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FGF-7 modulates ureteric bud growth and nephron number in the developing kidney

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SUMMARY

The importance of proportioning kidney size to body volume was established by clinical studies which demonstrated that in-born defecits of nephron number predispose the kidney to disease. As the kidney develops, the expanding ureteric bud or renal collecting system induces surrounding metanephric mesenchyme to proliferate and differentiate into nephrons. Thus, it is likely that nephron number is related to ureteric bud growth. The expression patterns of mRNAs encoding Fibroblast Growth Factor-7 (FGF-7) and its high affinity receptor suggested that FGF-7 signaling may play a role in regulating ureteric bud growth. To test this hypothesis we examined kidneys from FGF-7-null and wild-type mice. Results of these studies demonstrate that the developing ureteric bud and mature collecting system of FGF-7-null kidneys is markedly smaller than wild type. Furthermore, morphometric analyses indicate that mature FGF-7-null kidneys have 30±6% fewer nephrons than wild-type kidneys. In vitro experiments demonstrate that elevated levels of FGF-7 augment ureteric bud growth and increase the number of nephrons that form in rodent metanephric kidney organ cultures. Collectively, these results demonstrate that FGF-7 levels modulate the extent of ureteric bud growth during development and the number of nephrons that eventually form in the kidney.

Key words: Fibroblast Growth Factor-7, Kidney Development, Aquaporin-3

INTRODUCTION

Organogenesis is dependent on two fundamental embryonic processes: (1) the generation of organ specific cell types, and (2) the growth of organ size to match body volume. The importance of proportioning organ size to body volume is well illustrated in the developing human kidney, an organ characterized by 10^6 complex epithelial tubules or nephrons that filter blood to keep the volume and composition of body fluids constant. Clinical evidence indicates that even slight inborn deficits of nephron number correlate with the incidence of hypertension and renal failure (Brenner and Milford, 1993; MacKenzie and Brenner, 1995).

Nephrons derive from a domain of caudal intermediate mesoderm, the metanephric blastema (Saxen, 1987). The renal collecting system derives from the ureteric bud, which invades and subsequently branches within the metanephric blastema. During development, the branching ureteric bud tips induce surrounding nephron progenitors of the metanephric blastema to proliferate and differentiate into nephrons. Although the exact number of nephrons that form around each ureteric bud branch is unknown, the extent of ureteric bud growth and branching during development is believed to be proportional to the number of nephrons that eventually form (Saxen, 1987; Oliver, 1968; Stuart et al., 1995). Thus, nephron number is likely to be dependent on factors regulating ureteric bud growth during development.

Members of the *Fibroblast Growth Factor* (*FGF*) gene family have been implicated in regulating epithelial growth and branching during development (Guo et al., 1996; Alarid et al., 1994; Yi et al., 1995; Nguyen et al., 1996; Samakovlis et al., 1996; Sutherland et al., 1996; Bellusci et al., 1997). Several members of this multigene family including FGF-1, 2, 5 and 7 are expressed by or around the growing ureteric bud in the developing kidney (Barasch et al., 1997; Finch et al., 1995; Gonzalez et al., 1990; Haub and Goldfarb, 1991; Hebert et al., 1990**)**. Furthermore, the ureteric bud expresses mRNAs encoding at least two high affinity FGF-receptors (Finch et al., 1995; Stark et al., 1991). In this report we analyze the role of Fibroblast Growth Factor-7 (FGF-7) in kidney morphogenesis. The distribution of mRNAs encoding FGF-7 and its high affinity receptor, the Keratinocyte Growth Factor

Receptor (KGF-R), are consistent with this ligand-receptor complex mediating ureteric bud growth (Fig. 1). *FGF-7* mRNA is first detected in the developing urogenital system on embryonic day 14.5 (Mason et al., 1994). The cells that express *FGF-7* mRNA in the developing kidney are renal fibroblasts or stroma surrounding the growing ureter and collecting system. Recently, renal stroma has been shown to be required for ureteric bud growth by targeted deletion of the fork-head transcription factor, BF-2 (Hatini et al., 1996). *KGF-R* mRNA is expressed in the ureteric bud and its derivative, renal collecting tubules throughout development; it has not been detected in nephron progenitors (Finch et al., 1995). Thus, *FGF-7* is one member of the *FGF* gene family that exhibits spatial and temporal expression patterns consistent with a direct role in supporting ureteric bud growth. To elucidate the function of FGF-7 during kidney morphogenesis, we examined the kidneys of wild-type and FGF-7-null mice (Guo et al., 1996). Results of these studies demonstrate that the developing ureteric bud and mature renal collecting system of FGF-7-null kidneys are smaller than observed in age-matched wild-type organs. Extensive morphometric analyses demonstrate that kidneys of mature FGF-7-null animals have 30±6% fewer nephrons than age- and sex-matched wild-type kidneys. Conversely, exogenous FGF-7 augments ureteric bud growth in rodent metanephric kidney organ cultures and increases nephron number by approximately 50%. Thus in the absence of FGF-7, significant inborn nephron deficits occur in vivo, while elevated levels of FGF-7 increase nephron number in vitro. Further experiments demonstrate that elevated levels of FGF-7 modulate ureteric bud growth and differentiation in vitro. Collectively, these data indicate that FGF-7 is part of the signaling pathway controlling collecting system size and nephron number in the kidney during development.

