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Galectins in kidney development

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Galectins are a family of proteins with overlapping but distinct carbohydrate- binding specificities. They differ in cell-type and tissue distribution, and have various functions. Extracellularly several galectins can modulate cellular adhesive interactions and signalling pathways, effects that may be important in the establishment and maintenance of tissue organization during normal development. This review will summarise recent progress in defining the roles of galectins that are expressed in the kidney in normal development, and discuss the evidence linking aberrant expression of galectins with kidney disease.
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Introduction

The developing kidney has long been used to study the complex interactions that occur between diverse cell types in formation of branching epithelia, in epithelial conversion of mesodermal mesenchyme, in vasculogenesis and cell-type specific gene expression [1,2]. The development of the collecting duct system and the nephrons of the kidney proceeds in several stages, each requiring separate signalling pathways (Figure 1A). The first of these, occurring around embryonic day 11 in the mouse, involves bud formation from the Wolffian (or nephric) duct. Bud formation is induced by glial cell-derived neurotrophic factor GDNF, released from the mesenchyme and interacting with its ureteric receptors Ret and one or more of several glycosyl-phosphatidyl inositol (GPI)-linked proteins of the GFRα family. The ureteric bud invades the mesenchyme, inducing its condensation and epithelialization leading to formation of primitive nephron vesicles. Ureteric factors inducing mesenchymal conversion include FGF7, BMP7 and Wnt 11. Conversion is accelerated by Wnt4 that is produced in the induced mesenchyme. In turn, the mesenchyme induces branching and growth of the ureteric bud by expressing factors that include integrin $\alpha 8\beta 1$ and its receptor nephronectin associated with the Wolffian duct and the ureteric epithelium [3]. Additional signalling molecules required directly or indirectly for continuation of ureteric branching include Wnt 11 and BMP7, originating from the differentiating ureteric epithelium itself. Branching may also be induced by HGF or EGF receptor-ligands such as TGFα produced by stromal cells (Figure 1A). The ureteric tree is established after several rounds of branching, and each branch tip is free to fuse with a primitive nephron vesicle (Figure 1A) which then elongates and folds into the precursors of the mature proximal and distal tubules. Each proximal tip is finally invaginated by nearby capillaries to produce the primitive glomeruli. The cycle of reciprocal interactions between ureteric bud derivatives, the progenitors of the collecting ducts, and the mesenchymally-derived nephron tubules continue through gestation, leading to a stable, functioning kidney at or shortly after birth.

Cell–surface adhesive interactions are crucially important in normal kidney development [4–6]. In addition to $\alpha 8\beta 1$, integrin subunits $\alpha 2$, $\alpha 3$, $\alpha 6$, $\beta 1$ and $\beta 4$ together with their matrix receptors play vital roles in ureteric branching and tubulogenesis *in vivo*. Antibodies to laminin domains perturb renal development by blocking interactions with one or more of these integrins. Cadherins, calcium-dependent cell adhesion molecules, are crucial components in epithelialization and selective cell-cell aggregation defining boundaries in the developing kidney. Antibodies directed against cadherin sub-types disrupt formation of kidney tubules. Thus, normal kidney development requires the combined effects of many cell-cell and cell-matrix adhesion molecules, as well as numerous signalling pathways. These interacting events must be tightly controlled *in vivo* in order to co-ordinate and ultimately limit growth of the organ to its functional state.

Developmentally regulated glycosylation in the kidney

Many studies have shown that normal kidney development requires the involvement of various types of glycoconjugates. For example, it is sensitive to tunicamycin and other glycosylation

