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Comprehensive alpha, beta and delta cell transcriptomes reveal that ghrelin selectively activates delta cells and promotes somatostatin release from pancreatic islets

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ABSTRACT

Objective: Complex local crosstalk amongst endocrine cells within the islet ensures tight coordination of their endocrine output. This is illustrated by the recent demonstration that the negative feedback control by delta cells within pancreatic islets determines the homeostatic set-point for plasma glucose during mouse postnatal development. However, the close association of islet endocrine cells that facilitates paracrine crosstalk also complicates the distinction between effects mediated directly on beta cells from indirect effects mediated via local intermediates, such as somatostatin from delta cells.
Methods: To resolve this problem, we generated reporter mice that allow collection of pure pancreatic delta cells along with alpha and beta cells from the same islets and generated comprehensive transcriptomes for each islet endocrine cell type. These transcriptomes afford an unparalleled view of the receptors expressed by delta, alpha and beta cells, and allow the prediction of which signal targets which endocrine cell type with great accuracy.
Results: From these transcriptomes, we discovered that the ghrelin receptor is expressed exclusively by delta cells within the islet, which was confirmed by fluorescent in situ hybridization and qPCR. Indeed, ghrelin increases intracellular calcium in delta cells in intact mouse islets, measured by GCaMP6 and robustly potentiates glucose-stimulated somatostatin secretion on mouse and human islets in both static and perfusion assays. In contrast, des-acyl-ghrelin at the same dose had no effect on somatostatin secretion and did not block the actions of ghrelin.
Conclusions: These results offer a straightforward explanation for the well-known insulinostatic actions of ghrelin. Rather than engaging beta cells directly, ghrelin engages delta cells to promote local inhibitory feedback that attenuates insulin release. These findings illustrate the power of our approach to resolve some of the long-standing conundrums with regard to the rich feedback that occurs within the islet that is integral to islet physiology and therefore highly relevant to diabetes.

Keywords Ghrelin; Delta cell; Somatostatin release; Transcriptome; Beta cell; Alpha cell

1. INTRODUCTION

Pancreatic alpha and beta cells co-localize in an arrangement that facilitates accurate coordination of glucagon and insulin release from islets. While glucagon and insulin, along with Islet amyloid polypeptide (Iapp), are among the few islet signals that meet the classic definition of a hormone as a 'factor that is released into the general circulation to signal at a distant site in the body', a rich constellation of paracrine and neural interactions takes place within the islet to ensure tight control over their release [1–3]. Pancreatic delta cells are the third-most common endocrine cell type in the islets, and the somatostatin they release is an important inhibitor of both insulin and glucagon [4–6]. We recently described a novel negative feedback loop where the paracrine peptide Urocortin 3 (Ucn3) is co-released with insulin from beta cells and promotes glucose-stimulated somatostatin release via the type 2 corticotropin-releasing hormone (Crhr2) receptor expressed

The transcriptome data reported in this paper can be accessed at http://huisinglab.com/islet_txomes_2016/ and have been deposited in the Gene Expression Omnibus (GEO) repository under accession number GSE80673.

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Abbreviations: Crhr2, Corticotropin-releasing hormone receptor type 2; FISH, Fluorescent in situ hybridization; Ghsr, Growth hormone secretagogue receptor; GSSS, Glucose-stimulated somatostatin secretion; Iapp, Islet amyloid polypeptide; RPKM, Reads per kilobase gene model per million reads sequenced; Trpm2, Transient receptor potential melastatin 2; Ucn3, Urocortin 3; YFP, Yellow fluorescent protein

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by delta cells [7]. Somatostatin then attenuates further insulin secretion and helps to maintain stable and tight control over plasma glucose. The appearance of Ucn3 across the beta cell mass correlates with the well-known uptick in plasma glucose levels [8–10]. The onset of Ucn3 is directly responsible for this phenomenon by initiating delta cell-dependent feedback on insulin release, thus determining the homeostatic set-point for plasma glucose [7]. Delta cell-mediated feedback breaks down early in diabetes, which leads to marked increases in plasma glucose fluctuations that resemble the glycemic volatility that impacts patients across the diabetes spectrum [7].

While these observations highlight the physiological importance of delta cell-dependent feedback, we know relatively little about the cues that control delta cells. The intimate co-localization of islet endocrine cells has also made it difficult to distinguish direct effects on beta cells from indirect actions that are mediated by locally produced intermediaries. We previously generated a transgenic mIns1-H2b-mCherry reporter mouse in which beta cells are labeled by the nuclear expression of mCherry driven by the mIns1 promoter and crossed these to S100b-eGFP reporter mice that we serendipitously discovered to label alpha cells [11]. These reporter mice enabled the comparison of mouse and human beta cell transcriptomes but did not fully resolve the transcriptomes of pancreatic alpha and delta cells. To improve substantially on these prior observations and to determine the factors that directly engage delta cells, we now generated a set of triple transgenic mouse models in which mIns1-H2b-mCherry [11] beta cells are crossed to mice with alpha or delta cells are marked by YFP in a Cre-dependent fashion. We used these reporter mice to generate unbiased and comprehensive transcriptomes of pancreatic delta and alpha cells, along with beta cells from the same islets. Our transcriptomes are validated by the strong enrichment for a large panel of known alpha, beta, and delta cell markers in the appropriate cell type. We then use this information to determine the signals that directly engage the delta cell by virtue of selectively expressed receptors and analyze ghrelin stimulation of delta cells as an example to highlight the utility of our approach. We discovered that ghrelin acts directly on mouse and human delta cells to promote somatostatin release. Our observations offer a straightforward explanation for the well-known insulinostatic actions of ghrelin (e.g. [12–16]) and illustrate the physiological importance of delta cell-mediated feedback within pancreatic islets. An accurate understanding of ghrelin's mechanism of action within pancreatic islets is highly relevant given ghrelin's central role in the regulation of energy and glucose metabolism [17].

