RESEARCH ARTICLE

Nephron progenitor cells in development and renal disease: Renal Hypoplasia, Wilms Tumour and recovery from Acute Kidney Injury

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Abstract

The emergence of nephron progenitor cells (NPCs) in early embryonic life leads to the many rounds of nephrogenesis that result in a richly endowed kidney by the end of gestation. A delicate balance between NPC differentiation and self-renewal must be maintained to guarantee optimal nephron endowment. Genetic errors which disturb NPC cell fate can result in premature NPC depletion and renal hypoplasia/dysplasia or permit the β-catenin mutations that accompany malignant transformation into a Wilms tumor. Retention of a small population of NPCs scattered throughout the adult kidney are important for recovery from acute tubular injury later in life. In this review, we track the origin and characteristics of NPC, describe the phase of NPC priming prior to nephron induction and describe NPC differentiation during nephrogenesis. We then cover the role of NPC in human renal disease, including mechanisms by which quiescent NPCs repair the injured adult kidney and the human diseases linked to dysfunction of NPCs.

Keywords: Nephron progenitor cells (NPCs); Renal hypoplasia; Wilms tumour (WT); Acute kidney injury (AKI)

1. Introduction

Nephron progenitor cells (NPCs) represent a population of highly specialized cells in the developing kidney. When they receive the right inductive signal, a differentiation cascade is unleashed, and these cells go on to form the mature nephron structures of the kidneys (each linked to the collecting duct system). NPCs in lower organisms can generate new nephrons in response to injury throughout their entire lifespan. In contrast, the NPC pool in mammals is nearly depleted in the perinatal period; all nephrons are formed during embryogenesis and final nephron number is set before birth in humans.[1, 2] Although NPCs originate at an early embryonic stage, a small subset are maintained into postnatal life and play an important repair function in the mature kidney. In humans with acute injury to renal tubules, acute tubular necrosis (ATN) may be severe enough to cause cessation of urine output. Remarkably, kidney function can be restored, though recovery may take days to weeks. Recovery involves repopulation of damaged tubular segments by proliferation of injury-resistant NPCs scattered throughout the kidney.[3, 4] In this review, we cover the role of NPCs in both mammalian kidney development and renal disease.

2. Development of NPCs

2.1 Embryology of kidney development

Early in embryonic development, the inner cell mass differentially expresses stem cell genes and genes responsible for tissue specificity and commitment to certain cell fates, with high and low expression, respectively. Around E6.5 in mouse embryonic development, the primary germ layers (endoderm, mesoderm and ectoderm) begin to form.[5] Transcription factor Odd-skipped-related-1 (*Osr1*) marks the entire mesoderm at this stage, however, gradients of inhibitory growth factors in the lateral plate and paraxial mesoderm eventually restrict expression to the intermediate mesoderm.[6, 7] Organs derived from the intermediate mesoderm, including kidneys and gonads, fail to develop in *Osr1* knockout mice.[8, 9] Around E8.5, a subset of *Osr1*(+) intermediate mesoderm differentiate into the epithelial cells of the paired nephric ducts which express *Pax2*, *Pax8* and *Gata3.*[10, 11] At this stage, another subset of *Osr1*(+) intermediate mesoderm remain mesenchymal and begin expressing Wilms tumour 1 (*Wt1*) around E9.0.[12] *Osr1*(+);*Wt1*(+) cells form the metanephric mesenchyme which contain the NPC population. Similar to Osr1 knockout mice, kidneys fail to develop in *Wt1* knockout mice.[13] As the *Pax2*;*Pax8*;*Gata3* expressing nephric ducts extend caudally in the embryo, around E10.5, they reach the glial-derived neurotrophic factor (GDNF) secreting metanephric mesenchyme (27^{th}/28^{th} somite) which stimulates an outgrowth from the nephric duct. This outgrowth, the ureteric bud (UB), invades and induces the metanephric mesenchyme to condense around the UB tip.[14] This "cap" of cells represents the early "primed" NPC population prior to induction of nephrogenesis. The first UB branching event occurs where it forms the characteristic T-like structure around E11.5 in mouse kidney development.[15] Around this stage, the transcription factors *Cited1* and *Six2* are expressed in the cap mesenchyme and these cells represent the population of NPCs ready to initiate nephrogenesis.[16-23]

2.2 Origins of the NPC

Whereas mesenchymal stem cells are pluripotent, NPCs arise at E9.0 in mouse kidney when a subset of intermediate mesoderm cells begin to express the master transcription factor, *Wt1*, committing them to nephrogenesis. This unlocks the undifferentiated stem cell state in which many genes required for nephrogenesis are broadly suppressed by epigenetic silencing. This is organized by polycomb repressive complexes (PRC) which methylate histones, making many genes inaccessible to transcription machinery. Maintenance of self-renewal and stem-like states have been shown to be dependent on histone methylation in human embryonic stem cells (hESCs) and epidermal progenitor cells.[24, 25] WT1 is crucial for the transition from undifferentiated stem cell in *Osr1*(+) mesoderm to committed NPC in metanephric mesenchyme. Expression of this transcription factor suppresses gene silencing by inhibiting the catalytic subunit (EZH2) of the PRC. WT1 suppresses *EZH2* through at least 2 concerted mechanisms. WT1 binds directly to the *EZH2* promoter to suppress transcription and also engages microRNAs to suppress EZH2 translation.[26, 27] Thus, the onset of *Wt1* expression sets early NPC fate and commits the cells to nephrogenesis. WT1(+) NPCs will give rise to all the tubular segments from glomerulus to distal convoluted tubule.

