

Research Report
2010 / 2011

Neurosurgical Society of Australasia Inc

Chairman's Welcome

Dear Colleagues,

The Neurosurgical Society of Australasia Research Committee was formed less than a year ago with the broad aim of improving the profile and success of research for members and prospective members of the NSA.

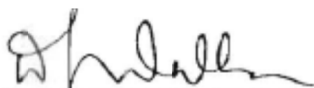
For many years, the neurosurgical community in Australia and New Zealand have been at the forefront of the field of medical research. Yet to some extent, particularly for those outside the NSA and those wishing to do neurosurgical research at the start of their careers, it has been difficult to know where research was being done, what was being done, who was doing it, and importantly, how a project could be funded.

This Annual Report summarises the activity of the Research Committee, including the evaluation of prospective research projects, promoting access to research funds, and promulgating the research activities of the NSA members. A survey of attitudes to research, designed by Dr Ben Jonker, has been conducted and the results will be published in the near future.

Ultimately we believe that an active and vibrant research community is important for the NSA as a whole and we thank the NSA Board for supporting us in our endeavour.

On behalf of the NSA Research Committee,

Kind Regards,



David Walker



Our Objective

The objective of the Neurosurgical Society of Australasia (the Society) is the study and advancement of the art and science of neurosurgery and research into the cause, prevention and cure of disease in human beings in the field of neurosurgery.

The Society has a Research Foundation established to promote basic and applied clinical research in the field of neurosurgery by Australian and New Zealand neurosurgeons and trainees. The Foundation is fully supported by the Society and its constituent members.

The Research Committee is responsible for setting the research agenda of the Society and ensuring the transparent allocation of research funds consistent with the research agenda with a focus on both basic science and clinical research. The Research Committee is also responsible for identifying potential funding sources to support neurosurgical research and dissemination of research information.

The Research Committee considers requests to support research surveys, projects and programs and the dissemination of research information to members.

Research Committee Members

We gratefully acknowledge the following individuals, being the members of the Society Research Committee as of 30 June 2011.

Chairman	David Walker	Queensland
Member	Kate Drummond	Victoria
Member	Benjamin Jonker	New South Wales
Member	Neville Knuckey	Western Australia
Member	Peter Reilly	South Australia
Member	Marcus Stoodley	New South Wales

Research Funds Allocated

With the support of our sponsors and partner organisations the Society Research Foundation awarded \$1,169,183 in scholarships and grants to support neurosurgical research in Australia and New Zealand for 2007 to 2011 inclusive. A further \$205,000 has been awarded for 2012. We would like to take this opportunity to sincerely thank our sponsors and partner organisations for their ongoing support for neurosurgical research in Australia and New Zealand.

Our 2010, 2011 and 2012 sponsors and partner organisations are shown opposite. The research projects supported are identified in this Annual Report.

Grants & Scholarships Awarded - 2010

NSA Research Scholarship \$55,000 - Sponsored by Medtronic

- ❖ Dr Nicholas Hall
- ❖ The application of mesenchymal stem cells in the spine – effects on fusion, disc regeneration and modeling a biomimetic disc

NSA Research Scholarship \$55,000

- ❖ Dr Gemma Olsson
- ❖ Identifying Signaling Pathways Driving Glioblastoma Multiforme-PI3 kinase, ERK, PKC and Src Cellular Signalling Pathways Drive Glioblastoma Multiforme Survival and Growth

NSA Research Scholarship \$50,000 - Sponsored by Synthes

- ❖ Dr Nova Thani
- ❖ Studying MRI frame-based stereotaxis with implanted guide tubes and stylettes

NSA Research Scholarship \$5,000 (top up of RACS Scholarship)

- ❖ Dr Miu Fei Lam
- ❖ The convection enhanced delivery of neuroprotective agents to the striatum

NSA Research Scholarship \$22,372 (top up NH&MRC) –Sponsored by Synthes

- ❖ Dr Adam Fowler
- ❖ The role of IQGAP in high grade glioma and its regulation by mir-124-A

NSA Research Grant \$35,000 –Sponsored by Synthes

- ❖ Dr Andrew Davidson
- ❖ The application of focused ultrasound and a vascular targeting strategy in an animal model of human brain AVM

NSA Research Grant \$35,000 –Sponsored by Stryker

- ❖ Professor Neville Knuckey
- ❖ In vitro assessment of potential neuroprotective proteins

NSA Research Grant \$35,000 –Sponsored by Medtronic

- ❖ Associate Professor Michael Murphy
- ❖ Interlukin-6 and oncostatin M protein expression in human glioblastoma multiforme



Grants & Scholarships Awarded - 2011

NSA Research Grant for \$35,000 –Sponsored by Medtronic

- ❖ Dr Andrew Davidson
- ❖ Molecular imaging of vascular targets in an animal model of brain AVM primed with focused ultrasound

NSA Research Scholarship for \$35,000 – Sponsored by Synthes

- ❖ Dr Katherine Holland
- ❖ Clinical and molecular factors determining recurrence and survival in glioblastoma multiforme

NSA Research Scholarship for \$35,000 – Sponsored by Synthes

- ❖ Dr Rupal Jayalath
- ❖ Assessment of chromosomal instability in glioblastoma and development of combinatorial therapy to delay therapy resistance in glioblastoma

NSA Research Scholarship for \$35,000

- ❖ Dr Iwan Bennett
- ❖ Vascular biomarkers in malignant glioma

NSA Research Scholarship for \$20,000

- ❖ Dr Jin Wee Tee
- ❖ Predictors of functional outcome of patients with traumatic spine fractures

NSA Research Scholarship for \$15,000 (top up of RACS Scholarship)

- ❖ Dr Johnny Wong
- ❖ Investigating the role of Aquaporin-4 in the development of post-traumatic syringomyelia
- ❖

Grants & Scholarships Awarded - 2012

Scholarship for \$50,000 – Funded by The Cure For Life Foundation

- ❖ Dr Iwan Bennett
- ❖ Vascular biomarkers in malignant glioma

Scholarship for \$50,000 – Funded by Neurosurgical Research Foundation (SA)

- ❖ Dr Adam Wells
- ❖ Establishing a surgical model of middle cerebral artery occlusive stroke in the sheep

NSA Research Scholarship \$35,000 –Sponsored by Synthes

- ❖ Dr David Oehme
- ❖ Novel approaches to the repair of degenerate and prolapsed lumbar intervertebral discs

NSA Research Scholarship \$35,000 –Sponsored by Lifehealthcare

- ❖ Dr Wayne Ng
- ❖ Investigation of PI3K/Akt pathway inhibitors using a mouse glioma stem cell bioluminescent

NSA Research Scholarship \$35,000 –Sponsored by Synthes

- ❖ Dr Stephen Byrne
- ❖ Viral pathogenesis in glioblastoma and its impact on virus-specific cellular immunity

The application of mesenchymal stem cells in the spine – effects on fusion, disc regeneration and modeling a biomimetic disc

Research Institution: The Ritchie Centre – Monash Institute of Medical Research

Other Investigators: Professor Graham Jenkin, Professor Peter Ghosh, Dr Tony Goldschlager

Scholarship: NSA Research Scholarship - Sponsored by Medtronic



Cervical Spine

The concept of adjacent segment disease in the cervical spine following anterior interbody fusion remains a controversial topic. We believe that this is likely to be a real entity and have evaluated the use of Mesenchymal Progenitor cells, (MPC's) a proprietary stem cell isolated from the perivascular niche with a high degree of potential for differentiation and renewal, to produce a 'biomimetic' disc made from hyaline cartilage produced by stem cell differentiation. We have previously demonstrated in vitro that the addition of Pentosan Polysulfate (PPS) a sulphated polysaccharide to MPC's produces chondrogenic differentiation. We have also demonstrated in the sheep (ovine) cervical spine animal model, which biomechanically and embryologically is very similar to the human spine, that cartilage can be produced in the intervertebral disc space.

This project aimed to build on the findings of a previous study undertaken by our group where MPC's and PPS added together in the ovine disc space inhibited bone formation and histologically produced significant amounts of hyaline cartilage. In this previous study the cartilaginous end plates of the vertebral bodies were left intact. In our study we aimed to evaluate how removal of the cartilaginous end plates, an operation much more akin to a normal anterior cervical decompression surgery, would affect the ability of the MPC + PPS combination to produce hyaline cartilage. In this micro environment, blood containing normal bone marrow stem cells would likely be exposed to the cage and matrix providing an environment more conducive to bone formation.

Although statistical analysis of our data is still awaited the clear impression from our 2 principle outcome measures of CT and histology suggests that in control groups containing no MPC's or PPS a combination of bone and fibrous tissue predominates. In groups containing MPC's and matrix alone a high proportion of bone growth predominates and in the group with MPC's and PPS bone growth is inhibited but without significant production of hyaline cartilage. These findings support previous animal trials performed by our group that show strong augmentation of bony fusion by MPC's added to a tricalcium phosphate-hydroxyapatite matrix. Our final results will be published following independent analysis and scoring by a board certified veterinary histopathologist and radiologist.

The findings of this research support ongoing work showing the value of MPC's in the setting of augmentation of bony fusion. A phase Ib human trial is currently in progress evaluating safety and efficacy of MPC's in multilevel cervical ACDF.

We have also performed a mini study using 2 intervertebral disc levels in 3 sheep with green fluorescent protein labelled MPC's and PPS. The fluorescent labelling is integrated into the cells DNA and so following differentiation and replication the progeny of the initial cell population can be identified. This study will provide valuable insight into how MPC's work in the intervertebral disc space.

Osteochondral defects in the knee study

Concurrently we performed a pre-clinical trial evaluating the repair of osteochondral defects in the stifle joint of mature ewes. This study group comprised of 6 sheep with a microfracture and either MPCs alone or MPCs + PPS on each of the animals 12 joints. The surgery consisted of an open lateral parapatellar approach followed by controlled microfracture on either side of the trochlear groove and leaving either a bleeding defect or loading a custom made scaffold into the defect. This was novel in that it was different to my more usual surgical operative repertoire. These animals were sacrificed at 6 months and underwent MRI and CT to evaluate cartilage production on the joint surface the specimens are currently in a process of lengthy decalcification to allow histological preparation.

Publications & Presentations

I submitted a number of applications to present work at national and international conferences. The first national presentation was recently well received and I was awarded a session best prize for the presentation and also the R C Bennett medal for best presentation of the conference. This was also published. I also recently presented at the annual Neurosurgical Society of Australasia meeting 2010 and presented at the 4th Asia Pacific Cervical Spine Society meeting.

