Role of keratinocyte growth factor in the pathogenesis of autosomal dominant polycystic kidney disease

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Abstract

Background. Previous studies have shown that the expression and distribution of keratinocyte growth factor (KGF), also known as FGF-7 (fibroblast growth factor-7) or HBGF-7 (heparin-binding growth factor-7), may be implicated in kidney cyst formation and expansion. However, there are no data on KGF expression in human autosomal dominant polycystic kidney disease (ADPKD) tissue, and it is unknown whether it affects ADPKD cyst-lining epithelial cell proliferation.

Methods. The expression and distribution of KGF and KGF receptor (KGFR) mRNA in ADPKD cystic and normal kidney tissues were examined using quantitative real-time polymerase chain reaction (PCR) and in situ hybridization. KGF and KGFR protein expression in the above tissues was analysed by immunohistochemistry and western blot. The effect of KGF on cyst-lining epithelial cell proliferation was assessed by MTT assay, and its effect on the cyst-lining epithelial cell cycle was analysed by flow cytometry. The effect of KGF on cyclin D1 and P21wafl gene expression in cyst-lining epithelial cells was also determined.

Results. KGF and KGFR mRNA expression in ADPKD cysts was higher than in normal kidney tissues. KGF and KGFR protein expression was also higher in ADPKD cysts and was localized to cyst-lining epithelial cells, tubular and interstitial cells. In vitro experiments revealed that KGF promoted cyst-lining epithelial cell proliferation, and decreased the ratio of G0/G1 phase but increased that of S phase. In response to KGF, the expression of the cyclin D1 gene in cyst-lining epithelial cells increased markedly while P21wafl expression decreased.

Conclusions. KGF and KGFR expression was upregulated in ADPKD kidney tissues. KGF stimulated the proliferation of cyst-lining epithelial cell in vitro by regulating the expression of cyclin D1 and P21wafl genes. KGF may play a role in pathogenesis of ADPKD.

Keywords: autosomal dominant polycystic kidney disease (ADPKD); cell cycle; cyclin; keratinocyte growth factor (KGF); proliferation

Introduction

Autosomal dominant polycystic kidney disease (ADPKD) is the most common hereditary renal disease in humans and has a prevalence of 1/400–1/1000 in the population [1]. It is characterized by progressive cyst development and enlargement that result in bilateral polycystic kidneys and end-stage renal failure in the majority of patients. Mutations in at least two genes are known to cause the disease, but the exact mechanism for the genesis and development of the cysts is unknown. Recent studies have shown that certain growth factors may play important roles in ADPKD pathogenesis by acting through autocrine or paracrine pathways [1]. Keratinocyte growth factor (KGF) is secreted via an autocrine pathway and binds to KGF receptors (KGFRs) located on epithelial cells. In a previous study using microarray analysis, we found that the expression of KGF mRNA was upregulated in ADPKD tissues [2]. In addition, cysts have been found in the kidney cortex, medulla and collecting tubes of KGF-overexpressing transgenic mice [3], and the overexpression of KGF in developing mouse kidneys led to the formation and enlargement of kidney cysts [4]. However, there have been no previous studies on KGF expression in human cystic renal tissue, nor is it known whether KGF stimulates cyst-lining epithelial...
KGF in pathogenesis of ADPKD

cell proliferation in ADPKD tissues. Therefore, the aim of the present study was to investigate the role of KGF in ADPKD pathogenesis by observing KGF expression in polycystic kidney tissue and by exploring the effect of KGF on cyst-lining epithelial cells.

Patients and methods

Subjects

All subjects with ADPKD were diagnosed by family history, clinical symptoms, imaging examination and gene mutation analysis. Samples of polycystic kidney tissues were obtained from 10 subjects (four men and six women, aged 53–69 years, mean 60.9±4.91) with ADPKD (eight subjects with PKD1 mutation and two subjects with PKD2 mutation), who had undergone unilateral nephrectomy or extensive cyst unroofing and drainage surgery in our hospital, from January to December, 2002. All subjects had renal function insufficiency with serum creatinine concentrations between 130 and 1168 μmol/l (mean 628.9±350.5). Normal kidney tissues were obtained from six subjects (two men and four women, aged 41–65 years, mean 55.7±8.73) with renal carcinoma, who had undergone nephrectomy during the same period. The samples were fixed in 4% paraformaldehyde for in situ hybridization assay or in 10% neutral formalin for immunostaining. Paraaffin sections (4 μm thick) were prepared after routine dehydration and paraaffin embedding.