2.3 NPC Priming

Although NPCs are committed to nephrogenesis at this stage, they are not yet up to the task. In 2005, Carroll et al. identified WNT9B as the inductive WNT-ligand and showed that it is produced by the adjacent UB.[28] A *Wt1*(+) cell line (M15), isolated by Hastie et al. from E10.5 mouse mesonephric mesenchyme, was found to be unresponsive to WNT9B.[29] In the interval between E10.5 and E11.5 which marks the arrival of the UB, early NPCs are primed to respond to the inductive signal. Additional transcription factors (EYA1, SIX2, CITED1, SALL1) mark the fully primed NPC which can unleashes the β-catenin signalling pathway in response to WNT9B.[17, 18, 20, 30, 31]

An essential part of NPC priming involves the expression of missing elements in the β-catenin pathway. When M15 cells were analyzed for constituents of the WNT/β-catenin pathway, they were found to lack some key elements present in mature *Cited1*(+) NPCs from the cap mesenchyme of E17 mouse kidney.[29] M15 cells lack both *Frizzled 5* (*Fzd5*) and R-spondin1 (*Rspo1*) mRNA expression. FZD5 is part of the WNT co-receptor complex expressed at the cell surface of NPCs in the cap mesenchyme; RSPO1 stabilizes the co-receptor complex and amplifies the canonical WNT-signal.[29] Addition of these missing components to M15 cells in vitro permitted a robust response (11-fold increase) to WNT9B. Vidal *et al* detected *Rspo1* and *Rspo3* transcripts in the nephrogenic zone of E14.5 mouse kidneys.[32] Knockout of R-spondin alone in mouse embryonic kidney cap mesenchyme gave no kidney phenotype but mice with double knockout of *Rspo1*;*Rspo3* in *Six2*(+) NPCs had hypoplastic/dysplastic kidneys.

2.4 WNT/β-catenin signalling and induction of NPCs

Once the NPCs are fully primed, they respond to WNT-signalling with robust activation of the β-catenin pathway, releasing the differentiation cascade and a burst of rapid cell division which drives nephrogenesis. Iglesias *et al* detected high levels of canonical WNT-signalling activity in both cap mesenchyme NPCs and UB cells of embryonic mouse kidneys (Figure 1).[33] β-catenin knockout in UB cells causes renal dysplasia in mouse kidneys.[34] β-catenin knockout in mouse metanephric mesenchyme resulted in small, dysplastic kidneys.[35]

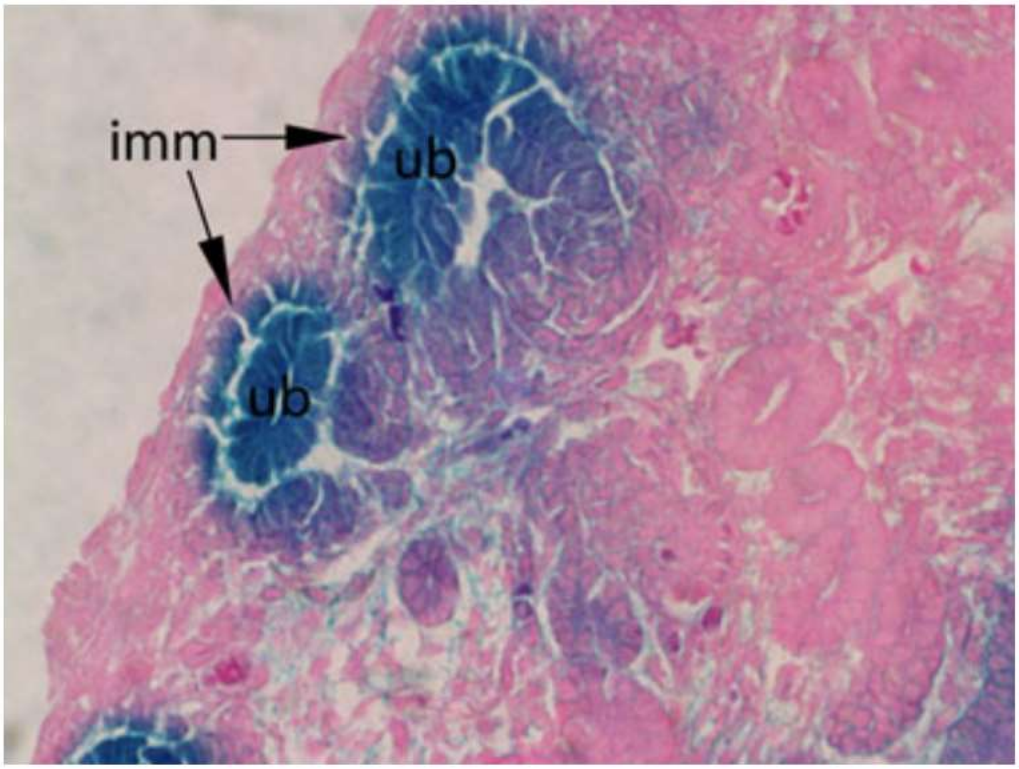

Figure 1. Active WNT/β-catenin signalling in embryonic mouse kidney. A section from an E15 kidney of the TOPGAL mouse. These mice contain the β-galactosidase gene driven by a TCF promoter (β-catenin responsive). Kidneys were stained with X-gal to detect the β-galactosidase signal. ub: ureteric bud epithelia; imm: induced metanephric mesenchymal cells. Republished from Iglesias et al 2014.[33]

