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Exocrine pancreas trans-differentiation to hepatocytes—A physiological response to elevated glucocorticoid *in vivo*

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ABSTRACT

Damage or ectopic expression of some growth factors can lead to the appearance of hepatocyte-like cells within the pancreas. Since glucocorticoids promote liver hepatocyte phenotype *in vitro*, the effect of glucocorticoid on pancreatic differentiation *in vivo* was examined. Treatment of rats with glucocorticoid for 25 days at levels that significantly inhibited weight gain resulted in the appearance of acinar cells expressing cytokeratin 7 and hepatocyte markers glutamine synthetase, carbamoyl phosphate synthetase and cytochrome P450 2E (the nomenclature employed is that given at http://drnelson.utmem.edu/CytochromeP450.html). Using a plastic pancreatic acinar cell line, this response was shown to be associated with changes in the regulation of WNT signalling-related gene expression and a repression of WNT signalling activity. These data suggest that a pathological response of the pancreas *in vivo* to elevated glucocorticoid is a differentiation of exocrine pancreatic cells or pancreatic progenitor cells to an hepatocyte-like phenotype.

1. Introduction

Glucocorticoids are a class of steroid hormone primarily secreted from the adrenal gland, which function in the regulation of intermediary metabolism (e.g. gluconeogenesis in the liver [1]). In the embryo, glucocorticoid concentrations are maintained at relatively low levels by placental 11β hydroxysteroid dehydrogenase type 2, which converts cortisol (or corticosterone in rodents) to inactive cortisone (11 dehydrocortisone in rodents) [2]. The late gestational development of the foetal hypothalamic–pituitary–adrenal (HPA) axis appears to have a maturation role on tissue development and differentiation, most notably in the lung [3–5]. The significance of physiological peri-natal exposure to glucocorticoid is underscored by the effects of abnormally elevated exposure at this time, which is a determinant in susceptibility to hypertension, insulin resistance, type 2 diabetes and cardiovascular disease in adult life [2].

The appearance of hepatic-like cells in the rodent acinar pancreas after extensive pancreatic necrosis [6,7] or in response to the over-expression of growth factors associated with wound healing [8,9] suggests that pancreatic "hepatocytes" are derived from ductal progenitor cells [10,11]. However, glucocorticoid treatment of rodent acinar cells *in vitro* also results in their trans-differentiation into hepatocyte-like cells [12–17], suggesting that acinar cells have the plasticity to trans-differentiate. However, this plasticity may be a specific response to *in vitro* culture environments which bear little resemblance to the *in vivo* situation. To examine this question, adult rats were treated with glucocorticoid and their pancreata examined for evidence of hepatocyte-like cells. We show for the first time that elevated glucocorticoid exposure leads to the presence of pancreatic hepatocytes in rats *in vivo*. Using a model "AR42J B-13" cell system we show that this response is likely associated with WNT signalling changes, a prominent pathway controlling embryonic development of tissues, including the liver.

2. Materials and methods

2.1. Animal studies

Male Sprague–Dawley rats (150–175 g body weight) were purchased from Harlan. Rats were administered 10 mg dexamethasone-21-phosphate (Dex-21-P)/kg body weight from a

Abbreviations: BMP-4, bone morphogenic protein-4; CK-7, cytokeratin 7; CPS, carbamoyl phosphate synthase I; CYP, cytochrome P450; DEX, dexamethasone; Dex-21-P, dexamethasone-21-phosphate; DKK, dickkopf; α-FP, α-foetoprotein; Fzd, frizzled receptor; GAPDH, glyceraldehyde phosphate dehydrogenase; GS, glutamine synthetase; GSK3, glycogen synthase kinase 3; γ-GT, γ-glutamyl transferase; H and E, haematoxylin and eosin; HEX1, hematopoietically expressed homeobox gene; Lrp5, low density lipoprotein related protein 5; PBS, phosphate buffered saline; sFRP, secreted frizzled-related protein; Tcf/Lef, T cell factor/lymphoid enhancer factor.

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5 mg/ml PBS stock by *i.p.* injection up to 3 times per week. Control rats received PBS vehicle (2 ml/kg body weight) by *i.p.* injection. Body weights were monitored daily and injections modified to ensure that animals did not lose weight. After 25 days, rats were killed by CO_2 asphixiation and cervical dislocation and tissues removed for analysis. Rat embryos were obtained from pregnant females (the time at which the vaginal plug found taken as the time of conception) after CO_2 asphyxiation and cervical dislocation. Embryos were pooled and snap frozen in liquid nitrogen prior to homogenisation and extraction of RNA.

Table 1
DNA oligonucleotide sequences employed in RT-PCR or PCR genotyping.

