β1-Integrin is required for kidney collecting duct morphogenesis and maintenance of renal function

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Departments of 1Pediatrics, 2Medicine, 3Radiology and 4Cellular Molecular Medicine, 5VA Healthcare San Diego, and the 4John and Rebecca Moores UCSD Cancer Center, School of Medicine, University of California, La Jolla, California

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Wu W, Kitamura S, Truong DM, Rieg T, Vallon Y, Sakurai H, Bush KT, Vera DR, Ross RS, Nigam SK. β1-Integrin is required for kidney collecting duct morphogenesis and maintenance of renal function. Am J Physiol Renal Physiol 297: F210–F217, 2009. First published May 13, 2009; doi:10.1152/ajprenal.90260.2008.—Deletion of integrin-β1 (Itgb1) in the kidney collecting system led to progressive renal dysfunction and polyuria. The defect in the concentrating ability of the kidney was concomitant with decreased medullary collecting duct expression of aquaporin-2 and arginine vasopressin receptor 2, while histological examination revealed hypoplastic renal medullary collecting ducts characterized by increased apoptosis, ectasia and cyst formation. In addition, a range of defects from small kidneys with cysts and dilated tubules to bilateral renal agenesis was observed. This was likely due to altered growth and branching morphogenesis of the ureteric bud (the progenitor tissue of the renal collecting system), despite the apparent ability of the ureteric bud-derived cells to induce differentiation of the metanephric mesenchyme. These data not only support a role for Itgb1 in the development of the renal collecting system but also raise the possibility that itgb1 links morphogenesis to terminal differentiation and ultimately collecting duct function and/or maintenance.

urinary bladder; metanephric mesenchyme; branching morphogenesis; cystic kidney disease; diabetes insipidus; renal failure; polyuria


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traced through the tail vein. Control and knockouts were placed in pairs in a prone position on the surface of a BioSpace planar scintigraphic camera. Images were collected for 50 min following the injection of the tracer. Volumetric computed tomography (CT) scans were performed at 90-µm³ isometric voxel resolution using an eXplore Locus RS rodent MicroCT scanner (GE Healthcare). Images were reconstructed with the manufacturer’s proprietary software. Both imaging experiments were conducted at the UCSD Imaging Core Facility.

Laser capture and qPCR. Whole kidneys were flash-frozen in liquid nitrogen. Frozen 10-µm kidney sections were collected. The kidney medulla was isolated using laser-microdissection with a MMI SL µCut Laser Capture Microdissection microscope (Molecular Machines and Industries, Rockledge, FL). RNA was then extracted from either the medulla or the whole kidney using an Ambion RNA microkit (Austin, TX) and then amplified into cDNA using the Applied Biosystems’ Fast Real-Time PCR 7500 (Foster City, CA). Primers for selected genes were designed using Primer 3 and then generated. qPCR was performed using Invitrogen Syber Green/Rox (Carlsbad, CA) and the Applied Biosystems’ Fast Real-Time PCR 7500 (Foster City, CA).

**RESULTS**

**Generation of collecting duct-specific Itgb1 deletion.** To determine the role of Itgb1 in the renal collecting system, LoxP-flanked Itgb1 mice were crossed with a transgenic line expressing Cre recombinase under the control of the UB-specific HoxB7 promoter (19, 29, 37). Itgb1LoxP/loxP HoxB7Cre+ mutants were then generated through mating with heterozygous Itgb1fl/+ HoxB7Cre+. Expected Mendelian frequencies were obtained. Heterozygous Itgb1fl+/loxP HoxB7Cre+ littersmates were used as controls. qPCR analysis of Itgb1 expression was performed using whole adult kidneys, as well as on microdissected renal medulla. There was little, if any, significant difference in the level of Itgb1 expression in whole kidney preparations of Itgb1fl/loxP HoxB7Cre+ mice (compared with littermate Itgb1fl+/loxP HoxB7Cre+) (Fig. 1), presumably due to the large amount of surrounding non-UB/collgecting duct-derived tissue. However, qPCR analysis of RNA isolated from laser capture, microdissected renal medullary tissue (which contains a greater proportion of tissue that is of collecting duct origin) revealed a substantial loss of Itgb1 expression in conditional knockout mice, compared with heterozygous control mice (Fig. 1).

