Untangling the signalling networks in cells, development and human disease

Cellular signalling pathways triggered by the transforming growth factor beta (TGFβ), bone morphogenetic protein (BMP) and Wnt proteins control a plethora of cellular processes throughout metazoan development and adult life. Their malfunctions cause a range of human disorders from bone defects to cancer progression. **Dr Gopal Sapkota**, from the University of Dundee's MRC Protein Phosphorylation and Ubiquitylation Unit, has a track record of success in uncovering some of the fundamental mechanisms underlying their signalling activity.

For growth and maintenance of many healthy tissues, TGFβ proteins serve as signals for inhibition of cell growth, promotion of cell migration, determination of cell fate and suppression of immune signalling. However, when these proteins are incorrectly expressed or regulated, the resulting breakdown in signalling has the potential to cause many diseases. For example, cancer cells which can overcome the growth inhibitory effects of TGFβ proteins are able to proliferate unchecked and, worse still, might turn these proteins against the body's immune system to metastasise unimpeded.

A CIRCUITOUS PATHWAY

TGFβ proteins are produced and discharged by many cells and can signal to any cell that has specific receptors for these. Upon engaging TGFβ proteins, the cell triggers an intracellular signalling cascade through the activation of SMAD proteins (a name coined from similar proteins first identified in fruit flies and nematodes). These proteins act as transcription factors, which regulate the expression of certain genes. The expression of specific gene sets determines the fate of the cell receiving TGFβ signals. The SMAD proteins are in turn regulated by crosstalk and feedback inputs from other signalling pathways, to provide a coordinated response to multiple signals that the cell receives at any given time. By investigating these molecular mechanisms of regulation, Dr Sapkota hopes to gain a better understanding of their activities and ultimately identify novel therapeutic targets for related diseases.

THE PATH THAT LEADS TO DESTRUCTION

In pursuit of this goal, Dr Sapkota and his research group have made many key discoveries relating to these and other associated cell signalling pathways. As recently as 2014, they identified a protein, which they termed PAWS1, that interacts with one of the SMAD proteins and acts as a transcriptional regulator of BMP (a TGFβ protein family member) signalling – a key target for their research. In doing so, they have shed light on a previously poorly understood mechanism of gene regulation by these proteins. The classic TGFβ pathway involves the association of SMAD transcription factors with the protein SMAD4, although SMAD4-independent signalling is also known to occur. This research has identified a novel mechanism by which this can take place. Not only that, but their research has also demonstrated that PAWS1 has wider effects on gene regulation than the BMP pathway alone, making it a critical target for further research.

Signalling pathways are often regulated by the degradation of factors produced to convey a signal within the cell. As such, one way of enhancing signalling is therefore by inhibiting this endogenous (from within the cell) degradation. This is certainly true of SMAD proteins, which are tightly regulated to ensure balanced cellular responses to TGFβ signals.

Work completed by Dr Sapkota and his team in 2013 identified how the TGFβ-induced recruitment of a specific enzyme called OTUB1 to an active SMAD complex prevented its ubiquitination (the addition of a small ubiquitin protein which targets proteins for degradation or relocation within the cell). Blocking this enzyme resulted in a significant loss of TGFβ signal, which could be rescued by disrupting proteasomal activity (the cellular machinery responsible for degrading unwanted proteins).

DESIGNATING THE DESTINATION

This enzyme, dubbed OTUB1 because of its association with ovarian tumours, has a wider role in DNA repair and is therefore required in the nucleus (the site of DNA storage in the cell). The team have discovered that phosphorylation (the addition of a phosphate molecule) at a specific location within the protein structure is responsible