Acknowledgements

Professor Graham Jenkin - The Ritchie Centre – Monash Institute of Medical Research

Professor Peter Ghosh - Mesoblast & Proteobiactives

Dr Tony Goldschlager – Neurosurgery Registrar Monash Medical Centre / The Ritchie Centre – Monash Institute of Medical Research

Identifying Signaling Pathways Driving Glioblastoma Multiforme-PI3 kinase, ERK, PKC and Src Cellular Signalling Pathways Drive Glioblastoma Multiforme Survival and Growth

Research Institution: Princess Alexandra Hospital

Scholarship: NSA Research Scholarship

Research Update

Human glioblastoma tumour tissue was collected from the operating theatre at Princess Alexandra (PA) Hospital for use in this research. A collaboration between our lab group and neurosurgeons at the PA has existed for a number of years to enable this. Only a small amount of tissue is able to be accessed each year in this way however and information such as survival as a surrogate of aggressiveness is often not available for some time. There exists a large amount of human tumour tissue in archival form and the next stage of this work will involve histopathological analysis of archival patient tissue in the form of tissue microarrays. Correlation with survival will then be possible and will add to protein analysis. Work on primary tumour tissue has shown by western blot analysis that there is variable activation of the above signalling pathways in individual tumours. Activation is conserved in primary and cultured tissue of the same tumour. If this activation can then be shown to be conserved in tissue treated in the same way as the archival tissue held by Queensland Health then we can justify using this tissue for analysis of these pathways. Due to the larger numbers of tumours able to be studied a statistically proven result is then more likely.

The rationale for studying signalling pathways rather than single proteins or genes is that whole genome sequencing of cancers, recently the focus of a global research effort, has yielded more mutations than previously expected. The Cancer Genome Atlas Group who performed systematic characterisation of genomes of GBM tumours from a 206 patient cohort revealed that unexpected finding the GBM tumours display significant mutational heterogeneity, with only a fraction of genes commonly mutated across patients. As a result it will be difficult to identify individual drivers of GBM disease based on genetic data.

Our approach is to identify core signalling pathways utilised by GBM in functional studies, and then confirm the activation and clinical significance of these pathways in a large patient cohort. If successful, the results of this study can be used to identify drivers of GBM disease for therapeutic intervention. These core pathways are highly conserved in cells and are activated by numerous cellular mechanisms to produce cellular proliferation, survival and growth. If these pathways are activated by upstream cellular mutations then these cellular mechanisms may drive disease in vivo.

There are many core pathways that have been implicated in the literature to be important in GBM and other cancers. The first part of this study looked at many and found five to be of interest.

Core pathways were pharmacologically inhibited in cultured tumour cells derived from primary human GBM tumours. Numerous pathways were inhibited using numerous pharmacological agents for each pathway.

The PI 3 kinase, ERK, PKC and Src pathways were found to be crucial for tumour survival and growth. Pathway activation has then been confirmed using primary GBM samples from patients by western blot. In twelve primary samples pathway activation was shown to be variable but present compared to controls for each of the four pathways of interest. Some tumours activation was stronger than others. Activation was then further confirmed in patient samples by immunohistochemistry. This is the role I was solely responsible for. This is the modality to be used for the next phase and we wanted confirmation that expression of pathway activation would correlate with other findings.

Ten patient samples were available for this and again variable pathway activation was confirmed and consistent with western blot results.

The small sample size and unknown survival of patients whose tumour tissue was utilised for these initial experiments warrants a larger study to see if there is significance to these results. An archival tissue set will be used.

Of particular interest and not surprisingly tumours displayed a significant degree of heterogeneity in pathway activation. We hope that this might relate to differing survival and the most interesting phase of this work will relate to quantitative correlation of pathway activation (we will use histochemical expression as a surrogate marker of this) with survival in a large cohort of patients. We hope to find that pathway activation corresponds to survival.


In the next phase of this work further confirmation of pathway activation in primary tumours will be performed using quantitative immunohistochemistry analysis of archival histopathological tissue in a microarray format from a cohort of 507 consecutive South East Queensland public patients. Pathway activation will be correlated to patient outcome, proliferative index and therapy regimen using sophisticated statistical matrices.

The patient cohort is similar to previously described GBM cohorts. Variability in pathway activation between tumours indicates differential usage of core signalling pathways across different patients.

In conclusion this initial work suggests that activation of core signalling pathways in GBM may identify drivers of GBM disease and may indicate novel therapeutic targets and rationally stratify patient treatment based on pathway activation.

The Future

Archival fixed paraffin embedded tissue has been collected from a cohort of public patients from three South East Queensland hospital where Neurosurgery occurs. This collection was granted by the appropriate governing body after ethics approval for the project was granted. Tissue samples were then considered for appropriateness to be used in a tissue microarray format based largely on tissue volume. Appropriate representative samples were then selected by a pathology registrar and these areas were used to produce four different blocks giving internal controls for each tumour. A total of 206 tumours and 13 healthy brain controls are available for protein analysis. This is from the cohort of patients whose survival and management analysis formed the basis of the work above and will provide survival information to the above information on protein pathway utilisation by tumours. It is hoped that a



correlation will be send and that this may form the basis for a therapeutic target. TMAs were produced with the assistance of the Molecular and Clinical Research Laboratory at the Princess Alexandra Hospital and were completed in December 2010. Staining will commence in the new year and analysis shortly after.

Publications and Presentations

The results of the clinical audit were presented to the NSA Annual Scientific Meeting (ASM) 2010. The results of the initial pilot tissue protein analysis were also presented to the NSA ASM and are summarized above.

Acknowledgments

Dr Angus Harding – Glioma Group Leader, Diamantina Institute, University of Queensland

Dr Sarah Olson – Neurosurgeon, Princess Alexandra Hospital

Molecular and Clinical Research Institute, Princess Alexandra Hospital

Interlukin-6 and oncostatin M protein expression in human glioblastoma multiforme

Other Investigator: David Moses

Research Institution: Centre for Clinical Neurosciences and Neurological Research

Research Grant: NSA Research Grant – Proudly Sponsored by Medtronic



Research Update

The aim of this project was to investigate oncostatin-M (Osm) and Interleukin-6 (IL-6) protein expression in surgical samples of human glioblastoma multiforme (GBM) by means of semi-quantitative western blotting.

We hypothesised that expression levels of IL-6 and OSM and the ratio of IL-6/OSM are altered in human GBM compared to normal human brain. We anticipate that IL-6 expression and the ratio of IL-6/OSM are elevated in GBM samples.

GBM is a primary brain cancer for which no effective cure currently exists. Similar to other organ cancers, uncontrolled cellular proliferation is a hallmark of this devastating condition. Previous studies with GBM cell lines and in rodent models suggest that Cytokine Interleukin-6 (IL-6) promotes proliferation of GBM cells. Experiments with GBM cell lines also showed that Cytokine Oncostatin M (OSM) inhibited proliferation and induced differentiation of GBM cells. Surprisingly, expression of IL-6 in human GBMs at the protein level has never been examined. Only one study has previously reported elevated OSM levels in a small number of GBM samples.

Our preliminary data lend further support to the hypothesis that IL-6 and OSM are relevant to GBM biology. We have demonstrated that IL-6 is a potent mitogen for human GBM-derived stem cells, whereas OSM opposes IL-6 driven mitogenic effect.

Based on existing data and our preliminary results, we propose further experiments to begin to understand the in vivo role of autocrine/paracrine IL-6 and OSM signaling in GBM biology and whether these Cytokines and their respective signaling pathways represent potential therapeutic targets. We propose to examine expression of IL-6 and OSM and their respective receptors IL-6r and OSMr at the protein level in surgical samples of human GBMs by means of semi-quantitative western blotting technique. Expression of IL-6, OSM and respective receptors in GBM will be compared to expression in normal human brain. Ratio of IL-6/OSM in GBMs and in normal human samples will be also derived and statistically analysed. Experiments that we propose here are essential to pave the way for development of therapeutic strategies focusing on manipulation of IL-6/OSM and their respective signaling pathways.

Studying MRI frame-based stereotaxis with implanted guide tubes and stylettes

Other Investigator: Mr Christopher Lind

Research Institution: Sir Charles Gairnder Hospital, Western Australia

Scholarship: NSA Research Scholarship - Sponsored by Synthes



Significant Changes

The aim of this research is to validate the use of 3-tesla magnetic resonance imaging (MRI)-directed frame-based stereotaxis in deep brain stimulation surgery and develop accurate methods of trajectory planning and deep brain stimulation (DBS) electrode contact analysis. We have adapted the MRI-directed implanted guide tube technique of Gill (Frenchey hospital, UK) which enables (a) high resolution stereotactic MRI under anaesthesia to clearly see small brain structures, (b) surgery under general anaesthesia without clinical examination or neurophysiology, (c) complete avoidance of cerebrospinal fluid leakage or brain shift, and (d) the ability to perform intra-operative MRI of an electrode surrogate made of carbothane inserted through a permanently implanted guide tube that obviates MRI related signal artefact.

The surgical nuances, specifically, assessing the accuracy of MRI-guided frame based stereotactic surgery has been the majority of work performed in the first semester together with the collection of data. Assessment of spatial accuracy of MRI and its implication for stereotactic surgery is proving a fruitful area of research with wide-ranging implications. Therefore, the major component of the research thesis would be formed on the subject of "surgical accuracy" in deep brain stimulation surgery. Data on the clinical and scientific outcomes of caudal zona incerta will also be continued and the effect of neuromodulation on tremor will form part of the thesis.

Research Update

Using the novel guide tube surgical technique for 23 consecutive stylette tracks for DBS, the specific aims were to: (1) assess the spatial fidelity of 3-tesla MRI in-vivo for stereotaxis; (2) assess the accuracy of frame based stereotaxis for delivering deep brain probes to brain structures visible on MRI; (3) measure the margin of error in MRI-based safe trajectory planning to validate its application accuracy; and (4) use the intra-operative MRI of stylettes as a gold standard against which to validate the commonly used postoperative CT /preoperative MRI image fusion technique for determining DBS electrode locations.

We have found good spatial accuracy using 3-tesla MRI for stereotaxis in our in vivo analysis. There was no surgically significant difference in the three dimensional accuracy of the probe at the periphery of the brain compared to the central targets, both 1.8 mm (95% CI: 1.5 mm, 2.1 mm); which indicates there is no significant MRI geometric distortion at different distances from the magnetic iso-centre. We have

demonstrated that a safe trajectory with 97.5% confidence of avoiding ventricles, blood vessels and eloquent cortical structures can be achieved using a virtual planning cylinder with a radius of 2.85 mm that encloses the entire DBS lead from entry point to target. Using our intra-operative MRI data, we have also shown that the commonly used CT/MRI fusion technique results in an error of $1.6 \text{ mm} \pm 0.2 \text{ mm}$ in identifying electrode contact points.

Our research provides evidence that MRI geometric distortion is not surgically significant for MRI-directed stereotaxis. We have proven that safe trajectory planning can be achieved in practice with a high degree of accuracy by factoring in measured margins of error. The implanted guide tube technique enables accurate electrode contact localisation for research of tiny brain structures visible on MRI. The commonly used CT/MRI fusion technique provides reasonable electrode localisation accuracy for clinical purposes but does not allow definite correlation with MRI-visible brain structures.

