J Am Soc Nephrol 12: 515–523, 2001

Galectin-3 Modulates Ureteric Bud Branching in Organ Culture of the Developing Mouse Kidney

SIMON L. BULLOCK,* TANYA M. JOHNSON,‡ QI BAO,† R. COLIN HUGHES,† PAUL J. D. WINYARD,‡ and ADRIAN S. WOOLF‡

*Divisions of *Mammalian Development and †Protein Structure, National Institute for Medical Research, London, and the ‡Nephro-Urology Unit, Institute of Child Health, London, United Kingdom.*

Abstract. Galectin-3 is a mammalian β-galactoside–specific lectin with functions in cell growth, adhesion, and neoplastic transformation. On the basis of expression patterns in humans, it is proposed that galectin-3 modulates fetal collecting duct growth. This article provides evidence that galectin-3 can modulate branching morphogenesis of the mouse ureteric bud/collecting duct lineage. With the use of immunohistochemistry, galectin-3 was not detected in early metanephrogenesis but was upregulated later in fetal kidney maturation when the protein was prominent in basal domains of medullary collecting ducts. Addition of galectin-3 to embryonic days 11 and 12 whole metanephric cultures inhibited ureteric bud branching, whereas galectin-1 did not perturb morphogenesis, nor did a galectin-3 mutant lacking wild-type high-affinity binding to extended oligosaccharides. Exogenous galectin-3 retarded conversion of renal mesenchyme to nephrons in whole metanephric explants but did not affect nephron induction by spinal cord in isolated renal mesenchymes. Finally, addition of a blocking antiserum to galectin-3 caused dilation and distortion of developing epithelia in embryonic day 12 metanephroi cultured for 1 wk. The upregulation of galectin-3 protein during kidney maturation, predominantly at sites where it could mediate cell/matrix interactions, seems to modulate growth of the ureteric tree.

Lectins are naturally occurring proteins that bind specific configurations of carbohydrate residues of glycoconjugates, and galectins are a family of calcium-independent soluble β-galactoside–binding lectins (1,2). Galectin-3 is a 30- to 42-kD molecule that is expressed by epithelia and immune cells in many mammalian species. Galectin-3 protein has been identified in the nucleus and cytoplasm and also attached to appropriately glycosylated cell surface and extracellular matrix ligands. Accordingly, a variety of functions have been proposed for the molecule, including a nuclear role in pre-mRNA splicing and proliferation (3), a cytoplasmic role inhibiting apoptosis (4), and an extracellular role modulating cell–cell and cell–substratum adhesions (5).

Galectin-3 is a two-domain molecule with an N-terminal proline-rich domain and a C-terminal carbohydrate recognition domain (1,2). Galectin-3 binds to simple β-galactosides such as lactosamine, Galβ1–4GlcNAc, and with higher affinity to polylactosamine chains (6–8). Addition of sialic acid, N-acetylgalactosamine or fucose substituents to the terminal galactose of a polylactosamine chain enhances galectin-3 binding still further (6–8). Amino acid residues Arg139 and Ser232 in the carbohydrate-binding pocket of galectin-3 are essential for binding to extended oligosaccharides (9).

Galectin-3 is expressed by hamster metanephroi (10) and during human nephrogenesis (11). In human ureteric bud branch tips, galectin-3 immunolocalizes to apical epithelial surfaces, but, as this lineage matures, it appears in a less-polarized distribution in cytoplasm and cell surface membranes, with high expression in fetal medullary collecting ducts. Postnatally, galectin-3 is downregulated but remains expressed by intercalated cells. The protein is expressed apically in ureteric bud–derived cysts in human dysplastic kidneys (11); the aberrant hyperproliferation of these epithelia correlates with expression of PAX2, a potentially oncogenic transcription factor, and BCL2, a survival molecule (12). Similar upregulation and location of galectin-3 occurs in collecting duct cysts in human autosomal recessive polycystic kidneys (11).

In culture, galectin-3 is synthesized and secreted by Madin Darby canine kidney (MDCK) cells (13). These form cysts in collagen gels, and galectin-3 co-localizes with laminin on the basal surface of these structures (14). Cyst growth is modulated by perturbing galectin-3 activity: blocking antibodies increase their expansion, whereas exogenously added galectin-3 slows their expansion. Ricin-resistant MDCK cells, which fail to transfer galactose residues during synthesis of glycoconjugates and lack extracellular galectin-3 receptors, undergo enhanced cystogenesis and abnormal morphogenesis (15). Therefore, *in vitro*, MDCK cell growth is regulated by galectin-3 interactions at basal membranes. In normal renal development, the changing distribution of the protein could regulate collecting duct growth while expression in cysts may reflect a failure of

Received February 2, 2000. Accepted August 30, 2000.
Correspondence to Professor Adrian S. Woolf, Nephro-Urology Unit, Institute of Child Health, 30 Guilford Street, London WC1N 1EH, UK. Phone: 44-20-7242-9789 x0712; Fax: 44-20-7916-0011; E-mail: a.woolf@ich.ucl.ac.uk
S.L.B. and T.M.J contributed equally to this article.

1046-6673/1203-0515
Journal of the American Society of Nephrology
Copyright © 2001 by the American Society of Nephrology

ureteric bud lineage maturation. We describe galectin-3 expression in mouse metanephroi and examine the bioactivity of this lectin in organ culture. Our evidence supports the hypothesis that galectin-3 modulates ureteric bud branching.

Materials and Methods

Animals and Reagents

We studied normal mouse strains, C56Bl/6J or (CBA x C57B10) F1, with similar results. The day of the vaginal plug was designated E0. Reagents were obtained from Sigma (Poole, UK) unless otherwise specified. We used primary rabbit polyclonal antibodies raised against hamster full-length galectin-3 (10) and laminin, a component of metanephric epithelial basement membranes (16). We used a mouse monoclonal antibody to calbindin-D28K, a cytoplasmic collecting duct marker (17). Secondary antibodies were conjugated with fluorescein-isothiocyanate (FITC) or Texas Red (TR). Other experiments used FITC-labeled Dolichos biflorus agglutinin (DBA), which binds to ureteric bud/collecting duct epithelia (18).

Reverse Transcription-PCR

RNA was extracted from organs, as described (19,20). Reverse transcription-PCR (RT-PCR) galectin-3 primers were as follows: 5'-AGC TTA TCC TGG CTC AAC TG-3' for sense primer, corresponding to nucleotides 405 to 424 of mouse galectin-3 (GENEMBL X16074), and 5'-CGG AGG TTC TTC ATC CGA TG-3' for the antisense primer, corresponding to nucleotides 853 to 872. β-Actin primers were as described (19). Thirty-five cycles of amplification were performed: 94°C for 1 min, 60°C for 1 min, 72°C for 3 min, and finally 72°C for 7 min. Seven μl of each reaction was electrophoresed through a 1% agarose gel and 467-bp galectin-3 and 540-bp β-actin products sought after ethidium bromide staining. Negative controls included reactions with water substituted for RNA or reverse transcriptase.