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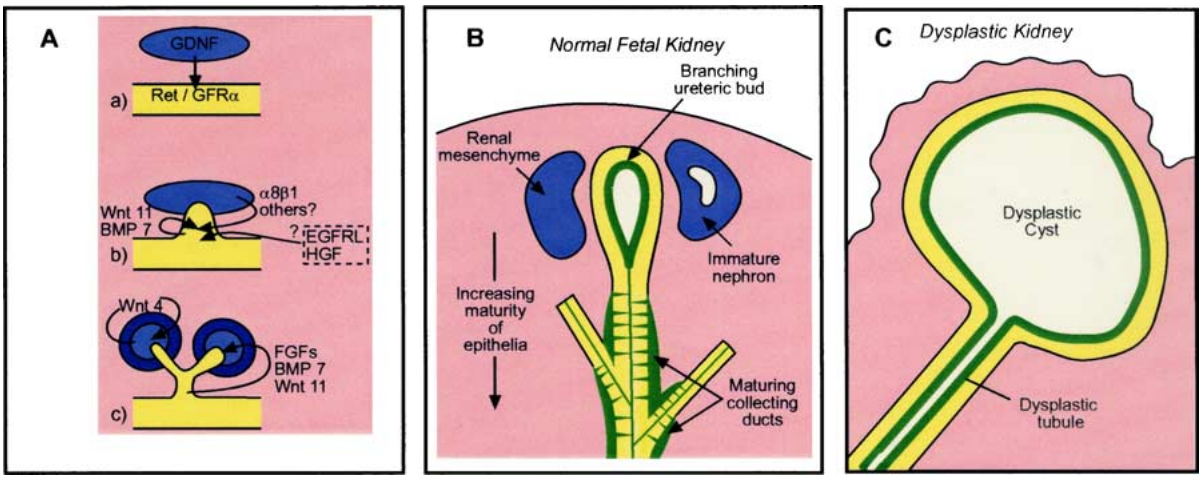

Figure 1. Summary of signalling pathways and galectin-3 expression in normal and cystic nephrogenesis. (A) The committed metanephric mesenchyme (light blue) is induced (a) by an unknown signal from the Wofffian duct epithelium (yellow) to produce GDNF that initiates formation of the ureteric bud through the Ret/GFRα complex (b). Factors such as integrin $\alpha 8\beta 1$ and others as yet undefined from the mesenchyme as well as factors such as Wnt11 and BMP7 from the ureteric bud itself, signal ureteric branching (c). Additional branching inducers may be HGF or EGF receptor ligands including TGFα. Ureteric signals including FGFs, BMP7 and Wnt11 induce production of endogenous mesenchymal Wnt4 that initiates tubulogenesis of the epithelialized component (light blue) of the mesenchyme. This selective list omits many other known signalling factors. (B) Cross section of fetal kidney showing apical galectin-3 (green) in branching tips and mainly basolateral staining in deeper maturing collecting ducts (yellow). The undifferentiated mesenchyme and maturing nephrons (light blue) are galectin-3 negative. (C) In human and mouse kidney malformations, both dysplastic tubules and cysts have a fetal, apical pattern of galectin-3 expression.

inhibitors [7,8]. Blocking antibodies directed against the Forsmann pentasacharide glycolipid [GalNAc α1,3 GalNAc β1,3 Gal α1,4 Gal β1,4 Glcβ1,1 ceramide] inhibit tubule formation by a collecting duct cell line, MDCK cells [9] and isolated chick kidney epithelial cells [10] in culture. Tubulogenesis also requires hyaluronic acid and its receptor CD44 [11]. Glycosaminoglycans are clearly implicated since inhibitors of proteoglycan sulphation block renal development [12,13]. Most strikingly, null mice lacking a heparan sulphate 2-O-transferase fail to progress with renal development beyond the first outgrowth of the Wolffian duct [14,15].

Distinct changes in cell surface glycoconjugates during kidney development have been demonstrated using plant lectins [16,17]. For example, in human or mouse kidney con A and wheat germ agglutinin (WGA) stain all tubules, whereas mature proximal tubules are characterised by reaction with Lotus tetragonolobus agglutinin (LTA). LTA positivity indicates the presence in proximal tubules of glycans with terminal α-fucosyl residues, including H—and Lewis^Y/Lewis^b blood group epitopes. Positive staining with peanut agglutinin (PNA) and soybean agglutinin (SBA) is an early marker of conversion of metanephric mesenchyme into the polarised nephron epithelium. PNA also marks a sub-set of cells in the mature collecting ducts. Ureteric bud derivatives such as collecting duct epithelia and connecting segments of distal tubules bind very specifically and uniformly to Dolichos biflorus agglutinin (DBA). DBA staining is first detectable around day 12 in the mouse embryonic kidney, at the beginning of extensive branching of the ureteric epithelium. Thus, the glycosylation pattern recognised by DBA is established in the ureteric epithelium at a very early stage in development. DBA, as well as other lectins such as Helix pomatia agglutinin (HPA) reacting rather specifically with branching ureteric epithelia, recognise glycans with terminal α-GalNAc residues. Hence, blood group A determinants are recognised by DBA [18] and antibodies against the A-blood group antigen react specifically with collecting tubules and convoluted distal tubules in kidney of A- and AB- individuals [19].

