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HNF4alpha expression amplifies the glucocorticoid-induced conversion of a human pancreatic cell line to an hepatocyte-like cell

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ABSTRACT

The pancreas and liver are closely related developmentally and trans-differentiation of cells from one tissue into the cells of the other has been documented to occur after injury or exposure to selected growth factors or glucocorticoid hormones. To generate a readily-expandable source of human hepatocyte-like (H-13) cells, the human pancreatic adenocarcinoma cell (HPAC) line was stably transfected with a construct encoding the variant 2 hepatocyte nuclear factor 4 α (HNF4α) using a piggyBac vector and transient expression of a transposase. Through induction of transgene HNF4α regulated via an upstream glucocorticoid response element in combination with existing modulating effects of glucocorticoid, H-13 cells were converted into quantitatively similar hepatocyte-like (H-13/H) cells based on expression of a variety of hepatocyte proteins. H-13/H cells also demonstrated the ability to store glycogen and lipids. These data provide proof of concept that regulated expression of genes associated with hepatocyte phenotype could be used to generate quantitatively functional human hepatocyte-like cells using a readily expandable cell source and simple culture protocol. This approach would have utility in Toxicology and Hepatology research.

1. Introduction

Hepatocytes are specialised cells that perform a variety of specific functions not performed by other cell types [1]. The mature hepatocyte phenotype is thought to be dependent on the expression levels and activities of transcription factors that co-ordinate the appropriate hepatic function-defining gene expression pattern. However, this program has been challenging to fully recapitulate in stem cell-derived hepatocytes in vitro, with cells bearing more comparability to foetal hepatocytes [2–4].

Hepatocyte de-differentiation is a paradigm for specialised cell responses to the in vitro culture environment. De-differentiation may be triggered as early as the cell isolation procedure and likely driven by multiple factors such as cell density, cell-cell interactions, cell-matrix interactions, loss of hormonal regulators and other factors in vitro [5–7]. Under very simple culture conditions (e.g. culture on plastic), hepatocytes may barely resemble their original phenotype within a few days of culture. However, evidence that rat hepatocyte de-differentiation may be markedly halted under these conditions exists since the proliferative AR42J-B13 cell line (B-13 cells) can be differentiated into non-proliferative – quantitatively equivalent – mature adult hepatocyte-like (B-13/H) cells in vitro through the addition of glucocorticoids [8–10]. Critically, once these cells differentiate into hepatocytes, they stably

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remain in this phenotype for several weeks and do not de-differentiate [10]. Understanding the apparent obliviousness of B-13/H cells to their environment and applying this to stem cells may be one way to overcome the reluctance of stem cells to fully differentiate into quantitatively equivalent, stable adult hepatocytes in vitro, and prevent their de-differentiation.

B-13 cells were originally derived from the rat pancreas and appear to behave as an immortal pancreatohepatobiliary progenitor cell line restricted to differentiation to B-13/H cells [11,12]. Differentiation is triggered by exposure to glucocorticoid which – via the glucocorticoid receptor (GR) – induces serine threonine kinase 1, β-catenin phosphorylation, transient wnt signalling repression, epigenetic changes and induction of hepatic transcription factors Cebpα, Cebpβ, Hnf1α and Hnf4α [8,10,13,14]. Recently, the human pancreatic adenocarcinoma cell line (HPAC) phenotype was shown to be suppressed by glucocorticoid exposure and to undergo a qualitatively similar expression of genes associated with hepatocytes (although the levels were relatively low compared to intact human liver [15]). In this communication, we report the amplification of hepatocyte gene expression in HPAC cells to protein levels quantitatively similar to human liver.

2. Materials and methods

2.1. Cells, tissues and constructs

HPAC cells were purchased from LGC Standards (Middlesex, UK). Human induced pluripotent stem cell (hiPSC)-derived hepatocytes were purchased from Definigen (Cambridge, UK) and maintained as described [16]. The empty PiggyBac vector containing a tetracycline regulatable promoter sequence (PB-TET) [17] was kindly provided by Dr Andras Nagy (University of Toronto, Canada). The transposase-encoding construct pCyL4320 [18] was obtained from the Wellcome Trust Sanger Institute (Cambridge, UK). Human liver and pancreas tissue was obtained with patient consent through the Newcastle Hepatopancreatobiliary Research Tissue Bank, with ethical approval by the Newcastle & North Tyneside 2 Research Ethics Committee. Human pancreas and liver tissue was taken from the margins of fresh tissue removed from patients due to the presence of tumours, but was histologically normal. Human acinar cells were prepared as previously outlined [19].

