The role of HNF1B in human pancreas development and diabetes

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Introduction

- Differentiation of human pluripotent stem cells (PSCs) along the the pancreatic and hepatic lineages presents a unique tool to explore the roles of specific genes in human pancreas and liver development and function
- Disease-specific iPSCs can be used as an in vitro platform for identification of new genes and pathways that contribute to diabetes pathogenesis and identification of novel therapeutic targets

HNF1B and endoderm development

- HNF1B → Homeodomain-containing transcription factor
- Widely distributed in embryonic tissues – kidney, pancreas, liver, biliary tract, genito-urinary tract...
- Expressed in early embryonic development at anterior definitive endoderm stage
- Key member of network of transcription factors controlling differentiation of the pancreatic progenitor cells that assemble the exocrine and endocrine pancreas

HNF1B gene mutation phenotypes

- **In mice:** Heterozygous mutations → no phenotype
  Homozygous mutations → embryonic lethal
- **In humans:** Heterozygous mutations → HNF1B-associated disease / RCAD / MODY5
- **Clinical Phenotype includes:**
  - Diabetes: β-cell dysfunction, hepatic insulin resistance
  - Pancreatic hypoplasia, exocrine insufficiency
  - Liver dysfunction and cholestasis
  - Renal developmental disease and genitourinary abnormalities

Aims and objectives

1) To establish an in vitro model of HNF1B-associated diabetes
a) Derivation of HNF1B mutant human PSC lines
- HNF1B knock out hESC lines using CRISPR-Cas9 nuclease system
- iPSCs from patients with HNF1B mutations

b) Pancreatic and hepatic differentiation and functional characterisation of HNF1B mutant cell lines

2) To determine the molecular mechanisms by which HNF1B gene mutations cause pancreatic hypoplasia and diabetes

Methods

CRISPR-Cas9 assisted bi-allelic targeting in human PSCs

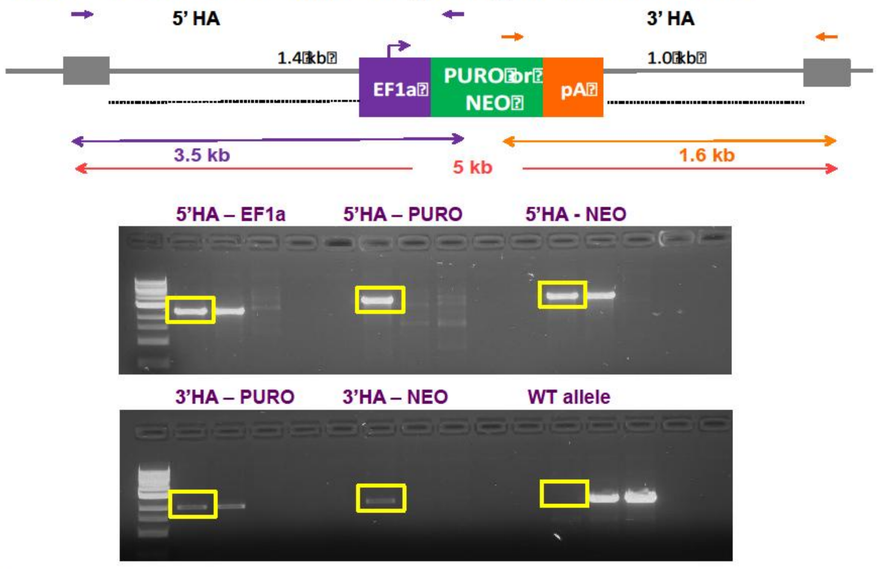

Figure 1: **PCR screening of targeted clones**. Example of a homozygous clone with insertion of puromycin and neomycin resistance markers highlighted in yellow boxes

Collection of patient samples

- Human Induced Pluripotent Stem Cells Initiative (HipSci) → UK national iPSC resource funded by the Wellcome Trust and MRC

Gene	Condition	Number of patient samples
HNF1A	MODY	8
HNF4A	MODY	3
HNF1B	MODY	11
ABCC8	Permanent and transient ND	3
KCNJ11	Permanent and transient ND	12
GATA6	Permanent ND and pancreatic agenesis	4
GCK	Permanent ND	2
INS	Permanent ND	7
GLIS3	Permanent ND	1
SLC19A2	Thiamine responsive megalobalstic anaemia (TRMA) syndrome	1
FOXP3	IPEX syndrome	1
STAT3	Autoimmune ND	1
MT-TL1	Maternally inherited diabetes and deafness (MIDD)	3
Unknown	Permanent ND +/- pancreatic agenesis	3

