From ES cells to urothelium

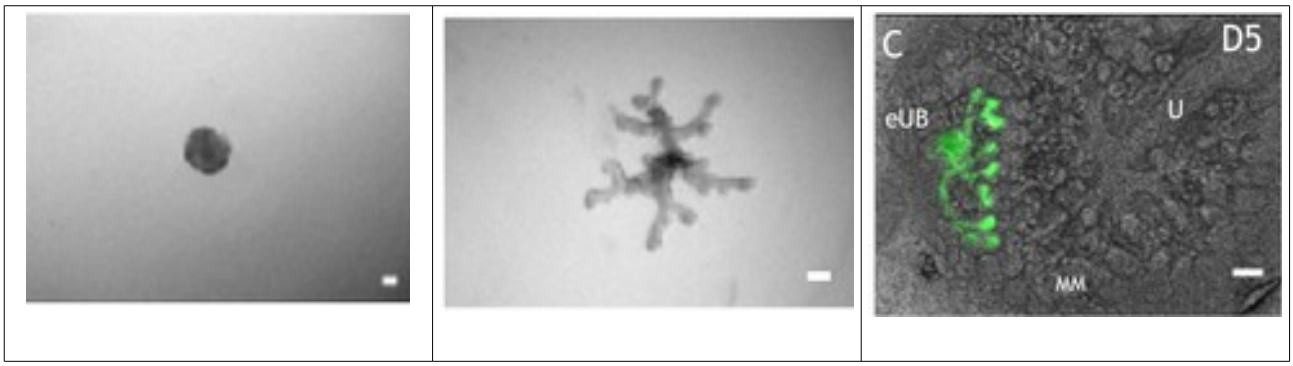
The goal of building a working kidney from stem cells has featured several times in this series of blogs; indeed, it has been one of the main themes in this lab for a decade. A kidney itself would not be of any use without a ureter to take the urine away, and a few years ago a then PhD student, Chris Mills, found a way to persuade a part of a developing kidney that it wanted to make the epithelial part of a ureter - the 'urothelium' - instead (see the earlier blog article, *Chris takes the you-know-what*). Now, May Sallam, a PhD student in her final year of study, has taken this further. Working with others in the lab, she has developed a way of making this ureter epithelium from embryonic stem (ES) cells. She has also shown that the epithelium she makes can organize supportive cells to cluster round it, and to turn into muscles that squeeze it with a rhythm very similar to the contraction seen in real ureters. Her work has just been accepted for publication in the *Journal of the American Society of Nephrology* (a leading kidney journal). Alas, COVID-19 lockdown means we cannot all gather for a lab celebration yet, but here is the story anyway.

Science usually proceeds by someone building something new on foundations laid by others, and this story is no exception. In 2017, Atsuhiro Taguchi and Ryuichi Nishinakamura, working in Kumamoto, Japan, published a method for turning ES cells (cells from the early embryo that can, in principle, make any part of the body) into a type of cell called ureteric bud. This is the progenitor tissue for both the tree-like urine collecting duct system inside the kidney, and for the ureter that drains that organ. Taguchi and Nishinakamura presented a compelling case that their engineered ureteric buds could make realistic kidney collecting ducts. Chris' experiments of a few years ago worked by persuading a branch of the ureteric bud tree in a developing kidney to make ureter instead of collecting ducts. It therefore seemed a logical possibility that it might be possible for us to persuade engineered 'ureteric buds', made from ES cells according to the 'Taguchi method', to make ureter tissue instead of collecting duct.

May first learned to reproduce the work of Taguchi and Nishinakamura exactly as they had published it. This is typical of the way that projects start, for two good reasons. First, it is a test that a protocol developed by other people far away is indeed reproducible. In our experience, they always are; discourse about science is full of panic about a 'replication crisis' but at least in the fields in which I work, I have yet to meet the problem for real. The second reason for repeating the original published experiments to make ureteric buds and then collecting duct is as a way of confirming, when it is our turn to publish, that we really have been following the earlier protocol

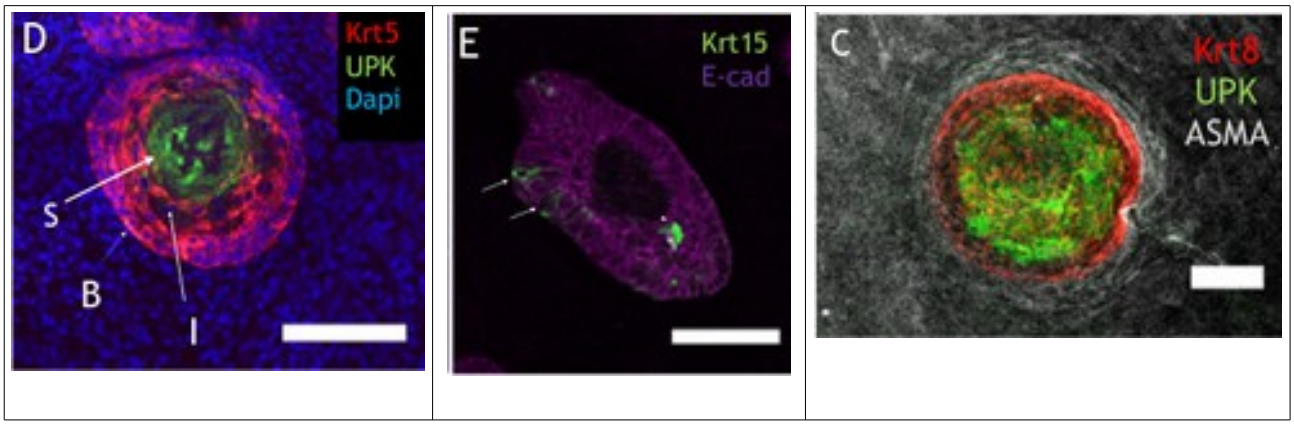
exactly, up to the point where half of our samples were switched to a new protocol to make ureter.

