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Bone Morphogenic Protein-7 Induces Mesenchymal to Epithelial Transition in Adult Renal Fibroblasts and Facilitates Regeneration of Injured Kidney*

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In the kidney, a unique plasticity exists between epithelial and mesenchymal cells. During kidney development, the metanephric mesenchyme contributes to emerging epithelium of the nephron via mesenchymal to epithelial transition (MET). In the injured adult kidney, renal epithelia contribute to the generation of fibroblasts via epithelial-mesenchymal transition, facilitating renal fibrosis. Recombinant human bone morphogenic protein (BMP)-7, a morphogen that is essential for the conversion of epithelia from condensing mesenchyme during kidney development, enhances the repair of tubular structures in the kidney. In this setting, BMP-7 inhibits epithelial-mesenchymal transition involving adult renal epithelial tubular cells and decreases secretion of type I collagen by adult renal fibroblasts. In search of a mechanism behind the ability of BMP-7 to repair damaged renal tubules, we hypothesized that systemic treatment with BMP-7 might induce MET involving adult renal fibroblasts in the injured kidney, generating functional epithelial cells. Here we report that BMP-7 induces formation of epithelial cell aggregates in adult renal fibroblasts associated with reacquisition of E-cadherin expression and decreased motility, mimicking the effect of BMP-7 on embryonic metanephric mesenchyme to generate epithelium. In addition, we provide evidence that BMP-7-mediated repair of renal injury is associated with MET involving adult renal interstitial fibroblasts in mouse models for renal fibrosis. Collectively, these findings suggest that adult renal fibroblasts might retain parts of their original embryonic imprint and plasticity, which can be re-engaged by systemic administration of BMP-7 to mediate repair of tubular injury in a fibrotic kidney.

A distinction between epithelial and mesenchymal cells was appreciated in the late 19th century based on their morphology and topic organization (1). Interconversion of mesenchymal and epithelial cells during embryonic development via epithelial-mesenchymal transitions (EMT)[1] and mesenchymal to epithelial transition (MET) are well established mechanisms in the development of multicellular organisms (2). Capacity of epithelial and mesenchymal cells to interconvert during development is essential, because formation of mesenchymal cells from epithelial cells (EMT) enables these cells to migrate through an extracellular environment and settle in distinct areas to form organs (3, 4).

In adults, mesenchymal and epithelial cells have been traditionally considered as terminally differentiated (2, 3). However, more recent studies demonstrate that EMT is a common event among adult epithelial cells (3, 4). It has now been established that adult epithelial cells of the kidney, eye, lung, peritoneum, and colon can undergo EMT in chronic inflammatory diseases associated with fibrosis, in addition to EMT associated with carcinogenesis in a broad range of epithelia (3, 4). In animal models of kidney fibrosis, about 30% of fibroblasts derive via EMT, and inhibition of EMT can prevent the progression of fibrosis, demonstrating that EMT is an important contributor to the progression of renal disease (5).

The kidney has evolved as a model system to study embryonic and adult cellular plasticity between epithelia and mesenchyme (2). Kidney development depends on a program of reciprocal inductive interactions between two mesodermal derivatives, the ureteric bud and the metanephric mesenchyme (6, 7). In mice, at ~10.5 days postcoitum, the ureteric bud contacts the metanephric mesenchyme, inducing these cells to condense and aggregate (6). Subsequently, this induced population undergoes mesenchymal to epithelial transition (MET) to form a comma-shaped node, which elongates to form an S-shaped tubular epithelial structure (6, 7). Further, morphogenesis and differentiation of this S-shaped tubule results in the formation of the glomerulus, the distal and proximal tubule elements associated with the mature nephron (6). In the adult kidney, tubular epithelial cells can convert into fibroblast via EMT (8). During kidney fibrosis, conversion of renal tubular epithelial cells into myofibroblasts/fibroblasts is considered unfavorable, since it leads to disruption of polarized renal tubular epithelial layers and an increase in fibrotic scar formation (8). Therefore, EMT in the kidney is of significant interest as a therapeutic target, and in this regard, inhibition of EMT by systemic administration of bone morphogenic protein (BMP)-7,

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[1] The abbreviations used are: EMT, epithelial to mesenchymal transition; BMP, bone morphogenic protein; rhBMP-7, recombinant human bone morphogenic protein-7; FSP1, fibroblast-specific protein-1; MET, mesenchymal to epithelial transition; NTN, nephrotoxic serum nephritis; IMDM, Iscove's modified Dulbecco's medium.

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results in regression of established lesions in the kidney and improved renal function (9).

