MRS detection of altered choline metabolism following HSP90 inhibition

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Purpose

Upregulated choline phospholipid metabolism is one of the hallmarks of cancer cells. While many traditional and targeted therapies have been shown to decrease phosphocholine (PC) levels in various cancer types, treatment with the HSP90 inhibitor 17-AAG has been shown to have the unique consequence of increasing PC levels (1). The goal of our study was to investigate the mechanism behind the observed increase in PC utilizing ^{13}C, ^{31}P and ^{1}H MRS to study intact cells and cell extracts.

Materials and Methods

The effects of HSP90 inhibition were studied in MCF-7 human breast cancer cells after treatment with 3μM 17-AAG for 48 hours. Cells were grown on Biosilon microcarrier beads (NUNC) in order to be loaded into a perfusion system for MRS studies as described previously (2). MRS studies were performed on a 500-MHz INOVA spectrometer (Varian). ^{31}P spectra were first acquired to confirm cell viability and quantify metabolite levels. Cells were then perfused with fresh culture medium containing 56μM [1,2-^{13}C]-choline (Cambridge Isotope Laboratories) over a period of 14 hours.^{13}C spectra were acquired during that time in 2-hour intervals with a 30° pulse and 3 second relaxation delay. At the end of the perfused cell experiments, cells were extracted from the beads, modifying the dual-phase extraction method (1). Cell extracts were investigated using a 600-MHz spectrometer (Varian). ^{13}C and ^{31}P spectra were acquired using a 30° pulse and 3 second relaxation delay. Choline kinase (ChoK) activity was measured by ^{1}H MRS as previously described (3). Phosphatidylcholine (PtdCho)-specific phospholipase C (PLC) activity was determined using the EnzChek Direct Phospholipase C Assay (Invitrogen). Fluorescence (485 nm excitation, 535 nm emission) was measured by SpectroFluor Plus spectrofluorometer (Tecan).

Results

Data from ^{31}P spectra of intact cells confirmed an increase in the total PC pool after treatment with 17-AAG. PC levels increased to 179±29% relative to control, from 12.1±2.6 to 22.1±4.7 fmol/cell (n=5, p=0.002) (Figure 1). After addition of [1,2-^{13}C]-choline to the culture medium, the conversion of choline to PC by choline kinase was monitored in ^{13}C spectra over a 14-hour period (Figure 2). The data showed an increase in the *de novo* synthesis of PC, as indicated both by final ^{13}C-labeled PC levels and by the initial pseudo rates (2) (Figure 3). The pseudo rate of PC synthesis increased with 17-AAG treatment to 178±30% relative to control, from 1.01±0.03 to 1.81±0.26 fmol/cell/hour (n=3, p=0.03). Additionally, the final amount of ^{13}C-labeled PC after 14 hours increased to 176±32% of control, from 11.1±1.6 fmol/cell in control cells to 19.3±3.1 fmol/cell in treated cells (n=3, p=0.03), reflecting labeling of approximately 90% of the total PC pool in both the control and 17-AAG treated cells. Initial results of a ChoK activity assay (n=1) also indicate an increase in ChoK activity to 121% of control following 17-AAG treatment. ^{31}P spectra from cell extracts did not show a significant change in the membrane phospholipid PtdCho (n=3, p=0.49), but glycerophosphocholine (GPC) levels increased from 1.3±0.3 to 3.0±0.9 fmol/cell (n=5, p=0.006). This suggested an increase in breakdown of PtdCho via phospholipase A (PLA) and lysophospholipase (LPL). Additionally, probing PLC activity to assess the effects of 17-AAG on the breakdown of PtdCho to PC, showed a significant increase in PLC activity to 114±5% (n=3, p=0.005) after 17-AAG treatment.

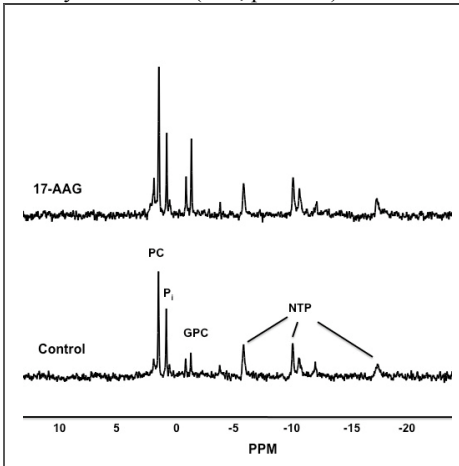

Figure 1: Representative ^{31}P spectra of control (bottom) and 17-AAG-treated (top) perfused cells, illustrating increased PC and GPC levels.

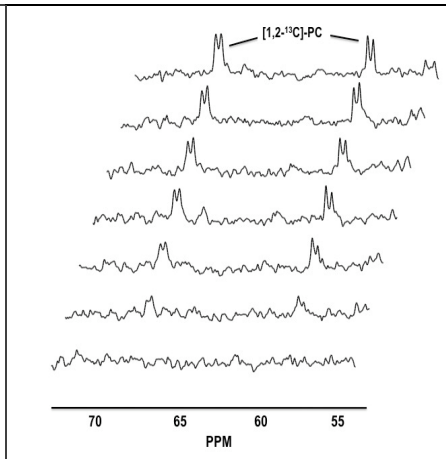

Figure 2: ^{13}C spectral array showing the buildup of [1,2-^{13}C]-phosphocholine in perfused MCF-7 cells over a 14 –hour period.

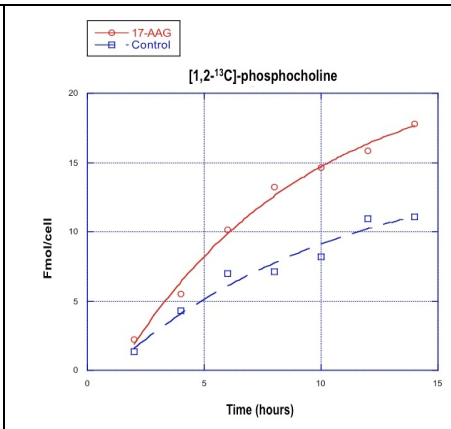

Figure 3: Fit of buildup of *de novo* [1,2-^{12}C]-phosphocholine in control (blue) and 17-AAG-treated (red) MCF-7 cells.

Discussion and Conclusions

Based on the results of our experiments, we hypothesize that the observed increase in PC levels in 17-AAG-treated cancer cells is due to an increase in the synthesis of PC from extracellular choline, along with increased breakdown of PtdCho via PLC. We further hypothesize that both synthesis and breakdown of PtdCho are upregulated, resulting in a constant PtdCho pool. Additional studies of the expression and activity of all enzymes involved in choline phospholipid metabolism and their link to 17-AAG targets will help to further clarify the mechanism for modulation of PC and choline metabolism by 17-AAG.

References: [1] Chung *et al*. J Natl Cancer Inst 2003, [2] Ronen *et al.* Biochim Biophys Acta 1991, [3] Iorio *et al.* Cancer Res 2005.

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