Pancreatic epithelial plasticity mediated by acinar cell transdifferentiation and generation of nestin-positive intermediates

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Summary

Epithelial metaplasia occurs when one predominant cell type in a tissue is replaced by another, and is frequently associated with an increased risk of subsequent neoplasia. In both mouse and human pancreas, acinar-to-ductal metaplasia has been implicated in the generation of cancer precursors. We show that pancreatic epithelial explants undergo spontaneous acinar-to-ductal metaplasia in response to EGFR signaling, and that this change in epithelial character is associated with the appearance of nestin-positive transitional cells. Lineage tracing involving Cre/lox-mediated genetic cell labeling reveals that acinar-to-ductal metaplasia represents a true transdifferentiation event, mediated by initial dedifferentiation of mature exocrine cells to generate a population of nestin-positive precursors, similar to those observed during early pancreatic development. These results demonstrate that a latent precursor potential resides within mature exocrine cells, and that this potential is regulated by EGF receptor signaling. In addition, these observations provide a novel example of rigorously documented transdifferentiation within mature mammalian epithelium, and suggest that plasticity of mature cell types may play a role in the generation of neoplastic precursors.

Key words: Pancreas, Metaplasia, Differentiation, Transdifferentiation, Stem cells, TGFα, Cancer, Mouse

Introduction

Metaplastic conversion is broadly defined as replacement of one predominant cell type by another within a multilineage tissue, and is frequently associated with an increased risk of subsequent neoplasia. As recently reviewed (Tosh and Slack, 2002), tissue metaplasia may result from a variety of cellular mechanisms. These include selective expansion of differentiated cell types ordinarily present in low abundance, trans-determination (or re-programming) of local tissue-specific stem cells, or by actual transdifferentiation of one mature cell type to another, either directly or via an undifferentiated intermediate. In adult mammalian tissues, the specific mechanisms underlying metaplastic events have rarely been rigorously defined, reflecting the challenges associated with confirming precursor-progeny relationships in a multilineage context. Although formal lineage tracing using either genetic or physical labeling of individual cell types has been accomplished in lower vertebrate and developing mammalian systems (Echeverri and Tanaka, 2002; Gu et al., 2002; Kawaguchi et al., 2002), and more recently in the context of islet neogenesis (Dor et al., 2004), these techniques are difficult to apply to the study of metaplastic conversion in mature mammalian epithelium. As a result, the cellular basis for most forms of epithelial metaplasia has not been identified, and no information is available regarding mechanisms of metaplasia responsible for generation of neoplastic precursors.

The exocrine pancreas undergoes metaplastic change in the setting of both chronic pancreatitis and pancreatic cancer. In these conditions, the pancreas changes from an acinar cell-predominant tissue to a tissue comprised predominantly of ductal epithelium. These metaplastic ducts have been postulated to arise either by outgrowth of normal ductal epithelium, by activation of pancreatic stem cells or by transdifferentiation of mature cell types (De Lisle and Logsdon, 1990; Githens et al., 1994; Rooman et al., 2000; Sphyris et al., 2005). Without direct lineage tracing in combination with molecular marker analysis, the cell of origin for metaplastic ductal epithelium has remained controversial. Elucidating this mechanism would represent a significant advance not only in understanding the plasticity of terminally

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differentiated tissues, but also in determining the cellular basis of pancreatic cancer, as metaplastic ducts have frequently been proposed to be the progenitors for pancreatic ductal adenocarcinoma (Lowenfels et al., 2000; Parsa et al., 1985; Song et al., 1999; Wagner et al., 2001; Wagner et al., 1998).

The metaplastic conversion of acinar cells to ductal cells can be recapitulated by culturing pancreatic epithelium in vitro. When exocrine epithelial explants are cultured in or on an appropriate matrix, loss of acinar cells is frequently associated with a reciprocal increase in ductal epithelium (De Lisle and Logsdon, 1990; Githens et al., 1994; Rooman et al., 2000; Sphyris et al., 2005). Although a variety of culture conditions have been shown to promote this acinar-to-ductal conversion, the molecular and cellular mechanisms are not known. We have used this in vitro metaplastic conversion to understand three basic processes underlying epithelial metaplasia in mammalian pancreas: (1) to identify autocrine and/or paracrine pathways regulating pancreatic metaplasia; (2) to identify the cell of origin for metaplastic ductal epithelium; and (3) to identify intermediary cell populations arising during this process. Using primary explant cultures and rigorous lineage tracing techniques, we demonstrate that acinar cells undergo conversion to metaplastic ductal epithelial cells in response to TGFα and EGFR signaling; that this represents a true transdifferentiation event involving conversion of terminally differentiated acinar cells to a ductal epithelial phenotype; and that this transdifferentiation occurs via intermediates that are nestin positive and simultaneously express both acinar and ductal markers. These results provide a novel example of rigorously documented transdifferentiation within a mature mammalian epithelium, and suggest that plasticity of fully differentiated epithelial cells may contribute to the generation of neoplastic precursors.

Materials and methods

Transgenic mouse lines and *in vivo* lineage labeling

Breeding and genotyping of MT-TGFα mice were performed as previously described (Song et al., 1999; Wagner et al., 2001). Villin-Cre transgenic mice (el Marjou et al., 2004) and Elastase-CreERT2 mice (D.A.S., unpublished) were crossed onto a *Gt(ROSA)26Sor*^{tm1Sor} (R26R) background (Soriano, 1999). For in vivo genetic labeling of acinar cells, Ela-CreERT2; R26R mice were treated with seven consecutive daily intraperitoneal injections of free base tamoxifen (Sigma; 1 mg per day in sunflower seed oil). Tissue for epithelial explant culture was harvested at a minimum of 1 week following final tamoxifen administration. Villin-Cre and Ela-CreERT2 transgenic mice were genotyped by PCR using primers directed at the Cre-coding region (Postic et al., 1999).

Preparation of epithelial explant cultures

Explant cultures of adult mouse pancreas were established by modification of previously published protocols (De Lisle and Logsdon, 1990; Githens et al., 1994; Wagner et al., 2002). Whole pancreas was harvested and digested in 0.2 mg/ml collagenase-P (Boehringer Mannheim, Mannheim, Germany) at 37°C. Following multiple washes with Hanks balanced salt solution (HBSS) supplemented with 5% fetal bovine serum (FBS), collagenase-digested pancreatic tissue was sequentially filtered through 500 μm and 105 μm polypropylene mesh (Spectrum Laboratories, Laguna, CA). The filtrate was passed through a 30% FBS cushion at 1000 rpm. The cellular pellet was resuspended in Waymouths MB 752/1 media or RPMI1640 media (Gibco BRL, Gaithersburg, MD) supplemented with penicillin G (1000 U/ml), streptomycin (100 μg/ml) with 1% heat-inactivated FBS (growth factor experiments) or 10% FBS (lineage tracing experiments). An equal volume of neutralized rat tail collagen type I (RTC) (Collaborative Biomedical Products, Bedford, MA) was added to the cellular suspension. The cellular/RTC suspension was supplemented with 0.1 mg/ml soybean trypsin inhibitor (Sigma Chemicals, St Louis, MO) and 1 μg/ml dexamethasone (Sigma). Cellular/RTC suspension (500 μl) was pipetted into each well of a 24-well plate (well diameter=16mm) (Corning, Corning, NY) pre-coated with 200 μl of RTC. After solidification of the RTC, media supplemented with penicillin G, streptomycin (100 μg/ml) and FBS (at above mentioned concentrations) were added. Cultures were maintained at 37°C and 5% CO_2 in air for up to 14 days. Explants harvested from non-transgenic mice were maintained in the presence or absence of recombinant human TGFα or HGF (R&D Systems). Where appropriate, explants were additionally treated with the EGF receptor inhibitors AG1478 (Sigma) or EKI-785 (generously provided by Philip Frost at Wyeth-Ayerst). Media supplemented with appropriate growth factors and/or inhibitors were exchanged on day 1 and day 3. For detection of β-gal activity, whole collagen gels were fixed in 0.2% glutaraldehyde/1% formaldehyde and stained in X-gal overnight at 37°C (Means et al., 2003).

