Cancer Breakthrough

Hon A/Prof Tracey Brown has been in the news this week as the scientist behind a cancer breakthrough. Dr Brown has developed a new drug delivery platform known as the Hyaluronan ChemoTransport (HyACT) Technology. The treatment makes chemotherapy up to 40 times more effective without an equivalent increase in chemotherapy side effects. It is currently being tested, with remarkable results, and overseas trials are planned to make it more widely available.

Those who are interested in the trial and have small cell lung cancer can contact: Peter Midolo, Research Manager, Monash Medical Centre on 03 9928 8195.

Synopsis

The basis of Dr Brown’s translational research at Monash University is using the naturally occurring polysaccharide, hyaluronan (HA) as a drug delivery vehicle that transports currently approved anti-cancer drugs to solid tumours which over-express activated CD44. The HA/drug extravasates into the tumour followed by active internalization of the HA/drug complex by tumour cells. The therapeutic end result of the increased drug uptake is increased efficacy and a reduction in the side-effects commonly observed when using currently approved cytotoxic drugs.

General Background on the Technology

In an attempt to increase the benefit associated with irinotecan-based treatment, and/or to reduce the dose-limiting toxicity often associated with this therapy, irinotecan has been formulated with the naturally ubiquitous polysaccharide, hyaluronan (HA), resulting in a proprietary product (HA-irinotecan). This product utilizes the unique physiochemical and biologic properties of HA as a macromolecular carrier of drugs to solid tumors. Several intrinsic characteristics of HA highlighted its potential as a drug delivery vehicle: i) the amphiphilic nature of HA enables it to form a large coiled meshwork at low concentrations making it an ideal vehicle for the solvation and entainment of smaller molecules; (ii) up-regulation of the HA receptor CD44 on malignant tissue where activation of the CD44 within the tumoral environment mediates HA internalization; and iii) HA is non-immunogenic and considered by regulatory bodies as a biologically-inert compound. After intravenous administration, the HA-derivitized drug rapidly enters the tumor and aggregates thereby forming a vascular microembolism within the tumor. of smaller molecules; (ii) up-regulation of the HA receptor CD44 on malignant tissue where activation of the CD44 within the tumoral environment mediates HA internalization; and ii) HA is non-immunogenic and considered by regulatory bodies as a biologically-inert compound.

Diagrammatic representation of the Mechanism of Action of the HyACT Technology

Continued Page 2
HyACT and Cancer Stem Cells

The target protein for the HyACT drugs is CD44. CD44 is a protein that is over-expressed on hyper-proliferative cancer cells and is also considered a reliable contributor to the CSC phenotype in breast, pancreatic, small cell and non-small cell lung cancer, colorectal and prostate cancer. In preclinical studies the Hyaluronan Laboratory demonstrated that HyACT drugs were capable of targeting chemotherapeutic drugs to cancer stems thereby overcoming treatment resistance and increasing tumour responses up to 40-fold. These data were presented at the American Academy of Clinical Research (AACR) in 2010 which prompted oncologists to consider using the lead HyACT drug, HA-Irinotecan as a cancer stem cell targeting therapy. Since 2010 Dr Brown’s team has completed the preclinical assessment of HA-Irinotecan in small cell lung cancer where it was substantiated that the HA-drugs could target and kill cancer stem cells.

Dr Vinod Ganju at Monash Medical Center has worked with the Hyaluronan Laboratory team and designed and initiated a randomized, Investigator-sponsored Phase II study in first-line, extensive stage small cell lung cancer patients where the primary end-point will be to quantitate the cancer stem cells (CD44+/CD133+/ALDH+/ABCG2+) before, during and after therapy. The secondary end-points will be to investigate the effect of the therapy on progression free survival and overall survival.

The Phase II trial was commenced 3 weeks ago and within 7 days after commencing therapy the initial two patients have demonstrated complete responses of metastatic lesions and 80-100% remission of the primary lung tumour. These data have obtained media attention because responses within such a short period is highly encouraging but also unexpected as normally such responses, if obtained would take >40 days in this patient demographic.

