Chapter 16
Proteomics and Islet Research

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Abstract The complementary disciplines of genomics and proteomics offer better insights into the molecular mechanisms of diseases. While genomics hunts for defining our static genetic substrate, proteomics explores the structure and function of proteins expressed by a cell or tissue type under specified conditions. In the past decade, proteomics has been revolutionized by the application of techniques such as two-dimensional gel electrophoresis (2DGE), mass spectrometry (MS), and protein arrays. These techniques have tremendous potential for biomarker development, target validation, diagnosis, prognosis, and optimization of treatment in medical care, especially in the field of islet and diabetes research. This chapter will highlight the contributions of proteomic technologies toward the dissection of complex network of signaling molecules regulating islet function, the identification of potential biomarkers, and the understanding of mechanisms involved in the pathogenesis of diabetes.

Keywords Proteomics · Islets · Two-dimensional gel electrophoresis · Mass spectrometry · Proteome · Glucolipotoxicity

16.1 Introduction

Pancreatic islets, the fascinating little magic box, because of their vital performance in blood glucose regulation have long been central focus of diabetes research. The essential illusion of these magical islets is the β-cell, a 'mysterious maiden' with bags full of insulin. Search for the understanding of the β-cells has given rise new ideas, imagination, and creativity in the worldwide scientific community, but till now not a single phenomenon of the β-cell has been fully understood. Every new

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M.S. Islam (ed.), *The Islets of Langerhans*, Advances in Experimental Medicine and Biology 654, DOI 10.1007/978-90-481-3271-3_16,

discovery tells a tale about the previous one – a little more, but the story seems a never ending one. In this promising journey of biomedical discovery, the completion of the human genome project has facilitated the entry of the biomedical researchers into a new dimension – the post-genomic era. This era is marked by an explosion of terms containing the suffix 'omics,' like the word genomics, transcriptomics, and metabolomics. One of the very stylish and trendy labels carrying the appellation 'omics' is *proteomics,* which attracted the attention of contemporary scientists and offered to fill the void left by the human genome project to gain an in-depth understanding of future disease prevention and innovation of novel drug targets. The contributions of proteomic technologies toward the insights of the pathophysiology of the pancreatic islet function will be discussed in following sections.

16.2 Proteome and Proteomics

All cells in the human body have essentially the same genetic information, and the genes possess only the information which is sequentially encoded to construct the final products – the proteins. These proteins are dynamic in nature and considered as the molecular engineers for a cell; their composition in a cell may vary at its different stages of development whereas the genes remain as the static component of a cell. A classic example is the caterpillar and its mature form, the butterfly; they have the same genetic makeup whereas their protein composition is quite different and it is the protein which is responsible for different shapes and forms of the organism. The renaissance of proteomics is due to the fact that proteins are expressed in quantities and physical forms that cannot be predicted from DNA and mRNA analysis [1, 2]. In addition, the diseased cells often produce proteins that healthy cells do not have and vice versa. Hence, scientists are aiming toward creating a complete catalogue of all the human proteins with an intention to uncover their interactions with one another [1, 3]. Their definitive goal is to discover biomarkers and to devise better drugs with fewer side effects. Significant progress has already made in biomarker discovery where several groups have announced that using proteomic techniques it is highly possible to make an accurate early diagnosis for cancers including ovarian, breast and prostate cancer [4–6].

In general, proteomics includes cataloging all the proteins present in a cell or tissue type at a specific time under specific conditions, quantitation, and functional characterization of these proteins to elucidate their relationships (protein–protein interaction networks) and functional roles and ultimately outlining their precise three-dimensional structures in order to find where the drugs might turn their activity on or off – the 'Achilles heels' [7–9]. The term proteome was coined as a linguistic equivalent to the concept of genome and first used in 1994 at the "Siena 2D Electrophoresis" meeting (9–11). It denotes the entire PROTEin complement to a genOME, expressed by a cell or tissue type, at a specific time in the development of the organism under specific conditions [12, 13]. While humans are estimated to have approximately 20,000–25,000 genes, alternate RNA splicing and posttranslational

modification may led to encoding as many as 250,000–1 million individual proteins or peptides. For example, more than 22 different isoforms of α-1-antitrypsin exist in human plasma [14]. In addition, the proteome undergoes dynamic changes as it continuously responds to autocrine, paracrine, and endocrine factors as well as exposure to any pathogen, changes in external environment, and during time course of disease and drug treatment. Various gene products, including microRNA [15], as well as epigenetic factors [16] also influence the expression levels of genes and their transcripts. As a consequence, the proteome is far more complex than the genome. Thus, the scale of protein discovery task is challenging and very large indeed. And multiple specialists from different fields must collaborate to provide a range of sophisticated tools to analyze nature's tremendous complexity. However, proteomics is still in an early stage and at the time when mRNA expression arrays are spreading like cell phones in industry and in academic institutions, systems for large-scale protein analysis are still novelties. The commonly available proteomic technologies to date are summarized in Table 16.1.

Table 16.1 Currently used proteomic technologies

Proteome profiling	**Emerging technologies**
1D gel electrophoresis	SILAC
2D gel electrophoresis	Imaging mass spectrometry (IMS)
2D-DIGE	Molecular scanner
MS-based methods	iTRAQ
SELDI-TOF	ICAT
MALDI-TOF	HysTag
CE-ESI-MS	Label-free LC-MS/MS quantitation
LC-MS	Protein chips:
Protein identification	*Spotted array-based tools:*
Mass spectrometry	Forward-phase arrays (FPA), e.g., antibody arrays, protein arrays
Mud-PIT or shotgun proteomics	Reverse-phase arrays (RPA)
2-D LC-MS/MS	*Microfluidic-based tools*
Protein function	
Yeast two hybrid	
Phase display	
Surface plasmon resonance analysis	
Immunoaffinity	
Structural proteomics	
X-ray crystallography	
NMR spectroscopy	
Electron tomography	
Immunoelectron microscopy	

2D-DIGE, two-dimensional differential in-gel electrophoresis; CE-ESI-MS, capillary electrophoresis electrospray ionization mass spectrometry; ICAT, isotope-coded affinity tags; iTRAQ, isobaric tagging for relative and absolute quantitation; LC-MS liquid chromatography mass spectrometry; Mud-PIT, multidimensional protein identification technology; MALDI-TOF, matrix-assisted laser desorption/ionization time of flight; NMR, nuclear magnetic resonance; SELDI-TOF, surface-enhanced laser desorption/ionization time of flight; SILAC, stable isotope labeling by amino acids in cell culture.

Over the years, proteomics has expanded to include profiling, quantitative, functional, and structural proteomics based on a broad range of technologies. *Protein profiling* involves identifying and making a list of the proteins present in a biological sample [17]. *Quantitative proteomics* discovers molecular physiology at the protein level and allows comparisons between samples by measuring relative changes in protein expression in response to external stimuli [18, 19]. *Functional proteomics* attempts to identify proteins in a cell, tissue, or organism that undergo changes in abundance, localization, or modification in response to a specific biological condition and discover their functions based on the presence of specific functional groups or based on their involvement in protein–ligand interactions [17, 20]. Similarly, pathways can be characterized as a cascade of specific protein interactions required to activate cellular functions. Functional proteomics thus focuses on understanding part of the wiring diagram of a cell. *Structural proteomics* attempts to determine the three-dimensional structure of proteins, the structure of protein complexes, and small molecule protein complexes. X-ray crystallography and NMR are its main approaches [21, 22].

