An improved primary culture system of pancreatic duct epithelial cells from Wistar rats

Ke-Ling Chen · Xue-Lian Zheng · Yuan Li · Lie Yang ·
Zong-Guang Zhou · Xiang-Yu Zhou · Bin Zhou · Rong Wang ·
Jing-Jing Jiang · Li-Hui Chen · Lan Zhan

Received: 5 December 2008 / Accepted: 7 June 2009 / Published online: 26 July 2009
© Springer Science+Business Media B.V. 2009

Abstract Pancreatic duct epithelial cells (PDEC) are involved in most common pancreatic diseases. Primary cultivation of PDEC is a prerequisite for in vitro studies, in which in vivo situations can be simulated and molecular mechanisms investigated better than in cultured cell lines. However, some problems still exist regarding rat PDEC primary cultivation. In this study, an improved primary culture system of rat PDEC is presented. Some modifications, especially regarding specimen chosen, digestive control and epithelium purification, were made to simplify the procedure, increase cell yield, and improve epithelium purification. Cultures were identified as PDEC by morphological characteristics, reverse transcription-polymerase chain reaction and immunocytochemistry staining with cytokeratin 19. In addition, growth characteristics of rat PDEC are described in detail. This improved technique, which is more efficient and cost-effective, will be useful for in vitro pancreatic studies.

Keywords Growth characteristics · Improved techniques · Pancreatic duct epithelial cells · Primary culture · Wistar rat

Abbreviation

PDEC	Pancreatic duct epithelial cells
BSS	Balanced Salt Solution
D-HBSS	Ca ²⁺ -Mg ²⁺ -free Hank's Balanced Salt Solution
FBS	Fetal bovine serum
IMEMZO	Richter's improved MEM medium with zinc option (cell culture medium)
EGF	Epidermal growth factor
CK-19	Cytokeratin 19
RT	Reverse transcribed
RT-PCR	Reverse transcription-polymerase chain reaction
PBS	Phosphate buffered saline

Introduction

Pancreatic duct epithelial cells (PDEC) are involved in most common pancreatic diseases, such as pancreatic cancer, pancreatitis, and diabetes mellitus (Grapin-Botton 2005). More than 90% of pancreatic cancers originate from PDEC (Jiaying et al. 2005; Hong et al. 2000; Ni et al. 1998; Adachi et al. 2006; Agbunag and Bar-Sagi 2004), and PDEC play an immune-protective role in the initiation of acute pancreatitis (Yuan et al. 2005). Recently, PDEC have been shown to

K.-L. Chen · X.-L. Zheng · Y. Li · L. Yang ·
Z.-G. Zhou (✉) · X.-Y. Zhou · B. Zhou ·
R. Wang · J.-J. Jiang · L.-H. Chen · L. Zhan
Institute of Digestive Surgery, State Key Laboratory of
Biotherapy, Sichuan University, No.1 on Ke-Yuan-Si-Lu,
Gao-Peng-Da-Dao, 610041 Chengdu, Sichuan, China
e-mail: zhou767@163.com; xhwkyjs@163.com

Z.-G. Zhou
Department of General Surgery III, West China Hospital,
Sichuan University, No.1 on Ke-Yuan-Si-Lu,
Gao-Peng-Da-Dao, 610041 Chengdu, Sichuan, China

potentially have therapeutic value for diabetes, as they can be induced to differentiate into islet cells (Bonner-Weir et al. 2000; Yatoh et al. 2007; D'Alessandro et al. 2007; Noguchi et al. 2006; Boretti and Gooch 2006; Jindal et al. 1997). Thus, clearly defining an in vitro primary culture system of normal ductal cells may provide a method that can simulate in vivo situations and in which molecular mechanisms underlying malignant transformation, inflammation initiation, and cell differentiation can be investigated. Often cultured cell lines used in in vitro studies have lost important components of the normal cell precursors, which can potentially confound results.

Rats are widely used as experimental model animals based on their high propagation rate, reasonable cost, and genomic comparability with humans. However, specific properties of the rat pancreatic duct, such as slender anatomy structure, few epithelium resources, and sensitive digestion identity, restrict its application in pancreatic studies. Many technical problems, such as accurate choice of experimental specimen, effective means to prevent contamination with mesenchymal cells, and appropriate control of digestive process still exist. Furthermore, a more detailed characterization of PDEC physiology based on a well-established system is needed.

The aim of the current study was to summarize and modify the current PDEC primary culture system with regard to existing problems and to propose some modifications of experimental design based on detailed observation of rat PDEC growth characteristics.

