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RESEARCH HIGHLIGHT

Lymphatic metastasis in non-small cell lung cancer: recent discoveries and novel therapeutic targets

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1 | LYMPHATIC METASTASIS AND LYMPHANGIOGENESIS IN NON-SMALL CELL LUNG CANCER

Lung cancer is the 2nd most common and 1st deadliest cancer worldwide [1]. It presents an enormous burden on society in China, because of its high incidence and mortality rate [2, 3]. About 80%-85% of lung cancers are non-small cell lung cancer (NSCLC). Lymphatic metastasis is a critical event in disease progression that influences clinical treatment and determines the prognosis for NSCLC [4].

Unlike the tightly joined smooth muscle cells and pericytes that make up the walls of blood vessels, lymphatic endothelial cells (LECs) of lymphatic capillaries loosely overlap due to the lack of a complete basement membrane. Due to the high interstitial pressure in tumor tissues and the loose structure of lymphatic capillaries, initial lymphatics are considered the most accessible route for tumor metastasis [5]. Tumors were found to acquire the characteristics of lymphatic metastasis mainly in two ways. One of them is that tumor cells passively enter the lymphatic system through the existing lymphatic vessels. Another is that tumor cells actively mediate the remodeling of the lymphatic system to escape from the primary site, and lymphangiogenesis plays a pivotal role in this lymphatic metastasis way [6].

Lymphangiogenesis has been observed in several cancer types, including NSCLC. It is an indispensable procedure involving lymphatic metastasis in NSCLC progression. Here, we outlined current understanding of the dysregulation of lymphangiogenic factors in NSCLC and discussed potential therapeutic strategies that target these factors during NSCLC progression.

List of abbreviations: NSCLC, non-small cell lung cancer; LEC, lymphatic endothelial cell; VEGF, vascular endothelial growth factor; VEGFR, vascular endothelial growth factor receptor; PGDH, prostaglandin dehydrogenase; CCBE-1, collagen and calcium-binding EGF domain-1; CCR7, C-C motif chemokine receptor 7; CCL21, C-C motif chemokine ligand 21; CXCR4, C-X-C motif chemokine receptor 4; IL, interleukin; EV, extracellular vesicle; AGAP2-AS1, AGAP2 antisense RNA 1; sVEGFR-2, soluble vascular endothelial growth factor receptor-2; miRNA, microRNA; MMP-2, matrix metalloproteinase-2; MMP-9, matrix metalloproteinase-9; PTEN, phosphatase and tensin homolog.

2 | MOLECULAR MECHANISMS OF LYMPHATIC METASTASIS AND LYMPHANGIOGENESIS IN NON-SMALL CELL LUNG CANCER

Several molecular mechanisms of lymphatic metastasis have been reported in lung cancer. Here, we highlighted some growth factors, inflammation factors, and extracellu-

lar vesicle components associated with NSCLC lymphatic metastasis and tumor-associated lymphangiogenesis.

The members of the vascular endothelial growth factor (VEGF) family are not only key regulators of tumor angiogenesis but also key mediators of tumor lymphangiogenesis. The VEGF-C/vascular endothelial growth factor receptor (VEGFR)-3 and VEGF-D/VEGFR-3 axes are the main drivers of tumor lymphangiogenesis [7]. In addition, some molecules can also play important roles in tumor lymphangiogenesis by regulating VEGF-C/VEGFR-3 or VEGF-D/VEGFR-3 axes. For instance, prostaglandin dehydrogenase (PGDH) can also enhance VEGF-D-mediated lymphangiogenesis by increasing prostaglandin synthesis [8]. Moreover, collagen and calcium-binding EGF domain-1 (CCBE-1) can enhance the proteolysis of VEGF-C to promote tube formation and migration of LECs [9]. Therefore, VEGF-C/D acts as a primary hub of tumor lymphangiogenesis. Other lymphangiogenic factors have also been identified currently, including fibroblast growth factor [10], platelet-derived growth factor [11], hepatocyte growth factor [12], epidermal growth factor [13], angiopoietins [14] and so on.

Apart from the lymphangiogenic factors mentioned above, some inflammatory factors, such as chemokines, cytokines and their receptors, also serve essential functions in the lymphatic metastasis of lung cancer. C-C motif chemokine receptor 7 (CCR7) is known to promote lymphatic metastasis of NSCLC cells. Sun *et al.* [15] found a strong positive correlation between lymphangiogenic factor VEGF-D and C-C motif chemokine ligand 21 (CCL21)/CCR7 chemokine axis. They found that CCL21/CCR7 drives lymphangiogenesis in NSCLC by inducing VEGF-D up-regulation via ERK/Akt pathway. In addition, Feng *et al.* [16] specifically knockdown the expression of VEGF-C in NSCLC cell lines and found that C-X-C motif chemokine receptor 4 (CXCR4) and CCR7 were involved in NSCLC progression by promoting lymphangiogenesis. By comparing the highly metastatic tumors to low metastatic counterparts, Watari *et al.* [17] found that interleukin (IL)-1α-driven lymphangiogenesis provided a pre-metastatic niche favorable for lymphatic metastasis through cross-talk with tumor-associated macrophages.