Kidney galectins

Of the fourteen or so galectins so far described in mammalian tissues, galectins-1, -3 and -9 have been positively identified in kidney cells. Galectin-1 is a prototype 14 K structure that carries a single carbohydrate-binding-domain (CRD), but exists as a non-covalent homodimer under physiological conditions. Galectin-9 is an approximately 36K protein that is constitutively bivalent, containing in tandem two non-homologous CRDs joined by a short linker sequence rich in proline and glycine. Galectin-3 is a chimaeric structure of similar size but containing a single C-terminal CRD and a unique, proline-rich N-terminal domain. Galectin-3 forms multimers at relatively high concentrations, mediated in part by N-terminal domain interactions, that can increase substantially its affinity for glycoconjugates. Another candidate kidney lectin is a 17 K protein [20] with a reported high homology to the prototypic galectin-5. This protein may indeed be galectin-5, or more likely a proteolytic C-terminal CRD fragment of galectin-9 which is homologous to galectin-5 CRD.

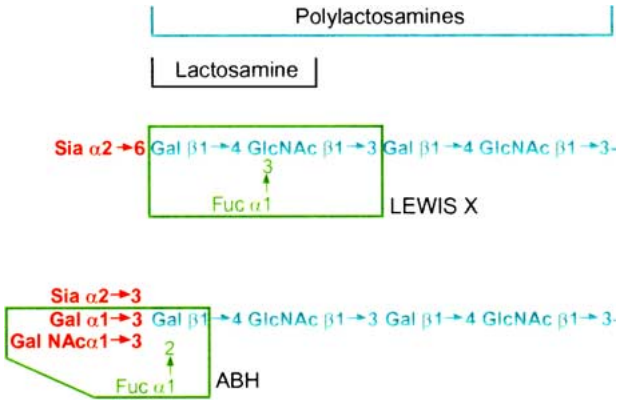

Figure 2. Carbohydrate-binding specificities of galectins. A minimum requirement for galectin carbohydrate recognition domain (CRD) binding is a terminal β-galactosyl residue, as in N-acetyllactosamine. Polylactosamine structures bind generally with higher affinity. Substitution of the terminal galactose residues by α2,6-sialic acid, or fucosylation of the penulimate N-acetylglucosamine residue as in Lewis^X-type epitopes, reduces binding. Terminal galactosides bearing 3-O-sialyl residues, or 2-O and 3-O-substituents as in the ABH-type epitopes , are recognized with high affinity by galectin-3 and galectin-9 N-terminal CRD, but not galectin-1 nor galectin-9 C-terminal CRD.

Structural and binding studies [21–24] have shown that, although the galectin CRDs have a related β-sandwich topology, their binding sites do vary substantially in agreement with known differences in sugar-binding affinities (Figure 2). In particular, although galectins-1, -3 and -9 have broadly similar affinities for simple structures such as Gal β1,4 Glc or Gal β1,4 GlcNAc, galectin-3 and galectin-9 N-terminal CRDs contain an extended binding site that can accommodate larger oligosaccharides. Extension at the non-reducing end of these disaccharides with Gal α1,3 (or GalNAc α1,3) and Fuc α1,2-units increases affinity to galectin-3 and galectin-9 N-terminal CRD but not galectin-1 nor galectin-9 C-terminal CRD. Hence, blood-group A and B epitopes are high-affinity ligands for the former CRDs, but bind relatively poorly to the latter. Similarly, α2,3-O-sialylation enhances affinity of oligosaccharides for galectin-3. Recently, we found that part of the specific interaction of galectin-3 with these extended oligosaccharides involves, in addition to the primary binding site in the CRD proper, some contribution from the unique N-terminal domain of the lectin [25]. Interestingly, phosphorylation of serine/threonine residues in the N-terminal domain greatly reduces binding of galectin-3 to oligosaccharides, suggesting one potentially important physiological mechanism for modulating binding activity [26]. By contrast, the length of the linker sequence of galectin-9 isoforms appears to have no influence on binding properties of N- or C-terminal CRDs [24].

