EP₂ receptor mediates PGE₂-induced cystogenesis of human renal epithelial cells

Gerard Elberg, Dorit Elberg, Teresa V. Lewis, Suresh Guruswamy, Lijuan Chen, Charlotte J. Logan, Michael D. Chan, and Martin A. Turman

1Department of Pediatrics and 2College of Pharmacy, University of Oklahoma Health Sciences Center, Oklahoma City, Oklahoma

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Prostaglandin E₂ (PGE₂) is a major lipid mediator in the kidney that exerts both autocrine and paracrine actions (39, 51). A potential role of PGE₂ in ADPKD is suggested by higher levels of PGE₂ during the progression of the disease (41), secretion of PGE₂ into the cyst fluid of human ADPKD kidneys (14), and inhibition of cyst fluid-induced cAMP formation with L-161982, a PGE₂ receptor 4 antagonist (2). PGE₂ activity is mediated by binding to four different G protein-coupled receptors named E-prostanoid (EP) receptors 1–4. Among them, EP₁ and EP₄ mediate their effect through coupling to G stimulatory proteins, stimulating the induction of cAMP formation and activation of protein kinase A (7, 13). Stimulation of EP₂ receptors also antagonizes vasopressin action, known to stimulate cAMP, through the induction of Rho activation and actin polymerization in collecting-duct principal cells (44). However, EP₃ receptor stimulation typically inhibits the formation of cAMP as well as Ca²⁺ release through G inhibitory pertussis toxin-sensitive protein (7). Interestingly, EP₃ mRNA has been shown to be upregulated in human ADPKD kidneys (38). The EP₁ receptor induces inositol 3-phosphate formation and consequently the release of Ca²⁺.

The cellular actions of PGE₂ are possibly regulated via the expression of different EP receptors located in different tubular segments and cell types in the kidney. The EP₁ receptor is expressed in the collecting ducts (7, 8, 18, 31), and EP₃ receptor is mainly located in distal tubules, collecting ducts, and glomeruli (8, 31). Whereas the EP₄ receptor is located in the collecting ducts and glomeruli in normal kidney, the presence of EP₂ receptor in the kidney is still elusive (7, 8, 31). The absence of EP₂ receptor in RTC remains an enigma, because experiments using EP₂ receptor knockout mice (24), in which the animals develop salt-sensitive hypertension, suggest a critical role for EP₂ receptor in sodium regulation in the kidney. Both the EP₂ and EP₄ receptors are identified by their vasodepressor effect, in which EP₂, but not EP₄, is sensitive to butaprost, a PGE₂ agonist (7). PGE₂ induces cAMP formation in CEC from ADPKD kidneys, which suggests a role for EP₂ or EP₄ receptor in mediating this effect (3). Also, it has been reported that L-161982, an EP₄ antagonist, partially inhibited PGE₂-induced cAMP formation (2). Because the particular EP receptor mediating PGE₂-induced cystogenesis has not been extensively characterized, our study aims to define PGE₂ receptor subtype and signaling involved in ADPKD.

Autosomal-dominant polycystic kidney disease (ADPKD) is a genetic disorder derived from mutations mostly of PKD1 and PKD2 genes, which encode for polycystin-1 and -2 (PC-1 and -2), respectively (47). ADPKD is characterized by abnormal growth of tubular epithelial cells that form multiple fluid-filled cysts (43). Various studies in vitro using renal epithelial tubular cells (RTC) and cystic epithelial cells (CEC) derived from normal and polycystic human kidneys indicate that the level of cAMP plays an important role in the regulation of cyst formation (20, 32, 55, 56). Therefore, various natural ligands of G protein-coupled receptors that are known to induce adenylyl cyclase and cAMP formation in the kidney possibly stimulate cyst enlargement and ADPKD progression.

Address for reprint requests and other correspondence: G. Elberg, Dept. of Pediatrics/Nephrology, The Univ. of Oklahoma Health Sciences Center, 940 N. E. 13th St., 2B2309, Oklahoma City, OK 73104 (e-mail: gerard-elberg@ouhsc.edu).

