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Activation of latent metastases in the lung after resection of a metastatic lymph node in a lymph node metastasis mouse model

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ABSTRACT

Iatrogenic induction of regional and distant cancer metastases is a risk associated with clinical resection of tumor-positive sentinel lymph nodes. However, there have been no studies of this risk in a mouse model of cancer metastasis. Here, we report that resection of a tumor-bearing subiliac lymph node (SiLN) enhanced lung metastasis in a mouse model of lymph node metastasis. Bioluminescence imaging revealed that metastatic tumor cells in the secondary lymph node continued to grow after resection of the SiLN, and that the probability of metastasis to the lungs was increased when the interval between SiLN inoculation and resection was reduced. Futhermore, histological analysis demonstrated that latents in the lung were stimulated to grow after resection of the SiLN. Fluorescence imaging indicated that the route of tumor cell dissemination from SiLN to the lung was the venous system located over the SiLN. We speculate that our mouse model will be useful for studying the mechanisms of tumor cell latency, with a view to improving the detection and treatment of latent metastases.

1. Introduction

Lymph node (LN) metastasis is an important prognostic factor for many malignant tumors, including breast, head and neck cancers, and plays an important role in the development of distant metastases to vital organs [1]. The treatment of LN metastasis with LN resection is limited to those patients who can tolerate surgery and where the risk of metastatic foci invading other major organs is minimal [2]. Although the area of dissection is determined by clinicopathological guidelines, dissection alone does not control local recurrence in progressive cases and thus is used together with adjuvant therapy. Although dissection of the primary tumor is beneficial, it may disturb metastatic homeostasis [3], resulting in the activation and rapid growth of latent tumors in distant metastases; this has been suggested to occur for several cancer types including breast [4], lung [5] and head and neck [6] cancers. Recurrence after dissection is a common phenomenon, but the underlying mechanisms are not fully understood. Removal of LNs that have an immunological anti-tumor immune role [7] may facilitate the rapid growth of distant latent metastases. However, there are no reports on the relationship between resection of LNs and the subsequent rapid growth of latent micrometastases, for reasons including the microscopic size of tumors, clinical accessibility and the lack of an experimental model.

We have developed mouse models of LN metastasis using MXH10/Mo-*lpr*/*lpr* (MXH10/Mo/lpr) [8]. These models have been used to map the lymphatic system [8], demonstrate the plausibility of administering local therapy to a metastatic LN [9], and shown that a lymphatic drug delivery system can be used to deliver agents to LNs outwit the dissection area [2].

In our study, MXH10/Mo/lpr mice were used as an experimental model of metastasis to show that dissection of a SiLN inoculated with tumor cells resulted in the rapid growth of latent lung tumors. For previously discussed reasons we used the nomenclature “SiLN” instead of “inguinal LN” [8,10].

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2. Materials and methods

The Institutional Animal Care and Use Committee of Tohoku University approved all *in vivo* studies.

2.1. Mice

MXH10/Mo/lpr mice (16–18 weeks of age) were bred under pathogen-free conditions in the Animal Research Institute, Tohoku University [8]. MXH10/Mo/lpr mice are unique; their most peripheral LNs grow to 10 mm in size at 2.5–3 months of age and the mice do not develop severe autoimmune diseases.

2.2. Cell culture

Malignant fibrous histiocytoma-like KM-Luc/GFP cells, stably expressing a fusion of luciferase and enhanced-green fluorescent protein genes and C3H/He mouse mammary carcinoma cells (FM3A-Luc), stably expressing the Luc gene, were used [8,11]. The relative growth rates of KM-Luc/GFP and FM3A-Luc cells were 3.8/day and 1.1/day, respectively.

2.3. Visualization of flows into the efferent LVs and the thoracoepigastric vein from the SiLN

Two kinds of dyes were used; India ink and 0.5 mM 5(6)-carboxyfluorescein (MW: 376; excitation: 492 nm; emission: 517 nm; Sigma-Aldrich Japan, Tokyo, Japan). The former was used to identify, visually, communication between the lymphatic and venous system via SiLN, while the latter was used to visualize dynamically the flow from the SiLN to the lymphatic and venous system. A 1-mL syringe connected to a 27-gauge standard double-wing needle (Terumo Co., Tokyo, Japan) was filled with the appropriate solution. To gain access to the LNs, a skin incision was performed under general anesthesia. The solution (60 μL) was injected near the center of the SiLN manually (India ink) or at a rate of 100 μL/min using a driven syringe pump (fluorescence solution). Images were obtained immediately after the injections using a stereomicroscope (SZX10, Olympus, Tokyo, Japan) for India ink or a fluorescence stereomicroscope (M165-FC; fluorescent filter: GFP2; excitation: 460–500 nm; emission: >510 nm; Leica, Bensheim, Germany) connected to a high-speed camera (Cool SNAP HQ2; Photometrics, Tokyo, Japan) for fluorescence solution [2].

