumors, can activate the body's specific immune response to fight against tumor cells. Bacillus Calmette-Guerin (BCG) has ushered in a new era of tumor vaccine therapy for BC, since its first application to superficial BC in 1976 [53]. Currently, intravesical immunotherapy with BCG remains the standard therapy for high-risk NMIBC. Although 80% of patients respond to BCG, more than half experience disease progression and relapse [54]. Interestingly, a single instillation of BCG before intravesical immunotherapy can enhance its therapeutic efficacy by the mechanism that BCG can disseminate to TDLNs and initiate IFNγ-secreting T cells [55]. Thus, targeting TDLNs may serve as an effective adjunct to BCG intravesical immunotherapy, aiming to enhance the clinical response induced by BCG.

In addition, TDLNs, as a critical reservoir of the systemic immune response, can fuel robust anti-tumor immune responses upon activation by exogenous antigens, offering opportunities for the development of novel tumor vaccine immunotherapies. In a murine BC model, local administration of *Helicobacter pylori* neutrophil activating protein (HP-NAP), which can trigger cytotoxic T helper 1 (Th1) response, reaches regional LNs, inducing $CD4^+$ and $CD8^+$ IFN-γ-secreting cells accumulation in LNs. IFN-γ mediates anti-angiogenic activity and reduces tumor vascularization, ultimately decreasing tumor growth [56, 57]. In the TDLNs of the MB49 mouse model expressing male antigens HY, HY-specific cytotoxic T lymphocytes (CTLs) are present and can be activated by vaccinia-expressed tumor antigen and GM-CSF, eliciting an immune response [58]. In addition, an in situ tumor vaccine strategy innovatively modified *Lactobacillus lactis* with αOX40 and resiquimod, which was delivered intratumorally, triggering immune responses in the tumor microenvironment (TME) and TDLNs as well as inhibiting tumor growth [59, 60].

Immune adjuvants are an essential component of tumor vaccines, enhancing the immune response generated against the vaccines. Developing adjuvants that generate potent and durable antitumor immunity is critical to the success of tumor vaccines. *Antrodia camphorate* (AC), a unique basidiomycete fungus that acts as an immunomodulatory adjuvant in conjunction with a HER-2/neu DNA vaccine, enhances the anti-tumor efficacy of the vaccine in BC-bearing mice [61]. Mechanistically, the HER-2/neu DNA-AC combination can activate DCs in TDLNs and increase the production of Th1 activation cytokines such as IL-12 and IFN-α, promoting T cell proliferation and the generation of Th1-like cell-mediated immune response. Furthermore, it can induce infiltration of $CD4^+$ and $CD8^+$ T cells into tumors. Ultimately, these immune responses exert significant anti-tumor effects. In addition, a novel skin-delivery IDO small interfering RNA (siRNA) serves as an immune adjuvant for HER-2/neu DNA vaccine, as it silences IDO expression in DCs, which migrate to TDLNs to trigger anti-tumor responses, thereby enhancing the efficacy of the HER-2/neu DNA vaccine [62]. Recently, a multi-adjuvant tumor vaccine therapy has been developed that combines STING and TLR 7/8 agonists with a vaccine to activate DCs in LNs, promoting antigen cross-presentation and antigen-specific $CD8^+$ T cell activation [63]. Delivering immune adjuvants effectively to TDLNs is a critical strategy to enhance tumor vaccine efficacy, as TDLNs serve as the primary sites for antigen presentation. TLR7/8 agonist encapsulated in poly (lactide-co-glycolide) nanoparticles can be successfully delivered to TDLNs and effectively activate DCs, triggering antigen-specific $CD8^+$ T cell expansion and CTL responses [64]. Moreover, a significant preventive and therapeutic effect as well as a reduction in systemic metastasis was observed in the BC model. Therefore, it is encouraging to develop engineering nanoparticle vaccines for BC that are effectively delivered to TDLNs to enhance antigen presentation and $CD8^+$ T cell priming.