In the plethora of proteomic technologies, two-dimensional gel electrophoresis (2DGE) remains as a cornerstone of protein profiling [23, 24]. The 2DGE separates proteins according to two independent parameters, isoelectric point (p*I*) in the first dimension and molecular mass (*M*r) in the second dimension by coupling isoelectric focusing (IEF) and sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS-PAGE) [25, 26]. Theoretically, 2DGE is capable of resolving up to 10,000 proteins simultaneously, with approximately 2,000 proteins being routine and detecting and quantifying protein amounts of less than 1 ng per spot [23, 24]. Despite the well-known limitations of the 2DGE approach, e.g., poor solubility of membrane proteins, limited dynamic range, difficulties in displaying and identifying low-abundant proteins, lack of reproducibility and automation, 2DGE will remain as a powerful and versatile tool for display and quantification of a majority of proteins in biological samples. The detailed technology, challenges as well as the application, potential and future of high-resolution 2DGE have been elegantly reviewed in several papers [23, 24, 27, 28]. However, gel-free high-throughput protein profiling techniques have leapt prominence and now become preferred method of choice including multidimensional protein identification technology (Mud-PIT) [29], molecular scanner [30], stable isotope labeling by amino acids in cell culture (SILAC) [31, 32], isotope-coded affinity tag (ICAT) [2], isobaric tagging for relative and absolute quantitation (iTRAQ) [33], protein microarrays [34–37], and HysTag reagent [38]. It should be noted that the use of these emerging techniques is limited to certain specialized and privileged laboratories. Also, the choice of a given proteomic approach depends on the type of biological question asked, since each proteomic technology is characterized by specific applications, technical advantages, and limitations. A typical gel-based proteomic work flow is schematically illustrated in Fig. 16.1. Peptide mass fingerprinting (PMF) and tandem mass spectrometry (peptide fragmentation to generate partial sequence, MS/MS) are commonly used for protein identification on two-dimensional proteomic patterns [39–42]. The recent progress in the sensitivity of mass spectrometry analysis has

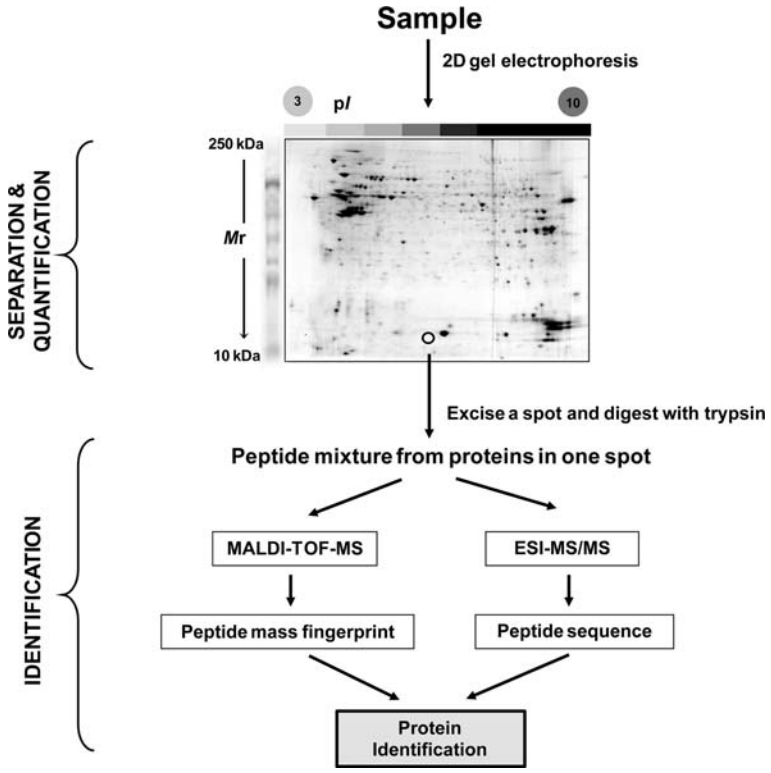


Fig. 16.1 A two-dimensional gel-based proteomic workflow. There are two principal steps. The *first* is separation and quantification of proteins in a sample using 2D gels. In the first dimension, proteins are separated in a pH gradient according to their molecular charge, known as isoelectric focusing. In the second dimension, the proteins are separated orthogonally by electrophoresis based on their molecular mass. The end result is a 2D gel with thousands of spots where individual spot represents a protein/peptide or a mixture. The *second* is identification of the separated proteins, typically using mass spectrometry (MS) techniques and bioinformatics. A protein spot can be excised from the 2D gel, digested with a protease and the peptides extracted. These peptides can then be analyzed using MS techniques such as matrix-assisted laser desorption/ionization time of flight (MALDI-TOF) and electrospray ionization tandem MS (ESI-MS/MS)

significantly increased the applicability of proteomic technologies [43] as protein identification and profiling tool as well as determining protein interactions and the type and location of posttranslational modifications [41, 44, 45]. Surface-enhanced laser desorption/ionization time of flight (SELDI-TOF) is a suitable technique for high-throughput proteomics analysis of complex mixtures of proteins where proteins are retained on solid-phase chromatographic surfaces with specific properties and are subsequently ionized and detected by TOF MS [46–48]. However, this system is limited for profiling low molecular weight proteins (<20 kDa) [47]. In another protein profiling strategy, commonly referred as 'bottom-up' *shotgun proteomics* (multidimensional LC-MS/MS or Mud-PIT), complex protein mixtures are digested

into peptides, followed by chromatographic separation of peptides prior to analysis by tandem mass spectrometry, and computer algorithms then map the peptides onto proteins to determine the original content of the mixture [49].

Quantifying changes in protein abundance between samples is a key goal of proteomics. Promising novel methods for high-throughput quantitation involve '*label-free*' approaches. Several studies have demonstrated that LC-MS peptide ions spectral peak intensities are directly proportional to the protein abundances in complex samples [50]. Another label-free method, termed spectral counting, compares the number of MS/MS spectra assigned to each protein [50, 51]. With controls for normalization between runs, label-free quantitation offers a simpler approach for analysis. Spectral sampling also enables ranking different proteins by their relative abundances, providing information that other methods cannot achieve [52].

In addition to the protein profiling and comparative proteomics, functional study of target proteins is essential in any successful proteomic study. Functional proteomic approaches are based on interactions of proteins or specific activities of proteins. Phage display is a powerful proteomic tool used to express proteins or domains of proteins [53, 54]. The system has played a pivotal role in mapping epitopes of monoclonal and polyclonal antibodies, defining amino acid substrate sequences, and identifying peptide ligands for drug research. Yeast two-hybrid system detects binary protein interactions by activating expression of a reporter gene upon direct binding between the two tested proteins [55, 56]. SELDI-TOF MS has also been used to characterize protein–protein interaction [47]. Recently, for studying the functions and interactions of proteins, protein microarrays have been developed in analogy to DNA microarrays which can also be applied for comparative studies of expression of large sets of proteins [57]. There are two major types of protein microarrays – forward (FPA) and reverse-phase array (RPA) [58, 59]. In forward protein arrays, thousands of recombinant antibodies carrying the desired specificities are arrayed on glass slides, which make it very well suited for high-throughput screening of biological samples for specific disease markers [60, 61]. The BD Clontech™ Ab Microarray 500 represents a significant step in that direction. With this array, over 500 specific proteins can be assayed to detect and compare expression level of both cytosolic and membrane-bound proteins representing a broad range of biological functions, including signal transduction, cell cycle regulation, gene transcription, and apoptosis. In contrast to using chips with immobilized antibodies to detect specific proteins, protein chips carrying the proteome of a specific organism or cell type can be made by cloning and purification of these proteins [62]. This protein microarray can then be screened on the basis of the ability of the chip to bind specific ligands or interact with specific proteins. The human ProtoArray® protein microarray (Invitrogen™) contains more than 8,000 full-length human proteins purified under native conditions. This high-content discovery tool provides highly sensitive and reproducible results enabling rapid and easy profiling of thousands of biochemical interaction. In reverse-phase microarrays, tissues [63], cell lysates [64], and serum samples [65] are spotted on the surface and probed with specific antibodies per analyte for a multiplex read-out. Thus, this analysis evaluates the expression level of defined target proteins in

multiple samples. Both forward- and reverse-phase protein microarrays are novel technologies in proteomics and offer great promise for use in clinical applications.

