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Transcriptional regulation of podocyte disease

Sumant S. Chugh, MD

Division of Nephrology, Department of Medicine, Northwestern University, Feinberg School of Medicine, Chicago, IL, 60611 USA

Abstract

The podocyte is a highly specialized visceral epithelial cell that forms the outermost layer of the glomerular capillary loop, and plays a critical role in the maintenance of the glomerular filtration barrier. Several transcriptional factors regulate podocyte function under normal and disease conditions. In this review, the role of WT1, Lmx1b, pod1, pax-2, kreisler, NF- κ B, smad7 and ZHX proteins in the development of podocyte disease is outlined. The regulation of several important podocyte genes, including transcriptional factors, by ZHX proteins, their predominant non-nuclear localization in the normal in vivo podocyte, and changes in ZHX expression related to the development of minimal change disease and focal and segmental glomerulosclerosis are discussed. Finally, some future therapeutic strategies for glomerular disease are proposed.

Introduction

With an ever increasing number of podocyte expressed genes being identified, there is growing interest in their role in the development of disease, and in their transcriptional regulation. Previous reviews on podocyte transcriptional factors have largely focused on kidney development.^{1,2} Over the past few years, several new members have joined the ranks, and substantial progress is being made in clarifying mechanisms of common glomerular disorders. This review focuses on transcriptional factors that have shown a promising role in the development of podocyte or glomerular disease.

WT1

Wilms Tumor 1 (WT1), a zinc finger protein, is the most complex of all podocyte expressed transcriptional factors. As a result of alternative splicing, alternative translational start sites and RNA editing, there are at least 24 different WT1 isoforms, though very few of these isoforms are likely to be expressed in the podocyte.³ Isoforms that lack the KTS sequence (–KTS isoform) are potent transcriptional activators and bind preferentially to DNA, whereas the +KTS isoform proteins may also have a role in RNA binding.^{4,5} Various degrees of loss of podocyte WT1 content⁶ or gene mutations⁷ are noted in specific forms of focal glomerulosclerosis (FGS). The most dramatic reduction in WT1 expression in human disease is seen in the collapsing variant of FGS. By contrast, WT1 levels are unchanged in minimal change disease and membranous nephropathy, though borderline reduction in WT1 mRNA expression may be noted in the proteinuric phase of passive Heymann nephritis.⁸ Three genes known to be directly regulated by WT1 in the podocyte include podocalyxin,⁹ nephrin⁵ and pax-2.¹⁰ In addition, WT1 mutations have been associated with the development of Denys

Address for correspondence: Sumant S. Chugh, MD, Division of Nephrology, Tarry 4-753 Northwestern University, Feinberg School of Medicine, 320 East Superior, Chicago IL 60611. s-chugh@northwestern.edu, Phone: (312)503-3072, Fax: (312)503-0622

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Drash syndrome (diffuse mesangial sclerosis)¹¹ and Frasier syndrome (steroid resistant FGS)¹². A large number of different type of WT1 mutant or deficient mice are available in literature¹³. WT1 knockout mice die at midgestation because of cardiac abnormality before the formation of kidneys. WT1 heterozygous mice appeared to be healthy on initial assessment and have up to 95% of normal WT1 mRNA levels,¹⁴ but about 40% of the same mice bred on a mixed genetic background appear to die from severe diffuse glomerulosclerosis within 13 months.¹⁵ WT1 null mice with a 470 Kb human YAC clone containing the full length WT1 gene survive beyond birth, and develop dosedependent renal disease.¹⁴ With one copy of the YAC clone (62% of normal WT1 mRNA levels), mice develop albuminuria at birth and diffuse mesangial sclerosis by day 10, that evolves into a crescentic glomerulonephritis and die by age 3 weeks. WT1 null mice transgenic with two copies of the YAC clone (70% normal wild type WT1 mRNA levels) develop albuminuria at 3 weeks, followed by diffuse mesangial sclerosis and 26% die of end stage renal disease by 5 months of age. In a Denys Drash syndrome mutant mouse (WT1^{tmT396}), the mutant protein (5% of total WT1) couples efficiently with the normal WT1 protein, and is sufficient to cause development of sclerotic glomeruli. Two additional transgenic mice were developed to express only the KTS+ WT1 isoform (the KTS mouse) or the KTS- isoform (Frasier mouse) are available. Data from these mice suggests that WT1 (+ KTS) isoform is important for the development of podocyte architecture and the integrity of the glomerular tuft.¹³ Two WT1 associated proteins, WT1-interacting protein (WTIP) and brain acid soluble protein 1 (BASP1), are also expressed in the podocyte and function as corepressors of WT1 transcriptional factor activity. Whereas BASP1 normally associates with WT1 in the nucleus,¹⁶ WTIP translocates from its normal site of expression in the slit diaphragm complex into the nucleus during development of disease.¹⁷

