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EDITORIAL

# Roles of cancer-associated fibroblast functional heterogeneity in shaping the lymphatic metastatic landscape: new insights and therapeutic strategies

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Lymph node (LN) metastasis is a process in which cancer cells travel from primary tumors to LNs *via* the lymphatic system, then proliferate and spread within the LNs. In most cancers, LN metastasis is a major mode of cancer dissemination, and a critical indicator of cancer progression and worsening prognosis[1]. The occurrence of LN metastasis indicates that the tumor has invaded the lymphatic system, thus markedly increasing the risk of further dissemination to distant body parts. This metastasis often requires invasive treatments, such as radiotherapy, chemotherapy and immunotherapy. Because cancer cells can spread *via* lymph fluid to other LNs or organs and shape an immunosuppressive microenvironment, the presence of LN metastasis affects the efficacy of immunotherapy. Moreover, LN metastasis is generally accompanied by series of symptoms that decrease patient quality of life, such as swelling, pain, and lymphedema. Therefore, exploring the molecular mechanisms underlying LN metastasis and developing new therapeutic targets based on these findings are crucial for improving patient outcomes.

Emerging evidence indicates that the shaping of the tumor microenvironment (TME) through the orchestration of numerous molecular events is crucial for tumor progression. Changes in the TME provide critical biochemical and biophysical cues, and mechanosensitive signaling, that alter stromal cellular adhesion and migration, and drive subsequent tumor metastasis[2]. Cancer-associated fibroblasts (CAFs) are functionally activated fibroblasts that extensively infiltrate the TME. CAFs play crucial roles in shaping the metastatic TME to favor tumor LN metastasis, by directly interacting with other cell types, secreting various cytokines *via* paracrine signaling, and producing exosomes containing biological molecules. CAFs are highly heterogeneous cells, and recent advancements in single-cell RNA sequencing (scRNA-seq) have partially elucidated this functional heterogeneity. On the basis of their secreted spectrum and marker gene expression, numerous CAF subsets have been discovered and shown to be involved in tumor metastasis by creating a metastatic landscape. The biological roles and mechanisms of CAF subpopulations in shaping the landscape of lymphatic metastasis markedly differ across tumor types. Importantly, therapeutic strategies designed to target different CAF subsets have exhibited high efficiency in inhibiting tumor LN metastasis in animal models[3]. In this editorial, we review current understanding of the molecular mechanisms through which CAF subsets shape the tumor lymphatic metastatic microenvironment and consequently induce tumor LN metastasis (**Figure 1**). We additionally discuss related targeted therapeutic strategies.

## Roles of CAF subsets in shaping the lymphatic metastatic landscape through physical interactions

Specific CAF subsets co-localize with other stromal cells in the TME and shape the lymphatic metastatic microenvironment

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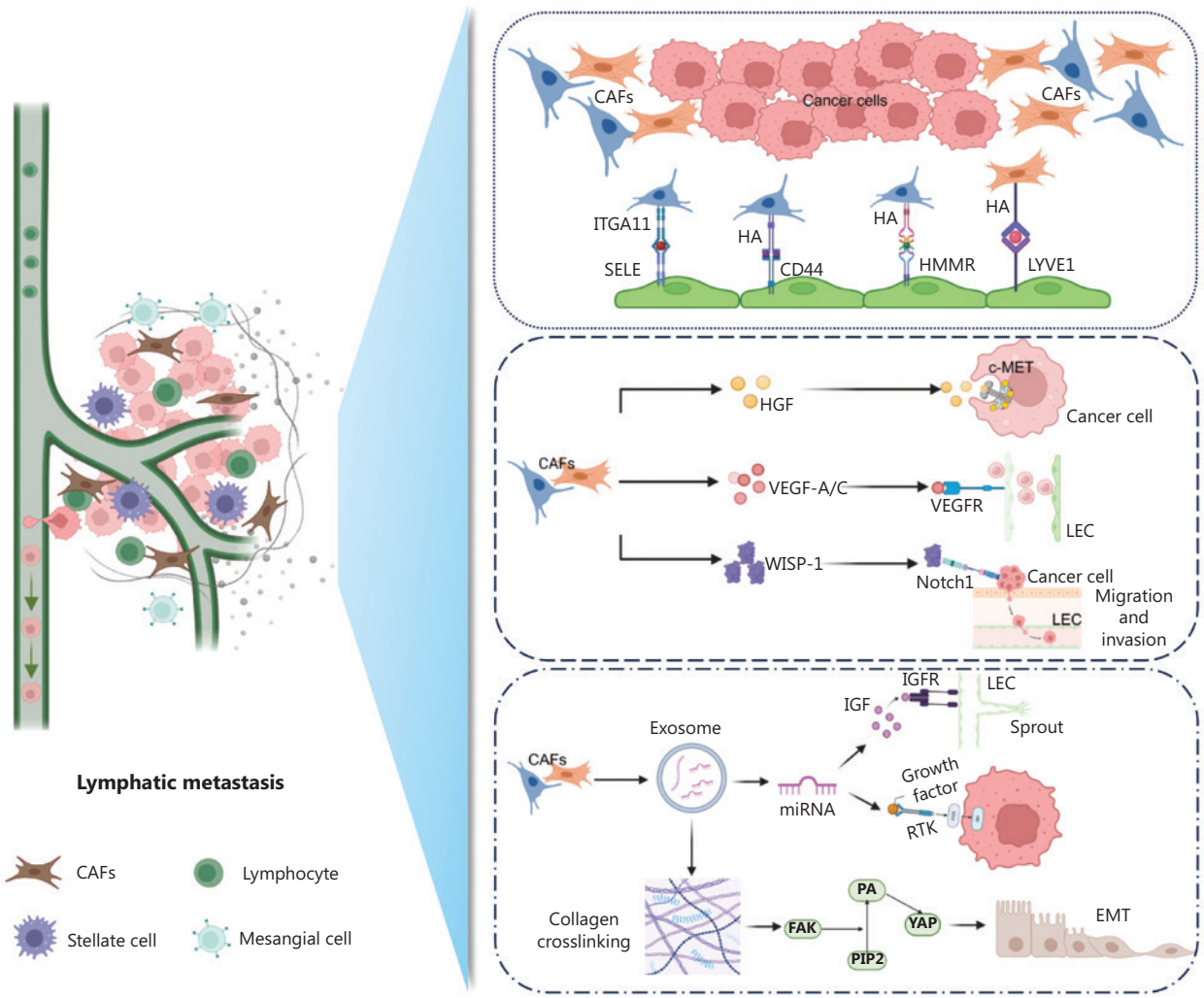


