Differentiation of Pancreatic Acinar Cells to Hepatocytes Requires an Intermediate Cell Type

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BACKGROUND & AIMS: The appearance of hepatic foci in pancreas has been well-documented in animal experiments and in patients with pancreatic cancer. We previously demonstrated that transdifferentiation of pancreatic exocrine cells to hepatocytes required members of the CCAAT enhancer binding protein family. Although the molecular basis of hepatic transdifferentiation is understood, the early cellular events remain to be defined. **METHODS:** Dexamethasone and oncostatin M were used to induce transdifferentiation of primary cultures of mouse acinar cells and exocrine cell lines into hepatocytes. Fluorescent-activated cell sorting was used to identify intermediate cell types and side-population characteristics. Cre-loxP-based lineage tracing was used to investigate whether acinar cells contribute directly to hepatocytes via intermediates that express adenosine triphosphate-binding cassette subfamily G member 2 (ABCG2). **RESULTS:** Lineage tracing studies showed that hepatocytes were derived directly from pancreatic cells via ABCG2-expressing intermediates. Exposure of cells to insulin increased Akt phosphorylation, ABCG2 expression, and hepatic transdifferentiation. Inhibition of the phosphoinositide 3-kinase pathway, through addition of LY294002 or overexpression of a dominant-negative form of Akt, was sufficient to prevent transdifferentiation. When ABCG2-expressing cells were incubated with glucagon-like-peptide 1 or epidermal growth factor, the intermediate cells could differentiate into insulin-producing β-like cells. **CONCLUSIONS: The phosphoinositide 3-kinase pathway is important in the transdifferentiation of acinar cells to hepatocytes and those hepatocytes arise from acinar cells via ABCG2-expressing intermediates. Furthermore, ABCG2-expressing cells are multipotent and able to differentiate into hepatocytes and insulin-producing β cells.**

Keywords: Pancreas; Metaplasia; Transdifferentiation; Plasticity.

Transdifferentiation (or metaplasia) is defined as the conversion of one differentiated cell type to another.[1] Metaplasia is clinically significant because it may predispose to neoplasia. It is therefore important to dissect the cellular and molecular mechanisms underlying metaplasia. One example of transdifferentiation is the conversion of pancreatic acinar cells to ductal cells. Hall et al and Vila et al revealed rapid transdifferentiation of acinar cells to ductal cells in primary culture.[2,3] Acinar-to-ductal transdifferentiation may occur in experimental pancreatitis and in the progression to pancreatic neoplasia.[4–7] Acinar cells can also transdifferentiate into nestin-positive intermediates[8] followed by differentiation into duct-like cells.

The appearance of hepatic foci in adult pancreas is another example of metaplasia that has been observed in rodents exposed to various experimental treatments and in human cancer patients.[9–12] Previous reports identified hepatocyte-like cells localized in either pancreatic tumors or in ductal adenocarcinomas.[11,12] We previously found that addition of glucocorticoid was sufficient to induce conversion of pancreatic exocrine cells to hepatocytes and identified CCAAT enhancer binding protein β (C/EBPβ) as a key gene in transdifferentiation of pancreatic cells to hepatocytes.[13,14] "Transdifferentiated hepatocytes" express proteins characteristic of mature hepatocytes including albumin, acute phase proteins, a liver specific calcium channel, and cytochrome P450s[13–17] and can support hepatitis B virus replication.[18] Although we know quite a lot about the phenotype of the transdifferentiated hepatocytes, the cellular basis underlying the pancreatic-to-hepatic conversion is less well understood.[17] Therefore, in the present study, we attempted to

Abbreviations used in this paper: ABCG2, ATP-binding cassette subfamily G member 2; AMY, amylase; bp, base pair; C/EBPβ, CCAAT enhancer binding protein β; CPA, carboxypeptidase A; CYP, cytochrome P450; CYP3A1, cytochrome p450 3A1; DEX, dexamethasone; Gln-Syn, glutamine synthetase; GLP-1, glucagon-like peptide 1; EGFP, enhanced green fluorescent protein; NIC, nicotinamide; RT-PCR, reverse-transcription polymerase chain reaction; SP, side-population; OSM, oncostatin M; PI3K, phosphoinositide 3-kinase; TFN, transferrin.

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investigate the early cellular events associated with the conversion of pancreatic acinar cells to hepatocytes.

We used a cell culture model together with lineage tracing techniques to understand three processes underlying the transdifferentiation of pancreatic cells to hepatocytes: (1) the cellular origin of transdifferentiated hepatocytes in the pancreas, (2) whether transdifferentiation occurs through an intermediate cell type, and (3) to dissect the signaling pathways regulating the conversion from a pancreatic to hepatic phenotype. We show that acinar cells can directly contribute to hepatocytes, via cells that express adenosine triphosphate (ATP)-binding cassette (ABC) transporter G2 (ABCG2), and that the phosphoinositide 3-kinase (PI3K) pathway plays a key role in regulating transdifferentiation of pancreatic acinar cells to hepatocytes.

