The objectives of this thesis were to clarify the distributions of lymph nodes (LNs) and lymph vessels (LVs), the communication between lymphatics and the surrounding veins, and the relationship between cancer metastasis and the lymphatic system in MXH10/Mo/lpr, a lympho-proliferative in-bred mouse, to extrapolate the findings to develop potential new clinical treatments.

In Chapter 1, overviews of cancer metastasis, the lymphatic system and the relationship between them are outlined. An extensive literature review strongly indicated that the lymphatic system facilitates cancer cell dissemination throughout the body, acting in concert with the venous system. It is clear that preclinical models of LN metastasis are indispensable to aid the understanding of the mechanisms involved in tumor cell trafficking, which are fundamental for developing new techniques to diagnose and treat LN metastasis.

However, the identification of LNs and LVs in mice is challenging with conventional imaging modalities, since the LN diameter in normal mice is 1–2 mm. In this thesis, a preclinical model of LN metastasis has been used involving MXH10/Mo/lpr/lpr (MXH10/Mo/lpr) inbred mice, which develop systemic swelling of LNs up to 10 mm in diameter.

In Chapter 2, the aim was to identify LNs and LVs in MXH10/Mo/lpr mice and establish one of the peripheral inter-LN routes that can induce regional lymphatic cancer metastasis. Twenty-two different LNs were found distributed in the peripheral, thoracic and abdominal regions, and 4 peripheral inter-LN vessels were identified, from the subiliac LN (SiLN) to the proper axillary LN (PALN), the parotid LN to the caudal deep cervical LN, and the popliteal LN to both the sciatic LN and the SiLN. Moreover, peripheral regional metastasis was induced by inoculating FM3A/Luc mouse breast cancer cells into the SiLN. This chapter unveils the anatomy of murine lymphatics to give new insights into the investigation of inter-LN metastasis of cancer, especially the mechanisms involved in the trafficking of cancer cells in inter-LN vessels. The lymphatic anatomy data will be useful in the quest for deepening our understanding of cancer cell trafficking and the mechanisms involved in cancer metastasis. The results also show, for the first time, that the MXH10/Mo/lpr mouse strain is an ‘investigator-friendly’ and reliable model of peripheral inter-LN cancer metastasis.

In Chapter 3, the communication pathways between the lymphatic and venous systems are clarified, with a focus on the anatomy of these communication routes in the axillary and subiliac regions. The communication pathways between the lymphatic and venous systems in the axillary and subiliac regions of mice were unequivocally identified. The efferent LVs of the PALN were demonstrated to communicate with the subclavian vein. Furthermore, it was shown that the thoracoepigastric vein (TV), which connects the subclavian vein and inferior vena cava, runs adjacent to the
SiLN and PALN, and receives venous blood from these LNs routed through small branches. The direction of blood flow in the TV occurred in two directions in the intermediate region between the PALN and SiLN; one to the subclavian vein, the other to the inferior vena cava. This research reveals the anatomy of the communication between the lymphatic and venous systems in the axillary and subiliac regions of the mouse, and provides further lymphatic-venous anatomy data relevant to the investigation of the trafficking routes of cancer cells in preclinical mouse models. The bi-directional flow of blood in the TV between the PALN and SiLN gives insight into tumor cell trafficking from regional areas to the whole body. It is proposed that the final form of LN metastasis should be recognized as “LN-mediated hematogenous metastasis” based on lympho-venous communication.

In Chapter 4, the surgical and non-surgical outcomes of an implanted LN tumor were explored, with a focus on regional cancer and distant metastasis. The tumor-bearing SiLN was resected to simulate clinical dissection of the sentinel lymph node (SLN). It was found that resection of a tumor-bearing SiLN enhanced lung metastasis in the mouse model. Bioluminescence imaging revealed that metastatic tumor cells in the down-stream LN continued to grow after the resection of the up-stream tumor-bearing SiLN, and that the probability of metastasis to the lungs was increased when the interval between SiLN inoculation and resection was reduced. Furthermore, histological analysis demonstrated that latent cancer cells in the lungs were stimulated to grow after resection of the SiLN. Fluorescence imaging indicated that the route of tumor cell dissemination from the SiLN to the lungs was via the venous system enveloping the SiLN. This part of the thesis confirmed two trafficking routes in the SiLN region: one towards the PALN via the LV, which is related to PALN lymphogenous metastasis, and the other towards the venous system via the TV, which is related to hematogenous metastasis to distant sites. The resection of a SiLN inoculated with tumor cells led to an accelerated growth of metastatic tumor cells in the lungs and ipsilateral PALN. This result is a timely reminder of the clinical risk of iatrogenic induction of regional and distant cancer metastases. This phenomenon provides new insights into the concept of “LN-mediated hematogenous metastasis” and is the starting point for tracing the activation process of distant dormant cancer cells.

In Chapter 5, it is concluded that the lymphatics can facilitate cancer cell dissemination, and that this is fundamental to the occurrence of cancer metastasis. Presently, two contrasting models are used to explain the formation of distant organ metastases: a lymphatic-independent hematogenous model and a lymphatic-dependent sequential model. The most crucial issue to resolve is whether metastatic cells come to a halt in LNs or continue to disseminate throughout the body by usurping either LN vascular vessels or efferent LVs to colonize distant organs via the blood circulation. To date, the detailed mechanisms involved in this important process remain a mystery. However, there is now little doubt that the interplay between tumors and the lymphatic system represents the main route used by solid cancers to spread.

Based on a study of MXH10/Mo/lpr mice, this dissertation shows the topography of 22 LNs and 4 peripheral lymphatic drainage routes, which can be used to explore peripheral lymphatic cancer metastasis in a preclinical setting. In addition, the thesis has clarified the communications between the lymphatic and venous systems in the axillary and subiliac regions, and revealed that the LV running from the SiLN to the PALN is capable of draining tumor cells. Moreover, the research in this thesis has also shown that lung metastatic foci could be rapidly activated following resection of a tumor-bearing SiLN. It is anticipated that this mouse model will be useful for studying LV imaging, lymphatic trafficking kinetics and the mechanisms of tumor-lymphatic system interplay during both regional and distant metastasis, with the aim of translating this basic research into enhanced clinical diagnosis and novel drug delivery systems.
Dr. Shao did the investigation of lymphatic network and cancer metastasis in Prof. Kodama's laboratory.

Dr. Shao and Prof. Kodama's family.