

Under the Microscope

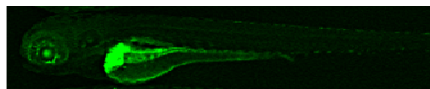
Research Highlights from the Ludwig Institute for Cancer Research Melbourne Tumour Biology Branch

Zebrafish feeling green around the guts.

The idea that some of the genes governing embryonic development of the gut are also involved in the formation of colorectal cancers has led to a great deal of interest in defining the changes in tissue organisation that occur during gut development and the genes that drive these events. Because scientists cannot study gut development in human embryos, instead they use experimental animal models. Surprisingly, the zebrafish, a small, tropical freshwater fish often found in the home aquarium, is an ideal animal model for studying the relationship between genes and gut development. Zebrafish breed rapidly and their embryos reach maturity in two to three months. Zebrafish embryos are transparent, allowing easy viewing of organ development in live animals.

Despite the transparency of zebrafish embryos, the developing gut is still difficult to study as it is located deep inside the embryo. To overcome this difficulty, members of the Colon Cell and Molecular Biology Laboratory have used a genetically modified zebrafish, called gutGFP, to allow them to visualise the cells in the developing gut. Genetic engineering was used to make the cells in the gut contain a protein, called GFP, which is normally found in jellyfish that glow in the dark, resulting in the guts of transparent zebrafish embryos fluorescing bright green under blue light.

The group studied the formation and shape of the developing fluorescent green gut. They found that, as is the case in humans, the zebrafish gut is formed from a specialised ribbon of cells called endoderm. In early gut development, all the cells in the endoderm appeared to be the same. However, soon afterwards, starting at the position of the future mouth, the ribbon of endoderm organised itself into a tube around a hollow called the lumen. Unlike tube formation in some other organs, formation of the gut lumen did not involve cell death. Next, the cells lining the gut reorganised themselves to form a single layer. Again, this process did not appear to involve programmed cell death. Later, gut cells began to acquire specialised functions. The three types of gut cells in the zebrafish were found to be essentially the same as those in the human gut. Most gut cells are used to absorb nutrients from digested food and these are called enterocytes. A small number of specialised gut cells, called goblet cells because of their goblet-like shape, secrete a lubricant into the gut lumen. A few gut cells, called enteroendocrine cells, become specialised in the production of hormones. Of all the cell types in the developing gut, these are the hardest to see due to their low numbers and small size. To overcome this, the group and their collaborators have genetically engineered



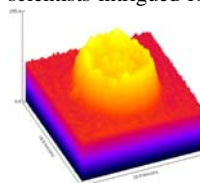
zebrafish so that the enteroendocrine cells are the only cells in the gut that are fluorescent green. They were then able to clearly visualise the enteroendocrine cells as they developed in the embryonic gut, and to show that they did indeed produce hormones such as glucagon and somatostatin.

Having systematically analysed and described the formation of the embryonic gut in zebrafish, members of the Colon Cell and Molecular Biology Laboratory have created a useful framework for the characterisation of zebrafish with mutations in genes that affect gut development.

Ng, A.N., de Jong-Curtain, T.A., Mawdsley, D.J., White, S.J., Shin, J., Appel, B., Dong, P.D., Stainier, D.J. and Heath, J.K. (2005). Formation of the digestive system in zebrafish: III Intestinal epithelium morphogenesis. *Dev. Biol.* **286**, 114-135.

How many EGF receptors does it take to send a signal?

The binding of a small protein hormone called EGF to its receptor on the cell surface sends a signal inside the cell, telling it to grow and divide. Amazingly, this relatively simple interaction between two proteins has kept scientists intrigued for nearly two decades. Early studies showed that EGF binding to EGF receptors caused them to form pairs. This became the standard working model for the mechanism of interaction between many growth factors and their receptors.



In 2003, members of the Melbourne branch of the LICR and their colleagues made this interaction visible with their 3-dimensional picture of an EGF-related growth factor in a complex with a section of the EGF receptor that is normally on the outside of the cell (Under the Microscope Issue 6). The picture showed two EGF receptors back-to-back with each other, and each with a growth factor molecule bound to their front.