2.4. Induction of metastasis to the proper axillary LN by injection of tumor cells into the subiliac LN

KM-Luc/GFP and FM3A-Luc cells passaged three times were used. KM-Luc/GFP (1.0×10^6 cells/mL) or FM3A-luc (1.35×10^7 cells/mL) cells were suspended in a mixture of 20 μL phosphate-buffered saline (PBS) and 40 μL of 400 mg/mL Matrigel (Collaborative Biomedical Products). The SiLN was exposed by incision of the overlying skin, and the lymphatic vessels (LVs) connecting the SiLN to the proper axillary LN (PALN) were clamped (DDP-09-151 clamp; Daddy D Pro, Pakistan). Prepared cells were injected into the cortical part of the SiLN using a 27-G injection needle. The needle was maintained in the same position for 5 min to solidify the Matrigel after removal of the needle. Then, the exposed SiLN was irrigated with 20 mL of saline and excess fluid aspirated (M-20 aspirator; Tokyo M.I. Company, Inc., Japan). Thereafter, the clamp was removed. Subsequently, the wound was sutured with 5–0 polyamide sutures.

2.5. Resection of the SiLN

Mice were anesthetized using an inhaled mixture of 2% isoflurane and oxygen. After depilation and skin disinfection, a minimal invasive approach was used for skin incision and exposure

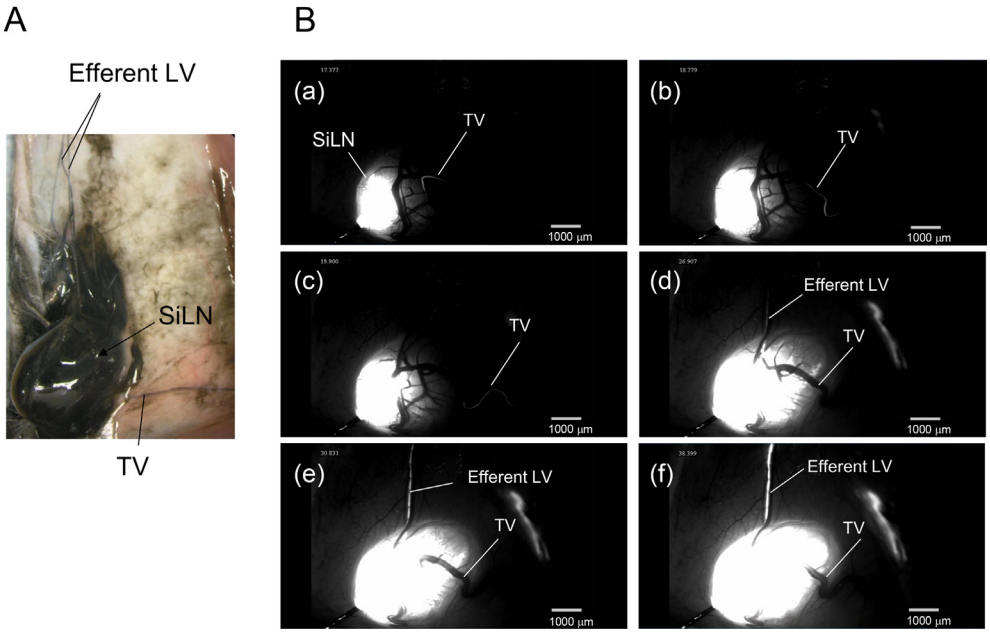

Fig. 1. Communication between the efferent LV and the TV via. SiLN. A. Communication between the efferent LVs and the TV via. SiLN. India ink that was injected into the SiLN flowed into the efferent LVs and the TV. B. Sequential frames from Media 1. Fluorescence solution flowed into the efferent LV and the TV of SiLN. The solution (60 μL) was injected near the center of the SiLN at a velocity of 50 μL/min using a driven syringe pump. (a) 17 s, (b) 19 s, (c) 20 s, (d) 27 s, (e) 31 s, (f) 38 s after the start of recording. Fluorescence solution that was injected into the SiLN flowed into the TV located on the SiLN in (a) and it flowed into the efferent LN in (d). The solution continued to flow from the SiLN into the efferent LVs and TV even in (f).