Diffusion kurtosis imaging for characterizing tumor heterogeneity in an intracranial rat glioblastoma model

Clémentine Lesbats | Claire Louise Kelly | Gabriela Czanner | Harish Poptani

The utility of diffusion kurtosis imaging (DKI) for assessing intra-tumor heterogeneity was evaluated in a rat model of glioblastoma multiforme. Longitudinal MRI including $T_2$-weighted and diffusion-weighted MRI (DWI) was performed on six female Fischer rats 8, 11 and 14 days after intracranial transplantation of F98 cells. $T_2$-weighted images were used to measure the tumor volumes and DWI images were used to compute diffusion tensor imaging (DTI) and DWI based parametric maps including mean diffusivity (MD), mean kurtosis (MK), axial diffusivity (AD), axial kurtosis, radial diffusivity, radial kurtosis, fractional anisotropy (FA) and kurtosis fractional anisotropy (KFA). Median values from the segmented normal contralateral cortex, tumor and edema from the diffusion parameters were compared at the three imaging time points to assess any changes in tumor heterogeneity over time. ex vivo DKI was also performed in a representative sample and compared with histology. Significant differences were observed between normal cortex, tumor and edema in both the DTI and DKI parameters. Notably, at the earliest time point MK and KFA were significantly different between normal cortex and tumor in comparison with MD or FA. Although a decreasing trend in MD, AD and FA values of the tumor were observed as the tumor grew, no significant changes in any of the DTI or DKI parameters were observed longitudinally. While DKI was equally sensitive to DTI in differentiating tumor from edema and normal brain, it was unable to detect longitudinal increases in intra-tumoral heterogeneity in the F98 model of glioblastoma multiforme.

KEYWORDS
brain tumor, diffusion kurtosis imaging, rat brain tumor, tumor heterogeneity

1 | INTRODUCTION

Glioblastoma multiforme (GBM) is the most frequently occurring central nervous system primary brain tumor with poor prognosis and a median survival rate of 15 months after diagnosis. Of the several rodent models of GBM, the rat orthotropic F98 model exhibits several traits of human...
Diffusion-weighted magnetic resonance imaging (DWI) has been widely used to quantify the random motion of water molecules in biological tissues. Standard analytical models processing diffusion-weighted MRI data for computation of the apparent diffusion coefficient (ADC) values assume water displacement in the tissue (voxel of interest) follows a Gaussian statistical distribution, similar to the water diffusion observed in homogeneous liquids. However, it is well known that the assumption of Gaussian distribution fails in vivo conditions due to the inherent heterogeneity from the presence of various tissue compartments, including different cell types, cell morphologies, extracellular matrix and blood. 

Diffusion kurtosis imaging (DKI) is a dimensionless metric that quantifies how much the water diffusion deviates from a Gaussian distribution due to cellular membranes, intra- and extracellular compartments and tissue structure. Thus, the diffusion of water molecules in homogeneous liquids will follow a Gaussian distribution with a kurtosis of zero. In tissues where diffusion is mostly hindered and restricted, water molecules will more likely diffuse short distances around the initial position in a time t, leading to a sharper statistical distribution and a positive kurtosis.

DKI has been used to assess white matter (WM) damage and myelin density. Preclinical DKI studies include infarct, traumatic brain injury and Alzheimer’s disease, type 2 diabetic ischemic stroke and acute alcohol intoxication.

DKI has also been reported to aid in assessing microstructural heterogeneity in tumors and its degree of diffusion restriction. It has been used in grading of human gliomas whereby higher mean kurtosis (MK) and lower mean diffusivity (MD) values were noted in high-grade solid tumors with increased cellularity. Increased cellularity and the presence of spindle-shaped cells led to a higher kurtosis and lower diffusivity in colorectal tumor xenografts.

Although promising, none of the published studies have assessed longitudinal changes in kurtosis parameters of the tumor for assessing changes in tumor tissue heterogeneity with regard to the microenvironment and cellular components as the tumor grows. Therefore, we performed a longitudinal study in a rat F98 brain tumor model to assess whether changes in DKI parameters can better assess tumor heterogeneity as the tumor volume increases over time.

