Asia-Pacific Kidney Development Workshop Abstracts

A1: “There and back again”: A journey through kidney development on the back of WT1

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Kidney development is orchestrated by a complex network of transcripition factors and signaling molecules. One of the key genes in this process is the Wilms’ tumor suppressor WT1, a developmental regulator that is mutated in a proportion of children suffering from nephroblastoma. WT1 acts at multiple steps during kidney formation including initation, mesenchyme to epithelial transition (MET) and podocyte differentiation. Over the years, my lab has used WT1 as an entry point to dissect kidney organogenesis and we have employed ChIP-Seq and RNA-Seq experiments to determine direct downstream targets. In this lecture I will provide an overview of our work, describe some of the signaling pathways that are regulated by WT1 (BMPs, FGFs, Rspondins) and that control the delicate balance of progenitor survival, proliferation and differentiation. I will describe our genetic studies in mice that identified key effectors of WT1 (SOXC genes) and helped to clarify the process of MET. Finally, I will discuss the role of WT1 in podocyte differentiation and outline some of the open questions that need to be addressed.

A2: Control of proliferation in the interstitium of the developing kidney

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Interstitial cells of the kidney play important roles in health and disease. On the one hand, they are essential for functions such as the production of the hormone erythropoietin. On the other hand, interstitial expansion is a hallmark of scarring and loss of kidney function. In contrast to the injured kidney, the developing kidney has the capacity to tightly control proliferation of interstitial cells to ensure that the organ is endowed with the appropriate number of these cells. Several studies have indicated that Wnt signalling from epithelial cells to the adjacent interstitium is required for their proliferation. However, the mechanism by which this proliferation is curtailed remains poorly understood. To address this question, we mapped regional variations in proliferation of the kidney interstitium, and asked which signalling pathways were active in regions where proliferation was reduced. Based on this spatial information, we found that TGFβ signalling was a strong candidate. TGFβ signals through two distinct intracellular pathways; MAPK and Smad. Using the interstitium-specific *Foxd1cre* deleter, we inactivated unique nodes in each of these pathways; *Map3k7* and *Smad4*. Mutants display distinct phenotypes in the neonatal period; *Foxd1cre*;*Map3k7* shows scarring of the glomerular mesangium and *Foxd1cre*;*Smad4* shows over-proliferation of renal interstitium. Loss of proliferation control in the interstitium is associated with deregulation of Wnt signalling in interstitial cells, and based on molecular marker analysis we propose a model in which TGFβ signalling regulates Wnt signalling at the transcriptional level.

A3: Generating Patterned Kidney Organoids for Modeling Development and Diseases

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Human pluripotent stem cells (hPSCs)-derived kidney organoids recapitulate complex developmental processes and tissue architectures, but the intrinsic limitations, such as variability and a lack of vasculature, have greatly hampered their application. Here we establish a highly efficient and versatile protocol for generating vascularized three-dimensional (3D) kidney organoids. We employ dynamic modulation of WNT signaling to control the relative proportion of proximal versus distal nephron segments, thereby producing a correlative level of VEGFA to define the resident vascular network. By single cell RNA-sequencing, we identify a subset of nephron progenitor cells as a potential source of the vasculature. Upon implantation into host mice, these kidney organoids undergo further structural and functional maturation, as demonstrated by the size-selective handling of dextran. Based on this differentiation platform, we establish an *in vitro* model of autosomal recessive polycystic kidney disease (ARPKD) by differentiating induced PSCs (iPSCs) from an ARPKD patient into 3D kidney organoids that develop tubule cysts in response to cAMP upregulation. The cystogenesis phenotype can be effectively prevented by gene correction or drug treatment. Our studies provide a versatile platform for studying human kidney development and diseases, and opens new avenues for modeling disease pathogenesis and performing patient-specific drug screening.

A4: Directing isolated GATA3+CDH1+ structures from kidney organoids to an improved ureteric epithelial identity

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In 2015 we described a protocol for generating iPSC-derived human kidney organoids which contain patterned nephron structures connected to CDH1+/GATA3+/PAX2+/CALB1+ ureteric epithelium[1]. However, there is no evidence of RET+/WNT11+ ureteric tips within our organoids. Indeed, recent single cell transcriptional analyses of both mouse and human fetal kidney have identified expression of many presumed ureteric epithelial marker genes, including GATA3, in ureteric epithelium but also the distal nephron and connecting segment. This raised questions about the true identity of the GATA3+ epithelium within human kidney organoids.

