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**HNF1α maintains pancreatic α and β cell functions in primary human islets**

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ABSTRACT

HNF1A haploinsufficiency underlies the most common form of human monogenic diabetes (HNF1A-MODY) and hypomorphic HNF1A variants confer type 2 diabetes risk, but a lack of experimental systems for interrogating mature human islets has limited our understanding of how the transcription factor HNF1α regulates adult islet function. Here, we combined conditional genetic targeting in human islet cells, RNA sequencing, chromatin mapping with Cleavage Under Targets & Release Using Nuclease (CUT&RUN), and transplantation-based assays to determine HNF1α-regulated mechanisms in adult human pancreatic α and β cells. Short hairpin RNA-mediated (shRNA) suppression of HNF1A in primary human pseudoislets led to blunted insulin output and dysregulated glucagon secretion after transplantation in mice, recapitulating phenotypes observed in diabetic patients. These deficits corresponded with altered expression of genes encoding factors critical for hormone secretion, including calcium channel subunits, ATPase transporters and extracellular matrix constituents. Additionally, HNF1A loss led to upregulation of transcriptional repressors, providing evidence for a mechanism of transcriptional de-repression through HNF1α. CUT&RUN mapping of HNF1α DNA-binding sites in primary human islets imputed a subset of HNF1α-regulated genes as direct targets. These data elucidate mechanistic links between HNF1A loss and diabetic phenotypes in mature human α and β cells.
INTRODUCTION

Diabetes mellitus is a pandemic disease of dysregulated glucose metabolism that arises from both acquired and genetic mechanisms. Understanding of diabetes genetics has been advanced by studies of monogenic diabetes, like Maturity Onset Diabetes of the Young (MODY) (1), and identification of causal variants of type 2 diabetes (T2D) through genome-wide association studies (2). The most common MODY form, HNF1A-MODY, results from mutations in HNF1A, which encodes the transcription factor HNF1α (3). Moreover, hypomorphic HNF1A variants confer increased T2D risk (4). Despite the strong association between HNF1A deficiency and human diabetes, the mechanisms by which HNF1α regulates mature human islet cell function remain incompletely understood.

Previous studies of HNF1A-MODY patients revealed impaired insulin secretion that improves with sulfonylurea treatment (5). These and studies in human stem cell models (6–10) strongly suggest a developmental role for HNF1α in islet β cell ontogeny and function, but the consequence of acute HNF1A loss in adult human islet β cells is unclear. HNF1A is also expressed in islet α cells (11, 12), and HNF1A-MODY patients have dysregulated glucagon secretion (13, 14). However, the impact of HNF1A loss in adult human α cells is unknown. Thus, the roles of HNF1α in maintaining functions of mature α and β cells in human islets have not been firmly established.

This knowledge gap reflects challenges in studying HNF1α loss of function in adult islet cells. In humans, HNF1A haploinsufficiency underlies HNF1A-MODY, but mice heterozygous for an Hnf1a null allele are not diabetic (15). Human studies using HNF1A deficient β-like cells derived from multipotent stem cells have provided valuable insights regarding HNF1α regulation during islet cell development (6–10), but progeny cells from these studies did not fully recapitulate adult
β cell functions, limiting conclusions about the roles of HNF1α in mature adult islets.

Characterization of islets from an HNF1A-MODY patient was recently reported (13), but conclusions in this case were inferred from studies of a single subject.

To elucidate how acute HNF1α loss leads to impaired function in mature human α and β cells, we used a pseudoislet-based strategy. Human 'pseudoislets' are formed by dispersion and re-aggregation of primary human islet cells, which permits efficient genetic targeting while maintaining cardinal features of primary islet cells (16). This approach enabled conditional genetic targeting, high throughput RNA sequencing (RNA-seq), Cleavage Under Targets & Release Using Nuclease (CUT&RUN) chromatin mapping, and transplantation-based functional assays of islet cells lacking HNF1α.

RESULTS

shRNA targeting of HNF1A expression in primary human islets

We used the pseudoislet system (Figure 1A) to achieve conditional HNF1A loss in primary human islets. Specifically, primary human islets were dispersed to permit efficient transduction either with lentivirus expressing short hairpin RNA (shRNA) that suppressed HNF1A (HNF1AKD; 'knockdown', KD), or lentivirus expressing non-targeting control shRNA (control). Afterwards, cells were re-aggregated to form pseudoislets (Methods). The lentiviral vector co-expressed a GFP transgene (Figure 1B), thereby marking transduced cells. By 5 days after infection, we observed GFP+ cells (Figure 1C-D) and significant reduction in HNF1A mRNA as measured by qRT-PCR (Figure 1E). We also observed reduced mRNA levels encoding genes thought to be regulated by HNF1α, including HNF4A, HNF1A-AS1, TMEM27, KCNJ11, and SLC2A2 (Figure 1F). While insulin mRNA levels were reduced after HNF1AKD, we did not
detect significant reduction of glucagon mRNA, or changes of insulin and glucagon protein expression (measured by ELISA) compared to controls (Supplemental Figure S1A-D). Western blot analysis confirmed significant reduction of HNF1α expression at the protein level in HNF1AKD versus control pseudoislets (Figure 1G-H). Thus, lentiviral shRNA targeting with a pseudoislet strategy achieved conditional loss of HNF1A in primary human islet cells.