Controls had normal-appearing kidneys, while the Itgb1fl/loxP HoxB7Cre+ knockouts displayed a wide range of kidney abnormalities ranging from bilateral agenesis to apparently normal-sized kidneys. Of the 25 mutants examined, 11 displayed gross renal hypoplasia, 1 had unilateral renal agenesis, 2 had bilateral renal agenesis, and the remaining 11 mutants had grossly normal-sized kidneys (Table 1). An additional 20 mutants were allowed to grow to term and then weaned; these survived into adulthood (8 wk of age). Thus, of the 45 mutants examined, only 2 displayed complete bilateral renal agenesis (~5% of the mutants, which did not survive either birth or the initial week; Table 1), while all the others developed into adulthood (8th wk), indicating the presence of at least one functioning kidney (as described below, many of these were found to exhibit a renal phenotype).

Adult Itgb1fl/loxP HoxB7Cre+ animals developed renal insufficiency. Adult Itgb1fl/loxP HoxB7Cre+ (along with Itgb1fl+/loxP HoxB7Cre+) animals maintained in metabolic cages at 8 wk of age were found to excrete more than 10 times the volume of urine than the Itgb1fl+/loxP HoxB7Cre+ mice (when allowed to drink water ad lib) (Fig. 2A, left; \( P < 0.05 \)). The urine of the knockouts remained relatively isosmotic with blood plasma (Fig. 2B). Water restriction for 12 h did not significantly reduce the urine output in Itgb1fl/loxP HoxB7Cre+, while the Itgb1fl+/loxP HoxB7Cre+ mice produced a smaller volume of urine with water restriction (Figs. 2A, right; \( P < 0.05 \)). Histological examination of mutant kidneys with polyuria and renal failure revealed a dramatically perturbed architecture of the kidney (Fig. 2, C–F). The cortex was characterized by relatively normal patches of renal cortex, containing clustered glomeruli, separated by larger areas containing multiple cystic structures which are likely to be dilated cortical collecting ducts. Tubular ectasia was also seen in the renal medulla (compare Fig. 2, C and D, vs. E and F) with dilated collecting ducts that were almost three times as wide in the mutant medulla (compare Fig. 2, D vs. F). The cells of the medullary collecting duct express the hormone vasopressin (AVP). The collecting ducts from the mutant medullary region were found to have significantly reduced expression of Aqp2 and vasopressin V2 receptor, Avpr2 (Fig. 2G). Given the

**Table 1. Phenotype distribution in β1-integrin-deficient mice**

<table>
<thead>
<tr>
<th>Genotype</th>
<th>E18-P1</th>
<th>Adult</th>
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<tbody>
<tr>
<td></td>
<td>Small kidney</td>
<td>Small body weight</td>
</tr>
<tr>
<td></td>
<td>(&lt;2/3 normal)</td>
<td>Total</td>
</tr>
<tr>
<td>Itgb1fl+/+/HoxB7Cre+ &amp;</td>
<td>101</td>
<td>72</td>
</tr>
<tr>
<td>Itgb1fl+/loxP HoxB7Cre+</td>
<td>46</td>
<td>28</td>
</tr>
<tr>
<td>Itgb1fl+/loxP HoxB7Cre+ (Het)</td>
<td>25</td>
<td>14 (3 agenesis)</td>
</tr>
<tr>
<td>Total</td>
<td>172</td>
<td>125</td>
</tr>
</tbody>
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*E18, embryonic day 18; P1, postpartum day 1; Het, heterozygous; KO, knockout.*
physiological function, defects in the medullary collecting ducts could provide an explanation for the observed polyuria phenotype in *Itgb1*−/−*HoxB7Cre*+ mice.

To investigate this further, blood and urine chemistries for the mutants and controls was performed (Table 2). Adults were found to have relatively normal plasma level of Na⁺ and K⁺ compared with controls. Furthermore, while the pH of the urine in the knockouts was not significantly changed, the plasma urea level was elevated and there was a clear and significant decrease in the blood hematocrit (HCT; 42% in knockouts vs. 52% in control) (Fig. 2H). There was no significant change in the blood creatinine level between mutant and control adults (Table 2); the fact that the urinary AVP-to-creatinine ratio (as a surrogate for plasma AVP levels) was enhanced in the mutants (Fig. 2I) indicates that the primary cause of polyuria is likely due to an inability to respond to AVP within the kidney, a role performed by cells of the collecting duct, derived from the UB. Moreover, the concentrating defect seems to be com-
parable to that found in Aqp2 and Avpr2 mutants (18, 29, 30, 36). Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/−</sup> were also noted to have an elevated plasma urea concentration. The fact that these knockouts have an abnormal medulla and exhibit polyuria supports the notion that the medullary defect itself precipitates the development of a urinary concentration defect in Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/−</sup> mice. Taken together, the data are consistent with chronic renal disease in the knockout.

