MR Characterization of an Experimental Invasive GBM Model

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Introduction Glioblastoma (GBM) is the most common adult brain tumor. Patients usually die within a year of diagnosis due to extensive tumor infiltration throughout the brain. The histologic features of GBM are florid angiogenesis in the metabolically active tumor, central necrosis, with a tumor rim that is hypercellular and has a high neovascularity with widespread neoplastic infiltration into white matter. The MR imaging characteristics of the tumor are low density center, enhancing rim, and dark on T1-weighted imaging and bright on T2-weighted imaging. Currently established animal models of GBM use decade old cell lines (e.g. U87, U373, U343, LN229) implanted into mouse brain but these tumors do not recapitulate all the authentic features seen in human GBM. A new experimental tumor model based on human tumor stem cell is being developed which contains all histological features of human GBM, including angiogenesis, mitosis, necrosis and invasion. We characterized this new brain tumor model in vivo using novel MRI techniques and compared its imaging features with a well established U87 GBM model.

Materials and Method The imaging experiments were performed on a 4.7T horizontal bore system interfaced with a Bruker console. Intraaxial brain tumors were formed by intracranial injection of experimental human tumor stem cell (hTSC) into SCID mice (n=11) with 3 of them expressing green fluorescence protein (GFP) and compared to intraaxial brain tumors from U87 cell line (n=3). The studies were approved by IACUC of the institutions involved. At the clinical end point of the mice, i.e. the mice is close to death, the mice were imaged by MRI and fluorescence imaging after anesthetized at 1.5% isofluorane with 95% oxygen.

T2-weighted images (T2w) (TR/TE_{eff}=2000ms/72ms) were acquired with a 2D fast spin echo sequence at 1mm slice thickness at 80μmx130μm in-plane resolution. Magnevist[Tm] Gd-DTPA was injected intraperitoneally at 0.7 mmol/kg and T1-weighted images (T1w) (TR/TE_{eff}=417ms/24.5ms) were acquired after 12 minutes delay to allow for optimal contrast enhancement. Tumor ROIs were drawn manually on the T1w with reference to tumor size/location from T2w. Neck muscle ROIs were drawn manually on the T1w post-Gd images. Neck muscle is a large tissue structure and can be easily identified that has high Gd extravasations rate compare to other tissues around the brain region. We proposed to use the relative tumor enhancement ratio (RTER), signal intensity of tumor divided by signal intensity of neck muscle, as a biomarker to grade the microvascular permeability of tumors.

Diffusion weighted imaging were acquired at the same slice location as T1 and T2 protocols with a standard 2D spin echo sequence with a diffusion weighting of b=0 and 1000 s/mm^2 at three orthogonal direction, triggered with respiratory gating at about 20 bpm. The effective TR was about 3000ms with a TE of 22ms, with a 175μm x175μm in-plane resolution. Apparent diffusion coefficient maps were computed with DTIstudio (1). After the MR imaging session, GFP expressing experimental brain tumors were excised and sliced to 1mm thickness for fluorescence imaging. Fluorescence imaging was acquired with NightOWL II LB983 (Berthold Technologies GmbH & Co. KG, Bad Wildbad, Germany) to image the distribution of active tumor cells and the image from fluorescence channel was overlaid on the corresponding T2-weighted MRI.

Results Fig. 1 showed the RTER plot of hTSC vs U87 tumors plotted as mean ± 1SD. There were two distinct subgroups in hTSC with one group more angiogenic and leakier than U87 and one group more invasive and less angiogenic/leaky than U87. Both subgroups were well separated from U87 indicating RTEF is a good biomarker to grade the microvascular permeability of the tumors. Fig. 2 showed a multimodal representation of the hTSC tumor with GFP expressing tumor overlaid on T2w and T1w post-Gd MRI. The horizontal T2w matched exactly to the GFP expressing region while the T1w has a slight mismatch which was due to distortion/slice orientation during tissue processing. Fig. 3 showed the MRI characteristics of a representative example from hTSC invasive subgroup. The tumor was bright in T2w, and with minimal enhancement in T1 post-Gd. There were no detectable ADC change compared to contralateral side which is different from U87 tumor (data not shown). Fig. 4 showed the H&E staining of U87 vs hTSC tumor. Fig. 4a showed the mass effect of U87 tumor "t" with a clear tumor boundary to the surrounding brain tissue "b" as shown by the arrows in Fig. 4c. Contrary to U87 tumor, hTSC tumor "t" was very invasive and grew along the subventricular zone infiltrating the white matter indicated by black arrows in Fig. 4b. There was no clear boundary between the tumor "t" and surround brain tissue "b" as shown in Fig. 4d.