Table 1: Skin biopsy samples collected from patients with monogenic forms of diabetes

	Stage 1	Stage 2	Stage 3	Stage 4		Stage 5
CDM	CDM-PVA	ADV-DMEM	ADV-DMEM	ADV-DMEM	ADV-DMEM	ADV-DMEM
A/F	A/F/B/Ly	RA/Nog/SB/F10	RA/Nog/F10/CYCP	F10	B27/ DAPT	B27
Day 0	Days 1-3	Days 4-6	Days 7-9	Day 10-12	Days 13-15	Days 16-18
hESCs hiPSCs →	Definitive Endoderm →	Dorsal Foregut →		Pancreatic Endoderm →		Endocrine Progenitors
OCT4	**SOX17**	**HNF1B**		**PDX1**		**NGN3**
NANOG	CXCR4	HNF4A		SOX9		
SOX2	GSC	FOXA2		HNF6		**CPEP**
	CER	HLXB9		NKX6.1		**SST**
		RFX6		PTF1A		**GCG**

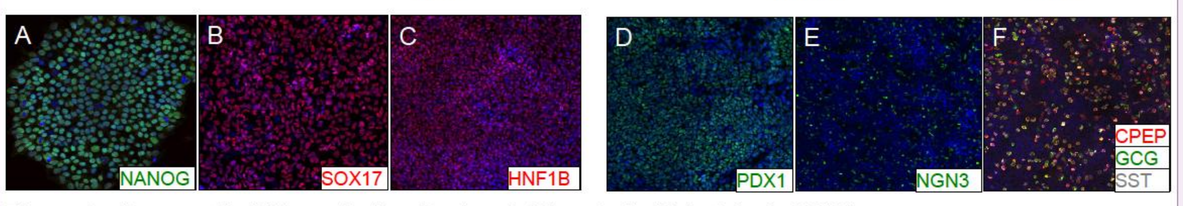

Figure 2: **Pancreatic Differentiation Protocol** *(Cho et al. Diabetologia 2012)*
A-F: Successive expression of markers showing patterning from pluripotency to definitive endoderm to foregut to pancreatic endoderm and hormone-expressing cells

Results

Homozygous knockout of HNF1B results in failure of pancreatic endoderm development *in vitro*