This year, members of the Cell Biophysics and Epithelial Biochemistry Laboratories have added more detail to this picture. They have used sophisticated, high-resolution fluorescence microscopy to look at the EGF receptor in its natural environment on the surface of a cell. In this situation, the majority of EGF receptors were paired, even when EGF was not bound. Binding of EGF caused most of the EGF receptors to organise themselves into groups of four, although some smaller groups also existed. Within these groups of four EGF receptors, measuring the distance between two fluorescently-labelled EGF molecules suggested that they were closer to each other than would be expected from the 3-dimensional picture described above. This suggests that two back-to-back EGF/EGF receptor complexes come together and stand side-by-side, placing two EGF molecules next to each other. The Cell Biophysics group speculates that, because it is the major form of the receptor when EGF is bound, a pair of back-to-back complexes is the active, signalling form. It seems that pairing off is not enough, and that it takes four EGF receptors and four EGF growth factors to send a signal inside the cell.

Clayton, A.H., Walker, F., Orchard, S.G., Henderson, C., Fuchs, D., Rothacker, J., Nice, E.C. and Burgess, A.W. (2005). Ligand induced dimer-tetramer transition during the activation of the cell surface epidermal growth factor receptor - A multidimensional microscopy analysis. *J. Biol. Chem.* **280**, 30392-30399.

A33 targets colon tumours.

The A33 antibody was isolated at the LICR and subsequently shown to recognise cells only in the large and small intestine and in colorectal cancers. It has shown promise in early phase clinical trials due to its ability to specifically target metastatic colorectal cancer.

A humanised version of A33 (huA33) was constructed to minimise adverse immune responses in patients and produced under GMP conditions by the LICR Biological Production Facility. Members of the Clinical Program at the Austin Hospital have described two Phase I clinical trials with radioactively-labelled huA33 (¹³¹I-huA33) in patients with advanced colon cancer and shown that it has many of the characteristics required by an anti-tumour agent. ¹³¹I-huA33 was able to rapidly and specifically target the radioactivity to colon tumours and was not toxic to patients at doses required to treat colon tumours. Contrary to most similar studies, they found that ¹³¹I-huA33 was evenly distributed in even large tumours after a single dose.

Chong, G., Lee, F.-T., Hopkins, W., Tebbutt, N.C., Cebon, J.S., Mountain, A., Chappell, B., Papenfuss, A., Schleyer, P., U, P.L., Murphy, R., Wirth, V., Smyth, F., Potasz, N., Poon, A., Davis, I.D., Saunder, T., O'Keefe, G.J., Burgess, A.W., Hoffman, E.W., Old, L.J. and Scott, A.M. (2005). Phase I Trial of ¹³¹I-huA33 in Patients with Advanced Colorectal Carcinoma. *Clin. Cancer Res.* **11**, 4818-4826.

Scott, A.M., Lee, F.-T., Jones, R., Hopkins, W., MacGregor, D., Cebon, J.S., Hannah, A., Chong, G., U, P.L., Rigopoulos, A., Sturrock, S., Murphy, R., Wirth, V., Murone, C., Smyth, F.E., Knight, S., Welt, S., Ritter, G., Richards, E., Nice, E.C., Burgess, A.W. and Old L.J. (2005). A Phase I Trial of Humanized Monoclonal Antibody A33 in Patients with Colorectal Carcinoma: Biodistribution, Pharmacokinetics and Quantitative Tumor Uptake. *Clin. Cancer Res.* **11**, 4810-4817.

Much ado about mAb806

The Tumour Targeting Laboratory has furthered their studies on mAb806 (see Under the Microscope Issues 3 & 11), an antibody that recognises the EGF receptor only when there is too much of it or when it is mutated in tumour cells. They found that the mutant receptor (de2-7EGFR) recognised by mAb806 had different sugar groups, containing more mannose, attached to it compared to the normal EGF receptors that reach the cell surface. Normally, high-mannose sugars are only found on immature forms of receptors as they are being synthesised inside the cell. This may help explain why mAb806 does not recognise the normal EGF receptor on non-cancer cells. The group has also shown that the anti-tumour activity of mAb806 can be further improved when used in combination with another antibody that recognises the EGFR, called mAb528.