## METHODS

### 2.1 Cell culture

F98 glioma cells (ATCC CRL-2937) were maintained as adherent monolayers cultured in Dulbecco Modified Eagles Medium (DMEM D6429, Sigma-Aldrich, St. Louis, MO, USA) containing 4.5 g/L glucose supplemented with 10% fetal bovine serum (FBS 10270–106, Gibco, Thermo Fisher Scientific, Waltham, MA, USA). The cells were maintained at 37°C in a 5% CO₂ humidified atmosphere. Cells were passaged twice-weekly at 1 x 10⁵ per T-75 flask and terminated after the fifth passage to avoid any chance of further mutations. Cells were tested bi-monthly for mycoplasma.

### 2.2 Brain tumor model

In vivo studies on rats were conducted in compliance with the UK Home Office (Animals Scientific Procedures Act 1986) and with the ethical approval of the local committee of the University of Liverpool. Six F344 female (100-120 g) Fischer rats (Charles River, Margate, UK) were injected with 50 000 F98 cells suspended in 5 μL serum-free DMEM culture medium. The injection was performed in an aseptic environment using sterile tools. The rat was maintained under surgical anesthesia using a 3% isoflurane in O2 gas mixture. Rats were given subcutaneous injections of antibiotics (5 mg/kg, 25 mg/mL enrofloxacin, 2.5% Baytril, Bayer, Leverkusen, Germany) and analgesia (0.3 mg/mL buprenorphine, Vetergesic, Ceva Animal Health, Amersham, UK) before the surgery, and 2 mL saline after the surgery. The rat was maintained in a three-point stereotaxic frame, the head was shaved and a small incision allowed access to the skull. A burr hole was drilled through the skull 3 mm right and 3 mm posterior from the bregma and the cells were injected 2.5 mm deep into the cerebral cortex. After the surgery, the skin was sutured, and the animal was returned to its cage for recovery. Three animals were housed together in a cage with stimulation objects and free access to food and water, which was provided ad libitum and the animals were kept in a 12-hour day/light cycle.

### 2.3 MRI acquisition

MRI scans were performed at 9.4 T on a Bruker Biospec (Bruker BioSpin, Ettlingen, Germany). Signal was generated using an 86 mm transmission birdcage coil and detected by a four-channel phased array surface coil. The rats were anesthetized with 2% isoflurane in O₂ and the respiration
rate and body temperature were monitored using an abdominal motion sensor and a rectal probe (SA Instruments, Stony Brook, NY, USA). The body temperature was maintained at 35°C by a hot water blanket and the respiration rate at 50-60 inspirations per minute. Each MRI experiment consisted of a localizer scan, followed by an anatomical T2-weighted sequence and a DWI sequence.

In vivo MR images were acquired longitudinally on days 8, 11 and 14 after inoculation of tumor cells to assess changes in the tumor microenvironment with DKI using a minimum of three time points (the early, mid and late tumor stages). These time points were also chosen to comply with UK Home Office guidelines of not subjecting the animal to undue stress of multiple anesthesia sessions or exceeding the severity limits on animal health. A multi-slice T2-weighted sequence was acquired to locate the tumor using a fast spin echo sequence with the following parameters: TE/TR = 33/5000 ms, RARE factor = 8, matrix = 256 x 256, FOV = 40 x 20 mm, 38 slices, scan duration = 2 minutes 38 seconds. DKI was performed using a respiratory-gated EPI-DTI sequence with the parameters: TE/TR = 23/2500 ms, five averages, four EPI segments, matrix = 128 x 64, FOV = 40 x 20 mm, 38 slices, voxel resolution = 0.3 x 0.3 x 0.3 mm³, δ/Δ = 4/11 ms, 15 directions, b-values = 0-1000-2000 s/mm², three b0 images, 27.5 minutes. The total scan duration for each experiment was ~ 60 minutes. Animals were rehydrated with 1 mL saline injected subcutaneously after each MRI session.

2.4 | Image processing and statistical analysis

The brain was manually segmented on the b = 0 s/mm² images from the diffusion-weighted datasets using ITK-SNAP (www.itksnap.org). Tumors were manually segmented on the T2-weighted images to assess tumoral growth. Tumor growth rate was calculated from the logarithm of the volume ratio from day 8 to day 14, and volumetric doubling time was then calculated using the exponential growth model. Diffusion and kurtosis parametric maps were calculated using the Diffusional Kurtosis Estimator (DKE) software (Medical University of South Carolina, SC, USA). A characteristic T2-weighted image of a typical rat 11 days after tumor cells injection and its corresponding parametric maps are shown in Figure 1. No corrections were made for geometric distortions or eddy current effects. Volumes of interest (VOIs) corresponding to the whole tumor, the whole peritumoral edema and the contralateral normal-appearing healthy brain parenchyma cortex were also segmented using ITK-SNAP and the binary masks were overlaid on the parametric maps. The contralateral normal brain healthy cortex VOI was segmented by selecting a region of left frontal cortex for every slice containing the tumor. As the contralateral normal brain microstructure is unlikely to change due to the presence of the tumor, it was used as a reference with the hypothesis that no significant changes in normal brain will be observed, while changes in tumor heterogeneity will lead to changes in DKI parameters. Care was taken to keep normal brain

