Novel staining method for visualization, isolation and processing of intracranial brain tumors

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ABSTRACT

BACKGROUND & OBJECTIVE: Studies using intracranial (IC) tumors are critical for the development of drugs in the treatment of brain cancer such as glioblastoma (GBM), and tumor harvest and processing are important when studying PK/PD, drug delivery, and tumor evolution. Typically, tumor dissection from the surrounding brain is dependent on distinct morphology and/or expression of fluorescent markers. However, genetic modification of fluorescent protein expression is not always feasible, especially for patient-derived xenograft (PDX) tumor models. Thus, we have developed a dye-based staining method to confidently visualize tumor in the mouse brain and assessed usability of isolated tumors for drug, DNA, and RNA analyses.

METHODOLOGY: Whole brains were harvested from mice with IC Mayo GBM PDX tumors and stored at -80ºC. Prior to staining, brains were transferred to -20ºC. Brains were cut into 1 mm thick coronal sections and transferred onto glass slides. Slices were covered with toluidine blue (TB) solution (0.05-10 mg/mL) for 45 s, followed by removal of excess stain and observation under white light. Regions of tumor increased in transparency, while normal brain remained opaque. This enabled dissection of tumor from brain for downstream analyses.

RESULTS: TB concentration was optimized to stain 1 mm thick sections of fresh-frozen brain, and the best contrast between tumor and normal brain was observed at 0.1-0.3 mg/mL. TB aided differential visualization of tumors in several PDX models – GBM12, GBM22, GBM43, GBM39 and GBM108 – suggesting broad applicability. To evaluate the accuracy of TB staining, brain with PDX tumor transduced with pSINeGFP/fLuc2 was observed by both TB staining and eGFP fluorescence. This analysis demonstrated excellent concordance across 6 animals with three distinct PDX models. Genomic DNA was isolated from tumor dissected under TB guidance and analyzed by PCR for primers specific for murine or human PTGER2. 50-70% of the DNA in tumor samples was detected to be of human origin while normal brain tissue had 90-95% of the mouse DNA, highlighting the specificity of TB stain for tumor detection. Tumors from brigimadlin treated GBM108 PDX were isolated under TB guidance and comparable drug and RNA levels were detected by LC-MS/MS and nanodrop as analyzed in GFP-assisted tumor samples, respectively.

CONCLUSIONS: Staining fresh-frozen, thick brain sections with toluidine blue enables visualization of tumor from normal brain regions that is compatible with varied downstream processes. Development of such staining assays can help make tumor processing faster and easier and is not dependent on genetic modification of tumor cells prior to experimentation.

TOLUIDINE BLUE STAINING TO VISUALIZE INTRACRANIAL TUMORS

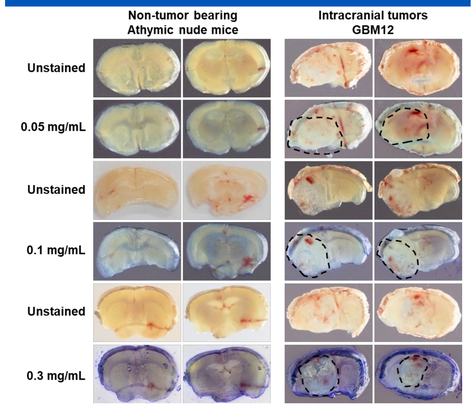


Figure 1. Intracranial tumors can be distinctly visualized from surrounding normal brain after staining with TB solution. TB is an acidophilus metachromatic nuclear stain used in histology and vital staining. It has been used to detect mucosal abnormalities and identifying carcinoma of the oral cavity.

BROAD APPLICABILITY ACROSS SEVERAL GBM PDX MODELS

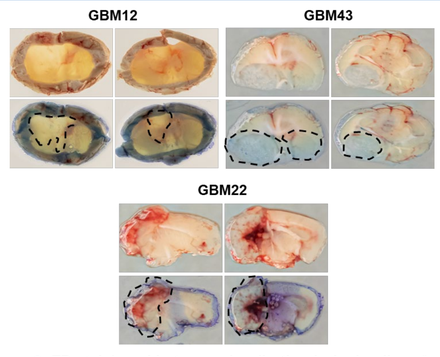
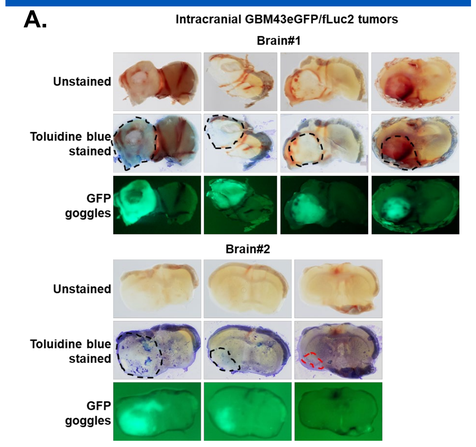