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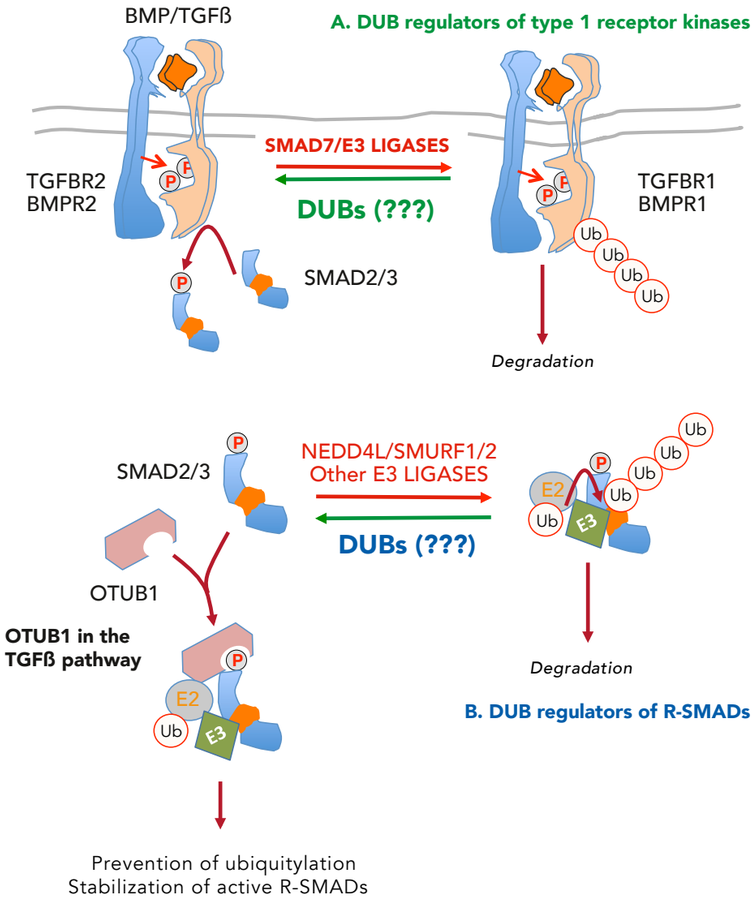
for enabling its localisation in the nucleus, where it can initiate DNA repair mechanisms. Widely expressed in tissues throughout the body, OTUB1's diverse effects can be attributed to this sort of modification. The Sapkota lab was successful in identifying the kinase (an enzyme that adds a phosphate on targets) responsible for this particular phosphorylation event, making it a potential therapeutic target. However, as Dr Sapkota points out himself: "Future investigations should keep in mind that [this kinase] can phosphorylate more than 300 proteins, and off-target effects are likely."

Prevention of protein degradation via the ubiquitination pathway is a key strand of the group's research. Two studies in particular have been successful in identifying deubiquitinating enzymes (DUBs) which are vital for the precise regulation of the TGFβ pathway. Complex feedback loops whereby transcriptional targets of TGFβ proteins promote the ubiquitination and subsequent degradation of the TGFβ receptors or other mediators have been elucidated. The presence of DUBs allows for this to be reversed, to enhance TGFβ signalling. As these pathways are vital for accurate embryogenesis (development of embryos to the foetal stage), efficient control is key to preventing developmental defects. Improper regulation is also a key feature of carcinogenesis (development of cancer), so identifying the main elements of the pathway gives a clearer picture of how this disruption occurs and where it might be mediated.



TRAILBLAZING TECHNIQUES

These are just a few of the significant advances the group led by Dr Sapkota have made in recent years. Alongside their collaborators, they are continuing to probe the cellular mechanisms underlying the TGFβ signalling pathway, as well as looking at other critical pathways in cellular homeostasis (maintenance of a stable equilibrium), such as the Wnt pathway. This group of signalling pathways pass signals into cells via surface receptors and intracellular proteins. Involved in gene transcription, cellular morphology (shape) and internal calcium concentration (itself related to the regulation of diverse cellular processes), this complex and interacting field of study is still not completely understood.

Untangling this complex web of interactions is not for the faint hearted. Dr Sapkota's team use the latest techniques in molecular biology and careful study design to unravel the effects of different signalling molecules and protein interactions. For example, Dr Sapkota says: "We are combining the rapid genome editing capability afforded by CRISPR/Cas9 with advanced knowledge of protein chemistry to engineer robust molecular tools capable of selectively targeting individual proteins." It is precisely these tools which have enabled Dr Sapkota and his research team to make such rapid progress in understanding the pathways' attributes, continually driving their research forward. As they uncover more functionality to the effects of post-translational modification (changes to a protein after it has been created by the cell), particularly around the reversible elements of phosphorylation and ubiquitination, the group is likely to identify further targets for therapeutic exploitation. Developing both the knowledge and the tools to address the problems of developmental disorders and the scourge of cancer is the ultimate goal of this ground-breaking research.

