Detection of LacZ and luciferase double gene expression in breast cancer xenograft by MRI and BLI

W. cui[1], L. Liu[1], and R. Mason[1]

[1]Radiology, UT Southwestern Medical Center, dallas, tx, United States

Introduction: There is great interest in developing reporter molecules for non-invasive assessment of transgene activity. Historically, the most valuable reporter gene has been lacZ, which generates β-galactosidase and is used in many molecular biology contexts. As such many colorimetric histological stains are available, but hitherto imaging approaches have lagged behind. We have previously shown that the "black stain", S-Gal™ had potential as a proton MRI reporter for β-gal. Upon cleavage by beta-galactosidase in the presence of ferric ions (Fe3+), the aglycone chelates iron to produce an intense black stain, which is not only visible, but also paramagnetic. We now report a novel double gene approach to detecting transgenic activity in tumors based on ^{1}H MRI of the S-Gal™ and bioluminescent imaging (BLI), which is a highly sensitive method to determine the location and relative intensity of luciferase gene expression in mice.

Methods: MCF7 wild type cells were stably transfected with phCMV/lacZ. These cells were further transfected using adenovirus with Luc gene and the highest expressing clones isolated, propagated and implanted in flanks of female nude mice. MRI was performed using a 4.7T Varian Inova system with T2* weighted images obtained before, and 2 min after the intratumoral injection of 50 mg/kg S-Gal-Na and 25 mg/kg ferric ammonium citrate (FAC) (TR=500 ms, TE=15 ms, Flip angle=20o, matrix=128x128). The whole abdomen was observed using a 2cm volume coil at 4.7 T. Luciferin-D (450 mg/kg) was administered S.C. in the neck region of anesthetized mice and BLI acquired 10 mins later over 2 mins with a charge-coupled device (CCD) camera.

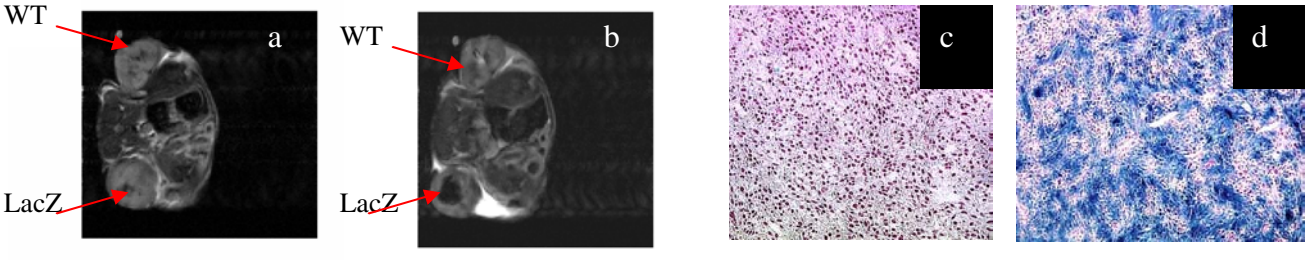

Figure1. β-gal expression was detected *in situ* in the living mice and confirmed by post mortem histology. (a) T2-weighted MRI pre-injection of S-GalTM and FAC; (b) post contrast: MCF7/WT tumorshowed minimal change, while MCF7/lacZ/luc tumor showed strong contrast. Histology using X-gal and Nuclear fast staining for (c) MCF7/WT and (d) MCF7/lacZ tumor.

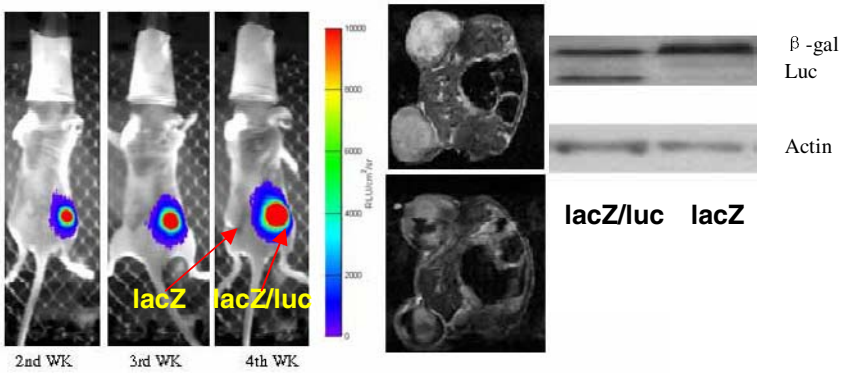

Figure 2. *In vivo* detection of LacZ and Luc gene expression by BLI and MRI (a) BLI time course showing tumor growth; (b) Detection of β-gal expression in both tumors by 1H MRI using S-Gal ™; (c) Western blot tumors confirming similar β-gal expression, but differential luc expression

Results: The paramagnetic black precipitate was detected rapidly in both MCF7/lacZ/Luc and MCF7/lacZ tumors by 1H MRI following direct intra tumor injection of S-Gal™. The wild type tumor showed minimal contrast. BLI showed a good linear correlation between relative intensity and tumor volume.

Conclusions: We have demonstrated combined MRI and BLI to detect LacZ/luc double gene expression in breast tumor xenografts. BLI is relatively cheap and facilitates high throughput interrogation, while MRI provides high spatial resolution. We believe this combined approach can become a valuable tool for assessing tumor growth (*e.g.*, constitutive Luc) together with *in situ* transfection.