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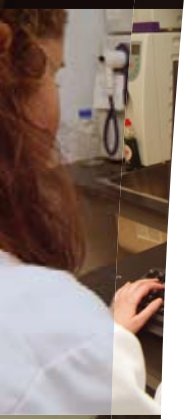
conway focus



Tracking the secretory pathway in cells

Secretion is a fundamental process essential to almost every cell type in the body; delivering hormones into the blood stream, digestive enzymes into the gut, and signalling molecules between cells.

Similar to any supply chain logistics, proteins and lipids are transported along a complex pathway from the point of manufacture through stages of packaging, labelling and transportation through the cell before delivery to the cell surface.

Technology did not permit scientists to catalogue this journey through cell organelles until recently. For the first time, using a combination of genetic and sophisticated microscopy techniques, over 8 million individual cells have been assessed to reveal those genes that influence the transport network or secretory pathway.

"This study is the first genome-wide assessment of the secretory process in a human cell system", explains Conway Fellow, Professor Jeremy Simpson, UCD School of Biology & Environmental Science, co-author of the research paper published in *Nature Cell Biology*.

"In order for us to understand the impact on the body when this fundamental process of secretion goes wrong, we must first decipher the functional network of membrane trafficking pathways within the cell.

Previous studies on the secretory process have been carried out in more simplistic organisms such as the fruit fly (Drosophila) or with a more narrow focus such as on a specific group of genes. Now, using high content screening, we have been able to target 22,000 human genes and track the journey of a specific, fluorescently-tagged protein as it travels through, and out of, over 8 million individual cells."

The UCD team, in collaboration with scientists in the European Molecular Biology Laboratory (EMBL) in Heidelberg, systematically silenced each of our 22,000 genes to observe to what extent this affected the cell's ability to transport a protein. They found that 15% of human genes somehow influence the secretory pathway including genes that provide a link to other events in and around the cell.

From analysis of more than 700,000 microscopy images, they found 554 proteins that influence secretion with more than 143 influencing the early stage of the secretory pathway or morphology of the Golgi, a cellular structure responsible for modifying proteins after they have been made. Membrane traffic pathways connect membrane bound organelles in a carefully ordered sequence to ensure the correct complement of proteins and lipids exists within the cell to maintain cellular balance or homeostasis.

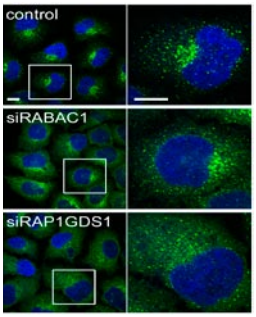
Newly synthesised proteins and lipids in the endoplasmic reticulum are modified as they pass along the secretory pathway through the Golgi apparatus to the cell surface.

The secretory pathway has the capacity to cope with a wide variety of cargo molecules, and as such utilises extensive regulatory machinery in the process. This study focuses on particular regulatory elements in the early stage of the pathway called the cytoplasmic coat protein complexes.

The major part of funding for this research has come from Science Foundation Ireland, the EU-funded network of excellence, 'Systems Microscopy', and the EU-FP6 MitoCheck consortium.

Reference:

Simpson, J.C et al. Genome-wide RNAi screening identifies human proteins with a regulatory function in the early secretory pathway. Nature Cell Biology, advance online publication 3 June 2012, doi: 10.1038/ncb2510. Data available at www.mitocheck.org



In cells where different genes are silenced (middle, bottom), the site where the secretory processes begins (green) changes compared to normal cells (top).

Director's Message

Welcome!

This issue coincides with preparations for the Euroscience Open Forum Dublin 2012 when those who most influence science, society and policy will arrive in the capital for the largest open forum of its kind. It is anticipated that the international conference will attract more than 5,000 scientists, business leaders, government officials and media.

UCD Conway Institute will have a presence at the event as part of the University's research showcase. We look forward to presenting the quality and breadth of our research programme as well as highlighting the core technology expertise and customised service available to academics and industrial partners in conjunction with our dedicated core technology directors and their staff.

As the international spotlight turns to focus on Irish science, I am delighted to see the impact of Conway research as our scientists continue to publish in highly regarded journals. In particular, I would like to congratulate Professor Jeremy Simpson on his *Nature Cell Biology* research article that is highlighted in this issue.

Professor Walter Kolch
Director

UCD DUBLIN
conway focus

Gremlin key to tackling pulmonary hypertension

UCD researchers have identified, for the first time, a molecular mechanism that accounts for the unique vascular remodelling of the pulmonary circulation in response to hypoxia or low levels of oxygen.

The findings, published in the journal, *Circulation* may focus efforts in tackling pulmonary hypertension, a feature of chronic hypoxic lung diseases such as chronic obstructive pulmonary disorder (COPD) and emphysema that significantly impacts on patient morbidity & mortality.

