1-12-2015

The Cell's Antenna and Bending with the Flow

Surya M. Nauli
Chapman University, nauli@chapman.edu

Kimberly F. Atkinson
Chapman University

Sarmed H. Kathem
Chapman University

The Cell's Antenna and Bending with the Flow

DRS SURYA M NAULI, KIMBERLY F ATKINSON & SARMED H KATHEM

The cell's antenna

Drs Surya M Nauli, **Kimberly F Atkinson** and **Sarmed H Kathem** describe their innovative research in primary cilia and the many international collaborations that have enabled their success

Could you begin by introducing the function of primary cilia within the body?

SMN: The ability of primary cilia to sense fluid flow is probably their best studied function. This enables ciliated cells to sense urine flow in the kidney, blood flow in the vasculature, hepatic bile in the liver, digestive fluid in the pancreas, lacunocanalicular fluid in bone or cartilage, and more.

Collaborating with Dr Wissam AbouAlaiwi at the University of Toledo, USA, we found that primary cilia can regulate cell division and DNA content, and help the cell to locate itself within the microenvironment – a process also known as planar cell polarity. Most recently, our laboratory has also found a chemical-specific sensory function of primary cilia in both vascular endothelia and renal epithelia.

Aside from the mechanosensory, chemosensory and regulatory cell division functions of primary cilia, other laboratories have also discovered the roles of primary cilia in gravitational sensing, regulating cell volume, identifying left-right body formation during embryogenesis, directing cell migration, and many others.

Why is it important to gain a solid understanding of the molecular and biophysical properties of mechanosensory and chemosensory primary cilia?

KFA: The importance of primary cilia has only just begun to be understood. When a cell loses sensory input from its antenna-like cilium, it cannot respond to environmental cues and changes. The mechanosensation of primary cilia occurs principally in response to fluid shear stress, while chemosensation refers to the cell's ability to sense chemical cues such as ligands in the blood or neurotransmitters in the synapse. Dysfunctional cilia are known to be the cause of a group of diseases termed ciliopathies, which include polycystic kidney disease (PKD), Bardet-Biedl syndrome, and primary ciliary dyskinaesia. These diseases are caused by mutated proteins in the primary cilium or centrosome. The association between cilia and centrioles has opened the possibility for cilia having a role in cell division and potentially being a good target in cancer therapy.

Can you describe the translational work you are undertaking? How are you developing ciliotherapies to help patients?

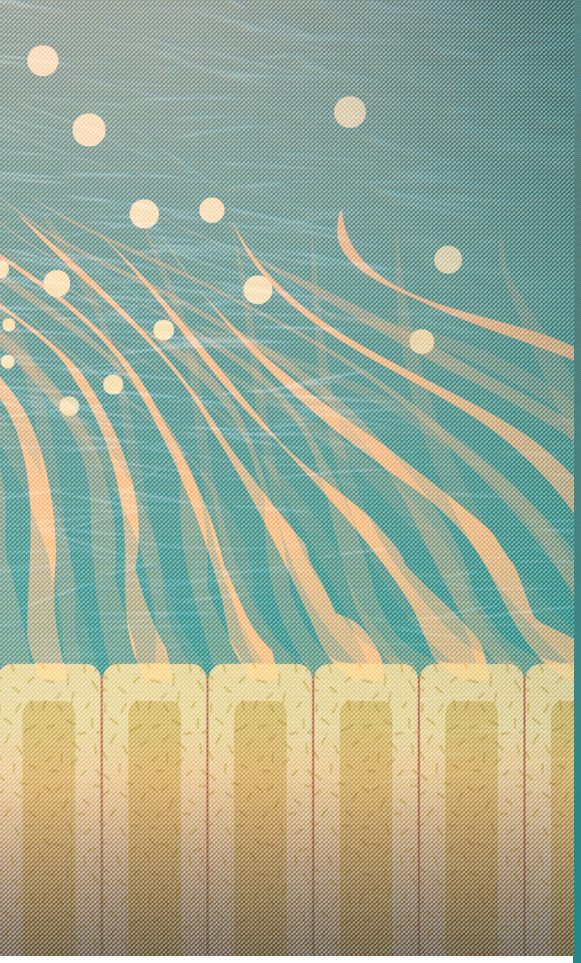
SHK: Our research focused on primary cilia has two goals. The first is to explore the molecular components of cilia-regulated signalling pathways. Understanding these would allow scientists to intervene in the molecular mechanisms in the hope of correcting abnormal molecular pathways of the cell. The second goal is to utilise the knowledge gained to directly target primary cilia. We achieve this by screening thousands of small molecules and asking if any of these compounds would affect primary cilia function or structure. Then, we examine whether and how any alteration would affect cilia-regulated signalling pathways in disease.

How did you come to work in this field? To what extent has collaboration shaped the research you have conducted?