16.3 Application of Proteomics in Islet Research

The accomplishment of human genome sequences has conferred the islet scientists with immense errands to assess the relative levels of expression of these gene products including the proteins and their posttranslational modifications in pancreatic islets. In the post-genomic era, to clarify the molecular mechanism of islet function in both normal and disease states, it is important to understand the entire gene products which regulate the phenotypes of islet cells and their ability to differentiate and secrete specific hormones. An important advantage of global protein expression profiling compared with individual gene or protein regulation studies is the ability to monitor changes in several functional groups simultaneously. It should be kept in mind that proteomics per se is not a hypothesis-driven experimental approach, but rather a hypothesis generating '*fishing-expedition*' where one explores the proteins that are not a priori expected to be associated with any pathophysiological conditions, which allows discovering novel proteins and signaling networks opening new research avenues. Since its introduction in 1994, the proteomic booms continue and got considerable attention of the islet researchers as well. Improvements of the core technologies, especially advancement of protein identification by mass spectrometry and bioinformatics tools, have recently encouraged the application of proteomics to unlock the secret of islet pathophysiology. It is indeed interesting to note that the most widely used protein separation technique, the 2DGE, has been employed in 1982 for insulin granule protein profiling [66]. In those early days more than 150 protein/peptide spots were detected in a 2DG of insulin secretory granule and some of the high molecular weight spots were presumed as glycoprotein. Lack of high-throughput protein identification method did not permit annotation of the granule proteins but provided an opportunity to study the functional properties of the insulin secretory granule and to dissect the molecular events of exocytosis. A similar proteomic approach has been utilized to explore the glucose-responsive granule proteins in ^{35}S-methionine-labeled rat islet and insulinoma cells and the study showed that biosynthesis of 25 granule proteins were stimulated 15–30-fold by glucose [67]. In a subsequent subproteomic study, almost after 25 years, Brunner et al. [68], separated the INS-1E granule proteins by 1-dimensional SDS-PAGE and identified 130 different proteins by LC-MS/MS.

16.3.1 Protein Profiling of Pancreatic Islets

A high-quality 2DGE reference map of the isolated pancreatic islets is essential for a 2DG-based comparative proteomics study and for generation of hypothesis. In the holy grail of protein profiling of pancreatic islets, Sanchez et al.

[69] did a pioneering work where they mapped 63 spots corresponding to 44 mouse islet protein entries. This protein map is available in the Swiss-2D database (http://us.expasy.org/ch2d/). Nicolls et al. [70] identified 88 proteins in total from mouse islets of which 18 were already identified by Sanchez and coworkers. Continued attempts in '*shooting at stars*' generated another mouse islet 2DGE reference map where 124 spots corresponded to 77 distinct proteins [71]. A reference map of rat insulinoma-derived clonal INS-1E β-cell proteins has also been constructed (Fig 16.2). This 2D map contains 686 valid spots, among which 118 spots corresponding to 63 different proteins have been successfully identified by MALDI-TOF MS and a combination of liquid chromatography and electrospray tandem MS (LC-ESI-MS/MS). Using 2DGE and MALDI-TOF MS the first protein map and database of human islets have been generated in 2005 where 130 spots corresponding to 66 different protein entries were successfully identified [72]. A high level of reproducibility was reported among the gels, and a total of 744

Fig. 16.2 2-D PAGE image of INS-1E proteins. Proteins (200 μg) were loaded onto an IPG strip (pH 3–10 NL) and subsequently separated by mass on a gradient (8–16%) SDS-PAGE gel. The gel was stained with colloidal Coomassie blue and the filtered image was generated by PDQuest software. Experimental masses and p*I*s are indicated. The gene names mark the location of the corresponding proteins on the gel. Aco2, aconitate hydratase, mitochondrial; Actb, β-actin; Ak2, adenylate kinase isoenzyme 2, mitochondrial; Alb, serum albumin; Aldoa, fructose-bisphosphate aldolase A; Anx2, annexin A2; Anxa4, annexin A4; Anxa5, annexin A5; Arhgdia, Rho GDP-dissociation inhibitor 1 (Rho-GDI α); Atp5a1, ATP synthase subunit α, mitochondrial; Atp6v1a, V-type proton ATPase catalytic subunit A; Calr, calreticulin; Cfl1, cofilin-1; Eef1a1, elongation factor 1-α 1; Eef1a2, elongation factor 1-α 2; Eno1, α-enolase; Gapd, glyceraldehyde-3-phosphate dehydrogenase; Gnb2, guanine nucleotide-binding protein $G_i/G_s/G_t$ subunit β-2; Grp58, protein disulfide isomerase A3; Grp75, stress-70 protein, mitochondrial (75 kDa glucose-regulated protein); Grp78, 78 kDa glucose-regulated protein; Hadha, trifunctional enzyme subunit α, mitochondrial; Hadhsc, hydroxyacyl coenzyme A dehydrogenase, mitochondrial; Hnrpa2b1, heterogeneous nuclear ribonucleoproteins A2/B1; Hnrpk, heterogeneous nuclear ribonucleoprotein K; Hnrpl, heterogenous nuclear ribonucleoprotein L; Hsc70, heat shock cognate 71 kDa protein (Hspa8); Hsp40, DnaJ homolog subfamily B member 1 (heat shock 40 kDa protein 1); Hsp60, 60 kDa heat shock protein, mitochondrial; Idh3a, isocitrate dehydrogenase [NAD] subunit α, mitochondrial; Ihd2, isocitrate dehydrogenase [NADP], mitochondrial; Krt8, keratin, type II cytoskeletal 8; Mdh1, malate dehydrogenase, cytoplasmic; Mdh2, malate dehydrogenase, mitochondrial; Nme2, nucleoside diphosphate kinase B; Orp150, 150 kDa oxygen-regulated protein (hypoxia up-regulated protein 1); Pdia1, protein disulfide isomerase; Pdia6, protein disulfide isomerase A6; Pebp, phosphatidylethanolamine-binding protein 1; Pfn1, profilin-1; Pgk1, phosphoglycerate kinase 1; Pgrmc1, membrane-associated progesterone receptor component 1; Phgdh, D-3-phosphoglycerate dehydrogenase; Pkm2, pyruvate kinase isozymes M1/M2; Ppia, peptidyl-prolyl *cis–trans* isomerase A (cyclophilin A); Prdx1, peroxiredoxin-1 (thioredoxin peroxidase 2); Rpsa, 40S ribosomal protein SA; Sod1, superoxide dismutase [Cu-Zn]; Stip1, stress-induced phosphoprotein 1; Tkt, transketolase; Tpm5, tropomyosin α-3 chain; Tra1, endoplasmin; Tuba, tubulin α; Tubb5, tubulin β-5 chain; Txndc4, thioredoxin domain-containing protein 4; Ubc, polyubiquitin; Uchl1, ubiquitin carboxyl-terminal hydrolase isozyme L1; Vcp, transitional endoplasmic reticulum ATPase; Vdac, voltage-dependent anion-selective channel protein; Ywhae, 14-3-3 protein ϵ; Ywhaz, 14-3-3 protein ζ/δ (protein kinase C inhibitor protein 1)

