Save Sight Institute Research Report 2004

Introduction

The research groups within the Save Sight Institute approach the problems of blindness from clinical and laboratory perspectives. Integrating with those exploring digital media, education and service, the groups work in close collaboration. They are listed alphabetically.

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The Save Sight Institute

Corneal Research Group

Corneal disease research in the Institute investigates the pathogenesis of the major blinding bacterial and viral corneal infections and aims to improve the outcome of corneal transplants through development of corneal storage techniques and refinement of corneal surgery.

Group Members

Kathy McClellan, MBBS PhD FRANZCO FRACS, *Unit Head*
Con Petsoglou, MBBS, MMed (Clin Epi) FRANZCO FRACS, *Lecturer*
Li Wen, MBBS MMed, *Research Officer*
Tarinee Sangiampornpanit, MBBS, *Corneal Fellow*
Raj Devasahayam, BAppSc, *Eye Bank Laboratory Manager*

Research Activity

A transparent cornea makes an important contribution to eyesight by allowing light to reach the retina and providing refractive power for focusing of images. Although the cornea and outer eye have a variety of well-developed protective mechanisms, the cornea remains vulnerable to infection. Blinding corneal disease may also be caused by developmental anomalies that result in progressive corneal opacity and distortion from birth.

Herpes simplex virus (HSV) infection causes corneal scarring and loss of vision; we are investigating its distribution in trigeminal ganglia and cornea as well as its role in suppurative keratitis.

We have determined the antibiotic susceptibilities of common ocular isolates. This information allows effective and rational antibiotic therapy of corneal infection.

Finding methods of corneal storage that extend the life of corneal transplants is the focus of research in the Eye Bank Laboratories.

Clinical research in corneal disease is developing better techniques of lamellar or partial thickness corneal grafting and refining corneal transplantation in infants and children.

Projects in 2004

The 22nd Cornea and Eye Bank Meeting

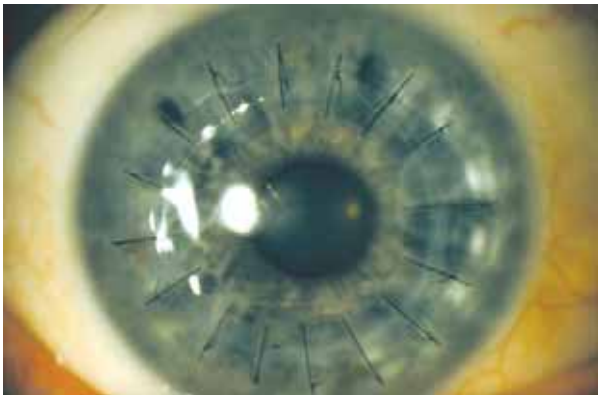
This meeting was hosted by the Save Sight Institute and Lions NSW Eye Bank. 2004 marks the 100th year since the first successful cornea transplant in Czechoslovakia in 1905. The meeting was attended by 85 Australian, New Zealand and international cornea specialists and Eye Bank staff. It provided an excellent opportunity for the discussion of present research into corneal diseases and eye banking.

Human herpes simplex virus (HSV) and herpes zoster virus

The distribution of these viruses in human ganglia and cornea, genome specificity in cornea and lid disease, prevalence in suppurative keratitis, role in corneal graft failure without rejection and detection in iridocorneal endothelial syndrome are all being researched.

Antibiotic susceptibilities

Antibiotic susceptibilities of corneal isolates and conjunctival flora have been determined in 125 organisms isolated from 80 patients presenting to the Sydney Eye Hospital Emergency Department during the past 12 months.



Corneal transplant

Corneal storage

Optimisation of the organ culture method of corneal storage and investigation of corneal metabolism during storage is in progress.

Infectious keratitis

Infectious keratitis is being studied in 3 groups of patients - contact lens wearers, the elderly and patients with corneal grafts - to determine risk factors, common pathogens and outcomes, with the aim of better preventing and managing this condition.

Pterygium

Lamellar keratoplasty is being investigated for the management of recurrent pterygium.

Corneal transplant in infants and children

This study was the first to demonstrate graft survival beyond 14 years amongst this group of young recipients.

Corneal confocal microscope

A corneal confocal microscope was acquired through the Sydney Eye Hospital Foundation. Research into the use of the microscope in the diagnosis, investigation and treatment of corneal diseases is being undertaken. Specific expertise and research into acanthamoeba keratitis has led to results being presented at conferences.

Digital Media Research Group

Research in this group is focused on exploiting the potential of the digital media, information technology (IT) and the communications revolution to:

•Create and implement state of the art outreach ophthalmic services for remote and rural communities.

•Develop cutting edge ophthalmic teaching services to provide excellence in education of future eye doctors.

•Empower the community through the internet by providing interactive access to information about

eye care and lifestyle choices which can help prevent eye disease.

Group Members

Frank Billson, MBBS FRANZCO FRACS FAC FRCOphth, *Unit Head*
I-van Ho, *Postgraduate student*

Research Activities

Teleophthalmology Initiative: The challenge of providing specialist eye care services outside the metropolitan centres of Australia is a significant one. This group is working to develop more effective ways of screening for eye disease in remote and rural areas.

Virtual Teaching Initiatives: The unit is researching initiatives to provide distance learning services and developing innovative ways for students to have clinical trial experience in a virtual arena before engaging in patient practice.

Interactive Community Services: As digital media increasingly become a delivery tool for community information and access to services, this group is focusing on improving ways of delivering Sight Saving Information to the community and developing simple interactive eye services to be accessed via e-media.

Projects in 2004

Virtual Ophthalmology Clinic

The unit's virtual ophthalmology program allows medical students to gain skills in history taking by making use of "virtual" clinical patients. The histories in the program's database, transcribed from real patient interviews and presented by interactive virtual actors, are programmed to reflect symptoms associated with common eye diseases and complaints.

The program mimics real interactions with patients in that students control the order in which they ask questions and gather information to make a diagnosis prior to an examination. The diagnosis is emailed to the student's supervisors before the student is allowed to continue the examination and higher levels of investigation. The strength of the program is that it allows the student to develop skills in interviewing and forming a diagnosis before practising on real patients. Its success as a teaching and self-development tool highlights the direction of future digital and distance learning initiatives.

The program is now deliverable via the internet. Recently the interface was partly redesigned for improved navigability and its networking capabilities were fine-tuned to enhance the program's utility across a wider range of environments. It is also envisaged that it be included in the course content for the Graduate Diploma and Masters Degree in Ophthalmic Science, a distance and onsite learning collaboration between the University of Sydney and the University of Otago, which began in 2004.

