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Unraveling pancreatic islet biology by quantitative proteomics

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Abstract

The pancreatic islets of Langerhans play a critical role in maintaining blood glucose homeostasis by secreting insulin and several other important peptide hormones. Impaired insulin secretion due to islet dysfunction is linked to the pathogenesis underlying both Type 1 and Type 2 diabetes. Over the past 5 years, emerging proteomic technologies have been applied to dissect the signaling pathways that regulate islet functions and gain an understanding of the mechanisms of islet dysfunction relevant to diabetes. Herein, we briefly review some of the recent quantitative proteomic studies involving pancreatic islets geared towards gaining a better understanding of islet biology relevant to metabolic diseases.

Keywords

diabetes; insulin resistance; pancreatic β cells; pancreatic islets; quantitative proteomics; signaling pathway

The islets of Langerhans are micro-organs localized within the pancreas that account for nearly 2% of the total pancreatic mass. These islets consist of at least five types of endocrine cells, including glucagon-producing α cells, insulin-producing β cells, somatostatin-producing δ cells, pancreatic polypeptide-producing pancreatic polypeptide cells and ghrelin-producing ϵ cells [1]. Among these cells, the insulin-producing β cells are the predominant cell type, contributing to approximately 70% of all islet cells [2], and the secreted insulin serves as the primary regulator of blood glucose homeostasis. Elevated blood glucose levels promote insulin synthesis, as well as its secretion from β cell secretory granules. In turn, the secreted insulin promotes glucose uptake in peripheral insulin-sensitive tissues, such as liver, muscle and adipose tissues, thus regulating blood glucose back to normal levels.

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Impaired insulin secretion from pancreatic β cells is one of the major factors associated with both Type 1 and Type 2 diabetes mellitus. Type 1 diabetes mellitus (T1DM) is characterized by a loss of insulin-producing β cells due to an autoimmune attack that often leads to complete insulin deficiency. On the other hand, Type 2 diabetes mellitus (T2DM) is characterized by a relative reduction in insulin secretion due to β -cell loss or β -cell dysfunction combined with insulin resistance in the peripheral tissues. A significant loss of β cell mass is characteristic in T1DM and late stages of T2DM [3–5]. There are several lines of evidence pointing to a variety of mechanisms for β cell loss, including autoimmune attack [6], lipotoxicity [7,8], glucose toxicity [9], amyloid deposition [10], glutamate toxicity [11] and altered insulin receptor signaling [12]. Importantly, a more detailed understanding of the molecular pathways associated with β -cell function and the development of diabetes will benefit the development of novel therapeutic strategies for diabetes.

Biological studies geared towards unraveling the molecular mechanisms of islet functions, such as regulation of insulin secretion and β -cell loss, have accelerated as a result of increasingly available genome sequences and continuously improving genomic and proteomic technologies. Benefiting from the recent advances in mass spectrometry (MS) and bioinformatics, proteomics is now capable of identifying and quantifying thousands of proteins from isolated pancreatic islets [13–17], islet-derived primary cell cultures [18], islet-related cell lines [19–21] and subcellular organelles from islet-related cell lines [22–24]. Herein, we review some of the studies involving isolated pancreatic islets, primarily focusing on advances afforded by quantitative proteomic applications in islet biology over the past 5 years. Earlier studies on islet proteomics were covered by a prior review [25]. Before delving into these studies, we will provide a brief overview of the quantitative proteomic technologies.

Quantitative proteomic technologies

Quantitative measurements of differential protein abundances are essential for elucidating molecular pathways associated with a given biological system, such as pancreatic islets. A number of quantitative MS-based strategies have been applied to study pancreatic islets, including gel-based approaches [26,27], as well as label-free [14,16] and stable isotope labeling [15,17] liquid chromatography (LC)-based approaches. In this section we briefly summarize approaches that either have been applied or are applicable to islet studies.

