Report for the Andrew McCartney Trust – February 2014

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

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Lay Summary:
Fat droplets are known to affect how cancer cells grow and survive. Our research is interested in finding out how the size and amount of these fat droplets changes in different types of brain tumours and as the cancer advances. In this project we developed a method that lets us see these fat droplets within tumour cells. We then counted the number of fat droplets in different brain tumour types. We found that there were many more fat droplets in high grade tumours compared to low grade tumours. This suggests that the amount of fat droplets in cancer cells is related to the severity of the tumour type, with more fat droplets found in tumours with a worse outcome. This is important as it means that these fat droplets may be affecting how cancer cells grow within tumours. This is the first study to look at these fat droplets using these methods in clinical brain tumour samples and it suggests that these methods may be useful in helping with clinical diagnosis. Our research also confirms these fat droplets are an important factor in brain tumour growth and severity and provides an interesting avenue for future research.

Aims:
The aim of this project was to investigate fat droplets in brain tumour cells. The first aim was 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 was 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.

Progress and Results:
Two brain tumour cell lines were grown in the laboratory and then processed into paraffin blocks. The quality of these cell lines after processing was assessed with a routine stain called H & E, and found to be good quality to continue the remaining work. Successful staining was carried out of the lipid droplets in the two brain tumour cell lines using a staining method called immunohistochemistry. This was carried out with a particular molecule called adipophilin. Adipophilin is located on the surface of lipid droplets in the cytoplasm of the cell, where we would expect to find it.

Adipophilin staining was carried out in a commercially sourced brain tumour tissue microarray (TMA). A TMA is a slide containing a selection of tissue from different types and grades of adult brain tumour. Once the TMA was stained with adipophilin, each piece of tissue on the tissue microarray was then scored based on how many tumour cells contained adipophilin staining and how strong the staining was. Results showed that grade four glioblastomas and anaplastic oligodendrogliomas contained the most lipids droplets. Over 60% of cancer cells in these tumours contained lipid droplets. This was significantly higher than the number seen in lower grade brain tumours. This confirms that high grade tumours with a poor clinical outcome do have significantly higher numbers of lipid droplets. Normal brain cells also contained some lipid droplets suggesting that these lipids play a role even in normal cells.

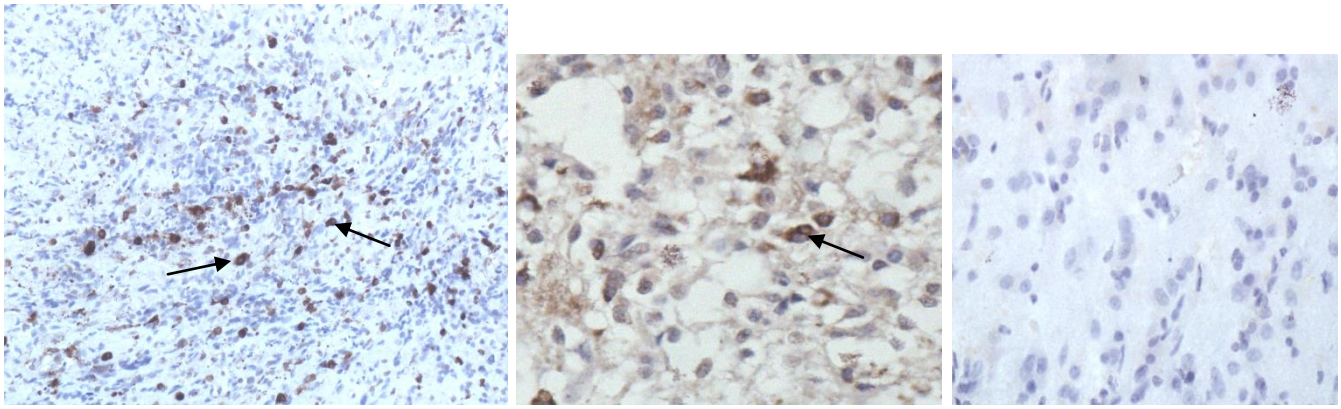

Figure 1. a. Adipophilin-stained lipid droplets in a grade four glioblastoma (the brown stained areas are lipid droplets within the tumour cells, see arrows for examples) b. Same picture with more detail at higher magnification. c. Normal brain cells with no lipid droplets (stained blue with H&E, no brown staining seen).

Following on from the successful localisation of lipid droplets within tumour cells, the second part of this project was to use high resolution fluorescent microscopy to label the lipid droplets in order to see them more clearly. Using this method it was hoped we could carry out double-labelling staining to investigate whether other crucial cancer-related proteins are found directly on the surface of lipid droplets. Although it was possible to label the lipid droplets in cell lines, unfortunately it proved difficult to label these with fluorescent methods. Multiple different staining methods were trialled to improve the quality of the fluorescent staining. However these methods were unsuccessful within the timeframe of this project. It is likely that this method to double-label lipid droplets will be successful, however it will require significantly more time and work on the methodology.

Summary of Main Findings:

We have proven that it is possible to label lipid droplets in paraffin embedded clinical tissue. Investigating lipid droplets in this manner in clinical brain tumour tissue has not been previously carried out. We have also found that the number of lipid droplets inside tumour cells increases with the severity of the tumour. The ability to detect lipid droplets in clinical tissue may prove very useful in helping to predict the prognosis and response to treatment in the future.

Outcomes:

- The method development and adipophilin staining in tumour cell lines was presented at the annual meeting of the Central Cellular Pathology Society in October 2013.
- It is anticipated that one good quality scientific journal paper will be submitted for publication within 6 months as a result of this project.
- There is potential to present this work at a suitable cancer research conference at some point in 2014.

Potential Future Research Projects:

- Following on directly from the current research it may be possible to correlate the number of stained lipid droplets in biopsied paediatric brain tumours with the amount of lipids detected in MRS scans at Birmingham Children's Hospital. It will also be possible to correlate lipid droplets with response to treatment and/ or patient outcome. This will provide further direct information on the relationship between lipids, severity of disease and patient outcome after brain tumour in children.
- Previous data has suggested an important link between a chemical called glutamine and lipid droplets within brain tumours. Further investigation could be undertaken on glutamine pathways within brain tumour cell lines. By growing cell lines with and without treatment targeting the glutamine pathway, lipid droplet formation could be stained by adipophilin using the same methods as the current project. 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 would provide crucial information on the relationship between lipids and glutamine in tumour cells.
- Further analysis of the surface of lipid droplets could be undertaken by continuing to work on the fluorescent methodology or by using different techniques. This work would likely follow on from the other projects, when a suitable target has been identified. Improved understanding of the molecules on the

surface of lipid droplets would provide important information on how these droplets contribute to the aggressiveness of particular tumours.