High-Resolution Fluorescence Microscopy and MRI Reveals Mechanism of Lymphatic Metastasis

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Introduction

Metastatic spread to regional lymph nodes is one of the earliest events of tumor cell dissemination, thus presenting a critical prognostic factor (1). Despite recent advances, many key questions about tumor cell spread via the lymphatic system remain unresolved (2). *In vivo* imaging of the metastatic process along the tumor drainage pathway would reveal the distinctive features of lymphatic metastasis. Tracking tumor cells along this pathway can provide clues on the mechanism of lymphatic invasion. In order to visualize microscopic alterations in the lymph node and lymphatic network of a living animal, high-resolution imaging methods should be applied. In this study we aimed to characterize the metastatic spread into the lymphatic system using combined high-resolution MRI and fluorescence microscopy in a spontaneous metastatic model, in which primary tumor cells spread into lymphatic network.

Methods

Highly efficient transfection of MDA-MB-231 human breast cancer cells with Red Fluorescent Protein (pDsRed2-N1, Clontech) yielded intense fluorescent breast cancer cells. Approval for all animal procedures was obtained in accordance with the Guiding Principles for the Care and Use of Research Animals of the Weizmann Institute of Science. Cells were inoculated into the mammary fat pad of female SCID mice as previously described (3). Initially, fluorescence microscopy was used to non-invasively detect and characterize the metastatic spread into lymph nodes. Mice were anesthetized and inspected under Zoom Stereo Microscope SZX-RFL-2 (Olympus), equipped with fluorescence illuminator and CCD camera Pixelfly QE (PCO). Intradermal injection of FITC-Dextran (Mw $5x10^5$, Sigma Aldrich) was used to mark lymphatic vessels. The excitation and emission red filter set was 510-550 nm and 590 nm (Long Pass), respectively. Green filter set was 460-490 nm for excitation and 510-550 nm for emission. The MR experiments were performed on a 4.7T Biospec spectrometer (Bruker). To localize the lymph node region, a coronal multislice T_2-weighted spin echo sequence was obtained, with 6 averages of TR/TE of 2515/23 ms, a field of view of 3x3 cm and a matrix of 256x256. Prior to imaging, mice were anesthetized with 1% isoflurane. Dynamic contrast-enhanced experiments were performed by a bolus injection of Gd-DTPA at a dose of 0.04 mmol/kg followed by slow infusion into the tail vein with Gd-DTPA, at a dose of 0.015 mmol/kg min for 100 min. A multislice 3D gradient echo sequence was acquired before and during the infusion with TR/TE of 18.3/4.3 ms, a flip angle of 30°, 4 averages and acquisition time of 2.5 min, using a field of view of 3x3x0.5 cm and a matrix of 256x128x16.

Results

Micrometastases in lymph nodes originating from the primary tumor were clearly observed by fluorescence microscopy. Using fluorescent videomicroscopy in living animal we recorded spontaneous metastasizing cells moving along the lymphatic flow, and monitored their development into lymph node metastases and to local metastases in lymph junctions. Prior knowledge of the presence and extent of lymph node metastases achieved by fluorescence microscopy, facilitated examination of lymph node metastases by MRI. We aimed to identify, at high-resolution, subanatomical features of the metastatic lymph node by Gd-DTPA contrast-enhanced MRI. MRI experiment consisted of localizing the lymph node by acquiring T2-weighted images followed by 3D-gradient-echo dynamic contrast-enhanced infusion protocol, designed to enhance drainage pathways in the lymph nodes. The infusion protocol also enabled acquisition of high spatial resolution images to resolve the microanatomy of invasion to the lymph node. In normal lymph nodes of naïve mice, the enhancement pattern following contrast-agent infusion corresponded to the natural drainage pathways in a lymph node, starting form the rim into the inner lymph node. In the metastatic lymph nodes, however, the drainage appeared to be blocked in a peripheral rim. The enhanced ring of 150-200 µm in the metastatic lymph node exactly correlated to a metastatic ring with the same dimensions seen by histopathology (Figure 1).

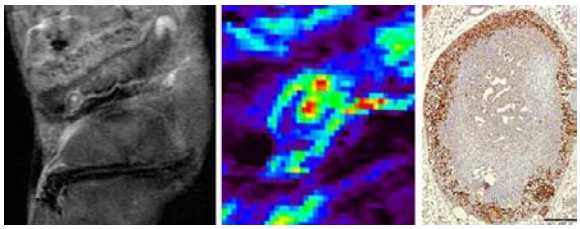

Figure 1: Metastatic lymph node: enhancement of the contrast-agent post-infusion was delimited to the outer ring of the lymph node as shown by the MR image 25 min post-infusion (left), max-intensity map (middle) and its correlation to a cytokeratin staining of the epithelial cancer cells (right). (High intensity in the center is the lymph node hillus, rich in blood vessels).

Discussion

A real-time insight into lymphatic metastasis, as it occurs, was achieved using combined fluorescence microscopy and MRI. By *In vivo* fluorescence microscopy we demonstrated that metastatic cells followed the natural pathway of lymphatic drainage and then gave rise to lymph node metastases and local metastases evolved from cell clusters in lymphatic junctions. Similarly, by MRI we observed that tumor cells occupying the lymphatic tissue might have blocked extravasation of the contrast-agent throughout the entire lymph node, thus interfering with the normal lymph flow. Previous studies of lymph node metastases in mice by MRI also showed no filling of the contrast agent in the metastatic lymph node using subcutaneous injection of dendrimer-based contrast agent (4). The deep insight we gained into lymphatic metastasis may improve our understanding of the process and will aid to design clinical imaging methods.

References

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