DETECTING REGION OF RECURRENCE OF BRAIN TUMORS BY TRACKING THE MOVEMENT OF BRAIN TUMOR CELLS ON A RAT MODEL USING MR-DTI

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INTRODUCTION

- Our overall hypothesis is that paths of elevated diffusion provide a preferred route for migration of cancer cells away from primary tumor and that this can be used to improve radiation treatment of brain cancer (eg. gliomas).
- Toward this end, we have developed a computational model of cell migration based upon MR-diffusion tensor imaging (DTI) to predict the microscopic spread of cancer in patients.
- Our objective in this work is to conduct in vivo and in vitro experiments to track rat glioma cancer cells with MRI to determine several key model parameters of our computational model including cancer cells' affinity for fibers and migration velocity.

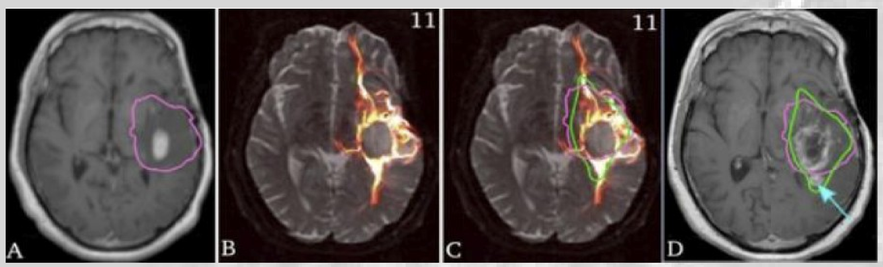

Fig1: [A] T1 weighted post-contrast image of a primary high grade glioma and the associated treatment plan (pink= 90% isodose curve). [B] [C] Map of cell concentrations (red=moderate; yellow/white=high) predicted from the model. Design of a proposed treatment plan (in green) that extends the margin into areas of predicted higher cell concentration by 1-2cm while reducing it in low-risk areas. [D] Follow-up image showing the location of the secondary tumor (arrow) outside the original treatment margin but within the proposed anisotropic treatment plan *(Krishnan AP et al.)*.

Hypothesis:

1. Through histological analysis we can demonstrate that tumor cells migrate farther from the site of engraftment along major fiber tracts compared to gray matter.
2. Our computational model applied to a rat brain DTI dataset can demonstrate elongated migration along major fibers compared to gray matter.

RESULTS

- The tumor cells fluoresced as expected and grew out of the injection site to a significant tumor size and showed early signs of spread. H&E stains showed high cell concentration around injection site predicting presence of tumor.

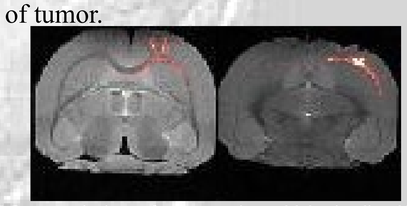

Fig5. Representative images from computation model showing slices 64 and 81. Slice 64 shows site of injection and slice 81 shows spread along fiber.

- Number of unique voxels traveled, major fiber voxels traveled and the linear distance were calculated for each cell with the initial and modified code. The modified rat random walk results were compared to the unmodified model.
- There exists a correlation between the total different voxels travelled by the cells(~total distance) and the fiber voxels travelled by the cells. Greater the unique voxels travelled, greater is the number of fiber voxels travelled. Anisotropic distribution of Linear Distance vs fiber voxel numbers showed higher correlation after change of FA values.

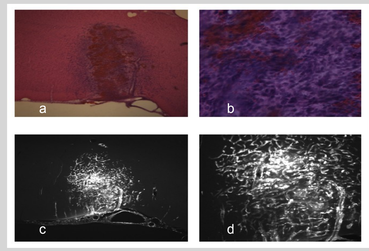

Fig6. Pictures at higher magnification showing Tumor Region using H&E stains (a & b) and GFP labels with fluorescent microscopy (c & d) .

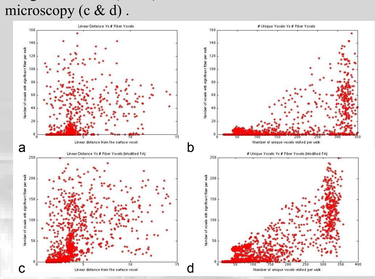

Fig7. Plots of Linear Distance and # Unique Voxels Vs # significant fiber voxels traveled by each cell for unmodified (a & b) and modified FA (c & d). # steps: 1000 ; #cells / voxel: 100 for all plots.

