Cell cycle control of cell death

Prolonged mitosis leads to cell death whereas normal mitosis does not. How can a cell distinguish between normal and prolonged mitosis to dictate cell fate?

New research led by Conway Fellow, Dr Margaret McGee suggests that an important cell death signal during prolonged mitotic arrest is the Cdk1/cyclin B1-dependent hyperphosphorylation of the Bcl-2 interacting mediator of cell death (Bim).

Research has highlighted a crucial role for Bim in paclitaxel (Taxol®)-mediated death of tumour cells. This chemotherapeutic agent disrupts the microtubule dynamics within cells leading to mitotic arrest and ultimately cell death.

Dr McGee and postgraduate student, Sean Mac Fhearraigh looked at molecular signals that connect cell cycle arrest to the cell death machinery in chronic myeloid leukaemia (CML) cells.

Explaining their findings, Dr McGee said, "Cell cycle progression is controlled by various cyclins and cyclin-dependent kinases (Cdks) and our research reveals that these kinases can also control cell death through the direct phosphorylation of Bim. We have shown a novel association between Bim and cyclin B1 during mitosis that mediates the phosphorylation of Bim by Cdk1.

We believe that hyperphosphorylation of Bim resulting from sustained Cdk1 activity during mitotic arrest may be an important molecular switch that promotes cell death during mitotic arrest.

Reference:
Mac Fhearraigh et al. cyclin B1 interacts with the BH3-only protein Bim and mediates its phosphorylation by Cdk1 during mitosis. Cell Cycle 10:22, 3886-3896; November 15, 2011

Awards for research excellence

Senior Research Fellow, Dr Darran O'Connor received the 9th St Luke's Young Investigator award from the Royal Academy of Medicine in Ireland for 'Antibody based proteomics: Fast tracking molecular diagnostics in oncology'.

This is based on research of a protein named cocaine-and-amphetamine-regulated transcript (CART), a potential breast cancer biomarker, by the SFI-funded strategic research cluster, Molecular Therapeutics for Cancer Ireland. Dr O'Connor and his colleagues examined breast cancer tissue in over 1,000 patients. They found that those patients with high levels of CART had a significantly poorer outcome when treated with tamoxifen compared to those patients with zero or low levels of the protein. In the future, it may be possible to establish the CART status of a patient's cancer in order to personalise their treatment regimen.

PhD student, Vishal Salunkhe received a $500 abstract achievement award at the 53rd American Society of Haematology (ASH) annual conference in San Diego. Working with Dr Patricia Maguire, Vishal focuses on transcription factor MEIS1, an important molecular switch from megakaryocyte to pro-platelet formation.

Dr James Jones, editor-in-chief IJMS; Prof Tom Walsh, past president RAMI; Dr Darran O'Connor, winner of the 9th St Luke's Young Investigators award; Dr Ken O'Halloran, general secretary RAMI

In brief: Researching the small in Nature

Two research groups associated with the Institute have recently reported research findings in Nature journals.

Conway Fellow, Dr Emma Teeling led a study on the only two populations in existence of the world's smallest mammal, the bumble bee bat, to determine the early drivers at play in the evolution of a species. Teeling and her team were in the unique position of being able to look at speciation 'in action' and found that geological distance has a primary role in limiting gene flow rather than echolocation divergence.

With the possibility that nanoparticles could offer new ways to deliver drugs into the body, Professor Kenneth Dawson's team explore the fate of these miniscule materials in cells. Their recent findings show that nanoparticles are not exported from cells but are split between daughter cells when the parent cell divides. A cell that divides frequently will dilute the nanoparticle dose with each cycle of cell division. The dose in each cell varies as the cell advances through the various phases of the cell cycle.

Reference:
Kim et al. Role of cell cycle on the cellular uptake and dilution of nanoparticles in a cell population Nature Nanotechnology (2012) doi:10.1038/nnano.2011.191

Puechmaille et al. The evolution of sensory divergence in the context of limited gene flow in the bumblebee bat Nature Communications (2011) doi:10.1038/ncomms1582

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Elaine Quinn
Communications & Education Officer
UCD Conway Institute of Biomolecular & Biomedical Research
University College Dublin
Belfield, Dublin 4
Ireland