![FIGURE 1](image_url)
VOI as big and as close as possible to the first imaging time point in each animal, and during longitudinal studies. Typical VOIs are shown in Figure 2B. Histograms of the parameter value distribution in the tumoral, edematous and cortical regions (Figure 2A,C) were generated using MATLAB (Mathworks, Natick, MA, USA). Mean, median and standard deviation values were calculated for each parameter using Origin (OriginLab, Northampton, MA, USA). A Wilcoxon signed-rank test was used to compare the tumor diffusion and kurtosis median values with the peritumoral edema and contralateral cortex. A Friedman test was used to compare the longitudinal data. A P-value of .05 or less was considered to be significantly different between the groups.

2.5 | Tissue collection

Animals were euthanized 1 day after the last MRI session using an overdose of 3 mL/kg pentobarbital sodium (Euthatal, Merial Animal Health, Harlow, UK) injected intraperitoneally. An incision was performed along the mid-ventral line through the abdomen to sever the aorta under the diaphragm. A midline thoracotomy gave access to the heart. A 25-gauge needle connected to an extension tube was clamped to the left ventricle of the heart to perfuse with 50 mL saline followed by 75 mL 4% Formalin (Sigma-Aldrich). Following fixation, brains were collected and suspended in 4% Formalin.

2.6 | Ex vivo MRI

Ex vivo MR images of the brain suspended in perfluoropolyether oil (Fomblin, Solvay, Brussels, Belgium) were acquired using the same $T_2$-weighted coronal fast spin echo sequence as the in vivo protocol except that 25 averages were used (scan duration = 1 hour 12 minutes). DWI was carried out using the same EPI-DTI sequence that was used in vivo with 25 averages (scan duration = 3 hours 26 minutes).

**FIGURE 2** A, mean diffusivity and C, mean kurtosis histograms in the tumor of a representative rat 14 days after tumor cell injection compared with the contralateral cortex and peritumoral edema and their corresponding B, mean diffusivity and D, mean kurtosis maps. The tumor (red), peritumoral edema (green) and contralateral cortex (blue) volumes of interest contours are illustrated on B, the MD and D, MK maps.
### 2.7 | Histology

The brain sample that was used for ex vivo DKI study was embedded in paraffin until sectioning after the DKI study. Hematoxylin and eosin (H&E) staining was performed on 4 μm coronal sections across the tumor. The sections closely matching the ex vivo imaging slice were qualitatively analyzed and the extent of cell density and cellular organization was based on visual assessment of staining.

### 3 | RESULTS

Figure 3 shows representative $T_2$-weighted MR images of a tumor-bearing rat brain, in which the developing tumor could be visualized 8, 11 and 14 days after inoculation of tumor cells. All six rats developed tumors in the right cortex, visible on the MRI scans from 1 week postsurgery. The tumor volume grew from $23.63 \pm 10.20 \text{ mm}^3$ (day 8) to $112.40 \pm 37.77 \text{ mm}^3$ (day 14). Based on these volumetric measurements, the growth rate was 0.116 days$^{-1}$. The tumor volume doubling time was 3.65 days ($n = 6$) in agreement with other F98 volumetric studies.\(^{26-28}\)

Figure 2 shows the MD and MK histograms in the tumoral, edematous and contralateral regions of a representative rat and their corresponding maps 14 days postimplantation. Higher MD is observed in the tumor compared with the contralateral cortex, but with overlapping distributions (Figure 2A). The peritumoral edema demonstrated higher MD than both the tumor and the contralateral cortex. The highest MK was observed in the tumoral region, whereas the lowest values were found in the edematous region (Figure 2C). However, the MK voxel distributions from the three regions were overlapping.

