Report for the Andrew McCartney Trust – June 2013

Project: *Immunohistochemical identification of lipid droplets in brain tumour cells and their association with cancer-related markers*

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Summary:

This project will investigate fat droplets in brain tumour cells. The first aim is to develop a staining method to stain fat droplets in tissue taken from different brain tumours. This method has not been done before in brain tumour tissue, and will provide clinically useful information on fat droplets in different types of brain tumours. The second aim is to use the same staining method to stain tumour cells grown from brain tumours to research different cancer-related molecules that may be located on the surface of fat droplets within brain tumour cells. This will allow us to investigate if there are particular molecules that are present on fat droplets that may cause the cell to become more aggressive or to respond to treatment differently.

Background:

Previous research funded by the Andrew McCartney Trust and carried out by Xiaoyan Pan discovered that different brain tumour cell lines contain different amounts and sizes of fat (lipid) droplets. This information may be important for determining how different brain tumour cells grow and how they respond to treatments.

Current Project:

Our new project aims to further investigate the presence of these lipid droplets in tumour cell lines using a different technique called immunohistochemistry. This technique uses a novel antibody called adipophilin that recognises the fat droplets and stains them in tumour cells. It is carried out on tumour cell lines that have been paraffin embedded in the same way as most tumour tissue is processed in hospital laboratories. Lipid droplet formation has not been routinely assessed in tumour tissue as the lipid droplet was thought to be lost during processing. However, it is now known the membrane around the droplets remains intact. Given the known importance of lipid in brain tumours, the ability to stain lipid droplets using this method may have an impact on clinical decision-making regarding treatment choice and long-term patient outcome. This method will also allow us to stain for other cancer-related markers on the surface of lipid droplets.

Progress to date:

Two brain tumour cell lines have been grown in the laboratory and then processed into paraffin blocks. The quality of these cell lines post-processing has been assessed with a routine stain called H&E, and found to be good quality to continue the remaining work.

A suitable antibody for adipophilin has been found and a staining protocol devised and optimised. The staining has been shown to be specific and of good quality according to published literature.

Successful staining of the lipid droplets in the two brain tumour cell lines using adipophilin has been carried out. This staining is localised to the lipid droplets in the cytoplasm of the cell, where we would expect to find it.

Work to be done:

Adipophilin staining of a commercially sourced brain tumour tissue microarray (TMA). A TMA is a slide containing a selection of tissue from different types of brain tumour (adult and paediatric).

Investigating lipid droplets in this manner in clinical brain tumour tissue has not been previously carried out.

With the successful localisation of lipid droplets in cell lines we can carry out double-labelling staining to investigate whether other crucial cancer related proteins are found directly on the surface of lipid droplets. This will provide important information on why lipid droplets accumulate in particular brain tumour cells and whether this is related to tumour type/aggressiveness. To do this the staining will be adapted and undertaken with florescent stain markers and specialised microscopy to allow us to visualise the lipid droplets better.

Previous data has already been collected from two different brain tumour cell lines that have been grown whilst deprived of glutamine, or treated with a drug that disrupts the glutamine pathway. The same cell lines will be grown again with and without treatment targeting the glutamine pathway, and lipid droplet formation will be stained by adipophilin using the same methods as above. These lipid droplets can then be examined to discover if their number and size are altered by disrupting glutamine pathways in brain tumour cells. Together this data will provide crucial information on the relationship between lipids and glutamine in tumour cells.

Expected Outcomes:

This data may be submitted as an abstract for the National Cancer Research Institute (NCRI) Cancer Conference taking place in Liverpool in November.

It is anticipated that 1-2 scientific papers will be prepared and submitted for publication by the completion of this project

Figure 1: Schematic Diagram of Lipid Droplets before

and after paraffin processing