

Polycystin-1 Expression in Fetal, Adult and Autosomal Dominant Polycystic Kidney

The mutation of the *PKD1* gene causes autosomal dominant polycystic kidney disease (ADPKD), and the *PKD1* gene encodes polycystin-1 (PC-1). PC-1 is thought to be a cell-cell/matrix adhesion receptor molecule at the cell surface that is widely expressed in the kidney. However, there are controversies about the role of PC-1 protein and its expression when using different antibodies to detect it. We used two PC-1 antibodies; C-20 (Santa Cruz, sc-10372) as the C-terminal antibody, and P-15 (Santa Cruz, sc-10307) as the N-terminal antibody. We evaluated the PC-1 expression by performing immunoblotting on the human embryonic kidney (HEK) 293 cells and the renal proximal tubular epithelial cell (RPTEC) lysates. We characterized the expression of PC-1 in the fetal, adult and polycystic kidneys tissues by performing immunohistochemistry. We confirmed the PC-1 expression in the HEK 293 cells and the RPTEC lysates, but the expression was very low. The PC-1 proteins were diffusely expressed in the tubular epithelial cells cytoplasm in the fetal and adult kidneys, and the PC-1 expression was more prominent in the proximal tubules of the fetal kidney. In the ADPKD kidney, the PC-1 proteins were heterogeneously and weakly expressed in the tubular or cyst lining epithelial cells. Our data suggests that the development of the kidney may regulate the expression of PC-1, and an altered PC-1 expression may contribute to cyst formation in ADPKD.

Key Words : Polycystic Kidney Diseases; Polycystin-1; Polycystic kidney disease 1 protein

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INTRODUCTION

Autosomal dominant polycystic kidney disease (ADPKD) is a common inherited disease that affects approximately 1 in 1,000 individuals. The disease's main clinical manifestations are bilateral renal cyst formation and hypertension, and the half of these patients reach end stage renal failure by the age of 60 yr (1). ADPKD is genetically heterogeneous and most of the afflicted families have mutations of either *PKD1* on chromosome 16 (-85%) or *PKD2* on chromosome 4 (-15%) (2, 3). *PKD1* encodes polycystin-1 (PC-1), which is an integral membrane protein of 4,302 amino acids with an expected molecular mass of 462 kDa (4, 5). The functions of the PC-1 remain unclear. PC-1 is widely expressed in the renal tubular epithelium and it is thought to be a cell-cell/matrix receptor molecule at the cell surface. PC-1 and PC-2 may heterodimerize to form a PC complex and this could function as the same signaling pathway (6, 7). However, there are controversies about the PC-1 expression and function when using different antibodies to study them. The large size and the low expression of PC-1 have made the study of PC-1 very difficult. There are also some doubts as to the specificity of the PC-1 antibodies (8, 9).

As an initial approach towards studying ADPKD and to

obtain more insight into PC-1 expression, we have carried out immunoblot and immunochemical analyses of the PC-1 expression in the tubular cells of fetal, adult and ADPKD kidneys.

MATERIALS AND METHODS

Immunoblot

We used human embryonic kidney (HEK) 293 cells and renal proximal tubular epithelial cells (RPTECs) for performing immunoblotting. The cells were grown to 70-90% confluence and they were then lysed in phosphate buffered saline that contained 0.5% Nonidet P-40. A large amount of protein (~400 µg each) was separated on 4% SDS-PAGE gel with or without boiling. The separated proteins were electrotransferred onto nitrocellulose membranes. The membranes were blocked with 5% nonfat dry milk for 1 hr and they were next incubated with primary antibodies diluted at 1:500. We used two PC-1 antibodies; C-20 (Santa Cruz, sc-10372, Santa Cruz, CA, U.S.A.) was used as the C-terminal antibody and P-15 (Santa Cruz, sc-10307, Santa Cruz, CA, U.S.A.) was used as the N-terminal antibody. After washing, the mem-

branes were incubated for 1 hr at room temperature with the peroxidase-labeled secondary antibody (anti-goat IgG-HRP, Santa Cruz) at 1:500 dilution. The membrane-bound antibodies were detected by using the enhanced chemiluminescence detection system (Amersham Biosciences, Bucks, U.K.).