The family of BMPs contains several different members and in general, BMPs control morphogenic pathways at different stages of development (10, 11). BMP-7, also sometimes referred to as osteogenic protein-1, was originally identified as a potent osteogenic factor purified from bone (12). Different studies have demonstrated a specific role for BMP-7 during mammalian kidney development (13, 14). Although BMP-7 is expressed in many organized regions of the early embryo, defects in BMP-7-deficient mice are largely restricted to kidney and eye (13, 14). BMP-7-deficient mice die shortly after birth due to severe kidney dysplasia (13, 14). In the developing kidney, BMP-7 is expressed initially in the ureteric bud (15). As development proceeds, expression is next observed in the metanephric mesenchyme and then in the early tubules derived from the mesenchyme (15). BMP-7-deficient kidneys show a gradual cessation of nephrogenesis, associated with a reduction of ureteric bud and the loss of metanephric mesenchyme, suggesting that BMP-7 activities are essential for morphogenesis during the later stages of kidney development (13, 14). BMP-7 is considered a principal regulator of MET in kidney development and a survival factor for the metanephric mesenchyme (16, 17). In adults, BMP-7 is robustly expressed in the kidney (18). However, both acute and chronic renal injury are associated with substantial decrease in the expression of BMP-7 (19–21). Several recent reports unequivocally demonstrate that administration of exogenous recombinant human BMP-7 (rhBMP-7) enhances recovery of the kidney in animal models of acute and chronic renal injury (9, 19, 20, 22–24). These studies collectively suggest that the therapeutic effect of rhBMP-7 is due to its direct effect on tubular epithelial cells and interstitial fibroblasts (8). In previous studies, we demonstrate that BMP-7 can inhibit EMT involving tubular epithelial cells, resulting in potential repair of injured tubules (9). In addition, we demonstrate that rhBMP-7 can reduce secretion of type I collagen from adult renal fibroblast (24). However, the precise action and mechanism of BMP-7 activity on fibroblasts are still unknown. Here we report that BMP-7 can induce MET and potentially facilitate the repair of tubular epithelial structures in injured kidneys.

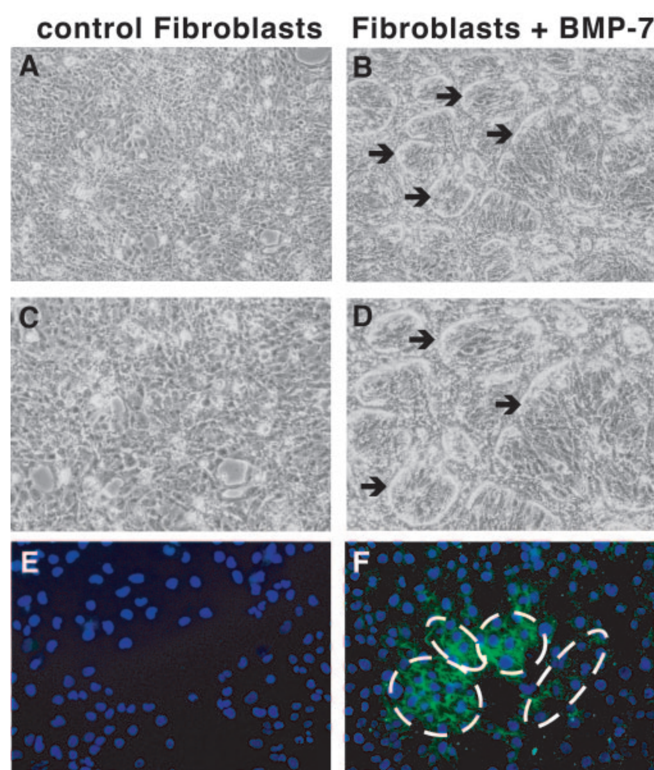

FIG. 1. **BMP-7 induces condensation of adult renal fibroblasts.** Human renal fibroblasts were grown for IMDM control medium (*A*, *C*, and *E*) or in medium containing 1000 ng/ml rhBMP-7 (*B*, *D*, *F*). BMP-7 treatment induced formation of cell aggregates within the confluent cell monolayer (*B* and *C*, *arrows*). *F*, fibroblasts within these islands displayed abundant expression of the epithelial marker E-cadherin (*green staining*) (the *circles* mark the outer border of the cell islands). *E*, without BMP-7 treatment, fibroblasts (4′,6-diamidino-2-phenylindole-stained nuclei are seen in *blue*), did not express E-cadherin. *A–D*, bright field microscopy. *E* and *F*, immunofluorescence staining. Original magnification was ×200 (*A* and *B*) or ×400 (*C–F*).

EXPERIMENTAL PROCEDURES

Cell Culture—The human renal fibroblast cell line TK173 was maintained in Dulbecco's modified Eagle's medium (Invitrogen) supplemented with 10% fetal bovine serum, 100 units/ml penicillin, and 100 units/ml streptomycin (25). The cells were cultured with 5% CO_2 and 95% humidity. For experiments, the medium was replaced with Iscove's modified Dulbecco's medium (IMDM; Invitrogen) without fetal bovine serum. In cell culture experiments, rhBMP-7, dissolved in 24 mM sodium acetate containing 1% mannitol, was used (Curis, Inc., Cambridge, MA) (24).