In conclusion, the central role of TDLNs in tumor immune responses makes them a critical target for developing novel cancer vaccine immunotherapies. Thorough research and utilization of TDLNs hold the potential to offer new hope for oncological treatment.

Adoptive cell therapy (ACT)

ACT is a highly prospective immunotherapy strategy in which collected immune cells from patients are expanded and genetically engineered in vitro, and then infused back into the patient's body to kill tumor cells [65]. Previous studies have shown that tumor-reactive cells isolated from SLNs of BC display immune function when activated in vitro, suggesting that SLNs will be a promising source for the expansion of immune cells [4, 5]. A clinical trial has successfully isolated tumor-specific T helper cells from the SLNs of six BC patients for ACT and observed anti-tumor responses and no significant adverse effects, preliminarily indicating that T-cell-based ACT is feasible and safe [66]. However, it did not investigate the patients' survival. Another study observed significantly prolonged overall survival in patients receiving ACT based on T cells derived from SLNs of BC, further demonstrating the efficacy and safety of this immunotherapy [67]. These findings emphasized the special value and significance of SLNs in the development of ACT. In addition, tumor-infiltrating lymphocytes (TILs) from LNs, which possess the ability to recognize and fight against tumor cells, can be applied to ACT and elicit an anti-tumor response [68, 69]. However, prior exposure to chemotherapy or BCG immunotherapy may affect the

efficacy, and the underlying mechanisms remain to be explored. Furthermore, mixed histology BC shows better responsiveness to TIL-based ACT. Therefore, patient assessment may be necessary before proceeding with TIL-based ACT to identify individuals suitable for this treatment.

Immune checkpoint blockade (ICB)

ICB blocks the interactions between tumor cells expressing immune checkpoints and immune cells, thereby interrupting the suppressive effects of tumor cells on immune cell activity. The discovery of PD-1/PD-L1 was a revolutionary breakthrough in tumor immunotherapy, sparking a research frenzy into immune checkpoints. Recently developed ICB therapies predominately focus on the TME, while the role of TDLNs in ICB therapy is often neglected. There is an abundant expression of PD-1 and its ligands PD-L1 and PD-L2 on the immune cells in TDLNs of non-metastatic BC, which suppress anti-tumor immune response and may help predict patient prognosis and response to immunotherapy [70, 71]. Besides, in non-metastatic melanoma, PD-1/PD-L1 interactions in TDLNs, rather than in the tumor, are closely associated with patient prognosis [22]. These laterally reflect that TDLNs occupy a pivotal position in ICB therapy. Blocking PD-1/PD-L1 can contribute to the localized accumulation of $CD8^+$ T cells in TDLNs, potentially enhancing the efficacy of anti-tumor therapy [23]. In addition, the combination of CD40 agonists and anti-PD-1 therapy can recruit $CD8^+$ TILs in TDLNs and induce IFNγ that coordinates the transition from MHCII-I^{low} TAMs to IFNβ-expressing $MHCII^{high}$ TAMs, thereby restoring the responsiveness of MIBC patients to anti-PD-1 therapy [72]. However, in urothelial carcinoma, $CD8^+$ T cells in TDLNs do not show significant changes after ICB, whereas $CD4^+$ T cells are activated to produce IFNγ, which is a critical player in the anti-tumor activity of ICB [73]. The mechanisms of ICB targeting TDLNs may be related to different tissue origins and individual patient differences, with the exact reasons remaining to be investigated.

