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Islet β–Cell Transcriptome and Integrated-omics

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Abstract

Purpose of Review—β cells represent one of many cell types in heterogeneous pancreatic islets and play the central role in maintaining glucose homeostasis, such that disrupting β cell function leads to diabetes. This review summarizes methods for isolating and characterizing β cells, and describes integrated "omics" approaches used to define the β cell by its transcriptome and proteome.

Recent Findings—RNA Sequencing and mass spectrometry-based protein identification have now identified RNA and protein profiles for mouse and human pancreatic islets and β cells, and for β cell lines. Recent publications have outlined these profiles and, more importantly, have begun to assign the presence or absence of specific genes and regulatory molecules to β cell function and dysfunction. Overall, researchers have focused on understanding the pathophysiology of diabetes by connecting genome, transcriptome, proteome, and regulatory RNA profiles with findings from genome wide association studies (GWAS).

Summary—Studies employing these relatively new techniques promise to identify specific genes or regulatory RNAs with altered expression as β cell function begins to deteriorate in the spiral toward the development of diabetes. The ultimate goal is to identify potential therapeutic targets to prevent β cell dysfunction and thereby better treat the individual with diabetes.

Keywords

β-cell transcriptome; β-cell function; β-cell dysfunction; gene expression; diabetes

Introduction

The human pancreas performs both endocrine and exocrine functions. Pancreatic endocrine functions are served by the Islets of Langerhans, a heterogeneous arrangement of endothelial cells and several different hormone secreting cells including α, β, δ, PP, and epsilon cells (1,2). The pancreatic islets play the major role of maintaining glucose homeostasis through the regulated release of insulin and glucagon from β and α cells, respectively. Type 1 diabetes (T1D) is caused by an autoimmune attack that results in β cell destruction and a consequent loss of endogenous insulin production (3). Type 2 diabetes (T2D) occurs as a result of resistance to insulin activity in muscle and adipose tissue, coupled with β cell dysfunction (4,5). Thus, the pancreatic β cell is a prime target for gene expression analysis in

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healthy, deteriorating, and diseased states. Until recently, however, β cell specific studies have been difficult due to the heterogeneous arrangement of pancreatic islet cellular composition.

In order to determine how gene expression controls β cell growth, development, response to environmental stimuli, and function (as well as dysfunction), studies should ideally focus on purified β cells. Previous efforts to obtain such purified β cells from heterogeneous islet cellular composition have relied upon flow cytometry based sorting methodologies and unique β cell characteristics such as side scatter and granularity (6,7), zinc-content (8-11), mice genetically engineered such that their β cells express marker genes (12-14), combinations of surface markers (15-17), and intracellular hormone staining (18). More recent technological advances in islet cell separation techniques, next generation sequencing, and mass spectrometry have improved our understanding of the pancreatic β cell at the DNA, RNA, and protein levels. This review will focus on how new results using these techniques have suggested previously unsuspected effects from the environment, lifestyle, differentiation factors, and regulatory RNA molecules. The control of gene expression at the transcriptome level and the resultant proteome together show how these factors control β cell function, proliferation development, and dysfunction resulting in diabetes (Figure 1).

Text of Review

β Cell Transcriptome

Transcriptome analyses from whole pancreatic islets or cultured β cell-lines using real-time PCR and microarrays (19-22) have long served as a surrogate for the β cell gene expression pattern. Advanced flow cytometry based methods for β cell sorting have improved the ability of researchers to measure cell-specific gene expression patterns (17,18). Most recently, the advent of next generation RNA Sequencing (RNASeq) (23) has provided an unbiased and high throughput method for examining gene expression profiles from enriched β cells (both rodent and human).

The most recent β cell transcriptome studies have focused on: 1.) Establishing the gene expression profile for each islet cell subtype (24-26), 2.) Determining the cellular location of each genome wide association screen (GWAS) gene identified from populations with T1D and T2D (25-27), and 3.) Assessing the effect of immune pressure on β cell health and gene expression patterns (27).

On average, β cells comprise approximately 30-75% of the endocrine pancreatic islet in humans, whereas in mice, β cells constitute about 60-80% of the islet cells (28). Transgenic mice expressing green fluorescent protein controlled by the mouse insulin I gene promoter (so called MIP-GFP mice) can be used to isolate a 95% pure β cell population based upon GFP expression (12). A limitation of such approaches is, of course, that the expressed transgene could influence the expression of other transcripts. With that caveat in mind, Ku et al. performed RNASeq on both whole islets and sorted GFP-positive β cells to identify genes specifically expressed in β cells (*24). Of the over 12,000 genes detected in the β cell, 43 demonstrated greater than 4-fold more gene expression in the β cell versus whole islets,

whereas approximately 1,400 whole islet-specific genes displayed a 4-fold decreased expression in the β cell enriched population. Interestingly, they identified 16 genes that were exclusively expressed in the enriched β cells and not in any other organ tissue.