Induction of EMT-dependent Crescentic Nephritis—The protocol for inducing crescentic glomerulonephritis in mice, modified for studies to avoid rapid lethality from acute renal failure while ensuring adequate tissue injury for analysis, was used as described in our previous publication (9, 26). Briefly, CD1 mice weighing ~30 g were preimmunized by subcutaneous injection of 200 μg of normal sheep IgG (Sigma) in Freund's complete adjuvant (Sigma). After 5 days, experimental mice were injected intravenously with 50 μl of NTS for 3 consecutive days (NTS was a generous gift from Dr. Salant (Boston University)). NTS was generated by immunization of sheep with rat glomeruli as described previously (26). Control groups (n = 6 mice/group) were sacrificed 1, 3, and 6 weeks post-NTS injection to obtain representative kidney specimen corresponding to the time course of NTN. Treatment with 300 μg/kg BMP-7 was started at 3 weeks (n = 6 mice/group) postinduction of NTN. For *in vivo* studies, recombinant human BMP-7 homodimer, noncovalently attached to prodomain protein (referred to as soluble rhBMP-7) was used (Curis, Inc., Cambridge, MA) as described previously (9). Treated mice were sacrificed 6 weeks postinduction of NTN, and kidneys and plasma were obtained for future analysis.

Histologic Assessment of Renal Injury—Tissue of kidneys, liver, heart, spleen, and brain was fixed in 4% paraformaldehyde and embedded in paraffin. Sections were stained with hematoxylin and eosin, Masson's trichrome stain, and periodic acid-Schiff stain. Extent of renal injury was estimated by morphometric assessment of the tubulointerstitial injury and glomerular damage in a double-blinded manner. The relative interstitial volume was evaluated by morphometric analysis using a 10-mm² graticule fitted into the microscope. The relative interstitial volume was defined as the cortical region outside tubules and glomeruli (27). Five randomly selected cortical areas, which included glomeruli, were evaluated for each animal (27). The relative interstitial volume was defined as the cortical region outside tubules and glomeruli.

Immunocytochemistry—For immunocytochemistry, human renal fibroblasts (TK173) were seeded into 4-well chamber slides (Nalge Nunc International, Naperville, IL) and stimulated according to the experimental protocol. At the end of incubation, cells were washed twice with phosphate-buffered saline and fixed with ethanol/acetic acid (50:50, v/v) at −20 °C for 20 min (28). The cells were subsequently washed again, blocked with 3% bovine serum albumin in phosphate-buffered saline/Tween for 20 min at room temperature, and incubated for 2 h with primary monoclonal antibodies to E-cadherin (Transduction Laboratories). Cells were then washed again and incubated for 1 h at room temperature with fluorescein isothiocyanate-conjugated secondary antibodies (Jackson Immunoresearch). The stained cells were covered with Vectashield® mounting medium containing 4′,6-diamidino-2-phenylindole (Vector Laboratories, Burlingame, CA) and coverslips. The staining was visualized by fluorescence microscopy, and representative pictures were documented using Zeiss Axiovision Software.

Immunohistochemistry—Immunohistochemical staining was performed as described previously (9). Briefly, 4-μm sections were obtained using a cryostat. Sections were fixed in 100% acetone at −20 °C for 10 min before the sections were incubated with primary antibodies to FSP1 and E-cadherin (Sigma) at 4 °C overnight. Subsequently, the