Publications and Presentations

Thus far, one publication, “High Frequency Pallidal Stimulation for Camptocormia in Parkinson’s disease” has been accepted in the Journal of Neurosurgery. Two further manuscripts “Accuracy of MRI-directed frame-based stereotaxis” and “A validation of post-operative CT and stereotactic MRI fusion for assessing deep brain stimulation electrodes” have been submitted for peer-review. A presentation was made at the 8th Asian Congress of Neurological Surgeons, Kuala Lumpur, Malaysia (2010).

Convection enhanced delivery of neuroprotective agents to the striatum

Other Investigators: Dr Meghan Thomas, Mr Christopher Lind

Research Institution: Western Australia Neurosurgical Service Sir Charles Gairrnder Hospital, Western Australia; School of Surgery, University of Western Australia; Edith Cowan University

Scholarship: NSA Research Scholarship & RACS Scholarship

Significant Changes

At the time of the last report, I have successfully set up a positive pressure infusion system with a continuous flow pump, which has reliably delivery known volumes into an agarose gel model. I have also been able to perform infusion line pressure monitoring during CED infusions. MRI monitoring enables volume of distribution estimation, it is limited by lag time and takes only intermittent snapshots during an infusion. Previous literature suggested the potential role of infusion line pressure as a surrogate measure of the convection pressure gradient, and therefore a valuable external continuous real-time indicator of the intra- and extra-cannula environments during attempted CED.

Research Update

The aim of the project is to characterise infusion line pressures during CED in an *in vitro* agarose gel model and an *in vivo* rodent striatal model. The hypothesis is infusion line pressure monitoring may be used to help characterise successful versus failed infusions during acute CED and thus enable the neurosurgeon to cease failing infusions earlier.

I have continued from my previous progress with the positive-pressure infusion system and confirmed reliability of the infusion line pressure monitoring set up according to previously published calibration of strain gauge pressure monitoring technique. Using this set up, I have also been able to achieve reliable stereotactic catheter targeting of rodent striatum after achieving general anaesthesia. I have acquired animal neuroscience laboratory knowledge and techniques including general anaesthesia of the animals, animal euthanasia, and processing of specimens. I have performed experiments to characterise the infusion line pressure in both the *in vitro* 0.6% agarose gel model and the *in vivo* rat striatum model, using a new "step" reflux preventing catheter designed by our collaborator and mentor Professor K Bankiewicz's surgical translational group in University of San Francisco, USA. This information has not been previously in peer-reviewed literature.

The results suggested promise in using real-time infusion-line pressure monitoring to distinguish during an earlier phase of CED infusions between potentially successful and failing infusions. The infusion line pressure profile of a typical CED infusion cannot be demonstrated as a hyperbolic curve over time, in the *in vitro* agarose gel model. At the end of the infusion, the pressure profile then decay exponentially. The result in the *in vitro* agarose gel model were useful to distinguish different infusion outcomes, and in particular, obstructions; and to a lesser extent, reflux infusions.

The same infusion line pressure profiles can be detected in the *in vivo* model in the rat striatum. The successful CED infusions again demonstrated a hyperbolic pattern over time, followed by an exponential decay at the cessation of infusion. This is in contrast to the linear pattern during failed infusions. However, the distinction is less obvious at a higher flow rate, most likely due to a short duration of infusion. No infusions in the rat striata refluxed. The difference between the pressure profiles in the rat striatum was less distinct due to a limitation imposed by the small size of the rat striatum. The rat striatum is too small to allow adequate testing of this technique.

Publications and Presentations

I have written the following:

1. Lam M, Thomas MG, Lind CRP. Convection-enhanced delivery: optimising neurosurgical delivery of therapeutic agents to the brain. ANZ Journal of Surgery (submitted and rejected 9/2010) – Professor Hall, Editor, ANZ J Surg, rejected on basis of lack of interest to general readership and recommended submission to specialised neurosurgical journal.
2. Lam M, Thomas MG, Lind CRP. Neurosurgical convection-enhanced delivery of treatments for Parkinson's disease. Journal of Clin Neurosci (accepted 1/2011).
3. Lam M, Thomas MG, Lind CRP. Infusion line pressure monitoring of convection-enhanced delivery. (Completing and to be submitted soon).

I have also presented in the following meetings:

1. Local experience in setting up convection-enhanced delivery (MMSci student presentation, WAMIR section, Combined Biological Science Meeting, Perth, 27/8/2010).
2. Convection-enhanced delivery in Parkinson's disease: Perth pre-clinical experience (Surgical Research Society 47th Annual Scientific Meeting, Adelaide, 19/11/2010).
3. Intrapallidal convection-enhanced delivery: use of infusion-line pressure monitoring in pre-clinical models (Poster presentation, University of Western Australia School of Surgery Research Symposium, Perth, 3/12/2010).

Acknowledgments

Royal Australasian College of Surgeons
Neurosurgical Society of Australasia
Professor K Bankiewicz, University of California, San Francisco, USA
Professor S Gill, Frenchay Hospital, Bristol, UK
McCusker Charitable Foundation

The role of IQGAP in high grade glioma and its regulation by mir-124-A

Other Investigators: Dr K McDonald, Dr M Messina, Professor B Robinson

Research Institution: Kolling Institute of Medical Research, University of Sydney

Scholarships: NSA Research Scholarship –Sponsored by Synthes and NH&MRC Scholarship



Research Update

In this research, the important role in modulating glioblastoma invasiveness by IQGAP1 was explored and an important, reciprocal and functional interaction of miR-124a was investigated. Whilst IQGAP1 has been proven to modulate invasion in a number of cancers, this research provides new evidence of its importance in glioblastoma. Both clinically and *in vitro*, this interplay between IQGAP1 and miR-124a showed important features that may, with further investigation, prove part of the solution to the huge clinical challenge, faced by patients, with this terrible disease.

A172 and T98G represent aggressive human glioblastoma cell lines with different p53 gene status. The biological significance on migration and invasion of altering IQGAP1 expression in glioblastoma cells was tested in experiments embracing multiple modalities. To reduce IQGAP1 expression, A172 and T98G cells were transfected with siRNA specifically targeting IQGAP1 (siIQGAP1) resulting in significant reduction in IQGAP1 mRNA and protein expression levels. To measure the effects of siIQGAP1 on cell migration, electrical cell substrate impedance sensing (ECIS) assays were used. Migration was significantly impeded in both cell lines with reduced IQGAP1 expression. The altered migratory capacity of siIQGAP1-transfected cells was independently confirmed using wound-healing assays with no major anti-proliferative effects observed. Invasion assays conducted following siRNA transfection displayed a significant reduction in numbers of invading cells in the IQGAP1 knockdown group, in both cell lines.

These remarkable results add to existing knowledge regarding the repertoire of molecules proven to have a role in assembly and regulation of invasive cellular substructures. The Rho-family GTPases, Cdc42, Rac1 and RhoA, are key regulators of actin cytoskeletal organization and have essential roles in many cellular processes, including cell migration. All three of these GTPases have also been shown to influence regulation of invasive cellular machinery albeit in different cell types. In U87MG glioma cells, depletion of Rac1 or its effector, synaptojanin 2 (a phosphoinositide phosphatase) using siRNA, inhibited invasiveness of cells through slices of rat brain tissue and through Matrigel™. In another study, endogenous active RhoA (RhoA-GTP) was shown to localize in protrusions of Src transformed NIH 3T3 fibroblasts. Thus RhoA appears to have a role in invasion in Src transformed cells. It is unlikely that Rac1, Cdc42 and RhoA would be simultaneously activated within invasive projections in any, one cell type (and this has not been investigated in glioblastoma) as RhoA and Rac1 have antagonistic activities that are spatially and temporally regulated.

The important results presented show that IQGAP1 as a member of the Ras-GAPs has the ability to negatively regulate glioma cellular migration and invasion *in vitro*. This is almost certainly due to their tissue-specific ability to bind Rho A and Cdc42 (amongst others) as cofactors of actin assembly in invasive cellular machinery. Mechanistically, this is an attractive modality of the action of IQGAP1 knockdown on glioma cell motility. A clearer understanding of the dynamics of Rac1, Cdc42 and RhoA and IQGAP1 activation and downregulation in (glioblastoma) cytoskeletal protrusions will help to clarify the roles of these proteins. Co-localization studies in a wider panel of glioblastoma cell lines would give important insight into the significant interplay of these molecules at invasive cellular fronts.

Another key interaction in which IQGAP1 participates is that of ARF6 (ADP ribosylation factor-6). ARF6 is a Ras-superfamily GTPase, found to function upstream of IQGAP1 and be involved in the regulation of plasma membrane recycling, exocytosis and actin polymerization. ARF6 is found in invasive cellular protrusions in melanoma and breast cancer cells. In LOX invasive melanoma cells, HGF stimulation enhanced protrusion activity that also coincided with activation of endogenous ARF6, whereas an ARF6 dominant negative mutant inhibited protrusion formation. Depletion of ARF6 with siRNA also blocked machinery formation in MDA-MB-231 breast cancer cells and in addition inhibited the invasion of cells through Matrigel™. Moreover, ARF6 expression levels correlated with invasiveness in a panel of breast cancer cell lines and *in vitro* invasiveness of cells could be inhibited in all cases by ARF6 knockdown. In 2009, important results came to light regarding ARF-6 signalling in glioblastoma. In this study by Hu et al[1], it was found that cellular depletion of ARF6 by small interfering RNA decreased Rac1 activation, impaired HGF-stimulated and serum-stimulated glioma cell migration *in vitro*, and markedly decreased the invasive capacity of invasive glioma in the brain. Upon stimulation of glioma cells with HGF, it was shown that IQGAP1 was recruited and overlapped with ARF6 at the leading edge of migrating cells and at the same time, cellular depletion of ARF6 abrogated this recruitment of IQGAP1 and attenuated the formation of surface protrusions. ARF6 forms complexes with Rac1 and IQGAP1 in glioma cells upon HGF stimulation, and knockdown of IQGAP1 significantly inhibited ARF6-induced Rac1 activation and cell migration. Taken together, this suggests that ARF6-mediated Rac1 activation is essential for glioma cell invasion via a signalling pathway that requires IQGAP1. In this thesis, ARF-6 and Rac1 activation was not assessed however it is reasonable to predict similar results to Hu et al and deserves investigation. One particularly attractive avenue of investigation would be to examine the interplay between VEGFR expression and IQGAP1, ARF6 and Rac1/RhoA. Given the wide body of evidence as to the importance of ARF-6 signalling in invasion, the results presented in this thesis cement the role that IQGAP1 plays in regulating invasion in glioblastoma in two further glioblastoma cell lines.

