Minireview

Experimental Nephrology

Exp Nephrol 1999;7:15–19

Received: April 30, 1998
Accepted: June 11, 1998

The Proximal Tubule Phenotype and Its Disruption in Acute Renal Failure and Polycystic Kidney Disease

Ralph Witzgall

Institute of Anatomy and Cell Biology I, University of Heidelberg, Germany

Key Words
Proximal tubule · Renal development · Polycystic kidney disease · Ischemia

Abstract
In light of recent developments in the fields of genetics, molecular, cell and developmental biology, the kidney is receiving increasing attention as a model system for organ development and human diseases. Gene disruption experiments have provided evidence for the essential role of a number of proteins in the earliest phase of nephron development, but very little is known about the identity of such proteins in more advanced stages. This minireview will focus on the proximal tubule and its role in the pathology of ischemic acute renal failure and polycystic kidney disease. Like all other nephron segments, the proximal tubule develops from the metanephrogenic mesenchyme. So far the only genetic model which affects the function of the proximal tubule is a strain of knockout mice with an inactivation of the *HNF1* gene. After ischemic renal damage the proximal tubule responds with a different genetic program than the distal tubule. Evidence from human polycystic kidney disease and several animal models of polycystic kidney disease suggests that proximal tubules are affected differently by polycystic kidney disease than distal tubules and collecting ducts.

The kidney is of the utmost importance for maintaining homeostasis. It handles a filtrate of about 200 liters per day, approximately two thirds of which is reabsorbed isoosmotically in the proximal tubule. The proximal tubule is also the major site for the reabsorption of glucose, amino acids and peptides. In order to fulfil its task, the proximal tubule is equipped with a specific set of proteins (transporters, channels, enzymes). The first section of this minireview will discuss how the proximal tubule differentiates in order to fulfil its tasks, whereas the following two sections will describe the specific response of the proximal tubule to pathogenic stimuli.

What 'Regulator' Genes Orchestrate the Differentiation of the Proximal Tubule?

The epithelial structures of the kidney originate from two distinct compartments, which develop in a mutually interactive fashion. The metanephrogenic mesenchyme gives rise to the nephrons, whereas the invading ureteric bud differentiates into the collecting ducts. Results from gene disruption experiments and the analysis of genetic diseases suggest that there are at least three stages of renal development, an 'inductive' phase, a 'morphogenetic' phase and a 'maturation' phase. It is clear that transcription factors play essential roles in each of these phases. During the inductive phase the ureteric bud invades the

KARGER

Fax + 41 61 306 12 34
E-Mail karger@karger.ch
www.karger.com

1018–7782/99/0071–0015$17.50/0

Accessible online at:
http://BioMedNet.com/karger

Ralph Witzgall
University of Heidelberg, Institute of Anatomy and Cell Biology I
Im Neuenheimer Feld 307, D–69120 Heidelberg (Germany)
Tel. +49 (0)6221 548686, Fax +49 (0)6221 544951
E-Mail ralph.witzgall@urz.uni-heidelberg.de

Table 1. Genes necessary for the proper development of the kidney

Developmental phase	Regulator genes
Inductive phase	*WT1, Pax2, Emx2*
Morphogenetic phase	?
Maturation phase	*HNF1*

Renal development proceeds in at least three phases which depend on regulator genes. In this review, genes are called regulators, when they encode transcription factors which are necessary (but maybe not sufficient) for a given phase. There may very well be more checkpoints or phases than the ones suggested above, but evidence from human genetic disorders and gene targeting studies in animals argues for at least those three phases. References are given in the text.

metanephrogenic mesenchyme and induces the metanephrogenic mesenchyme to start characteristic morphological changes. The inactivation of genes necessary for the inductive phase leads to renal agenesis; examples are the knockout mice with disruption of the genes coding for the transcription factors WT1 [1], Pax2 [2] and Emx2 [3].