Immunohistochemistry

Normal fetal kidney was obtained from autopsy (gestation age: 20 weeks, weight: 305 grams) after spontaneous abortion. Adult kidneys were obtained from renal tumor nephrectomy patients (a male 58 yr old and a male 63 yr old). The autosomal dominant polycystic kidneys were obtained from PKD1 patients (a male 53 yr old and a male 46 yr old) who had the typical clinical manifestations during transplant nephrectomy. All the tissues were embedded in optimal cutting temperature compound (Sakura Tissue Tek, Torrance, CA, U.S.A.) and stored at -70°C until further use.

Immunohistochemical staining was performed by using the streptavidin-biotin peroxidase method. Cryosections $5\ \mu\text{m}$ thick were cut, and the tissues were fixed in acetone at -20°C for 10 min and then washed with phosphate-buffered saline. After blocking the endogenous peroxidase activity, the primary antibodies (C-20 and P-15) were diluted to 1:100 and the sections were incubated for 1 hr with them at room temperature. After washing, the sections were incubated for 45 min with the secondary antibody (bionylated anti-goat Ig, Dako, Glostrup, Denmark). The reaction was developed with 3-amino-9-ethylcarbazol (AEC, red stain) or with 3,3'-diaminobenzidine (DAB, brown stain). The sections were counterstained with using hematoxylin.

RESULTS

Immunoblotting

We confirmed that the C-20 antibody was sensitive for

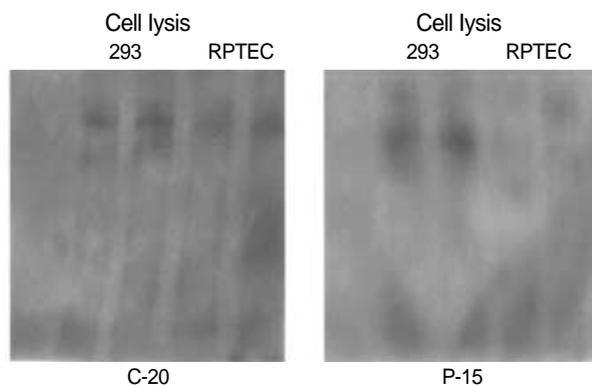


Fig. 1. Polycystin-1 in the HEK 293 cell and RPTEC is detected by using C-20 and P-15 antibodies in 4% SDS-PAGE gel. The polycystin-1 expression is very low.

detecting PC-1 by performing immunoprecipitation (10), and we confirmed the PC-1 expression in the HEK 293 cells and in the RPTEC lysates (Fig. 1). However, this was technically difficult because of the large size of the PC-1 molecule and its faint expression level.

Immunohistochemistry

In the fetal and adult kidneys, the staining was restricted to tubular epithelial cells cytoplasm (Fig. 2A to D). The patterns of expression were diffuse and cytoplasmic at the proximal tubules, distal tubules and collecting ducts. The glomerular tufts and interstitium displayed no staining. The expression in the fetal kidney was more prominent in the proximal tubules (Fig. 2A, B). In the ADPKD kidney, the PC-1 proteins were weakly expressed in the tubular or cyst-lining epithelial cells, as compared to that of the fetal and adult kidneys. The patterns of expression were heterogenous and cytoplasmic at the cystic epithelium (Fig. 3). The specific renal distribution of the PC-1 is summarized in Table 1.

DISCUSSION

The identification of PC-1, which is the major gene mutated protein seen in ADPKD, is a major step for discovering the pathogenesis in this common hereditary disease. However, there's been some controversy about the PC-1 expression when using different antibodies to detect it. The difficulties for studying PC-1 are due to the antibody specificity and the low levels of PC-1 expression in cells and tissues. Moreover, two thirds of the *PKD1* gene is duplicated on chromosome 16 and there are at least three homologous genes. Our study showed that PC-1 may be very weakly expressed in the renal tubular cells, as observed by immunoblotting, and it is widely expressed in the fetal and adult renal tubular epithelial cells cytoplasm, as observed by immunochemistry. Before this study, we confirmed that C-20 antibody was sensitive to detect the PKD-1 C-terminal by performing immunoprecipitation

Table 1. Summaries of the polycystin-1 expressions in the fetal, adult and autosomal polycystic kidneys

	C20 (C-terminal)	P15 (N-terminal)
Fetal kidney	GM -	GM -
	PT ++	PT ++
	DT +/-	DT +/-
	CT +/-	CT +/-
Adult kidney	GM -	GM -
	PT ++	PT ++
	DT +++	DT +++
	CT ++	CT ++
ADPKD kidney	cyst +	cyst +

GM, glomerulus; PT, proximal tubule; DT, distal tubule; CT, collecting duct.

