Noninvasive assessment of lymph node metastasis of melanoma using molecular MR reporter gene of ferritin

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Introduction

Application of MR reporter genes is a new strategy in molecular imaging based on over-expression of non-toxic proteins responsible for *in vivo* uptake of MRI-detectable probes [1-2]. Gene-based production of contrast agents for MR cell tracking has many advantages over the standard approach using exogenous administration of superparamagnetic particles, where the imaging signal does not reflect cell viability and quantity. Ferritin has been proposed as an endogenous MRI reporter for noninvasive imaging of gene expression in C6 glioma tumors [1] and for *in vivo* studies in the transgenic mice [2]. The potential of using MR reporter genes to study fate of cancer cells engrafted into subcutaneous area of mice has not been explored. We aim to develop genetically-based technique for molecular imaging of the MRI gene reporter ferritin to enable noninvasive assessment of lymph node metastasis of cancer cells after transplantation into subcutaneous area of mice.

Materials and Methods

Lentiviral vector was used to simultaneously generate MRI and fluorescent imaging via expression of both human ferritin H-subunit (hFTH) and enhanced green fluorescent protein (GFP). The transgene construct was stably transfected into B16F10 cell (a melanoma cell line). Expression of ferritin and GFP was monitored by Western blot analysis using monoclonal mouse hFTH-antibody. B16F10 cells were incubated with 100 mM and 200 mM ferric citrate in DMEM for 5 days. We measures the transverse relaxation rate (T2*) of the cell pellet of 1 x 10^7 using a 1.5 T MR scanner (GE health care). We used a MGRE sequence; NEX = 2, FA = 20°, TR = 800 msec, and 9 echoes raging from 4.2 to 58.3 msec. For the in vivo MRI we injected 1 x 10^6 B16F10 cells expressing hFTH/GFP into the dorsal subcutaneous area of Balb/c nuce mice (n = 6) to induce metastasis in the lymph node. In addition, normal B16F10 cells of 1 x 10^6 were also inoculated in the mice (n = 6). We also measured the transverse relaxation rate (T2*) of the main mass, and the brachial and axillary lymph nodes using a 9.4 T MR scanner (Brucker Biospin). We used a MGRE sequence; NEX = 2, FA = 90°, TR = 5000 msec, and 8 echoes raging from 3.12 to 34.4 msec. After MR imaging, in vivo and ex vivo fluorescent imaging of the main tumor and lymph nodes was obtained using optical imaging analyzer. To confirm lymph node metastasis of B16F10 cells and transgene expression, immunohistochemistry (IHC) and hematoxylin/eosin staining were performed.

Results and Discussion

The expression of GFP and hFTH was confirmed by Western blot analysis (Fig 1). B16F10 cells expressing hFTH/GFP showed significantly lower T2* relaxation rate than control cells (Fig 2). Main tumor with hFTH/GFP also showed significantly lower T2* relaxation rate than control, and the metastatic cells with hFTH/GFP revealed significantly lower T2* relaxation rate than control (Fig 3). However, the only main tumor with hFTH/GFP was demonstrated on in vivo optical imaging, and ex vivo optical imaging could demonstrate the lymph node metastasis with hFTH/GFP (Fig 4). Our study shows that hFTH/GFP transgene is a feasible modality for the investigation of cancer cell fate.

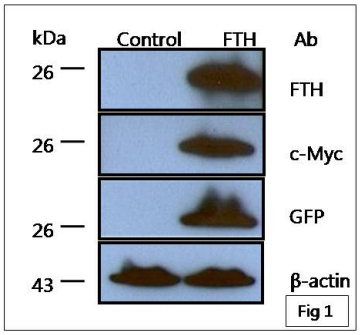


Fig 1. Western blot anlaysis with mouse monoclonal antibodies indicates the expression of FTH, c-Myc, and GFP. C-Myc is conjugated with FTH.

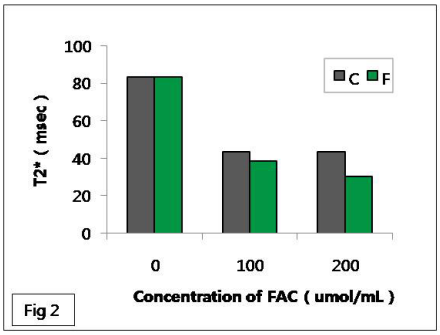
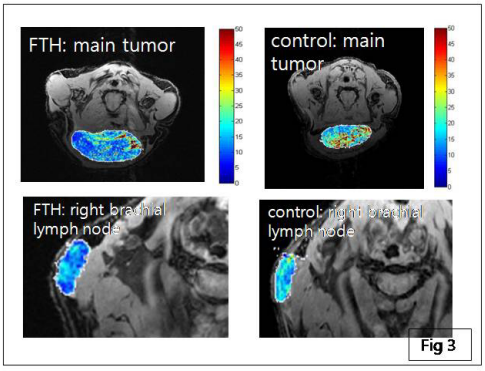


Fig 2. B16F10 cells expressing hFTH/GFP showed signifi cantly lower T2* relaxation rate than control cells.



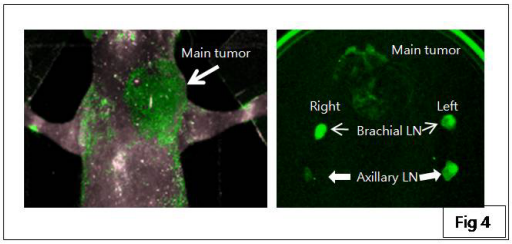


Fig 3. Main tumor with hFTH/GFP also showed significantly lower T2* relaxation rate than control, and the metastatic cells with hFT H/GFP in the right brachial lymph node revealed significantly low er T2* relaxation rate than control. Fig 4. The only main tumor wi th hFTH/GFP was demonstrated on in vivo optical imaging, and e x vivo optical imaging could demonstrate the lymph node metast asis with hFTH/GFP.

References

1. Cohen B, Dafni H, Meir G, Harmeliny A, Neeman M. Neoplasia. 2005;7:109–117.
2. Cohen B, Ziv K, Plaks V, et al. Nature medicine. 2007;13:498-503.