Research Article

The Global Transcriptional Response of Isolated Human Islets of Langerhans to Glucagon-Like Peptide-1 Receptor Agonist Liraglutide

Xiaoning Zhao, Yongming G. Tang, S. Vincent Wu, Charles Wang, Ricardo Perfetti, Nasif Khoury, Dehong Cai, Fang He, Xiaogang Su, Vay Liang W. Go, and Hongxiang Hui

1 Center of Metabolic Diseases, Beijiao Hospital, Southern Medical University, North 1838 Guangzhou Road, Guangzhou 510515, China
2 International Center for Metabolic Diseases, Southern Medical University (SMU), 8 Floor, Life Science Build, North 1838 Guangzhou Road, Guangzhou 510515, China
3 Department of Medicine, Cedar-Sinai Medical Center, Los Angeles, CA 90048, USA
4 Department of Medicine, VA Greater Los Angeles Health Care System, Los Angeles, CA 90073, USA
5 UCLA Center for Excellence in Pancreatic Diseases, David Geffen School of Medicine at UCLA, Los Angeles, CA 90095, USA
6 School Biotechnology, Southern Medical University, North 1838 Guangzhou Road, Guangzhou 510515, China

Correspondence should be addressed to Hongxiang Hui, huihongx@gmail.com

Received 2 August 2012; Accepted 20 August 2012

Academic Editors: H. Galbo, R. Laybutt, and A. Petryk

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GLP-1 and its analog have been used in diabetes treatment; however, the direct alteration of gene expression profile in human islets induced by GLP-1 has not been reported. In present study, transcriptional gene expression in the liraglutide-treated human islets was analyzed with 12 human U133A chips including 23,000 probe sets. The data compared between liraglutide and control groups showed a significant difference on glucose-induced insulin secretion, rather than viability. Microarray analysis identified 7000 genes expressed in human islets. Eighty genes were found to be modulated by liraglutide treatment. Furthermore, the products of these genes are proteins involved in binding capability, enzyme activity, transporter function, signal transduction, cell proliferation, apoptosis, and cell differentiation. Our data provides a set of information in the complex events, following the activation of the GLP-1 receptor in the islets of Langerhans.

1. Introduction

GLP-1 is synthesized by the intestinal L-cells and it has been known to stimulate insulin release in the postprandial state, to inhibit glucagon release, and to slow down the rate of gastric emptying, along with acid secretion [1]. In addition to these well-known functions, novel properties of GLP-1 on maintaining beta-cell mass have been identified recently. In glucose-intolerant Wistar rats, GLP-1 reverses an age-dependent beta-cell abnormalities, and this is associated with an expansion of beta-cell mass via islet cell neogenesis [2]. Similarly, Exendin-4, a GLP-1 receptor agonist, stimulates both beta-cell replication and neogenesis, resulting in increased beta-cell mass and improved glucose tolerance in diabetic rat created by partial subpancreatectomy [3]. A GLP-1-dependent differentiation of pancreatic precursor cells into mature beta-cells has also been proposed [4, 5]. Finally an inhibitory effect on islet cell apoptosis has been observed in the pancreas of animal models of diabetes, as well as in beta-cell lines and isolated human islets in culture [6–8]. All these data suggested that treatment of human islets with exogenous GLP-1 could improve function and survival of pancreatic islets. Indeed, it has been demonstrated by several groups that GLP-1 enhances beta-cell mass by both promoting islet cell neogenesis and inhibiting beta-cell apoptosis [8, 13]. However, the elucidation of the molecular and cellular
mechanisms regulating this complex set of diverse biological actions is still very marginally understood.

To evaluate GLP-1 efficacy on human islets and to identify GLP-1 induced gene profile as well as the related signal pathways, we cultured human islets isolated from cadaveric donors with liraglutide [9], a long-lasting GLP-1 analog and investigated the gene expression profile after the exposure of human islets to GLP-1 by microarray analysis. We demonstrated that in response to activation of the GLP-1 receptor, a coordinated expression of factors regulating biological process took place. This paper provides a preliminary map on the intracellular responses of islet cells to GLP-1.