This notion is further supported by BioSpace planar scintigraphic analysis, which revealed a reduction in the ability of the knockout to eliminate the contrast agent DPTA compared with control animals. Side-by-side comparison of the Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/−</sup> knockout and the Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/+</sup> (heterozygotes) controls revealed clear accumulation and concentration of the contrast agent in the kidneys and bladder of the heterozygotes (Fig. 3A).

Testing blood (range in parentheses) and urine samples were taken from 6 littermate pairs of heterozygous and homozygous KO mice, at 7–10 wk of age. BUN, blood urea nitrogen; SGPT, serum glutamic-pyruvic transaminase; AST, aspartate aminotransferase.

### Table 2. Blood and urine chemistry in β1-integrin-deficient mice

<table>
<thead>
<tr>
<th></th>
<th>Itgb1&lt;sup&gt;−/−&lt;/sup&gt; HoxB7Cre&lt;sup&gt;−/−&lt;/sup&gt;</th>
<th>Itgb1&lt;sup&gt;−/−&lt;/sup&gt; HoxB7Cre&lt;sup&gt;−/+&lt;/sup&gt;</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Blood</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Glucose (90–192 mg/dl)</td>
<td>187.2</td>
<td>143.3</td>
</tr>
<tr>
<td>BUN (18–29 mg/dl)</td>
<td>26.1</td>
<td>47.3</td>
</tr>
<tr>
<td>Creatinine (0.2–0.8 mg/dl)</td>
<td>0.2</td>
<td>0.25</td>
</tr>
<tr>
<td>Albumin (2.5–4.8 g/dl)</td>
<td>3.36</td>
<td>2.98</td>
</tr>
<tr>
<td>Globulin</td>
<td>1.72</td>
<td>1.52</td>
</tr>
<tr>
<td>Total protein (3.0–6.6 g/dl)</td>
<td>5.1</td>
<td>4.47</td>
</tr>
<tr>
<td>Bilirubin (0.1–0.9 mg/dl)</td>
<td>0.17</td>
<td>0.15</td>
</tr>
<tr>
<td>Sodium (126–182 meq/l)</td>
<td>156</td>
<td>158</td>
</tr>
<tr>
<td>Calcium (5.9–9.4 mg/dl)</td>
<td>10.06</td>
<td>8.73</td>
</tr>
<tr>
<td>Phosphorus (6.1–10.1 mg/dl)</td>
<td>11.2</td>
<td>9.37</td>
</tr>
<tr>
<td>SGPT (28–132 U/l)</td>
<td>81.2</td>
<td>51.8</td>
</tr>
<tr>
<td>Alkaline phosphatase (62-209 U/l)</td>
<td>149.5</td>
<td>158.5</td>
</tr>
<tr>
<td>Amylase (1,691–3,615 U/l)</td>
<td>963</td>
<td>911</td>
</tr>
<tr>
<td><strong>Urine</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Albumin, g/dl</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Alkaline phosphatase, U/l</td>
<td>&lt;5</td>
<td>&lt;5</td>
</tr>
<tr>
<td>ALT, U/l</td>
<td>83.8</td>
<td>78.1</td>
</tr>
<tr>
<td>Amylase, U/l</td>
<td>122.8</td>
<td>215.8</td>
</tr>
<tr>
<td>Bilirubin, total, mg/dl</td>
<td>&lt;0.1</td>
<td>&lt;0.1</td>
</tr>
<tr>
<td>Calcium, mg/dl</td>
<td>&lt;4.0</td>
<td>&lt;4.0</td>
</tr>
<tr>
<td>Creatinine, mg/dl</td>
<td>12.13</td>
<td>4.78*</td>
</tr>
<tr>
<td>Glucose, mg/dl</td>
<td>37.5</td>
<td>36.3</td>
</tr>
<tr>
<td>Total protein, g/dl</td>
<td>&lt;2.0</td>
<td>&lt;2.0</td>
</tr>
</tbody>
</table>

Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/−</sup> (knockouts), DPTA appears to be randomly distributed throughout the body, indicating a reduced capacity of the kidneys in the knockout animals to clear this agent from the blood (Fig. 3A). In addition, companion micro-CT scans were performed between the Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/−</sup> (knockouts) and Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/+</sup> (heterozygotes) control with labeled PAH. This examination revealed little uptake of the labeled PAH into the knockout kidneys compared with the kidneys from the control animals (Fig. 3, B and C). Semiquantitative analysis of these micro-CT scan images revealed that renal uptake of the labeled PAH was reduced ~85% compared with that of normal kidneys (Fig. 3D). Thus the function of the kidneys in these mutants is clearly and profoundly perturbed.