E: elaine.quinn@ucd.ie
T: (+353-1) 716 6706
F: (+353-1) 716 6701
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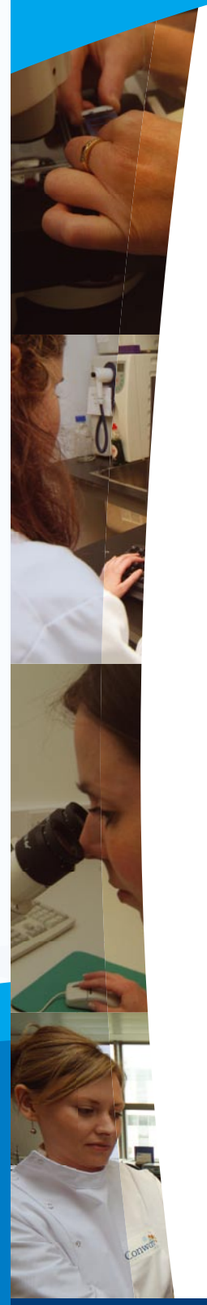


Unlikely oncogene accomplice identified

Cancer research has just taken a new twist. K-Ras, one of the most frequently mutated genes in human cancer, is known as an oncogene that drives the development of many prevalent human cancers. Now, research has unveiled an unexpected face of the non-mutated K-Ras as an accomplice of the mutant K-Ras oncogene.

The findings of research published recently in *Molecular Cell* demonstrate that mutant K-Ras transformation is supported by the wild type allele in colorectal carcinoma (CRC).

RAS mutations are seen in more than 30% of human cancers, of which KRAS mutations account for about 85%. K-Ras protein is the only family member that can activate apoptosis.

Led by Professor Walter Kolch, Director, Systems Biology Ireland & UCD Conway Institute, this international research collaboration reports that mutant K-Ras binds directly to the tumour suppressor gene RASSF1A and initiates the MST2-LATS1 signalling pathway to trigger apoptosis.

However, K-Ras counteracts this pro-apoptotic action by stimulating autocrine activation of the epidermal growth factor receptor (EGFR), which requires the wild-type (WT) K-Ras allele. Describing the research, Dr David Matallanas, group leader in Systems Biology Ireland said, "We were able to show that the ability to activate MST2 is specific to K-Ras and that when mutant K-Ras activates the MST2/LASTS1 pathway, the downstream effector is the tumour suppressor protein, p53.

Using a genetically engineered cell system, we were able to corroborate that the WT K-Ras counteracts the proapoptotic effects of mutant K-Ras".

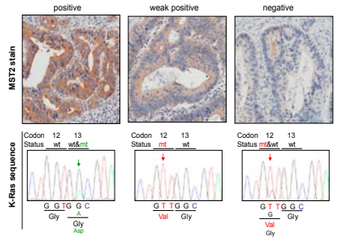
Given that KRAS is commonly mutated in colorectal carcinoma (CRC), the team looked at KRAS gene mutations and MST2 protein expression in 173 CRC patients. Their findings indicate that WT K-Ras allele is critical for anti-apoptotic EGFR signalling. Unless the WT K-ras allele is retained, MST2 expression and K-Ras mutations cannot co-exist.

Dr Matallanas explains, "When our CRC patient cohort was subdivided according to Dukes' staging, which measures the extent of tumour metastasis, we saw a highly significant association between KRAS mutations and loss of MST2 protein expression in Dukes' stages C and D. This is when the disease has advanced with local and distant metastases respectively.

With the loss of MST2 expression, late stage tumours do not require protective function of WT K-ras allele and EGFR.
Patients with Dukes' A stage CRC are successfully treated with surgery alone. However, 20-30% of Dukes' B stage patients relapse and perhaps these patients may benefit from EGFR inhibitory therapeutic regimens".

This research was supported through funding from Science Foundation Ireland, Cancer Research UK, EU FP6 STREP Growthstop, Kuwait Foundation for the Advancement of Sciences, Research Core Facility.

Reference:
Matallanas et al. Mutant K-Ras Activation of the Proapoptotic MST2 Pathway Is Antagonized by Wild-Type K-Ras. Molecular Cell 44, 893–906, December 23, 2011



Director's Message

Welcome!

As an Institute, we are justifiably proud of the calibre of our early-stage career scientists who continue to be recognised for the quality of their research. In particular, I extend congratulations to Dr Fiona McGillicuddy, the first funding recipient under the prestigious SFI-HRB-Wellcome Trust Biomedical Partnership, and to Dr Darran O'Connor who won the 9th St Luke's Young Investigator award from the Royal Academy of Medicine in Ireland.

On the occasion of their retirement, I wish Mr Paul Rooney and Mr Cormac O'Connell every success and thank them sincerely for their contribution to the Institute. Paul was integral to the establishment and smooth operation of the 1East laboratory while Cormac has been a vital part of the UCD electron microscopy facility that recently relocated to the Institute Imaging Core.

UCD Conway core technologies are a fundamental infrastructural resource that we are actively developing into research solution providers through the provision of competitive and comprehensive offerings to academic and industrial users. As evident in this issue, the services and expertise available are contributing to successful research outputs.