2D gel electrophoresis

Most of the early proteomic studies on islets employed 2DE, a well-established protein separation and visualization technique, for protein profiling and relative quantification [28–31]. The first dimension of 2DE separates proteins on an isoelectric focusing gel based on their isoelectric point, while the second dimension of 2DE separates proteins based on molecular weight [26]. After electrophoresis, the proteins on the gel are visualized and quantified by gel staining, such as Coomassie Brilliant Blue staining and SYPRO Ruby staining, and protein spots of interest are typically excised, digested and then identified using MS. A 2D-DIGE that utilizes fluorescent tags (Cy2, Cy3 and Cy5) to analyze multiple samples simultaneously can further improve protein quantification by 2DE [27,32]. While 2DE is still one of the commonly applied proteomic technologies in diabetes research [33–36], the technique has several inherent limitations, such as the laborious process of identifying proteins from individual gel spots by MS and the low sensitivity for detecting low-abundance proteins, as well as proteins with extreme molecular weight, isoelectric point or hydrophobicity.

Label-free LC–MS or LC–MS/MS quantification

Liquid chromatography coupled with MS or MS/MS affords a highly sensitive analytical tool with a wide dynamic range of detection for identifying and quantifying proteomic changes under different biological conditions [37,38]. Currently there are two general quantification strategies that do not require the use of an isotopic label to discern changes in protein abundance, commonly referred to as spectral counting and LC–MS intensity-based quantification. Both of these approaches have been applied in islets studies [14,16,39]. Spectral counting utilizes the number of MS/MS spectra that identify a given peptide or protein [40–42] as a measure of peptide or protein abundance. While this strategy is simple and straightforward, the quantification of low-abundance proteins is often unreliable because these proteins are typically identified by a small number of spectra. The second strategy utilizes peptide peak intensities or peak areas from LC–MS to quantify relative peptide and protein abundances among different conditions [38,43,44]. An example of the intensity-based LC–MS approach is the accurate mass and time (AMT) tag strategy [45,46], in which peptides are identified by matching the accurate mass and normalized elution time of each detected LC–MS feature to those in a previously established peptide reference database. Following accurate alignment of detected LC–MS features across different analyses, relative quantification of identified peptides is accomplished by comparing integrated MS peak areas among different samples. Similar quantitative approaches that rely on direct LC–MS measurements, feature alignment and peak identifications have also been reported [47–49].

Conceptually, label-free LC–MS quantitative methods have no limit to the number of samples that can be compared and are able to provide good proteome coverage; however, the reliability of label-free approaches is highly dependent on the LC–MS platform reproducibility, which can often be a challenge for analyzing a large-set of samples.

Stable isotope labeling-based quantification

In addition to label-free quantification, stable isotope labeling-based LC–MS quantification has been commonly applied to biological studies [50]. Common stable isotope labeling strategies include metabolic labeling (e.g., stable isotope labeling with amino acids in cell culture [51]), enzymatic labeling (e.g., trypsin-catalyzed ^{18}O labeling [52–54]) and isotopic tagging with chemical reactions (e.g., isobaric tags for relative and absolute quantification [iTRAQ] [55] and tandem mass tags [56]). iTRAQ was recently applied in proteomic studies of islets to identify factors associated with T2DM [11,13,50], as well as proteins associated with the islet-like cell differentiation [57].

Both iTRAQ and tandem mass tags, which were developed for multiplexed quantification of four to eight samples in a single LC–MS/MS experiment, are based on specific reactions of isobaric tags with the primary amine groups on peptides, such as, N-termini and lysine side chains [55,56]. Samples are labeled with different versions of isobaric tags and pooled prior to LC–MS/MS. The same peptides labeled with different isobaric tags have exactly the same mass and coelute precisely in LC separations. The quantitative information is obtained from the low-molecular-mass reporter ions with different masses that are generated upon MS/MS fragmentation. For example, in the case of quadruplexed iTRAQ labeling, the masses of reporter ions for different samples are 114, 115, 116 and 117. The intensities of these ions can be used for relative quantification of the peptides across four different conditions.

Compared with label-free LC–MS quantification, stable isotope labeling-based quantification provides more reliable quantitative results; however, the labeling may also lead to less proteome coverage, partially due to the increased sample complexity and complication of peptide fragmentation patterns introduced by the tagging [58].

Proteomic studies on islet biology

Table 1 illustrates the range of islet studies that have applied proteomic techniques to unravel islet biology. In this section, we review these studies with regard to islet proteome profiling, glucose-stimulated islet proteome response, mechanisms of β -cell failure, T2DM and insulin resistance, islet development and regeneration, and diabetic drug response. One of the concerns for MS-based islet proteome profiling is the potential contamination of exocrine acinar tissue in islet preparations as reported by Ahmed *et al.*, and it is important to take such potential contamination into consideration for data interpretation [59].