2. Materials and Methods

2.1. Materials. Liberase-HI purified enzyme blend was purchased from Boehringer Mannheim (Indianapolis, IN); dithizone was purchased from Sigma (St. Louis, MO). Human U133A C hips were obtained from Affymetrix, INC (Santa Clara, CA). Medium and RNA extraction kit were purchased from Qiagen (QIAGEN Inc., Valencia, C A). Live/Dead Viability/Cytotoxicity Kit and streptavidin-phycoerythrin were from Molecular Probe (Eugene, OR). GLP-1 was purchased from American Peptide Co. (Sunnyvale, CA). Ficoll Cobe 2991 was purchased from COBE BCT, Inc. (Lakewood, CO). Tissue culture plastic ware was obtained from Corning Coaster Corporation (Cambridge, MA). Fetal calf serum and SuperScript Choice system were purchased from Corning Coaster Corporation (Cambridge, MA). Fetal calf serum and SuperScript Choice system were obtained from Corning Coaster Corporation (Cambridge, MA). Medium and RNA extraction kit were collected by centrifugation at 1000 rpm for 3 min. RNA was isolated using the Qiagen RN easy Mini kit and immediately stored at −80°C.

2.2. Islet Isolation, Purification, and Quantification. Human pancreases were recovered from cadaveric donors. Pancreas were placed in cold UW (University of Wisconsin) solution on ice and immediately transported to the islet isolation laboratory for processing and selected with criteria of having at least 10 h of cold storage in UW. All organs were processed using identical isolation techniques of collagenase digestion and Ficoll purification. Brieﬂy, human pancreases were placed in a customized perfusion apparatus and injected with liberalase-HI. They were then transferred to a continuous digestion device for mechanical disrupt and enzymatic digestion until the majority of the islets were free from exocrine tissue. Samples of pancreatic tissue were evaluated for purity during the digestion after staining with dithizone.

Pancreatic digest containing endocrine and exocrine tissue was puriﬁed by continuous gradients centrifugation of Ficoll Cobe 2991. Islet-enriched layers were then selected and collected. Islet recovery after purification was assessed in duplication by counts of dithizone-stained aliquots of the ﬁnal suspension of tissue. The purity of the preparations was assessed by comparing the relative quantity of dithizone-stained endocrine tissue divided by the unstained tissue. The viability of isolated cells was 87 ± 6%, and their purity was ≥ 57 ± 4%.

2.3. Living/Dead Cell Determination. Cell viability was determined using a Living/Dead Viability/Cytotoxicity Kit. Brieﬂy, isolated islets were washed twice with PBS and 50 μL aliquots were placed in the 96-well plate. Then 10 μL of solution component A (Calcein AM) and component B (Ethidium homodimer-1) in Live/Dead Viability/Cytotoxicity Kit were added to each well. After 30 min, islets were examined under a microscope, Living cells were identified by a green staining (Calcein AM staining), while dead cells showed a brown nuclear staining (ethidium homodimer-1 staining).

2.4. Incubation of Isolated Islets with GLP-1. Isolated islets were cultured, in 75-mL ﬂasks, in the presence of M199 medium with 100 μg/mL penicillin, 50 μg/mL streptomycin, and 10% of fetal calf serum at 37°C under humidified conditions with 5% CO2. After 4 h, the islets were washed twice with M199 without FBS and antibiotics. They were then treated with 10 nM of GLP-1 in M199 medium containing 16 mM glucose (without F BS) for 22 h. Islet pellets were collected by centrifugation at 1000 rpm for 3 min. RNA was isolated by using the Qiagen RN easy Mini kit and immediately stored at −80°C.

2.5. Detection of Insulin Secretion. After 22 h of culturing in the presence of GLP-1, or vehicle, insulin released into the medium was measured by RIA (radioimmunoassay). Total cellular protein content was measured using the method of Bradford Assay. The amount of proteins measured served as a correction factor for determining the relative amount of insulin released into the culture medium by each individual culture condition.