Determining the effect of hyaluronan on the internalization and intracellular localization of doxorubicin in Hs578T breast cancer cells

The Hs578T breast cancer cell line was seeded at 60% confluence on sterile glass coverslips. After 8h the cells were exposed to 800nM doxorubicin or 800nM HA-Doxorubicin. Coverslips were harvested at 2 hours after application of the test compound. At each time point the media was removed, cells washed with PBS and the coverslip removed from the well and placed in phenol-red free growth media. The internalisation of doxorubicin or HA-Doxorubicin was monitored using an Olympus FV1000 spectral confocal microscope (absorbance and emission wavelength of 600-650nm) where cells were incubated at 37°C in a temperature controlled observation chamber. White arrows indicate the nuclear envelope and vesicle-associated localization of the HA-doxorubicin or doxorubicin. The accumulation of doxorubicin was evidently increased in CD44+ cell subpopulations when formulated with the CD44-targeted HA drug delivery vehicle.

General background on the technology continued

After intravenous administration, the HA-derivated drug rapidly enters the tumor and aggregates thereby forming a vascular microembolism within the tumor. The intra-tumoral drug depot persists resulting in increasing drug accumulation and retention. The proposed mechanism of action follows that the increased intra-tumoral drug concentration enables the increased internalization of the anti-cancer agent via a CD44-mediated mechanism, ultimately enhancing efficacy. A secondary effect is the diversion of the drug from healthy tissue leading to a reduction in some commonly observed treatment toxicities. Phase I and II studies formulating HA with irinotecan, 5-FU or doxorubicin have been completed, each demonstrating the safety and efficacy of these combinations. In the randomized Phase II colorectal cancer (CRC) trial, in the 76 patients the HA-Irinotecan formulation significantly increased the progression free survival of third line CRC patients [5.2 versus 2.4 months (p=0.02)] and time to treatment failure [4 versus 1.8 months (p=0.01). Median survival was 10.1 months for HA-Irinotecan versus 8.0 months for irinotecan-alone patients (p=0.20). Disease control occurred in 76% of HA-Irinotecan patients and 46% in irinotecan-alone patients (p=0.05). Based on these data a global Phase III study in 400 second and third line CRC patients will commence within 2 months at 60 sites within the UK, Australia, Poland, Bulgaria, Russia, Ukraine and Serbia. After interactions with the US FDA and European Medicines Agency it has been agreed that if the Phase III study reaches its primary endpoint of increased progression free survival then HA-Irinotecan can be approved for sale in both Europe and the US. Marketing of HA-Irinotecan will be a major milestone for the Hyaluronan Laboratory, Department of Biochemistry and Molecular Biology, Monash University where the HyACT technology was invented and developed.
Department of Biochemistry and Molecular Biology

Postgraduate Research Scholarship

The Department is instituting a Golden Jubilee Postgraduate Research Scholarship to celebrate the 50th anniversary of the founding of the Department at Monash University's Clayton campus in 1951 and the subsequent achievements of its staff and students.

The Scholarship will be offered annually (commencing 2012) to the Department's top-ranked recipient who has demonstrated outstanding academic merit in the most recent end-of-year Monash University Postgraduate Research Scholarship Round.

For further details, please visit the Biochem webpage at http://www.med.monash.edu.au/biochem/

Conditions of the award can be found at http://www.med.monash.edu.au/biochem/golden-jubilee-scholarship.html

Seminars in October

4 pm on Wednesdays in Building 13A, Lecture Theatre M2

October 5
Victoria Haritos (CSIRO)

Cellulase gene discovery and characterisation from Australian fauna: applications to biofuels production

October 6
Howard Riezman (University of Geneva)

Lipid Homeostasis and Function in Model Organisms and Cells

October 12
Kip Gabriel (Monash, Biochemistry Dept)

