REPROGRAMMING OF HUMAN PANCREATIC EXOCRINE CELLS TO BETA-LIKE CELLS

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**ABSTRACT**

Rodent acinar cells exhibit a remarkable plasticity as they can transdifferentiate to duct-like cells, hepatocyte-like cells and islet beta-like cells. We evaluated whether exocrine cells from adult human pancreas can similarly respond to pro-endocrine stimuli. Exocrine cells from adult human pancreas were transduced directly with lentiviruses expressing activated MAPK and STAT3 and cultured as monolayers or as 3D structures. Expression of STAT3 and MAPK in human exocrine cells activated expression of the pro-endocrine factor Neurogenin3 in 50 to 80% of transduced exocrine cells. However, the number of insulin-positive cells increased only in the exocrine cells grown initially in suspension prior to 3D culture. Lineage tracing identified human acinar cells as the source of Ngn3- and insulin-expressing cells. Long-term engraftment into immune-compromised mice increased the efficiency of reprogramming to insulin-positive cells. Our data demonstrate that exocrine cells from human pancreas can be reprogrammed to transplantable insulin-producing cells that acquire functionality. Given the large number of exocrine cells in a donor pancreas, this approach presents a novel strategy to expand cell therapy in type 1 diabetes.

**KEYWORDS:** acinar cells/beta cells/human pancreas/reprogramming/diabetes

**Abbreviations:** KRT19, Cytokeratin 19; CHYMO, Chymotrypsin; GCG, Glucagon; ICC, immunocytochemistry; IHC, immunohistochemistry; INS, Insulin; PTF1A, pancreas-specific transcription factor 1a; PDX1, pancreatic and duodenal homeobox 1; NEUROG3, Neurogenin 3; MAPK, mitogen-activated protein kinase; STAT3, signal transducer and activator of transcription 3; GFP, green fluorescent protein; CELA2A, chymotrypsin-like elastase 2A.
INTRODUCTION

To overcome the scarcity of endocrine beta cells for cell replacement therapy in patients with type 1 diabetes, additional sources of transplantable beta cells are needed. Reprogramming of non-endocrine pancreatic cells into beta cells offers one attractive approach. Exocrine cells comprise the vast majority of cells obtained from cadaveric donor pancreases, but are discarded following isolation of the endocrine islets, and thus could provide a large pool of cells for conversion to beta cells.

Historically pancreatic duct cells have been favored as potential source of new islet beta cells due to histological observations in the developing and adult human pancreas showing close association of duct and endocrine cells (1, 2) with cells detected expressing both duct and beta cell markers. Purification of human duct cells based on CA19.9 expression and subsequent 3D in vitro culture was shown to yield a limited number of insulin+ cells with an immature glucose-induced insulin response (3, 4). These reports have been contested later on suggesting that dedifferentiated islet beta cells may have been the source of these new insulin+ cells (5), leaving the differentiation potential of human exocrine duct cells currently unanswered.

Pancreatic acinar cells represent an alternative attractive population for exocrine-to-endocrine transdifferentiation due to their abundance and potential for plasticity. Rodent pancreatic acinar cells are shown to exhibit phenotypic instability in vitro and undergo a spontaneous ductal metaplasia following isolation (6, 7). These metaplastic acinar cells can adopt a duct-like (6, 8), hepatocyte-like (9) and beta-like phenotype (8, 10-12), depending on the stimuli provided. In contrast, similar plasticity has not been demonstrated for human pancreatic acinar cells, although they can undergo spontaneous metaplasia to duct-like cells in vitro (13) similar to what is observed in rodents. We previously showed that supplementation of the medium of cultured rat acinar cells with EGF and LIF (8, 10, 14) and triggering MAPK and STAT3 signal transduction (14) converts them to beta-like cells. Therefore we hypothesized that ectopic signaling through MAPK and STAT3 might convert human acinar cells to beta-like cells as well. The current study shows that ectopic expression of activated MAPK and STAT3 in human pancreatic acinar cells activates the pro-endocrine transcription factor Neurog3 and reprograms human acinar cells to insulin-positive beta-like cells able to ameliorate chemical diabetes.
**Materials & Methods**

**Human exocrine cells**

Ethical approval to use exocrine-enriched cells derived from donor organs was given by the Medical Ethical Committee of the University Hospital of the Vrije Universiteit Brussel (O.G. 016) to the Beta Cell Bank-University Hospital Brussels (permission 2010/193). The human exocrine fraction was obtained from heart-beating cadaveric non-diabetic donors as the discarded fraction from islet cell isolation for the purpose of clinical transplantation in type-1 diabetes patients. Human donors aged between 18 and 67 years (median age = 51 years) and had a male-to-female ratio of 1.3 (n=16). In the exocrine fraction, starting preparations contain approximately 60% acinar, 35% duct, 1-3% endocrine and 1-2% mesenchymal cells. After a culture period of at least 4 days, these preparations contain 1.5% of cells expressing endocrine cell markers and 90% expressing the duct cell-specific phenotypic markers Krt19 and carbohydrate antigen 19.9 (15).

**Animals**

All animal experimentation was performed in agreement with the regulations approved by the ethical committee of the Free University of Brussels. Eight-week old NOD.CB17-Prkdc<sup>scid</sup>/NCrCrl or C.B-17/IcrHsd-Prkdcs<sup>cid</sup>Lystbg-J mice and BALB/cAnNCrl-nuBR nude mice (Charles River Laboratories, L’Arberesle Cedex, France) weighing 22-28 g were used as recipients for transplantation.

**Lineage tracing**

Acinar lineage tracing was achieved by the combination of an adenovirus expressing Cre recombinase under control of a 550kb human pancreatic elastase 2A promoter fragment (Ad-Cela2A-Cre) and a lentivirus expressing either DsRed or LacZ preceded by a stop sequence flanked by loxP sites, under control of the constitutive active CMV promoter (Le-CMV-LSL-DsRed/LacZ). This allows for indefinite labeling of acinar cells.

**Experimental model**

Beta cell neogenesis was induced in exocrine cell cultures after a differentiation period of 7 days monolayer culture, 7 days 3D matrix culture or a sequential period of 10 days suspension followed by 7 days 3D matrix. The matrix constitutes undiluted Matrigel Matrix Growth Factor Reduced (Matrigel GFR, BD Biosciences). Human exocrine cells were transduced directly after isolation with lentiviruses expressing...
activated MAPK and STAT3. The cells were cultured in RPMI1640 medium supplemented with 1%FBS (Life Technologies).

**Reverse transcription polymerase chain reaction**

Total RNA was isolated using the RNeasy Mini Kit (Qiagen, 74106) and was transcribed and amplified as described by the manufacturer using blanks in each assay.

Quantitative polymerase chain reaction was performed using TaqMan Fast Universal PCR Master Mix (Applied Biosystems, 4364103) with selected primers (table1). Two replicate reactions were used for every sample. In addition, a positive control was used and all expression levels were normalized to the house-keeping gene PPIA (Peptidylpropyl isomerase A or Cyclophilin-A)(16). Nuclease-free water was run as negative control.

**Viral constructs**

Constitutive active STAT3 was generated by site-directed mutagenesis using the primer 5'-GCTATAAGATCATGGATTGTACCTGCATCCTGGTGTCTCC (kind gift from J.E. Darnell) (17). The Stat3<sup>CA</sup> was ligated in a SpeI/XmaI digested pTrip-CMV-eGFP-Nhelpoly-invPGK vector generating pTrip-CMV-eGFP-Nhelpoly-invPGK-STAT3<sup>CA</sup>. From the pTrip plasmid a lentivirus production is started following the described protocol.

Constitutive active Mapk1 was generated by constructing a fusion between native ERK2 and MEK1, and subsequential mutation of 4 leucine residues in the export signal region to alanine, generating a hyper-activated MAPK1. The pG5E4D38-CMV5-MAPK<sup>CA</sup> vector is a kind gift from M.H. Cobb (18). CMV-MAPK<sup>CA</sup> was transferred to a pTrip vector by SalI/NheI ligation, generating a pTrip-CMV-MAPK<sup>CA</sup>–ires-eGFP vector, which was used for lentivirus production.