First author and PhD candidate, Edwina Cahill explains that "diseases such as emphysema, chronic bronchitis and fibrosis of the lungs cause abnormally low levels of oxygen in the lung. When the lung is starved of oxygen, blood pressure in the arteries of the lung increases causing damage to blood vessels and making it more difficult for the heart to pump blood. In many cases, this can lead to heart failure and premature death".

While investigating the factors that contribute to high blood pressure in the arteries, the UCD team found that, with reduced oxygen levels in the lung, the body produces elevated quantities of a protein called gremlin, which suggests a link between elevated levels of gremlin and pulmonary hypertension.

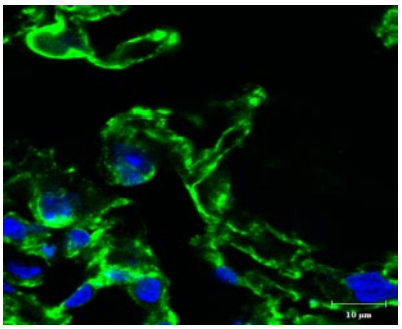
The research team focused on gremlin 1 given its ability to efficiently block the actions of the BMP signalling pathway in pulmonary hypertension.

Under hypoxic conditions, gremlin 1 expression is increased as is its secretion from the vascular endothelium. It inhibits BMP signalling and contributes to the development of pulmonary hypertension.

Conway Fellow, Professor Paul McLoughlin who led the project believes that, "Given that hypoxia induced increase in gremlin 1 is selective for the lung, it offers an attractive potential target for therapy as antagonising its action may have minimal effects in other organs."

This research was funded primarily through the Health Research Board and Science Foundation Ireland.

Reference
Cahill, E et al. Gremlin Plays a Key Role in the Pathogenesis of Pulmonary Hypertension. Circulation (2012) Published online before print January 13, 2012, doi: 10.1161/circulationaha.111.038125



Representative confocal image of immunofluorescent (FITC) staining of gremlin showing labelling in the alveolar wall is suggestive of localisation predominantly in the capillary endothelium.

Predicting toxicity in the drug development pipeline

It is not currently possible to predict accurately and at an early stage whether there are toxicity issues with candidate drugs. This shortcoming of existing toxicology evaluation methods can not only create a bottleneck in the drug development pipeline but can sometimes lead to the withdrawal of drugs from the market.

UCD researchers have reported in *Molecular & Cellular Proteomics* on a proof-of-principle study that may benefit the pharmaceutical industry in the future by providing a roadmap for large scale pre-clinical toxicology biomarker verification studies.

The study involved the molecular profiling of models that had been exposed to known toxic insults in an effort to derive the associated biomarker signatures.

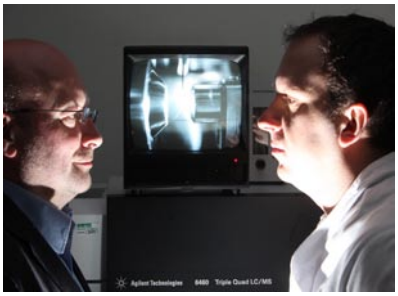
Forty-eight candidate biomarkers of liver toxicity were assembled from a discovery proteomics screen of liver in a hepatotoxicant treated rat model using label free liquid chromatography mass spectrometry (LC-MS); a previous transcriptomics study of the sample and from literature sources.

The team developed and optimised a selected reaction monitoring assay (SRM) in order to quantify the proteins in this putative biomarker panel. This revealed a panel highly enriched for proteins that had been changed significantly as a result of toxicant exposure.

Dr Ben Collins, first author and Agilent UCD Newman Fellow, explains, "The idea was to use transcriptomics and proteomics and to combine the data to provide earlier markers of toxicity. Although this study focused on one hepatotoxic compound, there is sufficient flexibility in the approach used to allow medium to high throughput for large scale verification studies involving large numbers of well-defined toxicants and ultimately for more sensitive toxicology evaluation for drugs under early development."

Team leader and corresponding author, Professor Steve Pennington adds, "We are now working to extend this approach to more readily accessible sample types such as blood and are applying it in other studies to develop diagnostics for chronic conditions such as cancer, cardiovascular disease and arthritis. With support from Agilent Technologies, we are now establishing a dedicated laboratory to undertake these SRM-based validation studies."

Reference
Collins, BC et al; Development of a pharmaceutical hepatotoxicity biomarker panel using a discovery to targeted proteomics approach. Molecular & Cellular Proteomics (2012) doi: 10.1074/mcp.M111.016493



Professor Steve Pennington pictured with Dr Ben Collins, Agilent UCD Newman Fellow (now based at the Institute of Molecular Systems Biology, ETH Zurich).

