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Role of Transcription Factors in Podocytes

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Abstract
Despite a wealth of information on structural proteins, comparatively little is known on the transcriptional regulation of podocyte structure and function. In this review we will highlight those transcription factors which, by gene inactivation or classical transgenic experiments, have been shown to be essential for podocytes or probably will turn out to be so. The tumor suppressor protein WT1 is not only indispensable for the initial stages of kidney development, but also very likely maintains the integrity of the fully differentiated podocyte. In the kidney, the LIM homeodomain transcription factor LMX1B is specifically synthesized in podocytes, and mutations in *LMX1B* lead to nail-patella syndrome and the associated nephropathy. Other transcription factors such as hypoxia-inducible factors and PAX2 are likely to play a role in podocytes, whereas the significance of others, e.g. of POD1 and CITED2, is more speculative at this point.

Introduction

The essential function of podocytes was irrevocably brought to nephrologists' attention with the identification of the *NPHS1* gene [1], the gene mutated in patients suffering from congenital nephrotic syndrome of the Finnish type. From then on the podocyte has not left center stage when it comes to the investigation of glomerular development and diseases. During development, podocytes differentiate from a cuboidal into an octopus-shaped cell with many large and small tentacles. Primary processes (the large tentacles) extend from the cell body and elaborate many fine secondary processes, the foot processes. By a mechanism so far shrouded in mystery, foot processes emanating from two different primary processes (but not from the same primary process!) are connected by a proteinaceous bridge called the slit diaphragm, one component of the glomerular filtration barrier. In the absence of the protein product of the *Nphs1* gene, nephrin, no slit diaphragms form [2, 3]. Both figuratively and literally speaking, nephrin has been the nucleus around which the slit diaphragm was built. Furthermore, the podocytes, together with glomerular endothelial cells, are also responsible for the synthesis of the glomerular basement membrane. Despite this increasing wealth of information on structural proteins produced by

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podocytes, comparatively little is known on the transcription factors regulating the synthesis of these proteins. Our review will highlight the current knowledge on gene regulation in podocytes. One of the challenges for the coming years will be to identify the target genes of transcription factors in podocytes, which will yield a deeper insight both into the regular development of podocytes and into podocyte diseases.

Transcription Factors with a Proven Role in Podocytes

FOXC2 (Mfh2)

The forkhead/winged-helix family of transcription factors plays essential roles in cell fate determination, proliferation and differentiation of multiple tissues and organs (reviewed in [4]). One member of this family, FOXC2, has been detected in developing and mature podocytes [5], and indeed podocytes in *Foxc2* knockout mice retain a columnar cell shape and do not elaborate foot processes and slit diaphragms. Attesting to the cross-talk between podocytes and glomerular endothelial cells, the latter lacked fenestrations. These defects closely resemble the phenotype observed in *Lmx1b* and *Pod1* knockout mice (see below). Transcript profiling and immunohistochemistry revealed that podocin, the α3 and α4 chains of collagen IV, and MafB synthesis were downregulated in glomeruli of *Foxc2* (–/–) mice [5]. The expression of *Lmx1b* and *Pod1* was normal in the *Foxc2* knockout mice, whereas that of *Cited2*, a novel podocyte marker and transcriptional coactivator (see below), was also downregulated [5]. These results suggest that FOXC2, LMX1B and POD1 function in independent pathways to regulate expression of these putative target genes.

LMX1B

Nail-patella syndrome (synonyms: HOOD syndrome or hereditary osteo-onychodysplasia, Turner-Kieser syndrome, Fong disease, Österreicher syndrome) is an autosomal-dominant genetic disease which has been named after the characteristic malformed finger and toe nails and the hypoplastic or absent kneecaps. Renal involvement was first described in 1967 [6], and symptoms may range from light proteinuria to ultimately chronic renal failure. Altogether the kidney may be affected in up to 40% of the patients [7]. Ultrastructurally, a thickened glomerular basement membrane with both electron-lucent areas [8, 9] and fibrillar inclusions resembling collagen [9] are considered pathognomonic. In addition, foot process effacement was observed for a certain percentage of podocytes [8, 9].

