Aquaporin-1 Facilitates Epithelial Cell Migration in Kidney Proximal Tubule

Mariko Hara-Chikuma and A.S. Verkman

Departments of Medicine and Physiology, Cardiovascular Research Institute, University of California, San Francisco, San Francisco, California

Aquaporin-1 (AQP1) is the principal water-transporting protein in cell plasma membranes in kidney proximal tubule, where it facilitates transepithelial water transport. Here, a novel role for AQP1 in kidney involving the migration of proximal tubule cells is reported. Migration was compared in primary cultures of proximal tubule cells from wild-type and AQP1 null mice. Cell cultures from AQP1 null mice were indistinguishable from those of wild-type mice in their appearance, growth/proliferation, and adhesiveness, although, as expected, they had reduced plasma membrane water permeability. Migration of AQP1-deficient cells was reduced by >50% compared with wild-type cells, as measured in a Boyden chamber in the presence of a chemotactic stimulus. Comparable slowing of migration of AQP1-deficient cells was also found in an *in vitro* scratch assay of wound healing, with reduced appearance of lamella-like membrane protrusions at the cell leading edge. Adenoviral-mediated expression of AQP1 in the AQP1-deficient cells, which increased their water permeability to that of wild-type cells, corrected their migration defect. The potential relevance of these *in vitro* findings to the intact kidney was tested in an *in vivo* model of acute tubular injury caused by 30 min of renal artery occlusion. At 3 to 5 d after ischemia-reperfusion, kidneys in AQP1 null mice showed remarkably greater tubular injury and cellular actin disorganization than kidneys in wild-type mice. These results provide evidence for the involvement of AQP1 in migration of proximal tubule cells and possibly in the response of the proximal tubule to injury.

J Am Soc Nephrol 17: 39–45, 2006. doi: 10.1681/ASN.2005080846

The water channel aquaporin-1 (AQP1) is expressed widely in mammalian epithelial and endothelial cell plasma membranes. AQP1 functions primarily as a passive water pore that increases water transport across cell membranes in response to an osmotic driving force. In the kidney, AQP1 is expressed at the apical and basolateral plasma membranes in epithelial cells of the proximal tubule and the thin descending limb of Henle and in the plasma membrane of endothelial cells in the outer medullary descending vasa recta (reviewed in ref. [1]). Targeted AQP1 gene disruption in mice greatly impairs urinary concentrating ability (2), with reduced baseline urine osmolality and inability to increase osmolality in response to water deprivation or vasopressin administration. A qualitatively similar defect in urinary concentrating ability is found in humans who lack AQP1 (3). Defective urinary concentrating function is the consequence of impaired countercurrent multiplication and exchange, resulting from reduced water permeability in thin descending limb of Henle and outer medullary descending vasa recta (4,5). In addition, AQP1 deficiency causes impairment in near-isosmolar fluid absorption in proximal tubule (6), resulting in marked luminal fluid hypotonicity in end proximal tubule as a consequence of active solute absorption and reduced transepithelial water permeability (7). AQP1-facilitated transcellular water transport thus has an important role in kidney, as well as in various extrarenal epithelia such as choroid plexus (8) and ciliary epithelium (9).

Recently, a new role of AQP1 in cell migration was suggested from the finding of impaired angiogenesis in AQP1 null mice (10). AQP1 is expressed strongly in tumor microvessels (11,12). We found slower growth of implanted tumors in mice that lacked AQP1 (compared with wild-type mice), with a much lower density of microvessels resulting in islands of viable tumor cells surrounded by necrotic tissue. Analysis of endothelial cell function in primary cell cultures indicated a selective defect in the migration of AQP1-deficient endothelial cells. From biophysical studies, we proposed that reduced water permeability in AQP1 deficiency impairs the formation of lamellipodia (membrane protrusions) in migrating cells. Because lamellipodia formation involves rapid local changes in ion fluxes and cell volume, which likely induce transmembrane water movement (13), reduced water transport at the tip of a lamellipodium might impair the dynamics of cell membrane protrusions and thus cell motility. On the basis of the finding that the migration of cultured fibroblasts and epithelial cells could be increased by transfection of AQP1 or AQP4 (10), we proposed that aquaporin-dependent cell migration might be a general phenomenon relevant to some nonendothelial cell types.

Here, we test the hypothesis that AQP-facilitated cell migration might be important in kidney. Cell migration in primary cultures of proximal tubule cells from wild-type and AQP1 null

Received August 11, 2005. Accepted October 10, 2005.

Published online ahead of print. Publication date available at www.jasn.org.

Address correspondence to: Dr. A.S. Verkman, Cardiovascular Research Institute, 1246 Health Sciences, East Tower, University of California, San Francisco, CA 94143-0521. Phone: 415-476-8530; Fax: 415-665-3847; E-mail: verkman@itsa.ucsf.edu

ISSN: 1046-6673/1701-0039

mice was compared, and *in vivo* experiments were done using a mouse model of acute renal injury caused by transient unilateral ischemia-reperfusion. We found significant impairment in the migration of AQP1-deficient proximal tubule cells *in vitro*, which was corrected by adenovirus-mediated reintroduction of AQP1, as well as remarkably more severe proximal tubule damage in kidneys in AQP1 null mice after ischemia-reperfusion. These data provide evidence for a novel role of AQP1 in kidney distinct from its well-established role in transcellular water transport and suggest the involvement of AQP1 in the pathophysiology of renal injury and repair.

