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● *Original Contribution*

SUPERSELECTIVE DRUG DELIVERY USING DOXORUBICIN-ENCAPSULATED LIPOSOMES AND ULTRASOUND IN A MOUSE MODEL OF LUNG METASTASIS ACTIVATION

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Abstract—Conventional treatment of lymph node metastasis involves dissection of the tumor and regional lymph nodes, but this may cause activation of latent metastatic tumor cells. However, there are few reports on animal models regarding the activation of latent metastatic tumor cells and effective methods of treating activated tumor cells. Here, we report the use of a superselective drug delivery system in a mouse model of lung metastasis in which activated tumor cells are treated with doxorubicin-encapsulated liposomes (DOX-LP) and ultrasound. The axillary lymph node was injected with DOX-LP and exposed to ultrasound so that the released DOX would be delivered from the axillary lymph node to the metastatic lung *via* the subclavian vein, heart and pulmonary artery. The size of the DOX-LP was optimized to a diameter of 460 nm using indocyanine green-encapsulated liposomes, and the ultrasound intensity was 0.5 W/cm^2. We found that compared with DOX or DOX-LP alone, the superselective drug delivery system was effective in the treatment of metastasis in both the lung and axillary lymph node. We anticipate that this superselective drug delivery system will be a starting point for the development of new techniques for treating lung metastasis in the clinical setting. Furthermore, the superselective drug delivery system may be used to screen novel drugs for the treatment of lung cancer and investigate the mechanisms of tumor cell activation after resection of a primary tumor or lymph nodes. (E-mail: kodama@tohoku.ac.jp)

Key Words: **Doxorubicin, Liposomes, Ultrasound, Lung metastasis, Drug delivery system.**

INTRODUCTION

Excision of a primary tumor is beneficial for the local control of cancer, but this procedure risks the promotion of metastasis *via* homeostatic mechanisms (Demicheli et al. 2008). In breast cancer (Tagliabue et al. 2003), head and neck cancer (Sano et al. 2013), lung cancer (Maniwa et al. 1998) and other tumors, tumor activation and rapid growth after resection of the primary lesion have been confirmed by clinical (Braunschweiger et al. 1982) and animal (Fisher et al. 1989; Van Dierendonck et al. 1991) experiments. Lymph node (LN) dissection is recommended in many cancer therapy guidelines, but this can induce the activation of latent tumor in distant organs (White et al. 2002). However, there is a paucity of data on the activation of tumor cells in distant organs caused by LN dissection, mainly because of the lack of suitable animal models. Our research group has developed a mouse model of lung metastasis activation using MXH10/Mo-*lpr/lpr* (MXH10/Mo/lpr) mice (Shao et al. 2015a). In this model, tumor cells are inoculated into the subiliac LN (SiLN) so that they are delivered to the proper axillary LN (PALN) *via* lymphatic vessels and to the lung *via* blood vessels. Metastatic tumor cells in the PALN continue to grow after resection of the SiLN, and the probability of metastasis to the lung is increased in the interval between SiLN inoculation and resection. In the present study, we report the use of a novel, superselective drug delivery system to treat lung metastasis in our mouse model using

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doxorubicin-encapsulated liposomes (DOX-LP) and ultrasound (US). The metastatic PALN was injected with DOX-LP and then exposed to US. The released DOX was delivered from the PALN to the lung *via* the subclavian vein, heart and pulmonary artery. It was found that compared with DOX or DOX-LP alone, the superselective drug delivery system is very effective at treating metastasis in the lung as well as in the PALN.

METHODS

All *in vivo* protocols were approved by the institutional animal care and use committee of Tohoku University.

Mice

MXH10/Mo/lpr mice (16–18 wk) were bred under specific pathogen-free conditions in the Animal Research Institute, Graduate School of Medicine, Tohoku University (Shao et al. 2013). Figure 1A is an anatomic drawing illustrating the features of the lymphatic and venous systems in an MXH10/Mo/lpr mouse (Takeda et al. 2017). The efferent lymphatic vessel of the PALN is connected to the subclavian vein (Shao et al. 2013). The thoracoepigastric vein runs adjacent to the SiLN and PALN and connects to the inferior vena cava and subclavian vein.