Agonistic antibody

Unlike the mechanism of ICB, which acts like releasing the brakes, agonistic antibodies function more like pressing the accelerator by activating specific receptors on the surface of immune cells to intensify their immune effects and kill tumor cells. CD40 is a member of the tumor necrosis factor receptor (TNFR) superfamily and is expressed on APCs. Sandin et al. reported that both local and systemic delivery of CD40 agonistic antibodies resulted in the accumulation of anti-CD40 antibodies in TDLNs and both were able to eradicate BC, but local delivery has relatively fewer side effects [74]. In addition, repeated anti-CD40 treatment can dose-dependently increase the expression of CD40 on the surface of B cells, DCs, and macrophages in TDLNs. Similarly, the study by Fransen et al. showed that local delivery of CD40 agonistic antibodies to TDLNs can effectively activate DCs, eliciting a robust systemic anti-tumor $CD8^+$ T cell response [75]. Thus, TDLNs may play a crucial role in local treatment with CD40 agonistic antibodies. Moreover, locally delivering CD40 agonistic antibodies can target DCs with high CD40 expression in the TME, inducing potent anti-tumor activity without evidence of systemic toxicity [76]. It is anticipated that local delivery of CD40 agonistic antibody may be an attractive therapeutic option for BC in the clinic.

Gene therapy

Stimulating the interaction between CD40 and CD40 ligand (CD40L) can promote the maturation of DCs and enhance their antigen-presenting capabilities [77]. A gene therapy transferred adenoviral vectors expressing CD40 ligand (AdCD40L) to BC, in which CD40-CD40L interaction initiates a Th1-associated response through activating DCs and inhibits the development and function of Tregs in the TDLNs, ultimately curing in situ BC in mice [78]. Malmström et al. pioneered a Phase I/IIa clinical trial to evaluate this gene therapy, demonstrating that local administration of AdCD40L is safe and evokes immune activation [79]. In addition to BC, AdCD40L therapy has demonstrated antitumor effects in hepatocellular carcinoma [80], melanoma [81], and brainstem tumors [82], making it a potential monotherapy or adjunctive treatment for various solid tumors.

Neoadjuvant chemotherapy (NAC)

During tumor treatment, chemotherapy demonstrates a multifaceted role that not only kills tumor cells directly but also activates anti-tumor immunity [83, 84]. Krantz et al. demonstrated that NAC exerted profound impacts on T cell subsets in SLNs [85]. Specifically, after NAC treatment, $CD8^+$ effector T-cell exhaustion was reduced and the cytotoxicity was increased, and $CD4^+$ T-cell clonal expansion was increased while the activity of Tregs was significantly suppressed. Whereas, this immunostimulatory effect was not observed in patients who did not respond to NAC. In addition, Alvaeus et al. reported the results of a prospective multicenter study that the amount of SLNs in patients who experienced disease progression after NAC and RC was significantly reduced compared to those with complete response and stable disease, implying a linkage between SLNs and prognosis [86]. As previously mentioned, SLNs are key sites for initiating anti-tumor immune responses, and enhancing the cytotoxicity of immune cells within them through NAC could be a promising therapeutic approach.

Table 2 Studies investigating the impact of LND on immunotherapy

Tumor type	Experimental model	LN management	Main finding	References
Melanoma	Mouse	TDLN surgical ablation or TDLN genetic deficiency	There was a reduction in tumor-specific CTL, and the radiation-induced antitumor effects were substantially weakened.	[88]
Colon adenocarcinoma, melanoma	Mouse	Bilateral TDLN resection post-immune-radiotherapy	Bilateral TDLN removal impaired the antitumor effects induced by immune-radiotherapy, which was attributed to reduced infiltration of $CD8^+$ T cells in the tumor and disrupted polarization of TAMs.	[89]
HNSCC	Mouse	Lymphablation pre ICB	Lymphablation can impair the host's immunity and the response to ICB, worsening OS.	[90]
Colorectal cancer (CRC)	Mouse	TDLN resection post-ICB	Resection of TDLNs can eliminate the anti-tumor effects induced by ICB therapy.	[23]
NSCLC	Human	LN resection pre ICB	Resection of more than 16 LNs was associated with a poor response to immunotherapy.	[91]
Metastatic lung cancer	Mouse	Intact TDLNs, partial resection, or complete resection	Performing a complete LND during tumor removal was detrimental to survival rates in metastatic lung cancer. Partial LND or early administration of aPD-1/aCD40 therapy can improve survival rates.	[94]
MSI-H/dMMR CRC	Human	LN resection	Excessive LND may exert a negative impact on the long-term prognosis of MSI-H/dMMR CRC patients.	[95]