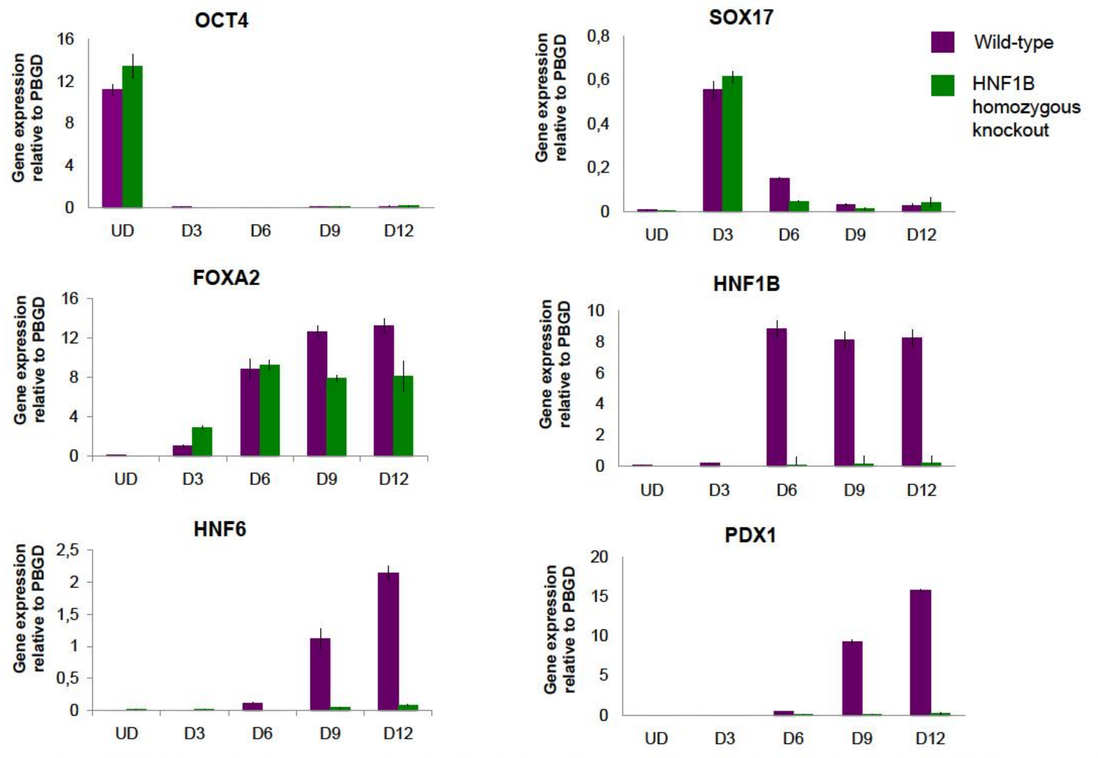

Figure 3: Expression of transcription factors during pancreatic differentiation of wild-type and HNF1B knockout human iPSCs
mRNA expression of pluripotency (OCT4), definitive endoderm (SOX17), dorsal foregut (HNF1B, FOXA2), pancreatic progenitor cell (PDX1, HNF6) markers in a human iPSC line chemically induced to undergo pancreatic differentiation, assessed by qRT-PCR in undifferentiated (UD) PSCs and at Day 3, 6, 9, 12 of differentiation. All genes were normalised to the housekeeping gene; PBGD. Bars represent mean +/- standard error of the mean (SEM).

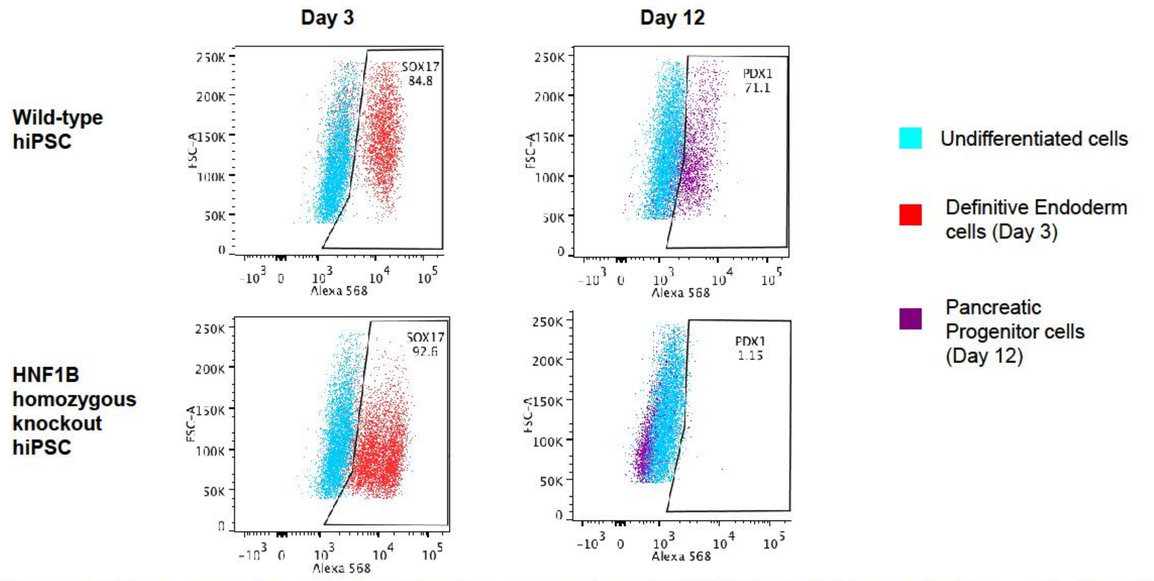

Figure 4: Flow cytometry analyses showing expression of SOX17 in definitive endoderm cells (Day 3) and PDX1 in pancreatic progenitor cells (Day 12)
Primary antibody stained undifferentiated controls were used as negative control to gate the positive population.

Conclusions

- HNF1B has an essential role in pancreatic progenitor cell specification
- Genetically engineered PSCs and iPSCs derived from patients with known genetic defects can be used to generate in vitro models of human disease
- Targeted differentiation to a relevant mature cell type can be used to study the functional molecular and cellular consequences of a defined genetic defect

ESPE 2015 102--P1
Diabetes and Insulin
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DOI: 10.3252/pso.eu.54espe.2015
Poster presented at: 54th Annual Meeting 1-3 October ESPE 2015 BARCELONA European Society for Paediatric Endocrinology