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MATERIALS AND METHODS

Cell cultures and culture conditions. RTC were isolated from human kidney cortex and were maintained as previously described by our laboratory (48, 49). Cells derived from different kidneys were identified by using individuals with ADPKD were isolated essentially as described (53, 55). Cells derived from different kidneys were identified by using different cell numbers. RTC and CEC were propagated in MEM (55). Cell cultures and culture conditions. RTC were isolated from different kidneys. Our study protocols were approved by the Institutional Review Board of the University of Oklahoma Health Sciences Center.

Three-dimensional gel system. RTC and CEC (0.15 x 10^5–0.5 x 10^5 cells/well) were suspended in a 4°C solution containing complete MEMα and a 1:1 (vol/vol) mixture of liquid rat-tail collagen I and Matrigel (Becton Dickinson, Franklin Lakes, NJ) and were poured into cell-culture inserts as previously described (19). Cells were treated as indicated in figure legends. Cysts were observed by phase-contrast microscopy and were counted before fixation by incubating gels in 10% formalin (Labsco, Louisville, KY). Permeabilization of cells was performed in PBS supplemented with 0.2% saponin and 1% BSA (Sigma), and nuclei were stained with 2 μg/ml 4',6-diamidino-2-phenylindole dihydrochloride (DAPI; Sigma) in PBS. Cysts were viewed with an IX50 inverted fluorescent microscope (Olympus, Melville, NY), and size was determined by using the SPOT program (Diagnostic Instruments, Sterling Heights, MI).

Quantitative real-time RT-PCR. The method for mRNA quantitation by real time RT-PCR was previously published (12). mRNA was treated with RNase-free DNase (Invitrogen), or extraction was performed by using an RNeasy Plus mini kit (Qiagen, Valencia, CA) that removes contaminating genomic DNA from the sample. RNA extraction from three-dimensional (3-D) gels was performed by using the TRIzol method (Invitrogen). mRNA was measured by using SYBR Green technology (Applied Biosystems, Foster City, CA) on an ABI PRISM 7000 sequence detection system (Applied Biosystems) and was calculated by using the formula 2^(-ΔΔCT) (29). The sequences of primers not published previously are shown in Table 1.

Transfection of EP receptors. cDNA for human EP1, EP2, EP3, and EP4 receptors were provided by the cDNA Resource Center (University of Missouri-Rolla). DNA plasmids were purified by using a maxiprep kit (Qiagen) according to the manufacturer’s instructions.

Table 1. Sequences of primers used for real-time RT-PCR analysis and not previously published (12)

<table>
<thead>
<tr>
<th>Forward Primer</th>
<th>Reverse Primer</th>
</tr>
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<tr>
<td>EP1</td>
<td>GGCACGTTTGCAGATATCAT</td>
</tr>
<tr>
<td>EP2</td>
<td>AGAGGCGGACATCTTTC</td>
</tr>
<tr>
<td>EP3</td>
<td>CGTTGGCTGTGGATTGTC</td>
</tr>
<tr>
<td>EP4</td>
<td>GCGACACCTGGCTCCTGA</td>
</tr>
<tr>
<td>Bax</td>
<td>CCAAGTGGCGGAGACTGA</td>
</tr>
<tr>
<td>Bcl-2</td>
<td>TGAATTGCTTCCGCGTATTTG</td>
</tr>
<tr>
<td>Cyclin D1</td>
<td>CTGGTCTTCATACCCAGGCG</td>
</tr>
<tr>
<td>Cyclin D2</td>
<td>GTCTCAATGTTTTGCAACTGCCAAA</td>
</tr>
<tr>
<td>PCNA</td>
<td>CGTCTACCTTCAAGAAGGTTT</td>
</tr>
<tr>
<td>MCM2</td>
<td>CCGCGCGTACGAAATGGA</td>
</tr>
</tbody>
</table>

EP, E-prostanoid receptor; PCNA, proliferating cell nuclear antigen; MCM2, minichromosome maintenance protein 2.