**Figure 1** Roles of CAFs in shaping the lymphatic metastatic landscape. Schematic illustration of various mechanisms through which CAF subsets shape the lymphatic metastatic landscape, including the physical interactions between CAF subsets and stromal cells, the stimulation of paracrine signaling *via* various cytokines, and the secretion of exosomes containing functional biological molecules.

through physical interactions with surface membrane proteins on other stromal cells. Zheng et al.[4] have reported that a CAF subset characterized by platelet derived growth factor receptor α (PDGFRα) and integrin α11 (ITGA11) directly interacts with lymphatic endothelial cells. Subsequently, a lymphatic metastatic landscape is induced, and lymphovascular invasion (LVI) and lymphatic metastasis of early-stage bladder cancer (BCa) are promoted. First, the authors used scRNA-seq to uncover CAF heterogeneity in early-stage BCa, and identified high infiltration of PDGFRα$^+$ITGA11$^+$ CAFs in LVI positive early-stage BCa. Further validation in a multicenter clinical cohort of 910 BCa cases indicated a correlation between PDGFRα$^+$ITGA11$^+$ CAFs and both LN metastasis and poor patient prognosis. Through spatial transcriptome sequencing, the authors found that PDGFRα$^+$ITGA11$^+$ CAFs are distributed primarily around newly formed lymphatic vessels and closely interact with lymphatic endothelial cells. Moreover, the recognition and binding of ITGA11 on PDGFRα$^+$ITGA11$^+$ CAFs to its receptor selectin E (SELE) on lymphatic endothelial cells was found to contribute to the attachment and physical interaction of PDGFRα$^+$ITGA11$^+$ CAFs to lymphatic endothelial cells in BCa. The ITGA11-SELE interaction activates the mitogen-activated protein kinase signaling pathway in lymphatic endothelial cells, thereby inducing a lymphatic

metastatic landscape characterized by uncontrolled lymphangiogenesis, and consequently promoting LVI formation and LN metastasis in BCa.

Recently, the collective invasion of tumor cells has been recognized as the common manner for cancer cell invasion during lymphatic metastasis. Physical interactions of CAF subsets with cancer cells have been found to enhance the collective invasion and metastasis of cancer cells. Labernadie et al. have demonstrated that direct interaction between N-cadherin and E-cadherin is crucial for the adhesion of the CAF subset to spheroids containing cancer cells, subsequent recruitment of β-catenin, and collective cancer cell invasion[5]. Yang et al.[6] have revealed that endosialin positive CAFs interact with macrophages *via* endosialin and CD68, thereby upregulating growth arrest specific 6 (GAS6) expression and macrophage polarization in hepatocellular carcinoma.

The direct physical interactions between cells provide favorable targets for the research and development of targeted drugs. However, currently, few targeted drugs are available for CAF surface proteins. This aspect requires further research in the future. In addition, the development of spatial combined scRNA-seq technology has provided a powerful technical means for studying the direct interactions between CAF subsets and stromal cells. Further identification of specific CAF subsets and their functional mechanisms through spatial combined scRNA-seq may be another major research direction.

