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Androgen receptor-deficient islet β-cells exhibit alteration in genetic markers of insulin secretion and inflammation. A transcriptome analysis in the male mouse

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Abstract

Aims—Testosterone action is mediated via the androgen receptor (AR). We have reported that male mice lacking AR selectively in β-cells (βARKO$^{-/y}$) develop decreased glucose-stimulated insulin secretion (GSIS), producing glucose intolerance. We showed that testosterone action on AR in β-cells amplifies the insulinotropic action of GLP-1 on its receptor via a cAMP- dependent protein kinase-A pathway.

Methods—To investigate AR-dependent gene networks in β-cells, we performed a high throughput whole transcriptome sequencing (RNA-Seq) in islets from male βARKO$^{-/y}$ and control mice.

Results—We identified 214 differentially expressed genes (DEGs) (158 up- and 56 down-regulated) with a false discovery rate (FDR) < 0.05 and a fold change (FC) > 2. Our analysis of individual transcripts revealed alterations in β-cell genes involved in cellular inflammation/stress and insulin secretion. Based on 312 DEGs with an FDR < 0.05, the pathway analysis revealed 23 significantly enriched pathways, including cytokine-cytokine receptor interaction, Jak-STAT signaling, insulin signaling, MAPK signaling, type 2 diabetes (T2D) and pancreatic secretion. The gene ontology analysis confirmed the results of the individual DEGs and the pathway analysis in showing enriched biological processes encompassing inflammation, ion transport, exocytosis and insulin secretion.

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Conclusions—AR-deficient islets exhibit altered expression of genes involved in inflammation and insulin secretion demonstrating the importance of androgen action in β-cell health in the male with implications for T2D development in men.

Keywords

Androgen receptor; Type 2 Diabetes; Pancreatic β-cell; RNA-Seq; transcriptome

1. Introduction

The aging of the U.S. male population will cause a large increase in the burden of clinically symptomatic androgen deficiency, which currently represents 18% of men over 70 years old (Araujo et al., 2007). In addition, prostate cancer is the most common malignancy in men, and androgen deprivation therapy (ADT), the standard of treatment, produces severe testosterone deficiency. Treatment of the metabolic complications of ADT is now considered a clinical challenge (Navarro, Allard, Xu, & Mauvais-Jarvis, 2015; Yu, Lin, Sparks, Yeh, & Chang, 2014; Zitzmann, 2009). The impact of testosterone deficiency on the development of visceral obesity and insulin resistance in men is well established (Navarro et al., 2015; Zitzmann, 2009). In contrast, and surprisingly, the role of testosterone deficiency in β-cell dysfunction remains poorly explored. This remarkable lack of knowledge is particularly surprising because previous research has implicated low testosterone levels in the pathogenesis of hyperglycemia in men (Mauvais-Jarvis, 2016a). Men with prostate cancer treated with ADT, and therefore exhibiting primary testosterone depletion, are predisposed to diabetes. In two large population-based studies of men with prostate cancer, ADT was associated with a 28% to 44% increased risk of incident T2D compared to controls (Keating, O'Malley, Freedland, & Smith, 2010; Keating, O'Malley, & Smith, 2006). Mice lacking AR globally are also hyperglycemic and exhibit decreased GSIS (Dubois et al., 2016).

To assess the role of AR in β-cell function in the male, we previously generated male mice lacking AR selectively in β-cells (βARKO$^{-/y}$). These mice develop decreased glucose-stimulated insulin secretion (GSIS) without alteration in β-cell mass but producing glucose intolerance (Navarro et al., 2016). When these mice are exposed to a western diet, they are hyperglycemic and hypoinsulinemic in the fasted and fed states. We reported that testosterone action on AR β-cells amplifies the insulinotropic action of GLP-1 on its receptor via a cAMP-dependent protein kinase-A pathway (Navarro et al., 2016). Thus, androgen deficiency predisposes to T2D via the combination of loss of androgen action in peripheral tissues producing insulin resistance and loss of androgen action in β-cells producing β-cell failure to compensate for insulin resistance (Mauvais-Jarvis, 2016b; Navarro et al., 2015; Navarro et al., 2016).

To gain further insight on the role of AR in male β-cells though AR-dependent gene networks, we performed a high throughput whole transcriptome sequencing (RNA-Seq) in islets from male βARKO$^{-/y}$ and control mice.