Fig. 1. Effect of prostaglandin E2 (PGE2) on cyst formation using cystic epithelial cells (CEC) in collagen/Matrigel 3-dimensional (3-D) gel culture. Left: cells were cultured in growth medium with and without FBS (serum-free medium, SFM) and in the absence or presence of 77 nM PGE2 for 15 days. Magnification, ×40. Bottom left: CEC were cultured in the presence of FBS and PGE2 for 27 days. Macroscopic size of cysts (arrow) is shown. Right: 4',6-diamidino-2-phenylindole dihydrochloride (DAPI) staining of nuclei in CEC forming cysts in presence or absence of PGE2 for 15 days. Magnification, ×100.
cysts. Magnification, growth medium for 14 days lost their tubular-like organization and form small (of nuclei reveal that cells incubated with 77 nM PGE2 in bottom collagen/Matrigel 3-D gel culture. Phase-contrast images (Table 2. Effect of PGE2 on cyst formation in collagen/Matrigel 3-D gel cultures

<table>
<thead>
<tr>
<th>Cell Type</th>
<th>No PGE2</th>
<th>With PGE2</th>
<th>Fold</th>
<th>No PGE2</th>
<th>With PGE2</th>
<th>Fold</th>
</tr>
</thead>
<tbody>
<tr>
<td>CEC2900</td>
<td>20.2 ± 3.8</td>
<td>35.3 ± 3.5</td>
<td>1.7</td>
<td>50</td>
<td>111</td>
<td>2.2</td>
</tr>
<tr>
<td>CEC41801</td>
<td>36.3 ± 3.3</td>
<td>93.6 ± 5.4</td>
<td>2.6</td>
<td>189.0</td>
<td>601</td>
<td>3.2</td>
</tr>
<tr>
<td>CEC72499</td>
<td>68.0 ± 13.0</td>
<td>108.6 ± 14.8</td>
<td>1.6</td>
<td>79</td>
<td>171</td>
<td>2.2</td>
</tr>
<tr>
<td>RTC12946</td>
<td>1.2 ± 0</td>
<td>8.2 ± 1</td>
<td>6.8</td>
<td>1</td>
<td>52</td>
<td>52</td>
</tr>
<tr>
<td>RTC72095</td>
<td>5.7 ± 1.0</td>
<td>14.3 ± 7.9</td>
<td>2.5</td>
<td>2</td>
<td>25</td>
<td>12.5</td>
</tr>
<tr>
<td>RTC91896</td>
<td>9.6 ± 1.8</td>
<td>17.9 ± 7.4</td>
<td>1.9</td>
<td>12</td>
<td>103</td>
<td>8.6</td>
</tr>
</tbody>
</table>

Values are means ± SE for size and means for number. Cells were grown in growth medium in the presence or absence of 77 nM PGE2 and were observed in 25 fields of view at magnification ×40. PG, prostaglandin; 3-D, three dimensional; CEC, cystic epithelial cells; RTC, renal tubular cells.
FBS; cyst formation was augmented by addition of PGE2 in medium supplemented with or without serum. The size of cysts was maximal when medium was supplemented with both FBS and PGE2. CEC cysts formed not only inside the gels, but some macroscopic cysts expanded out from the surface and were easily visible without magnification after prolonged culture. Cystic structures were also well documented by appearance of nuclei on staining with DAPI. The 3-D structure of cysts is clearly visible, with nuclei in the cyst walls lining a central cavity, which can be appreciated at different levels of focus.

Figure 2 shows that in the absence of PGE2, RTC formed tubular structures that can be visualized by phase-contrast microscopy and also by the linear appearance of nuclei on staining with DAPI. Under these same conditions, CEC formed cysts (Fig. 1). PGE2 treatment inhibited tubule formation and induced formation of small cysts. The effect of PGE2 on cyst size and number was determined in three different CEC and RTC cultures derived from different kidneys. Table 2 shows that RTC formed few cysts with a small average size compared with CEC in the absence of PGE2. PGE2 treatment increased cyst number by 9- to 52-fold in RTC and 2- to 3-fold in CEC. Note that the size and number of cysts formed by CEC in the absence of PGE2 was greater than for RTC even in the presence of PGE2. Therefore, PGE2 stimulates cyst formation in both CEC and RTC, but CEC have a greater propensity for cystogenesis, consistent with their derivation from ADPKD kidneys.