We have generated kidney organoids from human iPSCs harbouring fluorescent reporters mCherry, mCitrine and mTagBFP2 inserted into the endogenous GATA3, HNF4A and MAFB loci respectively, and using fluorescent activated cell sorting (FACs) isolated the GATA3+/mCherry+ epithelium. Culturing this population in conditions that promote maintenance and expansion of mouse ureteric bud-like structures[2] drove proliferation and branching of the GATA3+/mCherry+ cells. Analysis by reporter and immunofluorescence showed that the GATA3+/mCherry expression was maintained after several weeks of culture, dissociation and re-plating. RNAseq-based transcriptional profiling identified the cellular identity was more similar to distal tubule prior to culture, whereas a loss of SLC12A1 and induction of WNT11 and RET post-culture indicated a more ureteric epithelial identity. Single cell analysis showed that there was both a ureteric tip (RET) and putative medullary compartment (UPK2), as well as WNT9B expression consistent with that observed in human fetal kidney.

This analysis redefines the identity of the GATA3+/CDH1+ epithelium within our kidney organoids as an early connecting segment / distal tubule. However, appropriate culture conditions can transition this to a more collecting duct fate. The capacity to expand and propagate this ureteric epithelial population may represent a useful approach for the generation of collecting duct tissue.

1. Takasato, M. *et al. Kidney organoids from human iPS cells contain multiple lineages and model human nephrogenesis.* Nature **526**, 564–568 (2015).

2. Yuri, S., Nishikawa, M., Yanagawa, N., Jo, O. D. & Yanagawa, N. *In Vitro Propagation and Branching Morphogenesis from Single Ureteric Bud Cells.* Stem Cell Reports **8**, 401–416 (2017).

A5: Kidney organoids to model nephrotoxicity – advantages and limitations

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The clinical use of the chemotherapeutic drug cisplatin is limited by its severe nephrotoxic side effects. The development of reno-protective therapies for cisplatin-induced acute kidney injury (AKI) is hampered by a lack of understanding of cisplatin-mediated nephrotoxicity. In recent years, kidney organoids derived from human pluripotent stem cells have been proposed as a platform to study the complex cellular and molecular processes underlying AKI. We performed a comprehensive approach to test the applicability of kidney organoids generated in our lab to model cisplatin-induced AKI. We found that organoids treated with cisplatin display increased levels of the kidney injury marker KIM1, DNA damage and cell death in a dose-dependent manner. Co-localisation of the DNA double-strand break marker yH2AX with the different cell types in the organoids revealed that cisplatin predominantly affects proliferating cells suggesting a general cytotoxic effect reminiscent of the drug's impact on tumour cells. This result is contrary to cisplatin specifically damaging the proximal tubule *in vivo*. We measured low, fetal-like levels of expression of proximal tubule-specific cisplatin transporters in organoids, providing one explanation for this observation. We also detected several AKI biomarkers and cytokines in the culture medium of cisplatin-treated organoids, consistent with the induction of an AKI-like inflammatory response. Our work validates the use of kidney organoids for modelling the inflammatory aspects of cisplatin-induced AKI and supports the potential of this human cell-based system for developing improved therapies. However, due to the immature state of the current organoids, recapitulation of the full range of injury events in AKI is limited.

A6: Local tissue damage microenvironment induces distinct resident interstitial cell subsets to drive regional fibrotic responses after acute kidney injury

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After acute kidney injury (AKI), injury-induced epithelial activation of Sox9 drives the regeneration of the acutely damaged nephron tubular epithelia. Canonical Wnt/β-catenin signaling has also been linked to nephron tubular epithelial homeostasis, repair, and fibrotic processes, however, the precise cellular and molecular underpinnings of the pathway remains unclear. Thus, whether the two cellular pathways represent mutually exclusive cellular populations, or activation of Sox9-Wnt/β-catenin cross talk driving kidney repair after AKI remains unknown. Here, utilizing a series of genetic lineage-tracing strategies and clonal analysis, we identify and describe a distinct, resident cell-type that activates Wnt/β-catenin signaling. Neither the resident cells of the normal adult uninjured kidney, nor the acutely injured tubular epithelial cells akin to Sox9 displayed active Wnt/β-catenin signaling. After ischemic- or rhabdomyolysis-induced AKI, whilst immune cells induced Wnts, only the resident Pdgfrβ+ interstitial population of the outer medullary region, the region of maximal epithelial cell loss/damage after AKI, activated β-catenin signaling, with the descendants contributing to the majority of the subsequent regional αSMA myofibroblast population. Strikingly, none of the αSMA+Pdgfrβ+ interstitial cells residing around patchy injured cortices activated the pathway. Preliminary data suggest that the fate-map of αSMA+ cells that underwent removal of β-catenin activity revealed overall reduction in αSMA population after AKI, compared to the population with intact β-catenin activity, but blocking immune-cell Wnt secretion had no effect. Our studies highlight region-specific, molecular heterogeneity of αSMA+ myofibroblast population determined by functionally, distinct resident interstitial cell subset responses to tissue damage associated microenvironment. These findings have important implications for therapies aimed at modulating pathological interstitial remodeling after acute tissue damage.