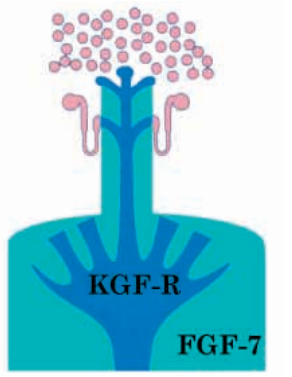

Fig. 1. Patterns of *FGF-7* and *KGF-R* mRNA expression in the developing kidney. *FGF-7* mRNA is localized to fibroblasts or stroma (turquoise) surrounding the ureter and developing renal collecting system. *KGF-R* mRNA is expressed in the ureter and branching ureteric bud (dark blue). *KGF-R* and *FGF-7* mRNAs are not expressed in nephron progenitors of the metanephric blastema or in differentiating nephron epithelia (pink). The diagram is based on data from Mason et al. (1994) and Finch et al. (1995).

MATERIALS AND METHODS

Tissue preparation and analysis

FGF-7-null mice (C57BL/6), supplied by L. Degenstein and E. Fuchs (University of Chicago), were bred and housed at the Cornell University Medical College Animal Facilities. Wild-type C57BL/6 mice of the same age and sex were purchased from Charles River. Animals were killed by CO_2 asphyxiation, and the kidneys isolated and fixed by immersion in Bouin's fixative. Tissue was embedded in paraffin and 10 μm serial sections prepared and stained with Hematoxylin and Eosin. Tissue volume (K_v) was determined for kidneys isolated from six wild-type and six FGF-7-null animals at stated ages. Embryonic kidneys analyzed were from separate litters. Volume determinations were made from serial-sectioned kidneys using the following measurements and equation:

$$Kv = \sum_{\text{first section}}^{\text{last section}} (\text{section surface area} \times \text{section thickness}),$$

where K_v = Kidney volume. Nephron density (nephron number/mm^3) was determined by counting glomeruli according to standard morphometric procedures (Bertram et al., 1992).

Metanephric kidney organ culture

Gestation day (E) 12.5-13 rat embryos were removed from timed pregnant Sprague-Dawley rats killed by CO_2 asphyxiation (Hilltop Laboratories). Kidney rudiments and ureteric buds were isolated and cultured as described previously (Qiao et al., 1995). Ureteric bud and metanephric kidney organ cultures were maintained in the absence or presence of given concentrations of FGF-1, 2, 4, 5 and 7, purchased from R&D systems.

Antibody and lectin staining

Aquaporin-3 antibody binding experiments were performed on frozen sections of kidney tissue isolated from wild-type and FGF-7-null animals and whole metanephric kidney organ cultures. Aquaporin-3 antibody was provided by Dr G. Frindt, Cornell University Medical College, and has been previously characterized (Ecelbarger et al., 1995). Frozen sections or kidney rudiments were fixed with 4% paraformaldehyde, permeabilized with 0.1% Saponin and incubated with Fetal Calf Serum (FCS) prior to application of AQP-3 antibody at a dilution of 1:100 for 2 hours at room temperature. Samples were then washed extensively in phosphate-buffered saline (PBS), and secondary FITC-conjugated goat anti-rabbit antibody (Sigma) was utilized to detect Aquaporin-3 antibody binding. Samples were examined with a Zeiss Axiophot fluorescence microscope after washing and post-fixation. No staining was detected when primary antibody incubation was omitted.

The lectin Dolichos Biflorus (DB) specifically binds to ureteric bud and renal collecting system epithelia (Holthöfer, 1983). FITC-conjugated DB (FITC-DB) was purchased from Zymed. Organ cultures were processed for FITC-DB binding as described previously (Qiao et al., 1995). Peanut Lectin Agglutinin (PNA; Zymed) specifically binds to glomeruli. Organ cultures were processed for RITC-PNA binding as described (Gilbert et al. 1994).