DRS SURYA M NAULI, KIMBERLY F ATKINSON & SARMED H KATHEM

Bending with the flow

Researchers at **Chapman University**, USA, have demonstrated the importance of the mechanosensory ability of primary cilia – microscopic hairs on the surface of cells – and led the way in developing novel treatments for ciliopathic diseases



SMN: We came to learn about the primary cilia while completing our National Institutes of Health (NIH) fellowships with Dr Jing Zhou at Harvard Medical School in the US, working on an exciting project related to PKD. Dr Zhou then introduced me to Dr Ken Spring, who was an outstanding scientist from NIH. He was the first to directly activate mechanical function of cilia using a micropipette. Dr Spring visited Boston many times to instruct me in using microscopy to study this mechanosensory function further. We were also lucky enough to be guided by Drs Don Ingber and Joe Bonventre, experts in cell mechanics and renal physiology. Our laboratory is currently working on the roles of primary cilia in the cardiovascular system. We have thus collaborated with our mentor and colleague, Dr William Pearce, who is a renowned cardiovascular physiologist. Having such wonderful mentors provided us with excellent experiences and enabled us to attain a successful research career in cilia biology.

Have any results surprised you thus far?

SMN: Our group has provided a potential new cellular mechanism for fenoldopam. It was previously known that fenoldopam-induced hypotension was achieved by increasing kidney filtration, but the mechanism by which this occurred was not understood. Our surprising data show that the fenoldopam receptor is exclusively located in the cilia. Activating this receptor increases cilia length, improving their function, which in turn induces vasodilation and increases the kidney (glomerular) filtration rate.

POLYCYSTIC KIDNEY DISEASE (PKD) is the most common life-threatening genetic disease worldwide – affecting about 12.5 million people. Patients with the condition have multiple fluid-filled cysts in their kidneys that lead to a massive enlargement of the organ and gradual worsening of its function, as well as complete failure in many cases. The lack of available treatment means patients may eventually require regular dialysis or a kidney transplant.

Known as a ciliopathy, PKD is caused by dysfunction of the primary cilia on the renal epithelial cells lining the ducts within the kidney. Primary cilia are microscopic hair-like structures found on the surface of most mammalian cells; however, until the 1990s they were not considered important for cell function. Unlike the motile cilia that cover certain types of cell and move in a coordinated manner, only one non-motile primary cilium is present on each cell, increasing its surface area for important roles in sensing a variety of chemical and mechanical signals.

Primary cilia are mechanosensitive; they bend in response to fluid-shear stress and provide information about the external fluid environment to the cell. Dr Surya M Nauli from Chapman University, USA, has spent many years investigating the roles of primary cilia in PKD and other diseases. In collaboration with his colleagues, Drs Sarmed H Kathem and Kimberly F Atkinson, his team is focusing on how interrupting a cell's ability to detect fluid flow leads to disease. "Fluid flow-induced intracellular signalling regulates various cellular functions like planar cell polarity, cell division and differentiation," Kathem elucidates. "As PKD patients have dysfunctional fluid flow mechanosensation, this signalling is also impaired and, consequently, disorientated renal cell divisions lead to the formation of cysts – the prominent feature of PKD." It is therefore vital to obtain a better understanding of primary cilial function in the body, with the ultimate aim of developing new ways of treating ciliopathies.

CILIAL FUNCTION

PKD is typically caused by a mutation in one of two genes, PKD1 or PKD2, which encode polycystin 1 (PC1) and PC2 respectively. Both of these proteins are found on the surface of the cell, localised on the primary cilium. PC1 has a long structure that reaches out into the cell's environment, while PC2 is a calcium-permeable cation channel. The two form a polycystin complex on the surface of the primary cilium, which, when activated, can signal inside the cell.

The team was able to uncover the link between these proteins and PKD. "Among other ciliary proteins, Nauli's research showed that PC1 and PC2 function as mechanosensory proteins," Kathem reveals. "Owing to the unique ciliary localisation of these proteins, when primary cilia are bent by the fluid flow, these ciliary proteins undergo a structural conformation change and evoke intracellular signals that regulate many cellular functions." Nauli's early work showed that blocking the function of either PC1 or PC2 was enough to stop kidney epithelial cells from perceiving the mechanical cues of fluid flow, which could lead to the regulation of tissue growth and result in the development of cysts.

Nauli and his colleagues succeeded in defining the roles that PC1 and PC2 play in primary cilia mechanosensation. The long extracellular domain of PC1 extends out into the cell's microenvironment and acts as a lever to amplify stresses at the cell surface, transmitting the signal to its tightly-associated PC2 channel. The channel then opens to allow calcium ions to enter the cell in a flow-induced manner, enabling the cell to understand the fluid dynamics of its external environment and inducing gene expression to regulate growth. This knowledge was very important for deepening the researchers' understanding of PKD and providing potential targets for future therapeutic development. "The search for therapy to treat cilia-associated diseases is, no doubt, a very complex process," Nauli explains. "It will take years or even decades to accomplish and validate its safety and effectiveness toward our patients, but we think that we are on the right path to tackle the challenges."