Tele-ophthalmic diabetic eye screening and treatment program

The tele-ophthalmology initiative in the Northern Territory continued in 2004. There has been a significant increase in the number of remote Aboriginal communities successfully screened for diabetic retinopathy.

A similar study is underway in the Macquarie Area Health region in NSW.

A fully equipped facility has been developed in the Dubbo Aboriginal Medical Co-Operative Centre. A skill-transfer program will continue and the data generated will form an important opportunity in the development of a database for analysis, working with the Aboriginal Centre to develop strategies that will improve access to and understanding of eye health among Aboriginal people. It will be possible to integrate the service, with Aboriginal agreement, with the Dubbo Rural Clinical School.

This facility was opened by the Minister for Health & Ageing, The Hon. Tony Abbott MP, on August 18th 2004.

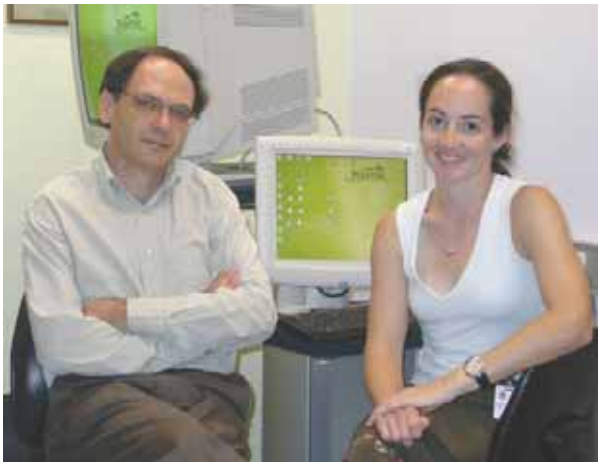
Electrophysiology and Glaucoma Research Group

Group Members

John Grigg, MBBS FRANZCO FRACS
Alex Klistorner, BMed PhD
Stuart Graham, MBBS MS FRANZCO FRACS
Frank Billson, MBBS FRANZCO FRACS FAC FRCOphth
Alessandra Martins, MBBS, *Postgraduate student*
Clare Fraser, MBBS, *Postgraduate student*
Chandra Balachandran, MBBS, *Postgraduate student*
Lisa Feldman, DipNursing, *Electrophysiology Technician*
Priya Narayan, *Electrophysiology Technician*
Asya Klistorner, *Electrophysiology Technician*

Research Activity

The electrophysiology section continues to provide clinical testing as well as research into new techniques for electrodiagnosis, especially for the detection of glaucoma. The department will soon be acquiring a new electrodiagnostic capability which will replace all our old standard testing equipment with state of the art technology for ERG/EOG/VEP recording. The Espion Diagnosys System comes programmed with the ISCEV standards as well as allowing modifications for research. It also has a hand-held mini ganzfeld which is useful for testing children. It will also enable testing of children without the need for sedation.



Dr Alex Klistorner and Dr Clare Fraser in front of the AccuMap

Projects in 2004

Development of Objective Perimetry – the Multifocal Visual Evoked Potential
Klistorner, Graham, Grigg, Billson.

In 2001 ObjectiVision Pty Ltd was set up as a collaboration between investigators at the Save Sight Institute and the Sydney University Business Liason Office to develop new techniques in vision testing. In 2002 the AccuMap V1.3 objective perimeter was launched as a new test for glaucoma that relied not upon the patient's subjective responses but on recording the tiny electrical signals generated in the brain, called visual evoked potentials, when the patient was viewing a stimulus on a computer screen. The initial AccuMap won 2 Australian design awards in 2002.

The AccuMap then was redesigned with a major software upgrade using the OPERA V2.0 platform. This was developed to include new noise detection parameters and make the interface much more user-friendly. The system received FDA approval late 2003, and was launched in the USA at the American Academy of Ophthalmology. Heidelberg Technologies have been contracted as US distributors.

At present there are 5 US trial sites involved in the multicenter AccuMap Early Glaucoma Detection Study, with Dr Klistorner and Dr Graham at the Save Sight Institute being co-ordinators. The results of this study will help confirm the effectiveness of the technique compared to conventional methods of detecting glaucoma. Dr Grigg and Prof Billson are principle investigators.

We have recently been awarded an NHMRC Development Grant for the extension of the technique to use virtual reality goggles as a means of presenting the stimulus. This will have the potential advantage of making the test portable, and standardising the test distance.

Development of a Blue/Yellow Multifocal Visual Evoked Potential
Martins, Balachandran.

This research investigates the use of blue-yellow stimuli for presenting the multifocal Visual Evoked Potential (mVEP) in an attempt to detect glaucoma at an earlier stage. The rationale for this is that in subjective perimetry blue/yellow testing has been shown to be more effective at detecting early disease. To date, the mVEP technique has used a black and white stimulus to demonstrate abnormal visual field defects in glaucoma cases. This research project has now developed an optimal design which maximises the mVEP amplitudes for the stimulus. The blue/yellow alternating checkerboard with isoluminantly matched checks has now been incorporated in the AccuMap system. This work was supported by funding from an ORIA grant in 2004.

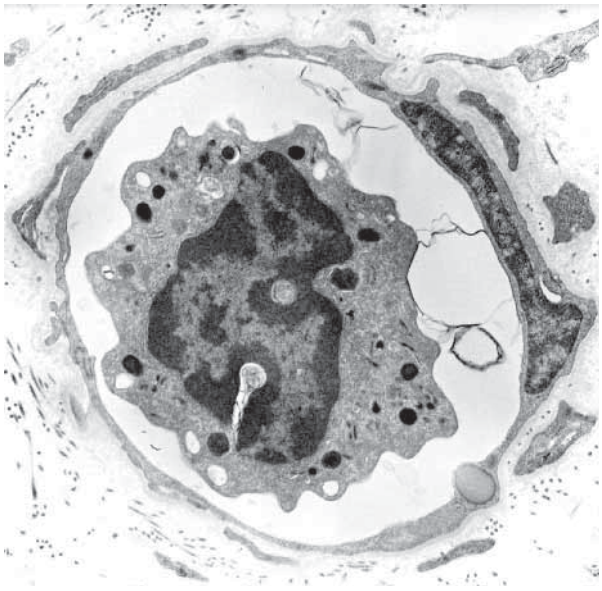
The test has been applied to normal subjects in order to create a normal blue-yellow database. Currently, the technique is being applied to established glaucoma patients and high risk suspects for glaucoma to establish its sensitivity in comparison with other diagnostic techniques such as Humphrey Visual Fields-the gold standard, black-white mVEP, Short-Wave Automated Perimetry and Optical Coherence Tomography.

