Murine Embryonic Stem Cell–Derived Pancreatic Acinar Cells Recapitulate Features of Early Pancreatic Differentiation

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Background & Aims: **Acinar cells constitute 90% of the pancreas epithelium, are polarized, and secrete digestive enzymes. These cells play a crucial role in pancreatitis and pancreatic cancer. However, there are limited models to study normal acinar cell differentiation in vitro. The aim of this work was to generate and characterize purified populations of pancreatic acinar cells from embryonic stem (ES) cells.** ***Methods:*** **Reporter ES cells (Ela-pur) were generated that stably expressed both β-galactosidase and puromycin resistance genes under the control of the elastase I promoter. Directed differentiation was achieved by incubation with conditioned media of cultured fetal pancreatic rudiments and adenoviral-mediated co-expression of p48/Ptf1a and Mist1, 2 basic helix-loop-helix transcription factors crucial for normal pancreatic acinar development and differentiation.** ***Results:*** **Selected cells expressed multiple markers of acinar cells, including digestive enzymes and proteins of the secretory pathway, indicating activation of a coordinated differentiation program. The genes coding for digestive enzymes were not regulated as a single module, thus recapitulating what occurs during in vivo pancreatic development. The generated cells displayed transient agonist-induced Ca^{2+} mobilization and showed a typical response to physiologic concentrations of secretagogues, including enzyme synthesis and secretion. Importantly, these effects did not imply the acquisition of a mixed acinar-ductal phenotype.** ***Conclusions:*** **These studies allow the generation of almost pure acinar-like cells from ES cells, providing a normal cell-based model for the study of the acinar differentiation program in vitro.**

Pancreatic acinar cells play a key role in digestion in vertebrates. The synthesis and secretion of digestive enzymes is finely regulated: many enzymes are synthesized as propeptides and stored in zymogen granules (ZG), the exocytosis of which is tightly controlled. Binding of secretagogues to the muscarinic 3 and cholecystokinin (CCK) receptors leads to Ca^{2+} release from the endoplasmic reticulum and triggers ZG secretion.[1]

Acinar cells also play an important role in exocrine pancreatic diseases, including acute and chronic pancreatitis and, possibly, ductal adenocarcinoma. Upon stress, acinar cells readily undergo a phenotypic switch, resulting in loss of differentiation and acquisition of duct-like features both in vivo and in vitro.[2–6] Together with their minimal proliferative potential, these effects hamper the study of acinar differentiation. Furthermore, there are very few acinar tumor cell lines and they lack differentiated features (ie, ZG). Because of their tumor origin, their study is also of questionable physiologic relevance. Therefore, the establishment of normal-cell–derived acinar cultures remains a high priority to better understand and manipulate acinar differentiation. In particular, it is important to better characterize and dissect substages of acinar differentiation during pancreatic development and regeneration to better interfere with their loss of differentiation properties. For instance, it generally is considered that genes coding for digestive enzymes are activated simultaneously and that their expression is under identical regulatory control mechanisms, yet conventional reverse-transcription polymerase chain reaction (RT-PCR) data support the notion that these genes are regulated sequentially.[7] Because nonquantitative RT-PCR (non–qRT-PCR) has technical limitations, the relative levels of expression of the genes coding for acinar enzymes at different developmental stages remains to be analyzed. This is important because in vivo tracing experiments evaluating the contribution of acinar cells to

Abbreviations used in this paper: Ad, adenovirus; Amyl, α-amylase; CPA, carboxypeptidase A; CCK, cholecystokinin; ChymoB, chymotrypsinogen B; CM, conditioned medium; EB, embryoid bodies; Ela1, elastase 1; ES, embryonic stem; qRT-PCR, quantitative reverse-transcription polymerase chain reaction; ZG, zymogen granules.

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other pancreatic cell types currently are performed using digestive enzyme gene promoters.