Once the metanephrogenic mesenchyme is induced, it epithelializes and is structured into the glomerulus, the proximal tubule, the descending and ascending thin limb and the distal tubule. For simplistic reasons, this phase shall be called the morphogenetic phase, but it may very well be that there are additional checkpoints at which the development of the nephron can stop. We do not know what genes control this phase and whether there are 'regulator' genes for each nephron segment (in the context of this review, a gene is called a regulator when it codes for a transcription factor which is indispensable for the development of a certain structure, although it may not be sufficient – at the inductive phase, *WT1, Pax2* and *Emx2* are all regulators). There are, however, examples of rare genetic diseases which suggest that such regulators exist for specific nephron segments. The rare human inheritable disorder renal tubular dysgenesis, which is characterized by the total absence of recognizable proximal tubules [4 and references therein], may be due to a mutation in such a regulator.

The last phase of nephron development is characterized by the acquisition of sets of proteins specific for each nephron segment, the maturation phase. Whether the maturation phase can be separated timewise from the morphogenetic phase must remain a matter of debate at present (to what extent is function also mirrored by structure?). In the case of the proximal tubule, there is at least one example which supports the assumption that the proximal tubule develops as a structure but lacks distinct functional characteristics. In mice, the disruption of the *HNF1* gene leads to a renal Fanconi-like syndrome characterized by polyuria, glucosuria, aminoaciduria and phosphaturia [5]. The brush border of those mice appeared normal by electron microscopy so that the HNF1 protein, which belongs to the homeodomain protein family of transcription factors, probably only regulates target genes coding for functional and not structural proteins (table 1).

What Is So Special about the Proximal Tubule in Ischemic Acute Renal Failure?

Ischemia as a model for acute renal failure has been used for several decades [for a recent review, see 6]. Already 20 years ago it could be demonstrated that the S3 segment of the proximal tubule is particularly sensitive to the ischemic insult, but nevertheless it possesses the ability to recover completely [7, 8]. Up to now, however, no satisfying explanation can be given for the specific response of the S3 segment. Hypoxia cannot be the only culprit because the whole kidney is cut off from blood supply. Results obtained over the last decade suggest that the S3 segment of the proximal tubule and the distal tubule respond with different genetic programs to ischemic damage (table 2). After ischemia, the proximal tubule expresses increased amounts of secreted proteins such as clusterin [9] and osteopontin [10, 22], injury molecules such as the heat shock proteins Hsp25 [11] and Hsp72 [12], the pro-apoptotic protein Bax and the anti-apoptotic protein Bcl-2 [13], and structural proteins such as the intermediate filament protein vimentin [9, 14] and KIM-1, a member of the immunoglobulin superfamily [14]. An increased expression of calcyclin [15] and RACK1 [16] probably reflects the important role of intracellular calcium in the regenerative process. The high proliferation rate in the S3 segment is mirrored by the transient expression of the protooncoprotein c-Fos and by the high levels of PCNA, a DNA polymerase δ-associated protein [9]. The distal tubule (and in some cases also the collecting duct), however, expresses the JE antigen, a member of the cytokine superfamily [17], the transcription factors Egr-1 [18], ATF3 [19], c-Fos [9, 20] and c-Jun [19, 20], the cell cycle inhibitor $p21^{WAF1/CIP1/SDI1}$ [21] and also calcyclin [15] and the secreted proteins clusterin [9] and osteopontin [10, 22]. The JE antigen, Egr-1, ATF3, c-Fos and c-Jun

Table 2. Proteins and/or mRNAs with increased expression levels after acute ischemic damage to the kidney

mRNA/protein	Proximal tubule	Distal tubule
Hsp25	+	
Hsp72	+	
Bax	+	
Bcl-2	+	
Vimentin	+	
KIM-1	+	
PCNA	+	
RACK1	+	
Clusterin	+	+
Osteopontin	+	+
Calcyclin	+	+
c-Fos	+	+
JE antigen		+
Egr-1		+
ATF3		+
c-Jun		+
$p21^{WAF1/CIP1/SDI1}$		+

The different nephron segments (and the collecting duct) display distinct but also overlapping responses to an acute ischemic insult (the emphasis in this review lies on the upregulation of genes, although there are also examples where genes are shut off). Looking at the data, it is clear that a Northern or Western blot from whole kidney extracts will provide only limited information because of the complex response patterns (mRNAs and proteins, which are expressed both in the proximal and distal tubule, may also follow different time courses of expression in the respective nephron segments). The various expression patterns may depend to some extent on the experimental designs. References are given in the text.