2. Material and Methods

2.1 Generation of Mutant Mice

The βARKO $^{/y}$ mouse was generated by crossing mice carrying the AR gene with floxed exon 2 on their X chromosome (AR^{lox}) with transgenic mice with the Cre recombinase expression under rat insulin II promoter (RIP-Cre, Jackson Laboratory) as previously described (Navarro et al., 2016).

2.2 Islet Isolation and RNA Preparation

Islet isolation was performed following pancreatic duct injection with collagenase as previously described (Tiano et al., 2011). Islets were isolated from 3 male $ARlox^{-/y}$ mice and 3 RIP-Cre mice and immediately frozen in liquid nitrogen (Fig. 1). Mice were at 12-week of age at the time of islet isolation, and were fed on the normal chow. The metabolic parameters of mice at the same age were previously described (Navarro et al., 2016). Total RNA was extracted using RNeasy Maxi Kit (Qiagen) following the manufacturer's recommendations, and the samples were sent to NUseq Core, Northwestern University for RNA sequencing.

2.3 RNA-Seq Analysis

The quality of DNA reads, in FASTQ format, was evaluated using FastQC (http://www.bioinformatics.babraham.ac.uk/projects/fastqc/). Adapters were removed and reads of inadequate quality were filtered. The raw read data was processed largely following the procedure described in (Trapnell et al., 2012). Briefly, the reads were aligned to the *Mus musculus* genome (mm10) using TopHat (v2.0.8b). Subsequently, the aligned reads, in conjunction with a gene annotation file for mm10 obtained from the University of California Santa Cruz (UCSC) website (http://genome.ucsc.edu/), were used to determine RNA expressions of annotated genes using Cufflinks (v2.1.1).

2.4 Single-Gene Analysis

For a transcript *g*, the expression level is estimated by the number of reads (C_g) mapped to the region of the transcript normalized by the length (L) of the transcript in nucleotides and the total number (N) of mapped reads of the mouse genome. If we use kilobase as the unit for L and million reads as the unit for N, this estimation is called reads per kilobase of transcript per million mapped reads (RPKM), which is the most widely used RNA-seq normalization method (Li, Piao, Shon, & Ryu, 2015). The individual transcript files generated by Cufflinks for each sample were merged into a single gene annotation file, which was then used to perform a DE analysis with the Cufflinks routine, Cuffdiff. Significant DEGs were determined by Cuffdiff using the procedure described in (Trapnell et al., 2012) based on a Benjamini-Hochberg false discovery rate (FDR) threshold of 0.05 (Reiner, Yekutieli, & Benjamini, 2003). Results of such differential expression analysis were processed with CummeRbund (Trapnell et al., 2012). The significant DEGs were separated into those that were up-regulated and those that were down-regulated.

2.5 Quantitative Reverse Transcription PCR (qRT-PCR)

Total RNA was extracted from MIN6 cells with RNeasy Plus Mini Kit (Qiagen) following the manufacturer's instructions. The quality and concentration of RNA were assessed by NanoDrop Spectrophotometer (Thermo Scientific). RNA was reverse transcribed into cDNA using iScript cDNA Synthesis Kit (Bio-Rad). Quantification of targeted genes was performed using iTaq Universal SYBRR Green Supermix (Bio-Rad) and the iCycler iQ Real Time PCR Detection System (Bio-Rad). Ct values were normalized to TBP and the relative gene expression was calculated with the $2^{-\Delta\Delta}$Ct method. Gene-specific KiCqStart primers were purchased from Sigma-Aldrich as listed in Supplemental Table 1.

2.6 Pathway and Gene Ontology (GO) Analysis

The list of 312 (225 up- and 87 down-regulated) significant DEGs (FDR < 0.05) were analyzed by applying (i) GeneCodis3 (http://genecodis.cnb.csic.es) (Tabas-Madrid, Nogales-Cadenas, & Pascual-Montano, 2012) and (ii) GeneTrail (http://genetrail.bioinf.uni-sb.de/) (Backes et al., 2007) software tools to identify significantly enriched Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and GO categories by over-representation analysis.