Multifocal Visual Evoked Potentials in Optic Neuritis
Fraser, Grigg, Garrick.

Multiple Sclerosis (MS) can damage the optic nerve leading to vision loss, this can be the first presentation of MS in 30% of patients. The resulting optic neuritis causes the loss of the protective layer surrounding the nerve fibres. Consequently there is a disruption in the electrical signal transmitted from the eye to the brain. Visual Evoked Potentials (VEPs) record the electrical responses in the brain that represent the transmission of this visual information from the eye.

Current VEP recordings only measure these responses from the central visual field and cannot differentiate non-specific exacerbations of optic neuritis from true recurrences. A pilot study conducted at the Save Sight Institute has shown that the multifocal VEP (mVEP), measured by AccuMap, can localise optic neuritis abnormalities to a specific visual field area and thus potentially demonstrate exacerbations of existing lesions versus new demyelination. We are continuing the testing of the AccuMap mVEP system of objective perimetry to detect changes associated with optic neuritis. The AccuMap will provide an objective measure of a patient's visual field, as well as information on the axonal function between the retina and the occipital visual cortex. We hope to use the AccuMap to detect subtle and more peripheral vision changes than conventional VEP, and to monitor recovery of nerve function. By studying the changes in mVEPs seen in acute optic neuritis and then following these changes over time we hope to develop the mVEP as a useful tool for monitoring the effects of the new treatments for MS on recovery of visual function. Old lesions can be identified, but a chronic inflammatory change (potentially reversible) in the optic nerve may be also be identified if it can be seen to resolve with new forms of treatment.

We also hope to detect sub-clinical remissions or new lesions within the visual pathways. This may allow more patients earlier access to interferon therapy. Furthermore the mVEP could be a sensitive tool to detect remyelination, and if this could be achieved, help evaluate future therapies.



Electron micrograph of a blood vessel in the eye

Eye Genetics Research Group

Group Members

Robyn Jamieson, MBBS FRACP PhD
John Grigg, MBBS FRANZCO FRACS
Frank Billson, MBBS FRANZCO FRACS FAC FRCOphth

Research Activity

Genetic eye disorders contribute to the causes of blindness and partial-sightedness for many with visual disability in our community. We are establishing a research program in genetic eye disease, with collaborative links with the Discipline of Paediatrics and Child Health at the Children's Hospital at Westmead and the Children's Medical Research Institute. The aim of this research is to open the way for new and better treatments and management for the causes of these conditions. Our research studies focus on developmental ocular conditions including cataracts (clouding of the lens), glaucoma (raised pressure in the eye), retinal anomalies (disorders affecting the back of the eye) and microphthalmia or anophthalmia (small or absent eye). All of these conditions can lead to visual disability or blindness and in all there are few or limited treatment options. Treatment is difficult both for initial management and also in the prevention of ongoing vision loss for the child as he or she grows and develops. Current treatments are often surgically orientated, and while such treatments may still be required, this research work aims to discover disease genes important in these conditions, and the functions of the proteins they encode. By understanding the detailed protein functions, we will then be able to develop better medical treatments to vastly improve the management of these conditions.

In this research program we are studying patients and families who have developmental eye conditions, who also have clues on their history or assessment to provide an entry point through which we can work to identify the underlying disease gene. These include families which are large and suitable for linkage analysis, and families where there are chromosome changes which are leading us to the underlying disease gene. We are also examining other patients and families seen through the Save Sight Institute for changes in these genes. Once we have identified the novel disease gene, we are then using the mouse as a model to understand the detail of the encoded protein and its functions in the eye.

Lens Research Group (Protein Chemistry)

Group Members

Roger Truscott, BSc PhD, *Unit Head, NHMRC Senior Research Fellow*
Peter Hains, BSc PhD, *Postdoctoral Fellow*
Karl Heys, BSc, *Postgraduate Student*

Research Activity

This group, originally from The University of Wollongong, joined the SSI in 2004 and began the process of moving research activities to the Sydney Eye Hospital Campus. Their work has ranged from investigating oxidative changes in nuclear cataract to more recent studies on the lens diffusion barrier. It is proposed that nuclear cataract commonly arises because of the age-related onset of a diffusion barrier in the lens. This 'barrier hypothesis' is now supported by a large body of data that links back to their earlier studies on protein oxidation in the lens nucleus. The lack of sufficient antioxidants, due to the barrier, and a consequent increase in protein modification resulting from increased reactive species, such as UV filters, leads to nuclear opacification. Much of this group's work is focused on testing this hypothesis. The protein chemistry group is set to expand their activities at SSI in 2005.

Projects in 2004

Posttranslational modification (PTM) in age-related cataract
Truscott, Hains.

The reason for the development of age-related nuclear cataract is not known. This disease is associated with major oxidation and colouration of the lens proteins. By working out the nature of these modifications we hope to be able to identify PTMs that have brought about this change in the properties of the lens. In this way we may be able to understand what causes cataract. Mass spectrometry is one of the sensitive techniques that

has been employed to enable these alterations in protein structure to be elucidated.

Ageing of human lens
Heys, Truscott, Hains.

Human lens crystallins are present for the duration of a person's lifetime. In this period they become modified and as a consequence alter their properties. We have been monitoring some of these changes and relating them to alterations in the physical and optical properties of the lens. In this way we hope to understand one aspect of the biochemistry of ageing. Lenses were examined for stiffness and then were dissected into various regions and each extracted sequentially with buffer, 4M and 7Murea. Each fraction, including the membrane fraction, was analysed by SDS PAGE, bands quantified by scanning, and then each protein band identified by in-gel tryptic digestion followed by mass spectrometry of the peptides.

Investigations into the lens barrier and its role in nuclear cataract
Truscott, Lam, McAvoy.

At middle age the lens nucleus becomes functionally uncoupled from the metabolically-active lens cortex. The resulting lack of adequate antioxidant defence, renders the nucleus susceptible to oxidation. The existence of the lens barrier has been confirmed by NMR imaging. The consequences of the onset of the lens barrier are profound. It is not only the impediment to entry into the nucleus that is a problem; restricted exit from the lens centre also has deleterious consequences. The barrier hypothesis is increasingly recognised as the basis for understanding nuclear cataract. Ongoing studies into human lenses from the Lions Eye Bank were aimed at more precisely identifying the onset and the molecular basis of the barrier. This work involved the detailed proteomic analysis of the structure, function and interactions of molecules that play key roles in cell-cell communication.

