# Mutants, mice & melanomas

A deeper understanding of the intricate relationship between connexins, pannexins and tissue function could usher in a new era of targeted therapeutics and organ restoration, as **Professor Dale Laird** explains

**You have been researching connexin assembly and gap junction turnover since 1992. How have your aims and objectives evolved over time?**

When I started my research as a postdoctoral fellow at the California Institute of Technology, only three human connexins (Cx) were known to exist; now there are 21 and the field has become increasingly more complex. We now know that most cells in the human body express two or more members of the Cx family, allowing for great diversity in channel types and meaning that our laboratory must consider and investigate how Cx family members work together and differently in various cell types. For example, Cx have a long life in the lens and mineralised bone, but in almost every other tissue their turnover is incredibly fast for an integral membrane protein. Our long term objectives continue to focus on how Cx regulate the organisation and function of tissues, as they are essential for proper organ formation and function.

**Could you elaborate on the cultured cells and three-dimensional tissue-relevant models you use to examine the mechanisms underlying certain human disease states?**

Cells grown in culture are great for investigating how a disease-linked Cx mutant may cause disease. We often start with cells that have no Cx and engineer them to express mutants, while asking whether the mutant has the functional capacity to make a gap junction channel. Functional assays include assessing if a cell can pass small fluorescent dyes or, working with our collaborator Dr Donglin Bai, if they are electrically-coupled. Since Cx mutants are often co-expressed with wild-type counterparts, we then ask whether the mutant inhibits the function of wild-type Cx.

These experiments are particularly important as they help us to understand why a patient might have disease in one organ but not in another. Three-dimensional tissue-relevant models, or organotypic cultures, recapitulate the complexity of the organ. In our laboratory, we routinely grow and differentiate keratinocytes on a collagen-base at a liquid-air interface, causing the keratinocytes to form a complete epidermis, including cornification, mimicking mammalian skin. This enables us to evaluate the role of skin disease-causing Cx mutants by engineering the keratinocytes to express the mutant prior to inducing their differentiation into epidermis.

**In what way do the genetically-modified mouse models complement your investigations?**

Genetically-modified mice are a great resource for investigating the link between Cx mutants and disease. We have been fortunate to have received animals from several colleagues around the world in which the mutant is co-expressed with its normal counterpart. These mice mimic the patient groups that have autosomal dominant Cx gene mutations and, importantly, we can either study them as whole animals, or isolate primary cells from different tissues and organs for further biochemical and cell function analysis.

**You have also established several bench-to-bedside studies using patient samples obtained from around the world. Could you offer a brief summary of this work?**

Some years ago we came to the realisation that the best experimental model for human genetic diseases are humans themselves. This raised the question as to what tissues or cells one might be able to obtain from patients with Cx-related diseases. Through clinical collaborations with Dr Jacinda Sampson (formally at the University of Utah) and Dr Ethylin Jabs (Mount Sinai Hospital, New York City) we were able to obtain skin biopsies from which primary human dermal fibroblasts were isolated and grown. In some cases, we were able to recruit the participation of unaffected family members into the study, using dermal fibroblasts as comparative controls. These primary human dermal fibroblasts, expressing the Cx of interest as well as the mutant, can be used indefinitely for numerous biochemical and functional tests where we attempt to rescue the cellular defects cause by the mutant Cx.

**What has your research revealed about the role of pannexins in malignant melanoma cancer cells?**

We discovered that Pannexin1 (PANX1) is highly up-regulated in malignant mouse melanomas; and upon targeted knockdown of Panx1, melanomas re-programme to become more melanocytic in nature. Most importantly, melanomas with reduced Panx1 grow more slowly in animal models and are less prone to metastasise. We are currently validating our mouse findings in human melanoma cell lines and patient tumours, and plan to devise strategies for blocking Panx1 channels, or reduce Panx1 levels, as a means of reducing the aggressive nature of malignant melanomas. Panx1 is an ideal target for drugs as it has motifs that are exposed on the outside of the cell.

# Closing the gap

A laboratory at the **University of Western Ontario**, Canada, is pioneering studies in cell biology which promise a new generation of therapies for patients suffering from gap junction diseases

**PART OF THE** normal functioning of human cells involves direct communication with their neighbours through channels called gap junctions, which are constructed from the protein connexin (Cx). There are 21 different types of Cx, and they play a major role in the normal tissue functioning of most major organs, with numerous human cell types expressing more than one member of the Cx family. Many diseases are accompanied either by cells failing to produce the correct number of gap junctions, or by Cx which contain mutations, inhibiting their function.

