Lymphangiogenesis and Lung Cancer

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THE LYMPHATICS: NEW TOOLS AND NEW RESEARCH AVENUES

The lymphatic vasculature maintains tissue homeostasis and sustains immune function by guiding leukocytes and activated antigen presenting cells toward the lymph nodes. The lymphatic system is also relevant in cancer progression because cancer cells frequently spread out of the original tumor through the lymphatic capillaries.[1] The detection of cancer cells in lymphatic vessels and regional lymph nodes is a key criterion in the staging of many human tumors, including lung cancer, and is used as decisive element for therapeutic intervention.[2] Although the clinical relevance of lymph node involvement is well known, the molecular mechanisms by which tumor cells spread via lymphatic vessels are less understood. In fact, the study of the lymphatic vasculature has long been hampered because of a lack of lymphatic-specific markers. Enormous progress has occurred in this field during the past decade through the identification of specific lymphatic endothelial cell markers such as VEGFR3, the receptor for vascular endothelial growth factors C and D, the hyaluronan receptor LYVE-1, or the mucin-like transmembrane protein podoplanin.[3] The availability of these markers has allowed the quantification of lymphatic vessel density in tumor biopsies and the isolation of lymphatic endothelial cells for in vitro studies.

LYMPHANGIOGENESIS AND CANCER: MOLECULAR REGULATORY PATHWAYS

Although the hemangiogenic activity of tumor cells was established decades ago,[4] relatively recent data have shown that tumors are also able to induce lymphangiogenesis through the production of pro-lymphangiogenic factors such as VEGF-C.[3] What is more relevant, a great number of clinicopathological studies show good correlation between lymphatic vessel density in human tumors and poor prognosis.[1] In fact, consensus criteria for the immunohistochemical evaluation of lymphangiogenesis in solid tumors have been recently proposed (Figure 1).[5]

The information gathered so far about the mechanisms involved in the regulation of lymphatic vessel growth shows that there are overlapping molecular players shared between hemangiogenesis and lymphangiogenesis. The main regulatory pathway specific for lymphatic endothelial cells is the axis formed by VEGFR-3 (also called Flt-4) and its ligands: VEGF-C and VEGFC-D. VEGFR3 was the first specific lymphatic endothelial cell marker discovered and shows high structural homology with VEGFR2, which is more directly involved in vascular angiogenesis.[6] VEGF-C and VEGF-D are secreted as immature pro-peptides and are proteolyzed by extracellular convertases.[7] Their processing increases their affinity for VEGFR3 and (less efficiently) for VEGFR2. Ligand binding induces VEGFR3 tyrosine auto-phosphorylation and the recruitment of intracellular signaling proteins, which promote lymphatic endothelial cell proliferation, migration, and survival.[7] Therefore, the expression and activation of the VEGFC-D/VEGFR3 pathway seem to be a key molecular link between cancer and lymphangiogenesis, making it a good target to prevent the metastatic spread of solid tumors. Nevertheless, newly published data suggest that the picture is far more complex. The identification of co-receptors for VEGF-C and VEGF-D such as neuropilin-2,[8] or the β1 and α9-integrins, suggests the existence of more intricate signaling pathways.[9] Recent in vivo studies have shown that the hemangiogenic factor VEGF-A also induces lymphangiogenesis and promotes tumor metastasis.[10] Furthermore, other growth factors such as angiopoietin, fibroblast growth factor 2, platelet-derived growth factor B, and hepatocyte growth factor[11] stimulate lymphatic vessel growth, suggesting that we have only spotted the tip of a complicated regulatory process.

Studies performed in animal models strongly suggest that tumor secreted VEGF-C is also capable of stimulating the motility and the ability of cancer cells to cross the lymphatic endothelial wall.[12] Nevertheless, there is still some degree of controversy regarding the relevance of either peritumoral and/or intratumoral lymphatic vessels in solid tumors. On one hand, several findings suggest that the functional lymphatics located in the tumor margin alone are sufficient for lymphatic metastasis.[13] On the other hand, increasing experimental evidence shows the existence of intratumoral lymphatics and suggests their association with poor survival.[14] The question of intra- versus peritumoral localization is interesting when evaluating tumors arising from organs, such as the lung, with heterogeneous lymphatic distribution. In the lung, the lymphatic vasculature extends as far as the bronchioles but fails to reach the alveoli.[15]