Evidence provided by this research and by other investigators[2], provides an important link to the prognostic importance of IQGAP1 in a clinical cohort of glioblastoma. Previous studies reported high levels of IQGAP1 protein to be prognostic of adverse survival in high-grade glioma [2]. In the current study, IQGAP1 over-expression was observed in 47% of the glioblastoma cohort. Increased expression and altered localization of IQGAP1 has been frequently observed in immortalised cancer cell lines and in various primary neoplasms [3-5] and is a key regulator of cell adhesion, migration and cell polarity. As a major regulator of IQGAP1 expression, the expression of miR-124a was assessed in several ATCC glioblastoma cell lines and found to be profoundly under-expressed compared to normal brain samples. This result is consistent with previously published studies in clinical glioblastoma samples and cancer cell lines[6-8]. To analyse the functional role of miR-124a in glioblastoma, we restored miR-124a expression in glioblastoma cells that lack the miR-124a transcript. Changes in cell proliferation, using two independent methodologies, did not reveal a major anti-proliferative effect. To test that miR-124a regulates tumour cell migration the motility of transfected A172 glioblastoma cell lines was examined. Approximately 24 hours post wound scraping of a confluent monolayer, nearly full wound closure was observed in the control cells. In contrast, wounds scraped in confluent cultures of miR-124a transfected

cells closed only partially. ECIS wound-repair assays confirmed a diminished capacity for miR-124a transfected cells to migrate. Transwell™ assays were conducted using a serum gradient demonstrating a reduced invasive capacity for miR-124a transfected cells. As a direct target of miR-124a, the cellular expression of IQGAP1 was significantly diminished. Clinical expression analysis revealed that miR-124a is significantly down regulated in 87% of glioblastoma in this cohort and that low levels of miR-124a are associated with shorter median survival. Significant down-regulation of miR-124a transcript in glioblastoma, oligodendroglioma and also medulloblastoma has previously been reported [6-8]. This current study advanced a step further by examining the relationship between miR-124a loss and survival. Although no significant differences in median survival were apparent, it was interesting that close to 20% of patients with high miR-124a expression survived longer than 2 years after their initial surgeries. Loss of miR-124a has been shown to be a marker of increased risk for developing cervical cancer [9] and with a higher relapse rate and mortality rate in patients with acute lymphoblastic leukaemia (ALL) [10]. In both of these clinical studies, loss of miR-124a was attributed to epigenetic silencing. Although this was not investigated in this thesis, it represents an important future avenue of investigation.

Among the hundreds of potential human genes as targets of miR-124a, IQGAP1, LAMC1 and ITGB1 were examined in detail. All three targets are involved in migration and all three were identified to be direct targets of miR-124a from a study that devised a biochemical method for identifying miRNA targets that combined RNA-induced silencing complex (RISC) purification with a microarray analysis of bound mRNAs[11] and in varied published high-throughput screening methods [11-16]. As shown in Chapter 4, over expressed miR-124a in A172 cells reduced expression of IQGAP1, LAMC1 and ITGB1. We further showed evidence of this reciprocal association between miR-124a and all three targets in our clinical cohort of 119 patients. Whilst the contribution of SNPs in miR-124a binding site of IQGAP1 still remains unquantified, it may represent new risk stratification if evaluated in a larger cohort of glioblastoma. Results attained cement miR-124a as having prognostic (if not predictive) importance. This effect could be mediated through three mRNA targets, IQGAP1, LAMC1 and ITGB1, all reported to play a role in migration. Less well defined, and a region for further investigation is the patterns of expression of integrin α V β 3 and β 5 in much larger cohorts of glioblastoma. It is reasonable, based on the work of this thesis, to examine other molecules in tandem with these, notably laminin γ 1 and miR-124a in much larger cohorts of glioblastoma. Interestingly, in our results, IHC staining pattern of laminin γ 1 mirrored IQGAP1.

There are strong links between laminin and integrin signalling as well as the links with IQGAP1, thus manipulating levels of miR-124a could be targeting a common and glioblastoma -specific, roadmap for the driving of cellular invasion.

Recognizing a potential role for α V integrins in glioma, CENTRIC (CilENGitide in combination with Temozolomide and Radiotherapy in newly diagnosed glioblastoma phase III randomized Clinical trial) is a multicentre, open label, controlled phase III study currently underway. Registration and recruitment is ongoing at the time of writing. As discussed in detail in Chapter 4, miR-124a occupies a unique position within glioblastoma as it has the potential to regulate three specific molecules driving glioblastoma invasion – notably IQGAP1, LAMC1 and ITGB1 providing a very attractive prediction of a powerful therapeutic synergy with cilengitide. Early *in vitro* results, have demonstrated an anti-proliferative synergy, based on target convergence, between cilengitide therapy and miR-124a over-expression in selected glioblastoma cell lines.

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Matar, E. Cook, R. Fowler, A. R. Biggs, M. Little, N. Wheeler, H. R. Robinson, B. McDonald, K. Post-contrast enhancement as a clinical indicator of prognosis in patients with anaplastic astrocytoma J Clin Neurosci. Volume 17, Issue 8 Epub 7/8/2010

Presentations

1. Clinical Oncology Society of Australasia "Best of the Best Oral Presentations" Gold Coast, November 2009
2. Surgical Research Society 46th Annual Scientific Meeting, Adelaide, South Australia – Oral Presentation – Awarded the Young Investigator Award for Royal Australasian College of Surgeons. Nov 2009
3. Combined Kolling, Sydney University and UTS Scientific Meeting – Awarded Sanofi-Aventis Award for Best Oral Presentation. Nov 2009
4. Association of Academic Surgeons / Society of University Surgeons Annual Scientific Conference, San Antonio, Texas, USA, Feb 2010 – International Guest Speaker.

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In vitro assessment of potential neuroprotective proteins

Other Investigator: Bruno P. Meloni

Research Grant: NSA Research Grant –Sponsored by Stryker



Research Update

The aims of this project involved further characterising several proteins we have identified to be differentially expressed in neuronal cultures following preconditioning, with the hypothesis that these proteins are neuroprotective.

The proteins we chose to characterise further were: citrate synthase (CS), heat shock protein 60 (HSP60), mitochondrial stress protein 70 (GRP75), nucleoside diphosphate kinase A (NDKA), prohibitin (PHB), fatty acid binding protein (FABP7), glucose regulated protein 78kd (GRP78), voltage dependent anion channel 1 (VDAC1), and a novel gene (NP1).

One of the first steps in order to accomplish this aim was to construct adenoviral expression vectors for each of these proteins. For two of these proteins this had already been accomplished (NDKA and FABP7) and for another protein (NP1) an additional vector was required to allow expression under the control of a RSV promoter; note, the RSV promoter is our standard promoter for neuronal work. In addition, the cDNA for GRP75 still had to be cloned and sequenced verified.

In the course of this project we have been successful in constructing and purifying all the adenoviral vectors listed in our project application, and hence have accomplished this major aim. These adenoviral vectors are now ready to allow assessment of the effects of specific protein over-expression on neuronal survival in our in vitro ischaemia-like injury models. We currently have a PhD student (Jonathan Teoh) who will be performing these experiments shortly.

To complement over-expression studies, a second major aim of our project was to establish the RNA interference (RNAi) technique in our laboratory in order to under-express our proteins of interest in neuronal cultures. This involved selecting and assessing commercially available double stranded RNAi constructs that bind to and degrade the mRNA that encode for our proteins of interest. Following the assessment of 1 - 3 RNAi constructs for each of our proteins of interest we were able to confirm by western analysis that at least one RNAi construct for each of our proteins could down-regulate the protein. Furthermore, in order to achieve optimal RNAi down-regulation of our proteins, a two time-point dosing regimen was developed. Hence, the second major aim of our project was achieved. These constructs are now in readiness for Mr Teoh to apply in our in vitro ischaemia injury models.

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We acknowledge the Australian Neuro-Muscular Research Institute for providing the facilities to undertake the work associated with this project. We would also like to acknowledge the following research staff that has contributed to the project in one-way or another: Jonathan Teoh, Jaclyn Chan, Izabella Greene and Sherif Boulos.

The application of focused ultrasound and a vascular targeting strategy in an animal model of human brain AVM

Other Investigators: Professor Marcus Stoodley, Professor Michael Morgan

Research Institution: Australian School of Advanced Medicine, Macquarie University

Research Grants: NSA Research Grant – Sponsored by Synthes



Research Update

Some of our research group's earlier work has been directed at understanding molecular changes that occur in endothelial cells of AVMs. Both the intrinsic changes found in AVMs, and the additional changes induced in AVM endothelial cells by the application of various "priming" agents (such as focused irradiation, or heat energy), have been studied. It may be possible to "switch on" a molecular change in the endothelial cell of an AVM that permits the use of vascular targeting agents in the treatment of AVMs.

Earlier *in vitro* studies investigating the gene and protein expression in endothelial cells following hyperthermia have identified several molecules that warrant further investigation as part of a ligand-directed vascular targeting strategy in the treatment of brain AVMs. In order to investigate these molecules *in vivo*, a strategy had to be devised that would allow the targeted heating of blood vessels using focused ultrasound.

During 2010, preliminary trials of a modified focused ultrasound device were performed. In these experiments, a thermistor probe was implanted in the wall of the common carotid artery of a male Sprague-Dawley rat and the vessel was targeted with a modified High Intensity Focused Ultrasound (HIFU) machine (Focus Surgery, Sonablate® 500). The device incorporated diagnostic ultrasound imaging using a 6MHz imaging probe, and a pulsed HIFU probe (3-100J per pulse) designed to emit continuous low-level focused ultrasound adjustable between 0-5W.

These experiments showed that it was possible to selectively heat a vessel to 40°C using focused ultrasound without inducing whole body hyperthermia. The treatment protocol identified in these preliminary studies was then repeated in an animal model of AVM.

In these experiments, a carotid-jugular fistula was created in male Sprague-Dawley rats. After 6 weeks maturation, it resembled a human AVM; it had an arterial feeder, a branching and reconnecting system of arterialized veins (the 'nidus'), and a draining vein. Our research group has previously shown that the adhesion molecule and thrombotic molecule expression of endothelial cells in this model closely resembles the expression in human AVMs.

Following focused ultrasound hyperthermia of the model AVM, the nidus was harvested and tissue sent to the Australian Proteome Analysis Facility (APAF) at Macquarie University, where Mass Spectrometry proteomic profiling was performed to demonstrate the differential protein expression in treated AVMs compared to untreated AVMs and normal vessels. Complete analysis of these results has not yet been completed; however, preliminary results confirm that focused ultrasound hyperthermia does produce differential protein expression in the animal model of AVM.

In the next phase of our research, *in vivo* localization studies are necessary to determine the specificity of these molecular changes prior to the institution of a systemic targeted therapy. These studies will involve a molecular imaging system that performs multispectral fluorochrome imaging with co-registered x-ray and white light images for improved localization of biological markers *in vivo*.

Ultimately, it may be possible to 'prime' the endothelial cells of human AVMs by the non-invasive application of heat energy, through the use of high-intensity focused ultrasound. This could then be followed by the administration of a vascular targeting agent directed specifically at these molecular changes, which could result in the selective thrombosis of AVM vessels.