Oligo ID	5′–3′ sequence	Annealling conditions °C (35 cycles)	Comments
rCYP2EUS rCYP2EDS	TCGACTACAATGACAAGAAGTGT CAAGATTGATGAATCTCTGGATCTC	42.0	Will amplify rat CYP2E1 (NM_031543.1) cDNA sequence of 525 bp
rALBUS1 rALBDS1	CGTCAGAGGATGAAGTGCTC CTTAGCAAGTCTCAGCAGCAG	57.0	Will amplify rat albumin (NM_134326.2) cDNA sequence of 471 bp
rCPSUS rCPSDS	ATACAACGGCACGTGATGAA GCTTAACTAGCAGGCGGATG	55.0	Will amplify rat CPS (NM_017072.1) cDNA sequence of 390 bp
rmAMYLASEUS rAMYLASEDS	CAAAATGGTTCTCCCAAGGA AAGGGCTCTGTCAGTAGGCA	57.0	Will amplify rat pancreatic amylase 2 (NM_031502.1) cDNA sequence of 224 bp
rmhGAPDHUS rmhGAPDHDS2	TGACATCAAGAAGGTGGTGAAG TCTTACTCCTTGGAGGCCATGT	50.0	Will amplify rat (NM_017008), human (NM_002046) or mouse (NM_008084) glyceraldehyde 3 phosphate dehydrogenase cDNA sequence of 243 bp
rα-FPUS2 rα-FPDS2	CTACCACTGTCTGGGATGAAGC TGTCTGGAAGCATTCCTCCATG	57.0	Will amplify rat α-foetoprotein (NM_012493.1) cDNA sequence of 633 bp
rγ-GTUS rγ-GTDS	CAGCTTTTGCCCAATACCACAACAG AACCACCTGACGTTCGAACGAC	52.0	Will amplify rat (NM_053840.2) γ-glutamyltransferase 1, cDNA sequence of 142 bp
rHEX1US rHEX1DS	GCGGAGCAGCCATGCAGTTCCCGCA CGGCCGATGACTGTCATCCAGCATTAAAA	53.0	Will amplify rat HEX (NM_024385.1) cDNA sequence of 840 bp
rCD44US rCD44DS	TCATGGACAAGGTTTGGTGGCA GGTAGGTCTGTGACTGATGTACAGTC	50.0	Will amplify rat CD44 (NM_012924.2) cDNA sequence of 421 bp
rWNT1US rWNT1DS	CCCAGCAGGAAAAACCTACA AAAAAGGCTGGCAGACAAGA	66.8	Will amplify rat wingless-type MMTV integration site family member 1 (NM_001105714.1) cDNA sequence of 260 bp
rWNT2bUS rWNT2bDS	CATCTGGAGGTTTGAAGGGA TGTCTGCAGTGAAGGGACAG	66.8	Will amplify predicted rat wingless-type MMTV integration site family, member 2B (XM_001066411.1) cDNA sequence of 230 bp
rWNT3aUS rWNT3aDS	ACTTAAGGATCCAATGGGGG GGCAAGTTCTGCCAAGAGTC	65.4	Will amplify rat wingless-type MMTV integration site family, member 3A (NM_001107005.1) cDNA sequence of 355 bp
rWNT5aUS rWNT5aDS	CCTTCGCCCAGGTTGTAATA AGTGTTGTCCACTGTGCTGC	65.4	Will amplify rat wingless-type MMTV integration site family, member 5A (NM_022631.1) cDNA sequence of 260 bp
rWNT9aUS rWNT9aDS	GGGAAAGAGCCTACCAGTCC GAGAAATCACAGGCTGCACA	66.8	Will amplify rat wingless-type MMTV integration site family, member 9A (NM_001105783.1) cDNA sequence of 354 bp
rDKK1US rDKK1DS	TTACTGTGGGGAAGGTCTGG CAGGGGAGTTCCATCAAGAA	66.8	Will amplify rat dickkopf homolog 1 (NM_001106350.1) cDNA sequence of 268 bp
rsFRPUS rsFRPDS	TCTTCCTCTGCTCGCTCTTC CACTGTTGTACCTTGGGGCT	66.8	Will amplify predicted rat secreted frizzled-related protein 1 (XM_001072532.1) cDNA sequence of 224 bp
rFzd1US rFzd1DS	GTTCTGCAAAAGCTTCCGTC CTTCCTCTACAGTCCGCAGG	67.0	Will amplify rat frizzled homolog 1 (NM_021266.3) cDNA sequence of 278 bp
rLrp5US rLrp5DS	TGCTGCACAGAACATTGTCA CCCAGTACAGCTTCTGCTCC	67.0	Will amplify rat low density lipoprotein receptor-related protein 5 (NM_001106321.2) cDNA sequence of 344 bp
rGSK3αUS rGSK3αDS	AGGTGGCTTACACCGACATC ATGTAGGACACGTTGGGCTC	66.8	Will amplify rat glycogen synthase kinase 3 alpha (NM_017344.1) cDNA sequence of 493 bp
rGSK3βUS rGSK3βDS	TCCGATTGCGGTATTTCTTC AATGTCTCGATGGCAGATCC	66.0	Will amplify rat glycogen synthase kinase 3 beta (NM_032080.1) cDNA sequence of 218 bp
rmβ-catenUS rmβ-catenDS	CTGACCAGTTCCCTCTTCA CCATCTCATGCTCCATCATA	67.0	Will amplify rat (NM_053357.2) and mouse (NM_007614.2) catenin (cadherin associated protein), beta 1 cDNA sequence of 187 bp and 187 bp, respectively
rTCF7US rTCF7DS	TGACCTACCTACTGGTGGGC GCTGAAGGATGAGTTCTGGC	66.0	TCF1—Will amplify predicted rat transcription factor 7, T-cell specific (XM_001073458.1) cDNA sequence of 217 bp
rLEFUS rLEF1DS	GACGAGCACTTTTCTCCAGG TCCCCTGAAAGTGAAGATGG	66.8	Will amplify rat lymphoid enhancer binding factor 1 (NM_130429.1) cDNA sequence of 224 bp
rTCF L1US rTCF L1DS	GGATTGGGTTGGCAGTTCTA CGCTTTGTACCTGCAGATGA	66.9	TCF3—Will amplify rat transcription factor 3 (XM_232064.4) cDNA sequence of 203 bp
rTCF7 L2US rTCF7 L2DS	GCCTCGTCATCTCCCAATTA TCCATATCCTGAGCCCAGAC	67.0	TCF4—Will amplify rat transcription factor 4 (NM_053369.1) cDNA sequence of 210 bp
rchibbyUS rchibbyDS	GGAAGTCGGCCTCTCTCTCT TCTGAAAGCATGTCCAGCAG	65.0	Will amplify rat chibby homolog 1 (NM_145676.1) cDNA sequence of 265 bp

2.2. Immunohistochemistry

Tissues were either snap frozen in liquid nitrogen or fixed in formalin and processed for immunohistochemistry as previously outlined [18]. Paraffin-embedded tissue sections were stained with haematoxylin and eosin (H and E) [18]. Immunostaining was performed essentially as previously outlined [19,20] with liver and pancreas tissue embedded within the same block and sections stained together on the same slide to ensure identical and controlled staining conditions.

2.3. Western blotting

Western blotting was performed essentially as previously outlined [20]. Anti-CYP2E1 was generously provided by Prof. Magnus Ingelman-Sundberg (Karolinska Institute, Sweden). Anti-sera to carbamoyl phosphate synthase-I (CPS) and albumin were purchased from Abcam (Cambridge, UK).

2.4. AR42J B-13 cell culture, treatments and transfection

AR42J B-13 cells were routinely cultured in Dulbecco's modified Eagle Medium (DMEM with 1000 mg/l glucose) supplemented with 10% (v/v) fetal calf serum, 80 units/ml penicillin and 80 μg/ml and sub-cultured using trypsin at a ratio of 3:1 by standard methods. All cells were incubated at 37 °C in an humidified incubator gassed with 5% CO_2 in air. Dexamethasone (DEX, Sigma Chem Co., Poole, UK) was added to medium from 1000-fold concentrated ethanol vehicle solvated stocks such that medium concentration was 10 nM, control cells were treated with ethanol alone. For paracetamol toxicity experiments, paracetamol was dissolved directly into the culture medium.