Materials and Methods

AQP1 Null Mice

AQP1 null mice were generated by targeted gene disruption as described (2). Protocols were approved by the UCSF Committee on Animal Research.

Primary Culture of Kidney Proximal Tubule Epithelial Cells

Proximal tubule cells were cultured from 1- to 2-mo-old wild-type and AQP1 null mice (in a CD1 genetic background) as described (14). Briefly, the renal cortices were minced and incubated for 30 min in Hanks' solution that contained 140 U/ml collagenase (Worthington, Lakewood, NJ) and 0.75 mg/ml trypsin inhibitor (Sigma, St. Louis, MO). After removal of undigested fragments that settled by gravity, the suspension was mixed with an equal volume of Hanks' solution that contained 10% FBS and centrifuged for 7 min at 50 × *g* (4°C). Cortical tubules were purified by gradient centrifugation in DMEM/F-12 that contained 40% Percoll for 20 min at 36,000 × *g* (4°C). The band that contained proximal tubules was collected and washed twice with DMEM/F-12. Cells were cultured in DMEM/F-12 that contained 2 mM glutamine, 15 mM HEPES, 5 μg/ml transferrin, 5 μg/ml insulin, and 50 nM hydrocortisone. These cells were previously shown to be predominantly of proximal tubule origin (14), and this was confirmed here by immunostaining using an anti-megalin antibody (Santa Cruz Biotechnology, Santa Cruz, CA).

Adenovirus-Mediated AQP1 Expression

A recombinant adenovirus vector encoding rat AQP1 (AQP1-Ad) was generated as described (15). Proximal tubule cells were infected with AQP1-Ad at a concentration of 100 PFU/cell. The medium was replaced after 1 h of incubation with virus at 37°C. Experiments were done at 48 to 72 h after viral infection.

Immunofluorescence and Immunoblot Analysis

Immunofluorescence staining was done on paraformaldehyde-fixed cells with anti-AQP1 primary antibody (Chemicon International, Temecula, CA) and anti-rabbit Cy3-conjugated IgG (Sigma) secondary antibody. For immunoblot analysis, AQP1 antibody and horseradish peroxidase–conjugated secondary anti-rabbit IgG antibody (DAKO, Carpinteria, CA) were used for detection by enhanced chemiluminescence (Amersham Pharmacia, Piscataway, NJ).

Osmotic Water Permeability

Cells that were grown on glass coverslips were incubated with 20 μM calcein acetoxymethyl ester (Molecular Probes, Eugene, OR) for 30 min at room temperature and then mounted in a perfusion chamber designed for rapid solution exchange (<100 ms). The time course of cytoplasmic calcein fluorescence, which changes reciprocally with cell volume (16), was monitored in response to exchanging perfusate between PBS (300 mOsm) and hypertonic PBS (600 mOsm) that contained 300 mM mannitol.

In Vitro *Migration and Wound Healing*

Migration was assayed using a modified Boyden chamber (Corning Costar, Cambridge, MA) that contained a polycarbonate transwell membrane filter (6.5 mm diameter, 8 μm pore size) (17) coated with 10 μg/ml fibronectin. Cells (10^4) were plated on the upper chamber in DMEM/F-12 that contained 1% FBS. The lower chamber contained DMEM/F-12 with 10% or 1% (control) FBS. Cells were incubated for 6 h at 37°C in 5% CO_2/95% air. Adherent cells were counted after staining with Coomassie blue, and migrated cells that remained on the bottom surface were counted after nonmigrated cells were scraped from the upper surface of the membrane with a cotton swab.

In vitro wound healing was assayed in confluent cell monolayers. Cells were scraped in an approximately 400-μm-wide strip of the cells using a standard 200-μl pipette tip (10). The wounded monolayers were washed twice to remove nonadherent cells. Wound healing was quantified as the average linear speed of the wound edges over 24 h. Cells were stained with phallotoxins conjugated to Alexa Fluor 488 (Molecular Probes) to count lamellipodia and to visualize the F-actin cytoskeleton. In some experiments, time-lapse photography of the wound edges was performed by phase-contrast microscopy (600 frames over 10 h).

Cell Size, Adhesion, and Proliferation

Cells were suspended in medium after trypsinization for measurement of cell diameter by transmission light microscopy at high magnification (×250). For measurements of cell adhesion, 96-well plates were coated at 4°C overnight with collagen type I (10 μg/ml; BD Biosciences, Bedford, MA), laminin (10 μg/ml, natural mouse laminin; Invitrogen, San Diego, CA), or human fibronectin (10 μg/ml; Roche Diagnostics, Indianapolis, IN). After rinsing, uncoated surfaces were blocked with PBS that contained 2% heat-denatured BSA for 1 h at 37°C. Cells (2×10^4 cells) were added to each well and incubated for 1 h at 37°C. After cells were washed twice with PBS to remove unattached cells, adhered cells were stained with Hoechst 33342 for 1.5 h, and the fluorescence of each well was measured (excitation 360 nm, emission 530 nm) as described (18). Cell proliferation was assayed using a commercial BrdU Cell Proliferation Assay Kit (Calbiochem, La Jolla, CA).