2.6. Microarray Analysis. Affymetrix arrays (Human U133A) were used for mRNA expression proﬁling. Experimental procedures for gene chips were performed according to the Affymetrix Gene Chip Expression Analysis Technical Manual. Brieﬂy: double-stranded cDNAs were synthesized using the SuperScript Choice system and an oligo-(dT)24 anchored T7 primer. Two samples (duplicate) of 5 μg of total RNA from each sample were used to start the synthesis of cDNA. Double-stranded cDNA products were puriﬁed by phenol:chloroform:isoamyl alcohol (25:24:1 saturated with 10 mM Tris-HC l, pH8.0/1 mM EDTA), followed by extraction, phase separation with Phase Lock Gels, and ethanol precipitation. Biotinylated RNA was synthesized using the BioArray high yield RNA transcript labeling kit. Biotinylated RNA products were puriﬁed using Qiagen
Table 1: Primers sequences used for RT-PCR.

| Genes                             | Primers sequence (5'-3')                        | U133A accession number and primer location on cDNA |
|-----------------------------------|-------------------------------------------------|---------------------------------------------------|
| BarH-like homeobox 1              | TGGGC TCTAACC TGGGAGACT                        | 219845_at                                         |
|                                   | GAGCTCAGGGTAGAGACTGTAGCTTC                     | 21F                                               |
|                                   |                                                 | 83R                                               |
| Chondroitin sulfate proteoglycan 2| TTTAAAAATTCCTCATCAGCAAAGG                      | 211571_s_at                                      |
| (versican)                        | TCATGTGGGATGATTTATTTGAATTTGTC                  | 67F                                               |
|                                   |                                                 | 19R                                               |
| Growth arrest-specific 2          | TGC TATGCTTTCAAGTAAAGTAAATTCAC                 | 205848_at                                        |
|                                   | CAGCCCTGTCCCAGGTATACAA                        | 64F                                               |
|                                   |                                                 | 148R                                              |
| Zinc finger protein 185 (LIM domain)| CGTTGGTGAGAGTGCTTGCT                             | 203585_at                                        |
| NK6 transcription factor related, locus 1 | AGAAGCAGGACTCGGAGACAGA                        | 221366_at                                        |
| (Drosophila)                     | TGCTGGACTTGTGCTTCAA                            | 1F                                               |
|                                   |                                                 | 138R                                              |

RNeasy columns and fragmented to a size of 30 to 200 nucleotides. A total of 15 μg biotinylated fragmented RNA was then hybridized with Affymetrix GeneChip arrays (Human, U133A). After washing, the arrays were stained with streptavidin-phycocerythrin, signal amplified by biotinylated antistreptavidin, and then scanned on an Agilent Gene array scanner. The intensity for each signal of the array was captured with the Affymetrix GeneChip Software (MAS 5.0), according to standard Affymetrix procedures. The mRNA abundance was determined based on the average of the differences between perfect match and intentional mismatch intensities for each probe family. Gene induction or downregulation was evaluated for statistical significance using the software provided by Silicon Genetics’ GeneSpring 5.0, Affymetrix DMT 3.0.

2.7. Data Conversion and Statistics. All array assays were used at least in duplicate and the results represented an average of duplicates to minimize the assay variation. Expression data obtained from image files by Affymetrix Microarray Suite 5.0 were scaled to 200 expression units as the median. Raw expression values were normalized within each chip by dividing the median expression value of each individual chip. For each gene, the expression values of the treated samples were further normalized across chips by dividing the mean expression values of the control samples. Statistical analysis was performed with MATLAB software and data was transformed by natural log format. Differentially expressed genes were selected by three methods: (1) t-test \( (P < 0.05) \); (2) a density score greater than 100 for positively identified genes; (3) more than twofold differences from control. All identified genes were annotated based on NetAffx database and functionally classified by gene ontology.