Cells were transfected with a renilla expression vector (RL-TK) under control of the thymidine kinase (TK) promoter from Promega (Southampton, UK) and either the T cell factor/lymphoid enhancer factor (TCF) "topflash" vector (TCF_6-TK) or "fopflash" vector (an identical vector without TCF response elements (-TK)) as control, using Effectene (Qiagen, Southampton, UK) at a ratio of 6:1 (TK plasmid: RL-TK plasmid). The TCF_6-TK vector contains a concatamer of 6 TCF response elements upstream of the TK promoter. Cells were treated 24 h after transfection, and harvested after a further 24 h. Luciferase and renilla activities were determined using the Dual-Luc kit (Promega, Southampton, UK) and a luminometer.

2.5. RT-PCR analysis of gene expression

Total RNA was purified from cells using Trizol (Invitrogen, Paisley, UK) and RT-PCR performed and analysed essentially as previously outlined [20] (primer sequences are given in Table 1).

2.6. Microarray analysis

Trizol-purified RNA was further purified using RNeasy kits (Qiagen) essentially according to the manufacturers instructions and 10 μg was converted to cDNA, labelled and hybridized to the Affymetrix Rat RG-U34A GeneChips as described [21]. Probesets intensities were averaged using Microarray Suite 5.0 (Affymetrix), the mean of each array was globally normalised to 500 and these data were imported into GeneSpring v6.0 (Silicon Genetics). The following normalisations were applied: data transformation of measurements below 0.01–0.01; per-chip and per-gene normalisations. Gene lists were created in GeneSpring by filtering for differences in fold expression between pair wise treatment combinations.

2.7. Statistics

The Student's *T* test (two tailed) was used to test for replicate statistical significance.

3. Results

To determine whether exogenous glucocorticoid treatment affects pancreatic differentiation, the synthetic glucocorticoid dexamethasone 21-phosphate (Dex-21-P) was administered over a 25-day period. This dose was calculated to significantly increase circulating glucocorticoid levels. Dexamethasone is a relatively pure and potent glucocorticoid. Both dexamethasone and its 11 keto form bind and activate the human GR [22]. Although these compounds also bind to the human mineralocorticoid receptor and promote translocation to the nucleus, dexamethasone only weakly transcriptionally activates the human mineralocorticoid receptor [22] and is likely to mediate its effects primarily via the GR. However the dose of Dex-21-P remains approximately 10-fold lower than the levels required to maximally induce the expression of glucocorticoid-inducible (PXR-dependent) cytochrome P450 3A1 (CYP3A1) [23]. Fig. 1A shows that Dex-21-P inhibited weight gain in the animals. Dex-21-P also resulted in a statistically significant 24% increase and 34% decrease in relative liver and pancreas organ weights respectively. Microscopic examination of the liver showed that the liver had accumulated fat but that there was not a significant depletion of liver glycogen in response to Dex-21-P (Fig. 1B). These data indicate that the Dex-21-P

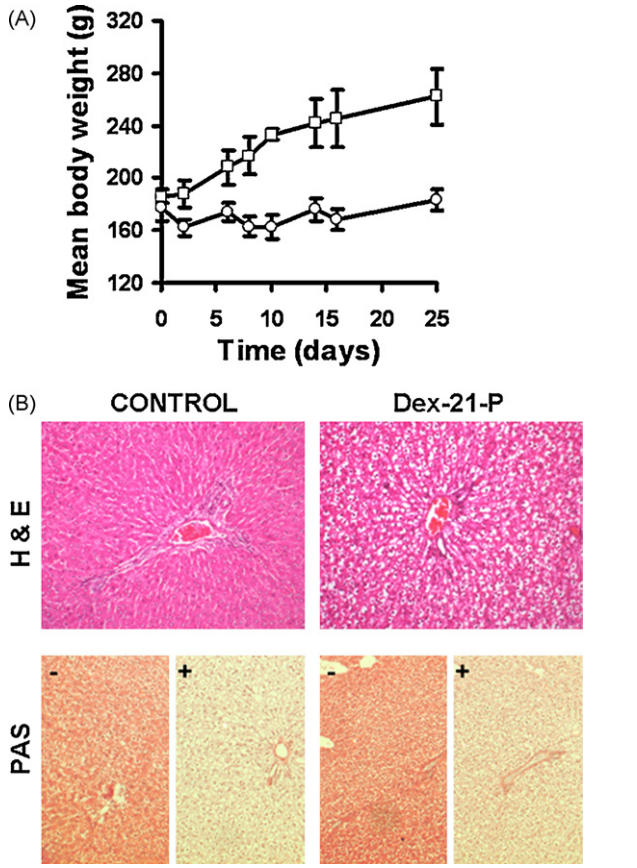

Fig. 1. Effects of Dex-21-P on rat body weight and liver histology. (A) Mean body weights and standard deviation for rats administered control (PBS vehicle, -□-) or Dex-21-P (-○-) as outlined in experimental procedures (5 rats/group). (B) Liver sections from PBS vehicle (CONTROL) and Dex-21-P treated rats after 25 days, upper panels H and E stains; lower panels PAS stains with (+) or without (−) prior diastase treatment.