RESULTS

FGF-7-null kidneys are smaller than wild type

FGF-7-null mice were previously constructed by targeted gene deletion of exon 1, a portion of the FGF-7 gene that is required for heparin association and subsequent receptor binding (Guo et al., 1996). *FGF-7* mRNA is not detected in any FGF-7-null tissues, including the kidney, as determined by RT-PCR. No significant differences in total body mass or crown-rump length were observed between FGF-7-null or wild-type animals. With

the exception of previously described hair abnormalities, no overt aberrations in phenotype were reported (Guo et al., 1996). The kidneys of FGF-7-null and wild-type mice of the same age, sex and genetic background (C57\BL) were examined.

By morphological criteria, sections of mature FGF-7-null kidneys could be consistently distinguished from wild type by the presence of a thin papilla, the portion of the kidney that contains the most distal collecting system segments (Fig. 2A,B). The distal collecting system of wild-type kidneys located at the tip of the renal papilla is lined by a high cuboidal to columnar epithelium (Fig. 2C). The papillary collecting tubules of mutant kidneys were few, as reflected by the thin papillary diameter (Fig. 2B). In contrast to the wild-type kidney, collecting tubule epithelia at the papillary tip of FGF-7-null kidneys exhibited a low cuboidal morphology (Fig. 2E).

Although the size and cytoarchitecture of the renal collecting system of FGF-7-null animals is markedly different from wild type, these animals were able to concentrate urine to 3,000 mOsm, identical to wild type (Schmidt-Neilsen and O'Dell, 1961). These physiological criteria indicate that FGF-7-null kidneys possess collecting tubules that can concentrate urine. Using antibodies directed against a collecting tubule-specific water channel, Aquaporin-3 (Ecelbarger et al., 1995), we found that FGF-7-null kidneys exhibit tubules with protein expression patterns consistent with water reabsorbing, mature collecting tubules (Fig. 2D,F).

In addition to a small collecting system size, the cortical zone of FGF-7-null kidneys was thinner than wild type (Fig. 2A,B). The cortical zone of the kidney contains proximal nephron segments and glomeruli; the thinned cortical zone of FGF-7-null kidneys suggests that these animals may possess

