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Research paper

Study of fluid dynamics reveals direct communications between lymphatic vessels and venous blood vessels at lymph nodes of mice

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ABSTRACT

Cancer cells metastasize to lymph nodes, with distant metastasis resulting in poor prognosis. The role of lymph node metastasis (LNM) in the spread of cancer to distant organs remain incompletely characterized. The visualization of flow dynamics in the lymphatic and blood vessels of MXH10/Mo-*lpr*/*lpr* mice, which develop systemic swelling of lymph nodes up to 10 mm in diameter, has revealed that lymph nodes have the potential to be a direct source of systemic metastasis. However, it is not known whether these fluid dynamics characteristics are universal phenomena present in other strains of laboratory mice. Here we show that the fluid dynamics observed in MXH10/Mo-*lpr*/*lpr* mice are the same as those observed in C57BL/6J, BALB/cAJcl and NOD/ShiJic-*scid*Jcl mice. Furthermore, when fluorescent solution was injected into a tumor-bearing lymph node, the flow dynamics observed in the efferent lymphatic vessels and thoracoepigastric vein depended on the type of tumor cell. Our results indicate that fluid dynamics in the lymphatic and blood vessels of MXH10/Mo-*lpr*/*lpr* mice are generalized phenomena seen in conventional laboratory mice. We anticipate our results can facilitate studies of the progression of lymphatic metastasis to hematogenous metastasis via lymph nodes and the early diagnosis and treatment of LNM.

1. Introduction

Metastasis is a critical event for cancer patients, and lymph node metastasis (LNM) is one of the most important factors predicting systemic or distant metastasis. More than 80% of cancer types have the potential to spread to distant organs through the lymphatic system (Leong et al., 2011). Lymphangiogenesis, the formation of new lymphatic vessels from pre-existing lymphatic vessels, plays an important early role in lymphatic metastasis (Skobe et al., 2001). In the classical view of lymphatic metastasis, metastatic tumor cells that invade lymphatic vessels reach the draining lymph nodes (LNs) near the primary tumor region. After growing in the draining LNs, the tumor cells continue to metastasize sequentially to downstream LNs. Finally, the tumor cells gain entry into the thoracic duct or subclavian vein (SV), resulting in systemic metastasis (Karaman and Detmar, 2014). There are two different theories of LN metastasis that consider the clinical merits of local control of breast cancer, namely systemic theory (Fisher and Fisher, 1966) and spectrum theory (Hellman, 1994). Systemic theory advocates that breast cancer is a systemic disease from the beginning and that LN dissection is useful only for prognostic information and does not affect overall survival (Quiet et al., 1996; Kawada and Taketo, 2011). Spectrum theory suggests that the source of distant metastasis is from not only the metastatic LN but also the primary tumor and that every site can be a source of systemic metastasis (Hellman, 1994). Although these theories advocate the importance of hematogenous routes and the source of the metastatic cells, the role of the metastatic LN is not fully understood. Mouse models of cancer metastasis potentially allow the detailed study of this process from the initiation of tumor development in LNs. However, it is difficult to identify LNs and lymphatic vessels in conventional mice such as BALB/c and SCID mice, because the LNs are only 1–2 mm in diameter and the lymphatic vessels are transparent. Recently, a new mouse model of LNM has been developed in our laboratory (Shao et al., 2013). This model uses MXH10/Mo-*lpr*/*lpr* (MXH10/Mo/lpr) mice that show systemic lymphadenopathy from 8 weeks of age due to accumulation of lpr-T cells (Nose et al., 2013). The axillary region of the MXH10/Mo/lpr mouse contains two LNs, the proper axillary LN (PALN) and the accessory axillary LN (AALN). The AALN and subiliac

Abbreviations: CIV, Common iliac vein; Cor, Cortex; ELV, Efferent lymphatic vessel; IVC, Inferior vena cava; LN, Lymph node; LNM, Lymph node metastasis; LV, Lymphatic vessel; Med, Medulla; MXH10/Mo/lpr, MXH10/Mo-*lpr*/*lpr*; PALN, Proper axillary lymph node; Paracor, Paracortex; SiLN, Subiliac lymph node; SV, Subclavian vein; TEV, Thoracoepigastric vein.

