Changes in choline metabolism as potential biomarkers of HSP90 inhibition in NEU/HER2-driven mammary carcinoma Oncomouse® cells

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Introduction Heat shock protein 90 (HSP90) is required for the stability and activity of many proteins, including NEU/HER2 and its downstream proteins, which have key roles in cell growth and survival. Amplification and overexpression of NEU/HER2 proto-oncogene is observed in 25% of human breast cancers (1). 17-AAG is a novel anticancer drug that inhibits HSP90 leading to combinatorial degradation of many oncogenic client proteins. Previous magnetic resonance spectroscopy (MRS) studies have shown that treatment of different cell lines with 17-AAG was associated with an increase in phosphocholine (PC) *in vitro* and *in vivo* in tumor xenografts (2). The NEU/HER2 oncogene product is an HSP90 client protein that is highly sensitive to 17-AAG (3). Hence, the aim of this study was to determine the MRS detectable metabolic alterations for HSP90 inhibition in cells isolated from a NEU/HER2-driven mammary carcinoma Oncomouse®.

Materials and Methods A cell line of NEU/HER2-induced mammary carcinoma cells was established from a tumor mass from a NEU/HER2-overexpressing Oncomouse® (4). Cells were treated with 17-AAG ($5xGI_{50}$) for 24h. HSP90 inhibition was verified using Western blotting for HSP90 client proteins. Control and 17AAG-treated cells ($5x10^7$) were extracted using the DPE method and the aqueous fractions were analyzed by ^1H and ^{31}P MRS at room temperature on a 500 MHz Bruker spectrometer using a 30° flip angle and a 1s relaxation delay. Metabolite levels were corrected for saturation and cell number. Cell cycle distribution following 17-AAG treatment was determined by flow cytometry. Results represent (average ± SEM, n = 8)

Results & Discussion The number of 17-AAG treated cells as a % of control showed a significant reduction to 53±5%, P=0.00003, consistent with decreased proliferation. Western blotting proved that the cell line we have established continues to overexpress NEU/HER2 which was almost depleted by 17-AAG treatment, confirming sensitivity to 17-AAG (Fig. 1A). Treatment with 17-AAG also reduced C-RAF and CDK4 and induced HSP70 expression (Fig. 1A). These results provide molecular evidence for HSP90 inhibition in the 17-AAG treated Oncomouse® cells. Flow cytometry analysis showed that 17-AAG induced a moderate accumulation of cells in G2/M phase and a decrease in the percentage of cells in S phase. ^{31}P and ^1H MRS were used to identify potential biomarkers of HSP90 inhibition in NEU/HER2-overexpressing Oncomouse® cells. Figure 1B&D illustrates ^{31}P and ^1H MR spectra of control and 17-AAG treated Oncomouse® cells. As summarized in Fig. 1C, ^{31}P MRS showed that 17-AAG treatment led to a significant 2-fold increase in the levels of PC, glycerophosphoethanolamine (GPE) and glycerophosphocholine (GPC) relative to controls. This is in line with our previous report showing an increase in PC levels following HSP90 inhibition in different cell lines (2). PC, GPE and GPC are intermediate metabolites in phospholipid metabolism and their accumulation could reflect an increase in phospholipid turnover. Furthermore, the ratio of all these metabolites to NTP, a parameter independent of cell number, also increased significantly (PC/NTP, up to 152±7%, P=0.0002), (GPE/NTP, up to 141±9%, P=0.003) and (GPC/NTP, up to 178±11%, P=0.0003) relative to controls. Hence, these ratios could serve as *in vivo* MRS biomarkers of HSP90 inhibition in cells/tumors driven by NEU/HER2 expression. Analysis of ^1H MR spectra confirmed the changes observed with ^{31}P MR including PC (up to 200±27%, P=0.02) and in GPC (up to 254±47%, P=0.03). Furthermore, an increase in the levels of choline (up to 250±22%, P=0.001) and total choline (tCho=Cho+PC+GPC, up to 214±22%, P=0.004) was also detected. This demonstrates the capacity of ^1H-MRS, a clinically well-established technique with higher sensitivity and wider applicability compared with ^{31}P MRS, to monitor response to HSP90 inhibition.

Conclusions Our data demonstrate a high degree of sensitivity of this clinically relevant NEU/HER2-driven cell model to HSP90 inhibition and suggest that the increase in choline-containing metabolites could serve as potential pharmacodynamic biomarkers for monitoring HSP90 inhibition in tumors driven by NEU/HER2 expression.

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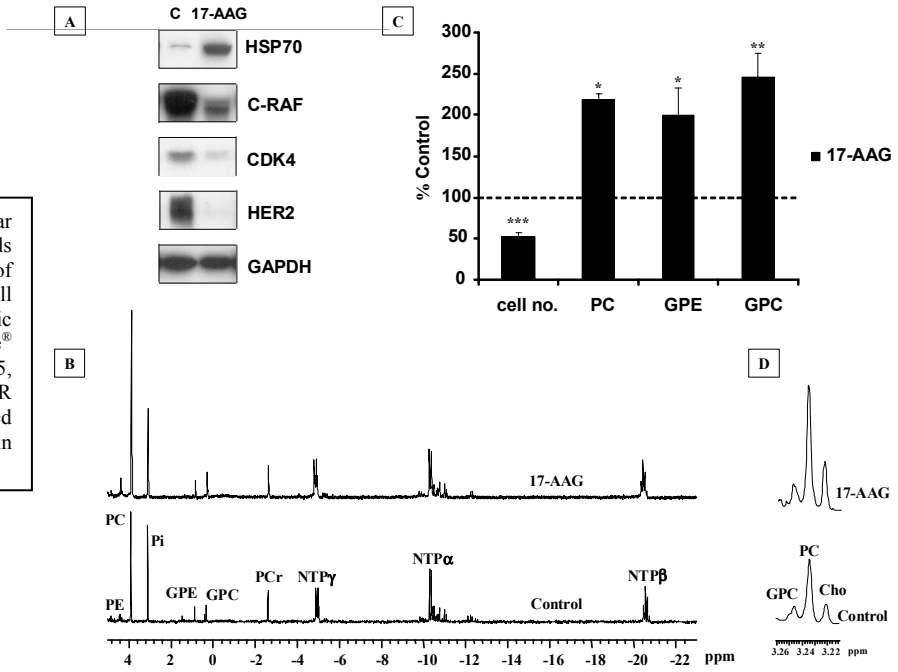

Fig. 1. A) Western blots showing molecular changes in 17-AAG treated Oncomouse® cells compared to controls. B) ^{31}P MR spectra of control and 17-AAG treated Oncomouse® cell extracts. C) ^{31}P MRS-detected metabolic changes following treatment of Oncomouse® cells with 17-AAG (*P<0.05, **P<0.005, ***P<0.00005). D) Expansion of ^1H MR spectra of control and 17-AAG treated Oncomouse® cell extracts showing changes in choline-containing metabolites.