Could you outline your background and how your research interests came to focus on the physiology of the bladder?

I completed my doctoral studies at the Women’s and Children’s Hospital of Adelaide in Australia, working with epithelial cells to try to understand the pathophysiology of cystic fibrosis. Hopes were very high for a cure when I started because the chloride channel responsible for the disease – the cystic fibrosis transmembrane conductance regulator (CFTR) – had just been cloned. Over the course of time, unfortunately, those hopes have diminished considerably as both the protein and the disease it causes have proven to be much more complex and difficult to unravel than originally anticipated.

I continued my studies on CFTR at the University of Pittsburgh when I joined Dr Ray Frizzell’s team and three years later I moved to Dr Mark Zeidel’s lab to begin studying the biophysics of membrane permeability and membrane transport molecules. My morphogenesis into a bladder physiologist began a few years later at the Beth Israel Deaconess Medical Center (BIDMC) in Boston when we established a collaboration with another group at the hospital led by Dr Raghu Kalluri. They had bred mice that lacked integrins in the kidney, and showed highly abnormal structures in the glomerulus and an inability to filter urine correctly. This motivated us to try a similar thing in the epithelium of the bladder.

What are integrins and how have they facilitated your approach to understand the function of the urothelium?

Integrins are cell adhesion molecules that connect the inner cytoskeleton of the cell to the extracellular environment through interactions with matrix molecules like collagen, laminin and fibronectin. We were prepared to see urothelial defects and possibly a loss of barrier function, which is one of the primary roles of the urothelium since it stops chemicals in urine leaking back into the blood. However, as usual, science or perhaps nature chose not to cooperate and we could find nothing structurally wrong with the urothelium at all. Instead, the ability of the mice to urinate was abnormal and they appeared not to sense bladder filling properly, a phenomenon we refer to as a mechanosensory defect. A greater understanding of how the bladder senses filling at the molecular level is where our primary research focus lies today.

How does Cre-lox deletion work? Can you describe this process?

Cre-lox deletion is an approach which allows the investigator to remove specific genes (and therefore proteins) in a target tissue. The critical requirement is that the targeted cell type possesses a gene that only it can express. By isolating the promotor for that cell-specific gene, and engineering it to sit next to the viral DNA-cleaving enzyme gene Cre (short for cyclisation recombination), one ensures that only the cell type with that active promotor expresses Cre. As an example, the uroplakin protein UP-II is expressed only in the urothelium and its gene promotor therefore makes a very useful tool for Cre-lox. When the Cre enzyme comes in contact with specific sequences of DNA known as lox P sites, it cleaves the DNA at two points in the sequence, removes the DNA in between, and connects the two lox P sites back together. By mating a strain of mouse that expresses tissue-specific Cre with another strain in which lox P sites have been engineered to surround the targeted gene (or some important part of the gene, say an exon), offspring may be generated, in which a very specific DNA excision has occurred, resulting in loss of that gene product.

You have collaborated with other institutions to form the Center for Interdisciplinary Research in Benign Urology (IRBU). How has this facilitated the progression of your project?

The Center involves collaboration between my group, the laboratory of Dr Mark Zeidel, who is Chair of Medicine at BIDMC, and geneticists and biostatisticians at the Jackson Laboratory in Maine led by Drs Gary Churchill and Cheryl Ackert-Bicknell. Its goals are not directly related to our integrin studies, but broadly aim to determine the genetic signatures that predispose mice to lower urinary tract symptoms with ageing. Interestingly, although we suspect that urinary tract problems may be heritable, it is almost impossible to establish whether this is actually true. This is because, firstly, the symptoms take so long to develop, and secondly, they are embarrassing and therefore often go unreported both to physicians and to other family members.

Do you plan to present your work at any forthcoming events or conferences?

I will be attending the American Urological Association conference in Orlando, Florida in May 2014 to present some of the IRBU Center data. I also plan to attend the Experimental Biology Conference in 2015, by which time we should have some new findings on the integrin signalling pathways, and on the vesicular nucleotide transporter knockout.
TERMINAL ILLNESSES SUCH as cancer and heart disease are highly visible, and rightly attract a lot of attention from the medical research community – but in social and psychological terms, their lethality is only part of their threat. Often, a fear of terminal illness is not only a fear of death, but also of the loss of independence, the loss of freedom, and of presenting a burden to others. In other words, a reduced quality of life might be perceived by patients as worse – or at least more immediate – than the loss of life itself. But these problems are not the sole preserve of terminal illnesses; while cancer and heart disease are examined from every angle, less lethal diseases, but ones that still present a great threat to the patient’s quality of life, are being largely overlooked.

One area that has received less attention than it deserves from researchers, despite the burdens it imposes, is the urinary tract. ‘Lower urinary tract symptoms’ is a catch-all term used to refer to diseases of the bladder, prostate, urethra and urethral sphincter, and it reflects the lack of specificity medicine brings to this region of the body. 10 per cent of nursing home admissions are precipitated by the loss of bladder control, a fact that adequately demonstrates the huge influence of these symptoms on independence and quality of life – but bladder control is only the beginning. Problems with pain, incontinence, frequency, urgency and even blockage all reside within the category of lower urinary tract symptoms – and patients often find these vaguely defined disorders to be very clear in terms of the extreme embarrassment and pain they cause. A sobering example of this is interstitial cystitis, which is thought to produce discomfort comparable to chronic cancer pain, and yet is so poorly understood that it can only be diagnosed by exclusion.