Table 3. Differential origin of renal cysts in human and animal models of polycystic kidney disease

Model	Proximal tubule	Collecting duct
Han:SPRD (*cy*/+) rat	+	
cpk/cpk mouse	$+^1$	$+^2$
bpk/bpk mouse	$+^1$	$+^2$
orpk/orpk mouse	$+^1$	$+^2$
Pkd1 knockout mouse	$+^1$	$+^2$
Bcl-2 knockout mouse	+	+
Human ADPKD	+	+
pcy/pcy mouse	$+^2$	$+^1$
Pkd2 knockout mouse		+
chi/chi rat		+

The table lists the origin of cysts in human and various animal models of polycystic kidney disease as far as it has been determined. In some models, cysts can develop in a consecutive fashion in two different locations, so that a '$+^1$' indicates the primary location of cysts, whereas a '$+^2$' indicates the subsequent location of cysts. It should not be concluded from this table, however, that under no circumstances do cysts originate from other locations than the one(s) listed, but such alternative origins are rarer or cyst formation from those origins is less prominent. References are given in the text.

are encoded by immediate-early genes, a family of genes activated after exposure to very diverse stimuli such as mitogens and stress, suggesting that not only the proximal tubule, but also the distal tubule and the collecting duct are injured, although it is puzzling that those proteins are not strongly expressed in the proximal tubule, where the injury is even more severe than in the distal tubule. Since the injury in the distal tubule is obviously not serious enough to cause marked cell death, the cells do not have to enter the cell cycle, an effect possibly mediated by $p21^{WAF1/CIP1/SDI1}$. But why is the injury so much more serious in the proximal tubule to cause pronounced cell death? One of the challenges in the future will be to localize the plethora of molecules implicated in cell injury in the kidney (e.g. stress-activated protein kinases, NF-κB) and correlate their expression pattern with functional data. Once we are equipped with this knowledge, we can try in a more targeted fashion to develop better therapeutic strategies for a clinical syndrome whose prognosis has not changed markedly over the last 40 years.

Are There 'Proximal' and 'Distal' Pathways to Polycystic Kidney Disease?

Autosomal dominant polycystic kidney disease affects in the order of 500,000 patients in the USA; it is one of the most common genetic diseases. Our understanding of the pathogenesis of this disease has been substantially furthered by several animal models of both autosomal dominant (ADPKD) and autosomal recessive polycystic kidney disease (ARPKD). The analysis of these animal models suggests that the different nephron segments and the collecting duct display a different degree of susceptibility to cyst formation (table 3). The *cpk/cpk* mouse was one of the first mouse models of polycystic kidney disease described; it displays an autosomal recessive pattern of inheritance. In the *cpk/cpk* mouse cyst formation begins in the proximal tubule and only later does cystic development also affect the collecting duct [23, 24]. This is very similar to the situation in the *Pkd1* knockout mouse [25] and in two other mouse models of ARPKD, the *bpk/bpk*

mouse [26] and *orpk/orpk* mouse (originally called *TgN737Rpw*) [27]. The analysis of cyst formation in the Han:SPRD *cy*/+ rat model of ADPKD has also clearly shown that cysts originate predominantly in the proximal tubule [28–30]. In contrast, in the *Pkd2* knockout mouse [31], in the *pcy/pcy* mouse model [32] and in the *chi/chi* rat model [33] of ARPKD the cysts appear to start mainly in the collecting ducts and in the distal tubules. It has to be mentioned, however, that in some of those studies only light and electron microscopy was used to determine the origin of the cysts – in later stages of cyst development, the cyst wall cells tend to lose their differentiation characteristics (e.g. loss of the brush border in the proximal tubule), and therefore it may be difficult to discriminate between different nephron segments and the collecting duct. Furthermore, at later time points cysts also cease to express markers specific for those nephron segments they originate from [30], therefore the absence of a marker cannot be taken as proof against a certain origin. The situation in some of the animal models mimics human polycystic kidney disease in as far as the renal cysts from ADPKD patients probably originate mainly in the proximal tubule and in the collecting duct [34, 35].