We next assessed hormone secretion after acute HNF1A loss. In static batch assays, insulin secretion from HNF1AKD pseudoislets was modestly but significantly reduced at 16.7 mM glucose 5 days after transduction (Supplemental Figure S1E). Likewise, glucagon secretion from HNF1AKD pseudoislets was blunted after stimulation with 1mM glucose+10mM L-Arginine in vitro (Supplemental Figure S1F).

**HNF1α deficiency leads to phenotypes in transplanted α and β cells**

We predicted that hormone secretion phenotypes could be more pronounced after prolonged HNF1A suppression, but the duration of pseudoislet culture is limited to approximately 6 days. To extend the duration of HNF1A suppression and phenotyping in islet cells, we transplanted control and HNF1AKD pseudoislets under the renal capsules of immunocompromised NOD scid IL2Rγnull (NSG) mice (Methods). Given previously reported species differences in HNF1A deficiency phenotypes (15), the potential variability introduced by chemically induced diabetes, and the ability to distinguish between circulating human and mouse insulin via ELISA, we transplanted human pseudoislets in nondiabetic mice. The goal of these studies was to quantify human graft insulin secretion phenotypes after several weeks of HNF1A loss; we did not aim to characterize impacts of grafts on host mouse metabolism. Four weeks after transplantation of human pseudoislets into nondiabetic NSG mice, we measured circulating human insulin (Figure...
After intraperitoneal glucose tolerance testing (IPGTT), we observed significant blunting of insulin secretion from transplanted HNF1AKD cells compared to controls (Figure 2B-D). Moreover, this deficit was ameliorated by treatment of transplanted grafts with glibenclamide (Supplemental Figure S2A-D), a sulfonylurea used in diabetic patients with mutations in HNF1A (17). After recovery of human grafts, immunostaining demonstrated that HNF1α expression was significantly reduced in HNF1AKD pseudoislets (Supplemental Figure S2E-G), confirming sustained HNF1A suppression after months in vivo. The total number of transduced (GFP+) cells recovered was similar between control and HNF1AKD grafts, suggesting that observed insulin secretion phenotypes were not due to differential graft survival (Supplemental Figure S2H). Thus, our transplantation studies revealed that conditional targeting and loss of HNF1A led to reduced human β cell insulin secretion.

Dysregulated glucagon secretion has also been documented in a subset of HNF1A-MODY patients (13, 14). To measure glucagon secretion from transplanted human islets, we previously developed a NSG immunocompromised mouse harboring a glucagon gene knockout (GKO-NSG) (18). Elimination of host glucagon production in GKO-NSG mice permits ELISA-based detection of glucagon from transplanted human α cells (Figure 2E). We transplanted HNF1AKD and control pseudoislets in GKO-NSG mice, then assessed glucagon secretion four weeks after transplantation. Glucose challenge (IPGTT) resulted in comparable levels of acute hyperglycemia in GKO-NSG mice transplanted with HNF1AKD or control pseudoislets (Figure 2F). As expected, glucagon secretion from control grafts decreased in response to hyperglycemia by 30 minutes after glucose injection (Figure 2G). In contrast, glucagon secretion was not suppressed in HNF1AKD grafts, and area under the curve (AUC) measures of net glucagon secretion were higher for HNF1AKD versus control grafts (Figure 2H). Thus, our
transplantation studies also revealed inappropriate glucagon output by human α cells after acute HNF1A loss.

In addition to glucagon hypersecretion at high glucose levels, studies of islets from a single HNF1A-MODY donor reported reduced glucagon secretion in conditions that normally stimulate glucagon output (13). To investigate effects of HNF1A loss on glucagon secretion during hypoglycemia, we performed intraperitoneal insulin tolerance tests (IP-ITT). IP-ITT led to acute hypoglycemia in GKO-NSG mice transplanted with HNF1AKD or control pseudoislets (Figure 2I). However, compared to controls, we observed blunted glucagon serum excursion in mice transplanted with HNF1AKD grafts (Figure 2J-K). In summary, our functional studies reveal that acute loss of HNF1A in adult primary islets phenocopied multiple hormone secretion defects observed in humans chronically deficient for HNF1A, including reduced insulin secretion, excessive glucagon output during hyperglycemia, and blunted glucagon secretion during hypoglycemia.