2.7 Statistical Analysis

Results are presented as mean ± SEM in Fig. 4. All statistical analyses were performed using the unpaired Student's *t* test. A P value less than 0.05 was considered statistically significant. ** P<0.01, *** P<0.001.

3. Results

3.1 Single-Gene Analysis

A total of 23,179 genes were annotated with RefSeq IDs. Of these, a fold change (FC) (defined as the relative ratio of gene expression between βARKO$^{-/y}$ to control islets) could be computed for 22,061 genes. Among these genes, 312 differentially expressed genes (DEGs) (225 up- and 87 down-regulated) were discovered at a false discovery rate (FDR) < 0.05. At FDR < 0.05 and FC > 2, a total of 214 significant DEGs (158 were up- and 56 down-regulated) were identified (Supplemental Table 2). Of these DEGs, 66 were associated with inflammation and stress (53 up- and 13 down-regulated) (Table 1), and 56 were associated with β-cell insulin secretion including metabolism, cAMP-PKA signaling, ion channels, Ras-related protein/GTPase, glucose metabolism, membrane polarization, and secreted factor (44 up- and 12 down-regulated) (Table 2). Thus, in βARKO$^{-/y}$ islets, 31% of the DEGs were associated with β-cell inflammation and stress, and 26% with insulin secretion (Fig. 2). We validated a set of individual gene expression results by qRT-PCR in cultured MIN-6 insulin-producing cells treated with the pure AR agonist dihydrotestosterone (DHT) (Fig. 3). Mirroring the results obtained in control and βARKO$^{-/y}$ islets, DHT suppressed the mRNA for hepatokine fibroblast growth factor 21 (*Fgf21*), the innate immune molecule lipocalin 2 (*Lcn2*), syntrophin, gamma 2 (*Sntg2*), G-protein-coupled receptor (GPR) 26 (*Gpr26*), and *Gpr119*. No effect of DHT was observed for dual oxidase 2 (*Duox2*), and the transient receptor potential cation channel, subfamily C, member 4 (*Trpc4*).

3.2 Pathway Analysis

Gene expression analyzed on a gene-by-gene basis ignores the underlying biological structure and diminishes the power of analysis, obscuring the presence of important biological signals (Haynes, Higdon, Stanberry, Collins, & Kolker, 2013). Thus, grouping genes by biological pathways is often the most relevant approach, because it takes into account the cooperative nature of genes and considers that genes involved in the same process are dysregulated together. Such an approach yields more robust results and may reveal novel insights about molecular mechanisms of disease (Lee, Chuang, Kim, Ideker, & Lee, 2008). The 312 DEGs at FDR < 0.05 were interpreted in a biological pathway context. Based on GeneCodis3 analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, 23 significantly enriched pathways were revealed (Table 3). The representative pathways are cytokine-cytokine receptor interaction (Kegg: 04060), Jak-STAT signaling pathways (Kegg: 04630), MAPK signaling pathway (Kegg: 04010), insulin signaling pathway (Kegg: 04910), and pancreatic secretion (Kegg: 04972). Based on the KEGG pathway results and our analysis of the literature, we combined these pathways and summarized them into three biologically relevant pathways: insulin secretion (Fig. 4A), stress/growth factor signaling (Fig. 4B), and inflammatory pathways (Fig. 4C).

3.3 Gene Ontology (GO) Analysis

Ontologies provide a formal representation of knowledge that is amenable to computational as well as human analysis, an obvious underpinning of systems biology (Thomas, Mi, & Lewis, 2007). The GO, like other formal ontologies, consists of a structured hierarchical controlled vocabulary for standardizing representations of gene and gene product attributes in relation to a large and growing context of biological knowledge (Boyle et al., 2004). Scientists have used GO terms to evaluate the characteristics of sets of genes (Consortium, 2017). The GO classifies gene functions into three categories: biological process (BP), cellular component (CC), and molecular function (MF) (Table 4). For the 312 significant DEGs, based on GeneCodis3 and GeneTrail analyses of GO categories, 43 BP, 17 CC, and 23 MF categories were identified (selection criteria: # genes in GO category ≥ 2 and FDR < 0.05 for both programs). For BP, representative categories included inflammatory response (GO: 0006954), ion transport (GO: 0006811), insulin secretion (GO: 0030073), negative regulation of signal transduction (GO: 0009968), apoptosis (GO: 0006915), cell adhesion (GO: 0007155), regulation of growth (GO: 0040008), and response to stress (GO: 0006950), indicating alteration in the β-cell function and stress. For CC, significantly enriched categories included extracellular region (GO: 0005576), integral to membrane (GO: 0016021), voltage-gated potassium channel complex (GO: 0008076), and integral to plasma membrane (GO: 00058887), revealing structural alterations in membrane proteins involved in insulin secretion. For MF, enriched categories were GTPase activity (GO: 0003924), calcium ion binding (GO: 0005509), GTP binding (GO: 0005525), hexokinase activity (GO: 0004396), transporter activity (GO: 0005215), ion channel activity (GO: 0005216), voltage-gated potassium channel activity (GO: 0005249), and potassium channel activity (GO: 0005267), also indicating functional changes in β-cell secretory capacity.