Analysis of lens membrane components as a function of age
Truscott, Heys, Blanksby (University of Wollongong).

Over the life span of an individual, the lipid composition of the cell membranes in the lens changes substantially. The consequences of this are unknown, but we believe that such changes may underpin the development of presbyopia – the inability to focus on nearby objects after age 50. In continuing studies, we characterized cortical and nuclear changes in lens lipid composition and related these to separate measures of lens stiffness obtained by Dynamic Mechanical Analysis.

Lens Research Group (Cellular and Developmental Biology)

Research is directed at identifying molecules and mechanisms that govern the behaviour of lens cells in health and disease. Our studies have identified molecules that play key roles in both normal and pathological lens development. Currently we are working to gain a better understanding of how these molecules are regulated in the eye. This is fundamental to identifying new therapeutics for retarding or preventing cataract, one of the most common (and costly) diseases of ageing.

Group Members

John McAvoy, BSc PhD, *Unit Co-Head*
Frank Lovicu, BSc PhD, *Unit Co-Head*
Richard Stump, BSc PhD, *Laboratory Manager*
Jessica Boros, BSc, *Research Assistant*
Sharon Ang, BSc, *Research Assistant*
Michael O'Connor, BSc, *Postgraduate Student*
Elizabeth Wederell, BSc, *Postgraduate Student*
laxmi Iyengar, BSc, *Postgraduate Student*
Yongjuan Chen, BSc, *Postgraduate Student*
Colin Chong, MBBS, *Postgraduate Student*
Kevin Wang, BSc, *Postgraduate Student*
Bramilla Patkunathan, BSc, *Postgraduate Student*

Research Activity

The lens transmits and focuses light onto the retina. To do this it needs to be transparent and to have appropriate refractive properties. This depends on the development and maintenance of highly ordered cellular architecture.

The lens consists of two forms of cells encapsulated within a basement membrane; (i) elongated fibre cells, grow to several millimetres in length, and are precisely aligned to form a regularly packed spheroidal mass, and (ii) cuboidal epithe-

lial cells form a single-layered sheet that covers the anterior surface of the fibres.

Whilst the fibre cells make up the bulk of the lens and mostly determine its optical properties, epithelial cells play a key role in maintaining an appropriate physiological environment within the lens. In addition, the epithelium contains the 'stem cells' that proliferate, migrate and differentiate into the new fibres that are progressively added to the fibre mass throughout life.

We have focussed our attention on growth factors because of their importance in regulating cell fates in developmental systems. Using a unique lens epithelial explant culture system we have identified members of the FGF growth factor family as inducers of lens cell proliferation, migration & differentiation; responses that are induced in a progressive dose-dependent manner. We have proposed that an anterior-posterior gradient of FGF determines lens polarity and growth patterns. A major thrust of research activity in our laboratory is aimed at testing this hypothesis.

Insights into the molecular basis of the major lens pathology, cataract, have also arisen from our growth factor studies. We have shown that members of the transforming growth factor beta (TGFß) family induce aberrant growth and differentiation of lens cells. This progressively leads to disruption of normal cellular architecture and opacification of the lens.

Cataract is the most common cause of blindness in the world today. Although surgery is generally effective, in many countries it cannot keep pace with the growing demand. Moreover, complications such as aberrant growth and differentiation of lens cells left behind after cataract surgery (most commonly referred to as posterior capsule opacification), require further treatment and add to the cost of cataract management. Because of its clinical significance it is vital to understand how TGFß is regulated in the eye and how it induces cataractous effects on the lens. This information is fundamental to understanding the molecular basis of cataract and devising strategies for prevention.

Projects in 2004

FGF signalling and lens cell proliferation and differentiation
Lovicu, Iyengar, Wang.

These studies were aimed at elucidating how FGF induces different cellular responses. We investigated the intracellular signalling cascades that are activated by FGF. We showed that the ERK1/2 (MAPK1/2) signalling cascade is involved in both proliferation and differentiation. Inhibition of ERK1/2 blocks cell proliferation; however, it blocks only the morphological (elongation), not the molecular (beta-and gamma-crystallin gene expression) aspects of differentiation. This indicates that fibre differentiation depends on activation of several intracellular signalling cascades. The dynamics of these growth factor-induced cascades were also studied in relation to the signaling cascades induced by the ocular media.

PDGF/IGF/EGF signalling and lens cell proliferation
Lovicu, Iyengar, Wang.

IGF/insulin and PDGF growth factor families can induce lens epithelial cells to undergo cell division. To determine if IGF and PDGF utilise the same signalling pathway(s) as FGF, we examined the role of ERK1/2 signalling in IGF- and PDGF-induced lens cell proliferation. Similar to FGF, both IGF and PDGF can induce lens cell proliferation, in an ERK-dependent fashion. However, PDGF activates ERK within 10 minutes compared to 20 minutes for IGF. Differences in the temporal activation of lens cell proliferation by IGF and PDGF may be due to differences in the mode by which these growth factors activate ERK1/2. The dynamics of these growth factor-induced cascades were studied in relation to the signaling cascades induced by the ocular media. Overall, these results support an important role for ERK signalling in growth factor-induced lens cell proliferation.

Integrin expression patterns in lens development
Wederell, McAvoy, de Iongh (University of Melbourne).

In earlier studies we identified key cell-matrix adhesion molecules in the lens. We were particularly interested in defining the changes in integrin gene expression during the transition of an epithelial cell into a fibre cell. The main findings were that alpha3 integrin, which is strongly expressed in epithelial cells, shuts off sharply as fibres begin to differentiate. In addition, alpha6A and alpha6B which are expressed in both epithelial cells and fibres, undergo a switch so that alpha6A is more strongly expressed in fibres than in epithelium. This indicates that different integrin family members may facilitate the different adhesion requirements of these two forms of lens cells to their substratum, the lens capsule.

Lens regeneration
O'Connor, Lovicu, McAvoy.

FGF is good at inducing fibre differentiation in lens epithelial explants. However, elongating cells in FGF-treated explants do not show the same degree of alignment with their neighbours as do elongating fibres in vivo. There is growing evidence to suggest that other, as yet unidentified factors, are involved in coordinating the differentiation of the epithelial 'stem cells' and their elongation into highly aligned fibre cells. Recent developments in tissue culture techniques pioneered by our laboratory are enabling a more rigorous investigation of the intraocular fluids in search of these factors. This raises the possibility of devising strategies that will promote lens reconstruction after cataract surgery.

Crim 1 expression and function in the lens
Lovicu, Boros, Little (University of Queensland), McAvoy.