Reagents and equipment

The cyst-lining epithelial cell line was produced in our laboratory [5]. The human proximal tubular epithelial cell line (HKC) was kindly provided by Dr Racusen (Johns Hopkins University Medical School, Baltimore, MD). Recombinant human KGF (rHuKGF) was purchased from PeproTech (Rocky Hill, NJ). Goat anti-human KGF polyclonal IgG and rabbit anti-human KGFR IgG were purchased from Santa Cruz Biotechnology Inc. (Santa Cruz, CA). The KGF and KGFR mRNA in situ hybridization kits, the SABC immunochemistry kit and MTT were purchased from Sigma (St Louis, MO).

In situ hybridization

In situ hybridization was performed according to the manufacturer’s instructions. Sections (4 μm thick) were hybridized with digoxigenin (Dig)-labelled oligonucleotide probes designed from the appropriate mRNA sequences. To increase the specificity of in situ hybridization, three probes were used in this study and their sequences were as follows: for KGF, (1) 5'-CGACA CACAACAAGG TATAG TTACA TGAGAAA-3', (2) 5'-ATCGA TTACAA GATCGG-3', (3) 5'-GTTC CATAATCAA AAGGG GATTC CTGTA-3'; and for KGFR, (1) 5'-CCTCT TCAGT TTAGT TGAGG TAATCC ACAT-3'; (2) 5'-ATCGA TTCTC ACTCAC ACAAA TGAGG-3'; and (3) 5'-CTCA ATCTGCC ACACA AAGGG GATTC CTGTA-3'. Human skin samples were used as the positive control and unlabelled probes as the negative control. Samples were visualized with 3,3'-diaminobenzidine (DAB) for microscopic observation and photography. The average optical density of staining was analysed by IDA-2000 digital image analysis system software.

Quantitative real-time PCR

Real-time polymerase chain reaction (PCR) was used to determine KGF mRNA and KGFR mRNA in ADPKD cyst tissues and in normal human kidney tissues. It was also used to measure the expression of the cell cycle regulatory genes cyclin D1 and P21wafl in cyst-lining epithelial cells and in HKCs. After homogenization of tissues and cells, the total RNA was extracted as described in the literature and cDNA was synthesized through reverse transcription [6]. Real-time quantitative PCR was performed and the quantity of mRNA was expressed as copy numbers/10^6 housekeeping gene GAPDH. The primers used in real-time PCR are listed in Table 1.

Tissue immunostaining

The expression of KGF and KGFR proteins in polycystic and normal kidney tissues was examined by streptomyacin avidin–peroxidase immunochemistry (SABC), as previously described [7]. Briefly, samples were cleaned with paraffin and rehydrated. Endogenous peroxidase activity was blocked by incubating sections in 3% H2O2 solution for 10 min. Slides filled with citric acid buffer were heated in a microwave oven for antigen retrieval. Sections were incubated with animal serum for 10 min at room temperature and then with the appropriate primary antibody (1:500 dilution) overnight at 4°C. After washing with phosphate-buffered saline (PBS),

<table>
<thead>
<tr>
<th>Gene</th>
<th>Primer sequence</th>
<th>Sense</th>
<th>Antisense</th>
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<tr>
<td>KGF</td>
<td>CCCTGACGCAACAACAAGAAGT</td>
<td>GTCCTTTTCTCTCCTGTTCC</td>
<td>CCAAACTCACAAGGCCACTG</td>
</tr>
<tr>
<td>KGFR</td>
<td>GGATGAGGAAGGAGGCAGCC</td>
<td>ATGAGTGGGAGGAGGCCAGG</td>
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</tr>
<tr>
<td>Cyclin D1</td>
<td>GATGCTGGAGGAGCTGCGAGA</td>
<td>ATGAGTGGGAGGAGGCCAGG</td>
<td>GAAGGCCCAGCAACTGGAAGT</td>
</tr>
<tr>
<td>P21wafl</td>
<td>AGAGGCCCAGCAACTGGAAGT</td>
<td>CGCGTTTGGAGGTGTAAGA</td>
<td>ATGCCAGTGGAGGCTTCCTCCGTAC</td>
</tr>
<tr>
<td>GAPDH (housekeeping gene)</td>
<td>GGTATCGTGGAAAGAACATGCAGAC</td>
<td>ATGCCAGTGGAGGCTTCCTCCGTAC</td>
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the sections were incubated with the secondary antibody for 20 min at 37°C, with avidin–peroxidase for 20 min at 37°C, and then colour was developed with DAB. After the sections were washed several times, they were counterstained with haematoxylin, dehydrated with ethanol, rinsed in xylene, and gum mounted for microscopic examination and photography. For negative controls, the primary antibody was replaced by PBS. Positive controls were tumour slides purchased from the Buoshide Company (Wuhan, China).