Materials and methods

Experimental animals

Four Wistar rats (Experimental Animal Center of Sichuan University, Chengdu, China), weighing 150–200 g, were used per experiment. All animal experiments were conducted according to the guidelines of the local Animal Use and Care Committees and executed according to the National Animal Welfare Law.

Optimization of culture medium

Complete medium: IMEMZO (Richter's improved MEM medium with zinc option) (Gibco, Carlsbad,

CA, USA) supplemented with $5 \mu\text{g mL}^{-1}$ insulin (Sigma, St. Louis, MO, USA), 100 ng mL^{-1} EGF (Chemicon, California), $4 \mu\text{g mL}^{-1}$ dexamethasone (Sigma, St. Louis, MO, USA), 100 ng mL^{-1} cholera toxin (Sigma, St. Louis, MO, USA), 100 ng mL^{-1} transferrin (Sigma, St. Louis, MO, USA), 20% FBS (fetal bovine serum) (Hyclone Company, South America), 500 U mL^{-1} penicillin, and $5 \mu\text{g mL}^{-1}$ streptomycin.

Minimal medium: IMEMZO supplemented with the above additives except 2% FBS, 100 U mL^{-1} penicillin, and $1 \mu\text{g mL}^{-1}$ streptomycin were used instead.

Digestive enzyme solution

The digestive enzyme solution contained 3 mixed enzymes: 0.1% collagenase type V, 0.1% hyaluronidase, 0.1% soybean trypsin inhibitor (all from Sigma, St. Louis, MO, USA).

Balanced salt solution (BSS)

D-HBSS (Ca^{2+} - Mg^{2+} -free Hank's Balanced Salt Solution) with 500 U mL^{-1} penicillin and $5 \mu\text{g mL}^{-1}$ streptomycin.

Isolation of rat PDEC

Under 10% pentobarbital anesthesia (5 mg g^{-1}), a laparotomy was performed after sterilization. Good exposure of the pancreas head was achieved by clamping the pyloric of the stomach and proximal duodenum at the same time. Thus, a diaphanous, light-yellow duct, with 1 mm diameter crossing the head of pancreas at a 30–45-degree angle could be visualized. The duct was dissociated and periductal tissue was removed along the main duct as precisely as possible while it was still attached to the duodenum (Fig. 1). The duct was then resected and placed into a conical centrifuge tube containing 5–6 mL ice-cold BSS.

The centrifuge tube was taken into the fume hood (Class II A/B3 biological safety cabinet, Thermo Forma) under sterile conditions. The ducts were transferred into a petri dish, washed 3 times with ice-cold BSS, and minced into 1–2 mm diameter fragments. The following modifications were made to the digestive control procedure (the new procedure was termed simplified segmental timing digestion). The fragments were placed in a 15 mL sterile conical

Fig. 1 Anatomical method. A assistant clamped the pyloric of the stomach and proximal duodenum while the head of pancreas exposed to us. Removed the periductal tissue and isolated the main duct (arrow) carefully

centrifuge tube with 2 mL of digestive solution and incubated in a water bath at 37 °C for 12 min with vigorous shaking every 3–4 min. The reaction was stopped by addition of 6–8 mL BSS and the duct fragments were allowed to settle for 5 min. The suspension, which included small pieces of pancreatic tissue, was discarded. An additional 1 mL of digestive solution was added to the pancreatic duct fragments and the digestion proceeded for 5 min. After digestion was complete, 0.5 mL FBS was added to quench the reaction. The bigger fragments settled in about 5 min, after which the suspension was discarded again. Further digestion of the large fragments was achieved by adding 1 mL mixed enzymes and incubating for 5 min. The reaction was quenched by adding 0.5 mL FBS and shaking vigorously (From this time on, the supernatant containing single cells and small clusters of PDEC was collected into a conical centrifuge tube). In order to collect the monolayer of epithelial cells, the ductal fragments were subjected to 3–4 successive 5 min enzyme digestions at 37 °C until no ductal fragments could be seen. The collected supernatant was centrifuged for 10 min at 286 g and washed by resuspending the pellet in BSS and centrifuging for 5 min at 161 g. The pellet was resuspended in complete pancreatic medium and seeded into a petri dish.