We localised a new gene, Crim1 (initially identified by colleagues at University of Queensland), during eye morphogenesis. Crim1 is particularly strongly expressed in the lens epithelium. During postnatal growth, expression is reduced in all ocular tissues and eventually it is only expressed in the lens. Investigating the functional significance of Crim1 in the lens in gain-of-function and loss-of-function mouse models forms the basis of an ongoing collaborative study with colleagues at the University of Queensland.

Wnt signalling in lens development
Stump, Chen, Ang, Reinten, Lovicu, Pinson (University of California, Berkeley, USA), McAvoy.

We studied the expression of members of the Wnt growth factor family and its receptors, the frizzleds (Fzs). Wnts are commonly involved in regulating expression of molecules involved in cell proliferation, communication and adhesion. We showed that multiple Wnts and Fzs are expressed in the lens. In addition, we showed that key Wnt/Fz signalling molecules, including those involved in Wnt/ß-catenin and Planar Cell Polarity pathways, are expressed in the lens. A mouse mutant that is deficient in a co-factor required for Wnt signaling does not develop a complete lens epithelium. This supported our hypothesis that Wnt signaling plays a key role in the formation and maintenance of the lens epithelial sheet.

Wnt signaling in TGFß-induced cataract
Chong, Ang, Lovicu, McAvoy.

Transforming growth factor-beta (TGFß) induces aberrant growth and differentiation in rodent lenses that is characteristic of some forms of human cataract. As changes in Wnt signalling are frequent key events in abnormal growth and differentiation in other systems, we investigated Wnt expression in established TGFß-induced cataract models. We showed that Wnt expression is altered in TGFß-induced rat and mouse cataract models. Levels of Wnt5a and Wnt7b protein and mRNA are distinctly upregulated in cataractous plaques of all the models we examined. This suggests that de-regulation of Wnt signalling is involved in regulating abnormal growth and differentiation processes in TGFß-induced cataracts and may play a role in human cataract, such as posterior capsule opacification.

Lithium stabilises the polarised lens epithelial phenotype and blocks TGFß-induced cataract
Stump, Ang, Lovicu, Pandey (Intraocular Implant Unit, Sydney Eye Hospital), McAvoy.

A common complication of cataract surgery is caused by epithelial mesenchymal transition (EMT) and aberrant growth of residual lens cells. As recent studies indicate a role for Wnt/ß-catenin

signalling in lens epithelial differentiation, we assessed the effects of lithium chloride (LiCl), which is often used to mimic aspects of ß-catenin signalling, on regulating the behaviour of cells in lens epithelial explants. In controls, cells depolarised, proliferated and migrated. In contrast, in LiCl-treated explants cells remained in discrete groups, did not proliferate or migrate and maintained their normal polarity and cobblestone-like packing. In controls, ß-catenin was initially localised to the cell margins but as cells depolarised and migrated, it became distributed throughout the cytoplasm and was particularly strong in nuclei. Many of these cells also showed activation of the TOP-green reporter indicating canonical ß-catenin signalling. By contrast, in polarised LiCl-treated cells, ß-catenin remained localised to cell margins and, as in vivo, there was no evidence of ß-catenin signalling. Significantly, the effects of lithium also extended to blocking the cataract-promoting effects of TGFß on both rat and human explants. This opens up possibilities for developing molecular approaches for maintaining the polarised epithelial phenotype and preventing aberrant growth that leads to cataract.

Vitronectin in lens development and cataract
Ang, Taliana (Invitrogen Corporation), Evans (CSIRO, West Ryde, NSW), McAvoy .

The lens capsule forms during lens morphogenesis and provides the substratum for lens cell attachment, growth and differentiation. Its primary extracellular matrix (ECM) constituents include type IV collagen, laminin, heparan sulphate proteoglycan, fibronectin and entactin/nidigen. Recent studies have shown that during the aberrant growth and differentiation seen in some forms of cataract, abnormal accumulations of ECM are deposited as lens cells undergo epithelial mesenchymal transition. One of the molecules identified within the resulting fibrotic plaques is vitronectin (Vn). We showed that Vn is expressed by lens epithelial cells and is present in lens capsule during development. Lens epithelial cells can attach and migrate on Vn; however, on this substratum, the cells undergo an epithelial-mesenchymal transition that is characteristic of cataract. This result raises questions as to how, or if, lens cells and Vn interact in normal development and how this relationship may be disrupted during cataract formation.

Retinal Research Groups

The retina, which detects light like the film of a camera, is an exquisitely complex and delicate part of the eye. Retinal diseases, such as diabetic retinopathy and age-related macular degeneration, are now the commonest causes of untreatable blindness in Australia.

The SSI has 3 research groups working on various aspects of retinal biology and pathology:

1) Retinal Development, Ageing & Eye Cancer
2) Retinal Therapeutics
3) Retinal Cell Death and Survival

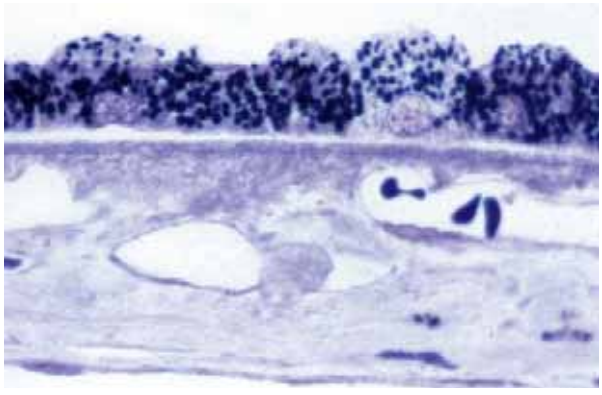
The groups work in close association – sharing information, insights and frequently joint publications.

Age-related macular degeneration is a common thread of research in terms of:

•Basic biology, development and changes in associated vasculature.

•The importance of therapy for sub-retinal new vessels in the blinding stage of this disease.

•The fact that genetic factors may be important in susceptibility to the disease.



Light micrograph of normal human retina

Retinal Development, Ageing & Eye Cancer Research Group

Research aims to shed light in 2 areas - Retinal Development and Eye Cancers - and to explore the inter-relationships between them.