**Western blot analysis**

The expression levels of KGF and KGFR protein in polycystic or normal kidney tissues were examined by western blot as previously described [8]. Briefly, protein was extracted from normal renal tissue or polycystic kidney tissue. Proteins were separated by 10% SDS-PAGE and transferred to nitrocellulose filters. The filters were blocked with a blocking buffer, incubated with goat anti-KGF polyclonal antibody or rabbit anti-KGFR polyclonal antibody (1:500 dilution) overnight at 4°C, followed by the addition of horseradish peroxidase-linked anti-goat or anti-rabbit IgG and enhanced chemiluminescence (ECL; Amersham Pharmacia Biotech) and visualization of the bands.

**In vitro cell growth assay**

Proliferation of cyst-lining epithelial cells or HKCs was examined by MTT assay as described in the literature [9]. After passage, cells at the logarithmic growth phase were plated in 96-well plates and incubated for 24 h in DMEM + F12 medium containing 5% FBS at 37°C under a 5% CO₂ atmosphere. After the growth medium was changed to serum-free medium and cultured for 24 h, the cells were synchronously in the quiescent phase. The cells were treated with rHuKGF at various concentrations (0, 0.01, 0.1, 1, 10, 20, 50 and 100 ng/ml) for 24, 48 or 72 h. Each experimental group included six wells, and all experiments were repeated six times. Trypan blue staining showed that >95% of cells were viable. At 4 h before the end of the incubation, 10 µl of 5 g/l MTT was added to each well. The OD value of each well was measured using a microculture plate reader with a test wavelength of 492 nm.

**Flow cytometry**

Flow cytometry was used to study the effect of KGF on the cyst-lining epithelial cell cycle. Cyst-lining epithelial cells at logarithmic growth phase were plated in 6-well plates and incubated for 48 h in serum-free DMEM + F12 medium containing various concentrations of rHuKGF (0, 0.1, 1, 10, 20 and 50 ng/ml) at 37°C under a 5% CO₂ atmosphere. After treatment with trypsin, single cell suspensions were washed by PBS, fixed with 70% cold ethanol and stored at −20°C overnight. The cells were then washed twice with the same volume of PBS and stained with propidium iodide solution for flow cytometry testing. The cell cycle was analysed with MCYCLE software.

**Statistical analysis**

Results are expressed as means ± SD. Paired t-tests and one-way analyses of variance (ANOVA) were used for statistical analysis. In all cases, P < 0.05 was considered statistically significant.

**Results**

**The expression of KGF and KGFR mRNA in normal and ADPKD kidney tissues**

Quantitative real-time PCR showed that KGF mRNA in normal human kidney tissue was 456 ± 252.55 copies/10⁶ GAPDH, KGFR mRNA was 1030 ± 479.26 copies/10⁶ GAPDH, KGF mRNA in ADPKD kidney tissues was 3720 ± 636.88 copies/10⁶ GAPDH and KGFR mRNA was 9810 ± 1417.22 copies/10⁶ GAPDH (n = 10). There were significant differences in the quantity of KGF mRNA (t = −8.252, P = 0.001) and KGFR mRNA (t = −10.165, P = 0.001) between ADPKD kidney tissues and normal kidney tissues. In situ hybridization showed that in normal kidney tissues, KGF mRNA was weakly expressed primarily in tubular, vascular, glomerular, parietal and splanchnic epithelium, and in mesangial cells, while KGFR mRNA was more strongly expressed primarily in tubular epithelial cells. In ADPKD tissues, KGF mRNA was localized to the cyst-lining epithelial cells and vascular endothelial cells, while KGFR mRNA was mostly expressed in the cyst-lining epithelial cells. KGF and KGFR gene expression was significantly stronger in ADPKD kidney tissues than in normal kidney tissues (Figure 1). IDA-2000 digital imaging software showed that the mean optical densities of KGF mRNA were 0.13 ± 0.02 and 0.19 ± 0.02 in normal and ADPKD kidney tissues, respectively, while KGFR mRNA mean densities were 0.47 ± 0.04 and 0.59 ± 0.08 in normal and ADPKD tissues, respectively. These differences were significant (P < 0.05).