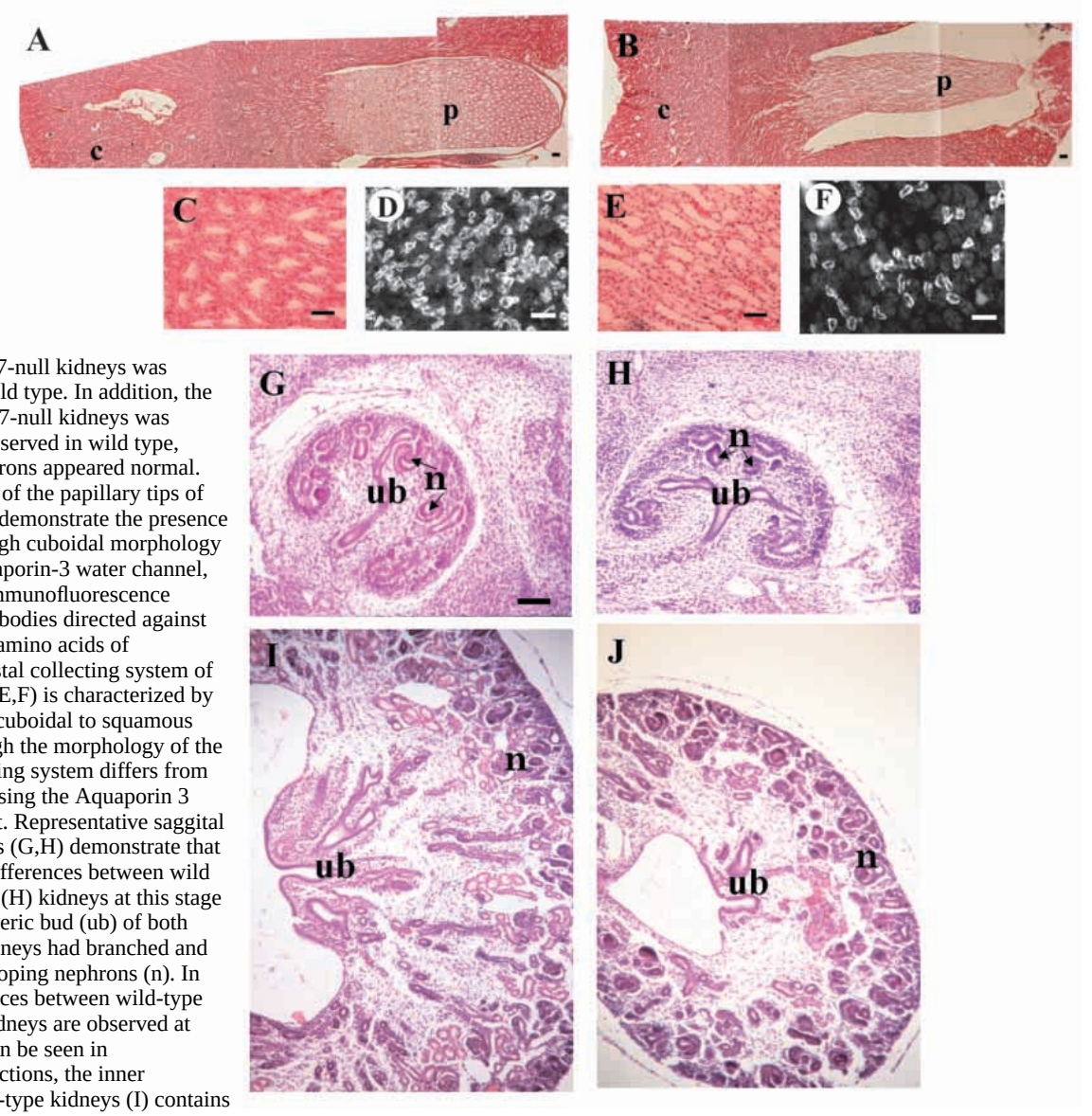

Fig. 2. The kidneys of FGF-7-null mice exhibit morphological abnormalities. Representative sections of kidneys from age-matched C57/BL wild-type and FGF-7-null mice. At 6 months of age, wild-type kidneys (A) could be readily distinguished from FGF-7-null kidneys (B). The renal papilla (p) of FGF-7-null kidneys was markedly thinner than wild type. In addition, the cortical zone (c) of FGF-7-null kidneys was markedly thinner than observed in wild type, although individual nephrons appeared normal. High power examination of the papillary tips of wild-type kidneys (C,D) demonstrate the presence of tubules exhibiting a high cuboidal morphology (C) that express the Aquaporin-3 water channel, as detected by indirect immunofluorescence microscopy utilizing antibodies directed against the 26 carboxy-terminal amino acids of Aquaporin 3 (D). The distal collecting system of the FGF-7-null kidneys (E,F) is characterized by tubules exhibiting a low cuboidal to squamous morphology (E). Although the morphology of the FGF-7-null distal collecting system differs from wild type, tubules expressing the Aquaporin 3 water channel are present. Representative saggital sections of E13.5 kidneys (G,H) demonstrate that there are no detectable differences between wild type (G) and FGF-7-null (H) kidneys at this stage of development. The ureteric bud (ub) of both wild-type and mutant kidneys had branched and was surrounded by developing nephrons (n). In contrast, marked differences between wild-type (I) and FGF-7-null (J) kidneys are observed at gestation day 16.5. As can be seen in representative saggital sections, the inner medullary region of wild-type kidneys (I) contains an extensively branched ureteric bud network (ub). Nephrons are present in the cortical region (n). The kidneys of E16.5 FGF-7-null embryos (J) are smaller than wild type. Few ureteric bud branches (ub) are present in the inner medullary region, although nephrons (n) are forming in the cortex. Bars, 100 μm.

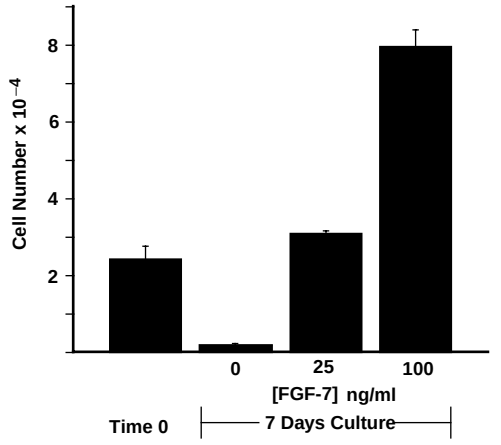

Fig. 3. FGF-7 supports the survival and growth of isolated ureteric buds. Ureteric buds were isolated from E13 rat kidney rudiments and cultured in the absence (control) or presence of given FGF-7 concentrations. The number of cells present in freshly isolated ureteric buds (Time 0) was compared to the cell number present in cultures maintained for 7 days in the presence of given FGF-7 levels. Each culture was established with 10 ureteric buds. Values are the average number of cells present in 5 separate cultures ± s.e.m.

fewer nephrons than wild type. The kidneys of six wild-type and six FGF-7-null animals were subjected to extensive morphometric analysis. Total kidney volume was calculated from serial sectioned material and nephron density determined by counting glomeruli, according to standard morphometric techniques (Bertram et al., 1992). Results of these analyses indicate that the average number of nephrons present in FGF-7-null kidneys was 30±6% fewer than the average number of nephrons present in wild-type age- and sex-matched control kidneys (Table 1). The decrease in nephron number observed in FGF-7-null animals is due to a decrease in total kidney tissue volume rather than to alterations in cortical nephron density (Table 1).