Looking at the evidence reviewed above, one has to wonder what makes the proximal tubule and/or the collecting duct so much more susceptible to cyst formation. One clue might come again from the study of knockout mice. The inactivation of the gene coding for tensin, a protein located in focal adhesions, leads to the development of cysts primarily in the proximal tubule, which is in line with the strong expression of tensin in the proximal tubules [36]. It may therefore be that the mutations in the various animal models and in the *PKD1* and *PKD2* genes affect structures (e.g. cell-cell contacts, cell-matrix contacts) which are less dispensable for the regular function of the proximal tubule and/or the collecting duct than for the regular function of other nephron segments. Mutations in such proteins would not affect the differentiation program of the proximal tubule and the collecting duct per se but could be interpreted as failing to provide a 'stop signal' so that the prospective cyst wall cells would continue to proliferate and form 'more lumen'. It is only at later time points that the cyst-lining cells dedifferentiate. The normal appearance of differentation markers in many studies, the high proliferation rate of cyst wall cells, and transgenic animal models with an increase in cellular proliferation would also argue in favor of such a model.

Concluding Remarks

This minireview has tried to provide some new insight and provoking thoughts into our concepts of how to look at certain aspects of the kidney. Spontaneous and recombinant animal models for human diseases together with the molecular analysis of those diseases have already provided a lot of insight into pathogenic mechanisms. If the past years are a sign of what is yet to come, then the kidney will continue to develop into one of the model organs for many lines of research.

Acknowledgements

I want to apologize to all those whose articles could not be quoted due to space constraints. Projects in my laboratory are carried out with financial support from the Fritz Thyssen Foundation and the Forschungsfonds der Fakultät für Klinische Medizin Mannheim der Universität Heidelberg. I also want to emphasize my very close collaboration with Norbert Gretz and thank the members of my laboratory for their effort.

References

1 Kreidberg JA, Sariola H, Loring JM, Maeda M, Pelletier J, Housman D, Jaenisch R: WT-1 is required for early kidney development. Cell 1993;74:679–691.

2 Torres M, Gómez-Pardo E, Dressler GR, Gruss P: *Pax-2* controls multiple steps of urogenital development. Development 1995;121: 4057–4065.

3 Miyamoto N, Yoshida M, Kuratani S, Matsuo I, Aizawa S: Defects of urogenital development in mice lacking *Emx2*. Development 1997;124: 1653–1664.

4 Genest DR, Lage JM: Absence of normal-appearing proximal tubules in the fetal and neonatal kidney: Prevalence and significance. Hum Pathol 1991;22:147–153.

5 Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, Babinet C, Yaniv M: Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. Cell 1996;84:575–585.

6 Thadhani R, Pascual M, Bonventre JV: Acute renal failure. N Engl J Med 1996;334:1448–1460.

7 Donohoe JF, Venkatachalam MA, Bernard DB, Levinsky NG: Tubular leakage and obstruction after renal ischemia: Structural-functional correlations. Kidney Int 1978;13:208–222.

8 Venkatachalam MA, Bernard DB, Donohoe JF, Levinsky NG: Ischemic damage and repair in the rat proximal tubule: Differences among the S_1, S_2, and S_3 segments. Kidney Int 1978; 14:31–49.