The Taguchi method has many steps, but they can be divided into three phases. In the first, ES cells are exposed to a series of signalling environments that mimic those that would be experienced by a cell that happened to find itself in the part of the embryo that would make ureteric bud. In the second, these cells are placed in a 3D gel (left-hand figure, below; the gel is transparent). The gel has with signalling proteins known to drive ureteric bud branching (many, many years ago, when I was about the age that May is now, I played a part if discovering these). The outcome is that the



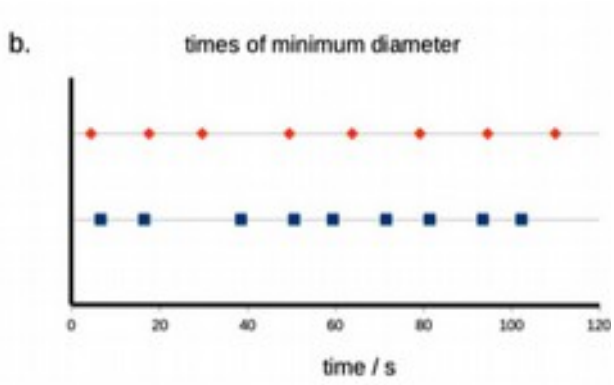
cells make a highly branched structure in the gel (middle photo). In the final phase, the tip of one of these branches is manually isolated, and combined with metanephrogenic mesenchyme, essentially a bunch of stem cells that make the other parts of the kidney. The right-hand photo, above, shows a version of this in which the engineered ureteric bud ('eUB' on the photo) has been grafted into an whole kidney, and is making its own little tree. That tree is green because our ES cells were engineered to make Green Fluorescent Protein, so that we could easily track them in experiments like these.

May's next experiment was to surround her engineered ureteric buds not with mesenchyme from the early kidney, but with mesenchyme that would usually surroung a developing ureter. This mesenchyme is a rich source of signalling proteins such as BMP4, that Chris found to be able to turn ureteric buds into ureter epithelium. When she did this, May found that the engineered ureteric buds did not branch, but instead made a multi-layer epithelium characteristic of the ureter, and that the layers expressed protein markers found in the various types of urothelial cell, with everything being arranged in its expected place in the layers. In the pictures on the next page, the left-hand image shows a layered structure with Krt5-positive basal cells, UPK-positive superficial (urine-facing) cells, and occasional Krt15 cells scattered through the basal cells. There are more pictures in our paper, showing more markers. Striking in the right-hand photo over the page is the presence



in the cells surrounding the engineered urothelium - cells of the host, not the graft - of smooth muscle actin ('ASMA' in the legend). These cells did not make this to begin with. They seem to have organized themselves to wrap around the ES-cell-derived urothelium and to start making proteins characteristic of muscle.

There was a reason that May stained for this smooth muscle marker. When she was looking at her cultures, she was sure she saw them move! To prove the point, she used the microscope camera to make a movie of them, and excitedly sent me the footage. Sure enough, these self-organized 'tissues' twitched with a fairly steady rhythm. I sat down with a very large cup of tea and worked through the movies, and images of natural embryonic ureters undergoing their peristalsis, and plotted out the contractions against time. The rhythms were remarkably similar: this graph shows contractions in the engineered construct (red) and in a natural ureter (blue). There are two interesting things about this, I think. The first is a clear implication that the developing urothelium can send signals to surrounding cells that cause them to organize into active smooth muscles. We have some guesses about what these signals are, but do not know for sure. The other is a verification of an old idea, that muscles of the ureter have their own 'pacemaker' that can establish rhythmical contractions without connection to the central nervous system. In our simple culture system, there was no nervous system, so an inbuilt pacemaker is the only thing that can have



driven those contractions.

The next step in this series of experiments is to make the muscle cells from ES cells too. Mona, a post-doctoral fellow in the lab, is making good progress with this (or at least she was, until COVID-19 threw its spanner in the works), and I look forward to telling the story in a blog when her paper is accepted for publication.

Jamie Davies, East Lothian, June 2020

Links

(I will add a link to May's paper as soon as it is published online).