In vivo 19F MRS Studies of the Efficacy of Gene Therapy for Human Glioma Xenografts Using Adenovirus-Mediated Transfer of the Fusion CD/UPRT Gene

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Introduction Gene-directed enzyme prodrug therapy (GDEPT) is a promising strategy for the cancer treatment and has entered clinic trials. The mechanism of this therapy involves the gene transfer of non-mammalian enzyme(s) into tumor cells, which converts an inactive prodrug into cytotoxic metabolites to kill tumor cells. Mammalian cells lack cytosine deaminase (CD) that is needed to convert the nontoxic 5-FC into cytotoxic 5-FU. Therefore various adenoviral vectors carrying CD or bifunctional CD/UPRT fusion gene have been constructed [1] for 5-FC/GDEPT. It is desirable that the efficacy of transgene expression, the pharmacokinetics of the prodrug, and the physiological alteration at the cellular level can be monitored noninvasively. Stegman et al.[2] in 1999 first demonstrated that ^{19}F-MRS can be a useful technique to follow the *in vivo* 5-FC conversion to 5-FU in human HT29 cells, a model pre-engineered to stably express CD enzyme. We describe herein the *in vivo* ^{19}F MRS studies using replicative-incompetent adenovirus for the transfer of yeast CD or CD/UPRT genes into human brain glioma D54MG cells. The *in vivo* treatment protocol employed in this study mimics the clinical situation by first intratumoral injection of the viral vectors for three consecutive days and followed with i.p. injection of 5-FC. Serial ^{19}F MRS spectra were acquired immediately to monitor the gene expression and efficacy of the treatment.

Methods and materials Human glioma D54MG and 293 cells were obtained from ATCC. Cells were grown at 37°C in Dulbecco's minimal essential medium (DMEM-F12). Replication incompetent adenoviral vectors carrying a cytomegalovirus (CMV)-driven transcription unit of the yeast CD (Ad5.CMV.CD) or CD/UPRT (Ad5.CMV.CD.UPRT) gene were constructed. For in *vitro* ^{19}F MRS, D54MG cells (3x10^6) were infected with viruses at various viral particle concentrations (multiplicity of infection, MOI). At 24 hr post infection, medium in each flask was replaced with 2 ml of DMEM-F12 containing 5-FC (2 mM). The cells were then cultured at 37°C, and the medium and cells were collected separately at different times and subjected to ^{19}F-MRS (SF = 188.4 MHz) analysis using a horizontal-bore 4.7T MRS/MRI system. Faraday shielded solenoidal coils (^{19}F/^{1}H) were home-constructed. The concentrations and chemical shifts were referenced to an external NaF solution. For *in vivo* ^{19}F MRS, about 5x10^6 cells were inoculated at the flank of athymic mice (supplied by Harlan Sprague). Tumor size was measured and the volume was calculated. The treatment protocol started as tumors sizes reached 200-500 mm^3 as follows: the Ad5.CMV.CD.UPRT virus was injected intratumorly once a day for three consecutive days, and a 5-FC solution (0.5 mL) with a dose of 500 mg/kg body weight was injected i.p. into rodents at day 1 (n=6) or day 4 (n=4) post the last viral injection. Mice were then anesthetized with isofluorine and serial ^{19}F-MRS spectra were acquired within 3-5 hrs. The same protocol was followed for 7-9 days to access the virus activity.

Results and discussion *In vitro* ^{19}F-MRS results indicate that glioma D54MG cells infected with either Ad5.CMV.CD or Ad5.CMV.CD.UPRT demonstrated CD or CD/UPRT expression and their efficacies of conversion of 5-FC to 5-FU were similar. 5-FU was further converted to its toxic derivative 5-fluorouridine-5'-monophosphate (FdUMP) and other fluorinated nucleotides and nucleosides (FNuc) by tumor's own UPRT enzyme and by the expression from the CD/UPRT gene. The tumor group infected with Ad5.CMV.CD.UPRT virus exhibited Fnuc levels at least 5 times higher than that of the Ad5.CMV.CD treated tumors. In addition, the 5-FC to 5-FU conversion rate in D54MG glioma cells treated with both viral vectors are found proportional to their viral particle concentrations *in vitro*, as indicated in Figure 1. *In vivo* conversion of 5-FC to 5-FU was observed in 9 of the 10 mice studied, however, FNuc was detected only among 6 of them. Figure 2 shows a typical stacked plot of the *in vivo* serial ^{19}F MRS spectra from a s.c. glioma D54MG solid tumor infected with Ad5.CMV.CD.UPRT virus followed with i.p. 5-FC injection. Each spectrum was averaged over a 15 min interval. As shown in Figure 2, the 5-FC peaked at ca. 35 min post 5-FC injection, while the 5-FU concentration reached a maximum at ca. 50 min and both became undetectable within 4-5 hr. The FNuc signal first appeared in about 1 hr, increased steadily and remained detectable 6-24 hr post 5-FC injection. Importantly, the FNuc concentration of individual mouse tumors correlates well with the treatment response (*versa* tumor mass volume), i.e. the higher the FNuc level and the longer the FNuc remaining in the tumor, the tumor growth delayed longer (one totally diminished). The ^{19}F signals were quantified with respect to an external NaF solution. The pharmacokinetic changes of fluorinated metabolites measured in response to 5-FC treatment given at different days post viral injections were similar. These results also indicate that the virus enzymatic activity from the CD/UPRT gene expression *in vivo* reached a maximum at day 3 ± 1 and gradually decreased till completely inactive at day 8 ± 1 post the last virus injection (n=9).

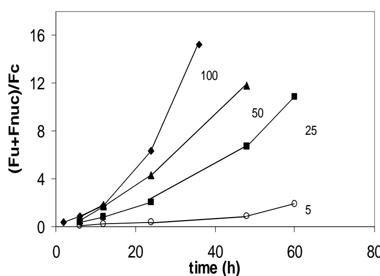

Figure 1. Dependence of the enzymatic activity on the viral vector concentrations

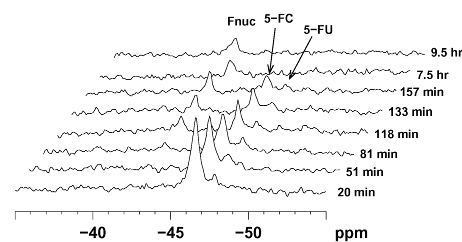

Figure 2. Serial *in vivo* ^{19}F spectra of a 5-FC treated s.c. D54MG solid tumor.

In summary, we have developed *in vivo* ^{19}F MRS technique to non-invasively monitor the efficacy of gene-directed enzyme prodrug therapy using adenovirus-mediated transfer of the fusion CD/UPRT gene, which may be translated into human patients. Replicative-incompetent adenoviral vectors containing yeast CD or CD/UPRT genes have been constructed. ^{19}F MRS results demonstrate that both CD and CD/UPRT genes efficiently expressed in virus-infected human glioma D54MG cells, as evidenced by the temporal changes of 5-FC, 5-FU and FNuc levels. The enzymatic activity peaks at day 3 post viral injections and lasts for about 8 days *in vivo*, while the *in vitro* ones increase with a MOI-dependent manner.

References 1) a) Hirschowitz ES et al. *Hum Gene Ther* **1995**, 6, 1055; b) Erbs P et al. *Cancer Res* **2000**, 60, 3813; c) Chung-Faye GA et al. *Gene Therap* **2001**; 8, 1547; 2) Stegman LD et al. *Proc Natl Acad Sci* **1999**, 96, 9821.