



Lincoln's Inn Fields

Lymphatic Development

www.london-research-institute.org.uk/taijamakinen

Group Leader **Taija Makinen**

Postdoctoral Scientists

Eleni Bazigou
Florence Tatin

Graduate Student

Sophie Lutter

Scientific Officer

Sherry Xie

The lymphatic vasculature constitutes an intricate network of vessels critical for fluid homeostasis, immune surveillance and fat absorption. In cancer, the metastatic tumour cells can exploit the lymphatic vasculature and spread via the lymphatic vessels to the lymph nodes. Our goals are to understand the cellular and molecular processes that form and maintain the lymphatic vessels and to use this knowledge to gain insights into the mechanisms underlying pathological conditions involving lymphatic vasculature, such as tumour metastasis and lymphoedema.

The lymphatic capillaries form a network of blind-ended vessels, which are distributed throughout the tissue spaces in almost all organs. These vessels collect the extravasated tissue fluid and drain via collecting vessels first into lymph nodes and then to larger lymphatic ducts, which connect to the venous system. Skeletal motion, arterial pulsation and respiration cause movements that generate pressure gradients required for the maintenance of fluid flow. In addition, smooth muscles cells around the collecting vessels cause contractions while luminal valves prevent backflow.

Studies using genetically modified mice have recently provided insight into the developmental processes and molecular mechanisms regulating lymphangiogenesis. Vascular endothelial growth factor-C (VEGF-C), acting through its receptor VEGFR-3, has been established as a key regulator of lymphatic endothelial cell proliferation and lymphatic vessel

sprouting needed for the establishment of the embryonic lymphatic vascular network (Figure 1a). This primary vessel network is subsequently remodelled during late embryonic and early postnatal development. Our previous studies have shown that the remodelling processes involve expansion and transformation of a uniformly sized primary capillary plexus into a hierarchical vascular tree, composed of lymphatic capillaries and collecting vessels. This remodelling involves changes in vessel morphology, branching pattern and diameter, and requires the formation of new vessels via sprouting from the pre-existing vasculature, the specification of collecting vessels via recruitment of smooth muscle cells and the formation of luminal valves (Figure 1a). While significant progress has been made in understanding the developmental processes and genes that are controlling the initial development of embryonic lymphatic vessels, lymphatic vessel remodelling and maturation are only beginning to be understood. By using a combination of mouse genetics and *in vitro* studies of primary lymphatic endothelial cells we aim at addressing how the functional network of highly specialised lymphatic vessels is established.

Developing tools for targeting and visualising the lymphatic endothelium *in vivo*

Tissue-specific and inducible methods are necessary to allow functional studies specifically targeting the lymphatic endothelium, however, currently these studies are hampered by lack of suitable mouse models. We have therefore generated transgenic mice that allow gene deletion and/or overexpression specifically in lymphatic endothelial cells *in vivo* using the Cre/loxP recombination system. We are also generating transgenic mouse lines expressing fluorescent reporter proteins specifically in lymphatic endothelia. These mice will be used to visualise lymphatic vessels *in vivo* to gain insights into the processes of developmental as well as tumour-induced lymphangiogenesis and the entry of metastatic cells into the lymphatic vessels.

Collecting lymphatic vessels and lymphoedema

Lymphoedema is a progressive and lifelong condition characterised by gross swelling of the affected limb,

