Inhibition of bFGF-receptor type 2 increases kidney damage and suppresses nephrogenic protein expression after ischemic acute renal failure

Sandra Villanueva, Carlos Cespedes, Alexis A. Gonzalez, Eric Roessler, and Carlos P. Vio

Laboratorio de Fisiología, Pontificia Universidad Católica de Chile, Santiago; Laboratorio de Fisiología Integrativa y Molecular, Universidad de Los Andes, Santiago; and Laboratorio de Nefrología, Pontificia Universidad Católica de Chile, Santiago, Chile

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Villanueva S, Cespedes C, Gonzalez AA, Roessler E, Vio CP. Inhibition of bFGF-receptor type 2 increases kidney damage and suppresses nephrogenic protein expression after ischemic acute renal failure. Am J Physiol Regul Integr Comp Physiol 294: R819–R828, 2008. First published January 9, 2008; doi:10.1152/ajpregu.00273.2007.—Recovery from acute renal failure (ARF) requires the replacement of injured cells by new cells that are able to restore tubule epithelial integrity. We have recently described the expression of nephrogenic proteins (Vimentin, neural cell adhesion molecule, basic fibroblast growth factor (bFGF), Pax-2, bone morphogen protein-7, Noggin, Smad 1-5-8, p-Smad, hypoxia-inducible factor-1α, vascular endothelial growth factor), in a time frame similar to that observed in kidney development, after ischemic ARF induced in an ischemia-reperfusion (I/R) model. Furthermore, we show that bFGF, a morphogen involved in mesenchyme/epithelial transition in kidney development, induces a reexpression of morphogenic proteins in an earlier time frame and accelerates the recovery process after renal damage. Herein, we confirm that renal morphogenes are modulated by bFGF and hypothesized that a decrease in bFGF receptor 2 (bFGFR2) levels by the use of antisense oligonucleotides diminishes the expression of morphogenes. Male Sprague-Dawley rats submitted to ischemic injury were injected with 112 μg/kg bFGFR2 antisense oligonucleotide (bFGFR2-ASO) followed by reperfusion. Rats were killed, and the expression of nephrogenic proteins and renal marker damage was analyzed by immunohistochemistry and immunoblot. Animals subjected to I/R treated with bFGFR2-ASO showed a significant reduction in morphogen levels (P < 0.05). In addition, we observed an increase in markers of renal damage: macrophages (ED-1) and interstitial α-smooth muscle actin. These results confirm that bFGF participates in the recovery process and that treatment with bFGFR2-ASO induces an altered expression of morphogen proteins.

We have hypothesized that renal regeneration may recapitulate part of the kidney genetic program elicited during organogenesis, including apoptosis (2, 36). In this context, we have described the reexpression of several morphogenes in the regeneration phase of ATN in a temporal pattern similar to that observed during kidney development (36). These observations open the possibility that morphogenes could be involved in the recovery process.

The nephrogenic proteins reported after ischemic ATN are the mesenchymal proteins Ncam (1), WT-1 (42), and Vimentin (43) and the epithelial proteins Pax-2 (8, 17, 29), which play a crucial role during early metanephric development (29). Similarly, bone morphogen protein 7 (BMP-7) (11), its antagonist Noggin (7, 35), and their transcription factors Smad 1 and 5 (21) are first expressed by embryo mesenchymal cells, are downregulated in the adult (41), and are reexpressed in the recovery phase of ischemic ATN induced by I/R in the same sequential pattern as that observed during kidney development (36).

An important protein secreted at early stages of kidney development is the basic fibroblast growth factor (bFGF). bFGF is necessary to promote mesenchymal cell condensation (18), inhibition of apoptosis, transition epithelial-mesenchymal (34) and early tubulogenesis (27, 30).

Previously, we have evaluated the effect of bFGF in the recovery of ATN using a recombinant protein. We reported that kidney damage was reduced, and the reexpression pattern of the morphogenes and transcription factors analyzed was earlier in time and greater in intensity. These results show that bFGF can be involved in the recovery process and suggest that a bFGF treatment can accelerate the repair after ischemic damage in rat kidneys (37).