4. Discussion

Using islets from adult male βARKO$^{-/y}$ mice, we identified 214 dysregulated genes involved in β-cell insulin secretion and stress, confirming that AR plays a vital role in male β-cell health. A third of these genes are coding for proteins mediating or responding to inflammation and cellular stress, demonstrating that islets with prolonged AR deficiency are injured and suffering. These include genes coding for *Fgf21* (Wente et al., 2006), *Lcn2* (Chang, Kim, Ko, Jo, & Kim, 2013), the member of the tumor necrosis factor receptor superfamily osteoprotegerin (*tnfrsf11b*) (Maruyama et al., 2006; Reid & Holen, 2009), chemokine ligands 5 and 10 (*Cxcl5* and *Cxcl10*) (Nunemaker et al., 2014; Schulthess et al., 2009), several interferon (IFN)-gamma-induced guanylate-binding proteins (*Gbp4, Gbp 5, Gbp 6, Gbp 8, Gbp 9, Gbp 10,* and *Gbp 11*) (Kim et al., 2016), intra islet pro-inflammatory cytokines and associated receptors like interleukin-1β (*Il1b*), the interleukin 22 receptor-α1 (*Il22ra1*) (Shioya, Andoh, Kakinoki, Nishida, & Fujiyama, 2008), the IL-1 receptor antagonist (*Il1rn*) (Dayer-Metroz, Wollheim, Seckinger, & Dayer, 1989) and interleukin-10 (*Il10*) (Russell & Morgan, 2014). The coagulation factor XIII, A1 subunit (*F13a1*) has also been implicated in chronic low-grade inflammatory islets in T2D subjects (Sharma et al., 2015).

The second finding is that 20% of dysregulated genes are involved in β-cell function. These include genes coding for GPRs such as *Gpr161* (Bachmann et al., 2016), *Gpr126* (Mogha et al., 2013), *Gpr26* (Zhang et al., 2011), ion channels altering membrane polarization like the potassium inwardly-rectifying channel, subfamily J, member 5 (*kcnj5*), the potassium voltage-gated channel, subfamily Q, member 1 (*kcnq1*) (33), and *trpc4* (Islam, 2011), as well as proteins involved in β-cell exocytosis machinery such as the Ca(2+)-sensor synaptotagmin-10 (*Syt10*) (Cao, Maximov, & Sudhof, 2011), the GTP binding protein rabphilin 3a (*Rph3a*) (Arribas, Regazzi, Garcia, Wollheim, & De Camilli, 1997), heparan sulfate (glucosamine) 3-O-sulfotransferase 1 (*Hs3st1*) (Takahashi, Ohashi, & Nata, 2012) and enzymes involved in glucose metabolism, hexokinase 2 (*hk2*), hexokinase domain containing 1 (*hkdc1*) (Ludvik et al., 2016), glucokinase binding protein 6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3 (*Pfkfb3*) (Arden et al., 2008) and zinc transport in βcells like the zinc transporter, member 4 (*Slc39a4*) (Hardy et al., 2015).