Bacterial Toxins Targeted to Mitochondria

October 19
NOT PROFs

Dr Luc Furic:
Survival signalling in prostate cancer: eIF4E at the crossroads of PI3K and MAPK pathways

Dr Alison Thorburn:
A Streptococcus Pneumoniae-based Immunoregulatory Therapy for Asthma

LATEST MEMBERS OF STAFF

<table>
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<tr>
<th>Lab Head</th>
<th>New Staff Member</th>
<th>Position</th>
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<td>Prof Jamie Rossjohn</td>
<td>Jennifer Ly Huynh</td>
<td>Personal Assistant</td>
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<tr>
<td>Dr Tracey Brown</td>
<td>Olivia Watson</td>
<td>Technical Assistant</td>
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NOTDRS in conjunction with the Monash Institute for Medical Research (MIMR) student committee held a bowling night at Strike Bowling on Chapel St on Friday 23rd September. Around 30 students and young staff from both institutes showed off their bowling style, and the opportunity was there to meet fellow students from MIMR.

Natalie Rynkiewicz
President

Mouse hair follicles imaged in 3 dimensions by confocal microscopy.

Courtesy Prof Ian Smyth
Monash's Clayton campus has been a second 'home' for many years. Being born and bred in Melbourne's Eastern suburbs it won't surprise many that I attended Monash (Clayton) as an undergraduate. I came with little idea of what I wanted to do in the future except a Science degree. After taking first year biology I was hooked on genetics and molecular biology and ended up majoring in Biochemistry and Genetics. My Honours year and PhD were completed in the "molecular biology" group of the Biochemistry Department, led by Tony Linnane, which at that time focussed exclusively on questions of mitochondrial biogenesis using yeast as a model system. At that time (mid 1970s) we barely understood that the mitochondrial genome was circular let alone what it encoded and my project dealt with characterising mitochondrial mutants.

I submitted my thesis in December 1979 and a few days later my wife Eileen and I left Melbourne for Iowa in the US midwest and the University of Iowa (Iowa City). We went from high 30s to about minus 40°C when you factor in wind chill. It took a week to feel warm even indoors. I joined Carol Newton's lab in the Dept of Zoology (now Biology) working on characterising the replication origins of a ring derivative of yeast chromosome III (after I managed to figure out how to isolate some of that structure intact!). My position involved some teaching and I found my self in front of one of 25 sections of first year biology students (sessions ran everyday of the week including Sunday!) Iowa City was a great place to live; a typical US college town where people did not lock their back doors or their cars. During this time the opportunity to attend a Cold Spring Harbor Yeast meeting proved invaluable for establishing contacts and collaborations.

In 1982 we left Iowa to return to Melbourne prior to the birth of our first child. It wasn't my deliberate intention to return to Monash, but I did so being one of a small group of inaugural awardees of the Vice-Chancellor's Research Fellowship. I was funded to work on construction of yeast expression vectors for expression in yeast of heterologous proteins of therapeutic interest including the interferons. Remember that this was pre PCR; cloning, cutting and pasting of DNA were not anywhere as routine and kit driven as they are now. After two and half years as a Fellow I accepted a position as Lecturer and stared my journey up the academic staff ladder. Newly appointed junior staff did not get the pick of lecturing topics and I did stints delivering to second-year students some of what would still be regarded as the least interesting topics in Biochemistry. I was promoted to Senior Lecturer in 1989, to Associate Professor in 1996 and then to Professor in 2005.

Involvement in graduate matters

Twenty years ago I was appointed Convenor of the Departmental Graduate Matters Committee. I was delegated complete responsibility for administration of graduate matters. Perhaps to the surprise of some I decided the position entailed more than this and sought to maintain a high level of pastoral as well as administrative oversight of students and their progression through candidature. This was not always easy at certain times! I am particularly proud of my role in initiating and implementing in 1995 our annual Departmental Student Research Conference which focuses on the achievements of our third year research students. Later on I became Associate Dean (Research Degrees) with responsibility for higher degree research training in the Faculty of Medicine. In 2004 I took on my current role as Deputy Chair of the University’s Research Graduate School Committee and notionally spend 40% of my time in this role. Thanks to the support of my students I have been honoured to receive a Special Commendation, Vice-Chancellor’s Awards for Postgraduate Supervision (2006) and be awarded the Monash Postgraduate Association, Supervisor of the Year (2008).