Ischemia-Reperfusion Model of Acute Renal Injury

Mice were anesthetized with ketamine and xylazine, injected intraperitoneally, and maintained at 37°C using a warming pad. The left renal pedicle was identified and clamped for 30 min using a vascular clamp. The clamp was removed, and reperfusion was confirmed visually. The incision was closed by suture, and the mice were allowed to recover. Kidneys were harvested at the specified time points and fixed in formalin for paraffin sectioning or 4% paraformaldehyde for frozen sections. For measurement of proliferation, in some experiments mice received an intraperitoneal injection of 5-bromo-2′-deoxyuridine (BrdU) (100 μg/g body wt; Sigma) in saline 2 h before being killed.

Paraffin-embedded sections were stained with periodic acid-Schiff reagent (Sigma) and hematoxylin. Tubular injury was evaluated in kidney sections at 5 d after ischemia-reperfusion by two investigators who were blinded to genotype information. A minimum of 100 proximal tubules from five mice were examined, and the number of tubules with >5 μm inner diameter (dilated tubules) were counted, based on established procedures (19,20). The percentage of dilated tubules was computed. Cell proliferation was measured in paraffin-embedded sections by staining with BrdU antibody (Abcam, Cambridge, MA) followed by biotinylated anti-mouse IgG and horseradish peroxidase–

conjugated ABC reagent (Vector Laboratories, Burlingame, CA). F-actin in frozen sections was stained by Alexa Fluor 488–conjugated phalloidin (Molecular Probes).

Results

Characterization of Proximal Tubule Cell Cultures

Proximal tubule epithelial cells were cultured from kidney cortex of adult wild-type and AQP1 null mice. The primary cell cultures were indistinguishable in their appearance by phase-contrast light microscopy (Figure 1A, left). Megalin immunostaining indicated that >98% of cells in the cultures were of proximal tubule origin (Figure 1A, right). AQP1 immunostaining in cells that were cultured from kidneys of wild-type mice showed AQP1 protein expression in a diffuse intracellular vesicular and plasma membrane pattern, with no staining in cells from AQP1-deficient kidneys (Figure 1B). Infection of AQP1-deficient cells by AQP1-Ad at a multiplicity of infection of 100 PFU/cell produced AQP1 protein expression in approximately 90% of the cells. Immunoblot analysis showed AQP1 protein expression with the expected molecular size of approximately 28 kD in wild-type and AQP1-Ad infected cells (Figure 1C).

Osmotic water permeability was measured by cell volume changes in response to osmotic challenge using the calcein fluorescence quenching method. Figure 1D (left) shows the reversible time course of cell volume in response to changing perfusate osmolality between 300 and 600 mOsm. The osmotically induced cell volume changes were slower in the AQP1-deficient cells compared with wild-type cells and AQP1-Ad–infected AQP1 null cells. Fitted reciprocal exponential time constants (proportional to water permeability) were reduced by approximately two-fold in the AQP1-deficient compared with wild-type cells (Figure 1D, right). AQP1-Ad infection in AQP1 null cells restored water permeability to approximately that in uninfected wild-type cells. It is interesting that water permeability in AQP1-Ad–infected wild-type cells was similar to that of uninfected wild-type cells despite greater AQP1 protein expression by immunoblot analysis, which may be caused by AQP1 protein mistargeting in the virus-treated cells.

Proximal Tubule Cell Migration In Vitro

In vitro assays of wound healing and transwell migration were done in the primary cultures of proximal tubule cells from wild-type and AQP1 null mice, as well as in AQP1-Ad adenovirus "corrected" cells from AQP1 null mice. Cell migration toward FBS was assayed using a modified Boyden chamber. Adherent cells (before scraping) and migrated cells on the bottom surface of transwell membrane (after scraping) were