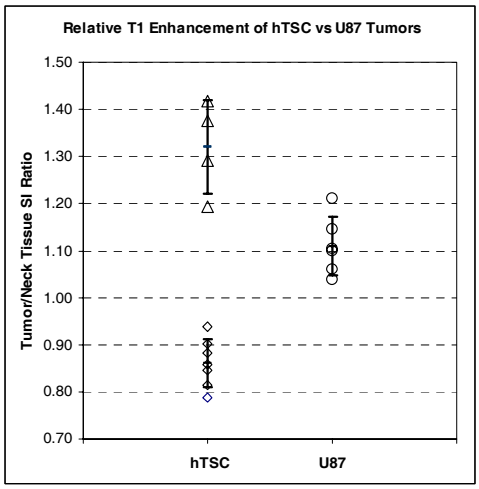

Fig. 1 Relative tumor enhancement ratio of hTSC and U87 tumors. The upper hTSC group is more angiogenic and leakier than U87 and the lower hTSC group is more invasive and less angiogenic/leaky.

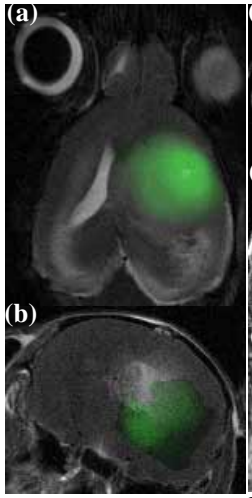

Fig. 2 GFP positive tumor overlaid on a (a) horizontal T2w image and on a (b) coronal T1w post-Gd image.

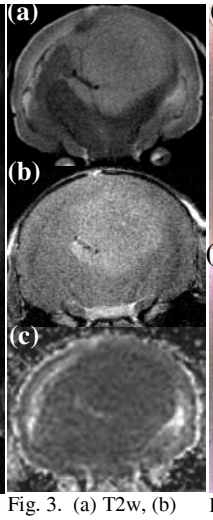

Fig. 3. (a) T2w, (b) T1w, and (c) ADC map at the same slice location.

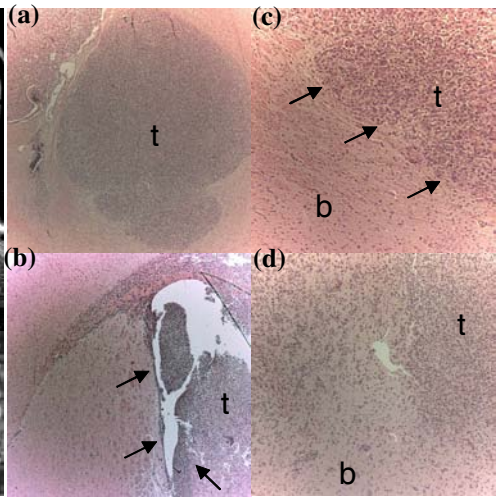

Fig. 4. H&E staining of (a, c) U87 tumor and (b, d) hTSC tumor. Note in (d) the tumor cells infiltrate into brain and no clear brain -tumor boundary can be seen.

Discussion We characterize the MRI features of an experimental human tumor stem cell mouse model with MRI and fluorescence imaging. Two subgroups of hTSC have been identified with a proposed biomarker, RTER, and it can separate both subgroups with traditional U87 GBM tumor. The dark T1w and bright T2w findings were consistent with known diagnostic criteria. In the invasive hTSC subgroup, hazy T1 enhancement was observed in the tumor region identified by T2w while no ADC change was observed compared to adjacent and contralateral brain tissues, which maybe attributed to the invasive nature of this hTSC model. It is suggested that ADC map may not be very helpful in detecting the invasive region of human GBM and RTER deserve further validation as a biomarker to grade tumor microvascular permeability.

References

1. Jiang H, van Zijl PC, Kim J, Pearlson GD, Mori S. Comput Methods Programs Biomed. 2006 Feb;81(2):106-116.