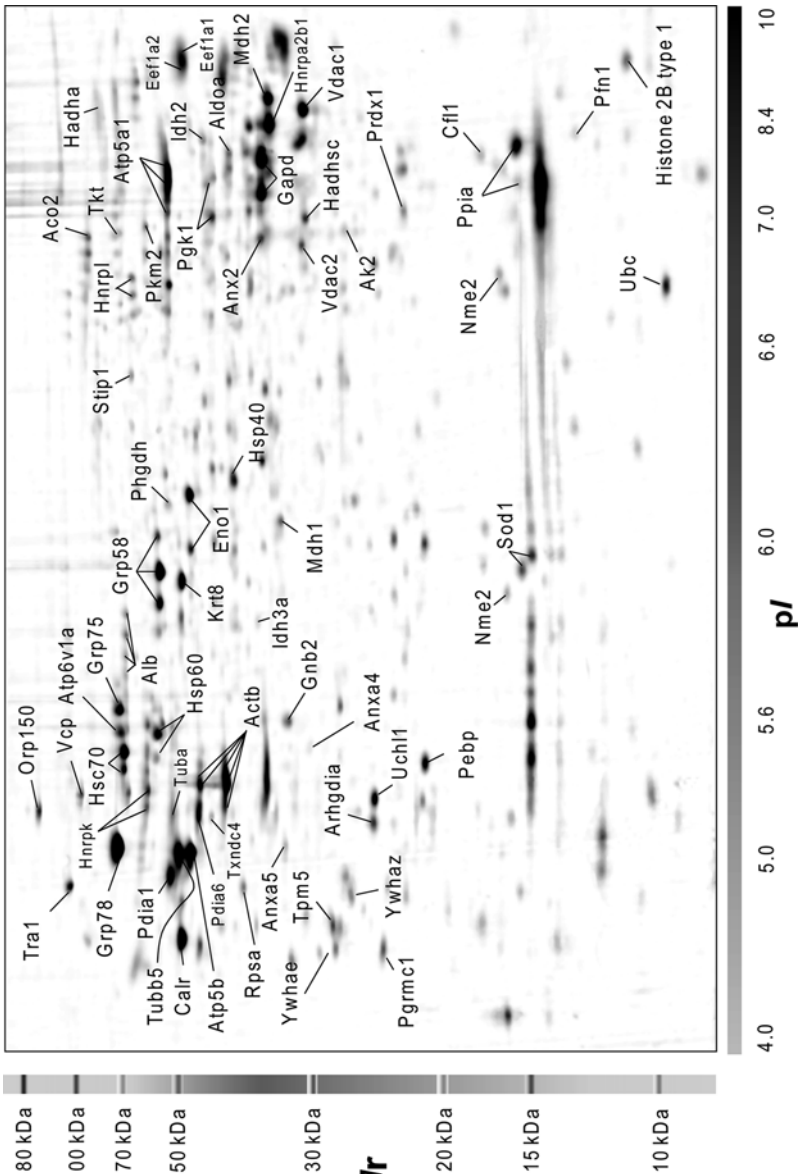


Fig. 16.2 (continued)

protein spots were detected [72]. All the protein profiling studies [69–72] using 2DGE categorized the identified proteins according to cellular location and function. Any attempt to compare these studies renders déjà vu since a number of prevailing proteins were repeatedly reported and most proteins fell into the cytosolic category followed by mitochondrial and endoplasmic reticulum (reviewed by [73]). In aforementioned studies a large part of the proteins have either chaperone (e.g., protein disulfide isomerase, PDI; calreticulin; 78 kDa glucose-regulated protein, GRP78; 58 kDa glucose-regulated protein, GRP58; endoplasmin) or metabolic (e.g., α enolase, transketolase, pyruvate kinase, and hydroxyacyl-CoA dehydrogenase, SCHAD) functions. However, every laboratory blessed with the successful application of 2DGE has its own protocol for protein extraction, isoelectric focusing, and SDS-PAGE. Therefore, a reference map produced by one group cannot necessarily be useful for any other group interested in comparative islet proteomics. Moreover, since introduction in SWISS-2D database, the islet proteome map has not been updated assigning identification of more protein spots. Therefore, the technical hurdle remains for the laborious protein identification procedure even if one follows a similar protocol.

Recent advances in mass spectrometry techniques allowed use of strong cation exchange fractionation coupled with reversed phase LC-MS/MS and characterization of 2,612 proteins in the mouse islet proteome [74]. Using nano-UPLC coupled to ESI-MS/MS more than thousand proteins have been identified in mouse islet (unpublished data). A 2D LC-MS/MS study of the human islets characterized 3,365 proteins covering multiple signaling pathways in human islets including integrin signaling and MAP kinase, NF-κβ, and JAK/STAT pathways [75]. Combined genomic and proteomic techniques have been employed for profiling of glucagon secreting α-cells [76]. While a total of 5,945 gene products were detected in α-cells by the gene chips alone, only 1,651 proteins were identified with high confidence using shotgun proteomics and rigorous database searching. Seven hundred sixty-two cross-mapped gene product pairs (both the gene and the corresponding protein) were jointly detected by both platforms. Conversely, 126 gene products were detected exclusively by proteomics, being somehow missed by the gene chip platform [76]. In recent years the growing number of islet proteomic data necessitates development of bioinformatics tools for easy data handling and data mining to assign subcellular location, functional properties, molecular networks, and known potential posttranslational modifications. It is becoming essential to create a common platform for islet proteomic users integrating molecular, cellular, phenotypic, and clinical information with experimental genetic and proteomics data.

An important feature of proteomics is that protein isoforms generated by posttranslational modifications can be separated by 2DGE. Among the hundreds of different types of protein modifications, reversible protein phosphorylation is a key regulatory mechanism of cellular signaling processes [77–79]. To detect global phosphoproteome profiles of islets, the advantages of the fluorescent dye Pro-Q Diamond, which is suitable for the fluorescent detection of phosphoserine-, phosphothreonine-, and phosphotyrosine-containing proteins on 2D gels directly [80], have been exploited and 90 different phosphorylated proteins were detected

on the 2D map (unpublished data). However, vanishingly small amounts of phosphorylated proteins in cells and lack of robotic picker in our laboratory precluded spot cutting and identification of most of the spots. Only a few, including ATP synthase α chain, elongation factor 1-α, actin, γ-aminobutyric-acid receptor α-3 subunit, and α-2-HS-glycoprotein could be successfully identified. Further isolation and purification of phosphoproteins and increasing the loading amount by pooling islet samples will possibly increase the chances for better identification for comparative studies to elucidate how posttranslational modifications regulate insulin secretion. LC-MS/MS analysis for posttranslational modifications of mouse islet proteome identified relatively abundant secretion-regulatory proteins including chromogranin A and secretogranin-2 [74]. Then again, it is just the very minute tip of the phosphoproteome iceberg.