2.2. Cell culture

All cell lines were routinely cultured in low glucose (1 g/L) Dulbecco's Modified Eagle's Medium (DMEM) containing 10% (v/v) fetal calf serum (FCS), 100units/ml penicillin, 100 μg/ml streptomycin and 0.584 g/L L-glutamine in an humidified incubator at 37 °C and an atmosphere of 5% CO_2 in air. HiPSC-derived hepatocytes were maintained as outlined [16] HPAC, B-13 and H-13 cells were treated with dexamethasone (DEX) through addition of 1000-fold molar ethanol solvated stocks, with controls treated with 0.1% (v/v) ethanol vehicle as control.

2.3. Recombinant DNA and cloning

The GRE4 and minimal promoter sequence from the glucocorticoid receptor inducible reporter gene construct GRE4-pGL4.28 [14] was amplified using a proof reading polymerase by PCR with primers incorporating 5′ PacI (ATTTCTCTGGCTTAATTAACCGGTACCTGAGC) and a 3′ SacII (ACCAACAGCCGCGGATTGCCAAGCTGGAAGTCGAGCTTC) restriction sites respectively. The blunt ended PCR product was cloned into pCR TOPO (Invitrogen) and sequence checked. The TET-CMV promoter sequence in the PB-TET construct (PiggyBac construct without the pluripotency-inducing genes [17] was restricted with PacI and SacII to remove the tetracycline regulatory sequence and ligated with the GRE4 and minimal promoter regulatory sequence to generate the PB-GRE4prom construct. HNF4α transcript variant 2 (NM_000457.4) was cloned by RT-PCR using the upstream 5′-CACCATGGGACTCTCCAAAACCCTCGTCGACATGGAC and downstream primers 5′-CGGCTTGCTAGATAACTTCCTGCTTGGTGATGGTCGGCTG (containing the CACC + ATG start codon and stop codon respectively). A single PCR product of 1436bp was amplified, ligated into pENTR (Life technologies) and clones sequence checked prior to a sub-cloning the HNF4α sequence into PB-GRE4prom with LR clonase to generate PB-GRE4prom-hHNF4α.

2.4. Gene expression studies

RNA and protein extracts were prepared and RT-PCR performed as previously outlined [13,14] using primers described in Table 1. Western blotting, immunocytochemistry and immunohistochemistry were performed as described [13,14].

2.5. Adenoviral-mediated gene expression

Replication-deficient recombinant adenovirus were generously provided by Harry Heimberg, Vrije Universiteit, Brussels (Ad-null); Emma Regardsoe, University of Oxford, UK (Adv-GFP); Hiroshi Sakaue, Department of Nutrition and Metabolism, Institute of Health Biosciences The University of Tokushima Graduate School 3-18-15 Kuramoto-cho, Tokushima, Japan (CEBPβ LIP; CEBPβ LAP); Marco Pontoglio and Benoit Viollet, Institute Cochin, INSERM U1016/CNRS UMR 8104/Université Paris-Descartes (HNF1α) and Ramiro Jover Unidad Mixta Hepatologia Experimental & CIBERehd, Departamento de Bioquimica y Biologia Molecular, Universidad de Valencia, Spain (AdV-HNF4α).

2.6. Glycogen analysis

Glycogen was stained in formalin-fixed cells using periodic acid Schiff stain essentially as previously described [20]. For quantitative analyses, a colorimetric method was used, using purified rabbit glycogen as standard [20].

2.7. Lipid analysis

Triglyceride within cells was identified by oil red O staining essentially as already described [21]. After examination, the oil red O stain was extracted from the cells by incubation with 100% isopropanol and quantification through absorbance at 515 nm. The total cell protein was then determined using the Lowry method.

3. Results and discussion

Previous experience with rat B-13 cells has demonstrated that the hepatocyte-like B-13/H cells derived therefrom show good comparability with primary rat hepatocytes with respect to several cytochrome P450s and sensitivities to drug/chemical toxicity [8–10,12–14]. Exposing HPAC cells to glucocorticoid had a variety of effects also observed when B-13 cells were treated with glucocorticoid, including an inhibition in proliferation, phenotypic changes and low level expression of a variety of hepatocyte-specific transcripts (e.g. albumin) [15]. However, when comparing the response of B-13 and HPAC cells to DEX, it can be seen – based on cytochrome P450 2E1 (CYP2E1) or carbamoyl phosphate synthetase I (CPSI) expression - that the HPAC cells lack the robust quantitatively comparable conversion to hepatocyte-like cells seen with B-13 cells (Fig. 1A). In order to determine whether a minority

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