2.5 Self-renewal of NPCs

In humans, the metanephric kidney begins to function by about 10 weeks of age but UB branching and successive generations of nephrons are continued until 36-38 weeks gestation. Since each generation of nephrons consumes NPCs, it is important to replenish the NPC pool throughout the embryonic period. NPC survival and self-renewal are driven by low levels of β-catenin, stimulated by WNT9B from the UB.[36] Failure to maintain the NPC pool throughout nephrogenesis results in reduced nephron number, smaller kidney size and is associated

with an increased risk of developing renal insufficiency in later life.[37] Differentiation of NPCs to form the renal vesicle (RV) appears to require additional signals which amplify the level of β-catenin signalling. An inner layer of NPCs develop intimate contact with WNT11-expressing cells at the distal UB tip and appear to modify their polarity and behaviour.[38] Although movement of uninduced NPCs is likely random, it has been proposed that the induced NPCs may move directionally along the underarm of the UB branch. There, they are exposed to additional signals which result in high levels of β-catenin signalling.[39] This induces both a burst of proliferation[40] and simultaneous differentiation into the RV.[36, 39] One proposed mechanism postulates stromal cell influence on NPC behaviour in the underarm niche of the UB (Figure 2). It is plausible that defects in the regulation of these NPC events could be involved in some forms of renal dysplasia but have not been reported in humans to date.

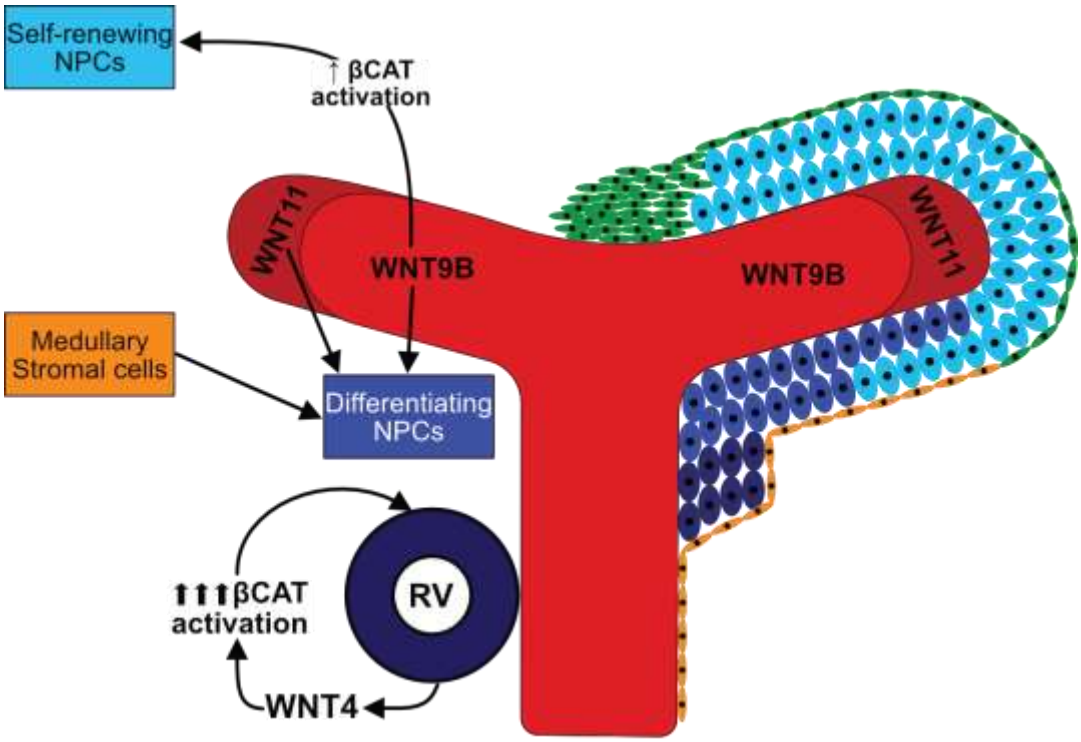

Figure 2. Schematic of canonical WNT-signalling in NPCs. Self-renewing NPCs (light blue); differentiating NPCs (medium blue); renal vesicle (RV - dark blue); ureteric bud (red); distal ureteric bud tip (dark red); cortical stromal cells (green); medullary stromal cells (orange). Modified from Ramalingam et al 2018.[36]

2.6 NPC transcription factors in kidney development and human renal disease

2.6.1 SALL1

In *Six2*(+) cells, *Sall1* expression promotes self-renewal and positively regulates NPC genes *Cited1* and *Osr1*.[31] *Sall1* deletion in mice resulted in depletion of the NPC pool, decreased survival of differentiated cells and severe renal dysgenesis. *SALL1* mutations have been identified in individuals with