Because kidney formation is mostly normal at birth during ADPKD (33) and cysts form from tubular epithelial cells after birth, it was of interest to determine whether PGE2 affects
well-organized RTC tubules in 3-D matrix. Figure 3 shows that addition of PGE2 to already formed tubules results in tubule disintegration into patches that expand to form cysts. Staining of nuclei demonstrates that the tubules expand and cells become organized in a circular manner, forming cysts. Therefore, our results indicate that PGE2 induces cyst formation in RTC independent of mutations in both PC-1 or PC-2, but CEC have a greater propensity toward forming more and larger cysts.

Characterization of the EP receptor subtypes present in CEC. To determine the different EP receptor subtypes present in CEC, we initially analyzed the mRNA expression of four different EP receptors. Figure 4 shows that CEC express high levels of EP2 receptor mRNA and low levels of EP1 and EP4, whereas EP3 was not detected. The expression of EP2 mRNA is particularly surprising because previous studies suggested the absence of EP2 receptor in RTC of human tissue. To further confirm the presence of EP2 receptor protein in human kidneys, we performed Western blot analysis using an EP2 antibody generated by genetic immunization. We did not find other commercially available antibodies to be specific or sensitive enough to be used in Western blot and immunohistochemical analysis. The specificity of this antibody to EP2 receptor, but not to other PGE2 receptor subtypes, was demonstrated by analyzing extracts of CHO cells transfected with the different receptor subtypes. Figure 5 shows that cells transfected with EP2 receptor exhibit a 65-kDa protein band that does not appear in other CHO extracts. We also observed a protein band of similar molecular weight detected by EP2 antibody in membranes from ADPKD kidney.

We next performed immunohistochemical-staining analysis with the same antibody for EP2 receptor that was used for immunoblot analysis. mlGlG was used as a negative control. Figure 6 shows that EP2 receptors are present on the CEC-lining cysts in polycystic kidney tissue from patients with ADPKD. Staining was not evident in blood vessels (Fig. 6, middle). EP2 receptors are mostly expressed on the apical side of the cysts, which implies potential direct contact with PGE2 secreted into the cyst fluid during progression of ADPKD. Therefore, our study indicates that EP2 receptors are expressed in CEC and may play an important role in the cystogenic potential of PGE2 activity.

Pharmacological and functional characterization of EP2 receptors in CEC. To demonstrate the functionality of the EP2 receptors, we analyzed the effect of pharmacological analogs of PGE2 to modulate cAMP levels in CEC. Figure 7 shows the effect of PGE2 and butaprost free acid, a selective agonist for EP2 receptors, on cAMP formation in CEC. The dose-response curves for PGE2 and butaprost free acid yielded ED50 of 37.4 ± 1.7 and 212.5 ± 75.8 nM (means ± SE, n = 3 and 4), respectively. This difference in effective concentration of PGE2 and butaprost free acid may correspond to differences in binding affinity observed in COS-1 cells transfected with the cloned rabbit EP2 receptor (17). Stimulation of cAMP with 77 nM PGE2, 5 μM butaprost free acid, and 10 μM forskolin was 23.8 ± 4.5-, 24.2 ± 4.8-, and 5.1 ± 1.3-fold over basal, respectively (means ± SE, n = 3). Therefore, maximal stimulation was similar for PGE2 and butaprost free acid, whereas the effect of forskolin was lower, possibly because forskolin needs time to permeate into cells, where it activates adenylyl cyclase.

We also used AH-6809, an antagonist of EP1 and EP2 receptor but not EP4 receptor (26). Because EP2 and EP4 receptors but not EP1 mediate an increase in cAMP, the effect of AH-6809 on cAMP is specific for EP2. Figure 7 shows that AH-6809 inhibits PGE2 stimulation of cAMP formation in

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Fig. 6. Immunohistochemical staining analysis of EP2 receptor expression in human polycystic kidney from a patient with ADPKD. mlGlG is used as negative control. Marked area in middle is enlarged at right. Top: EP2 receptor antibody reacts with epithelial cells lining cysts. Magnification, ×100. Middle: EP2 receptor is not expressed in blood vessels. Magnification, ×100. Bottom: EP2 receptor expression in cysts. Magnification, ×400.
CEC, which substantiates the conclusion that the PGE\textsubscript{2} effect is mediated by EP\textsubscript{2} receptor.