Research

For about a decade my principal research effort was directed towards understanding the structural and functional organisation of the yeast mitochondrial ATP synthase (mtATPase) complex. This molecular machine is critical for cellular energy production, especially as the body turns over its own weight in ATP each day. Determining its complete structure and mechanism of action remains one of the great challenges of research in biology. We made contributions regarding: the mtATPase subunits encoded by the mitochondrial genome which have a critical role in function of the proton channel in ATP synthesis; the non-membrane proteins that make structural and functional connections between the catalytic component and proton channel of the complex; and the importance of the correct arrangement of mtATPase complexes within the mitochondrial inner membrane for the genesis and/or maintenance of mitochondrial cristae and morphology. The latter aspect applied innovative approaches based on the use of fluorescent proteins developed in collaboration with Mark Prescott. A highlight during this period was the award in 1998 of an Australian Academy of Science, Bede Morris Fellowship to work with collaborators based in Bordeaux. My stay just so happened to coincide with the French defeating Brazil in the world cup final and consequent healthy consumption of red wine from the nearby St Emilion vineyards.

About a decade ago I became interested in how mitochondria are turned over, which I saw as a logical progression from considering aspects of their biogenesis. This in turn led to consideration of the possible role of autophagy in mitochondrial turnover and quality control. In collaboration with Mark Prescott, a 'discovery pipeline' has been established to systematically screen a number of different yeast gene libraries and identify the molecular components and networks required for the regulation of autophagy of mitochondria (mitophagy).

In mammals autophagy is involved in a variety of processes such as programmed cell death and development of different tissue-specific functions, as well as pathological conditions including infection by pathogenic bacteria or viruses. Together with Ben Adler and John Boyce (ARC Centre of Excellence in Structural and Functional Microbial Genomics) the molecular mechanisms by which the soil bacterium, Burkholderia pseudomallei, avoids destruction by autophagy are being investigated. In humans this infection leads to Melioidosis, a disease with high rates of mortality which is endemic in tropical and subtropical areas (including Australia). We have found that an autophagy-related pathway, LC3-associated phagocytosis (LAP), provides a level of defence for macrophage cells against invading B. pseudomallei. However LAP is relatively ineffective and most bacteria escape to the cytosol where they efficiently evade subsequent capture by canonical autophagy. We are seeking to identify bacterial proteins that act as effectors to modulate host cell biology with a view to eventually being able to overcome the failure of autophagy in host cell defence.
POSTGRADUATE MATTERS

Faculty of Medicine Research Supervisor Accreditation Level 1 Workshops - Semester 2, 2011

Online Registration now open. Please note, Your Monash login details will be required.

*Accreditation is compulsory for staff intending to supervise postgraduate research students (with more than 25% supervision load)

Intending research supervisors need to complete a series of nine modules. Approximately 80 per cent of the requirements of these nine modules can be covered by attendance to both of the Faculty’s Research Supervisor Accreditation Training workshops.

Dates and time of workshops

The accreditation program is split into 2 workshops (attendance to both workshops is required) for prospective supervisors (Monash staff members only- your staff ID number will be required) from all departments within the Faculty of Medicine, Nursing & Health Sciences.

• Workshop 1 will be held on Friday 4th November 2011
• Workshop 2 will be held on Wednesday 9th November 2011
• Registrations from 9.20am, with each workshop concluding around 12.40pm
• Venue: S2 Lecture Theatre, Building 25, Science Precinct, Clayton Campus

You will need to have a mentor allocated to you by your department or centre before you may attend the workshop. These workshops are run free of charge.