Lmx1b

LIM homeobox transcription factor 1, beta (Lmx1b) is mutated in patients with Nail-Patella syndrome.¹⁸⁻²⁰ In the kidney, Lmx1b is expressed in podocytes. Lmx1b protein has two zinc-binding LIM domains at the amino terminus and a homeodomain in the middle. The LIM domains interact with other proteins, while the homeodomain binds to DNA. Mutations in the *LMX1B* gene mostly lead to the absence or inactivation of the homeodomain, so that the mutated protein is unable to recognize its target genes. Lmx1b in podocytes regulates the function of *COL4A3* and *COL4A4* genes by binding to their common regulatory site. Consequently, the level of expression of these two genes is significantly reduced in Lmx1b ^{-/-} glomeruli.^{21,22} Lmx1b also binds to the *NPHS2* and *CD2AP* promoters, and significantly reduced expression of these genes is noted in Lmx1b ^{-/-} glomeruli. The role of Lmx1b in other forms of human glomerular disease has yet to be clarified.

Pod1

Pod1 (capsulin, epicardin) is a basic-helix-loop-helix protein expressed in developing, and adult, podocytes and in several other organs.²³ Pod1 null mice die at birth as a result of heart and lung defects, and have a complex kidney phenotype.²⁴ The number of glomeruli is reduced due to reduced ureteric bud branches, and glomerular differentiation is arrested at the capillary loop stage, with a single capillary loop in many glomeruli. Columnar podocytes with rudimentary foot processes are noted. The precise role of Pod1 in podocyte development, and any potential role in adult podocyte disease has yet to be clarified. The expression of Pod1 may play an important role in vascular remodeling during glomerular development at a stage when endothelial cells are undergoing differentiation and branching. Interestingly, ZHX1 is marginally downregulated in Pod1 ^{-/-} mice.^{24(supplemental data)} A single study shows downregulation of Pod1 mRNA expression in a new model of rapidly developing non-HIV collapsing glomerulopathy.⁸

Pax-2

Pax-2 is an early regulator of kidney development that is first expressed in the developing kidney in the ureteric bud and the metanephric mesenchyme, that eventually differentiates into tubular epithelium and the podocytes.²⁵ Following the expression of WT1 in the s-shaped body, podocyte pax-2 is repressed and in the fully developed normal glomerulus, pax-2 expression is noted in parietal epithelium only. WT1 appears to downregulate pax-2 expression in the podocyte, whereas in mesenchymal cells, modulation of WT1 expression by pax-2 has also been noted.²⁶ The regulation of WT1 by pax-2 is however more complex, since pax-2 represses WT1 expression in the presence of groucho / transducin-like enhancer (TLE4) proteins, and activates it in the absence of these proteins.²⁷ Transgenic expression of pax-2 results in the development of nephrotic syndrome at birth and death within 1 - 3 days of life.²⁸ Podocyte specific transgenic expression results in glomerular collapse soon after birth and death within a few days.²⁷ By contrast, controlled transgenic podocyte – specific expression of Pax 2 in adult mice results in the development of FGS 3 months after the induction of Pax 2 expression. In patients with collapsing glomerulopathy, increased expression of Pax 2 is noted in the cells that tend to crowd the urinary space around the glomerular tuft.^{29,30} It is equally possible that these cells are parietal cells, that normally express pax-2, or that there is re-expression of pax-2 in de-differentated and proliferating podocytes due to loss of WT1 expression, since these cells tend to be WT1 negative. An interesting middle ground for this contentious issue may actually be a class of cells that express some podocyte proteins, but are normally interspersed with the parietal epithelium.³¹

Kreisler

Kreisler (maf-1 or mafb) is expressed in podocytes in developing and newborn mice.³² Homozygous mutant mice die within 24 hours of birth. The mice appear to be proteinuric at birth, and the podocyte foot processes are effaced in the capillary loop stage of glomerular development.³³ There is a paucity of data investigating the role of kreisler in the fully developed kidney. Gene expression profiling in the diabetic KK/Ta mouse reveals kreisler / maf-1 to be one of the genes present in the vicinity of a quantitative trait locus for the development of albuminuria.³⁴

NF-κB and Smad7

Nuclear Factor - kappa B (NF-κB) and Smad7 are two transcriptional factors that are widely expressed, and appear to play a significant role in selected animal models of glomerular disease. TGFβ transgenic mice develop progressive glomerulosclerosis preceded by podocyte apoptosis. Increased expression of Smad7 in damaged podocytes in these mice is felt to inhibit the nuclear translocation and consequently the anti-apoptotic properties of NF-κB.³⁵ Recent evidence from HIV-transgenic mice, that develop severe focal sclerosis, suggests that NF-κB may also have pro-apoptotic properties, since a persistent state of NF-κB activation in the podocytes and other renal epithelia induces apoptosis by increasing the expression of both Fas and Fas – ligand.³⁶