Over 40 years after initial linkage of nail-patella syndrome to the AB0 blood group locus [10], mutations in the *LMX1B* gene have been found to be responsible for the disease [11–13]. *LMX1B* codes for a transcription factor with two zinc-binding LIM domains at its NH_2-terminus, a DNA-binding homeodomain in the middle and a putative activation domain at the COOH-terminus. LIM domains mediate protein-protein interactions (for review see [14, 15]). This also is the case for LMX1B, which through its LIM domains interacts with the helix-loop-helix protein E47 [16] and the transcriptional adapter protein LDB1 (synonyms: NLI, CLIM2) [17]. It is somewhat controversial whether LDB1 exerts a positive or a negative regulatory effect on LMX1B, this crucial question should be resolved with the identification of LMX1B target genes in podocytes. Our own observations indicate that in the podocyte only the interaction between LMX1B and LDB1, but not that between LMX1B and E47, is of functional significance [17a].

Due to the limited availability of human material, the *Lmx1b* knockout mice have been an extremely valuable tool to examine the role of LMX1B in podocytes more closely. In the absence of Lmx1b, podocytes elaborate only rudimentary, if any, foot processes [18, 19]. No slit diaphragms were observed between podocytes, neighboring cells are rather connected by a structure resembling an adherens junction [18, 19]. In addition, the glomerular basement membrane was split and endothelial fenestrations were greatly reduced [19]. These developmental defects are accompanied by characteristic molecular changes. Not only are the α3 and the α4 chains of collagen IV absent from the glomerular basement membrane ([20] and our own unpublished observations), but the *Nphs2* gene is no longer expressed either [18, 19] (fig. 1). The loss of expression of these genes could possibly be explained by the binding of LMX1B to AT-rich sequences in the first intron of the *COL4A4* gene [20] and in the promoter region of the *NPHS2* gene [18, 19]. It also has to be mentioned, however, that the *NPHS2*, *COL4A3* and *COL4A4* genes are still expressed in podocytes of patients with nail-patella syndrome [21].

MafB (Maf-1, Kreisler)

MafB belongs to the basic leucine zipper family of transcription factors which play essential functions in a number of cellular contexts (reviewed in [22]). They bind specific recognition elements (MAREs) and homo- and heterodimerize with other transcription factors to posi-