RNA-seq identifies transcriptome changes after HNF1A loss in mature β cells

HNF1α is a transcriptional regulator (3). Thus, to investigate the mechanisms underlying islet phenotypes we observed after HNF1A loss, we performed RNA-seq to characterize the β cell transcriptome after HNF1A knockdown (Figure 3A). To isolate HNF1AKD β cells, we used fluorescence-activated cell sorting (FACS) to purify lentivirus-infected GFP+ cells expressing NTPDase3, a human β cell surface marker (19). The fraction of endocrine cells (HPi2+) expressing GFP was similar between control and HNF1AKD samples (Figure 3B). Enrichment of insulin (INS) mRNA in NTPDase3+ fractions was confirmed by qRT-PCR (Supplemental Figure S3A-B). We produced and sequenced RNA-seq libraries of GFP+ NTPDase3+ cells from
4 human donors. Principal Component Analysis (PCA) showed clustering of samples by donor, consistent with prior studies (11, 20, 21), and separation of HNF1AKD and control samples (Supplemental Figure S3C-D). The DESeq2 algorithm (22) identified 1,605 differentially expressed genes (DEGs) in β cells after HNF1A loss (Supplemental Table S3). This included 800 genes with significantly reduced mRNA, including HNF1A (Figure 3C), and 805 with increased mRNA levels (Figure 3D-E).

Gene Ontology (GO) pathway analysis of genes downregulated after HNF1A loss identified known regulators of hormone secretion (e.g. CHGA, UCN3), extracellular matrix (ECM) organization (ADAMTS2, COL6A2), cell-cell signaling (GIPR, GLP1R), glucose homeostasis (G6PC2, SLC2A2), and endocrine pancreas development (MAFB, TM4SF4) (Figure 3F). Additionally, downregulated genes were significantly enriched for KEGG pathways related to MODY (HNF4A, INS, NEUROD1, GCK) and cAMP signaling (ADCY1, PDE3B) (Figure 3H). By contrast, genes upregulated upon HNF1A loss were related to type I interferon responses (IRF7, ISG20) and transcription repressor activity (GZF1, SCML1) (Figure 3G). These results suggest HNF1A is necessary in mature islet cells to maintain the expression of hundreds of crucial genes encoding hallmark regulators of human β cell function.

RNA-seq identifies adult human α cell transcriptome changes after HNF1A loss

Our evidence of α cell dysregulation after HNF1AKD (Figure 2F-K) and prior studies (13, 23) indicate that HNF1α is required for α cell function. However, little is known about HNF1α gene regulation in mature human α cells, aside from studies of a single patient-derived sample (13). Here we used RNA-seq to investigate the HNF1A-dependent human α cell transcriptome. To isolate HNF1AKD α cells, we used FACS to enrich for transduced GFP+ cells expressing the
human α cell marker CD26 (11, 24) (Figure 4A). Glucagon (GCG) enrichment in the CD26+ cell fraction was verified by qRT-PCR (Supplemental Figure S3A-B). Consistent with prior reports (11), analysis of RNA-seq libraries generated from α cell fractions revealed that HNF1A mRNA was higher in control α versus β cells (Figure 4B, Figure 3C). Similar to β cells, we achieved greater than 50% suppression of HNF1A in α cells (Figure 4B).

DESeq2 analysis of HNF1AKD versus control RNA-seq libraries demonstrated 447 downregulated DEGs and 230 upregulated DEGs after HNF1A loss in α cells (Figure 4C, Supplemental Table S4). More than half of α cell DEGs (365/677) were also dysregulated in β cells (Figure 4D). These included genes encoding previously characterized HNF1α targets (TM4SF4, HNF4A) and pancreatic islet regulators with species-specific expression patterns (MAFB, UCN3). GO term analysis of this 'overlapping' gene set highlighted shared pathways related to type I interferon signaling (IFNAR2, ISG20), collagen-containing ECM (COL6A2, COL1A1), and peptide hormone secretion (CACNA1D, SLC5A1) (Figure 4E).

GO term analysis of DEGs also identified α cell-specific changes after HNF1A loss, including enrichment of pathways related to cell adhesion (COL6A1, ADAMTS4) and hormone secretion (e.g., the α cell-enriched factor ABCC4) (Figure 4F). Additional GO pathways enriched in α cell-specific DEGs included voltage-gated calcium (Ca2+) channel constituents and ATPase-coupled transmembrane transport (Figure 4F). While CACNA1D and ATP2A3 expression were significantly downregulated in both β and α cells after HNF1A loss, expression of several additional voltage-gated Ca2+ channel (CACNA1A, CACNG4, CACNA1H) and ATPase-coupled transporter (ABCC4, ABCG2, ABCA3, ATP8A1) genes was significantly changed in α but not β cells (Figure 4G). Furthermore, patch-clamp electrophysiology studies revealed reduced Ca2+ currents in HFN1AKD versus control α cells, but not β cells, at 5 days after transduction.
(Supplemental Figure S4A-C). Thus, our studies provide evidence for HNF1α direct or indirect regulation of hundreds of human pancreatic α cell genes, including genes not previously reported as HNF1α-dependent, and a subset known to govern crucial physiologic processes that regulate hormone secretion.