Perera, R.M., Narita, Y., Furnari, F.B., Gan, H., Murone, C., Ahlqvist, M., Luwor, R.B., Burgess, A.W., Stockert, E., Jungbluth, A.A., Old, L.J., Tamaki, K., Scott, A.M. and Johns, T.G. (2005). Treatment of Human Tumour Xenografts with Monoclonal Antibody 806 in Combination with a Prototypical Epidermal Growth Factor Receptor-Specific Antibody Generates Enhanced Antitumor Activity. *Clin. Cancer Res.* **11**, 6390-6399.

Johns, T.G., Mellman, I., Cartwright, G.A., Ritter, G., Old, L.J., Burgess, A.W. and Scott, A.M. (2005). The antitumor monoclonal antibody 806 recognizes a high-mannose form of the EGF receptor that reaches the cell surface when cells over-express the receptor. *FASEB J.* **19**, 780-782.

Other highlights

Members of the Joint Proteomics Research Laboratory have detailed a method for an entirely liquid-based 2-dimensional separation of complex mixtures of proteins using free-flow electrophoresis and reversed-phase HPLC. This rapid and reproducible procedure allows simplification of protein mixtures and increases the number of proteins that can be identified in the mixture using proteomic methods.

Moritz, R.L. and Simpson, R.J. (2005). Liquid-based free-flow electrophoresis-reversed-phase HPLC: a proteomic tool. *Nature Methods* **2**, 863-73.

Members of the Epithelial Biochemistry Laboratory and the Joint Proteomics Services Facility have described the use of a Biosensor for purifying proteins that bind to specific domains of either E-cadherin or APC for identification using proteomic methods.

Catimel, B., Rothacker, J., Catimel, J., Faux, M.C., Ross, J.L., Connolly, L., Clippingdale, A.B., Burgess, A.W. and Nice, E. (2005). Biosensor-Based Micro-Affinity Purification for the Proteomic Analysis of Protein Complexes. *J. Proteome Res.* **4**, 1646-1656.

Recent Research Publications

For a full listing of our research publications during the last 4 months, please see the publications database on our website at http://www.ludwig.edu.au/publications/pub_database.cfm

What's Happening?

- Anita Skandarajah, an MD student in the Joint Proteomics Research Laboratory, was awarded the Campbell Penfold Prize, worth \$1000, for Best Presentation at the RMH Surgical Research Dinner in December this year.
- Eugene Kapp, from the Joint Proteomics Research Laboratory, won a US\$2000 award for his presentation on the independent analysis of the data from the HUPO Plasma Proteome Project at the HUPO 4th Annual World Congress in Munich in September this year.
- Ken Pang, a PhD student in the T Cell laboratory, won the BD Science Communication award at the Australasian Society of Immunology annual conference in Melbourne in December this year for his presentation entitled "Discovery of novel non-coding RNA genes in CD8+ T cells".
- Prof. Richard Simpson was a principal investigator on 3 successful grants this year including a 5 year NH&MRC Medical Bioinformatics, Genomics and Proteomics Program Grant to support proteomic profiling of dendritic cells in collaboration with colleagues at WEHI, a \$2 million NH&MRC Enabling Grant to establish the Australian Proteomics Computational Facility, a shared computing cluster for all proteomics centres in Australia, and a \$1 million Australian Cancer Research Foundation grant for the purchase of a new FTIR mass spectrometer to accelerate the identification of potential colon cancer biomarkers.
- Dr. Andrew Clayton was part of a consortium that received a \$1 million Linkage Infrastructure Equipment and Facilities grant from the Australian Research Council to establish a dedicated facility for the study of the structure and interactions of membrane proteins. A/Prof. Margaret Hibbs received a 3 year NH&MRC Project Grant to further her work on the role of Lyn and SHIP-1 in leukaemia. A/Prof. Ed Nice, Dr. Peter Gibbs and A/Prof. Jonathan Cebon obtained a Cancer Council Victoria grant for the development of a generic biosensor platform for cancer biomarker screening. Dr. Weisan Chen of the T Cell Laboratory also received a 3 year Cancer Council Victoria grant.
- Brad McColl and Kumi Kugathasan (Angiogenesis Laboratory), Ben Hogan and Meredith Crowhurst (Cytokine Biology Laboratory), Sara-Jane Beavitt (Signal Transduction Laboratory) and Adam Cole and Robert Moritz (Joint Proteomics Laboratory) were all awarded their PhDs this year.
- Andrew Badrock from the Colon Molecular and Cell Biology Laboratory and Joe Wei from the T Cell Laboratory both received First Class Honours this year.