A7: Non-mammalian models of kidney development and regeneration

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Non-mammalian models have been central to our understanding of basic processes involved in kidney specification and morphogenesis. In particular, collective cell migration was shown to be important in both early pronephros development and its maturation. Our results suggest that collective migration is indeed central to pronephros maturation, controlling both kidney cell shape and number as well as the overall nephron morphology. Little is known about the molecular events underlying this nephron collective cell migration. In order to better understand the migration mechanism, we performed a small molecule screen of kidney cell migration in transgenic zebrafish embryos. Our initial results indicate that the underlying molecular events are similar to collective migration in other normal and pathological processes such as cancer invasion. This study highlights the power of zebrafish as an in vivo screening platform for novel drug discovery.

While pronephros remains a powerful model to study nephron development and repair, non-mammalian metanephric kidney remains under-investigated. In our survey of post-embryonic nephrogenesis in reptiles we found that in many species metanephric kidneys continue to generate new nephrons throughout the life of an individual. In particular, using alligator as a model, we found no evidence of switching from nephrogenesis to hypertrophy even in larger adults. This persistence of nephrogenesis as a predominant mechanism by which kidney increases its functional capacity is accompanied by persistence of Six2 positive cell population. Together these results emphasize the importance of non-mammalian models for our understanding of human kidney development, regeneration and its evolution.

A8: Do epigenetic events underpin potential new treatment targets in cystic kidney disease?

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Autosomal Dominant Polycystic Kidney Disease (ADPKD) is the most common heritable renal disease, causing enlargement of the kidneys due to the development of fluid-filled cysts. While end-stage kidney disease due to ADPKD typically occurs in the 4th to 5th decade of life, cysts are thought to initiate *in utero* during development. A germline mutation in at least one of the genes *PKD1, PKD2, GANAB* or *DNAJB11* is required for the development of ADPKD, with additional somatic mutations and/or gene dosage effects hypothesised to lead to cystogenesis. However, the underlying mechanisms initiating and progressing cyst growth are not well understood. The limited treatment options for ADPKD underscores the need for a better understanding of these processes.

Many similarities have been noted between cyst development in ADPKD and neoplasia. DNA methylation is known to be altered in neoplasia and is a target for several FDA-approved therapies. To extend the limited data available on DNA methylation in ADPKD, we have assessed the methylome of human ADPKD cystic kidneys. Using reduced representation bisulfite sequencing (RRBS), single nucleotide resolution methylomes of ADPKD and non-ADPKD kidney samples were generated for analysis. In contrast to previously published data, we have shown that ADPKD tissue shows a statistically significant global hypomethylation. The identification of several differentially methylated loci, two of which have altered expression levels in ADPKD, reveals potential new pathways of interest in promoting cyst growth. Assessment of these loci using RNAi and *in vitro* cyst growth assays will further ascertain their roles in promoting cyst growth and may reveal novel pathways for therapy development.

A9: From Networks to Function – Computational Models of Organogenesis

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One of the major challenges in biology concerns the integration of data across length and time scales into a consistent framework: how do macroscopic properties and functionalities arise from the molecular regulatory networks and how do they evolve? Morphogenesis provides an excellent model system to study how simple molecular networks robustly control complex pattern forming processes. In my talk, I will focus on lung and kidney branching morphogenesis and discuss how chemical signaling and mechanical constraints shape the developing organs. Using light-sheet microscopy, we can observe epithelial dynamics during branching at cellular resolution, and I will discuss the physical principles of epithelial organization.