## Roles of CAF subsets in shaping the lymphatic metastatic landscape through paracrine signaling

Beyond physical interactions, CAF secrete many cytokines *via* paracrine signaling that in turn shape the lymphatic metastatic microenvironment. A CAF subset, α-SMA$^+$ CAFs, has been found to be highly enriched in LN metastasis positive BCa tissues, and to be associated with lymphatic metastasis and poor prognosis among patients[7]. Molecular mechanism studies have revealed that α-SMA$^+$ CAFs induce lymphangiogenesis, thereby shaping the lymphatic metastatic landscape by secreting hepatocyte growth factor (HGF) and ultimately promoting BCa lymphatic metastasis. Another study by Cadamuro and colleagues has investigated the mechanisms of lymphatic metastasis in cholangiocarcinoma and explored the roles of CAFs in this process[8]. CAFs are closely adjacent to lymphatic endothelial cells and secrete vascular endothelial growth factor (VEGF)-A and VEGF-C, which in turn stimulate VEGF receptor (VEGFR) 2 and VEGFR3 on lymphatic endothelial cells, thus increasing lymphatic endothelial cell monolayer permeability and lymphangiogenesis, and enhancing LN metastasis of cholangiocarcinoma. In an animal model, navitoclax-induced CAF depletion has been found to suppress lymphatic vascularization and to inhibit lymphatic metastasis in cholangiocarcinoma.

LN metastasis is a multistep biological process. Beyond the lymphatic network extension, changes in tumor cell behavior to acquire invasion and cloning ability in LNs are also crucial for tumor lymphatic metastasis. Increasing studies are providing new insights into the mechanisms through which CAFs secrete a variety of bioactive molecules promoting tumor cell growth and invasion. A FAP$^{High}$ CD29$^{Med\text{-}High}$ αSMA$^{High}$ PDPN$^{High}$ PDGFRβ$^{High}$ CAF subset in breast cancer has been found to enhance lymphatic metastasis by sustaining tumor cell invasion[9]. Moreover, CAFs derived from colorectal cancer (CRC) have been found to facilitate the adhesion and migration of CRC cells, both *in vitro* and *in vivo*, by upregulating CD44 expression[10]. This process is enhanced by the secretion of HGF from CAFs, which activates the HGF/c-MET signaling pathway in CRC cells; these findings underscore the critical role of CAF-mediated paracrine signaling in promoting CRC metastasis. Another study has demonstrated that CAFs, after exposure to apoptotic cancer cells, influence tumor behavior through paracrine signaling mechanisms[11]. This finding is evidenced by the ability of conditioned medium from lung CAFs exposed to apoptotic cells to modulate the migration and invasion of cancer cells and CAFs themselves, primarily through the Notch1-WISP-1 signaling pathway. Furthermore, in the context of intrahepatic cholangiocarcinoma, a distinct CAF subset with high hyaluronan synthase 2 (HAS2) expression has been identified to engage in the regulation of intrahepatic cholangiocarcinoma progression. HAS2-expressing CAFs interact with tumor cells, macrophages, and endothelial cells by recognizing HA receptors, including CD44, hyaluronan mediated motility receptor (HMMR), and lymphatic vessel endothelial hyaluronan receptor 1 (LYVE1)[12]. These findings highlight the complex roles of CAFs in the TME, where their interactions with tumor cells influence cancer progression and metastasis, and may suggest potential therapeutic targets in these paracrine pathways. The development of multi-omics technologies may increase understanding of the roles of paracrine signaling in CAF subsets in shaping the lymphatic metastatic landscape.

For example, scRNA-seq can reveal the molecular features of single CAF cells, and bulk-RNA sequencing, flow cytometry, and mass cytometry are crucial for screening of paracrine signaling in CAF subpopulations that shape the lymphatic metastatic landscape.

## Roles of CAF subsets in shaping the lymphatic metastatic landscape through exosome secretion

Exosomes are small extracellular vesicles secreted by various cell types, including immune cells, stem cells, cancer cells, and CAFs. Generally, exosomes from CAFs, immune cells, stem cells, and cancer cells are nano-sized lipid bilayer microvesicles ranging from 30 to 150 nm in diameter. These exosomes play critical roles in intercellular communication by transporting a diverse array of molecules such as proteins, nucleic acids, and lipids from donor cells to recipient cells, thereby influencing various cellular processes such as cell proliferation, differentiation, immune responses, and tissue repair[13]. CAFs secrete exosomes to shape the lymphatic metastatic microenvironment and promote LN metastasis. Chen et al. have shown that CAFs exert their biological effects in shaping the metastatic microenvironment primarily through decreased secretion of exosomal miR-100-5p[14]. Exosomes derived from CAFs have low levels of miR-100-5p, which facilitate the proliferation, migration, invasion, and tube formation of lymphatic endothelial cells. Consequently lymphangiogenesis and LN metastasis are promoted in esophageal squamous cell carcinoma (ESCC) through decreased inhibition of the PI3K/AKT axis.