Transitioning from mouse to human islets, Bramswig et al. used surface antibodies to greatly enrich α, β, and exocrine cell populations for transcriptome analysis. These results provide a census of genes expressed in each cell population along with their relative, and in some cases apparently cell-specific gene expression (*25). In another study, Nica et al. used Newport Green staining to compare gene expression profiles of enriched human β cell populations to both the non-β cell and whole islet fractions. They applied a mathematical modeling strategy based upon these populations in an effort to account for α and δ cells contaminating the β cells, and specifically identified 614 islet genes not expressed in the β cell and 526 genes unique to the β cell (**26). As previously speculated based upon the innervated nature of pancreatic islets and other β cell characteristics, the functional annotation analysis found that the β cell specific gene expression pattern demonstrated numerous similarities to neuronal cells (29,30).

GWAS studies examining single nucleotide polymorphisms have identified over 70 candidate genes potentially linked to T1D or to T2D (31-33). RNASeq has permitted tissue specific, high throughput detection of mRNA from those genetic loci. Using RNASeq followed by real time PCR confirmation, over 60% of the identified T1D genes (such as the insulin gene), have been localized to the pancreatic β cell (26,**27). In T2D, greater than 90% of the associated genes, including VEGFA and SLC30A8, are specifically expressed in the β cell (26).

In other studies, overexpression of inflammatory, innate, and autoimmune response genes have been found in affected islets from individuals with T1D (34). Upon exposure to cytokines, the islet responded by increasing expression of apoptosis and inflammation related genes and T1D candidate gene expression was altered specifically in the β cell (27). These findings further establish the link between the endocrine β cell and the body's immune system.

Regulatory RNAs in the β Cell

A network of transiently expressed transcription factors control pancreatic organogenesis and subsequent differentiation, including the development and proliferation of the endocrine pancreas (including the β cell subpopulation). Non-coding RNAs have been implicated as major regulators of gene expression and include both small microRNA (miRNA) and long noncoding RNA (lncRNA) (35,36).

MicroRNAs have been intricately studied in mouse pancreatic development and maintenance. miR-375 has been emphasized as a key miRNA regulating α and β cell mass, insulin secretion, and therefore total body glucose homeostasis (37). Furthermore, miR-375 expression has recently been reported in an enriched human β cell population, highlighting its likely importance in β cell health (**38,**39). When looking at human miRNA profiles, 366 miRNAs were found to be expressed in the human islet, and 85% were expressed in both whole islets and β cells. As compared with α cells, β cells expressed 19-fold more of

these miRNAs (39), and that expression was inhibited to a greater degree by α-cell specific transcription factors. Klein et al. overexpressed β cell specific miRNAs in α-TC cells and inhibited those same putative β cell miRNAs in MIN6 cells (a mouse β cell line). They reported that overexpressing β cell miRNAs decreased glucagon mRNA expression by silencing α cell specific transcription factors, whereas suppressing the β cell specific miRNAs increased the levels of α-cell transcription factors (39). Similarly, Barbagallo et al. showed that suppressing miRNA expression in α-TC cells in response to environmental stress resulted in overexpression of targets (such as IGF1R, IRS-1 and ERK-1) and prevented apoptosis (40). Overall, these recent studies show that miRNAs play a key role in promoting the β cell phenotype by preventing expression of many genes that impair proper β cell function, previously termed "β cell disallowed genes" by Schuit et al (41). A study by van de Bunt et al. [**38] showing how islet specific miRNA target sequences matched GWAS-identified genes and variations associated with T2D demonstrates that repressing specific genes is required for proper β-cell function. Given the sequence specific regulatory function of miRNAs, variation in the target sequence appears to alter miRNA regulatory mechanisms and may well contribute to T2D pathogenesis.