1. Menshykau, D., et al. (2019). *Image-based modeling of kidney branching morphogenesis reveals GDNF-RET based Turing-type mechanism and pattern-modulating WNT11 feedback.* Nat Commun 10(1): 239.
2. Kokic, M., et al. (2019). *Minimisation of surface energy drives apical epithelial organisation and gives rise to Lewis' law.* bioRxiv
3. Vetter, R., et al. (2019). *Aboave-Weaire's law in epithelia results from an angle constraint in contiguous polygonal lattices.* bioRxiv

A10: Growth and patterning of the developing mouse kidney

Short, K.M.[1,2], LeFevre, J.G.[3], Combes, A.N.[4,5], Lisnyak, V.[1], Jones,L.K.[1,2], Lamberton, T.[3], Michos, O.[6], Graf, D.[7,] Little, M.H.[4,5,8], Hamilton, N.A.[3], Smyth, I.M. [1,2,9]

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Kidney growth is mediated by the interaction of ureteric bud and metanephric mesenchyme derivative cells, driving epithelial branching morphogenesis and nephrogenesis. To understand the nature of how the future collecting duct system is built during renal development, we mathematically interrogated 3D branching data from whole embryonic mouse kidneys. The "delay" models we developed help explain the majority of normal kidney branching patterns which exhibit an asymmetric "balance" where from a single branch point, more tips are one side than the other. These models define the level of asymmetry typically seen in normal branching, and suggest the degree of balance is determined by locally operating growth mechanisms arising from positional suppression of bifurcation at the tips. Analysis of branching in *TgfB2*$^{+/-}$, *Spry1*$^{-/-}$ and *Bmp7*$^{-/-}$ kidneys revealed that changes in kidney branching morphogenesis decreased balance, increasing asymmetry, and are explained by changes in tip to tip spacing at the surface of the kidney. With this mode of branching identified, an alternative "unifying theory" has been suggested which supposedly explains mammary, prostate and kidney branching morphogenesis with a single rule – with one primary tenet being stochastic cessation of proliferation of UB tips after nephrogenesis. We have shown that kidney morphogenesis does not fit the proposed "unifying" model, by profiling the impact of nephron formation and maturation on elaboration of the ureteric bud during kidney development. We have found a distinct absence of random branch termination events within the kidney or evidence that nephrogenesis impacts the branching program or cell proliferation in either tip or progenitor cell niches. Instead, organogenesis proceeds in a manner indifferent to the development of these structures. Hence, stochastic cessation of branching is not a unifying developmental feature in all branching organs.

A11: Nephron progenitor commitment is a stochastic process influenced by cell migration

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Nephrons in the mammalian kidney are formed from self-renewing mesenchymal progenitors that reside within a niche defined by the cap mesenchyme and ureteric epithelial tip. As the mouse kidney develops, progenitor cells differentiate in response to inductive cues and exit the niche to form nephrons. We previously showed that the cap mesenchyme population is highly dynamic, migrating both within and between domains in response to niche cues. More recently we have examined how these migrating progenitors commit to form nephrons in a coordinated, spatially defined manner.

We identified a subset of cells that express *Wnt4*, an early marker of nephron progenitor commitment, but migrate back into the progenitor population where they accumulate over time. Single cell RNA-seq and computational modelling of returning cells reveal that nephron progenitors may traverse the transcriptional hierarchy between self-renewal and commitment in either direction. Our findings suggest that nephron progenitor commitment is a stochastic process in which migration events dictate the duration of exposure to spatially defined commitment cues. Plasticity within the progenitor population may help to ensure robust regulation of nephrogenesis as niches grow and remodel during organogenesis.

We are now extending this work by developing methods to time-lapse image entire nephron progenitor domains at single cell resolution. By correlating this population-scale migration data with individual cell fate outcomes, we hope to better characterise the events that precede nephrogenesis.

A12: Using single cell analysis to investigate kidney development and inform renal regeneration

Combes, A.N.[1,2], Phipson, B.[1,3], Zappia, L.[1,4], Lawlor, K.[1], Moreau, J.[1], Er, P.X.[1], Oshlack, A.[1,4], Little, M.H.[1,2,3]

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Human kidney organoids hold promise for disease modelling and drug screening. However, the utility of stem cell-derived kidney tissues will depend on how faithfully these replicate the cellular complexity and identity of normal kidney tissue. We performed an integrated analysis of single cell datasets from human kidney organoids and human fetal kidney to assess similarities and differences between component cell types. Clusters in the combined dataset contained cells from both organoid and fetal kidney with transcriptional congruence for key stromal, endothelial and nephron cell type-specific markers. 'Off-target' neural, glial and muscle progenitor populations were also evident in organoids. Some previously characterised cell types were missing from this specific organoid dataset or were less mature than in prior work, highlighting experimental variability[1]. We then profiled the developing mouse kidney to better understand the signalling pathways that regulate the maintenance and differentiation of cell types in the major renal lineages. Our analysis provides improved resolution of stromal sub populations, nephron progenitor heterogeneity, and marker specificity. We identified transcriptional congruence between the ureteric epithelium and distal nephron, showing that several markers thought to identify the ureteric epithelium are not specific to that cell type. Receptor-ligand and pathway analysis associated known and new pathways with progenitor maintenance and differentiation of nephron and ureteric epithelium cell types[2]. Together this work affirms the fidelity of cell types present within kidney organoids and provides a roadmap to refine production of specific renal cell types in vitro.