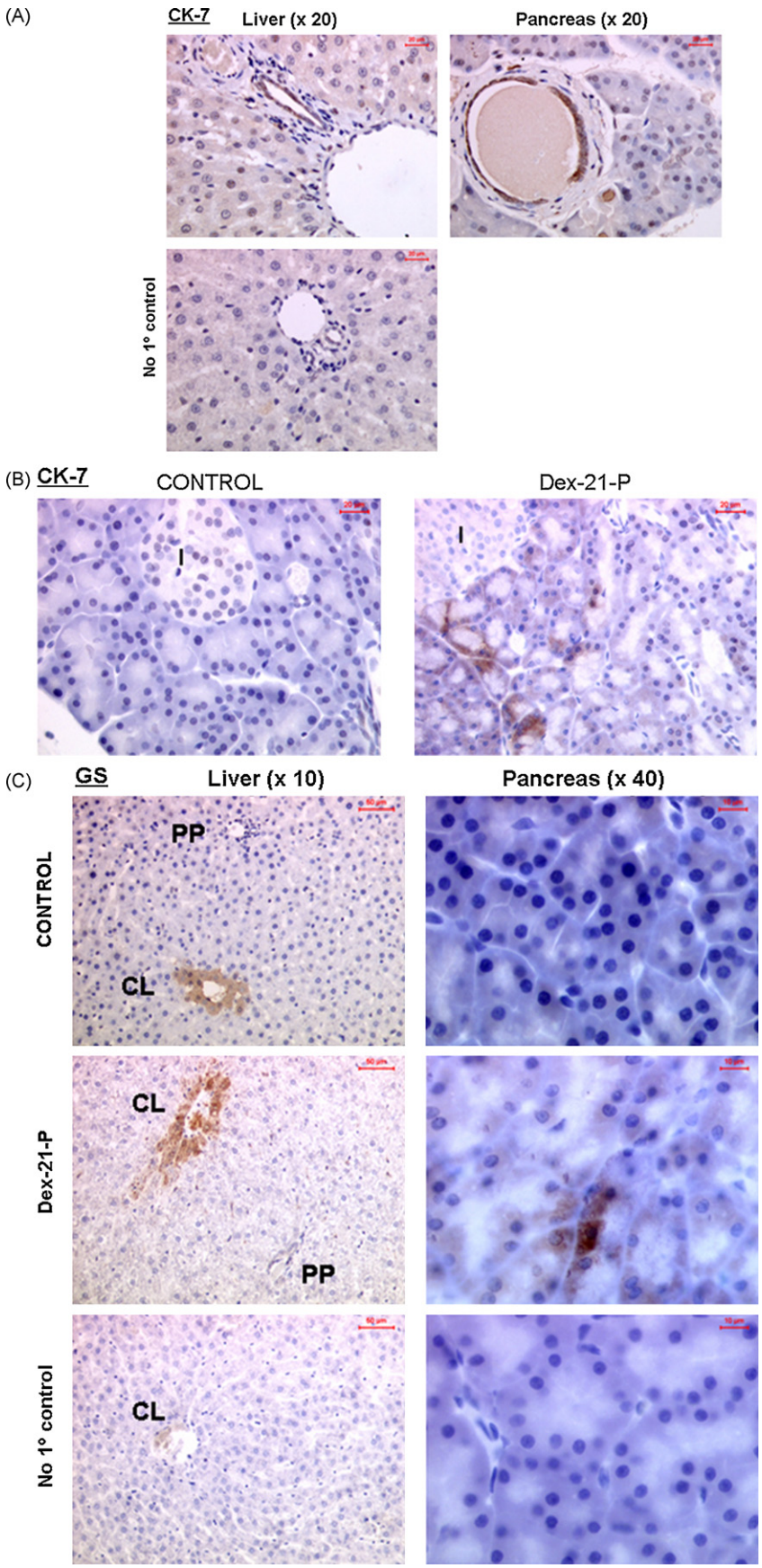

Fig. 2. Effect of Dex-21-P treatment on ductal and hepatic marker gene expression in the pancreas. (A) Rat liver and pancreas sections immunostained for CK-7. No. 1° control—sections stained without addition of anti-CK-7 anti-sera. (B) Pancreas sections from PBS treated (control) and Dex-21-P treated rats immunostained for CK-7. I, pancreatic islet. (C) Liver and pancreas sections from PBS treated (control) and Dex-21-P treated rats immunostained for GS. CL, centrilobular region of liver lobule; PP, periportal region of lobule. No. 1° control—sections stained without addition of anti-GS anti-sera.