2.8. Real-Time PCR. The expression of selected genes identified by Affymetrix GeneChip analysis was further verified by real-time PCR (with TaqMan technology) on an ABI Prism 7700 Sequence Detection System. PCR primers and TaqMan probes were designed with Primer Express V1.5 software, based on gene sequences downloaded from the GenBank or NetAffx web sites. PCR primers used for the real time PCR are listed in Table 1. TaqMan probes were labeled with 6-carboxy-fluorescein (6-FAM) as the reporter dye and 6-carboxy-tetramethyl-rhodamine (TAMRA) as the quencher dye. Real-time PCR was performed in a two-step process. In the first step, sample RNA (0.1 μg) or reference RNA was reverse transcribed in a volume of 100 μL containing TaqMan RT buffer, 5.5 mM MgCl₂, 500 μM of each dNTP, 2.5 μM random hexamers, 0.4 U/μL RNase inhibitor, and 1.25 U/μL MultiScribe Reverse Transcriptase at 25°C for 10 min, 48°C for 30 min, and 95°C for 5 min. In the second step, real-time PCR was carried out in a MicroAmp Optical 96-well plate using TaqMan Gold PCR reagents. Each well contained 5 μL of reverse-transcribed cDNA, TaqMan buffer A, 5.5 mM MgCl₂, 200 μM each of dATP/dCTP/dGTP/dTTP, 400 μM dUTP, 900 nM each of forward and reverse primers, 250 nM TaqMan probe, 0.01 U/μL AmpErase UNG, and 0.025 U/μL AmpliTaq Gold DNA polymerase in a total volume of 50 μL. The thermal cycling conditions for real-time PCR were: a) 50°C for 2 min, b) 95°C for 10 min, and c) 40 cycles of melting (95°C, 15 sec) and annealing/extension (60°C, 60 sec). PCR reactions were monitored in real time using the ABI P RISM 7700 Sequence Detector. A standard curve for each target gene was generated with reference RNA. Relative quantification of gene expression was determined using the standard curve method as described in ABI’s User Bulletin #2.
3. Results

3.1. Islet Viability and Responsiveness to GLP-1. Islets viability was determined by the Living/Dead Viability/Cytotoxicity assay. Living cells were identified by a green staining (Calcein AM staining), while a brown nuclear staining distinguished the dead cells (Ethidium homodimer-1 staining). We observed a majority (>90%) of islet cells viable, with no significant difference in the proportion of live versus dead cells between islets cultured in the presence of GLP-1, and control groups (Figure 1(a)).

The concentration of insulin in the medium, measured by RIA, and its abundance, normalized for by the total concentration of proteins in each individual culture are listed in Figure 1(b). There was $28.87 \pm 3.29$ ng insulin/mg of protein in the culture medium of islets grown in the presence of GLP-1 versus a $15.43 \pm 3.6$ ng insulin/mg of protein in the culture medium of islets grown in the control culture (no GLP-1). Statistic analysis indicates the difference between two status is significant ($P < 0.01$).

3.2. Genes Regulated by GLP-1. A total of 24 microarrays were employed to evaluate the gene expression profile associated with the exposure of human islets to GLP-1. After 22 h induction in a medium containing 10 nM GLP-1, more than 7000 gene transcripts were detected. Among them, the 80 genes (1%) with differential expression by 2-fold or more (up- or downregulation) are listed in Tables 1 and 2. The top 5 genes found upregulated by GLP-1 are: S EMA3C (sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C), 3.172-folds; RBBP6 (retinoblastoma binding protein 6], 3.147-folds; BARX1 (BarH-like homeobox 1), 3.122-folds; S100A9 (S100 calcium binding protein A9 (calgranulin B)), 2.940-folds; and DNM1L (adhesion glycoprotein), 2.935-folds, respectively. The top 5 genes found down-regulated by GLP-1 are: KCNJ15 (potassium inwardly-rectifying channel, subfamily J, member 15), 0.306-folds; UTS2 (urotensin 2), 0.299-folds; SLC7A6 (solute carrier family 7 (cationic amino acid transporter, y+ system), member 6), 0.260-folds; HSA9947 (putative ATPase), 0.231-folds; and ARHGEF9 (Cdc42 guanine nucleotide exchange factor (GEF) 9), 0.169-folds.

3.3. Validation of Microarray Data by Real-Time PCR. The alteration of gene expression by GLP-1 induction, as observed by microarray analysis, was further validated by real-time PCR. RNA samples from the same islet preparations were split and used for both microarray analysis and real-time PCR. We verified mRNA expression of 5 genes, two upregulated genes (BarH-like homeobox 1 and chondroitin sulfate proteoglycan) and three downregulated genes (growth arrest-specific 2, zinc finger protein 185, and NK6 transcription factor), responding GLP-1 induction. We were able to confirm the consistent results from real-time PCR and from microarray analysis (Figure 2).
Table 2: (a) Upregulated genes selected from GLP-1 treated islet, (b) Downregulated genes selected from GLP-1 treated islet.