Combining both single vectors to a single pTrip-CMV-MAPK<sup>CA</sup>–ires-eGFP-Nhelpoly-invPGK- STAT3<sup>CA</sup> vector, generated a bicistronic vector expressing both MAPK<sup>CA</sup> and STAT3<sup>CA</sup>.

**Immunostaining**

Immunocytochemistry was performed in 24-well plates. Immunohistochemistry was performed on paraffin sections (15). The different antibodies used can be found in table2.

**Microscopy**

All images were acquired with a Zeiss LSM710 NLO TiSa multiphoton confocal
microscope using Zeiss Zen2011 software (Carl Zeiss NV-SA, Zaventem, Belgium). All pictures were analyzed with VolocityLE software (Improvision, Coventry, UK).

**Transplantation**

Cells were detached using 0,25% Collagenase-V (Sigma, 9001-12-1) for the monolayers. After exposing the left kidney, a small incision was made in the kidney capsule. The cells were collected in a catheter and delivered under the kidney capsule using a microdispenser pipet (Mitutoyo). An average of 300,000 human exocrine cells was engrafted under the kidney capsule. Per human donor 3 mice were engrafted and 6 independent donors were used for transplantation.

**Metabolic studies**

Blood glucose levels were monitored in tail vein samples (Glucocard Memory Strips; A.Menarini Diagnostics Benelux, Zaventem, Belgium). Mice were fasted during 6 hours and injected intraperitoneally (i.p.) with glucose (2 g per kg body weight) for glucose tolerance tests, and blood glucose concentration was measured from tail vein blood with a portable glucometer. Plasma c-peptide concentration was determined with the Human C-peptide ELISA kit (Millipore). For GSIS analysis, pancreatic islets were isolated by collagenase digestion, handpicked and pooled. Secreted insulin levels were determined at low (2h at 2.5 mM Glucose) and high (2h at 20 mM Glucose) concentrations. The level was determined with the Human Insulin ELISA kit (Millipore).

**Statistical analysis**

Graphpad Prism version 5.0b was used to create the graphs and perform the statistics (Graphpad Prism, La Jolla, CA). Results compared to their control set at 1 were analyzed using a one-sample Student t-test. When two treatment groups were compared a 2-tailed Student t-test was used. Mean values are presented as the mean ± standard error of the mean (SEM). The number of independent experiments is indicated in the text. N-values represent independent human donors.
RESULTS

Overexpression of MAPK<sup>CA</sup> and STAT3<sup>CA</sup> activates an endocrine differentiation program in monolayers of cultured exocrine cells.

Pancreatic cell populations consisting of acinar, centroacinar, duct, and few endocrine/mesenchymal cells from human donor pancreata were transduced with LeMS<sup>CA</sup>, a bicistronic lentivirus constitutively overexpressing active MAPK and STAT3, and subsequently cultured as adherent monolayers (Figure 1A). In the LeMS<sup>CA</sup>-infected cell cultures, levels of NEUROG3, PAX4 and NKX2.2, mRNAs encoding transcription factors functioning in endocrine progenitor cells, were increased compared to control-transduced cells (LeGFP) (Figure 2A, protocol 1). NKX6.1 mRNA, however, significantly decreased and INS mRNA remained similar to control. At a transduction efficiency of 48.1±2.1% (n=4), Ngn3 protein was detected in 38.3±1.4% (n=4) of transfected exocrine cells (Figure 2D). A small fraction of the Ngn3<sup>+</sup>cells co-expressed Pdx1 protein (7.6±0.8%; n=4), but insulin<sup>+</sup> cells were not increased (Figure 2E). All Ngn3<sup>+</sup> and Pdx1<sup>+</sup> cells analysed co-expressed GFP, indicating the presence of LeMS<sup>CA</sup> virus. In control LeGFP-transduced cells no Ngn3 or Pdx1 expression was detected (Figure S1A,B).

LeMS<sup>CA</sup>-transduced cells also contained higher levels of mRNA encoding acinar cell-specific markers relative to LeGFP-transduced cells (Figure 2B, protocol 1), despite similar marked decreases in phenotypically stable acinar cells in both cultures (Figure S1C). In LeMS<sup>CA</sup> cells, the levels of ductal mRNAs FOXA2 and SOX9 were significantly decreased (Figure 2C, protocol 1), while expression of ONECUT1, a previously described regulator of Neurog3 expression in rodents (19), was increased (Figure 2C, protocol 1). All cells, independent of their state of transduction, expressed the duct markers Krt19 and Sox9 by immunostaining, but no acinar cell-specific proteins (Figure S1D).

In an attempt to increase endocrine differentiation, we examined the effect of overexpressing either MAPK<sup>CA</sup> (M<sup>CA</sup>) or STAT3<sup>CA</sup> (S<sup>CA</sup>) alone prior to the combination of MAPK<sup>CA</sup> + STAT3<sup>CA</sup> (MS<sup>CA</sup>). Three days of STAT3<sup>CA</sup> followed by 7 days of MS<sup>CA</sup> (LeS<sup>CA</sup><sub>3d</sub>MS<sup>CA</sup><sub>7d</sub>) did not further increase Ngn3 expression and lowered Pdx1 expression as compared to LeMS<sup>CA</sup> only (Figure S2A). However, overexpression of MAPK<sup>CA</sup> prior to MS<sup>CA</sup> (LeM<sup>CA</sup><sub>3d</sub>MS<sup>CA</sup><sub>7d</sub>) markedly increased endocrine gene
expression (Figure 1B and 2A, protocol 2). Compared to LeMS\textsuperscript{CA} cells, (Figure 2A protocol 1 and 2) the amount of \textit{NKX6.1}, \textit{PDX1} and \textit{INS} mRNA significantly increased in LeM\textsuperscript{CA}_{3d}MS\textsuperscript{CA}_{7d}, suggestive of on-going beta cell differentiation (n=9; p<0.01). While the number of Ngn3\textsuperscript{+} cells remained similar (39.9±2.4% vs 38.3±1.4% in LeMS\textsuperscript{CA} cells) (Figure 1D-F), the number of LeM\textsuperscript{CA}_{3d}MS\textsuperscript{CA}_{7d} cells with Pdx1 protein was increased (28.0±2.5% vs. 7.6±0.8% LeMS\textsuperscript{CA} cells; Figure 2E-F), indicating that pre-treatment with MAPK\textsuperscript{CA} did not hamper activation of a pro-endocrine program. However, despite the presence of high endogenous Pdx1, the number of hormone-producing cells did not increase (Figure 2D).

Expression level of acinar cell-specific mRNAs was comparable in LeMS\textsuperscript{CA} and LeM\textsuperscript{CA}_{3d}MS\textsuperscript{CA}_{7d} cells (Figure 2B). The expression of ONECUT1 mRNA was markedly increased in LeM\textsuperscript{CA}_{3d}MS\textsuperscript{CA}_{7d} cells while FOXA2 transcripts significantly decreased. The majority of Pdx1\textsuperscript{+} cells still displayed a duct-like phenotype, expressing Krt19 and Sox9, the latter at low levels (Figure S2B).

Ectopic expression of MAPK\textsuperscript{CA} and STAT3\textsuperscript{CA} thus demonstrates the potential of human exocrine cells to respond to this specific signalling by initiating a pro-endocrine differentiation program, similar to what has previously been described in rodent cells (8, 10, 14) albeit without the ability to complete endocrine differentiation under these conditions.