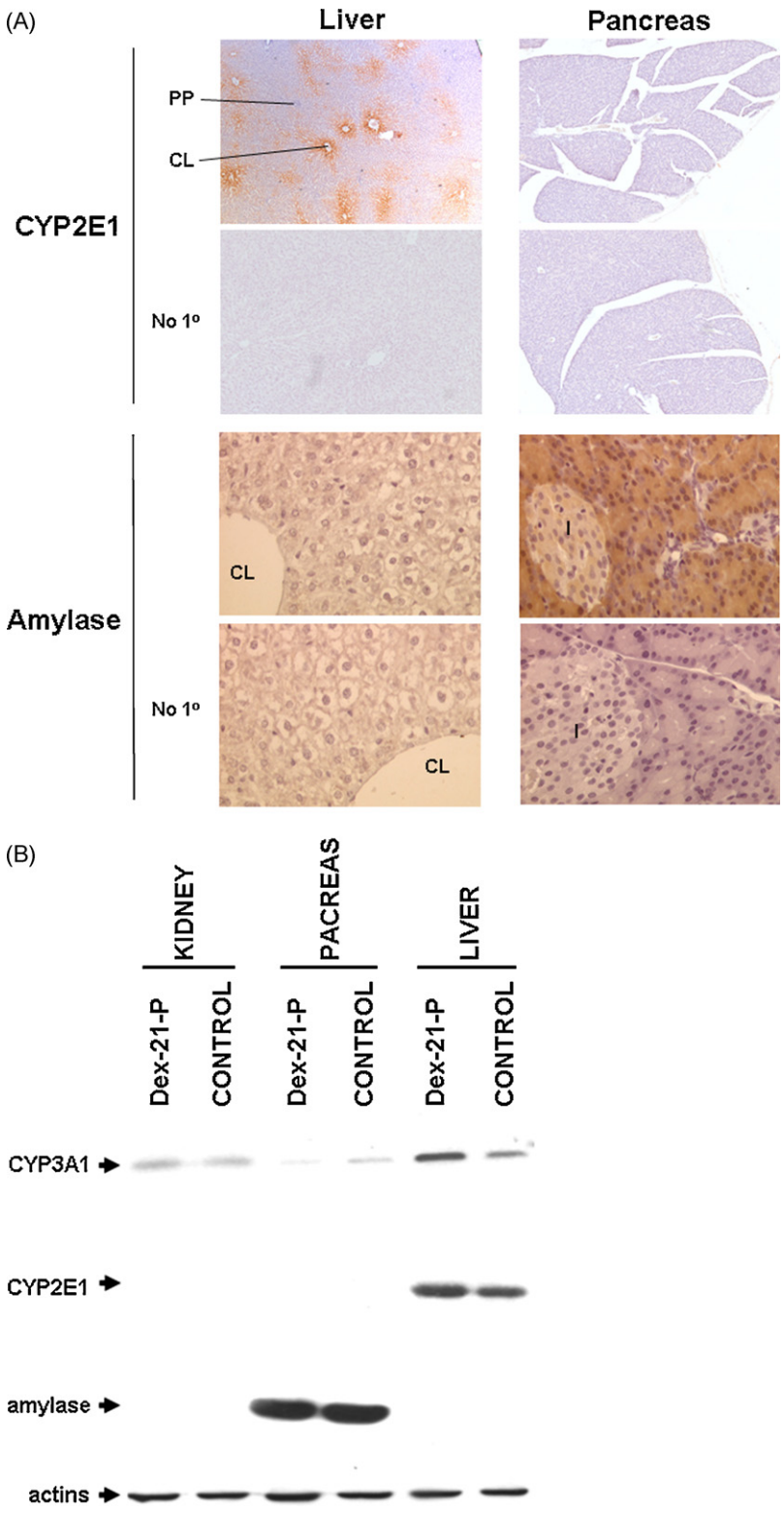

Fig. 3. Gross effect of Dex-21-P treatment on CYP2E1 expression in the pancreas. (A) Low power field of liver and pancreas sections immunostained for CYP2E1 or amylase. Sections stained without addition of primary antibody indicated as "No. 1°". I, pancreatic islet; PP, periportal region of liver; CL, centrilobular region of liver lobule. (B) Western blot of homogenised tissues prepared from rats treated for 25 days with PBS vehicle (CONTROL) or Dex-21-P. 15 μg of total protein were loaded in each lane and probed for the expression of the indicated protein.

treatment resulted in significant metabolic and physiological alterations.

Macroscopically, no obvious differences where observed between the pancreata from control and Dex-21-P-treated rats. Sections of pancreas where initially examined for the expression the ductal marker cytokeratin 7 (CK-7) [24]. In control animals, expression was restricted to pancreatic ductal cells and bile duct cells in the liver (Fig. 2A). However, Fig. 2B shows that acinar cells within the pancreas expressed CK-7 after Dex-21-P treatment. Glutamine synthetase (GS) is expressed in several tissues including the liver (but

with expression markedly restricted to downstream centrilobular hepatocytes, see Fig. 2C). GS expression is strongly compartmentalized from liver-specific carbamoyl phosphate synthase I (CPS) that is expressed in the periportal region [25]. Both GS (Fig. 2C) and CPS (data not included) were expressed within selected areas of the acinar region in Dex-21-P treated animals.

Using the highly specific CYP2E1 anti-sera (Fig. 3A and B), it can be seen that Dex-21-P treatment did not cause a widespread change in the levels of CYP2E1 or amylase (an exocrine pancreatic cell marker). However, Fig. 4A clearly indicates the presence within pancreatic acinar tissue of cells staining positive for CYP2E1 in Dex-21-P treated animals. Under the staining conditions employed, the hepatic lobule staining patterns observed in Fig. 3A were evident suggesting that positive cells in the pancreas are expressing "high" centrilobular levels of CYP2E1. Blinded examination of sections from 5 rats in each group indicate that there was a significant – above background – number of CYP2E1-positive pancreatic acinar/heptocyte-like cells in Dex-21-P-treated rats (Fig. 4B).

WNT signalling is a prominent pathway regulating development and differentiation and is known to impact on liver development [26]. Since relatively few cells within the pancreas trans-differentiate into hepatocyte-like cells *in vivo* in response to Dex-21-P and such changes were not detectable in whole tissue homogenates (Fig. 3B), a model of this process was used to examine the potential mechanism(s) involved. The AR42J B-13 cell line is unusual in that it readily trans-differentiates into hepatocyte-like cells *in vitro* in response to glucocorticoid [11–16] (see Fig. 5). To