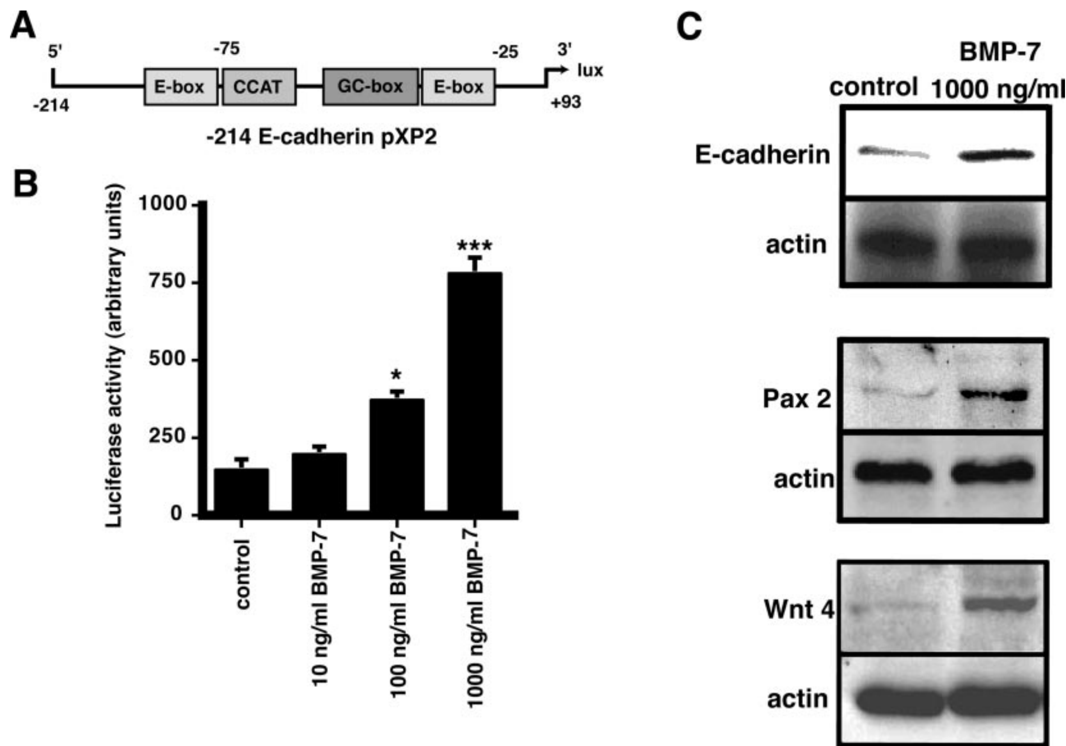

FIG. 2. **Regulation of E-cadherin expression by BMP-7 in adult renal fibroblasts.** *A*, schematic representation of the proximal E-cadherin promoter fragment (−214/+93), indicating the E-boxes, the CCAAT box, and the GC-rich region. *B*, adult renal fibroblasts (TK173) were transfected with the indicated promoter fragment construct, treated with BMP-7 (0.1–1000 ng/ml) for 24 h prior to lysis, and analyzed for luciferase activity. BMP-7 up-regulated E-cadherin promoter activity in a dose-dependent manner with a maximum induction at a concentration of 1000 ng/ml. *C*, adult renal fibroblasts were treated with BMP-7, and the expression of E-cadherin, Pax2, and Wnt4 was analyzed by immunoblot. The blots were stripped and reprobed with specific antibodies to actin to control for equal loading. BMP-7-mediated increase of E-cadherin protein was associated with increased expression of Wnt4 and Pax2. The experiments were repeated three separate times. Representative blots are shown here.

slides were washed three times in Tris-buffered saline and incubated with donkey-derived secondary antibodies conjugated with rhodamine or fluorescein isothiocyanate (Jackson Immunoresearch, West Grove, PA). After four washes with Tris-buffered saline, the slides were mounted with Vectashield mounting medium (Vector Laboratories) and coverslipped. The staining was analyzed using a fluorescence microscope (Zeiss). FSP1 antibody was a gift from Dr. Eric G. Neilson.

Analysis of Mouse E-cadherin Promoter Fragment Activity in Epithelial Cells—For analysis of mouse E-cadherin promoter activity, a fragment containing the regulatory region of the E-cadherin promoter was ligated into the pXP2 luciferase vector (29). This truncated construct (−214 E-cadherin pXP2) displayed enhanced specificity for epithelial cells compared with the full-length promoter fragment, which exhibits activity in epithelial cells as well as in fibroblasts (30, 31). This construct (−214Ecad/luc)was used to transfect TK173 adult renal fibroblasts, and stable clones were obtained. TK173 cells were co-transfected with 5 μg each of −214 E-cadherin pXP2 construct and pNeoβ-gal plasmid (Stratagene, La Jolla, CA) using a calcium phosphate co-precipitation method. Cells were tested for luciferase activity, staining with antibodies to luciferase (Promega, Madison, WI), and β-galactosidase activity was assessed by an *o*-nitrophenyl-β-galactopyranoside substrate-based assay kit (Stratagene). Single-cell-derived cell lines were established by limited dilution in selection medium that contained 1000 μg/ml G418 (31). A selection of cells that contained the −214 E-cadherin pXP2 construct was confirmed by staining with antibody to luciferase. Stable transfected TK173 cells were stimulated in IMDM that contained BMP-7 in a 6-well plate when they were 50–60% confluent for 24 h. Measurement of luciferase activity was performed using the Bright-Glo™ luciferase assay system (Promega) according to the manufacturer's recommendations.

Migration Assay—Migration assays using a Boyden chamber were performed as described previously (32). Briefly, polyvinyl-pyrrolidone-free polycarbonate membranes with 8-μm pores (Neuro Probes, Inc.) were coated with type I collagen on both sides (50 μg/ml). The bottom wells of chamber were filled with IMDM medium containing supplements according to the specific experimental protocol. Wells were covered with the coated membrane sheet, and 20,000-fibroblasts/well, which had been serum-starved for 24 h, were added into the upper chamber. The Boyden chamber was incubated for 6 h at 37 °C to allow possible migration of cells through the membrane into the lower chamber. Membranes were stained with Hema3® stain according to the manufacturer's recommendations (Biochemical Sciences, Inc., Swedesboro, NJ). Cells that migrated through the membrane were counted using a counting grid, which was fitted into an eyepiece of a phase-contrast microscope. All experiments were repeated at least three times.

Under-agarose Cell Spreading Assay—Under-agarose cell motility assays were performed as described with modifications (33). 6-Well plates were coated with fibronectin for 1 h at 37 °C. 1-cm-thick agarose gels (2% low melt agarose in Dulbecco's modified Eagle's medium) were cast into each well. After the gel had solidified, we punched a central hole (~1 cm) surrounded by four additional holes (~0.5 cm). Adult renal fibroblasts were stained with green (PKH67; Sigma) or red (PKH2626; Sigma) fluorescent dyes according to the manufacturer's recommendations. Equal uptake of the dyes was confirmed by fluorescence microscopy, before the cells were treated for 48 h with rhBMP-7 or maintained in serum-free IMDM control medium. 3000 cells/100 μl of serum-free medium were plated into the central cavity in the center of the gel, and the surrounding holes were each filled with 100 μl of medium containing 10% fetal calf serum to create a chemotactic gradient. Plates were incubated for 48 h in a cell culture incubator under standard conditions to allow the cells to migrate. The cells were fixed in 4% paraformaldehyde, and the cells were visualized under a fluorescent microscope (Zeiss Axiovert). Cells that migrated from the central hole underneath the gel were counted using a fluorescent microscope.