ZHX family

Only a limited number of target genes have been identified for the transcriptional factors discussed above. By contrast, the zinc fingers and homeoboxes (ZHX) family of transcriptional factors regulate a large number of structurally and functionally important podocyte-expressed genes.³⁷ ZHX proteins contain two C2H2-type zinc finger domains and five Hox-like homeobox domains. All three known ZHX family members (ZHX1, ZHX2 and ZHX3) are expressed in the in vivo podocyte. Of the ZHX target genes identified to date (Table 1), 70% are regulated by only one of three family members. About 30% of the genes are regulated by two members of the family (sometimes in the opposite direction), and only one published gene

(*ENPEP* or aminopeptidase A) is known to be regulated by all three ZHX proteins. Full length rat ZHX3 was first cloned from a downregulated gene fragment noted in proteinuric glomeruli from rats injected with γ 2-nephrotoxic serum.^{37,38} ZHX proteins have some unique properties that give them a key role in the development of glomerular disease. Only a small percentage of ZHX proteins (5-10% for ZHX2, ZHX3; 20% for ZHX1) are located in normal podocyte nuclei. This predominant non-nuclear localization suggests that the majority of the protein is transcriptionally inactive at baseline. All ZHX proteins have two nuclear localization signals, but remain sequestered in the non-nuclear compartment predominantly because of heterodimer formation. Therefore, loss of heterodimerization, as would be seen during an increase or decrease in the expression of a single ZHX family member, or from altered protein : protein interactions, results in the nuclear migration of the upregulated ZHX protein or the binding partner of the downregulated ZHX protein (Figure 1). The effect of ZHX protein on the promoters of their target genes is likely to be influenced by the DNA binding motif (e.g. CdxA for ZHX3), the sequence around this motif, and by the concentration of the ZHX protein in the nucleus. In vitro overexpression of a ZHX protein generates a higher nuclear concentration of that protein than knockdown of its binding partner, and this may result in a qualitative and / or quantitative difference in the expression of target genes. Also, ZHX proteins have a negative feedback effect on their own promoters, but not that of other family members, and this absence of cross-regulation permits free ZHX proteins to regulate target genes without altering the expression of their binding partners. Finally, it is possible that the zinc finger region and the homeobox region of these proteins may regulate different genes, since posttranslational cleavage of ZHX proteins into two fragments has been documented at the very least for ZHX3.

The expression of ZHX proteins have been studied longitudinally in animal models of human glomerular disease,^{8,37} and in human kidney biopsies.³⁷ ZHX3 is transiently downregulated prior to the development of overt proteinuria in young rats with puromycin aminonucleoside nephrosis (PAN, single intravenous dose of 15 mg/ 100 grams puromycin aminonucleoside). Due on loss of heterodimerization, small amounts of ZHX1 or ZHX2 (most likely, ZHX1 > ZHX2, in view of lack of WT1 involvement) enter the nucleus at this stage. This is followed by a gradual recovery of ZHX3 expression that coincides with the development of proteinuria. The mRNA expression of ZHX1 and ZHX2 remains unchanged. Increased podocyte nuclear ZHX3 staining is noted in the early stages of proteinuria, suggesting that ZHX3 protein synthesized during recovery of ZHX3 expression enters the nucleus. Increased podocyte nuclear expression of ZHX3 is also noted in biopsies from patients with human minimal change disease. In a study of about 28 podocyte expressed genes using quantitative PCR,³⁷ transient knockdown of ZHX3 in cultured glomerular epithelial cells (GECs) produced a gene expression profile in the recovery phase that was qualitatively identical to the gene expression profile in proteinuric rat glomeruli in early PAN. By contrast, sustained severe knockdown of ZHX3 in these cells mostly alters a different set of genes, largely as a result of entry of ZHX1 and ZHX2 into the nucleus following loss of heterodimerization. Of interest, three other transcriptional factors, WT1, Lmx1b (both ZHX2 target genes) and Pax2 (regulated by ZHX1), are downregulated following severe ZHX3 knockdown. The recognition of *WT1* regulation by ZHX2, pending confirmation using promoter-reporter constructs, is a key advance in WT1 biology, since very little is known about the regulation of WT1 expression in podocytes.