In the present study, we hypothesized that a decrease in bFGF receptor 2 (bFGFR2) levels, using an antisense oligonucleotide (ASO), will diminish the expression of morphogenes and that this deregulation would contribute to the delay of morphogen expression, impairing the recovery of kidney submitted to I/R.

MATERIALS AND METHODS

Animals. Adult male Sprague-Dawley rats (220–250 g) were housed and maintained at the University animal care facilities; food and water were supplied ad libitum in a 12:12-h light-dark cycle. All experimental procedures were in accordance with institutional and international standards for the human care and use of laboratory animals (Animal Welfare Assurance Publication A5427-01, Office for

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Protection from Research Risks, Division of Animal Welfare, National Institutes of Health) as previously described (36, 37). All protocols were reviewed and approved by the Universidad P Universidad Católica de Chile.

Renal I/R injury and bFGFR2-ASO treatment. An established model of renal I/R injury was performed (36, 37); this mimics structural and functional consequences of renal ischemia, including the presence of apoptotic tubular epithelial cell (4). Animals (n = 5 for each I/R group) were anesthetized with ketamine-xylazine (25:2.5 mg/kg ip), and body temperature was maintained at 37°C. Both kidneys were exposed by a flank incision, and both renal arteries were occluded with nontraumatic vascular clamps for 30 min. After 30 min of clamping, the left kidney was injected intrarenally with bFGFR2-ASO (112 μg/kg) (9) in a total volume of 200 μl and was considered as treated; the right kidney was injected with the same volume of saline and served as a control. After the injection, clamps were removed, renal blood flow was reestablished, and the incisions were sutured. A group of sham animals (n = 5) was included; these animals were subjected to the same surgical procedure and conditions, without clamping the renal arteries. A third group was included corresponding to animals injected intrarenally with bFGFR2-ASO in both kidneys; these animals were used to analyze renal function. Rats were allowed to recover in a warm room with water and food ad libitum. The rats were killed under anesthesia (ketamine-xylazine) at 24, 48, 72, and 96 h after reperfusion; both kidneys were removed and processed for immunohistochemistry and Western blot.

Fig. 1. Effect of antisense oligonucleotide (ASO) on basic fibroblast growth factor receptor 2 (bFGFR2) expression. PCR and Western blot were performed in kidney samples collected at 24, 48, 72, and 96 h after ischemia-reperfusion (I/R; n = 5 for each I/R group). Kidneys were injected with saline (I/R + S) or with bFGFR2-ASO (I/R + bFGFR2-ASO). An important inhibition in the bFGFR2 mRNA and protein was observed in kidney treated with ASO compared with kidneys injected with saline at 24 to 96 h after I/R. GAPDH, glyceraldehyde-3-phosphate dehydrogenase.

Fig. 2. Visualization of bFGFR2-ASO. The visualization of bFGFR2-ASO was performed using streptavidin peroxidase conjugate and was developed with 3,3′-diaminobenzidine (DAB). The nuclear stain in kidneys injected with bFGFR2-ASO was observed at 24 (B), 48 (C), 72 (D), and 96 (E) h after I/R. The nuclear stain was not observed in control kidneys injected with saline (A). Scale bar = 25 μm. The arrows indicate localization of nuclear stain for each time point.
**ASO treatment.** ASO phosphorothioate including 23 bases (TGTT-TGGCAGGACAGTGAGCCA) was synthesized by solid phase and biotinylated. The sequence was designed to hybridize with the 3'-region of the rat bFGFR2 mRNA promoter. The lyophilized product was diluted in 0.9% saline sodium chloride and injected in the medulla area with a 30-gauge needle (37). A dose of 112 μg/kg was administered in a total volume of 200 μl. Preliminary data using methylene blue dye or Bouin’s solution demonstrated that the volume used allowed extensive diffusion through the tissue.