\textbf{Transplantation of human exocrine cells overexpressing MS\textsuperscript{CA} allows for further endocrine differentiation of 2D exocrine cell cultures.}

Since endocrine progenitor cells have already been shown to mature \textit{in vivo} to functional beta cells (20), we evaluated the capacity of an in vivo environment to provide critical maturation signals missing in vitro. Following monolayer culture, LeM\textsuperscript{CA}_{3d}MS\textsuperscript{CA}_{7d} or LeGFP cells were transplanted under the kidney capsule of immune-compromised mice to study their potential for maturation to endocrine cells (Figure 1B). When harvested 42 days after engraftment, very few Neurog3\textsuperscript{+} cells remained (Figure 3A,D) while the number of Pdx1\textsuperscript{+} cells persisted (n=5) and Krt19\textsuperscript{+} cells marginally decreased (84.3±2.6% vs. 92.7±0.8% prior to transplantation) (Figure 3D). The LeM\textsuperscript{CA}_{3d}MS\textsuperscript{CA}_{7d} grafts contained both glucagon\textsuperscript{+} (Figure 3B) cells and insulin\textsuperscript{+} cells (Figure 3C) but the LeGFP grafts did not. The number of insulin\textsuperscript{+} cells
significantly increased following engraftment (0.44±0.06% before vs. 1.17±0.20% after transplantation; p<0.05) (Figure 3C,D) while no insulin+ cells were observed among grafted LeGFP cells. The insulin+ cells did not stain for Krt19, but only a few displayed nuclear MafA, indicative of incomplete maturation to functional beta cells(21, 22). Rare insulin+MafA+ cells were observed. Together, these observations resemble transient expression of Ngn3 during pancreas development and support the hypothesis that engraftment of LeMCA3dMSCA7d exocrine cells from human pancreas further stimulates endocrine differentiation.

**Overexpression of MS** 

In an attempt to reproduce the endocrine cell differentiation observed *in vivo* in cultured cells and knowing that exocrine LeMCA3dMSCA7d cells in 2D culture did not differentiate efficiently, we cultured the cells in 3D matrix for 8 days (LeMSCA3d) (Figure 1C), which promotes alterations in cell polarity and cell-cell contact that stimulate differentiation (23, 24)(25). Because suspension culture of rodent acinar cells allows their dedifferentiation (rather than transdifferentiation to duct-like cells) and subsequent differentiation to insulin+ cells (8, 26), we examined the effects of suspension pre-culture by keeping freshly isolated, lentivirus-transduced exocrine cells in free-floating suspension culture for 10 days followed by 3D matrix culture for 8 days (LeMSCAFF/3D) (Figure 1D).

LeMSCA3d and LeMSCAFF/3D cells contained significantly more NEUROG3 and NEUROD1 mRNA compared to LeGFP3d cells while the level of Pdx1 and insulin transcripts remained similar (Figure 4A). Compared to LeGFPFF/3D, LeMSCAFF/3D cells contained significantly more NKX6.1, NEUROD1 and ISL1 transcripts (p<0.05). A significant increase in NEUROD1 expression was already observed in the LeMSCA3d culture condition, however, this increase is even more pronounced in LeMSCAFF/3D, suggesting improved endocrine differentiation, a conclusion supported by increased abundance of insulin transcripts and protein when a free-floating culture period was included (Figure 4A,E).
Compared to LeGFP control cells, levels of duct cell-specific transcripts did not change (Figure 4B). All cells expressed Krt19 at the protein level (Figure 4C,E). The acinar cell-specific transcript PTF1A increased in LeMS\textsuperscript{CA} cells (Figure 4B) without an associated rise in the mRNA encoding acinar cell-specific proteins amylase, chymotrypsin, Mist1 and Ptf1a.

Ngn3\textsuperscript{+} cells were detected in LeMS\textsuperscript{CA}\textsubscript{3D} (4.6±1.3%) and LeMS\textsuperscript{CA}\textsubscript{FF/3D} (7.7±3.0%) but not in LeGFP\textsubscript{3D} or LeGFP\textsubscript{FF/3D} cultures (Figure 4C,D). The number of Pdx1\textsuperscript{+} cells was significantly increased in both LeMS\textsuperscript{CA}\textsubscript{3D} (10.7±2.0%, p<0.01) and LeMS\textsuperscript{CA}\textsubscript{FF/3D} cells (6.1±2.6%, p<0.05) as compared to LeGFP control cells (Figure 4D,F); and insulin\textsuperscript{+} cells were even more dramatically increased in Le-MS\textsuperscript{CA}\textsubscript{FF/3D} cells (7.3±2.6% (n=7)) vs. Le-MS\textsuperscript{CA}\textsubscript{3D} (0.83±0.40% in (n=8)) and Le-GFP\textsubscript{FF/3D} (0.06±0.06% (n=10); p<0.05) (Figure 4D,F).

KRT19, PTF1A and NEUROG3 mRNA levels were confirmed by conventional RT-PCR and indicate that the acinar cells do adopt a duct-like phenotype during culture (Figure S3), as previously reported (13),(27) and that at least a subpopulation of LeMS\textsuperscript{CA}\textsubscript{FF/3D} cells initiate an endocrine differentiation program (Figure S3). Unlike the pre-existing beta cells, the insulin\textsuperscript{+} LeMS\textsuperscript{CA}\textsubscript{FF/3D} cells did not stain for MafA by immunohistochemistry, suggesting that the newly formed insulin\textsuperscript{+} cells are not fully mature (Figure S4A). The new insulin\textsuperscript{+} cells did not derive by proliferation of pre-existing beta cells: no insulin\textsuperscript{+} cells expressed the proliferation marker Ki67 (Figure S5).

Unlike the exocrine cell cultures, transduction of human islets with LeMS\textsuperscript{CA} did not activate expression of Neurog3 (Figure S4). In addition, both transduced (GFP\textsuperscript{+}) and non-transduced (GFP\textsuperscript{−}) beta cells expressed MafA, Pdx1 and insulin, showing that viral transduction does not prevent their expression.

**Short-term transplantation of LeMS\textsuperscript{CA}\textsubscript{FF} cells promotes endocrine differentiation.** Because the LeMS\textsuperscript{CA}\textsubscript{FF/3D\textsuperscript{+}}-transduced human exocrine cells yielded the most efficient endocrine differentiation, we tested whether substituting 3D culture by engraftment could further improve the efficiency of differentiation. LeMS\textsuperscript{CA}\textsubscript{FF} or LeGFP\textsubscript{FF} cells were transplanted under the kidney capsule of immune-compromised mice and 35 days later the graft-bearing kidney was harvested for analysis (Figure 1E). The amount of
GFP\(^{+}\)insulin\(^{+}\) cells strongly increased in LeMS\(^{CA}\)\(^{FF}\) grafts compared to LeGFP\(^{FF}\) controls (Figure 5A,D). The grafts showed a near total loss of Ngn3\(^{+}\) cells while Pdx1 was present in all human cells (Figure 5B,D). A markedly higher expression of Pdx1 was observed in 4.2±0.7% cells of LeMS\(^{CA}\)\(^{FF}\) grafts (Pdx1\(^{high}\) cells), a significant increase (p<0.001; n=5) compared to control grafts and concordant with transplanted LeMS\(^{CA}\) cells after 2D culture. The majority of the grafted cells are Krt19\(^{+}\), except for the hormone\(^{+}\) cells (Figure 5A,C). The fraction of insulin\(^{+}\) cells increased significantly (5.6±1.1% in LeMS\(^{CA}\)\(^{FF}\) vs. 0.5±0.3% in LeGFP\(^{FF}\); p<0.001, n=10) (Figure 5D) and was similar to that obtained in LeMS\(^{CA}\)\(^{FF}/3D\) cells in vitro (Figure 4E). In absolute numbers however, the yield of insulin\(^{+}\) cells remained rather low.

**New human beta cells derive from reprogrammed acinar cells.**

For unbiased tracing of the fate of the insulin\(^{+}\) LeMS\(^{CA}\)\(^{FF}/3D\) cells, a lentivirus-mediated, Cre-lox-based reporter expression was used. First, infection with AdCela2A\(^{Cre}\) virus ensured acinar-specific expression of Cre recombinase under control of the Elastase 2A gene promoter. When combined with a second virus that, upon Cre-mediated excision of the loxSTOPlox (LSL) signal, constitutively expressed a DsRed reporter under control of the cytomegalovirus promoter (LeCMV-LSL-DsRed), acinar cells could be permanently traced (Figure 1F). The fate of acinar cells from human pancreas was traced in LeMS\(^{CA}\)\(^{FF}/3D\) cells as these showed the most efficient endocrine differentiation. The analysis was performed on cells co-expressing the lineage tracer DsRed and the LeGFP- or LeMS\(^{CA}\)-derived GFP (Figure 6A). Insulin\(^{+}\)DsRed\(^{+}\) (20.8±1.4% of all Insulin\(^{+}\) cells; n=4) or Ngn3\(^{+}\)DsRed\(^{+}\) (16.3±1.1% of all Ngn3\(^{+}\) cells; n=4) cells were readily detected among LeMS\(^{CA}\)\(^{FF}/3D\) but never among LeGFP\(^{FF}/3D\) cells (Figure 6A,B). Acinar cells can thus be reprogrammed to insulin\(^{+}\) cells when transduced with activated MAPK and STAT3 and cultured under free-floating/3D conditions. These observations demonstrate the plasticity of acinar cells and indicate that under appropriate conditions human acinar cells can be re-specified to insulin\(^{+}\) cells following transient expression of Ngn3 (14).