Statistical Analysis—All values are expressed as mean ± S.E. unless specified. Analysis of variance was used to determine statistical differences between groups using Sigma-Stat™ software (Jandel Scientific, San Rafael, CA). Further analysis was carried out using *t* test with Bonferroni correction to identify significant differences. A level of $p < 0.05$ was considered statistically significant.

RESULTS

BMP-7 decreases the expression of type I collagen in adult renal fibroblasts (24). In experiments here, treatment of adult

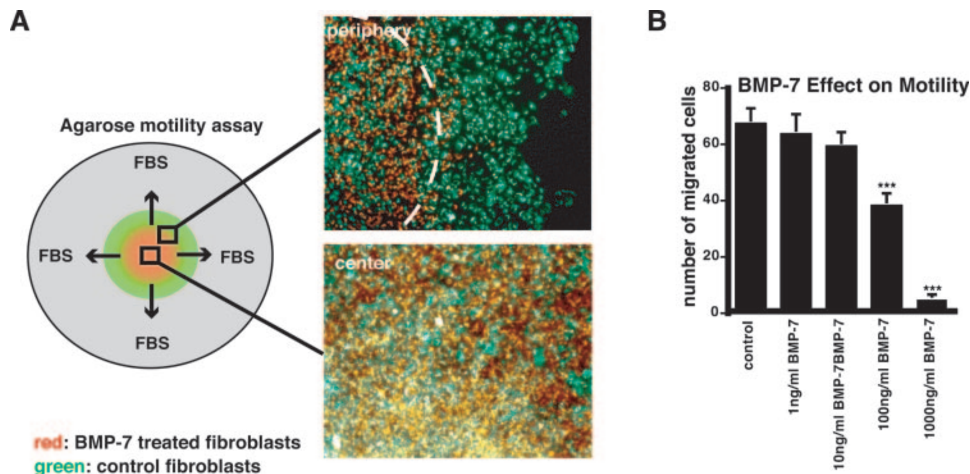

FIG. 3. **Regulation of adult renal fibroblasts motility by BMP-7.** *A*, schematic illustration of the under-agarose cell motility assay. Agarose gels were cast into 6-well plate, and a central hole surrounded by 4 holes was punched into each well. A suspension of untreated fibroblasts (stained in *green*) and of BMP-7 treated fibroblasts (stained in *red*) was filled in the central hole, whereas the surrounding holes were filled with media containing 10% fetal bovine serum (*FBS*) to create a chemotactic gradient. The fibroblasts were allowed to migrate under the gel for 48 h toward the chemotactic gradient. The *images* display representative areas of the periphery (*upper image*) and the center region of the central hole. The *dashed line* indicates the edge of the center hole; cells that are to the *right* of the line have migrated underneath the gel toward the chemotactic gradient. Direct comparison of untreated control fibroblasts (*green*) and BMP-7-treated fibroblasts (*red*) demonstrates that BMP-7 decreased the migratory capacity of adult renal fibroblasts. The BMP-7-treated cells remained mainly in the center region of the hole. *B*, the *bar graph* summarizes cell counts of fibroblasts per visual field at ×400 magnification that have migrated underneath the gel. Experiments were performed three independent times in triplicate.

renal fibroblasts with BMP-7 at the optimum dose of 1000 ng/ml resulted in the formation of epithelial cell aggregates within the 90–100% confluent fibroblast monolayer (Fig. 1, *B* and *D*). Immunocytochemistry analysis of BMP-7-treated fibroblasts revealed that cells within the aggregated/condensed areas expressed abundant levels of the epithelial marker E-cadherin (Fig. 1*F)* and cytokeratin (data not shown) as compared with the untreated fibroblasts (Fig. 1*E*). E-cadherin staining was diffusely distributed within the cells as well as some distribution within the typical junctional regions, suggesting that the phenotype represents an early stage of MET.

Several different studies have demonstrated that E-cadherin is an important determinant for maintenance of the epithelial phenotype (2, 34). E-cadherin is an epithelial cell-specific intercellular adhesion molecule, which by itself can induce MET if overexpressed in mesenchymal cells (35). Such studies have led to the speculation that E-cadherin is a potential epithelial master gene (2). In order to further analyze the regulation of E-cadherin expression in adult renal fibroblasts, we performed stable transfections using a cDNA fragment of the mouse E-cadherin promoter (−214/+93) conjugated to the luciferase gene (*lux*), which contains all of the reported key regulatory elements for function in epithelial cells (Fig. 2*A*) (30, 36). The −214/+93 mouse E-cadherin promoter construct (−214 E-cadherin pXP2) is active only in epithelial cells with minimal activity in fibroblasts, as compared with the full-length promoter, which displays robust activity in both epithelial cells and fibroblasts (30, 31). In dose-response studies, rhBMP-7 induces E-cadherin promoter activity with maximal induction (5.3-fold) observed at 1000 ng/ml in TK173 fibroblast cells (Fig. 2*B*). Similar levels of induction (2-fold) of E-cadherin promoter activity, associated with epithelial-mesenchymal plasticity, has been reported previously (9, 36, 37).