(a)

| Symbol | Name | Chromosome location | FC  | P (t-test) |
|--------|------|---------------------|-----|------------|
| Homo sapiens cDNA FLJ39067 fis, clone NT2RP7014910. | Sema domain, immunoglobulin domain (Ig), short basic domain, secreted, (semaphorin) 3C | 7q21-q31 | 3.240 | 0.027 |
| SEM A3C | 16p12-p11.2 | 3.172 | 0.031 |
| RBBP6 | BarH-like homeobox 1 | 9q12 | 3.122 | 0.020 |
| BARX1 | S100 calcium binding protein A9 (calgranulin B) | 1q21 | 2.940 | 0.006 |
| S100A9 | Adhesion glycoprotein | 3.240 | 0.027 |
| DNM-1 | Homo sapiens cDNA FLJ14046 fis, clone HEMBA1006461. | 2.829 | 0.016 |
| Homo sapiens cDNA FLJ10906 fis, clone OVARC1000035. | 2.829 | 0.016 |
| Sec15B protein | 2p12 | 2.776 | 0.026 |
| SEC15B | 6p22-p21.3 | 2.773 | 0.001 |
| Histone 1, H3i | 1q32.1 | 2.504 | 0.017 |
| HIST1H3I | 17q11-q21 | 2.500 | 0.000 |
| PRLR | Chemokine (C–C motif) ligand 3 | 17q11-q21 | 2.500 | 0.000 |
| CYLD | 1q32.1 | 2.504 | 0.017 |
| S100A4 | Sulfotransferase family, cytosolic, 1C, member 1 | 2q11.1-q11.2 | 2.586 | 0.034 |
| SULT1C1 | Homo sapiens, clone IMAGE: 4702418, mRNA | 2.522 | 0.016 |
| Homo sapiens, clone IMAGE: 4471726, mRNA | 2.522 | 0.016 |
| PTPN7 | Apolipoprotein B mRNA editing enzyme, catalytic polypeptide-like 3G | 2q13.1-q13.2 | 2.310 | 0.020 |
| APOBEC3G | Transient receptor potential cation channel, subfamily M, member 3 | 9q21.11 | 2.265 | 0.027 |
| TRPM3 | Homo sapiens mRNA, cDNA DKFZp434E2423 (from clone DKFZp434E2423) | 17q22-q23 | 2.201 | 0.020 |
| PNUTL2 | Peanut-like 2 (Drosophila) | 17q22-q23 | 2.201 | 0.020 |
| CSGP2 | 5q14.3 | 2.198 | 0.037 |
| COL14A1 | 8q23 | 2.189 | 0.025 |
| MGC27165 | 14 | 2.111 | 0.021 |
| Chromosome 16 open reading frame 5 | 16p13.3 | 2.106 | 0.016 |
| C16orf5 | 11q24.2 | 2.089 | 0.024 |
| FLJ23342 | SBBI31 protein | 5q23.2 | 2.085 | 0.029 |
| FLJ10140 | 7q32 | 2.082 | 0.040 |
| KIAA0999 | Hypothetical protein FLJ10140 | 22q13 | 2.077 | 0.029 |
| KIAA0999 | Homo sapiens mRNA full length insert cDNA clone EUROIMAGE 362780. | 11q23.3 | 2.048 | 0.015 |

(b)