**The distribution of KGF and KGFR proteins in normal and ADPKD kidney tissues**

The immunohistochemistry results confirmed the in situ hybridization findings. IDA-2000 digital imaging software showed that KGF mean optical densities were 0.13 ± 0.02 and 0.21 ± 0.04 in normal and ADPKD tissues, respectively, while KGFR mean optical densities were 0.40 ± 0.03 and 0.50 ± 0.05 in normal and ADPKD tissues, respectively. These differences were statistically significant (P < 0.05) (Figure 2). Cytimmunohistochemistry revealed KGF in the apical and bilateral surfaces as well as in the cytoplasm.

**Western blot of KGF and KGFR proteins in normal and ADPKD kidney tissues**

Western blot assay showed that the expression of KGF and KGFR proteins in ADPKD kidney tissues was markedly increased compared with that in normal kidney tissues (Figure 3).
The effect of KGF on the proliferation of cyst-lining epithelial cells or HKCs

KGF had no cytotoxic effects on cyst-lining epithelial cells or HKCs within the tested concentration range. Treatment of cyst-lining epithelial cells and HKCs with various concentrations of KGF for 24 h resulted in dose-dependent increases in cell proliferation that peaked at 50 ng/ml (comparison of the different concentrations: $F = 308.904, P = 0.000$). High concentrations of KGF clearly stimulated proliferation after 48 h, and, after 72 h, only cells treated with the highest concentrations of KGF showed enhanced proliferation. The difference in proliferation between different time points was statistically significant ($F = 396.650, P = 0.000$). Analysis by ANOVA showed that the stimulating effect of KGF on the proliferation of HKCs was weaker than that on AKPKD cyst-lining epithelial cells ($F = 210.055, P = 0.000$). (Table 2 and Figure 4)

The effect of KGF on the cyst-lining epithelial cell cycle

Flow cytometry showed that with increases in KGF concentration, the ratio of $G_0/G_1$ phase decreased while the ratio of $S$ phase increased, and there was no marked change in the ratio of $G_2$ phase (Table 3). These results suggest that KGF induced cyst-lining epithelial cells from the $G_0/G_1$ phase to the $S$ phase.

The effect of KGF on the expression of cyclin D1 and P21wafl genes in cyst-lining epithelial cells

After stimulation with KGF, the expression of cyclin D1 mRNA was significantly upregulated in the experimental group ($78600 \pm 7676.8$ copies/$10^6$ GAPDH), which is in contrast to the control group ($54600 \pm 4243.5$ copies/$10^6$ GAPDH). The expression of P21wafl mRNA was obviously downregulated in the experimental group ($7400 \pm 790.5$ copies/$10^6$ GAPDH) compared with the control group ($10795 \pm 1076.5$ copies/$10^6$ GAPDH).

Discussion

Although previous studies have shown that the main pathological change of ADPKD is hyperplasia of cyst-lining epithelial cells, the cause is still under investigation. KGF was first identified in the conditioned medium of a human embryonic lung fibroblast cell line [7] as a 28 kDa heparin-binding member of the fibroblast growth factor (FGF) family (alternative designation: fibroblast growth factor-7, FGF-7 or heparin-binding growth factor-7, HBGF-7) that
induces proliferation of a wide variety of epithelial cells. There are at least 22 members of the FGF family in vertebrates, ranging in molecular mass from 17 to 34 kDa and sharing 13–71% amino acid identity [10]. The cloning of the signal-transducing receptors for FGFs has revealed a tyrosine kinase gene family with at least four members (FGFR1, IIIb and IIIc; FGFR2, IIIb and IIIc; FGFR3, IIIb and IIIc; and FGFR4). These four cell surface FGF receptors (FGFRs) bind members of the FGF family with varying affinities [10]. Upon receipt of the extracellular signals (FGFs), these receptor tyrosine kinases (RTKs) activate the Ras/MAPK signalling pathway. KGFR is a splice variant of FGFR2 (FGFR2-IIIb), which is expressed exclusively in epithelial tissues. In addition to KGF, FGFR binds FGF-1, FGF-3 and FGF-10. However, KGF only binds to the KGFR. KGF has been found to be the cytokine responsible for mediating the interaction between mesenchymal and epithelial cells. This interaction acts specifically through KGFs on epithelial cell membranes. In this manner, KGF is able to accelerate epithelial cell proliferation, migration and differentiation. Previous work revealed that KGF mRNA is expressed in mesenchymal cells and corresponding epithelial cells of tissues or organs, such as the skin, respiratory, gastrointestinal and urinogenital tracts, where an interaction occurs.