Formation of E-cadherin-expressing epithelial cell aggregates, as observed in our studies, mimics BMP-7-mediated condensation of the embryonic metanephric mesenchyme, which represents the initial step in the process that leads to formation of the tubular epithelium (16, 21, 38, 39). To confirm this notion, we examined the cells for the expression of Pax2 and Wnt4. Wnt4 encodes a secreted molecule, which is a putative early marker for epithelium formation (38). In the developing kidney, it appears in the condensing mesenchyme and persists in primary vesicles, but it is down-regulated in the S-shaped bodies (38). The epithelium of the adult nephron does not express Wnt4 (38). Pax2 is expressed in induced mesenchyme and in the early epithelial derivates, and it is rapidly down-regulated in mature kidney (40). Incubation of adult renal fibroblasts with the optimum dose of BMP-7 (1000 ng/ml) induced expression of both Pax2 and Wnt4, further suggesting that BMP-7 induces formation of epithelial cell aggregates in adult renal fibroblasts, as it does in the embryonic metanephric condensing mesenchyme (38).

Because fibroblasts are highly mobile, whereas epithelial cells are not, regulation of cell motility is an important aspect of the plasticity between epithelium and mesenchymal cells (2–4, 41). In order to determine the functional consequence of MET involving adult renal fibroblasts, we evaluated the effect of BMP-7 treatment on cell motility.

We first used a two-compartment Boyden chamber migration assay, which allows evaluation of haptotactic migration (migration from the upper compartment into the bottom compartment due to direct stimulation of the cells in the upper compartment) and chemotactic migration (migration of cells from the upper chamber into the bottom chamber induced by a growth factor gradient from the bottom compartment) (32). Direct stimulation with rhBMP-7 in the upper chamber reduced haptotactic migration of adult renal fibroblasts in a dose-dependent manner. However, this effect was statistically insignificant (data not shown). rhBMP-7 used as a chemoattractant in the bottom chamber had no effect on migration of adult renal fibroblasts (data not shown). In the Boyden chamber assay, a single cell suspension is allowed to migrate for 6 h; therefore, an inhibitory effect on cell motility was probably not realized, since 6 h may be too short a period for MET to occur and for epithelial cell junctions to assemble.

In order to further validate the role of BMP-7 on the motility of human renal fibroblasts, we next used an under-agarose cell motility assay, which allows evaluation of cell motility over a 48-h period. We treated human renal fibroblasts for 48 h with BMP-7 to induce MET. We then stained BMP-7-treated fibro-

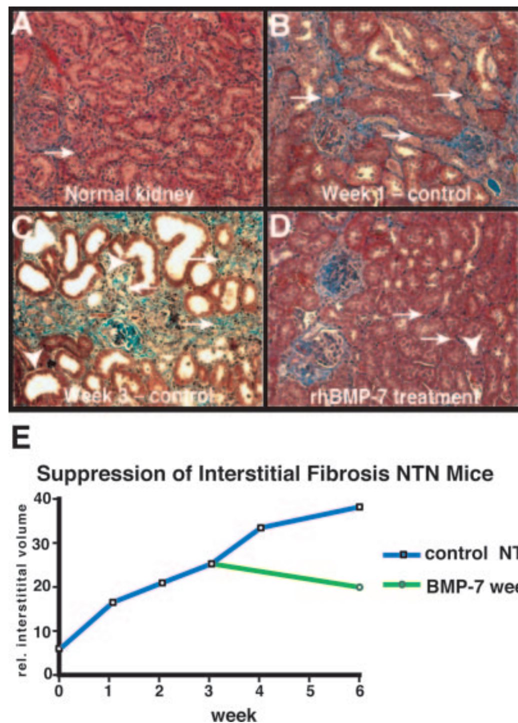

FIG. 4. **Reversal of chronic renal injury by BMP-7 in a mouse model of nephrotoxic serum nephritis.** *A*, normal mouse kidney. The *arrow* points to normal interstitium between tubules with tubular epithelial cells. *B*, kidneys of untreated control mice 1 week after NTS injection. At this stage, the kidneys display moderate interstitial disease associated with widening of the interstitial area (*arrows*). The *blue color* represents ECM accumulation as detected with Masson's trichrome stain. *C*, kidneys of untreated control mouse 3 weeks after NTS injection. The *arrows* show widening of the interstitial space, representative of the fibroblast accumulation and inflammatory cells. Injury of tubular epithelial cells results in tubular atrophy (*arrowheads*) *D*, kidneys of rhBMP-7-treated mice, 6 weeks after NTN. The rhBMP-7 treatment was started 3 weeks after initiation of disease (compare with Fig. 4*C*). The kidney specimen displayed vastly decreased interstitial space (*arrows*) and improved tubular structure (*arrowhead*). *E*, summary of morphometric assessment of interstitial volume. Progression of interstitial fibrosis was associated with an increasing interstitial volume until week 6 of the disease. The interstitial volume was decreased as compared with the untreated control after 6 weeks of disease.

