

Assessment of metastatic potential of 67NR and 4T1 tumors with selective multiple-quantum coherence transfer

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Introduction

Reliable methods that assess the metastatic potential of a tumor will enable appropriate treatment at the early stage of growth. The driving hypothesis of this study is that tumor lactate level may be considered as a prognostic marker on whether the tumor is “non-metastatic” or “metastatic”. Tumor lactate accumulation is caused by both aerobic glycolysis, a hallmark of cancer, and by normal anaerobic glycolysis in the hypoxic region of tumors. This study employs MRSI and PET imaging techniques to investigate the glycolytic activity of mouse tumors. We hypothesize that lactate production is correlated with LDH-A (lactate dehydrogenase A) activities. Moreover, we correlated the *in vivo* measurements of lactate concentration with ¹⁸F-FDG uptake.

Materials and methods

Two isogenic tumorigenic breast cancer lines (67NR and 4T1) derived from one spontaneous tumor in the BALB/cfC₃H mouse were used in this study. 67NR cells form primary tumors, but no metastases. 4T1 cells are able to complete all steps of metastasis and efficiently form metastatic macroscopic nodules in lung and other organs (1). Cells were injected subcutaneously into the mammary fat pad of athymic nu/nu female mice (NCI). Lactate signal was acquired using the selective multiple-quantum coherence transfer (selMQC) editing sequence in combination with chemical shift imaging (CSI) (2). The spectra were then calibrated by means of the phantom replacement technique to determine the *in vivo* lactate concentration. Additionally, tumors were imaged using ¹⁸F-FDG PET. To characterize the LDH-A expression in 67NR and 4T1 cells, we used semiquantitative RT-PCR and immunoblotting analysis. The tumor sections were analyzed by immunochemical staining.

Results

Figs. 1 and 2A show results of the longitudinal *in vivo* lactate production study of 67NR and 4T1 tumors in mice. The distribution of lactate signals in orthotopic tumors of different phenotype and different sizes is presented on Fig.1 and reflects the heterogeneity of the tumors. Despite the similar growth curves (Fig.3), the non-metastatic phenotype (67NR) and metastatic phenotype (4T1) tumors have different patterns in the lactate concentration changes as they grow. The 67NR tumors have very low lactate level at the small sizes (<100 mm³); the level increases rapidly and is relatively constant at large sizes (300-800 mm³) (Fig. 2A). In contrast, 4T1 tumors exhibit relatively high lactate at small sizes and their lactate level decrease at large sizes (Fig.2A). The 4T1 tumors undergo significant necrosis during the growth and the loss of viable tissue may explain the decrease in lactate concentration. ¹⁸F-FDG microPET imaging demonstrates no significant variation across different phenotypes at medium (100-300 mm³) and bigger sizes (300-800 mm³) (Fig.2B). *In vitro* measurements of LDH activity, LDH-A expression and glucose utilization, as well as lactate production have characterized 4T1 breast cancer cells as typical Warburg phenotype cancer cells, while 67NR cells display more as oxidative phosphorylation phenotype.

Discussion

The abnormal high lactate level at the early stage of the tumor growth may be a potential marker of metastasis. Lactate detection by selMQC may provide a noninvasive approach for early diagnosis of certain type of metastatic tumors.

References

1. C. J. Aslakson, et. al., Cancer Res. **52**, 1399 (1992); 2. Q. He, et al., J. Magn. Reson. B **106**, 203 (1995).

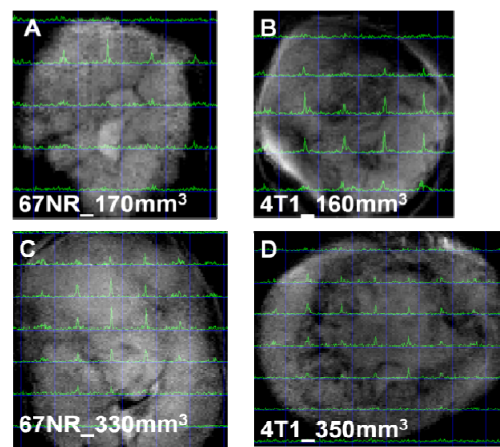


Fig.1 Lactate distribution at different 4T1 and 67NR tumor volumes.

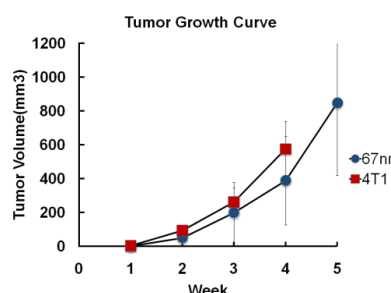


Fig.3 Tumors growth curve.

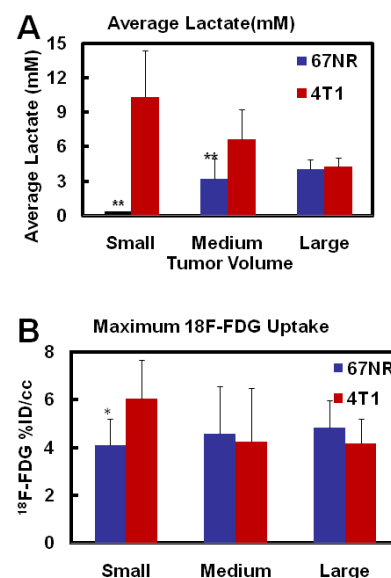


Fig.2 (A) Lactate concentration of 67NR and 4T1 tumors at small (<100mm³), medium(100-300mm³) and large sizes (300-800 mm³) tumors. (B) Maximum ¹⁸F-FDG uptake in the same tumors determined by microPET imaging the same tumors volumes.