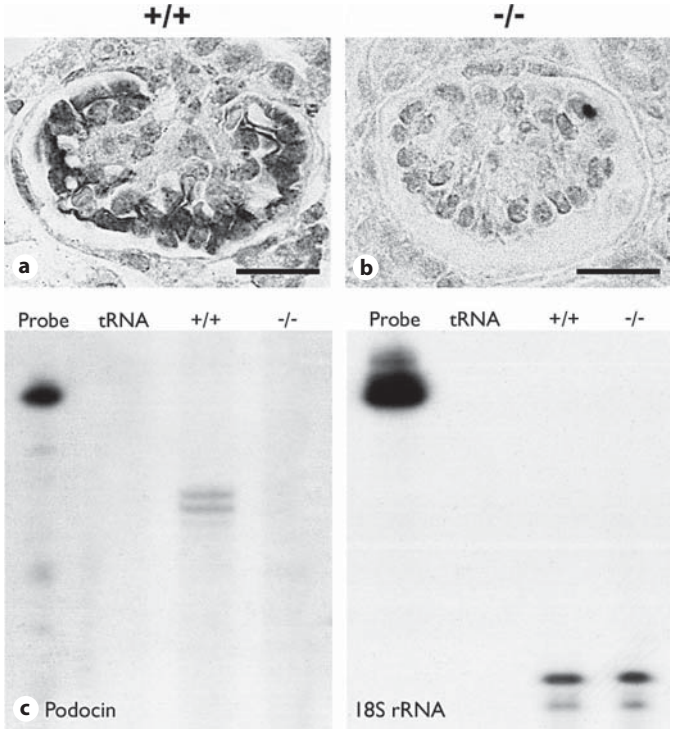

Fig. 1. Synthesis of podocin protein and mRNA in newborn *Lmx1b* (–/–) mice. Podocin protein (**a**) and mRNA (**c**) were present only in podocytes of wild-type mice, but not in those of homozygous knockout mice (**b**, **c**). **a**, **b** Immunohistochemical staining of newborn mouse kidneys with an anti-podocin antiserum. **c** RNAse protection assay with podocin antisense RNA (tRNA served as a negative control). A protection assay with a probe directed against 18S rRNA shows that the RNA concentration was determined correctly. Taken with permission from Rohr et al. [19]. Bar = 20 μm (**a**, **b**).

tively or negatively regulate transcription. In the kidney, *MafB* is specifically expressed in developing podocytes, apparently dependent on the presence of another transcription factor, Pod1 [23]. The inactivation of *MafB* results in the loss of podocyte foot processes and is associated with the downregulation of the *Nphs1*, *Nphs2* and *Cd2ap* genes [23, 24], which encode essential components of the podocyte slit diaphragm. It remains to be demonstrated, however, whether these genes represent direct or indirect targets of MafB.

WT1

Wilms' tumor or nephroblastoma is the most common solid childhood tumor. It arises by mutations in several different genes, only one of which, *WT1*, has been cloned so far [25–28]. *WT1* (Wilms' tumor gene 1) belongs to the tumor suppressor gene family and codes for a protein with 4 typical zinc fingers of the C_2H_2 class. Several alternative splice variants of WT1 have been described which for example differ by the presence or absence of the 3-amino acid peptide 'NH_2-LysThrSer-COOH' (KTS in the single letter code) between the third and fourth zinc finger [29]. The DNA-binding characteristics of WT1 depend on the presence of this peptide [30] (for a more extensive discussion of the DNA-binding properties of WT1 also see [31]). By in situ hybridization and immunohistochemistry the WT1 mRNA and protein could be detected from the earliest stages of nephron development (in the condensed metanephrogenic mesenchyme), later on its expression is restricted to the podocytes [32–37].

In addition to Wilms' tumors at least three other human renal diseases are caused by mutations in *WT1*. Denys-Drash syndrome, which is characterized by pseudohermaphroditism, nephropathy and a predisposition to Wilms' tumor, is believed to be caused by a dominant-negative mechanism. The mutations in patients with Denys-Drash syndrome affect the zinc finger domain of WT1 and lead to the inactivation or loss of the zinc fingers [38–40], and heterozygous knock-in mice mimicking a mutation found in Denys-Drash patients also develop sclerotic glomeruli [41]. The cause of WAGR syndrome (Wilms' tumor, aniridia, genitourinary malformations, mental retardation) is a large deletion in the chromosomal location 11p13 and involves the *PAX6* gene as well [42]. Finally, in patients with Frasier syndrome, a very rare disease with pseudohermaphroditism and progressive glomerulopathy, the splice donor site in intron 9 is mutated. This mutation results in a higher ratio of the (–KTS) over the (+KTS) splice form and emphasizes the importance of a balance between the two splice forms [43]. Such an interpretation is corroborated by findings in mice in which the ratio of the +KTS and –KTS isoforms was altered. Approximately two thirds of heterozygous $Wt1^{wt/-KTS}$ mice [mice with one wild-type allele and one allele which only encoded the (–KTS) isoform] developed focal-segmental glomerular sclerosis after 2–3 months, and homozygous $Wt1^{-KTS/-KTS}$ and $Wt1^{+KTS/+KTS}$ even died within 24 h after birth, in either case showing pronounced podocyte defects [44].

