In Vivo Cell Tracking using Micron-sized Iron Oxide Particle (MPIO) Labeling in Rat Model of Liver Tumor Metastasis

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Introduction

Hepatocellular carcinoma (HCC) is one of the most common and aggressive human malignancies with high rate of recurrence and extrahepatic metastases that spread to other parts of the body such as lungs and lymph nodes [1]. To improve prognosis and treatment of HCC, information regarding the biology of metastasis of this disease must be determined. However, our current understanding of metastases of HCC is still rather limited. Techniques that allow monitoring the growth and metastasis of cancer cells longitudinally and noninvasively are desired for investigating HCC metastases and optimizing therapeutic paradigms in treating metastases of liver cancer. Magnetic resonance imaging (MRI), with the use of iron-containing contrast agents, is a suitable candidate among many imaging techniques. In this study, we showed that hepatoma cell line labeled with micron-sized iron oxide particles (MPIOs), a sensitive negative contrast agent, can be monitored longitudinally after being directly injected into the circulation via portal vein in an experimental rat model of liver tumor metastasis.

Methods

Cell labeling and animal model: Hepatoma cell line McA-RH7777 (CRL1601, ATCC, Manassas, VA) was labeled with 0.9-μm superparamagnetic styrene-divinyl benzene inert polymer-coated micron-sized iron oxide particles (MPIOs; Bangs Laboratory, Fishers, IN) of concentration 2.5×10^8 particles/mL for 24 hours. To remove free particles after labeling, the labeled cells were washed thoroughly with PBS and then density centrifugation through Ficoll-Paque PLUS was used [2]. The labeling efficiency was >90% with ~15-20 particles/cell and viability after labeling was >85% using Trypan blue staining. Inbred male Buffalo rats (200 to 250 g, N = 2 animals) were anesthetized with an intraperitoneal (i.p.) injection of 40 mg/kg pentobarbital sodium. The abdomen was shaved and a midline incision was made. Rats were injected with 5×10^6 MPIO-labeled hepatoma cells in 500 μL PBS via the right portal vein.

MRI: MRI scans were performed on a PharmaScan 70/16 7 T scanner (Bruker, Germany) using a 60 mm diameter volume coil for liver imaging and a 38 mm diameter volume coil for brain imaging. For liver imaging, T_2*-weighted images were acquired coronally by multislice 2D gradient echo flow-compensated (GEFC) sequences with respiratory gating and TR = 1 respiratory cycle ~ 1000 ms, TE = 3.8 ms, FA = 40º and T_2-weighted images were acquired coronally by multislice 2D fast spin echo (RARE) sequences with TR = 2200 ms, TE = 40 ms, RARE factor = 8. Both T_2*- and T_2-weighted sequences were acquired with FOV = 3.84×3.84 cm, slice thickness = 0.5 mm, number of slices = 15, acquisition matrix = 192×192, resolution = 0.2×0.2×0.5 mm^3, total scan time ~ 30 min/10 min for T_2*-/T_2-weighted sequences. For brain imaging, T_2*-weighted images were acquired sagittally by multislice 2D gradient echo (GE) sequences with TR = 500 ms, TE = 20 ms, FA = 40º, FOV = 2.55×2.55 cm, slice thickness = 0.38 mm, number of slices = 16, acquisition matrix = 256×256, resolution = 0.1×0.1×0.38 mm^3, total scan time ~ 16 min. Note that high resolution imaging was required in order to track the MPIO-labeled cells [3]. For longitudinal study the animals were scanned on the day of injection (day 0) and 7, 10, 14, 18 days after injection.

Results

Figs. 1a and 1b show the T_2*-weighted coronal images of liver at day 0 and day 7 respectively. Punctuated dark contrast spots due to the MPIO-labeled hepatoma cells were observed in the liver. The contrast of labeled cells was observed to be decreasing over the time studied. Fig. 1c and 1d show the T_2*- and T_2-weighted coronal images of liver at day 18 at which multiple small tumor nodules appeared throughout the liver (Fig. 1d). Note that short TE was required in T_2*-weighted sequences because at long TE the rapid dephasing of blood signal would also result in hypointensities. Figs. 1e – 1g show the T_2*-weighted sagittal images of brain at day 0, 7, and 14 respectively. The number of hypointense signals in the brain decreased with time indicating that some labeled hepatoma cells were gradually cleared. No tumor was found in the brains of the rats studied likely due to the short observation time or relatively rare occurrence of liver cancer metastasis in brain [4]. The MPIO-labeled hepatoma cells observed in the liver and brain were arrested within microvasculature primarily due to the size restriction mechanism [5].