Two galectins CL-14 and -16 are present in chicken kidney. CL-16 appears to be a chick homologue of galectin-1, since it is a homodimer under normal conditions and has a similar mode of sugar binding. CL-14 exists normally as a monomer, analogous to mammalian galectin-5, and has a rather different binding specificity. Neither chick lectin binds ABH-type structures [27].

Glycoconjugates bearing high-affinity glycans for galectins include components of the extracellular matrix, such as some isoforms of fibronectin, laminin and tenascin, as well as cell surface adhesion molecules such as integrin subunits and ancillary proteins such as CD98 antigen involved in integrin modulation [28]. Galectin-1, -3 and -8, are implicated in modulation of cell-cell and cell-matrix interactions, either negatively or in some conditions positively most likely through cross-linking of cell surface and matrix components by multivalent lectins. Antagonistic effects on adhesive events would have an influence indirectly in down-stream activities of adherent cells, including their proliferation, differentiation and metabolic activities. Synergistic effects and cross-linking of extracellular glycoconjugates are likely to be important in supporting cell structure and motility, as well as in signalling, for example in immunological activation and cytokine release [29].

Roles in normal kidney

Chicken galectins

In the chick, the expression of CL14 and CL16 increases to a maximum by embryonic day 12 (E12), the time at which the avian metanephric kidney begins to develop. The levels of both galectins is maintained throughout kidney development but is decreased rapidly after birth, especially so for CL14 [30]. Expression of both galectins is highest in the proximal tubules, with lesser expression in maturing collecting ducts. Although considerable lectin is present cytoplasmically, a significant proportion of CL16 in particular is found at the cell surface and within the basement membranes of the forming tulules [31]. Here it may play a role in attaching the cells to extracellular structures containing high-affinity ligands such as laminin. Interestingly, treatment of cultures of isolated chick kidney rudiments with sugar inhibitors of galectins, such as thiodigalactoside, induces a distorted tubular organization; the cells lose their normal cuboidal shape, the basal localization of their nuclei is destroyed and the internal lumen of the tubules is lost. Some or all of these effects may be related to a break-down in cell adhesions supporting normal tissue organization.

Galectin-1

Galectin-1, the nearest equivalent of the chick galectin CL16, is expressed widely in mesodermally–derived cells in the developing mouse embryo, including kidney [32]. In kidney, these cells are responsible for development of the mesodermal mesenchyme that in turn gives rise to the non-ureteric tubular epithelia. A human cell-line, probably originating from proximal tubules, contains galectin-1 in agreement with this finding [33]. By contrast, endodermal cell types, that are the progenitors of the ureteic epithelia including collecting ducts, are negative. These findings suggest that galectin-1 is predominantly located

in the connective tissues of organs such as the kidney, and may be important in the functioning of cells responsible for laying down extracellular matrices and tissue organization. Any function of galectin-1 appears to be transient, however, since its expression is markedly down-regulated in the adult kidney [34].

Galectin-3 in branching morphogenesis

Galectin-3 expression is rather tightly regulated in human [35] and mouse [36] kidney, as shown by RT-PCR and Western blotting. In mice the first transcripts appear around embryonic day12: protein expression is maximal around E16, is reduced sharply after birth and is barely detectable in adult kidney. In the new–born kidney galectin-3 expression is confined to derivatives of the ureteric bud *i.e*. collecting ducts and connecting segments of distal tubules. These findings suggest some predominant role for galectin-3 in growth of the ureteric tree during early development.