Histochemistry

Organs were fixed in 2% paraformaldehyde, paraffin embedded, and sectioned at 4 μm. After quenching endogenous peroxidase with 3% hydrogen peroxide in methanol for 30 min, sections were incubated at 37°C with anti–galectin-3 antibodies followed by secondary antibody and a streptavidin-biotin-peroxidase system (11). Negative controls comprised omission of primary antibody, substitution with preimmune serum, or preabsorption of primary antibody with recombinant galectin-3. Nuclei were stained with hematoxylin or methyl green, and some samples were stained with periodic acid-Schiff (PAS) to define proximal tubule brush borders. Cultured metanephroi were processed by three methods. Some were fixed in ice-cold methanol for 10 min, washed in phosphate-buffered saline (PBS) containing 0.1% Triton X-100, and reacted with calbindin-D28K (1:200) or laminin antibodies (1:1000) at 4°C. They were incubated with secondary antibodies (TR-conjugated for calbindin D28K and FITC-conjugated for laminin) for 4 h, defining ureteric bud branches (calbindin-D28K *positive*/laminin *positive*) and mesenchyme-derived nephrons (calbindin-D28K *negative*/laminin *positive*). Others were exposed to FITC-DBA (50 μg/ml in PBS) at 4°C overnight. These samples were mounted in Vectashield (Vector Laboratories, Burlingame, CA) and viewed with a confocal laser scanning microscope (Leica, Heidelberg, Germany) or a Zeiss Axiophot epifluorescence microscope (Oberkochen, Germany). Other cultured metanephroi were fixed in paraformaldehyde, paraffin embedded, and sectioned, as described above.

Organ Culture

Embryonic day 11 (E11) and E12 metanephroi were explanted onto Millicell-CM filters (Millipore Corporation, Bedford, MA) or 1 μm Nuclepore filters (Corning Costar Corporation, Cambridge, MA) at the interface between air/5% CO_2 atmosphere and medium and cultured at 37°C in control medium comprising Dulbecco's modified Eagle's medium (DMEM)-F12, 5% fetal calf serum, 1 mM glutamine, and antibiotics, as described (19,20).

In one set of experiments with E11 or E12 organs, medium was supplemented with galectins (1 μM) as follows: (*1*) recombinant hamster galectin-3 (21); (*2*) a recombinant protein, Δ1-93, containing the C-terminal domain but lacking most of the N-terminal domain (22): this fragment retains carbohydrate-binding specificity comparable to full-length galectin-3; (*3*) a mutant protein, SS, of full-length galectin-3 in which mutagenesis of residue Arg139 to serine abolishes the preferred binding of galectin-3 to extended oligosaccharides (9); and (*4*) galectin-1, a molecule that constitutively lacks the critical Arg139 residue in its binding pocket and exhibits a similar binding specificity to the SS mutant (7,8). A medium change, with or without additives, was performed every day during the organ culture period. Metanephroi were examined daily under a dissecting and/or phase-contrast microscope for up to 4 d. In some experiments, exogenous galectin-3 was removed after 2 d by washing in PBS, and culture continued for an additional 2 d in control medium. Branch tips and nephrons formed in culture were examined by whole-mount immunostaining: the numbers of branch tips in each group were expressed as a mean ± SD. To examine directly the effects of galectins on mesenchymal differentiation, we placed isolated mesenchymes from single E11 metanephroi adjacent to the dorsal edge of an E11 spinal cord segment (23) and incubated them in the presence or absence of exogenous galectins (1 μM). Isolated mesenchymes cultured without spinal cord failed to differentiate into nephrons (not shown).

We also examined effects of galectin-3 blocking antibody on E12 organs cultured for 7 d. We used dilutions of antibody (1:20 to 1:50) known to be bioactive in MDCK cyst assays (14); preimmune rabbit antiserum, in the same dilutions, was used for controls. A medium change was performed every day during the organ culture period. Preliminary experiments demonstrated that culture of E16 to 17 organs, which already express abundant galectin-3 protein, resulted in necrosis within 24 h because of their large size; hence, they were not suitable for blocking experiments. Therefore, we elected to explant E12 organs and culture them for up to 1 wk. This longer-term culture strategy using earlier-stage organs permitted *ex vivo* differentiation of nephrons and collecting ducts and upregulation of galectin-3 expression by collecting ducts with good tissue viability, providing a "window of opportunity" in which galectin-3 antisera could be added and potential effects could be recorded. Explants were photographed at 3 and 7 d and examined in paraffin-embedded sections at 7 d. Ureteric bud branch tips and glomeruli were not quantified because these explants acquired considerable complexity over a week in culture.

MDCK Assays

MDCK type II cells, trypsinized from monolayer cultures, were resuspended in ice-cold 2.4 mg/ml collagen type I solution at 1×10^5 cells/ml and dispensed into multiwell plates (1 ml/well; Costar). After gelation at 37°C for 45 min, cells were grown in DMEM with 10% fetal calf serum for up to 2 mo (14). Some cultures were grown throughout with galectins, as described in the Results section. Cyst growth was assessed by measuring the maximum diameter of randomly selected spherical cysts, as described (14). Results for each experimental group, comprising 10 fields in triplicate wells, were expressed as mean ± SD.

Results

Galectin-3 Expression in the Metanephros

Figure 1 demonstrates one of three representative RT-PCR assays. Galectin-3 transcripts were not detected in E11 metanephroi; similar results were obtained for E12 organs (not shown). In contrast, the appropriate RT-PCR product was detected in E14, E17, neonatal, and adult organs. Transcripts for β-actin, a housekeeping gene, were detected at all times.

The E11 metanephros contained mesenchyme surrounding a just-branched ureteric bud, while at E12, mesenchymal condensates had formed near branch tips; galectin-3 immunostaining was absent in these stages (not shown). At E16, immunostaining was noted in tubules in the superficial cortex and in larger diameter tubules in the deep cortex (Figure 2, A and B). In the E17 nephrogenic zone (Figure 2C), stems of ureteric bud branches expressed galectin-3, whereas their tips had barely detectable staining. Deep in the E17 cortex, collecting ducts expressed galectin-3 (Figure 2D), some with cytoplasmic or weak basal staining, others with a subset of cells with intense cytoplasmic immunostaining. Large medullary collecting ducts displayed intense galectin-3 staining basally (Figure 2E). Epithelia lining the E19 papilla also expressed the lectin (Figure 2F). The impression that galectin-3 immunostaining was restricted to the ureteric bud/collecting duct lineage was supported by PAS counterstaining, which confirmed that proximal tubules were galectin-3 negative (not shown). No immunostaining was noted in glomeruli. Postnatally, galectin-3 protein was detected in a uniform apical distribution in cortical tubules that were PAS negative and that were narrow in diameter, *i.e.*, they were not proximal tubules; these galectin-3–expressing cortical tubules were often located adjacent to glomeruli, suggesting that they were distal convoluted tubules. Galectin-3 was also immunolocalized to deep medullary collecting ducts and the urothelium of the renal papilla (not shown).