Townes-Brocks syndrome (TBS). Several organ systems are affected in individuals with TBS, however, a commonly observed phenotype includes renal hypoplasia, dysplasia and kidney failure.[41, 42] Botzenhart et al describe 56 *SALL1* mutations, with 46 of them located in a small stretch within exon 2 of the gene and 10 of them associated with a renal phenotype.[43] Faguer et al describe two additional *SALL1* mutations in TBS patients, both occurring in the hotspot region in exon 2. One mutation identified was a frameshift mutation caused by an insertion (c.981_982insTGGC) and the second mutation was a frameshift mutation caused by an insertion-deletion (c.1451_1458delACAGGTTCinsT) with both mutations predicted to generate non-functional, truncated proteins.[44] Both individuals in their report displayed renal hypodysplasia and chronic renal failure. A frameshift mutation resulting in formation of a premature stop codon in *SALL1* was reported (c.3414–3415delAT (p.T1138fs1152X)) in an individual with an isolated renal phenotype who developed bilateral renal hypoplasia.[45] Additional mutations are listed in Table 1.

Table 1: Human renal hypoplasia/dysplasia causing mutations in key NPC genes

Gene	Mutation (DNA sequence change)	Amino acid change	Reference
BMP4	c.130G>T	p.G44*	[46]
	c.272C>G	p.S91C	[47]
	c.347C>G	p.T116S	
	c.450C>G	p.N150K	
EYA1	c.1179insGGG	p.394insG	
	Partial gene deletion	Loss of function	
	c.1360T>C	p.S454P	[48]
	c.1414T>G	P.L472R	
	c.823C>T	Premature stop codon	
	c.1251T>CC	Frameshift	
	c.755InsC	Frameshift	
	c.1555InsTTGT	Frameshift	
	c.1359InsC	Frameshift	
	c.1372T>AGAGC	Frameshift	
	c.1599 +5 G>C	Aberrant Splicing	
	c.1498 +2 T>G	Aberrant Splicing	
	Exon 11-16 del20-37Kb	Large deletion	
	Exon 9 del5.6Kb	Large deletion	
	Exon 11-15 del5.8-7Kb	Large deletion	
	c.1377-2A>G	-	Boys Town National Research Hospital (unpublished)
	c.303C>A	p.Tyr101*	
	c.619G>T	p.G207*	[49]
	c.806delC	p.A269fs	
	c.1048_1050+1del	-	

	c.1319G>A	p.R440Q	
	c.1487_1488del	p.V496fs	
	c.727G>T	p.E243*	50
	IVS15-3C>T (SNP)	-	
	c.920delG	p.G307fs	51
	c.1475+1G>C	-	52
	c.1381delA	p.R461fs467*	53
	c.966+5G>A	-	54
	c.1474_1475insC	p.R492Pfs*40	55
	c.319G>A	p.Gly107S	56
	c.430C>T	p.Gln144*	
	c.586_596dup (+)636_644delInsTG	p.[Ser200IlefsX12 + Ser213GlyfsX112]	
	c.616dupT	p.Tyr206LeufsX50	
	c.989A>T	p.Glu330Val	
	c.1100+1G>C	-	
	c.1216_1219dup	p.Arg407Glnfs*13	
	c.1231_1232dupAT	p.Tyr412Serfs*24	
	c.1372_1375dupTCCC	p.Arg459Leufs*41	
	c.1425delA	p.Leu476Trpfs*9	
	c.1434dup	p.Val479Serfs*20	
	c.1542_1546delAAAAG	p.Arg514Serfs*83	
	c.1554T>G	p.Tyr518*	
	c.1655dup	p.His552Glnfs*47	
	c.1678T>C	p.*560Gln	
	c.104−?_461+?del	-	
	Entire gene deletion	-	
	c.880C>T	p.R294*	57
	c.1459T>C	p.S487P	
	c.1604_1607del	p.E535fs	
	c.1691delC	p.A564fs	
	c.1220G>A	p.Arg407Gln	
	c.781C>T	Premature stop codon	58
	c.1501delAAAG	Frameshift	
	c.1592delC	Frameshift	
	c.1442T>C	-	59
	c.1042-1G>A	-	Molecular Otolaryngology Research Laboratory
	c.124_432del309	p.V42_Q185del	60
	c.1050+1G>C	-	61
	c.1286A>G	p.D429G	
	c.1420-1421delCC	Premature stop codon	62
	c.497T>A	p.Y163*	63
	c.1107T>A	p. Y370*	
	IVS9-2A>G	Aberrant splicing	
	IVS14- 1G>A	Aberrant splicing	
	c.952G>T	Aberrant splicing	

