

# Tracking Metastatic Tumor Cells in Lymphatics in Mice Xenograft Model by MR Imaging

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## Purpose

To enrich our understanding on the mechanism of tumor lymphatic metastases, we developed a model system for tracking metastatic tumor cells in lymphatic system with MR cellular imaging in live mice and hypotheses that by observing the inaction between tumor cells and lymphatic system, we could dig into the fundamental mechanical aspects of tumor lymphatic metastasis.

## Methods

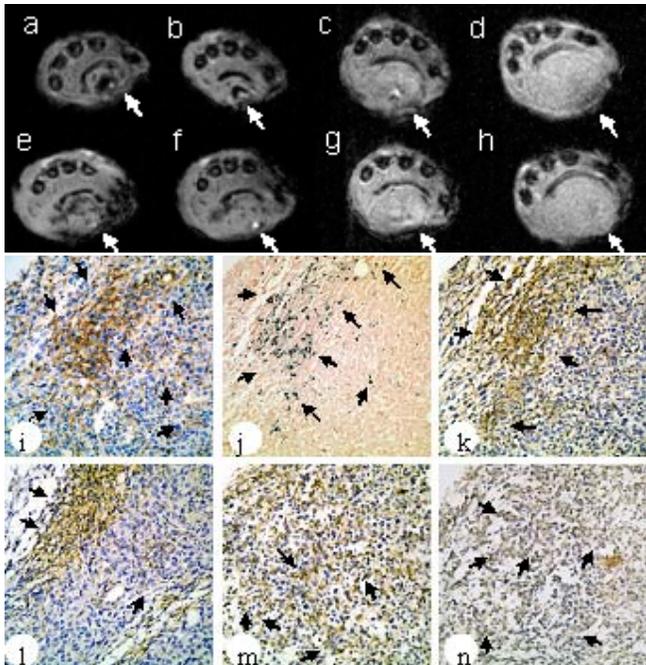
Human colorectal cancer LOVO cells were labeled with ultrasmall superparamagnetic iron oxide (USPIO) nanoparticles. The labeling efficiency was evaluated by Prussian blue staining. Thirty-six nude mice were divided into 6 groups, and then inoculated subcutaneously with labeled and unlabeled LOVO cells ( $3 \times 10^8$  cells/0.1ml) in foot pad, groin or axillary area, respectively. Serial 7T MR imaging of the tumors and surrounding regions was performed in following 4 weeks. After imaging, tumor tissues and regional lymph nodes were collected and subjected to immunohistologic analysis, which include hematoxylin and eosin (H&E) staining, Prussian blue (PB) staining, CD68 staining and lymphatic vessel endothelial hyaluronan receptor (LYVE-1) staining, CD31 staining, and VEGF-C staining.

## Results

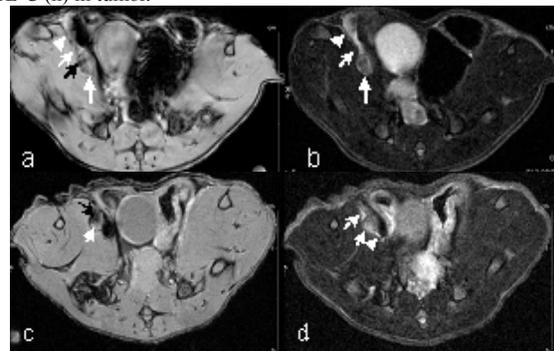
MR T2/ T2\* weighted image showed the primary tumor growth and the draining lymphatic architecture, as well as the USPIO labeled tumor cells metastasized into regional lymph node at 8 days P.I. MRIs also revealed information on sentinel lymph node mapping with high-resolution anatomical information. Histological finding confirmed in vivo MR imaging results and revealed lymphangiogenesis, angiogenesis, infiltration of macrophage, and expression of VEGF-C in tumor and sentinel lymph nodes as well.

## Conclusion

This technology provides a powerful tool for tracking USPIO-labeled cancer cells in the lymphatics by MR cellular imaging. There was a close relationship between tumor lymphatic metastasis with lymphangiogenesis.



**Figure 1.** MRI images of tumor implantation in foot pad at 1, 3, 5, 7 days P.I.(a-h). It demonstrated hypointense regions (a-d white arrows) in where USPIO labeled tumor cells were located, then the low signal increased gradually P.I. The increase in signal of the peripheral parts was more rapid than that of central parts of the tumor. There were no hypointense regions in primary tumor in the control group (e-h). Prussian blue staining (j) and CK20 staining (i) were positive in the tumor in study group. There were lymphangiogenesis (k), angiogenesis (l), infiltration of macrophage (m), and expression of VEGF-C (n) in tumor.



**Figure 2.** At 8 days, lymphatic network structure draining from the primary tumor (white arrowheads) were identified, metastatic tumor cells (black dotted arrows) could be imaged in draining lymph nodes (white arrows) simultaneously on T2- (a) and T2\*- (b)

weighted images in the groin area inoculation group. The size of micrometastasis (black dotted arrow) was  $0.4 \times 0.8 \text{ mm}^2$  in regional lymph node. MRI allowed clear delineation of the afferent (white dotted arrows,d) and efferent lymphatic vessels (white arrowheads,d) of lymph nodes (white arrows,c,d) in which micrometastasis (black dotted arrows,c) was detected at 8 days P.I. in the groin area inoculation group. The size of the lymphatic vessel is 0.2mm.

## References

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- [2] Jonathan P. Sleeman, Wilko Thiele. Int. J. Cancer 2009; 125: 2747-2756