Report to the ANZBMS Council on travel undertaken with funding from the Christine and T. Jack Martin Travel Grant

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Last year at the Annual Scientific Meeting of the Australian and New Zealand Bone and Mineral Society I was awarded the Christine and T. Jack Martin Travel Grant. These funds enabled me to visit several prestigious international laboratories to present my PhD research and develop new experiments and learn new methods. Also, I attended the 30th European Symposium on Calcified Tissues where I presented my recent research and received a Novartis Young Investigator Award.

30th European Symposium on Calcified Tissues

The European Calcified Tissue Society (ECTS) is the major organisation in Europe for researchers and clinicians working in the field of calcified tissues and related subjects. Around 2000 delegates, both clinicians and basic scientists from a wide range of disciplines attended the ECTS meeting this year in Rome, May 8th - 12th. The meeting included seven symposia on all major areas of bone research: Osteoblasts, Sex Hormones, Osteoclasts, Genetics, Hormones, Other Bone Diseases, and Osteoporosis Treatment; six workshops on diverse topics such as Nanotechnology, Skeletal response to Mechanical load, Inflammation in Bone and Children's Bone Diseases; and nine Oral Communication Sessions. In addition 440 posters were presented during the meeting.

I was invited to give an oral presentation describing my recent research into the mutation in OPG. My paper entitled 'Characterisation of an inactivating mutation in Osteoprotegerin' was awarded a Novartis Young Investigator Award.

A highlight of the meeting was the Symposium on Osteoclasts with presentations by David Lacey of Amgen Inc. and Lorenz Hofbauer from Philipps-University, Germany both concerning the RANKL/RANK/OPG system. The presentation by David Lacey was very informative and summarised much of the research undertaken at Amgen in the development of OPG as a therapeutic. Dr Lacey gave more in-depth information about the results of the two Phase 1 clinical studies, the first in osteoporotic women and the second study of patients with breast cancer or multiple myeloma related bone metastases. Also for the first time it was revealed that Amgen have been developing RANKL antibodies as a therapy for both osteoporosis and bone metastases. The RANKL antibodies are specific for a particular domain of RANKL and do not cross-react with the related molecules CD40L, TRAIL or TNF . Initial murine studies using the antibodies indicate effective inhibition of bone resorption. Dr Hofbauer presented the relevance of the RANKL/RANK/OPG system in clinical practise, in pathogenesis and in treatment of disease and use of the RANKL:OPG ratio as a biochemical marker. Dr Hofbauer also outlined the effect of different molecules to increase or decrease expression of OPG and RANKL including 17 -estradiol, raloxifene, phytoestrogens and rapamycin. The involvement of RANKL in rheumatoid arthritis and breast cancer and multiple myeloma related bone metastases was described, as well as RANKL blockade through OPG therapy to prevent bone erosion.

Another highlight was the Workshop and Symposium on Children's Bone Disease. Five types of bone disorders were described including the clinical features and genetic causes. Particularly interesting presentations during this session were given by Dr Nick Bishop on Osteogenesis Imperfecta, Dr Jens Bollerslev on Autosomal Dominant Osteopetrosis and Dr Wim van Hul on Sclerosing Bone Dysplasias. The workshop on Nanotechnology and Bone was also very intriguing. A presentation by Dr Wilson Poon from Edinburgh University described the future of smart silicon chips for biology. The oligonucleotide chips currently used in microarrays are an example of a 'passive' system, Dr Poon outlined how 'smart silicon' chips of the future will be 'active' systems allowing real-time manipulation of hybridisation or binding by the use of multiple electrodes to alter the local charge at the chip surface.

Other interesting presentations were the Keynote Address by Pekka Kannus from Finland on 'Pathogenesis and Prevention of Fractures among Elderly People', and a presentation by Dr Tim Skerry at the workshop on Skeletal Response to Mechanical Load about Neurotransmitter function in bone.

Laboratory Visits

Principal Laboratories

Amgen Inc, Thousand Oaks California USA

Research at Amgen is focused on five therapeutic areas: Cancer Biology, Inflammation, Metabolic Disorders, Neurology, and Haematology. Potential targets are identified using genomics, microarrays and bioinformatics, and academic collaborations. Research relating to bone disease is pursued by both the Inflammation and Metabolic Disease research groups. The Inflammation research group is strongly focused on rheumatoid arthritis (RA). The Metabolic Disease group as well as research into diabetes has a strong interest in bone and joint diseases, including osteoporosis and osteoarthritis.