Dysregulated genes seem to fall into two categories. Some are detrimental to β-cell health and could be instrumental in impairing GSIS. For example, genome-wide association studies identified *Kcnq1* and *Rasgrp1* (RAS guanyl releasing protein 1) as susceptible genes for T2DM (Yasuda et al., 2008; Zeng et al., 2016) and βARKO$^{-/y}$ islets exhibit increased expression of both. *Kcnq1* impairs insulin secretion by enhancing the β-cell potassium currents (Yamagata et al., 2011). Increased expression of *Nr0b2* in βARKO$^{-/y}$ islets, coding the orphan nuclear receptor small heterodimer partner (SHP), is also expected to impair insulin gene transcription and decrease GSIS (Park et al., 2007) while increased *Car2* expression (carbonic anhydrase 2) (Yamato, Tashiro, & Miyazaki, 2013) is a genetic marker of poor GSIS, and increased *Sostdc1* (sclerostin domain containing 1) expression inhibits Bmp and Wnt which impairs β-cell function (Henley, Gooding, Economides, & Gannon, 2012). Other adverse upregulated genes include *lcn2*, induced in β-cells by inflammatory cytokines (Chang et al., 2013), the chemokines (*Cxcl5* and *Cxcl10*) increased in islets from

T2D humans and rodents and which are known to impair β-cell function and survival (Nunemaker et al., 2014; Schulthess et al., 2009), and multiple GBPs that activate the inflammasome and produce β-cell inflammation (Kim et al., 2016).

In contrast, another set of dysregulated genes seems to be part of a concerted compensatory mechanism attempting to preserve β-cell function from the deleterious effect of the AR knockout. For example, increased expression of *Fgf21* (Wente et al., 2006) *or Il1rn* (IL-1β receptor antagonist) (Dayer-Metroz et al., 1989) is expected to protect islet function and survival during inflammation, and the increased *Hs3st1* expression is expected to enhance GSIS (Takahashi et al., 2012). Other adaptive mechanisms include increased expression of genes coding for proteins that could enhance GSIS by increasing β-cell glucose metabolism (*Hk2, Pfkfb3*) (Ludvik et al., 2016), cAMP production (*Gpr119, Gpr26, GPR126, Gpr161,* activating transcription factor 3, (*Atf3*)), insulin vesicle exocytosis (*Syt10, Rph3a*), and β-cell membrane depolarization (*Trpc4*) (Islam, 2011).

Our pathway analysis revealed 23 significantly enriched pathways that we combined into two biologically relevant pathways, inflammatory pathways and insulin secretion, confirming our observation from individually dysregulated genes. Ontologies used to evaluate the characteristics of differentially expressed genes in βARKO$^{-/y}$ islets were also enriched for GO terms “response to stress,” “inflammatory response,” “apoptosis,” “insulin secretion,” “ion transport,” and “cell adhesion.” Taken together, these results of GO analysis confirmed the results of the pathway analysis and our individual gene evaluation that AR deficiency promotes β-cell dysfunction and inflammation. Consistent with our findings, testosterone protects early apoptotic damage induced by streptozotocin in male rat pancreas through AR suggesting that AR activation may protect male islets from inflammation (Morimoto et al., 2005; Palomar-Morales, Morimoto, Mendoza-Rodriguez, & Cerbon, 2010). In addition, neuronal specific AR-deficient mice exhibit hypothalamic inflammation via activation of nuclear factor-κB (Yu et al., 2013) which promotes obesity, insulin resistance and glucose intolerance.

A limitation of the present study is that we did not validate all our individual gene expression results by qRT-PCR. However, previous studies have reported high consistencies between RNA-seq and qRT-PCR results (Trost et al., 2015). RNA-seq shows both high reproducibility and low frequency of false positives (Richard et al., 2010) and has been used for transcriptional profiling of specific cell types or tissues at unprecedented precision (Schmid et al., 2012).

In conclusion, a transcriptome analysis of islets from adult male βARKO$^{-/y}$ mice revealed alterations in genes involved in inflammation and insulin secretion, demonstrating the importance of androgen action in β-cell health in males, with implications for the development of T2D in androgen deficient men.

Supplementary Material

Refer to Web version on PubMed Central for supplementary material.