Assessment of cell death and cell proliferation

Cell viability was determined by Trypan Blue exclusion. Immediately prior to plating, an aliquot of cells were mixed with an equal volume of 0.4% Trypan Blue stain (Gibco) for 10 minutes, washed and counted for the number of blue (dead) and non-blue cells (alive). After 3 days of culture, cells suspended in collagen were digested with 25 μg/ml collagenase P for 10 minutes, washed and stained with Trypan Blue as above. Four different experiments were performed, with 1000-2000 cells counted in each sample. Results are presented as mean±s.e.m. For assessment of cell proliferation, BrdU was added to the culture medium throughout the 5 days of culture, and explants were similarly analyzed for BrdU incorporation using immunofluorescence.

RNA extraction and semi-quantitative RT-PCR analysis of nestin expression from total cellular RNA was performed using TRIZOL Reagent (Life Technologies, Rockville, MD). cDNA was prepared by random priming from 1 μg of total RNA using a First-Strand cDNA Synthesis kit (Life Technologies, Rockville, MD) according to the manufacturer's instructions. Amplification was carried out in 50 μl of reaction mixture containing dNTP (200 μM each), 30 pmol of each of the primers and 2.5 U of Taq DNA polymerase (Qiagen). For nestin, Gapdh and β-actin amplifications, 5 μl of cDNA template was amplified using the following primer pairs: nestin (annealing temp: 59°C), forwards 5′-GCT GGA ACA GAG ATT GGA AGG C-3′ and backwards 5′-TCA AGG GTA TTA GGC AAG GGG G-3′; GAPDH (annealing temp: 58°C) forwards 5′-TGT TCC AGT ATG ACT CCA CTC ACG-3′ and backwards 5′-GCC CTT CCA CAA TGC CAA AG-3′; β-actin (annealing temp: 59°C), forwards 5′-GCT CGT CGT CGA CAA CGG CTC-3′ and backwards 5′-CAA ACA TGA TCT GGG TCA TCT TCT-3′.

PCR product accumulation was assessed at 20, 25 and 30 cycles of amplification in order to confirm linear detection of PCR product. The expected and observed amplification product sizes were as follows: nestin, 372bp; GAPDH, 384 bp; β-actin, 359 bp.

Immunostaining

The following antibodies were used for immunofluorescence analysis: rabbit polyclonal anti-nestin (gift from Dr R. McKay), mouse monoclonal anti-nestin (Pharmingen), goat polyclonal anti-amylase (Santa Cruz Biotechnology), rabbit polyclonal anti-cytokeratin, wide spectrum (Dako), rabbit polyclonal anti-carbonic anhydrase II (Chemicon) and sheep polyclonal keratin 19 (The Binding Site). For immunofluorescent labeling of explanted pancreatic tissue, collagen gels containing explanted pancreas were fixed in 4:1 methanol:DMSO overnight, 4°C, then washed and stored at –20°C in 100% methanol. Cultures in collagen disks were rehydrated, washed in PBS, then

PBSBT (PBS+ 0.5% tritonX-100 + 2% BSA). Disks were blocked with 5% normal donkey serum in PBSBT for 2 hours at room temperature, then incubated sequentially with the primary and secondary antibodies diluted in PBSBT, overnight at 4°C. Following each antibody, disks were washed extensively in PBT (PBS + 0.5% tritonX-100). After the final overnight incubation, the cultures were washed twice in PBT, three times in PBS, then counterstained with YoPro nuclear dye (Molecular Probes) and washed in PBS. Images were captured on a Zeiss LSM-510 Meta confocal microscope at an optical depth of 1 μm. Immunoperoxidase staining was performed on paraffin-embedded tissue that was sectioned at depths of 2 or 5 μm, using the Vectastain ABC Elite kit (Vector Labs) as indicated by manufacturer. Antibodies used were rabbit anti-amylase (Sigma) and rabbit anti-cytokeratin, wide spectrum (Dako). For quantitative analysis of immunohistochemically and histochemically stained cells, all quantification is presented as mean±s.e.m. For each analysis, cells were counted from at least three independent experiments for a total of 900 to 1600 cells counted per analysis.

Results

Acinar-to-ductal metaplasia requires EGF receptor tyrosine kinase activity

A number of studies have demonstrated that acinar cell-enriched pancreatic epithelial explants can give rise to ductal structures following culture in or on collagen matrix, mimicking in vivo acinar-to-ductal metaplasia (De Lisle and Logsdon, 1990; Githens et al., 1994; Rooman et al., 2000; Sphyris et al., 2005). However, the factors influencing this process are not clear. Based on the ability of EGFR ligands to induce acinar-to-ductal metaplasia in vivo (Means et al., 2003; Sandgren et al., 1990; Song et al., 1999; Wagner et al., 2002), we tested the ability of EGFR signaling to induce metaplastic conversion of exocrine epithelial explants. Acinar cell clusters with associated terminal ductal elements were isolated from wild-type mice or mice that expressed a TGFα transgene under regulation of a metallothionein (MT) promoter (Sandgren et al., 1990; Song et al., 1999). To minimize the effects of other growth factors, the explants were cultured in a low (1%) serum environment, and identical culture conditions were maintained for tissue harvested from both non-transgenic and transgenic mice. MT-TGFα transgenic mice develop progressive pancreatic acinar-to-ductal metaplasia in vivo, with eventual generation of neoplastic ductal lesions (Song et al., 1999; Wagner et al., 1998). When isolated at 5-6 weeks of age (prior to the onset of in vivo metaplasia in MT-TGFα mice), freshly harvested explants from MT-TGFα transgenics and non-transgenic littermates were morphologically identical. Tissue from both transgenic and non-transgenic mice consisted of clusters of pancreatic acini with associated terminal ductal epithelium (Fig. 1A-C). When explanted in a low (1%) serum environment, explants of non-transgenic pancreas maintained a

