Julia's eggsperiments

Regular readers of these blogs will know that one of the long-term goals of this lab is to build a working kidney from stem cells. We pioneered the first 'organoids' of kidneys back in 2010, and over the years we have found ways to add to their realism. We can now make kidneys, admittedly very small and immature ones, that have all of their urine-making and urine-carrying tubes arranged properly round a tree-like urine collecting system that makes an exit via a ureter. It has been obvious for some time that the next really big challenge is to make an equally realistic 'tree' of blood vessels to serve the organ. The main function of kidneys is, after all, to filter blood.

Making capillaries in kidney organoids is relatively easy, but making anything larger is not. If an embryonic kidney that already has larger blood vessels is removed from a mouse embryo, the large vessels quickly degenerate, leaving only the capillaries as survivors. The probable reason is that all large blood vessels measure the flow within them and use this measurement to make decisions about whether to grow, maintain themselves, modify themselves or degenerate. And of course, in an organ sitting in a dish, there is no blood flow.

We do not do experiments in living animals, but one promising alternative is to use fertilized chick eggs. These form a rich blood vessel system just under the shell, on a part of the egg called the chorioallantoic membrane (everyone just calls it the 'CAM'). The chicken egg CAM has long been a site used by cancer biologists to graft pieces of human tumour to study how they command a blood supply, and it has been used in the past by kidney researchers to ask whether kidney blood vessels arise from the kidney itself or come in from outside (the answer to that one turned out to be complicated, and not a simple either/ or). Maybe, therefore, it would be worth revisiting CAM culture to see if ingrowing chick egg-derived vessels can make a realistic network of blood vessels in a grafted kidney rudiment. This would at least tell us whether the kidney rudiment contains enough information, in the form of a molecular 'map', to tell incoming vessels where to go. If it does, the next step would be to do the same thing with organoids.

The experiments were done by Julia Tarnick, a one-time PhD student in this lab who has now graduated and is a post-doctoral fellow, still in this lab for a while longer I am pleased to say. She learned to graft mouse embryonic kidney rudiments on to the CAM, and showed that they did indeed attract vessels from their host. The kidneys developed well and made glomeruli, the filtration units that filter blood. The source of the tiny blood vessels in the glomeruli was slightly surprising -

around 65% of the glomeruli contained vessels coming from the kidney itself, not the CAM. These could be distinguished because they stained for a mouse antigen present in mouse blood vessels only, not in vessels of the chick CAM. There were also large vessels in the kidneys, and these came from the CAM and carried flowing blood (pumped by the heart of the chick embryo elsewhere in the egg). To ask whether the vessels from the CAM connected with the graft-derived small vessels, Julia injected a dye into the CAM blood system, and took videos and photographs as it entered the grafted kidney and then flowed into the small vessels derived from the mouse kidney tissue. Clearly, then, the CAM could provide large vessels with flowing blood, and could connect to the graft to give that flowing blood too.

That was the good news. The less good news is that even the larger vessels from the CAM remained stubbornly immature, not developing the thick layers of muscle that arteries are meant to have. Equally annoyingly, already-formed large vessels in older mouse kidneys still degenerated when the kidneys were grafted on to the CAM: they could not connect to the flowing blood in the CAM vessels early enough to save them. And, also annoyingly, the incoming vessels of the CAM did not make an orderly tree of the kind produced in normal kidney development, but entered from everywhere and made what could best be described as a disorderly mess.

Though she was no doubt disappointed, Julia did the right thing and wrote the whole thing up, because publishing what does not work is important in saving other people from going down the same blind alley, and also because every piece of information may contribute to someone, somewhere, figuring out what we should do to get the vessels to develop in a proper tree. A link to the paper appears below.

Jamie Davies, Edinburgh, June 2022

Links:

Julia's paper: https://journals.biologists.com/bio/article/11/7/bio059459/275910/Introducing-blood-flow-in-kidney-explants-by