1 Combes, A.N., Zappia, L., Er, P.X., Oshlack, A. & Little, M.H. (2019) *Single-cell analysis reveals congruence between kidney organoids and human fetal kidney*. Genome Med **11**, 3.

2 Combes, A.N., Phipson, B., Lawlor, K.T., Dorison, A., Patrick, R., Zappia, L., Harvey, R.P., Oshlack, A., Little, M.H. (2019) *Single cell analysis of the developing mouse kidney provides deeper insight into marker gene expression and ligand-receptor crosstalk.* Development **146**, 12.

A13: GDNF regulates renal development through control of the progenitor populations

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The prevalence of kidney diseases has increased tremendously during previous decades, which places an enormous economic burden on society. Congenital renal defects, ranging from dysplasia to aplasia and cancer, are the most common cause for kidney failure in children. Glial cell-line derived neurotrophic factor (GDNF) activated Ret signaling is necessary for kidney induction, but the exact mechanisms and cellular behaviors it regulates during renal differentiation remain unclear. Here, we used a new *Gdnf* overexpression model, which leads to increased, but spatially unchanged expression of endogenous mRNA and protein. We observed expanded ureteric buds, thinner renal cortex and cyst formation in the collecting ducts of these mice. Further characterization discovered that GDNF positively regulates collecting duct progenitors and also appears to repress elongation of ureter trunk. Moreover, we also discovered reduced nephron counts, which likely are caused by the impact of excess GDNF on nephrogenesis, a possibility that we are currently exploring. Particularly, postnatal nephrogenesis is sustained longer with the excess GDNF expression though the nephron progenitor population is greatly diminished in embryonic kidneys. Taken together, our results suggest that GDNF dosage is critical for renal progenitor regulation and determination of final location of kidneys. Our discoveries may propel new diagnostics and therapies by augmenting the basic comprehension of renal development.

A14: Interrogating kidney neurovascular function during development

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The renal vasculature and nerves modulate blood flow and filtration, tubular reabsorption, and hormone production. Despite their important physiological roles, little is known about how the kidney neurovascular networks are established during development. Additionally, the functional contribution of these networks to the process of kidney development has not been adequately addressed. We found that kidney innervation closely follows vascularization and they rely on the netrin signalling pathway for their proper positioning. In the absence of stromal progenitor-derived netrin signals, neurovascular networks are aberrantly patterned and kidney development is significantly disrupted. We predict that the vascular endothelial cells and axons which innervate the kidney mediate development through the release of signalling molecules. Interrogation of expression data reveals the kidney endothelium produces the growth factor *Igf1.* Putative recipient cells that express *Igf1r* include the ureteric epithelium, nephron progenitors, developing nephrons, and endothelium itself. Our current efforts are focused on a vascular-specific removal of *Igf1* to verify its role in kidney development. Ablation of renal nerves leads to smaller kidneys suggesting they also release important developmental regulators. Candidate factors include norepinephrine which modulates the development of other organs including the pancreas and heart and we are currently altering this pathway in the developing kidney and human iPSC-derived kidney organoids. Together, our studies have revealed the importance of proper neurovascular patterning and function to kidney development. Efforts to generate kidneys de novo and promote regeneration in vivo will rely on the knowledge of how neurovascular networks are properly established and influence the nephrogenic program.

A15: Using diagnostic genomics to identify novel kidney disease genes – the Kidgen experience

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KidGen is a collaboration between Clinical, Diagnostic and Research teams based in Australia whose aim is to better understand the causes of inherited kidney disease. By developing Renal Genetics Clinics in hospitals in every Australian State, we work with patients and diagnostic providers to try to determine the genetic basis of kidney diseases. Having identified potentially causative genetic lesions we then seek to model and verify these changes in biological model systems, in so doing furthering our understanding of kidney development and disease. I will discuss the successes and challenges experienced during the establishment of this program, focussing particularly on the modelling of potentially causative patient variants in organoid and organismal models. I will also discuss the identification and validation of a novel causative mutation in a basement membrane protein which results in a multisystem developmental disorder with renal involvement.

