^{13}C MRS detection of increased choline metabolism following HDAC inhibition

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Introduction.

Aberrant choline phospholipid metabolism in cancer cells and solid tumors has been extensively investigated by magnetic resonance spectroscopy (MRS) (1). We have previously shown that inhibition of cell and tumor growth by HSP90 inhibition results in an unexpected increase in phosphocholine (PC) and total choline (tCho) levels as detected by ^{31}P and ^{1}H MRS, respectively. This observation was made following treatment either with an HSP90 inhibitor (2) or with a histone deacetylase inhibitor (HDACI) resulting in HSP90 inhibition (3). In an effort to understand the underlying mechanism of the increase in PC, we propose to determine how choline metabolism is modulated by these inhibitors. To this end, we used ^{13}C MRS combined with 1-^{13}C-labeled choline to monitor choline uptake and metabolism, in control and HDACI-treated cells. To monitor total metabolite pools, ^{1}H and ^{31}P MR spectra were also recorded.

Materials and Methods.

PC3 human prostate cancer cells were cultured to 60% confluence in DMEM/F-12, supplemented as described previously (3). For MRS experiments, the cells were treated as described earlier (3) with the fluorinated derivative of the clinically relevant HDAC inhibitor, suberoylanilide hydroxamic acid (SAHA), in fresh culture medium containing 64μM of 1-^{13}C choline (Cambridge Isotope Laboratories Inc., MA). After 24 hrs of incubation, the cells were extracted using the dual-phase extraction method (2). ^{1}H, ^{13}C and ^{31}P MR spectra of the water soluble metabolites and the lipid fractions were recorded on a DRX500 spectrometer (Bruker, Germany), using 90° flip angle and a relaxation delay of 5s for ^{1}H MRS and 30° flip angle and a relaxation delay of 3s for ^{13}C and ^{31}P MRS. Metabolite levels were determined by integration and normalization to an external reference and to cell number.

Results.

Fig.1a shows typical ^{31}P spectra of water soluble metabolites in control and SAHA treated cells, indicating a 154% increase in PC levels, from 7±0.9 to 11.4±0.2 fmol/cell following SAHA treatment. At the same time, tCho levels increased by 133% (data not shown. n=3, p=0.01). Glycerophosphocholine (GPC) levels are hardly detected in this cell line but when detected an increase of 134% is observed (n=2). Importantly, the total cellular Phosphatidylcholine (PtdCho) levels did not increase significantly (p=0.69) as illustrated by the ^{31}P spectra of lipid fractions in Fig.1b. Fig.s 2a and 2b illustrate typical ^{13}C spectra of the water soluble metabolites and lipid fractions, respectively. The data show that an increase of 186% in labeled PC (n=3, p=0.02) and an increase of 143% in labeled PtdCho levels (n=3, p=0.05) occur following SAHA treatment, indicating an increase in the *de novo* synthesis of both of these metabolites.

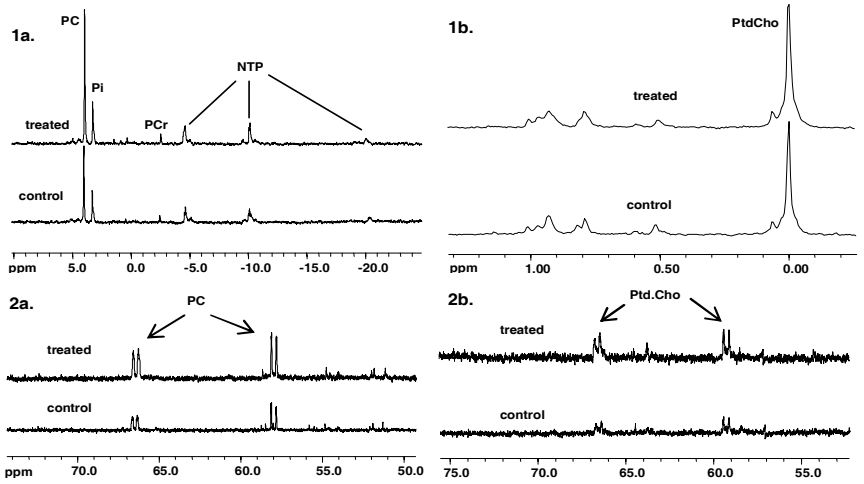

Figure 1. Typical ^{31}P spectra of a. water soluble metabolites and b. lipid fractions of control and SAHA treated cells

Figure 2. Typical ^{13}C spectra of a. water soluble metabolites and b. lipid fractions of control and SAHA treated cells

Discussion and Conclusion.

Based on these findings, we hypothesize that following SAHA treatment, the rates of PC and PtdCho synthesis are increased as evidenced by the higher ^{13}C labeled pool sizes. However, the fact that the total PtdCho pool remains constant and the observed increase in GPC indicate that the rate of PtdCho breakdown is probably increased as well, possibly also contributing to the total PC pool. Further quantitative studies need to be performed to monitor PtdCho breakdown and to assess enzyme expression and activity levels in control and SAHA treated cells.

References: 1. Podo F. (1999) NMR in Biomedicine, 12:413–39, 2. Chung, Y. L., Troy, H., Banerji, U., Jackson, L. E., Walton, M. I., Stubbs, M., Griffiths, J. R., Judson, I. R., Leach, M. O., Workman, P., and Ronen, S. M. (2003) Journal of the National Cancer Institute, 95: 1624-1633, 3. Sankaranarayanapillai, M., Tong, W.P., Maxwell, D.S., Pal, A., Pang, J., Bornmann W.G., Gelovani, J.G., Ronen, S.M. (2006) Molecular Cancer Therapeutics, 5: 1325-1334.

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