Osteoprotegerin (OPG) was discovered by Amgen's Genomics Program and has been investigated at Amgen for use as a therapeutic for osteoporosis, and a preventative of bone erosion in arthritis and bone metastases.

As Amgen has been studying OPG for approximately 10 years, visiting the production and protein analysis groups at Amgen was an ideal opportunity for me to discuss my investigations into the mutation in OPG and the difficulties I have had in the production and purification of the mutant OPG. I was hosted by Dr Scott Simonet the head of the research group that first identified OPG and characterised its function. We discussed how OPG was first identified and the process of its development as a therapeutic. There were two main research groups I interacted with, the protein analysis group both using Biacore surface plasmon resonance technology and structure analysis techniques, and also the protein production and purification group.

The primary reason to visit Amgen was their familiarity with OPG and with the surface plasmon resonance technology of Biacore™. The protein analysis group at Amgen utilise this technology extensively, replacing other common techniques such as ELISA, radio-ligand binding, concentration determination and affinity testing. From this group I met with Dr Luke Li head of the group, Dr Shinchiru Okazaki and Dr Ching Chen. Dr Chen was very helpful in analysing the results of the Biacore™ experiments we have carried out in Auckland investigating the different binding behaviour of the wtOPG and OPG 182 to RANKL. Dr Chen provided her insight into the differences between the two basic binding curves and the results of the kinetic and steady state analyses. We designed a further set of experiments to confirm the results. These experiments are designed to reduce the complexity of the binding interaction.

From the process group I met with Dr Grant Shimamoto and Dr Randy Heacht. We discussed the problems I have had in the production of wtOPG and OPG 182. We have determined methods to improve the purification procedure and analysis using the current production system and also formulated an alternative production protocol for any future investigations in the lab requiring the production of proteins. Also during the visit we discussed how scale up of production was considered during the research phase for proteins. I was shown the production facility for large scale production of proteins for research and toxicology testing and also the pilot plant. It was very interesting to compare the two different facilities, for *E. coli* and mammalian cell culture.

Nuffield Orthopaedic Research Centre, University of Oxford, Oxford UK

Raj Thakker is the May Professor of Clinical Medicine at Oxford University. His research group comprises 15 researchers including DPhil students, post-docs and research fellows. The group has previously identified the genetic cause of several metabolic disorders. Each of the disorders is an entry point for the analysis of the biochemical pathway involved. The group is now focussing on developing cellular models that will characterise these pathways. At the Nuffield Orthopaedic Centre I spent most of the day with Fiona Wu a DPhil student and Dr Paul Christie a senior member of the lab. I discussed with them and other researchers in the lab how they would use the mouse knock out models to achieve functional characterisation of mutations linked to disorders. An alternative animal model used in the lab is chemical mutagenesis using the alkylating agent ethylnitrosurea (ENU). ENU treatment leads to mice with varying phenotype and those most similar to a certain disease i.e. development of kidney stones can be investigated to determine the biochemical pathway involved.

I presented the OPG project to several research groups at the research centre and there was a lot of interest in the project with many questions about the phenotype and treatment of the patients.

Later in the day Professor Jack Martin gave the first lecture in the GlaxoSmithKine lecture series at Sommerville College on Skeletal Complications of Cancer. This was a prestigious lecture, attended by many academic staff

from the University of Oxford. The topic was very interesting, focussing on the various hypotheses surrounding why some cancers (breast/prostate) have the highest incidence of metastasis to bone. Professor Martin described the chemotaxic signals that lead to cancer cells 'homing' to bone, and also the 'seed and soil' hypothesis of Steven Paget whereby a receptive environment in bone allows cancer cells to promote bone resorption and form a secondary tumour.

Bone Research Group, University of Aberdeen, Aberdeen UK
There are six major research areas within the bone research group headed by Professor Stuart Ralston: Genetics of bone disease, Nitric oxide in bone, Mechanisms of bisphosphonate action, Biology of the osteoclast, Cell adhesion, Cancer associated bone loss.