Primary cultivation of PDEC

The isolated cells were plated in a 60 mm petri dish. After 2 h initial incubation in a humidified 5% CO₂

atmosphere at 37 °C, the subculture was added to another petri dish that was pretreated with polylysine (Sigma, St. Louis, MO, USA) at 4 °C overnight to facilitate the adhesion of cells. Cells were left to adhere and grow on the dish undisturbed at 37 °C and 5% CO₂ for 48–72 h, then the nonadherent cells were removed by aspirating the medium; the minimal medium was replenished for the adherent cells. Media (using the minimal medium) were changed every 2 days and the growth characteristics of the cells were observed daily. Cytokeratin 19 (CK-19) was used as specific epithelia marker to characterize the cultured cells.

Morphological studies

Morphologic characteristics were studied by optical microscopy (C-5060, OLYMPUS, Japan) and transmission electron microscope (H-600 IV, HITACHI, Japan). Electron micrographs were performed by the standard published procedure for adherent cells.

RT-PCR amplification and primers

Total RNA was extracted from the cultured cells in the petri dish using Trizol (Invitrogen, Carlsbad, CA, USA). RNA was reverse transcribed (RT) into cDNA and 1 µL of the RT product was amplified by PCR (iCycler iQ, Bio-Rad, USA) in a 25 µL reaction system for 40 cycles as follows: 94 °C for 30 s, 55 °C for 30 s, and 60 °C for 1 min. Primers were designed and synthesized by Invitrogen Co. (Shanghai, China). Primers for CK-19 polymerase chain reaction (PCR) were as follows: CK19F, 5'-TTAGTG CCCTGAGGAGCCA-3'; CK19R, 5'-CTGGACCTT ACGTCGGAGT-3'. (yielding a 248 bp PCR product) The amplified products were resolved by electrophoresis in 2.0% agarose gels and visualized by goldview staining.

Immunocytochemistry

Immunocytochemistry was carried out according to the standard protocol (Öberg-Welsh et al. 1997). Briefly, 3 days after initial plating the cultured cells were stained with an epithelial cell specific marker. A 1:10 dilution of the primary CK-19 antibody (Dako Co., Carpinteria, CA) was used and the cells were visualized by reaction for 5–10 min in DAB solution (Zhongshan Co., Beijing, China).

Results

This study provided an improved PDEC primary cultivation system with some modifications based on current methodologies and our experimental practices, mainly regarding specimen chosen, digestive control, and epithelium purification. All the experiment had been repeated at least three times to ensure the reproducibility and cell yield, which was really well reproducible.

Cultured cells were identified as PDEC by the following:

Morphological characteristics

The morphologic characteristics of cultured cells under light microscope were readily recognized as tight clusters of epithelium-like cells islands. These cells assumed a polygonal shape and grew in a cobblestone arrangement (Fig. 2). Transmission electron micrographs revealed features of epithelial cells including numerous surface microvillous processes with core rootlets and tight-junctional complexes (Fig. 3a). Cells possessed abundant intracellular vacuoles, smooth endoplasmic reticulum, and well-developed Golgi complexes. Furthermore, desmosome junctions formed between adjacent cells and even the

Fig. 2 The morphologic characteristics under light microscope. These cells assumed a polygonal shape and grew in a cobblestone arrangement. Cells grew from the adherent clumps and formed monolayer plaques of cells with typical epithelial morphology. Original magnification 200 $\times$. Scale bar: 50 μ m

intermediate filaments involved were particularly prominent (Fig. 3b).

RT-PCR

Cultures could be characterized by CK 19 expression, which is a specific cytoskeletal structure of simple epithelia, including pancreatic ductal cells. The amplified PCR products separated on agarose gels stained with goldview, showing that they were of the expected size (248 bp). The result of RT-PCR showed significant mRNA expression for CK-19 in the cultured cells (Fig. 4).

Immunocytochemistry

More than 95% of the cells stained positive in the immunocytochemistry analyses. This indicated that the available cells were PDEC with high purification (Fig. 5).