FGF-7 levels modulate kidney size during development in vivo

Detectable differences in FGF-7-null and wild-type embryonic kidney or ureteric bud size were not observed from E11.5 to E13.5 (data not shown). At E13.5, the ureteric bud of both FGF-7-null and wild-type embryonic kidneys had undergone approximately 3-4 rounds of branching and nephrogenesis was observed in the surrounding metanephric blastema (Fig. 2G,H). In contrast, on E16, the size of the ureteric bud compartment of FGF-7-null kidneys was markedly smaller than wild type (Fig. 2I,J). In representative wild-type kidney sections, the inner medullary region was characterized by an extensively branched ureteric bud or collecting system network. The inner medullary region of FGF-7-null E16.5 kidneys contained few ureteric bud branches. Furthermore, differences in the volume of FGF-7-null and wild-type embryonic kidneys were detected at this stage of development (Table 1). These data indicate that FGF-7 levels influence ureteric bud and kidney size during embryonic development.

Elevated levels of FGF-7 modulate ureteric bud growth, maturation and nephron number in vitro

Ureteric buds were isolated from E13 rat kidney rudiments and cultured with DMEM/10% FCS on nitrocellulose filters in the absence or presence of increasing concentrations of exogenous FGF-7. Although isolated ureteric buds cultured at the air/medium interface in DMEM/FCS died, ureteric buds maintained in the presence of exogenous FGF-7 formed a monolayer of viable cells that exhibited dose-dependent growth (Fig. 3). In addition, isolated ureteric buds suspended in a complex extracellular matrix, Matrigel, died unless supplemented with exogenous FGF-7. FGF-7 supported dose-dependent ureteric bud growth in Matrigel; however, normal branching morphogenesis did not occur (data not shown). Neither FGF-1, 2, 4 nor 5 at concentrations from 10-500 ng/ml supported isolated ureteric bud growth or viability in vitro (data not shown).

E12.5-13 rat kidney rudiments containing both the ureteric bud and surrounding metanephric mesenchyme were cultured in the absence or presence of 100 ng/ml FGF 1, 2, 4, 5 or 7 (data not shown). Only cultures maintained in the presence of FGF-7 exhibited gross alterations of the ureteric bud compartment. To study the effect of elevated FGF-7 levels further, metanephric kidney organ cultures were maintained in the absence of added FGF-7, transiently maintained with elevated levels of FGF-7 or maintained with elevated levels of FGF-7 for the entire culture period (Fig. 4). Examination of organ cultures stained with FITC-DB to visualize the ureteric bud compartment suggested that elevated levels of FGF-7 increased the size of the ureteric bud compartment during development in vitro (Fig. 4A,C,E,G,I). To confirm this qualitative observation, we counted the number of ureteric bud epithelia present in cultures maintained in the absence or presence of exogenous FGF-7 (Table 2). Although saturating concentrations of FGF-7 did not significantly change the total number of cells present in cultured rudiments, the number of ureteric bud epithelia identified by FITC-DB staining increased approximately 1.5- and 2.5-fold in cultures maintained with FGF-7 for 4 days and 10 days, respectively (Table 2). Thus, exogenous, elevated FGF-7 levels increased the size of the ureteric bud compartment during kidney development in vitro.

The total number of nephrons that formed in cultured kidney

Table 1. FGF-7-null kidneys are smaller than wild type

Age	Genotype	Volume (mm^3)	Glomeruli/ mm^3	Nephron number
6 months	+/+	89±6.8	318±37	28,000±855
	–/–	68±7.8	282±19	20,203±746
E16.5	+/+	9.53±1	nd	nd
	–/–	6.72±2	nd	nd

Kidneys from six FGF-7-null and six wild-type mice of the same sex and genetic background were analyzed at given ages. Each E16.5 embryo was from a different litter.

Values presented are the mean ± s.e.m.

Significant differences in nephron number were detected between FGF-7-null and wild-type kidneys at 6 months of age and between FGF-7-null and wild-type kidney volume at 6 months of age and on E16.5 (P<0.01; Student's t-test).