Islet proteome profiling

Although the application of 2DE in early proteomic profiling of islets revealed thousands of islet protein spots, only small sets of proteins of interest were excised for identification by MS [35,59–61]. Later studies attained significantly improved islet proteome coverage through the use of 2D LC (i.e., strong cation exchange chromatography coupled with reversed phase LC)–MS/MS. For example, initial LC–MS/MS-based profiling of the human islet proteome by Metz *et al.* resulted in approximately 3400 confident protein identifications [13]. Similarly, application of 2D LC–MS/MS to profile the mouse islet proteome resulted in 2612 proteins identified with at least two unique peptides [14]. Figure 1 shows the coverage of insulin receptor signaling pathways obtained from this dataset, which illustrates the ability of LC–MS/MS-based proteomics to detect a good percentage of signaling proteins (~45%) in a major signaling pathway. Moreover, a comparison of the islet proteome with the proteomes of eight other mouse tissues (i.e., brain, liver, heart, muscle, kidney, lung, placenta and adipocyte) revealed 133 proteins either specifically or predominantly expressed in pancreatic islets; 68 of which have never been identified in islets previously. In addition to known peptide hormones (insulin, glucagon, peptide YY, pancreatic polypeptide and urocortin-3), these islet-specific proteins included many proteins known to be involved in the regulation of hormone secretion (chromogranins, secretogranins, syntaxins and synaptotagmins), providing confidence in the approach. These observations were consistent with the islet specific transcriptome [62]. Importantly, these islet-specific proteins are potentially interesting candidates for further detailed biological studies in order to investigate their roles in islet biology and their relevance to metabolic diseases.

More recently, LC–MS/MS using an LTQ-Orbitrap mass spectrometer demonstrated sufficient sensitivity for profiling single pancreatic islets [16]; nearly 2000 proteins were identified in single islets. By analyzing pooled islets in the same study, 6873 proteins were confidently identified, representing the most comprehensive islet proteome coverage to date [16]. In general, this extensive islet proteome coverage provides a valuable resource with respect to the proteins and potential signaling pathways that regulate islet function. This information contributes towards the understanding of normal islet function and the pathogenesis of diabetes.

Glucose-stimulated islet proteome response

Since the major function of islets is the secretion of hormones in response to alterations in the concentrations of metabolites (e.g., glucose and free fatty acids), the identification of protein factors or pathways associated with glucose-stimulated insulin secretion is crucial for understanding the underlying mechanisms of diabetes. For this reason, significant efforts have been centered on studying glucose-responsive proteins in islets and islet-related cell lines. In the early 1990s, 2DE studies by Collins *et al.* revealed numerous islet proteins altered after glucose stimulation; however, these glucose-responsive proteins were not identified at that time [63,64]. More than a decade later, Ahmed *et al.* applied 2DE coupled

with MALDI-TOF-MS to characterize changes in the global mouse islet proteome following stimulation with 11 mM glucose [59]. Among the approximately 1000 protein spots observed, 379 of them were differentially expressed. A total of 77 proteins corresponding to 124 protein spots were identified using MS, including α enolase, endoplasmic, glucose-regulated proteins, heat shock proteins, peroxiredoxins, prohormone convertase 2, protein disulfide isomerase and superoxide dismutase. These results indicated that after glucose stimulation, the activity of insulin synthesis, granular mobilization and maturation and stress response are enhanced in mouse islets.

Hu *et al.* used a 2D-DIGE approach to identify the islet proteins related to insulin secretion activated by glucose stimulation [65]. Based on a previous observation that α 2A adrenergic receptor (α 2AAR) attenuates glucose-stimulated insulin release from islet β cells [66], this study compared proteomic differences between islets from α 2AAR knockout mice (α 2AAR KO) and wild-type mice. The authors were able to identify a small set of proteins significantly upregulated in α 2AAR KO mice, including bile salt activated lipase (Bsd1), pancreatic lipase related protein 1 (Plrp1), D-3-phosphoglycerate dehydrogenase (3-Pgdh), pancreatic triglyceride lipase (Ptl), carboxypeptidase B1 (Cpb1) and A1 (Cpa1) and peroxiredoxin-4 (Prdx4). Many of the identified upregulated proteins were involved in biosynthesis, enzyme secretion and other pancreatic functions; however, several of these proteins, such as Bsd1, Ptl, Cpb1 and Cpa1, are known as digestive enzymes, potentially from pancreatic acinar tissue [59].