Furthermore, the following normal growth characteristics of rat PDEC were observed:

After resuspending the pelleted cells in complete medium and plating them in a petri dish, mainly clumps of small round epithelial cells were present. Occasionally, the clumps of epithelial cells were contaminated with some erythrocytes, mesenchymal cells, and probably small clumps of granulated acinar cells (Fig. 6). After 24 h of culture, several clumps of epithelial cells had attached to the plate; these were allowed to grow undisturbed. After 2 days of culture, more clumps had attached to the culture plate and the cells from the previously adhered clumps proliferated and formed monolayer plaques of cells. Epithelial cells proliferated only from detached colonies of cells and not from single cells. The proliferating cells formed cobblestone-like monolayers, which is typical for cultured epithelial cells (Fig. 7). For the next 3 days, the attached clumps continued to proliferate. The monolayers were approximately 50% confluent, which was about 5 times more confluent than at the previous day (Fig. 8). At this stage, PDEC cultures were in an adequate condition for characterization of cell morphology and staining for epithelial cell specific markers. Over the next 1–2 days, the epithelial cells were in the bloom growth condition and the layers of duct epithelial cells were nearly confluent. Over

Fig. 3 Transmission electron micrographs of rat PDEC. Cells possess numerous surface microvillous (*arrow*) and tight-junctional complexes (*), abundant intracellular vacuoles, smooth endoplasmic reticulum, and well-development Golgi complexes (**a**). Original magnification 10,000 $\times$. Desmosome junctions (*arrows*) formed between adjacent cells and even the intermediate filaments involved were particularly prominent (**b**). Original magnification 25,000 $\times$

Fig. 4 PCR result. The amplified PCR products showing the size of 248 bp

Fig. 5 Immunocytochemistry result. Cells were stained positive in the immunocytochemistry analyses. While the cytomembrane was stained by CK19 antibody, the nucleus was counterstained by hematoxylin. Original magnification 200 $\times$. Scale bar: 50 μ m

these 2 days, more than 80% of the petri dish was covered with epithelial cells (Fig. 9). At the 6–7 day time point, the proliferation continued but some of the attached cells began to detach and float in the medium. After 8 days, the viability of the cells decreased and many vacuoles appeared (Fig. 10). On day 12 after isolation, most of the duct epithelial cells were dead and by 14–15 days after isolation, all the PDECs had died.

Discussion

Pancreatic duct epithelial cells are involved in most pancreatic diseases that have severely threatened human health for a hundred years. An optimal *in vitro* system of PDEC primary cultivation is necessary for studying the physiology and pharmacology of pancreatic diseases. Here, we established an improved primary culture system of rat PDEC. Cultured cells

Fig. 6 Collected suspension with clumps of cells. In the suspension, there were mainly clumps of small round epithelial cells or individual ones (*arrows*) with unavoidably few erythrocytes and mesenchymal cells. Original magnification 200 $\times$. Scale bar: 50 μ m

Fig. 8 Growth characteristic at the third day. The plaques proliferated quickly at the stage of 50% confluency. Original magnification 40 $\times$. Scale bar: 250 μ m

Fig. 7 Growth characteristic at the second day. Cells grew from the adherent clumps and formed monolayer plaques with epithelial morphology. Original magnification 100 $\times$. Scale bar: 100 μ m

Fig. 9 Growth characteristic at the fourth to fifth day. The epithelial cells were in the bloom growth and more than 90% of the Peri dish was covered with epithelial cells. Original magnification 40 $\times$. Scale bar: 250 μ m

were identified as PDEC by morphological observation and expression of the specific epithelial marker CK19. We summarized and modified the current methodologies, specifically regarding specimen chosen, digestive control, and epithelium purification. These modifications resulted in simplification of the procedure, increased cell yield and improved rate of epithelium purification.

First, we provided an easier and quicker method to perform the specimen anatomy. In our system, only pancreatic ducts were used. In earlier studies, researchers used the entire pancreatic tissue (Tsao and Duguid

1987; Githens and Whelan 1983; Heimann and Githens 1991). The problem with this method is that, despite the addition of soybean trypsin inhibitor, release of enzymes from acinar cells can potentially harm the duct epithelial cells. In addition, the risk of contamination with mesenchymal cells is increased if all the pancreatic tissue is used. Researchers have recognized these problems and in the late 1990s began to isolate the main duct from pancreatic tissue. The main duct was isolated in a dish after the entire pancreas was dissected (Agbunag and Bar-Sagi 2004; Agbunag et al. 2005; Öberg-Welsh et al. 1997).

Fig. 10 Growth characteristic at the eighth day. Many vacuolizations (arrows) appeared. Original magnification 200 $\times$. Scale bar: 50 μ m

However, the pancreas shrunk without the strain after it was dissected from periorgans, which made it difficult to remove the periductal tissue. In the method described in this study, periductal tissue was removed as precisely as possible before it was dissected from the periorgans.

