Detection of transgene expression using hyperpolarized ^{13}C urea and diffusion-weighted MRS

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Target Audience: Basic researchers who are interested in molecular imaging.

Purpose: To assess the potential of a gene reporter system, based on a urea transporter and diffusion-weighted ^{13}C MRS of hyperpolarized [^{13}C]urea.

Methods: SCID mice were implanted subcutaneously with either unmodified HEK 293T cells, or the same cells expressing a urea transporter (UTB) and red fluorescent protein (mStrawberry) (figure 1A). After injection of hyperpolarized [^{13}C]urea[1], a diffusion-weighted spin-echo sequence (TR = 200 ms, TE = 52 ms, b = 5-1067 s/mm^2, δ = 6 ms, Δ = 10.5 ms)[2] was used to measure urea diffusion in control and UTB-expressing tissue at 7 T. For comparison, water diffusion data were acquired using a ^{1}H spin-echo sequence (TR = 1000 ms, TE = 30 ms, b = 10-1345 s/mm^2, δ = 6 ms, Δ = 14 ms). Apparent diffusion coefficients (ADC) were estimated using a mono-exponential diffusion equation. For ^{13}C diffusion data, echo signals were normalized to the corresponding FID signal before fitting[2]. Tumor cellularity was assessed using hemotoxylin and eosin staining.

Results: The ADC of hyperpolarized urea was 21% lower in tissue expressing the urea transporter when compared with with control tissue ($p<0.05$, 1-tailed t-test, n = 6 in each group), (figure 1C). No difference in water ADC or cellularity between these tissues was found, indicating that they were similar in composition (figures 1B and 1D).

Discussion: Expression of a urea transporter led to a lower urea ADC, most likely due to transport of urea into the intracellular space. However, the difference between control and UTB-expressing xenografts was relatively small, possibly due to high concentrations of the urea in the interstitial space, where it may also be expected to have a lower ADC. Diffusion analysis using an extended range of b-values could be used to further assess the distribution of urea between the intra- and extracellular spaces, although this experiment will be limited by the rapid loss of hyperpolarized signal.

Conclusion: Expression of the urea transporter, by mediating cell uptake of urea, lowers the ADC of hyperpolarized ^{13}C urea in tissue and thus the approach has the potential to be used as an MR-based gene reporter *in vivo*.

References:

[1] Golman et al. Proc Natl Acad Sci U S A 100(18):10435-9, 2003.

[2] Kettunen et al. MRM 70(5):1200-9, 2013.

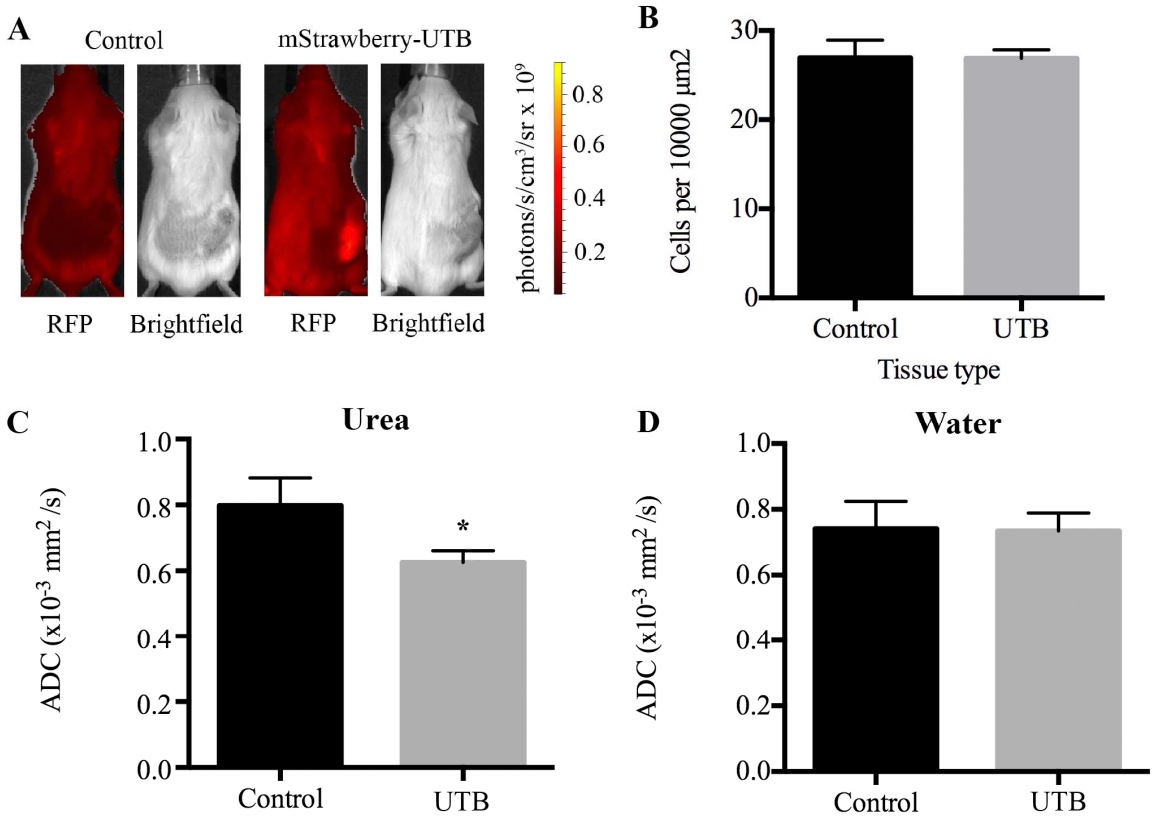

Figure 1. (A) Fluorescence imaging confirming transgene expression (mStrawberry-E2A-UTB) in implanted xenografts. **(B)** Histological analysis of xenograft tissue *ex vivo* showed comparable cellularity. **(C)** Hyperpolarised [^{13}C]urea showed lower diffusion in cells expression UTB. **(D)** Water diffusion in both xenograft types was comparable.