Identifying proteins critical to bovine embryo survival

UCD researchers led by Conway Fellow, Professor Mark Crowe describe, for the first time, the protein landscape of the uterine environment in any domesticated species before implantation.

With more than 30% of reproductive failure in mammals arising due to the loss of an embryo in the preimplantation period, it is vital to understand how the uterine environment functions to support the embryo during the early establishment of pregnancy.

As the sole supply of vitamins, minerals, enzymes and other nutrients for the developing conceptus before implantation, the histotroph (uterine secretions) is critical for survival of the early embryo and may be a promising source of biomarkers of uterine function.

Describing the study, Dr Giuliano Elia, UCD Conway Proteomics Core Director said, "Using label-free liquid chromatography-tandem mass spectrometry, we performed shotgun proteomics of the bovine histotroph proteome at two key preimplantation stages of the estrous cycle in high fertility cattle. We examined the proteomic changes occurring between days 7 and 13 to identify specific proteins likely to contribute to embryo survival."

Functional analysis of 34 differentially expressed proteins revealed distinct biological roles believed to be involved in early pregnancy. These included remodelling of the uterine environment for implantation; nutrient metabolism; embryo growth, development & protection; maintenance of uterine health and maternal immune modulation.

A Science Foundation Ireland strategic research centre (SRC) award funded this research.

Reference
Mullen, M et al. Proteomic characterisation of histotroph during the preimplantation phase of the estrous cycle in cattle. Journal of Proteome Research (2012) 11, 3004-3018 doi/10.1021/pr300144q

Nature Communications; from bovines to brains

Problems with diagnosing bovine TB in cattle infected with the common livestock disease, liver fluke, may be contributing to the spread of bovine TB, according to new research published in *Nature Communications* involving Conway Fellow, Professor Grace Mulcahy.

A study of 3,000 dairy herds in England and Wales found that liver fluke infection reduces the sensitivity of skin tests used to diagnose bovine TB (BTB).

Consequently, cattle infected with both liver fluke and BTB may not be identified by existing bovine TB surveillance schemes and may continue transmitting BTB if they are moved from their farm of origin.

In the same issue, a collaborative research study that included Conway Fellow, Professor Sean Callanan outlined a method of safely manipulating blood vessels in the brain to reduce cerebral oedema.

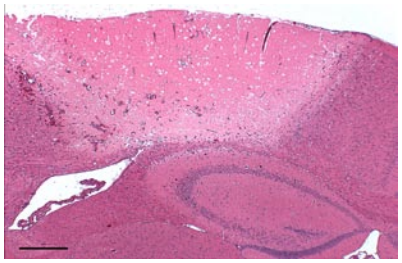
By allowing periodic opening of tight junction channels between cells lining the blood vessels in the brain, the blood vessels in the brain become marginally and reversibly permeable to tiny molecules. This allows the fluid in the brain, largely comprising water, to efficiently drain back into the blood.

The team also observed cognitive improvement in murine models of focal cerebral oedema after administering short interfering RNA directed against claudin-5.

These observations may have consequences for early intervention in cases of traumatic brain injury, or indeed any neurological condition where cerebral oedema is the hallmark pathology.

Reference
Claridge, J et al. Fasciola hepatica is associated with the failure to detect bovine tuberculosis in dairy cattle. Nature Communications 22 May 2012 doi: 10.1038/ncomms1840

Campbell, M et al. Targeted suppression of claudin-5 decreases cerebral oedema and improves cognitive outcome following traumatic brain injury. Nature Communications 22 May 2012 doi: 10.1038/ncomms1852



H&E stained section showing a region of cerebrum revealing cerebral damage to the level of the hippocampus. Courtesy of Brian Cloak, UCD

New test can better predict successful IVF embryos

Research from Conway Fellows, Dr Lorraine Brennan and Dr Fionnuala McAuliffe outlines a new way to measure the potential success rate of an embryo before it is transferred back into the womb during in vitro fertilisation (IVF).

The fluid within a woman's ovaries that surrounds the egg or oocyte holds metabolic information that can improve predictions on which embryo is more likely to lead to pregnancy.

"We analysed samples of the follicular fluid surrounding the immature ovum or egg before it was retrieved for IVF," says Dr Brennan. "There were clear metabolic differences between the follicular fluids from women who successfully achieved pregnancy as a result of IVF to the fluids from the women who did not."

An accurate and validated method of embryo assessment and selection for transfer might allow implementation of single embryo transfer as the standard procedure while maintaining a high pregnancy rate and minimising multiple pregnancies.

Reference
Wallace M et al. An investigation into the relationship between the metabolic profile of follicular fluid, oocyte developmental potential and implantation outcome. Fertility and Sterility 97; 5, 1078-1084.e8 May 2012

Carcinogen exposure causes cilia loss in renal cells

The primary cilium is a highly specialised sensory and signal transduction hub that plays a central role in diverse cellular processes such as differentiation, polarity and cell cycle progression. Primary cilia are absent in several cancer cell types such as breast and pancreatic cancers.