Effects of Galectin-3 Added to Organ Culture

E11 metanephroi in control medium underwent extensive morphogenesis over 4 d: the ureteric bud, which had branched once *in vivo*, produced approximately five further branching events over this period, and mesenchyme formed numerous nephrons. In cultures that had been treated with 1 μM galectin-3, a decrease in ureteric branch tips was visible after whole-mount immunostaining (Figure 3). In a typical quantified experiment assessed at 3 d, there were 86 $\pm$ 13 tips in control cultures *versus* 23 $\pm$ 5 (27% of controls) in lectin-treated cultures (Figure 4A). With the use of daily inverted microscope observations of individual organs, an inhibitory effect on branching of galectin-3 was visible after 2 d (not shown). Moreover, the inhibition was largely reversible: after 2 d of galectin-3 treatment followed by 2 d without exogenous lectin, five of five explants had recovered and the mean branching was 80% of the controls (Figure 3; data not shown). Although not formally quantified, qualitatively similar results were obtained with E12 metanephric cultures after visualizing ureteric bud derivatives with FITC-DBA (not shown). We examined the properties of galectin-3 required for branching inhibition (Figure 4A). Δ1-93 protein (1 μM), containing the C-terminal domain but lacking most of the N-terminal domain of galectin-3 (22), significantly inhibited branching. In contrast, neither the galectin-3 SS mutant (9) nor galectin-1 inhibited branching at the same concentrations. We also tested these galectins, at the same concentrations used in metanephric cultures, in the MDCK cyst assay (Figure 4B). Galectin-3 effectively slowed cyst expansion. The carbohydrate recognition fragment Δ1-193 protein (22) was also inhibitory, whereas, at equivalent concentrations, the galectin-3 SS mutant or galectin-1 was not.

Most (17 of 23) E11 metanephroi that were treated with galectin-3 failed to form nephrons, and in the minority of cases in which nephrons did form, their numbers were reduced. Although nephrons were not formally quantified, this effect is depicted in Figure 3. The Δ1-193 protein behaved similarly to intact galectin-3, but neither the SS mutant nor galectin-1 had a discernible effect on nephron formation (not shown). We also examined E12 explants cultured for 4 d: unlike small E11 explants, these organs were large enough to process reproducibly by paraffin embedding and histology. In controls, ureteric bud branches were flanked by nephron condensates and vesicles (Figure 5A), whereas glomeruli formed deeper in the explants (Figure 5C). However, in organs that were exposed to 1 μM galectin-3, ureteric branches appeared distorted, and although condensed mesenchyme was observed, these organs lacked mature nephrons (Figure 5, B and D). To distinguish whether galectin-3 inhibited mesenchymal to epithelial transformation directly or indirectly, we cultured isolated mesenchymes with a potent inducer, the spinal cord. Cultures differentiated similarly in the presence or absence of 1 μM galectin-3: after 3 d, all mesenchymes formed laminin-positive epithelial tubules (Figure 6).

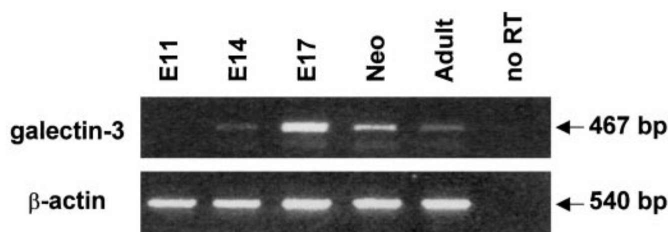

Figure 1. Galectin-3 mRNA. Representative results from three experiments. As assessed by reverse transcription-PCR (RT-PCR), galectin-3 transcripts were absent in embryonic day 11 (E11) metanephroi. The appropriate RT-PCR product (467 bp) was detected in E14, E17, neonatal, and adult organs. Transcripts for β-actin (540 bp) were detected at all times. No products were generated when reverse transcriptase was omitted (No RT: far right lane).

Effects of Galectin-3–Blocking Antisera in Organ Culture

Control E12 explants in 1:20 preimmune rabbit antiserum (n = 25, two experiments) grew over 7 d into increasingly complex structures that contained numerous glomeruli with normal tubule morphogenesis (Figure 7, A through C). By the end of the experiment, galectin-3 immunohistochemistry revealed a similar pattern to E16 to 17 organs *in vivo* (not shown). When