	c.982C>T	p.R328*	64
	c.164C>T	p.T55M	65
	c.348delA	p.G117fs	
	c.402C>A	p.Y134*	
	c.450_451del	p.G151fs	
	c.553C>T	p.Q185*	
	c.592G>T	p.G198*	
	c.634C>T	p.Q212*	
	c.638A>T	p.Q213L	
	c.640-15G>A	new splice acceptor	
	c.777dupA	p.E260fs	
	c.851C>G	p.S284*	
	c.863_866del	p.K288fs	
	c.967-1G>A	-	
	c.1050+1G>T	-	
	c.1081C>T	p.R361*	
	c.1138_1140+1delinsCTTC	-	
	c.1377_1378delinsAT	p.K460*	
	c.1476-2A>G	-	
	c.1496delT	p.L499*	
	c.1510C>T	p.Q504*	
	c.1579T>A	p.Y527N	
	c.1591A>T	p.K531*	
	c.1649T>A	p.V550E	
	c.1657_1659del	p.V553del	
	c.1697dupA	p.H567fs	
	c.790 C>T	p. R265*	66
	c.1680A>C	p.*559Y	
	c.1649 T>C	p.L549P	
	c.387insT	-	
	c.868-1G>A	-	
	c.1041+1G>T	-	
	c.1498+2T>G	-	
	c.1042-13 23bp inv	-	
	c.889C>T	p.R297*	
	c.1140+1G>T	-	
	c.1773C>G	p.Y591*	67
	c.1141-1G>A	p.E380fs*387	
	c.1360+4A>G	p.G454fs*461	
	c.920delG	p.R307fs*365	
	c.1627C>T	p.Q543*	68
	c.867+5G>A	p.Y244Sfs*8	69
	c.1261+5G>A	-	
	c.1397dupT	p.L466Ffs*33	
	c.579C>G	p.Y193*	70
	c.678C>G	p.Y226*	
	c.297delCCGTACGG	Frameshift	71

	c.870InsGT	Frameshift	
	c.967A>T	pR323*	[72]
OSR1	rs12329305	-	[73]
SALL1	c.981_982insTGGC	-	[44]
	c.1451_1458delACAGGTTCinsT	-	
	c.3414–3415delAT	p.T1138fs1152*	[45]
	c.817delG	-	[74]
	c.814C>T	-	[43]
	c.995delC	-	
	c.1119_1197del79	-	
	c.1134delT	-	
	c.1174_1175delCT	-	
	c.1273delC	-	
	c.1516_1517dupAT	-	
	c.3249_3255del7	-	
SIX1	c.679G>T	p.D227Y	[45]
	c.619insG	-	
	c.397-399delGGA	p.delE133	[75]
	c.328C>T	p.R110W	[76]
SIX2	c.402C>T	p.L43F	[47]
	c.997C>T	p.P241L	
	c.1100–1101GG>AA	p.D276N	
WNT9B	c.949G>A	p.G317R	[77]
	c.11dup	p.P5Afs*52	

2.6.2 EYA1

EYA1 is expressed in cap mesenchyme NPCs and functions as a transcriptional co-activator. NPC-specific knockout of *Eya1* in mice resulted in premature differentiation of the NPC population.[30] *Six2* expression was minimal in *Eya1* knockout NPCs suggesting *Eya1* is essential for expression of *Six2* to maintain the NPC pool in an undifferentiated state. *EYA1* was identified as a causative gene for Branchio-Oto-Renal (BOR) syndrome in 1997.[78] As the name suggests, the syndrome is associated with branchial, otic and renal anomalies. Individuals with BOR syndrome display a phenotype ranging from renal hypoplasia to bilateral agenesis. Over 100 *EYA1* mutations have been reported, accounting for ~40% of all BOR syndrome cases.[52, 56, 65] *SIX1* and *SIX5* have also been associated with BOR, however, these account for only 5% of cases.[76, 79] Additional mutations are listed in Table 1.

2.6.3 SIX2

Knockout of *Six2* in mice results in severe renal hypoplasia due to compromise of the NPC pool.[22] Similarly, *SIX2* mutations cause renal hypoplasia in humans. Three missense mutations in *SIX2* (c.402C>T (p.L43F); c.997C>T (p.P241L); c.1100–1101GG>AA (p.D276N)) were described by Weber et al in 5 patients with renal hypoplasia, dysplasia and/or vesico-ureteral reflux.[47] These mutations occurred in highly conserved amino acids in the six domain of SIX genes or the SIX2 specific C-terminal domain.

2.6.4 SIX1

Like SIX2, SIX1 is essential for NPC survival and differentiation. Interestingly, Xu et al analyzed the metanephric mesenchyme of *Six1*-null mice and observed a significant reduction in *Six2* expression.[80] *SIX1* mutations have been linked to BOR syndrome in several individuals. Ruf et al describe a family with an in-frame deletion in *SIX1* (c.397-399delGGA) resulting in the deletion of a glutamate amino acid.[75] The patient described developed one hypoplastic/dysplastic kidney with vesicoureteral reflux and renal failure. *SIX1* mutations (c.679G>T (p.D227Y)) were reported in two siblings who also had a frameshift mutation in *PAX2*.[45] One individual developed bilateral renal hypoplasia and the second developed bilateral renal dysplasia. The father of the children who passed on the *PAX2* variant had isolated renal hypodysplasia and chronic renal insufficiency. The mother carried the *SIX1* variant, however, had no symptoms. Additional mutations are listed in Table 1.