| Symbol | Name | Chromosome location | FC  | P (t-test) |
|--------|------|---------------------|-----|------------|
| GAS2 | Growth arrest-specific 2 | 11p14.3-15.2 | 0.498 | 0.045 |
| SUOX | Sulfite oxidase | 12q13.13 | 0.496 | 0.034 |
| KIAA0469 | KIAA0469 gene product | 1p36.23 | 0.496 | 0.026 |
| DNAJB6 | DnaJ (Hsp40) homolog, subfamily B, member 6 | 7q36.3 | 0.495 | 0.022 |
| Symbol | Name | Chromosome location | FC | P (t-test) |
|--------|------|---------------------|----|-----------|
| RBM 9  | RNA binding motif protein 9 | 22q13.1 | 0.490 | 0.034 |
| MYB    | v-myb myeloblastosis viral oncogene homolog (avian) | 6q22-q23 | 0.488 | 0.030 |
| CLASP2 | Cytoplasmic linker associated protein 2 | 3p 22.2 | 0.487 | 0.047 |
| MGC5297| Hypothetical protein M GC5297 | 5p15.3-p15.2 | 0.486 | 0.039 |
| PPP2R4 | Protein phosphatase 2A, regulatory subunit B' (PR 53) | 9q34 | 0.484 | 0.031 |
| PIP5K1C| Phosphatidylinositol-4-phosphate 5-kinase, type I, gamma | 19p13.3 | 0.482 | 0.022 |
| ZFHX1B | Zinc finger homeobox 1b | 2q22 | 0.479 | 0.028 |
| ADCY9  | Adenylate cyclase 9 | 16p13.3 | 0.463 | 0.043 |
| FLJ12788| Hypothetical protein FLJ12788 | 2p12 | 0.458 | 0.015 |
| TM 6SF1 | Trans membrane 6 superfamily member 1 | 15q24-q26 | 0.447 | 0.043 |
| TBC1E  | Tubulin-specific chaperone e | 1q42.3 | 0.446 | 0.035 |
| GTF2H2 | General transcription factor IIH, polypeptide 2, 44kDa | 5q12.2-q13.3 | 0.438 | 0.007 |
| PRO1331| Hypothetical protein PRO1331 | 5q33.3 | 0.437 | 0.024 |
| ZNF185 | Zinc finger protein 185 (LIM domain) | Xq28 | 0.428 | 0.021 |
| KIAA0460 | KIAA0460 protein | 1q21.2 | 0.427 | 0.021 |
| DKFZP434B168 | DKFZP434B168 protein | 1p36.13-q42.3 | 0.426 | 0.042 |
| FCMD   | Fukuyama type congenital muscular dystrophy (fukutin) | 9q31-q33 | 0.415 | 0.045 |
| ABCF2  | ATP-binding cassette, subfamily F (GCN20), member 2 | 7q36 | 0.414 | 0.012 |
| LM O7  | LIM domain only 7 | 13q21.33 | 0.413 | 0.039 |
| SULF1  | Sulfatase 1 | 8q13.1 | 0.412 | 0.033 |
| KIAA0090| KIAA0090 protein | 1p36.13 | 0.411 | 0.003 |
| POR    | P450 (cytochrome) oxidoreductase | 7q11.2 | 0.407 | 0.015 |
| SE70-2 | Cutaneous T-cell lymphoma tumor antigen se70-2 | 13q22.1 | 0.406 | 0.030 |
| NKX6-1 | NK6 transcription factor related, locus 1 (Drosophila) | 4q21.2-q22 | 0.403 | 0.006 |
| LOC51231| VRK3 for vaccinia related kinase 3 | 19q13 | 0.403 | 0.002 |
| BSYL   | Bystin-like | 6p21.1 | 0.401 | 0.032 |
| HIP1R  | Huntingtin interacting protein-1-related | 12q24 | 0.396 | 0.012 |
| PPP2R4 | Protein phosphatase 2A, regulatory subunit B' (PR 53) | 9q34 | 0.387 | 0.046 |
| WASL   | Wiskott-Aldrich syndrome-like | 7q31.3 | 0.383 | 0.032 |
| TIMM 17A| Translocase of inner mitochondrial membrane 17 homolog A (yeast) | 1q32.1 | 0.378 | 0.032 |
| ARL4   | ADP-ribosylation factor-like 4 | 7p21-p15.3 | 0.375 | 0.002 |
| LOC51204| Clone HQ0477 PRO0477p | 17q24.1 | 0.363 | 0.023 |
| KIAA1023| KIAA1023 protein | 7p22.3 | 0.357 | 0.017 |
| CPT1A  | carnitine palmitoyl transferase 1A (liver) | 11q13.1-q13.2 | 0.351 | 0.021 |
| RAD51C | RAD51 homolog C (S. cerevisiae) | 17q22-q23 | 0.347 | 0.022 |
| ITGB3  | integrin, beta 3 (platelet glycoprotein IIIa, antigen CD61) | 17q21.32 | 0.345 | 0.008 |
| MGC4309| hypothetical protein M GC4309 | 1q32.1 | 0.336 | 0.007 |
| ARHGEF9| Cdc42 guanine nucleotide exchange factor (GEF) 9 | Xq11.1 | 0.169 | 0.019 |