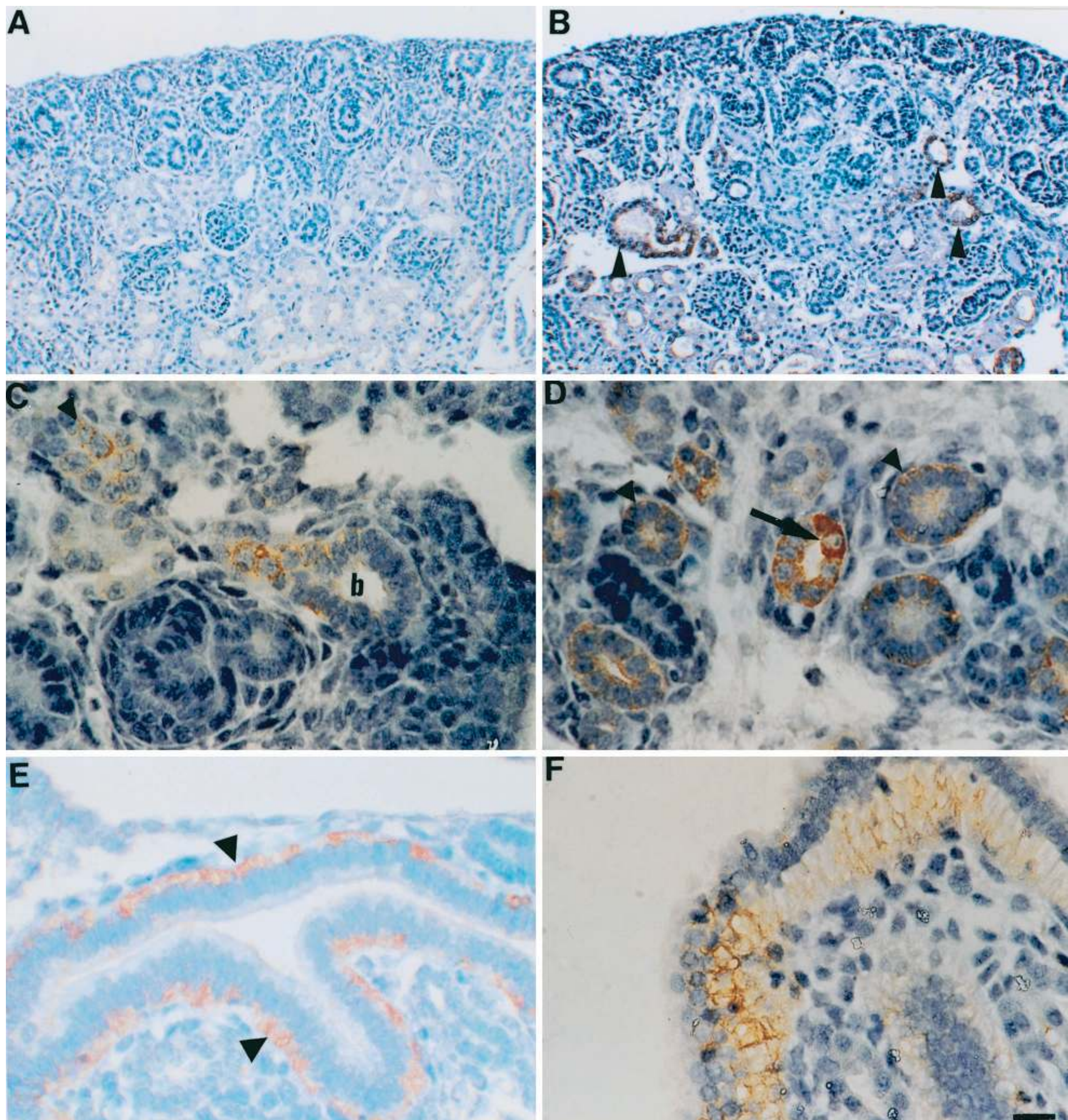

Figure 2. Galectin-3 immunohistochemistry. A and B were an E16 metanephros, C-E were E17 organs and F an E19 organ. A, B, and E were counterstained with methyl green and others with hematoxylin. A was a negative control with galectin-3 antibody prereacted with galectin-3. B through H were probed with galectin-3 antibody. (A) No reaction in the negative control. (B) A subset of tubules (arrowheads) immunostained brown for galectin-3. (C) Nephrogenic zone showing that the stem of a ureteric bud branch expressed galectin-3 (arrowhead), whereas its bulbous tip (b) had barely detectable immunostaining. (D) Deep in the cortex, two types of tubule expressed galectin-3: some (arrowheads) displayed cytoplasmic or weak basal staining, whereas others contained a subset of cells that intensely expressed galectin-3 (arrow). (E) Branches of medullary collecting ducts displayed galectin-3 immunostaining basally (arrowheads). (F) Papillary epithelium expressed galectin-3. Bar: 60 μm in A and B; 10 μm in C through F.

organs were cultured in 1:20 galectin-3 antiserum (n = 20, two experiments), organs appeared less compact and the formation of structures that resembled dilated tubules was noted at the periphery of the organ at 3 d (Figure 7D); this malformation was maintained until day 7 (Figure 7E). Figure 7F depicts a tissue section on the last day of blockade, showing a dilated epithelial tubule on the edge of the organ, resembling a small cyst, surrounded by undifferentiated tissue lacking glomeruli. Most tubules in these organs stained with DBA agglutinin (data not shown), suggesting that they were ureteric bud-derived

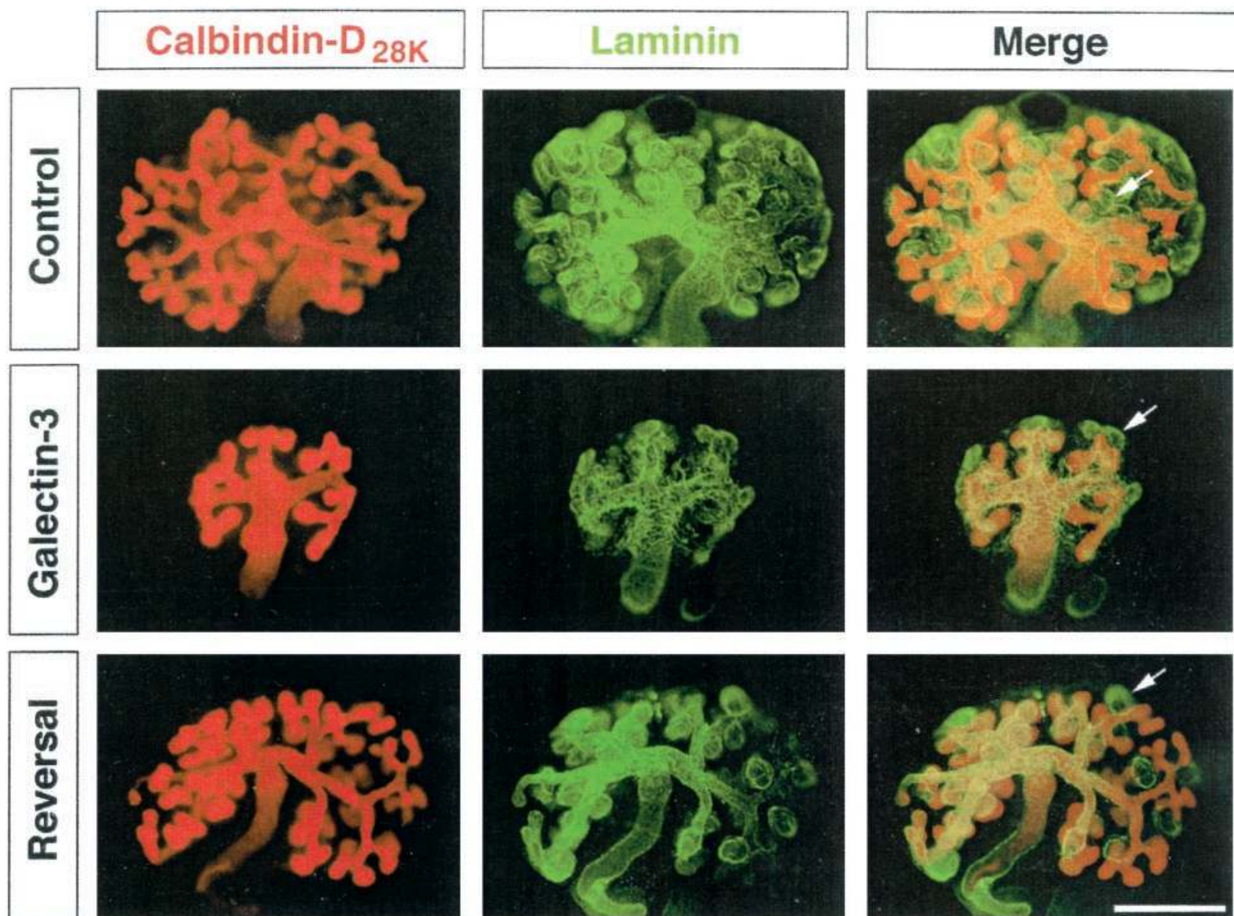

Figure 3. Effects of galectin-3 on cultured E11 metanephroi. E11 explants were cultured for 4 d in the absence (Control, $n = 13$) or presence of 1 μM galectin-3 (Galectin-3, $n = 9$). In other cultures, galectin-3 was removed after 2 d and culture continued in control medium for 2 d (Reversal, $n = 5$). Cultures were stained with antibodies against calbindin-D28K and laminin followed respectively by secondary antibodies conjugated with Texas red (red) or FITC (green). Merged images are shown in frames on right. In galectin-3–treated cultures, there was a decrease in ureteric branches (calbinin-D28K *positive*/laminin *positive* structures) after 4 d, whereas a relatively normal branching pattern was attained after withdrawal of galectin-3. Exogenous galectin-3 also impaired nephron generation (arrows), as evidenced by the relative deficiency of calbindin-D28K *negative*/laminin *positive* structures. Bar, 500 μm.

structures. When control medium was substituted for blocking antisera on day 3 ($n = 25$, two experiments), relatively normal growth resumed in the last 4 d of culture, with organs becoming more compact with formation of glomeruli and normal caliber tubules (Figure 7, G through I). Qualitatively similar but lesser tubule malformations were generated with 1:100 blocking antisera ($n = 10$; not shown).