(10). To verify the specificity of the immunohistochemical staining, we performed immunoblot analysis of the kidney cells (HEK 293 cells and RPTECs), the kidney tissues (fetal and adults) and the ADPKD tissues. We were unable to detect the PC-1 band in the kidney tissues and the ADPKD tissues. This is suggested that the PC-1 is either not expressed at detectable levels in normal and ADPKD kidneys, or it is lost during sample preparation. However, we were able to detect low levels of PC-1 in the kidney cells. According to the previous reports (8, 9), many experiments have failed to detect the PC-1 band. Immunoblotting of PC-1 was technically

difficult because of the large size of the PC-1 molecule and the faint level of expression.

A number of published papers have described the PC-1 expression in normal renal and cystic tissue. Van Adelsberg et al. (11) have reported that in the early fetal kidney, PC-1 was localized to the plasma membranes of the ureteric buds and the S-shaped bodies. However, in the late fetal kidney and in the ADPKD kidney, the majority of PC-1 staining was intracellular. They detected several bands in the fetal kidney and no bands were detected in the adult kidney by immunoblotting. Gene et al. (12, 13) found no major differences bet-

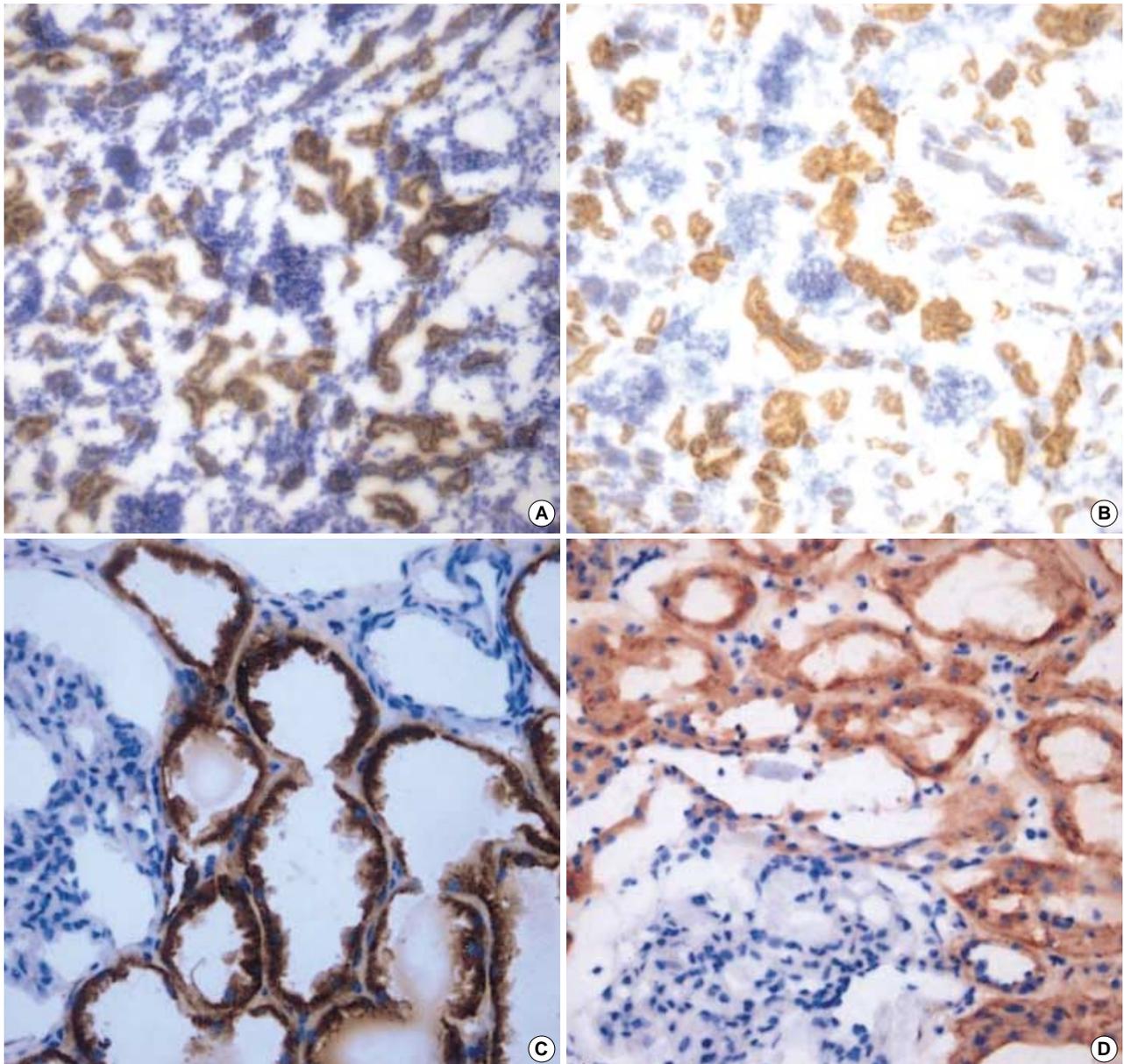


Fig. 2. The immunohistochemical expression of polycystin-1 in the fetal (A and B, $\times 200$) and adult (C and D, $\times 400$) kidneys with using goat polyclonal C-20 (A and C) and P-15 antibodies (B and D). The expression of polycystin-1 shows a diffuse cytoplasmic pattern at the tubules, and it is negative at the glomerular tufts and interstitium. The expression in the fetal kidney is less prominent in the collecting ducts and distal tubules than in the proximal tubules.