The importance of the *WT1* gene for renal development is emphasized by the phenotype of the *Wt1* knockout mice. *Wt1* (–/–) embryos die in utero with renal agenesis due to a defect in the metanephrogenic mesenchyme, consistent with the expression pattern described above [45]. Several pieces of data support the assumption that WT1 also exerts a role in the fully differentiated podocyte, al-

though ultimate evidence is still lacking. (1) Patients with Denys-Drash syndrome and Frasier syndrome develop sclerotic glomeruli. (2) *Wt1* knockout mice can be rescued by a YAC transgene containing the human *WT1* locus and survive until birth. Although kidneys are present in these mice, fully differentiated podocytes are not observed [46]. (3) Genetically engineered mice with reduced levels of WT1 develop glomerulosclerosis, and podocytes synthesize reduced amounts of nephrin and podocalyxin [47]. (4) WT1 binds to sequences in the promoter regions of the *Podxl* gene (encoding podocalyxin) [48] and of the *NPHS1*/*Nphs1* gene (encoding nephrin) [49, 50], furthermore it activates the respective reporter constructs. Induction of the endogenous *NPHS1*/*Nphs1* gene, however, was only described in one report [50] but not in another [48], which may be due to the use of different cell lines.

Transcription Factors with a Likely Role in Podocytes

Hypoxia-Inducible Factors

Hypoxia-inducible factors (HIFs) are heterodimeric proteins of the basic helix-loop-helix/PAS family of transcription factors. While the β subunit is not regulated by the oxygen tension, the α subunit is sensitive to oxygen levels (reviewed in [51]). Under normoxia, the oxygen-dependent prolyl hydroxylation of HIF-α subunits promotes an interaction with the von Hippel-Lindau protein, which subsequently leads to HIF-α ubiquitination and its degradation by the 26S proteasome. In addition, the oxygen-dependent hydroxylation of a conserved asparagine residue within the COOH-terminal transactivation domain of HIF-α prevents its interaction with the p300/CBP transcriptional co-activator. Under hypoxic conditions, however, HIF-α is stabilized and interacts with p300/CBP, thus leading to the transcriptional activation of its target genes [51]. HIFs were detected in podocytes of newborn mouse kidneys both on the mRNA and protein level [52]. Potential HIF target genes in podocytes are the ones encoding vascular endothelial growth factor (VEGF-A) [52, 53] and the G-protein-coupled chemokine receptor Cxcr4 [53]. Podocyte-specific ablation of the von Hippel-Lindau gene and the concurrent upregulation of HIFs is sufficient to induce rapidly progressive glomerulonephritis, for which the upregulation of Cxcr4 is at least partly responsible [53].

LIM1

LIM1 (synonym LHX1) and LMX1B share several features. Both proteins belong to the LIM-homeodomain family of transcription factors, and as LMX1B, LIM1 is involved in renal development [54] and has been shown to interact with the cofactor LDB1 [55]. *Lim1* is expressed in comma- and S-shaped bodies, but then its expression decreases in the mature glomerulus [54, 56], which is in contrast to *Lmx1b* whose expression is maintained in the mature glomerulus. *Lim1* knockout mice suffer from renal agenesis [57], and subsequent analysis has demonstrated that LIM1 is required at multiple steps of kidney development; by inference from chimera experiments LIM1 may also be involved in podocyte development [54].

PAX2

PAX2 belongs to the paired box-family of proteins that contain only a truncated homeodomain. It can act both as a transcriptional activator [58] and repressor [59] of the *WT1* gene, the latter activity probably depends on its interaction with proteins of the Groucho/TLE family [60, 61]. During renal development PAX2 is expressed both in the derivatives of the metanephrogenic mesenchyme and of the ureteric bud [62, 63]. Its expression declines as the S-shaped body evolves and is absent in the mature podocyte, possibly due to transcriptional repression by WT-1 [63]. This downregulation may be essential for proper glomerular development because the synthesis of PAX2 in transgenic mice leads to podocyte damage [61, 64]. Definitive evidence for the importance of PAX2 for podocyte development is lacking because *Pax2* (–/–) mice altogether lack kidneys [65]. Interestingly, a physical interaction has been demonstrated between PAX2 and LMX1B [66], suggesting that PAX2 and LMX1B act together or modulate their mutual activities. Although the functional significance of the LMX1B/PAX2 interaction remains to be demonstrated, it would be interesting to investigate whether PAX2 is aberrantly expressed in podocytes of patients suffering from nail-patella syndrome.