Acknowledgments

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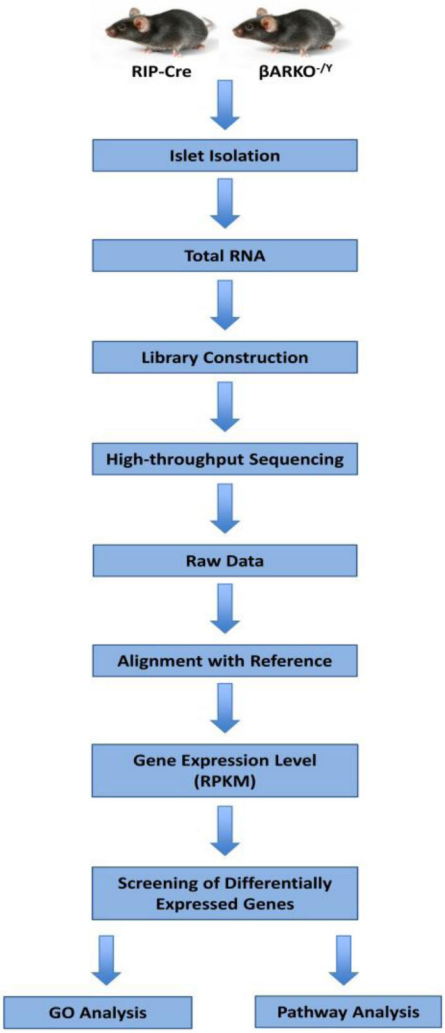