The visualization of ASO was performed using streptavidin peroxidase conjugate and was developed with 3,3’-diaminobenzidine (DAB).

**Tissue processing and immunohistochemical analysis.** Immunohistochemical studies in Paraplast-embedded sections were performed as previously described (36, 37, 38). For cryosections, the kidney sections (3–4 mm thick) were processed as recently described (36).

Immunolocalization studies were performed using an indirect immunoperoxidase technique (36, 37). Briefly, the tissue sections were incubated with the primary antibody overnight at room temperature, followed by incubation with the corresponding secondary antibody and with the peroxidase-antiperoxidase (PAP) complex. Peroxidase activity was detected by incubation of the sections with 0.1% (wt/vol) DAB and 0.03% (vol/vol) hydrogen peroxide. For some specific antibodies, immunoreactivity was revealed using a secondary antibody conjugated to alkaline phosphatase in the presence of nitroblue tetrazolium/5-bromo-4-chloro-3-indolyl-phosphate (4.5:3.5 ml). Preliminary data using methylene blue dye or Bouin’s solution demonstrated that the volume used allowed extensive diffusion through the tissue.

**Protein extraction and immunoblotting.** Whole kidney sections (~1 mm thick) were homogenized as previously described (36), and the protein concentration was determined through the Bradford method with the reagent obtained from Bio-Rad (Richmond, CA).

**Fig. 3. Evidence of tissue damage in hypoxic kidney induced by I/R.** Immunohistochemistry was performed in kidney samples collected at 96 h after I/R (n = 5 for each I/R group) in sham kidney (A–C) injected with saline (D–F) or with bFGFR2-ASO (G–I). A clear induction of renal damage markers such as macrophages (ED-1) (H), or myofibroblast α-smooth muscle actin (α-SMA) (I) was observed in the interstitial space from the inner and outer medulla in kidney injected with bFGFR2-ASO. This stain was also observed with lower intensity in kidney injected with saline (E and F). Staining for ED-1 and α-SMA was performed using peroxidase and was revealed with DAB (brown color reaction). The tissue damage was evaluated by periodic acid-Schiff (PAS) staining. PAS staining (A, D, and G) and brush-border and epithelial flattening are shown in kidney injected with bFGFR2-ASO (H). Indicating that I/R can prolong the renal damage in kidney injected with bFGFR2-ASO. Opposite results were observed in kidney injected with saline at 96 h (D), with morphology closer to normal kidney (A). Scale bar = 100 μm. The arrows indicate the localization of the corresponding marker for each panel.
Western blotting was performed as described by Harlow and Lane (16). For SDS-PAGE, proteins were mixed with sample buffer (100 mM Tris-HCl, pH 6.8, 200 mM dithiothreitol, 4% SDS, 0.2% bromphenol blue, and 20% glycerol), transferred to nitrocellulose membranes, and blocked as previously described (36). After being blocked, the membranes were probed with the corresponding antibody, washed with Tris-buffered saline-0.1% Tween 20, and incubated with horse-radish peroxidase-conjugated secondary antibody for 1 h at room temperature. Immunoreactivity was detected using the enhanced chemiluminescence technique obtained from Perkin-Elmer, Life Sciences (Boston, MA). The positive controls for all nephrogenes were obtained from sham-operated rat kidneys.

Detection and quantification of renal cell apoptosis by “in situ” end labeling of fragmented DNA. Apoptotic cells in kidney tissue slices were visualized using the Apop Tag Fluorescein In Situ Apoptosis Detection Kit by the indirect TUNEL method from Chemicon (Temecula, CA) following the manufacturer’s protocol.

Determination of functional and tissue damage. As previously described (36), functional damage was assessed through serum creatinine levels (33), and tissue damage was evaluated using periodic acid-Schiff (PAS) staining and immunolocalization of interstitial macrophages (ED-1) and myofibroblasts (α-SMA).