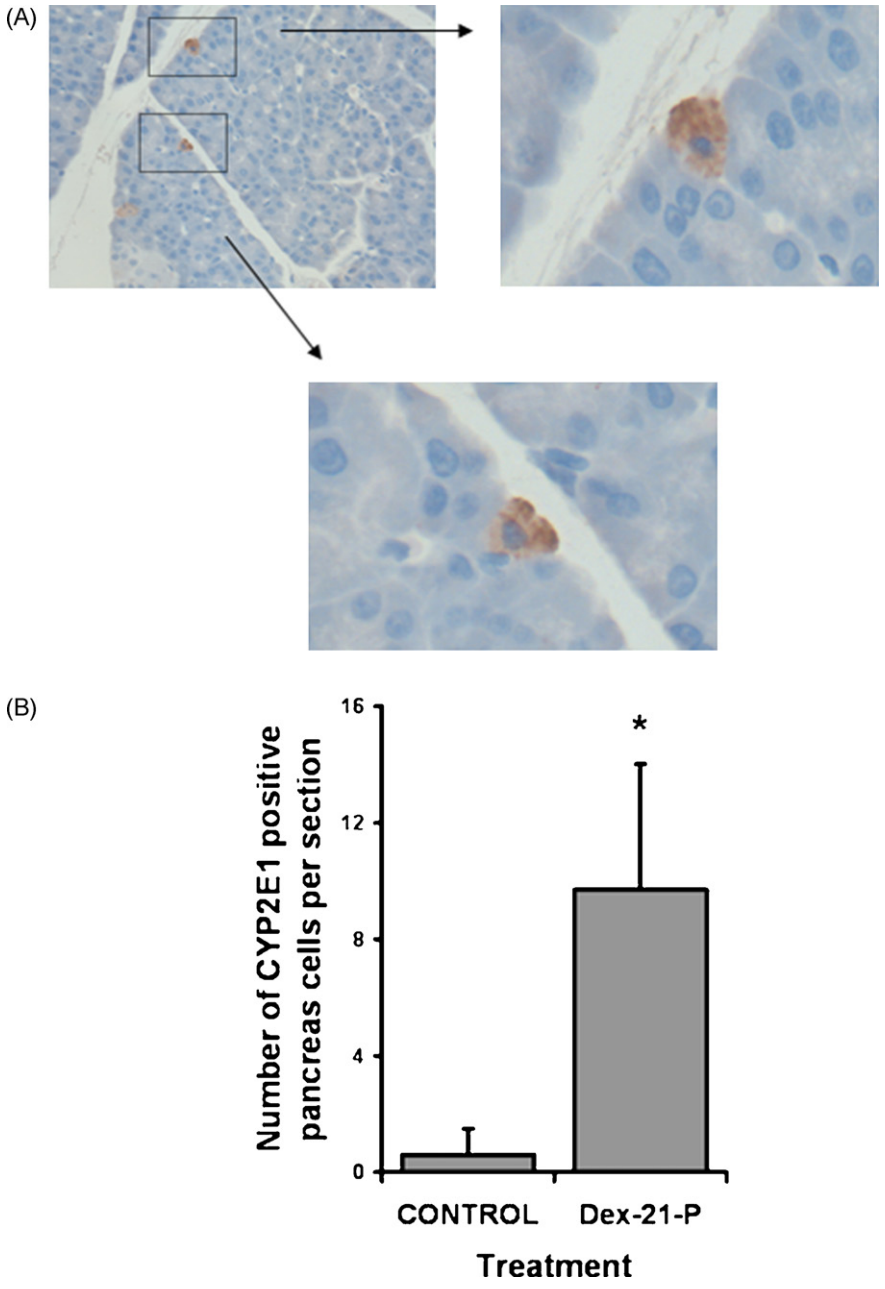

Fig. 4. Identification of pancreatic hepatocytes in Dex-21-P treated rats. (A) Selected fields from sections of pancreas from Dex-21-P treated rats stained with CYP2E1 antisera. Box outlines indicate field of view magnified in adjacent photomicrographs. (B) Numbers of CYP2E1 positive pancreas cells identified in PBS vehicle (control) and Dex-21-P treated rats. Data are expressed as the mean and standard deviation number of cells visible in section tissue from 5 animals per treatment. *Significantly different $P > 95\%$ from control using Student's T test (two tailed). All results are typical of at least 5 animals per treatment.

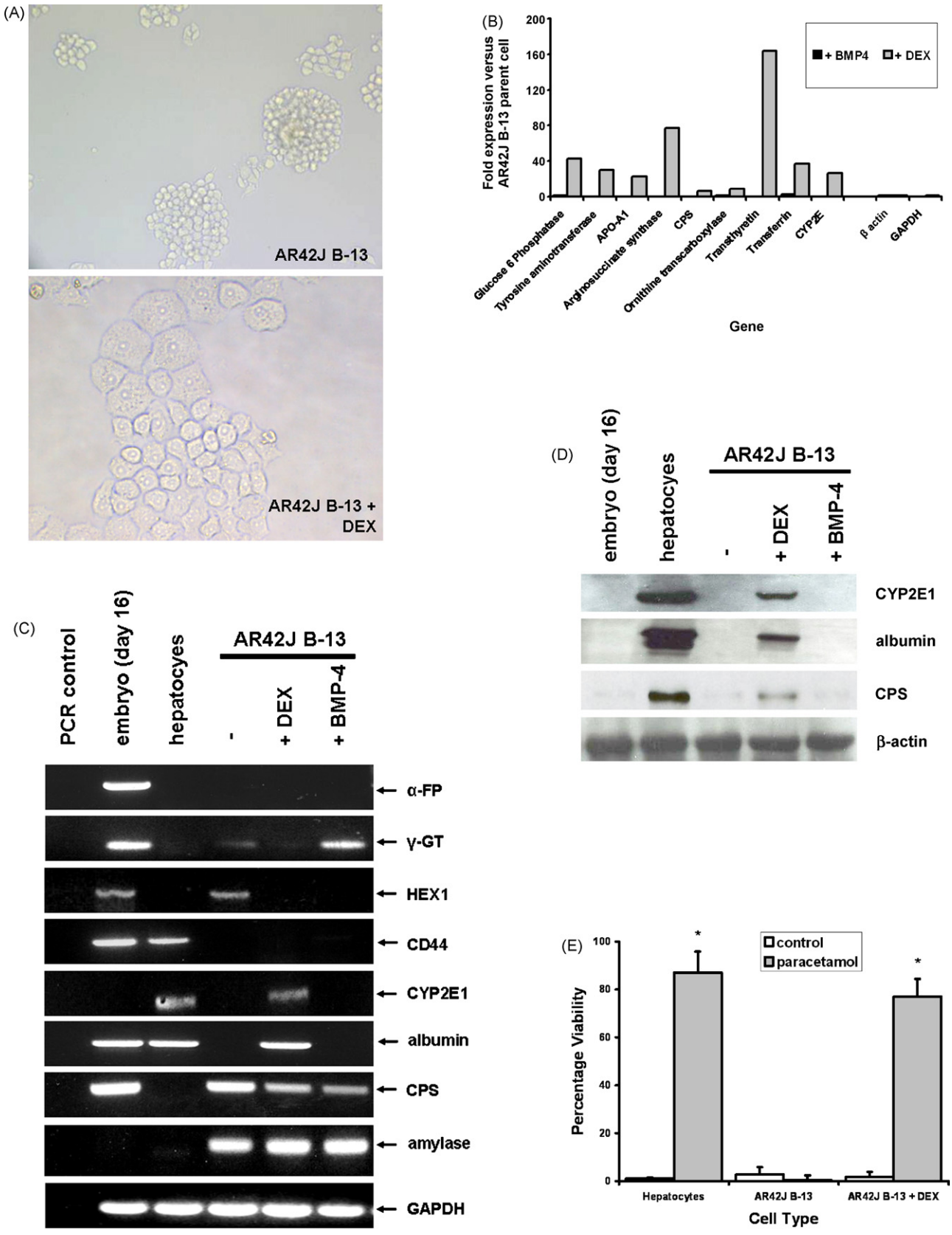

Fig. 5. AR42J B-13 cells trans-differentiate into hepatocyte-like cells in response to glucocorticoid treatment. AR42J B-13 cells were treated with DEX or 10 ng/ml BMP-4 for between 9 and 14 days. (A) Typical phenotype of untreated and DEX-treated cells. (B) RNA was analysed by microarray and data are expressed as fold change versus untreated AR42J B-13 cells for the indicated gene after BMP-4 (black bars) or DEX (grey bars) treatment. (C) RT-PCR analysis for the indicated mRNA transcript performed. PCR control, amplification without input RNA. (D) Western blot for the indicated protein (20 μg protein/lane). (E) Effect of 20 mM paracetamol on cell viability as determined by trypan blue exclusion after 24 h treatment. Data are the mean and standard deviation of 3 wells from the same experiment, *significantly different $P > 95\%$ from control using Student's T test (two tailed). All data are typical of at least 4 separate experiments.

date, trans-differentiation has only been associated with an induction of C/EBPβ [11]. The role of WNTs in this process has not been examined.