**Note:** The table continues with additional entries not shown here.
Figure 2: Results comparison between microarray and real-time-PCR. Five selected transcripts among those identified by microarray analysis were validated by the real-time-PCR analysis. The three transcripts selected were: BarH-like homeobox 1 (BARH); chondroitin sulfate proteoglycan (CSP); growth arrest-specific 2 (GAS); zinc finger protein 185 (LIM domain) (ZFP); NK6 transcription factor (NK6T). The graph shows the folds of induction or suppression as derived by the two methods. The data are expressed as the means ± SE of three independent analyses.

Figure 3: Functional clustering of islet genes regulated by GLP-1.

3.4. Clustering of Genes Regulated by GLP-1. Among the 80 genes regulated by GLP-1, 66 were with known molecular functions, 34 were linked to cellular structural components; 73 were involved in various biological processes. Further analysis demonstrated that genes encoding for proteins, with protein-DNA or protein-protein binding capacity (n = 25) and enzyme activity (n = 17) were in two main subgroups (Figure 3). Genes encoding proteins with intracellular structure (n = 30) represented a main class for cellular component synthesis (Figure 4). Genes involved in

*Some genes are listed as a part of more than one subgroup.
physiological process \( (n = 35) \), including metabolism, response to stress, and response to external stimuli and the genes involved in cellular processes \( (n = 22) \), as cell growth and cell–cell interaction, represented the genes with known biological processes (Figure 5). Functional analysis showed that GLP-1 widely modulated the expression of genes linked with cell adhesion/extracellular matrix, cell cycle, cytoskeleton/structural, enzymatic activity and metabolism, growth factors/hormones/cytokines, nucleotide processing, protein processing, receptors, signal transduction, transcription factors, and transporter proteins.

4. Discussion

We report the identification of a large set of genes whose expression is regulated by the binding of GLP-1 to its receptor localized on human islets. The elucidation of this complex gene-regulatory network is essential for a better understanding of the physiological and pharmacological effects of GLP-1, as well as for deciphering the mechanism(s) by which this peptide has such a diverse repertoire of actions on islet cells. The effect of GLP-1 on islet cells has been investigated with rodent cell lines, as well as murine and human islets [10, 11]. From these researches, most data suggested that GLP-1 promoted beta-cell mass by increasing beta-cell proliferation, inhibiting beta-cell apoptosis, and inducing the differentiation of beta-cell from progenitor cells. While the in vivo application of GLP-1 on the diabetic and aging animals suggested that multifactors might be involved and sounded complex on the mechanism, the in vitro treatment of GLP-1 on the isolated islets provided more direct evidence of GLP-1 effect on islets. Farrila et al. demonstrated that GLP-1 added to freshly isolated human islets preserved the cell morphology and function and was able to inhibit cell apoptosis [12]. This effect is associated with a higher expression of Bcl-2 and a lower expression of active Caspase 3. Similarly, Mancosu et al. found that high glucose concentration and glucagon-like peptide 1 (GLP-1) were associated with the maintenance of either the insulin secretory patterns from the incubated monolayer cells, or the transcriptional marker expression associated with beta-cell like phenotypes [13]. Upregulated expression of PDX-1, PAX4, Glut-2, and GK was also detected in their long cultured islets, in the presence of GLP-1.