The interstitial α-SMA immunoreactive area was determined by image analysis using Simple PCI software from Compix (Crannberry Township, PA). The tissue sections were observed and photographed using a Nikon optiphot microscope and dxm 1200 digital camera (Nikon, Tokyo, Japan). The values corresponding to total immunostained (brown) cells were averaged and expressed as the mean absolute values and the mean percentage of stained cell area per field (0.064 mm²) with a modification of a previously described method [40].

Statistical analysis. The differences were assessed with the non-parametric test of Mann-Whitney for pairwise comparisons when overall significance was detected. The significance level was defined at P < 0.05.

RESULTS

Effect of ASO on the bFGFR2 expression. Levels of bFGFR2 mRNA and protein were analyzed by RT-PCR and Western blot in kidney submitted to I/R and injected with bFGFR2-ASO or saline. After 24 h of I/R, an important inhibition of bFGFR2 mRNA and protein was observed in kidney treated with bFGFR2-ASO compared with kidneys injected with saline (Fig. 1). This inhibition was maintained at 48, 72, and 96 h after I/R (Fig. 1).

Visualization of bFGFR2-ASO. The visualization of bFGFR2-ASO was performed using streptavidin peroxidase conjugate and was developed with DAB. We observed a nuclear stain in kidneys injected with bFGFR2-ASO at 24 to 96 h after I/R. This stain was not observed in control kidneys injected with saline (Fig. 2).

Determination of tissue damage. Renal functional damage by I/R was assessed by serum creatinine levels, which in rats subjected to the I/R protocol and injected with saline was 0.6 ± 0.1, 0.4 ± 0.1, 0.4 ± 0.05, and 0.4 ± 0.02 mg/dl at 24, 48, 72, and 96 h, respectively. In animals subjected to the I/R protocol and injected with FGFR2-ASO, the creatinine levels observed were higher: 1.1 ± 0.5, 0.9 ± 0.1, 0.9 ± 0.2, and 0.9 ± 0.3 mg/dl at 24, 48, 72, and 96 h, respectively.

The PAS staining of renal sections injected with saline showed a histological damage consistent with ATN at 24 h after I/R (data not shown); however, these alterations were not detected at 96 h after I/R (Fig. 3D) where we observed near-normal renal histology (Fig. 3A). In contrast, the bFGFR2-ASO-treated kidney showed an altered morphology at 24 h after I/R that was maintained to 96 h after I/R; these alterations were characterized by flattening of the brush-border epithelia and were consistent with ATN (Fig. 3G).

The renal damage markers showed an increased number of macrophages (ED-1) and interstitial α-SMA at 24 and 96 h after I/R in bFGFR2-ASO-treated kidneys (α-SMA: 18,839 μm² and ED-1: 3,132 μm²; Fig. 3, H and I). Conversely, kidneys injected with saline showed a lower number.
Detection of markers induced by hypoxia: HIF-1α and VEGF. As reported previously, following renal artery clamping and injection with saline, a strong nuclear accumulation of HIF-1α occurred within the first 30 min. HIF-1α staining increased and peaked at 48 h after I/R in the kidney injected with saline (Figs. 5.1A and 6C); from 48 h onward, the number of HIF-1α positive cell nuclei steadily declined, disappearing at 96 h (Fig. 5.1A). In kidneys treated with bFGFR2-ASO, HIF-1α immunostaining was observed at 24–48 h after I/R, but with a reduction in the number of positive cells and shorting in the staining area (Fig. 6E). The expression of HIF-1α was observed in papillary collecting ducts, the thick ascending limb, and proximal tubular cells, which are mainly localized in the inner and outer medulla of kidneys (Fig. 6, A, C, and E).