**Direct targets of HNF1α identified by CUT&RUN**

CUT&RUN assesses transcription factor DNA binding sites in situ (25), and we used this assay to identify direct HNF1α target genes in primary human islet cells. To overcome low endogenous islet expression of HNF1α and the reduced yields inherent to primary pancreatic samples, we used lentiviral transduction to express a transgene encoding human HNF1A tagged with the FLAG immuno-epitope in human islet cells (Figure 5A). DNA bound by HNF1α-FLAG protein was enriched with an anti-FLAG antibody and sequenced (Methods). This approach captured HNF1α-FLAG-bound and immediately adjacent DNA regions. We used HOMER (26) to identify genomic regions captured by HNF1α-FLAG as previously reported (21). We observed enriched read densities in HNF1α-FLAG CUT&RUN DNA peak centers compared to minimal enrichment at these sites for IgG control samples (Supplemental Figure S5A-B). HOMER analysis identified that HNF1α-FLAG-bound genomic peaks were significantly enriched for the HNF1α binding motif (Figure 5B) and other transcription factor motifs previously observed in pancreatic islet enhancer clusters (27), including PDX1 (Figure 5B) and NKX6.1 (Supplemental Figure S5C).

Using the GREAT algorithm (28), we associated HNF1α-FLAG-bound regions to 5,569 proximate genes. To prioritize direct regulatory targets of HNF1α, we compared these genes to DEGs identified by RNA-seq after HNF1A loss. Of 1,917 DEGs in HNF1AKD α or β cells, 637
were also present in the HNF1α-FLAG CUT&RUN gene set (Figure 5C; Supplemental Table S5). The concordance between HNF1α-FLAG-bound regions and DEGs after HNF1A loss provides evidence that our CUT&RUN approach identified direct HNF1α targets.

Consistent with reports that HNF1α functions as a transcriptional activator (7, 29, 30), 68% (434/637) of the putative HNF1α targets identified by the intersection of our HNF1α-FLAG CUT&RUN and HNF1AKD RNA-seq gene sets were downregulated upon HNF1A loss (Figure 5D). These downregulated genes were enriched for GO pathways related to cell-cell signaling (CASP, DPP4), hormone secretion (ABCC8, KCNJ11), ECM (COL6A3, ADAMTS2), glucose homeostasis (G6PC2, GCK), ion transmembrane transport (ATP2A3, CACNA1D), and endocrine pancreas development (HNF4A, MAFB). As expected, HNF1α-FLAG-bound genomic regions localized to presumptive accessible promoter and enhancer regions, as revealed by colocalization with transposase integration sites and histone marks reported in prior ATAC-seq and ChIP-seq studies (Figure 5E-F) (27, 31). These findings support a model for direct HNF1α activation of genes, including those encoding factors essential for mature α and β cell function (Figure 5G).

By contrast, the 203 (32%) putative HNF1α targets upregulated after HNF1A loss were broadly related to negative gene regulation (NR1D1, GZF1, HBP1, SCML1) (Figure 5D). These findings implicate HNF1α as a direct negative regulator of transcriptional repressors, suggesting that HNF1α de-repression of transcriptional networks is another mechanism for maintaining human islet cell function (Figure 5G).

Comparing islet transcriptomes after acute HNF1A loss and in congenital HNF1A-MODY

To assess the applicability of studying acute HNF1A suppression in human pseudoislets to understanding HNF1A-MODY, we compared HNF1α gene targets identified in this study with
islet RNA-seq datasets from a subject with HNF1A-MODY (13). The majority of HNF1α targets (368/637, 58%) we identified with CUT&RUN and RNA-Seq were differentially expressed in the RNA-seq data obtained from human HNF1A-MODY islet cells. Pearson correlation analysis of normalized gene expression levels of these putative targets in our adult HNF1AKD α cells versus the HNF1A-MODY donor α cells revealed a significant positive correlation ($r=0.42$, $P=2.2E-16$). Similarly, we observed a positive correlation in gene expression levels of putative HNF1α targets between HNF1AKD and HNF1A-MODY donor β cells ($r=0.32$, $P=5.3E-16$). This concordance between our data and those previously reported (13) is readily visualized in heatmaps of HNF1α target genes in α (Figure 6A) and β cells (Figure 6B). Notably, many of the genes common to our dataset and the prior study were associated with ECM organization ($COL5A1$, $ADAM22$), ion transmembrane transport ($ATP2A3$, $SLC30A8$), glucose metabolism ($SLC2A2$, $G6PC2$), hormone secretion ($KCNJ11$, $CACNA1D$), and transcriptional repression ($SCML1$, $HBPI$, $BACH2$). Thus, our observations demonstrate some concordance of DEGs in α and β cells of islets from a congenital HNF1A-MODY donor and from acute conditional $HNF1A$ loss-of-function in primary human islets.

**DISCUSSION**

Work here addresses knowledge gaps about the roles of HNF1α in mature pancreatic islet α and β cells. Prior studies of constitutive $HNF1A$ deficiency have shaped our understanding of HNF1α roles in β-like cells derived from human stem cell lines (6–10). These cells retain features of fetal β cells; moreover, $HNF1A$ targeting in these systems led to constitutive deficiency. Thus, while this prior work has broadened our understanding of HNF1α roles in islet β cell development, inferences about the roles of HNF1α in adult β cells are weakened by
features of these stem cell models. Additionally, HNF1α function in mature α cells cannot be assessed in these systems. Here, we used conditional lentiviral shRNA targeting of HNF1A to investigate functions of HNF1α in primary human adult α and β cells, an approach for HNF1A study not previously reported. We observed dysregulated function and gene expression after acute HNF1A loss, and our transcriptome studies revealed that HNF1α regulates genes critical for establishing and maintaining characteristic islet cell features, like glucose metabolism and hormone secretion. These findings contribute to our understanding of adult islet cell gene regulation by HNF1α.