A number of tubular markers were examined by RT-PCR. No significant changes were observed in the expression of the glomerular markers Nephrin and Podocin, or the proximal tubular marker Oat3, and thick ascending limb of Henle’s loop marker Thp when their expression levels were normalized to that of Gapdh (data not shown). However, a significant increase in terminal transferase-mediated dUTP nick-end labeling-positive cells was observed in the cortex (Fig. 4, A and C) and associated with the dilated tubules in the medulla of mutant kidneys (Fig. 4, B and C). In addition, there was a significant increase in cellular proliferation, as evidenced by cells positive for Ki67 (a cellular marker for proliferation) in the mutant kidney cortex, probably associated with proximal tubular structures (Fig. 4, D and F), but not in the medulla (Fig. 4, E and F).

A medullary defect and severe polyuria become evident in the mature Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/−</sup> kidney. As indicated in Table 1, the majority of mutants were born with gross renal hypoplasia. The kidneys failed to attain the size of the wild-type or heterozygous kidneys during postnatal development and were still significantly smaller at both 1 and 2 wk of age (Fig. 5, A–C). On closer examination, late-stage developing Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/−</sup> kidneys were small, primarily due to the reduced size of the inner medulla (Fig. 5, D and F), revealed by conjugated DB staining specific for collecting duct/UB-derived structures, (which is normally densely populated by cells of the collecting ducts) as well as the expression of some extracellular matrix- and tissue-specific markers. At a higher magnification, DB-positive cells were found lining the medullary tubular structures of the remaining collecting ducts (Fig. 5, E and G). In addition, the basement membrane of the UB-derived tubules in the Itgb1<sup>−/−</sup>HoxB7Cre<sup>−/−</sup> kidney were found to express an epithelial marker, collagen IV (Fig. 5H), a
ligand of β1-integrin receptors, in a pattern similar to that seen in Itgb1f/fHoxB7Cre+ control kidney (data not shown). Moreover, the localization of occludin along the apical aspect of the tubular cells in Itgb1f/fHoxB7Cre+ mice indicates that these cells retain their epithelial nature and possess tight junctions (Fig. 5I). These findings suggested that the mutant UB-derived cells were competent to maintain polarity through the normal establishment of a basolateral matrix and an apical tight junction. Similarly, epithelial cells of the mesenchyme, which form the future nephron, did not express DB and were found to express a proximal tubular marker, Aqp1, at the apical surface (Fig. 5J); thus polarized proximal tubular cells were induced in the Itgb1f/fHoxB7Cre+ mice.

**DISCUSSION**

We have conditionally deleted the integrin receptor β1 in epithelial cells comprising the UB and its derivatives, and thus specifically from the renal collecting system. The following were observed: 1) progressive renal impairment; 2) inability to concentrate urine; 3) decreased expression of Avpr2 and Aqp2; and 4) compromised formation and/or maintenance of the medullary collecting ducts. The results demonstrate the importance of Itgb1 in the growth and development of the renal collecting system, while supporting a role for it in the differentiation and/or maintenance of the medullary collecting ducts.

As previously described, defects in the medullary region of the kidney, particularly in the medullary collecting ducts, are observed in Itga3 knockouts. Since the only identified partner for Itga3 in the UB (the progenitor tissue of the renal collecting system) is the β1-integrin subunit, the fact that the Itgb1f/fHoxB7Cre+ mice exhibit a phenotype somewhat similar to that seen in Itga3 knockouts supports a key role for integrin α3β1 in the development of the renal collecting system (a role which cannot be completely compensated for by other integrin receptors found in this tissue). In adult kidneys, tubular epithelial cells in the medulla exhibited a significant increase in apoptosis. Cells in the medulla, especially the deeper medulla, are exposed to more stressful physiologic conditions (i.e., higher osmolarity) than in other parts of the kidney (15). Therefore, it is possible that the lack of Itgb1 results in a lower tolerance to stress in cells in this region, manifesting as increased apoptosis. Moreover, there was decreased expression of Aqp2 and Avpr2 in the medullary region of the adult mutant kidney. Although increased apoptosis in this region could cause a reduction in the collecting duct cells expressing these markers in the medulla, an alternative explanation is that Itgb1-mediated signaling may be required for proper development/differentiation of the developing UB cells and medullary collecting duct cells, either during development or as the apoptotic cells are replaced in the adult kidney.