Group Members

Michele Madigan, BOptom PhD, *Unit Co-Head*
Max Conway, PhD FRACO FRACS, *Unit Co-Head*
Jan Provis, BSc PhD, *SSI Associate (ANU)*
Diana van Driel, BSc, *Senior Research Assistant*
Elisa E Cornish, PhD, *Research Assistant (P/T)*
Alexandra Allende, MBBS, *Postgraduate Student*
Trong Van Pham, MD, *Postgraduate Student*
Kenneth Lai, BMedSci, *Postgraduate Student*

Member advising on clinical aspects:

Meidong Zhu, MD PhD, *Research Fellow*

Research Activity

The human 'macula' is a region of the retina that includes several specialisations not present in non-primate retina and which together enable us to see detail. Understanding the development and ageing of the macula and how this unique structure makes humans vulnerable to degenerative disease is the principle aim of our research into development and ageing. The fovea is at the geometric centre of the macula and is identifiable by a number of structural modifications including: (1) an absence of a direct blood supply from the retinal circulation, (2) the presence of a pit which thins the retina locally, (3) a focal concentration of a class of colour sensitive photoreceptors (cones), and (4) the inclusion of specialised circuitry which conserves the electrical responses of individual cones. Few groups internationally are applying modern molecular tools to the investigation of primate retinal development and human retinal disease. During 2001 we have developed new approaches to investigate the biology of the macula. Our focus is to identify unique molecular profiles associated with the unique anatomy and physiology of the macula and fovea.

Retinoblastoma (Rb) and ocular melanoma are the most common primary intraocular eye cancers in children and adults respectively. Better understanding of the pathogenesis of these tumours involves understanding the normal processes of development and ageing in the retina and choroid, as well as the mechanisms controlling proliferation, cell death and angiogenesis within tumours. Combined clinical and laboratory research provides an understanding of the biology of these tumours and improves the rationale for treatment. This is important given the morbidity associated with enucleation, the side effects of therapies, particularly radiation, and the high incidence of metastases in ocular melanoma.

Projects in 2004

Growth factors in retinal development
Allende, Cornish, Natoli (ANU), Madigan, Provis (ANU).

Fibroblast growth factors (FGFs) regulate a number of cell functions including morphological differentiation (ie changes in cell shape). At the fovea – the part of the retina that enables us to discriminate fine detail – cones are highly elongated and very narrow, enabling them to pack at high density. During ageing and in macular degeneration this morphological specialisation is lost and is associated with a decline in visual acuity. Our studies show that during development members of the FGF family are expressed on cone photoreceptors in a pattern consistent with a role in cone differentiation. In particular, cone photoreceptors uniquely express one of the receptors with high specificity for only one member of the FGF family.

Blood vessel growth during foveal development
Allende, Madigan, Provis (ANU).

Other studies show that certain members of the Transforming Growth Factor (TGF) family are expressed in the developing retina in a gradient that suggests they may have a role in definition of the macula. We are testing the hypothesis that TGFß is highly expressed in this region and inhibits angiogenesis.

Retinal microglia and candidate receptors for HIV-1

Pham, McCluskey (St Vincents Hospital), Penfold (ANU), van Driel, Madigan

Microglial cells are the primary antigen presenting cells in the retina and can harbour viral antigens that may damage neural tissue via the release of neurotoxins. All cells bearing CD4 molecules and co-receptors (members of the chemokine receptor and Fcgamma receptor families) are potential targets for the human immunodeficiency virus (HIV-1). These studies examined cultured human retinal microglia and frozen sections of human retinas for expression of candidate HIV-1 binding receptors, including CD4, CC chemokine receptor 5 (CCR5) and Fcgamma receptors. Human retinal microglial cells are found to express detectable levels of CD4, CD16, CD64 and CCR5 in vitro and Fcgamma receptor I (CD64) in situ. As such, human retinal microglia may be a potential reservoir for HIV-1 infection and be involved in the pathogenesis of HIV retinopathy.

In vivo effects of retinoids on tumour growth in an Rb mouse model

Madigan, Conway, van Quill (Ocular Oncology, UCSF, San Francisco), O'Brien (Ocular Oncology, UCSF, San Francisco).

Collaborative studies with Professor O'Brien are investigating the efficacy of various retinoids in controlling intraocular tumour growth in a mouse model of Rb. These agents can induce tumour cell differentiation and inhibit tumour growth in some instances. When used in combination with chemotherapy and radiation, some of these agents may reduce the dose of potentially cytotoxic therapies required when treating tumours.

Targeted immunoradiotherapy of ocular melanoma

Li, Madigan, Conway, Billson, Allen (St George Hospital, UNSW).

Laboratory research has focused on using select antibodies or proteins to target radiotherapy more precisely to melanoma cells to deal with both primary and metastatic disease. We have recently been studying a melanoma specific antigen NG2 (using Mab 9.2.27) which may have potential as a target for radiotherapy treatment. Preliminary studies show that the majority of choroidal melanomas and ocular melanoma cell lines (from Prof Jager, Netherlands) express NG2 immunoreactivity. In vitro studies indicate specific cytotoxicity of alpha-immunoconjugates (213Bi-9.2.27) for NG2-positive melanoma cell lines, suggesting potential use in local therapy either alone or in combination with plaque radiation.

Matrix metalloproteinases in ocular melanoma

Madigan, Lai, Crouch (SEALS Anatomical Pathology, Prince of Wales Hospital), Conway, Jager (Leiden Medical Centre).

Matrix metalloproteinases (MMPs) are a family of enzymes involved in degradation of extracellular matrix components such as collagen, laminin and proteoglycans. MMPs are involved in normal development, wound healing, new blood vessel growth and tumour invasion and metastases. Our studies in ocular melanoma indicate that MMP-2, -9 and MT1-MMP are involved in tumour angiogenesis. We have also found that ocular melanoma cells express EMMPRIN, an inducer of MMP activity. The juxtaposition of EMMPRIN-positive tumour cells and MMP-2 expressing fibroblasts at tumour edges provides insight into the mechanisms underlying ocular melanoma growth. In vitro studies support these findings, where co-cultures of fibroblasts and melanoma cells display enhanced MMP-2 activity compared to fibroblasts or melanoma cells grown alone.

The effect of UV on growth of uveal melanoma

Lai, Di Girolamo (UNSW) and Madigan.

Epidemiological studies suggest that exposure to UV radiation has a role in the pathogenesis of uveal melanoma. Preliminary studies have investigated the effects of UV-B radiation on ocular melanoma cell growth, viability and MMP-production. Melanoma cells appear to be very sensitive to UV-B radiation and cell death is induced even at very low doses (~5mJ/cm2). However, lower doses of UV-B do not appear to induce significant effects on MMP production by ocular melanoma cells. Whether UV-B can induce changes in primary choroidal melanocytes important for transformation to melanomas, as seen for example in cutaneous melanoma, is currently being investigated.

Development of an artificial eye
Conway, Ben-Nissan (UTS).