After suppression of HNF1A, we observed defects in insulin and glucagon secretion reminiscent of phenotypes in humans with HNF1A deficiency, including HNF1A-MODY patients (13, 14) and subjects with T2D (4, 32). In contrast to recent growth in our understanding of mechanisms underlying HNF1A-deficient β cell dysfunction, little is known about the basis of phenotypes in α cells lacking HNF1A (13, 23). Work here revealed that α cell HNF1α is required to maintain expression of genes encoding known regulators of ECM organization, Ca^{2+} signaling, and ATPase-coupled transport. HNF1A-dependent expression of subsets of these genes was previously noted in human β cells or whole islets, but not in purified α cells (6–10, 33). Prior publications have linked ECM signaling to regulation of insulin secretion (34, 35), and our work provides evidence that HNF1α promotes normal ECM dynamics in both β and α cells. Ca^{2+} influx is another well-established component of islet cell hormone secretion (36), and our studies support that HNF1α regulates voltage-gated Ca^{2+} channel subunit expression and Ca^{2+} channel currents in mature α cells. Store-operated Ca^{2+} flux has also been proposed to regulate glucagon secretion via intracellular sequestration of Ca^{2+} through sarco(endo)plasmic reticulum ATPases (37, 38). We found that several genes related to ATPase-coupled transport were
downregulated after HNF1AKD in α cells, including \textit{ATP2A3} and \textit{ATP2C2}, which encode ATP-driven Ca\textsuperscript{2+} transporters (39, 40). These findings suggest that dysregulation of ATPase function and Ca\textsuperscript{2+} transport may contribute to anomalous glucagon secretion from α cells in \textit{HNF1A}-deficient diabetes.

To identify direct genetic targets of HNF1α in human islet cells, we used CUT&RUN after mis-expression of FLAG-tagged HNF1α. We recognize that results after misexpression of tagged HNF1α should be interpreted cautiously. Therefore, we also integrated CUT&RUN with DEG analysis after \textit{HNF1A} suppression, and this combined approach increased confidence in 'calling' direct genetic targets of HNF1α imputed by CUT&RUN. Thus, we identified hundreds of putative direct genetic targets of HNF1α that are critical to mature α and β cell functions.

Our analysis also provides index evidence that HNF1α may both activate and de-repress gene networks in human islets (\textbf{Figure 5G}). While HNF1α is well-known as a transcriptional activator (7, 29, 30), gene expression studies here and in prior reports demonstrate that HNF1α deficiency results in both decreased and increased gene expression (6, 7, 13, 30). Direct transcriptional repression by HNF1α has been observed in hepatocytes (41), but it has not previously been reported in islets. Here, we report that approximately one-third of HNF1α target genes were upregulated after \textit{HNF1A} loss. Notably, many of these upregulated targets encode transcriptional repressors with previously characterized roles in repressing cell proliferation (HBP1) (42), homeotic gene expression (SCML1) (43) and antioxidant response pathways (BACH2) (44). Dynamic transcriptional de-repression is critical for multiple physiological processes, including endocrine cell differentiation (45). Our findings support the view that islet phenotypes from \textit{HNF1A} deficiency could reflect loss of transcriptional activation \textit{and} repression in HNF1α-dependent genetic pathways.
In summary, our study identifies genetic targets of HNF1α regulation in primary human islets and correlates loss of HNF1A with dysregulated gene expression and functional deficits in α and β cells. A subset of these features phenocopy those in humans with diabetes from HNF1A deficiency. We demonstrate that HNF1α maintains genetic pathways crucial for regulated hormone secretion and de-represses pathways that may be necessary for mature islet function. These findings advance our understanding of HNF1A-dependent mechanisms that maintain adult human α and β cell function.

METHODS

Human Islet Procurement

Deidentified, nondiabetic human islets were obtained through the Integrated Islet Distribution Network, International Institute for the Advancement of Medicine, University of California San Francisco, and Alberta Diabetes Institute IsletCore (www.isletcore.ca). Supplemental Table S1 contains donor details.

Constructs and Lentivirus Production

Lentiviral constructs for shRNA targeting exon 4 of HNF1A were obtained from Dharmacon. plenti-CMV-HNF1A-cMyc-DDK was used in CUT&RUN experiments (OriGene RC211201L1). Lentiviruses were produced by transfection of HEK293T cells with lentiviral constructs and pMD2.G and psPAX2 packaging constructs (Addgene). TurboFect reagents were used for transfection (ThermoScientific) and supernatants were purified using PEG-it (System Biosciences).

Human Pseudoislet Generation
Human pseudoislets were generated as previously described (16, 20, 21). Briefly, intact human islets were dispersed into single cells by enzymatic digestion (Accumax, Invitrogen) and transduced with $1 \times 10^9$ viral units/mL lentivirus. Transduced cells were cultured in ultra-low attachment cell culture plates (Corning) for 5 days prior to analysis.

