Vilhelm Magnus Laboratory for Neurosurgical Research

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Research area and aims
Vilhelm Magnus Laboratory for Neurosurgical Research is a research group of the Oslo University Hospital and encompasses the Neurosurgical Departments at Ullevål University Hospital (UUS), Rikshospitalet-Radiumhospitalet Medical Center (RR), and the University of Oslo. The goal of the laboratory is to build a bridge between the basic biological sciences and clinical neurosurgery, to explore the biology underlying neurosurgical conditions, and to facilitate translation of new knowledge from the basic research disciplines into the clinic. Research efforts therefore encompass both normal brain cell development and disease states such as tumours. Investigations aim to understand these processes and develop methods to treat disease as well as promote cell replacement to heal damaged brain tissue.

Stem cells from the adult human brain
A central dogma in neuroscience has been that the mature brain is unable to produce new neurons. Towards the end of the 20th century, studies in birds and rodents came to question this doctrine as new markers for labeling neurons combined with techniques for identifying cells that had been born in adult life, suggested that new neurons sometimes may develop later in life in some species.

At the turn of the century, these findings were to some degree extended to the human brain, as a few research groups had been able to culture immature cells from the human ventricular wall and hippocampus. It was still not known, however, whether it would be possible to differentiate these cells into functional neurons, i.e. cells with typical neuronal action potentials with the ability to communicate via synapses.

The putative existence of an adult human brain stem cell type with the ability to proliferate and differentiate into mature neurons created huge interest as one could now envisage treatment of neurological diseases with either transplantation of stem cells that have been expanded in vitro or by mobilization of endogenous progenitor cells. The work on developing functional neurons from cells from the human ventricular wall was started in Professor Langmoen’s laboratory at the Karolinska Institute in Stockholm by Morten Moe and Mercy Varghese. Human tissue was harve-
sted from the wall of the lateral ventricle in temporal lobe specimens resected due to epilepsy. In keeping with earlier results from other groups they were able to expand stem cells from the ventricular wall as cell clusters (neurospheres) in vitro. Following dissociation and exposure to differentiation cues (mainly withdrawal of growth factors and addition of serum) these cells went through characteristic steps of morphological and electrophysiological development and developed into the three principal building blocks of the brain:

1. Astrocytes
2. Oligodendrocytes
3. Neurons

Our group was first to demonstrate that it is possible to transform immature progenitor cells from the adult human ventricular zone into functional neurons, i.e. cells with typical neuronal action potentials and the ability to communicate through synapses (Fig. 1). Essentially, our group was able to develop a small nervous system from a single human stem cell in vitro. This ‘nervous system’, consisting of glial cells as well as a large number of neurons, communicated via synapses. These results were selected from more than 15000 abstracts for presentation at the press conference of the Society for Neuroscience seven years ago. See Westerlund U et al Exp Cell Res 2003, Moe MC et al Brain, 2005 and Neurosurgery 2005.

For stem cells to be useful in the clinical situation, it must be demonstrable that after transplanting them to another adult brain they can survive and integrate into the recipient neuronal circuitry. Using rats with a selective lesion of the hippocampal CA1-region (a small part of cerebral gray matter), Håvard Ølstørn and Morten Moe demonstrated that stem cells from the adult human brain are not only able to survive in the rat brain, but also selectively target and migrate to the area with the lesion (Ølstørn H et al Neurosurgery, 2007).

Use of specific antibodies against human nuclei (HuN) demonstrated survival of the transplanted cells and showed that the grafted cells frequently express the immature marker human nestin. Less frequently, the cells expressed the immature neuronal marker doublecortin and the glial marker GFAP. Ølstørn and Moe further showed that by using ‘predifferentiation’, i.e. “pushing” the cells in a neuronal direction prior to transplant, it was possible to significantly enhance the development of neurons following transplantation. This study for the first time showed that stem cells from the adult human brain are able to survive and differentiate in another adult brain. The first part of the study secured Ølstørn the first prize at the Scandinavian Neurosurgical Society’s Annual Congress in 2005. See Olstorn H et al Neurosurgery, 2011.