As most of these studies were based on 2DE-MS, the number of glucose-responsive proteins identified was limited by the laborious protein identification process from individual gel spots. The application of LC-MS/MS-based methodologies has the potential to reveal additional glucose-responsive proteins [16].

Mechanisms of β -cell failure

Type 1 diabetes results from irreversible selective destruction of the insulin-producing β cells within the islets. The gradual development of β -cell dysfunction can lead to β -cell failure, with approximately 70–80% loss of β cell mass at the time of diagnosis [4]. Currently, the most accepted hypothesis for β -cell failure in T1DM is that β cells are destroyed by an autoimmune attack. In this hypothesis, the immune system invades the islet space, releases toxic cytokines such as IL-1 β , IFN- γ and TNF- α , consequently triggering the apoptosis of β cells [67]. However, the exact molecular mechanism behind this hypothesis is still unclear. Application of quantitative proteomics has the potential to significantly improve our understanding of the mechanisms of β -cell failure and provide potential candidates for therapeutic interventions.

Most of the T1DM-related quantitative proteomic studies on islets to date have been performed using islets isolated from animal models [68] and β cell derived cell lines [69], and involved the use of 2DE-based approaches. For example, Andersen *et al.* applied 2DE to compare the rat islet proteome change after exposure to IL-1 β and identified 33 proteins as being differentially regulated [70]. In a subsequent study that utilized 2DE to profile 35S-methionine-labeled proteins from IL-1 β -treated rat islets, abundance alterations for 89 proteins were reported [71]. In 2006, Karlsson *et al.* also used 2DE to compare rat islet proteome changes after IL-1 β treatment and found that gal-3 was the most highly upregulated protein [72]. Further investigation revealed that over expression of gal-3 protected β cells against IL-1 β toxicity by completely blocking JNK phosphorylation, which is essential for IL-1-mediated apoptosis. A haplotype comprising three coding single nucleotide polymorphisms showed significantly increased transmission to unaffected offspring in 257 T1DM families and this result was replicated in an independent set of 170 T1DM families [72]. These data suggest gal-3 as a candidate gene/protein that promotes

T1DM susceptibility; however, the effect of gal-3 may not be limited to T1DM, since it was also reported to be associated with amyotrophic lateral sclerosis [73].

In a more recent study, Xie *et al.* generated a mouse model by multiple injections of low-dose streptozotocin to mimic the β -cell failure as observed in T1DM [35,74]. They subsequently analyzed the proteomic changes in the islets using 2DE and MALDI-TOF-MS/MS. Seven proteins were observed to be significantly altered in diabetic mice – that is, ATP synthase subunit β (Atpb), calreticulin (Crtc), lithostathine 1 (Lit1) and 2 (Lit2), Prdx4, ubiquinol-cytochrome-c reductase complex core protein I (Uqcrl) and voltage-dependent anion-selective channel protein 1 (Vdac1).

Besides those proteomic studies on drug-induced β -cell failure, Johnson *et al.* found that physiological concentrations of insulin can protect islet cells from apoptosis through the antiapoptotic transcription factor Pdx1 [36]. Further 2D-DIGE study on human islets identified 36 protein spots significantly changed by insulin stimulation. More importantly, biological studies on these candidates revealed that Bridge-1 was a positive regulator of Pdx1 after low-concentration insulin stimulation.

Collectively, these data suggest that the balance between β cell proliferation and cell death is important for the maintenance of islet mass and function, and that oxidative stress plays an important role in diabetes [35].