The median parametric values of the whole tumor excluding the edema, the peritumoral edema and the contralateral cortex volumes of interest (as shown in Figure 2B) for the six rats are shown as scatterplots in Figures 4 and 5 at days 8, 11 and 14. Table 1 lists the mean and standard deviation values of the diffusivity and kurtosis parameters in the six rats.

Figure 2 shows the MD and MK histograms in the tumoral, edematous and contralateral regions of a representative rat and their corresponding maps 14 days postimplantation. Higher MD is observed in the tumor compared with the contralateral cortex, but with overlapping distributions (Figure 2A). The peritumoral edema demonstrated higher MD than both the tumor and the contralateral cortex. The highest MK was observed in the tumoral region, whereas the lowest values were found in the edematous region (Figure 2C). However, the MK voxel distributions from the three regions were overlapping.

The median parametric values of the whole tumor excluding the edema, the peritumoral edema and the contralateral cortex volumes of interest (as shown in Figure 2B) for the six rats are shown as scatterplots in Figures 4 and 5 at days 8, 11 and 14. Table 1 lists the mean and standard deviation values of the diffusivity and kurtosis parameters in the six rats.

Tumors were observed on the MD maps with a concentric hyperintense structure composed of the peritumoral edema and the necrotic core (Figure 2B). The tumor appears hyperintense on the MK maps (Figure 2D). The axial (AD) and radial diffusivity (RD) maps showed a concentric structure similar to that observed on the MD maps formed of high diffusivity in the peritumoral edema and necrotic core. Likewise, the axial
FIGURE 4  Comparison boxplots of the median values of mean diffusivity (MD) (A, left) and mean kurtosis (MK) (B, left) in the six rats in the tumor (red), peritumoral edema (yellow) and contralateral cortex (blue) (* P < .05), and representative MD and MK maps at days 8, 11 and 14 (right). Filled diamond indicates individual data point from each animal, and empty square indicate the median value.

FIGURE 5  Boxplots of A, the axial diffusivity, B, radial diffusivity, C, fractional anisotropy, D, axial kurtosis, E, radial kurtosis and F, kurtosis fractional anisotropy in the tumor, the contralateral healthy cortex and the edema, at days 8, 11 and 14 postimplantation (* P < .05). Filled diamond indicates individual data point from each animal, empty square indicate the median value.
### Table 1

Mean ± standard deviation for all diffusion and kurtosis parameters in the tumor, edema and contralateral cortex volumes-of-interest of the six rats (#: significant difference between the tumor and the contralateral cortex, $: significant difference between the tumor and the edema). Mean and standard deviation from the ex vivo data were taken from the volume of interest in one representative rat.