VEGF is an angiogenic signaling molecule induced by hypoxia. VEGF showed a marked expression 24 h after I/R in the kidney injected with saline (Fig. 5.1B and 6D) and de-

![Fig. 5. Immunoblot of nephrogenic proteins in acute renal failure (ARF) induced by I/R. 5.1: expression of endothelial cell markers in kidney regeneration was studied by immunoblot for proteins: hypoxia-inducible factor (HIF)-1α (A) and vascular endothelial growth factor (VEGF, B) at 24, 48, 72, and 96 h after 30 min ischemia in kidney injected with saline or bFGFR2-ASO. An increase in HIF-1α and VEGF levels was observed in kidney injected with saline, with a maximum at 48 h, followed by a decrease that reached control levels at 96 h. Kidneys injected with bFGFR2-ASO showed a reduced expression of these markers at 24 and 48 h after I/R, followed by an increase at 72–96 h after I/R, but with levels that are lower than control kidney injected with saline. 5.2: Expression of metanephric mesenchymal (MM) and early epithelial markers in kidney regeneration was studied by immunoblot for morphogenic proteins neural cell adhesion molecule (Ncam, C), Pax-2 (D), and Vimentin (E). Samples were collected at 24, 48, 72, and 96 h after ischemia. Kidneys injected with saline showed an increased expression of MM markers Ncam, Pax-2, and Vimentin with a maximum at 48 h after ischemia. Expression levels of these markers decreased at 72 h and returned to basal level at 96 h. Kidneys injected with bFGFR2-ASO showed a reduced expression of these markers at 24 and 48 h after I/R. Only after 72–96 h after I/R was an increase in expression observed, which is inferior to that observed in kidney injected with saline. 5.3: expression of epithelial and tubular markers in kidney regeneration was studied by immunoblot for morphogenic proteins bone morphogen protein (BMP)-7 (F) and bFGFR2 (G). Samples were collected at 24, 48, 72, and 96 h after kidney ischemia. An increase in epithelial and tubular proteins can be observed with a maximum at 48 h and a decrease at 72 h after ischemia in kidney injected with saline. In kidney injected with bFGFR2-ASO was observed as diminishing in these markers at 24 h, maintained at 48 h after I/R, followed by a decrease at 72–96 h after I/R. The expression observed in kidney injected with bFGFR2-ASO was inferior that observed in kidney injected with saline. 5.4: expression of the transcription pathway for BMP-7 was studied by immunoblot for morphogenic proteins BMP-7 (H), Noggin (I), and the 1-5-8 nonphosphorylated (J) and phosphorylated (K) form of the transcription factor Smad. Samples were collected at 24, 48, 72, and 96 h after kidney ischemia. A maximum increase in BMP-7 and Noggin protein levels was observed at 24 h followed by a decrease at 72–96 h after ischemia in kidney injected with saline. In contrast, kidneys injected with bFGFR2-ASO showed decreased expression of both markers at 24 h, with a peak at 24–48 h followed by a limited increase at 72–96 h after I/R. Expression levels of kidney injected with bFGFR2-ASO are inferior to that observed in kidneys injected with saline. Smad proteins show an increase with a maximum at 48–72 h and decreased at 96 h after ischemia in kidneys injected with saline. However, kidneys injected with bFGFR2-ASO showed undetectable protein levels (n = 5 for each I/R).
In kidneys injected with saline, the highest expression after I/R was observed at 24–72 h for BMP-7, Ncam, and Noggin and at 48 h for bFGF. The expression of these proteins decreased at 96 h after I/R (Figs. 5.2, 5.3, and 5.4) in a time fashion similar to previous reports (36). These proteins were mainly localized to the inner and outer medulla; bFGFR and BMP-7 were localized to proximal tubule cells; Ncam and Noggin were localized in the peritubular area (Figs. 7, E and F, and 8, E and F).