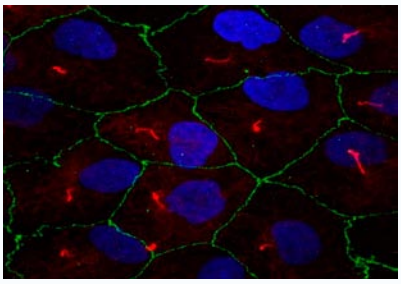
Research from the UCD Renal Disease Research Group (www.ucd.ie/renal) led by Conway Fellow, Dr Tara McMorrow and collaborators, Professor Michael Ryan and Dr Oliver Blacque, has demonstrated that known renal carcinogens disrupt cilia structure and function in tubular epithelial cells.

In a study, recently published in the *American Journal of Physiology-Renal Physiology*, the team demonstrated that exposure to carcinogens (ochratoxin A and potassium bromate) significantly reduced the percentage of ciliated renal cells. Transcriptomic analysis identified several important signalling pathways involved in mediating these effects.

The work was carried out in conjunction with scientists in Austria, Switzerland and The Netherlands as part of the EU FP6 project, CarcinoGENOMICS.

Dr McMorrow plans to expand this research by characterising mechanisms of primary cilium loss during carcinogenesis and explore the application of this research as a novel screening method for potential chemical carcinogenicity.

Reference
Radford, R et al. Carcinogens induce loss of the primary cilium in human renal proximal tubular epithelial cells independently of effects on the cell cycle. Am J Physiol Renal Physiol (2012) 302(8):F905-16.



Hair-like structures known as cilia (red), located on the surface of kidney epithelial cells, may be damaged or lost by carcinogen exposure.

New insight to CO2 sensing and signalling

There is a Jekyll and Hyde quality to impact of hypercapnia or elevated levels of CO_2 in the body. In inflammatory conditions such as chronic obstructive lung disorder (COPD) or cystic fibrosis, hypercapnia is associated with a worse prognosis for patients as it results in them being susceptible to infection.

In contrast, hypercapnia and associated acidosis can be associated with improved patient outcome in instances where clinicians reduce mechanical damage to the lungs of patients with acute respiratory distress syndrome using a therapeutic hypoventilation strategy.

With CO_2 gaining recognition as an intracellular signalling molecule that can affect both inflammatory and immune responses, it is becoming increasing important to decipher the exact molecular pathway by which it operates.

New findings from researchers led by Conway Fellow, Professor Cormac Taylor provide the beginnings of a molecular understanding of how elevated CO_2 can affect immune/inflammatory signalling.

Senior author, Dr Eoin Cummins explains, "We identified a novel modulation of a key innate immunity/inflammatory pathway (NF-κB). A key transcription factor in this pathway, RelB is processed and localised differentially under conditions of elevated CO_2 that may influence NF-κB dependent signalling."

The group used murine and human models of lung injury to show that RelB is cleaved and sent to the nucleus of cells in response to elevated arterial CO_2.

Science Foundation Ireland funded this research including a short term travel fellowship to Dr Cummins to Northwestern University, USA.

Reference
Oliver, KM et al. Hypercapnia Induces Cleavage And Nuclear Localisation Of RelLB Giving Insight Into CO2 Sensing And Signalling. Journal of Biological Chemistry (2012) doi:10.1074/jbc.M112.347971

Recognising excellence in research

Conway scientists at every level were recognised for their research through personal and funding awards in the last quarter.

Conway Fellow, Dr John O'Connor was selected by the Royal Academy of Medicine in Ireland to deliver the 2012 Conway Review Lecture and receive the Academy silver medal in recognition of his work on the effect pro-inflammatory agents have on synaptic signalling before and during hypoxic insults to neurons.

Research Fellow, Dr David Croucher received funding under the first Science Foundation Ireland starting investigator research grant (SIRG) programme with co-funding from the International Marie Curie COFUND scheme. David will look at the fundamental behaviour of signalling pathways in breast cancer using systems biology approaches.

Research Fellow, Dr Silke Ryan won the 2012 James B. Skatrud new investigator award at the annual conference of the American Thoracic Society. Silke works on obstructive sleep apnoea syndrome and the role of intermittent hypoxia in the development of cardiovascular complications in OSAS by selectively activating inflammatory pathways.

Postdoctoral Fellow, Dr Maria Prencipe won the 2012 DAMC Young Investigators Research Symposium laboratory-based oral presentation award. Maria's research focuses on finding potential therapeutic targets for treatment-resistant prostate cancer cells and she presented work on a candidate biomarker, serum response factor (SRF).

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