Discussion

Galectin-3 expression was not detected at E11 to 12, the earliest stages of mouse metanephric development, whereas mRNA and protein were present later in metanephrogenesis. Galectin-3 protein was restricted to ureteric bud derivatives, including collecting ducts and urothelium of the renal pelvis. These results are in accordance with findings in human (11) and hamster (10) nephrogenesis and MDCK cells (14). In actively branching ureteric bud branch tips, galectin-3 was barely detectable; instead, it was expressed strongly and basally in maturing medullary collecting ducts. Immunoreactivity was also noted in smaller cortical collecting ducts, and in some of these tubules a subset of cells had intense cytoplasmic staining; on the basis of human studies, these may be differentiating intercalated cells (11). These patterns suggest multiple roles for galectin-3 in the ureteric bud lineage.

Exogenous galectin-3 perturbed branching in E11 and E12 explants. This inhibition occurred at relatively low concentrations and was largely reversible, hence unlikely to be due to unspecific cytotoxicity. Perhaps addition of exogenous galectin-3 to basal surfaces of ureteric bud epithelium, analogous to the pattern of endogenous protein in maturing medullary collecting ducts, converts ureteric tips to a more trunklike fate, unable to produce or receive the inducing signals. This action might be mediated in several ways.

One possibility is that galectin-3 affects growth signaling pathways. Candidate molecules that promote branching and that originate from ureteric bud lineage epithelia include Wnt 11 (23) and bone morphogenetic protein 7 (24), whereas mesenchymal-derived signals include glial cell line–derived neu-

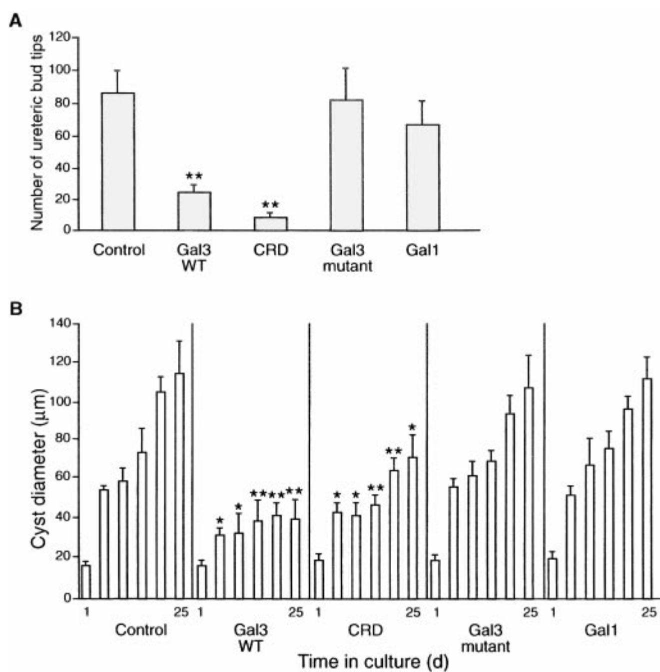

Figure 4. Quantification of ureteric bud branching and Madin Darby canine kidney (MDCK) cyst growth. (A) E11 metanephroi were cultured for 3 d in the absence (Control) or presence of 1 μM wild-type galectin-3 (Gal3 WT), Δ1-93 protein carbohydrate recognition domain (CRD), a mutant SS galectin-3 (Gal3 mutant) and galectin-1 (Gal1). Means $\pm$ SD of ureteric bud branch tips were counted in cultured explants ($n = 11$ for each set). (B) MDCK cells were cultured as cysts in collagen type I gels for 1, 5, 10, 15, 20, and 25 d. Media contained additives as in A. The maximal diameter (mean $\pm$ SD) of at least 10 separate cysts in triplicate cultures were measured. Comparisons between controls and treated cultures at similar time points are designated $*P < 0.05$ and $**P < 0.01$ (*t* test) with unmarked bars representing no significant difference.

rotrophic factor (GDNF) (19), fibroblast growth factor 7 (25), and integrin $\alpha 8\beta 1$ (26). Such branch-inducing pathways might be inhibited by galectin-3, perhaps by modulation of ureteric bud cell-surface receptors that bind these signaling molecules. Another explanation for the effects of galectin-3 comes from the observation that the lectin slows MDCK cyst expansion. Indeed, the inhibitory actions on metanephric branching and MDCK cyst expansion are mediated by a galectin-3 domain that permits high-affinity binding to glycoproteins that carry extended glycan chains. Expansion and branching of the ureteric bud epithelium and enlargement of MDCK cysts in gels probably require a delicate balance between the relative strength of cell–cell and cell–matrix adhesions. Of note, metanephric growth is tunicamycin sensitive (27), implicating glycosylation of adhesive proteins, whereas other experiments show that galectin-3 binds matrix components, such as laminins (22,28) and cell-surface integrins including $\beta 1$ integrins (29,30), and these same classes of molecules are implicated in branching *in vivo* and MDCK tubulogenesis *in vitro* (16,31–35). Therefore, extracellular galectin-3 might modulate metanephric growth by cross linking laminins to membrane-bound receptors.

The above experiments added galectin-3 during the earliest days of E11/12 metanephric growth in culture when there may be little, if any, endogenous galectin-3 protein. These studies represent a relatively pure, albeit artificial, situation of galectin-3 excess. Therefore, we attempted to ascertain whether endogenous galectin-3 had a role in metanephrogenesis by blocking its activity with antiserum when the protein was upregulated *ex vivo* during extended culture of E12 explants. We cultured E12 metanephroi for 7 d in dilutions of antiserum that enhance MDCK cyst expansion (14). Explant growth was altered in a reproducible and characteristic manner. Controls, exposed to preimmune sera, grew as compact structures with generation of normal caliber, slender tubules as well as glomeruli. In contrast, organs with galectin-3 blockade increased in area but became less compact and were composed of distorted tubules, some of which were abnormally large in cross section, superficially resembling small cysts. We believe that these are distorted ureteric bud branches, based on their peripheral distribution and that they bound DBA lectin, which are overgrowing, since our preliminary results demonstrated that $>$90% of nuclei in the walls of these structures express proliferating cell nuclear antigen (12). Because galectin-3 blockade was partially reversible, it was unlikely to be an unspecific toxic effect. We speculate that when the activity of endogenous metanephric galectin-3 is blocked, collecting duct epithelial growth is uninhibited and the normal pattern of branching morphogenesis is also lost.

Hence, an excess and also a deficiency of galectin-3 each leads to abnormal growth; other examples illustrate that not only "too little" but also "too much" of a molecule can perturb renal development. One example is the PAX2 transcription factor, a molecule expressed in the ureteric bud/collecting duct and early nephron lineages and downregulated as maturation proceeds. PAX2 deficiency leads to urinary tract growth failure. whereas overexpression is associated with another type of malformation, cyst formation (12,36,37). Another example is provided by RET, the GDNF receptor expressed by ureteric bud branch tips. When RET signaling is downregulated, *e.g.*, by antibody blockade in organ culture (19), branching is deficient and nephron generation is also inhibited as a secondary effect. Conversely, when a constitutively active RET protein is expressed by the whole ureteric tree, another malformation is generated: nodules caused by deregulated branching (38).