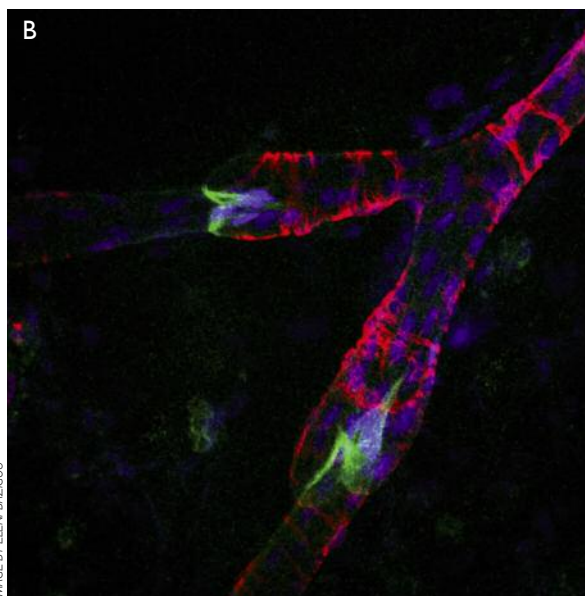
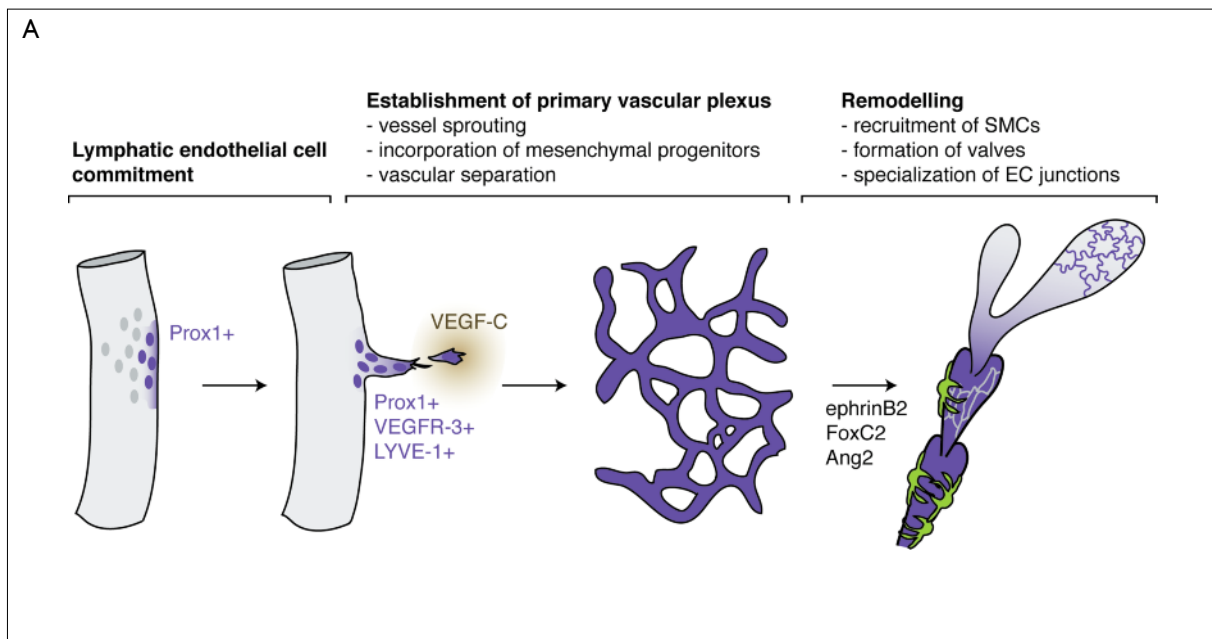


Figure 1. a) Schematic figure of lymphatic vascular development. b) Confocal image showing a luminal valve (green immunofluorescence) of a collecting lymphatic vessel in mouse skin. The nuclei of lymphatic endothelial cells are shown in blue and the smooth muscle cells are stained in red.

accompanied by fibrosis and susceptibility to infections. Currently no effective treatment for lymphoedema exists but the management of symptoms relies on remedial massage and restrictive bandaging. Inherited or genetic mutations, which compromise the development or function of the lymphatic vessels, may subsequently lead to lymphoedema. However, the vast majority of all lymphoedemas is due to damage to the collecting lymphatic vessels, most often caused by surgery or radiation therapy. A recent study, which used a mouse model of axillary lymph node dissection, suggested that growth factor-induced regeneration and maturation of lymphatic vessels might provide a basis for future therapy of lymphoedema (Tammela *et al.*, Nature Med. 2007). However, before such strategies can be efficiently employed, a better understanding of the molecular regulation of collecting lymphatic vessel development and of the pathophysiological mechanisms involved is needed.

Luminal valves present in the collecting vessels (Figure 1b) ensure unidirectional fluid flow and the functionality of the entire lymphatic vascular system, highlighted by the lack or insufficient function of lymphatic valves as one of the underlying causes of human lymphoedema. We found that one member of the integrin-family, integrin- $\alpha 9$, is predominantly expressed in the endothelial cells of the lymphatic valve. Interestingly, integrin- $\alpha 9$ deficient mice displayed specific defects in the formation of luminal valves, which resulted in retrograde lymphatic flow and impaired fluid transport. Together with ephrinB2 and FoxC2, which we (Makinen *et al.*, Genes Dev. 2005) and others (Petrova *et al.*, Nature Med. 2004) have previously shown to play an important functions in lymphatic remodelling, these three genes represent the only known regulators of lymphatic valve morphogenesis to-date. Future research will address the intracellular signalling mechanisms critical for lymphatic valve morphogenesis. Towards this aim we will study ephrinB2 and integrin- $\alpha 9$ -mediated functions in lymphatic endothelial cells via identification and functional characterisation of molecular interactors and downstream signalling pathways.

Publications listed on page 124