Phase Contrast MR Microscopy to Study Glioma Invasion in Vivo at High Field

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Introduction:

Mouse models for human brain tumors are important tools to gain understanding about tumor formation, tumor physiology, as well as the assessment of longitudinal effects of drug action. As the brain is composed of a variety of cell type that vary strongly according to their anatomical location, an accurate assessment of the implantation site plays an important role in the investigation of growth characteristics and physiology of tumors especially with respect to the different compartments of the brain [1]. So far, cells are typically labeled for MR imaging using iron oxide particles [2] or more sophisticated labels such as perfluorocarbon nanoparticles for ^{19}F imaging [3]. This study was performed to determine to what extend high-resolution MR microscopy enables to accurately determine the early fate of implanted glioma cells and the injection tract under *in vivo* conditions. The presented approach based on special contrast techniques that exploit the MR signal phase can be an efficient alternative to replace the need of cell labeling that might potentially alter cellular characteristics.

Material and methods: In order to study the role of PTBP1 in glioma [4], *in vivo* experiments were performed using shRNA to knockdown PTBP1 using lentiviral mediated infection. SNB19 glioma cell lines were infected with PTBP1 shRNA and intracranially injected into immunocompromised mice. In order to confirm that the cells were correctly injected and to investigate their migrational dynamics, high-resolution brain imaging was performed at different time points after injection (7, 30 and 60 days respectively). Experiments were performed at 7T on a Bruker Biospec System. A cryogenically-cooled quadrature-resonator (Bruker, Ettlingen, Germany) was used as transceiver. High-resolution morphological T_2 weighted (RARE, TR/TE = 3800/18 ms, acquisition time 10 min) and 2D gradient echo (GE) flow compensated images were acquired. The parameters for the GE image were: *TR* = 1000 ms, *TE* = 16 ms, flip angle=72 degrees, resolution **35 x 35 x 250 µm^3**, within a short **acquisition time of 7 min**; the flip angle was adjusted to Ernst angle considering T_1 = 1600 ms (mouse cerebral cortex). Images were processed offline with custom-made software developed in Matlab to reconstruct both magnitude and phase images. After Fourier transformation, in order to preserve the phase information, the complex images acquired with each individual channel were combined using the adaptive reconstruction method proposed by Walsh [5]. The background field inhomogeneity resulting from imperfect shimming was removed firstly by a low pass filter of the complex image (Gaussian filter equal to the image size) and secondly by complex division of the complex image by the low pass filtered image on a voxel by voxel basis. Prior to MRI, histology was performed on paraffin-embedded sections using Hematoxylin-Eosin staining from mice injected with the same SNB19 glioma cells infected with PTBP1 shRNA.

Results:

Superior contrast compared to the magnitude of both GE and T_2 weighted data was obtained on the high-pass filtered phase images enabling to detect groups of tumor cells (bright spots) with higher contrast compared to the host tissue. Figure 1 displays axial views of the mouse brain acquired at 7 (**a**) and 30 days (**d**) after injection revealing the injection site extending from cortex to striatum and the modality of cell migration through the cortex to the meninges. After 7 days, MRI shows the presence of cells along the cortex, several injected cells being located close to the ventricular walls (see red arrows), others located through the meninges as shown in figure 1**c**. As seen in Fig 1**b**, the cell location is not clearly delineated on the T_2 weighted images. After 30 days, MRI shows several injected cells into the striatum and the tumor located in the upper part of the brain (Fig. **1d**, red arrow). The location of the tumor outside the meninges with its projections through the cortex in confirmed by histology as shown in figure **1e**.

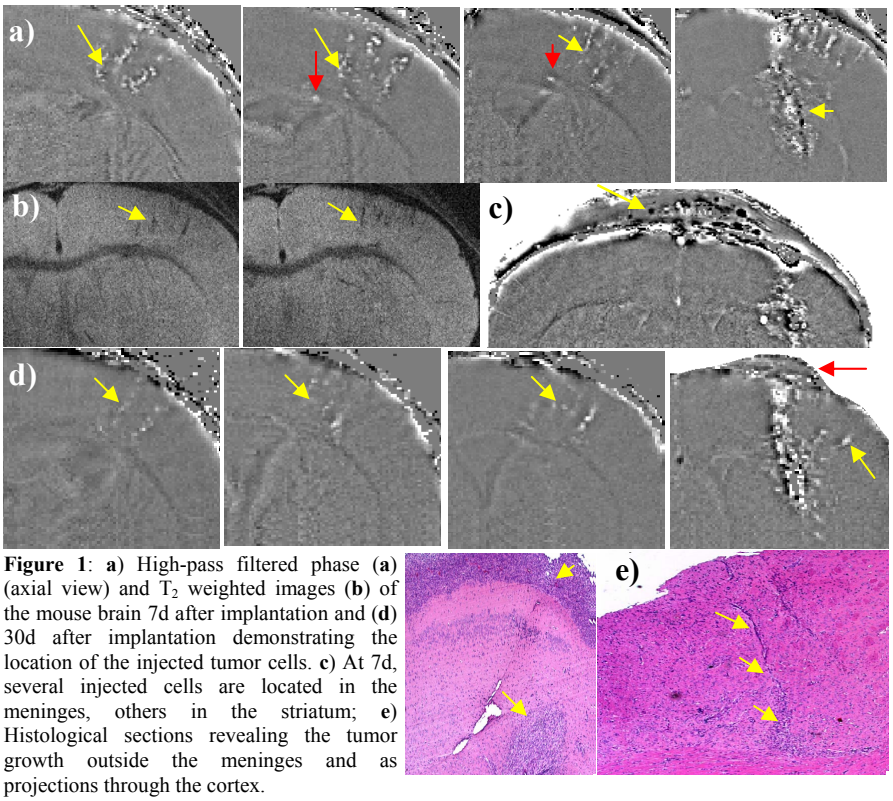

Figure 1: **a**) High-pass filtered phase (**a**) (axial view) and T_2 weighted images (**b**) of the mouse brain 7d after implantation and (**d**) 30d after implantation demonstrating the location of the injected tumor cells. **c**) At 7d, several injected cells are located in the meninges, others in the striatum; **e**) Histological sections revealing the tumor growth outside the meninges and as projections through the cortex.

Discussions and Outlook

The phase contrast MR microscopy results were in good agreement with histological findings. The histological analysis of brain sections revealed that tumors from cells infected with shPTBP1 mostly grew as a compact mass with sharp edges and projections towards the cortex and outside the meninges, possibly following the injection tract. Since tumors in mice injected with control cells grew as expected in the striatum, the here presented results suggest that PTBP1 knockdown could mostly affect the invasive capability of glioma cells. In summary, the present investigation demonstrates that the detection sensitivity of MRI combined with the superior contrast provided by the phase contrast technique is an ideally suited approach for non-invasive observation of non-labeled tumor cells at high spatial and temporal resolution.

References: [1] Fomchenko EI, Holland EC. Clin Cancer Res 2006 ; 12(18): 5288-97. [2] Srinivas M, et al, 2010;28(7):363-70. [3] Himmelreich U *et al*, *Neuroimage* 2006: 32(3) :1142–1149. [4] [4] Cheung HC et al, BMC Genomics, 2008; [5] Walsh et *al*, Magn Reson Med. 2000; 43:682-690.