|                  | MD x 10^{-3} (μm²/ms) | AD x 10^{-3} (μm²/ms) | RD x 10^{-3} (μm²/ms) | FA   | MK    | AK    | RK    | KFA  |
|------------------|------------------------|------------------------|------------------------|------|-------|-------|-------|------|
| **Day 8**        |                        |                        |                        |      |       |       |       |      |
| Tumor            | 0.78 ± 0.04            | 0.90 ± 0.06            | 0.71 ± 0.04            | 0.16 ± 0.04 | 0.87 ± 0.05 | 0.89 ± 0.07 | 0.86 ± 0.09 | 0.49 ± 0.15 |
| Edema            | 0.87 ± 0.06 $          | 1.03 ± 0.08            | 0.79 ± 0.06 $          | 0.19 ± 0.02 | 0.76 ± 0.03 $ | 0.78 ± 0.06 $ | 0.75 ± 0.05 $ | 0.50 ± 0.16 |
| Contralateral cortex | 0.71 ± 0.03 #           | 0.84 ± 0.03 #          | 0.64 ± 0.03 #          | 0.17 ± 0.03 | 0.78 ± 0.09 # | 0.75 ± 0.26 | 0.70 ± 0.17 # | 0.60 ± 0.18 # |
| **Day 11**       |                        |                        |                        |      |       |       |       |      |
| Tumor            | 0.75 ± 0.04            | 0.86 ± 0.05            | 0.70 ± 0.03            | 0.15 ± 0.03 | 0.84 ± 0.06 | 0.91 ± 0.79 | 0.79 ± 0.11 | 0.47 ± 0.10 |
| Edema            | 0.88 ± 0.03 $          | 1.03 ± 0.08            | 0.79 ± 0.03 $          | 0.22 ± 0.02 $ | 0.74 ± 0.04 $ | 0.79 ± 0.03 $ | 0.71 ± 0.08 $ | 0.50 ± 0.09 |
| Contralateral cortex | 0.68 ± 0.04 #          | 0.78 ± 0.04            | 0.61 ± 0.04 #          | 0.19 ± 0.05 | 0.75 ± 0.07 # | 0.92 ± 0.02 | 0.67 ± 0.12 # | 0.62 ± 0.11 # |
| **Day 14**       |                        |                        |                        |      |       |       |       |      |
| Tumor            | 0.74 ± 0.05            | 0.83 ± 0.03            | 0.67 ± 0.02            | 0.14 ± 0.03 | 0.82 ± 0.08 | 0.88 ± 0.06 | 0.77 ± 0.08 | 0.49 ± 0.06 |
| Edema            | 0.89 ± 0.04 $          | 1.07 ± 0.05 $          | 0.80 ± 0.04 $          | 0.22 ± 0.02 $ | 0.73 ± 0.02 $ | 0.74 ± 0.04 $ | 0.72 ± 0.06 | 0.46 ± 0.06 |
| Contralateral cortex | 0.67 ± 0.01 #          | 0.82 ± 0.03            | 0.59 ± 0.01 #          | 0.22 ± 0.02 # | 0.72 ± 0.06 # | 0.83 ± 0.06 | 0.73 ± 0.11 | 0.62 ± 0.05 # |
| **Ex vivo**      |                        |                        |                        |      |       |       |       |      |
| Tumor            | 0.55 ± 0.08            | 0.63 ± 0.08            | 0.51 ± 0.08            | 0.18 ± 0.07 | 0.90 ± 0.15 | 0.86 ± 0.14 | 0.83 ± 0.18 | 0.42 ± 0.08 |
| Contralateral cortex | 0.31 ± 0.01            | 0.37 ± 0.02            | 0.28 ± 0.01            | 0.23 ± 0.03 | 1.65 ± 0.12 | 1.61 ± 0.17 | 1.56 ± 0.16 | 0.50 ± 0.05 |
Kurtosis (AK) maps demonstrated hyperintense tumors. On the other hand, the radial kurtosis (RK) maps did not provide a clear definition of the tumor edges.

### 3.1 | Tumor vs. contralateral cortex

A Wilcoxon signed-rank test showed a significantly higher MD in the tumor compared with the contralateral cortex from day 11 ($Z = 2.097, P = .036$) (Figure 4A). Similar to MD, the RD was significantly higher in the tumor than the contralateral cortex, as illustrated in Figure 5B. No significant difference was observed between the tumor AD and the contralateral values (Figure 5A). The fractional anisotropy (FA) was significantly lower in the tumor on day 14 (Figure 5C).

The MK was significantly higher in the tumor compared with the contralateral cortex ($Z = 2.097, P = .036$ for all time points) (Figure 4B). Median RK was also significantly higher in the tumor compared with the contralateral cortex on days 8 and 11 (Figure 5E), whereas the tumor AK was not significantly different (Figure 5D). Kurtosis fractional anisotropy (KFA) was significantly lower in the tumor compared with the contralateral cortex at all time points (Figure 5F).

### 3.2 | Tumor vs. peritumoral edema

MD was significantly higher in the edema compared with the tumor and the contralateral cortex from day 8 ($Z = 2.097, P = .036$) (Figure 4A). RD was also significantly higher in the edema compared with the tumor (Figure 5B), and AD was significantly higher only on day 14 (Figure 5A). FA was significantly greater in the edema compared with the tumor (Figure 5C). MK was significantly lower in the edema compared with the tumor ($Z = 2.097, P = .036$) (Figure 4B). Significant differences were observed for AK at all time points (Figure 5D), and RK at days 8 and 11 (Figure 5E). KFA did not show any significant difference between the tumor and the peritumoral edema (Figure 5F).

**FIGURE 6** A, FA and B, KFA maps of an ex vivo rat brain and C, corresponding 10X H&E staining. 20X magnification on D, the edematous region, E, the tumor edge, F, the tumor center and G, the contralateral cortex. The red arrows indicate the tumor on the FA and KFA maps.
3.3 | Longitudinal changes in imaging parameters

The Friedman test showed no significant changes in tumor MD ($\chi^2 = 3, df = 2, P = .22$) or MK ($\chi^2 = 1.33, df = 2, P = .51$) values with time as tumor growth occurred. None of the other diffusivity and kurtosis parameters displayed any significant change with time and tumor growth.

