^{19}F MR spectroscopy can be used to detect histone deacetylase inhibition

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Introduction. Histone deacetylase (HDAC) inhibitors are emerging as a new and exciting class of anti-neoplastic agents. Initial clinical trials have been promising and treatment with HDAC inhibitors results in inhibition of cell proliferation and induction of differentiation or apoptosis (1,2). Our goal is to develop a non-invasive magnetic resonance spectroscopy (MRS) method to determine inhibition of HDAC *in vivo*. To this end, we have used a fluorinated HDAC substrate. We show that cleavage of the substrate by recombinant HDAC can be monitored *in vitro* using ^{19}F MRS. We further show that the substrate permeates cells and that the levels of substrate observed by MRS are significantly higher in cells treated with an HDAC inhibitor compared to untreated controls correlating with inhibition of HDAC activity. ^{19}F MRS combined with a cell permeable fluorinated HDAC substrate can therefore serve as a direct marker of HDAC activity in cells and potentially *in vivo*.

Materials and Methods. To assess cleavage of the HDAC substrate Boc-Lys(Tfa)-OH by MRS, 0.6mM was incubated with 14U recombinant HDAC-8. ^{19}F MRS was used to monitor decrease of Boc-Lys(Tfa)-OH and buildup of the cleavage product trifluoroacetic acid (TFA) in real time by acquiring spectra at 10 min intervals on a 300 MHz Bruker spectrometer using a 30 deg. flip angle and 3 s relaxation delay. To monitor HDAC activity in cells by MRS, PC3 human prostate cancer cells were incubated for 8 or 24 h with 1mM Boc-Lys(Tfa)-OH in the presence or absence of 6.7 μM of the HDAC inhibitor trichostatin A (TSA). Cells were then extracted using a dual phase extraction method (3) and ^{19}F MR spectra of the water-soluble metabolites recorded as above. ^{31}P MR spectra were recorded on a 500 MHz Bruker spectrometer using a 30 deg. flip angle and 3 s relaxation delay. The effect of Boc-Lys(Tfa)-OH and TSA on cell proliferation and viability were determined using the WST-1 assay as well as cell counts. The effect of Boc-Lys(Tfa)-OH and TSA on HDAC activity was determined using a fluorescence based activity assay.

Results and discussion. Figure 1 illustrates cleavage of Boc-Lys(Tfa)-OH by HDAC-8 *in vitro* and formation of TFA, confirming that Boc-Lys(Tfa)-OH is a substrate of HDAC, and that its metabolism can be monitored by ^{19}F MRS. To test the applicability of this method to tumor cells, we investigated PC3 cells following treatment with the HDAC inhibitor TSA. 1mM Boc-Lys(Tfa)-OH did not significantly affect cell proliferation or HDAC activity. Treatment with TSA for 24 h lead to a significant drop in HDAC activity down to 16% relative to control. Inhibition in cell growth was also observed, but did not reach statistical significance at 6.7 μM TSA. Figure 2 illustrates the ^{19}F MR spectra of PC3 cells. It demonstrates that Boc-Lys(Tfa)-OH is detectable in cells using ^{19}F MRS. It further indicates that following a 24 h incubation with TSA an increase in Boc-Lys(Tfa)-OH (HDAC substrate) levels can be detected. Specifically, Boc-Lys(Tfa)-OH levels increased significantly from 7 fmol/cell to 13 fmol/cell following inhibition of HDAC. Preliminary results also indicate that the increase in substrate can be detected within 8 h of incubation with TSA. Interestingly, no TFA could be detected in the cells, probably indicating that the acid was either bound to protein, or transported out of the cell. ^{31}P MR spectra were not altered by any treatment. This study indicates that ^{19}F MRS of fluorinated HDAC substrates could be used to non-invasively monitor HDAC activity in cells, and possibly *in vivo*.

References 1. Drummond et al., Annu. Rev. Pharmacol Toxicol. 45:495, 2005. 2. Vigusin et al., Anti-cancer drugs 13:1, 2002 3. Ronen et al., Br. J. Cancer 80:1035 1999.

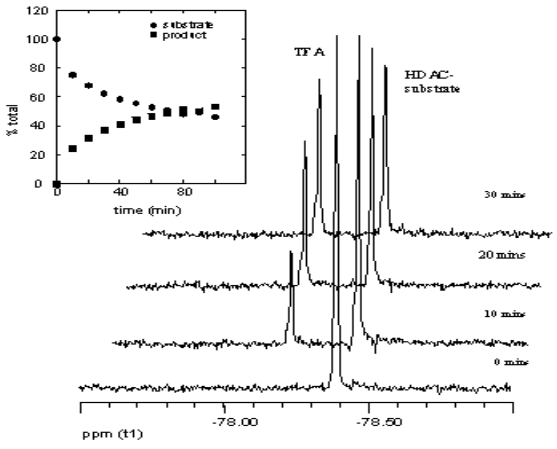

Figure 1 ^{19}F MRS spectra of Boc-Lys-(Tfa)-OH (HDAC substrate) breakdown by HDAC-8 producing TFA

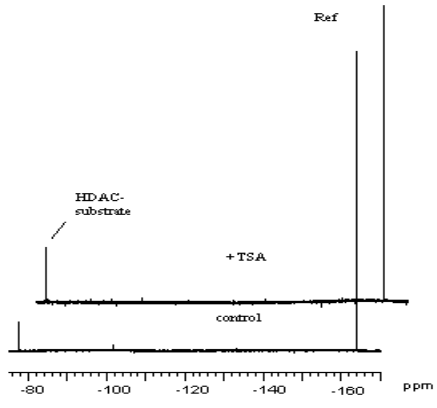

Figure 2. ^{19}F MRS spectra of PC3 cell extracts grown in the presence of HDAC substrate alone or together with the HDAC inhibitor TSA