Table 2. Elevated levels of FGF-7 increase ureteric bud size in vitro

Culture conditions	Ureteric bud cell number
Control	5,145±549
Transient FGF-7	8,717.5±962
Continuous FGF-7	12,785±1,648

E12.5-13 rat kidney rudiments were cultured for 10 days in the absence of added FGF-7 (Control), in the presence of 100 ng/ml FGF-7 for the first 4 days of culture (Transient FGF-7) or with 100 ng/ml FGF-7 for the entire culture period (Continuous FGF-7). Rudiments were dissociated into single cell preparations, total rudiment cell number and number of FITC-Dolichos Bifloris-stained ureteric bud epithelia/rudiment counted. The average total cell number/rudiment was 57,000±5,230. Significant differences in total rudiment cell number were not observed. However, the number of ureteric bud epithelia increased in the presence of elevated FGF-7 levels.

Values are the average number of ureteric bud epithelia present in rudiments ± s.e.m. A minimum of 24 rudiments were analyzed for each culture condition.

rudiments was quantified by counting glomeruli tagged with Peanut Lectin Agglutinin (Gilbert et al., 1994). Nephron number was increased by 50±12% in cultures transiently exposed to exogenous FGF-7 as compared to cultures maintained without added factor (Figs 4B,D, 5). Thus, transient exposure of organ cultures to FGF-7 resulted in an increased renal collecting system size and a concomitant increase in nephron number.

Although continuous exposure of organ cultures to elevated levels of FGF-7 resulted in an increased number of ureteric bud epithelia, nephrogenesis was partially inhibited (Figs 4F, 5). Furthermore, ureteric bud architecture was markedly altered in cultures continuously exposed to FGF-7 (Fig. 4E,G). The ureteric bud of cultures transiently treated with factor or maintained without it, stained intensely with FITC-DB and exhibited an arborized network of slender tubules with terminal branched ampullae (Fig. 4A,C,I). Rudiments exposed to FGF-7 throughout the culture period exhibited a dilated, sac-like ureteric bud compartment that had variable intensities of DB staining (Fig. 4E,G). These results demonstrate that continuous exposure of kidney rudiments to elevated levels of exogenous FGF-7 alters the lectin staining properties and architecture of the developing renal collecting system in addition to limiting nephrogenesis.

Previous experiments in which FGF-7 levels were modulated in vivo or in vitro demonstrate that this growth factor alters the growth and differentiation of KGF-R-expressing epithelia (Guo et al., 1993; Alarid et al., 1994; Yi et al., 1995; Nguyen et al., 1996). Thus, we tested whether elevated levels of FGF-7 effect ureteric bud differentiation in vitro. The ureteric bud differentiates into mature collecting tubules, as indicated by the upregulation of mature collecting system proteins (Yamamoto et al., 1997). Proteins expressed by the mature collecting system such as Aquaporin-3, a water channel, are not expressed in the ureteric bud early in kidney development (Ecelbarger et al., 1995; Yamamoto et al., 1997). Later, collecting tubule differentiation antigens are expressed in the slender tubular portion of the ureteric bud but not at its immature, growing ampullary tips. Kidney rudiments cultured in the presence or absence of exogenous FGF-7 were assayed for Aquaporin-3 expression to determine if continuous, elevated levels of FGF-7 perturb ureteric bud differentiation into collecting tubule epithelia (Fig. 4H,J). In cultures maintained in the absence of added FGF-7, all DB-positive ureteric bud epithelia co-expressed Aquaporin-3 (Fig. 4I,J). Cultures maintained with elevated levels of exogenous FGF-7 exhibited a dilated, DB-positive ureteric bud network that did not label with antibodies directed against Aquaporin-3 (Fig. 4G,H). Collectively, these data indicate that elevated levels of FGF-7 in vitro stimulate ureteric bud growth and delay ureteric bud differentiation into the collecting system.

DISCUSSION

Comparative anatomical studies suggest that the extent of ureteric bud growth and branching during development is proportional to the number of nephrons that eventually form in the kidney (Oliver, 1968). Although several ligands, receptors and transcription factors, including GDNF, c-RET, Pax-2, BF-2 and WT-1, have been shown to be required for ureteric bud morphogenesis in vivo, animals lacking functional expression of these genes also exhibit severe defects in nephrogenesis (Kreidberg et al., 1993; Schuchardt et al., 1994; Torres et al., 1995; Hatini et al., 1996; Pichel et al., 1996; Moore et al., 1996; Sanchez et al., 1996). Thus, the relationship between ureteric bud growth and final nephron number within the kidney is difficult to address using these animal models.