If successful, then the results of this study could revolutionise the treatment of large, difficult to treat AVMs. Eventually, the entire treatment procedure could potentially be performed non-invasively, and with a lower risk profile and greater efficacy than current invasive treatments.

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Presentations:

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2. Morgan MK, Davidson AS: Decision making in the management of cerebral AVM. **Macquarie Neurosurgery 2010 Research and Clinical Symposium**, Sydney, 2010
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4. Davidson AS: A novel approach to the treatment of human brain arteriovenous malformations. **ASAM Seminar Series**, Macquarie University 2011

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Clinical and molecular factors determining recurrence and survival in glioblastoma multiforme

Other Investigator: Andrew Morokoff

Research Institution: Royal Melbourne Hospital

Scholarship: NSA Research Scholarship –Sponsored by Synthes



Significant Changes

This research originally hypothesised that there is an inter-relationship between clinical and exogenous patient factors and molecular factors in determining survival and prognosis in glioblastoma multiforme and that glioma stem cells are the key cells related to tumour recurrence and outcome and as such, they contain particular molecular features (for example EGFR, PI3K, PTEN, Akt activation) that correlate with survival and could be used as prognostic predictors.

This retrospective study has utilised the existing database of glioma patients obtained through the Royal Melbourne Hospital brain tumour research group.

Additionally, in collaboration with the Peter MacCallum Hospital “Foundation Sequencing Project”, genetic analysis of twenty-one neurosphere-forming glioma stem cell lines has identified numerous biologically significant genetic variants. Gene mutations found in two new and novel genetic pathways predominate: the Wnt-Beta-catenin and Salvador-Warts-Hippo pathways. The Wnt-Beta-catenin pathway constitutes a putative stem cell survival pathway, whilst the Salvador-Warts-Hippo pathway is a potent tissue growth inhibitor, implicated in many solid organ malignancies. Involvement of these two pathways in gliomagenesis and in vitro neurosphere formation has been only separately reported in the literature to date.

In light of these findings, the focus of the project has expanded to investigate further the genetic signature underpinning this subset of gliomas with neurosphere-forming capacity:

Existing hypotheses have therefore been expanded to include the following:

1. That there exists a subset of aggressive high grade glioma with capacity for gliomasphere formation, and potentially poor prognosis;
2. That this subset of aggressive tumours are driven by a distinct genetic footprint predominantly linked to the Wnt-Beta-catenin and Salvador-Watts-Hippo pathways; and
3. That the genetic signature of gliomasphere-forming tumours is more closely representative of true tumour biology (compared to cell lines or bulk specimens).

Research Update

The next stage of the project will include performance of immunohistochemistry, in conjunction with the Royal Melbourne Hospital Anatomical Pathology Department, and immunofluorescence on the glioma stem cells, targeting YAP localisation (nuclear and cytoplasmic) in order to confirm the significance of Hippo pathway derangement in glioma stem cells. Such experiments, investigating derangement of the Hippo pathway in glioma stem cells, have not previously been described in the literature to date. It is anticipated that this will be complete in the next two to three months. Further validation of gene variants identified in genetic sequencing will be undertaken in collaboration with the Peter MacCallum Cancer Institute.

Acknowledgements

Peter MacCallum Cancer Institute

Assessment of chromosomal instability in glioblastoma and development of combinatorial therapy to delay therapy resistance in glioblastoma

Other Investigators: Dr Angus Harding and Dr Sarah Olson

Research Institution: Diamantina Institute, Princess Alexandra Hospital, University of Queensland

Scholarship: NSA Research Scholarship – Sponsored by Synthes



Significant Changes

Glioblastoma is the most common primary brain tumour in adults and affects 2-3 people in 100,000 in the general population. Current treatment for glioblastoma combines maximal surgical resection with focal radiation and chemotherapy. Despite aggressive therapeutic regime, patients diagnosed with glioblastoma have a median survival of 9-15 months. One factor limiting long term survival of glioblastoma is the emergence of therapy resistance in tumour cells due to mutations.

Briefly, the research plan is to treat patient tumour lines with single agents or sequential treatments with two or more therapies, and compare the rate of adaptation in single versus multi-therapy regimes. We predict that multi-therapy regimes will significantly reduce the adaptive capacity of patient tumour lines.

Research Update

Chromosomal instability (CIN) is a characteristic of cancer biology including in glioblastoma. Since the initial descriptions of cancer cells, many studies have shown that tumours can be capable of very rapid evolution and adaptation to conventional therapy, and this is the primary cause of acquired therapy resistance. Those cells that acquire survival advantage through random genetic or heritable epigenetic changes are selected through multiple rounds of clonal expansion. We hypothesise that these changes greatly increase genomic heterogeneity within tumours, and this in turn drives the rapid emergence of therapy resistant cells in cancer, including in glioblastoma. In support of this hypothesis, CIN generates many of the genomic changes present in brain tumours, including aneuploidy (abnormal chromosomal numbers), translocations, inversions, interstitial deletions, amplifications and loss of heterozygosity. In addition, it is established that patients with tumours containing a CIN expression signature have poor prognosis relative to patients with more genetically stable tumours.

Current treatment for glioblastoma combines maximal surgical resection with a protocol of radiation and concurrent temozolomide. The hypothesis tested in Aim 2 of the project is that sequential treatment of tumours with differing drug classes targeting diverse cellular pathways will kill different sub-populations of tumour cells, reducing the overall genomic heterogeneity within the tumour and therefore reducing the ability of the tumour to evolve therapy resistance. If this approach works to significantly delay the emergence of therapy resistance in our experimental models, it will lay the foundation for the design of more sophisticated and novel multi-drug regimes based on the inhibition of tumour evolution.

Publications and Presentations

Preliminary findings of this research will be presented at the Neurosurgical Society of Australasia Annual Scientific Meeting in September 2011.

Vascular biomarkers in malignant glioma

Other Investigator: Andrew Morokoff

Research Institution: University of Melbourne

Scholarship: NSA Research Scholarship

Significant Changes

It has become apparent that despite identical histopathological appearances, tumours falling under the diagnostic banner of glioblastoma multiforme (GBM) may have greatly varied clinical courses and responses to treatment. One recent example comes from the 5-year analysis of the EORTC-NCIC trial which identified a subgroup of patients with significantly improved long-term survival receiving adjuvant and concomitant chemo-radiotherapy. A surrogate marker, MGMT methylation status, was found to be the strongest predictor of benefit from temozolomide chemotherapy.

Research Update

Surrogate markers of tumour vascularity are also likely to be useful in GBM. Not only is glioblastoma amongst the most vascular tumours known, but the progression and growth of these aggressive solid tumours is reliant on their ability to obtain and maintain an adequate blood supply. In fact, this is the rationale behind the introduction of some newer chemotherapeutic agents used in the treatment of GBM, which target tumour angiogenesis. Vascular biomarkers are also expected to be of significant clinical value rather than solely a research tool. Circulating cells involved in neovascularisation such as vascular endothelial cells and endothelial progenitor cells can be procured from peripheral blood samples. Advanced MRI techniques have been developed which have been shown to correlate with tumour microvessel density, and can be added to routine glioma protocols without significant addition to time and resources. As such, vascular biomarkers can be made easily available to treating clinicians, at multiple time-points and in a relatively non-invasive fashion.

To determine the usefulness of these surrogate markers in GBM, the Vascular Biomarkers in Malignant Glioma project was initiated at the University of Melbourne via its Department of Surgery, and in close collaboration with the Neurosurgical Units of the Royal Melbourne Hospital and the Melbourne Private Hospital. Nineteen patients were enrolled into a pilot study assessing the pre- and post-operative levels of circulating endothelial cells in GBM patients. This study demonstrated that these cells could be reliably identified from peripheral blood samples, and that levels in GBM patients were elevated compared to normal controls. A consistent post-operative decrease in circulating cell numbers was also uncovered.

Using research-specific imaging software, volumetric analysis of pre- and post-operative MRIs have been obtained from the pilot-study data and included in a submission for publication. In-house software has been developed for the quantitative analysis of perfusion-weighted MRI scans, and analysis of the available data in pilot-study patients is being prepared for publication. These techniques will be applied to a retrospective quantitative analysis of a 2-year database of glioblastoma patients imaged with perfusion MRI at the Royal Melbourne Hospital. Ethics approval has also been obtained for the further

enrollment of GBM patients into the Vascular Biomarkers in Malignant Glioma project. As with the initial pilot study, their pre- and post-operative peripheral blood and MRI data are being recorded. This entire cohort of patients will be closely followed, and follow-up peripheral blood and MRI data obtained to identify any significant correlates with ongoing treatment and disease progression. This 2-year project is expected to reach conclusion in early 2013.

Publications and Posters

An abstract was submitted for the Royal Melbourne Hospital Research Week and accepted for posted presentation in May 2011.

Predictors of functional outcome of patients with traumatic spine fractures

Other Investigators: Professor Jeffrey Rosenfeld, Professor Russell Gruen, Mr Patrick Chan

Research Institution: The Alfred Hospital

Scholarship: NSA Research Scholarship

Research Update

The research study is being undertaken to identify the predictive factors in determining functional outcome in patients with spinal column trauma. The research aims to identify patient variables presenting to all three spine trauma centres in Victoria with spinal column trauma, in terms of demographics, presentation, comorbidities, injury patterns, treatment and outcomes; to assess, using multivariate analysis, predictors of outcome; and to develop and pilot a prototype spinal column injuries registry and novel treatment-guiding scoring system.

The successful set-up of the Victorian Spine Trauma Registry is the crucial step to the successful completion of my research initiative. I have been able to complete the pilot study assessing the feasibility of such a registry. I have been able to test the logistics and identify dataset issues prior to its launch. The results of this pilot will be presented at scientific meetings and submitted for publication.

In summary, the pilot showed that data collection using our minimum dataset and with the aid of the dataset dictionary is quick yet uncompromising on data quality (five minutes). It also proved the robustness of the collected data by the successful utilisation of the collected data in performing a Compliance Study on the SLIC and TLICS spine trauma algorithms.

I conclude from the pilot that a Victorian Spine Trauma Registry is feasible and a necessary process to guide the development and implementation of best clinical practice guidelines.

I am also currently performing a systematic review on all spine trauma registries to investigate the authenticity of their classification and assess their relevance by means of performance indicators. This will be crucial in assisting the extrapolation of research, logistics and financial estimates for the registry.

The successful pilot ushers along the inception of the Victorian Spine Trauma Registry. In order to achieve the project aims, collection of twelve months of clinical data and at least six months of outcome data will be collected.

