Evaluation of Variability in Bioluminescence Measurements in Orthotopic Bladder Tumors with Dynamic Contrast Enhanced MRI (DCE – MRI)

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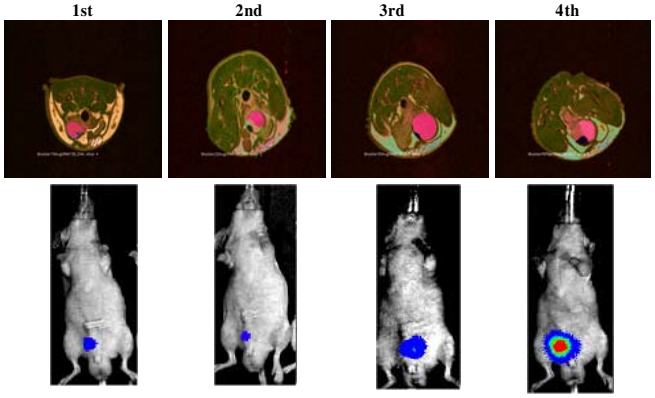
Purpose: Recently, bioluminescence (BLI) has emerged as a potential technique for validating the results of cell culture research *in vivo* using orthotopic models of cancer (1). However, in vivo use of the technique has been somewhat limited by confounding physiological factors, such as hypoxia, vascularity and intervening tumor tissue which result in poor correlation between tumor volume measurements and BLI in several tumor cell lines, including bladder (2). The uptake of Gd-DTPA in dynamic contrast-enhanced magnetic resonance imaging (DCE-MRI) is related to the blood perfusion, vascular density and permeability (3) while multi-gradient echo (MGE) sequences are sensitive to tissue oxygenation levels through R2* values (4). The current research attempts to use these imaging techniques to explain this lack of correlation, by evaluating the Gd-DTPA concentration and the oxygenation and perfusion status of these tumors.

Materials and Methods: Orthotopic bladder tumors were established in 5-week-old nude mice (n=20) with 100,000 KU7 or 500,000 253J-BV cells that had been previously transduced with a lentiviral luciferase construct. The mice were imaged weekly after intra-peritoneal injection of luciferin with the In Vivo Imaging System (Xenogen). All MR imaging (Bruker Biospin 4.7T) was performed on the same day as the BLI and dynamic contrast studies using 0.2 mmol/kg Gd-DTPA (Magnevist; Berlex Laboratories, Inc.) were performed for the final two time points. Pre-contrast T1-weighted (TR/TE: 700.0 ms/8.5 ms, FOV: 4.0 cm x 3.0 cm, Matrix: 256 x 192) and, T2-weighted (TR/TE: 4000 ms/70 ms, ETL: 12, Echo-spacing: 13.659 ms, FOV: 4.0 cm x 3.0 cm, Matrix: 256 x 192) were acquired. An MGE sequence (TE: 2 – 47 ms with spacing of 5ms, TR: 800 ms, FOV: 4.0 cm x 3.0 cm, Matrix: 128 x 96) was used to estimate the R2* values. To enhance lesion contrast, T1 and T2 images were fused via the color scaling method using IDL software (RSI, Boulder, CO) and regions-of-interest (ROI) were drawn along the tumor perimeter for each slice. The dynamic contrast enhancement curve for each slice was obtained from the averaged signal intensities. Quantitative pharmacokinetic analysis was performed, using the Patlak model (5) implemented on IDL software. Mice were sacrificed at four weeks 45 minutes after intraperitoneal injection of 60-mg/kg piminidazole. Immunohistochemistry using a monoclonal antibody specific to piminidazole adducts to detect hypoxia and hematoxylin and eosin staining was performed to assess necrosis.

Results: KU7 xenograft bioluminescence correlation coefficients (R) to MR tumor volume for day 7,14, 21 and 28 were 0.29, 0.42, - 0.06 and 0.05 respectively. The tumor weight correlation to BLI was poor, while it was significant with MR(R = 0.87 $p < 0.05$). In contrast, 253J-BV xenograft bioluminescence correlation coefficients (R) to MR tumor volume for day 7,14, 21 and 28 were 0.13, 0.02, 0.70 and 0.93 respectively. The tumor weight correlation was good with both BLI (R = 0.75) and MR volumes (R = 0.84). In the KU7 group, the BLI measurements showed significant correlation with the maximum Gd-DTPA concentration and total concentration over time ($p < .05$). The tumor tripled in volume between 1st and 2nd week for the KU7 mice while it remained stable for the 253J-BV. Oxygenation levels were less in the KU7 xenografts, as seen by the R2* values in comparison to the 253J-BV. This was confirmed on histology, which showed extensive hypoxic and necrotic areas. The plasma volume (Vp) of the KU7 decreased as the tumor grew, while it kept increasing with tumor size for the 253J-BV.

Conclusion: This study shows that researchers need to be careful when evaluating therapeutic effects in orthotopic models on the basis of serial BLI measurements. Changes in tumor size, poor functional vasculature and hypoxic levels can contribute significantly to the intensity of the signal in unexpected ways. We have shown in these cell lines that DCE-MRI and MGE sequences assists in characterizing tumor behavior, and selecting cell lines which are suitable to be monitored in orthotopic models by BLI. Similar analysis would be insightful in research of drug delivery, gene therapy and evaluating treatment effectiveness.



References

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Figure 1. *Upper panel* shows tumor in the fused T1 and T2 images from a 253J-BV mouse for the 1st(day 7), 2nd (day 14), 3rd (day 21) and 4th (day 28) time point, *Lower panel* shows the serial BLI images for the same mouse at the same time points.