Monitoring Tumor Microvasculature Development in a Rodent Model of Human GBM using DCE-MRI

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INTRODUCTION

Glioblastoma multiforme (GBM) is the most common and anaplastic brain tumor, characterized by complicated angiogenesis, increased vascular permeability and focal necrosis [1]. The formation of new vessels is a general finding in all solid tumors; however, animal experiments have shown that different cancer cell lines implanted at the same site recruit vessels with different microvascular architecture [2]. Although these cell lines do not disseminate diffusely into the brain, they are a useful tool for studying angiogenesis in animal models [3], and in particular, for understanding the exact mechanism regulating the vascular formation. Previous studies have shown the ability of dynamic contrast-enhanced MRI (DCE-MRI) for microvasculature evaluation in human brain tumors and that a strong correlation between tumor grade and relative cerebral blood volume (rCBV) exists [3-6]. More recently studies have shown that relative vascular permeability and rCBV may also be correlated when examining high-grade GBMs in human patients [3]. Although DCE-MRI has shown a great deal of promise in human cases, little work has been done to investigate the vascular characteristics of GBM tumors in rodent models at high field strengths. The objective of this study was to determine the ability of DCE-MRI for measuring vascular changes during tumor development in a rat model of surgically implanted human GBM tumor cells.

MATERIALS AND METHODS

Tumor Implantation: All experiments were conducted on immunodeficient female nude rats (Charles River, Wilmington, MA). Intracranial tumor surgical implantation took place once each rat reached the specified target weight and age. U251-GBM tumor cells (5 x 10^5 cells in 10 µL PBS with 10% FBS) were administered using a syringe (Hamilton, Reno, NV), into the right hemisphere of the rat brain, while the animals were held in place by a stereotactic frame (Kopf Instruments, Tujuna, CA). After an initial one week recovery period following the surgery, animals were scanned twice a week for a total of 12 weeks. During surgery and subsequent MRI scanning, animals were anesthetized with 3% isoflurane in 3 L/min O_2, and were maintained on 1.5% isoflurane in 1 L/min O_2.

MRI Data Acquisition: MR experiments were performed on a 7T small animal MRI system (Bruker BioSpin, Ettlingen, Germany), equipped with an actively-shielded gradient set, with a maximum gradient strength of 400 mT/m. A 72 mm birdcage coil was used for signal excitation and reception. Conventional T_2-weighted RARE images (TE = 40 ms, TR = 4000 ms, slice thickness = 1 mm, matrix = 256 x 192) were acquired in axial and sagittal orientations (FOV = 3 x 3 cm^2) to assess differences in anatomy and to assist in positioning of the EPI slices. Prior to EPI data acquisition, all 1st and 2nd order shim coil currents were adjusted using the FASTMAP technique [7], for the entire brain region (cube size = 16 x 16 x 16 mm^3). DCE perfusion images were acquired with a T_2*-weighted single-shot SE-EPI sequence (TE = 50 ms, TR = 1500 ms, FOV = 3 x 3 cm^2, matrix = 64 x 64) with contiguous slices (slice thickness 2 mm) used to cover the entire brain region. Gadolinium-DTPA was administered via tail vein injection (0.1 mmol/kg) over a period of 4 repetitions, out of 100 total repetitions (temporal resolution = 3 sec/rep). Perfusion maps (rCBV) were computed using in-house written Matlab software (MathWorks, Natick, MA), based on the theory and methodology presented in reference [8].

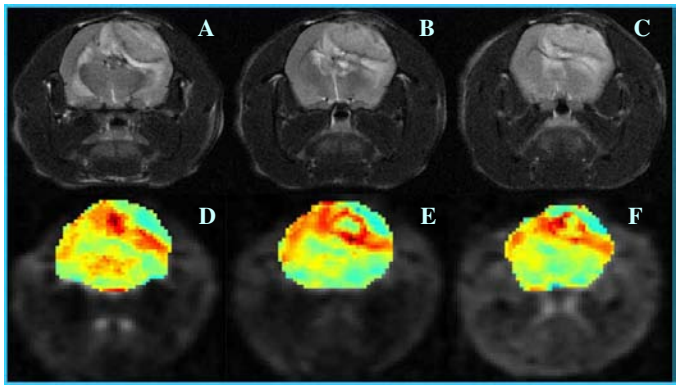

Figure 1: T_2-weighted RARE images of a GBM tumor in a rat brain (A-C) and the corresponding rCBV maps (D-F)

RESULTS AND DISCUSSION

A total of nine animals were subjected to serial DCE-MRI examination following intracranial tumor implantation surgery. On average, the tumors grew quickly reaching their largest size within 8 weeks after implantation, and began to show signs of necrosis immediately following. Among the studied animals, large inter-tumor and intra-tumor heterogeneities were seen from the DCE-MRI perfusion data. Figure 1 illustrates three T_2-weighted RARE anatomical slices (A-C) and the corresponding rCBV perfusion maps (D-F) within the tumor region from a rat brain with a U251-GBM tumor 8 weeks after implantation. In this example, the three rCBV maps show a ring of higher rCBV values with a region of lower rCBV values developing near the core of the tumor. Throughout the studied animals, distinct patterns of rCBV changes were noticeable throughout the tumor development, which helped to illustrate the predominant direction of tumor progression, as well as the detailed corresponding changes in tumor microvasculature. Figure 2 illustrates an enlarged region from figure 1 (E), further illustrating the substantial intra-tumor rCBV heterogeneity and the associated signal-vs-time curves from three different locations. Using the same imaging parameters, age matched control animals were also studied at the same time points. Unlike the tumor animals, each control animal showed a very consistent and controlled pattern of rCBV distributions, for the various brain regions throughout the duration of the experiment. DCE-MRI is a commonly used technique for measuring cerebral perfusion and evaluating the microvasculature distribution within various brain tissues. The results from this study show that the quantification of perfusion parameters can provide detailed local microenvironment information throughout tumor progression, and that DCE-MRI at high field strengths is suitable method for examining vascular changes during tumor development using animal models of human GBM tumors.

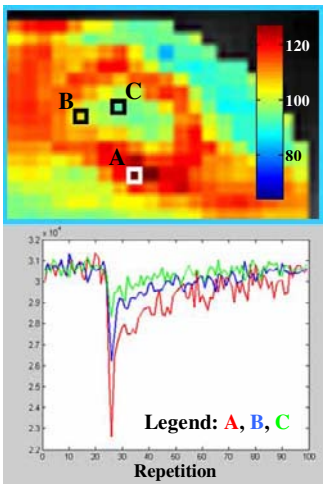

Figure 2: Zoomed rCBV map (top) and the corresponding signal-vs-time curves (bottom)

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