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Q&A

How are the complexities of cell signalling best explained to a layman?

Cells are the building blocks of all living organisms. In multicellular organisms, cells and tissues constantly communicate with each other in order to maintain a healthy state. When cells and tissues receive specific signals, which can be anything from chemicals and proteins (such as TGFβ) to pathogens and environmental factors, they initiate complex intracellular signalling events to help them mount a suitable response. The breakdown in cellular communication processes often leads to many diseases, including cancer. Therefore, understanding precisely how cells sense and process specific signals and mount an appropriate response is key to identifying and overcoming any miscommunication.

What are the main challenges in uncovering these interconnected pathways?

Trying to untangle the interconnected signalling networks that control the behaviour and fate of every cell in different tissues and microenvironments within an organism at the molecular level is incredibly challenging and requires concerted effort from many scientists around the world and multidisciplinary approaches. We know much about how individual linear signalling networks function within individual cells. However, figuring out how these are integrated and regulated in the context of whole organisms remains challenging. Tackling these challenges requires a mammoth effort from scientists from all disciplines and as such, our lab focusses on resolving a few pieces of this enormous jigsaw puzzle!

What has been the highlight so far of your extensive achievements?

In recent years, we have uncovered some key deubiquitinating enzymes that protect intracellular mediators of TGFβ/BMP signals from destruction. More recently, we have developed an affinity-directed protein missile system (AdPROM) that allows us to selectively destroy target proteins inside the cells. This technology is not only useful for researchers to uncover protein function but also offers potential to expedite drug discovery, especially for targets where conventional drug strategies have failed. We are particularly excited by our recent identification of the previously uncharacterised protein PAWS1 not only as regulator of non-canonical BMP signalling but also as critical regulator of Wnt signalling.

How have collaborations helped you to achieve your goals?

My research group has actively sought, pursued and greatly benefitted from both national and international collaborations. The expertise that our collaborators bring is critical in allowing us to address our research questions in a multidisciplinary fashion and explore the biological significance of our discoveries in unique biological contexts. We have ongoing collaborations with colleagues from Dundee University as well as Crick Institute, Oxford University and Harvard University. Additionally, the ongoing collaboration between MRC-PPU and leading pharmaceutical companies has allowed us to communicate our findings directly with the pharmaceutical companies in order to translate our findings into potential drug discovery projects.

What is next in this fast-moving field of research?

These are exciting times to be involved in biomedical research! Recent breakthroughs in biotechnology, such as CRISPR/Cas9 genome editing technology, advanced genome sequencing and bioinformatics tools, advanced protein chemistry tools, and many others, allow us to investigate complex cellular signalling processes and understand how they malfunction in diseases at break-neck speeds. At this rate, the next decade will see significant advances in our understanding of many human diseases, such as cancer and neurodegeneration, and better and improved therapies. The practical challenges we face are ensuring that bright young minds continue to harness these challenges into the future and the policymakers continue to value and support biomedical research.

Detail

RESEARCH OBJECTIVES

Dr Sapkota's research focuses on elucidating the signalling networks involved within cells and human disease. More specifically, his lab aims to determine the molecular function and regulation of the TGFβ/BMP and Wnt pathways in cells and human diseases.

FUNDING

Medical Research Council (MRC)

COLLABORATORS

- Sir Jim Smith (Crick Institute)
- Alex Bullock (Oxford)

BIO

Dr Sapkota grew up in Nepal before studying Biochemistry at Bath University. He later obtained a PhD in Biochemistry at Dundee University before moving to New York to pursue a Postdoctoral Fellowship at the Memorial Sloan Kettering Cancer Center. Following this, he joined the School of Life Sciences at Dundee University as a Programme Leader.

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