ween human and mouse PC-1 expression. They detected about a 400 kDa PC-1 band in the fetal and adult mouse and human kidneys. PC-1 was noted to be localized at the plasma membrane and PC-1 expression was seen in most (~90%) of the ADPKD cysts. Ong *et al.* (14) reported that in adult PKD1 tissue, the majority of cysts (~80%) showed PC-1 expression, although PC-1 staining was absent in a variable, but significant minority of the cysts (~20%). Leeuwen *et al.* (15) found that the mutant *Pkd1* mice that had a reduced *Pkd1* gene expression showed polycystic kidney disease. They generated a novel mouse model with a hypomorphic *Pkd1* allele, and the pathologic features of this mouse were similar to the human

ADPKD phenotype. Therefore, the reduced PC-1 expression of the normal allele may lead to ADPKD. Recently, Roitbak *et al.* (16) have reported that PC-1 formed a complex with E-cadherin and β -catenin at both the cell membrane and intracellularly. Enhanced phosphorylation of PC-1 changed its subcellular localization and its ability to form protein complexes. The plasma membrane expression of PC-1 was diminished in the ADPKD cells.

There have been different results reported by different research groups. However, researchers have reached some consensus. First, the PC-1 expression is temporally and spatially regulated during renal development. Second, the PC-1 is