Fig. 5B demonstrates that a range of hepatocyte markers were significantly elevated in AR42J B-13 cells after treatment with dexamethasone (DEX) whereas bone morphogenetic protein 4 (BMP-4), which promotes the development of vascular-like structures in AR42J B-13 cells (not shown) had no effect on the expression of these genes. These data are supported by Fig. 5C and D which demonstrate that hepatic markers CYP2E1, albumin and carbamoyl phosphate synthase I (CPS) were expressed in AR42J B-13 cells after DEX treatment. Interestingly, the endodermal marker HEX1

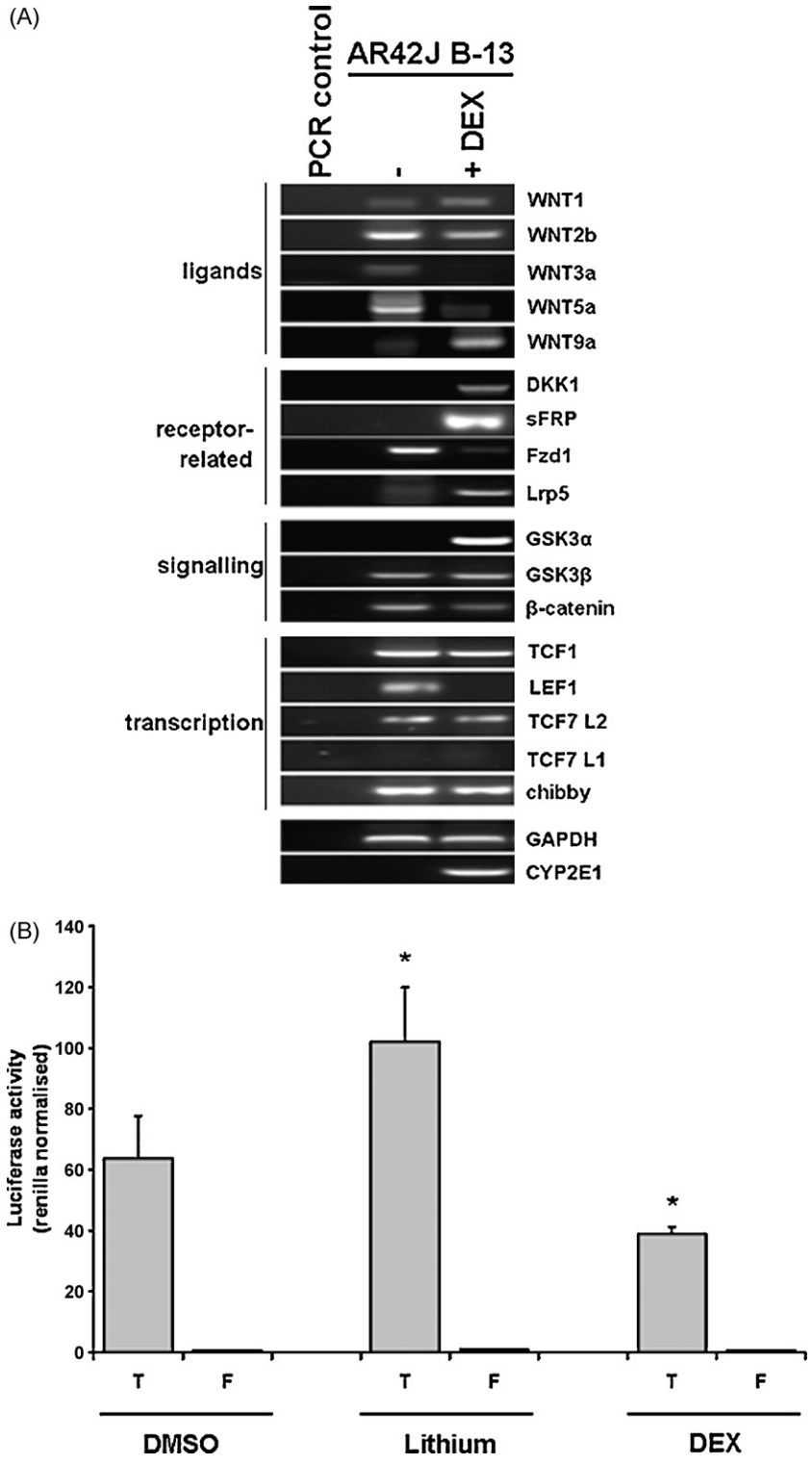

Fig. 6. WNT signalling changes in AR42J B-13 cell trans-differentiation into hepatocytes. (A) RT-PCR analysis for the indicated mRNA transcript performed. PCR control, amplification without input RNA. (B) AR42J B-13 cells were transfected with either topflash (T) or foplash (F) vectors and treated with 0.1% (v/v) DMSO vehicle control or 10 mM lithium or 10 nM dexamethasone and reporter gene expression determined. *Significantly different $P > 95\%$ from DMSO control using Student's T test (two tailed). Data are the mean of 3 separate transfections from the same experiment. All results typical of at least 3 separate experiments.

[27] was constitutively expressed in AR42J B-13 cells and down-regulated after treatment with DEX (Fig. 5C). The foetal hepatic marker α-foetoprotein (α-FP) was not expressed (Fig. 5C). "Small hepatocytes" are a population of hepatocytes within adult liver reported to be proliferative *in vitro* (in contrast to mature adult hepatocytes) [28,29]. Recent work has identified that the surface marker CD44 is specifically expressed in small hepatocytes *in vitro* [30]. Fig. 5C indicates that CD44 mRNA was readily detectable in embryo and adult hepatocyte RNA but was not expressed in AR42J B-13 cells with or without treatment with DEX or BMP-4. Fig. 5D confirms that the hepatic markers CYP2E1, albumin and CPS were expressed at high levels since the more quantitative technique of Western blotting demonstrates that DEX treatment resulted in levels of proteins similar to those present in freshly isolated rat hepatocytes. DEX treatment therefore resulted in an acquired sensitivity to paracetamol in AR42J B-13 cells – as observed in cultured hepatocytes – indicating that CYP2E1 expression (which activates paracetamol to an hepatotoxic product [31]) was not limited to protein expression but to functional activity (Fig. 5E).

These data therefore show that DEX promoted a co-ordinated trans-differentiation of AR42J B-13 cells into hepatocyte-like cells and that changes were not limited to morphological changes but included a functional hepatocyte-like phenotype.