However, most of the therapies remain in the early stages of basic research, and many intriguing ideas will face significant challenges in implementation. On one hand, the lack of sufficient clinical data on the efficacy and safety will greatly hinder the further development and progress of these therapies. On the other hand, the route of administration may lead to varying therapeutic outcomes across different therapies. For instance, it has been observed that local delivery of CD40 agonistic antibodies results in fewer side effects, but this has yet to be thoroughly investigated in other immunotherapies.

Preserving TDLNs for immunotherapy: a promising direction

Immunotherapy aims to initiate an immune response; however, when TDLNs are removed, the critical sites where T cells can survive and be activated are eliminated. This undoubtedly compromises host anti-tumor immunity, thereby eliminating the host's response to immunotherapy. In the era of immuno-oncology, it is high time to consider preserving TDLNs for immunotherapy, which may represent a new direction and trigger new thinking in oncological treatment [87].

In recent years, researchers have increasingly focused on the impact of LND on immunotherapy, with relevant studies in full swing and achieving impressive results (Table 2). An early study reported that LND not only diminished the radiation-induced anti-tumor effects but also contributed to a significant reduction in the proportion of CTLs within the tumor [88]. Liu et al. revealed that in subcutaneous tumor models of mice, bilateral removal of TDLNs significantly weakened the antitumor effects induced by immune-radiotherapy and impaired $CD8^+$ T cell infiltration and cytotoxicity within the tumor [89]. Besides, the polarization of TAMs in the TME depended on the presence of intact bilateral TDLNs. Therefore, maintaining the integrity of bilateral TDLNs is essential for both immunotherapy and radiotherapy. In addition, Saddawi-Konefka et al. found that in head and neck squamous cell carcinoma (HNSCC), lymphablation can abrogate the tumor response to ICB, which was attributed to the loss of conventional type I DCs and type I interferon signaling that were essential for ICB efficacy [90]. Similarly, Fransen et al. reported that the dissection of TDLNs in tumor-bearing mice can eliminate the tumor regression effect induced by ICB [23]. These studies provide compelling evidence for developing TDLN-preserving strategies. Recently, Deng et al. conducted a multi-institution retrospective cohort study, which found that in non-small cell lung cancer (NSCLC) patients, an elevated count (cut-off value: 16) of dissected LN (DLN) was associated with a poorer response to postoperative adjuvant immunotherapy [91]. Notably, preserving LNs with a higher proportion of central memory $CD8^+$ T cells tended to achieve better outcomes in immunotherapy. Therefore, excessive LND can negatively impact the efficacy of immunotherapy for NSCLC, contradicting the common belief that more extensive LND is always better. The implementation of immune-directed TDLN preservation treatments is expected to benefit patient prognosis and requires further validation through long-term studies in the future.

However, TDLNs are significantly heterogeneous and not all TDLNs retain their immune potential as demonstrated in a landmark study on patients with HNSCC, who received ICB before undergoing tumor and LN resection [92]. The researchers reported that in uninvolved regional LNs post-ICB, the abundance of

progenitor-exhausted $CD8^+$ T cells (Tpex) in the T cell zone significantly decreased and localized near DCs, while the proportion of proliferating intermediate-exhausted $CD8^+$ T cells (Tex-int) increased, coinciding with the anti-tumor immune response, but this immune effect was impaired when tumor cells metastasize to LNs. These results highlighted the central role of uninvolved TDLNs in mediating responses to ICB, potentially creating new opportunities for next-generation immunotherapies that focus on harnessing these responses [93]. Therefore, a precise rather than blindly exhaustive LND strategy should be employed, aiming to completely and accurately remove all LN metastases while appropriately preserving the immune potential of the TDLNs.