9 Witzgall R, Brown D, Schwarz C, Bonventre JV: Localization of proliferating cell nuclear antigen, vimentin, c-Fos, and clusterin in the postischemic kidney: Evidence for a heterogeneous genetic response among nephron segments, and a large pool of mitotically active and dedifferentiated cells. J Clin Invest 1994; 93:2175–2188.
10 Padanilam BJ, Martin DR, Hammerman MR: Insulin-like growth factor I-enhanced renal expression of osteopontin after acute ischemic injury in rats. Endocrinology 1996;137:2133–2140.
11 Aufricht C, Ardito T, Thulin G, Kashgarian M, Siegel NJ, van Why SK: Heat-shock protein 25 induction and redistribution during actin reorganization after renal ischemia. Am J Physiol 1998;274:F215–F222.
12 van Why SK, Hildebrandt F, Ardito T, Mann AS, Siegel NJ, Kashgarian M: Induction and intracellular localization of HSP-72 after renal ischemia. Am J Physiol 1992;263:F769–775.
13 Basile DP, Liapis H, Hammerman MR: Expression of bcl-2 and bax in regenerating rat renal tubules following ischemic injury. Am J Physiol 1997;272:F640–F647.
14 Ichimura T, Bonventre JV, Bailly V, Wei H, Hession CA, Cate RL, Sanicola M: Kidney injury molecule-1 (KIM-1), a putative epithelial cell adhesion molecule containing a novel immunoglobulin domain, is up-regulated in renal cells after injury. J Biol Chem 1998;273: 4135–4142.
15 Lewington AJP, Padanilam BJ, Hammerman MR: Induction of calcyclin after ischemic injury to rat kidney. Am J Physiol 1997;273:F380–F385.
16 Padanilam BJ, Hammerman MR: Ischemia-induced receptor for activated C kinase (RACK1) expression in rat kidneys. Am J Physiol 1997;272:F160–F166.
17 Safirstein R, Megyesi J, Saggi SJ, Price PM, Poon M, Rollins BJ, Taubman MB: Expression of cytokine-like genes JE and KC is increased during renal ischemia. Am J Physiol 1991;261: F1095–1101.
18 Bonventre JV, Sukhatme VP, Bamberger M, Ouellette AJ, Brown D: Localization of the protein product of the immediate early growth response gene, Egr-1, in the kidney after ischemia and reperfusion. Cell Regul 1991;2:251–260.
19 Yin T, Sandhu G, Wolfgang CD, Burrier A, Webb RL, Rigel DF, Hai T, Whelan J: Tissue-specific pattern of stress kinase activation in ischemic/reperfused heart and kidney. J Biol Chem 1997;272:19943–19950.
20 Megyesi J, Di Mari J, Udvarhelyi N, Price PM, Safirstein R: DNA synthesis is dissociated form the immediate-early gene response in the post-ischemic kidney. Kidney Int 1995;48: 1451–1458.
21 Megyesi J, Udvarhelyi N, Safirstein RL, Price PM: The p53-independent activation of transcription of $p21^{WAF1/CIP1/SDI1}$ after acute renal failure. Am J Physiol 1996;271:F1211–F1216.
22 Kleinman JG, Worchester EM, Beshensky AM, Sheridan AM, Bonventre JV, Brown D: Upregulation of osteopontin expression by ischemia in rat kidney. Ann NY Acad Sci 1995;760:321–323.
23 Nidess R, Koch WE, Fried FA, McFarland E, Mandell J: Development of the embryonic murine kidney in normal and congenital polycystic kidney disease: Characterization of approximatic tubular degenerative process as the first observable light microscopic defect. J Urol 1984;131:156–162.
24 Avner ED, Studnicki FE, Young MC, Sweeney WE Jr, Piesco NP, Ellis D, Fettermann GH: Congenital murine polycystic kidney disease. I. The ontogeny of tubular cyst formation. Pediatr Nephrol 1987;1:587–596.
25 Lu W, Peissel B, Babakhanlou H, Pavlova A, Geng L, Fan X, Larson C, Brent G, Zhou J: Perinatal lethality with kidney and pancreas defects in mice with a targetted *Pkd1* mutation. Nat Genet 1997;17:179–181.
26 Nauta J, Ozawa Y, Sweeney WE Jr, Rutledge JC, Avner ED: Renal and biliary abnormalities in a new murine model of autosomal recessive polycystic kidney disease. Pediatr Nephrol 1993;7:163–172.
27 Moyer JH, Lee-Tischler MJ, Kwon H-Y, Schrick JJ, Avner ED, Sweeney WE, Godfrey VL, Cacheiro NLA, Wilkinson JE, Woychik RP: Candidate gene associated with a mutation causing recessive polycystic kidney disease in mice. Science 1994;264:1329–1333.
28 Cowley BD Jr, Gudapaty S, Kraybill AL, Barash BD, Harding MA, Calvet JP, Gattone VH II: Autosomal-dominant polycystic kidney disease in the rat. Kidney Int 1993;43:522–534.
29 Schäfer K, Gretz N, Bader M, Oberbäumer I, Eckardt K-U, Kriz W, Bachmann S: Characterization of the Han-SPRD rat model for hereditary polycystic kidney disease. Kidney Int 1994;46:134–152.
30 Obermüller N, Gretz N, Kriz W, van der Woude FJ, Reilly RF, Murer H, Biber J, Witzgall R: Differentiation and cell polarity during renal cyst formation in the Han:SPRD (*cy*/+) rat, a model for ADPKD. Am J Physiol 1997; 273:F357–F371.
31 Wu G, d'Agati V, Cai Y, Markowitz G, Park JH, Reynolds DM, Maeda Y, Le TC, Hou H Jr, Kucherlapati R, Edelmann W, Somlo S: Somatic inactivation of *Pkd2* results in polycystic kidney disease. Cell 1998;93:177–188.
32 Takahashi H, Calvet JP, Dittemore-Hoover D, Yoshida K, Grantham JJ, Gattone VH: A hereditary model of slowly progressive polycystic kidney disease in the mouse. J Am Soc Nephrol 1991;1:980–989.
33 Inage Z, Kikkawa Y, Minato M, Owada M, Kitagawa T, Ohno K, Kondo K, Ueda Y, Iidaka K: Autosomal recessive polycystic kidney in rats. Nephron 1991;59:637–640.
34 Bachinsky DR, Sabolic I, Emmanouel DS, Jefferson DM, Carone FA, Brown D, Perrone RD: Water channel expression in human ADPKD kidneys. Am J Physiol 1995;268:F398–F403.
35 Devuyst O, Burrow CR, Smith BL, Agre P, Knepper MA, Wilson PD: Expression of aquaporins-1 and -2 during nephrogenesis and in autosomal dominant polycystic kidney disease. Am J Physiol 1996;271:F169–F183.
36 Lo SH, Yu Q-C, Degenstein L, Chen LB, Fuchs E: progressive kidney degeneration in mice lacking tensin. J Cell Biol 1997;136:1349–1361.