Much like the miRNA clusters that occur within small regions of genomic DNA and are transcribed together (42-44), lncRNAs with tissue-specific expression patterns are found adjacent to protein coding genes, especially transcription factors (45-47). In addition to mRNA profiles, transcriptome analysis by next generation sequencing has also discovered, identified, and cataloged lncRNA that are specifically expressed in whole islet or enriched β cell populations (24,25,**48). In whole islets and in enriched β cells, lncRNAs appear to be much more tissue- or cell-specific than protein coding genes (24,48). Two separate studies by Ku et al. and Morán et al. show that lncRNAs are co-located in the same genomic region as specific islet and β cell proteins and transcription factors and include intergenic and antisense RNA located near FOXA2, GATA1, HNF1A, INSM1, ISL1, MAFB, NEUROD1, NKX2-2, NKX6-1, PAX6, PCSK1, PDX1, and RFX6 (24,48). Further, lncRNA expression may be more prominent during endocrine differentiation and maturation versus early development, and lncRNAs may serve as potential biomarkers in embryonic or induced pluripotent stem (iPS) cell differentiation into β cells (48). Last, like miRNAs, many lncRNAs appear to be associated with T2D-gene variants, and the regulatory effect on gene expression may contribute to β cell dysfunction and diabetes (48).

β Cell Proteome

Similar to what RNASeq has allowed for RNA analysis, mass spectrometry serves as an unbiased method to detect nearly all proteins expressed in a particular cell type: at baseline, in response to environmental conditions, or in the disease state (49,50). The proteome serves as the ultimate functional result of miRNA and lncRNA regulation on β cell gene expression. As our understanding of the genomics of β cell biology advances, characterizing the β cell proteome has become even more critical to promote understanding β cell function and dysfunction.

Specific proteomic studies of whole islets, cultured cell lines, and enriched β cells have used two approaches: 2-dimensional gel electrophoresis (2DGE) combined with mass

spectrometry (MS) (51) and liquid chromatography in line with tandem MS (LC-MS/MS) (52). Ahmed et al. generated the first proteomic gel maps of isolated human islets, which opened the door to studying whole-cell protein expression across multiple conditions and disease states (53). More recently, the same group used 2DGE/MS to demonstrate altered expression of 75 mitochondrial proteins in the INS1E rat β cell line subjected to chronic hyperglycemia (54). Similarly, Maris and colleagues identified 74 differentially expressed proteins in INS1E cells cultured in hyperglycemic conditions, suggesting potential targets responsible for β cell dysfunction from chronic high glucose exposure (55). Due to their frequent use as a model system for studying insulin secretion and β cell dysfunction, D'Hertog et al. developed a 2DGE/MS proteomic reference map for INS1E cells (56). In the MIN6 mouse β cell line, Diraison et al. used 2DGE/MS to demonstrate that translationally controlled tumor protein (TCTP) is glucose regulated, suppressed by cell stress, and that it therefore appeared to play a protective role for β cells during hypoglycemia (57). In studying islet cell differentiation from islet and bone marrow-derived mesenchymal stem cells (MSCs), Zanini et al. showed that different sources of MSCs did not yield identical proteomic profiles when the cells were differentiated into islet-like cells (58). Coppola and colleagues generated a cytokine-resistant mouse β cell line and profiled protein differences between the cytokine sensitive and resistant β cells in order to better understand the autoimmune destruction of β cells in T1D (59). Specifically relevant to T1D studies, proteomics has been used to identify potential antibody targets for therapeutics (60). For instance, Massa et al. attempted to identify new autoantigen targets by blotting human pancreatic islet cells against sera from patients with T1D (*61). Finally, Maris et al used 2DGE/MS to highlight the changes in the β cell proteome induced by the fatty acid palmitate, implicating it as a cause of β cell dysfunction (62).

Sample availability and quantity are often limiting factors for β cell biologists (and in particular for human β cell studies), but newer LC-MS/MS based methods have recently been employed to resolve the β cell proteome (52,63). The first analysis of the human islet proteome by LC-MS/MS by Metz and colleagues identified an unprecedented 3,365 proteins (64). Waanders et al. later identified 6,873 unique proteins using mouse pancreatic islets, requiring only 3-4,000 cells in total (65). Using these general proteomic profiles and whole human islets, Schrimpe-Rutledge et al. identified 256 proteins that are upregulated or downregulated after chronic hypoglycemia (*66). Similar profiling of rat β cells showed that both regulators of protein biosynthesis and glycolytic enzymes were upregulated during hypoglycemia (67). Consistent with observations about the neuronal nature of the β cell, Schvartz and colleagues used INS1E cells grown in hyperglycemic conditions and identified, as one of 11 proteins upregulated by glucose stress, neuronal pentraxin 1 (NP1), a protein previously thought to be expressed only in the brain (68).

