^{1}H NMR demonstrates that oleic acid (18:1) and linoleic acid (18:2) accumulate during cell death in human Primitive Neuroectodermal Tumour cells.

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INTRODUCTION:

Several studies have reported an increase in ^{1}H NMR visible lipids in response to successful cancer treatment. Identification of the NMR lipids can potentially provide information about involvement of lipid metabolism and lipid molecules in cell death pathways. In this study, two human primitive neuroectodermal tumour cell lines were treated with cisplatin. The accumulation of cytoplasmic lipid droplets (LD) was monitored by microscopy during cell death.1D (^{1}H) and 2D (HSQC) HR-Magic Angle Spinning NMR was performed on untreated and treated whole cells to investigate the changes in NMR spectral patterns and to indentify chemical species of accumulated lipids. ^{1}H NMR was performed on isolated LDs and their extracts from untreated and treated cells.

METHODS

Cell culture and chemotherapeutic treatment: DAOY and PFSK-1 cells were cultured in DMEM F:12 (Invitrogen) supplemented with 10% (v/v) foetal calf serum (PAA), 1% 200mM L-glutamine and 1% MEM non essential amino acid solution. Cisplatin (Sigma Aldrich), at working concentrations as indicated, was freshly made each time before use.

Nile red and DAPI staining: 4µg/ml Nile red and 0.4ug/ml DAPI was used to stain the cells after the cytospin.

Isolation and Extraction: Sucrose gradient ultracentrifugation was used to isolate LDs from whole cell homogenate. Methanol and chloroform extraction was performed on isolated LDs.

HR-MAS NMR: NMR experiments were performed on whole cell pellets with a Bruker 500-MHz spectrometer using a HR-MAS probe (Bruker Biospin). A rotor speed of 4800 Hz and temperature of 4 ^{o}C were used for all experiments. ^{1}H NMR spectra on isolated LDs and their extracts were performed on the same spectrometer with a cryo-probe.

RESULTS

Cell death, LD accumulation and isolated LDs: Larger LDs accumulated and fragmentation of the nuclei occurred in 10µM-cisplatin 48h treated DAOY cells and 50µM-cisplatin 48h treated PFSK-1 cells. The morphology of the LDs from untreated DAOY and PFSK-1 cells was unchanged compared with LDs in whole cells.

Whole cell NMR: ^{1}H HR-MAS NMR spectra of DAOY cells with and without cisplatin exposure are shown (Fig 1). The spectra were normalized to the macromolecule peak at 3.0ppm. An increase in peaks from unsaturated and saturated lipids is evident after 48h exposure (Fig. 1). Similar findings were present for PFSK-1 cells

Whole cell HSQC: The HR-MAS HSQC spectrum of DAOY cells treated with 10µM cisplatin for 48h is shown.(Fig. 2) The resonance from CH= was assigned to oleic acid (18:1) and linoleic acid (18:2) according to the literature and HSQC spectra acquired from standard oleic acid and linoleic acid samples.

Isolated LD NMR: ^{1}H spectra from isolated LDs of treated and untreated DAOY and PFSK-1 cells are shown in Fig 3. The lipid profiles are the same as those from the whole cell experiments. An increase in unsaturated lipids was detected in the LD extracts of treated cells.

CONCLUSION:

An increase in ^{1}H NMR detectable lipids was seen in cisplatin responding DAOY and PFSK-1 cells and was associated with the accumulation of cytoplasmic LDs. The increased lipid peaks at 2.8 and 5.4ppm confirmed the accumulation of unsaturated lipids. HSQC spectroscopy indentified oleic acid and linoleic acid as the two major unsaturated fatty acids accumulating during cisplatin-induced cell death in DAOY and PFSK-1 cells. The increase of unsaturated lipid peaks in isolated LD spectra suggests these fatty acids are likely to be located in cytoplasmic LDs.

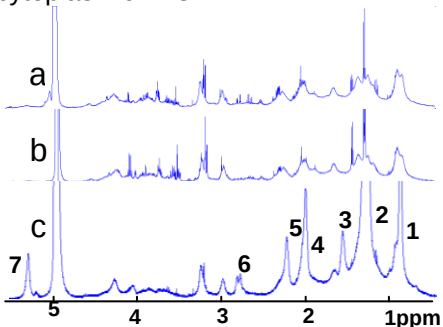

Figure 1 HR-MAS spectra of DAOY a, control 0h b,control 48h c 10µMcisplatin 48h (Lipid signals labeled on the bottom spectrum are as follows:1-C**H**3, 2–CH2-C**H**2-CH2, 3-C**H**2-CH2-C=O, 4-C**H**2-CH2-CH=, 5-CH2-C**H**2-C=O, 6-CH2-C**H**2-CH=, 7=C**H**)

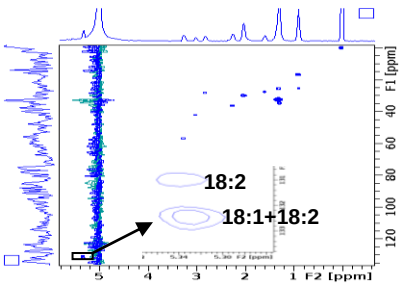

Figure 2 HSQC spectrum of DAOY 48h-treated whole cells

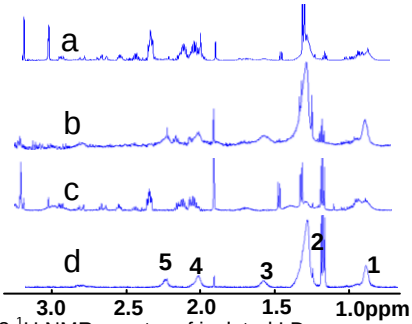

Figure 3 ^{1}H NMR spectra of isolated LDs a, PFKS-1 b, 48h treated PFSK-1 c DAOY, d 48h treated PFSK-1 peaks labelled are as follows:1-C**H**3, 2–CH2-C**H**2-CH2, 3-C**H**2-CH2-C=O, 4-C**H**2-CH2-CH=, 5-CH2-C**H**2-C=O

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