**qRT-PCR**

RNA was isolated from whole pseudoislets using the PicoPure RNA isolation kit (Life Technologies). cDNA was synthesized using the Maxima first strand kit (ThermoScientific), and gene expression was assessed by PCR using TaqMan gene expression mix (ThermoScientific) and probes listed in Supplemental Table S2.

**Western Blot Analysis**

Whole-cell protein extracts were obtained from 500 pseudoislet per sample through lysis in RIPA buffer (Thermo Scientific) containing 1X protease inhibitor cocktail (Roche). Protein concentrations were quantified using a NanoDrop spectrophotometer; 40 μg of total protein was mixed with sample buffer [4× Laemmli Buffer (Bio-Rad), 10% β-mercaptoethanol (Sigma-Aldrich)] and boiled for 5 min at 95°C. Samples were run on a 4–15% Mini-PROTEAN TGX Precast Gel (BioRad) for 60 min at 100 V in Tris-glycine-SDS buffer. Samples were then transferred to a polyvinylidene difluoride (PVDF) membrane at 180mA for 40 min in Tris-glycine-methanol buffer. The PVDF membrane was blocked in 5% milk in phosphate-buffered saline containing 0.1% Tween-20 for 1 hr at room temperature. Incubation with a primary antibody against HNF1α (1:150) was performed at 4°C overnight followed by incubation with an HRP-conjugated anti-rabbit secondary antibody (1:750) for 1 hr at room temperature (see Supplemental Table S2 for antibody details). HRP signal was detected on X-ray films by chemiluminescent substrate (Thermo Fisher 34577). Blots were stripped using
Restore Western Blot Stripping Buffer (Thermo Scientific) and reprobed using an HRP-conjugated antibody against β-actin (1:50,000). ImageJ was used to quantify protein bands; HNF1α band intensity was normalized to β-actin (housekeeping gene) band intensity for each sample.

**Transplantation and in vivo assessment of pseudoislet function.**

Batches of 1000 pseudoislets were transplanted under the renal capsule of 3-month-old male NOD scid IL2Rγnull (“NSG,” The Jackson Laboratory 005557) or GKO-NSG (18) mice using a micro-capillary tube, as described previously (18). Four weeks later, mice received an intraperitoneal (IP) injection of 3g glucose/kg body weight. For sulfonylurea sensitivity testing, NSG mice received 2.5mg glibenclamide (Sigma-Aldrich G0639)/kg body weight via a single intraperitoneal injection 6 weeks after transplantation of pseudoislets. For insulin tolerance tests, GKO-NSG mice received 0.5U Humulin R (Lilly)/kg body weight via intraperitoneal injection. Blood samples were collected via the tail vein; glucose and hormones were measured using a glucometer (True Metrix) and ELISA kits (Mercodia).

**Patch-clamp electrophysiology studies**

Single-cell patch-clamp studies were performed as described previously (46). Pseudoislets were dissociated to single cells and cultured in 5.5mM glucose media for 1-3 days. Prior to whole-cell patch-clamping, media was changed to a bath solution containing: 118mM NaCl, 20mM Tetraethylammonium-Cl, 5.6mM KCl, 1.2mM MgCl2, 2.6mM CaCl2, 5mM HEPES, and 5mM glucose (pH 7.4 with NaOH) in a heated chamber (32–35 °C). Patch-clamping was performed using fire polished thin wall borosilicate pipettes coated with Sylgard (3-5mOhm) containing: 125mM Cs-glutamate, 10mM CsCl, 10mM NaCl, 1mM MgCl2, 0.05mM EGTA, 5mM HEPES, 0.1mM cAMP, and 3mM MgATP (pH 7.15 with CsOH). Data were recorded using a HEKA...
EPC10 amplifier and PatchMaster Software (HEKA Instruments Inc, Germany) within 5min of break-in. The stability of seal (>10 GOhm) and access resistance (<15 MOhm) throughout the experiment were assessed for quality control. FitMaster (HEKA Instruments Inc) was used for data analysis.

**Extracellular Staining and FACS of human islet cells**

Pseudoislets were dispersed into single cells, stained with the LIVE/DEAD Fixable Near-IR kit (Life Technologies), and washed with cell staining buffer (BioLegend). The following primary-secondary conjugated antibodies were used: HPi2-PE/Cy7, NTPDase3-647, and CD26-PE (Supplemental Table S2). Labeled cells were sorted on a special order five-laser FACS Aria II (BD Biosciences) using a 100-μm nozzle, with compensation controls and doublet removal. Sorted cells were collected in 100μL of FACS buffer with RiboLock RNase inhibitor (ThermoScientific).