In bFGFR2-ASO-treated kidneys, low expression for mesenchymal and epithelial markers Ncam, Pax-2, Vimentin, and Noggin was observed at 24 h after I/R and was maintained out to 72–96 h (Figs. 5.2 and 5.4). This expression pattern in the recovery phase was consistent with the maintenance of cellular damage (Figs. 7, I–L, and 8J). For the tubular markers BMP-7 and bFGFR, a downward expression was observed at each time point; in the case of BMP-7, proteins peaked at 72 h and decreased at 96 h after I/R (Figs. 5.3F and 8F). In summary, we observed that all morphogenic protein levels tested were diminished in kidneys treated with bFGFR2-ASO compared with kidneys injected with saline.

BMP-7, a survival factor for undifferentiated mesenchyme (12), is antagonized by Noggin protein (7, 35) and phosphorylates the transcription factors Smad 1 and 5 (21). The transcription factor Smad (total and phosphorylated) was studied in kidneys injected with bFGFR2-ASO, the expression level was decreased at 96 h after I/R (Figs. 5.3F and 8F). In the case of BMP-7, proteins peaked at 72 h and decreased at 96 h after I/R (Figs. 5.3F and 8F).

Levels of markers for differentiation in kidneys submitted to I/R. To study the effect of ATN induced by I/R on kidneys injected with bFGFR2-ASO or saline, freshly prepared extracts from inner/outer medulla kidney sections were analyzed with the corresponding antibodies to evaluate the expression of nephrogenic proteins. Western blots were run with all samples (n = 5) in each time period, and selected blots are shown as being representative from each group. Compared with kidney homogenates injected with saline, the kidneys treated with bFGFR2-ASO showed diminished protein levels similar to that observed by immunohistochemistry in proximal tubular cells (Fig. 5.1–5.4) (P < 0.05). In summary, at 24–48 h after I/R, diminished levels of HIF-1α, VEGF, bFGF, Ncam, Pax-2, Vimentin, Noggin, BMP-7, Smad 1-5-8, and p-Smad levels were representative from each group. Compared with kidney homogenates injected with saline, the kidneys treated with bFGFR2-ASO showed diminished protein levels similar to that observed by immunohistochemistry in proximal tubular cells.
were observed. This reduction was also observed in samples obtained 72–96 h after I/R (Figs. 5.1–5.4).

**DISCUSSION**

Embryonic kidney development is characterized by proliferation of undifferentiated cells, followed by later differentiation of daughter cells into specific cell phenotypes. A similar sequence of events can be observed during the regeneration process, opening the possibility that renal regeneration may recapitulate part of the kidney genetic program during organogenesis, including apoptosis (2, 36). To test this hypothesis, we have previously studied the effect of recombinant bFGF added exogenously in a model of ATN induced by I/R. Recombinant bFGF promoted acceleration in the repair process and prevented damage in kidney submitted to I/R (37). In this study, we performed complementary analysis and evaluated the effect of the inhibition of bFGFR2-ASO on the repair process and the subsequent morphogenic induction in kidney damage.

Apoptosis is a programmed mode of cell death that plays an important role in the pathogenesis of the ischemic ATN (25). Apoptosis and the presence of markers of kidney damage, including macrophages (ED-1), interstitial α-SMA, and PAS staining, are methods used to evaluate the cellular damage induced by ischemia (36). Ischemic kidneys treated with bFGFR2-ASO showed a significant increase in the number of tubular TUNEL positive cells, a strong immunoreactivity to ED-1 and α-SMA, and morphological characteristics in agreement with an important damage in tubular cells at 24 h and maintained at 96 h after I/R. This is significant, considering that in control kidneys these characteristics were maintained for only 24–48 h, indicating that damage was more extensive in the presence of bFGFR2-ASO. In addition, we did not detected mitosis when evaluated by PAS staining, in agreement with previous reports (36, 37). Together, these results suggest that bFGFR2-ASO may increase the renal tubular apoptosis triggered by ischemia. An important observation was that the number of TUNEL
positive cells was maintained, whereas the expression of morphogenetic proteins and cellular mitosis was inhibited. These data show that the inhibition of bFGF can increase apoptotic cell death leading to a delayed or incomplete recovery of the kidney. In addition, we showed that the reexpression of morphogenes can be induced only when cellular death is not present (36, 37).