The current study also demonstrates that exogenous galectin-3 downregulated mesenchymal to epithelial transformation in whole cultured metanephroi. However, it had no effect on tubules induced by spinal cord in isolated E11 renal mesenchymes. Thus, exogenous galectin-3 is likely to perturb mesenchyme induction in whole-organ culture indirectly, perhaps by impairing bud-derived mesenchyme growth factors (39). We also observed that antisera to galectin-3 prevented formation of glomeruli after 7 d of organ culture. We postulate that this specific effect is also likely to be secondary to perturbed bud development, especially because we failed to immunolocalize galectin-3 protein to the mesenchymal/nephron lineage.

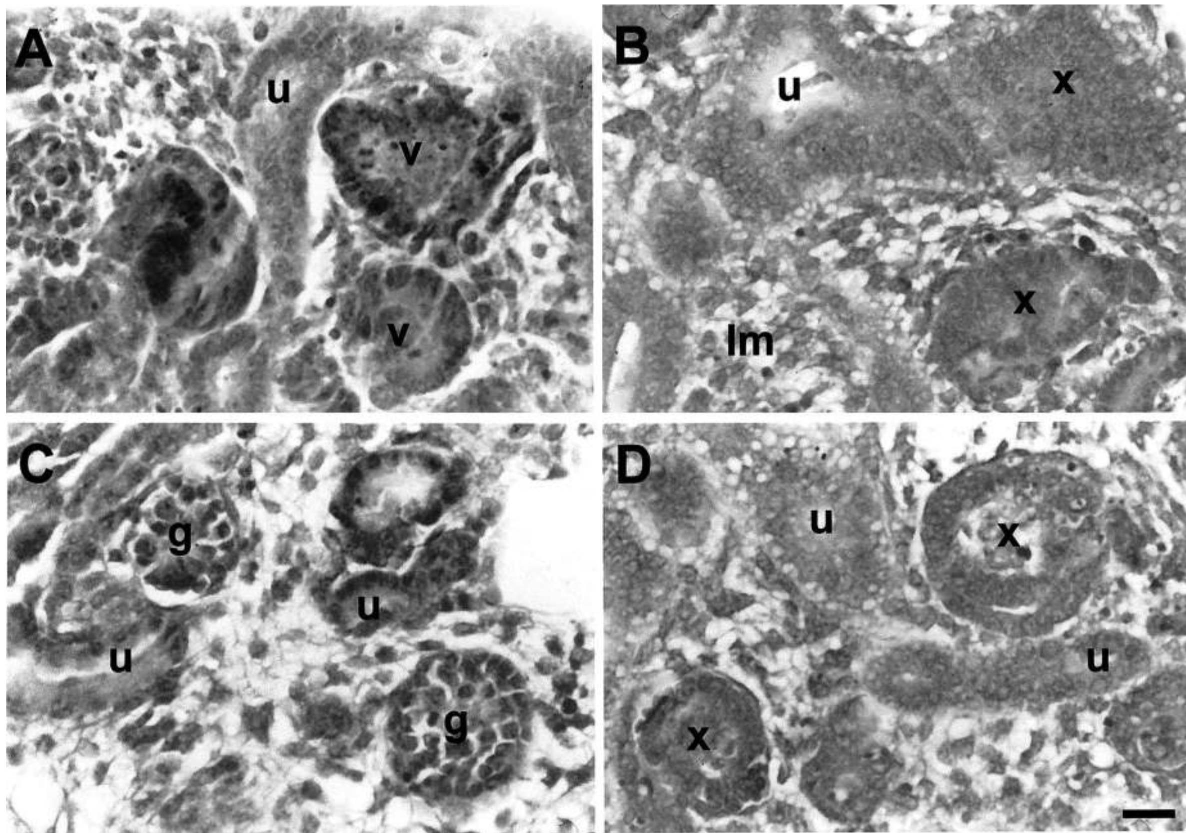

Figure 5. Galectin-3 effects on nephron formation. E12 metanephros after 4 d of culture: sections stained with hematoxylin. (A and C) Control medium. Note ureteric bud branches (u) surrounded by nephron vesicles (v) in the outer part of the explant (A) and glomeruli (g) deeper in the organ (C). (B and D) Views at comparable levels as A and C but in an explant exposed to 1 μM galectin-3 for 4 d. In B, some mesenchyme aggregated into condensates, but these failed to develop lumens (x). Loose mesenchyme was also noted (lm). D is a deeper section of the explant; note malformed mesenchymal condensates (x) and the absence of glomeruli. Bars, 10 μm.

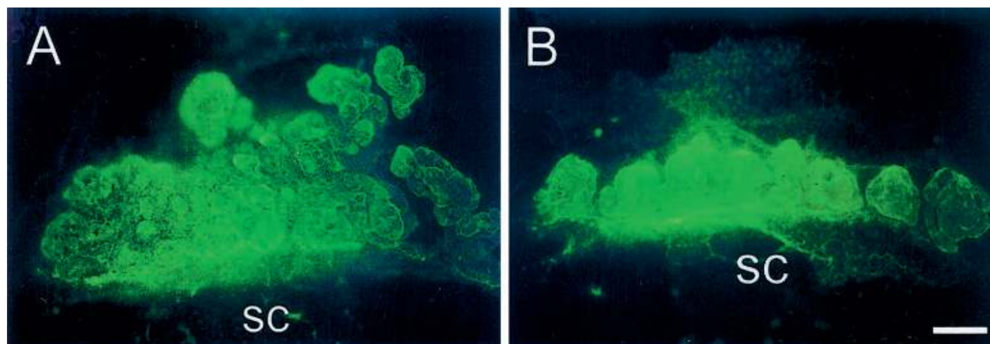

Figure 6. Induction of nephrons in isolated mesenchyme. Representative pictures from four experiments in each data set. Isolated mesenchyme from single E11 metanephroi were cocultured atop spinal cord (sc) for 3 d in the presence (A) or absence (B) of 1 μM galectin-3. Whole mounts were reacted with laminin antibody detected by FITC-conjugated second antibody; nephrons formed in both conditions. Bar, 10 μm.