16.3.2 Comparative and Quantitative Islet Proteomics

The ability of the islet of Langerhans to respond with proper insulin release when the ambient glucose concentration is changed is of fundamental importance for glucose homeostasis [81]. In diabetes mellitus this ability is impaired with reduction in both first- and second-phase insulin secretion [82, 83] which leads to postprandial hyperglycemia. In the search for islet-derived factors responsible for the deranged insulin secretion, isolated islets have typically been cultured under different conditions, and it is well documented that elevated glucose concentrations (11 mM) during culture are essential for maintaining islet β-cell functions [84]. Individual islets from the NOD and *ob*/*ob* mouse, which are animal models of type 1 and type 2 diabetes, respectively [85, 86], have demonstrated improved glucose-stimulated insulin secretion (GSIS) after exposure to high glucose in culture medium [87, 88]. Such beneficial effects on GSIS have been correlated to changes in expression of individual proteins like glucokinase, glucose transporter 2, and uncoupling protein 2 [89–91]. However, molecular details of the phenotypic shift in response to elevated glucose are to a large extent unknown. Since GSIS is a multifactorial event, approaches capable of determining multiple proteins simultaneously are essential for the elucidation of molecular mechanisms responsible for changes in GSIS. 2DGE and MS have been employed to characterize changes in global islet protein expressions related to exposing islets to high glucose [71]. In this proteomic study, the prohormone convertase 2 and cytokeratin 8 appeared as distinct spots on 2D gels of islets exposed to high glucose, but the proteins were barely visible on gels of freshly isolated islets [71]. The observed glucose-induced changes in global protein expression pattern suggested that enhanced insulin synthesis, restoration of insulin content and granule pools, and increased chaperone activity and antioxidants are important mechanisms underlying the augmented secretory effect of glucose in mouse islets. In comparison to other discrete hypothesis-driven studies, this report, for first time, showed orchestrated changes of multiple islet proteins that may contribute to the enhanced GSIS observed in these islets [71]. From this

proteomic study, it is unclear how glucose-induced increase in cytokeratin interacts with kinesin–microtubule system and contributes, if any, in enhanced glucose responsiveness. However, it has been conjectured that kinesin-dependent interaction of cytokeratin with microtubules is mediated by the insulin granules where cytokeratins can interact with various lipids of the insulin granules, which are anchored to microtubules through kinesin interaction. In support of this view, oligonucleotide microarray studies showed an increase in cytokeratin 19 gene in pancreatic β-cells exposed to high (25 mM) glucose compared to low glucose (5.5 mM) for 24 h [92]. Increase in cytokeratins level in different cultured cells has also been reported [93, 94]. This type II cytoskeletal 8 protein (KRT8) has been detected on 2D maps of glucose-responding mouse islets, INS-1E cells [95], and human islets [72]. In search for glucose-responsive proteins, a 65 kDa protein has been detected on 2D map of mouse islets [96] and glucose-induced synthesis of this protein was blocked by D-mannoheptulose, a specific blocker of glucose phosphorylation and metabolism. However, isolation and characterization of this protein has not been performed. Among the 2,000 different islet protein spots, 1.5% was reported to be regulated by glucose in physiological concentration range [97]. In another study, depolarization induced Ca^{2+} influx and insulin release was found to be highly correlated with phosphorylation of a 60 kDa protein [98]. Identification of this phosphoprotein revealed an intermediate filament protein of the keratin class in hamster insulinoma cells and in pancreatic islets [99]. This cytokeratin protein exists in both phosphorylated and unphosphorylated state and corresponds to the gel position of KRT8 detected by Ahmed et al. [71, 72, 95]. The gel position of the unidentified glucose-responsive 65 kDa protein also matches with the KRT8. In support of the suggestion that cytokeratin may be involved in the regulation of insulin release, cytokeratins 7, 8, 18, and 19 were localized to adult endocrine pancreas and insulinoma cells by immunohistochemistry and immunoblot analysis [99–102], and it has been well documented that disturbances in cytoskeleton of the pancreatic β-cells drastically reduced their insulin secretory function and lifetime [103].

Comparative proteomics of glucose-responsive and glucose-nonresponsive MIN-6 cells using 2D-differential in-gel electrophoresis (DIGE) [104] also contributed to the understanding of the proteins involved in GSIS. Similar to the findings of Ahmed et al. [71], they showed that glucose-nonresponsive cells have lower ER chaperone proteins (e.g., PDI, GRP78, endoplasmin, endoplasmic reticulum protein 29) and decreased antioxidative enzymes (e.g., carbonyl reductase 3, peroxiredoxin 4, and superoxide dismutase 1) suggesting proper protein folding and protection against oxidative stress are required for glucose-stimulated insulin release from pancreatic β-cells. To dissect the molecular events associated with β-cell dysfunction and development of diabetes, Lu et al. [105] characterized global islet protein and gene expression changes in diabetic MKR mice and compared with nondiabetic control mice. Using iTRAQ, 159 proteins were found to be differentially expressed in MKR; marked up-regulation of protein biosynthesis and endoplasmic reticulum stress pathways and parallel down-regulation in insulin processing/secretion, energy utilization, and metabolism were observed. One hundred fifty-four of the differentially expressed proteins were able to be mapped to probe IDs

on the microarray. In this study about 45.2% of the differentiated proteins showed concordant changes (i.e., changes in the same direction) in mRNA, 0.6% were discordant (i.e., having higher protein expression but lower mRNA expression), and notably 54.2% showed changes in the proteome but not in the transcriptome. Similar approaches have been used for better understanding of the cellular and molecular functions of the signaling pathway of insulin synthesis and release in human β-cells [106]. Of the 97 differentially expressed proteins involved in improved insulin release, the changes in protein and mRNA expression for 49 proteins (50.5%) were in the same direction, while they moved oppositely for 14 proteins (14.4%). Thirty-four of the 97 differentially expressed proteins were identified by protein expression but not by mRNA expression. The proteomic and genomic data indeed supplement each other and suggest a posttranscriptional and/or posttranslational regulation of a substantial number of differentially expressed proteins is involved in islet function.

Imaging mass spectrometry (IMS) has been applied to identify differential expression of peptides in thin tissue section of pancreas of control and *ob*/*ob* mice [107]. Improvement and successful application of the IMS may lead to the discovery of new disorder-specific peptide biomarkers with potential applications in disease diagnosis. Protein expression profiling in fetal rat islets after protein restriction during gestation expanded our knowledge in the pathogenesis of type 1 and type 2 diabetes [108].

16.3.3 Glucolipotoxicity and Islet Proteomics

Whereas glucose is the most important physiological stimulus for insulin secretion, chronic hyperglycemia causes desensitization and impairment of insulin release in response to glucose [109–112]. Similarly, a high-fat intake, particularly if rich in saturated fatty acids, is associated with impaired insulin sensitivity and secretion and development of type 2 diabetes [113]. It is commonly accepted that acute exposure (1–3 h) of pancreatic islets to free fatty acid leads to stimulation of GSIS both in vitro [114–117] and in vivo [118–120]. However, the impact of long-term (>6 h) FFA exposure remains controversial [118, 121, 122]. The discrepancies may depend on the circulating free fatty acid levels and also on the percentage of unsaturation of the fatty acids [123, 124]. It has been proposed that an increased FFA concentration alone is insufficient to induce β-cell failure and that an elevation of FFAs combined with high glucose is required to result in β-cell malfunction [125, 126], possibly as a result of accumulation of harmful lipid metabolites, e.g., ceramide in the cytoplasm [127, 128]. This in turn is believed to interfere with the ability of the β-cells to respond to glucose with enhanced insulin secretion. Although the concept of glucolipotoxicity has become very popular and often debated, the underlying causes as well as functional consequences remain poorly defined. The main dietary fatty acids palmitate and oleate modulate the immediate early response genes, *c-fos* and *nur-77*, and a number of late genes of fatty acid metabolism including acetyl