**Longterm engraftment of LeMS\(^{CA}\)\(^{FF}\) cells generates functional grafts capable of producing c-peptide and responding to increased glucose levels.**

Following engraftment of LeMS\(^{CA}\)\(^{FF}\) cells under the kidney capsule of immune-compromised mice (Figure 1E) a significant rise in circulating human c-peptide could
be observed from day 90 onwards (Figure 7A) (n=4; p<0.05) whereas control mice never show a change in c-peptide levels. Injection of alloxan to destroy the endogenous rodent beta cells further increased the basal circulating c-peptide in LeMS\textsuperscript{CA}\textsubscript{FF} mice. Prior to alloxan injection, the blood glucose levels in LeMS\textsuperscript{CA}\textsubscript{FF} and LeGFP\textsubscript{FF} mice were similar. Following destruction of the endogenous beta cells, LeGFP\textsubscript{FF} mice displayed a sharp increase in glycemia while LeMS\textsuperscript{CA}\textsubscript{FF} mice were able to attenuate and partially control the expected rise in blood glucose levels (on day 206 16.6±0.5 mmol/l in LeMS\textsuperscript{CA}\textsubscript{FF} vs. 32.7±0.3 mmol/l in LeGFP\textsubscript{FF}; p<0.01, n=6) (Figure 7B). Upon nephrectomy of the graft-bearing kidney, LeMS\textsuperscript{CA}\textsubscript{FF} mice became severely hyperglycemic and indistinguishable from controls (on day 212 31.9±0.6 mmol/l in LeMS\textsuperscript{CA}\textsubscript{FF} vs. 33.0±0.2 mmol/l in LeGFP\textsubscript{FF}; p>0.05, n=6). Intra-peritoneal glucose tolerance test performed on day 208 demonstrated improved glucose tolerance of LeMS\textsuperscript{CA}\textsubscript{FF} mice compared to LeGFP\textsubscript{FF} mice. LeMS\textsuperscript{CA}\textsubscript{FF} mice remained however more glucose intolerant compared to mice with mature human islet grafts (especially at 90 and 120 minutes post glucose injection) (n=6; p<0.05)(Figure 7C). Isolated grafts showed glucose responsiveness in vitro with a 5-fold increase in secreted insulin levels when comparing low vs high glucose conditions (n=3; p<0.05) (Figure S6A). The beta-like cells in the grafts however did not proliferate (0.1±0.03% Ki67\textsuperscript{+}Insulin\textsuperscript{+} cells in LeMS\textsuperscript{CA}\textsubscript{FF} grafts; n=6) (Figure S6B). However, graft functionality was further demonstrated by serial transplantation with retrieved grafts able to attenuate blood glucose levels in secondary recipient mice (Figure S6C).

**Longterm engraftment of LeMS\textsuperscript{CA}\textsubscript{FF} cells generates insulin\textsuperscript{+} islet-like clusters.**

Engraftment of LeMS\textsuperscript{CA}\textsubscript{FF} cells under the kidney capsule of immune-compromised mice during 210 days generates stable grafts readily detectable by GFP expression (Figure 7D). Upon closer examination a substantial amount of insulin\textsuperscript{+} cells was detected (8.0±0.1%; n=6) (Figure 7E). Whereas 29.6±1.2% of all cytokeratin19\textsuperscript{+} cells contained GFP, indicating expression of activated MAPK and STAT3 (n=6), insulin\textsuperscript{+} cells seemed to have a higher prevalence of GFP (65.5±9.0% GFP\textsuperscript{+}insulin\textsuperscript{+} cells; n=6) (Figure 7E-F). In addition, acinar-specific genetic lineage tracing (AdCela2A\textsuperscript{Cre}/LeCMV-LSL-LacZ) (Figure 1F) revealed that although 27.1±1.2% of Krt19\textsuperscript{+} cells (n=6) contained the acinar beta-galactosidase (βgal) label, the fraction of insulin\textsuperscript{+} cells expressing this genetic tracer was significantly higher (61.7±8.8%;
n=6)(Figure 7E-F). Insulin+ cells were organised in islet-like clusters and expressed high levels of Pdx1 (Figure 7F).
DISCUSSION

Rodent acinar cells demonstrate a remarkable plasticity in culture that can be manipulated to transdifferentiate them to insulin$^+$ cells for replacement therapy in diabetes. This reprogramming can be induced in cultured acinar cells from rat and mice by the combination of, respectively EGF+LIF (8) and EGF+nicotinamide (28) and \textit{in vivo} in mouse pancreas by ectopic expression of 3 transcription factors, Neurog3, Pdx1 and MafA (29) or by EGF+CNTF treatment of diabetic mice (30). In the present study we focus on translating these original findings from rodent to man. We hypothesised that MAPK and STAT3 signalling, known effectors of EGF and LIF, may regulate the dedifferentiation of human acinar cells to a progenitor state and the subsequent redifferentiation to endocrine cells (10, 14).

Our data indicate that human acinar cells can undergo reprogramming upon introduction of activated MAPK + STAT3 (MS$^{CA}$). In accordance with previous reports (13, 26), acinar cells rapidly lost their identity during \textit{in vitro} culture and the majority adopted a phenotype resembling pancreatic duct cells. However, in contrast to earlier studies, the key developmental transcription factor Neurogenin-3 (Neurog3) (31-33) was re-expressed in MS$^{CA}$ cells. The expression of Neurog3 was transient when MS$^{CA}$ cells were transplanted. When human exocrine cells were transduced with MS$^{CA}$ and cultured as 2D monolayers they initiated a pro-endocrine differentiation program. The endocrine differentiation was not completed, however, as the amount of insulin$^+$ and glucagon$^+$ cells did not increase. Compared to culture in free-floating aggregates (34) exocrine cells in 2D lost their Pdx1 expression but by sequential transduction with LeMAPK and LeSTAT3 the number of Pdx1$^+$ cells in 2D cultures increased again. By altering the culture conditions from 2D monolayer to 3D matrigel, we observed enhanced endocrine differentiation of MS$^{CA}$ cells. Other studies have previously reported that alterations in cell polarity and cell-cell contact positively affect cell differentiation (23, 24). By combining free-floating culture with 3D matrigel culture, we further stimulated endocrine differentiation as illustrated by increased numbers of Neurog3$^+$ and Pdx1$^+$insulin$^+$ cells. The appearance of insulin$^+$ cells indicates that under these conditions of culture human exocrine cells transduced with MS$^{CA}$ are prone to terminal endocrine differentiation rather than acino-ductal transdifferentiation. Possibly the free-floating pre-culture allows the acinar cells to adopt a state similar to embryonic
pancreas progenitors, making these cells more susceptible to pro-endocrine signalling (34). In contrast to previous reports (35, 36) the presence of activated MAPK did not activate the cell cycle as the fraction of proliferating cells was low in these cultures and did not differ from control cells.

When adult islet beta cells were subjected to LeMS\textsuperscript{CA\_FF/3D} treatment, Neurog3 expression was not activated. In addition, the expression of insulin, MafA and Pdx1 in adult islet beta cells was not influenced by LeMS\textsuperscript{CA}. These observations confirm the hypothesis that activated MAPK and STAT3 can convert human exocrine cells into beta-like cells, and pre-existing beta cells are unlikely to be the source of the Neurog3\textsuperscript{+} or newly formed insulin\textsuperscript{+} cells.