RT-PCR screens confirm that AR42J B-13 cells expressed 15 out of the 19 WNT genes at the mRNA level (data not shown) suggesting that AR42J B-13 cells likely regulate WNT signalling in an autocrine manner. Focussing on WNTs predicted to play a role in liver development [26], it can be seen that AR42J B-13 cells expressed WNT1, WNT2b, WNT3a and WNT5a mRNAs and that the levels were repressed by DEX treatment (Fig. 6A). In contrast, AR42J B-13 cells up-regulated WNT9a in response to DEX treatment (Fig. 6A). WNT proteins interact with frizzled receptors (Fzd) [32], an interaction which is modified by a range of proteins, including dikkopf (DKK) – which inhibits Fzd co-receptor protein low density lipoprotein related protein 5 (Lrp5) [33] – and secreted frizzled-related protein (sFRP), which bind to WNTs and antagonizes WNT function [34]. Fig. 6A shows that Fzd was down-regulated and both DKK and sFRP up-regulated by DEX treatment in AR42J B-13 cells.

Fzd receptor activation leads to glycogen synthase kinase 3 (GSK3) phosphorylation and inactivation, blocking its constitutive phosphorylation of β-catenin which targets it for degradation [32]. Fig. 6A shows that GSK3α mRNA was induced whereas GSK3β mRNA expression is relatively unaffected by DEX treatment. The levels of β-catenin mRNA were suppressed by DEX treatment (Fig. 6A).

The end-point for WNT signalling is the activity of the Tcf/Lef transcription factors, which are regulated by the levels of nuclear β-catenin [32]. Fig. 6A shows that 3 of the 4 Tcf/Lef transcription factors were expressed in AR42J B-13 cells and expression was either retained or repressed by DEX treatment (Fig. 6A). Chibby interacts with the C-terminal region of β-catenin and inhibits β-catenin-mediated transcriptional activation [35]. Expression of chibby was maintained in AR42J B-13/H cells (Fig. 6A).

Fig. 6B confirms that WNT signalling was functional in AR42J B-13 cells since there was a significantly higher level of topflash-dependent reporter gene expression compared AR42J B-13 cells transfected with fopflash. Treatment with the GSK3 inhibitor lithium significantly increased topflash reporter gene expression, indicating that the canonical mechanism of Tcf/Lef regulation was functional in AR42J B-13 cells (Fig. 6B). DEX treatment significantly reduced topflash reporter gene expression prior to any overt phenotypic change (Fig. 5A), suggesting that repression of WNT signalling is an early event in AR42J B-13 trans-differentiation to hepatocyte-like cells.

4. Discussion

The data in this paper demonstrate for the first time that adult rat pancreatic acinar tissue trans-differentiates into hepatocyte-like cells in response to elevated glucocorticoid exposure *in vivo*. Steroids have important regulatory effects on tissue differentiation through their ability to imprint adult tissue expression patterns *via* neonatal exposure. One of the best characterised examples is the effect of androgen exposure in the neonate on sexually dimorphic liver cytochrome P450 gene expression in the adult [36]. Glucocorticoids have potent effects upon tissue development, most notably in lung maturation [3–5]. Less is known about the precise role of glucocorticoid in hepatocyte differentiation although empirical observations over many years has resulted in its common inclusion in culture media designed to maintain primary hepatocyte differentiation or promote stem cell differentiation into hepatocytes *in vitro*. Recent research has shown that pancreatic acinar cells can be re-programmed into islet beta cells through the over-expression of 3 transcription factors and that this alteration did not require a regression to a plastic progenitor-like state [37]. Our data suggest that glucocorticoid exposure may achieve a similar outcome although the cells are re-programmed to an hepatic state.

The AR42J B-13 pancreatic cell line has been shown to trans-differentiate into hepatocyte-like cells *in vitro* in response to glucocorticoid treatment [12–16]. This phenomenon is unique to this cell line and therefore could be a response specific to the *in vitro* environment and/or in any case, an aberrant un-physiological response to glucocorticoid. The data presented in this paper indicate that this response is a real physiological response of either some acinar cells or a resident progenitor cell to high but clinically relevant concentrations of glucocorticoid. This paper therefore demonstrates that glucocorticoid hormone levels regulate cell differentiation and that elevated levels alone are sufficient to re-program the differentiation of some cells. This may have important clinical implications in patients in which glucocorticoid homeostasis is lost (e.g. Cushing's disease) or for patients who require long term systemic glucocorticoid therapy.

Elevated systemic glucocorticoid due to Cushing's disease or *via* therapy frequently results in diabetes, indicating that glucocorticoids have effects on the pancreas [38,39]. Little is known about the effects on the exocrine pancreas (e.g. malabsorption) although these may be masked by the appearance of diabetes. However, a significant proportion of ageing transgenic mice with chronic elevated circulating glucocorticoid initially show a range of Cushing's disease symptoms (such as weight gain and hair loss) but then begin to rapidly lose weight [40]. This weight loss is associated with marked exocrine pancreatic trans-differentiation into hepatocyte-like cells which may lead to a loss of exocrine function and malabsorption of dietary nutrients although this remains to be determined.

The expression of WNT signalling-related genes suggests that the WNT pathway is functional in AR42J B-13 cells and results in the constitutive activation of Tcf/Lef transcriptional activity, confirmed by promoter-reporter gene transfection experiments. However, it is likely that the AR42J B-13 cell represents a plastic intermediate of the acinar cell and that acinar cells which trans-differentiate to hepatocyte-like cells have to re-program to a AR42J B-13 phenotype before differentiation to hepatocyte-like cells. The changes in expression of many of the genes associated with WNT signalling support a silencing of WNT activity in AR42J B-13/H cells. WNT expression and WNT receptor (Fzd) expression generally decrease whereas antagonists of WNT receptor activity increase when the cells trans-differentiate into AR42J B-13/H cells. GSK3 expression also increased in AR42J B-13/H cells. Although expression of mRNA gives no indication of protein activity, increase in GSK3 expression is more likely to promote β-catenin phosphorylation, which targets

β-catenin for degradation resulting in reduced Tcf/Lef activation. Tcf/Lef mRNA expression was also reduced.

The data in this paper therefore show that elevated glucocorticoid exposure can lead to a reprogramming of adult rat pancreatic acinar cells into hepatocyte-like cells and suggest that WNT signalling may play a pivotal part in the process.

Conflict of interest

We here declare that there are no conflicts of interest for any of the authors or the institution.

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