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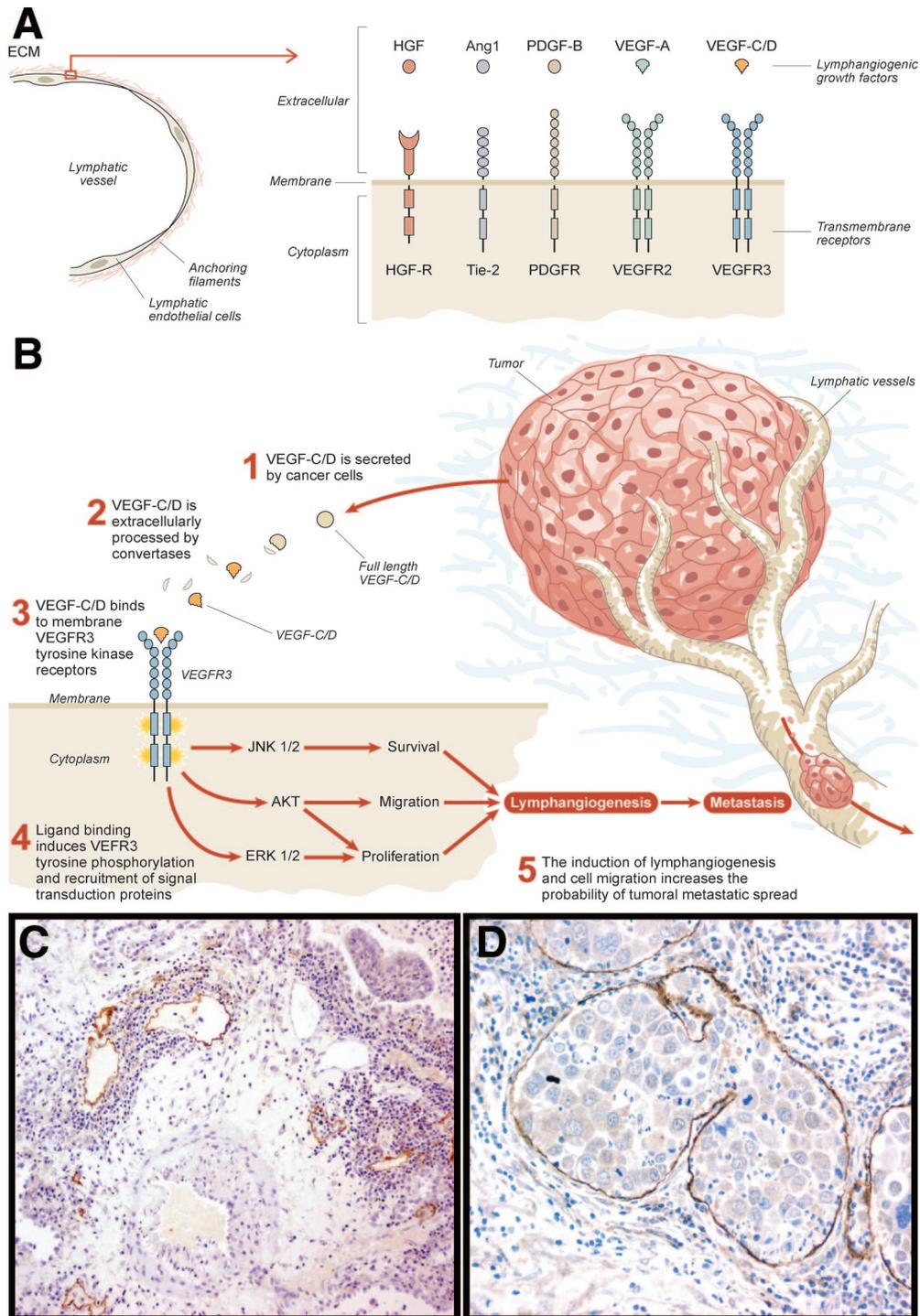
FIGURE 1. *A*, Endothelial growth factors and receptors involved in lymphatic endothelial cell growth signaling. *B*, Binding of VEGF-C/D to VEGFR3 leads to its phosphorylation and to the activation of proliferation, migration, and survival pathways. *C*, *D*, Immunohistochemical staining of lymphatic vessels using anti-podoplanin antibody in human lung adenocarcinoma samples. *Left*, Tumor marginal lymphatic vessels. *Right*, Adenocarcinoma cells within the lumen of a lymphatic vessel.

LYMPHANGIOGENESIS IN LUNG CANCER

Expression studies demonstrated that the levels of VEGF-C and VEGF-D mRNA and protein are elevated in small cell lung cancer cell lines.[16] Interestingly, the evalua- tion of VEGF-C, VEGF-D, and VEGFR3 expression in clinicopathological samples suggest that they are frequently overexpressed in non-small cell lung cancer (NSCLC) cells. This increase is associated with lower survival rates.[17] More-

over, serum VEGF-C determination in NSCLC has been used as a marker for lymph node metastasis, and its evaluation, along with computed tomographic examination, has been proposed as a complement for accurate lymph node staging.[18] Despite evidence on the presence of an active proliferating network of lymphatic vessels in the invading front of lung adenocarcinomas, a number of questions about lymphangiogenesis in lung cancer remains: among others, the presence of intratumoral lymphatics[19] or the existence of an association between its heterogeneous lymphatic support and the frequency of metastatic spread. Finally, it is not clear to what extent the relevance of lymphatic vasculature in NSCLC changes with histological type, tumor size, or location within the lung.

LYMPHANGIOGENESIS INHIBITION

Several approaches have been used for the therapeutic targeting of tumoral lymphatic growth in preclinical and clinical studies. Some of them used specific antibodies developed against VEGF-C, VEGF-D, or VEGFR3 to neutralize their activity.[20] Alternatively, soluble versions of VEGFR3 receptor have been developed and used in preclinical trials.[20] For example, the overexpression of soluble VEGFR-3 by lung cancer cells reduced tumoral lymphatic vessels and the incidence of metastasis to sentinel lymph nodes in animal models.[21] Besides, some small molecule inhibitors of VEGFR2 kinase activity that have also been shown to inhibit VEGFR3 kinase activity are currently being tested in clinical trials. Examples of these multiple inhibitors are BAY 43-9006 (sorafenib), PTK787/ZK222584 (Vatalanib), CEP-7055, and indolinones such as MAE87 and MAZ51.[22]

The potential clinical benefit of anti-lymphangiogenesis molecular targeted approaches is promising. A combined therapy to block both hemangiogenesis and lymphangiogenesis may even be considered. Nevertheless, some potential adverse effects, mainly in hematopoiesis and tissue edema, are strong reasons to act cautiously when translating them into clinical practice.

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