Exosomes from CAFs also regulate tumor cell biological behaviors. Zhang et al. have revealed that CAFs secrete exosomes containing miR-522, which in turn downregulate ALOX15 expression, decrease lipid-ROS accumulation in tumor cells, and result in chemo-resistance in gastric cancer[15]. In addition, upregulation of miR-20a expression has been shown to facilitate tumorigenesis and metastasis of non-small cell lung cancer[16]. CAFs have been found to deliver miR-20a to tumor cells, thereby inhibiting PTEN expression, activating the PI3K/AKT signaling pathway, and enhancing tumor progression. Furthermore, CAFs release exosomes that preferentially bind the extracellular matrix and induce collagen crosslinking through lysyl oxidase (LOX), thereby promoting epithelial-to-mesenchymal transition in oral squamous cell carcinoma *via* activating the FAK/paxillin/YAP pathway in tumor cells[17]. Extracellular matrix remodeling with high stiffness also mechanically stimulates tumor cells to secrete high levels of fibronectin 1 (FN1) and matrix metalloproteinase 9 (MMP9), thus shaping a metastatic landscape facilitating tumor progression[18]. Among the various roles of CAF-derived exosomes in stimulating tumor metastasis, the precise mechanisms controlling the targeting of these exosomes to different cells are unclear and worthy of further investigation.

## Treatment applications of targeting CAF subsets

Emerging studies indicate that CAF subsets play important roles in tumor progression, and the development of tumor treatment strategies targeting CAF subsets is a major current research topic. Targeting specific subpopulations of CAFs is a critical avenue in cancer therapy, particularly for addressing tumor progression and metastasis. However, most clinical trials of treatment strategies targeting CAFs have ended in failure or even the progression of cancer due to the heterogeneity in CAFs. Therefore, comprehensive exploration of the roles of CAF functional heterogeneity in shaping the lymphatic metastatic landscape, and identification of the underlying crucial CAF subsets, might promote the development of novel therapeutic strategies targeting CAF subsets to block tumor LN metastasis. Recent advancements have enabled the classification of CAF subtypes according to distinct molecular signatures, the expression of surface markers including FAP and α-SMA, and different functional roles within the TME. These classifications have facilitated the development of targeted therapies aimed at disrupting the pathological interactions between CAFs and tumor cells. For example, on the basis of the important role of PDGFRα$^+$ITGA11$^+$ CAFs in promoting LVI and LN metastasis of early-stage BCa, Zheng et al.[4] have developed ITGA11 and CHI3L1 neutralizing antibodies that inhibit PDGFRα$^+$ITGA11$^+$ CAFs. These antibodies have shown high efficiency in suppressing the formation of LVI and LN metastasis in a patient-derived xenograft model. Moreover, inhibitors specifically targeting FAP-expressing CAFs have shown potential in preclinical models, by altering the immunosuppressive and pro-tumorigenic conditions fostered by these fibroblasts[19]. Additionally, therapeutic strategies that modulate signaling pathways, such as PDGFβ–PDGFRβ and HGF/c-MET, which are commonly activated in CAFs, have been explored to inhibit tumor-promoting effects[20]. However,

the specificity and safety of these interventions remain critical challenges, because indiscriminate targeting of fibroblasts could potentially disrupt normal tissue architecture and repair mechanisms. Ongoing studies continue to focus on refining therapeutic strategies to selectively target cancer-promoting CAFs, while minimizing adverse effects and improving clinical outcomes in cancer patients.

## Conclusions and future perspectives

In this editorial, we summarized current understanding of molecular mechanisms and therapeutic strategies for targeting the tumor lymphatic metastatic microenvironment shaped by CAFs in the TME. Nevertheless, several aspects related to the roles of CAFs, the primary stromal cells and functional components within the TME, in cancer progression remain to be resolved. First, the identification of more specific biomarkers for CAF subtypes associated with tumor lymphatic metastasis requires further exploration. Second, the origins of different CAF subsets and the developmental relationships between them must be elucidated. Additionally, current therapeutic approaches targeting CAFs lack specificity and often result in various adverse effects, thereby necessitating the development of more precise targeting strategies. Finally, translating basic research into clinical applications remains a major challenge, thus highlighting the need for better research models that accurately simulate the conditions in humans.

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## Conflict of interest statement

The authors declare no competing interests.

## Author contributions

Conceived and designed the analysis: Tianxin Lin, Changhao Chen. Wrote the paper: Hanhao Zheng, Daiyin Liu, Zhicong Liu, Mingjie An, Yuming Luo.

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