Unique Structural Patterns in Rat Brain Tumors Revealed by High-Resolution Diffusion Tensor MRI

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Introduction: Malignant gliomas are the most common primary brain tumors. To find treatments, rat brain tumor models and several cell lines have been widely used. Established rat glioma cell lines (e.g., 9L and F98) have the tendency to display a noninvasive growth pattern in animals, while primary glioma xenografts derived from patients maintain genetic fidelity and growth patterns more analogous to those of human tumors, including invasive growth and tumor necrosis. MRI has been used to study brain tumors in rat brain models. Conventional MRI, such as T_1W and T_2W imaging, lacks contrast to differentiate substructures inside tumors and visualizes tumors as homogenous regions. Diffusion tensor imaging (DTI) is a unique technique that uses the microscopic motion of water molecules to probe tissue 3D microstructures. It can characterize the 3D properties of tissue water diffusion by providing several types of information, including the extent and anisotropy of diffusion, and its predominant orientation. DTI can be used to detect the existence of well-ordered structures at the microscopic level, and several groups have used DTI to study the influence of brain tumors on surrounding axonal tracts and possible migration of tumor cells along neuronal tracts. In this study, we used DTI to study the tumor microstructure of three rat brain tumor models, 9L, F98, and primary glioma xenografts derived from patients.

Methods: Total 15 rats were used in this study with 5 rats for each cell line. In vivo MRI was performed on a 4.7T MR system. For each rat, six diffusion weighted (DW) images (b=1000 s/mm^2) and one non-diffusion weighted (b= 50 s/mm^2) image were acquired with a multiple spin echo sequence (TEs = 27/37/47/57 ms, TR = 2 s, NA=4, Δ = 14 ms, δ = 6 ms, resolution = 0.3 x 0.3 x 2 mm^3, horizontal slices, diffusion gradient direction: [0.707, 0.707, 0], [0.707, 0, 0.707], [0, 0.707, 0.707], [-0.707, 0.707, 0], [0.707, 0, -0.707], [0, -0.707, 0.707]). T_2W images were acquired (RARE, TE = 64 ms, TR = 3 s, ETL = 4, NA = 2, resolution = 0.15 x 0.15 x 2 mm^3). After in vivo MRI, rats were perfused and fixed with 4% paraformaldehyde, and the brain specimens were imaged on a 9.4T MR system. We acquired six DW (b=1000 s/mm^2) and one B_0 images (3D multiple spin echo, TE/TR = 30/700 ms, ETL = 6, NA =2, Δ = 14 ms, δ = 6 ms, resolution = 0.16 x 0.16 x 0.16 mm^3, the same diffusion gradient direction as in vivo). T_2W images were also acquired (RARE, TE/TR = 50/900 ms, flip angle= 40°, NA = 4). Diffusion tensors were calculated using a Log-linear fitting method, along with FA and the predominant orientation of water diffusion. After MRI, hematoxylin and eosin-stained histology was performed.

Results: Figure 1 shows typical in vivo MR images from the three different brain tumor models. In the T_2W images, regions with tumors have higher signal intensities than the normal brain tissue. The FA maps of all three models show previously unreported contrast patterns within the tumors. Inside the tumor regions defined by the T_2W images, the FA maps show central foci of low diffusion anisotropy (center, region 1) surrounded peripherally by a high diffusion anisotropy region (rim, region 2). The presence of high diffusion anisotropy within the tumors shows that water diffusion in the surrounding regions is coherently, not randomly distributed, which indicates the existence of highly ordered sub-structures inside these tumors. Furthermore, the predominant orientation of water diffusion in the rims, as illustrated by the direction encoded color-maps (DEC) and schematic diagrams, forms a circular pattern in the 9L and F98 models, i.e., water molecules are more likely to move circumferentially around the centers of low diffusion anisotropy. While in the human xenograft models, the peripheral region has a radial pattern, i.e., water molecules appear to move from the central core outward. The patterns were preserved as the tumors grew. Ex vivo DTI results (Fig. 1 ex vivo) with high SNR and spatial resolution confirmed the in vivo findings, and demonstrate that the distinctive patterns of water diffusion observed in vivo is not limited to a particular horizontal slice but throughout the entire tumor. We observed similar microstructure patterns of tumor cell organization in histology sections.

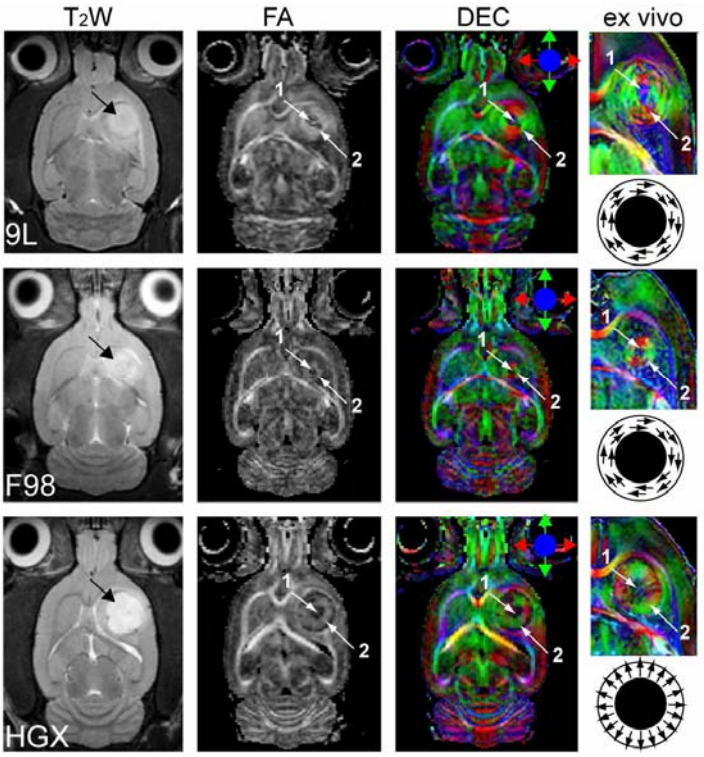

Fig. 1. In vivo horizontal T_2W, FA, DEC and ex vivo DEC images acquired from three rat brain tumor models (9L, F98 and human glioma xenografts (HGX)). The location of tumor can be clearly appreciated in T_2W images (indicated by black arrows). The color-scheme used in the DEC images is red: medial-lateral; green: rostral-caudal; blue: dorsal-ventral. Schematic diagrams under the ex vivo results illustrate the primary direction of water diffusion as measured by DTI. Inside the tumor, the dark tumor centers with low FA are labeled as region 1, while the rims with high FA are labeled as region 2.

Discussion & Conclusions: We have shown that DTI can reveal structural organizations within experimental gliomas. The observed difference in the predominant orientation of diffusion between the established cell lines (9L and F98) and primary human glioma xenografts derived directly from patients is primary due to their difference in tissue microstructure. These DTI patterns appear to reflect distinct patterns of interstitial fluid flow and possibly directional cell growth and invasion. Although the exact mechanism of the tissue contrast observed here is still under investigation, the results may provide a new way to distinguish different types of tumors and to monitor the growth of malignant gliomas in vivo at the cellular level.

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