MATERIALS AND METHODS

Imaging DTI: Ex-vivo DTI performed on fixed rat brain with 117 x 163 x 117 µm resolution.
Tractography performed on MR-DTI to demonstrate major fiber bundles.

Injection: Lewis Rats were stereotactically injected with BEHAB/Brevican CNS-1 cells. All injections were on right hand side of skull. 2mm anterior and 2.4mm/1.15 mm lateral to bregma targeting white and grey matter regions.

MRI: Animals sacrificed after 11 days after injection. T1 weighted images were taken with resolution of 164 x 117 x 117 µm.

Histology and Staining:

The brain was sectioned at 40µm thickness.

- Every first section was mounted for GFP imaging.
- Every second section stained with H&E.
- Every Third section was stored for future use.

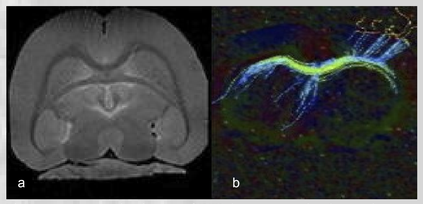

Fig2. a. representative image of DTI b. tractography done using Camino.

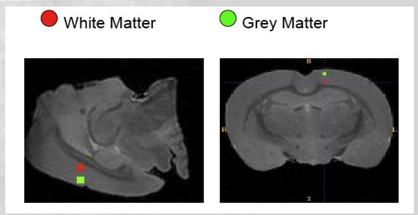

Fig3. Injection sites in different views.

Computational Model:

- Simulated cells seeded in every voxel on 3D tumor surface
- Migration of each cell was simulated independently.
- Uncertainty to estimate principal diffusion direction (PDD) was varied with fractional anisotropy (FA) value
- Initial Model: FA values of 0-0.3, 0.3-0.6 and 0.6-1 were assigned for ±35°, ±20° and ±10° uncertainty ranges in PDD angles.
- Modification: FAs of 0-0.2, 0.2-0.4 and 0.4-1 for ±35°, ±20° and ±10° PDD angles were assigned to better suit available fiber information.
- Direction of migration was selected using a random number generator.
- The output values were constrained to be Gaussian-distributed within the PDD angle uncertainty range.
- At the end of number of steps, the probability of tumor recurrence at any voxel was defined as the number of cells passing through each voxel.
- Random walk model run with tumor seed points located at injection sites (White and Grey Matter). The cells were allowed to walk for 1000 steps with a step size of ¼th voxel width.

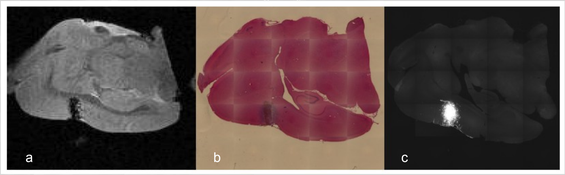

Fig4. Images showing injection site in a. MRI b. H&E and c. GFP Imaging.

DISCUSSION AND CONCLUSION

Animal Studies:

- Injected CNS-1cells formed tumor of significant size and show early signs of spread.
- Red regions are either necrotic or are blood cells from leaky vessels.
- The void in MRI was possibly due to micro bubbles or from haemoglobin in blood cells from leaky vessels.

Computation Model:

- Greater the number of major fiber voxels travelled, greater is the total distance travelled.
- Larger linear distances were covered by cells that travelled primarily on fiber voxels.
- The cells that have low linear distances but high count of fiber voxels were likely cells on the curved fibers.

Future Directions:

- Animal studies to be repeated by increasing the number of cells and days of incubation before animal sacrifice.
- Monitor tumor growth with invivo MR imaging before sacrifice.
- Inject Super Paramagnetic Iron Oxide (SPIO) labeled cells into Lewis Rat and check feasibility of visualization of cells with MRI.
- Expected Result: More Tumor spread along the Fiber tracks.
- Parameters of Computation model will be modified to identify values that closely relate to animal histology imaging results.

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We thank the Gateway for Cancer Research Foundation, Clinical and Translational Science Institute (Rochester) and the Rochester Center for Brain Imaging for funding.

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