Note Added in Proof

In contrast to the earlier report [1], a renal Fanconi-like syndrome in the *HNF1* knockout mouse was not observed in a subsequent study [2].

Polycystic kidney disease also develops in *Bcl-2* and *AP-2β* knockout mice. No experimental support was presented for the location of cysts in the kidneys of *AP-2β* knockout mice [3], whereas it could be shown that cysts in *Bcl-2* knockout mice arose in the proximal tubule, distal tubule and collecting duct [4].

1 Pontoglio M, Barra J, Hadchouel M, Doyen A, Kress C, Bach JP, Babinet C, Yaniv M: Hepatocyte nuclear factor 1 inactivation results in hepatic dysfunction, phenylketonuria, and renal Fanconi syndrome. Cell 1996;84:575–585.
2 Lee Y-C, Sauer B, Gonzalez FJ: Laron dwarfism and non-insulin-dependent diabetes mellitus in the *Hnf-1α* knockout mouse. Mol Cell Biol 1998;18:3059–3068.
3 Moser M, Pscherer A, Roth C, Becker J, Mücher G, Zerres K, Dixkens C, Weis J, Guay-Woodford L, Buettner R, Fässler R: Enhanced apoptotic cell death of renal epithelial cells in mice lacking transcription factor AP-2β. Genes Dev 1997;11:1938–1948.
4 Sorenson CM, Padanilam BJ, Hammerman MR: Abnormal postpartum renal development and cystogenesis in the bcl-2 (−/−) mouse. Am J Physiol 1996;271:F184–F193.