Innovative scientific techniques

The Vilhelm Magnus lab now has an extensive scientific and student staff dedicated to developing and utilising the best up-to-date molecular cellular biological techniques in fulfilment of its broad aims of understanding brain cell development and disease and thereupon working towards treatment innovations. PhD students get to explore the various aspects of particular techniques and subsequently apply them to experimental questions. Screening methods such as microarrays (Cecilie Sanberg) and proteomics (Linda Paulson) can provide data to be used to delineate differences between normal and cancerous tissue. Statistical and bioinformatic analysis then suggests candidate genes for follow up. These then need to be confirmed using molecular techniques such as quantitative PCR to confirm transcription of mRNA followed by Western blot (Zanina Grieg) to confirm protein involvement. FACs (facilitated cell sorting and cytometry, Einar Vik-Mo, Mrinal Joel) is a very versatile technology that can give specific measurements of cell sub-group behaviour within cell populations and allows for elaborate assessment of experimental parameters. The ability for distinction of many wavelengths of fluorophore as well as the development of sophisticated antibody-fluorophore conjugates means that data relating to cell cycle, division

Fig. 1
Dual patch-clamp recording from neighboring neuron-like cells, demonstrating synaptic communication between the cells
rate, phenotype and even transitory signalling pathway activation can be assessed within control and experimental cell populations. As well xenotransplantation of cell types to chick embryo (Mrinal Joel) or immune-compromised (SCID) mice (Awais Mughal, Artem Faizullin) provide powerful experimental tools that combine the prowess of surgeon and cell biologist alike.

The ability to knock out or down candidate gene expression is a powerful tool for discerning biological roles as well as involvement in disease pathology. The lab (under the expert guidance of Biljana Stangeland) has developed the expertise to employ lentiviral methodology whereby designer constructs can produce targeted short interfering (Si) and short hairpin (Sh) RNA for transcriptional block as well as express label such as GFP in tandem allowing identification and tracking of transduced cells. Lentiviral constructs integrate permanently into the genome of experimental cell lines giving faithful tracking of cell progeny. As well this most sophisticated molecular cell technology provides scientific rigor and convincing inference of mechanism.

Stem cells and brain cancer

Brain cancers in principle always recur despite apparent complete removal under the operating microscopic and subsequent adjuvant therapy. This is particularly true for the most common intracranial tumour type, the glioblastoma (GBM), where 50 percent of treated patients die within one year from diagnosis. In parallel with results emerging from other research institutions, our group has shown that only a subpopulation of cells in brain cancers have the ability to proliferate and initiate new tumours following transplantation to immunodeficient mice. This cell population infiltrates surrounding brain tissue, appears resistant to both irradiation and chemotherapy, and is the likely explanation for recurrence.

In a leading study, Mercy Varghese and Morten Moe showed that these cells share a number of the properties of normal neural stem cells of the adult human brain. Håvard Ølstørn demonstrated that stem cells isolated from tumours could reproduce those tumours in immunodeficient mice, whereas stem cells from normal human brain did not result in tumours (Fig. 2) (Varghese M et al, Neurosurgery, 2008).

Both normal and tumour stem cells showed a high proliferation rate when cultured to do so. Interestingly, the proliferation rate fell dramatically also in tumour stem cells when they were induced to differentiate. Normal and tumour stem cells showed a similar pattern of differentiation, i.e. in neuronal and glial directions, although differentiated cells from the tumour were clearly abnormal morphologically and differentiation in itself progressed much faster. Einar Vik-Mo performed a number of experiments studying the effect that in vitro culture of tumour stem cells has on the cells’ ability to form tumours, to differentiate and to undergo genotypic and expression changes (Vik-Mo et al, Neurosurgery, 2008).
Oncol. 2010). He has also explored the cellular organization of neuro- and tumoursheres, looking at the cellular heterogeneity of such spheres (Vik-Mo et al, Exp Cell Res. 2011 ). By sorting tumour cells based on surface antigens, we hope to establish methods for better identification of the progenitor population.

We have also used this technology and experience to establish a clinical protocol (Einar Vik-Mo, Birthe Mikkelsen). This protocol is designed to harness the patients' own immunity. The inclusion of patients into the “Phase I/II trial of vaccine therapy with hTERT, survivin and tumour stem cell derived mRNA- transfected dendritic cells in patients receiving standard therapy for glioblastoma” started in February 2009. So far 22 patients have been recruited to the study. This clinical trial is backed up from the collaboration through the Cancer Stem Cell Innovation Center (SFI CAST) and is a collaboration with the Neurosurgical department, Avd. for klinisk krefftorskning, Avd. for cellterapi, and Avd. for immunologi, Institutt for krefftorskning, Radiumhospitalet and the Oncological department at Oslo University Hospital.