CoA carboxylase and fatty acid synthase [129]. By analyzing global gene expression profiles in chronic fatty acid-treated MIN6 cells, it was found that the major groups of genes regulated by fatty acids are metabolic enzymes, transcription factors, and genes controlling distal secretory processes [130]. However, in another study long-term lipid infusion in normal rats showed little influence on broad spectrum of islet-associated genes [131]. A series of selected 'candidate genes' have also been studied recently [132]. The insulin (Ins1) and Glut2 transcript levels were significantly down-regulated in the presence of both palmitate and oleate. Transcription of the mitochondrial acyl-CoA transporter carnitine palmitoyltransferase I (CPT I) was up-regulated almost 4-fold. In contrast to previous findings [133–135], the uncoupling protein UCP-2 was up-regulated 2-fold in the presence of high glucose but no additional effect by FFAs was detected [132]. Therefore, it has been suggested that the failure of glucose to stimulate insulin secretion from FFA-pretreated islets is conceivably not due to increased uncoupling and reduced ATP generation [132]. However, conflicting opinion also exists since Western blot analysis indicates that high glucose and fatty acid synergistically impaired the production of ATP in β-cells through reduction of ATP synthase β-subunit protein expression [136]. Interestingly we have found that the expression of ATP synthase subunit α (1.21-fold) and ATP synthase subunit β (1.16-fold) was significantly increased ($p < 0.05$) in islets isolated from high-fat-fed mice (unpublished data). In this proteomic study, compared to control mice, islets from high-fat-fed mice showed differential expression of 1,008 proteins. In accordance with the previous findings of fatty acid-induced inhibition of insulin gene transcription [132], insulin-degrading enzymes [137] were highly over-expressed in islets isolated from high-fat-fed mice whereas both insulin 1 precursor and glucagon precursor were down-regulated. Top 10 down-regulated proteins in high-fat-diet islets include ARF (ADP ribosylation factor) GTPase-activating protein GIT1, flavin adenine dinucleotide (FAD) synthetase, CPT I, laminin subunit β2 precursor, γ-aminobutyric acid receptor subunit α-3, vesicle transport protein SEC20, reticulon 1, early endosome antigen 1, β-1,4-mannosyl-glycoprotein 4-β-N-acetylglucosaminyltransferase and tudor domain-containing protein 5. The largely down-regulated proteins include kelch-like protein 8, leucine-rich repeat containing protein 8D, transcription factor E3, ras-related protein Rab 11B, Na^+–K^+ ATPase subunit α2 precursor, putative ATP-dependent RNA helicase DHX33, SCHAD, F-actin capping protein subunit β, arylacetamide deacetylase, and type I inositol 3,4 bisphosphate 4 phosphatase. The vast amount of lipotoxicity proteomic data contains many novel proteins and opens new avenues for islet researchers. A recent SELDI-TOF analysis of INS-1E cells exposed to 0.5 mM palmitate for 48 h in the presence of high glucose (20 mM) identified calmodulin as palmitate-regulated protein (Sol EM, personal communication).

In a pioneering glucotoxicity proteomic study, Collins et al. [97] used 2DGE of ^{35}S-methionine-labeled islet proteins that were exposed in vivo or in vitro to either low or high glucose. Approximately 2,000 protein spots were detected on 2D gels and 1.5 and 1.6% detectable proteins showed differential expression in response to prolonged glucose load in vitro and in vivo model, respectively. Lack of mass spectrometry did not allow protein identification of those glucose-responsive proteins.

Schuit et al. [138], purified rat β-cells and performed 2DGE of ^{35}S-methionine-labeled proteins synthesized over 4 h at 10 mM glucose after 10 days culture in low (6 mM) or high (20 mM) glucose. They distinguished two patterns of β-cell proteome change between 6 and 20 mM glucose. In one pattern two spots corresponding to proinsulin were increased almost 9-fold in the presence of high glucose. Similar to this finding, on the 2DG map of INS-1E cells, proinsulin appeared as two spots. However, while one spot showed almost 2-fold up-regulation in the presence of high glucose (25 mM) the other spot was 5-fold down-regulated by high glucose compared to exposure to low glucose (5.5 mM, unpublished data). The other pattern described by Schuit et al. [138] showed suppression of translation of multiple spots close to pH 7 on 2D gels when the β-cells were exposed to 20 mM glucose. However, the identities of these protein spots were not determined. SELDI-TOF analysis of the different mitochondrial samples from INS-1E cells incubated for 5 days at 5.5, 11, 20, and 27 mM glucose showed 34 differentially expressed peaks among the samples [139]. Such changes in expression of proteins were correlated with impairment of GSIS. Nevertheless, no identification of the differentially expressed peptides has been carried out. Comparison of INS1-E mitochondrial 2DG proteome revealed 75 spots showing 2-fold or more significant changes ($p < 0.05$) in relative abundance in the presence of 20 mM glucose compared to the cells exposed to 5.5 mM glucose. Thirty-three protein spots appear only on the mitochondrial map of the INS-1E cells exposed to 5.5 mM glucose. Mitochondrial protein spots down-regulated in glucotoxic conditions include ATP synthase α chain and δ chain, stress-70 protein, mitochondrial (75 kDa glucose-regulated protein; GRP 75; HSPA9), malate dehydrogenase, aconitase, trifunctional enzyme β subunit and NADH-cytochrome b5 reductase, and voltage-dependent anion-selective channel protein (VDAC) 2. There were up-regulation of protein spots corresponding to heat shock protein 60, mitochondrial (HSP60) and 10 kDa heat shock protein, mitochondrial (HSP10). Typical to 2D map single protein appeared in multiple spots and several proteins co-migrated. For example, on the mitochondrial 2D map five different spots corresponding to VDAC1 appeared at same molecular weight but having different p*I*. Three spots showed over-expression in response to high glucose and two other spots were down-regulated. Changes in expression of a single isoform (spots) of a protein on 2DG do not necessarily signify alteration in total protein amount. Therefore, caution should be undertaken before concluding expression level of a protein on 2DG without validating the data with Western blot or other methods. In addition to the mitochondrial proteins, other differentially expressed proteins in glucotoxic condition includes proinsulin, calreticulin, protein disulfide isomerase A6 (PDIA6), PKC substrate 60.1 kDa protein, hypoxia up-regulated protein 1 (ORP150), endoplasmin, heat shock cognate 71 kDa protein (HSPA8), heterogeneous nuclear ribonucleoproteins D0 and A2/B1, lamin B1, histones H2B, H3.3, and H4 and elongation factor 1-α-1. With label-free LC-MS/MS approach 353 proteins were found to be differentially expressed in INS-1E cells exposed to 25 mM glucose compared to the cells cultured in the presence of 5.5 mM glucose (unpublished data). Ingenuity pathways analysis (IPA) revealed strong association of differentially expressed proteins with energy production, lipid