Investigating the role of Aquaporin-4 in the development of post-traumatic syringomyelia

Other Investigator: Professor Marcus Stoodley

Research Institution: Australian School of Advanced Medicine

Scholarship: NSA Scholarship and RACS Scholarship

Research Update

Syringomyelia is a serious neurological condition where high-pressure fluid-filled cysts (syrinxes) form within the spinal cord. As these cysts enlarge, they damage the spinal cord tissue and result in neurological symptoms and deficits, such as pain, paralysis and loss of sensation. Syringomyelia occurs in association with many conditions, the most common being: Chiari I malformation (51%), spinal cord injury (11%), tumours (10%) and scarring around the spinal cord after haemorrhage or infection (6%). In spinal cord injury patients, additional losses of neurological function can transform patients from disabled but independent people into patients requiring assistance with basic daily tasks. Up to 28% of spinal injury patients develop syringomyelia months to years after the original injury.

Syrinx formation and enlargement have been assumed to be related to abnormalities of cerebrospinal fluid (CSF) hydrodynamics. Early theories proposed that a communication exists between a syrinx and the fourth ventricle, and that CSF enters the cord via this connection. It is now recognised that most syrinxes have no communication with the central canal or the fourth ventricle. Regardless of the pathological type, there is flow of fluid into and out of a syrinx. In an enlarging syrinx, inflow must exceed outflow.

Aquaporins are a family of water channel proteins involved in water regulation. To date, over 13 types of aquaporins have been identified in mammals. Aquaporins are located in various organs in the body, such as the collecting ducts in the kidneys, salivary glands and even skin, implicating their roles in water transport and secretion. Within the central nervous system, Aquaporin-1 has been localised in the choroid plexus and may play a role in CSF production, while Aquaporin-9 has been weakly detected in some astrocytes and ependymal cells in the spinal cord. However, Aquaporin-4 has the most prominent expression and is considered to be the primary water channel found in the central nervous system. In addition, Aquaporin-4 is the only water channel protein that is present in the human central nervous system.

The overall aim of the project is to determine the effects of Aquaporin-4 inhibition on the development of syringomyelia. It is hypothesised that the inhibition of Aquaporin-4 expression at the initial syrinx induction will decrease the subsequent development of syringomyelia and that inhibition of Aquaporin-4 expression in pre-existing syrinxes will reduce the size of the syrinxes.

Understanding syringomyelia pathophysiology

Other Investigators: Professor Lynne Bilston, Ms Sarah Hemley, Dr Johnny Wong, Dr Shaokoon Cheng, Professor Nigel Jones, Professor Anne Cunningham

Research Institution: Macquarie University, NeuRA, University of Adelaide, Sydney Children's Hospital

Overview of Research

Syringomyelia is a serious neurological condition where high-pressure fluid-filled cysts (syrinxes) form within the spinal cord. As these cysts enlarge, they damage the spinal cord tissue and result in neurological symptoms and deficits. Treatment of syringomyelia is generally unsatisfactory, especially once neurological deficits have developed: most series report long-term failure rates of approximately 50%. Apart from simple shunt diversion of syrinx fluid, the mechanism by which surgical treatments effect even a temporary reduction in syrinx size is unknown. Post-traumatic syringomyelia has proven particularly difficult to treat, because the injured spinal cord is at substantial risk of further mechanical or vascular damage with any surgery and there are high rates of shunt malfunction and recurrent arachnoiditis. A major impediment to the development of improved treatment for syringomyelia is that the mechanism of syrinx formation remains unknown.

At the most basic level, the volume of fluid and pressure in a syrinx depends on the flow of fluid into and out of it, and the tissue properties surrounding the syrinx. An enlarging syrinx must have inflow exceed outflow and enlargement may occur due to either increased inflow or decreased outflow. Recent work has shed some light on these factors, including our work on syringomyelia pathogenesis and mechanical properties of spinal cord, CSF and dura. We were the first to demonstrate that there is a normal flow of CSF from the subarachnoid space, through perivascular spaces and interstitial spaces into the central canal. We then demonstrated perivascular flow of CSF from the subarachnoid space into enlarging syrinxes. We also showed that there is an increase in perivascular CSF flow in the presence of focal arachnoiditis, and used computational modelling to show that focal arachnoiditis causes an increase in pulse pressure in the subarachnoid space that might contribute to perivascular fluid inflow. In a computational modelling study, we recently demonstrated that Chiari malformation might result in a delay of pulse transmission from the head into the spinal subarachnoid space resulting in a decoupling of pulsations in the cord blood vessels and the subarachnoid space.

CSF from the subarachnoid space might not be the only source of fluid in syringomyelia, especially in the extracanalicular type. We found that blood-spinal cord barrier is disrupted around post-traumatic syrinxes and that fluid flows across the disrupted barrier into the interstitial space around these syrinxes.

Despite its likely importance in controlling syrinx size, to date there are no published studies of fluid outflow pathways in syringomyelia. In a current project, we are studying the outflow pathways in a sheep syrinx model by using CSF tracers injected under ultrasound guidance. Information gained from the animal studies will be used in poroelastic computational models of fluid outflow.

Current Aims

We aim to gain new information regarding the three basic factors related to syringomyelia pathogenesis: fluid inflow, fluid outflow, and surrounding tissue changes. We are studying these factors using an integrated set of experimental studies and computational models, with the following aims:

Fluid inflow

1. Determine the effects of pulsation decoupling on perivascular flow;
2. Measure pulse pressures and wave transmission in an animal model of arachnoiditis and syringomyelia;
3. Determine whether attenuating blood-spinal cord barrier breakdown reduces syrinx formation.

Fluid outflow

1. Determine the outflow pathway in extracanalicular syringomyelia;
2. Determine factors that can alter resistance to outflow through the cord parenchyma.

Tissue properties

1. Determine the effects of glial scar formation on tissue properties around syrinxes;
2. Determine the effects of tethering (arachnoiditis) on tissue mechanical behaviour around syrinxes.

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Column of Hope

Quality services and improvement initiative in spinal surgery

Other Investigators: Dr David Walker, Ms Beth Morrison RN

Research Institution: NEWRO Foundation, Brizbrain and Spine

Overview of Research

Recording objective and comparable outcomes from spinal surgery is problematic. The Surgical Outcome Survey (SOS) is a web-based program developed by our group designed to assess short and long term outcomes of lumbar and cervical spinal conditions from the patient and surgeon perspectives. It creates a standardised, paperless and automated system for assessing results; it can be modified for research purposes and it could create a national database to improve standards and outcomes.

Developing new treatments for brain AVMs

Other Investigators: Dr Andrew Davidson, Professor Michael Morgan, Dr Robert Smee, Associate Professor Mark Molloy, Dr Andrew Katsifis, Dr Hong Duong

Research Institution: Macquarie University

Overview of Research

Arteriovenous malformations (AVMs) of the brain are devastating congenital lesions that are the most common cause of haemorrhagic stroke in children and young adults. Although most small AVMs are curable, over 90% of large lesions are untreatable with current techniques. Our overall goal is to develop a new AVM treatment that is safe and rapid and can be applied to large lesions. We have established an animal model and have data to support the hypothesis that modification of the endothelial cells lining AVM vessels will enable a targeted treatment to induce selective thrombosis (“vascular targeting”). It is necessary to modify the AVM endothelium first, because the constitutive phenotype is not sufficiently different from normal endothelium to allow selective targeting. We have shown that selective modification can be achieved with stereotactic radiosurgery. Radiation-induced changes in endothelial molecular expression have been identified based on our understanding of endothelial biology and we have demonstrated that these can be harnessed to induce thrombosis. We are currently also working on focused ultrasound as an alternative method of selective endothelial modification.

Prior to developing and trialing vascular targeting agents, it is important to undertake a comprehensive analysis of endothelial membrane changes after radiosurgery so that all potential targets can be identified and investigated. We are nearing completion of microarray and proteomic analyses and have identified candidate targets.

For each potential target identified, our aims are:

Aim 1. Determine the gross anatomical distribution of expression in the animal model using *in vivo* molecular imaging.

Aim 2. Determine the cellular location of expression in the animal model using confocal microscopy.

Aim 3. Quantify the temporal changes in expression in the animal model using *in vivo* molecular imaging, real-time PCR, and Western blotting.

Aim 4. Validate the changes in the animal model using primary human AVM endothelial cultures.

At the completion of this project we will have identified and characterised radiation-induced AVM endothelial molecular changes. Appropriate targets will be selected for future vascular targeting trials. Vascular targeting agents will be developed by conjugating antibodies directed at the selected target molecules with pro-thrombotic molecules such as tissue factor. The efficacy of vascular targeting will be assessed in the animal model using angiography, Doppler ultrasound, and histological analysis.

Significance

The significance of this work is the potential for rapid translation into therapies for currently untreatable brain AVMs. In addition, successful development of a radiosurgery-vascular targeting technique has potential for wider application, such as for brain tumours.

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Pilot study of the use of Celecoxib in the treatment of low grade gliomas

Research Institution: NEWRO Foundation, Brizbrain and Spine

Overview of Research

Low grade astrocytomas and oligodendrogliomas are slow growing tumours which almost inevitably progress to malignant cancers. There is no known treatment that prevents or even significantly reduces progression. Because COX-2 inhibitors have been shown to reduce the incidence of polyps in patients with familial colon cancer, and that they also been shown to inhibit astrocytoma growth in preclinical and early clinical studies, it is reasonable to hypothesize that administration of celecoxib to patients with low grade astrocytomas, may result in decreased progression to higher grades of tumour.

Low grade astrocytomas (LGAs) progress via stepwise genetic changes into higher grades of tumour. Patients who are diagnosed with a low grade astrocytoma have a median survival of around 5 years and around 75 % eventually die of their disease, most from tumour progression. For oligodendrogliomas, prognosis is linked strongly to the status of chromosomes 1p and 19q. Patients whose tumours do not have loss of these chromosomes have a survival similar to those with low grade astrocytomas, if not worse (1) (2). In a recent study, 43% of patients with low grade astrocytoma (LGA), oligoastrocytoma (LOA) or oligodendroglioma (LGO) recurred, and more than 2/3s had progressed to a more malignant grade (3), with a mean time to progression of around 5 years. Other studies have shown an even higher rate of progression, and shorter time to progression (4).

There is no convincing evidence that the treatment modalities currently available for these tumours (ie LGAs or oligodendroglial tumours without chromosome 1p/19q loss), ie surgery, radiotherapy and /or cytotoxic chemotherapy, influence the time to progression. Celecoxib has been shown to reduce the number of colorectal polyps in familial adenomatous polyposis. The use of celecoxib in patients with low grade astrocytoma and oligodendrogliomas may similarly reduce the rate of progression to malignant astrocytoma.

Approximately half of all newly diagnosed brain tumours are of glial cell origin, and low grade astrocytomas and oligodendrogliomas represent up to ¼ of these. This corresponds to about 1800 and 150 new cases per year in North America and Australia respectively. Most of these will progress to malignant gliomas and eventually result in the death of the patient. Unfortunately, no current treatment has been proven to influence this natural history.

