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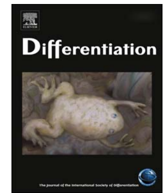

Glucocorticoid-induced pancreatic-hepatic trans-differentiation in a human cell line in vitro

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ABSTRACT

The rodent pancreatic AR42J-B13 (B-13) cell line differentiates into non-replicative hepatocyte-like cells in response to glucocorticoid mediated via the glucocorticoid receptor (GR). The aims of this study were to identify a human cell line that responds similarly and investigate the mechanisms underpinning any alteration in differentiation. Exposing the human pancreatic adenocarcinoma (HPAC) cell line to 1–10 μM concentrations of dexamethasone (DEX) resulted an inhibition of proliferation, suppressed carcinoembryonic antigen expression, limited expression of pancreatic acinar and hepatic gene expression and significant induction of the constitutively-expressed hepatic CYP3A5 mRNA transcript. These changes were associated with a pulse of genomic DNA methylation and suppressed notch signalling activity. HPAC cells expressed high levels of GR transcript in contrast to other nuclear receptors – such as the glucocorticoid-activated pregnane X receptor (PXR) – and GR transcriptional function was activated by DEX in HPAC cells. Expression of selected hepatocyte transcripts in response to DEX was blocked by co-treatment with the GR antagonist RU486. These data indicate that the HPAC response to glucocorticoid exposure includes an inhibition in proliferation, alterations in notch signalling and a limited change in the expression of genes associated with an acinar and hepatic phenotype. This is the first demonstration of a human cell responding to similarly to the rodent B-13 cell regarding formation of hepatocyte-like cells in response to glucocorticoid. Identifying and modulating the ablating factor(s) may enhance the hepatocyte-like forming capacity of HPAC cells after exposure to glucocorticoid and generate an unlimited in vitro supply of human hepatocytes for toxicology studies and a variety of clinical applications.

1. Introduction

The pancreas and liver are embryonic endoderm-derived tissues with a close developmental relationship (in that hepatocytes and biliary epithelial cells are derived from a population of embryonic pancreas cells (Deutsch et al., 2001)). Both pancreas and liver tissues have endocrine and exocrine functions with ductal progenitor cells in each tissue generating the endocrine (e.g. islet cells in pancreas) and exocrine (i.e. acinar cells in pancreas) tissue. Hepatocytes in the liver perform both endocrine (e.g. albumin secretion) and exocrine (bile acid excretion) functions (Wallace et al., 2008).

At present, limited supplies of human liver, the costs of hepatocyte isolation and difficulties in maintenance of function in vitro limit use of hepatocytes in cell-based studies (Wallace et al., 2010a). Considerable effort has been directed towards generating hepatocytes from either embryonic stem cells or induced pluripotent stem cells (Sullivan et al., 2010; Rashid et al., 2010; Hannan et al., 2013). Improvements in protocols have increased the proportion of cells differentiating toward hepatocytes but there remain a number of key hurdles to overcome before they will have significant basic or clinical utility. These include a lack of maturity, such that the cells remain at best, functionally equivalent to foetal hepatocytes and that they de-differentiate in vitro from a difficult-to-predict peak in function. There is also currently a requirement to follow complex differentiation protocols and the high costs associated with their production limit uptake and use (Wallace et al., 2010a; Szkolnicka et al., 2013).

An expandable rat pancreatic exocrine-like and/or ductal progenitor cell line – AR42J-B13 (henceforth referred to as B-13 cells) – to our knowledge, is unique in its quantitative ability to differentiate into hepatocyte-like cells that remain stably differentiated for many weeks on simple culture substrata. B-13 cells differentiate into non-replicative functional hepatocyte-like (B-13/H) cells after exposure to a single simple

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hormone (glucocorticoid), with many functions at levels quantitatively similar to the levels in freshly isolated rat hepatocytes (Shen et al., 2000; Marek et al., 2003; Wallace et al., 2010a; Probert et al., 2015).

It is known that pathological conditions lead to the appearance of hepatocyte-like cells in the pancreas (Zaret and Grompe, 2008). For an extensive review of rodent and human data with regard to this issue, see Probert et al. (2015). Since the proliferation of human pancreatic adenocarcinoma (HPAC) cells is inhibited after exposure to glucocorticoid (Gower et al., 1994; Norman et al., 1994), we hypothesised that this cell line could form the basis of a human equivalent to the B-13 cell, capable of conversion into functional hepatocyte-like cells. This paper demonstrates for the first time, that glucocorticoid exposure and the inhibition in proliferation, is also associated with alterations in notch signalling and a change in the expression of genes associated with acinar and hepatic phenotypes.

2. Results

2.1. Glucocorticoid exposure inhibits HPAC proliferation and promotes an epithelial phenotype in HPAC cells

Supplementary Fig. 1A demonstrates that exposing HPAC cells to DEX resulted in a dose-dependent reduction in nuclear proliferating cell nuclear antigen (PCNA) expression within 2 days of exposure and an inhibition in proliferation (Supplementary Fig. 1B) – as previously reported (Gower et al., 1994; Norman et al., 1994) – with a 50% inhibitory concentration between 1 and 10 μM. DEX exposure also resulted in a morphological change to a more epithelial phenotype (Supplementary Fig. 1B) and to a reduction – or complete loss in most cells – in expression of the carcinoembryonic antigen (CEA) (Supplementary Fig. 1C). A similar effect was also seen with the ductal marker cytokeratin 19 (Supplementary Fig. 1D).