Some evidence supporting this conclusion comes from experiments with a collecting duct-derived cell-line MDCK cells. MDCK cells grown *in vitro* within three-dimensional gels of type 1 collagen form small aggregates that eventually sort out into polarized cysts, with a clearly defined central lumen. Polarization requires the adhesive interactions between lateral domains of contiguous cells, involving cadherins, and the tight attachment of the cells at basal surfaces to the extracellular substratum mediated by integrins (especially $\alpha 2\beta 1$) and ancillary proteins [4].The extracellular matrix in the MDCK cultures is the exogenous collagen , as well as a basement membrane containing laminin produced by the cell themselves. The cysts expand in a well-regulated manner with time in culture, events requiring the transient breaking of adhesive contacts in the dividing cells followed by reformation of these contacts once mitosis is finished. Treatment of these cyst cultures with hepatocyte growth factor (HGF)/scatter factor induces the cysts to elongate by localized cell division into tubule-like structure that eventually fuse and form a large syncytium. Immunocytochemical studies show that galectin-3 is highly expressed at sites of cell-cell and cell- matrix adhesion in the body of the cysts, but it is absent in the cells engaged in initiating and elongating the tubular structures growing from the cyst [37]. Treatment of cyst cultures with blocking antibodies directed against galectin-3 speeds up the growth of the cysts during prolonged culture, while addition of high concentrations of recombinant lectin slows down the process. Therefore, it appears that endogenous galectin-3 is a negative regulator of cyst growth and tubulogenesis in the MDCK system, most likely by strengthening cell-cell and cell-substratum adhesions at lateral and basal surfaces respectively [28,37]. Indeed, a ricin-resistant MDCK mutant cell-line grows significantly faster than the wild-type cells in the cyst culture system and form tubular outgrowths in the absence of HGF, in line with the absence in these cells of galactosylated glycoconjugate receptors for galectin-3 [38].

The underlying processes involved in growth of MDCK cysts are very analogous to the formation of epithelial sheets and tubules occurring during kidney development. Hence it is reasonable to propose some similar function for galectin-3 in normal kidney. Recent results with human and mouse kidney development are consistent with this hypothesis [35,36]. Notably, galectin-3 expression is very low at the time of initiation and the first branching of the ureteric bud around E11/12 (Figure 1A). This is a time of rapid cell proliferation and migration, events requiring epithelial plasticity and probably inconsistent with a high level of galectin-3 expression as deduced from the MDCK experiments. As the ureteric epithelium matures into medullary collecting ducts the stabilization of adhesive contacts increases, simultaneously with up-regulation of galectin-3. In agreement with these proposals, galectin-3 is found at the apical domains of ureteric branches, where presumably it is functionless, but is highly expressed baso-laterally on maturing collecting ducts (Figure 1B). Strikingly, in explant cultures of kidney rudiments taken from E11 mouse embryos, ectopic exposure to galectin-3 by addition of recombinant protein effectively blocks the normal ureteric branching observed in control cultures over several days [36]. This effect is specific, since neither galectin-1 nor mutant galectin-3 lacking the wild-type affinity for extended glycans [22] affects branching. Galectin-3 also specifically impairs cultures of E11 or 12 kidney rudiments in forming nephron vesicles as well as glomeruli, the end-stage of nephron development (Figure 1B). This is most likely an indirect effect of excess galectin-3 on the ability of the ureteric epithelium to induce epithelial conversion of the renal mesenchyme (Figure 1B). First, galectin-3 is not expressed in the mesenchyme. Secondly, the isolated mesenchyme readily forms nephron vesicles when co-cultured with a heterologous inducer, namely spinal cord, and exogenous galectin-3 has no effect on this process [36]. Possibly, galectin-3 expressed ectopically at the baso-lateral surfaces of ureteric tips acts to limit the release and activity of growth factors such as FGFs, BM7 and Wnt11 (Figure 1A).

The requirement for wild-type high-affinity binding to extended oligosaccharides is interesting. It may be related to the presence in maturing collecting ducts of DBA-binding glycoconjugates, given the overlap in binding specificity of DBA and galectin-3 for terminal α-GalNAc residues as in A-type structures. However, the nature of the ureteric cell surface receptors for galectin-3 are presently unknown. Similarly, the mechanism by which secretion and expression of galectin-3 at apical or basolateral domains of polarized epithelia is regulated remains to be determined [39].

Branching morphogenesis is a feature of several developing tissues, including pancreas and lung. The basic pattern of bud initiation, bud splitting, tubule elongation and terminal differentiation is followed in each case. Is galectin-3 involved also in these processes? Galectin-3 appears to play a role in mammary gland involution [40], a process somewhat similar to epithelial remodelling occurring during branching morphogenesis. It is also expressed early in normal human lung development (M

Kasper, personal communication). It may be significant that putative galectin-3 ligands including cell surface β-galactosides and H-type carbohydrates have been implicated in epithelial cell proliferation during mammary [41] and prostatic [42] gland morphogenesis respectively.