Microarray technology
Cecilie Sandberg has used microarray technology to compare the global gene expression in normal stem cells and tumour stem cells, in order to identify possible targets for treatment and to better understand the biology of the cell population that escapes current treatment and causes recurrences. The results of this comparison study show a significant upregulation in tumour stem cells of genes connected to regulation of focal adhesion, actin cytoskeleton, axon guidance as well as the Wnt signalling pathway. Putative target genes have been confirmed at the protein level using immunohistochemistry and Western blot. This work is currently submitted for publication. Currently, the genes’ roles in glioma are investigated using shRNA-knockdown based technology and its effect on proliferation, apoptosis and sphere-forming capacity. The roles of the possible targets in the Wnt pathway are investigated by Kirsten Strømme.

Biljana Stangeland, investigated a set of 20 genes that were up-regulated in GBM tumour cultures using C. Sandberg’s micro-array data. The study compared gene expression in nine primary GBM stem cell cultures to that in five primary neural stem cell cultures from the adult human brain. Rigerous bioinformatic filtering identified 20 genes whose RNA expression levels were very high in the primary Glioma stem cell cultures but were undetectable in normal adult brain stem cells. The identified genes are involved in cell-cycle/division, epigenetic regulation, signaling and down-regulation of tumour-suppressors. Several of the candidate genes are implicated in cancer (breast, ovary and colon), while others have no known associations to cancer or have unknown functions. A total of nine new GBM primary cultures were further analyzed by real-time polymerase chain reaction (qPCR), Western blot and Immunohistochemistry on tissue sections and cell cultures. qPCR confirmed the increased expression of 17 of the candidate genes. These results were in good accordance with the information provided by public data bases (Rembrant, TCGA). Western blot performed on 17 candidates showed increased protein levels in 9 cases thus demarcating these as best candidates for molecular targeting. The rest of the proteins featured aberrant isoforms, probably due to alternate splicing, modifications, cleavage or degradation. Further bioinformatic analysis identified a subset of 5 candidates that are particularly highly up-regulated in GBM when compared to low-grade gliomas. To explore the functional importance of the potential target genes the lab established lentiviral-based shRNA delivery and started to test the potential of gene knock-downs (KD’s) to inhibit growth of tumour cells. Preliminary results look very promising with publications in preparation.

Wnt downstream genes are potential targets for a cancer therapy-role of FAM84B
Using a mouse model with conditionally activated beta-catenin, Stangeland and co-workers identified that FAM84B, a member of FAM84 family, is a downstream target of the Wnt pathway. FAM84B was upregulated in the brains of animals that contained conditional gain of function of beta-catenin during embryonic development. FAM84B was also upregulated in neurosphere cultures stimulated with Wnt3A. FAM84B was highly upregulated in all tested glioblastoma cultures at both the RNA and protein levels. Using lentiviral short hairpin technology knock-down of the FAM84B gene was performed.

Decline of expression of the full length FAM84B protein resulted in a very significant reduction in the sphere forming ability of resulting tumours. Further analysis revealed decline of the activated form of beta-catenin in the knockdowncultures when compared to the original tumours and to non-silencing controls. Currently cells are being transplanted to SCID mice to measure tumorigenicity.

Aquaporins or water-channels are targets indicated by Cecilie’s work. Former medical student, Guri Fossdal investigated the expression of these in tumour stem cells and their differentiated counterparts. Aquaporin 9 was shown to be associated with glioblastoma by Realtime PCR, immunohistochemistry and Western blot (Fossdal et al, TSWJ Cell Biology, 2011).
Awais Mughal started as a PhD student in the group in 2009. His project is also generated by Cecilie’s microarray data focusing on upregulated gene candidates confirmed by Biljana Stangeland. A selection of gene candidates that may serve as clinical targets is being investigated with immunohistochemistry and Western Blots to evaluate protein expression. Again Lentiviral based shRNA-technology is applied to silence genes of interest and establish stable Knock-Down cultures. The gene silencing efficiency is measured on mRNA- and protein level and the cultures are subjected to functional studies. In vitro functional studies investigate effects on proliferation, apoptosis and spheroformation. The biological importance of these findings is then further investigated using our xenotransplantation model in immunodeficient mice. Mrinal Joel is using this same methodology and has established a role for the PBK gene in glioblastoma.