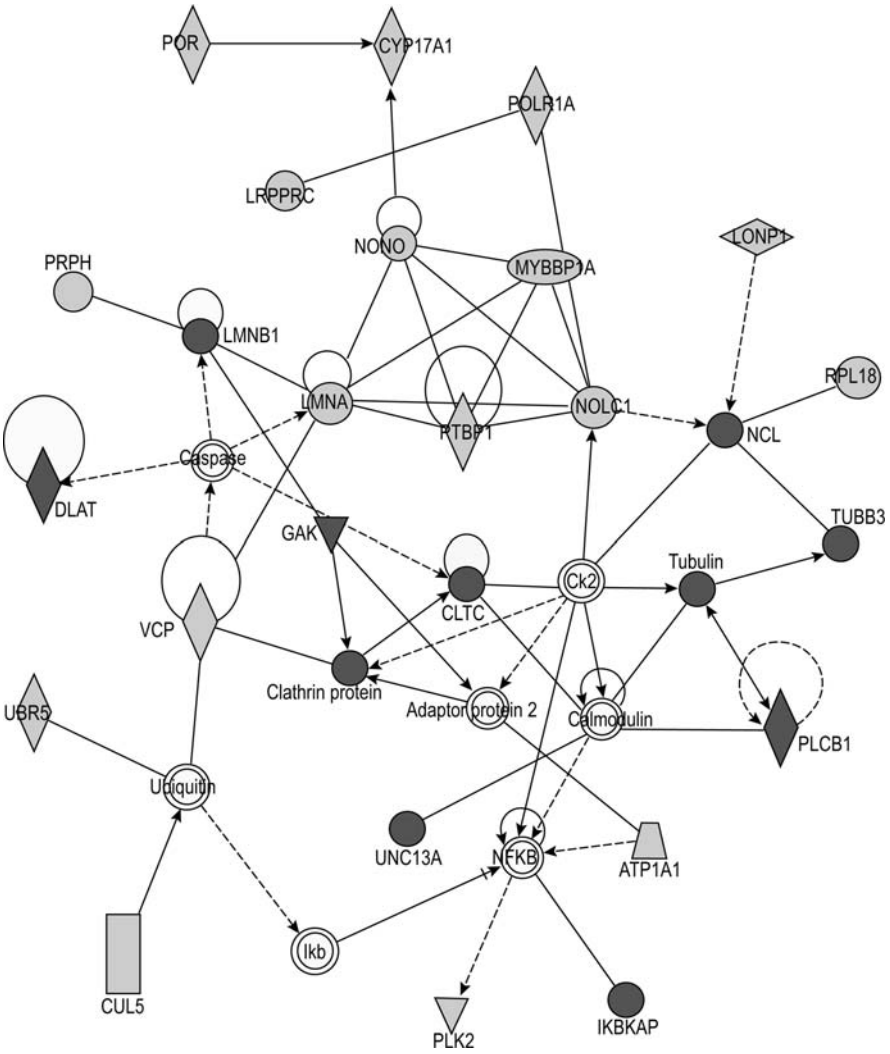
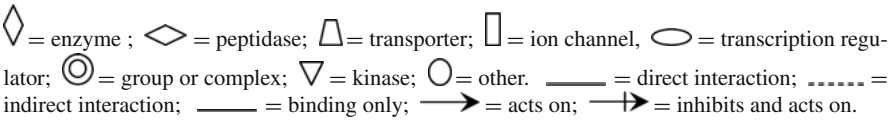


Fig. 16.3 Ingenuity pathway network obtained on a set of differentially regulated proteins detected in INS-1E cells exposed to 25 mM glucose compared to the cells cultured in the presence of 5.5 mM glucose. Proteins with a gray background were down-regulated by high glucose while other interacting proteins with a dark background were up-regulated. ATP1A1, ATPase, Na^+–K^+ transporting, α1 polypeptide; CK2, casein kinase 2; CLTC, clathrin, heavy chain; CUL5, cullin 5; CYP17A1, cytochrome P450, family 17, subfamily A, polypeptide 1; DLAT, dihydrolipoamide *S*-acetyltransferase; GAK, cyclin G-associated kinase; Ikb, inhibitor of nuclear factor of κ light polypeptide gene enhancer in B-cells, beta; IKBKAP, inhibitor of kappa light polypeptide enhancer in B-cells, kinase complex-associated protein; LMNA, lamin A/C; LMNB1, lamin B1; LONP1, lon peptidase 1, mitochondrial; LRPPRC, leucine-rich PPR-motif containing; MYBBP1A, MYB-binding protein (P160) 1a (p53-activated protein-2); NCL, nucleolin; NFKB, nuclear factor of κ light polypeptide gene enhancer in B-cells; NOLC1, nucleolar and

metabolism, protein synthesis, DNA replication, recombination and repair, cell signaling, and metabolic disease. Using IPA we mapped biological networks affected by the differentially expressed proteins between 5.5 mM and 25 mM glucose-exposed INS-1E cells. Figure 16.3 shows the network involved in endocrine system development and function, lipid metabolism, and small molecule biochemistry. In INS-1E cells exposed to 25 mM glucose, N-methylpurine DNA glycosylase (MPG) showed significant (> 2-fold) up-regulation while carboxypeptidase E (CPE) was 4-fold down-regulated. Other substantially down-regulated proteins in response to high glucose exposure included chromogranin A (CGA), membrane-associated guanylate kinase (MAGI1), ubiquitin protein ligase E3 component n-recognin 5 (UBR5), and mitofusin (MFN). Although fold change is a commonly used criterion in quantitative proteomics, it does not provide an estimation of false-positive and false-negative rates that are often likely in a large-scale quantitative proteomic analysis such as in label-free LC-MS/MS quantitation. It is therefore essential for the islet researchers to adopt effective significance analysis of proteomic data which is particularly useful in the estimation of false discovery rates [140]. The proteomic data from 2DG and LC-MS/MS analysis of the glucotoxic studies provide a comprehensive overview of the orchestrated changes in expression of multiple proteins involved in nutrient metabolism, energy production, nucleic acid metabolism, cellular defense, glycoprotein folding, molecular transport, protein trafficking, RNA damage and repair, DNA replication, apoptosis signaling, and mDNA stability. Farnandez et al. [141] have correlated proteomic data with metabolomic findings in glucotoxic conditions in INS-1 β-cell line. While 75 proteins showed differential expression in the presence of high glucose, only 5 of those proteins were found to be involved in the observed metabolomic alterations, suggesting allosteric regulation and/or posttranslational modifications are more important determinants of metabolite levels than enzyme expression at the protein level [141]. Combined SELDI-TOF and 2DGE approach identified 11 different proteins coupled to altered insulin release in response to high glucose (20 mM) (Sol EM, personal communication).

Fig. 16.3 (continued) coiled-body phosphoprotein 1; NONO, non-POU domain containing, octamer-binding protein; PLCB1, phospholipase C, β 1; PLK2, polo-like kinase 2; POLR1A, polymerase (RNA) I polypeptide A; POR, P450 (cytochrome) oxidoreductase; PRPH, peripherin; PTBP1, polypyrimidine tract-binding protein 1 (heterogeneous nuclear ribonucleoprotein I); RPL18, ribosomal protein L18; TUBB3, tubulin β-3; UBR5, ubiquitin protein ligase E3 component n-recognin 5; UNC13A, unc-13 homolog A; VCP, valosin-containing protein.