A16: Generating anephric, immune-compatible sheep for kidney complementation

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Farm animals have been proposed as hosts to grow human organs for xenotransplantation. This concept involves genetically disrupting target organ development in the livestock host, followed by complementation with human cells that populate the vacant niche and generate the transplantable organ. In mice, spalt-like transcription factor 1 (*Sall1*) functions as a master regulator for kidney development[1]. To generate anephric, immune-compatible hosts for organ complementation, we targeted *SALL1*, as well as two major xenoantigens involved in hyperacute immune reaction: galactose-α(1,3)-galactose (α-Gal) and N-glycolylneuraminic acid (Neu5Gc). We chose sheep as the host species because they have a similar kidney anatomy to humans but are perceived as culturally more acceptable organ donors than pigs.

Using CRISPR/Cas9 editing, we simultaneously disrupted *SALL1* and the genes underlying α-Gal and Neu5Gc formation, α(1,3)galactosyl transferase (*GGTA*) and cytidine monophosphate-N-acetylneuraminic acid hydroxylase (*CMAH*), respectively. Ovine fetal fibroblasts (OFFs) were transfected with three plasmids, each containing CRISPR guide RNA sequences for a different target gene. Following transient antibiotic selection and expansion, OFFs showed 97%, 93%, and 89% editing in *SALL1, GGTA*, and *CMAH*, respectively, by TIDE analysis (https://tide.nki.nl/). Single putatively edited OFFs were seeded, resulting in 20 clonal strains with biallelic mutations for all three genes. One triple knockout (KO) cell strain without plasmid integration and no observed mutations at the top three predicted off-target sites for each gene (http://crispor.tefor.net/) was used for somatic cell cloning. For complementation, male triple KO host embryos were aggregated with cloned female double KO donor embryos ($SALL1^{+/+}/GGTA^{-/-}/CMAH^{-/-}$), carrying a red fluorescent protein (RFP) reporter to trace the donor genotype ($SALL1^{+/+} \leftrightarrow SALL^{-/-}$ chimaeras). Non-chimaeric cloned double KO embryos with and without RFP served as wild-type controls for normal kidney development. Following embryo transfer into surrogate ewes, fetuses will be recovered during early gestation to analyse their kidney phenotype and potential rescue of renal agenesis in chimaeras.

1. Goto, T., Hara, H., Sanbo, M., Hirabayashi, M. (2019). *Generation of pluripotent stem cell-derived mouse kidneys in Sall1-targeted anephric rats*. Nat Commun, 10(1), 451.

A17: CCM3-STK complex controls kidney water reabsorption by maintaining water permeable epithelial fate

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CCM3, aka PDCD10, is a gene found to be associated with human disease, Cerebral Cavernous Malformations (CCMs). CCM3 forms complex with GCKIII kinases (STK24, STK25 and MST3). Studies in c. elegans and drosophila suggested CCM3-GCKIII complex play critical role in maintain epithelial integrity. To investigate the role of CCM3-GCKIII in epithelial system in mammalian, we specifically deleted CCM-STK expression in kidney distal tubules. We found loss of function of CCM3 or STK24/STK25 in distal tubule leads to polyuria and increased water consumption. However, the expression of water channels remain unchanged with the exception of AQP4 significantly increases. The physiological responses to AVP system remain intact in these mice. Gene expression profiling study revealed urothelium markers and tight junction proteins are upregulated in epithelial cell lacking CCM3 or STKs starting from embryonic stage. Clusters of Sprr and keratin genes are also progressively increased in postnatal animals. Scanning EM and transmission EM studies revealed that lacking CCM3 dramatically increased cell surface protrusions. This study identified an unexpected role of CCM-STKs in controlling kidney water reabsorption by suppressing the adoption of a urothelial like water-proven fate by renal epithelium.

A18: Adverse impact of preterm birth and/or intrauterine growth restriction on kidney development: implications to the lifelong renal health of Indigenous Australians

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Low birth weight is linked to renal disease later in life and this link is particularly strong in Indigenous Australians. Our research aims to investigate how low birth weight, due to preterm birth and/or intrauterine growth restriction (IUGR), adversely impacts the developing human kidneys and explores whether the kidneys of Indigenous Australian infants are more vulnerable to these early life renal insults.