Discussion

Punctuated dark contrast spots due to the MPIO-labeled hepatoma cells injected via portal vein were observed in livers and brains. The preliminary results in this study illustrated the feasibility of detecting and tracking of MPIO-labeled hepatoma cells injected via portal vein in this rat model of liver tumor metastasis. A recent study showed that MRI can quantify the proportion of cancer cells that ultimately cleared, developed into tumors, or remain dormant in brain in a mouse model of breast cancer metastasis [6]. However, this is challenging in liver due to the respiratory motion and rich vasculature. Previous studies showed that the activation state of the endothelium can influence whether cancer cells arrest by adhesive interactions in pre-capillary vessels or by size restriction in smaller capillaries in liver [7,8]. The protocols we used in this study could possibly be applied to investigate the interactions between tumor cells and the endothelium, for example, by comparing the local labeled cell density in liver between cytokine interleukin (IL)-1α-treated and non-treated groups.

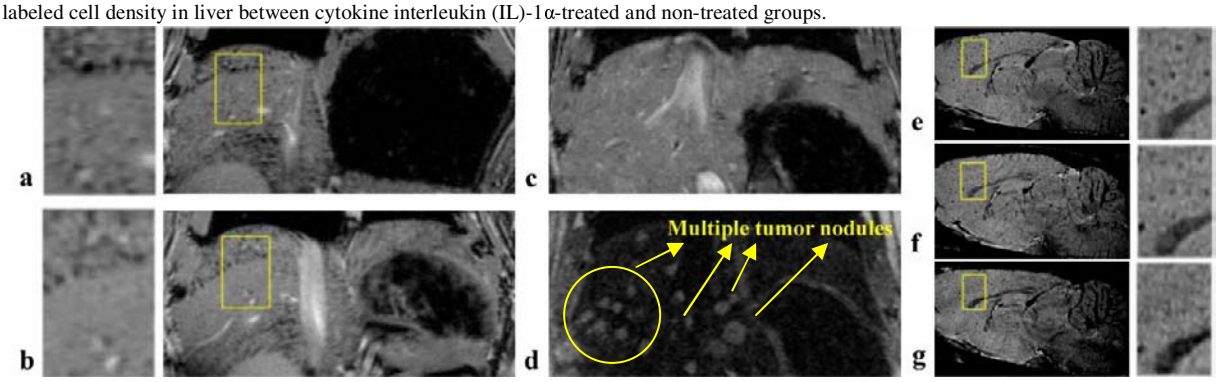

Fig. 1 Representative T_2*-weighted liver images of rats at day 0 (**a**), day 7 (**b**), and day 18 (**c**) after portal vein injection of 5×10^6 MPIO-labeled hepatoma cells. Punctuated dark contrast spots due to the MPIO-labeled hepatoma cells were observed on day 0 and 7 but not clearly on day 18. Multiple small tumor nodules can be observed throughout the liver in T_2-weighted image at day 18 (**d**). Representative brain images at day 0 (**e**), day 7 (**f**), and day 14 (**g**). The MPIO-labeled hepatoma cells observed in the liver and brain were arrested within microvasculature primarily due to the size restriction mechanism. Insets in (**a**,**b**,**e-g**) are the magnified view of the corresponding yellow boxes.

References [**1**] S Katyal et al, Radiology 2000;216:698-703. [**2**] EM Shapiro et al, MRM 2006;55:242-249. [**3**] EM Shapiro et al, PNAS 2004;101:10901-10906. [**4**] J Seinfeld et al, J Neuroncol. 2006;76:93-98. [**5**] AF Chambers et al, Nat Rev Cancer 2002;2:563-572. [**6**] C Heyn et al, MRM 2006;56:1001-1010. [**7**] FW Orr et la, Surg Oncol Clin N Am. 2001;10:357-381. [**8**] P Gassmann et al, Onkologie 2004;27:577-582.