This notion is consistent with alterations in cell proliferation seen in other conditional deletions of Itgb1. For example, conditional deletion of Itgb1 in the epidermal layer of the skin led to an ~70% decrease in keratinocyte proliferation (5, 27). Specific deletion of Itgb1 from chondrocytes or the mammary gland resulted in decreased proliferation both in vivo and in vitro (1, 17, 23). In these cases, cell cycle progression of Itgb1-deficient cells was slower than that observed in wild-type epithelial cells, suggesting that deletion of Itgb1 from the UB and UB-derived structures might have a similar effect in the kidney, both during development and in the adult. Thus the appearance of hypoplastic kidneys could be the result of slower growth and branching of the UB during kidney organogenesis. Cyst formation and tubular ectasia could be due to defective collecting duct morphogenesis or obstruction. It is noteworthy
that UB, IMCD, and other renal epithelial cells, as well as the isolated UB itself, are very sensitive to extracellular matrix composition when (2, 6, 26, 31–33, 38) grown in three-dimensional cultures. In fact, many of the receptors for these matrix components include β1-integrin as a subunit.

Many mutants developed renal impairment by 8 wk of age with lower HCT. Although it remains to be definitively determined, the lower HCT in the mutant mice is likely due to reduced Epo production in the mutant kidneys. Recently, a collecting duct-specific deletion of \( Aqp2 \) was reported (29). This animal exhibits nephrogenic diabetes insipidus to a degree comparable to that reported here. In these \( Aqp2 \) mice, however, neither the renal impairment nor the medullary hypoplasia (fallout) that is seen in the \( Itgb1^{+/+} \) mice was observed. Thus it is unlikely that the observed nephrogenic diabetes insipidus phenotype can fully explain the cause of renal impairment in the \( Itgb1^{+/+} \) mice. Mutants with significantly diminished nephron number are also not reported to develop this degree of progressive renal dysplasia or any defects in water handling (3, 7, 22, 24). It is possible that the increased apoptosis in the medullary collecting duct contributes to renal impairment, although this remains to be determined.

We also observed decreased expression of genes that play key roles in water handling, \( Aqp2 \) and \( Avpr2 \). During normal development, there are significant increases found in expression of both genes, \( Aqp2 \) and \( Avr2 \), around the time of birth, reaching adult levels of expression by the time of weaning. Thus it is also possible that \( Itgb1 \) plays a role in the differentiation of the collecting duct cells, and in its reduction/absence, medullary collecting duct cells have perturbed function. This result of epithelial cell differentiation occurring in the mutant
mice is similar to that which occurs following conditional deletion of Itgb1 from intestinal epithelium, where a defect in epithelial differentiation was observed (13). Furthermore, conditional deletion in mammary epithelium also led to abnormal differentiation such that these mice do not produce milk in response to prolactin (23). Both of these cases, along with our own data, point toward an essential role of Itgb1 in establishing and/or maintaining differentiation of polarized epithelial cells, such as those comprising the renal collecting ducts. In collecting duct cells, Itgb1 signaling may play an important role in regulating water channel and vasopressin receptor expression. If vasopressin itself is necessary for collecting system morphogenesis or maintenance, this could lead to a vicious cycle, resulting in progressive renal dysfunction. This is consistent with the data that the severe water handling defect seems to precede renal impairment. The remaining function may be attributable to incomplete penetration of the mutation.

Finally, we found increased medullary apoptosis in mature knockout kidneys. This is consistent with the data that the severe water handling defect seems to precede renal impairment. This is not generally reported in Itgb1 conditional knockouts. In Itgb1 deletion in Schwann cells (11) or mammary epithelial cells, increased apoptosis is not observed although chondrocytes lacking Itgb1 expression show increased apoptosis. In our study, increased apoptosis was observed only in adult kidney. It may be that Itgb1 is not critical for survival of certain cells in ordinary circumstances but may be required for survival in stressful environments such as the cartilage (low blood flow) or kidney medulla (high osmolarity). Furthermore, as discussed, if vasopressin functions in collecting system morphogenesis and/or maintenance, the decrease in expression of its receptor in the knockout may further compromise cell survival, especially under stress.

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REFERENCES