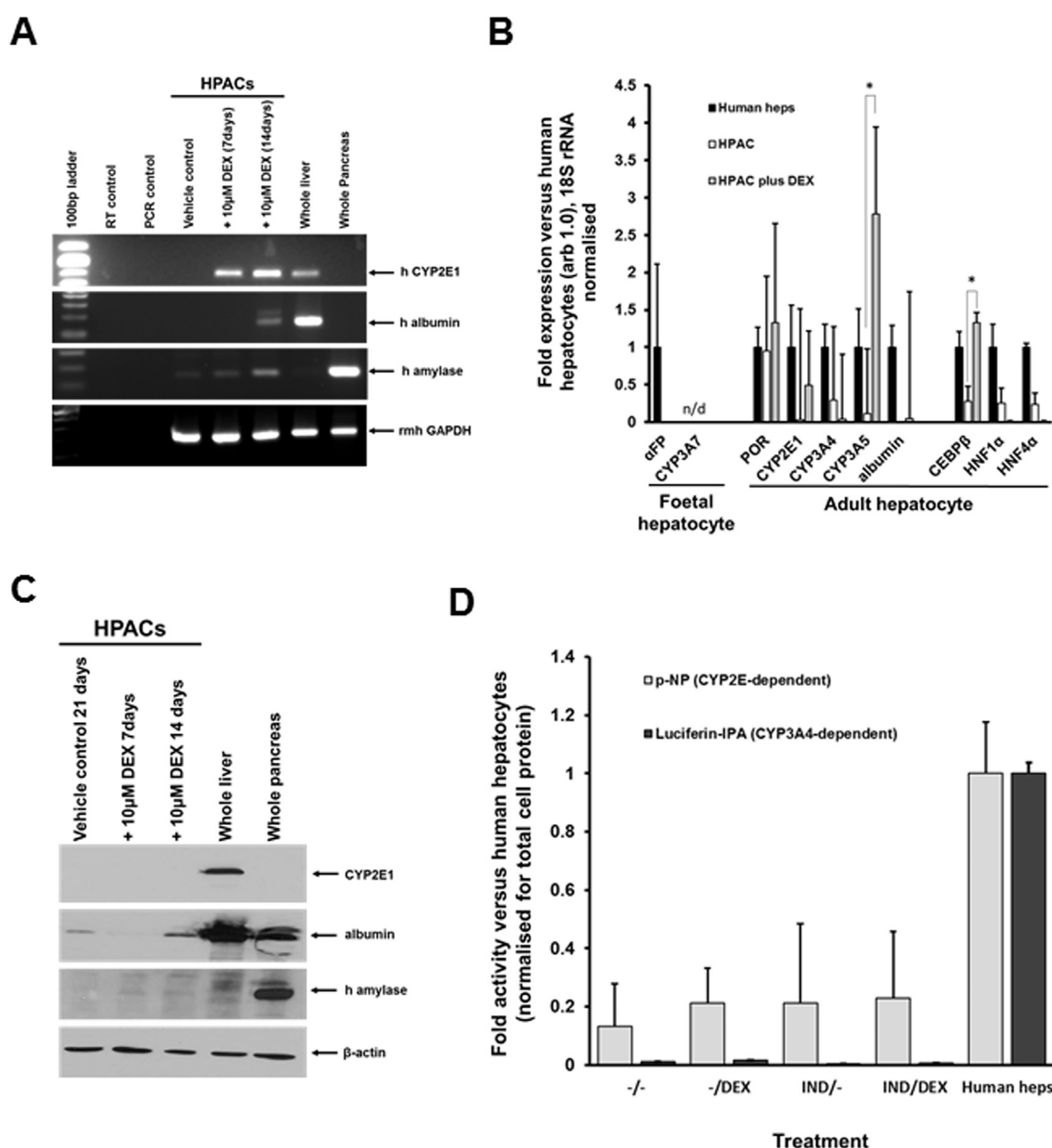

Fig. 1. ***Effect of DEX exposure on the expression of hepatocyte transcripts, proteins and activities in HPAC cells.*** **A**, RT-PCR for the indicated transcripts in HPAC cells, results typical of 7 separate experiments. **B**, qRT-PCR analysis for the expression of the indicated transcripts in RNA isolated from either HPAC cells treated with 10 μM DEX for 14 days or human liver, expressed relative to their levels in HPAC cells (set equal to 1). Data are the mean of 3 separate determinations from RNA pooled from 3 human livers and 7 separate HPAC experiments. **C**, Western blot for the indicated protein, data typical of 6 separate determinations. **D**, Cytochrome P450-dependent enzyme activities in HPAC cells treated as indicated. Data are the mean and standard deviation of 3 separate HPAC experiments or 3 separate human hepatocyte preparations. **D**, CYP2E and CYP3A4 enzyme activities in HPAC cells. HPAC cells were treated 10 μM DEX for 14 days (DEX), and for the last 5 days with a cocktail of cytochrome P450 inducers (IND) before isolation of cells and assays. Activities are normalized to protein, expressed relative to human hepatocytes (Human heps, mean and SD of 3 determination from the same donor cells) and are the mean and SD of 3 separate experiments.

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