The consequence of some ocular diseases, especially tumours, may be the loss of an eye with significant functional and psychological sequelae. Hydroxyapatite is currently used to replace eyes lost due to any cause, however it may be associated with problems including the potential for extrusion, infection, reduced motility and loss of cosmesis over time. The group has developed a novel porous bioceramic with improved biointegration and mechanical properties, which promises better function and cosmesis over existing implants. The results of the physical studies have recently been published; in vivo evaluation is in progress.

Retinal Therapeutics Research Group

This group aims to understand how the permeability of the retinal blood vessels is controlled in order to identify new and improved treatments for swelling of the retina, which is a common cause of loss of vision in conditions such as diabetic retinopathy. The group includes both clinical and laboratory research units.

Group Members

Mark Gillies, MBBS PhD FRACO, *Unit Director*

Clinical Research Unit:
Maria Males, RN BN BA GDip (Acute Care Nurs) *Clinical Research Officer*
Haipha Ali, BSc (Applied Vision Sciences, Orthoptics)
Meidong Zhu, MD PhD, *Senior Research Fellow*
Christine Gaston, MBBS, *Clinical Research Officer*

Laboratory Research Unit:
Martin Windsor, BSc PhD, *Senior Research Fellow*
Li Wen, MBBS MMed, *Research Officer*
Bryony Stracey, *Technical Officer*
Svetlana Cherepanoff, BMedSci MBBS, *Postgraduate Student*
Goff Quin, MBBS, *Postgraduate Student*
Marina Tretiach, BAppSc, *Postgraduate Student*
Jenny Wyndham, BSc DipEd MS MPH, *Postgraduate Student*

Research Activity

Clinical Research Unit

The Retinal Therapeutics Research Unit is an internationally certified clinical trial centre that conducts randomised clinical trials and other clinical and basic research in retinal disease. In 2004, four pharmaceutical company sponsored and three investigator initiated clinical trials were being conducted by the Unit. These studies addressed loss of vision in age-related macular degeneration and diabetic retinopathy.

Laboratory Research Unit

Retinal vascular diseases caused by abnormal leakage of fluid through small blood vessel walls (capillaries) are among the commonest causes of blindness – especially in diabetic retinopathy, retinal vein occlusion, ocular inflammation and the wet form of AMD. The Retinal Therapeutics Laboratory Research Unit studies the bimolecular determinants of leakiness in retinal capillaries using laboratory and animal models. The group also conducts preclinical studies into pharmacological and other therapies for the treatment of retinal vascular disease.

Projects in 2004

Clinical Projects

A randomised clinical trial of intravitreal triamcinolone for refractory diabetic macular oedema
Gillies, Zhu, Ali, Males, Sirimaharaj (Visiting Fellow), Sutter (Visiting Fellow).

Macular oedema is the leading cause of vision impairment from diabetic retinopathy. Laser treatment has been proven effective in reducing the risk of visual loss from diabetic macular oedema and is widely employed, but suffers the deficiency that it is inherently destructive. Progressive loss of vision occurs in up to 26% of patients with diabetic macular oedema despite laser treatment. There have been some uncontrolled and anecdotal reports recently that an intravitreal injection of triamcinolone (IVTA) may result in reduction of diabetic macular oedema and improved visual acuity. This project is a prospective, double-masked, placebo-controlled randomised clinical

trial to conduct a 2-year, single-centre, clinical trial of the efficacy and safety of intravitreal injections of triamcinolone acetonide for diabetic macular oedema that has failed laser treatment. Sixty-nine eyes of 43 patients have entered into the study. The results of this study will be directly relevant to patients with type I diabetes since it will provide data for use by clinicians that may reduce the risk of blindness from diabetic retinopathy.

Chorioretinal venous anastomosis for non-ischaemic central retinal vein occlusion
McAllister (Lions Eye Institute, WA), Gillies, Mitchell (Westmead Hospital), Zhu, Ali, Males.

Central retinal vein occlusion (CRVO) is a frequent cause of visual loss, especially in the elderly. Obstruction of central retinal vein may be partial or complete and can produce non-ischemic or ischemic CRVOs. The non-ischemic form initially is more common and vision is reduced due to retinal oedema. Even where the obstruction is eventually overcome, the prognosis for full return of central vision is poor due to macular damage from chronic oedema. A technique of venous anastomosis between the retina and choroid, using high intensity laser as a means of bypassing the obstruction has been created and that has been able to improve the success rate to 60% in a small trial over 5 years. The current project is a 3 year prospective, multicenter, randomised clinical trial to examine whether the use of high powered laser to create retinal vein bypass will benefit patients with a blockage of the central retinal vein. SSI is one of the main centre for the trial. We have enrolled 23 patients and 20 patients have reached their endpoints. The data analysis is ongoing.

Intravitreal triamcinolone versus laser for macular oedema secondary to retinal vein occlusion
Gillies, Larson (Visiting Fellow), Zhu, Ali, Males.

An occlusion of a vessel in the retina (retinal vein occlusion) leads to damage of blood vessels, causing them to leak resulting in oedema of the macula. The macular oedema is the main cause of loss of vision in eyes with retinal vein occlusion. Laser treatment can in some patients improve the visual acuity, but does not work in all cases. We have conducted a randomised clinical trial which has demonstrated that, at least in the short term, injection of a steroid, triamcinolone, into the eye reduces macular oedema and improves vision in 2/3 patients with macular oedema caused by diabetes that had persisted despite laser treatment. In many cases the oedema completely disappeared. This project is to conduct a 2 year, prospective, multi-centre, double-masked, exploratory, randomised, placebo-controlled trial in patients with branch retinal vein occlusion in order to assess if intravitreal triamcinolone is superior to laser treatment. Variables related to the progression or resolution of macular oedema will be examined in the study eye of all patients to determine the effects of treatment. In the meantime, we are recruiting patients.

Reduction in occurrence of centre-threatening diabetic macular edema (Sponsored by Eli Lilly)
Gillies.

Ruboxystaurin is a new class of drug that inhibits an enzyme, pkc, which is thought to be involved in leakage of the retinal blood vessels. We are testing whether this drug, which is taken as a tablet, can reduce the need for laser treatment in patients with diabetic retinopathy. This study will enrol eligible patients throughout 2005

A phase IIIb randomised, double-masked, active controlled, dose-ranging, multi-centre comparative trial, in parallel groups, to compare the safety and efficacy of intravitreal injections of eye001 (pegaptanib sodium, macugen) given every 6 weeks for 102 weeks, to pdt with visudyne, in patients with exudative age-related macular degeneration (AMD)
(Sponsored by Eyetech Pharmaceuticals)
Gillies, Males, Zhu, Luo, Ali, Sirimaharaj.