Genetically-modified mice lacking even one type of Cx exhibit various developmental problems and a greater susceptibility to disease. Mutations of specific Cx genes have been linked to human disorders including heart defects, neurodegeneration, skeletal abnormalities, skin disease, stroke, epilepsy and hereditary neurosensory deafness. These 'gap junction diseases' are typically hereditary. Though in some cases they can occur spontaneously; it is estimated that there are hundreds of thousands of affected patients. Research which helps us to build a more complete understanding of the mechanisms, events and pathways related to the lifecycle and function of Cx is therefore a crucial step toward the potential treatment of these disorders, which in turn could result in considerable economic and health benefits.

## CONNEXINS IN CANCER

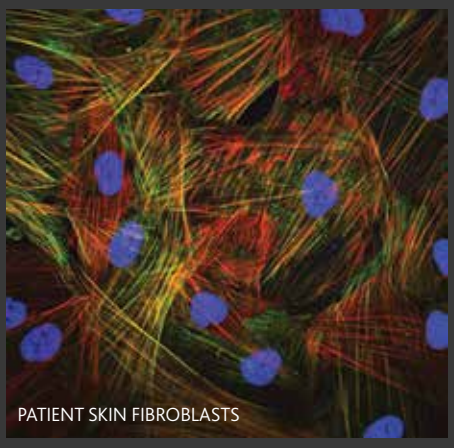
Professor Dale Laird is based at the University of Western Ontario's Department of Anatomy and Cell Biology. He is the Canada Research Chair in Gap Junctions and Disease. Since 1992, his laboratory has been pioneering cell biology studies focusing on Cx assembly and gap junction turnover, using cultured cells and three-dimensional models to examine the mechanisms underlying various associated human disease states. These studies are complemented by the use of genetically-modified mouse models which harbour mutations, mimicking human skin and developmental diseases. Laird's research has led to fascinating insights into the function of gap junctions, including insights into the long-suspected tumour-suppressor properties of certain members of the Cx family. "Studies in recent decades have shown that connexins are often lost in primary tumours, while the restoration of their expression sees the slowing of tumour growth, in culture and in mice," he explains. "We have shown that connexin expression causes breast tumour cells to revert to a more normal-like phenotype."

These are encouraging findings but they should be viewed with a degree of caution: while various studies have shown that mice lacking specific connexins are more susceptible to induced tumour formation in the lungs and liver, other studies have suggested that in some late stage tumours, Cx expression may facilitate metastatic disease providing an advantage to the tumour.

Taking these findings into consideration, Laird, along with his colleague Dr Christian Naus, published a 2010 article in *Nature Reviews Cancer* defining Cx as 'conditional tumour suppressors' dependent on the type of tumour and the stage of the disease. The findings suggest that potential cancer treatments should be combinatorial in nature, taking account of the fact that while enhanced Cx expression might be sensible in early stage disease, it might be, conversely, the inhibition of Cx which proves beneficial in late stage metastatic cases.

## CONSTANT RENEWAL

A relatively early discovery was that Cx and gap junctions have a life span of only a few hours, contrasting starkly with the majority of plasma membrane proteins that typically last for days. This means that nearly all the gap junctions existing in an individual today will have been replaced by tomorrow. While we do not yet fully



PATIENT SKIN FIBROBLASTS

## INTELLIGENCE

### CANADA RESEARCH CHAIR IN GAP JUNCTIONS AND DISEASE

### OBJECTIVES

To determine how mutations in connexin (Cx) genes lead to diseased organs and develop strategies to rescue organ function caused by malfunctioning Cx; and to use preclinical models to assess the value in targeting Cx and pannexin large-pore channels in cancer prevention and treatment.

### KEY COLLABORATORS

**Dr Donglin Bai**, University of Western Ontario, Canada

**Dr Gerald Kidder**, University of Western Ontario, Canada

**Dr Christian Naus,** University of British Columbia, Canada

### FUNDING

Canadian Institutes of Health Research

Canadian Cancer Society

Canadian Breast Cancer Foundation

Tier I Canada Research Chair

### CONTACT

**Professor Dale Laird, PhD**

Department of Anatomy and Cell Biology
University of Western Ontario
Dental Science Building
Room 00077
London
Ontario
N6A 5C1
Canada

**T** +1 519 661 2111 x 86827
**E** dale.laird@schulich.uwo.ca

**www.uwo.ca/anatomy/laird/index.html**

**PROFESSOR DALE LAIRD** completed his PhD in Biochemistry from the University of British Columbia, Canada, and a Postdoctoral Fellowship at the California Institute of Technology, USA, before becoming a full professor at the University of Western Ontario, Canada. He has been the recipient of the Premier's Research Excellence Award, the Medical Research Council Scientist Award, a Tier 1 Canada Research Chair, Faculty Scholar Award and the Dean's Research Excellence Award.

understand why this high turnover occurs, it is clear that our cells are very good at regulating the amount of direct communication they have with their neighbours. For example, when a woman is about to enter labour, her uterus will build 10 times more gap junctions within the 24 hours prior to its onset. Helping to synchronise smooth muscle contraction, this excess of gap junctions is then removed just as quickly once the child is born.