Ludwig Awards

The 25th Anniversary of the Ludwig Institute Melbourne Branch, under the directorship of Professor Antony Burgess, was celebrated in style on Thursday 15th December this year in the Carousel Park Marquee at the Royal Melbourne Zoo. There were many tributes to Prof. Burgess and his outstanding leadership, broad medical and scientific vision, altruism, good will, quiet determination and tireless commitment to the Institute and its staff over the last 25 years. Tony Burgess showcased many of the events of the last 25 years and highlighted the discovery of A33 and its utilisation to create intestinal-specific mouse models and target human colorectal tumours, the discovery of VEGF-D and the melanoma vaccine trials as Melbourne branch discoveries that are making a difference. He also looked ahead to the future in which the comprehensive Cancer Centre Network and Olivia Newton John Cancer Centre will assist diagnosis and treatment of new cancer patients. The director was presented with a personalised lab. coat and a certificate guaranteeing 2 weeks uninterrupted work in his own lab., in addition to some books. Today, the Melbourne Branch is the largest of the Institute's nine branches around the world and the only one in Australia. Numerous guests at the 25th anniversary including current and previous staff, affiliates, collaborators and supporters were present to help celebrate this special occasion.

The *George Hodgson Medal for 2005* was jointly awarded to Assoc. Prof. Marc Achen and Assoc. Director Steve Stacker. Prof. George Hodgson was director of the Peter MacCallum Institute for many years and had a wealth of knowledge about all things scientific. He was



someone who was "besotted by science for its own sake" and had a particular interest in stem cells. This commemorative medal is awarded to the person who has made the most creative contribution to the Melbourne branch over the last 5 years. It was jointly awarded to Steve and Marc for their successful research into the

biology and processing of the growth factors that control the lymphatic system, namely the VEGFs. This research partnership has had a considerable impact on the field and elucidated the role of these molecules in tumour spread around the body. Marc described the award as a great honour, citing George Hodgson as an inspirational scientist and acknowledging the tremendous research partnership between himself and Steve. Steve also emphasised his fruitful collaboration with Marc and was pleased to receive such a prestigious award for the "most enjoyable science of my career". Steve also spoke about discussing the initial idea, some eight years ago, of further exploring the field of vascular factors and angiogenesis with George Hodgson, who could not have been more encouraging and excited by the possibilities. He also thanked all the people in the lab; the Research Assistants, Post-docs and Students for doing the experiments, overcoming the failures and for their energy and enthusiasm. A final thank you was given to the director, Prof. Tony Burgess, for all his support over the years.

The *Ludwig Institute Student Medal 2005* was awarded to Dr. Ken Pang. This award acknowledges the achievements, commitment and enthusiasm of our talented and dedicated PhD students and is awarded on the basis of a final year PhD student's contribution to published papers, patents and/or clinical protocols. Ken is jointly supervised by Dr.



Weisan Chen of the T Cell Laboratory and Professor John Mattick, director of the Institute for Molecular Biosciences in Queensland. Ken has undertaken his PhD in two quite different fields and managed to juggle them most effectively. The first is in the area of CD8+ T cells and is highly relevant to vaccine development and better ways of mobilising the immune system to fight cancer. The second area of his research concerns the role of non-coding RNAs. Ken has developed a non-coding RNA microarray and the bioinformatics required to mine it. His database (published in Trends in Genetics and Genome Research) has been accessed over 200,000 times from 14,000 locations globally in the last year! He has six publications from his PhD including three first-author publications and two co-authored articles in "Science".

Long Service Awards- Mike Rubira and Greg Thege received awards for 20 years service and Zhanqi Liu received an award for 10 years service.