3.4 | Ex vivo diffusivity and kurtosis

Ex vivo MRI scans and corresponding H&E slices of a representative brain are shown in Figure 6. A reduced FA was observed in the necrotic center of the tumor and in the tumor surroundings (Figure 6A, Table 1). KFA followed the same trend (Figure 6B, Table 1). Comparing the histological section with the similar slice section on MRI demonstrated a dense tumor (the visual appearance of higher staining reflecting increased cell density) on H&E staining (Figure 6F). The necrotic center was hollow due to the fixation and dehydration processes. The tumor edge seemed to have elevated cellular density compared with the contralateral cortex (Figure 6E).

4 | DISCUSSION

In this study, we investigated the utility of diffusion kurtosis to probe intra-tumoral heterogeneity in a rat model of intracranial glioblastoma. Although diffusion kurtosis demonstrated significant differences between the tumor, the peritumoral edema and the contralateral cortex from the early stage onwards, none of the parameters changed significantly as the tumor grew.

We observed an increased MD and MK in the tumor in comparison with the contralateral cortex. The increased MD may be due to increased extracellular diffusivity, or a significant increase in intracellular water diffusion due to cellular swelling. An increased mean diffusivity in tumors relative to the contralateral cortex was reported in rats with F98 and C6 glioma, despite some discrepancy exists, as another study reported decreased MD in C6 gliomas. MRI diffusion parameters, such as MD and FA, have shown potential for predicting tumor grade, treatment monitoring and prognosis. The F98 tumors exhibited higher MK values compared with the contralateral cortex, similar to some human studies reporting higher MK in high grade tumors compared with lower grade glioma. However, higher MK has also been associated with inflammation and glial activity in a rat model of traumatic brain injury. Hempel et al reported that MK was a robust parameter for WHO classification of human gliomas. In fact, the highest MK values were measured in IDHWT glioblastoma described by an increased cellularity, cellular heterogeneity, hemorrhage, necrosis and microvasculature proliferation, and the lowest MK values were observed in IDHmut because of their low cell density and homogeneity. The high MK observed in the F98 glioma in our study might originate from the high cell density of the tumoral rim and the heterogeneity of the necrotic core, which was verified by H&E staining, whereby very high cell density was observed in the tumor, and a slightly increased cell density was observed in the peritumoral area compared with the contralateral cortex.

RD and AD exhibited the same trend as the MD values in the tumor and the contralateral cortex. Previous studies also reported higher RD in F98, 9L and GBM22 rat tumors. We observed that RK was higher in the tumor compared with the contralateral cortex on days 8 and 11 whereas AK was not significantly different between the tumor and normal brain.

A lower FA value was observed in the tumor compared with normal brain in our study suggesting a more chaotic cellular organization in the tumor. In an earlier study, higher FA in the tumor rim than in the tumor core was reported in the F98 model. As the tumor size increased, the necrotic core grew to become the major part of the tumor VOI by day 14, thereby contributing predominantly to the whole tumor diffusion anisotropy measurements in our study. Similar to our observations, increases in FA have been reported in human tumors from grade II to IV gliomas. Lower KFA from the F98 tumor, especially from the necrotic center, indicates a much lower degree of tissue organization. KFA, which represents the anisotropy of the kurtosis tensor, has been recently proposed as a useful microstructural contrast. Although this metric is more appropriate for WM analysis in the case of several crossing fiber orientations in the same voxel, it also seems to be of interest for gray matter (GM) microstructure, as elevated KFA was observed in tissues with low anisotropy such as the thalamus and lenticular nucleus, where the cells are organized in oriented structures (e.g., lamina, nuclei). The variability in normal brain VOI parameters (Figure 5) was larger than expected, especially in the FA, RK and KFA values. The fact that this variability is not observed in all the parameters suggests that there might be some variability in the selection of the VOI, leading to different GM/WM ratios, and that FA, KFA and RK are probably more sensitive to these subtle alterations than the other DTI and DKI parameters. We observed decreased KFA in the tumoral tissue, suggesting a lower degree of overall tissue organization, which was noted in H&E stains showing high cellular density in the tumor with heterogeneity due to the necrotic cores.