T2DM & insulin resistance

Type 2 diabetes is characterized by two major defects: β -cell dysfunction and insulin resistance in peripheral tissues. The exact alterations in molecular pathways associated with β -cell dysfunction in insulin-resistant and diabetic states are not clear. Proteomic studies of human islets are generally challenged by the availability of islets from living donors, and potentially large interindividual variation. As a result, most of the islet proteomic studies involving T2DM and insulin resistance tend to utilize islets from insulin-resistant or diabetic animal models, such as Zucker fatty (ZF) and Zucker diabetic fatty rats (ZDF) [15], high-fat-fed mice [75], muscle IGF-1 receptor–lysine–arginine (MKR) mice [17,76] and lep/lep mice [77]. A common feature of these animal models is that all models have manifested insulin resistance, and often exhibit islet dysfunction as it occurs in the early stages of diabetes.

Lu *et al.* used iTRAQ and an mRNA microarray approach to identify islet protein factors associated with the development of T2DM by using a 10-week-old MKR mouse model [17]. Among the 590 identified islet proteins, 159 were differentially expressed in MKR compared with control islets. Some previously reported proteins associated with insulin-secretion pathways or T2DM such as glucose transporter 2, Dnajc3, vesicle-associated membrane protein 2 (Vamp2), ras-related protein (Rab3a) and prohormone convertases (Pc1, Pc3) were significantly altered in the MKR islets. Many proteins associated with protein folding, endoplasmic reticulum (ER)-associated protein degradation and mitochondrial energy metabolism were also identified as differentially expressed. The comparison between proteomic data and mRNA microarray data suggested that approximately 54% of differentially expressed proteins in MKR islets showed changes in the proteome but not the transcriptome, suggesting that post-transcriptional regulation played an important role in disease development. More recently, the same group applied iTRAQ and LC-MS/MS to identify mitochondrial proteomic changes in MKR mouse islets [76]. A total of 36 mitochondrial proteins, including inner membrane proteins of the electron transport chain, were differentially expressed in 10-week-old MKR mice, indicating that mitochondrial dysfunction played a key role in the development of T2DM.

In another recent study, Han *et al.* compared islet proteomes from Zucker Lean (ZL), ZF and ZDF rats, representing control, obese and obese/diabetes conditions, respectively [15]. A total of 54 and 58 proteins were observed as differentially expressed in ZDF versus ZL rats and in ZF versus ZL rats, respectively. Impaired insulin secretion, mitochondrial dysfunction, dysregulation of triglyceride/free fatty acid cycling and lipotoxicity and microvascular dysfunction were proposed as potential factors mediating the progress from insulin resistance to T2DM [15].

Figure 2 illustrates the significant alterations in protein abundances in protein biosynthesis, ER stress, microvascular endothelial dysfunction, mitochondrial dysfunction, impaired hormone secretion and impaired glucose sensitivity, all of which are potential contributing factors to islet dysfunction and T2DM. Similar results were observed from our own study of pancreatic islets [ZHOU ET AL., UNPUBLISHED DATA] from insulin-resistant mice, including high-fat diet mice.

In a study performed with human islets, Nyblom *et al.* used LC-MS and SELDI methodologies to compare the differences in the proteome between islets isolated from patients with T2DM and age- and weight-matched controls [78]. In this study, approximately 20 proteins were observed as significantly changed in T2DM and several pathways, including cell arrest and apoptosis, immune-response and cell proliferation and regeneration were suggested to be activated in the islets of T2DM patients.

Altogether these studies have provided important insights into the pathways and factors induced by insulin resistance, as well as the factors linked to the transition from insulin resistance to β cell dysfunction in diabetes.

Islet development & regeneration

Islet transplantation has long been recognized as a potentially useful therapeutic strategy for both T1DM and advanced T2DM; however, successful islet transplantation still faces many, challenges despite significant recent advances [79]. A better understanding of the molecular mechanisms of islet development and regeneration may lead to new strategies for islet cell generation and transplantation. To this end, Hong *et al.* applied 2DE-MS to discover protein factors associated with the EGF-stimulated growth of neonatal porcine pancreatic cell clusters that are potential alternative sources of cells for islet transplantation [18]. They found that EGF-stimulated proliferation was mediated by the activation of the MAPK and PI3K pathways.

