Does Piezo1 play a role in vascular survival in ex-vivo kidneys?

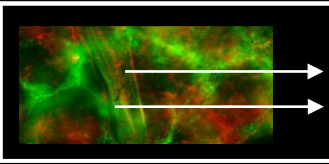
Hannah Collier, Jamie Davies

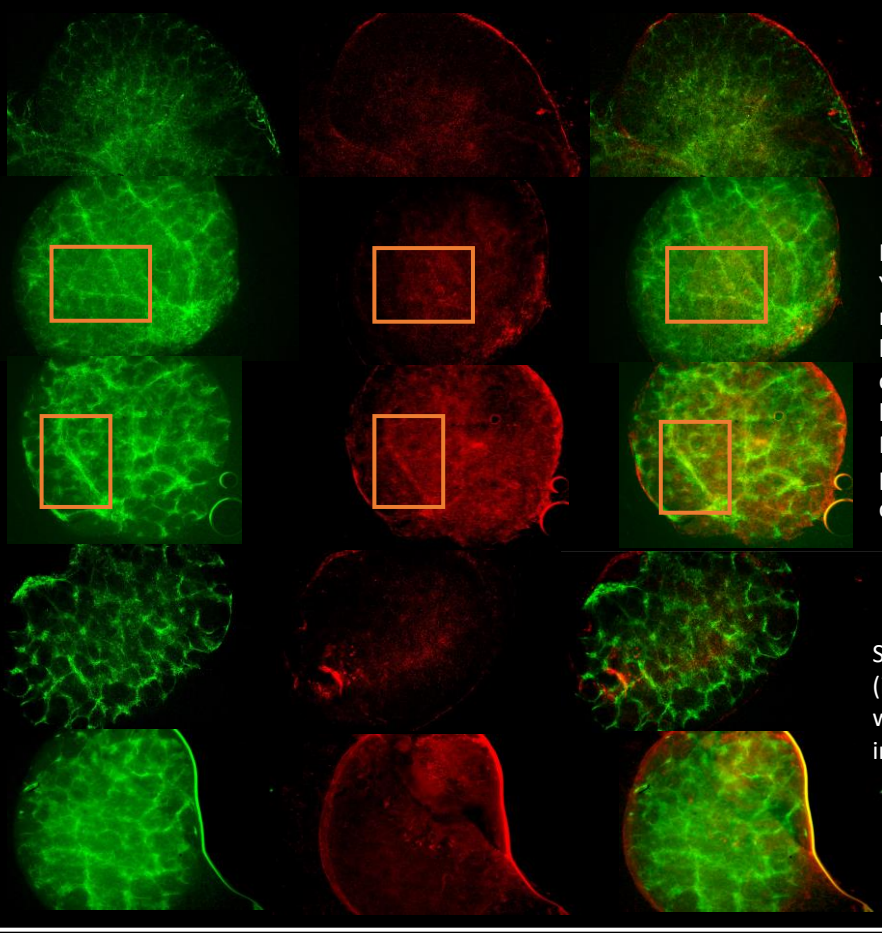
Introduction:
In the development of kidney organoids, renal tubules (such as nephrons and collecting ducts) and capillary networks develop well but larger vessels (such as veins and arteries) do not. When taken out of the embryo and cultured *in vitro*, arteries regress. This is thought to be due to a lack of blood flow resulting in the death of larger blood vessels. If vessels do not have blood flowing through them, they will die off, so that vessel networks can be remodelled to where blood flow is needed.

Piezo1 is a mechanoreceptor in blood vessels that is activated by fluid sheer stress from blood flow (Lhomme *et al.*, 2019). The drug Yoda1 is an agonist of Piezo1, therefore could perhaps be used to simulate blood flow through large vessels, preventing regression, and improving blood vessel survival in ex-vivo kidneys.

Methods:
Microdissection of E14.5 mouse embryos to extract kidney rudiments (Davies, 2010).
Culture kidney rudiments on Transwell Membrane at the air medium interface (Davies, 2010). The medium contains varying concentrations of Yoda1 (in DMSO) in kidney culture medium: 0µM (DMSO vehicle control), 10µM, and 20 µM.
After incubation for either 22 or 46 hours, kidney rudiments are fixed using 100% prechilled methanol at -20°C and stained using primary and fluorescently-tagged secondary antibodies. Kidneys are imaged using fluorescence microscopy.

Results:





Capillaries (stained in green) are formed but have no smooth muscle recruitment (stained in red). Therefore, large blood vessel survival was not exhibited in the control experiments.

In both 22hr incubations with Yoda1, smooth muscle recruitment was seen along blood vessels, as shown in the orange boxes. This indicates large blood vessel survival. Recruitment was more prominent with a higher concentration of Yoda1.

Smooth muscle recruitment (i.e. large blood vessel survival) was not visible in the 46hr incubations with Yoda1.

Conclusion:
This suggests that Yoda1 improves the survival of large blood vessels in ex-vivo kidneys. However, it only keeps the blood vessels alive for longer, not indefinitely: after 22 hours the large vessels were still alive but regressed during the 46-hour incubation. A higher concentration of the drug is more effective as 10µM of Yoda1 did not have as powerful effects on vessel survival as 20µM of the drug.

This experiment shows that Yoda1 may be useful in prolonging the lifespan of large vessels in ex-vivo kidneys. This is valuable in the development of kidney organoids, as it contributes to the understanding that a lack of blood flow is causative in the low survival rate of blood vessels in *in vitro* cultured kidneys. However, the activation of Piezo1 with Yoda1 only enabled blood vessels to survive for longer, not forever, suggesting that a lack of fluid sheer stress might only be one of a multitude of physiological variables that cause blood vessel regression in ex-vivo kidneys.

This experiment was only run twice as there was a two-week delay in embryo delivery during the six-week placement. Therefore, further experiments need to be performed to confirm the results and determine the optimum concentration of the drug and the maximum duration that vessels can be kept alive by intervention with Yoda1.

References:
Lhomme A, Gilbert G, Pele T, Deweirdt J, Henrion D, Baudrimont I, Campagnac M, Marthan R, Guibert C, Ducret T, Savineau JP, Quignard JF (2019) 'Stretch-activated Piezo1 Channel in Endothelial Cells Relaxes Mouse Intrapulmonary Arteries', *Am J Respir Cell Mol Biol*, Jun;60(6), pp. 650-658.
Davies, J.A. (2010) 'The Embryonic Kidney: Isolation, Organ Culture, Immunostaining and RNA Interference.' in: Ward, A., Tosh, D. (eds) *Mouse Cell Culture. Methods in Molecular Biology*, vol 633. Humana Press.