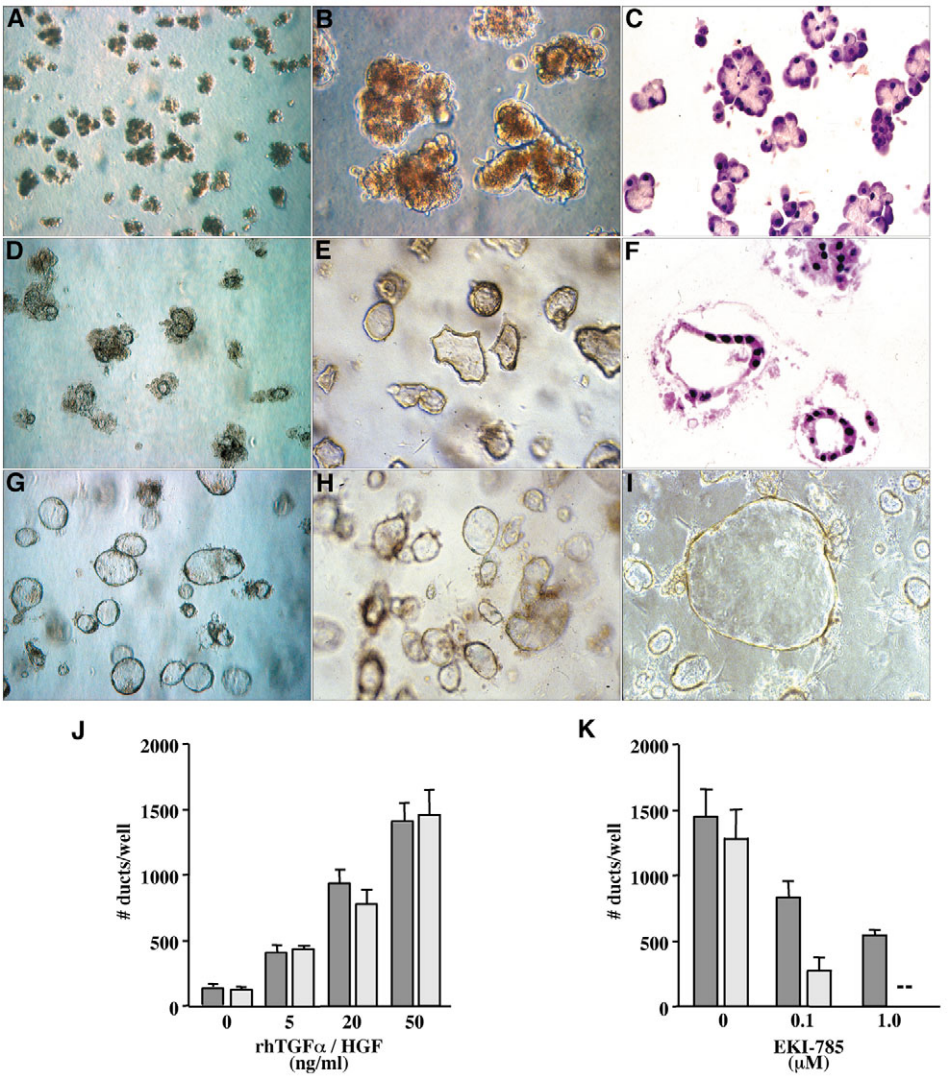

Fig. 1. Acinar-to-ductal metaplasia in primary explant cultures of mouse pancreas. (A,B) Phase contrast images of freshly harvested (day 0) non-transgenic pancreatic explant. (C) Hematoxylin and Eosin-stained section of non-transgenic day 0 explant. (D) Untreated non-transgenic day 5 explant. (E) Untreated MT-TGFα day 5 explant cultured in the absence of exogenous growth factors. (F) Hematoxylin and Eosin-stained section of expanded duct-like epithelium arising from day 5 MT-TGFα pancreas. (G) Non-transgenic day 5 explant cultured with 50 ng/ml rhTGFα. (H) Non-transgenic day 5 explant cultured with 50 ng/ml rhHGF. (I) Non-transgenic day 14 explant cultured with 50 ng/ml rhTGFα. (J) Quantification of duct-like structures formed following culture for 5 days with variable concentrations of either rhHGF (dark bars) or rhTGFα (light bars). (K) Effect of EGF receptor-specific tyrosine kinase inhibitor EKI-785 on expansion of duct-like epithelium by either 50 ng/ml rhHGF (dark bars) or 50 ng/ml rhTGFα (light bars). Values indicate mean±s.e.m. for three separate experiments; – – indicates no ductal structures observed.

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predominantly acinar cell identity, as indicated by columnar morphology, basal nuclei and apical zymogen granules, until loss of cell viability (Fig. 1D). By contrast, explant cultures of MT-TGFα pancreas developed progressive conversion from an acinar cell-predominant phenotype to a ductal epithelial phenotype characterized by large cystic structures lined by cuboidal and simple squamous epithelia (Fig. 1E,F). These observations were replicated in explants derived from at least five mice in both the transgenic and non-transgenic groups, in which at least four independent explants were derived from each mouse. The presence of metaplasia in explant cultures of MT-TGFα pancreas was uniformly observed. These observations indicate that expression of TGFα is sufficient to induce conversion from an epithelium comprised predominantly of acinar cells to a metaplastic ductal epithelium, in vitro as well as in vivo.

The similarity of this in vitro response to the in vivo ductal metaplasia observed in MT-TGFα mice suggests that the ability of TGFα to induce pancreatic ductal metaplasia may represent a direct effect on target epithelium, rather than an indirect effect induced by longstanding overexpression. However, because pancreatic epithelium from MT-TGFα mice may have experienced downstream effects of TGFα signaling prior to isolation, we tested whether soluble recombinant human TGFα (rhTGFα) was sufficient to induce the conversion of acinar cell-predominant epithelium to ductal cell-predominant epithelium. Acinar-enriched epithelium was isolated from wild-type mice and treated with rhTGFα or control media. As noted above, wild-type explants that were maintained in low serum without exogenous TGFα did not give rise to ductal structures. However, ductal cysts arose as a dose-dependent response to soluble rhTGFα. This response was observed in explants derived from more than 20 mice, with quadruplicate explants generated for each condition. TGFα-dependent conversion to a duct-like epithelium was observed in over 90% of all preparations, with only an occasional preparation failing to generate metaplastic epithelium, probably related to diminished viability at the time of explant initiation. The ductal epithelium induced by rhTGFα was similar to that observed in epithelial explants harvested from MT-TGFα mice (Fig. 1G,I,J; see Movie 1 in the supplementary material).

Based on the ability of hepatocyte growth factor (HGF) to regulate epithelial differentiation and morphology in a variety of settings (Brinkmann et al., 1995), as well as the recent implication of the HGF receptor as an a marker of pancreatic epithelial precursors (Suzuki et al., 2004), we next tested this growth factor for its ability to induce acinar-to-ductal metaplasia. Similar to the effects of rhTGFα, treatment of epithelial explants with human recombinant HGF also induced loss of acinar cells, and replacement by metaplastic ductal epithelium in a dose-dependent manner (Fig. 1H,J).

These data demonstrate that two growth factors, acting on different tyrosine kinase receptors, each induce similar effects on pancreatic epithelial explants. In many systems, signaling through EGFR is influenced by other receptors that mediate the cleavage and activation of EGFR ligands (Prenzel et al., 1999; Uchiyama-Tanaka et al., 2002). Therefore, we examined the requirement of EGFR signaling in the generation of metaplastic epithelium in response to either TGFα or HGF. For these experiments, acinar cultures were treated with either TGFα or HGF in the presence or absence of the EGFR/erbB2 tyrosine kinase inhibitors EKI-785 (Discafani et al., 1999) and AG1478 (Lin et al., 1997). As expected, these inhibitors caused a dose-dependent decrease in TGFα-induced ductal metaplasia, with complete inhibition observed at a concentration of 1.0 μM EKI-785 (Fig. 1K). By contrast, EKI-785 had a limited effect on generation of metaplastic epithelium by HGF, with even 1.0 μM EKI-785 unable to completely abolish the response to HGF (Fig. 1K). At a concentration of 5.0 μM, AG1478 showed an identical effect (data not shown), inhibiting TGFα-induced but not HGF-induced acinar-to-ductal metaplasia. The limited inhibition of HGF activity in response to EGFR inhibitors suggests that HGF may act partially upstream of EGFR; more importantly, these data indicate that separate signaling pathways, perhaps converging downstream, can independently induce a metaplastic change in pancreatic epithelial differentiation.

Metaplastic ductal epithelium arises by acinar cell transdifferentiation

Because acinar cells are lost during ductal metaplasia of the pancreas, it has frequently been proposed that transdifferentiated acinar cells represent the source of metaplastic ductal epithelium. However, tracing the fate of acinar cells either in vivo or in vitro has been complicated by extensive cell death, which typically occurs during the process of acinar-to-ductal metaplasia. To trace the fate of any remaining acinar cells, large number of cells needed to be labeled in an entirely cell type-specific manner. To generate such a label, we developed two different methods for acinar-specific recombination of the R26R reporter allele in *Gt(ROSA)26Sor*^{tm1Sor} (R26R) mice (Soriano, 1999). The R26R *lacZ* reporter allele is silent until a transcriptional stop cassette is excised by Cre-mediated recombination. Once recombined, the *lacZ* gene is expressed from the ubiquitously active Rosa26 locus, even if Cre is subsequently lost from the cell. As expression of β-gal enzymatic activity results from genomic recombination of the R26R allele, it represents a heritable genetic trait that will be durably expressed throughout the life of the cell, and also passed on to any progeny cells. Thus, acinar-specific activation of the R26R allele provides an indelible marker of both acinar cells and of any cells that arise from acinar cells.

To obtain acinar-specific recombination of the R26R allele, mice expressing a Villin-Cre transgene were crossed onto the R26R reporter line. Although the Villin-Cre transgene induced recombination in multiple tissues including intestine and kidney, β-gal activity in pancreatic tissue from Villin-Cre;R26R mice was strictly confined to acinar cells. No β-gal activity was seen in other pancreatic cell types, including islet and ductal cells (Fig. 2A-D; see Fig. S1A,B in the supplementary material). Even the terminal intercalated ducts most closely associated with acini were negative for β-gal activity. In addition, no β-gal activity was seen in single transgenic mice carrying either the Villin-Cre transgene or the R26R allele alone (data not shown).