Secreted factors have long been recognized as key regulators of lymphangiogenesis. With the discovery of the emerging roles of extracellular vesicles (EVs) in cancer biology, especially in tumor angiogenesis, EVs are gradually becoming the focal point of studies related to tumor lymphangiogenesis. EV-microRNAs (miRNAs) have proved to be key diagnostic and prognostic biomarkers of lymphatic metastasis in lung cancer. For instance, the overexpression of EV-miR-106b in serum was frequently observed in NSCLC patients with lymphatic metastasis. It promotes lymphatic metastasis by upregulating the expression of matrix metalloproteinase-2/matrix metalloproteinase-9 (MMP-2/MMP-9) and downregulating phosphatase and tensin homolog (PTEN) [18]. Additionally, EV-miR-378 upregulation has been reported to indicate lymphatic metastasis in NSCLC [19]. EV-miR-203a-3p was also shown to be significantly increased in lymph node-positive NSCLC patients [20]. Moreover, uregulated EV-derived circular RNAs and long non-coding RNAs, such as circR-0056285 and AGAP2 antisense RNA 1 (AGAP2-AS1), have been explored to diagnose lymphatic metastasis in NSCLC [21, 22]. These findings indicated the potency of EVs as tools for understanding the pathology and improving clinical management of lung cancer.

3 | TARGETED THERAPY AGAINST LYMPHATIC METASTASIS IN NON-SMALL CELL LUNG CANCER

The systemic treatment for lymph node-positive NSCLC patients has dramatically changed in the last decade. In addition to chemotherapy, targeted treatment and immunotherapy have been widely used in lymph node-positive NSCLC. Although these treatment options were shown to greatly impact the short-term survival of NSCLC, the low response rate and easy development of drug resistance seriously affect the long-term survival of patients with NSCLC. Targeted treatment specific to NSCLC lymphangiogenesis and lymphatic metastasis have received great attentions.

To date, anti-lymphangiogenic therapy of NSCLC is still in the initial stage. Qin *et al.* [23] retrospectively analyzed the incidence of new metastatic lesions in advanced NSCLC patients treated with anlotinib, a receptor tyrosine kinase inhibitor. They found that anlotinib significantly suppressed new metastatic lesion formation in patients with advanced NSCLC (new metastatic lesion rate: 18.18% in anlotinib arm vs. 31.82% in the placebo arm). Their study further revealed a critical role for anlotinib in lymphangiogenesis and lymphatic metastasis in NSCLC mouse models. They reported that anlotinib inhibited human LECs growth, migration, and lymphangiogenesis both in vitro and in vivo by disrupting the phosphorylation of VEGFR-3. This study provides new insights into the promising therapeutic potential of anlotinib in the treatment aimed at lymphatic metastasis in NSCLC.

Although there is a lack of treatment data on NSCLC, some factors may be promising targets for lymph node-positive NSCLC. The introduction of soluble VEGFR-2 (sVEGFR-2), one of the receptors of the VEGF family, inhibited lymphangiogenesis and suppressed lymphatic metastasis in NSCLC mouse models by inhibiting VEGF-C

[24]. In recent years, inhibitors targeting other molecules are also being investigated in the lymphangiogenesis of NSCLC. Itraconazole, a potent inhibitor of endothelial cell proliferation, was reported to play a key role in NSCLC mice with malignant pleural effusion by suppressing lymphangiogenesis, demonstrating the potential of itraconazole in treating patients with lymph node-positive NSCLC [25]. However, more clinical trials are warranted to confirm its efficacy in NSCLC.

4 | CONCLUSION AND FUTURE PERSPECTIVES

Lymphatic metastasis is a key prognostic factor in NSCLC. The treatment of lymph node-positive NSCLC patients is important in clinical practice. Current studies focused on the molecular mechanisms of lymphatic metastasis have greatly improved the discovery of therapeutic targets. Future studies will focus on investigating more precise drugs aimed at lymphatic metastasis in NSCLC and identifying predictive biomarkers for lymph node-positive NSCLC.

Targeted therapy aimed at lymphatic metastasis in lung cancer is still in its infancy. Existing inhibitors of lymphangiogenesis remain to be verified in lung cancer. Nanoparticles targeting key exosome components will become novel and promising therapeutic strategies in lung cancer. As more and more therapeutic targets being discovered, the therapeutic effects of targeted drugs on lymphatic metastasis in lung cancer are worth further investigation.

DECLARATIONS

AUTHOR CONTRIBUTIONS

All authors contributed substantially to the conception of this work and drafted the article. All authors read and approved the final manuscript.

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COMPETING INTERESTS

The author declares no competing interests.

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