**RNA-seq library preparation and data analysis**

Approximately 5,000 sorted live β or α cells were used for each RNA-seq library construction. RNA was isolated using the PicoPure RNA isolation kit (Life Technologies). The SMART-seq v4 Ultra Low input RNA kit (Clontech) was used to amplify cDNA, and libraries were generated using the Nextera XT DNA Library Preparation Kit (Illumina). Barcoded libraries were sequenced as PE150 reads on the Illumina NovaSeq 6000 platform. All libraries had >30 million reads, and FastQC v0.11.9 was used for quality control. Barcodes were trimmed using Trim Galore v0.5.0. Reads were aligned to the human genome index (GRCh38, Ensembl release 104) using STAR v2.6.1d (47). Estimated Counts and Transcripts per million (TPM) were quantified using RSEM v1.3.1 (48). Differentially expressed genes were detected using the DESeq2 R package (22), controlling for donor differences; \( P \)-adjusted cutoff of 0.05 and fold change
threshold of 1.5 were used. g:Profiler version e106_eg53_p16_65fcd97 was used for gene set enrichment analysis (49). RNA-seq datasets from HNF1A-MODY donor islets were obtained from Haliyur et al. (13). Pearson’s correlation analysis was performed on Z-transformed average HNF1AKD TPMs (versus 4 controls) and Z-transformed values of the HNF1A-MODY donor (n=1 HNF1A-MODY donor versus 5 controls) for each cell type.

**CUT&RUN Assay and Library Preparation**

CUT&RUN was performed on 500,000 dispersed HNF1α-FLAG pseudoislet cells per condition using CUTANA™ ChIC/CUT&RUN protocol v3.1. Nuclei were extracted with nuclear extraction buffer (20mM HEPES–KOH pH 7.9; 10mM KCl; 0.1% Triton X-100; 20% glycerol; 1mM MnCl₂; 0.5mM spermidine; 1x Halt protease inhibitor, ThermoScientific) for 10min on ice and immobilized onto Concanavalin-A beads (EpiCypher). After blocking and washes, samples were incubated with 0.5µg of rabbit anti-FLAG or rabbit anti-IgG antibodies (Supplemental Table S2) overnight at 4°C. pAG-MNase (EpiCypher) was added to nuclei (1:20) and incubated at RT for 10min. Targeted chromatin digestion was induced by adding 100mM CaCl₂ and nutating for 2hrs at 4°C. DNA fragments were purified using the CUTANA™ ChIC/CUT&RUN kit, according to the manufacturer’s instructions. DNA was resuspended in 0.1X TE and used for library preparation with the CUTANA™CUT&RUN Library Prep Kit (Epicypher 14-1001), according to the v1 manual. Libraries were sequenced as PE150 reads on the NovaSeq platform.

**CUT&RUN Data Analysis**

All libraries had >25 million reads. Reads were trimmed and aligned using CUT&RUNtools (50). Trimmomatic was used for trimming (51), Bowtie2 for alignment (52), and HOMER for peak calling using macs2.narrow outputs (26). P-values for motif enrichment were generated by HOMER software. Genome browser tracks were generated from mapped reads using the
“makeUCSCfile” command. The GREAT algorithm was used for gene annotation, using default parameters (28).

Statistics

The number of biological replicates, measure of central tendency/deviation, and statistical test used for analysis are detailed in figure legends. Graphs and statistical analysis were produced using GraphPad Prism (v9) and R (v4.1.1). Cytometry data were graphed using FlowJo (v10.8). Venn Diagrams and heat maps were generated in R. Browser tracks were generated using the UCSC genome browser (53) and method graphics were created with BioRender.com.

Study Approval

Animal studies were approved by Stanford’s Administrative Panel on Laboratory Animal Care (APLAC 29985). Human pancreatic samples were deidentified, and therefore not considered as human subject research by the Stanford Institutional Review Board.

Data Availability

The RNA-seq and CUT&RUN sequencing data from this publication have been deposited in NCBI’s Gene Expression Omnibus (GEO) (54) and are accessible through GEO Series accession number GSE246230. Values for all data points shown in graphs are in the Supporting Data Values file.