Professor Walter Kolch
Director

conway focus

Core technologies provide valuable support

Integral to the Institute infrastructure, UCD Conway core technologies provide a comprehensive level of service and expertise that is unrivalled within Ireland. Across the four core areas of genomics, proteomics, imaging and flow cytometry, the directors and their technical staff adopt a problem-solving approach to the research questions posed by scientists and provide support from experimental design to publication.

Conway Fellow, Professor Donal F. O'Shea of the Centre for Synthesis & Chemical Biology recently published findings in the *Journal of the American Chemical Society* of a study carried out in collaboration with Dr Dimitri Scholz, Director of the Imaging Core.

The research focused on creating the next generation of smart fluorescent probes that are not only highly sensitive but capable of switching their fluorescent signal from [off] to [on] in response to specific biological stimuli.

This would help to overcome the limitation of non-specific background fluorescence often encountered when using fluorescent imaging. It would allow researchers to use continuous real-time imaging rather than images captured at a specific point in time when background fluorescence has been cleared.

Professor O'Shea and his team describe a new near-infrared, [off] to [on] fluorescent switchable nanoparticle construct that can switch on its fluorescence after cellular uptake but remains switched off outside of the cell and demonstrated its usage in vitro using endocytosis as an example of a fundamental cellular process.

'We were able to demonstrate in movie format (http://www.youtube.com/watch?v=FjipbGTf8w4) the ability of these fluorescence responsive nanoconstructs to allow real-time continuous imaging of their uptake into cells. We now hope to expand our concepts to real-time imaging in vivo', said Professor O'Shea.

Conway Fellow, Dr Albert Smolenski from the School of Medicine & Medical Science recently published findings in *Blood* of work carried out in collaboration with Dr Giuliano Elia and Dr Alfonso Blanco, Directors of the Proteomics and Flow Cytometry Cores respectively.

Their research study demonstrates that the protein known as regulator of G-protein signalling 18 (RGP18) is a target in both platelet activating and inhibitory signalling pathways.

For the first time, they describe RGB18 as a new substrate of protein kinase A (PKA) and protein kinase GI (PKGI). The 30kDa substrate was identified using mass spectrometry analysis after initial evidence of its existence was seen as cross-reactivity of a phosphorylation site-specific antibody.

Dr Smolenski explains, "After identifying RGS18, we went on to define the functional outcomes of RGS18 phosphorylation at the molecular & cellular levels. The regulated interaction of 14-3-3γ protein with RGS18 through binding to either phosphorylated serines 49 or 218 may represent a switch in the control of calcium signalling in platelets."

Reference
Palma et al. Cellular Uptake Mediated Off/On Responsive Near-Infrared Fluorescent Nanoparticles. J. Am. Chem. Soc., 2011, 133 (49), pp 19618–19621

Gegenbauer et al. Regulator of G-protein signalling 18 integrates activating and inhibitory signalling in platelets. Prepublished online as Blood First Edition paper, Jan 10 2012; DOI 10.1182/blood-2011-11-390369

Prestigious award for Conway scientist

Dr Fiona McGillicuddy wins the first award under the SFI-HRB Wellcome Trust Biomedical Partnership. She will receive €750,000 over five years to investigate the links between obesity, 'good cholesterol', diet and coronary artery disease.

The research project will examine how obesity affects the function of high-density lipoprotein (HDL) or 'good cholesterol'. HDL plays an important role in removing cholesterol from the body, which in turn reduces the risk of heart attack brought on by cardiovascular disease.

"First we want to determine whether being obese affects HDL's ability to eliminate cholesterol from the body", says Dr McGillicuddy, from the Nutrigenomics Research Group led by Conway Fellow, Professor Helen Roche. "We then want to determine whether the type of diet that causes the obesity also plays a role in elevating the risk of coronary artery disease.

For example, you can be obese from eating a high-carbohydrate diet, or you can be obese from eating a high-saturated fat diet. We want to see how these very different diets affect HDL function, and consequently the risk of coronary disease.

We also know that during obesity as the fat mass (adipose tissue) expands it becomes inflamed. We plan to investigate whether this adipose tissue inflammation actually reduces the ability of HDL to remove cholesterol from the body. We will examine whether new therapies that reduce fat inflammation can also improve HDL function and reduce the risk of coronary disease despite the presence of obesity."

Dr Fiona McGillicuddy

Molecular Trafficking Pathways in Cilia

Gaining insight at the molecular level of the intracellular trafficking pathways operating in cilia may shed light on the causes of multisymptomatic cilia-related disorders such as polycystic kidney disease, Bardet-Biedl syndrome and retinitis pigmentosa.

New research by UCD researchers led by Conway Fellow, Dr Oliver Blacque in collaboration with colleagues in Brandeis University, USA has shown that endocytic membrane transport genes facilitate protein and membrane transport in C. elegans sensory cilia.