16.3.4 Type 1 Diabetes and Islet Proteomics

Type 1 diabetes (T1D) is an autoimmune disorder characterized by selective destruction of insulin-producing β-cells in the pancreas resulting from the action of environmental factors on genetically predisposed individuals [142]. The prevailing view for the pathogenesis of type 1 diabetes is that an autoimmune reaction, where cytokines play an important role, causes destruction of the β-cells [143]. Numerous reports have demonstrated both in rodent and in human islets that interleukin-1β (IL-1β) alone or in combination with interferon-γ (IFN-γ) and tumor necrosis factor α (TNF-α) affects the transcription and translation of genes, which have been implicated in β-cell destruction [144]. To search for novel proteins involved in cytokine-induced destruction of β-cells 2DGE has been used [145]. This approach has detected up-regulation of 29 proteins on 2DG image of rat islets exposed to IL-1β compared to control islets, and addition of nicotinamide reduced the up-regulation of 16 IL-1β-induced proteins [145]. In a subsequent study [146], on 2D gels of ^{35}S-methionine-labeled rat islets 52 spots were up-regulated, 47 down-regulated, and 6 synthesized de novo by IL-1β. Among these 105 differentially expressed proteins, 23 protein spots were found to be significantly affected when nitric oxide (NO) production was prevented, suggesting a major role of NO-independent IL-1β-mediated regulation of gene expression [147]. Mass spectrometric analysis allowed identification of 15 proteins, which were most profoundly altered by cytokine treatment [147]. Also, on the transcription level similar approaches have been employed to search for genes involved in the cytokine-induced alterations [148]. Both these powerful approaches have yielded important information about putative genes/proteins involved in the development of the disease. Larsen et al. [149] identified 57 different proteins from IL-1β-exposed rat islets and categorized them into several functional groups including (1) energy transduction; (2) glycolytic pathway; (3) protein synthesis, chaperones, and protein folding; and (4) signal transduction. Results of this differential expression analysis suggest that islet exposure to cytokines induces a complex pattern in β-cells comprising protective (e.g., up-regulation of stress proteins) as well as deleterious (e.g., iNOS induction and NO production) events [150]. The overall picture of the proteomic studies of type 1 diabetes is complex and do not allow us to predict which protein changes may be considered ‘primary’ or ‘secondary’ in importance, time, and sequence [149]. An integrative analysis method was developed combining genetic interactions using type 1 diabetes genome scan data and a high-confidence human protein interaction network [151]. Using this network analysis of the differentially expressed proteins in INS-1E cells exposed to cytokines, 42 of the differentially expressed proteins constituted a significant interaction network suggesting extensive cross talk between the different proteins and the pathways in which they are involved with some proteins such as the chaperones GRP78, HSPA8, and GRP75 and the RNA synthesis/turnover proteins placed at the center of different networks. In fact all these islet proteomic studies strongly suggest a protective role of the chaperones in regulating β-cell dysfunction.

16.3.5 Pharmacoproteomics and Pancreatic Islets

A potential application of proteomics in islet research is the detection of molecular alterations in diabetes and further characterization of existing or new drug [152]. One of the prime targets for the treatment of diabetes is to enhance the insulin sensitivity so that the tissues can precisely utilize glucose and keep its plasma level within physiological limit. Rosiglitazone, a member of the thiazolidinedione (TZD) class of antidiabetic agents, improves insulin sensitivity both in liver and in peripheral tissues. TZDs bind to and activate the peroxisome proliferator-activated receptor (PPARγ) and regulate the coordinated expression of multiple genes that integrate the control of energy, glucose, and lipid homeostasis, therefore contribute to increased insulin sensitivity. Rosiglitazone has been shown to prevent islet cell hyperplasia and protects islets from toxic agents [153–155]. In an elegant study using 2DGE, Sanchez et al. [156] compared protein expression profiles of pancreatic islets from obese diabetic C57BL/6J *lep*/*lep* mice and their lean littermates treated with rosiglitazone. They identified 9 differentially expressed proteins between lean and obese, diabetic, untreated mice. The expression levels of four of those nine proteins (tropomyosin 1, profilin, profilin fragment, and fatty acid-binding protein) were significantly modulated by rosiglitazone treatment of the obese mice. In a second set of experiments designed to identify proteins potentially associated with a low islet cell mass, they compared the islet protein expression between C57BL/6J and C57BL/Ks mice. The C57BL/Ks mice have a 2-fold less islet cell mass as compared with the C57BL/6J [157] and, as a consequence, were more susceptible to diabetes [158, 159]. Thirty-one proteins were found to be differentially expressed between the two mouse models and two of them, tropomyosin 1 and profilin, showed the same differential pattern between C57BL/Ks and obese diabetic C57BL/6J *lep*/*lep* mice. Taken together, these results suggest that actin-binding proteins could play an important role in defective islet function. We have a long way to go for the development of novel actin-modulating drugs for treatment of diabetes similar to microtubule-interacting or microtubule-stabilizing drugs developed for cancer treatment [160, 161]. In a recent study, the effects of imidazolines have been tested on rat islet proteome [162] with the optimism that if it were possible to develop one of the them into a drug. This compound may be effective without risk of insulin shock from hypersecretion in subjects with low or normal blood glucose as imidazolines increase insulin release selectively at high glucose concentrations [163]. The 2DG analysis revealed 53 differentially expressed proteins between imidazoline-treated and imidazoline-nontreated islets. Of special interest among the differentially expressed proteins are those involved in protecting cells from misfolded proteins (HSP60, PDI, and calreticulin), Ca^{2+} binding (calgizzarin, calcyclin, and annexin A1), and metabolism or signaling (pyruvate kinase, α enolase, and protein kinase C inhibitor 1). However, elucidation of exact mechanism of action of imidazolines and validation of targets require further studies.

Natural medicinal plant extracts and active components have antidiabetic activity [164], and the extracellular polysaccharides (EPS) obtained from mycelia culture

of *Phellinus baumii* has strong hypoglycemic activity. Proteomic study provided insights into the mechanism of antidiabetic activity of the EPS in type 1 diabetes [165]. The 2DGE image analysis and mass spectrometry identified 10 down-regulated and 16 up-regulated proteins in streptozotocin-treated diabetic mice islets. The altered level of all these differentially expressed proteins was partially or fully restored to normal level by EPS treatment. The interesting down-regulated proteins in diabetic model include cholesterol esterase, PDI and islet regenerating protein, whereas the up-regulated proteins were Cu/Zn superoxide dismutase, carbonyl reductase, GRP58, hydroxymethylglutaryl-CoA synthase, similar to α glucosidase II, α subunit, and putative human mitogen-activated protein kinase activator with WD repeats-binding protein. One advantage of this study is that the proteomic data was indeed supported by transcriptomics. It would be interesting to know how alteration of certain specific protein targets modulates the development and progress of type 1 diabetes. In a recent study, using proteomic approaches it has been demonstrated that Rho-GDI-α/JNK pathway might be the focus of therapeutic target for the prevention of mycophenolic acid-induced islet apoptosis [166].

16.4 Conclusion

During the last decade state-of-the-art proteomic technologies including the 2DGE and label-free LC-MS/MS quantitation have been applied to dissect the pathophysiology of islet function in an increasingly manner. A vast array of proteomics data has emerged from these studies providing molecular and comprehensive snapshot of complex disease process involving the pancreatic islet cells – but just like a trace of light through an age-old dark cave, coming from the gleaming endless ocean. Careful analysis and powerful bioinformatic tools are still required for functional summary of the data sets and generation of novel hypothesis. These proteomic studies are indeed very early steps toward better understanding of the mechanism of pathophysiology of diabetes and providing new approaches for the prevention and treatment of the disease. Almost no functional proteomics has been performed in islet research. However, improvement and easy availability of high-throughput proteomic techniques will hopefully draw the attention of more islet biologist and generate significant functional data. An important feature of diabetes is that it is a progressive condition. Pancreatic β-cell function, in particular, shows a progressive decline in the pre-diabetic phase and in established diabetes. To clearly define islet function, therefore, we need to measure it over a period of time amalgamating multiple platforms and involving cell biologists, physiologists, geneticists, and biochemists working together with proteomics specialists. A large-scale study will allow this, together with the detection of changes in islet protein patterns and other metabolic traits will lead to a better understanding of how susceptible gene variants and their protein products predispose to diabetes. This will also help to explore novel biomarkers to predict future diabetes, for better understanding of the pathophysiology of diabetes, to reveal drug targets, as well as to optimize the selection of molecules that interact with these targets.

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