Second, the digestive control was also modified and this modified procedure was called ‘simplified segmental timing digestion’. This modified digestive procedure both simplified the process and increased the cell yield. In the 1980s, Githens et al. (1981, 1987) and Githens and Whelan (1983) established the explant culture system, which was known as ‘classical culture system’ and was widely accepted during that period. Nevertheless, explant cultivation takes several weeks, although it is still the most cost-effective method. Therefore, more recently, researchers have preferred the enzyme digestion method over the explant method. In early studies, researchers performed ‘entire time digestion’ of the pancreatic ducts in a traditional fashion (Tsao and Duguid 1987; Öberg-Welsh et al. 1997; Heimann and Githens 1991). Our experience has shown that this entire time digestion resulted in the formation of gels that enveloped the cells, thus preventing their adherence to the culture plate. In recent years, Agbunag and Bar-Sagi (2004) and Agbunag et al. (2005) proposed to divide the digestive process into several steps, which resulted in a pronounced increase in cell yield. However, the drawback to this procedure was that many enzymes and manipulation steps were needed, which made the

system difficult to follow, especially in the beginning. In order to simplify the procedure, we used a mixed enzyme solution containing 0.1% collagenase type V, 0.1% hyaluronidase, and 0.1% soybean trypsin inhibitor throughout the entire digestion process. This mixed enzyme solution could be prepared in advance and stored at -20°C . Our new protocol could reduce the overall cost and the risk of contamination when the enzymes are changed. During the first 17 min of the digestion, the suspension contained many mesenchymal cells and acinar cells around the duct that needed to be removed, and the subsequent 3–4 digestions were performed to produce suitable cell clumps that could result in an increased cell yield of approximately 60% over the ‘entire time digestion’ (4×10^4 – 5×10^4 cells can be obtained per rat by this new method, while less than 1.5×10^4 cells can be yielded per rat by using the ‘entire time digestion’ method).

Finally, the following measures used together improved the epithelium purification:

- (a) Pancreatic duct rather than the whole pancreas was used, which effectively decreased the level of contamination with other pancreatic cells.
- (b) Minimal medium with a low FBS concentration was used 48–72 h after plating, which inhibited the growth of mesenchymal cells but not epithelial cells. Epithelial cells grew well in a medium that included EGF, insulin, dexamethasone, transferrin, and cholera toxin.
- (c) The subcultures were transferred into another petri dish 2 h after the initial plating. Upon initial plating, other pancreatic cells were also present in addition to the ductal cells. The primary concern is contamination from mesenchymal cells, since erythrocytes and acinar cells will naturally die off after a period of time. However, mesenchymal cells attach to culture plate more rapidly than epithelial cells and thus they can be preferentially removed by transferring the medium of the primary cultures 2 h after the initial plating.

According to the immunocytochemistry analysis result, more than 95% cells following this procedure were PDEC while only 80–90% could be obtained by previous methods.

Meanwhile, some suggestions about experimental design were proposed according to the growth characteristics of rat PDEC.

PDEC, like all normal diploid cell types, have a finite life span in vitro. In the first 24 h after seeding the cells, some clumps were not firmly attached to the plate and these were left undisturbed. From the second day on, the PDECs entered the exponential growth period. On the third to fourth day, PDECs were growing optimally, and this was the best time to treat with drugs and perform immunocytochemistry or morphological observation on the cells. The fifth to sixth day after plating, cells were in quantitation amounts, which is a appropriate time for passage and RNA or protein extraction. After 7 or 8 days, the viability of the cultures began to decrease. Cells in this condition were not useful for further manipulations. Thus, the life span of rat PDEC is not as long as that of large animals, such as dog, cow, pig or human (Stoner et al. 1978; Cotton 1998; Hootman and Logsdon 1988; Oda et al. 1996; Trautmann et al. 1993). Therefore, it is recommended to complete all manipulations with the cells by 8 days following the initial plating.

In conclusion, this modified rat primary PDEC cultivation provided an improved system with accurate and quick specimen anatomy, simple but high cell yield digestive control, and fast and complete epithelium purification. This improved technique and these detailed descriptions of rat PDEC growth characteristics will be useful for in vitro studies of pancreatic diseases.

Acknowledgments Financial support from National Nature-Science Grants 30400434, 30571811, 30830100 and the assistance of Guan-Qiao You, Hong-Ying Chen, Jian-Ping Yin are gratefully acknowledged.