2.6.5 OSR1

Osr1 expression marks the intermediate mesoderm lineage which gives rise to NPCs; Osr1 knockout in mice results in renal agenesis.[8, 9] In humans, a hypomorphic *OSR1* variant (rs12329305) is associated with an 11.8% reduction in kidney volume in the newborn.[73] The reduction in kidney volume was accompanied by a significant increase in cord blood cystatin C levels, suggesting these individuals have reduced kidney function. Lozić et al found an association between the same *OSR1* variant and congenital anomalies of kidney and urinary tract (CAKUT).[81]

2.7 NPC growth factors in kidney development and renal disease

2.7.1 BMP4

BMP4 is expressed in the developing metanephric mesenchyme and acts as an inhibitor of GDNF to prevent ectopic UB outgrowths in the early stages of kidney development.[82, 83] *Bmp4* knockout mice exhibit a number of CAKUT phenotypes, including renal hypoplasia/dysplasia.[82] Three missense mutations in the prodomain of *BMP4* were identified in 5 individuals (c.272C>G (p.S91C); c.347C>G (p.T116S); c.450C>G (p.N150K)).[47] Three individuals in this study developed renal hypoplasia or dysplasia and one individual had renal agenesis. Another missense mutation in *BMP4* was identified resulting in formation of a premature stop codon (c.130G>T (p.G44*)) in a patient who developed renal hypoplasia requiring renal replacement therapy.[46]

2.7.2 WNT9B

Mutations of *WNT9B*, compromise NPC induction and are associated with abnormal kidney development. In mice, knockout of *Wnt9b* resulted in loss of the NPC population, causing severe renal hypoplasia.[28] Lemire et al identified two novel *WNT9B* variants in two unrelated families.[77] The proband from the first family had a homozygous missense mutation c.949G>A (p.G317R) in *WNT9B* and developed bilateral renal cystic dysplasia and chronic kidney disease. Two previous pregnancies were terminated due to development of severe bilateral renal agenesis and oligohydramnios. Both fetuses had a homozygous c.949G>A (p.G317R) mutation. In the second family, the proband had a homozygous c.11dup (p.P5Afs*52) mutation

in *WNT9B* and developed renal hypoplasia/dysplasia with progressive renal insufficiency. The parents of this proband gave birth to 2 other infants who died on day 1 and day 20 of life. The genotypes of the siblings were not characterized; however, oligohydramnios was confirmed in one sibling.

3. Malignant transformation of NPCs (Wilms tumour)

3.1 Genetics of hereditary Wilms tumours

Wilms tumour (WT) is an embryonal tumour that occurs in the first few years of life

Hereditary forms of WT (Drash syndrome and WAGR syndrome) led to the discover of the WT1 gene.[84] *WT1* mutations are detected in approximately 20% of WTs and the current understanding of WT indicates that NPCs harbouring a germline *WT1* mutation are susceptible to somatic mutations which eliminate the trans *WT1* allele.[85] In an NPC, lacking WT1 expression, responsiveness to WNT9B is precluded. The clonal progeny of a mutant NPC form undifferentiated clusters in the kidney, termed nephrogenic rests (Figure 3). These cell clusters show no β-catenin signalling activity and undergo malignant transformation frequently associated with a new constitutively-active β-catenin gene (*CTNNB1*) missense mutation. Although nephrogenic rest cells are prone to malignant transformation, they can be found in normal kidneys where they usually involute during the first year of life.[86] Nephrogenic rests look phenotypically like NPCs; WTs characteristically retain expression of NPC transcription factors such as *CITED1* and *SIX2*.[87]

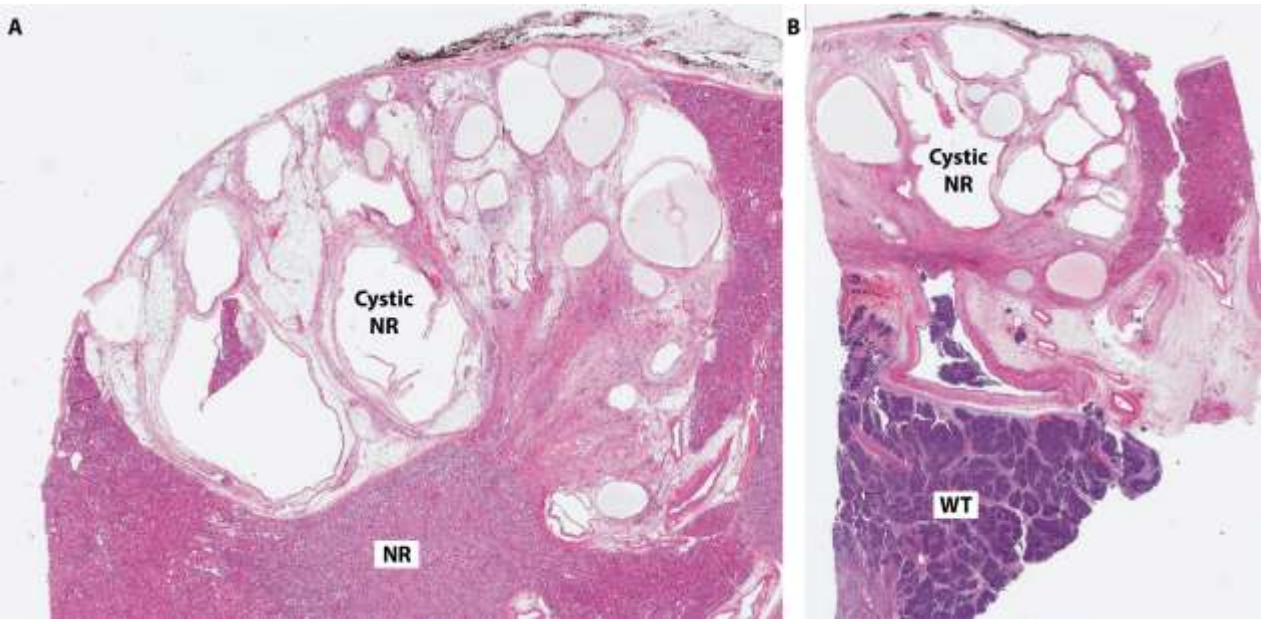

Figure 3. A) Cystic nephrogenic rest (NR) in a patient with a germline WT1 missense mutation and Drash Syndrome; **B)** Malignant Wilms tumour (WT) with darker staining sits adjacent to the NR.