Galectin-3 in collecting duct differentiation

Recent evidence implicates galectin-3 in regulating the polarity of surface expression of collecting duct proteins. As mentioned before, galectin-3 expression is very low in adult kidney. It appears to be confined to the cytoplasm of a subset of collecting duct cells known as intercalated A-cells [35]. Two morphologically distinct types of intercalated cells, occurring in mature collecting ducts, are responsible for acid-base exchange. A-cells secrete hydrogen ions into the lumen and contain an apical H^+-ATPase and a basal Cl^-/HCO_3^- exchanger. The polarity of these transport proteins is reversed in B-cells that secrete bicarbonate ions. These cell-types appear to be inter-convertible *in vivo*, for example in response to an acid load.

A clonal cell line established from rabbit kidney cortex, when seeded at low cell density and grown over several days to confluency, shows characteristics of B-intercalated cells. However at high seeding densities under conditions where confluency is quickly established, an A-type monolayer results. The key event regulating these different polarities appears to be the entrapment in the basal extracellular matrix of the "A-like"cells of a 230 kDa glycoprotein called hensin [43]. Remarkably, when grown from low density to confluency on a substratum composed of the detergent-insoluble residues of an "A-like" cell monolayer , the cells adopt an "A-like"rather than "B-like" appearance. Both cell types synthesize and secrete hensin, but only "A-like" cells produce soluble dimers and trimers of the protein and incorporate it into the extracellular matrix. This latter process appears to require additional oligomerization of hensin , and the involvement of galectin-3 [44]. Galectin-3 is present in both cell types. However, it appears to be secreted only from the apical domain of "B-type"cells but from apical and basolateral domains of "A-type" cells. The similarity to the domain-specific secretion and expression patterns in the developing ureteric tree is striking. Although galectin-3 secreted from basal domains of "A-like" cells apparently does not complex with the soluble hensin fraction, it can be extracted from the isolated extracellular matrix of "A-type" monolayers in association with oligomeric hensin. Hence galectin-3 appears to fix in some manner hensin oligomers into the extracelluar matrix, perhaps forming a substratum that clusters putative hensin receptors at the basal surface of the cells and initiating some signalling cascade involved in the A- to B-interconversion.

The mechanism of this process is unknown, in particular how culture conditions appear to affect intracellular trafficking of galectin-3. It has been suggested that one function of galectin-3 may be simply to "bundle" hensin into insoluble fibres for efficient incorporation into the matrix underlying confluent A-cells [44]. However, it is also possible that these proteins form higher order complexes with additional matrix components *in situ*, for example ternary complexes with laminin. The interaction of galectin-3 with hensin is insensitive to sugar haptens [44], possibly indicating some involvement of the N-terminal domain in protein-protein interactions and allowing the simultaneous binding to laminin through the lectin CRD.

Galectin-9

The tandem-repeat galectin-9 has been cloned from mouse [45,46] and rat [47] kidney. In mice, galectin-9 expression increases steadily from low levels at E12, through late embryonic development and postnatally, and reaches high and constant levels in adult kidney. Therefore, the developmental dynamics of galectin-9 is very different to galectin-3 and suggest that any role for galectin-9 is restricted to the fully functional kidney. In adult kidney highest expression of galectin-9 occurs in tubular epithelia of the renal cortex, mainly proximal tubules. Expression in medullary collecting ducts is undetectable.

Recently, it has been proposed that galectin-9 participates in transport of uric acid across plasma membranes [47,48]. This hypothesis implies integration of galectin-9 in cell membranes, since usually channel proteins are integral multi-span membrane components. Some preliminary evidence for a membrane spanning segment in galectin-9 has been put forward [49], and the recombinant protein can apparently be incorporated into synthetic lipid bilayers which then appears to support urate transport. However, transfection of kidney cells with galectin-9 constructs fails to augment endogenous urate excretion. The lack of a recognisable transmembrane or signal sequence in galectin-9 might suggest that the lectin may function in some ancillary role in a functional channel in living cells, mediated by more conventional renal transport proteins [50]. Transport systems capable of reabsorbing uric acid are present in adult kidney proximal tubules, and the possible role of galectin-9 in this activity is an interesting new development.