Previous studies have demonstrated that hypoxia can induce the expression of specific proteins involved in the repair process (2, 36). The induction of HIF-1α is an early event in the sequence of cellular changes following the interruption of blood flow and has an important role in the initiation of subsequent reactions. In this study, the kidneys injected with bFGFR2-ASO showed lower levels of HIF-1α at 72–96 h after I/R; this was considerably later than previous reports (36, 37). HIF-1α may play an important role in the balance between cell death and/or survival and thus induce the expression of morphogenetic proteins to help in the recovery process (36).

Although ATN has been tightly linked to tubular epithelia cell injury, there are also vascular factors involved, as observed by the damage of peritubular capillaries in rats subjected to renal ischemia (3, 6, 26). Considering this data, we analyzed the expression of VEGF, which is known to be regulated by HIF-1α. VEGF is essential for vasculogenesis and angiogenesis and allows various cellular types to survive and proliferate under extreme stress conditions, such as hypoxia (15, 19, 20). A significant decrease in VEGF expression was detected in kidneys treated with bFGFR2-ASO when compared with previously reported data (36, 37). Because of the considerable impact of VEGF on angiogenesis (5), it is possible that bFGFR2-ASO could inhibit endothelial function and thus slow the repair process. The upregulation of VEGF by FGF has been described (27); therefore, the bFGFR2 inhibition by ASO may contribute to the reduction of VEGF levels.

Ncam, bFGF, and BMP-7 are known to play a crucial role during early metanephric kidney development (17). After kid-
ney injury induced by I/R, these proteins are locally restricted and reexpressed in the regenerating proximal tubules (36, 37) and are overexpressed in kidneys injected with recombinant bFGF (37). Our results in kidney injected with saline confirm the previous reports (17, 36), but in kidney treated with bFGFR2-ASO the abundance of these morphogens was lower. Because previous reports have shown an upregulation of BMP by FGF (12), it is possible that the inhibition of FGFR can reduce the expression of these proteins.

Vimentin, a marker of mesenchymal cells and fully dedifferentiated renal epithelia, is not present in healthy adult tubule, but its reexpression occurs during tubular regeneration and proliferation (36, 43). The low resulting levels of these proteins in kidneys treated with bFGFR2-ASO show that in kidney cells the nephrogenic proteins may be required for repair and that a state of differentiation similar to that present in renal early development (36, 37) is absent in kidney treated with bFGFR2-ASO.

An interaction between BMP and FGF has been reported in early development, and a reexpression of BMP and its antagonist Noggin after I/R has been described (36, 37). Herein, in kidneys treated with bFGFR2-ASO both BMP and Noggin were expressed in lower amounts. The relevance of this data is related to the regulatory function of Noggin and BMP. Noggin could be generating specific signals to determine differentiation of a particular cell type, or it could make cell groups sensitive to a specific morphogen, such as BMP. Furthermore, this indicates that Noggin may be involved in kidney development and in the regeneration process after kidney damage. In addition, previous reports have shown the upregulation of Noggin, BMP, and their transcription factors (Smad) by bFGF (10, 14, 24). Therefore, inhibition of bFGF by bFGFR2-ASO may explain the reduced expression of these proteins.

BMP signaling is mediated by Smad and Smad in kidney development (41). Smad and Smad are reexpressed after kidney damage, with a maximum in a time coincident with the highest levels of Noggin and BMP. Its expression decreases in a similar fashion as epitheliogenic proteins, similar to what has been reported in embryonic development (36). Based on the observed patterns of expression, we speculate that Smads may play specific roles in the recovery process of ATN during kidney damage, replicating its expression profile observed during kidney development.

Perspectives and Significance

Our results demonstrate that, during the recovery process, bFGF can be reexpressed to restore mature kidney function in a process similar to nephrogenesis during embryonic development. These findings suggest that the repair process is inhibited by the treatment with bFGFR2-ASO.

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