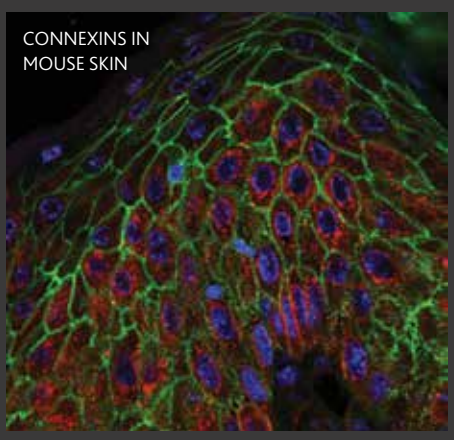
In 2000, a new family of mammalian proteins was identified. Called pannexins (Px), they were sequentially homologous with innexins – the gap junction proteins in invertebrates – leading researchers to suspect that Px might also be capable of forming gap junctions. "We decided to invest in this new family of channel-forming proteins, proceeding to clone and sequence the three mouse Px family members," Laird reveals. "We raised site-specific antibodies for each, and created expression vectors that encoded untagged and epitope-tagged forms of pannexins."

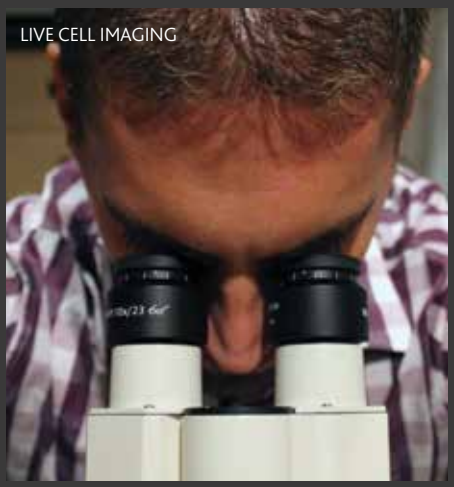
As the data showed, the investment paid off; while Px have many characteristics distinct from Cx, they are also capable of forming large-pore channels in many cell types, which allow molecules in and out of cells. The lab has capitalised on this success and some 50 per cent of its research now focuses on better understanding these promising channel proteins and their role in health and disease.

### OUTSTANDING TEAM

Laird is effusive in his appreciation of the efforts of his team of talented, motivated personnel and the various seminal discoveries for which they are responsible. "I want to stress that any of my lab's accomplishments belong to the outstanding team of researchers, trainees and students with whom I have worked over the past 21 years!" he enthuses. "We continue to seek viable therapeutic targets through our grass-roots approach to Cx and Px biology."

The lab was one of the first to study Cx trafficking and turnover in living cells – turnover which we now know is precisely regulated by a large interactome of proteins. The group has also shed light on the mechanisms through which Cx mutants selectively cause disease, illuminated its role in cancer and contributed extensively to an understanding of how these unique channels function in health and various devastating diseases.


CONNEXINS IN MOUSE SKIN


LIVE CELL IMAGING

As with most laboratories, funding continues to be a significant challenge and, as the researchers begin to work increasingly with cells from patients with Cx-related diseases, recruiting sufficient numbers of participants into their research programmes is ever more difficult. Laird is also acutely aware that translational research, which has more immediacy of impact on clinical care, must continue to be a focus, while being sensitive to the fact that paradigm-shifting, groundbreaking discoveries often arise from more basic science where a clinical application is not immediately obvious.

### ORGAN RESTORATION

In the long-term, the lab is aiming to develop strategies which compensate for the cellular defects caused by Cx mutations. The team is only just beginning to understand why certain Cx mutants cause disease in one tissue while leaving a second organ unaffected, despite being highly expressed in both. The data yielded so far suggest that the answer seems to lie in how mutant Cx interacts with, and impairs the function of, other co-expressed Cx. The lab is now working toward co-expressing Cx family members which do not interact with the mutant as a means of restoring a high level of gap junction communication, while attempting to knock out the mutant and leaving its co-expressed, non-mutated Cx counterpart untouched. Both of these approaches have clinical relevance, heralding the advent of novel therapeutics enabling the restoration of organ function – repairing a hearing defect perhaps, or alleviating a skin disease.

It is exciting work that could lead to a multitude of improvements in patient care. Yet despite the promise of such applications and his lab's various successes, Laird remains modest, retaining a tangible sense of the importance of conscientious research and its place within the international scientific community. "I feel it is necessary to engage on the world-stage at all levels," he concludes, "including policy reform that facilitates and fosters ethically-sound research."

Western