There is enormous potential in studying ZHX proteins in the near future. The precise subcellular localization in the non-nuclear compartment is currently under investigation. Additional studies are required to identify additional changes in gene expression induced by ZHX proteins during the development of minimal change disease (MCD) and FGS. In vitro changes in gene expression during recovery from transient ZHX3 knockdown mimic the early proteinuric phase of experimental MCD, whereas sustained knockdown of ZHX3 results in downregulation of WT1, which is consistently observed in severe forms of FGS, including collapsing glomerulopathy. This would suggest that transient downregulation of ZHX3 may be a critical

component of MCD, whereas sustained downregulation of ZHX3 may contribute to the development of FGS. Additional support for these observations comes from previous studies in rats injected with puromycin aminonucleoside. A single injection of puromycin aminonucleoside results in the development of MCD, whereas repeated injections result in FGS.³⁹ It is possible that repeated injection of puromycin aminonucleoside may prevent the recovery of ZHX3 expression that is normally noted following a single intravenous injection, and may effectively convert the recovery phase into a sustained downregulation, and hence the FGS gene expression profile. Therefore, whereas all ZHX proteins are likely to contribute to the MCD - FGS spectrum, the gatekeeper role appears to have been assigned to ZHX3.

The importance of studying ZHX2 in greater detail comes from its ability to influence the expression of WT1 and Lmx1b. Whereas the established diseases related to these two transcriptional factors have been discussed, further studies on the effect of ZHX2 on WT1 and Lmx1b expression in other forms of human glomerular disease are required.

The importance of studying ZHX1 comes from the multitude of protein-protein interactions that have been documented (mostly by yeast two-hybrid studies) for this protein.^{40(supplemental tables)} Whereas most of these proteins may not be expressed in the podocyte, there is potential for some of these proteins to be actively involved in the pathogenesis of podocyte disease. BHLHB2 (Stra13) is known to alter the cellular redox state in cultured GECs.⁴¹ The interaction of ZHX1 with vimentin, a critical component of intermediate filaments and microtubules present in the podocyte cell body and major foot processes, suggests that ZHX proteins may be displaced towards the nucleus during structural changes in the diseased podocyte. Interaction with P53 could also influence a variety of cellular functions. Since podocytes share a large number of specialized proteins with neurons, it is likely that some of these other proteins may eventually also be described in the podocyte. Downregulation of pax-2 following overexpression of ZHX1 in cultured GECs (Table 1) has the potential of explaining some of the changes in pax-2 expression noted in human glomerular disease.

Conclusion

With continuing advancement in microarray array – based technology, large scale identification of additional target genes of podocyte expressed transcriptional factors is on the horizon. Finally, blockage of the nuclear migration of ZHX proteins is an attractive future therapeutic target for human glomerular disease, especially if subsequent studies show a significant contribution to the development of other forms of glomerular disease, including diabetic nephropathy and lupus nephritis.

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Abbreviations

WT1	Wilms Tumor 1
FGS	focal glomerulosclerosis
WTIP	WT1-interacting protein

BASP1	brain acid soluble protein 1
Lmx1b	LIM homeobox transcription factor 1, beta
NPHS2 or podocin	nephrosis 2, idiopathic, steroid-resistant
CD2AP	CD2-associated protein
TLE4	transducin-like enhancer
NF-κB	Nuclear Factor - kappa B
ZHX	zinc fingers and homeoboxes
PAN	puromycin aminonucleoside nephrosis
MCD	minimal change disease
BHLHB2	basic helix-loop-helix domain containing, class B, 2
GECs	glomerular epithelial cells

Figure 1. Migration of ZHX proteins into the podocyte nucleus during development of disease
 All ZHX proteins have two nuclear localization signals. ZHX proteins exist mostly as heterodimers in the non-nuclear compartment (90-95% for ZHX2, ZHX3; 80% for ZHX1) in the normal in vivo podocyte. During the development of disease, loss of heterodimerization resulting from an increase or decrease in the cellular content of a single family member due to changes in gene expression, or as a result of cleavage of ZHX heterodimers due to altered protein : protein interaction, leads to the migration of individual ZHX proteins into the podocyte nucleus.

Table 1

List of changes in podocyte gene expression in cultured GECs following overexpression of individual ZHX proteins for 72 hours (derived from reference 37)

ZHX1	ZHX2	ZHX3
↓ZO-1 ↓FAT1 ↓COL4A3 ↓aminopeptidase A ↓dystroglycan ↓PAX2	↑nephrin ↓nephl ↓CD2AP ↓COL4A4 ↓entactin ↓aminopeptidase A ↓podocalyxin ↓beta 1 integrin ↑angiopoietin 2 ↓WT1 ↓Lmx1b	↓nephrin ↓ZO-1 ↓CD2AP ↓P Cadherin ↓cathepsin L ↓alpha actinin 4 ↓COL4A3 ↓COL4A4 ↓aminopeptidase A ↓dystroglycan ↓B7.1 ↓angiopoietin 2 ↓VEGF A