To determine whether acinar cells could transdifferentiate into ductal cells, acinar-enriched epithelial explants were isolated from pancreas of Villin-Cre;R26R mice and cultured in the presence of rhTGFα. At the time of isolation, β-gal staining was observed exclusively in acinar cells, with

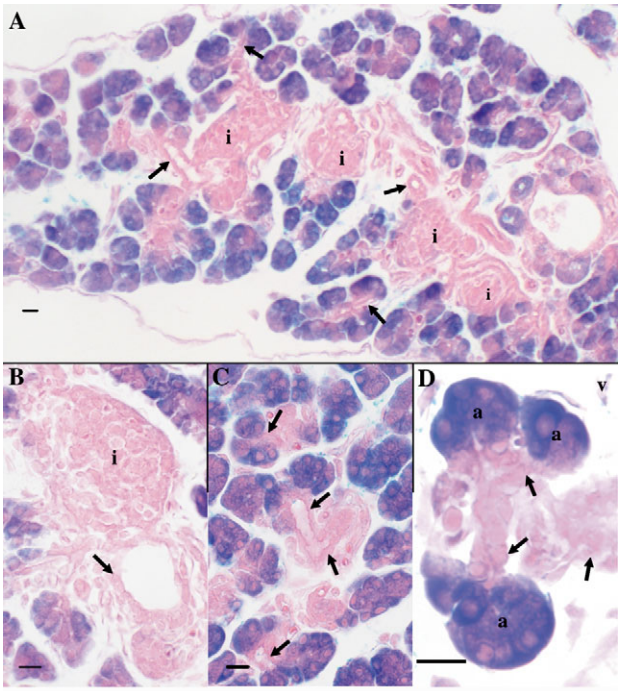

Fig. 2. Acinar cell-specific recombination of the R26R reporter allele in Villin-Cre pancreas. (A) Low magnification view of neonatal pancreas from a Villin-Cre; R26R pup stained for β-gal activity (blue) and counterstained with Eosin. (B) High-magnification view showing lack of β-gal activity in islet and large duct. (C) High-magnification view showing an intralobular duct with no β-gal activity and all cells with a clear acinar morphology positive for β-gal. (D) High magnification view showing no β-gal staining in terminal intercalated ducts that are most closely associated with acini. β-Gal activity is lacking in the large blood vessel in the upper right corner. In all panels, β-gal activity is present in all acinar cells. Scale bars: 10 μm. Arrows indicate pancreatic ducts. a, acinus; i, islet; v, blood vessel.

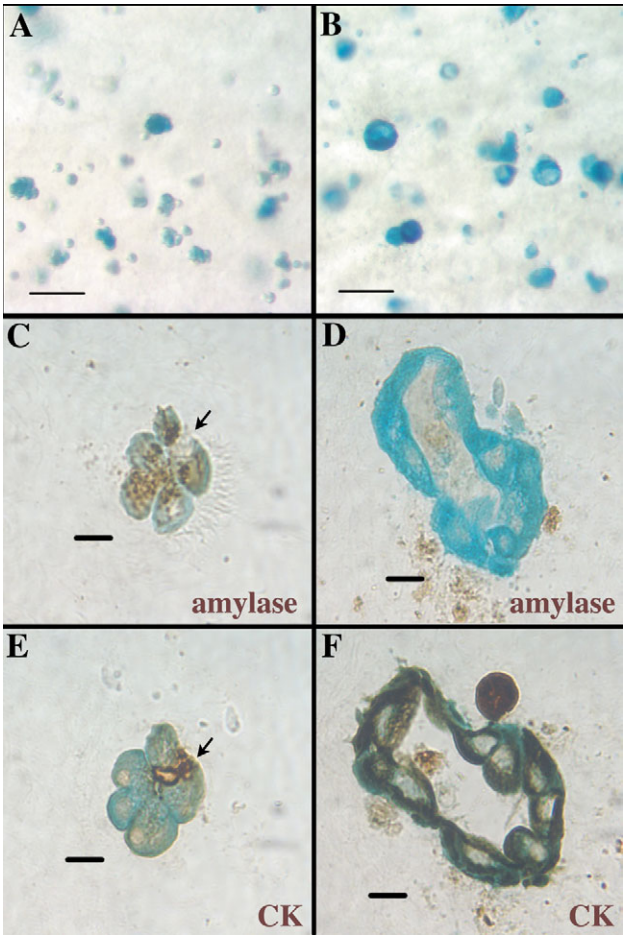

Fig. 3. Villin-Cre-based lineage tracing reveals that acinar cells transdifferentiate into ductal cells. Pancreatic epithelium from Villin-Cre;R26R mice was isolated and fixed immediately after plating (A,C,E) or after culture in the presence of TGFα for 5 days (B,D,F). On day 0, most cells (A) contained β-gal activity (blue) and were amylase positive (C) and cytokeratin negative (E), confirming that β-gal expression was confined to acinar cells. A small percentage of cells did not display β-gal activity, and most of these cells were positive for the ductal cytokeratins (arrows in C and E). Following 5 days of TGFα treatment, most cells were still β-gal positive (B). No intact amylase-positive cells were observed (D) and β-gal activity was present in cells expressing ductal cytokeratins (F), indicating that the β-gal-expressing acinar cells had transdifferentiated into ductal cells. Scale bars: 100 μm in A,B; 10 μm in C-F.

98.0±0.6% of β-gal-positive cells also staining positive for amylase (Fig. 3A,C). No β-gal activity was observed in cells expressing duct-specific keratins (Fig. 3E). Cytokeratin-positive ductal epithelial cells represented 15.0±2.8% of the initial cell population in freshly harvested explants, and probably represented terminal intercalated ductal epithelium based on characteristic squamous morphology and location in the center of acinar cell clusters. These results confirm our in vivo observation that Villin-Cre;R26R provides a genetic lineage label specific to acinar cells and their progeny.

After culturing Villin-Cre; R26R epithelial explants for 5 days in the presence of TGFα, cystic ductal epithelial structures formed that were positive for β-gal activity (Fig. 3B,D,F). Although most cells at the start of culture were amylase positive, we found no amylase expression by day 5 of culture. Rather, the majority of cells were found to express duct-specific keratins. The majority of these keratin-positive cells (88.4±2.3%) were also positive for β-gal staining, indicating an activated R26R allele. As the R26R allele could only be activated in Villin-Cre-expressing acinar cells, these data demonstrate that β-gal-positive ductal epithelium arose by transdifferentiation of acinar cells into ductal cells. (Fig. 3F).

Although Villin-Cre;R26R lineage tracing revealed that acinar cells could transdifferentiate into ductal cells, it was possible that the initially isolated epithelium contained small numbers of β-gal-positive, amylase-positive cells that were not fully differentiated and thus capable of changing their differentiation pathway. To assure that a small number of uncharacterized cells did not represent the source of metaplastic ductal epithelium, we examined the amount of proliferation occurring during the culture period. BrdU was added to the culture medium throughout the 5 days of culture, and explants were then assayed for the number of cells that had undergone proliferation, as determined by BrdU incorporation. During the 5 days of culture, only 10.3±3.5% of cells incorporated BrdU. Thus, it is unlikely that expansion of ductal

epithelium occurred by proliferation of a small population of uncharacterized cells. Rather, it appears that the majority of acinar cells are capable of undergoing transdifferentiation without obligate intervening cell division. Similar observations have recently been reported by Sphyris and colleagues (Sphyris et al., 2005).

Although acinar-to-ductal transdifferentiation was associated with infrequent cell proliferation, many acinar cells were also lost because of cell death. The extent of cell death varied widely from experiment to experiment, owing to differences in viability both at the time of harvest and during culture. Using Trypan Blue exclusion as an indicator of living cells, we found that 5.5-27.5% (mean 14.1±4.9%) of cells were dead (Trypan Blue positive) immediately following isolation. By 3 days of culture, dead cells accounted for 32-65% (mean 45.5±7.1) of total cells. Preliminary experiments involving immunostaining for cleaved caspase 3, a marker of apoptosis, indicated that the majority of this cell death was not apoptotic, suggesting necrosis as the predominant mechanism of cell death in this system (data not shown).