These hair-like projections from eukaryotic cell surfaces bear the responsibility for motility, sensation and developmental signalling. The functional integrity of cilia depends on intracellular transport pathways.

With no protein synthesis occurring in cilia, proteins must be trafficked to the organelle. Roles for exocytosis in regulating ciliary protein and membrane transport have been described previously but until now there was only limited evidence that endocytosis events are also involved.

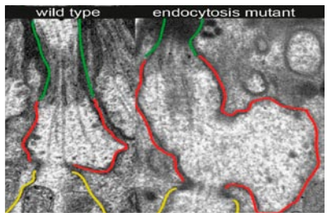
During endocytosis, plasma membrane and associated proteins bud off the cell surface as internalised vesicles, a process important for various cellular functions such as regulation of membrane protein activities and uptake of extracellular molecules.

Dr Oliver Blacque said, "This is the first demonstration in any multicellular organism of a functional connection between endocytosis and cilia. It provides a springboard for future work into how molecular transport systems organise the various structures and functions of cilia.

With ciliary defects implicated in more than a dozen multisymptomatic diseases, for which more than 80 genes are now associated, our work helps to understand how intracellular trafficking pathways contribute to disease aetiology."

Science Foundation Ireland and the EU FP7 funded Dr Blacque's research.

Reference
Kaplan et al., Endocytosis Genes Facilitate Protein and Membrane Transport in C. elegans Sensory Cilia, Current Biology (2012), doi:10.1016/j.cub.2012.01.060



Transmission electron microscopy image showing the ciliated dendritic ending of one sensory neuron in C. elegans. In worms with disrupted clathrin adaptor 2 complexes (endocytic mutant), the periciliary membrane (red) that exists immediately beneath the ciliary membrane (green) is massively expanded compared to wild type worms, indicating a defect in periciliary membrane recycling.

Ubiquitination role in gene silencing

The 2004 Nobel Prize in Chemistry recognised a landmark discovery that the generation of polyubiquitin chains on target proteins is a signal for protein destruction. Now, evidence suggests that protein ubiquitination is actually a more versatile process and has non-proteolytic functions.

The study in *PLoS Computational Biology* led by Professor Boris Kholodenko, Conway Fellow & Deputy Director, Systems Biology Ireland shows a flexible role for the process of ubiquitination in silencing gene expression.

Polycomb proteins interact with chromatin to silence genes. This process involves an essential step where the ubiquitin ligase, Ring1B monoubiquitinates histone H2A. Ring1B has a binding partner, Bmi1 that facilitates the self-ubiquitination of Ring1B and protects both proteins from rapid destruction.

Overexpression of Bmi1 is often seen in various human cancers and Bmi1 may protect cells from apoptosis by suppressing tumour suppressor and pro-apoptotic genes.

This work presents a computational model of the Ring1B/Bmi1 ubiquitination system. In partnership, it acts like an analog-digital converter to produce discrete signals that can distinctly affect gene silencing and potentially trigger different cell fates.

Dr Lan Nguyen explains, "The system can generate abrupt switches where increased Bmi1 turns on Ring1B ubiquitination activity and silences gene expression, for example. It can also display multistable dynamics, oscillations and overshoots with differing consequences for the cell.

These various digital responses can also trigger the creation of a biological memory through hysteresis. When a system that depends on both its current and past environment is stimulated, it 'remembers' the stimulus and will only require a lower level of stimulus when subsequently exposed".

Nobel laureate, Professor Aaron Ciechanover collaborated on the research that was funded by Science Foundation Ireland and the National Institutes of Health.

Reference
Switches, Excitable Responses and Oscillations in the Ring1B/Bmi1 Ubiquitination System. Nguyen et al. PLos Computational Biology Dec 2011 doi/10.1371/journal.pcbi.1002317

Research Frontiers in CLASS

Since the start of the 2011/12 academic year, a quarterly *Research Frontiers* session has been added to the weekly Conway Lecture & Seminar Series (CLASS). Up to four Conway Fellows are invited to make a summary presentation on a research proposal for which they have recently secured a funding award and all awardees in the previous quarter are acknowledged.

Professor Walter Kolch, Director says, "Securing competitive research funding in difficult economic times is extremely challenging for our researchers so it is vitally important that we celebrate all grant award successes and keep our research community informed of ongoing work."

At the inaugural session in September 2011, Professors Ulla Knaus and Johan Ericsson (SFI PI awards) and Dr John Baugh (HRB Research Training Fellowships) presented. Professors Cormac Taylor and Des Higgins (SFI PI awards) and Dr Oliver Blacque (SFI PI Career Advancement award) spoke in January 2012.