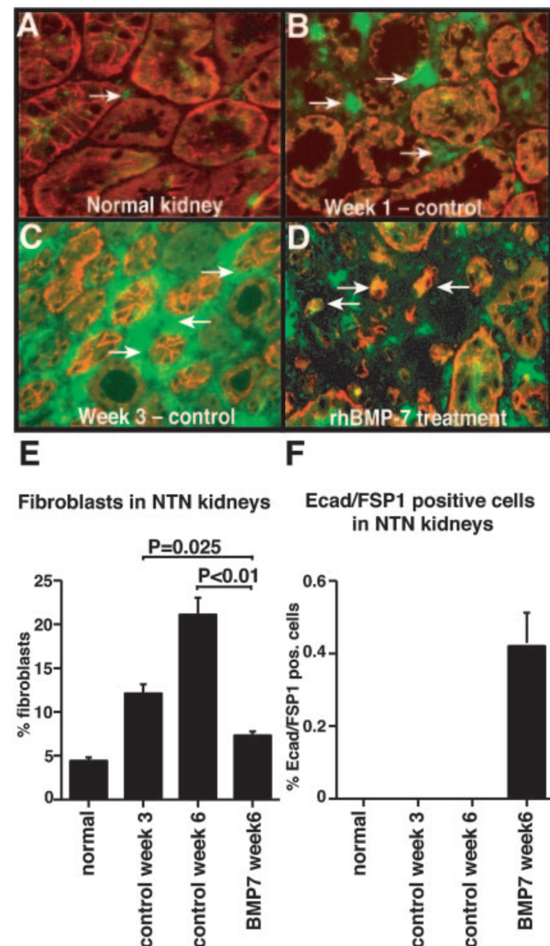

FIG. 5. **BMP-7 mediated in an *in vivo* renal injury model.** *A–D*, immunofluorescence double staining for E-cadherin (*red*) and FSP1 (*green*). *A*, in normal mouse kidney tubular epithelial cells express E-cadherin (*A*, *red staining*), whereas interstitial fibroblasts can be detected by FSP1 staining (*A*, *arrows*). In the normal mouse kidney, cells that co-express FSP1 and E-cadherin are not present. *B*, after the first week of NTN, the kidneys display increased numbers of FSP1-positive fibroblasts. Tubules with decreased levels of E-cadherin staining appear. Cells that co-express E-cadherin and FSP1 are not detectable. *C*, after 3 weeks post-NTS-injection, tubular cells with *de novo* expression of FSP1 and diminished expression of E-cadherin, indicating EMT, are robustly present. FSP1-positive fibroblasts are increased in the interstitium. Cells that co-express E-cadherin and FSP1 cannot be found within the interstitium. *D*, administration of rhBMP-7 (starting after 3 weeks of NTN, until after week 6; compare *D* with *C*) restored normal tubular architecture with E-cadherin expression. A novel population of cells that co-express E-cadherin and FSP1, indicating an intermediate phenotype of epithelial cell and fibroblasts, is now present within the interstitium (*arrows*). *E*, fibroblasts were identified by staining with FSP1 antibody, and the percentage of fibroblasts of total cells within the kidney cortex was assessed. The graph displays the average of six different kidneys in each group. The progression of fibrosis in the untreated kidneys was associated with a significant increase of interstitial fibroblasts. Treatment with rhBMP-7 resulted in a significant reduction of fibroblasts as compared with untreated control. *F*, kidneys were evaluated for the presence of cells in the interstitium that stained positive for both E-cadherin (*Ecad*) and FSP1. The *graph* summarizes the average of six different kidneys in each group. E-cadherin/FSP1 double-positive interstitial cells were exclusively present in the BMP-7-treated kidneys.

blasts with a red fluorescent cell tracker dye and untreated control fibroblasts with green fluorescent dye. Both cell populations were then mixed at equal number, and the cell suspension was placed into the middle hole in an agarose-filled tissue culture dish. The cells were then allowed to migrate from the center toward a fetal calf serum gradient for 48 h, allowing for direct comparison between the BMP-7-treated (*red*) fibroblasts and the untreated (*green*) control fibroblasts (Fig. 3). Whereas the BMP-7-treated fibroblasts remained in the center of the well, the control fibroblasts migrated toward the fetal bovine serum gradient (Fig. 3). In summary, these findings suggest that BMP-7 induces the formation of epithelial cell aggregates in adult renal fibroblasts in association with decreased cell motility.

