Concurrent use of in vivo 19F and 31P MRS at 9.4T to monitor the pathway for 5FC conversion to Fluorinated Nucleotides and FUDP-sugars in a Human Glioma Xenograft Treated with Ad5.CMV.CDUPRT/5FC Therapy

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Introduction In vivo ^{19}F MRS has been shown to sensitively detect the conversion of prodrug 5-FC to therapeutic 5-FU and subsequent cytotoxic fluorinated nucleotides in tumors (1-3). Concurrent measurement with ^{31}P MRS provides additional metabolites of α- and β-FUDP-sugars that have been suggested as markers of cell differentiation status (4). It has been known that at least three intracellular anabolic pathways of 5-fluorouracil lead to cell death by inhibiting RNA and/or DNA syntheses (1). Another pathway is believed to relate to a decrease in glycoprotein synthesis because of the formation of FUDP-sugars and its incorporation into membranes (4). Here, we extend our work to concurrent *in vivo* ^{19}F and ^{31}P-MRS study at high field 9.4T to investigate the cytotoxity mechanisms of fluorinated nucleotides and FUDP-sugars of Ad5.CMV.CDUPRT/5-FC therapy. The *in vivo* treatment protocol mimics the clinical method. High-field 9.4T in vivo ^{31}P MRS has the advantage of increased chemical shift dispersion of the two diphosphodiesters (DPDE) (or FUDP-sugars) elevated after CDUPRT/5FC treatment. The elevated FUDP-sugars peaks are in consistent with the increasingly accumulated FNuct in glioma tumors observed by ^{19}F MRS.

Methods and Materials: Human glioma D54MG cells were grown at 37°C in DMEM-F12. CMV-driven transcription unit of the yeast Ad5.CD or Ad5.CDUPRT were constructed. For in *vitro* ^{19}F and ^{31}P MRS, D54MG cells were first infected with viruses for 24 hours. Medium in each flask was then replaced with 2 ml of DMEM-F12 containing 5-FC (2 mM) and the cells were cultured at 37°C. At different time points of incubation, medium and cells were collected separately for MRS studies using a Bruker 9.4T/20 system. For *in vivo* MRS, about 5x10^6 cells were inoculated at the flank of athymic mice. As tumors sizes reached 200-500 mm^3, the Ad5.CDUPRT virus was injected intratumorily for three consecutive days. Starting at day 1 (n=11) post the last viral injection, a 5-FC solution (0.5 mL) with a dose of 500 mg/kg body weight was injected i.p. into rodents. Mice were then anesthetized with isofluorine and serial ^{19}F-MRS spectra were acquired within 3-5 hrs. ^{31}P-MRS was performed before virus infection, before 1st 5FC injection (control) and 6-8hr after daily 5FC injection. The same protocol was repeated for 7 days post virus infection.

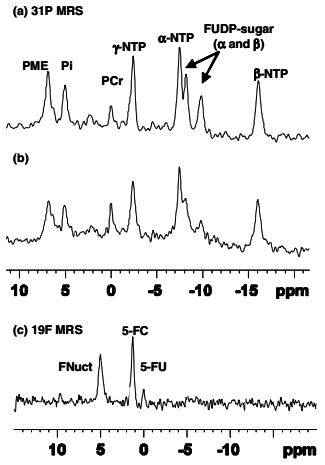

Fig. 1 In vivo ^{31}P MRS spectra of a s.c. tumor 8 h (a) and before (b) after 5-FC injection; in vivo ^{19}F spectrum of the same tumor 1.5 h after 5-FC

Results and Discussion: Figure 1 shows the in vivo ^{31}P-MRS spectra of a s.c. glioma solid tumor infected with Ad5.CD.UPRT virus (control) and 8hr after 5-FC injection. With the advantage of high field 9.4T, the two diphosphodiesters peaks were clearly resolved. A marked increase of these DPDE peaks in vivo was observed after first 5FC (500 mg/kg dose) treatment in solid D54MG tumor for the first time, which were tentatively assigned as FUDP-sugar (α and β) (5,6). In parallel with the increases of DPDE peaks in solid tumors, in vitro ^{31}P-MRS spectrum of D54MG cells infected with Ad5.CDUPRT viruses and then incubated with 2 mM 5FC for 24h shows similar increase as compared to the control spectrum (Fig. 2). The increased FUDP-sugar peaks observed by in vivo ^{31}P-MRS are concurrent with the increase of FNuct as shown by ^{19}F spectrum in Figure 1c, which represents a typical *in vivo* ^{19}F spectrum at 9.4T from the same tumor infected with virus followed with i.p. 5-FC injection. The prodrug 5-FC, therapeutic 5-FU and its fluorinated nucleotides (FNuct) were clearly resolved. The various fluorinated nucleotides (FUMP, FUDP, FUTP, and FUDP-sugars, etc) from 5-FU anabolism could not be resolved even at 9.4T. The pharmacokinetics of 5-FC conversion to 5-FU and FNuct were readily quantified by ^{19}F MRS. Concurrent analyses of ^{31}P-MRS data, the various mechanisms lead to cell death are estimated. Furthermore, we found that the FNuct concentration of individual tumors correlates well with the treatment response. In general, ^{31}P-MRS showed a decrease of PME and a slight alkaline shift of pH when tumors responses to the treatments. In conclusion, we have shown for the first time the observation of FUDP-sugars in vivo via the concurrent ^{31}P-MRS studies and their cytotoxic mechanisms in tumors treated with Ad5.CMV.CDUPRT/5FC therapy can be elucidated.

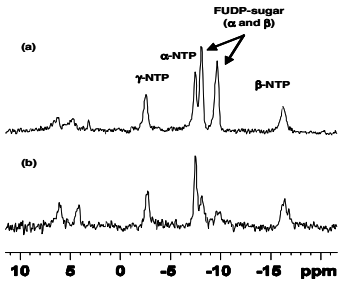

Fig. 2 ^{31}P spectra of (a) virus-infected D54MG cells after incubated with 5-FC (2mM) for 24h and (b) no 5FC .

References (1) Martino R et al Current Drug Metabolism 2000,1, 271-303; **(2)** Stegman LD et al. *Proc Natl Acad Sci* 1999, 96, 9821; **(3)** Ng, TC et al The 3rd Annu Mtg of Molecular Imaging,, St. Louis, MO, Sept. 9-12, 2004; **(4)** Wice BM et al. J Biol Chem 1985, 260, 139-146; **(5)** Shedd SF et al NMR Biomed. 1993, 6,254-263; **(6)** Chen T-B et al NMR Biomed. 1999, 12, 157-167