3.2 β-catenin (CTNNB1) mutations in Wilms Tumour

Approximately 15% of all WTs contain activating mutations in *CTNNB1*.[88] The most common *CTNNB1* mutations alters the critical serine 45 residue targeted by GSK3β and the β-catenin destruction complex.[89, 90] This mutation permits constitutive activation of the canonical WNT-signalling pathway, driving unregulated NPC growth and proliferation since β-catenin can no longer be targeted for ubiquitination and proteasomal degradation.[91] Interestingly, Maiti et al. showed that 95% of WTs analyzed in their study had both *WT1* and *CTNNB1* mutations.[88] Fukuzawa et al analyzed WT tissue and the neighbouring nephrogenic rests and detected *WT1* mutations in both compartments. In contrast, *CTNNB1* mutations were only detected in tumour tissue, suggesting *CTNNB1* mutations are a late mutational event in the progression of Wilms tumorigenesis in *WT1*-null cells.[92] Huang et al generated a mouse model in which *WT1*-knockout was targeted to either *Cited1*(+) NPCs, *Six2*(+) NPCs or *Foxd1*(+) stromal cells. Seventy-three percent (8/11) of *Cited1* mutants generated WTs in contrast to 45% (9/20) of *Six2* mutants. No tumours were generated in the *Foxd1* mutants. This suggests the NPC are particularly susceptible to malignant transformation when they lose WT1 expression.[93]

Multiple reports have identified multifocal WT cases in which each tumour harboured a unique activating β-catenin mutation, suggesting a random somatic event.[94, 95] This observation also suggests that there may be a selective pressure for this mutational event in WT1-null cells. In WT without activating mutations in *CTNNB1*, other mechanisms that result in nuclear localization/activation of β-catenin are seen. In a subset of tumours analyzed by Su et al, nuclear translocation of β-catenin was detected in 53% (10/19) of tumours, even though only 15.8% (3/19) of tumours had a mutation in *CTNNB1*.[96]

A plausible mechanism for the increased incidence of *CTNNB1* mutations in *WT1*-null cells involves the regulation of *EZH2* by WT1. Akpa et al showed that expression of WT1 normally suppresses *EZH2*, allowing expression of *CTNNB1*.[27] Thus, loss of *WT1* in NPCs precludes normal β-catenin, needed for the response to ureteric bud WNT9B. It is unclear whether the frequent occurrence of activating *CTNNB1* mutations is a random mutational event that drives rapid cell division or whether loss of *WT1* somehow destabilizes the NPC genome.

3.3 Genomic stability in the NPC

Although *WT1*-null WTs do not exhibit an obvious mutator phenotype, it is conceivable that loss of *WT1* creates a low level of genomic instability which drives frequent mutations in specific genes. Given the important role *WT1* plays in setting the NPC cell fate early in kidney development, loss of *WT1* may alter expression of specific DNA-repair genes, thus explaining the incidence of common co-mutations in *WT1*-null WTs. Mutations in well-known DNA repair genes are rare in WT,[97-99] however, the possibility remains that gene expression of specific repair genes could be altered at either the transcriptional or epigenetic level in WT1-null cells.

4. NPCs and repair of kidney injury

Although NPCs play a crucial role in primary nephrogenesis prior to birth, a small subset of embryonic NPCs were found to be retained by the mature kidney.[100] Unlike zebrafish which can generate new nephrons throughout adult life, mammalian kidneys cannot form new nephrons after the perinatal period. However, the observations by Sagrinati suggest that adult NPCs are retained to repair renal tubules following acute injury later in life. It is a common occurrence in hospital intensive care units to observe complete shutdown of urine output in patients who have experienced hypotensive shock or exposure to nephrotoxic drugs. Renal biopsy shows structural damage to both the renal glomerulus and renal tubules, particularly the renal proximal tubule. Anuria may persist for weeks, requiring dialysis and other supportive measures. However, in most cases, there is a dramatic turn of events which leads to restoration of urine output and general recovery of renal function, to baseline levels. The mechanism by which renal tubules are repaired is not well understood and could involve the adult NPCs described by Sagrinati et al.

In their report from 2006, Sagrinati et al showed that NPCs retained in the adult kidney express stem cell markers CD24/CD133. Others have added additional NPC markers such as Vimentin and VCAM1.[101, 102] These cells have the ability to self-renew and differentiate into both podocytes and tubular epithelial cells.[103] As development of the embryonic kidney proceeds, the number of these cells fall but remain detectable in the urinary pole of Bowman's capsule, the renal proximal tubule and in some segments of the distal nephron (Figure 4).[4] Analysis of human kidneys with acute tubular necrosis (ATN) identified an increased number of CD24(+) cells in the injured tubules compared to control.[104] Additionally, 85% of the CD24(+) cells in ATN biopsy samples were proliferating, suggesting a contribution to the repair of damaged tubules. Isolated CD24/CD133(+) were also shown to be more resistant to cell death compared to CD24/CD133(-) cells.[3] Using a confetti immunofluorescent reporter driven by the *Pax2* promoter to mark NPCs, Lazzeri et al showed that large stretches of damaged epithelium were repopulated from single NPC clones.[4] Single cell analysis showed that NPCs represent about 1 in 16 renal proximal tubule cells in the adult human kidney.[102]