PKD, HYPERTENSION AND ANEURYSM

In addition to cyst formation in their kidneys, patients with PKD often develop high blood pressure, which manifests around a decade earlier than in non-PKD patients, as well as other cardiovascular conditions such as aneurysm. Interestingly, this is unlikely to be due to the failing kidney. "PKD patients have hypertension that emerges early in life, even before renal function deterioration, which supports the hypothesis that hypertension in PKD is not solely the result of a cystic kidney, but rather stems from inherited endothelial dysfunction," Kathem explains. The endothelial cells lining the blood vessels are known to be able to sense fluid shear stress. The mechanostimulation of the cells causes an influx of calcium to initiate the biosynthesis and release of nitric oxide, which controls vascular contraction and therefore regulates blood pressure. The same influx of

SENSORY ROLES OF PRIMARY CILIA: CILIOPATHY AND CILIOTHERAPY

OBJECTIVES

To better understand the role of primary cilial function in the body with the overarching objective of developing new ciliotherapies for conditions such as polycystic kidney disease (PKD).

KEY COLLABORATORS

Dr Wissam AbouAlaiwi, University of Toledo, USA • **Dr Jing Zhou**; **Dr Don Ingber**; **Dr Joe Bonventre**, Harvard Medical School, USA • **Dr Ken Spring**, National Institutes of Health (NIH), USA (retired) • **Dr William Pearce**, Loma Linda Medical School, USA

FUNDING

Department of Defense (PR130153), USA

Chapman University School of Pharmacy, USA

CONTACT

Dr Surya M Nauli
Professor

Chapman University School of Pharmacy
Department of Biomedical and Pharmaceutical Sciences
9401 Jeronimo Road, Suite 272
Irvine, California 92618
USA

T +1 714 516 5485
E nauli@chapman.edu; snauli@uci.edu

www.chapman.edu/our-faculty/surya-nauli

SURYA M NAULI received his PhD in Pharmacology from Loma Linda Medical School, USA. Specific areas currently under investigation in his laboratory include molecular, cellular and biophysical studies of primary cilia; chemical screening for pharmacological candidates *in vitro*; and development of a more efficient *in vivo* system to further characterise the cilia-targeting therapy in vascular and kidney diseases. Surya's research and clinical teams are currently completing a clinical study strategised for a targeted therapy in hypertensive patients.

KIMBERLY F ATKINSON earned her PhD in Molecular Medicine and Translational Science from Wake Forest School of Medicine, USA. She is currently working on the role of primary cilia in human disease. Potential avenues of research include the involvement of primary cilia in ciliopathies such as PKD and targeting the centrosome and primary cilia in cancer therapy.

SARMED H KATHEM completed his PhD in Pharmacology in 2012 with a thesis focusing on the roles of primary cilia. He was immediately appointed as a tenured faculty member in the College of Pharmacy, University of Baghdad, Iraq. Kathem's research includes *in vitro* and *in vivo* models and clinical studies. His clinical expertise includes PKD, hypertension and ciliotherapy.

CHAPMAN UNIVERSITY

calcium also initiates a complex signalling cascade to regulate survivin expression and aneurysm formation.

Nauli and his colleagues used mice lacking PC1, PC2 or primary cilia to show that primary cilia are responsible for the initial mechanosensation of the endothelial cells, using the same PC1-PC2 signalling complex seen in the kidney epithelial cells. Cells from PKD patients confirmed that this protein is missing from cilia in their vascular endothelium – which are therefore unable to transmit information about increased blood flow – do not release nitric oxide to stimulate vasodilation and do not properly maintain blood vessel architecture. This important discovery explains the hypertension and aneurysm of PKD patients, and provides further evidence to show that primary cilia are mechanosensory microcompartments important for a range of biological activities. "The different cell types in the kidney and vasculature perform different functions, and having abnormal cilia will have different physiological effects," points out Atkinson. "The underlying problem is the inability of these cells to respond to mechanical stimulation of fluid flow, thereby affecting renal morphology, vascular architecture, vascular tone and blood pressure."

CHEMOSENSATORY TREATMENT

The length of primary cilia is very important for their ability to function, which is why they are highly regulated. In the kidneys, cilia are longer in wider tubules than in narrower ones. Nauli discovered the molecular interactions that regulate ciliary length by rearranging the cell's structural elements. He and Kathem began to screen a range of compounds that might alter cilial length in order to improve their function. One promising candidate was fenoldopam, a drug currently used to treat hypertension, which stimulated the ciliary chemosensory pathway. When Nauli and his team tested the drug on a PKD mouse model, both the length of cilia in the vascular endothelium and the level of nitric oxide in the blood increased. Clinical trials in PKD patients showed that dopamine agonist treatment led to a reduction in blood pressure, a groundbreaking result that provided the first example of a ciliotherapy capable of restoring primary cilia function in ciliopathic patients. "Our studies further show that ciliotherapy is a promising way to treat cilia-associated diseases, and we are still working on a more efficient agent that could restore cilia function and thus relieve hypertension," Kathem elaborates.

The group's research has highlighted the importance of primary cilial function by providing a deeper understanding of the ciliopathies that can lead to disease. Nauli and his team are working on the basic biology of these structures and the translation of this knowledge into therapeutics for the future. "Understanding the molecular and biophysical basis of cilia function is key to the development of drugs that target the primary cilia to treat disease," Atkinson concludes.