Fig. 1.
Flow chart of the RNA-Seq experiment

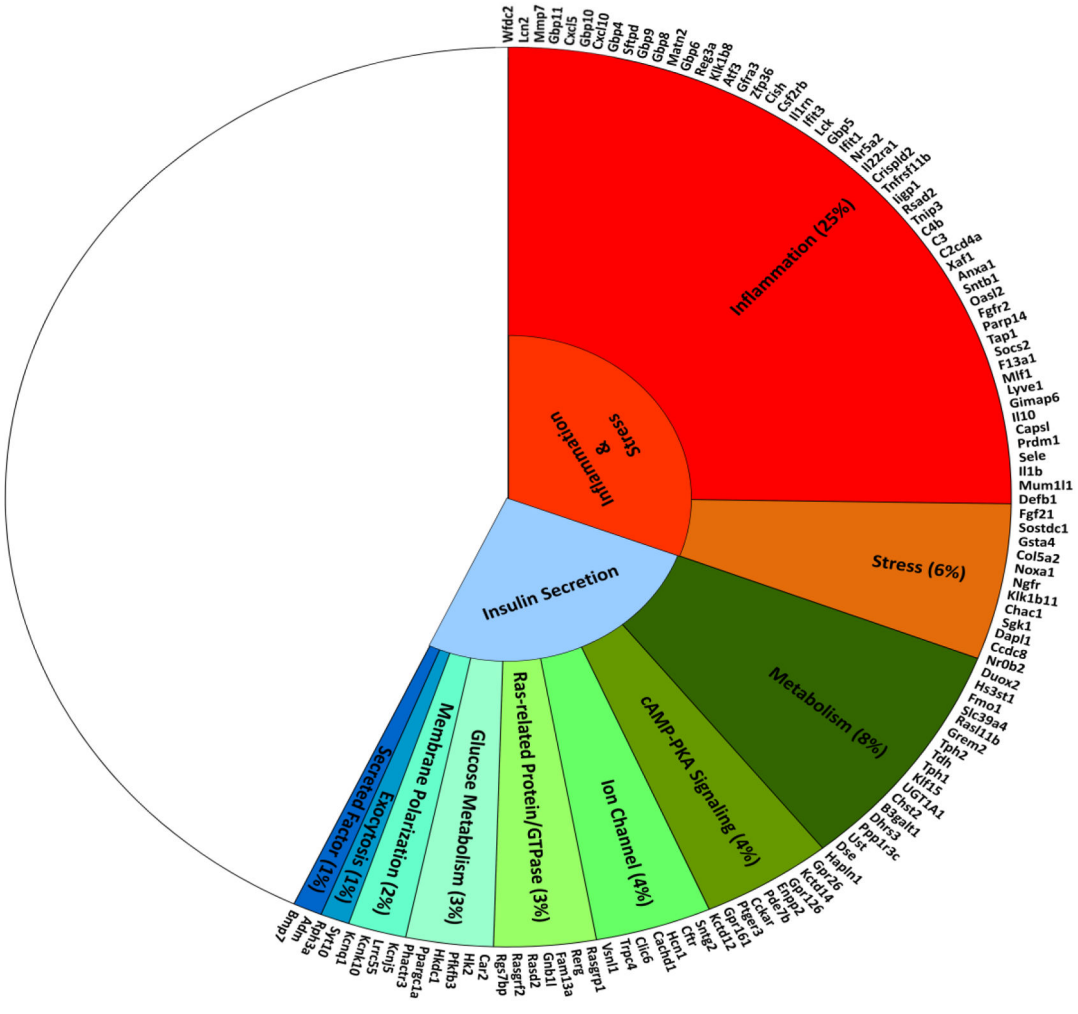

Fig. 2. Single-gene analysis pie chart

Dysregulated genes were involved in inflammation and stress, as well as insulin secretion.

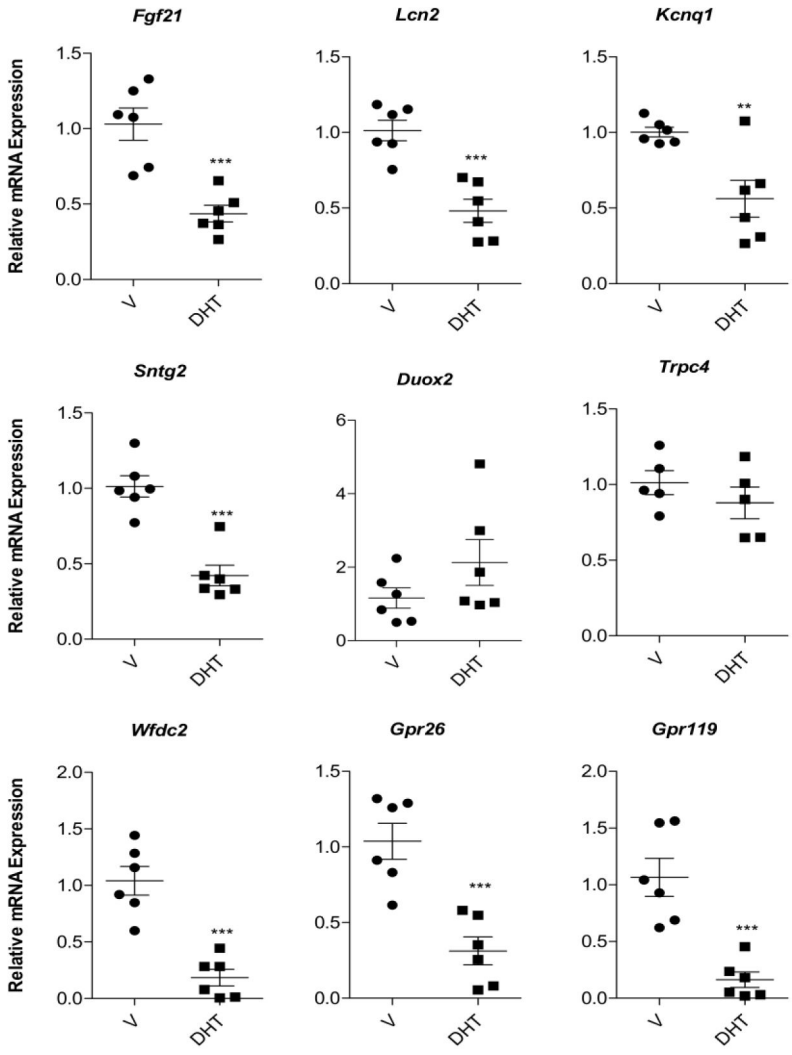

Fig. 3. qRT-PCR validation of RNA-seq analysis

Min6 cells were treated with vehicle or DHT for 24 minutes/24 hours. mRNA expression of target gene was normalized to that of TBP.