Both morphological and stereological studies in autopsied kidneys from human infants and in vivo studies of renal function and kidney size in newborn infants (Indigenous and non-Indigenous) have been conducted.

Our findings in preterm infants show a high number of abnormal glomeruli in the outer renal cortex; glomerular and tubular function are significantly affected by gestational age at birth and postnatal age. IUGR is associated with a reduced number of glomerular generations within the developing kidneys, increased urine protein excretion, and reduced kidney volume in preterm neonates at one month of age. Indigenous infants are more vulnerable to renal impairment following preterm birth, with significantly higher serum creatinine, fractional excretion of sodium, urine albumin, β-2 microglobulin and cystatin C than non-Indigenous infants when controlling for gestational/postnatal age, sex and birth weight Z-score. However, postnatal kidney growth of Indigenous infants in the first month of life was not different to non-Indigenous infants. Current BSA was the strongest correlate of kidney size across all preterm infants; every 1 unit increase in birth weight Z-score was associated with a 65% increase in kidney volume.

In conclusion, our findings clearly demonstrate early life renal impairment following IUGR and/or preterm birth, and that Indigenous infants are more vulnerable to renal impairment following preterm birth. The greater vulnerability to these early life renal insults is a likely contributor to the strong correlation between low birth weight and adult renal disease in the Indigenous population.

A19: WT1 mediates the unfolded protein response via NFE2L1 interaction

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The Wilms tumour suppressor 1 (WT1) gene encodes a zinc finger transcription factor that is essential for normal kidney development as well as oncogenesis. Many cancers overproduce the WT1 gene and in the case of blood cancers (leukaemias), this is associated with poor outcome. Despite its important role in cancer, little is known about how WT1 promotes cancer and this makes it difficult to develop new therapies.
Here, we report a novel mechanism on how WT1 regulates cell survival through physical protein interaction. Using yeast two hybridisation, we have identified the transcription factor NFE2L1 as a novel WT1 interacting partner. Functionally, WT1 physically binds to NFE2L1 and sequesters it from dimerising with its co-factor. Consequently, WT1-NFE2L1 interaction suppresses the transactivation of the downstream proteasome subunit genes. This leads to a deficiency in proteasome activity which in turns causes ER stress and the activation of the prosurvival unfolded protein response in cancer cells. Our study provides a functional link between WT1 and cancer growth via modulation of the proteasome and paves the way to developing targeted therapies for cancers that rely on WT1.

A20: Discovery of a new WT1-related urogenital syndrome in New Zealand

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Renal disease is a major healthcare burden worldwide and is often caused by damage to podocytes - specialised cells making up the kidney blood filters (glomeruli). We have identified a NZ family with a novel syndrome of glomeruli scarring and reproductive tract defects. From exome sequencing we found a DNA variant in the WT1 gene, encoding a transcription factor required for podocyte identity and urogenital development. Using CRISPR/Cas9 gene editing we introduced the WT1 variant into human induced pluripotent stem cells and matured these into kidney organoids (mini balls of kidney tissue). Analysis of podocytes from the kidney organoids revealed a differentiation delay and a downregulation of podocyte genes consistent with the WT1 variant being causal for the syndrome. In parallel, we used the zebrafish system to test the efficacy of compounds that can ameliorate podocyte injury and identified a molecule capable of rescuing the expression of podocyte genes when added to WT1-variant kidney organoids. Taken together, our work brings together human genetics, kidney organoids, and zebrafish to expand the spectrum of WT1-related urogenital disorders and paves the way to developing new therapeutics for renal disease.

Poster Abstracts

No.	Title	Presenter	Institutions
A21	Impact of the mesenchyme on kidney branching morphogenesis	Lisa Conrad	[1]Dept of Biosystems Science and Engineering, Swiss Federal Institute of Technology in Zurich, Basel, Switzerland, [2]Swiss Institute of Bioinformatics, Basel, Switzerland
A22	Shear Force and Kidney Organoids	Callum Tatton	Dept of Molecular Medicine and Pathology, University of Auckland, NZ
A23	Vascular roles during kidney development and implications for function	Samuel Honeycutt	Dept of Cell Biology and Physiology, UNC Kidney Center, University of North Carolina at Chapel Hill, USA