References

- Adachi T, Tajima Y, Kuroki T et al (2006) Bile-reflux into the pancreatic ducts is associated with the development of intraductal papillary carcinoma in hamsters. *J Surg Res* 136:106–111
- Agbunag C, Bar-Sagi D (2004) Oncogenic K-ras drives cell cycle progression and phenotypic conversion of primary pancreatic duct epithelial cells. *Cancer Res* 64: 5659–5663
- Agbunag C, Lee KE, Buontempo S et al (2005) Pancreatic duct epithelial isolation and cultivation in two-dimensional and three-dimensional culture systems. *Method Enzymol* 407:703–710
- Bonner-Weir S, Taneja M, Weir GC et al (2000) In vitro cultivation of human islets from expanded ductal tissue. *Proc Natl Acad Sci* 97:7999–8004
- Boretti MI, Gooch KJ (2006) Induced cell clustering enhances islet beta cell formation from human cultures enriched for pancreatic ductal epithelial cells. *Tissue Eng* 12: 939–948
- Cotton CU (1998) Ion-transport properties of cultured bovine pancreatic duct epithelial cells. *Pancreas* 17:247–255
- D'Alessandro JS, Lu K, Fung BP et al (2007) Rapid and efficient in vitro generation of pancreatic islet progenitor cells from nonendocrine epithelial cells in the adult human pancreas. *Stem Cells Dev* 16:75–89
- Githens S, Whelan JF (1983) Isolation and culture of hamster pancreatic ducts. *J Tissue Cult Methods* 8:97–102
- Githens S, Holmquist DR, Whelan JF et al (1981) Morphologic and biochemical characteristics of isolated and cultured pancreatic ducts. *Cancer* 47:1505–1512
- Githens S, Finley JJ, Patke CL et al (1987) Biochemical and histochemical characterization of cultured rat and hamster pancreatic ducts. *Pancreas* 2:427–438
- Grapin-Botton A (2005) Ductal cells of the pancreas. *Int J Biochem Cell B* 37:504–510
- Heimann TG, Githens S (1991) Rat pancreatic duct epithelium cultured on a porous support coated with extracellular matrix. *Pancreas* 6:514–521
- Hong OY, Lun-jun M, Catherine L et al (2000) Immortal human pancreatic duct epithelial cell lines with near normal genotype and phenotype. *Am J Pathol* 157:1623–1631
- Hootman SR, Logsdon CD (1988) Isolation and monolayer culture of guinea pig pancreatic duct epithelial cells. *In Vitro Cell Dev Biol* 24:566–574
- Jiaying Q, Jiangong N, Ming L et al (2005) In vitro modeling of human pancreatic duct epithelial cell transformation defines gene expression changes induced by K-ras oncogenic activation in pancreatic carcinogenesis. *Cancer Res* 65:5045–5053
- Jindal RM, McDaniel HM, Filo RS et al (1997) Stem cell potential of pancreatic duct-epithelial cells. *Transplant P* 29:1502
- Ni L, Toru F, Masao K et al (1998) Comparative phenotypic studies of duct epithelial cell lines derived from normal human pancreas and pancreatic carcinoma. *Am J Pathol* 153:263–269
- Noguchi H, Xu G, Matsumoto S et al (2006) Induction of pancreatic stem/progenitor cells into insulin-producing cells by adenoviral-mediated gene transfer technology. *Cell Transplant* 15:929–938
- Öberg-Welsh C, Sandler S, Andersson A et al (1997) Effects of vascular endothelial growth factor on pancreatic duct cell replication and the insulin production of fetal islet-like cell clusters in vitro. *Mol Cell Endocrinol* 126:125–132
- Oda D, Savard CE, Nguyen TD et al (1996) Dog pancreatic duct epithelial cells: long-term culture and characterization. *Am J Pathol* 148:977–985
- Stoner GD, Harris CC, Bostwick DG et al (1978) Isolation and characterization of epithelial cells from bovine pancreatic duct. *In Vitro* 14:581–590

- Trautmann B, Schlitt HJ, Hahn EG et al (1993) Isolation, culture, and characterization of human pancreatic duct cells. *Pancreas* 8:248–254
- Tsao M-S, Duguid WP (1987) Establishment of propagable epithelial cell lines from normal adult rat pancreas. *Exp Cell Res* 168:365–375
- Yatoh S, Dodge R, Akashi T et al (2007) Differentiation of affinity-purified human pancreatic duct cells to beta-cells. *Diabetes* 56:1802–1809
- Yuan L, Zong-Guang Z, Qing-Jie X et al (2005) Toll-like receptor 4 detected in exocrine pancreas and the change of expression in cerulein-induced pancreatitis. *Pancreas* 30:375–381