Transports of delight

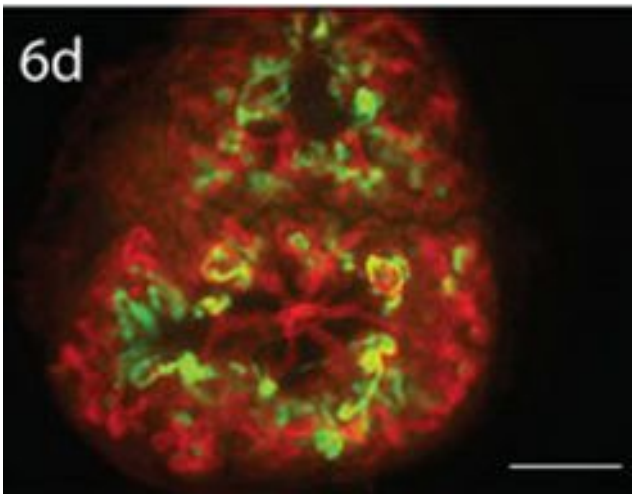
One of the main thrusts of work in this lab – if you can call a series of tentative, faltering steps with many wrong turns and re-tracings, a 'thrust' – is the project to make working, transplantable kidneys from stem cells. Back in 2010 we had our first real success in this, finding a way to take mixtures of dispersed mouse renogenic (kidney-creating) stem cells and culture them so that they would self-organize into a jumble of realistic kidney parts. A 'jumble' in the sense that while each individual part (eg nephron) looked fine, the overall arrangement of parts so important to kidney function was missing. A year later we developed an improved method that resulted in a more realistic arrangement, with developing nephrons (roughly speaking, the parts of the kidney that make urine) arranged, as they should be, in relation to a single, tree-like collecting duct system, and a year after that we could push the maturation of our lab-created kidneys to the stage of their having distinct cortex and medulla (as kidneys should), and loops of Henle. Many other things, like a ureter and a blood system, remain to be added and we are still a very long way from being able to apply any of this technology medically.

One question that is already valid, though, is the simple one of 'do they work'? Clearly these kidneys are far too small to sustain the life of even a small animal, which would be the ultimate test of function, but we can test at least a few basic elements of kidney physiology in culture without having to do animal work at all (regular readers will know that this laboratory does not do experiments in living animals). There are two really 'big' aspects to kidney physiology: filtration and active transport. Filtration is the process by which small molecules from blood pass through a fine filter into the urine space, and is the process mimicked well by dialysis machines. In real kidneys, molecules such as foods and salts that the body needs not to lose are recovered by active transport along the nephron. In addition, some specific organic molecules, typically toxins of various kinds, are expelled by active transport much more efficiently than they would be by filtration. In addition to these two 'big' functions, kidneys have other important functions such as the production of hormones to regulate blood pressure, production of red blood cells, etc, and some metabolism of poisons and drugs.

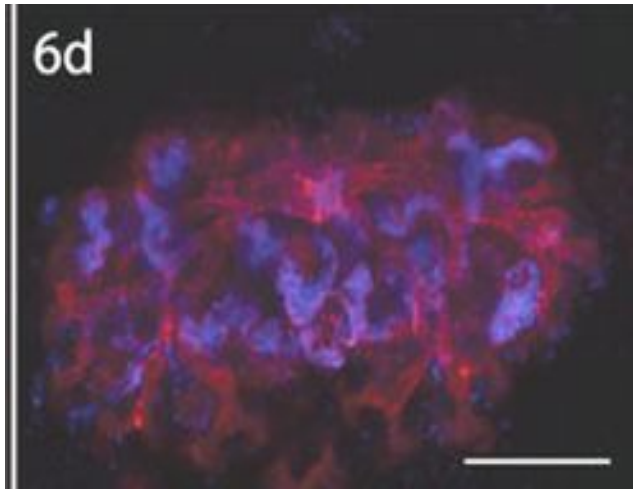
Testing filtration is difficult outside the body because it needs the kidney to be tested to a working blood system. It is therefore not the easiest place to start. Active transport, on the other hand, has no

particular requirement for blood flow as long as the tissues are getting enough oxygen and food to be happy, which are are in our culture systems. What is more, it is possible to purchase fluorescent versions of molecles that are transported actively in adult kidneys. We therefore reasoned that, if active transport is working, it should be possible to add a dilute solution of one of these fluorescent molecules to the nutrient medium surrounding our engineered kidneys and watch the tubules concentrate the fluorescence inside themselves. Furthermore, we thought, it should be possible to treat the cultures with specific drugs that block distinct transport systems, to verify that any transport we see is happening as it would in an adult kidney. Of course these were not very original thoughts – techniques like this have been used to study cell from adult kidneys for years.

Dr. Melanie Lawrence, a postdoctoral fellow in the lab and already skilled in kidney culture, took on this task with the help of C-Hong Chang, a PhD student just finishing his time at the lab who was keen to pass on his kidney engineering skills. Melanie's weekly news updates of each new type of transport detected and the response to each drug used, delivered in her gentle and distinctive Canadian accent and illustrated with stunning projection slides, became regular feature of our lab meetings for some months. Not only did she verify that these active transport systems are indeed up-and-running in our engineered kidneys; she also determined the time-course in which they are acquired in the development of natural kidneys, to show that the order of events is very similar.



A typical image from the paper. The red colour shows all tubules in the kidney. The green is a fluorescent organic anion that is transported actively into, and therefore concentrated in, proximal convoluted tubules.



Another typical image, with the same red stain and a blue-labelled organic cation, again being concentrated mainly in the proximal convoluted tubules. Images like this may look peculiar to the uninitiated, but they cause much excitement in lab meetings and, I am pleased to say, conferences.

We have just published the experiments (see links below): having verified that the basic physiology of active transport is working, we need to think of ways to test mechanisms of water conservation, sensitivity to hormonal control systems and, of course, filtration. Please wish us luck.

Jamie Davies,
Edinburgh,
March 2015

Links:

The paper being discussed: http://www.nature.com/articles/srep09092

The original 2010 paper: http://www.sciencedirect.com/science/article/pii/S0085253815542737

The 2011 improvement: https://www.ncbi.nlm.nih.gov/pmc/articles/PMC3142442/