We further determined whether activation of EP\textsubscript{2} receptors contributes to cystogenesis in CEC cultures. For this purpose, CEC were cultured in a 3-D matrix in the presence or absence of PGE\textsubscript{2}, butaprost free acid, and forskolin, a pharmacological adenylate cyclase activator. Figure 8 shows that PGE\textsubscript{2}, butaprost free acid, and forskolin stimulate cyst formation. This result indicates that the mechanism of cystogenesis induced by PGE\textsubscript{2} is likely to involve EP\textsubscript{2} receptors and formation of cAMP.

To further demonstrate that EP\textsubscript{2} receptors mediate the PGE\textsubscript{2} effect, we used RNAi to reduce endogenous levels of EP\textsubscript{2} receptors. Figure 9, top shows the effect of two different EP\textsubscript{2} siRNAs on stimulation of cAMP. EP\textsubscript{2} RNAi greatly reduced cAMP levels in CEC treated with PGE\textsubscript{2} and butaprost free acid. Therefore, these results demonstrate that EP\textsubscript{2} receptor expression mediates PGE\textsubscript{2} and butaprost free acid effect on cAMP formation. The effect of siRNA on EP\textsubscript{2} receptor expression was also analyzed by real-time RT-PCR analysis (Fig. 9, middle). Because we demonstrated previously that various treatments may modulate the expression of reference genes (12), we used cyclophilin A and 18S as two different reference genes to confirm mRNA quantitation. Our results indicate that the two different siRNAs reduced EP\textsubscript{2} receptor mRNA expression. In addition, we also analyzed the effect of PGE\textsubscript{2} treatment on EP\textsubscript{2} receptor expression. PGE\textsubscript{2} treatment induced EP\textsubscript{2} receptor expression by fourfold. This effect was specific because EP\textsubscript{2} siRNAs abolished it (Fig. 9, middle). We further demonstrated that PGE\textsubscript{2}, butaprost free acid, and forskolin similarly stimulated EP\textsubscript{2} receptor expression. Inhibition by siRNA indicates specificity of this effect (Fig. 9, bottom). We further demonstrated that PGE\textsubscript{2}, butaprost free acid, and forskolin-mediated cAMP induction stimulate expression of EP\textsubscript{2} receptor. This result implies possible regulation of EP\textsubscript{2} receptor expression by PGE\textsubscript{2} and cAMP-mediated signaling in the kidney.

PGE\textsubscript{2}-induced cystogenesis involves apoptosis but not proliferation. Both proliferation and apoptosis are induced during cystogenesis (6). To determine the mechanism by which PGE\textsubscript{2}...
epithelial cells (37). Because both cell growth and fluid secretion play a major role in cyst formation (16, 32), theoretically, EP2 receptor expression in the normal human kidney (results not shown). Our study addresses the role and mechanism by which PGE2 activity is involved in ADPKD. Because PGE2 stimulates different receptors mediating distinct signaling pathways, it is important to determine specific receptor subtype(s) responsible for PGE2 activity in CEC. Our study provides biochemical and functional evidence demonstrating for the first time that EP2 receptor is expressed in the human ADPKD kidney and directly mediates PGE2 activity on cyst formation.

Previous studies showed diffuse expression with no specific localization of EP3 receptor mRNA as detected by in situ hybridization in the human and rabbit kidney (8, 17). RT-PCR analysis reveals EP2 receptor mRNA expression in the glomeruli and medullary collecting ducts in rabbit kidney (17). In contrast, EP2 receptor mRNA expression and butaprost-induced cAMP are reported in the tubular cells of the rat medulla (22, 52). Also, it has been reported that micromolar concentrations of L-161982, an EP4 antagonist, partially inhibited PGE2-induced cAMP formation in CEC (2). However, the K_i for binding to EP2 and EP4 receptors is 21 μM and 24 nM, respectively (30), and an almost maximal inhibition of PGE2 effect mediated by EP4 was observed at 10 nM concentration of L-161982 (15). Therefore, it is possible that higher concentrations of L-161982 also inhibit EP2-mediated activation. EP2 receptor in human kidney was detected by immunoblot analysis and has been localized in the renal vasculature but not RTC by immunohistochemistry (31). Using a different antibody, we were able to detect EP2 receptor by immunoblot analysis but were unable to determine the specific localization for EP2 receptor in the normal human kidney (results not shown). However, we clearly observed EP2 receptor on the apical side of cysts in human ADPKD kidneys but not in the renal vasculature in ADPKD kidney (Fig. 6). It is possible that PGE2 secretion into cyst fluid induces EP2 receptor in ADPKD kidney because PGE2 induced EP2 receptor mRNA expression by three- to fourfold in CEC primary cultures (Fig. 9). Therefore, our results also imply that the effect of PGE2 mediated by EP2 receptor and cAMP signaling protects CEC from apoptosis. They imply a mechanism that involves regulation of Bcl-2 expression. The lack of treatment’s effect on expression of PCNA and MCM2, a marker for DNA replication, further confirms that PGE2 did not affect the rate of proliferation.

