博士論文概要

多発性嚢胞腎症ラットの膵臓の形態と機能

大学院医学系研究科
健康社会医学専攻　健康増進医学講座　健康栄養医学分野
博士課程 4 年　　衣　　蘭娟
指導教員　　石黒　　洋

[Introduction]

The epithelial cells lining the pancreatic ducts have primary cilia that extend from the apical membrane towards the lumen. Although the role of primary cilia in the pancreas is not known, studies on polycystic kidney disease (PKD) in the past decade disclose the role of primary cilia in morphogenesis and function of the epithelial tissues. Autosomal recessive polycystic kidney disease (ARPKD) is a neonatal form of PKD and is caused by mutation in *polycystic kidney and hepatic disease 1* (*PKHD1*) gene that encodes a large transmembrane protein, fibrocystin. Fibrocystin is localized to the primary cilium and basal body. The polycystic kidney (PCK) rat is a model of ARPKD with a mutation (IVS35-2A→T) in *Pkhd1*, a rat ortholog of human PKHD1 gene. To clarify the role of primary cilia in the pancreatic duct, we examined the structure and function of the exocrine pancreas of PCK rats.

[Materials and Methods]

Animals. The Filial 2 (F2) generations of F1 hybrid of the PCK and wild-type (WT) SD rats were used.

Duct structure. The pancreas was removed and the pancreatic ductal tree was isolated by removing acinar tissue using sharpened needles under a dissection microscope.

Immunohistochemistry. The rats were killed by intracardiac perfusion. The pancreas was stained with rabbit anti-AQP1. Standard protocol was used for hematoxylin and eosin (HE) staining.

Pancreatic and biliary secretion in anesthetized rats. The biliopancreatic duct (BPD) and the common bile duct were canulated separately to collect pancreatic juice and bile.

Effect of hydrostatic pressure on basal secretion. The outlet of the silastic tube for collecting pancreatic juice was attached to a vertical manipulator. The initial position (0 cm H_2O) was set at the level of the pancreas. The outlet was raised to 1, 2, 3, 4, and 8 cm every 3 min and the fluid level within the tube was recorded.

Data analysis. Statistical significance was evaluated with Student's *t* test.

[Results]

Anatomy. The kidneys and liver were markedly enlarged in PCK rats. When the pancreatic weight was normalized by the body weight, PCK rats had significantly ($P<0.05$) larger pancreas than WT rats. The BPD, the main pancreatic duct (MPD) of the splenic lobe, and the branches of PCK rats were significantly dilated compared with the WT duct. The lumen of the ducts of PCK rats was irregular compared with the smooth straight ducts of WT rats. There was no apparent cyst formation.

Microscopic anatomy. The interlobular ducts in PCK rats were surrounded by thick periductal connective tissues. The epithelial lining of BPD in PCK rats was irregular with many bulges toward the lumen. AQP1 immunoreactivity of the duct cells along the dilated BPD, MPD, and interlobular ducts in PCK rats was more intense than that in WT rats.

Pancreatic secretion in anesthetized rats. Under stimulation with the lowest dose of secretin (0.03 nmol kg^{-1} h^{-1}), the fluid secretory rate in PCK rats was significantly ($P<0.05$) less than that in WT rats. The differences became smaller (~20%) at higher doses of secretin. The amylase response to carbamylcholine in PCK rats was not significantly different from those in WT rats.

Effects of intraductal pressure on basal fluid secretion in anesthetized rats. The distensibility of the pancreatic duct system was examined. Basal secretion continued against the hydrostatic pressure of 4 cm H_2O in WT rats and there was no back flow of the juice into the pancreatic duct up to 8 cm H_2O.

In contrast, significant back flow was observed at >3 cm H_2O in PCK rats.

[Discussion]

We studied the potential role of primary cilia of pancreatic duct cells by examining morphology and secretory function of the pancreas of the PCK rat, in which the function of primary cilia was affected by a genetic mutation of ciliary protein, fibrocystin. Unlike the kidneys and liver no cyst was formed in the pancreas. The interlobular ducts in PCK rats were surrounded by thick periductal connective tissues, which is similar to human autopsy observation. Pancreatic acini appeared to be normal and enzyme secretion in response to cholinergic stimulation was unaffected. The most prominent structual change in PCK rats was irregular dilatation of the pancreatic duct, especially of larger ducts with enhanced AQP1 expression in epithelial cells. The pancreatic duct system exhibited higher distensibility to a low physiological pressure, which may in part explain low basal and secretin-stimulated fluid secretion. These findings suggest the pancreatic ductal structure and function are dependent on intact fibrocystin.

[Conclusion]

These findings suggest that fibrocystin/primary cilia-dependent mechanisms may play a role in the regulation of pancreatic ductal structure and fluid secretion.