A major reason to visit this group was to learn their method of RNA extraction from whole bone samples. Dr Tracy Stewart and Dr Val Mann have developed the protocols used in the laboratory for DNA and RNA extraction from bone biopsy samples. Dr Tracey Stewart gave me an overview of this method and showed me practical tips to improve yield and purity. This new method will be very important for the bone group in Auckland in the investigation of altered gene transcription at the site of bone disease.

I also spent a lot of time with two researchers from the Paget's disease research group. Dr Lynne Hocking was the main researcher in the identification of susceptibility loci on chromosomes 2q36, 5q35 and 10p13 and also the identification of mutations in sequestosome 1. Dr Hocking is currently planning a project to identify the functional significance of the mis-sense mutations in the ubiquitin binding domain. Dr Anna Daroszewska is working on the functional significance of an OPG polymorphism that has been linked to low BMD. I discussed with them their approaches to produce the proteins and determine any functional changes.

I presented my PhD research to the Paget's disease group and was very interested in the questions asked about the genetic analysis and the differences between the patients and the OPG knock-out mice. We also discussed the change in bone formation that occurs in patients with OPG deficiency or RANK over-activation.

Additional Laboratories

Derynck Lab, Department of Growth and Development, UCSF
Unfortunately Rik Derynck was not very well the day I visited his research group, however we did have a discussion of the methods from my PhD research utilised in his lab. I also presented my research into the OPG mutation to the group. The lab consists of 14 post-docs and a PhD student on rotation. I was very interested in the projects within the lab to manipulate mesechymal cell differentiation. Recently the group has published the observation that BMP and retinoic acid signalling co-operate to induce preadipocytes to form osteoblasts. This investigation could have applications in surgery for bone engraftments to use patient derived adipocytes to reduce the amount of bone required to replace that removed e.g. jaw tumour removal.

Bourne Lab, Department of Medicine and of Cellular and Molecular Pharmacology, UCSF
The visit to the Bourne Lab was very interesting. Over two days I met with Professor Bourne and members of his lab individually to discuss my project and the research of each person.

The lab consists of 8 post-docs a technician and lab manager. The focus of the lab is chemotaxis, specifically of neutrophils. The cell source for the lab is a leukaemia cell-line with neutrophil precursor phenotype. These cells can be chemically treated to induce the neutrophil precursors to differentiate to neutrophils which are studied in the lab.
The process of chemotaxis is controlled by a G-protein coupled receptor with downstream signalling events leading to the formation of a distinct 'front' (pseudopod) and 'back'. Using G protein inhibitors, specific PIP3 isoform inhibitors and dominant negative or positive transfection systems the lab is elucidating the pathways that cause the cell to polarise and maintain its 'compass'.

Overall Outcomes

It was very beneficial to speak with researchers at Amgen who are very familiar with OPG protein and also the SPR technology of Biacore. The help of Dr Chen to offer her thoughts on the altered binding behaviour of OPG 182 and her help to design further experiments will directly affect my PhD thesis and the second paper describing the characterisation of the OPG 182 mutation. The discussions with Dr Shimamoto and Dr Heacht will influence the approach taken by the Bone Group in Auckland in the production of recombinant proteins.

The animal model approach used in Professor Raj Thakker's group to investigate the function of genetic mutations in human metabolic disorders was very interesting and these methods could be very useful in the investigation of other bone disorders within our laboratory. Similarly, the new whole bone RNA extraction method I learnt at the Bone Group in Aberdeen will be very useful, both in my own research and to other members in the Bone Group in Auckland.

Presenting at the ECTS meeting was a great experience and I was honoured with a Young Investigator Award. The questions and discussion after my presentation, and also at the laboratories I visited, have identified points I will need to address in my PhD thesis regarding the differences between the phenotype of OPG knock-out mouse and the patients reported with OPG deficiency. Also the meeting highlighted for me the diverse range of research interests within the field of bone research. I was able to learn more about areas of research I may pursue in post-doctoral studies.

I would like to thank Merck, Sharpe and Dohme and The Australian and New Zealand Bone and Mineral Society for providing me with funds which enabled me to visit these prestigious laboratories and attend the ECTS conference. I feel very privileged to have received this award.

Thank you once again

Catherine Middleton-Hardie