Measuring Brain Tumor Growth: A Combined BLI / MRI Strategy

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Introduction: In oncology research, small-animal models are critical for the development of effective therapeutic strategies. A key to the effectiveness of these pre-clinical investigations is having methods to accurately monitor tumor growth and response to therapy *in vivo*. Magnetic Resonance Imaging (MRI) offers excellent anatomic tumor localization and accurate volumetric measurement, however it is less well suited to high throughput studies, and it does not address the need to assess tumor growth very early following implantation. Bioluminescence imaging (BLI) offers an efficient method for identifying tumors early, monitoring their growth, and measuring response to therapeutic intervention in larger numbers of animals.[1,2,3] A number of studies have shown a strong correlation between bioluminescence measurements and tumor volumes.[4,5] However, a comprehensive study of these combined techniques, including limitations, has not been previously described. In our experience with small-animal models of malignant brain tumors using the DBT cell model, we have observed specific instances in which the BLI data do not directly correlate with the corresponding anatomic images visualized using MR imaging. In this work, we describe combined BLI and MRI studies to characterize tumor growth and development in a mouse model of high-grade brain tumors and suggest a procedure by which small-animal MR imaging can be added to BLI to augment the effectiveness of this imaging modality. Together, these techniques offer a method by which multi-modality imaging may improve the accuracy of longitudinal *in vivo* studies without dramatically increasing the cost or time required for such studies.

Methods:

***Tumor Implantation*:** Tumors were implanted in forty mice with DBT cells transfected with firefly luciferase according to methods described previously[6].

Bioluminescence Imaging Mice were anesthetized, injected with D-luciferin at 50 mg/ml i.p. (Xenogen, Alameda, CA), and imaged ten minutes later with the IVIS Imaging System (Xenogen) for 1–120 s, bin size 2. To quantify bioluminescence, identical circular regions of interest encircled the entire head of each animal, and the integrated flux of photons (photons per second) within each region of interest was determined by using the LIVING IMAGES software package (Xenogen).

Magnetic Resonance Imaging Images were collected in an Oxford Instruments 4.7-Tesla magnet (33 cm, clear bore) equipped with 15-cm inner diameter, actively shielded gradient coils (maximum gradient, 18 G/cm; rise time, 100 μs). The magnet/gradients are interfaced with a Varian INOVA console, and data were collected using a 1.5-cm OD surface coil (receive) and a 9-cm ID Helmholtz coil (transmit). The mice were anesthetized with isoflurane/O_2 [3% (v/v)], and maintained on isoflurane/O_2 [1.5% (v/v)] throughout the experiments. Mice were injected intraperitoneally with 500 μl Omniscan contrast agent, diluted 1:10 in sterile saline, 15 minutes prior to being placed in the magnet. T1-weighted, gradient-echo multi-slice images (coronal and horizontal views) were collected (TR = .125 s, TE = .0025 s, FOV (coronal) = 1.5 × 1.5 cm^2, FOV (horizontal) = 3 × 3 cm^2, slice thickness = 0.5 mm). In addition, T2-weighted, multi-slice spin-echo coronal images were collected for each animal (TR = 1.5 s; TE = .05 s; FOV = 1.5 × 1.5 cm^2; thickness = 0.5 mm).

Results and Discussion: Four groups of ten mice each were injected with 50,000 luciferase-positive DBT cells and serially imaged with both MRI and BLI. The first two groups were used to establish a correlation between optical signal and tumor volumes. The second two groups were used to determine whether early optical signal could be used to predict subsequent (exponential) tumor growth. While the majority of our mice showed excellent correlations between quantitative BLI and tumor volumes as measured by MRI, some mice did not fit this correlative projection. Serial BLI and MR volumetric measurements were performed for each animal in the study. We identified two particular conditions under which mice displayed high BLI signal, but did not have correlating large tumor volumes: mice with hydrocephalus (Figure 1), and mice with intracranial hematomas (Figure 2).