Engraftment of LeMS\textsuperscript{CA\_FF} cells during an extended period of 210 days allowed the cells to acquire functionality. Under normoglycemic conditions these mice displayed an increase in circulating human c-peptide starting around day 90 post engraftment. Upon chemical destruction of the endogenous rodent beta cell population, c-peptide levels further spiked and the sharp increase in blood glucose levels in controls was attenuated in LeMS\textsuperscript{CA\_FF} mice. In addition these animals showed an improved glucose tolerance, suggesting that these LeMS\textsuperscript{CA\_FF} cells formed stable grafts able to respond to change in glycemia. Removal of the graft unequivocally identified the human cells as source of c-peptide and control over blood glucose levels. Moreover, serial transplantation revealed that these stable acinar-derived grafts could partially correct blood glucose levels when transplanted into a new diabetic recipient mouse.

Genetic lineage tracing based on acinar cell-specific expression of a fluorescent reporter revealed that human acinar cells can not only give rise to duct-like cells (previously documented (13)) but also to cells expressing Neurog3 and insulin following the overexpression of MAPK and STAT3 in specific pro-endocrine culture conditions. A recent report demonstrated that human acinar cells in culture can give rise to insulin\textsuperscript{+} cells following introduction of Neurog3, Pdx1 and MafA followed by a series of (epigenetic) signalling events (37), thus extrapolating the initial findings documented in mice \textit{in vivo} (38). The present report, however, is the first to show that human acinar cells can initiate pro-endocrine differentiation by activated signalling
without introduction of transcription factors and thus opening the possibility of inducing endocrine differentiation with a combination of growth factors.

Duct cells have previously been shown to harbour the potential for endocrine differentiation (3, 4, 39). When ectopic Neurog3 is introduced in human duct cultures, these cells adopt an endocrine-like fate (39). Lack of lineage tracing in this mixed exocrine population does not allow the assessment of potential acinar contribution to this phenomenon. Others reported that adherent culture of human duct cells using overlay matrigel coating generates insulin+ islets-like structures (3, 4). These starting preparations did not include lineage tracing, and follow-up studies using beta cell-depleted exocrine preparations failed to reproduce these findings (5). Although the exocrine fractions used here initially contains a mixed population including mature duct cells, and human duct cells have previously been suggested to give rise to new beta cells (3, 4), the absence of duct-specific lineage tracing does not allow us to speculate on the differentiation potential of human duct cells in the current study.

The number of Ngn3+ cells in MS<sup>CA</sup> cells was substantial but the absolute amount of insulin+ cells remained low in vitro, even under the most optimal culture conditions tested. Shortterm engraftment of these cells only modestly improved endocrine differentiation. We did observe increased expression of MafA in some of the beta-like cells after shortterm engraftment, suggesting improved in vivo maturation of the insulin+ cells rather than ongoing differentiation of exo- to endocrine cells. In contrast, longterm engraftment of MS<sup>CA</sup> cells appeared to provoke a significant increase in human insulin+ cells, which are now organised in structures resembling islets. Interestingly, under these conditions, GFP expression seems to be enriched in beta-like cells. This observation is suggestive of a preferential endocrine differentiation of MS<sup>CA</sup> cells following more than 200 days in vivo. Moreover, not only did we observe an increase in beta-like cell numbers, genetic lineage tracing also showed that more than 60% of these insulin+ cells originated from human acinar cells, compared to 30% of the duct-like population of the grafts.

The current report demonstrates for the first time that human acinar cells can adopt a beta-like phenotype without the need to introduce a combination of pancreatic transcription factors. This acino-insular reprogramming depends on 3D growth,
activation of MAPK/STAT3 signalling and an intermediate Neurog3+ step. Given the volume of human acinar cells discarded upon clinical islet isolation, this approach may present an interesting strategy to increase the amount of transplantable beta cells provided the efficiency of cell type conversion can be improved and the involvement of viruses avoided.
References


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Author contributions
Design: ML and LBa; Execution of experiments: ML, LBa, GL and YH; Analyses: ML and LBa; Interpretation of results: ML, MSG, HH and LBa; Writing: ML, MSG, HH and LBA; Project management: HH, LBo and LBa.

Conflict of Interest
The authors declare no conflict of interest.
Figure legends

Figure 1

Schematic overviews of different culture systems

(A-B) Schematic overview of the monolayer culture system

(A) Transduction of human exocrine cells obtained from cadaveric donors with a lentivirus overexpressing activated MAPK and STAT3 (LeMS\textsuperscript{CA}) and subsequent formation of adherent monolayer cultures. The cells are exposed to LeMS\textsuperscript{CA} for 24 hours after which the excess virus is washed away. The cells are kept for 7 days in multiwall plates to allow monolayer formation.

(B) Overexpression of MAPK\textsuperscript{CA} for 3 days prior to combined overexpression of MAPK\textsuperscript{CA} -STAT3\textsuperscript{CA} (LeM\textsuperscript{CA}\textsubscript{3d}MS\textsuperscript{CA}\textsubscript{7d}). The cells are exposed to Le-MAPK\textsuperscript{CA} for 24 hours, followed by removal of the excess virus, and cells are kept in fresh medium for 2 more days. On the third day the cells are transduced with Le-MAPK\textsuperscript{CA} -STAT3\textsuperscript{CA}. The medium is changed after 24 hours and the cells are kept in multiwall plates with fresh medium for 7 days.

To allow for potential further differentiation, the LeM\textsuperscript{CA}\textsubscript{3d}MS\textsuperscript{CA}\textsubscript{7d} condition is transplanted under the kidney capsule of immune-deficient mice. The animals are kept for 42 days after which the graft-bearing kidney is removed and the graft is recovered for further analysis.

(C-E) Schematic overview of the 3D, FF/3D and FF/in vivo culture systems

(C) Transduction of human exocrine cells with a lentivirus overexpressing activated MAPK and STAT3 and subsequent 3D matrigel culture (LeMS\textsuperscript{CA}\textsubscript{3D}). The cells are exposed to LeMS\textsuperscript{CA} for 24 hours followed by removal of the excess virus. The cells are kept in 3D matrigel for 8 days.

(D) Transduction of human exocrine cells with a lentivirus overexpressing activated MAPK and STAT3 and subsequent free floating/3D matrigel culture (LeMS\textsuperscript{CA}\textsubscript{FF/3D}). The cells are exposed to LeMS\textsuperscript{CA} for 24 hours followed by removal of the excess virus. The cells are kept in free floating culture for approximately 10 days after which they are transferred in 3D matrigel for 8 days.

(E) The free floating cells (LeMS\textsuperscript{CA}\textsubscript{FF}) are transplanted under the kidney capsule of immune-deficient mice to allow potential further differentiation. The animals are kept
for 42 days after which the graft-bearing kidney is removed and the graft is recovered for further analysis.

(F) Schematic overview of the acinar-specific genetic lineage tracing experiment
The human exocrine cells are exposed to the three viruses (Ad-Ela-Cre, Le-CMV-LSL-DsRed, LeMS<sup>CA</sup>) at the same time for 24 hours after which the excess virus is removed. The cells are then kept in free-floating culture for approximately 10 days after which they are transferred in 3D matrigel for 8 days.

**Figure 2**
**Overexpression of MAPK<sup>CA</sup> and STAT3<sup>CA</sup> promotes endocrine differentiation in vitro.**
(A-C) Gene expression profile of endocrine markers (A), acinar markers (B) and ductal markers(C). LeMS conditions are normalized to control conditions (LeGFP) set at 1 (red line).

**Protocol 1**: original protocol with 7d combined STAT3<sup>CA</sup>/MAPK<sup>CA</sup> (LeMS<sup>CA</sup>) (n=6; *p<0.05; **p<0.01). **Protocol 2**: sequential treatment 3d MAPK<sup>CA</sup> followed by 7d combined STAT3<sup>CA</sup>/MAPK<sup>CA</sup> (LeM<sup>CA</sup><sub>3d</sub>MS<sup>CA</sup><sub>7d</sub>) (n=9; *p<0.05; **p<0.01). Endocrine genes INS, PDX1 and NKX6.1 are significantly upregulated in protocol 2 whereas exocrine genes MIST1 and FOXA2 were downregulated compared to protocol 1. The trend on endocrine genes combined with the increase in ONECUT1 transcripts suggests an accelerated endocrine differentiation in protocol 2.