A STARTING POINT

The problem is that, far from being able to approach the problems of lower urinary tract dysfunction, science does not yet have a complete understanding of the role played by various cells, pathways and receptors in the normal function of the system. More research is needed in order to provide even a starting point for the investigation of these diseases – and as time goes on, the pressure to provide solutions will only grow. Already, 35 million Americans are known to suffer from overactive bladder syndrome alone; an ageing population will only serve to further highlight these kinds of health problems over the coming decades.

One team doing its part to find out more about the function of the lower urinary tract is led by Dr Warren Hill of the Harvard Medical School in Boston, Massachusetts. Relying largely on rodent models, the Harvard lab aims to determine the function of various protein families present in the bladder, and the role they play in mechanosensation. As Hill clarifies: “Normal urinary storage and voiding function requires the coordinated actions of many cell types, including smooth muscle cells, urothelium, and neurons,” – and the dysfunction of any of these components could therefore lead to lower urinary tract symptoms. Hill’s work focuses on the urothelium, the epithelium lining the inner surface of the bladder, which may be a far more complex group of cells than previously thought.
INTELLIGENCE

THE ROLE OF INTEGRINS IN BLADDER UROTHELIUM

OBJECTIVES

- To elucidate the molecular basis for diseases resulting in abnormal bladder function, including incontinence, overactive bladder, complications of diabetes and interstitial cystitis/painful bladder syndrome
- To investigate several classes of protein with putative mechanosensory function, such as integrins, ectonucleotidases, purinergic receptors and the vesicular nucleotide transporter
- To understand how genetics in mice influence the onset of lower urinary tract symptoms with ageing

KEY COLLABORATORS

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Dr Gary A Churchill; Dr Cheryl Ackert-Bicknell, The Jackson Laboratory, Bar Harbor, Maine, USA

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WARREN HILL has an extensive research background in epithelial physiology and within the last five years has focused on urothelial and purinergic signalling in the bladder using genetically engineered mice as animal models. His primary interest involves mechanosensation and striving to understand how the bladder senses and communicates its fullness and how the distinctive layers and cells of the bladder communicate that information to each other.

INTEGRIN AND INCONTINENCE

The bladder’s mechanosensory system is responsible for determining when the organ is full and urine must be voided, a process known to involve the nerves, interstitium, muscle and urothelium. Miscommunication between these components results in syndromes such as overactive bladder, and the researchers at Harvard Medical School recently performed a study aimed at discovering exactly how the urothelium is involved in this communication breakdown; their results were quite surprising. Using Cre-lox P deletion, they created β1-integrin knockout mice – mice lacking a specific intercellular protein usually found in the urothelium that is instrumental in the cellular sensation of force. The hypothesis was that if these mice showed abnormal bladder function, then it would suggest the urothelium was in some part a sensory tissue.

Indeed, the results demonstrated that the knockout mice were incontinent – they ‘leaked’ continually over the floor of their cage, and pressure recordings within the bladder showed that they were overactive as well. In short, the deletion of integrin in the urothelium had a dramatic impact on bladder function, strongly suggesting the urothelium is not an impermeable bag for storing urine, but a dynamic sensory tissue able to gather and communicate information about its environment. How signals arising in the urothelium translate to overactive bladder syndrome is still a mystery, but Hill suggests: “It could be due to potent signalling molecules such as adenosine triphosphate (ATP) being released inappropriately and affecting nerve stimulation”. This study may also motivate investigations into whether people with lower urinary tract symptoms suffer integrin signalling defects.

LOOKING CLOSER

For the Boston team, one of the most provocative findings from the integrin study was that the integrin knockout mice released twice as much ATP into their bladders as regular mice – and this discovery has led to a number of subsequent congruent studies. For decades it has been known that the urothelium releases ATP when it stretches, but excessive release could play a role in urinary disorders. To test whether this is the case, the researchers are now beginning to examine the role of vesicular nucleotide transporters (VNUTs) in the urothelium. VNUTs are proteins that transport ATP created within the cell to the extracellular environment, using lipid bubbles called vesicles as vehicles. By knocking out VNUTs, the scientists hope to determine the role of ATP signalling in bladder control – and whether cancelling it can bring the incontinent integrin-deficient mice back to normal functionality.

Other studies performed by the lab have been concerned with characterising new purinergic receptors within the smooth muscle tissue. ATP is associated with purinergic signalling, but other related molecules such as uridine-5’-triphosphate (UTP) are as well – and the researchers’ studies have shown that enzymes able to convert these signal molecules into different ones by ‘chopping them up’ and altering their structure are present on the smooth muscle cells of the urothelium. For example, whereas ATP causes the muscle to contract, adenosine alone causes the muscle to relax – so by removing the triphosphate, the enzyme can control the signal. This revelation has led the team to hypothesise that there is a purinergic network in the bladder, which fine tunes the timing of bladder contraction and relaxation.

THE ROAD TO TRANSLATION

In themselves, these studies aim to acquire a basic understanding of the lower urinary tract, rather than any kind of immediate translational solution for patients – but Hill and his collaborators are hopeful that this is a potential outcome that could be achieved with time, and with studies in human populations linking integrin function with an overactive bladder. Despite this possibility, however, Hill remains definite about where the focus of urologists should lie: “This may be my particular hobby horse, but I can’t stress enough how important it is that we continue to define the normal physiology of the lower urinary tract. How can one begin to treat the abnormal with no understanding of how it got that way?”