Vascularizing the kidney in the embryo and organoid: questioning assumptions about renal vasculogenesis

PERSPECTIVES

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Vascularizing the kidney in the embryo and organoid: questioning assumptions about renal vasculogenesis

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Despite much debate, there remains no clear consensus on how the kidney becomes vascularized. In general, blood vessels form by one of two processes, vasculogenesis (assembly of vessels *de novo* from mesodermal precursors), and angiogenesis (branching of new vessels from existing ones). In principle, renal endothelia may assemble through *vasculogenesis-only* (improbable and not supported), *angiogenesis-only* (conceivable but overlooked), or a *combination* of both processes (the option favoured by most accounts; for a summary of pertinent reviews since 1995 see http://dx.doi.org/10.7488/ds/2307).

Though the *combination* option is favoured, opinions are inconsistent regarding the relative contribution of each mechanism. We challenge this prevailing viewpoint and argue that the renal vasculature (specifically, the endothelial component) instead develops through *angiogenesis-only*.

An early proponent for kidney vasculogenesis was Herring (1900)[1], who speculated that glomerular capillaries are generated from *in situ* precursors within the cleft of the S-shaped nephron. This concept persists, despite electron microscopy and immunohistochemistry images suggesting that these early glomerular capillaries instead stem from local, pre-existing vessels[2,3]. Of course, still images only show cellular arrangement in the kidney at a single moment (at the point of fixation) and cannot prove or disprove dynamic processes such as angiogenesis or vasculogenesis. Here, time-lapse imaging of cultured embryonic kidneys has provided insights, showing fluorescence-tagged endothelia forming via angiogenesis[4] and migrating from pre-existing vessels into the S-shaped cleft[5] (Figure 1A).

More recent support for vasculogenesis comes from cross-transplantation experiments. In transplanted embryonic kidneys, both host- and graft-derived vessels usually developed, and observations of graft-derived vessels were taken as support for vasculogenesis. However, these studies assumed that the transplanted kidney rudiments were avascular, even when using embryonic day 12-12.5 (E12-E12.5) mouse kidneys. We find these to be highly vascularized (Figure 1B). In fact, even the earliest kidney contains blood vessels (E11; Figure 1C). More significantly, even if truly

avascular kidneys were transplanted and vasculogenesis occurred, this does not necessarily mean that it is the normal mechanism of kidney vascularisation. Transplanted kidneys are put into artificial environments that may encourage vasculogenesis – it is unclear how this relates to 'natural' development and we suggest that investigating kidney vascularization during normal development *in vivo* might be more revealing.

In vivo, mouse kidney vascularization starts by E11, when systemically connected vessels wrap around the ureteric bud[6] (the precursor of collecting ducts and ureter). Over the next day, blood vessels continue to disperse throughout the kidney mesenchyme, in connection with extrinsic vessels[6] (Figure 1B). Later, vessels at the border of the kidney form polygonal networks around populations of nephron progenitor cells[6] (they organise this way in human embryos too[7] - https://transparent-human-embryo.com/?p=1001; Figure 1D-E), while cortical and medullary vessels are simultaneously developing.

Based on our data, normal kidney vascularization relies on growth and remodelling of pre-existing vessels (angiogenesis) and does not depend on vasculogenesis at any point. When assessing the entire 3D vascular tree, isolated endothelia are never observed[6]. Even when immunostaining for stem cell leukemia (SCL), a marker of the most primitive endothelial progenitors[8], staining is observed only within mature vessels that are traceable to the renal artery[6]. Other studies, however, suggest that a small population of renal endothelia are stroma-derived[9], and the mechanisms by which these cells contribute to vascular development should be explored.

Uncovering the mechanisms of kidney vascularization may allow us to better recapitulate developmental processes to vascularize kidney organoids. Though organoids are generally improving, they lack anatomically realistic vascular systems. Based on the 'angiogenesis-only' hypothesis, we suggest that, rather than trying to induce vasculogenesis, methodological advances should promote invasion of renal organoids by exogenous vessels, both in culture and transplantation settings.

Illustrating the need for kidney organoid invasion by exogenous vessels, van den Berg et al[10] generated pluripotent stem cell-derived kidney organoids and demonstrated that long periods of culture (where exogenous vessels are lacking) led to gradual loss

of endothelia, whereas transplantation into pre-vascularised host tissue (beneath the murine renal capsule) led to the development of a functional perfused vasculature.

van den Berg et al[10] proposed that maturation of large kidney organoids in culture is limited due to hypoxia and metabolic deficiencies. To facilitate continued maturation in culture, it may be possible to generate perfused engineered vessels that can invade the organoid. Perfused vessels could supply oxygen and nutrients to the organoid, while also generating shear-forces to encourage vessel maturation. This could be achieved by connecting engineered vessels to a pumping system where the flow-input could be stringently controlled.

Improved *in vitro* vascularization and maturation prior to transplantation would allow for the engraftment of a functionally enhanced organoid. Upon transplantation, host-derived blood vessels can invade and connect with kidney organoid-derived vessels[10]. If a vascularized organoid with functioning glomeruli and nephrons can be transplanted, and its flow quickly re-established via anastomoses with the host's vasculature, the regenerative potential of kidney organoids may begin to be realised.

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DISCLOSURES

None to declare.

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Figure 1. Kidney vascularization. **(A)** Earliest glomerular endothelia forming into the cleft of an S-shaped nephron from pre-existing vessels in time-lapse culture (white arrowhead; adapted from[5]). **(B)** Model of early kidney vascularization *in vivo*. Some argue that the first vessels form via vasculogenesis, but our results suggest otherwise[6]. UB, ureteric bud; WD, Wolffian duct; PWM, peri-Wolffian mesenchyme. Blood vessels shown in red. **(C)** The E11 kidney is vascularized by systemically connected vessels (these vessels carry erythrocytes and connect to major arteries; black arrowhead), surrounding the ureteric bud (adapted from[6]), which calls into question whether avascular kidneys can be dissected at any age. Scale bar: 100 μm. **(D)** Representative image of the vascular network at the periphery of the E16.5 kidney. Some argue that these peripheral vessels form via vasculogenesis; however, they carry blood, are always enclosed by basement membrane, and connect with pre-existing vessels that can be traced to renal arteries[6]. Scale bar: 50 μm. **(E)** As with mouse embryonic kidneys, peripheral vessels in human embryonic kidneys arrange as polygonal networks around nephron progenitors (prepared from data accessed at https://transparent-human-embryo.com[7]).