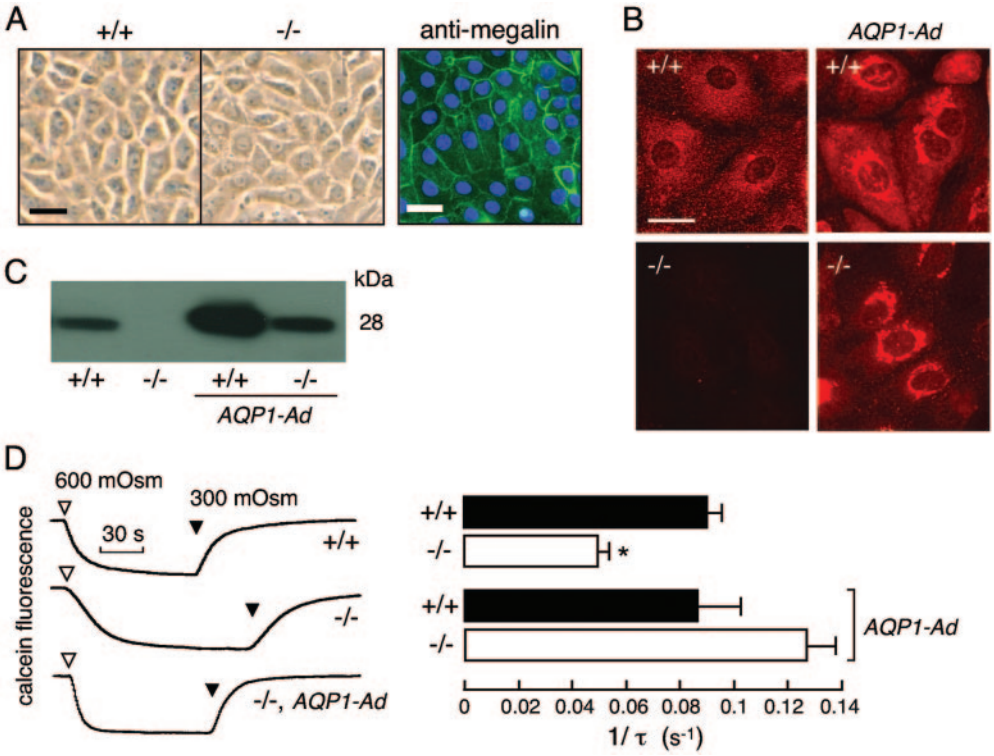

Figure 1. Characterization of proximal tubule cell cultures. (A, left) Phase-contrast micrographs of primary cultures of proximal tubule cells from wild-type (+/+) and AQP1 null (−/−) mice. (A, right) Immunostaining of cells from wild-type mouse for the proximal tubule cell marker megalin (green, with DAPI-stained blue nuclei). Bar = 20 μm. (B) Aquaporin 1 (AQP1) immunostaining (red). Where indicated, cells were infected with adenovirus encoding AQP1 (AQP1-Ad) as described in Materials and Methods. Bar = 10 μm. (C) AQP1 immunoblot of cell homogenates (10 μg protein/lane). (D) Osmotic water permeability of proximal tubule cells measured by calcein fluorescence quenching. (Left) Representative time course data showing responses to rapid changes in perfusate osmolality between 300 and 600 mOsm. (Right) Reciprocal exponential time constants (τ^{-1}) in five separate sets of measurements (SE, $^*P < 0.01$), which are proportional to osmotic water permeability.

quantified (Figure 2A). Migration of AQP1-deficient proximal tubule cells toward 10% serum was reduced significantly compared with wild-type cells, with low migration toward 1% serum in both wild-type and AQP1-deficient cells (Figure 2B). AQP1 replacement by AQP1-Ad infection corrected the migration defect in AQP1-deficient cells to approximately that in wild-type cells. Cell size and proliferation were similar in wild-type and AQP1-deficient cells (Figure 2, C and D), indicating that the slower migration in AQP1-deficient cells was not due to a difference in cell size or proliferation. No differences in cell adhesion to collagen I, fibronectin, or laminin were found, suggesting that reduced migration was also not due to altered integrin-dependent adhesion (Figure 2E) (21,22). In support of this conclusion, immunostaining for integrins $\alpha 3$, $\beta 1$, and $\beta 3$ were similar in wild-type and AQP1-deficient cells (data not shown).

Figure 3A shows delayed wound closure in AQP1-deficient proximal tubule cells at 24 h after creation of linear wounds in confluent cell monolayers. The closure speed of the wound edge was significantly slowed in AQP1-deficient compared with wild-type cells (Figure 3B). Time-lapse phase-contrast microscopy of wound closed showed different migratory behaviors of wild-type *versus* AQP1-deficient proximal tubule cells (supplemental videos are available online only). During migration the wild-type cells formed broad lamellipodia with persistent movements toward the wound area and more rapid migration compared with the AQP1-deficient cells. The slowed speed of wound closure in the AQP1 null cells was corrected by infection with AQP1-Ad under conditions that in Figure 1D normalized their osmotic water permeability.

F-actin staining with fluorescence phallotoxins showed cell protrusions (lamella) in many proximal tubule cells during wound closure (Figure 3C, top left), with a smaller number of AQP1-deficient cells having protrusions. Quantitative analysis of lamella, expressed as the ratio of lamella area per length of cell (at the leading edge), showed a significant reduction in lamella area in the AQP1-deficient cells (Figure 3C, right). F-actin staining was similar in areas of wild-type and AQP1 null cells not including lamella, suggesting that delay in wound healing in AQP1-deficient cells was not due to a cytoskeletal abnormality (Figure 3C, bottom left) (21,22).