(D) Immunocytochemical analysis of Neurog3 and insulin expression after the original 7d protocol (LeMS<sup>CA</sup>) and the sequential 3d MAPK<sup>CA</sup> followed by 7d combined STAT3<sup>CA</sup>/MAPK<sup>CA</sup> (LeM<sup>CA</sup><sub>3d</sub>MS<sup>CA</sup><sub>7d</sub>). Neurog3<sup>+</sup> cells were readily detected in LeMS<sup>CA</sup> and the fraction of Ngn3 expressing cells slightly increased in LeM<sup>CA</sup><sub>3d</sub>MS<sup>CA</sup><sub>7d</sub>. No insulin<sup>+</sup> cells could be detected in LeMS<sup>CA</sup> or LeM<sup>CA</sup><sub>3d</sub>MS<sup>CA</sup><sub>7d</sub>.

(E) Immunocytochemical analysis of Pdx1 and Neurog3 after LeMS<sup>CA</sup> and LeM<sup>CA</sup><sub>3d</sub>MS<sup>CA</sup><sub>7d</sub>. The number of Pdx1 expressing cells is markedly increased in LeM<sup>CA</sup><sub>3d</sub>MS<sup>CA</sup><sub>7d</sub> compared to the LeMS<sup>CA</sup> condition. The majority of the Pdx1<sup>+</sup> cells co-express Neurog3.

(F) Quantification of the proportion of transduced cells (EGFP<sup>+</sup>) expressing Neurog3 or Pdx1. The increase in the percentage of Neurog3<sup>+</sup> is comparable in both protocols (40±2% in LeM<sup>CA</sup><sub>3d</sub>MS<sup>CA</sup><sub>7d</sub> vs 38±1% in LeMS<sup>CA</sup>; n=4; p>0.05), however an increase
can be detected in the percentage of PDX1+ cells with LeMCA_{3d}MSCA_{7d} compared to LeMSCA (28±3% in LeMCA_{3d}MSCA_{7d} vs 8±1% in LeMSCA; n=4; *p<0.05; **p<0.01).

**Figure 3**

Engraftment of LeMCA_{3d}MSCA_{7d} monolayer-cultured human exocrine cells allows for in vivo maturation.

(A-C) Immunohistochemical (IHC) analysis of the graft-bearing kidney of immune-deficient mice transplanted with the human cells transduced with LeMCA_{3d}MSCA_{7d};

(A) In contrast to the in vitro cultures, a near total loss of Ngn3 expression is observed after transplantation of the LeMCA_{3d}MSCA_{7d} cells, whereas Pdx1 remains detectable in the majority of the epithelial cells. Bar: 35 um.

(B-C) All transplanted human cells remain Krt19+, except for the hormone+ cells; (B) Gcg+ cells were detected dispersed within the transplanted epithelial cells. (C) Following transplantation, insulin+ cells appear within the graft. When analyzing the beta cell marker Mafa, we observed only few insulin+ cells co-expressing MafA, indicative of their immature nature.

(D) Quantification of the protein expression after the transduction protocol LeMCA_{3d}MSCA_{7d} prior to transplantation (in vitro) and after 42 days under the kidney capsule (in vivo). The most striking observation is the appearance of insulin+ cells after in vivo transplantation combined wit the disappearance of Neurog3+ cells.

**Figure 4**

Overexpression of MAPKCA and STAT3CA promotes re-expression of Ngn3 and Pdx1 and endocrine differentiation in 3D matrix cultures in vitro.

(A-B) Gene expression profile of endocrine markers (A) and exocrine markers (B). LeMS conditions are normalized to control conditions (LeGFP) set at 1 (red line). In LeMSCA_{3d} condition we observed a significant increase in NEUROD and NEUROG3 transcripts compared to controls. These observations were mirrored in the LeMSCA_{FF/3D} condition with additional increase in INS, NKX6.1 and ISL1 transcripts. Both the LeMSCA_{3d} and LeMSCA_{FF/3D} condition showed a modest but significant increase in PTF1A transcripts (n=5) (*p<0.05; **p<0.01).

(C-E) Immunocytological analysis after 3D culture (LeMSCA_{3d}) and the free floating culture followed by 3D culture (LeMSCA_{FF/3D}). (C) Both in the LeMSCA_{3d} and
LeMS\textsuperscript{CA,FF}/3D condition Neurog3\textsuperscript{+} cells can be detected in transduced (EGFP\textsuperscript{+}) cells. The majority of these Ngn3\textsuperscript{+} cells co-express the ductal marker Krt19. Cells expressing Neurog3 protein were never observed in the corresponding control conditions (LeGFP\textsubscript{3D} and LeGFP\textsubscript{FF}/3D). (D) In the LeMS\textsuperscript{CA,3D} and LeMS\textsuperscript{CA,FF}/3D condition cells expressing high levels of Pdx1 protein can be detected. A fraction of these cells displayed co-expression with Neurog3. Cells expressing high Pdx1 protein were only rarely observed in the corresponding control conditions (LeGFP\textsubscript{3D} and LeGFP\textsubscript{FF}/3D). (E) LeMS\textsuperscript{CA,3D} and LeMS\textsuperscript{CA,FF}/3D conditions show for the first time the presence of INS\textsuperscript{+} cells in vitro. These insulin\textsuperscript{+} cells do not express the ductal marker Krt19. (F) Quantification of the proportion of cells expressing the different phenotypical markers. A significant increase in the number of insulin\textsuperscript{+} cells can be observed in the LeMS\textsuperscript{CA,FF}/3D condition (7.3±2.6% in LeMS\textsuperscript{CA,FF}/3D (n=7) vs 0.83±0.40% in LeMS\textsuperscript{CA,3D} (n=8) and 0.06±0.06% in LeGFP\textsubscript{FF}/3D (n=10); *p<0.05; **p<0.01). The percentage of Neurog3\textsuperscript{+} and Pdx1\textsuperscript{+} cells is significantly increased in both LeMS\textsuperscript{CA,3D} and LeMS\textsuperscript{CA,FF}/3D conditions compared to controls (*p<0.05; ***p<0.001).

Figure 5

Shortterm transplantation of LeMS\textsuperscript{CA,FF} cells yields a limited number of insulin\textsuperscript{+} cells.

(A-C) Immunohistochemical analysis of LeMS\textsuperscript{CA,FF} human exocrine cells following 42-day engraftment under the kidney capsule of immune-deficient mice. (A) In contrast to the cell preparation prior to transplantation, insulin\textsuperscript{+} cells can be observed in LeMS\textsuperscript{CA,FF} condition after engraftment in vivo. LeGFP\textsubscript{FF} controls never showed an increase in the fraction of insulin\textsuperscript{+} cells (B) Pdx1 expression is prominent in the majority of the Krt19\textsuperscript{+} epithelial cells both in LeGFP\textsubscript{FF} and LeMS\textsuperscript{CA,FF} conditions (the latter contains more cells with high Pdx1 expression). However, compared to the cell preparation prior to transplantation, we observed a near total loss of Neurog3\textsuperscript{+} cells after engraftment in the LeMS\textsuperscript{CA,FF} condition. LeGFP\textsubscript{FF} conditions never show Neurog3\textsuperscript{+} cells. (C) Hormone\textsuperscript{+} cells (INS\textsuperscript{+} or GCG\textsuperscript{+}) do not express the ductal marker Krt19. (D) Quantification of the fraction of cells expressing insulin, Neurog3 or high levels of Pdx1 protein in LeGFP\textsubscript{FF} and LeMS\textsuperscript{CA,FF} conditions. A significant increase in the number of insulin and Pdx1 expressing cells could be observed in the transplanted
LeMS$^{CA}_{FF}$ condition (5.6±1.1% insulin+ cells (n=10); 4.2±0.7% Pdx1+ cells (n=5); ***p<0.001).

