Report Prepared for the Andrew Macartney Trust Fund - July 2011

Project: Lipids and metabolites detected by magnetic resonance spectroscopy as biomarkers in brain tumours.
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Background and Accomplishments
We have found that saturated fats are present in aggressive tumours but that when successfully treated, the tumours accumulate unsaturated fats. We have developed a method to isolate these fats from tumour cells grown in the laboratory allowing them to be analysed in detail. In the past 4 months, we have performed complex experiments which have started to identify the fats which are present, mainly oleic and linoleic acid. We have also identified a new chemical (UDP-GalNAc) which accumulates when tumour cells die and plays a key role in altering fats. These findings are giving us important insights into how brain tumours work and ideas on how to treat them, which we are investigating.

Publications:

Conference Abstracts
Presented at 19th Annual Meeting and Exhibition of the International Society of Magnetic Resonance in Medicine (ISMRM) in Montreal Canada:

The alteration of the lipids in cytoplasmic lipid droplets after cisplatin treatment in human brain tumour cells, X Pan, M Wilson, C McConville, T. N. Arvanitis, J.L Griffin, R. A. Kauppinen and A. C. Peet (Abstract No:4262) Poster presentation

An in vitro metabonomic study detects increases in UDP-GlcNAc and UDP-GalNAc, as early phase markers of cisplatin treatment response in brain tumour cells X Pan, M Wilson, C McConville, T. N. Arvanitis, J.L Griffin, R. A. Kauppinen and A. C. Peet (Abstract No: 252) Oral presentation.

Submitted to British Chapter of ISMRM, Manchester 2011:

^{1}H NMR demonstrates that oleic acid (18:1) and linoleic acid (18:2) accumulate during cell death in human Primitive Neuroectodermal Tumour cells. Xiaoyan Pan, Martin Wilson Carmel McConivelle, Theodoros N. Arvanitis, Julian L. Griffin, Risto A. Kauppinen and Andrew C. Peet

Paper Publication
An in vitro metabonomic study detects increases in UDP-GlcNAc and UDP-GalNAc, as early phase markers of cisplatin treatment response in brain tumour cells, X. Pan, M. Wilson, L. Mirbahai, C. McConville, T. N. Arvanitis, J.L. Griffin, R. A. Kauppinen and and A.C. Peet, Journal of Proteome Research

The Details:
Progress in four months (March-June2011)

Experiments

1. High Resolution-Magic Angle Spinning NMR spectra on whole cell pellets of the brain tumour cell lines without drug added were collected to compare with those from cells with cisplatin added to them.
2. The concentration of cisplatin was increased to cause cell death in the cell line PFSK-1 to validate the increase of accumulating unsaturated fatty acids in drug-responding tumour cells.
3. HSQC experiments were performed on metabolite extracts of U87-MG and the standard samples of two UDP compounds to confirm the assignment.

Data analysis

1. Spectral analysis was undertaken on the ^{1}H NMR spectra of lipid extracts of isolated lipid droplets (LDs) from drug responding and non-responding cells. The analysis focused on measuring the signal intensity. The increase in the lipid peak of LDs, especially in unsaturated lipids was determined from spectra of drug-responding cells. The analysis was performed on at least 3 repeats of each group to validate the observation.
2. A comparison was made of the increase in the unsaturated lipid peak between whole cell lipids and LDs to confirm the active involvement of LDs in tumour cell death pathways.
3. All data collected so far was reviewed to indentify the gaps in the data and instruct the lab work.

Dissemination

Apart from the publications already noted, work has been undertaken on the preparation of a manuscript establishing the correlation between lipid droplet size and the intensity of the lipid droplet peak. A further paper has been drafted on the lipid composition of isolated lipid droplets. These papers, together with one establishing the changes in lipid droplet composition in treated cells will form the basis of Xiaoyan's PhD thesis.

Future work

1. To identify lipids in isolated lipid droplets using HSQC NMR
2. Investigate the effects of nutrient deprivation on tumour metabolism