In Vivo *Response to Acute Renal Tubular Injury*

A well-established ischemia-reperfusion injury model of acute tubular injury was used to investigate the potential *in vivo* relevance of impaired proximal tubule cell migration in AQP1 deficiency. Renal architecture was indistinguishable in wild-type *versus* AQP1 null control mice (Figure 4A, left). Unilateral renal ischemia-reperfusion (30 min) produced marked pathologic changes in the kidney, although there were no clinical

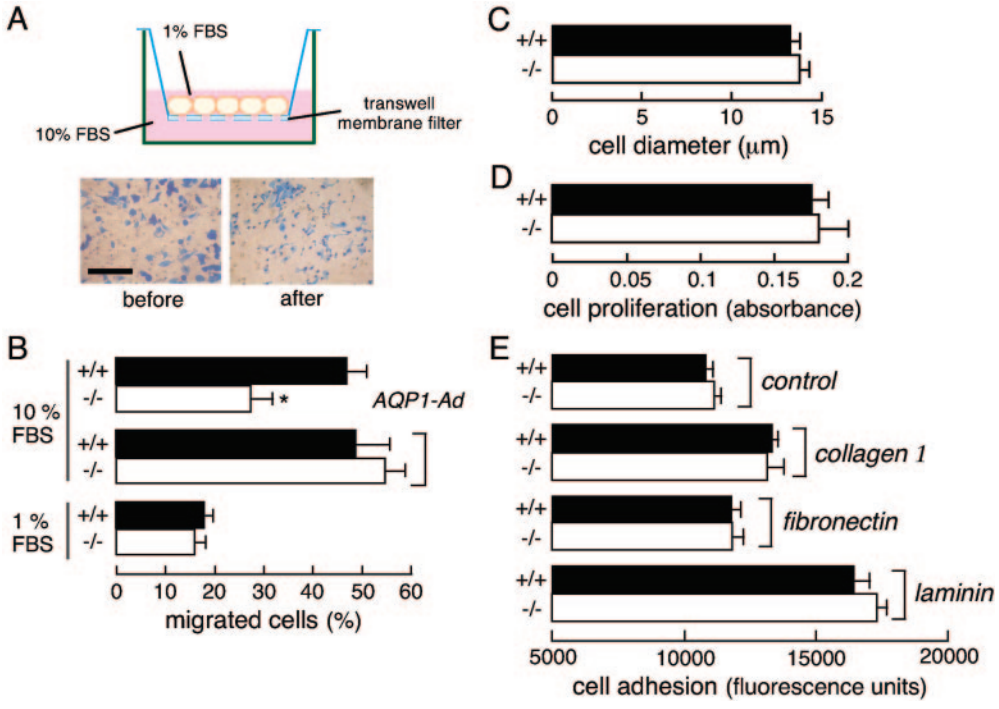

Figure 2. In vitro Boyden chamber assay of proximal tubule cell migration. (A, top) Schematic of modified Boyden chamber that contained a transwell porous membrane filter. (A, bottom) Photographs of adherent proximal tubule cells on transwell filters before scraping (left) and migrated cells (on the bottom filter surface) after scraping (right). Bar = 100 μm. (B) Percentage of migrated cells at 6 h after cell plating (SE, six sets of experiments, $*P < 0.01$). The bottom chamber contained 10% or 1% FBS. (C) Cell diameter measured in freshly trypsinized proximal tubule cells (SE, 20 cells measured per genotype). (D) Cell proliferation measured by 5-bromo-2′-deoxyuridine (BrdU) incorporation (absorbance at 520 nm; SE, six sets of experiments). (E) Cell adhesion to type I collagen, fibronectin, and laminin measured by fluorescence (SE, six sets of experiments). See Materials and Methods for details. In C through E, differences are NS.

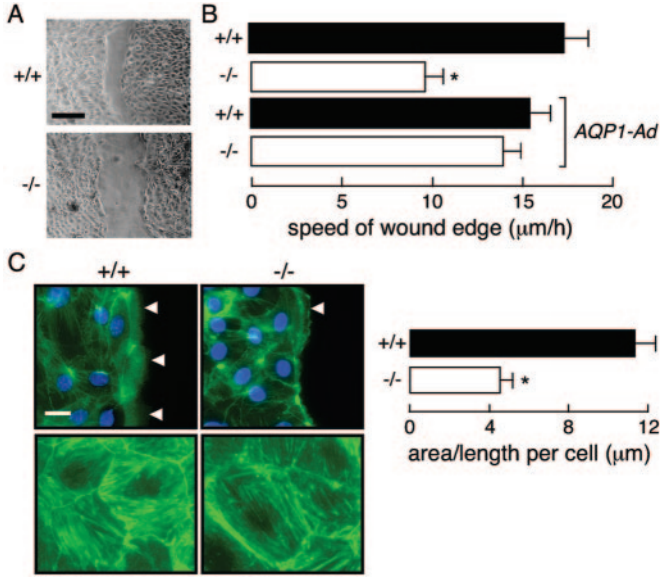

Figure 3. In vitro wound closure model to study proximal tubule cell migration. (A) Light micrographs of wounded cell monolayers showing delayed wound closure at 24 h in AQP1-deficient proximal tubule cells. Bar = 100 μm. (B) Speed of wound edge in wild-type and AQP1 null cells without and with AQP1-Ad infection (SE, six sets of experiments, $^*P < 0.01$). (C, left) Fluorescence micrographs of cells stained with phallotoxins showing protrusions at the leading edge (top, white arrowheads) and the F-actin cytoskeleton (bottom). Bar = 20 μm. (C, right) Area of protrusions per length of cells at the wound edge (SE, 100 cells analyzed, $^*P < 0.01$).