AUTHOR CONTRIBUTIONS

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REFERENCES


**Figure 1.** shRNA-mediated 'knockdown' (KD) of *HNF1A* in primary human islets. (A) Formation of pseudoislets for downstream assays; transduction with lentivirus, followed by reaggregation over 5 days in culture. (B) Schematic of lentiviral constructs coding for shRNA and a GFP reporter (tGFP); Control-shRNA= non-targeting (“control”), HNF1A-shRNA= HNF1A-targeting (“HNF1AKD”). (C,D) Blue light (488nm) images of human (C) control and (D) HNF1AKD pseudoislets, scale bars=1000μm. (E, F) mRNA expression of (E) *HNF1A* and (F) putative HNF1α targets in HNF1AKD relative to control pseudoislets; statistics performed on deltaCT values (n=4-8 donors per gene). (G) Western blot analysis of HNF1α protein expression in control (CTL) and HNF1AKD (KD) pseudoislets (n=3) and (H) quantification of blot intensities normalized to the housekeeping gene β-actin (ACTB). Data are presented as mean values ± SEM. Two-tailed t-tests were used to generate *P*-values; *P<0.05, **P<0.01, ***P<0.00001.
Figure 2. HNF1A suppression leads to dysregulated insulin and glucagon secretion after one month in vivo. (A) Experimental approach for control and HNF1AKD pseudoislet transplantation under kidney capsules of NSG mice and characterization of graft phenotypes; 1000 pseudoislets were transplanted per mouse. (B) Blood glucose, (C) plasma human insulin levels, and (D) area under the curve (AUC) of insulin excursion upon intraperitoneal (IP) glucose challenge after transplantation of pseudoislets to NSG mice (n=4 mice, 3 human donors). (E) Schematic of pseudoislet transplantation to glucagon knockout mice on an NSG background (GKO-NSG) for characterization of glucagon phenotypes; 1000 pseudoislets were transplanted per mouse. (F, I) Blood glucose, (G, J) plasma glucagon levels, and (H, K) AUC of glucagon excursion upon IP (F-H) glucose or (I-K) insulin challenge after transplantation to GKO-NSG mice (n=4 mice, 4 human donors). Data are mean values ± SEM. Two-tailed t-tests were used to generate P-values; *P<0.05, **P<0.01.
Figure 3. RNA-seq of HNF1AKD β cells shows that HNF1α regulates insulin secretion, metabolism, developmental pathways, and cell-cell signaling in β cells. (A) Schematic of FACS scheme for isolation of transduced live β cells (HPi2+GFP+NTPDase3+) from control and HNF1AKD pseudoislets for downstream RNA sequencing (n=4 donors). (B) Fraction of endocrine (HPi2+) cells expressing GFP in sorted samples. (C) HNF1A transcripts per million (TPM) in sequenced samples. (D) Differential expression analysis revealed significantly up- and down-regulated genes after HNF1AKD in β cells; thresholds: Fold change (FC)= 1.5, adjusted P-value= 0.05. (E) Heatmap of differentially expressed genes (DEGs) in β cells after HNF1AKD. Significantly (F) downregulated and (G) upregulated gene ontology (GO) pathways and (H) downregulated KEGG pathways in HNF1AKD relative to control β cells. P= Benjamini-Hochberg adjusted P-value; all P<0.05.
Figure 4. RNA-seq of HNF1AKD α cells identifies dysregulation of calcium channel complexes and ATPase-coupled transmembrane transport, as well as hormone secretion pathways shared with β cells. (A) Schematic of methods for isolation of transduced α cells (HPi2+GFP+CD26+) from control and HNF1AKD pseudoislets for downstream RNA sequencing (n=4 donors); left hand image is brightfield and right hand image is blue light (488nm) of human HNF1AKD pseudoislets, scale bars=1000μm. (B) HNF1A transcripts per million (TPM) in sequenced α cell samples. (C) Differentially expressed gene (DEG) analysis revealed significantly up- and down-regulated genes after HNF1AKD in α cells; thresholds: FC=1.5, adjusted $P$-value=0.05. (D) Venn diagram comparing α versus β cell DEGs revealed shared and cell-specific consequences of HNF1AKD. Gene ontology (GO) pathways of (E) shared and (F) α cell enriched DEG sets. (G) Boxplots displaying TPM of select DEGs. $P$= Benjamini-Hochberg adjusted $P$-value; *$P<0.05$; all $P<0.05$ for GO pathways.
Figure 5. CUT&RUN identifies direct binding targets of HNF1α in primary human islet cells. (A) Schematic of methods: pseudoislets expressing HNF1α-FLAG were used for CUT&RUN with anti-FLAG or anti-IgG (control) antibody (n=3 donors). (B) Enriched motifs in the HNF1α-FLAG CUT&RUN peaks (versus IgG controls). (C) Venn diagram of genes associated with HNF1α-FLAG peaks identified by CUT&RUN (HNF1α-FLAG CUT&RUN) versus HNF1AKD differentially expressed genes in primary islet cells identified by RNA-seq (HNF1AKD RNA-seq). (D) Gene ontology (GO) pathway analysis of overlapping genes from panel C, subset into genes that were downregulated or upregulated in RNA-seq analysis. (E, F) UCSC Genome Browser tracks showing genomic regions associated with HNF1α-FLAG CUT&RUN peaks near the genes (E) TM4SF4 and (F) CACNA1D; HNF1α-FLAG CUT&RUN enriched peaks identified by Genomic Regions Enrichment of Annotations Tool (GREAT) are highlighted in dashed boxes, and regulated genes are depicted below IgG control tracks. Accessible chromatin regions in human islets are shown by ATAC-seq and ChIP-seq (H3K427ac, H3K4me1, and H3Kme3) tracks (Pasquali et al. 2014). (G) Schematic depicting HNF1α’s dual role as a transcriptional activator and repressor in pancreatic islet cells.
Figure 6. Comparison of HNF1α targets in primary human islets with HNF1A-MODY adult donor datasets demonstrates conserved HNF1α regulatory pathways that are critical for mature islet cell function. (A-B) Heatmaps showing relative expression of genes in (A) α and (B) β cells isolated from an HNF1A+/-T260M donor (MODY) versus healthy control donors (C1-C5) (data from Haliyur et al. 2019); genes depicted were top differentially expressed genes in primary islet HNF1AKD RNA-seq data also identified in HNF1α-FLAG CUT&RUN data (putative adult HNF1α targets).