A21: Impact of the mesenchyme on kidney branching morphogenesis

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Branching morphogenesis is a developmental process that gives rise to the epithelial trees of organs such as the kidney and the lung. The branching pattern and regulatory gene networks, however, differ between organs. Previous work in our group has shown that a ligand-receptor-based Turing mechanism quantitatively recapitulates both lung and kidney branching in wild type and mutants1, but is based on FGF10 in the lung and GDNF in the kidney. This suggests a common mechanism for the control of branching morphogenesis, despite the differences in the molecular implementation.
We now seek to understand the origin of the different branching patterns in lungs and kidneys. Tissue-recombination experiments can be used to study the interactions between tissues of different organs and to perturb patterning and morphogenesis. We are using live microscopy of recombined tissue explants to test the impact of the lung mesenchyme on ureteric bud branching morphogenesis. By quantifying relevant morphometric parameters, we confirm published observations that the branching pattern and morphology of recombined explants of lung mesenchyme and ureteric bud combines features of kidney and lung morphogenesis. We now combine culture experiments and our in silico image-based modelling pipeline to define the regulatory mechanism that results in the organ-specific branching behaviours.

1. Menshykau, D., Michos, O., Lang, C., Conrad, L., McMahon, A.P., and Iber, D. (2019*). Image-based modeling of kidney branching morphogenesis reveals GDNF-RET based Turing-type mechanism and pattern-modulating WNT11 feedback.* Nature Communications 10, 239.

A22: Shear Force and Kidney Organoids

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Kidney disease is a significant health burden magnified by the poor regenerative capacity of nephrons upon injury, resulting in a deterioration of kidney functions. Kidney organoids have emerged as a useful tool for modelling kidney diseases and development but are currently limited by their immature fetal-like expression patterns. Vascularisation and the conduction of related shear forces upon developing kidney tissue are important for renal tissue maturation. Replication of these processes *in vitro* has been shown to be beneficial but is at the cost of greater expense and complexity[1]. We aim to replicate this with a simpler methodology in the interests of making such techniques more accessible. Briefly, organoids generated from human induced pluripotent stem cells[2] were cultured from day 4 in either 6-well plates on a shaker or within bioreactors (at a speed of 150rpm or 450rpm) and maintained for a further 22 days. Nephron maturation and vascularisation were monitored by quantifying specific renal markers using qPCR and immunohistochemical staining. Organoids cultured in moderate or high sheer force displayed an increase in distal tubule markers with concomitant downregulation of glomerular markers. By contrast, the degree of vascularisation in the organoids remained unchanged by the shear force. These results suggest that sheer force is important for the segmentation of the distal nephron in organoids, mirroring in-vivo experience. Furthermore, these indicate that the shear force alone is insufficient in inducing further vascularisation/maturation. Inclusion of other factors that mirror the in-vivo circulatory haemodynamics might be necessary to improve the maturation of organoids cultured in vitro.

1. Homan, K. A. et al. (2019). *Flow-enhanced vascularization and maturation of kidney organoids in vitro*. Nat. Methods **16**, 255–262.
2. Przepiorski, A. et al. (2018). *A Simple Bioreactor-Based Method to Generate Kidney Organoids from Pluripotent Stem Cells.* Stem Cell Reports **11**, 470–484.

A23: Vascular roles during kidney development and implications for function

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Kidney development is a tightly coordinated process which leads to the establishment of mature cells that mediate physiological functions. The renal endothelium is critical to maintaining physiological homeostasis and recent studies have begun characterizing the process of kidney vascularization. However, despite these advancements, we know little about how vascularization is regulated during kidney development. During embryogenesis, vascularization is in part guided by the secreted chemotactic guidance cue netrin-1. Netrin-1 (*Ntn1*) is expressed by the stromal progenitors of the developing kidney suggesting it may play a similar role during renal development. We found that conditional deletion of *Ntn1* from stromal progenitors results in aberrant vascular patterning. Utilizing a novel vascular labeling and imaging strategy which we developed, we find that abnormal arterial patterns persist into adulthood. Preliminary data suggests they may also have altered erythropoietin production thereby linking vascular pattern to proper physiological function. Additionally, the developing *Ntn1* mutant kidneys are hypoplastic and show reduced proliferation rates. We hypothesize that the endothelial cells of the kidney vasculature produce angiocrine signaling factors which regulate development. Using publicly available single cell RNA-seq data we have identified a number of signaling molecules specifically expressed by the kidney endothelium. We are currently investigating the implications to kidney development by vascular-specific removal of one of these factors, *Igf1*. Together, our studies will provide novel insights into proper vascular patterning of the kidney and the implications for development and physiological function. These findings will inform efforts to generate kidneys de novo where integrating functional vascular networks will be critical.