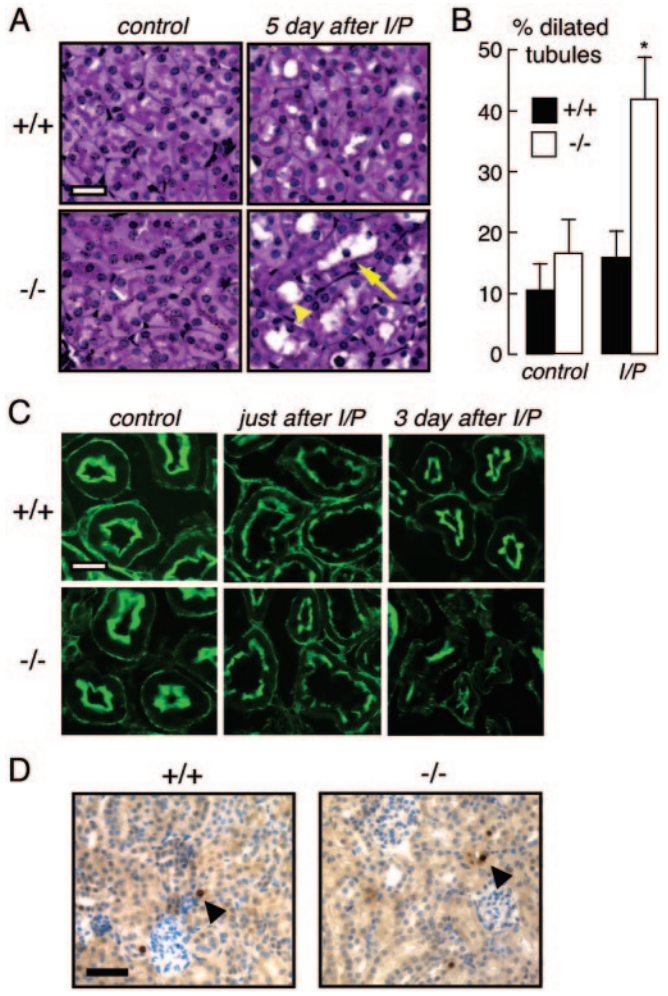

Figure 4. Ischemia-reperfusion model of acute renal tubular injury. (A) Periodic acid-Schiff and hematoxylin staining of kidney sections from wild-type and AQP1 null control mice (left) and 5 d after (right) ischemic/reperfusion (I/P). Bar = 20 μm. Arrowhead, dilated tubule; arrow, degenerated tubule. (B) Percentage of proximal tubules with inner diameter >5 μm (SE, >100 proximal tubules examined per mouse, five mice per group, $^*P < 0.05$). (C) F-actin staining kidneys of wild-type and AQP1 null control mice (left) and just after (middle) and 3 d after (right) I/P. Bar = 20 μm. (D) BrdU staining at 5 d after I/P. Arrow, BrdU-positive staining. Bar = 50 μm.

signs of disease or electrolyte/renal function abnormalities (because contralateral kidney was not damaged).

Figure 4A (right) showed remarkably greater tubule dilation and degeneration in AQP1 null *versus* wild-type kidneys at 5 d after ischemia-reperfusion. The percentage of proximal tubules with inner diameter >5 μm, which has been taken as a semiquantitative index of proximal tubule damage (19,20), was remarkably greater in AQP1 null mice (Figure 4B). Immunostaining showed F-actin expression concentrated at the brush border in control kidneys from wild-type and AQP1 null mice (Figure 4C, left). Just after ischemia-reperfusion, nearly all tubules in both types of mice were thinned and dilated, with disappearance of the brush border and a discontinuous F-actin distribution. Although the F-actin component of the brush border was largely normalized in kidneys of wild-type mice at 3 d after ischemia-reperfusion, kidneys of AQP1 null mice showed poor F-actin organization. At 5 d after ischemia-reperfusion, the percentage of proximal tubules with at least one BrdU-positive cell was similar in wild-type and AQP1 null mice (4.5 ± 1.6 *versus* 4.7 ± 0.9%; $n = 5$ slides; Figure 4D), indicating a similar proliferation rate.

Discussion

The principal finding of this study was impaired migration of proximal tubule epithelial cells that lack AQP1. The reduced migration was seen in both transwell filter and wound-healing assays and was associated with a reduced area of cell protrusions. The impaired migration of AQP1-deficient proximal tubule cells could not be accounted for by differences in their size, shape, adherence, or proliferation. We propose that reduced water permeability in lamella-like cell protrusions seen in migrating AQP1-deficient proximal tubule cells accounts for their reduced mobility. Indeed, the impaired migration of AQP1-deficient cells could be corrected by restoring their cell plasma membrane water permeability to that of wild-type cells by AQP1-Ad. Unilateral ischemia-reperfusion studies showing impairment in restoration of normal proximal tubule structure in AQP1 deficiency provided *in vivo* evidence for involvement of AQP1 in the response of the kidney to acute injury.