To apply for a place at the workshops, please register online:
http://www.mrgs.monash.edu.au/seminars/medicine/

*Registrations close Thursday 27 October 2011

Queries: Please contact Phyllis Di Palma, (Research Degrees Office, Faculty of Medicine, Nursing & Health Sciences) on ext 20047 or email Phyllis.DiPalma@med.monash.edu.au

For further biochemistry news, please visit our website: www.med.monash.edu.au/biochem
During my undergraduate studies at Monash University, I undertook two summer vacation scholarships in Professor James Whisstock’s laboratory, located in the Department of Biochemistry and Molecular Biology. After the completion of my Bachelor of Science degree (majoring in Biochemistry and Genetics), I was offered an honours project in the Whisstock laboratory, under the co-supervision of Dr Ruby Law and Mrs Qingwei Zhang. This project focused on the Serine Protease Inhibitor (Serpin) Tengpin, found in the thermophilic, anaerobic bacteria *Thermoanaerobacter tengcongensis*, which grows in an extreme hot spring environment in Tengcong (China). Serpins from thermophilic organisms are of special scientific interest, because while eukaryotic serpins are extremely labile (i.e. upon mild heating they spontaneously fold to an inactive latent state or polymerize), thermophilic serpins can function and remain stable at high temperatures. Importantly, in humans, serpin polymerization is the molecular basis for many diseases (termed “serpinopathies”) caused by the formation of misfolded serpin polymers or serpin protein aggregates. Therefore, our studies on serpins from thermophilic organisms were conducted to give insight into how these serpins evolved to overcome the inherent metastability of the serpin fold, in order to be able to function in extreme heat.

Our investigation indicated that in Tengpin, the first 56 amino acids at the N-terminus (which is not part of the serpin domain) have a critical role in its folding and thermal stability. The structural determination / comparison of both native and latent Tengpin conformations, combined with equilibrium refolding experiments and extensive truncation / mutagenesis studies, revealed crucial interactions by the N-terminus extension are involved in maintaining Tengpin in its metastable, native (inhibitory) conformation, and prevents spontaneous folding to the latent (non-inhibitory) state. This is similar to the role played by a co-factor called vitronectin in stabilizing the human serpin Plasminogen Activator Inhibitor-1. Part of my honours project on Tengpin contributed to a paper published in the journal EMBO reports in 2007. (Zhang et al., EMBO reports 8: 658-663).

After successfully completing my honours year, I started work as a Research Assistant in the Whisstock laboratory, and have been working there since mid 2007. During this time part of my research has been investigating the structure and function of the MACPF/CDC superfamily of pore forming toxins. Examples of this superfamily include bacterial virulence factors (e.g. Cholesterol Dependent Cytolysins, CDCs) and proteins of the immune system (also called the Membrane Attack Complex proteins and perforin, MACPF). CDC toxins are secreted by a wide variety of Gram-positive bacteria (e.g. *Clostridium perfringens*, *Bacillus anthracis* and *Streptococcus pneumoniae*), to aid in tissue or cell invasion. In comparison, MACPF proteins (eg. complement proteins C6, C7, C8α, C8β and C9, as well as perforin), perform critical roles in the defense against bacterial and viral infections, as well as performing roles in tumour surveillance. The MACPF/CDCs are synthesized as monomeric soluble proteins, and upon binding to their target membrane, these monomers firstly oligomerized, and then change conformation. This conformational change results in two α-helical regions (TMH1 and TMH2) unwinding to form four amphipatic β-strands, which then insert through the target cell membrane, thereby forming a pore.

Currently I am working on two different MACPF proteins, to better understand the molecular mechanism of membrane insertion, and also determine the specific interactions MACPF proteins use to target and bind to cellular membranes. One of the proteins I am working on is called pleurotolyisin; it is originally isolated from a carnivorous oyster mushroom called *Pleurotus ostreatus*. Under the supervision of Dr Michelle Dunstone, Dr Tamas Hatfaludi and collaborative work with Professor Rodney Tweten (University of Oklahoma, College of Medicine), our laboratory has conducted experiments to determine the membrane spanning regions of pleurotolyisin. Via site-directed mutagenesis, many unique cysteines were introduced into the putative membrane-spanning regions; these cysteines were used for florescence labeling and membrane binding analysis.