In another study, Jin *et al.* used iTRAQ and mRNA microarrays to identify proteins involved in islet-like differentiation of a human β cell line [57]. A number of proteins involved in cell cycle, cell structure and developmental processes were significantly downregulated, while proteins involved in lipid, fatty acid, steroid and nucleotide metabolism were upregulated, suggesting these molecular functions are likely to be associated with β -cell differentiation. To identify bioactive human peptides that might trigger islet neogenesis, Levetan *et al.* evaluated the peptide sequences within a variety of mammalian pancreas-specific regenerating genes from GenBank and all available proteomic databases to develop large-scale protein-to-protein interaction maps, which led to the discovery of human pro-islet peptide as a potential factor for islet neogenesis [80].

In general, these studies provide important information for developing new strategies to manipulate the process of islet development or regeneration.

Diabetic drug response

Another objective of proteomic studies on islets centers on the development of novel therapeutic strategies to treat diabetes. For example, Sanchez *et al.* used 2DE to investigate the effect of rosiglitazone on islet protein differential expression and found that the modulation of actin-binding protein levels by rosiglitazone may be involved in the protection of islet cell structure and function [77,81]. Jagerbrink *et al.* used 2DE to investigate changes in the islet proteome in response to an imidazoline derivative BL1128 and discovered 22 proteins with abundance changes, including four upregulated calcium-binding proteins – that is, calreticulin, calgizzarin, calcyclin and annexin I [34]. The upregulation of calcium-binding proteins is potentially linked to BL1128-stimulated insulin release at high glucose concentrations. While these studies provide initial insights into the islet response to potential therapeutic candidates, additional biological information remains to be acquired by using proteomic methodologies other than 2DE, which limits the number of identified proteins.

Conclusion

Recent proteomic studies of pancreatic islets have provided insights into the complexity of the islet proteome and the molecular pathways that regulate islet function, including a significant number of proteins that exhibit changes in expression levels in response to different biological or disease conditions. From these studies, it is becoming clear that mitochondrial dysfunction and impaired hormone secretion are two important processes that are linked to islet dysfunction. Further proteomic investigations of the mechanisms leading to islet dysfunction, as well as the functional roles of specific key proteins, should help pave the way for the development of novel therapeutic strategies for treating diabetes.

Expert commentary

Given the essential role of pancreatic islets in the regulation of glucose homeostasis, there is a major interest in gaining an understanding of the molecular mechanisms that modulate insulin secretion, islet cell proliferation, survival and apoptosis. The rapidly evolving science of proteomics has enabled investigation of some of these mechanisms at the proteome level, and several studies performed over the past decade have afforded new insights into the molecular pathways that regulate islet functions. Nevertheless, many of these studies were limited by the proteome coverage that could be achieved by the existing proteomic technology. This is especially true considering that a majority of the published proteomic studies on islets are based on 2DE, where often only a small number of proteins were identified. Although recent LC-MS/MS-based applications have significantly expanded coverage of the proteome, many important signaling proteins or transcription factors have yet to be identified. Moreover, it is well recognized that alterations in posttranslational protein modifications such as phosphorylation play a potentially even more important role in regulating cellular functions compared with alterations in protein abundances. In spite of its importance, proteomic analysis of protein phosphorylation in pancreatic islets has not yet been reported, most likely due to limitations associated with islet sample size and instrument sensitivity. Lastly, while proteomics is advantageous for measuring a large number of proteins simultaneously, this technology is often regarded as a discovery or hypothesis-generating tool. To make full use of this approach, selecting essential protein factors for further detailed functional studies following initial proteomic discovery of a large number of differentially expressed proteins remains a significant challenge. Further advances in bioinformatics and a larger knowledgebase will likely facilitate a more effective discovery of the key protein factors relevant to disease functions.

Five-year view

Within the next 5 years, we anticipate a significantly broader interest in studying the molecular mechanisms of islet hormone secretion, islet cell regeneration and islet cell death as a result of more advanced quantitative proteomic strategies, such as multidimensional LC–MS/MS [13,14], coupled with either label-free or stable isotope labeling-based quantification. Further improvements in the sensitivity of proteomic technologies will allow the application of subcellular fractionation approaches, such as mitochondrial enrichment [76] and secretory granule separation [22,24], to identify low-abundance signaling proteins. Moreover, studies will start to explore post-translational protein modifications, such as phosphorylation [82], oxidation [83], ubiquitination [84,85] and sumoylation [86] in pancreatic islets to reveal the respective roles of such modifications in regulating islet biology. We also foresee targeted quantitative proteomic technologies, such as selected reaction monitoring-MS [87] becoming an important tool for validating therapeutic candidates discovered in large-scale proteomic efforts. With these integrated efforts, one might expect the discovery of novel factors or therapeutic targets for treating both T1DM and T2DM.