Mrinal is also studying transplantation of GFP-transduced brain tumour stem cells to an embryonic environment using the chick embryo model. She investigates the behaviour and differentiation potential of tumour cells when placed in this environment. So far, interestingly, tumour cells display a more restricted activity in an embryonic environment compared to adult. Further studies will be based on the analyses of proliferation, cell death and differentiation ability of these cells into other cell types. Co-culture studies of the tumour cells with the cells from the central nervous system of chick embryo are also under investigation to examine these effects. Another of Mrinal’s lines of investigation has been the use of phosphoflow cytometry as a method to map phosphoprotein signal transduction networks in GBM.

Proteomic studies
Proteins upregulated in tumour stem cells were investigated by former Post-Doc Linda Paulson. She compared human normal stem cells with tumour stem cells. This may reveal why the two types of cells behave differently in the brain and therefore may help in designing treatments that have a major impact on clinical practice.

She adapted a method called SILAC proteomics. SILAC is a straightforward approach for in vivo incorporation of a label into proteins for mass spectrometry-based quantification. SILAC relies on metabolic incorporation of a given ‘light’ or ‘heavy’ form of an amino acid into the proteins; amino acids with substituted stable isotopic nuclei (e.g. containing deuterium, 13C, or 15N). Thus in an experiment, two cell populations are grown in culture media that are identical except that one of them contains a ‘light’ and the other a ‘heavy’ form of a particular amino acid. When the labeled analogue of an amino acid is supplied to cells in culture instead of the natural amino acid, it is incorporated into all newly synthesized proteins. The process is efficient and reproducible as the incorporation of the isotope label is 100% and does not affect protein behaviour. By adding stable, non-radioactive isotopic forms of amino acids to media when growing cells, it is possible to get a mass difference of 6 kDa in the same protein from different samples. Thus we can quantify relative protein abundance as cellular and metabolic processes occur. Experimental results achieve high fidelity with minimal bias, allowing relative quantitation of even small changes in specific protein abundance. Data obtained by Linda are currently being incorporated in manuscripts in preparation.

Håvard K. Skjellegrind started as a PhD student in 2010. He is doing live imaging of single cells, aiming to identify the “true stem cells” in heterogeneous normal stem cell and tumour stem cell cultures. He has already published on mitochondrial membrane potential in brain cells and at present is also examining this behaviour in single cells as a potential marker for “stemness”.

Towards tissue repair
An avenue where adult human neural stem cells offer tremendous promise is in the treatment of degenerative diseases such as Parkinson’s disease. Parkinson’s disease is characterized by loss of pigmented dopamine-secreting cells in the substantia nigra. Though transplantation of embryonic stem cell- or fetal stem cell-derived dopaminergic cells has shown promising results, the results have not been consistent. Furthermore, the ethical issues regarding the use of the aforementioned cell types, limit their clinical use. Dopaminergic cells derived from adult human brain stem cells have obvious benefits: firstly, they pose no ethical challenges and secondly, they make autologous transplantation possible. We are investigating the potential of adult human brain stem cells to develop into dopaminergic neurons. PhD graduate Mercy Varghese obtained preliminary results that in vitro, adult human brain stem cells can develop into neurons expressing tyrosine hydroxylase, a rate-limiting enzyme in dopamine synthesis. This has been confirmed by Wayne Murrell and Emily Telmo.

Stem cells isolated from regions external to the brain.
Mercy Varghese and coworkers isolated neural progenitors from the adult human filum terminale. This terminal end of the spinal cord has been referred to as a fibrovascular tag without neurogenic potential and of no clinical significance. Similar to brain stem cells mentioned earlier these cells from
the filum terminale generated functional neurons capable of firing action potentials. When transplanted into the adult central nervous system, the filum terminale-derived neural progenitors survived, differentiated and showed targeted migration to a site of injury. See Varghese M et al Stem Cells and Development, 2008.