Conclusions

Galectin-3 protein is not expressed at the earliest stages of metanephric development, and indeed expression of the lectin during this period might be incompatible with the rapid branching of the ureteric tree and nephrogenesis induced by ureteric bud branch tips. We suggest that galectin-3 acts to limit ureteric outgrowth and nephrogenesis at later stages in development, when expression is upregulated. However, further experiments are needed to define the mechanism of these effects. Recent evidence has implicated a 16-kD galectin in the maintenance of epithelial phenotype during chick mesonephric development (40), and other galectins are present in the kidney (41). Further studies may reveal many diverse functions for these carbohydrate-binding proteins in renal development and disease (11). In the future, experiments in which galectins are over- or underexpressed in the developing kidneys of geneti-

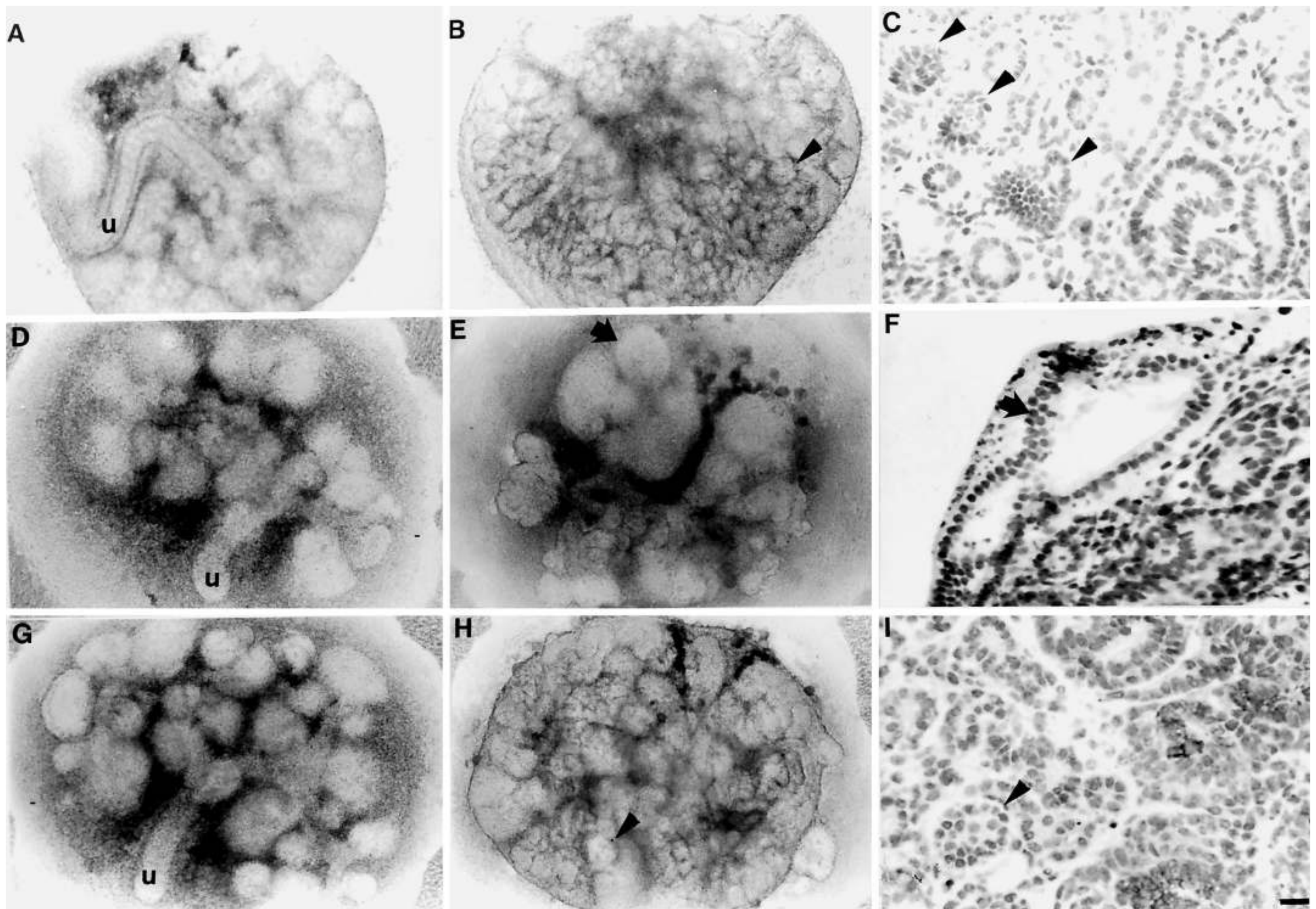

Figure 7. Effects of galectin-3–blocking antisera on E12 explants. Whole-mount photographs of E12 metanephroi cultured for 3 (A, D, and G) and 7 (B, E, and H) d. (C, F, and I) Representative hematoxylin-stained sections from organs harvested at 7 d. A was grown for 3 d in control media supplemented with preimmune serum. B is the same organ at 7 d; note its compact structure with increasing internal complexity. (C) A tissue section from the organ in B, demonstrating numerous tubules and glomeruli. (D) An organ grown for 3 d in galectin-3–blocking antibody; note the disturbance of morphogenesis with a less compact structure and formation of dilated ducts. When the organ in D continued culture in antiserum for 7 d (E), the malformation was maintained. (F) A tissue section of this organ, showing a dilated tubule in the periphery of the organ and a lack of glomeruli. (G and H) One organ cultured for 3 d in antiserum and then switched for an additional 4 d to control medium, respectively. Note resumption of relatively normal gross (H) and microscopic (I) appearances by day 7. u, main stem of ureteric bud; arrowheads, glomeruli; arrows, dilated tubules in organ periphery. Bars: 50 μm in A, B, D, E, G, and H; 10 μm in C, F, and I.

cally engineered mice may provide a further perspective to the possible *in vivo* roles of these molecules.

Acknowledgments

We thank the National Kidney Research Fund for support of S.B. and T.M.J. and a project grant 3/2/99 to A.S.W. and P.J.D.W. We thank Dr. D. N. W. Cooper (UCSF, San Francisco, CA) for the gift of galectin-1.

References

1. Barondes SH, Cooper DN, Gitt MA, Leffler H: Galectins. Structure and function of a large family of animal lectins. *J Biol Chem* 269: 20807–20810, 1994
2. Hughes RC: The galectin family of mammalian carbohydrate-binding molecules. *Biochem Soc Trans* 25: 1194–1198, 1997
3. Dagher SF, Wang JL, Patterson RJ: Identification of galectin-3 as a factor in pre-mRNA splicing. *Proc Natl Acad Sci USA* 92: 1213–1217, 1995
4. Akahani S, Nangia-Makker P, Inohara H, Kim HR, Raz A: Galectin-3: A novel antiapoptotic molecule with a functional BH1 (NWGR) domain of Bcl-2 family. *Cancer Res* 57: 5272–5276, 1997
5. Perillo NL, Marcus ME, Baum LG: Galectins: Versatile modulators of cell adhesion, cell proliferation and cell death. *J Mol Med* 76: 402–412, 1998
6. Sato S, Hughes RC: Binding specificity of a baby hamster kidney lectin for H type I and II chains, polylactosamine glycans, and appropriately glycosylated forms of laminin and fibronectin. *J Biol Chem* 267: 6983–6990, 1992
7. Leffler H, Barondes SH: Specificity of binding of three soluble rat lung lectins to substituted and unsubstituted mammalian beta-galactosides. *J Biol Chem* 261: 10119–10126, 1986