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Key issues

- Pancreatic islets play an essential role in maintaining blood glucose homeostasis; islet dysfunction is linked to both Type 1 and Type 2 diabetes.
- Proteomic studies of islets have revealed alterations in many proteins under conditions of insulin resistance, diabetes, glucose stimulation or drug treatment.
- Early islet studies that employed 2DE to separate proteins only provided a small number of protein identifications. Studies using liquid chromatography–MS/MS have illustrated the potential for increased proteome coverage.
- Future islet studies are expected to include protein phosphorylation and other posttranslational modifications involved in cell signaling.
- Key protein factors may be identified as therapeutic targets for treating diabetes.

Figure 1. Coverage of the insulin receptor signaling pathways

Proteins identified are highlighted as pink. Undetected proteins and low-molecular-weight chemical compounds are shown in green.

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Figure 2. Proteomic alterations observed from studies using insulin-resistant and diabetic animal models

Data were summarized from proteomic studies in diabetic animal models [15,17,76]. A maximum of ten representative proteins were presented for each category.

Pink background: upregulated proteins; green background: downregulated proteins.

Table 1

List of selected literature reports on proteomics studies involving pancreatic islets.

Study (year)	Species	Condition	Biological replicates	Technique	Altered proteins	Ref.
<i>Islet proteome profiling</i>						
Petyuk <i>et al.</i> (2008)	Mouse	NA	8 pooled in 1	2DLC-MS/MS		[14]
Metz <i>et al.</i> (2006)	Human	NA	5 pooled in 1	2DLC-MS/MS		[13]
Ahmed <i>et al.</i> (2005)	Human	NA	5	2DE		[60]
<i>Glucose-stimulated islet proteome response</i>						
Martens <i>et al.</i> (2010)	Rat	Glucose	3	LC-MS/MS	93	[39]
Waanders <i>et al.</i> (2009)	Mouse	Glucose	1	LC-MS/MS	142	[16]
Ahmed <i>et al.</i> (2005)	Mouse	Glucose	6	2DE	77	[59]
<i>β-cell failure</i>						
Karlsson <i>et al.</i> (2006)	Human, rat	IL-1 β	3	2DE	1	[72]
Xie <i>et al.</i> (2008)	Mouse	Streptozotocin	3	2DE	7	[35]
Johnson <i>et al.</i> (2006)	Human	Insulin	4	2D-DIGE	36	[36]
<i>T2DM & insulin resistance</i>						
Lu <i>et al.</i> (2010)	Mouse	MKR	3 pools	iTRAQ, 2D LC-MS/MS	36	[76]
Lu <i>et al.</i> (2008)	Mouse	MKR	3 pools	iTRAQ, 2D LC-MS/MS	159	[17]
Han <i>et al.</i> (2010)	Rat	Zucker rat	3	iTRAQ, 2D LC-MS/MS	112	[15]
Qiu <i>et al.</i> (2005)	Mouse	High fat	3	2DE	4	[75]
Nyblom <i>et al.</i> (2009)	Human	T2DM	5	LC-MS/MS, SELDI	31 from SELDI, 20 from LC-MS	[78]
<i>Islet development & regeneration</i>						
Jin <i>et al.</i> (2009)	Human	Development	2	iTRAQ, 2D LC-MS/MS	97/91	[57]
Hong <i>et al.</i> (2005)	Pig	Development	4	2DE	13	[18]
<i>Diabetic drug response</i>						
Kim <i>et al.</i> (2008)	Rat	EPS	6	2DE	34	[33]
Jagerbrink <i>et al.</i> (2007)	Rat	Imidazoline	6	2DE	22	[34]

EPS: Extracellular polysaccharide; iTRAQ: Isobaric tags for relative and absolute quantification; LC: Liquid chromatography; MKR: Muscle IGF-1 receptor-lysine-arginine; MS: Mass spectrometry; NA: Not applicable; T2DM: Type 2 diabetes.