In this study, we observed that the expression of most proliferation-related genes was associated with GLP-1 induction. These GLP-1 upregulated, proliferation-related genes include: the prolactin receptor, which has been proposed to regulate islet mass expansion during pregnancy [14]; retinoblastoma binding protein-6 (RBBP6) is a multifunctional protein found ostensibly in all eukaryotes but not in bacteria, which is implicated in a diverse set of cellular functions including mRNA metabolism, regulation of the cell cycle, tumourigenesis and development. [15]; CSPG2, which regulates cell motility and growth under P53 activation [16]; S100A4 can act as a novel cardiac growth and survival factor and may have regenerative effects in injured myocardium [17]; CYLD, which acts as tumor suppressor gene [18]; SEMA3a, which inhibits the binding of the VEGF with 165-amino acids (VEGF-165) to np1 and was reported to inhibit
angiogenesis [19]. We were unable to observe the significant alteration of bcl-2, P ik3, and lrs2 reported by others groups with cell line and/or animal models [20, 21]. Antiapoptosis is another important biological event modulated by GLP-1 [22]. Our microarray analyses observed 3 apoptosis-related genes (CYLD, RBBP6, and PNUTL2) upregulated, and one (GAS2) downregulated. CYLD (the familial cylindromatosis tumor suppressor gene) enhances the activation of the transcription factor NFkapa-B [23], RBBP6, (which binds to the retinoblastoma gene product pRB) [24], and PNUTL2 (which is an apoptosis-related protein in the TGF-beta signaling pathway) [25]. GAS2 plays an important role in apoptosis by acting as a cell-death gene substrate for caspases [26]. These data suggest a conflict function of GLP-1 on apoptosis regulation. Increasing CYLD promotes apoptosis and increasing PNUTL enhances cell death via TGF. On the other hand, increased expression of RBBP6 and suppressed expression of GAS2 revealed an antiapoptosis effect of GLP-1.

These observations contradictory on apoptosis and differentiation might indicate complicated, but balanced functions between gene groups (pro- and antiapoptosis), rather than a simple and one direction to lead cell-death. They also indicate that cellular function regulation, such as apoptosis, differentiation, and so forth, are more complicated than what we speculated previously. To understand such a complicated regulation and balance, more advanced methodologies, such as gene expression profiling, may provide unique capacities or advantages to overview the cellular response to specific stimuli. Most of islet differentiation factors are transcriptional factors, and several transcripts of these factors in GLP-1 treated islets have been identified in this study. GLP-1 increased the expression of BARX1, PAX4, and decreased the expression of MYB, NKx6–1, FCMD, and ZNF185. The homoebox gene Barx1 is highly expressed in prospective stomach mesenchyme and is required to specify this organ [27], while PAX4 plays a role in the differentiation and development of pancreatic islet cells [28]. This is consistent with Brun’s observation of that Pax4 expresses in human pancreatic islets and is activated during mitosis and by GLP-1 treatment [29]. MYB is essential for mammary tumorigenesis and its upregulation is associated with estrogen receptor (ER)-positive breast cancer [30]; NKx6–1 is a popular transcription factor involved in differentiation and development of pancreatic islet β-cells [31]. Significantly elevated expression of INSM1 was reported to be associated with both the AR42J cell line and the primary cultured mouse acinar cells differentiation into insulin-positive cells.
[32]. However in our observations, the expression of NKx6-1 was downregulated in GLP-1 treated islets. Similarly, the reduced expression of FCMD, which was reported to be involved in brain development [33], and ZNF185, a tumor-suppressor protein associated with actin-cytoskeleton [34] and reported in prostate cancer, was observed in our studies. Beyond our expectation, we were unable to detect the expression of PDX-1, although it is among the most extensively studied pancreatic transcriptional factor [35], responding to GLP-1 induction.

This study provides, the first time, the gene expression profile of human islets regulated by GLP-1 induction. The data presented here further supports the complex and diversity effects of GLP-1 in the regulation of protein governing, beta-cell mass control, and metabolism. The elucidation of the signal pathway triggered by GLP-1 may provide a scientific basis for molecular target identification, new drugs design, and diabetes disorder treatment.

Acknowledgments

This research was partially supported by NovoNordisk and China 973 Program (no. 2011CB504006), Songshan Lake Sci. & Tech. Industry Park Fund (no.2010B025 & no. 2010B026), Ph.D. Programs Foundation of Ministry of Education of China (no. 20104433110014), Guangdong Science and Technology Research Fund (no. 2010B090400041).

Conflict of Interests

The authors declare that there is no conflict of interests.

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