Wayne Murrell joined the Vilhelm Magnus lab as Senior Scientist at the end of 2007. Wayne is from Australia and for the previous seven years led laboratory research at Griffith University, Brisbane, on neural stem cells derived from a region of the peripheral nervous system, the olfactory mucosa. Wayne is first author of a paper Multipotent stem cells from adult olfactory mucosa (Murrell et al, Dev Dyn 2005) which demonstrates the potential to generate neurospheres, direct their differentiation towards neuronal and non-neuronal lineages and suggests autologous tissue repair is a real possibility. This paper was pivotal in gaining support from the Australian Federal Government for the establishment of an Australian National Adult Stem Cell Centre in Brisbane working on human-derived neural stem cells for potential to treat human disease. Wayne has broad experience in the fields of molecular, developmental and cell biology. In recent times he has investigated stem cell transplant in various animal models of disease including Parkinson’s, heart attack, disc degeneration and motor neuron diseases (Murrell et al, Stem Cells 2008, Spine J 2009, Dis Model Mech 2010, N Engl J Med 2010). Now he helps guide the efforts of the researchers of the Vilhelm Magnus lab. Any effective tissue replacement therapy will require capability to produce authentic stem/progenitor cells in bulk quantities whilst maintaining cell integrity.

We have now developed culture methods for the rapid isolation, maintenance and proliferation of undifferentiated adult stem cells. Many suggestions for improvement made by former Post-Doc John Bianco have been quantitated systematically by Wayne Murrell and Emily Telmo. Today, a robust technique for the culture of these cells in billions is now established. At present, studies are being performed to assess the fate of these stem cells and their differentiation potential. Directed differentiation of these cells towards a specific fate such as down the dopaminergic pathway to obtain dopaminergic neurons is being examined. This work is currently in preparation for publication.

What are the cellular processes of healing? What is involved in tissue repair? Surgical ablation of vital brain tissue or the damage accidents cause to the spine and subsequent paraplegia are both relevant scenarios for cell replacement therapy. As well treatments of neurological disorders such as Parkinson’s disease, amyotrophic lateral sclerosis and Alzheimer’s are desirable. We have shown that stem cells exist throughout the adult human nervous system. Human neural stem cells can be manipulated to differentiate to defined phenotypes in vitro. Thus we believe that human neural stem cells can repair damaged neural tissue. Are neural stem cells from different regions equivalent? Or can they be induced to be interchangeable? These are fundamental questions pertaining to any attempt at human neural tissue repair. For instance could cells from the filum terminale or the olfactory mucosa be used to repair the human brain?

Olfactory mucosa has been shown a potential source for autologous stem cell therapy for Parkinson’s disease (Murrell et al, Stem Cells, 2008). Parkinson’s disease is a complex disorder characterised by degeneration of dopaminergic neurons in the substantia nigra in the brain. Stem cell transplantation is aimed at replacing dopaminergic neurons because the most successful drug therapies affect these neurons and their synaptic targets. Murrell et al. have shown that neural progenitors can be grown from the olfactory organ of persons even with Parkinson’s disease. These neural progenitors proliferated and generated dopaminergic cells in vitro. They also generated dopaminergic cells when transplanted into the brain and reduced the behavioural asymmetry induced by ablation of the dopaminergic neurons in the rat model of Parkinson’s disease. These results indicate that Parkinson’s patients could provide their own source of neuronal progenitors for cell transplantation therapies.

“The transplantation of adult human neural stem cells in rat model of Parkinson’s disease” is an ongoing aim of the lab (Wayne Murrell, Emily Telmo, Artem Faizullin). The specific aims of this study are: 1. To investigate the ability of transplanted neural stem cells to reduce the signs of disease in Parkinsonian rats. 2. To estimate and compare the therapeutic efficacy of neural stem cells transplants from different sources (olfactory mucosa, subventricular zone and filum terminale). 3. To test the hypothesis that neural stem cell transplants will produce dopaminergic neurons in the striatum and/or will promote increased innervation of grafted striatum by surviving dopaminergic afferents.

Artem Fayzullin joined the Vilhelm Magnus Lab in 2010. As well as working on transplantation of normal human stem cells he is acquiring expertise in cell filming from Håvard Skjellegrind and will record cell movements of brain cancer stem cells as a contribution to the aims of CAST.

This report therefore summarizes the Vilhelm Magnus lab’s current work on the cell biology of the human brain and nervous system. We seek to broaden knowledge of cell development encompassing the biological concepts of
stem cells, cancer stem cells and the development of tumours, cell physiology of anesthesia, and as well the future development of cell replacement therapy.

**Awards and publications**

Einar Vik-Mo was awarded his well deserved PhD during early 2011. Our group achieved seven refereed publications during 2011.

**International collaborators of the Vilhelm Magnus lab:**

David Tirrell, California Institute of Technology, Los Angeles, USA  
Charles Liu, University of Southern California, Los Angeles, USA  
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Yasuhiro Watanabe, Tottori University, Japan  
Winston Hide, Harvard University, MA  
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