Fig. 2. KGF and KGFR protein expression in normal and ADPKD kidney tissues assessed by immunohistochemistry using the SABC method. Original magnification ×400. (A) KGF expression in tubular epithelial cells and in vascular endothelial cells from normal kidney tissues. (B) KGF expression in cyst-lining epithelial cells and vascular endothelial cells from ADPKD kidney tissues. (C) KGFR expression in tubular epithelial cells from normal kidney tissues. (D) KGFR expression in cyst-lining epithelial cells from ADPKD kidney tissues.

Fig. 3. Western blot of KGF and KGFR proteins in ADPKD and normal kidney tissues. (A) KGF protein expression. (B) KGFR protein expression. 1, ADPKD tissues; 2, normal kidney tissues.
between the two kinds of cells [8]. It was documented recently that KGF and KGFR mRNA expression was detected in human ovarian and prostate tumour epithelial cells [11], implying that KGF stimulated the proliferation of carcinoma cells by an autocrine mechanism. In a previous study, Nguyen and colleagues [3] successfully detected the expression of human KGF on hepatocytes during the later period of mouse gestation using a human apolipoprotein E (ApoE) gene promoter and its associated liver-specific enhancer, and also found morphological abnormalities in several organs that expressed KGFR. The most striking phenotypic aberration in the ApoE-hKGF transgenic embryos was a marked hyperplasia and cystic dilation of the cortical and medullary kidney collecting duct system, which is a phenotype that resembles infantile polycystic kidney disease in humans [3]. If the overexpression of KGF and overactivation of KGFR prove to be pathogenic factors for kidney cyst development, then the local expression of KGF and KGFR in PKD kidney tissues may be of clinical interest.

In the present study, we sought to investigate the expression and effect of KGF and KGFR in ADPKD renal tissue. First, by using in situ hybridization and immunohistochemistry combined with image analysis for localization and semi-quantitative examination, we found that KGF mRNA was expressed in the tubular, vascular, glomerular, parietal and splanchnic epithelia, and in mesangial cells from normal kidney tissues, and in the cyst-lining epithelium and vascular...
endothelium from ADPKD polycystic kidney tissues. KGF gene expression was weak in normal kidney tissues but showed significant upregulation in ADPKD tissues. KGFR gene expression was higher than that of KGF in both tissues, but was still significantly higher in ADPKD tissue than in normal kidney. Using quantitative real-time PCR, we demonstrated that the expression of KGF mRNA and KGFR mRNA in ADPKD renal cyst tissues was higher than in normal human kidney tissues. Furthermore, by using western blot to detect the expression of KGF and KGFR proteins in normal and ADPKD kidney tissues, we discovered that the expression of KGF and KGFR was significantly increased in ADPKD kidney tissues, indicating that the increased levels of KGF and KGFR mRNA in ADPKD tissues were translated into increased KGF and KGFR protein in cyst-lining epithelial cells. We also found that the expression of KGF and KGFR was also stronger in cyst-lining epithelial cells than in HKCs cultured in vitro, and that the distribution of KGF and KGFRs in cultured cyst-lining epithelial cells was similar to that in ADPKD kidney tissue (data not shown), indicating that the character of KGF and KGFR expression was steady in the in vivo and in vitro experiments. This suggests that KGF may participate in the genesis and development of ADPKD, perhaps by binding to KGFR on the membrane of cyst-lining epithelial cells (via the paracrine pathway), and in this way plays its biological role as a cytokine. However, it is additionally possible that an autocrine pathway is involved in the process.