Acinar cells originate from multipotent precursors located in the foregut giving rise to all pancreatic cell types. In mice, exocrine pancreas specification occurs at embryonic day 10 (E10). Cells with acinar features are distinguished at E14, ZGs accumulate from E16 to birth, and full maturation occurs postnatally.[8–10] However, little is known about the precise molecular events heralding acinar differentiation. To date, few transcription factors regulating exocrine differentiation have been identified: the basic helix-loop-helix proteins p48/Ptf1a and Mist1, and RBPL. Ptf1a messenger RNA (mRNA) is first detected around E9.5 in pancreatic primordia and, in the adult, becomes restricted to acinar cells. Ptf1a originally was described as part of the heterotrimeric transcription complex PTF1 involved in digestive enzyme gene expression.[11,12] Ptf1a also is essential for pancreas formation and for the development of all pancreatic cell lineages.[13–15] In its absence, foregut endoderm precursors assume an intestinal fate.[14] Ptf1a also promotes ectopic pancreas fates from endoderm tissue.[16–18] Mist1 is expressed in a wide array of secretory tissues and, in the adult pancreas, is detected only in acinar cells.[19,20] *Mist1* inactivation or inhibition of Mist1 function results in a severe impairment of acinar organization, including loss of gap junctions, structural alterations of secretory granules, and acinar-ductal metaplasia.[20,21] Therefore, Mist1 is necessary for proper cell polarization and maintenance of acinar identity. RBPL is essential for the high transcriptional activity of the PTF1 complex independently of Notch signaling.[22] Interestingly, RBPL is expressed in the developing pancreas tips at E14.5,[23] when exocrine precursors expand and differentiate, confirming a role in acinar differentiation during the secondary transition.[24]

Embryonic stem (ES) cells, derived from the inner-mass of pre-implantation embryos, can differentiate into cells of the 3 germ layers, making them an excellent tool for differentiation studies in vitro. Via embryoid body (EB) formation, endodermal precursors can be specified into endocrine and exocrine lineages in a process that partially recapitulates early pancreatic development.[25,26] Despite promising advances,[27] this process is very inefficient. Here, we aimed to generate ES cells that had activated a pancreatic acinar differentiation program to apply this knowledge to study exocrine pancreatic diseases. Our strategy relied on the ability of ES cells to respond to soluble factors involved in pancreatic embryogenesis in conjunction with expression of multiple exocrine transcription factors and genetic selection. This system allowed us to isolate normal cell populations displaying immature acinar phenotypes. These cells reproduce specific substages of acinar cell differentiation and should be valuable for studying the exocrine differentiation program.

Materials and Methods

ES Cell Culture and Transfection

CGR8 ES cells were maintained as published.[25] A reporter gene construct containing the -500/+8EI rat elastase I (Ela1) enhancer/promoter[28] was generated by subcloning the -500 fragment-driven puromycin resistance complementary DNA (cDNA) into pKS plasmid. An IresLacZ-mouse phosphoglycerate-kinase promoter-driven hygromycin resistance cassette (a gift from E. Maandag, The Netherlands Cancer Institute, Amsterdam, The Netherlands) was inserted downstream. Cells were electroporated (260V, 500 μF) using a Bio-Rad (Hercules, CA) gene pulser and selected using 200 μg/mL hygromycin to establish Ela-pur-IresLacZ clones (Ela-pur). Pancreatic acinar tumor cells (AR42J and 266-6) as well as NIH3T3 cells, were transfected as controls.

Adenoviral Generation and Gene Transduction

Adenoviral vectors were obtained as described.[29] Briefly, full-length cDNAs encoding rat Ptf1a[30] or mouse Mist1[31] were inserted into the pAd-shuttle-cytomegalovirus vector[32]; recombinant adenoviruses were produced by the Laboratory of Gene Therapy (Gene Vector Production Network, Nantes, France) using the Ad-Easy system.[32] Infections were performed as described in the Supplementary Materials and Methods section (see Supplementary material online at www.gastrojournal.org).

In Vitro Differentiation and Selection Procedure

To direct differentiation, Ela-pur ES clones were allowed to aggregate in bacterial Petri dishes (3.3×10^4 cells/mL) in medium supplemented with 3% fetal bovine serum without leukemia inhibitory factor. After 7 days, 30–50 EBs were plated in gelatin-coated, 6-well culture dishes for 7 additional days in 10% fetal bovine serum–supplemented medium (*differentiation step*). In some experiments, conditioned medium (CM) from E16.5 fetal pancreas cultures was added during this 14-day period (1:1 dilution with normal medium).[25] Medium was changed every 2 days. In addition, cells were infected 36 hours after EB plating with adenoviruses as described in the Supplementary Materials and Methods section. For ES–Ela-pur selection, differentiated cultures were maintained in differentiation medium plus puromycin (0.8 μg/mL) (Calbiochem, Darmstadt, Germany). After 2 weeks of selection, cells were re-infected with adenoviruses and cultured for an additional week (*selection step*, 35 days).

Molecular, Ultrastructural, and Functional Characterization of Selected Ela-Pur Cells

Cells were processed by immunocytochemistry, X-Gal staining, and electron microscopy as described