Identifying biomarkers reflecting the action of the Hsp90 inhibitor 17AAG in malignant human melanoma cells prior to clinical evaluation

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Introduction: 17AAG is a heat shock protein 90 (Hsp90) molecular chaperone inhibitor that leads to simultaneous depletion of several oncogenic proteins such as c-Raf, Akt, mutant p53 and Cdk-4 that are critical for driving the cancer phenotype thereby eliminating mechanisms of resistance. Hsp90 inhibition also causes up-regulation of Hsp70. 17AAG has shown promising anti-tumour activity both in *in vitro* and *in vivo* preclinical models, has undergone phase I clinical trial and will shortly be undergoing phase II clinical evaluation in patients with metastatic melanoma at our centre. Using MRS, we have shown that inhibition with 17AAG correlated with modulation of phospholipid metabolism in human breast as well as colon carcinoma cells *in vitro* and *in vivo* (1, 2). The aims of this study were to a) test whether our MR observations could be extended to human melanoma cells *in vitro*, and b) assess the dependence of any observed metabolic changes on the timing of treatment and concentration of drug. This information could be useful to aid planning and monitoring of clinical trials.

Methods: We used SKMEL5 and SKMEL28 human melanoma cells which had different oncoprotein status including WT B-Raf/ WT p53 and mutant B-Raf/ mutant p53 respectively. The IC_{50} values for 17AAG in SKMEL5 and SKMEL28 were 14 nM and 20 nM respectively. Both cell lines were treated for 48h with equipotent concentrations of 17AAG (NCI) equal to 140 nM and 100 nM respectively. SKMEL28 cells were further treated with 10 nM and 50 nM 17AAG for 48h, and with 100 nM 17AAG for 6h, 16h and 24h. Drug action was verified using Western blotting for c-Raf and Hsp70. Control and 17AAG-treated cells were subjected to a dual phase extraction method and the aqueous fractions analysed by ^{31}P MRS using a 500MHz Bruker spectrometer, a 2s repletion time and a 30° flip angle. Metabolite levels were corrected for saturation, cell number and volume.

Results & Discussion: Treatment for 48h with 140 nM and 100 nM 17AAG in SKMEL5 and SKMEL28 cells, respectively, was associated with c-Raf depletion and Hsp70 induction as well as a significant drop in cell number per flask to 36±2% and 42±8%, respectively, relative to control ($p \leq 0.001$). In SKMEL28 cells, ^{31}P MR analysis showed a significant increase in levels of phosphocholine (PC), glycerophosphocholine (GPC) and glycerophosphoethanolamine (GPE) to 197±14%, 237±54%, 159±27% respectively ($n=4$, $p \leq 0.02$) while nucleotide triphosphate (NTP) content remained unchanged. An increase in PC levels to 363±62% was also observed in SKMEL5 cells, however no apparent variations were recorded in NTP, GPC or GPE levels (n=2).

We further assessed the time course of the metabolic changes induced in an effort to correlate these with the timing of effects on specific 17AAG molecular biomarker proteins and potentially investigate the molecular mechanism behind the effects on the ^{31}P MR spectrum. Interestingly at 6h, 16h and 24h post-treatment with 100 nM 17AAG in SKMEL28 cells, and despite the detectable drop in c-Raf and increase in Hsp70 level at these earlier time points (Figure 1A), no significant alterations were observed in any of the ^{31}P-containing metabolites which were recorded at 48h ($n \geq 3$, Figure 1B). This time lag could suggest the existence of multiple steps between the molecular response to 17AAG and the downstream effects on phospholipid metabolism.

To test the dependence of the observed changes on the concentration of 17AAG used in the treatment, SKMEL28 cells were also treated with 10 nM and 50 nM 17AAG for 48h. This was associated with a drop in c-Raf and increase in Hsp70 that was detectable at 50 nM but not at 10 nM 17AAG (Figure 2A). However no alterations were recorded in PC, GPC, GPE or NTP levels following treatment with either concentration of 17AAG (Figure 2B, n=2).

In conclusion, we have shown that inhibition of Hsp90 with 17AAG in human melanoma cells correlates with modulation of phospholipid metabolites that is time- and concentration-dependent. In addition, the observed changes seem to be associated with late-occurring downstream effects of 17AAG which may involve alterations in gene expression of enzymes modulating phospholipid metabolism. This hypothesis requires more detailed investigation. More importantly, these findings could have implications for the design and monitoring of planned clinical trials with 17AAG.

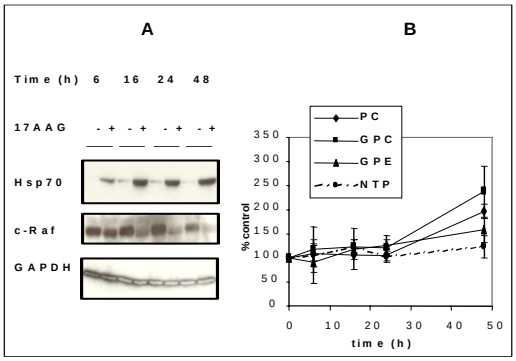

Figure 1: Time course of inhibition with 100 nM 17AAG in SKMEL28 human melanoma cells on A) Hsp70 and Raf-1 levels & B) the cellular content of the ^{31}P-containing aqueous metabolites.

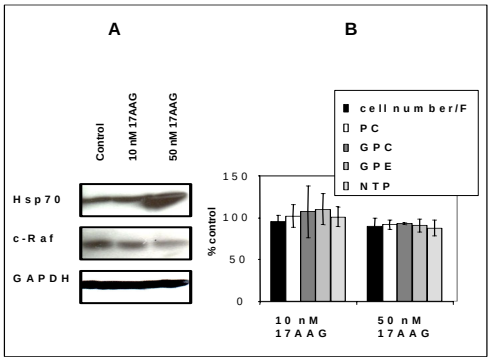

Figure 2: Effect of treatment with 10 nM and 50 nM 17AAG for 48h in SKMEL28 human melanoma cells on A) Hsp70 and Raf-1 levels & B) cell number per flask, PC, GPC, GPE and NTP levels.

References: 1) M. Beloueche et al (2002) Proc. ISMRM , Hawaii, USA, abstract no. 29. 2) Chung, Y-L et al (2003) JNCI *95* :1624-1633.

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