**Figure 6**

Genetic lineage tracing of human acinar cells reveals acinar to beta cell transdifferentiation

To trace the fate of adult human acinar cells in our culture system, the cells were transduced with Cre recombinase under control of the acinar-specific Elastase 2A promoter and the reporter construct CMV-LSL-nls-DsRed.

**A** Original acinar cells identified by the presence of the DsRed fluorescent protein were readily detected in both LeGFP$_{FF/3D}$ and LeMS$^{CA}_{FF/3D}$ conditions. However, insulin$^+$/DsRed$^+$ cells were only detected in LeMS$^{CA}_{FF/3D}$ condition, never in the LeGFP$_{FF/3D}$ control. Every insulin$^+$/DsRed$^+$ cell in the latter condition also contained the GFP label indicative of transduction with MAPK$^{CA}$-STAT3$^{CA}$.

**B** Neurog3$^+$ cells containing the acinar lineage tracer DsRed were observed in the LeMS$^{CA}_{FF/3D}$ condition only, as Neurog3$^+$ cells were never detected in the control condition.

**Figure 7**

Longterm engraftment generates acinar-derived islet-like clusters in vivo.

**A** Circulating human c-peptide levels in mice engrafted with either LeGFP$_{FF}$ or LeMS$^{CA}_{FF}$ cells. Significantly increased levels of c-peptide are detected in LeMS$^{CA}_{FF}$ mice from day 90 post engraftment. C-peptide levels become stable around day 148 (0.30±0.02 ng/ml in LeMS$^{CA}_{FF}$ vs. 0.08±0.02 ng/ml in LeGFP$_{FF}$; p<0.01, n=6). Post alloxan injection, c-peptide levels in LeMS$^{CA}_{FF}$ mice further increase to 0.38±0.02 ng/ml. No human c-peptide could be detected after nephrectomy of the graft-bearing kidney at day 210.

**B** Blood glucose levels in mice engrafted with either LeGFP$_{FF}$ or LeMS$^{CA}_{FF}$ cells. Prior to alloxan injection, both groups are indistinguishable (on day 190 6.3±0.2 mmol/l in LeMS$^{CA}_{FF}$ vs. 5.8±0.2 mmol/l in LeGFP$_{FF}$; p>0.05, n=6). Post alloxan injection, blood glucose levels of LeMS$^{CA}_{FF}$ mice remain significantly lower compared to LeGFP mice (on day 206 16.6±0.5 mmol/l in LeMS$^{CA}_{FF}$ vs. 32.7±0.3 mmol/l in LeGFP$_{FF}$; p<0.01, n=6). Removal of the graft-bearing kidney provoked an acute reversal to
hyperglycemia in LeMS\textsuperscript{CA}\textsubscript{FF} mice (on day 212 31.9±0.6 mmol/l vs. 33.0±0.2 mmol/l in LeGFP\textsubscript{FF}; p<0.01, n=6).

(C) I.p. glucose tolerance test (IPGTT). 2g glucose per kg BW was injected and clearance in blood was measured at indicated time points to indirectly measure the glucose responsiveness of insulin secretion by beta cells (*: p<0.05; n=6 each).

(D) 210 days post engraftment of LeMS\textsuperscript{CA}\textsubscript{FF} cells results in stable grafts visualized by GFP expression.

(E) Immunohistochemical analyses showed an increased fraction of insulin\textsuperscript{+} cells in LeMS\textsuperscript{CA}\textsubscript{FF} conditions to almost 10%. Whereas GFP was detected in only 30% of the Krt19\textsuperscript{+} cells at the time of analysis, GFP was found in over 60% of the insulin\textsuperscript{+} cells, indicating a preferential differentiation of LeMS\textsuperscript{CA}\textsubscript{FF} transduced cells to a beta-like phenotype. Genetic lineage tracing using the acinar-specific Elastase 2A promoter and the reporter construct CMV-LSL-LacZ revealed that 61% of the insulin\textsuperscript{+} cells originated from acinar cells, while the same holds true for 30% of the Krt19\textsuperscript{+} cells.

(F) LeGFP\textsubscript{FF} control grafts lacked expression of insulin\textsuperscript{+} cells but many cells expressed both GFP and β-galactosidase (βgal) demonstrating acino-ductal metaplasia. LeMS\textsuperscript{CA}\textsubscript{FF} grafts however contained clusters of cells devoid of Krt19 expression while clearly positive for GFP and the acinar-specific βgal label. Closer examination demonstrated the expression of insulin and Pdx1 in these LeMS\textsuperscript{CA}\textsubscript{FF} beta-like cells. (scale bars=100 um; 25 um; 30um)

Figure S1

Immunocytochemical analyses of LeGFP and LeMS\textsuperscript{CA} in monolayer culture conditions.

(A) Immunocytochemical analysis of Neurog3 and insulin expression in the control conditions (LeGFP) and after the original 7d protocol (LeMS\textsuperscript{CA}). Neurog3\textsuperscript{+} cells were only detected in LeMS\textsuperscript{CA}. No insulin\textsuperscript{+} cells could be detected in LeGFP or in LeMS\textsuperscript{CA} conditions.

(B) Immunocytochemical analysis of Neurog3 and Pdx1 expression in the control conditions (LeGFP) and after the original 7d protocol (LeMS\textsuperscript{CA}). Neurog3\textsuperscript{+} and Pdx1\textsuperscript{+} cells were only detected in LeMS\textsuperscript{CA} condition, with the majority of the Pdx1\textsuperscript{+} cells co-expressing Neurog3.

(C) All cells in both the LeGFP and the LeMS\textsuperscript{CA} condition express the ductal markers Krt19 and Sox9.
(D) All epithelial cells are Krt19+ and no CHYMO+ can be found in LeGFP or in LeMCA conditions, confirming the loss of the acinar phenotype during culture.

**Figure S2**

Immunocytochemical analysis shows a decrease in Pdx1 expression in LeS_C3dLeMCA_7d compared to LeMCA and confirms a ductal phenotype in both LeGFP and LeMCA_3dLeMCA_7d.

(A) Immunocytochemical analysis of Neurog3 and Pdx1 expression after the original 7d protocol (LeMCA) compared to the sequential treatment of 3 days STAT3CA followed by 7 days STAT3CA/MAPKCA (LeS_C3dMS_C7d). Neurog3+ cells were detected in both conditions, however a loss of Pdx1 expression can be observed in LeS_C3dMS_C7d.

(B) All cells in the Le-GFP as well as LeMCA_3d+LeMCA_7d condition express the ductal markers Krt19 and Sox9.

**Figure S3**

Conventional PCR analysis confirms a switch from acinar to ductal phenotype during culture and highlights the NEUROG3 expression following MAPKCA/STAT3CA introduction.

Conventional PCR confirms a high expression of KRT19 and no expression of PTF1A in our cultures. The results for NEUROG3 confirm our results on qPCR and protein level that in our treatment conditions LeMSCA_3D and LeMSCA_FF/3D some of the cells go through an Neurog3 positive state, which was never observed in the control conditions.

**Figure S4**

Expression pattern of MafA, Neurog3 and Pdx1 in adult endocrine cells after LeMSCA_FF/3D

(A) Adult human islets cultured first free-floating followed by 3D matrigel cultures (CTR_FF/3D) maintain a high expression level of both Pdx1 and MafA in the majority of the cells. No viral infection was performed on these islets.

(B) Freshly isolated adult human islets were infected with LeMSCA and cultured first as free-floating followed by 3D matrigel culture, in an identical setup as the human
exocrine cells. LeMS$^{CA}_{FF;3D}$ did not provoke the expression of Neurog3 in these adult endocrine cells, and the majority of the cells expressed high levels of Pdx1. In these human islets, insulin and Pdx1 expression is no longer restricted to the transduced (GFP$^+$) cells, highlighting the difference with the exocrine LeMS$^{CA}_{FF;3D}$ cultures where only in the GFP$^+$ cells a high expression of Neurog3 can be noticed and Insulin$^+$ cells are observed, but the majority fail to express MafA.

