**Progress Report**

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Date: 03/10/2016

Title of Project: **Can expression of the NFI pathway be used to treat primary human GBM?**

***Summary: (approximately 1,000 words)***
Glioblastoma (GBM) is a devastating primary brain cancer that affects around 800 Australians annually. Despite surgery, radiotherapy and chemotherapy, 1 in 2 patients diagnosed with GBM will not survive for more than 14 months and fewer than 1 in 10 patients will survive for five years. This short life expectancy highlights the need for novel and effective therapies for the treatment and cure of this deadly disease. We are developing a potential new therapeutic strategy, focussing on the glial origin of GBM, to complement the current treatment following tumour resection. Glial cells are essential for normal brain development and function, as they play important roles in regulating synapse formation and function as well as supplying nutrients to neurons. GBM tumours originate from this cell type. If we can reactivate the glial differentiation programme that normally drives the production of glial cells in the brain, we can potentially induce the differentiation of cancer cells and prevent further tumour growth, independent of the underlying molecular events driving disease progression.

Nuclear Factor One (NFI) is a family of genes required for normal glial biology transcription. Transcription factors are master regulators of gene expression, thereby coordinating the expression of other genes during developmental processes such as proliferation and differentiation. In GBM, these genes are often deleted or mutated. As NFI genes drive the differentiation of glial cells during brain development, they may act as tumour suppressors in GBM. NFI expression directly correlates with clinical outcome (less NFI = shorter patient survival) and for the gene NFIB, decreased gene copy number is associated with adverse disease progression and clinical outcome for patients. In cultured GBM cells, re-introduction of NFI stops cancer cell division and promotes the transition of these cells into glial cells, as such effectively reversing the cancer. Therefore, we propose that activating NFI expression will provide a novel complementary strategy for clinical treatment of GBM.

In this project, we are testing the hypothesis that activating NFI in primary human GBM tumours will stop their growth and reverses the cancer. Therefore, we have established a new pre-clinical model. We are now growing human primary GBM

tumours in mice on which we can do clinical testing. In short, we obtain primary human tumours directly from the operating theatres of Brisbane hospitals via the Wesley Medical Research Tissue Bank. These tumours are than injected and maintained in mice as so-called xenografts. Currently we have different xenografted tumours available for experiments and are generating more to create a xenograft bank for researchers.

*Hypothesis vs Findings*

Using immunofluorescence analysis, we have determined that in human tumours NFIB is mainly expressed in the tumour cells that are not proliferating, and more proliferative tumours have less NFIB expressing cells. To increase expression of NFIB in the dividing tumour cells, we have optimized a technique called *in vivo* electroporation, in which NFIB-expressing or control DNA constructs are injected into the tumour and a small electric current is applied over the tumour. This current pulls the DNA construct into the tumour cells. In the case of the NFIB-expressing construct, this will lead to expression of NFIB in these cells. As the DNA constructs also express a fluorescent marker, we can readily identify which cells are treated with NFIB (See figure).

We are now able to manipulate NFIB in established tumours that closely mimic the GBM tumours in human patients. We already tested NFIB treatment in xenografted tumours for two patients. Based on immunohistochemical staining and flow cytometry data, we confirmed that NFIB-treated cells within the tumour show less proliferation and less tumour characteristics. Although we need to further analyse the treatment on tumours from other patients, our data unequivocally proves that activation of NFIB in established tumours can stop tumour growth.

*Unanswered Questions*

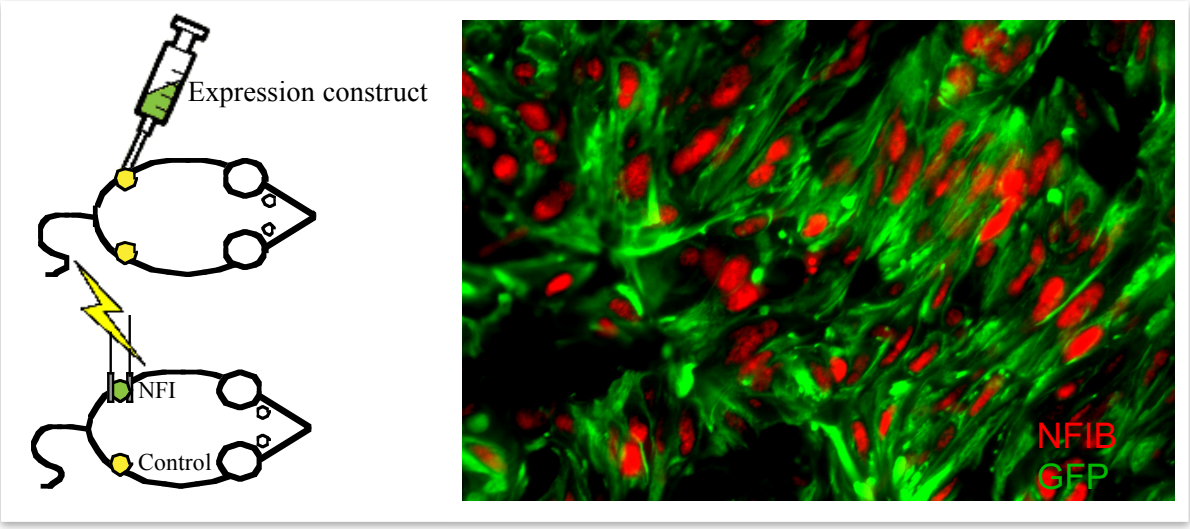
Recently published findings from our group showed that not all human tumours respond similar to NFIB treatment. While the tumours from the most aggressive subtypes displayed growth reduction when implanted in mice, some less aggressive tumours did not respond to NFIB treatment, and some even grew faster. Therefore, we need to understand why some tumours do not respond to NFIB treatment and find a way to diagnose the patients who will benefit from this treatment. Therefore, we are expanding our research using the now established xenografts and electroporation method to understand the molecular mechanism that determines whether NFIB treatment results in reduced or increased tumour growth. With the data generated using the Brain Foundation gift, I have been able to obtain a Scott Canner Young Researcher Grant from Tour de Cure to fund this line of research.

Furthermore, together with researchers within the Australian Institute for Bioengineering and Nanotechnology and the Centre for Advanced Imaging at the University of Queensland, we have now started to investigate how NFIB can be administered and how to monitor its effect on tumour growth. We are testing the use

of nanoparticles as an targeted drug-delivery method. Finally, we are imaging the progression of tumours when the level of NFIB is altered to verify its effectiveness.

*What these research outcomes mean*

Given the poor outcome and low quality of life of surviving patients with GBM, alternative and radical approaches are required to target this devastating desease. Current research has focused mainly on targets involved in driving tumour cell proliferation, which may differ between subtypes of GBM. In our approach we regard GBM as a disruption in the normal development of glia in the brain. NFI plays an important role during normal brain development, as it induces the transition from proliferation to glial differentiation. Based on our current results, we can conclude NFIB activation is able to act as a tumour suppressor as it stops proliferation of tumour cells of human tumours. Although we need to further investigate how to identify patients that will respond to this treatment and how to deliver NFIB to the tumour cells in human patients, our research has provided the critical proof-of-principle that NFIB or other glia differentiation genes can be developed into successful clinical therapeutic agents.




*An overview of the electroporation technique to introduce NFIB expression in human tumour xenografts. Human tumours are grow as xenografts under the skin of mice. To induce NFIB, an expression construct is injected into the tumour. Subsequently, this DNA construct is pulled into the tumour cells by an electric current. The tumours cells will now expressed NFIB (depicted in red) as well as a green fluorescent marker (GFP; in green) to easily identify treated cells.*