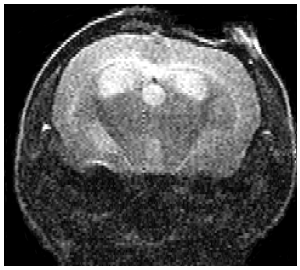


Figure 1: Spin-echo MR image of a mouse with high optical signal (2.25 × 10^8 photons per second) but no significant tumor volume. Instead, this mouse displays extensive hydrocephalus.

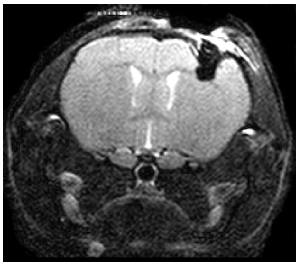
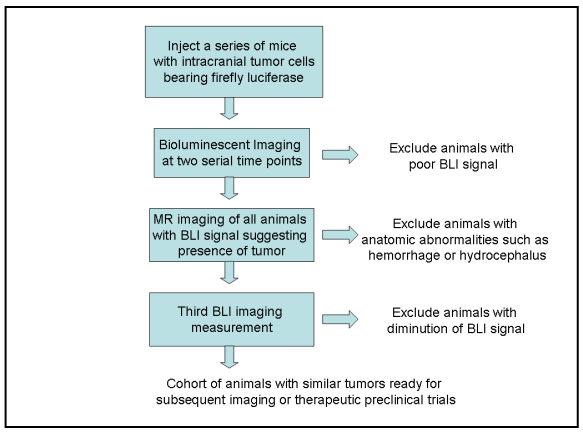


Figure 2: Spin-echo image of a mouse with high optical signal (9.4 × 10^7 photons per second) and intraparenchymal hemorrhage at the site of injection, but no visible intracranial tumor.



At late stages of exponential tumor growth in animals with growing solid tumor, optical signal tended to plateau discordantly with tumor growth. This leveling of the BLI signal is attributed predominately to the development of hemorrhage and necrosis within the tumor bed, a finding which was confirmed by MR (Figures 3, left and right). These experiences, as well as our success with identification of tumor volumes using a combination of BLI and MR imaging, lead us to suggest a method for combining these two imaging modalities to best select animals with similar growth rates and to limit confounding factors related to anatomic factors such as hemorrhage or hydrocephalus. This method is summarized in the flow chart below. This combination of anatomic MR imaging and bioluminescence imaging provides a multidisciplinary approach for selecting cohorts of animals having similar patterns of tumor growth for subsequent use in therapeutic preclinical trials.

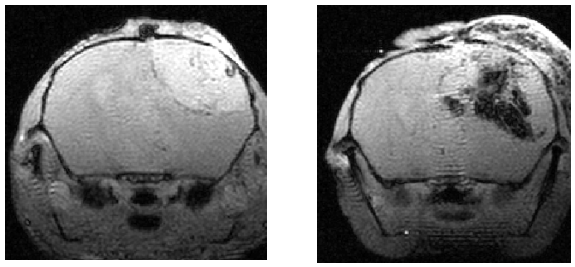


Figure 3, left and right: Contrast agent-enhanced, gradient-echo images of mouse with a DBT cell tumor at post operative days 15 and 19, respectively. Note the significant enhancing solid tumor at day 15 that changes to a partially necrotic and hemorrhagic tumor by day 19. While the tumor volume increases from 75 mm^3 to 210 mm^3, the optical signal declined slightly from 1.2 × 10^9 photons per second to 9.8 × 10^8 photons per second.

References: 1) Dinca EB, Sarkari JN et al. J Neurosurg. 107: 610-613, 2007. 2) Jenkins DE, Oei Y et al. Clinical and Exp Metastasis 20: 733-744, 2003. 3) Szentirmai O, Baker CH, et al. Neurosurgery 58: 365-372, 2006. 4) Rhetmtulla A, Stegman LD, et al. Neoplasia 2: 491-495, 2000. 5) Rubin JB, Kung AL, et al. Proc Natl Acad Sci USA 100: 13513-13618, 2003. 6) Jost SC, Wanebo JE et al. Neurosurgery 60: 360-371, 2007.