Transcription Factors with a Possible Role in Podocytes

CITED2 (Mrg1)

CITED2 (CBP/p300-interacting transactivator with glutamic acid- and aspartic acid-rich tail) belongs to a family of transcriptional cofactors (CITED1 to CITED4) lacking a DNA binding domain, and which are characterized by their ability to interact with the coactivator CBP/p300. In doing so, they can compete with many transcription factors for their interaction with CBP/p300. For instance,

CITED2 acts as a negative regulator of hypoxia-driven transcription through competition with HIF-1α for CBP/p300 interaction (see HIFs above). Interestingly, CITED2 expression itself is positively regulated by HIF-1α during hypoxia, and CITED2 is thus part of a negative feedback loop in the response to hypoxia [67]. In the kidney, CITED2 is expressed in differentiating podocytes from the S-shaped stage onwards, and its expression is downregulated in *Foxc2* (–/–) mice [5]. Although *Cited2* knockout mice have been generated, no kidney phenotype has been described so far and the function of CITED2 in podocytes remains to be examined. Since *Cited2* (–/–) mice display a clear increase in VEGF synthesis in response to hypoxia [68], it would be of interest to investigate the role of CITED2 in the regulation of HIFs in podocytes.

Lrrfip1 (GCF2/Trip)

Lrrfip1 is a poorly characterized RING finger protein that acts as a transcriptional repressor for a variety of genes. In the kidney Lrrfip1 has been detected in glomeruli and was proposed as a candidate podocyte marker [5]. Its expression is downregulated in *Pod1* (–/–) mice [69], and *Lrrfip1* as such represents a potential POD1 target gene.

MATH6

MATH6 belongs to the family of basic helix-loop-helix transcription factors and is produced in multiple tissues. In the developing kidney it was found in the metanephric mesenchyme-derived cell populations, and it became restricted to podocytes in the adult kidney [70]. The synthesis of Math6 is downregulated in HIV-associated nephropathy (HIVAN), a collapsing glomerulopathy characterized by podocyte dedifferentiation, together with that of nephrin and synaptopodin, suggesting a potential role of MATH6 in the differentiation and/or maintenance of podocytes [70].

POD1

Like MATH6, POD1 is a transcription factor of the basic helix-loop-helix family, and just as with other family members it can activate or repress transcription through heterologous interactions [71]. In the kidney, *Pod1* is expressed in developing and mature podocytes [72, 73]. Knockout experiments have demonstrated that Pod1 is required for tubular and glomerular differentiation [73], so that podocytes in *Pod1* (–/–) mice retain a columnar shape and form only few foot processes [73]. Somewhat surprisingly, however, chimera experiments showed the presence of fully differentiated *Pod1* (–/–) podocytes, thus indicating that POD1 also acts in a non-cell autonomous manner. Definitive evidence on the importance of POD1 in podocytes will require its podocyte-specific inactivation. POD1 target genes involved in podocyte differentiation remain unknown. Several genes differentially regulated in isolated glomeruli from *Pod1* knockout mice were recently identified using Affymetrix arrays [69]. Among the putative genes positively regulated by POD1 were *NPHS2*, *COL4A3* and *Lrrfip1* (see above). Furthermore, MafB is undetectable in podocytes from *Pod1* knockout mice [23]. It remains to be shown whether these genes represent direct or indirect targets.

Retinoic Acid Receptors

All-trans retinoic acid, a vitamin A derivative, regulates transcription through binding to retinoic acid and retinoid X receptors, and subsequent recognition of retinoic acid response elements (RAREs) within the promoter region of target genes (reviewed in [74]). All-trans retinoic acid has been shown to induce podocyte differentiation in vitro and in vivo, as monitored by foot process formation, decreased cell proliferation and upregulation of nephrin and podocin [75]. The *NPHS1* promoter contains three RAREs and is responsive to all-trans retinoic acid in a reporter assay [76], thus supporting direct regulation by all-trans retinoic acid. Furthermore, all-trans retinoic acid was effective in the treatment of the puromycin aminonucleoside model of podocyte injury [76, 77].

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