**Figure S5**

**Proliferation analysis of free-floating human acinar cells following transplantation under the kidney capsule.**

(A) Immunocytochemical analysis of Krt19, Ki67 and insulin expression of the recovered graft. Only rarely Ki67 positive cells could be found in LeGFP$_{FF}$ and LeMS$^{CA}_{FF}$. No double positive insulin$^+$Ki67$^+$ cells could be detected under these conditions. All human epithelial cells transplanted express Krt19 with the exception of insulin$^+$ cells.

(B) Quantification of the proportion of cells expressing the different phenotypical markers. Very few Ki67$^+$ cells can be found in LeGFP$_{FF}$ (1,9±0,5%; n=8) as well as LeMS$^{CA}_{FF}$ (2,8±0,6%; n=11). No double positive INS$^+$Ki67$^+$ cells could be detected under these conditions.

**Figure S6**

**Longterm human acinar grafts are glucose responsive, quiescent and attenuate hyperglycemia by serial transplantation in diabetic recipient mice.**

(A) Glucose-stimulated insulin secretion on isolated LeMS$^{CA}_{FF}$ grafts showed a 5-fold increase in secreted insulin levels (0.1±0.01 ng/mL medium at 2.5 mM glucose and 0.5±0.04 ng/mL medium at 20 mM glucose; n=3; p<0.05).

(B) Quantification of the absolute number of proliferating insulin$^+$ cells in the LeMS$^{CA}_{FF}$ grafts. Only 2.2±0.5 Ki67$^+$insulin$^+$ cells could be detected on a total of 1692±66 insulin$^+$ cells per graft (n=4).

(C) After the first transplantation (discussed in Figure 7)(left panel) the graft-bearing kidney was removed and the grafts were isolated and serially transplanted under the kidney capsule of new diabetes recipient mice (right panel). Control mice that never received a graft (CTR$_{ungrafted}$) remained severely hyperglycemic post alloxan injection
(32.1±0.2 mmol/l on day 25; n=3) whereas mice that received a LeMS\textsuperscript{CA}FF graft (LeMS\textsuperscript{CA}FF\textsuperscript{2nd}) showed a significant decrease in blood glucose levels 24h after transplantation (19.2±1.2 mmol/l vs 32.4±0.7 mmol/l in CTR\textsubscript{ungrafted} mice on day 9; n=3; p<0.05). The blood glucose levels remained stable in LeMS\textsuperscript{CA}FF\textsuperscript{2nd} mice until the termination on day 25 (20.6±1.5 mmol/l on day 25; n=3; p<0.05).
**Figure 1**

A. LeMSCA

- **day -1**
- Incubate human cells at 37°C for 24 hours
- Plate cells in suspension
- Monolayer in multi-well plate

- **day 0**
- Remove virus

- **7 days**

B. LeMSCA3dMSCA7d

- **day -1**
- Incubate human cells at 37°C for 24 hours
- Plate cells in 3D matrigel

- **day 0**
- Remove virus

- **2 days**

- **day 3**
- Remove virus

- **7 days**

C. LeMSCA3D

- **day -1**
- Incubate human cells at 37°C for 24 hours
- Plate cells in 3D matrigel

- **day 0**
- Culture in 3D matrigel for 8 days

D. LeMSCA_FF/3D

- **day -1**
- Incubate human cells at 37°C for 24 hours
- Plate cells in suspension
- Plate cells in 3D matrigel

- **day 0**
- Culture in 3D matrigel for 8 days

E. LeMSCA_FF

- **day -1**
- Incubate human cells at 37°C for 24 hours
- Plate cells in suspension

- **day 0**
- Harvest cells for transplantation

- **9-11 days**

F. LeMSCA_FF/3D

- **day -1**
- Incubate human cells at 37°C for 24 hours
- Plate cells in suspension

- **day 0**
- Plate cells in 3D matrigel

- **9-11 days**

- **8 days**

**Transplantation**

- Transplant cultivated cells under the kidney capsule
- Keep the animals for 42 days
- Harvest kidney for analysis

**42-210 days**

**Harvest kidney for analysis**

**Keep the animals for 42 days**

**Transplant cultivated cells under the kidney capsule**

**Harvest kidney for analysis**

- Keep the animals for 42 days

- Harvest kidney for analysis

**LeMSCA**

- LeMSCA
- Remove virus

- **LeMSCA3dMSCA7d**
- Remove virus
- Remove virus

**LeMSCA3D**

- LeMSCA
- Remove virus

**LeMSCA_FF/3D**

- LeMSCA
- Remove virus

**LeMSCA_FF**

- LeMSCA
- Remove virus

**Ad-CELA2A-Cre**

- Ad-CELA2A-Cre
- Remove virus

**LeMSCAFF/3D**

- LeMSCAFF
- Remove virus

**LeMSCAFF**

- LeMSCAFF
- Remove virus

**LeMSCAFF/3D**

- LeMSCAFF
- Remove virus

**LeMSCAFF**

- LeMSCAFF
- Remove virus

**Harvest kidney for analysis**

- LeMSCA
- Remove virus

**LeMSCA3dMSCA7d**

- LeMSCA
- Remove virus
- Remove virus

**LeMSCA3D**

- LeMSCA
- Remove virus

**LeMSCA_FF/3D**

- LeMSCA
- Remove virus

**LeMSCA_FF**

- LeMSCA
- Remove virus

- **LeMSCAFF/3D**
- Remove virus

**LeMSCAFF**

- LeMSCAFF
- Remove virus

**LeMSCAFF/3D**

- LeMSCAFF
- Remove virus

**LeMSCAFF**

- LeMSCAFF
- Remove virus

**Harvest kidney for analysis**

- LeMSCA
- Remove virus
Figure 3

A

Neurog3 Pdx1 DNA

B

DNA MafA Krt19 Gcg

C

DNA MafA Krt19 Ins

D

In vitro

In vivo
Figure 4

A  Endocrine markers

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<tr>
<td>GFP</td>
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B  Exocrine markers

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<td>Cftb</td>
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<td>Onec1t</td>
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C  Immunofluorescence images

D  Immunofluorescence images

E  Immunofluorescence images

F  Bar graphs

- **: p < 0.01
- ***: p < 0.001
- *: p < 0.05

Endocrine markers: Neurog3, Pdx1, Insulin
Exocrine markers: Krt19, Ptf1a, Cad, Cftb, Onec1t

Insulin: Le\textsubscript{3D} > Le\textsubscript{FF/3D}
Neurog3: Le\textsubscript{3D} > Le\textsubscript{FF/3D}
Pdx1: Le\textsubscript{3D} > Le\textsubscript{FF/3D}
Figure 5

A | DNA GFP Ins Krt19 | GFP Ins Krt19 | Ins Krt19
---|------------------|--------------|--------------
| DNA GFP Ins Krt19 | GFP Ins Krt19 | Ins Krt19

B | DNA Neurog3 Pdx1 Krt19 | Neurog3 Pdx1 Krt19 | Neurog3 Krt19
---|------------------|--------------|--------------
| DNA Neurog3 Pdx1 Krt19 | Neurog3 Krt19

C | DNA Krt19 Ins Gcg | Krt19 Ins Gcg | Ins Gcg
---|------------------|--------------|--------------
| DNA Krt19 Ins Gcg | Ins Gcg

D | LeGFPFF | LeMSCAFF
---|--------|--------
| LeGFPFF | LeMSCAFF

Insulin | Neurog3 | Pdx1 high
---|--------|--------
*** | *** | ***
Figure 6

**A**  
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<th>Ins</th>
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**B**  
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<th>Neurog3</th>
<th>Neurog3</th>
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Figure 7

A

human c-peptide (ng/ml)

B

Glycemia (mmol/l)

C

Glycemia (mmol/l)

time (m)

D

E

F

DNA GFP | Gal Krt19

DNA GFP | Gal Pdx1

DNA insulin | Gal Pdx1

GFP | Gal Krt19

GFP | Gal insulin

GFP | Gal

insulin

AdCela2Cre/LeLSLLacZ

LeMSCAFF

LeMSCAFF

LeMSCAFF

LeMSCAFF