**DISCUSSION**

To further analyze the effect of PGE2, butaprost free acid, and forskolin on proliferation and apoptosis, we performed real-time RT-PCR analysis for mRNA expression of marker genes in 3-D gels. Figure 11 shows that expression of the antiapoptotic factor Bcl-2 and cell cycle-regulated cyclin D3 are upregulated by treatment of PGE2, butaprost free acid, and forskolin. However, expression of proapoptotic factor Bax, cell cycle-regulated cyclin D1, cell proliferation-related PCNA and minichromosome maintenance protein 2 (MCM2), and negative control cyclophilin are not changed. These results indicate that the effect of PGE2 mediated by EP2 receptor and cAMP signaling protects CEC from apoptosis. They imply a mechanism that involves regulation of Bcl-2 expression. The lack of treatment’s effect on expression of PCNA and MCM2, a marker for DNA replication, further confirms that PGE2 did not affect the rate of proliferation.

**Fig. 9.** Inhibition of EP2 receptor expression by RNA interference prevents PGE2 signaling. CEC were transfected with control or 2 different EP2 receptor small interfering RNAs (siRNAs). Left: following 2 days incubation in SFM, cells were treated with or without 77 nM PGE2, 5 μM butaprost free acid, or 10 μM forskolin for 30 min and were analyzed for cAMP formation. cAMP concentration of control without treatment was under detection sensitivity of standard curve. Values represent means ± SD (n = 2). Middle: following transfection, cells were treated with or without 77 nM PGE2 for 1 day in SFM. Then cells were analyzed for EP2 receptor mRNA expression relative to 18S or cyclophilin A (CYP A) used as endogenous control genes. Values represent means ± SE (n = 3). Bottom: following transfection with EP2 receptor siRNA-1, cells were treated with or without 77 nM PGE2, 5 μM butaprost free-acid, or 10 μM forskolin for 2 days in SFM. Then cells were analyzed for EP2 receptor mRNA expression relative to 18S used as endogenous control gene. Values represent means ± SE (n = 3).
EP2 and EP4 receptors may play a role in this process. It has been established that EP4 receptor mediates PGE2 effect in rabbit collecting ducts (36); however, none of these receptors has been clearly characterized in cortical tubular epithelial cells. Our data also provide functional evidence for a role of EP2 receptor in CEC by using receptor-selective agonists/antagonists and siRNA (Figs. 7–9). Therefore, our study supports a model in which PGE2 secreted into cyst fluid binds to EP2 receptor, modulates EP2 receptor expression, and stimulates cAMP formation, thus contributing to cystogenesis.

To further determine the mechanism by which PGE2 stimulates cyst formation, we determined whether PGE2 stimulates proliferation or apoptosis of epithelial cells lining cysts in 3-D matrix. Although CEC were highly proliferative (70%) and no difference was found following PGE2 treatment, reduction of apoptosis was observed. Notably, the effect of PGE2 on cyst formation was obtained in medium containing mitogens such as EGF and insulin with (Fig. 1 and Table 2) and without FBS (Figs. 1 and 8). Therefore the high rate of proliferation in CEC (measured in the presence of FBS) is consistent with the presence of mitogenic factors in 3-D culture medium. Previous studies using monolayer cultures suggest that cAMP signaling stimulates proliferation in CEC while having the opposite or no effect in RTC (20, 55). However, stimulation of cAMP-dependent signaling induces transepithelial monolayer chloride transport and cyst formation in 3-D collagen I gel cultures of both cell types (32, 53, 56). In this model, it has also been shown that cAMP promotes RTC to form cysts only in the presence of EGF (32, 56), which further indicates that the mitogenic effects of cAMP agonist observed in monolayer cultures do not necessarily reflect on cyst formation in 3-D cultures. The high percentage of apoptotic cells observed (37%) on CEC-lining cysts in 3-D cultures is consistent with data showing numerous apoptotic nuclei in human polycystic kidney (54).