In recent years, subcellular β cell proteomics has become a popular target for study. Danzer et al. used a cell surface protein isolation method combined with LC-MS/MS to characterize the proteome of membrane-resident glycoproteins in both MIN6 cells and human whole islets (69). Han et al. used INS1E cells to identify 683 novel β cell phosphorylation sites from over 1,419 identified phosphoproteins (70). Also using INS1E cells, Schvartz et al. were able to identify not one, but three separate proteomic profiles exclusive to mature insulin secretory vesicles (ISCs), immature ISCs, and proteins common to both ISCs and the

rest of the cell (71). In all of these studies, the proteins identified have great potential as therapeutic targets to prevent or overcome β cell dysfunction.

Conclusion

Recent technological advances have made it possible to separate the heterogeneous cells comprising an islet into the α, β, and δ cell subpopulations thereby making it possible to independently investigate the gene expression profiles and regulatory mechanisms specific to β cells (and other cell types). Similarly, advanced technologies to discover and catalog mRNA, miRNA, lncRNA, and proteins specific to β cells in an unbiased fashion will identify potential therapeutic targets responsible for β cell function, differentiation, proliferation, or dysfunction. As we better characterize the β cell, the techniques promise to identify transcripts, regulatory RNAs, and/or proteins that control how environmental, genetics, and epigenetic factors contribute to diabetes pathogenesis. Scientists hope to apply these findings to determine what molecules are necessary to promote embryonic or iPS cells to differentiate into insulin producing β cells, to identify biomarkers of β cell dysfunction or autoimmune destruction, and to understand how β cells and the other endocrine cell types maintain glucose homeostasis.

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Abbreviations

T1D	(Type 1 Diabetes)
T2D	(Type 2 Diabetes)
(RNASeq)	RNA Sequencing
(GWAS)	Genome wide association study
(MIP-GFP)	Transgenic mice expressing green fluorescent protein controlled by the mouse insulin I gene promoter
(miRNA)	microRNA
(lncRNA)	long noncoding RNA
(iPS) cells	induced pluripotent stem
(2DGE)	2-dimensional gel electrophoresis
(LC-MS/MS)	liquid chromatography inline with tandem mass spectrometry
(TCTP)	translationally controlled tumor protein
(NP1)	neuronal pentraxin 1

(ISCs)	insulin secretory vesicles

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Key points

- Pancreatic islets are very complex ‘miniorgans’ composed of many different cell types: endocrine and nonendocrine. Flow cytometry and antibody staining techniques enable the separation of highly enriched subpopulations (particularly β cells) for detailed analysis.
- Next generation sequencing and mass spectrometry analysis have generated β cell specific mRNA, miRNA, lncRNA, and protein profiles.
- β-cell transcriptome and proteome results show how associated DNA variants can alter gene expression and regulatory mechanisms.
- β cell expression profiling efforts are beginning to identify genes, regulatory RNAs, and proteins that are differentially expressed in diseased versus healthy cells.
- Expression profiles at multiple stages of development should help guide the directed differentiation of embryonic and induced pluripotent stem cells to glucose sensitive, insulin secreting β cells.

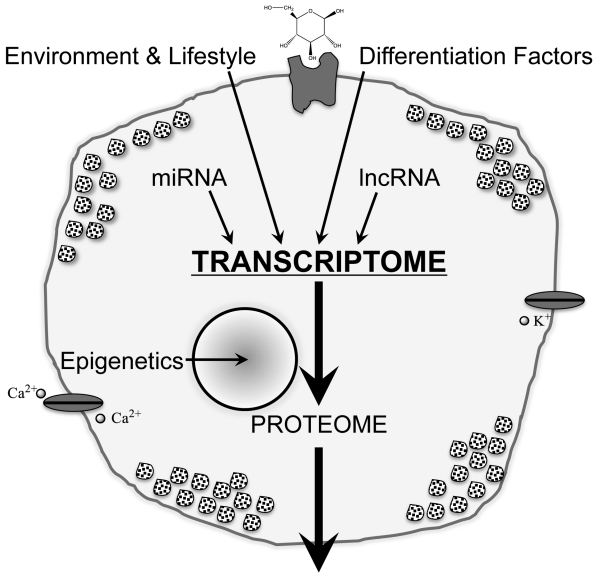

Figure 1. Factors controlling the β cell transcriptome

External and internal factors control β cell gene expression as represented by the mRNA transcriptome. The connection between the transcriptome and the expressed proteins are manifested by the β cell phenotype.

SOURCE: ORIGINAL