Material and Methods

Reagents

Dexamethasone (DEX), LY294002, verapamil, glucagon-like peptide 1 (GLP-1), nicotinamide (NIC), and insulin were from Sigma–Aldrich (St. Louis, MO). Recombinant human oncostatin M (OSM) and epidermal growth factor (EGF) were obtained from R&D Systems Inc (Minneapolis, MN).

Cell Lines and Culture Conditions

AR42J-B13 cells are a subclone of AR42J cells which were originally provided by Dr I. Kojima (Gumma University). AR42J cells were obtained from the Bioresource Collection and Research Center (Hsinchu, Taiwan). Both cell lines were maintained in Dulbecco's modified Eagle medium (Hyclone, Logan, Utah) containing penicillin, streptomycin, and 10% fetal bovine serum (Hyclone). DEX (1 μmol/L) was added as indicated in the text and the medium changed every 2 days. Insulin was added as a solution in HEPES or in Earle's balanced salt solution (EBSS) buffer at a final concentration of 10–100 μg/mL. OSM was added at a final concentration of 10 ng/mL. Anti-insulin receptor antibodies (Santa Cruz Biotechnology, Santa Cruz, CA) or control rabbit IgG (Santa Cruz Biotechnology) was added at a concentration of 0.5–2 μg/mL followed by incubation with DEX+OSM. When differentiating the intermediary cell population to insulin-producing β cells, GLP-1, NIC, and EGF were added at a final concentration of 10 nmol/L, 10 mmol/L, and 20 ng/mL, respectively.

Isolation and Culture of Pancreatic Exocrine Cells

Isolation of mouse and rat exocrine cells was performed as described previously,[15,19] with some modifications and the detailed method is described in the Supplementary Materials and Methods.

Establishment of Transgenic Mice and Genetic Labelling of Acinar Cells

All animal experiments were approved by the Academia Sinica Institutional Animal Care and Utilization Committee. The Elas-CreER construct was obtained from Dr Y. Dor (The Hebrew University).[20] Z/EG mice (B6.Cg-Tg(ACTB-Bgeo/GFP)21Lbe) bearing a Cre-dependent enhanced green fluorescent protein (EGFP) reporter transgene[21] were imported from The Jackson Laboratory. Elas-CreER transgenic mice were generated at the Level Transgenic Center (Taipei, Taiwan). Five founders were identified that showed acinar-specific expression of enhanced green fluorescent protein (EGFP) when crossed with Z/EG. For labeling acinar cells, 4-week-old Elas-CreER; Z/EG mice were injected intraperitoneally with free base tamoxifen (20 mg/mL in corn oil; Sigma–Aldrich) three times per week (3 injections of 2 mg each). EGFP-labeled acinar cells were harvested at a minimum of 6 days following final tamoxifen administration.

Isolation of Side-Population Cells

Cells were trypsinized and resuspended in Hanks' balanced saline solution containing 2% fetal bovine serum followed by incubation with Hoechst 33342 dye (7.5 μg/mL) (Sigma–Aldrich) at 37°C for 1 hour with or without 100 μmol/L verapamil. To analyze viability, cells were resuspended in ice-cold Hanks' balanced saline solution containing 2 μg/mL propidium iodide (Sigma–Aldrich) and filtered through a 40-μm strainer (BD-Clontech, Mountain View, CA). Sorting and side-population (SP) analyses were carried out using a FACS Vantage SE flow cytometer or a FACSAria cell sorter (Becton Dickinson, Franklin Lakes, NJ).

Results

Role of the PI3K/Akt Signalling Pathway in Hepatic Transdifferentiation

We previously established an in vitro model for the transdifferentiation of the pancreatic cell line AR42J-B13 to hepatocytes based on addition of DEX.[13] These cells express the exocrine markers amylase (AMY) and carboxypeptidase A (CPA), similar to adult acinar cells (Figure 1*A* and *F*). Addition of 1 μmol/L DEX and 10 ng/mL OSM induced the liver enriched transcription factor C/EBPβ and the hepatocyte markers transferrin (TFN) and glutamine synthetase (Gln-Syn). Induction of the hepatic phenotype was accompanied by a loss of CPA expression (Figure 1*B–D*). At day 3, some cells lost AMY and started to coexpress TFN and Gln-Syn (Figure 1*C*). At day 5, expression of cytochrome p450 3A1 (CYP3A1) was observed in most cells. At this stage, only a few cells expressed CPA and AMY. Immunocytochemical analysis and real-time quantitative reverse-transcription polymerase chain reaction (RT-PCR) indicated that the levels of liver markers such as CYP3A1 and TFN were similar to