In order to further implicate mature acinar cells as the source of metaplastic epithelium, we performed additional experiments using an inducible Cre system, so that genetic labeling of acinar cells could be delayed until adulthood. For these experiments, acinar cells were genetically labeled using a tamoxifen-inducible Cre driven by the Elastase (Ela) promoter. Ela-CreERT2 transgenic mice express a fusion protein comprised of Cre recombinase and a modified estrogen receptor ligand binding domain (ERT2) under control of the acinar cell-selective elastase promoter (D.A.S., unpublished). Under normal conditions, this fusion CreERT2 is inactive, apparently owing to cytoplasmic sequestration by heat-shock proteins. However, upon tamoxifen binding, the CreERT2 fusion protein translocates to the nucleus, resulting in effective Cre activity. When Ela-CreERT2 transgenic mice were crossed with R26R mice (Soriano, 1999) no leaky *lacZ* expression was noted in the absence of tamoxifen induction (D.A.S., unpublished). Following intraperitoneal tamoxifen injection, pancreatic tissue from Ela-CreERT2; R26R mice demonstrated acinar cell-selective β-gal activity, with no activity observed in inter- or intra-lobular ducts, islets or stroma (D.A.S., unpublished; see Fig. S1 in the supplementary material). Activity in acinar cells was mosaic, inducing recombination of the R26R reporter in ~40% of acinar cells, suggesting that, under the conditions employed, tamoxifen was only able to induce Cre activity in a limited number of cells. Following a seven-day in vivo 'pulse' of tamoxifen and a subsequent seven-day 'chase' period to ensure complete tamoxifen clearance, epithelial explants were harvested and subjected to TGFα-induced acinar-to-ductal metaplasia followed by staining for β-gal activity. Consistent with observations made on intact tissue, freshly harvested epithelial explants from tamoxifen-treated mice displayed a mosaic distribution of β-gal activity restricted to acinar cells (Fig. 4A,B). When epithelial explants were isolated and cultured in the presence of TGFα, the duct-like epithelial cells that arose were found to stain with X-gal in a similar mosaic pattern (Fig. 4C-F), confirming the acinar cell origin of metaplastic ductal epithelium and identifying

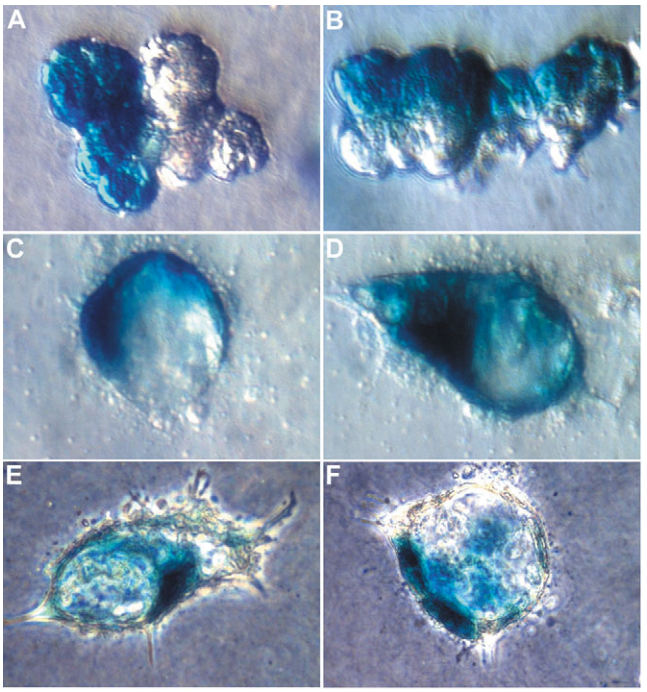

Fig. 4. Ela-CreERT2-based lineage tracing confirms acinar cell origin of metaplastic epithelium. (A,B) Phase-contrast images of X-gal stained Ela-CreERT2; R26R epithelium on initiation of explant culture, demonstrating mosaic expression of β-gal reporter in acinar cells. (C-F) Phase-contrast (C,D) and bright-field (E,F) images of X-gal stained Ela-CreERT2; R26R epithelium on day 4 of culture. β-Gal reporter marks acinar cell origin of metaplastic epithelium.

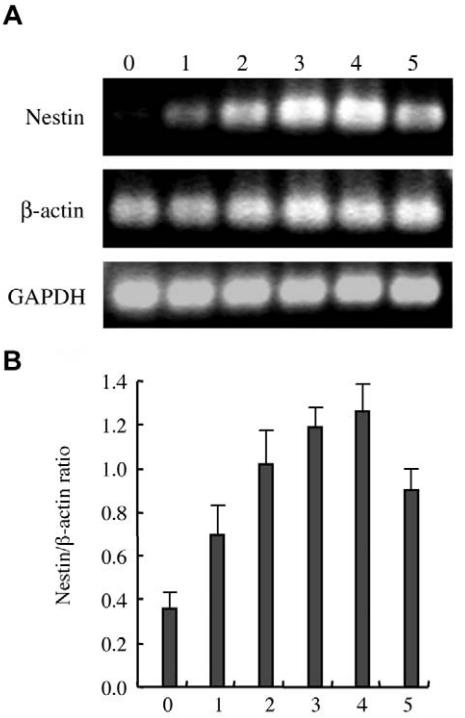

Fig. 5. Activation of nestin expression during TGFα-induced acinar-to-ductal metaplasia. (A) Semi-quantitative RT-PCR demonstrating induction of nestin expression relative to β-actin and GAPDH loading controls. Lane numbers indicate days in culture. (B) Quantification of RT-PCR results from three separate experiments. Values on *x*-axis indicate days in culture.

transdifferentiation as an active mechanism driving tissue metaplasia in this system.

Acinar cell transdifferentiation occurs via nestin-positive intermediates

Previous in vivo evaluations have suggested that metaplastic ductal epithelium shares features in common with precursor cells found in the embryonic pancreas (Miyamoto et al., 2003; Song et al., 1999). Based on the emerging role of nestin as a marker of precursor cells in developing exocrine pancreas (Esni et al., 2004; Delacour et al., 2004), we evaluated nestin expression at various stages of TGFα-induced acinar-to-ductal metaplasia. Using semi-quantitative RT-PCR, we detected little to no nestin expression in freshly harvested explant tissue (Fig. 5A,B). In the absence of TGFα, nestin transcripts remained undetectable throughout the culture period (data not shown). Following addition of TGFα, upregulated nestin expression became discernible within 24 hours, and continued to rise during progression of acinar-to-ductal metaplasia, with peak transcript levels detected on days 3 and 4 (Fig. 5A,B). In addition to detection of nestin transcripts, we also evaluated nestin protein production in conjunction with markers of acinar and ductal differentiation, using confocal microscopy. Consistent with the RT-PCR results, little to no nestin protein was detected by immunofluorescent staining of freshly harvested pancreatic explants using two different anti-nestin antibodies (Fig. 6A,D; data not shown). By contrast, nestin immunoreactivity was detected in a majority of cells following 2 days in culture with TGFα (Fig. 6B,E) and persisted at lower levels throughout the 5-day culture period (Fig. 6C,F; see also Fig. S2 in the supplementary material).