Galectins in kidney disease

Cystic disease

In autosomal polycystic kidney disease (PKD), the normal renal tubular appearance is progressively replaced by multiple large cysts, surrounded by a flattened epithelium. Many abnormalities at the cellular level have been described, suggesting that the cells lining the cysts lose the characteristics of a normal mature epithelium. Often these cystic epithelia show an abnormal expression of extracellular matrix components, including heparan sulphate proteoglycans and cell surface components such as adhesion molecules and ion transport proteins. Galectin-3 also falls into this category. In human recessive PKD the cells lining the large cysts deriving from collecting ducts and associated dysplastic tubules contain high amounts of galectin-3: much of this is expressed in the cytoplasm and at the lumenal surface (Figure 1C). Baso-lateral surfaces of cyst cells are negative [35].

Similar results are found for the *cpk* mouse, an animal model of the recessive form of human PKD (PJD Winyard and AS Woolf, personal communication). Given these observations and the data collected for normal expression of galectin-3 in renal development, it seems reasonable to predict that the abnormal distribution of the lectin in cystic kidneys may be functionally linked to the cystic phenotype. This idea fits with the hypothesis that galectin-3 expressed at basolateral domains stabilizes cell-cell and cell-matrix interactions that maintain normal epithelial cell polarity. Indeed, when kidney rudiments from normal E12 mouse embryos are kept in culture with blocking antibodies to galectin-3, the organs that develop over 4–7 days appear less compact, and the tubular structures more dilated than control kidneys cultured for comparable times [36].

The possible interactions during normal development of epithelial galectin-3 with the extracellular glycoproteins polycystin-1 and -2 [51] and fibrocystin [52], mutations in which are closely linked with the dominant and recessive forms of human PKD respectively, are important questions for future research. Although the functions of the polycystins and fibrocystin are still not fully understood, the present evidence suggests roles in cell adhesion and signalling and in terminal differentiation of kidney tubules. Notably, the polycystin-1/-2 complex is found at lateral borders of polarized kidney cells and fibrocystin is prominently expressed in collecting ducts at sites available for interactions with galectin-3. It is also relevant that mutations in these genes leads to cystic disease in other ductal structures, especially pancreas and liver. Interestingly, galectin–3 is expressed in the ductal complexes formed during chronic pancreatitis, suggesting some role in pseudoductular hyperplasia and duct enlargement in this disease [53].

Inflammatory disease

The presence of galectin-3 in inflammatory cells is well-established [29]. Its expression and secretion is increased during monocyte differentiation into mature macrophages.It has also been shown to function extracellularly in activating other inflammatory cells including mast cells, neutrophils and eosinophils and in promoting their extravasation from the circulation into inflammatory sites [54–56]. Several studies suggest galectin-3 has multiple roles in cell injury and regeneration in various types of renal inflammatory disease.

Administration of a single dose of nephrotoxic anti-Thy1.1 serum to rats results in complement-mediated lysis of mesangial cells that express Thy1.1, followed by a severe proliferative glomerulonephritis. This disease is characterized by glomerular inflammation and mesangial proliferation, parietal epithelial cell metaplasia and formation of glomerular crescents due to infiltrating monocytic cells. Later stages involve up-regulation of matrix synthesis and a progressive fibrosis involving the crescents and the peritubuar interstitia. Galectin-3 expression is rapidly increased in the early stages of experimental nephritis in this model [57]. Expression is first found in the macrophages infiltrating the glomeruli. It is also found at basal surfaces of *macula densa* distal tubules from which it could be secreted into the intra-glomerular space occupied by mesangial cells. These cells do not synthesise galectin-3, but do express surface receptors that support binding and endocytosis of the lectin. Galectin-3 added exogenously *in vitro* is mitogenic for mesangial cells, and its secretion from adjacent distal tubules or macrophages may be an important factor in mesangial hyper-proliferation during the acute phase of the disease. Treatment *in vitro* of mesangial cells with galectin-3 also induces increased matrix synthesis that *in vivo* may contribute to the build-up of collagens, laminin and other matrix components in the developing crescents. Interestingly, another study shows [58] that treatment of rats with high amounts of exogenous galectin-3 or galectin-1, but not galectin-9, at an early stage of experimental nephritis significantly reduces the glomerular infiltration of ED-1 positive macrophages, and decreases crescent formation. Possibly, this effect is due to blockade of integrin-mediated interactions of the macrophages with ICAM-1 expressed on the glomerular endothelium. At later stages of anti-Thy1.1 nephritis activated macrophages expressing the ED-1 antigen infiltrate the interstitial space [57]. Galectin-3 is strongly expressed at the surface of these cells, and may assist in their migration through a collagen-rich interstitial matrix [28]. Eventually, galectin-3 expression becomes normalized in all areas, in association with the regeneration of a normal morphology.