There is good reason to believe that COX-2 inhibitors, such as celecoxib (Celebrex®) may provide an effective adjunct in the treatment of malignant glioma. Upregulation of the COX-2 enzyme in different cancers has prompted laboratory and clinical studies with COX-2 inhibitors in cancer (5) based on the premise that COX-2 upregulation promotes oncogenesis. Studies have led to the Food and Drug Administration (FDA) in the USA to approve the use of celecoxib for patients with familial adenomatous polyposis (FAP) (6).

COX-2 overexpression has been demonstrated in human gliomas (7) and a correlation between COX-2 expression and shorter survival has been shown (8, 9). In preclinical studies, COX-2 inhibition has reduced cell proliferation and tumour growth in vitro (13). In a rat model of malignant glioma, Nam et al (10) showed that oral celecoxib induced decreased tumour cell proliferation and increased apoptosis.

A recent phase II study in patients with recurrent malignant glioma has suggested a positive effect when celecoxib (400mg bd continuously for up to 48 weeks) is combined with a second line chemotherapy agent, irinotecan (11). This combination was also safe. Another group combined continuous low-dose temozolomide and another COX-2 inhibitor, rofecoxib, in patients with incompletely resected glioblastoma and observed this regime to be safe and maintained a good of life (12).

Others have presented results showing that celecoxib enhances brain tumour cell radiosensitivity, leading to increased cell death (13). This group has also shown that, in a rat model, celecoxib and temozolomide combined provides more effective control of glioma growth than either treatment alone (14).

Not only is there evidence that COX-2 inhibition acts on glioma cells independently, and together with chemotherapy or radiotherapy, as discussed above, there is evidence that COX-2 inhibition may enhance the effectiveness of immunotherapy, such as dendritic cell vaccination (15, 16).

Safety Issues Regarding Trial Design

COX-2 inhibitors have come under scrutiny because of reports of increased risk of serious cardiovascular events after long-term use (17-19), secondary to selective inhibition of prostacyclin thereby creating a prothrombotic state (18). However, several other trials have not demonstrated an increased cardiovascular risk with administration of COX-2 inhibitors (20), including in patients with brain tumours (12). Given this uncertainty, in this trial, the following safeguards have been created (21):

1. Patients in the trial will be those considered to have a low cardiovascular risk : age <50y, no prior history of cardiovascular events, normal baseline ECG and serum lipids, non-smokers.
2. Patients randomised to the celecoxib group will also take low dose aspirin (100mg daily).
3. Once daily dosage of celecoxib, rather than twice daily, since it is considered that it is the sustained inhibition of prostacyclin that occurs with twice daily administration that contributes to cardiovascular risk.

Trial Purpose, Hypotheses and Objectives

Purpose

The purpose of this study is to test the safety and efficacy of celecoxib in patients with diffuse low grade gliomas.

Hypotheses

We hypothesize that the administration of celecoxib to these patients is safe and results in a improved progression free survival.

Objectives

1. The primary objective is to assess the safety of celecoxib when given to patients with diffuse low grade gliomas.
2. The secondary objective is to assess the efficacy of celecoxib to patients with diffuse low grade gliomas in improving the progression free survival of these patients.

TRIAL DESIGN

Endpoints

Primary endpoint

- The primary endpoint outcome will be safety, as assessed by the incidence of adverse events that are considered associated by the investigational drug.

Secondary endpoint

- The secondary endpoint will assess efficacy by improving progression free survival of drug therapy by neurological exam, Karnofsky Performance Score, quality of life assessment and 3 monthly MRI's.

Design

This is a pilot study that will enrol 10 patients diagnosed with diffuse low grade gliomas. Patients that meet the study entry criteria will be required to take celecoxib daily for a period of 1 year.

Making sense of brain tumour

Other Investigator: Dr Tamara Ownsworth (principal investigator)

Research Institution: Department of Neuropsychology, Griffith University

Overview of Research

Brief Overview of Program

The Making Sense of Brain Tumour (MSoBT) Program was developed after pilot research in 2007-2008 highlighted that the psychological support needs of individuals with brain tumour and their caregivers are often overlooked. The need for specialised counselling and rehabilitation was a specific recommendation out of the findings. The MSoBT program provides 10 sessions of home-based counselling and rehabilitation and aims to improve psychological well-being and everyday functioning of individuals with primary brain tumour and their family members. We are evaluating this program using a waitlist control design (RCT).

Participants

To date, 30 individuals with brain tumour and four relatives of individuals with brain tumour have enquired about participating in the project. Of these 34 enquiries, 9 did not participate in the project for a combination of reasons (e.g., the individual with brain tumour declined a need for psychological support /rehabilitation, the individual became too unwell and was hospitalised).

As a result 25 individuals have consented to take part in the program. Of these participants:

- 8 participants have completed the program AND 6-month follow-up assessments
- 7 participants have completed the program and post-treatment assessment
- 6 participants have received initial assessments and are currently undertaking the treatment program.
- 4 participants will commence their programs in July 2011.

Details of Participants:

- 59% male, 41% female; Mean age 43.9 years (SD = 9.37)
- Tumour type: 52% Benign tumours; 48% Malignant tumours
 - Pituitary x 4
 - Oligodendroglioma x 4 (3 x Grade 2, 1 x Grade 3)
 - Colloid cyst of 3rd ventricle x 2
 - Mesial Temporal Tumour x1, Ganglioglioma x 1
 - Glioblastoma Multiform x 8
 - Oligoastrocytoma
 - Glioma x 3 (3 x Grade 2)

The Focus of the Counselling and Rehabilitation Program

The most common treatment goals identified by participants include:

- Manage effects of brain tumour, for example, cognitive difficulties (e.g., difficulties with memory, concentration, organisation), loss of motivation and fatigue
- Reduce social avoidance and isolation
- Improve stress coping skills
- Address fears /concerns of participant's children
- Enhance preparedness for unexpected outcomes and end of life issues (e.g., family care plan, advanced health directive, guardianship)
- Enhance couple relationship functioning
- Assist with return-to-work
- Manage low mood, anxiety regarding uncertainty of future and prospect of death (grief and loss)
- Communication difficulties and augmentative language strategies for aphasia
- Intimacy and sex-related difficulties
- Manage anger and impulsivity issues

Preliminary Outcomes and Clinical Learning

In supporting people to work on the above treatment goals we are developing a series of specialised therapy resources for people with brain tumour and their family members. Due to the complex cognitive and behavioural effects, it is important to modify various conventional approaches to psychoeducation and counselling. For example, the use of written session summaries to emphasise key points, explaining concepts through visual representation, use of personal biographies (e.g., the story of my brain tumour), teaching people to develop memory prompt to practice techniques (e.g., mindfulness). The materials used in therapy are being collated into modules and we will have developed a very comprehensive set of clinical resources by the end of the program.

One of the biggest challenges experienced so far, is how to meaningfully involve caregivers in the program. Some caregivers wish to be actively involved from the outset, attending each session, whilst others are reluctant initially but then spontaneously decide to be part of a later session. A further challenge is that individuals with brain tumour are often unwell and need to cancel sessions (sometimes due to being hospitalised).

The clinicians are learning to be flexible with the therapy agenda, so that initial goals may be reformulated and the program adjusted to incorporate different support needs.

Some examples of qualitative feedback from participants:

Very constructive, very therapeutic. Dual reward for both me and my wife. We are very happy with the support we have received from the program.

Very successful, it helped me achieve goals I had, and taught me strategies to address the others in the long term (e.g. future direction goals).

I thought it was very rewarding, made me understand some things - recognise some of my reactions to things, like anxiety and avoidance

Very positive feedback was also provided at the Brain Tumour Forum (May 12th), where seven people and their partners approached Tamara Ownsworth after her talk to discuss how the program has impacted their lives and their ability to manage the effects of the tumour.

Some consistent feedback is that people often wish that the program could be longer – or that ongoing support be available ‘in case I need it’ rather than finishing after 10 sessions. We ensure that people have a range of support options provided at the end of the program. We are developing a better understanding of people’s more long-term support needs as the program continues.

Based on the first 14 individuals who completed the program, there is evidence of statistically significant benefits to mental health (reduction in symptoms of depression and anxiety), functional well-being (increased activity participation) and quality of life (existential well-being, social and psychological well-being). There are insufficient follow-up data as yet to determine whether such benefits are maintained 6 months after the program finishes.

Presentations

Early results have been presented at international meetings.

Acknowledgements

Ms Vivien Biggs, Neuro-Oncology Nurse Practitioner, Brizbrain and Spine

Immunological Profiling of HCMV-specific T cell responses in patients with Glioblastoma Multiforme: Implications for adoptive immunotherapy

Other Investigator: Associate Professor Rajiv Khurana, Dr Tania Crough

Research Institution: NEWRO Foundation, Brizbrain and Spine, Queensland Institute of Medical Research

Overview of Research

A number of studies have proposed a potential role for viral infections in the etiology of GBM; however, the evidence to support this hypothesis remained inconclusive. Of particular interest is the association between HCMV infection and GBMs. In spite of previously conflicting reports, two recent studies have provided very convincing evidence to link HCMV infection with GBMs. Using highly sensitive immunohistochemical and in situ hybridization technologies, HCMV antigen and DNA was detected in 21/21 (100%) cases of glioblastoma, 9/12 (75%) cases of anaplastic gliomas and 14/17 (82%) cases of low-grade gliomas (see Fig. 1). Expression of immediate-early-1 (IE-1) protein was detected in the nucleus of anaplastic and low-grade gliomas, while GBMs showed nuclear and cytoplasmic staining which is commonly observed in latently infected cells. These observations have also been confirmed by another group who showed that HCMV DNA and protein expression was detected in a high percentage (>90%) of GBM tumours. Furthermore, majority of newly diagnosed GBM (80%) showed circulating HCMV DNA in the peripheral blood, while healthy seropositive individuals and non GBM patients showed no detectable viral DNA, suggesting that either shedding of viral DNA from HCMV infected tumour cells or systemic reactivation of virus. Taken together, these observations strongly support the hypothesis that HCMV is associated with malignant GBM. Regardless of the potential role of HCMV in the pathogenesis of GBM, the expression of HCMV proteins in these malignant cells provides an opportunity to target these virally-encoded antigens as a target for T cell-based immunotherapy. Indeed a pilot study based on autologous dendritic-cell vaccination as adjunctive therapy in GBM has been initiated by a group at the University of California and preliminary results based on a single patient from this ongoing study has shown that after vaccination, a significant increase in the pp65-specific T cell response was observed. The vaccination was not associated with any systemic symptoms. Local lymphadenopathy was observed at the time of the expansion of the human CMV-specific T cells. More importantly, this study highlighted the relative ease of eliciting an immune response against viral antigens which is in contrast to the difficulty of immunization against "self" tumor antigens.