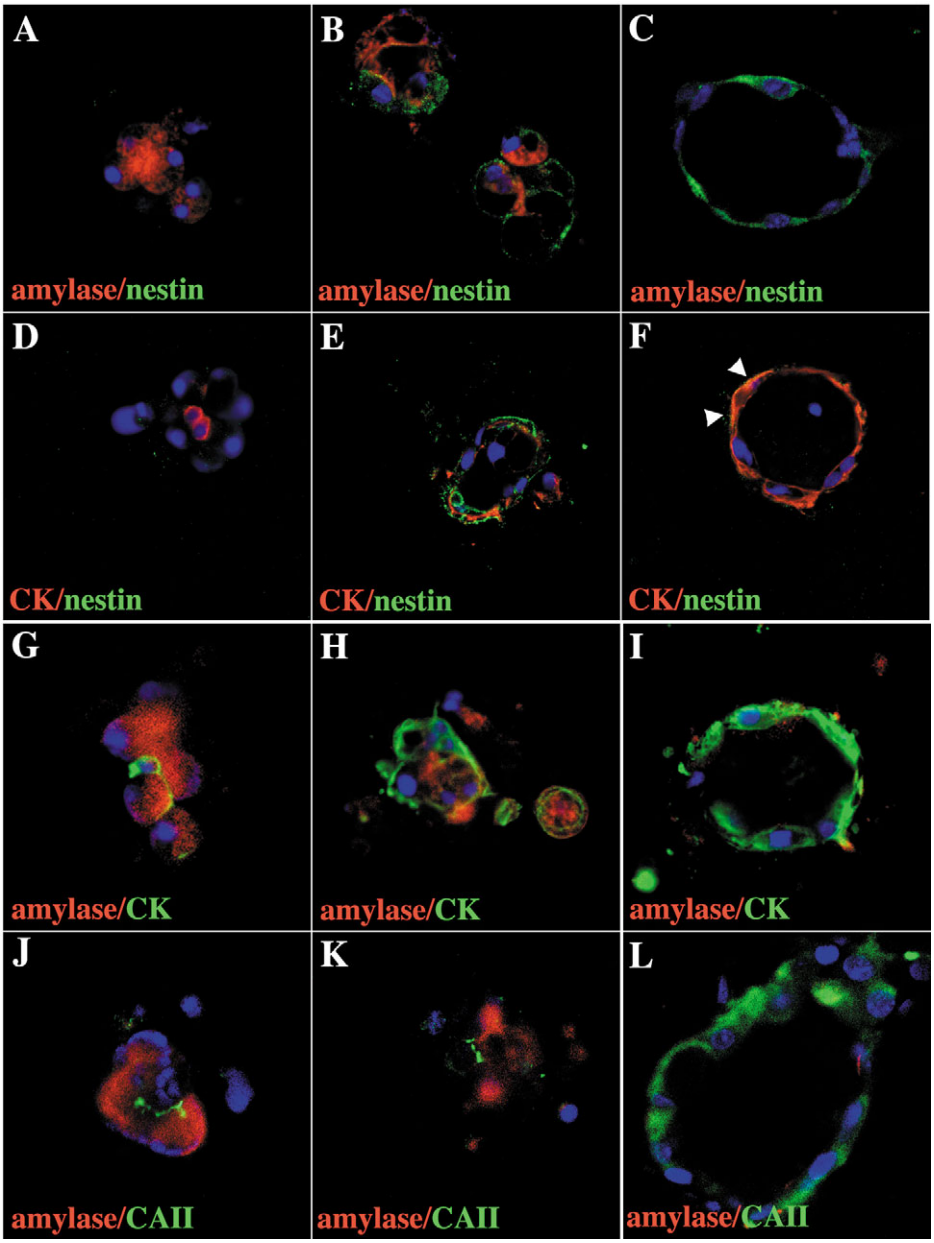

Fig. 6. TGFα-induced acinar-to-ductal metaplasia proceeds through nestin-positive intermediates. Images from day 0 (A,D,G,J), day 2 (B,E,H,K) and day 5 (C,F,I,L) TGFα-treated non-transgenic pancreatic explants, double immunolabeled for: (A-C) nestin (green) and amylase (red); (D-F) nestin (green) and ductal cytokeratins (red). Owing to high cytokeratin and low nestin expression, composite color is orange rather than yellow (arrowheads); unmerged images are provided in Fig. S2 in the supplementary material. (G-I) Ductal cytokeratins (green) and amylase (red); (J-L) carbonic anhydrase II (green) and amylase (red). Nuclei are labeled in blue. On day 0, there were no nestin-positive cells and no cells co-expressing acinar and ductal markers. Following only 2 days of culture, cells had reduced levels of amylase and were beginning to acquire ductal cytokeratins. Following 5 days of culture, amylase protein was no longer detected, and cells had acquired expression of both ductal cytokeratins and carbonic anhydrase II. All images are presented at identical magnifications.

The onset of nestin expression coincided with the dual expression of acinar-specific amylase and duct-specific cytokeratins. Reflecting in vivo expression patterns, amylase and cytokeratins were not co-expressed at the time of initial explant isolation (Fig. 6G). However, by day 2 of culture in TGFα, cell clusters that were beginning to form expanded lumena contained many cells that co-expressed amylase and ductal cytokeratins (Fig. 6H). By day 5 of culture, there were no remaining amylase-positive cells and most cells within cystic clusters expressed ductal cytokeratins (Fig. 6I). However, although ductal cytokeratins began to be expressed prior to the loss of amylase protein, another marker of ductal epithelium, carbonic anhydrase II, was not detected until after the loss of

amylase (Fig. 6J-L). These findings suggest that metaplastic conversion from an amylase-expressing, acinar cell-predominant population to a cytokeratin-expressing duct cell-predominant population occurs via nestin-positive intermediates that gradually lose amylase expression while progressively gaining cytokeratin and then carbonic anhydrase II expression.

Discussion

The results presented here identify differentiated acinar cells as cells of origin for metaplastic ductal epithelium in exocrine pancreas, and raise the possibility that transdifferentiation of mature cell types may represent a more general mechanism for initiating metaplasia/neoplasia sequences in other epithelial tissues. In previous studies of acinar-to-ductal metaplasia (De Lisle and Logsdon, 1990; Githens et al., 1994; Rooman et al., 2000), the acinar cell origin of metaplastic ductal epithelium was proposed based on the high proportion of acinar cells in the initial cultures and/or on the low amount of proliferation observed during acinar-to-ductal conversion. However, given the close juxtaposition of intercalated ducts to acini and the observed cell death of many acinar cells during culture, the possibility that metaplastic ductal epithelium arose from contaminating intercalated ducts could not be excluded. Through the use of genetic lineage labeling and molecular identification of both precursor and progeny cells carrying that label, our studies provide compelling evidence that metaplastic ductal epithelium is generated by transdifferentiation of mature acinar cells.

This conclusion is further supported by our analysis of cell death and cell proliferation. Some 90% of emerging ductal elements failed to incorporate BrdU during the 5-day culture period, suggesting that expansion of this ductal epithelium did not require cell proliferation. Although we did see extensive cell death, common to most primary cell cultures, the most viable cell isolates had only one-third of cells dying by day 3 of culture, a time at which loss of acinar identity and acquisition of a ductal phenotype are already observed. Owing to the significant extent of cell death observed during acinar-to-ductal metaplasia, it is possible that not all acinar cells have the capacity to transdifferentiate. However, the ability of 35-67% of cells to survive and undergo ductal differentiation suggests that a rather large subset of acinar cells carry this capability.

Transdifferentiation in other tissues

During development, undifferentiated, tissue-specific progenitor cells retain considerable plasticity, as evidenced by multiple examples of transdetermination, in which local tissue-specific progenitors undergo reprogramming to generate a variety of alternate cell fates. In developing mouse pancreas, transdetermination from a pancreatic to an intestinal cell fate has been demonstrated by following the fate of presumptive pancreatic progenitors in the presence and absence of the bHLH transcription factor, Ptf1a/p48. These studies have demonstrated that, in the absence of functional Ptf1a/p48 protein, cells normally fated to become pancreas instead become mature duodenal cells (Kawaguchi et al., 2002). Similary, *Drosophila* imaginal discs and avian limb buds also appear to be characterized by a considerable capacity for transdetermination (Maves and Schubiger, 1999; Takeuchi et al., 1999).

In contrast to these examples of plasticity involving undifferentiated cell types, transdifferentiation represents the conversion of one fully differentiated cell type to another (Shen et al., 2003). To date, rigorously documented examples of transdifferentiation occurring in the context of adult epithelium have largely been confined to amphibian species, with lens regeneration by retinal pigmented epithelial cells representing the most frequently cited example (reviewed by Eguchi and Kodama, 1993). Transdifferentiation is also apparent during appendage regeneration in urodele amphibians, in which tissue within the wound blastema undergoes dedifferentiation followed by multiple rounds of cell division, with subsequent redifferentiation along a variety of lineage pathways. For example, labeling of individual radial glial cells following tail amputation in axolotl salamanders has demonstrated transdifferentiation of these ectodermally derived cells to generate new muscle and cartilage (Echeverri and Tanaka, 2002).