2. RESEARCH DESIGN AND METHODS

2.1. Biological materials and ethics statements

All mouse procedures were approved by the UC Davis or the Salk Institute for Biological Studies Institutional Animals Care and Use Committee and were performed in compliance with the Animal Welfare Act and the Institute for Laboratory Animal Research (ILAR) Guide to the Care and Use of Laboratory Animals. Animals were maintained on a 12-h light/12-h dark cycle with free access to water and standard rodent chow. Static and dynamic hormone secretion experiments were carried out on C57BL/6NHsd mice, between 8 and 16 weeks of age, from Harlan (Indianapolis, IN). We obtained human islets via the Integrated Islet Distribution Program; the receipt was declared exempt from IRB review under 45 CRR 46.101 (b) category (4).

2.2. Immunofluorescence and FISH

Immunofluorescence was conducted as previously described [7,8]. Insulin was detected using guinea pig anti-insulin (Dako #A0564; 1:500), somatostatin using sheep anti-somatostatin (American Research Products Inc. #13-2366, 1:1000), Ucn3 using rabbit anti-Ucn3 (#6570; in house, 1:2000), Glucagon using rabbit anti-glucagon (Abcam #ab11022-1; 1:200), and YFP using goat anti-GFP (Rockland 600-101-215; 1:1000). All secondary antibodies were obtained from Jackson Laboratories Inc. RNA FISH was conducted using RNAscope probes for *Ghsr* and *Sst* (Advanced Cell Diagnostics) according to the manufacturers instructions.

2.3. Islet isolation and FACS sorting

Islet isolation was conducted as previously described [7,11,18]. Islets from mIns1-H2b-mCherry [11] (deposited with the Jackson laboratories as strain #28589) × Rosa-LSL-YFP [19] × Sst-Cre [20] or Gcg-Cre [21] triple transgenic animals were pooled by sex in 2 (Sst-Cre) or 3 (Gcg-Cre) replicate groups of a dozen animals. FACS sorting was conducted as described previously [7,11] with each sample collected directly in Trizol to ensure immediate cell lysis and preservation of RNA integrity.

2.4. Next generation sequencing and bioinformatics

RNA was isolated from Trizol-preserved samples by chloroform extraction and cleaned up over an RNeasy microcolumn essentially as previously described [11]. RNA quality was verified by Tapestation (Agilent, Santa Clara, CA). Indexed sequencing libraries were constructed using the TruSeq RNA sample Prep Kit v2 (Illumina Inc. San Diego, CA), sequenced at 50 cycles, and single read on an Illumina HiSeq 2000 platform. Results were validated by qPCR using Sybr chemistry and the primers listed in Table 1. Sequencing reads were mapped to the mouse genome version GenCode M5 (GRCm38.p3) using STAR v2.4 [22]. On average over 33 million reads were sequenced for each library with 89.9% of sequenced reads aligning (>63% unique alignment overall). FeatureCounts [23] was used to create count tables of the sorted bam files using reads aligning to RefSeq-defined exons. EdgeR version 3.12.0 [24] was used to conduct pairwise statistical comparisons. Wordles of transcript abundance were generated on wordle.net. Single cell RNAseq data from [25] were used to generate the violin plots in Figure 2C. Cells that had an RPKM value > 10 k of either *Sst*, or *Ins2*, or *Gcg* were defined as delta, beta, or alpha cells, respectively.

2.5. Functional imaging by GCaMP6

Sst-Cre mice and LSL-GCaMP6 mice (Jackson laboratories strain #24106) were crossed for functional imaging. Intact islets from bitransgenic offspring were plated on poly-D lysine-coated number 1.5 35 mm glass cover slip tissue culture dishes (Mattek) and maintained at 37 °C 5% CO_2 in RPMI 10% FBS, 5.5 mM glucose with pen/strep.

Table 1 – qPCR primer information.

Ref Seq ID	Gene	Primer	Sequence 5′ → 3′	Amplicon size (bp)
NM_008100	Gcg	qrodGcg.fwu1	TCACAGGGCACATTCACCAG	121
		qrodGcg.rvu1	CATCATGACGTTTGGCAATGTT	
NM_001185084	Ins2	qrodIns2.fwu1	GCTCTCTACCTGGTGTGTGGG	128
		qrodIns2.rvu1	CAAGGTCTGAAGGTCACCTGC	
NM_009215	Sst	qrodSst.fwu1	GACCCCAGACTCCGTCAGTTT	112
		qrodSst.rvu1	TCTCTGTCTGGTTGGGCTCG	
NM_021488	Ghsr	qmGhsr.fwu1	GACCAGAACCACAAACAGACAG	113
		qmGhsr.rvu1	GGCTCGAAAGACTTGGAAAA	
NM_013556	Hprt	qmHPRT.fwu	TCCTCCTCAGACCGCTTTT	90
		qmHPRT.rvu	CCTGGTTCATCATCGCTAATC	