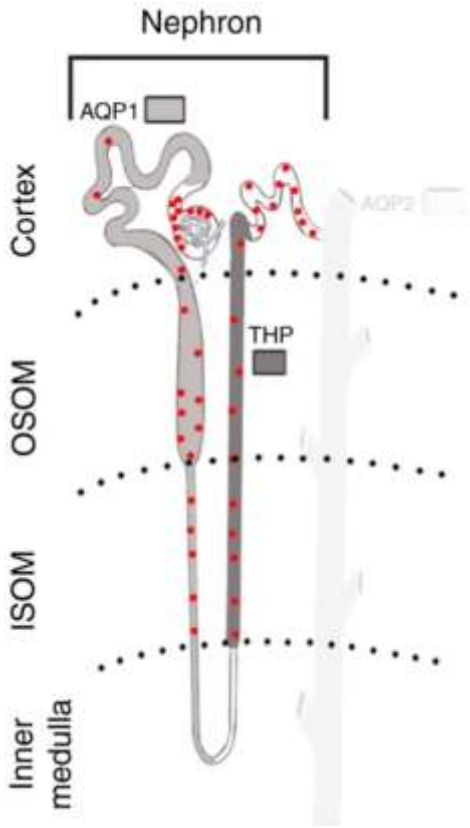

Figure 4. Schematic of NPCs from the *Pax2*(+) lineage within the mature nephron structure (red dots). Aquaporin-1 (AQP1) positive proximal tubules (light grey); Tamm–Horsfall Protein (THP) positive distal tubules (dark grey); OSOM: outer stripe of outer medulla; ISOM: inner stripe of outer medulla. Adapted from Lazzeri et al 2018.[4]

4.1 Therapy of acute renal injury with exogenous NPCs

Interestingly, Sagrinati et al and Lazzeri et al showed that CD24/CD133(+) NPCs could be harvested from both human fetal and adult kidneys and infused into mice with glycerol induced acute tubular necrosis (ATN) to accelerate recovery. The exogenous cells integrate into damaged segments of the renal tubular epithelium and significantly reduced blood urea nitrogen (BUN) and renal fibrosis.[100, 103] Zhang et al showed that integration of exogenous CD24(+) NPCs from embryonic mouse kidney into glycerol injured adult mice is highly dependent on the integrity of β-catenin signalling.[105] Pre-treatment of CD24+ cells with a canonical WNT-signalling inhibitor (IWR-1) prior to infusion resulted in a significant decrease in the number of integrated cells. Preliminary data from our lab showed that early unprimed NPCs from E10.5 mouse kidney are unable to integrate into glycerol-injured renal tubules (data not published).

Several groups have investigated the ability of mesenchymal stem cells (MSCs) to repopulate the damaged kidney. Amniotic fluid and bone marrow derived mesenchymal stem cells (MSCs) have been tested

extensively in mouse models with AKI. Rota et al infused MSCs from amniotic fluid and Morigi et al tested MSCs from bone marrow. Both improved recovery of AKI compared to untreated mice. However, 90% of exogenous cells were not integrated into the tubular epithelium.[106, 107] Thus, the beneficial effects of MSC therapy are thought to occur through paracrine secretion of protective growth factors and cytokines rather than repopulation of the damaged tubule.

Several groups have tested the ability of human urine-derived cells to treat immunodeficient mice with cisplatin or ischemic AKI. Human urine-derived cells improved repair when analyzing blood biomarkers and tubular injury.[108-111] Similar results were observed in a diabetic nephropathy mouse model.[112] Although these cell types improved repair, closer examination of the published immunofluorescence and immunocytochemistry images again showed minimal cell integration into tubular epithelial cells. Therefore, it is likely that these cell types primarily exert their protective function through paracrine secretion of growth factors/cytokines, similar to amniotic fluid and bone marrow derived MSCs.

Arcolino et al. recently described a protocol permitting the isolation of nephron progenitor-like cells from urine of newborn babies (gestational age of 31-36 weeks).[113] Cell sorting of neonatal urine samples using cell surface markers including CD24, CD133 and SIX2 isolates a putative NPC-like population. Culture of these cells in podocyte differentiation-medium for 7 days upregulated transcript levels of podocyte markers *PODOCALIXYN*, *SYNAPTOPODIN*, *CD2AP* and *NEPHRIN*. In cisplatin-treated proximal tubules cells, caspase 3 levels were significantly reduced by co-culture with NP-like cells derived from neonatal urine. However, the evidence for repair AKI by exogenous NPC from perinatal urine remains elusive.

5. Conclusions

This review provides an update on the biology of NPCs during embryonic kidney development and summarizes recent evidence that mutation of NPC genes contribute to human syndromes of kidney hypoplasia or dysplasia. In contrast, loss of the master NPC gene, *WT1*, disturbs NPC fate and leads to Wilms tumor. Finally, it is tempting to hope that infusion of exogenous NPC may, one day, be useful in repopulating the human nephron after acute kidney injury.

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