This relationship can be assessed by experimentally manipulating the levels of a growth factor that directly modulates ureteric bud growth during kidney development. *FGF-7* mRNA has been detected in the developing kidney by E14.5 and *KGF-R* mRNA is expressed specifically by the developing ureteric bud and collecting system throughout kidney morphogenesis (Fig. 1; Mason et al., 1994; Finch et al., 1995). In vitro experiments presented in this study demonstrate that FGF-7 can substitute for metanephric mesenchyme in keeping isolated ureteric buds viable and growing. Ureteric bud survival and growth in vitro appears to be specific for FGF-7. No other members of the FGF family tested, including FGF-1, FGF-2, FGF-4 or FGF-5, supported isolated ureteric bud viability or growth. Recent evidence indicates that FGF-10 binds with high affinity to KGF-R and supports lung bud branching in vitro (Igarashi et al., 1998; Bellusci et al., 1997). However, we were unable to test this member of the FGF family since it is not commercially available. Further metanephric kidney organ culture experiments demonstrated that continuous, elevated levels of FGF-7 delay the differentiation of the ureteric bud into collecting tubule epithelia, as determined by the inhibition of Aquaporin-3 expression. Thus, FGF-7 plays a role in ureteric bud epithelial survival, proliferation and differentiation in vitro. FGF-7 binds with high affinity to only one known fibroblast growth factor receptor, KGF-R; thus activation of KGF-R most likely mediates FGF-7-dependent ureteric bud growth in vitro (Rubin et al., 1995; Ornitz et al., 1996). FGF-1 also binds to KGF-R; however, this member of the FGF family did not support ureteric bud growth or viability in vitro, perhaps due to the absence of heparin-cofactors that are known to mediate FGF-1 binding (Green et al., 1996).