Although frequently reported, instances of transdifferentiation in mammalian systems are more difficult to evaluate. These examples frequently involve changes in differentiation of clonal cell lines in response to a variety of genetic and epigenetic influences. In these systems, transdifferentiation has typically been defined by clonal dilution or individual examination of isolated cells undergoing biochemical and morphological conversion to a different cell type. For example, mouse myoblasts have been reported to transdifferentiate into adipocytes (Hu et al., 1995), murine melanoma cells to glial cells (Slutsky et al., 2003) and rat acinar cell carcinoma cells to hepatocytes (Shen et al., 2003; Shen et al., 2000). However, it remains unclear how well these single cell observations faithfully recapitulate events occurring in an intact epithelium, and it is notable that each of these examples appears to involve input cells with a heightened progenitor potential. In the case of mouse myoblasts, the observed phenomenon might better be described as transdetermination of a tissue-specific precursor. Similarly, the observation of apparent transdifferentiation in mouse melanoma and rat acinar cell carcinoma cells might simply reflect heightened plasticity associated with the malignant phenotype.

Acinar cell transdifferentiation as the mechanism for acinar-to-ductal metaplasia

In the current study, we have shown that fully differentiated exocrine cells in adult mouse pancreas are capable of undergoing transdifferentiation, and that this transdifferentiation event represents the cellular mechanism for induction of acinar-to-ductal metaplasia. We have used morphological and molecular characterization as well as genetic labeling to clearly define a direct lineage relationship between acinar cell precursors and their ductal progeny. We have shown that this genetic lineage label is initially expressed exclusively by fully differentiated acinar cells, as judged both by columnar acinar morphology and by co-labeling with two molecular markers, amylase and carboxypeptidase A (Fig. 3; data not shown). We have also rigorously defined a ductal epithelial phenotype within the final population of β-gal-labeled cells, based on classical cuboidal or simple squamous morphology

Development

and expression of both ductal cytokeratins and carbonic anhydrase II. The direct lineage relationship between these cell types was clearly established by tracing heritable β-gal activity arising from acinar cell-specific recombination of the R26R reporter allele, accomplished by both the Villin-Cre and Elastase-CreERT2 transgenes. Thus, we can firmly conclude that mature acinar cells can transdifferentiate to form metaplastic ductal epithelium. We further conclude, based on an observed low frequency of cellular proliferation, that this transdifferentiation does not require intervening cell division. Based on previously established criteria (Eguchi and Kodama, 1993; Shen et al., 2003), we conclude that the current results represent a unique example of rigorously documented transdifferentiation occurring in mature mammalian epithelium.

Acinar-to-ductal transdifferentiation occurs via a dedifferentiated intermediate cell type

During lens or tail regeneration in amphibia, transdifferentiation typically proceeds by way of dedifferentiated intermediates (Echeverri and Tanaka, 2002; Eguchi and Kodama, 1993). In the current study, the detection of nestin expression in intermediary cells suggests that acinar cell transdifferentiation may involve a similar undifferentiated intermediate. Although the use of nestin as a label for undifferentiated pancreatic epithelial progenitors remains controversial, recent studies have confirmed that nestin-expressing epithelial cells are indeed present during early pancreatic development, and that these cells represent the cell of origin for differentiated exocrine cells (Delacour et al., 2004; Esni et al., 2004). Moreover, it appears that EGF receptor activation in developing mouse pancreas acts to maintain these undifferentiated nestin-positive precursors at the expense of differentiated acinar cells (Esni et al., 2004). The ability of TGFα to reactivate nestin expression in mature amylase-positive acinar cells may therefore represent an adult recapitulation of these embryonic events. In any case, the re-emergence of nestin expression in mature acinar cells suggests that fully differentiated pancreatic epithelial cells may act as latent or facultative precursors. However, unlike transdifferentiation in urodeles, we did not detect completely undifferentiated intermediate cell types in our system. Rather, transdifferentiating acinar cells displayed concomitant expression of nestin and acquisition of a ductal marker (cytokeratins) before the complete loss of acinar markers. Although the retention or non-retention of multiple lineage markers may simply be a function of marker protein stability, the rapid progression and relative lack of proliferation in our system further distinguish this form of transdifferentiation from that observed during urodele tail regeneration (Echeverri and Tanaka, 2002).

Recently, it has been reported that human β-cells are capable of generating new β-cells through a process of epithelial-to-mesenchymal transition (Gershengorn et al., 2004). This event apparently involves β-cell dedifferentiation to generate nestin-positive islet precursor cells displaying mesenchymal features. Under appropriate conditions, these precursors subsequently redifferentiate, producing new β-cells. In our system, however, nestin-positive cells retained expression of epithelial markers, and maintained an organized epithelial architecture. Although acinar cell transdifferentiation therefore cannot be considered a formal example of epithelial-mesenchymal transition, we cannot entirely exclude the possibility that a transient mesenchymal state is also present in our system.

Acinar cell transdifferentiation and the presence or absence of dedicated stem cells in adult pancreas

Rather than relying exclusively on a dedicated precursor population, adult pancreatic tissue appears capable of recruiting differentiated cell types as a source of novel and/or replacement cells. In the case of endocrine pancreas, recent Cre/lox-based lineage tracing studies have suggested that, both in the course of normal renewal as well as during accelerated islet neogenesis following partial pancreatectomy, new β-cells are generated from a pool of pre-existing, fully differentiated cells defined by the ability to express an insulin-CreER transgene (Dor et al., 2004). The current data support a similar ability for differentiated acinar cells to assume a precursor function in exocrine pancreas. Although the mechanism for generation of metaplastic ductal epithelium may certainly differ from the mechanisms employed for renewal of normal ductal epithelium, the results suggest that fully differentiated acinar cells retain a latent precursor potential. This model is consistent with the view that precursor activity may not necessarily be limited to a discrete population of undifferentiated, pluripotent cells within a given tissue, but rather might be considered an inducible biologic function of fully differentiated cells (Blau et al., 2001; Shen et al., 2000).

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Supplementary material

Supplementary material for this article is available at
http://dev.biologists.org/cgi/content/full/132/16/3767/DC1

References

Blau, H. M., Brazelton, T. R. and Weimann, J. M. (2001). The evolving concept of a stem cell: entity or function? *Cell* **105**, 829-841.

Brinkmann, V., Foroutan, H., Sachs, M., Weidner, K. M. and Birchmeier, W. (1995). Hepatocyte growth factor/scatter factor induces a variety of tissue-specific morphogenic programs in epithelial cells. *J. Cell Biol.* **131**, 1573-1586.

De Lisle, R. C. and Logsdon, C. D. (1990). Pancreatic acinar cells in culture: expression of acinar and ductal antigens in a growth-related manner. *Eur. J. Cell Biol.* **51**, 64-75.

Delacour, A., Nepote, V., Trumpp, A. and Herrera, P. L. (2004). Nestin expression in pancreatic exocrine cell lineages. *Mech. Dev.* **121**, 3-14.

Discafani, C. M., Carroll, M. L., Floyd, M. B., Jr, Hollander, I. J., Husain, Z., Johnson, B. D., Kitchen, D., May, M. K., Malo, M. S., Minnick, A. A., Jr et al. (1999). Irreversible inhibition of epidermal growth factor receptor tyrosine kinase with in vivo activity by N-[4-[(3-bromophenyl)amino]-6-quinazolinyl]-2-butynamide (CL-387,785). *Biochem. Pharmacol.* **57**, 917-925.

Dor, Y., Brown, J., Martinez, O. I. and Melton, D. A. (2004). Adult pancreatic beta-cells are formed by self-duplication rather than stem-cell differentiation. *Nature* **429**, 41-46.