This is a multicentre, international, placebo controlled trial studying the safety & efficacy of an anti-vascular endothelin growth factor aptamer injected into the eye in patients with wet age-related macular degeneration.

A phase II randomised, controlled, double-masked, dose-finding, multi-centre, comparative trial, in parallel groups, to establish the safety and preliminary efficacy of intravitreal injections of EYE001 (Anti-VEGF Pegylated Aptamer), given every 6 weeks for 12 to 30 weeks to patients with clinically significant diabetic macular edema (CSME) involving the centre of the macula (Sponsored by Eyetech Pharmaceuticals)
Gillies, Males, Zhu, Ali.

This is a multicentre, international, placebo controlled trial studying the safety & efficacy of an anti-vascular endothelin growth factor aptamer injected into the eye in patients with diabetic macular oedema. Three Sydney Eye Hospital patients were enrolled and completed their treatment phase. Post treatment monitoring continues in this ongoing trial.

A Phase II, multicenter, randomised, double-masked, active treatment-controlled study of the efficacy and safety of RhuFab V2 (Ranibizumab) compared with Verteporfin (VisudyneR) photodynamic therapy in subjects with predominantly classic subfoveal neovascular age-related macular degeneration (Sponsored by Novartis)
Gillies.

Similar to Macugen, RhuFab also inhibits a growth factor (VEGF) that is thought to cause abnormal blood vessel growth in wet macular degeneration. This study is ongoing.

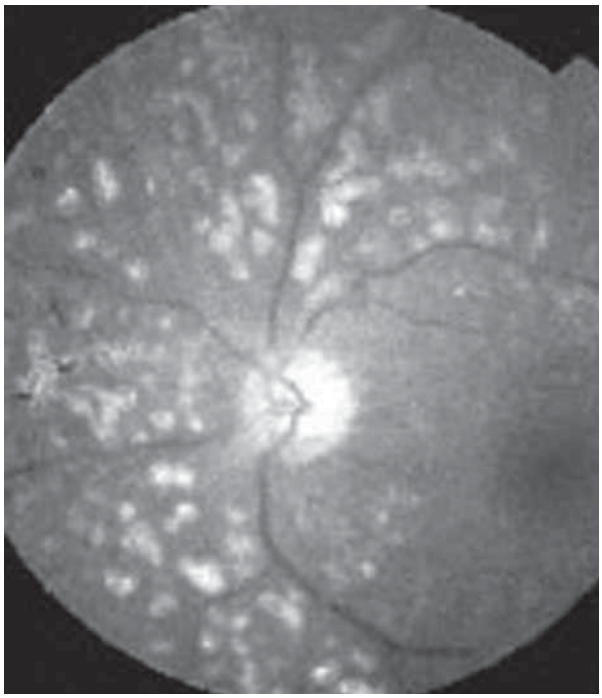
Laboratory Projects

The effect of paravascular cells on retinal permeability
Tretiach, Gillies.

It is likely that cells situated near the retinal blood vessels can influence permeability. In this project, Müller cells and pericytes have been co-cultured in our laboratory model of retinal vascular permeability. In addition to assessing the effects of these cells on vascular permeability, the effects of various conditions that mimic human disease such as low oxygen levels have also been assessed.

The effect of retinal laser treatment and pharmacological intervention on the permeability of retinal vessels
Quin, Windsor, Tretiach, Gillies.

While laser treatment of retinal swelling is often a very effective treatment, its mode of action is not at all understood. We propose that laser treatment induces the secretion of a protein that stabilises the "blood-retinal barrier". Preliminary laboratory experiments in which various retinal cell types are added to the vascular permeability assay suggest that this may indeed be the case. An animal model of early leak in the diabetic rat retina has been characterised to explore the effects of retinal laser treatment more fully. The identification of a barrier-restoring factor may have a therapeutic potential.



Fundus photo of the retina in diabetic retinopathy

The role of oxidative damage in diabetic retinopathy
Van Reyk, Gillies.

Whilst it is suspected that oxidative stress may contribute to diabetic retinopathy, the identification of those antioxidants that could be developed as a treatment is hampered by the lack of understanding of which of the many potential oxidative pathways in the retina is involved, and precisely what is being damaged. We are using high performance

liquid chromatography to detect the target types of oxidative damage to retinal proteins. This will identify the critical pathways involved which will then be targeted by specific antioxidants in animal studies.

The role of 'matrix metalloproteinase (MMPs) in the control of retinal vascular permeability
Wyndham, Gillies.

MMPs are a group of enzymes that can digest extracellular materials as well as cell surface receptors. The presence of MMPs was characterised in retinal vascular cells. The results indicate that MMP activity may be involved in the development of retinal swelling in diabetes, and that matrix metalloproteinase inhibitors may represent a new treatment for diabetic retinopathy

Autoantibodies in macular degeneration
Cherepanoff, Gillies.

Antibodies directed against retina are known to occur in the age related macular degeneration. This project will determine whether these antibodies have any effect on clinical outcome. A range of patient sera is in the process of being screened and the results will be correlated with the visual outcomes.

Connective tissue growth factor and retinal vascular disease
Wen, Gillies.

CTGF is a recently characterised growth factor which has an activity consistent with the regulation of blood vessel growth. We have found that retinal vascular cells become leakier when treated with CTGF. We have also found that CTGF increases the sensitivity of choroidal vascular cells to the leak-inducing factor VEGF. These findings suggest that CTGF may have a role in the development of vascular eye disease.

Retinal Cell Death and Survival Research Group

This group, now located in the Research School of Biological Sciences at the Australian National University, has been associated with the Save Sight Institute since 2002. The group has several collaborative projects with Save Sight Institute researchers and a growing track record of joint publications.

Group Members

Jonathan Stone, BSc PhD FAA, *Unit Head*
Krizstina Valter, MD PhD, *Senior Research Officer*
Diana van Driel, BSc, *Senior Research Assistant*

Research Activity

Research concerns the mechanisms which control the death and survival of neurones in degenerative retinal disease.

The group has made a series of findings novel in the understanding of photoreceptor death and survival in a range of rodent models and in the human.

New techniques and collaborations have been developed to take the analysis of the mechanisms of photoreceptor disease to the molecular level and to include the epidemiology of human disease.

Clinical trials have been organised to deploy some of this knowledge.

Projects in 2004

Current joint projects include the examination of mitochondrial specialisations in the normal and degenerative retina, and clinical trials of light restriction.