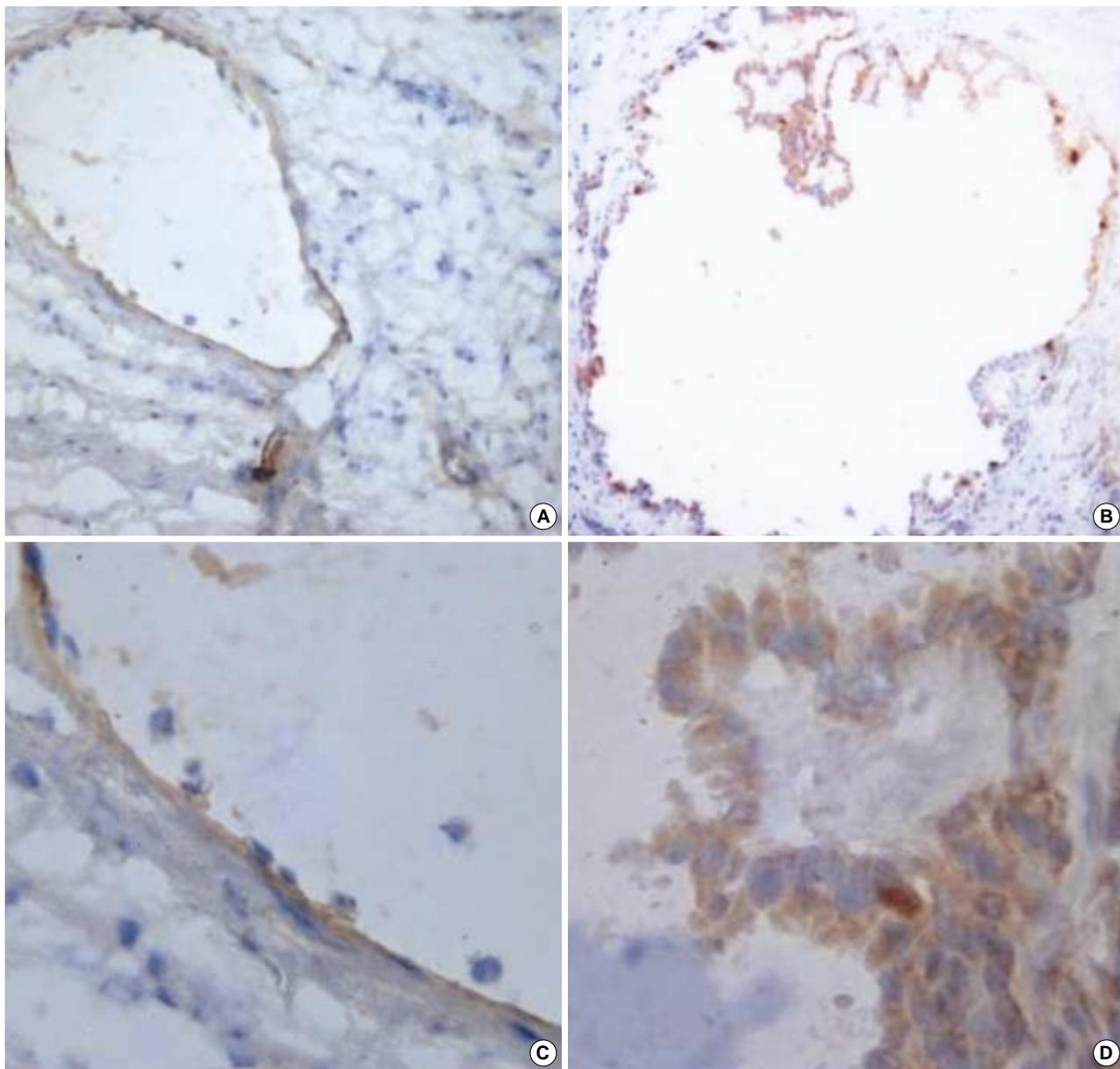


Fig. 3. The immunohistochemical expression of polycystin-1 in the autosomal dominant polycystic kidney with using goat polyclonal C-20 (A, $\times 400$ and C, $\times 1,000$) and P-15 antibodies (B, $\times 200$ and D, $\times 1,000$). The ADPKD kidney shows a weak and heterogenous expression of polycystin-1 in the tubular or cystic lining epithelial cells compared to that of the fetal and adult kidneys.

expressed in the tubular epithelium and in the ADPKD cyst epithelium. Third, the subcellular localization of PC-1 is mainly on the cell surface membrane and it may be changed in some conditions. Our results showed similar patterns of PC-1 expression for the fetal, adult and ADPKD kidneys, but our immunohistochemical study showed a cytoplasmic staining pattern of PC-1. However, in the HEK 293 cells grown to 80% confluency, we found that the PC-1 was localized on the cell surface membrane by performing immunofluorescent staining with using C-20 antibody (10). There is a possible explanation for this result. These PC-1 antibodies are sensitive, but they are not specific for detecting the PC-1. The epitope recognized by these antibodies may be not the full length, but rather, they are only a fraction of the PC-1.

PC-1 is the PKD 1 gene translated protein, and it is a 4,302 amino acids glycoprotein with an expected molecular mass of 460 kDa. Experimental studies have shown that PC-1 is a highly glycosylated 520 kDa polypeptide that is present in the plasma membrane (6, 7). By performing amino acid sequence homology analysis, PC-1 was found to be a membrane receptor that is capable of binding and interacting with ligands. However, the exact function and binding ligands of PC-1 are not yet known. Recent research have shown that PC-1 is also localized to the renal cilia and to the protein-mediated mechanosensation in the primary cilium of the kidney (17, 18).

In summary, we have found by performing immunoblotting that PC-1 is expressed at a very low level in the renal tubular cells. However, PC-1 is widely expressed in the fetal and adult renal tubular epithelial cells cytoplasm, as was noted upon performing the immunohistochemistry. In the ADPKD kidney, the PC-1 proteins were heterogeneously and weakly expressed in the tubular or cyst lining epithelial cells. A reduced PC-1 expression may well be associated with ADPKD. Our data suggests that the development of kidney may regulate the expression of PC-1, and the altered PC-1 expression may contribute to cyst formation seen in ADPKD.

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