Evaluation of the effects of Herpes Simplex Virus Thymidine Kinase (HSV-TK) Overexpression on 5-fluoro-2'-deoxy-uridine Metabolism in Tumor Xenografts using *In vivo* ^{19}F MR Spectroscopy

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Introduction: The antitumor activity of 5-fluoro-2'-deoxy-uridine (FUdR) is related to its metabolic conversion to 5-fluorouracil (5-FU) with the consequent production of cytotoxic fluoronucleotides (FNuc) which can be activated by the action of thymidine kinase (TK) [1]. Previous reports have shown the *in vivo* use of ^{19}F MRS to measure pharmacokinetics and drug metabolism of FUdR [1]. The purpose of this study was to use tumor models consisting of HSV-TK fused to green fluorescent protein (GFP) for optical and MR imaging of gene expression and FUdR metabolism.

Methods: Growth-inhibition studies were performed *in vitro* by growing R3327-AT, R3327-AT/TK [2] and RG2, RG2-TK [3] cells in media containing increasing concentrations of FUdR for 96h. After 4h incubation with MTS the absorbance was read at 490nm and IC_{50} values were calculated from individual inhibition curves. For *in vivo* studies, two million cells were injected subcutaneously into the hind leg of male athymic nu/nu mice. When tumor sizes reached a size between 200 and 400mm^3, ^{19}F MRS was performed and, in R3327-AT/TK tumors, the expression of the fusion protein, GFP-TK was evaluated by *in vivo* fluorescence imaging. MR was performed using a Bruker 7T spectrometer. An i.v. catheter was inserted into the tail vein of mice to allow the administration of 300mg/kg FUdR to the mouse in the magnet. Isoflurane was used for anesthesia. A homebuilt two-turn radiofrequency solenoid coil with a diameter of 15mm was used. An external reference standard of 75mmol/L NaF was added for quantitation. Body temperature was maintained at 37°C by use of a waterbath which also minimizes magnetic susceptibility effects [4]. ^{19}F MR spectra were obtained sequentially using a singlepulse sequence (Paravision 4.0, Bruker) for up-to 2h after administration of FUdR. Acquisition variables included a 60° pulse angle, a repetition time (T_R) of 1s, an acquisition size of 1048 and 600 scans (temporal resolution ~ 10min). The MR time domain was Fourier transformed after applying a line broadening of 40Hz and analyzed using the software XsOs NMR. Spectral assignments of peaks observed were based upon chemical shifts as previously described [5].

Results: Cell proliferation inhibitory studies showed that R3327-AT/TK cells were minimally but significantly more sensitive to FUdR than the parental cell line (Table 1). RG2-TK cells were significantly more sensitive to FUdR than its parental cell line (Table 1). *In vivo*, R3327-AT/TK-GFP tumors exhibited strong fluorescence (Fig. 1). Figure 2 shows ^{19}F MR spectra with a 10 minute temporal resolution for up-to 2h following administration of a 300mg/kg i.v. bolus. In the MR spectra two signals could be resolved: one centered at 0ppm representing what we assigned to be FUdR and one centered at 0.83ppm (unassigned), which disappeared after 40 minutes. Resonance signals for 5-FU was observed in some tumors and its major catabolite α-fluoro-β-alanine (FBAL, -20.5ppm) and fluoro-β-ureido-propanoic acid (FUPA, -22.3ppm) were observed in all samples. No FNuc was detected.

Table 1: Sensitivity to FUdR

Cell line	96h IC_{50} (μg/ml)
R3327-AT.1	9.10 ± 2.79 *
R3327AT.1/TK	4.58 ± 1.51 *
RG2	149.5 ± 2.12 †
RG2-TK	0.16 ± 0.02 †

*The ability of FUdR to inhibit cell growth was significantly higher in the TK-transfected cell line than in the parental cell line.(*P=0.03, †P<0.01 T-test for independent samples). Each value represents mean ± SD*

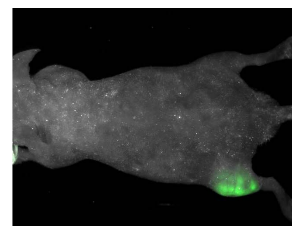

Figure 1: Representative *in vivo* fluorescent image showing GFP Expression of a R3327-AT/TK-GFP tumor

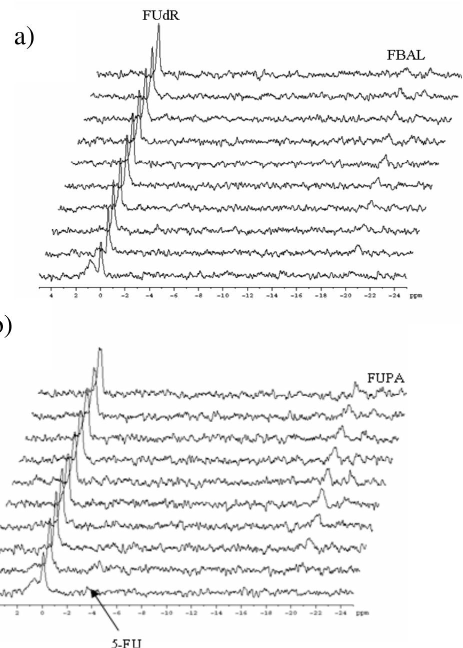

Figure 2: a.) R3327-AT.1 (379 mm^3) and b.) R3327-AT.1/TK (371 mm^3) over the period 20-110 min following administration of FUdR.

Conclusion: FUdR metabolism measured by ^{19}F-MRS showed similar results for R3327-AT and R3327-AT/TK and minimal enhancement in tumor sensitivity suggesting similar pharmacokinetics of FUdR in this tumor model. Studies with RG2 and RG2-TK where TK overexpression induced a major change in sensitivity *in vitro* are ongoing to determine if the enhanced cytotoxic activity in RG2-TK is reflected by altered FUdR metabolism measured by *in vivo* ^{19}F MR spectroscopy.

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