Our group has been working on the developing novel immunotherapeutic strategies for the treatment of HCMV diseases. One such strategy is based on the patented polyepitope technology developed by our group. We have designed a HCMV polyepitope construct which is designed to encode multiple epitopes restricted through a range of HLA class I and class II alleles. This polyepitope construct has been cloned in a novel replication-deficient chimeric adenoviral vector (Ad5f35) and allows rapid expansion of HCMV-specific T cells (see our publication in *Eur. J. Immunol.* 2005. 35: 996–1007). It is important to emphasize that the use of this polyepitope construct has already been approved by the Australian Gene

and related Therapies Research Advisory Panel (GTRAP). Furthermore, we have successfully used this adenoviral vector in another clinical trial which is aimed to expand EBV-specific T cells from nasopharyngeal carcinoma patients.

In this proposed clinical trial we intend to enrol 10 patients with recurrent GBM with an aim to expand autologous HCMV-specific T cells and then adoptively transfer these effector cells in these patients.

We have conducted extensive *ex vivo* profiling of human cytomegalovirus (HCMV)-specific CD8⁺ T-cell responses in a cohort of patients with glioblastoma multiforme (GBM). Of the patients analysed, approximately half exhibited serological evidence of past infection with HCMV. However, immunohistochemical staining for HCMV-encoded proteins showed presence of HCMV in the tumour biopsy sections in the majority of patients, including those who were serologically negative. Although no HCMV-specific CD8⁺ T cell responses could be detected in the serologically negative patients, virus-specific CD8⁺ T cell responses were detected in all seropositive GBM patients. Using MHC-peptide multimers, the frequency of HCMV-specific T cells in the patients detected ranged from 0.1 to 21.6% of CD8⁺ T cells. Assessment of these cells for the expression of CD27 and CD57 molecules showed a high proportion of CD57⁺ cells, consistent with a more terminally differentiated T cell phenotype. The polyfunctional analysis of the HCMV-specific T cells from GBM patients revealed that the majority of these cells produced multiple cytokines (MIP-1 β , TNF- α and IFN- γ) and displayed strong cytolytic function (CD107a mobilization) following stimulation with HCMV peptide epitopes. *In vitro* culture of HCMV-specific CD8⁺ T cells from GBM patients using synthetic HCMV peptide epitopes dramatically increased the number of cytokine producing cells. These expanded cells are being administered for adoptive therapy to GBM patients in a phase I clinical trial currently being conducted. Four patients have received treatment so far and the results are very promising.

Presentations

Early results have been presented at national and international meetings.

Acknowledgements

Ms Vivien Biggs, Neuro-Oncology Nurse Practitioner, Brizbrain and Spine

Phase I trial of dendritic cell vaccination and celecoxib with postoperative radiotherapy and temozolomide for treatment of malignant glioma

Other Investigator: Dr Chris Schmidt, Dr Alejandro Lopez

Research Institution: NEWRO Foundation, Brizbrain and Spine, Qld Institute of Medical Research

Overview of Research

We have been planning this project since 2008, when we completed our initial Dendritic Cell Vaccination study which showed an interesting response to chemotherapy after vaccination. A variety of logistical and technical issues have delayed the start date until late 2011.

Protocol Synopsis

Project Phase : Phase I

Primary Objective :

To study the safety, and effect of vaccination on a variety of in vitro and in vivo immunological parameters, and to correlate these with clinical effects, when vaccination and celecoxib are given concurrently with radiotherapy and chemotherapy.

Study Design : Open

Planned Total sample size : Sufficient patients will be enrolled so that 10 patients complete the priming vaccination phase (first 6 injections).

Patient entry criteria :Malignant glioma (WHO classification grade III or IV); age 18-75, performance status ECOG 0, 1 or 2; absence of concurrent infectious diseases; accessibility of tumour for resection; macroscopic resection of tumour (i.e. at operation, it was felt by the surgeon that all visible tumour was removed); normal haematological parameters; absence of autoimmune disease or other significant neoplasms; absence of pregnancy

Formulation :Autologous dendritic cells derived by culture of blood precursors in Granulocyte Macrophage-Colony Stimulating factor (GM-CSF) and Interleukin 4 (IL-4) are pulsed with antigens derived from lethally irradiated, fresh, autologous glioma cells, and matured by further culture in macrophage conditioned medium (MoCM). Cells are cryopreserved in a "thaw-and-inject" formulation.

Vaccine Dosages and Timing :Vaccination will commence toward the end of combined radiotherapy and chemotherapy. Vaccines will be delivered at 2 weekly intervals for the first 6 injections, then 4-6 weekly thereafter. 106 DC will be injected intradermally in each vaccination.

Route of Administration :

Intradermally into the abdomen

Parameters of :

Vaccine Tolerability

Patient tolerance will be determined by the incidence and grade of reported adverse events, and the intensity of local and systemic immune reactions

Clinical Effects

1. Changes in residual tumour will be quantitated or assessed by clinical and radiological examination where possible, ie MRI scans of the head.
2. Histological assessment of biopsies or resected tumours where appropriate.

Immunology (optional)

1. Immediate and delayed-type hypersensitivity reactions to vaccine, documented by measurement (area of erythema and area of induration) and photography, at peak response following vaccination.
2. Analysis of peripheral blood cells (before V1 and after V6) for :
 - a. Overall immune function, using the proliferative and cytokine response to mitogen and standard recall antigens.
 - b. Autologous glioma-specific cytotoxic T lymphocyte (CTL). When available, tumour infiltrating lymphocytes (TILs) from initial and possible subsequent biopsies will be examined for their cytotoxic potential.
 - c. Cytokine production from peripheral blood lymphocytes in response to stimulation with autologous glioma cells or with purified myelin basic protein (MBP).

Study Procedure

1. Prior to operation : Extent of disease in eligible patients will be clinically assessed by patient history and examination, and MRI scan of the head. ECOG status will be determined. Consent will be obtained to process and retain tumour tissue.
2. At the time of tumour resection: Tumour tissue will be taken and sent to the Department of Anatomical Pathology to confirm histology as a first step. Excess tumour tissue will be taken for processing. Tumour tissue will be processed by sieving to obtain single cells as a source of antigen, for culture. Where possible, a sample will also be retained for histology and immunohistochemistry and tumour-infiltrating leucocyte (TIL) analysis.
3. A postoperative MRI scan will be performed within the first 72 hours.
4. After confirmation of the histology (usually 2-4 days postoperatively), appropriate patients will be approached and the protocol explained. Consent will be obtained to participate in the trial.
5. Peripheral blood will be taken for DC culture by apheresis.
6. Postoperative radiotherapy with concurrent oral chemotherapy (temozolomide) will be given as per standard treatment. Temozolomide will be continued post-radiotherapy as per standard therapy.
7. Celecoxib (COX-2 inhibitor) will be administered orally during the period of radiotherapy and low-dose temozolomide therapy at a dosage of 400mg twice daily and continue for a total of 8 weeks.
8. Vaccination will begin between approximately 6 weeks postoperatively, ie in the 2nd last week of postoperative radiotherapy with concomitant temozolomide. Vaccinations will be given every 2 weeks for 6 vaccinations, then every 4-6 weeks thereafter, until :
 - a. There is evidence of progressive disease or recurrent disease clinically or on MRI which precludes further active treatment; or
 - b. If recurrence or progression does not occur, vaccinations will continue for up to 12 vaccinations; or
 - c. The patient wishes to discontinue vaccination and involvement in the trial

Molecular Imaging of Vascular Targets in an Animal Model of Brain AVM Primed with Focused Ultrasound

Other Investigators: Professor Marcus Stoodley, Professor Michael Morgan

Research Institution: Australian School of Advanced Medicine, Macquarie University

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Hypothesis

Earlier in vitro and in vivo studies investigating the gene and surface protein expression in endothelial cells following focused ultrasound hyperthermia have identified several molecules that warrant further investigation as part of a ligand-directed vascular targeting strategy in the treatment of brain AVMs. However, prior to the institution of a systemic targeted therapy, in vivo localization studies are necessary to determine the specificity of these molecular changes.

We hypothesize that AVM endothelial cells 'primed' by the application of focused ultrasound hyperthermia express surface molecular changes that allow discrimination from normal endothelial cells, thereby providing unique molecular targets that can be used for a systemic vascular targeting therapy.

Significance

Arteriovenous malformations (AVMs) of the brain are complex lesions that involve high-flow arteriovenous shunting through single or multiple direct arteriovenous connections in the absence of a capillary bed. Brain AVMs may present with haemorrhage, seizure, headache, or as an incidental finding. Haemorrhage from brain AVMs accounts for only 2% of all strokes, but is over-represented in young patients, being responsible for 38% of intracerebral haemorrhages in patients aged 15-45 years.

The surgical management of small AVMs is highly effective, and can be achieved with low morbidity and mortality. However, the management of large AVMs is plagued with difficulty. Surgery is associated with high complication rates, and the effectiveness of radiosurgery declines with increasing AVM size. As a result, a significant number of young patients harbour AVMs that remain untreatable using current techniques.

Recent work has demonstrated that tumour blood vessels are different to normal blood vessels. Researchers are taking advantage of these differences to develop vascular targeting agents to disrupt tumour blood supply and lead to tumour cell death. Some preliminary work has been performed on cerebral vascular malformations in an attempt to define the molecular changes that exist in cerebral cavernous malformations and arteriovenous malformations. However, at this time, no marker is specific enough for vascular targeting therapies to be directed towards AVMs.

Morphological changes in AVM blood vessels have been demonstrated following focused irradiation, and more recent work is attempting to address the molecular changes that occur following ionising radiation. However, a major limitation of radiotherapy in AVM management has been the marginal dose required to treat large lesions. One solution may be to use radiosensitizers in an attempt to reduce the marginal dose required for therapeutic effect.

Our research is based on an alternative source of 'sensitization' -the use of sub-lethal heat energy. By avoiding ionising radiation, it may be possible to avoid the toxic effects of focused irradiation in the treatment of large AVMs. Although little work has been done on the effects of heat on AVMs, it is possible that some of the techniques used in the field of hyperthermic oncology may be able to be utilised to induce selective changes in the endothelial cells of AVMs, rendering them susceptible to the effects of specific vascular targeting agents.

Some of our earlier work has been directed at understanding the molecular changes that occur in the endothelial cells of AVMs. Both the intrinsic changes found in AVMs, and the additional changes induced in AVM endothelial cells by the application of various 'priming' agents (such as focused irradiation, or heat energy), have been studied. It may be possible to 'switch on' a molecular change in the endothelial cell of an AVM that permits the use of vascular targeting agents in the treatment of AVMs.

Ultimately, it may be possible to 'prime' the endothelial cells of human AVMs by the non-invasive application of heat energy, through the use of high-intensity focused ultrasound. This could then be followed by the administration of a vascular targeting agent directed specifically at these molecular changes, which could result in the selective thrombosis of AVM vessels. If successful, then the results of this study could revolutionise the treatment of large, difficult to treat AVMs.

Eventually, the entire treatment procedure could potentially be performed non-invasively, and with a lower risk profile and greater efficacy than current invasive treatments.