The primary cultures of proximal tubule cells were indistinguishable in their size, morphology, proliferation, and adherence, although, as expected, the cells from AQP1 null mice had reduced osmotic water permeability compared with cells from wild-type mice. However, the approximately two-fold difference in water permeability in the wild-type *versus* AQP1-deficient cultured cells is less than the 4.5-fold difference in transepithelial osmotic water permeability found in perfused proximal tubules that were isolated from wild-type *versus* AQP1 null mice (7). There are several possible explanations for this difference, such as the effects of cell isolation from tubules and culture on a solid support. Indeed, the greater intracellular *versus* plasma membrane AQP1 staining in the cultures (Figure 1B) is somewhat different from that seen in sections of kidney, where AQP1 is seen most strongly at the brush border, with lesser apparent staining of the basolateral membrane and subapical intracellular vesicles (23). In any case, our culture model recapitulated at least qualitatively the reduced water permeability in AQP1 deficiency, which could be restored by AQP1-Ad adenovirus infection. The greater AQP1-facilitated water permeability in proximal tubule *in vivo* may well enhance the differences in cell migration seen in the primary cell cultures.

Several lines of evidence support the possibility that the association between AQP expression and cell migration is related to the AQP water-transporting function. Our laboratory reported that AQP1 deletion impairs tumor angiogenesis by inhibiting endothelial cell migration (10). Chen *et al.* (24) recently reported that AQP1-mediated localized water influx was involved in membrane protrusion formation during *Cryptosporidium parvum* cellular invasion. Loitto *et al.* (25) reported evidence for involvement of AQP9 in lamellipodia formation and motility in neutrophils. Taken together with previous studies, the involvement of AQP in cell migration may be related to rapid water movement that occurs during the formation of membrane protrusions and cell shape changes. AQP1-facilitated cell migration thus might contribute to tubule repair and regeneration after acute renal failure and possibly in the local extension and metastasis of kidney-derived tumors that express AQP.

We chose an ischemia-reperfusion model of acute renal injury to investigate the potential *in vivo* relevance of impaired migration of AQP1-deficient proximal tubule cells in the cell culture models. Ischemia-reperfusion in rodents causes renal proximal tubule cell injury and death, leading to acute renal failure. *In vivo* and *in vitro* studies have shown that tubule cell migration, proliferation, and repair are the three major processes in the structural and functional regeneration of tubules after acute injury (26). We found delayed restoration of normal proximal tubule architecture after acute injury *in vivo*. On the basis of the cell culture experiments showing impaired cell migration but not proliferation in AQP1 deficiency, we propose that the delayed restoration of proximal tubule structure and function in AQP1 null mice is caused by a primary defect in cell migration. This conclusion is supported by the unimpaired tubule cell proliferation in AQP1 null kidneys after injury *in vivo* and the abnormal cellular actin architecture. However, visualization of impaired proximal tubule cell migration in the *in vivo* kidney is not possible, so the conclusion of impaired proximal tubule cell migration in the intact kidney remains inferential rather than direct.

In summary, our data provide evidence for a novel role of AQP1 in kidney in proximal tubule cell migration, which is distinct from its established role in transepithelial and transendothelial water transport and in the urinary concentrating mechanism. AQP1-facilitated migration of epithelial cells in proximal tubule, in epithelial cells in thin descending limb of Henle, and in endothelial cells in vasa recta may be important in the renal response to various types of injury. However, AQP1-dependent kidney cell migration would not be expected to be important in renal morphogenesis because little renal AQP1 is expressed in the prenatal kidney (27). It remains to be tested whether increasing AQP1 in kidney cells *in vivo*, by small-molecule inducers or gene delivery, might accelerate the recovery of renal function after acute tubular injury.

Acknowledgments

This work was supported by grants DK35124, DK72517, EY13574, EB00415, HL59198, and HL73856 from the National Institutes of Health and by Research Development Program and Drug Discovery grants from the Cystic Fibrosis Foundation.

We thank Dr. Yuanlin Song for help in establishing the surgical procedure for transient renal ischemia, Dr. Eugene Solenov for help in water permeability measurements, Dr. Baoxue Yang for histologic examination, Drs. Marios Papadopoulos and Samira Saadoun for advice on migration studies, and Liman Qian for mouse breeding and genotype analysis.

References

1. Verkman AS: Renal concentrating and diluting function in deficiency of specific aquaporin genes. *Exp Nephrol* 10: 235–240, 2002
2. Ma T, Yang B, Gillespie A, Carlson EJ, Epstein CJ, Verkman AS: Severely impaired urinary concentrating ability in transgenic mice lacking aquaporin-1 water channels. *J Biol Chem* 273: 4296–4299, 1998
3. Preston GM, Smith BL, Zeidel ML, Moulds JJ, Agre P: Mutations in aquaporin-1 in phenotypically normal humans without functional CHIP water channels. *Science* 265: 1585–1587, 1994
4. Chou CL, Knepper MA, Hoek AN, Brown D, Yang B, Ma T, Verkman AS: Reduced water permeability and altered ultrastructure in thin descending limb of Henle in aquaporin-1 null mice. *J Clin Invest* 103: 491–496, 1999
5. Pallone TL, Edwards A, Ma T, Silldorff EP, Verkman AS: Requirement of aquaporin-1 for NaCl-driven water transport across descending vasa recta. *J Clin Invest* 105: 215–222, 2000
6. Schnermann J, Chou CL, Ma T, Traynor T, Knepper MA, Verkman AS: Defective proximal tubular fluid reabsorption in transgenic aquaporin-1 null mice. *Proc Natl Acad Sci U S A* 95: 9660–9664, 1998
7. Vallon V, Verkman AS, Schnermann J: Luminal hypotonicity in proximal tubules of aquaporin-1-knockout mice. *Am J Physiol Renal Physiol* 278: F1030–F1033, 2000
8. Oshio K, Watanabe H, Song Y, Verkman AS, Manley GT:

Reduced cerebrospinal fluid production and intracranial pressure in mice lacking choroid plexus water channel aquaporin-1. *FASEB J* 19: 76–78, 2005
9. Zhang D, Vetrivel L, Verkman AS: Aquaporin deletion in mice reduces intraocular pressure and aqueous fluid production. *J Gen Physiol* 119: 561–569, 2002
10. Saadoun S, Papadopoulos MC, Hara-Chikuma M, Verkman AS: Impairment of angiogenesis and cell migration by targeted aquaporin-1 gene disruption. *Nature* 434: 786–792, 2005
11. Endo M, Jain RK, Witwer B, Brown D: Water channel (aquaporin 1) expression and distribution in mammary carcinomas and glioblastomas. *Microvasc Res* 58: 89–98, 1999
12. Vacca A, Frigeri A, Ribatti D, Nicchia GP, Nico B, Ria R, Svelto M, Dammacco F: Microvessel overexpression of aquaporin 1 parallels bone marrow angiogenesis in patients with active multiple myeloma. *Br J Haematol* 113: 415–421, 2001
13. Schwab A: Function and spatial distribution of ion channels and transporters in cell migration. *Am J Physiol Renal Physiol* 280: F739–F747, 2001
14. Sheridan AM, Schwartz JH, Kroshian VM, Tercyak AM, Laraia J, Masino S, Lieberthal W: Renal mouse proximal tubular cells are more susceptible than MDCK cells to chemical anoxia. *Am J Physiol* 265: F342–F350, 1993
15. Yang B, Ma T, Dong JY, Verkman AS: Partial correction of the urinary concentrating defect in aquaporin-1 null mice by adenovirus-mediated gene delivery. *Hum Gene Ther* 11: 567–575, 2000
16. Solenov E, Watanabe H, Manley GT, Verkman AS: Sevenfold-reduced osmotic water permeability in primary astrocyte cultures from AQP-4-deficient mice, measured by a fluorescence quenching method. *Am J Physiol Cell Physiol* 286: C426–C432, 2004
17. Troyanovsky B, Levchenko T, Mansson G, Matvijenko O, Holmgren L: Angiomotin: An angiostatin binding protein that regulates endothelial cell migration and tube formation. *J Cell Biol* 152: 1247–1254, 2001
18. Mizushima H, Takamura H, Miyagi Y, Kikkawa Y, Yamanaka N, Yasumitsu H, Misugi K, Miyazaki K: Identification of integrin-dependent and -independent cell adhesion domains in COOH-terminal globular region of laminin-5 alpha 3 chain. *Cell Growth Differ* 8: 979–987, 1997
19. Faarup P, Holstein-Rathlou NH, Norgaard T, Hegedus V: Early segmental changes in ischemic acute tubular necrosis of the rat kidney. *APMIS* 112: 192–200, 2004
20. Sato W, Takei Y, Yuzawa Y, Matsuo S, Kadomatsu K, Muramatsu T: Midkine antisense oligodeoxyribonucleotide inhibits renal damage induced by ischemic reperfusion. *Kidney Int* 67: 1330–1339, 2005
21. Nigam S, Weston CE, Liu CH, Simon EE: The actin cytoskeleton and integrin expression in the recovery of cell adhesion after oxidant stress to a proximal tubule cell line (JTC-12). *J Am Soc Nephrol* 9: 1787–1797, 1998
22. Tian YC, Phillips AO: TGF-beta1-mediated inhibition of HK-2 cell migration. *J Am Soc Nephrol* 14: 631–640, 2003
23. Sabolic I, Valenti G, Verbavatz JM, Van Hoek AN, Verkman AS, Ausiello DA, Brown D: Localization of the CHIP28 water channel in rat kidney. *Am J Physiol* 263: C1225–C1233, 1992
24. Chen XM, O'Hara SP, Huang BQ, Splinter PL, Nelson JB, LaRusso NF: Localized glucose and water influx facilitates Cryptosporidium parvum cellular invasion by means of modulation of host-cell membrane protrusion. *Proc Natl Acad Sci U S A* 102: 6338–6343, 2005
25. Loitto VM, Forslund T, Sundqvist T, Magnusson KE, Gustafsson M: Neutrophil leukocyte motility requires directed water influx. *J Leukoc Biol* 71: 212–222, 2002
26. Nony PA, Schnellmann RG: Mechanisms of renal cell repair and regeneration after acute renal failure. *J Pharmacol Exp Ther* 304: 905–912, 2003
27. Smith BL, Baumgarten R, Nielsen S, Raben D, Zeidel ML, Agre P: Concurrent expression of erythroid and renal aquaporin CHIP and appearance of water channel activity in perinatal rats. *J Clin Invest* 92: 2035–2041, 1993

Supplemental information for this article is available online at http://www.jasn.org/