Although FGF-7 is required for isolated ureteric bud

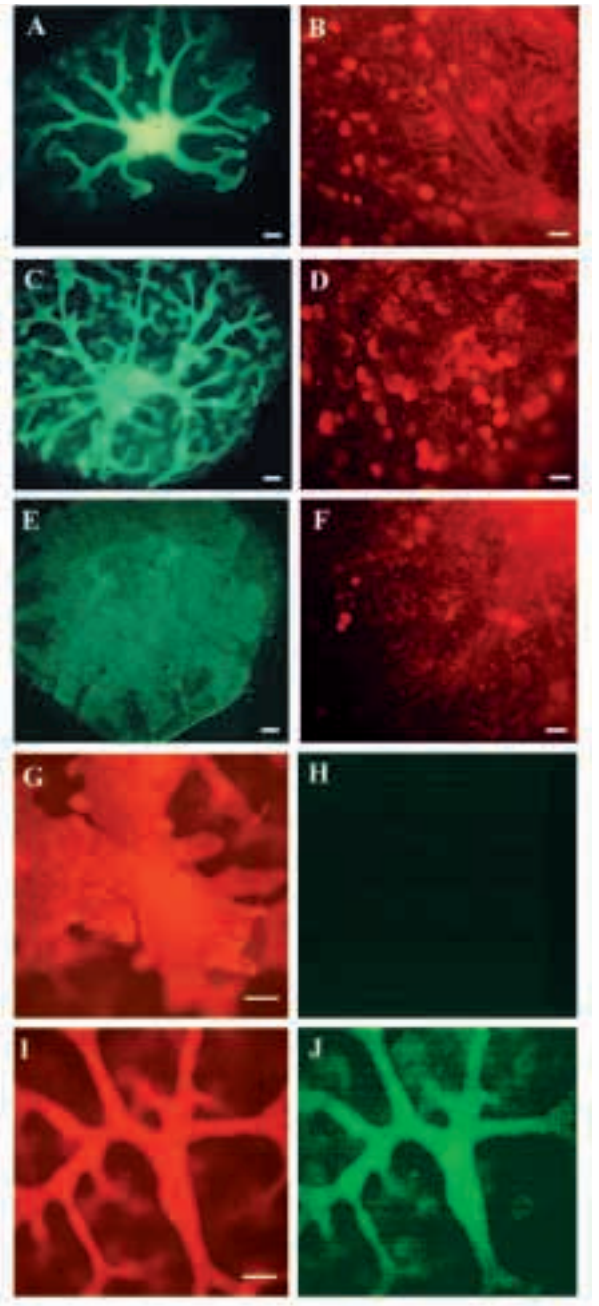

Fig. 4. Exogenous FGF-7 modulates ureteric bud differentiation, growth and nephron number in vitro. E12.5-13 rat kidney rudiments containing both the ureteric bud and surrounding metanephric blastema were cultured for 10 days in the absence of added FGF-7 (*n*=24; A,B,I,J), in the presence of 100 ng/ml exogenous FGF-7 for the first 4 days of culture (transient FGF-7; *n*=24; C,D), or with the added factor for the entire culture period (continuous FGF-7; *n*=24; E-H). Cultures were fixed, permeabilized and processed for visualization of the ureteric bud network by FITC- (A,C,E,) or RITC- (G,I) Dolichos Bifloris (DB) staining. Glomeruli were dectected by RITC-Peanut Lectin staining (B,D,F). Aquaporin 3 was detected by immunofluorescence microscopy using antibodies directed against the carboxy-terminal 26 amino acids of the protein and FITC-labeled secondary antibody (H,J). The ureteric bud network of representative control (A,I) and transiently treated cultures (C) is characterized by slender tubules with dilated, branched terminal ampullae. Incubation with elevated levels of FGF-7 for the entire culture period results in ureteric bud dilation and an absence of slender tubules (E,G). Elevated levels of FGF-7 also modulated the number of nephrons that form in vitro (see Fig. 5). More glomeruli were present in cultures transiently treated with FGF-7 (D) than control cultures maintained without added factor (B). Glomerular number was dramatically decreased in cultures maintained with elevated levels of FGF-7 for the entire culture period (F). In addition, the RITC-DB-positive ureteric bud network did not express detectable levels of Aquaporin-3, a collecting system terminal differentiation antigen (G,H). In control cultures, RITC-DB and Aquaporin-3 antibody staining was co-localized to the ureteric bud network (I,J). Bars, 100 μm (A-F); 30 μm (G-J).

viability and growth in vitro, it is clear that other factors are able to support these processes in vivo. *FGF-7* mRNA has not been detected in the developing urogenital system until E14.5. Thus, ureteric bud growth from E11.5-13.5 is regulated by factors other than FGF-7. In addition, FGF-7-null mice develop a renal collecting system, although it is markedly smaller than in wild type. A marked decrease in size of the FGF-7-null renal collecting system was observed on E16.5. In addition, the volume of FGF-7-null kidneys was significantly smaller than that of wild-type kidneys at this stage of development. We did not detect an increased number of apoptotic ureteric bud cells in FGF-7-null embryonic kidneys as compared to wild type. Thus, the decreased size of the FGF-7-null ureteric bud and renal collecting system appears to be due to less extensive growth during development. Collectively, these data suggest that FGF-7 is required for the full extent of ureteric bud growth that occurs in the kidney during development. However, it is likely that other members of the FGF gene family expressed in the developing kidney can substitute for FGF-7, as transgenic mice expressing a soluble dominant-negative FGF-receptor (FGFR2-b) construct exhibit severe renal abnormalities, including renal agenesis and dysgenesis (Celli et al., 1998).

In this study, we discovered that FGF-7-null animals exhibit

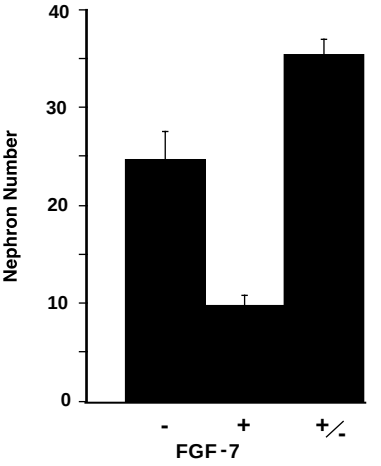

Fig. 5. Exogenous FGF-7 modulates the number of nephrons that form in organ culture. Metanephric kidney rudiments were cultured as described in Fig. 4 and nephron number determined by counting RITC-Peanut Lectin-labeled glomeruli. –, Control (no FGF-7); +, Continuous FGF-7; ±, Transient FGF-7. Nephron number, expressed as the mean ± s.e.m., was determined for a minimum of 15 rudiments/culture condition.

a reduced number of nephrons in addition to having a small collecting system size. Conversely, we observed that kidney rudiments transiently exposed to elevated levels of FGF-7 exhibited an increase in nephron number and collecting system size. These results demonstrate that the extent of ureteric bud growth during development is proportional to nephron number, and support previous comparative anatomical studies (Oliver, 1968). When metanephric kidney organ cultures were continuously exposed to elevated levels of FGF-7 nephrogenesis was partially inhibited. We are currently unable to determine whether elevated levels of FGF-7 inhibit nephrogenesis in vitro, however, by directly perturbing nephron progenitor differentiation or by perturbing the ability of the ureteric bud to initiate nephrogenesis.

In conclusion, we show that transient exposure of metanephric kidney rudiments to elevated levels of FGF-7 results in augmented ureteric bud growth and increased nephron number. As compared to wild-type animals, FGF-7-null animals exhibit reduced ureteric bud growth during development, and at maturity have a small renal collecting system and decreased nephron number. Thus, the phenotype of FGF-7-null animals is one of kidney size, not differentiation. This finding has broad clinical implications as slight inborn deficits of nephron number are predictive of renal failure (Brenner and Milford, 1993; MacKenzie and Brenner, 1995). Analysis of renal function in FGF-7-null animals as they age will be a useful model system with which to elucidate the mechanisms by which in-born nephron deficits predispose the kidney to disease.

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