8. Sparrow CP, Leffler H, Barondes SH: Multiple soluble β-galactoside-binding lectins from human lung. *J Biol Chem* 262: 7383–7390, 1987
9. Henrick K, Bawumia S, Barboni EAM, Mehul B, Hughes RC: Identification of subsites in the galectins involved in sugar-binding at the non-reducing end of the central galactose of oligosaccharide ligands: Sequence analysis, homology modelling and mutagenesis studies of hamster galectin-3. *Glycobiology* 8: 45–57, 1998
10. Foddy L, Stamatoglou SC, Hughes RC: An endogenous carbohydrate-binding protein of baby hamster kidney (BHK21 C13) cells. Temporal changes in cellular expression in the developing kidney. *J Cell Sci* 97: 139–148, 1990
11. Winyard PJD, Bao Q, Hughes RC, Woolf AS: Epithelial galectin-3 during human nephrogenesis and childhood cystic diseases. *J Am Soc Nephrol* 8: 1647–1657, 1997
12. Winyard PJD, Risdon RA, Sams VR, Dressler G, Woolf AS: The PAX2 transcription factor is expressed in cystic and hyperproliferative dysplastic epithelia in human kidney malformations. *J Clin Invest* 98: 451–459, 1996
13. Sato S, Burdett I, Hughes RC: Secretion of the baby hamster kidney 30-kDa galactose-binding lectin from polarized and non-polarized cells: A pathway independent of the endoplasmic reticulum-Golgi complex. *Exp Cell Res* 207: 8–18, 1993
14. Bao Q, Hughes RC: Galectin-3 expression and effect on cyst enlargement and tubulogenesis in kidney epithelial MDCK cells cultured in three-dimensional matrices in vitro. *J Cell Sci* 108: 2791–2800, 1995
15. Bao Q, Hughes RC: Galectin-3 and polarized growth within collagen gels of wild-type and ricin-resistant MDCK renal epithelial cells. *Glycobiology* 9: 489–495, 1999
16. Klein G, Langegger M, Timpl R, Ekblom P: Role of laminin A chain in the development of epithelial cell polarity. *Cell* 55: 331–341, 1988
17. Davies J: Control of calbinin-D28K expression in developing mouse kidney. *Dev Dyn* 199: 45–51, 1994
18. Qiao J, Cohen D, Herzlinger D: The metanephric blastema differentiates into collecting system and nephron epithelia in vitro. *Development* 121: 3207–3214, 1995
19. Towers PR, Woolf AS, Hardman P: Glial cell line-derived neurotrophic factor stimulates ureteric bud outgrowth and enhances survival of ureteric bud cells in vitro. *Exp Nephrol* 6: 337–351, 1998
20. Woolf AS, Kolatsi-Joannou M, Hardman P, Andermarcher E, Moorby C, Fine LG, Jat PS, Noble MD, Gherardi E: Roles of hepatocyte growth factor/scatter factor and the met receptor in the early development of the metanephros. *J Cell Biol* 128: 171–184, 1995
21. Mehul B, Bawumia S, Martin SR, Hughes RC: Structure of baby hamster kidney carbohydrate-binding protein CBP30, an S-type animal lectin. *J Biol Chem* 269: 18250–18258, 1994
22. Barboni EAM, Bawumia S, Hughes RC: Kinetic measurements of binding of galectin-3 to a laminin substratum. *Glycocon J* 16: 365–373, 1999
23. Kispert A, Vaino S, McMahon AP: Wnt-4 is a mesenchymal signal for epithelial transformation of metanephric mesenchyme in the developing kidney. *Development* 125: 4225–4234, 1998
24. Godin RE, Takaesu NT, Robertson EJ, Dudley AT: Regulation of BMP7 expression during kidney development. *Development* 125: 3473–3482, 1998
25. Qiao J, Uzzo R, Obara-Ishihara T, Degenstein L, Fuchs E, Herzlinger D: FGF-7 modulates ureteric bud growth and nephron number in the developing kidney. *Development* 126: 547–554, 1999
26. Muller U, Wang D, Denda S, Meneses JJ, Pederson RA, Reichardt LF: Integrin $\alpha 8\beta 1$ is critically important for epithelial-mesenchymal interactions during kidney morphogenesis. *Cell* 88: 603–613, 1997
27. Ekblom P, Nordling S, Saxen L, Rasilo ML, Renkonen O: Cell interactions leading to kidney tubule determination are tunicamycin sensitive. *Cell Differ* 8: 347–352, 1979
28. Massa SM, Cooper DNW, Leffler H, Barondes SH: L-29, an endogenous lectin, binds to glycoconjugate ligands with positive cooperativity. *Biochem* 32: 260–267, 1993
29. Dong S, Hughes RC: Macrophage surface glycoproteins binding to galectin-3 (Mac-2 antigen). *Glycocon J* 14: 267–274, 1997
30. Ochieng J, Leite-Browning ML, Warfield P: Regulation of cellular adhesion to extracellular matrix proteins by galectin-3. *Biochem Biophys Res Commun* 246: 788–791, 1998
31. Kreidberg JA, Donovan MJ, Goldstein SL, Rennke H, Shepherd K, Jones RC, Jaenisch R: $\alpha 3\beta 1$ integrin has a crucial role in kidney and lung organogenesis. *Development* 122: 3537–3547, 1996
32. Saelman EUM, Keeley PJ, Santoro SA: Loss of MDCK cell $\alpha 2\beta 1$ integrin expression results in reduced cyst formation, failure of hepatocyte growth factor/scatter factor-induced branching morphogenesis and apoptosis. *J Cell Sci* 108: 3531–3540, 1995
33. Falk M, Salmivirta K, Durbeej M, Larsson E, Ekblom M, Vestweber D, Ekblom P: Integrin $\alpha 6\beta 1$ is involved in kidney tubulogenesis in vitro. *J Cell Sci* 109: 2801–2810, 1996
34. Sorokin L, Sonnenberg A, Aumailley M, Timpl R, Ekblom P: Recognition of the laminin E8 cell-binding site by an integrin possessing the $\alpha 6$ subunit is essential for epithelial polarization in developing kidney tubules. *J Cell Biol* 111: 1265–1273, 1990
35. Sorokin L, Conzelmann S, Ekblom P, Battaglia C, Aumailly M, Timpl R: Monoclonal antibodies against laminin A chain fragment E3 and their effects on binding to cells and proteoglycan on kidney development. *Exp Cell Res* 201: 137–144, 1992
36. Dressler GR, Woolf AS: Pax2 in development and renal disease. *Int J Dev Biol* 43: 463–468, 1999
37. Torres M, Gomez-Pardo E, Dressler GR, Gruss P: Pax2 controls multiple steps of urogenital development. *Development* 121: 4057–4065, 1995
38. Srinivas S, Wu Z, Chen CM, D'Agati V, Constantini F: Dominant effects of RET receptor misexpression and ligand-independent RET signaling on ureteric bud development. *Development* 126: 1375–1386, 1999
39. Karavanova ID, Dove LF, Resau JH, Perantoni AO: Conditioned medium from a rat ureteric bud cell line in combination with bFGF induces complete differentiation of isolated metanephric mesenchyme. *Development* 122: 4159–4167, 1996
40. Murphy KM, Zalik SE: Endogenous galectins and effect of galectin hapten inhibitors on the differentiation of the chick mesonephros. *Dev Dyn* 215: 248–263, 1999
41. Burger A, Filsinger S, Cooper DNW, Hansch GM: Expression of the 14 kDa galactose binding protein, galectin-1, on human tubular epithelial cells. *Kidney Int* 50: 754–759, 1996