Previously, it has been reported that PGE2 binding to the EP2 receptor promotes cell survival through an antiapoptotic mechanism in radiated intestinal epithelial cells (21, 46). Endothelial cells from EP2 receptor knockout mice were more susceptible to apoptosis than cells from wild-type animals (23). Two other recent studies demonstrated that PGE2 through EP2 receptor protects mouse embryonic stem cells exposed to H2O2 (28) and human lung fibroblasts incubated with cigarette smoke extract (42) from apoptosis. Although it has been proposed that PGE2 induces cell growth, our study indicates for the first time that PGE2 promotes cystogenesis of CEC through protection against apoptosis. It has been shown that both epithelial cell apoptosis and proliferation are dysregulated in ADPKD (11, 27). Our study demonstrates that PGE2 could reduce cyst-lining epithelial cell apoptosis and simultaneously increase the ratio of Bcl-2 to Bax that commonly is used to measure apoptotic susceptibility (9). PGE2 has been shown to induce Bcl-2 expression in human colon cancer cells (40).
ingly, loss of Bcl-2 of knocked-out mice results in the development of polycystic kidney disease, provoking renal failure (50).

We also observed that PGE2, butaprost free acid, and forskolin induced mRNA expression of cyclin D3 but not cyclin D1. Functions of D-type cyclins commonly overlap in activating the cyclin-dependent kinases CDK4 and CDK6 in G1 and thereby promote the cell’s entrance into S phase (1). However, lack of a treatment effect on PCNA expression that is mainly in the late G1 and S phases of the mitotic cycle suggest that induction of cyclin D3 did not significantly affect cell proliferation in the presence of highly mitogenic culture medium. Expression of MCM2, a marker for proliferation involved in DNA replication (35), further confirmed that PGE2 did not affect the rate of proliferation. Cyclin D1 and D3 are not fully redundant, and a previous study indicated a distinct role of cyclin D3 in induction and/or maintenance of terminal differentiation (1). Our result implies that PGE2 activity is dependent on high apoptotic activity in CEC. This conclusion is further supported by a study showing that inhibition of the proapoptotic caspase-3, which decreases apoptosis and proliferation of CEC, were cultured in growth medium in absence or presence of 77 nM PGE2,5 M butaprost free acid, or forskolin. cAMP promotes growth and tubulogenesis in MDCK cells.

In the context of the mutation of PC-1 leading to ADPKD, conserved sequences on the cytoplasmic part of PC-1 are related to G-protein activation and mediate interaction with G inhibitory proteins and regulator of G-protein signaling 7 (10, 25, 34). Mutations of PC-1 may possibly alter G inhibitory protein-mediated inhibition of cAMP formation and be implicated in the process of cystogenesis. Our study indicates that PGE2 induces cyst formation but also inhibits tubule formation in RTC derived from the normal kidney. This result suggests that genetic conditions and environment restrict cyst formation in normal kidney but not in ADPKD kidney. Secretion of PGE2 into cyst fluid in ADPKD kidney potentially plays a major role in the progression of the disease (2, 14).

In conclusion, our results suggest that PGE2 secreted into cyst fluid binds to adjacent EP2 receptors located on the apical side of cysts and stimulates EP2 receptor expression. PGE2 binding to EP2 receptor leads to cAMP signaling and cystogenesis by a mechanism that involves protection of CEC from apoptosis. Therefore, blockage of the EP2 receptor may have significant implications for developing clinically relevant therapeutic strategies for ADPKD.

ACKNOWLEDGMENTS
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REFERENCES