Echeverri, K. and Tanaka, E. M. (2002). Ectoderm to mesoderm lineage switching during axolotl tail regeneration. *Science* **298**, 1993-1996.
Eguchi, G. and Kodama, R. (1993). Transdifferentiation. *Curr. Opin. Cell Biol.* **5**, 1023-1028.
el Marjou, F., Janssen, K. P., Chang, B. H., Li, M., Hindie, V., Chan, L., Louvard, D., Chambon, P., Metzger, D. and Robine, S. (2004). Tissue-specific and inducible Cre-mediated recombination in the gut epithelium. *Genesis* **39**, 186-193.
Esni, F., Stoffers, D. A., Takeuchi, T. and Leach, S. D. (2004). Origin of exocrine pancreatic cells from nestin-positive precursors in developing mouse pancreas. *Mech. Dev.* **121**, 15-25.
Gershengorn, M. C., Hardikar, A. A., Wei, C., Geras-Raaka, E., Marcus-Samuels, B. and Raaka, B. M. (2004). Epithelial-to-mesenchymal transition generates proliferative human islet precursor cells. *Science* **306**, 2261-2264.
Githens, S., Schexnayder, J. A., Moses, R. L., Denning, G. M., Smith, J. J. and Frazier, M. L. (1994). Mouse pancreatic acinar/ductular tissue gives rise to epithelial cultures that are morphologically, biochemically, and functionally indistinguishable from interlobular duct cell cultures. *In Vitro Cell Dev. Biol. Anim.* **30A**, 622-635.
Gu, G., Dubauskaite, J. and Melton, D. A. (2002). Direct evidence for the pancreatic lineage: NGN3+ cells are islet progenitors and are distinct from duct progenitors. *Development* **129**, 2447-2457.
Hu, E., Tontonoz, P. and Spiegelman, B. M. (1995). Transdifferentiation of myoblasts by the adipogenic transcription factors PPAR gamma and C/EBP alpha. *Proc. Natl. Acad. Sci. USA* **92**, 9856-9860.
Kawaguchi, Y., Cooper, B., Gannon, M., Ray, M., MacDonald, R. J. and Wright, C. V. (2002). The role of the transcriptional regulator Ptf1a in converting intestinal to pancreatic progenitors. *Nat. Genet.* **32**, 128-134.
Lin, R. Z., Hu, Z. W., Chin, J. H. and Hoffman, B. B. (1997). Heat shock activates c-Src tyrosine kinases and phosphatidylinositol 3-kinase in NIH3T3 fibroblasts. *J. Biol. Chem.* **272**, 31196-31202.
Lowenfels, A. B., Maisonneuve, P. and Whitcomb, D. C. (2000). Risk factors for cancer in hereditary pancreatitis. International Hereditary Pancreatitis Study Group. *Med. Clin. North Am.* **84**, 565-573.
Maves, L. and Schubiger, G. (1999). Cell determination and transdetermination in Drosophila imaginal discs. *Curr. Top. Dev. Biol.* **43**, 115-151.
Means, A. L., Ray, K. C., Singh, A. B., Washington, M. K., Whitehead, R. H., Harris, R. C., Jr, Wright, C. V., Coffey, R. J., Jr and Leach, S. D. (2003). Overexpression of heparin-binding EGF-like growth factor in mouse pancreas results in fibrosis and epithelial metaplasia. *Gastroenterology* **124**, 1020-1036.
Miyamoto, Y., Maitra, A., Ghosh, B., Zechner, U., Argani, P., Iacobuzio-Donahue, C. A., Sriuranpong, V., Iso, T., Meszoely, I. M., Wolfe, M. S. et al. (2003). Notch mediates TGFalpha-induced changes in epithelial differentiation during pancreatic tumorigenesis. *Cancer Cell* **3**, 565-576.
Parsa, I., Longnecker, D. S., Scarpelli, D. G., Pour, P., Reddy, J. K. and Lefkowitz, M. (1985). Ductal metaplasia of human exocrine pancreas and its association with carcinoma. *Cancer Res.* **45**, 1285-1290.
Postic, C., Shiota, M., Niswender, K. D., Jetton, T. L., Chen, Y., Moates, J. M., Shelton, K. D., Lindner, J., Cherrington, A. D. and Magnuson, M. A. (1999). Dual roles for glucokinase in glucose homeostasis as determined by liver and pancreatic beta cell-specific gene knock-outs using Cre recombinase. *J. Biol. Chem.* **274**, 305-315.
Prenzel, N., Zwick, E., Daub, H., Leserer, M., Abraham, R., Wallasch, C. and Ullrich, A. (1999). EGF receptor transactivation by G-protein-coupled receptors requires metalloproteinase cleavage of proHB-EGF. *Nature* **402**, 884-888.
Rooman, I., Heremans, Y., Heimberg, H. and Bouwens, L. (2000). Modulation of rat pancreatic acinoductal transdifferentiation and expression of PDX-1 in vitro. *Diabetologia* **43**, 907-914.
Sandgren, E. P., Luetteke, N. C., Palmiter, R. D., Brinster, R. L. and Lee, D. C. (1990). Overexpression of TGF alpha in transgenic mice: induction of epithelial hyperplasia, pancreatic metaplasia, and carcinoma of the breast. *Cell* **61**, 1121-1135.
Shen, C. N., Slack, J. M. and Tosh, D. (2000). Molecular basis of transdifferentiation of pancreas to liver. *Nat. Cell Biol.* **2**, 879-887.
Shen, C. N., Horb, M. E., Slack, J. M. and Tosh, D. (2003). Transdifferentiation of pancreas to liver. *Mech. Dev.* **120**, 107-116.
Slutsky, S. G., Kamaraju, A. K., Levy, A. M., Chebath, J. and Revel, M. (2003). Activation of myelin genes during transdifferentiation from melanoma to glial cell phenotype. *J. Biol. Chem.* **278**, 8960-8968.
Song, S. Y., Gannon, M., Washington, M. K., Scoggins, C. R., Meszoely, I. M., Goldenring, J. R., Marino, C. R., Sandgren, E. P., Coffey, R. J., Jr, Wright, C. V. et al. (1999). Expansion of Pdx1-expressing pancreatic epithelium and islet neogenesis in transgenic mice overexpressing transforming growth factor alpha. *Gastroenterology* **117**, 1416-1426.
Soriano, P. (1999). Generalized lacZ expression with the ROSA26 Cre reporter strain. *Nat. Genet.* **21**, 70-71.
Sphyris, N., Logsdon, C. D. and Harrison, D. J. (2005). Improved retention of zymogen granules in cultured murine pancreatic acinar cells and induction of acinar-ductal transdifferentiation in vitro. *Pancreas* **30**, 148-157.
Suzuki, A., Nakauchi, H. and Taniguchi, H. (2004). Prospective isolation of multipotent pancreatic progenitors using flow-cytometric cell sorting. *Diabetes* **53**, 2143-2152.
Takeuchi, J. K., Koshiba-Takeuchi, K., Matsumoto, K., Vogel-Hopker, A., Naitoh-Matsuo, M., Ogura, K., Takahashi, N., Yasuda, K. and Ogura, T. (1999). Tbx5 and Tbx4 genes determine the wing/leg identity of limb buds. *Nature* **398**, 810-814.
Tosh, D. and Slack, J. M. (2002). How cells change their phenotype. *Nat. Rev. Mol. Cell Biol.* **3**, 187-194.
Uchiyama-Tanaka, Y., Matsubara, H., Mori, Y., Kosaki, A., Kishimoto, N., Amano, K., Higashiyama, S. and Iwasaka, T. (2002). Involvement of HB-EGF and EGF receptor transactivation in TGF-beta-mediated fibronectin expression in mesangial cells. *Kidney Int.* **62**, 799-808.
Wagner, M., Luhrs, H., Kloppel, G., Adler, G. and Schmid, R. M. (1998). Malignant transformation of duct-like cells originating from acini in transforming growth factor transgenic mice. *Gastroenterology* **115**, 1254-1262.
Wagner, M., Greten, F. R., Weber, C. K., Koschnick, S., Mattfeldt, T., Deppert, W., Kern, H., Adler, G. and Schmid, R. M. (2001). A murine tumor progression model for pancreatic cancer recapitulating the genetic alterations of the human disease. *Genes Dev.* **15**, 286-293.
Wagner, M., Weber, C. K., Bressau, F., Greten, F. R., Stagge, V., Ebert, M., Leach, S. D., Adler, G. and Schmid, R. M. (2002). Transgenic overexpression of amphiregulin induces a mitogenic response selectively in pancreatic duct cells. *Gastroenterology* **122**, 1898-1912.