Preparation of indocyanine green- and doxorubicin-encapsulated liposomes

Preparation of a thin lipid film and encapsulation of indocyanine green (ICG) were carried out as described previously (Mikada et al. 2017; Miura et al. 2016). The lipid film was composed of 1,2-distearoyl-*sn*-glycero-3-phosphatidylcholine (DSPC; MC8080, NOF Co., Tokyo, Japan) and 1,2-distearoyl-sn-glycerol-3-phosphatidylethanolamine-methoxy-polyethylenglycol (DSPE-PEG[2000-OMe]; DSPE-020 CN, NOF Co.) in a ratio of 94:6 mol/mol. ICG-encapsulated liposomes of different sizes (ICG-LP1 and

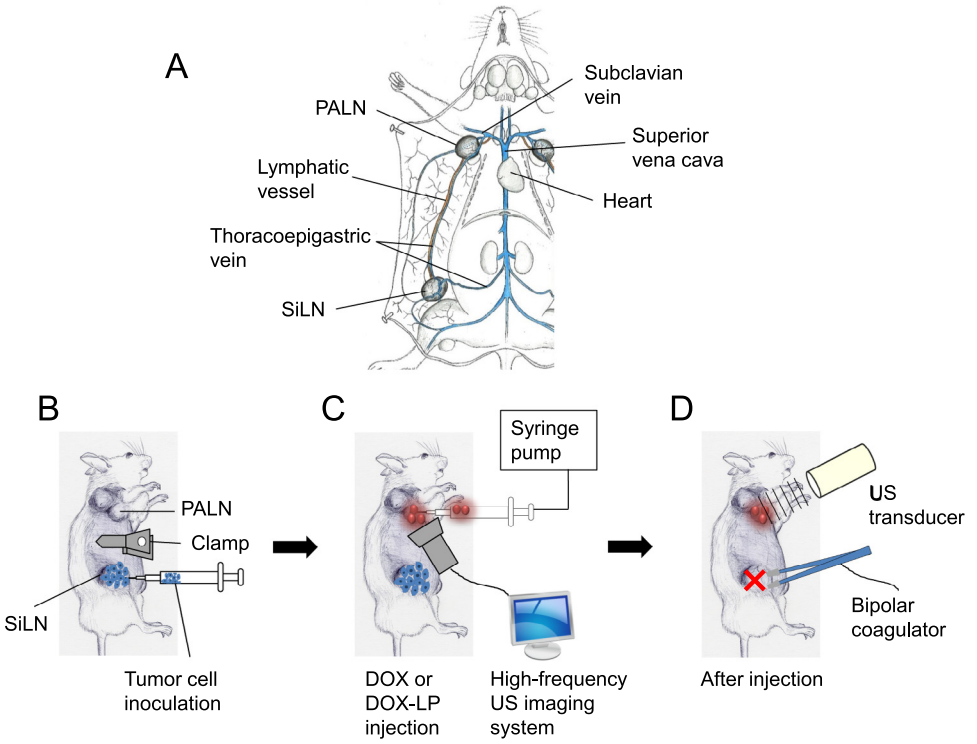

Fig. 1. Experimental procedure. (A) Lymphatic and vascular system in MXH10/Mo/lpr mice (Takeda et al. 2017). The efferent lymphatic vessel of the PALN is connected to the subclavian vein (Shao et al. 2013). The thoracoepigastric vein runs adjacent to the SiLN and PALN and connects to the inferior vena cava and subclavian vein. (B) Induction of metastasis in the PALN. The area between the SiLN and PALN was clamped to prevent flow from the SiLN to the PALN *via* lymphatic vessels and the thoracoepigastric vein. Cells were manually injected into the middle of the SiLN through an incision. (C) Injection of DOX or DOX-LP into the SiLN. At 6 h post-inoculation of tumor cells, DOX alone or DOX-LP was injected into the PALN under US guidance at the rate of 50 μL/min using a syringe pump. (D) Delivery of DOX to the lung and activation of metastatic tumor cells in the lung. The PALN was exposed to 1-MHz US. A bipolar coagulator and surgical microscope were used to facilitate the SiLN resection process. After excision of the SiLN, the surgical area was thoroughly washed with saline to remove any tumor cells that had leaked from the tumor-bearing SiLN. The skin wound was closed with sutures. DOX = doxorubicin; DOX-LP = DOX-encapsulated liposomes; PALN = proper axillary lymph node; SiLN = subiliac lymph node; US = ultrasound.