The other protein I am studying is called perforin. Recently Dr Ruby Law from our laboratory solved the structure of the mouse perforin monomer (Law et al., 2010. Nature 468: 447-451). This paper revealed the molecular mechanism of perforin’s pore formation by X-ray crystal data coupled with cryo-electron microscopy reconstruction of the perforin pore. However, what still remains unknown is the interactions perforin uses to bind to its target membrane. Studies on perforin have revealed the C2 domain (a calcium-dependent phospholipid binding domain) is responsible for the initial interaction with the cell membrane. Therefore to investigate these cellular interactions, under the supervision of Dr Ruby Law and Dr Daouda Traore, our laboratory has isolated the C2 domain of mouse perforin. We have also cloned, expressed and purified the perforin-like C2 domains of *Scophtamus maximus* and *Sparus aurata*. The intact C2 domains of these two species of fish are active proteins involved in immune response, and can therefore be considered as good models for studying the structural basis of calcium and membrane binding of the perforin C2 domains. We recently solved the crystal structures of these proteins, revealing they match the conserved architecture of the C2 domain (consisting of eight antiparallel β-strands connected by flexible loops, to form a β-sandwich). We also identified in our structures, the fully occupied calcium binding sites. Not only are these calcium binding sites important in stabilizing flexible loops in the structure, they are possibly involved in interactions with the negative head group of membrane phospholipids. Although some progress has been made, still very little is known about the molecular mechanism of C2 domain binding to cellular membranes. Our laboratory is trying to address this question, by the co-crystalization of C2 domains with a wide variety of lipids.

During the course of my career I have been privileged to be able to attend many local protein conferences, including the Melbourne Protein Group, the Lorne Protein Conference, and present at poster on my work conducted into Heat Shock Protein 47 at the International Proteolysis Society conference. I would like to take this opportunity to express my many thanks to Professor James Whisstock for his supervision and allowing me to work in his laboratory. I would also like to thank my laboratory supervisors Dr Ruby Law, Dr Daouda Traore, Dr Michelle Dunstone, Dr Tamas Hatfaludi, and all members of the Whisstock laboratory for their help and guidance over the past 5 years.
STUDENT SOCIETY

NOT DRS

Necessary Outlets for Tertiary Doctoral Research Students

To find out more about NOTDRS please visit:

Or find us on facebook:
86877543416

Ben Lang - Treasurer
Susie Berkowicz - Communications officer
Miranda Wills - 2nd year rep
Stephen Scally - 1st year rep
Min Yap - Secretary
Natalie Rynkiewicz - President

OHS MATTERS

IMPORTANT ANNOUNCEMENT

Contacting Emergency Services

In the event of an emergency where emergency services (ambulance, police, fire brigade) are required, immediately DIAL (0) 000 from a university phone and notify the relevant service followed by security 333 directly after to arrange an escort. All emergency services always report to the Monash Security Building upon arrival for escort. Security has advised there have been several instances where they have not been notified resulting in time being wasted trying to determine which building requires assistance.

OHS Student Representative

The Biochem OHS Committee is looking for nominations for the position of Student Representative. Any PhD student willing to take on this role can apply to the committee by sending their nomination to Craig Don Paul.

QUICK OVERVIEW OF WHAT TO DO WHEN AN EMERGENCY ARISES:

1. Remain CALM...
2. Yell out for a First Aider (don't go looking for one yourself, get someone else to go looking)
3. First Aiders: Read MSDS before treating any chemical injury
4. First Aiders: Call Med Centre if necessary ext. 53175
5. First Aiders: Call the Safety Officer and/or Safety Representative as soon as possible
### RECENT PUBLICATIONS

| 8 | Nagley, P., *Department of Biochemistry and Molecular Biology, Monash University*. Australian Biochemist 2011. 42(2): p. 43 |

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