In the surgical treatment of MIBC, radical cystectomy (RC) concurrent with pelvic lymph node dissection (PLND) following NAC has been the standard-of-care therapy. PLND in BC treatment has gained widespread recognition. Zaffuto et al. investigated the changes in the rate of PLND over recent years, showing a significant increase from 72.3% in 2004 to 85.9% in 2014 [96], indicating broad acceptance of this procedure. Accumulating studies have demonstrated the multifaceted oncological benefits of PLND for BC patients [97, 98]. A study based on the Surveillance, Epidemiology, and End Results (SEER) database analyzed 1,376 BC patients and concluded that the 5-year survival rate of patients who underwent PLND was significantly higher compared to those without PLND [98].

However, 58% of patients who underwent RC and PLND experienced early complications [3], posing significant challenges for the implementation of PLND. In addition, previous studies suggested that extended LND prolonged recurrence-free survival (RFS) as well as reduced metastasis in BC [16, 17, 99]. However, recent clinical trial results have raised doubts about the benefits of extended LND in BC. A clinical trial conducted by Gschwend et al. enrolled 401 patients to investigate the extent of LND on the oncologic outcome of BC and showed that extended LND did not demonstrate a statistically significant advantage compared to limited LND (NCT01215071) [18]. Nevertheless, this trial included T1G3 stage patients with a low LN positivity rate, which may lead to negative trial results. Another randomized phase III clinical trial comparing extended LND with standard LND for disease-free survival (DFS) and OS has recently been completed (NCT01224665), indicating that although extended LND increased the LN detection rate, it did not show a significant advantage in DFS or OS, but may increase perioperative morbidity and mortality [19, 100]. These impressive results make us pause and reconsider the value and significance of extended LND in BC. Furthermore, some studies have confirmed a strong correlation between the count of resected LNs and patient prognosis in BC, recommending the resection of 9 to 16 LNs [18, 101, 102]. The number of LNs resected is not necessarily the more, the better. Disruption of the integrity of the lymphatic system may also lead to complications such as lymph fluid accumulation and lymphatic vessel embolism, further impacting its immune function [103]. Therefore, proper LND is of significant clinical importance for improving patient prognosis and reducing surgical complications. However, no studies report that the survival outcomes of extended LND in BC are associated with the immune dysfunction of TDLNs, which may be a potential direction for future research. In addition, a recent study found that the risk of LN infiltration in MIBC patients who responded completely to NAC was only 3.2%, which was negligible, so pelvic LND (PLND) might be potentially omitted in these patients [104]. Therefore, NAC not only elicits potent anti-tumor immunity in TDLNs but also offers a valuable opportunity to preserve TDLNs.

With the advent of precision medicine, prior to surgery, assessing whether tumor cells have metastasized to the TDLNs is crucial for preserving the immunologically active TDLNs and maximizing their therapeutic potential. More precise diagnostic methods are needed to accurately determine whether TDLN metastasis has occurred. Currently, methods for diagnosing LN metastasis in BC include imaging diagnostics [105], molecular markers in tissue specimens [106, 107], and liquid biopsy [108]. Recently, Wu et al. developed a model for detecting LN metastasis based on artificial intelligence, whose diagnostic sensitivity far surpassed that of pathologists, particularly in identifying micrometastases [109]. It offered advantages such as accuracy, automation, and versatility, holding substantial potential for clinical application. Additionally, there are differences in cell populations among various LNs, contributing to functional heterogeneity and variability in immune responses [110]. The rapidly advancing single-cell multi-omics technologies are expected to be game-changers in the field of immuno-oncology, providing potent tools for deciphering cell heterogeneity, discovering novel cell types, and exploring the mechanisms of tumor metastasis and progression [111]. Thus, it is anticipated that a new personalized TDLN management strategy, in which precisely identifying involved TDLNs through advanced diagnostic methods and preserving healthy TDLNs to activate an immune response, may become a conventional option for future comprehensive oncological treatment.