Administration of rhBMP-7 accelerates recovery of acute renal failure and inhibits progression of fibrosis in the kidney (9, 19, 22–24, 42). We have previously demonstrated that administration of rhBMP-7 results in regression of chronic renal disease associated with restoration of the tubular epithelium architecture (9). In these studies, we could demonstrate that BMP-7 protects tubular epithelium by inhibition and potential reversal of EMT (9). We speculated that MET of fibroblasts could mediate decrease of interstitial fibroblasts and additionally contribute to the repair of renal tubular epithelial structures. In order to test this hypothesis, we used a mouse model of nephrotoxic serum nephritis that, when untreated, leads to severe renal fibrosis after 6 weeks (9). This model is responsive to rhBMP-7 therapy and leads to regression of renal fibrosis and repair of damaged tubular structures (9). We initiated treatment with 300 μg/kg rhBMP-7 (established as optimal therapeutic dosage in previous

studies) after 3 weeks of disease, when fibrotic lesions have been established and we evaluated the kidneys after 3 weeks of treatment (9). Treatment with rhBMP-7 starting from week 3 resulted in improved excretory renal function, decrease of interstitial fibroblasts, and repair of tubular injury (Fig. 4). Immunofluorescence double staining with FSP1 antibodies (fibroblast marker) and E-cadherin (epithelial marker) confirmed that in the normal kidney, E-cadherin was exclusively expressed by epithelial cells and FSP1 was exclusively expressed by fibroblasts (Fig. 5). Progression of the disease was associated with an increase in the number of interstitial fibroblasts (that express FSP1 but not E-cadherin) and EMT of epithelial cells (indicated by co-expression of E-cadherin and FSP1 in cells within the tubular epithelium) (Fig. 5). BMP-7-induced repair of renal injury was associated with a significant decrease of interstitial fibroblasts (Fig. 5). In addition, decrease of fibroblasts and improvement of tubular architecture were associated with detection of a novel cell population that co-expressed FSP1 and E-cadherin within the interstitium, indicating a cell phenotype that reveals it as a fibroblast and also an epithelial cell (Fig. 5). Such cells were not present in the untreated control kidneys and only present in the BMP-7-treated kidneys, in association with decreased interstial fibroblasts and improved tubular structure. These findings suggest that these novel cells are probably interstitial fibroblasts responding to rhBMP-7 treatment by undergoing MET.

DISCUSSION

It is known that during kidney development, the metanephric mesenchyme converts to epithelium (MET) and that during adult kidney disease renal epithelia can become fibroblasts via EMT (2, 3, 43, 44). Whereas the adult kidney possesses substantial intrinsic capacity to repair epithelial damage, the potential of adult renal fibroblasts to contribute to epithelial regeneration by converting into epithelial cells has not been explored.

BMP-7 is required for formation of epithelia during kidney development, and several studies have demonstrated that treatment with BMP-7 enhances the repair of tubular epithelial injury (9, 45). Whereas we previously demonstrated that BMP-7 inhibits EMT involving adult renal epithelial cells, we now provide evidence that BMP-7 induces formation of epithelial aggregates in adult renal fibroblasts, which mirrors the influence of BMP-7 on embryonic metanephric mesenchyme. These findings suggest that adult renal fibroblasts have retained at least some part of their embryonic epithelial imprint and plasticity, which can be potentially utilized to mediate repair of kidney injury by generating epithelial cells.

Acute renal failure is associated with re-expression of Pax-2, a principal regulator of epithelial development in the kidney, within the tubular epithelium (46). In addition, renal injury is associated with increased expression of Wnt-4, mainly in interstitial fibroblasts (47). Furthermore, it is well established that renal injury is associated with decreased BMP-7 expression, a regulator of MET during embryonic development (19, 20, 22, 24). These studies provide evidence that embryonic developmental programs are still likely to be active in the adult injured kidney.

In our studies, BMP-7 induces phenotypic changes in adult renal fibroblasts, which mimics the effect of BMP-7 on embryonic metanephric mesenchymal cells (16, 17, 21, 38). Decreased type I collagen expression, decreased motility, increased E-cadherin expression, and formation of cell aggregates represent an early stage of MET (17, 38, 39, 43). This is further validated by increased expression of Pax2 and Wnt4, both of which are present within condensing metanephric mesenchyme but are lost during tubule formation (38). Because BMP-7 induces formation of epithelial aggregates in both embryonic and adult renal mesenchymal cells, further studies are required if combinations of growth factors such as fibroblast growth factor-2, tumor necrosis factor-α, and leukemia inhibitory factor, which induce formation of tubules in the metanephric mesenchyme, are required to induce similar changes in adult renal fibroblasts (38).

Administration of exogenous BMP-7 mediates repair of chronic lesions in a mouse model of nephrotoxic serum nephritis. Here we provide evidence that BMP-7-mediated repair is associated with potential MET involving interstitial fibroblasts. Whereas the method of immunofluorescence double staining does not exclude the possibility that the cells detected here are epithelial cells undergoing EMT, this is highly unlikely for the following reasons. First, we could never detect E-cadherin/FSP1 double-positive cells in the untreated NTN control mice. Second, treatment with BMP-7 mediates repair of renal injury, and enhanced EMT would be counterproductive. Third, our *in vitro* results confirm that BMP-7 induces expression of E-cadherin in adult renal fibroblasts.

An unequivocal role for MET in the repair of renal injury remains unclear. It also remains unclear whether all of the renal fibroblasts retain the capacity to acquire an epithelial phenotype or whether only a subpopulation of fibroblasts maintain their embryonic plasticity. Nevertheless, our studies offer evidence for the capacity of adult fibroblasts to undergo MET and probably contribute to the emergence of healthy epithelial cells in the repaired renal tubular structures.

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