Yamasaki et al. [12] found that ADPKD rat renal tubular epithelial cells incubated in vitro showed an increased sensitivity to exogenous KGF, suggesting that KGF may lead to renal tubular epithelial cell hyperplasia during ADPKD pathogenesis. In our study, the cyst-lining epithelial cell line was derived from renal cystic tissues from ADPKD patients, and it retained the biological characteristics of primary cultured cells. By using these cells as the test group, HKCs as controls, and recombinant human KGF as the stimulator, we were able to explore further the effect of KGF on cyst-lining epithelial cells at the molecular and cytological levels. We found that KGF caused a remarkable time- and dose-dependent stimulation of cyst-lining epithelial cell proliferation, whereas, under the same conditions, HKCs showed less stimulation. This indicates that exogenous KGF may exert significant stimulatory effects on cyst-lining epithelial cell proliferation, and suggests that the difference in cell type sensitivity may be related to the differential expression of KGFR, which is more highly expressed in cyst-lining epithelial cells than in HKCs.

The cell cycle is the final pathway for cell proliferation, and is regulated at various checkpoints, of which the G1–S phase represents the most important step [13]. At this checkpoint, extra- and intracellular signals are integrated and transmitted into cells to determine whether they enter the cell division phase, apoptosis or the quiescence G0 phase. Cyclin D1 is an important cell cycle regulatory protein at G1 phase, whose expression is enhanced by the binding of growth factors to their receptors. Stimulation by cyclin-dependent kinase (CDK) causes phosphorylation of cyclin D1 and subsequent enhancement of expression of some related genes [14, 15], whose products induce the cells to pass the G1–S checkpoint and begin automatic division. Blockage of cyclin D1 will prevent cells from entering S phase. P21waf1 is an important CDK inhibitor. It can inhibit the activity of CDK or the cyclin D1–CDK complex, and can dephosphorylate cyclin D1 and inhibit cellular proliferation [16]. By using the flow cytometry technique, we found that within the concentration range of 0–100 ng/ml, KGF decreased the ratio of the G0/G1 phase and increased that of the S phase in a dose-dependent fashion. This result suggests that KGF had a stimulatory effect on the proliferation of ADPKD cyst-lining epithelial cells by regulating the cells at G1 phase. Furthermore, quantitative real-time PCR revealed the expression of c cyclin D1 and P21waf1 genes in ADPKD cyst-lining epithelial cells, suggesting that the cyclin D1 and P21waf1 genes were regulatory genes for ADPKD cyst-lining epithelial cells at G1 phase. Stimulation by KGF caused a marked upregulation of the cyclin D1 gene and a down-regulation of P21waf1, suggesting that KGF stimulated the proliferation of ADPKD cyst-lining epithelial cells by regulating the expression of cyclin D1 and of P21waf1 genes, and subsequently inducing cells to pass the G1–S phase checkpoint.

In a previous report, Bhunia and colleagues [17] showed that expression of polycystin-1 (PKD1) activates the JAK–STAT pathway, thereby upregulating P21waf1 and inducing cell cycle arrest in G0/G1 phase. They also showed that polycystin-2 (PKD2) was required as an essential cofactor in this process [17]. These results combined with our findings indicate that P21waf1 may be the common regulatory factor for PKD1/2 mutation or for KGF in ADPKD pathogenesis.

A potential bias in our study is that all of the ADPKD patients had renal function insufficiency, and therefore uraemic toxins may have had an impact on the expression and distribution of KGF and KGFR in cystic kidney tissues. Therefore, two subjects in this series with near normal creatinine concentrations (130 and 246 µmol/l) were picked out for detailed analysis of their kidney tissue samples. There appeared to be no significant differences between these two subjects and the other eight subjects in the expression and distribution of KGF and KGFR (data not shown), suggesting that the impact of uraemic toxins on KGF and KGFR may have been limited in this study. However, the small sample size in our study made it difficult to determine whether toxins played a role in KGF/KGFR expression and distribution in ADPKD kidney tissues.

In conclusion, cyst-lining epithelial cells showed increased production of KGF through possible paracrine and certain autocrine pathways. KGF may bind to an increased number of KGFRs on cyst-lining
epithelial cell membranes and cause stimulation of cell proliferation. As a possible mechanism for continuous proliferation of cyst-lining epithelial cells, stimulation of KGF may upregulate the expression of the cyclin D1 gene and downregulate the P21wafl gene, and consequently induce the cells to pass the G1–S phase checkpoint.

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Conflict of interest statement. None declared.

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