Figure 2. TB staining aids tumor visualization in brain slices from several Mayo GBM PDXs.

VALIDATION OF STAINING METHOD



PCR Oligo Primers

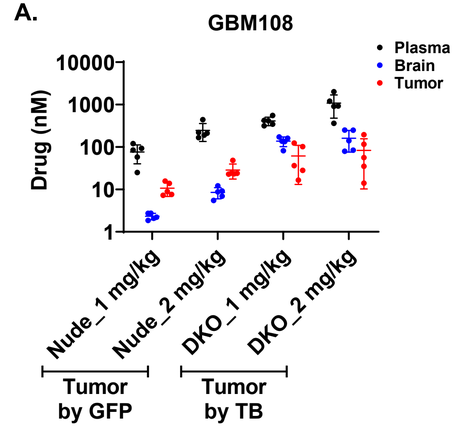
Species	Forward Primer	Common Reverse Primer	Amplicon (bp)
Human	gctgcttctcattgtctcgg	gccaggagaatgaggtggtc	189
Mouse	cctgctgcttatcgtgcgtg	same reverse primer listed above	189
Human + Mouse	tacctgcagctgtacgccac	same reverse primer listed above	215

Real-Time qPCR Probes

Species	Fluorophore	Sequence	Quencher
Human	NED	tgctgcttctcattgtctcg	MGBNFQ
Mouse	VIC	cctgctgcttatcgtgcgtg	MGBNFQ

Figure 3. Validation of TB guided tumor visualization and harvest. A. GBM43eGFP/fLuc2 intracranial tumor area delineated by TB staining matched with the tumor area detected by GFP fluorescence using GFP goggles. **B.** Tumor and normal brain samples from diverse PDX models, collected under TB guidance, showed abundant presence of human and mouse PTGER2 genes, respectively, as determined by PCR analysis of genomic DNA. Each dot on the plot represents sample from an individual mouse.

DOWNSTREAM PROCESSING OF DISSECTED TUMOR



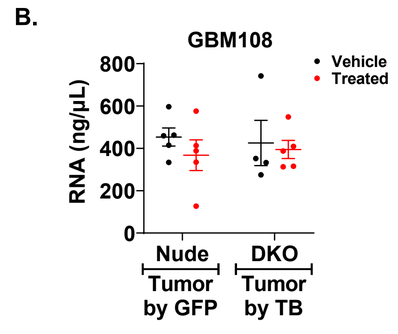


Figure 4. Processing of tumors harvested under guidance of TB staining for various downstream analyses. A. Using LC-MS/MS, concentration of drug was measured in the plasma, normal brain and tumor samples (TB or GFP guided) collected after brigimadlin treatment of nude mice with GBM108 parental tumor or double knockout (DKO) mice lacking efflux pumps with intracranial GBM108eGFP/fLuc2 tumor. **B.** Using nanodrop, concentration of RNA was determined in tumor samples collected by GFP signal or by TB staining from the untreated or brigimadlin treated GBM108eGFP/fLuc2 or GBM108 parental tumors, respectively. Each dot is data from an individual mouse.

SUMMARY & CONCLUSIONS

- TB stain enables confident visualization and precise harvesting of intracranial tumors, distinctly separating them from the surrounding normal brain.
- TB staining method is broadly applicable to visualize several distinct GBM PDX tumors.
- TB staining facilitates tumor visualization without relying on genetic modifications of tumor cells, which could introduce unintended alterations in tumor behavior.

FUTURE DIRECTIONS

- TB staining exhibits varied patterns, requiring further improvements to ensure consistent, reliable outcomes and a more user-friendly approach.
- Exploring whether TB staining can enhance the visualization of brain metastatic tumors with multiple foci of varying sizes would be particularly intriguing.

REFERENCES

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