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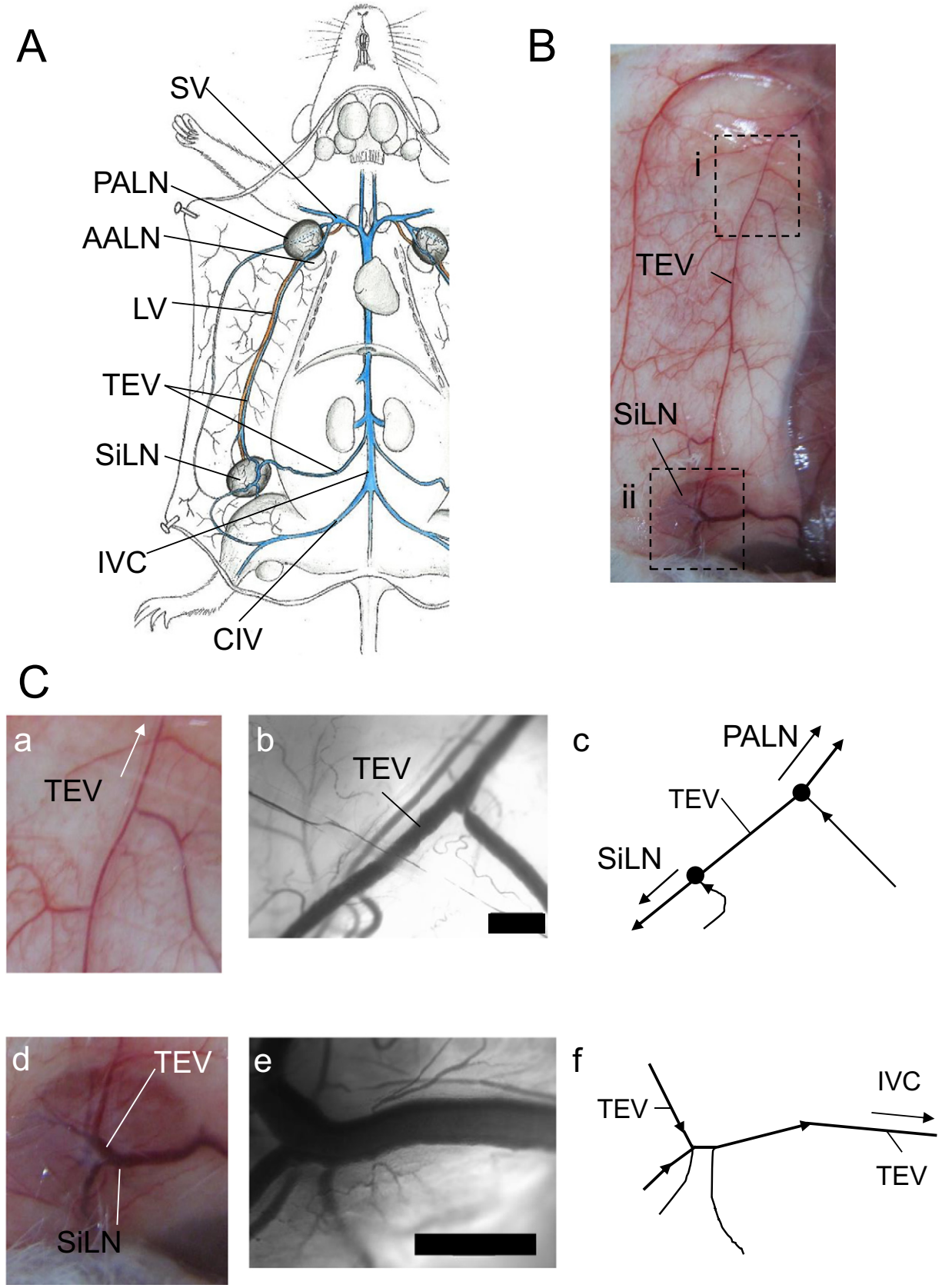

Fig. 1. Lymphatic and blood vascular systems in MXH10/Mo/lpr mice. A. Anatomical drawing illustrating important features of the venous system in an MXH10/Mo/lpr mouse (Shao et al., 2015b). AALN: accessory axillary lymph node, CIV: common iliac vein, IVC: inferior vena cava, LV: lymphatic vessel, PALN: proper axillary lymph node, SiLN: subiliac lymph node, SV: subclavian vein, TEV: thoracoepigastric vein. B. Blood vascular system between the SiLN and PALN in MXH10/Mo/lpr mice. (i) Region containing diverging flow. (ii) TEV over the SiLN. C. Enlarged images of regions (i) and (ii) in B. (a, b, c) Flow dynamics in the section with diverging flow. Two veins are connected to the TEV. Flow in the TEV split into two directions, one toward the PALN and the other toward the SiLN (see Media 1). (d, e, f) Flow dynamics in the region of the TEV over the SiLN. The TEV adjacent to the SiLN connected to the IVC (see Media 2). Scale bar in (b) and (e): 300 μm.