The peritumoral edema displayed higher MD due to increased extracellular water. The increased water diffusion in all directions causes a significant decrease in diffusional kurtosis compared with the contralateral cortex, but also relative to the tumor. Furthermore, the peritumoral edema FA was always significantly higher than that of the tumor. An increased peritumoral edema FA and increased MD were also described in several F98 and 9 L rat glioma studies. However, an increased FA and decreased ADC (MD) in the area surrounding the tumor was reported by Kim et al and by Lope-Piedrafita et al in 9 L, F98 and C6 rat glioma, assumed to be caused by the compression of the surrounding cells to an
The increased diffusional kurtosis in F98 tumors, and a decrease in the peritumoral edema, were observed compared with normal brain, although no changes in DKI parameters were noted as the tumor grew, indicating that this technique may not be able to observe the microstructural tumor heterogeneity in the F98 model.

ACKNOWLEDGEMENTS

Dr Arthur Taylor, University of Liverpool, is acknowledged for providing the F98 cells and the protocol for tumor transplantation. Imaging studies were performed at the Centre for Preclinical Imaging, which was funded in part by a Medical Research Council (MRC) grant (MR/L012707/1).

ORCID

Harish Poptani https://orcid.org/0000-0002-0593-3235

REFERENCES

1. Hanif F, Muzaffar K, Perveen K, Malhi SM, Simjee SU. Glioblastoma multiforme: a review of its epidemiology and pathogenesis through clinical presentation and treatment. Asian Pacific J Cancer Prevent. 2017;18(1):3-9.
2. Barth RF, Kaur B. Rat brain tumor models in experimental neuro-oncology: the C6, 9L, T9, RG2, F98, BT4C, RT-2 and CNS-1 gliomas. J Neurooncol. 2009;94(3):299-312.
3. Ortensi B, Setti M, Osti D, Pellicci G. Cancer stem cell contribution to glioblastoma invasiveness. Stem Cell Res Ther. 2013;4(1):18. https://doi.org/10.1186/s13287-016-0241-6.
4. Coquery N, Serduc R, Rémy C, Barbier EL, Lemasson B. Cluster versus ROI analysis to assess combined antiangiogenic therapy and radiotherapy in the F98 rat-glioma model. NMR Biomed. 2018;31(8):1-9. e3933.
5. Côte J, Savard M, Bovenzi V, et al. Selective tumor blood–brain barrier opening with the kinin B2 receptor agonist [Phe8(ψCH2NH)Arg9]-BK in a F98 glioma rat model: an MRI study. Neuropeptides. 2010;44(2):177-185.
6. Bolcaen J, Descamps B, Acou M, et al. In vivo DCE-MRI for the discrimination between glioblastoma and radiation necrosis in rats. Mol Imaging Biol. 2017;19(6):857-866.
41. Kopelman R, Lee Koo YE, Philbert M, et al. Multifunctional nanoparticle platforms for in vivo MRI enhancement and photodynamic therapy of a rat brain cancer. *J Magn Magn Mater.* 2005;293(1):404-410.

42. Yan X, Zhou M, Ying L, et al. Evaluation of optimized b-value sampling schemas for diffusion kurtosis imaging with an application to stroke patient data. *Comput Med Imaging Graph.* 2013;37(4):272-280.

43. Sun PZ, Wang Y, Mandeville E, Chan S-T, Lo EH, Ji X. Validation of fast diffusion kurtosis MRI for imaging acute ischemia in a rodent model of stroke. *NMR Biomed.* 2014;27(11):1413-1418.

44. Lätt J, Nilsson M, Wirestam R, et al. In vivo visualization of displacement-distribution-derived parameters in q-space imaging. *Magn Reson Imaging.* 2008;26(1):77-87.

45. Reynaud O, Winters KV, Hoang DM, Wadghiri YZ, Novikov DS, Kim SG. Surface-to-volume ratio mapping of tumor microstructure using oscillating gradient diffusion weighted imaging. *Magn Reson Med.* 2016;76(1):237-247.

46. Hope TR, White NS, Kuperman J, et al. Demonstration of non-Gaussian restricted diffusion in tumor cells using diffusion time-dependent diffusion-weighted magnetic resonance imaging contrast. *Front Oncol.* 2016;6:179. https://doi.org/10.3389/fonc.2016.00179

**How to cite this article:** Lesbats C, Kelly CL, Czanner G, Poptani H. Diffusion kurtosis imaging for characterizing tumor heterogeneity in an intracranial rat glioblastoma model. *NMR in Biomedicine.* 2020;33:e4386. [https://doi.org/10.1002/nbm.4386](https://doi.org/10.1002/nbm.4386)