Dear Editor:

The ductal epithelium is a minor component of pancreatic tissue, comprising only 3–4% of the rodent and guinea pig pancreas by volume (2,3). The duct system, however, serves a major function as the site of bicarbonate and fluid secretion from the pancreas (3,25). Cells in the interlobular and main ducts are believed to play a major role in carcinogenesis, and, based on this belief, several pancreatic cancer models have been developed in the rodent (17,19). Cells in the intralobular ducts are important because they may have the ability to differentiate into islet and/or acinar cells (18). Cell transformation leading to islet cell neogenesis and new islet formation in the postnatal period, however, is not well characterized. As a consequence, there are practical reasons to clarify whether a pool of multipotent cells persists in the pancreas in postnatal life and the location of these cells. Further elucidation of these questions requires the development of an experimental model.

Our laboratory has reported an in vivo model of islet cell neogenesis, induced by cellophane wrapping of the head of the hamster pancreas in order to create partial obstruction of the duct system (12). The ductal epithelium is a minor component of pancreatic tissue, comprising only 3–4% of the rodent and guinea pig pancreas by volume (2,3). The duct system, however, serves a major function as the site of bicarbonate and fluid secretion from the pancreas (3,25).

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A number of methods for adult pancreatic duct epithelial isolation and culture have been reported, either as monolayers from cow, human, rat, hamster, and guinea pig (12–14,26,27), or as intact duct fragments from rat and hamster (6–8). These conventional methods yield populations of main and interlobular duct epithelium. Reliable methods for the large scale isolation and culture of intralobular pancreatic ductules is required to clarify the molecular mechanisms involved.

A number of methods for adult pancreatic duct epithelial isolation and culture have been reported, either as monolayers from cow, human, rat, hamster, and guinea pig (12–14,26,27), or as intact duct fragments from rat and hamster (6–8). These conventional methods yield populations of main and interlobular duct epithelium. Reliable methods for the large scale isolation and culture of the terminal segments of the pancreatic duct system are not available.

For these reasons, we developed a process for the isolation and culture of the terminal segments of the pancreatic duct system of the Syrian golden hamster as epithelial cysts suspended in a rat-tail collagen matrix and cultured in DMEM:F12 media and 10% Nu-Serum supplemented with cholera toxin (CT, 100 ng/ml) and epidermal growth factor (EGF, 10 ng/ml). The ductules in this culture system arise from within partially digested fragments of acinar tissue, and a very high yield of ductular epithelium is achieved. From the stage of acinar fragments to duct epithelial cyst formation, the primary cultures were observed through an inverted microscope and photographed serially over the first 10 d. The material plated into primary culture on Day 0 consists of clumps of acinar debris, the occasional islet, and a few fragments of larger ducts or blood vessels (Fig. 1 a). Light microscopy (Fig. 1 b) confirms that the cell aggregates consist mostly of acinar tissue with intercalated or intralobular ductules. In addition, there are fragments of larger interlobular ducts and islets that are completely devoid of adjacent acinar tissue.

From 1–3 d, only a few cystic structures appear either on the periphery of large clumps of acinar tissue, or as separate cysts without any linkage to acinar fragments. These cysts are derived from the fragments of intralobular ducts that are observed at the time of plating, and tend to form very distended spherical structures on longer culture. Light microscopic examination of these structures shows the cyst wall to be composed of a single layer of columnar or pseudostratified columnar epithelium. At the ultrastructural level, some of these cells contain secretory vacuoles near the apical plasma membrane that resemble mucus. Acinar cells show evidence of degranulation and intracellular vacuolation.

Small cysts are present in the midst of clumps of acinar debris after 3–4 d (Fig. 1 c,d). Histologic examination shows that the wall of these cysts consists of a single layer of cubical or flattened epithelium.

From 4–6 d, there is progressive necrosis of acinar cells adjacent to the areas in which cysts are forming (Fig. 1 d,e). The number of small cystic areas surrounding by surviving acinar cells increases. Fibroblasts now appear, but remain few in number and are preferentially located at the periphery of the forming cysts. Between Days 7–10, the necrosis of acinar tissue is widespread and there is a continuing increase in the number of cystic areas. There is enlargement of cysts already formed (Fig. 1 e,f). On Day 12, approximately 3200 cysts/g tissue can be harvested from the primary culture. The secondary cultures consist of mixtures of small cysts with flattened or cuboidal epithelial cells and large cysts with columnar epithelium. There is progressive loss of the remaining acinar tissue, and very little fibroblastic contamination is noted. Tertiary cultures contain pure duct epithelial cysts of both types.

The ultrastructural appearance of the epithelium of the cysts resembles that of duct epithelium in vivo and that of cultured duct fragments. Apical microvilli and lateral interdigitations of the plasma membrane are present, cells are interconnected by tight junctions, and there is no basal lamina (Fig. 2). With longer time in culture, epithelial cells in the smaller cysts that form within the acinar tissue also assume a more primitive appearance, with few cytoplasmic organelles and little apical membrane specialization. Autoradiographic analysis of DNA synthesis with tritiated thymidine shows that on Day 2, very few cells incorporate the radiolabel (Fig. 3), and these are found predominantly within the centroacinar units. After 6 d, when duct cysts are well formed (Fig. 4), over 85% of the cells that comprise the newly formed ductular epithelium are labeled, demonstrating a significant proliferative capability.
Each pancreas was injected in situ with 2 ml of a digestive medium (pH 7.4) enriched with Hanks' balanced salt solution (HBSS), 1.0 mg/ml collagenase (type XI), and 0.1 mg/ml a-chymotrypsin. The HBSS was supplemented with 0.2 mg/ml of bovine serum albumin (BSA) (fraction V) and 0.1 mg/ml of soybean trypsin inhibitor. The pancreas was removed and, after stationary digestion at 37°C for 30 min, it was dispersed by vortex. After washing, the tissue was pipetted onto a 140-μm sieve. The retained fragments were harvested by rinsing the inverted sieve and were suspended in a solution of rat tail collagen that was prepared according to Richards et al. (20). To each plate was added 2 ml of DME:F12 medium supplemented with 10% NuSerum, insulin (1 μg/ml), dexamethasone (1 μM), soybean trypsin inhibitor (0.1 mg/ml), cholera toxin (100 ng/ml), and EGF (10 ng/ml). The cultures were incubated in an atmosphere of 5% carbon dioxide in air at 37°C. a, Time-lapsed photograph of collagenase digest of pancreas embedded in collagen in primary culture. Day 0. Cellular debris with fragments of acinar tissue (arrows) ×64. b, Pancreatic acinar fragments showing minimal vacuolation. Day 0. H&E ×201. c, Time-lapsed photograph of collagenase digest of pancreas embedded in collagen on Day 4. The two clumps of acinar tissue (arrows) begin to demonstrate marked cystic changes. ×64. d, Pancreatic acinar fragments. There is central necrosis in the larger fragment (*). Some smaller fragments show increasing cytoplasmic vacuolation, while cystic structures appear to be developing from within other areas. Day 4. H&E ×128. e, Time-lapsed photograph of collagenase digest of pancreas embedded in collagen on Day 8. ×64. f, Ductular formation. Well-formed ductules now present and are lined by a single layer of flattened ductal epithelium. Day 8. H&E ×128.