Galectin-3 transcription and expression is also greatly up-regulated in a model of ischemia/reperfusion injury in the rat [58]. Interestingly, however, in this model the lectin is first expressed in proximal convoluted tubules that are mainly impaired by the ischemia. These results, together with the finding of galectin-3 in the crescentic parietal epithelial cells in experimental glomeruonephritis discussed above, shows that the lectin can be expressed ectopically in proximal tubules and their derivatives, although it is not normally expressed at high levels in these tubules. Since the lectin is found at the basal surface of the injured ischemic proximal tubules, it may be involved in modulating attachments of these cells to basement membranes. In the acute phase galectin-3 may down-regulate adhesive interactions, resulting in accelerated detachment of necrotic cells from basement membranes. At a later stage, when galectin-3 is maximally expressed, this anti-adhesive effect may be reversed, restoring cell-matrix interactions and regenerating a more normal tubular morphology during resolution of the acute phase of the disease. Regeneration may also be assisted by galectin-3 positive cells, identified as ED1-positive activated macrophages,that appear in the interstitial spaces as in the resolution phase of the proliferative glomerulonephritis model.

In general, these studies indicate that galectin-3 plays several roles in kidney inflammatory disease: in the influx of inflammatory cells into glomeruli in the initial development of renal dysfunction, in promoting cellular hyper-proliferation, in the fibrotic process directly by increasing the synthesis of matrix components or perhaps indirectly by inducing secretion of other

pro-fibrotic mediators, in monocyte/macrophage interstitial recruitment and function during late stages of disease progression and resolution.

Diabetes

Diabetic nephropathy is characterized by thickening of glomerular basement membranes and glomerular hyperfiltration, followed by matrix deposition in the mesangium. As mentioned above galectin-3 is normally not expressed in glomeruli, and specifically mesangial cells. However, it is strongly expressed in these sites in diabetes, and appears to be involved in the pathogenesis of diabetic glomerular disease [59,60] by virtue of its activity as a cell surface receptor for advanced glycation end-products (AGE). Formation of AGE is greatly accelerated in diabetes and these products are strongly implicated in loss of renal function in the disease. Null mice lacking galectin-3, and treated with streptozotocin, accumulate increased levels of glomerular AGEs and develop a severe diabetic glomerulopathy much more rapidly than control mice carrying a normal galectin-3 gene. This strongly suggests that the lectin functions *in vivo* to clear glomerular AGE, in conjunction with other AGE-receptors.

Conclusions

The present evidence shows that galectins are expressed widely in the kidney. Expression is regulated developmentally and in the adult kidney is highly specific to certain cell-types. In the case of galectin-3, there appear to be separate roles that come into play at discrete stages in development. Multiple roles for the galectins are expected, given their potential for interactions with various receptors bearing high-affinity glycans, that may themselves be expressed in a regulated fashion during development or in the functioning kidney. Suprisingly, gene-targetting studies so far have failed to reveal a profound kidney phenotype in galectin1- or -3 null mice [59,61]. However, it quite likely that more detailed analysis might reveal some defects in the fine-tuning of kidney development in the galectin-deficient mice. Perhaps the large number of inducers and other growth factors that have been implicated in normal kidney development (see Figure 1A for a partial list) may indicate a fail-safe redundancy necessary for a vital developmental process. This appears to be the case for several integrin subunits implicated in kidney development [5].

Studies on the association of galectins with various aspects of kidney disease are still at an early stage. However, sufficient is already available to suggest galectins, especially galectin-3, are a promising target for the alleviation of inflammatory and fibrotic kidney disease. Ectopic over- expression of galectin-3 may also be a useful approach in the regeneration of a normal architecture in cystic epithelia and in diabetic nephropathy. Undoubtedly, further studies will reveal many diverse functions for these interesting carbohydrate-binding proteins in normal kidney development and disease.

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