Conclusions

During BC progression, the TDLN microenvironment undergoes profound changes from anti-tumor immunity to immune evasion. Therefore, mobilizing immune cells within TDLNs to reactivate anti-tumor immunity

is a key goal of current TDLN-targeted immunotherapy, and as a result, multiple immunotherapeutic strategies have been developed. However, TDLNs are removed as a metastatic pathway in tumor procedures. While this can reduce LN metastasis, it may also diminish the effectiveness of immunotherapy. The trade-off between reducing tumor LN metastasis and preserving immune resources in TDLNs needs to be reweighed. Given the therapeutic potential of TDLNs, we need to consider altering the current treatment paradigm and shifting towards TDLN-preserving approaches. A rational combination and sequencing of immunotherapy and standard therapy will trigger robust and sustained immune responses, thereby maximizing tumor control and eradication.

Abbreviations

BC	Bladder cancer
NMBIC	Non-muscle invasive bladder cancer
MBIC	Muscle invasive bladder cancer
LN	Lymph node
TDLNs	Tumor-draining lymph nodes
SLNs	Sentinel lymph nodes
OS	Overall survival
LND	Lymph node dissection
EVs	Extracellular vesicles
HA	Hyaluronic acid
APCs	Antigen-presenting cells
LECs	Lymphatic endothelial cells
lncRNA	Long noncoding RNA
LNMAT-2	Lymph node metastasis-associated transcript 2
hnRNPA2B1	Heterogeneous nuclear ribonucleoprotein A2B1
PROX1	Prospero homeobox 1
H3K4me3	Trimethylation of lysine 4 on histone H3
hnRNPA1	Heterogeneous nuclear ribonucleoprotein A1
VEGF-C	Vascular endothelial growth factor-C
VEGFR3	VEGF receptor 3
ELNAT1	EV-mediated LN-associated transcript 1
UBC9	Ubiquitin carrier protein 9
SUMOylation	Small ubiquitin-like modifier binding
CAFs	Cancer-associated fibroblasts
Tc1	Type 1 $CD8^+$ T cells
MHC-I	Major histocompatibility complex class I
Tregs	Regulatory T cells
GM-CSF	Granulocyte-macrophage-colony-stimulating factor
NK cell	Natural killer cell
TAMs	Tumor-associated macrophages
EMT	Epithelial-mesenchymal transition
DCs	Dendritic cells
IDO	Indoleamine 2,3-dioxygenase
BCG	Bacillus Calmette-Guerin
HP-NAP	Helicobacter pylori neutrophil activating protein
Th1	T helper 1
CTLs	Cytotoxic T lymphocytes
TME	Tumor microenvironment
AC	Antrodia camphorate
siRNA	Small interfering RNA
ACT	Adoptive cell therapy
TILs	Tumor-infiltrating lymphocytes
ICB	Immune checkpoint blockade
TNFR	Tumor necrosis factor receptor
CD40L	CD40 ligand
AdCD40L	Adenoviral vectors expressing CD40 ligand
NAC	Neoadjuvant chemotherapy
HNSCC	Head and neck squamous cell carcinoma
NSCLC	Non-small cell lung cancer
DLNs	Dissected LNs
Tpex	Progenitor-exhausted $CD8^+$ T cells
Tex-int	Intermediate-exhausted $CD8^+$ T cells
CRC	Colorectal cancer
RC	Radical cystectomy
PLND	Pelvic lymph node dissection
SEER	Surveillance, Epidemiology, and End Results
DFS	Disease-free survival

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Y.R., H.Q.G., and T.H.L. acquired the funding. X.Y., X.Y.W. wrote this paper and drew the figures. X.Y.H. collected the references and reviewed the article. Each author reviewed the final version of the article before giving their approval for publication. X.Y. and X.Y.W. made equal contributions to the manuscript.

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Declarations

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