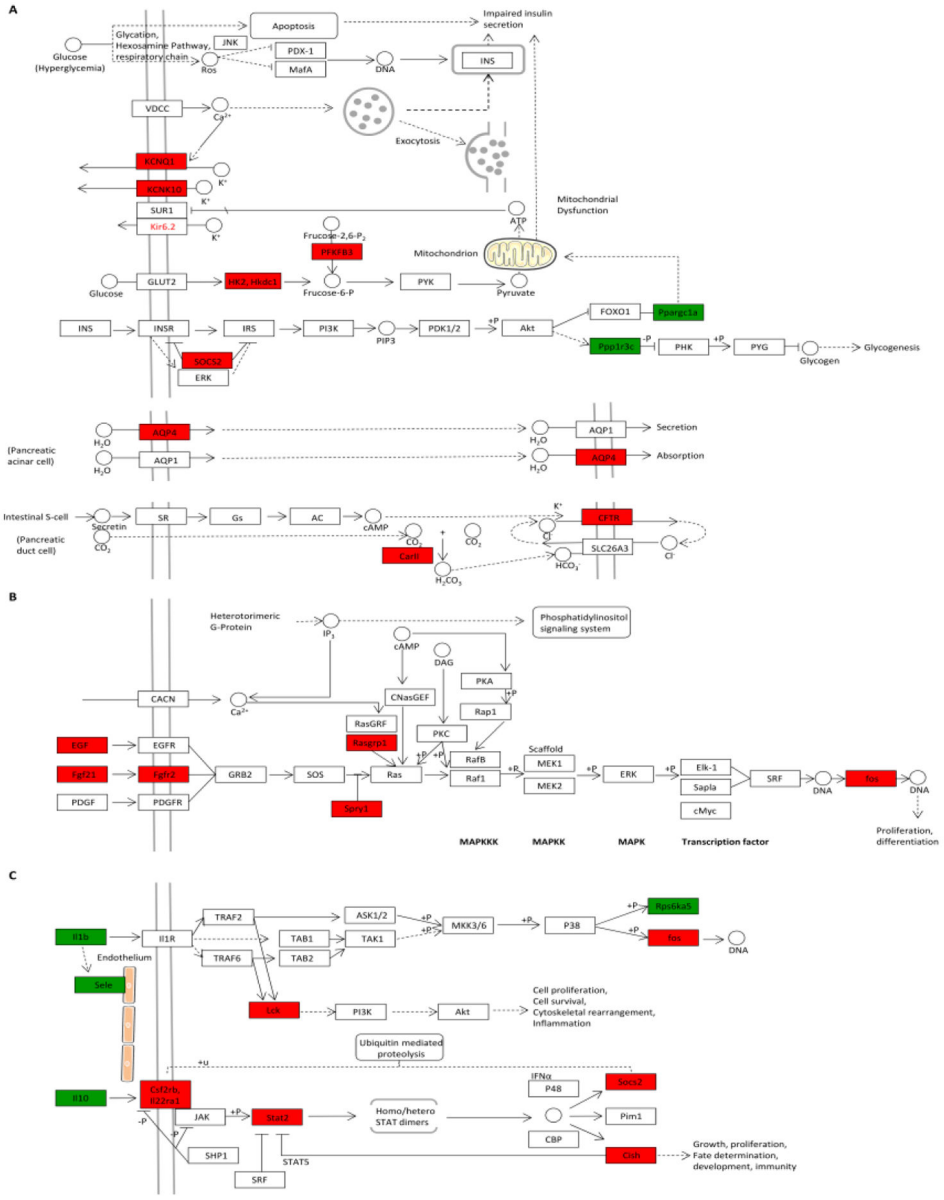

Fig. 4.

Based on GeneCodis3 analysis of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways, 23 significantly enriched pathways were revealed. We combined these pathways and summarized them into three biologically relevant pathways: **(A)** insulin secretion, **(B)** stress/growth factor signaling, and **(C)** inflammatory pathway. Red color represents up-regulated genes and green color represents down-regulated genes.

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Table 1

Single-gene Analysis

The table highlights 52 significant DEGs which involve inflammatory response and stress.*

Gene Symbol	Full Name	RefSeq ID	Fold Change	FDR-adjusted p-value	Annotation
Wfdc2	WAP four-disulfide core domain 2	NM_026323	11.09	4.24E-02	Inflammation
Lcn2	lipocalin 2	NM_008491	10.04	4.28E-03	Inflammation
Mmp7	matrix metallopeptidase 7	NM_010810	7.80	1.02E-02	Inflammation
Gbp11	predicted gene, EG634650; guanylate-binding protein RIKEN cDNA 5830443L24 gene 11;	NM_001039647	7.17	4.28E-03	Inflammation
Cxcl5	similar to LPS-induced CXC chemokine; chemokine (C-X-C motif) ligand 5	NM_009141	6.16	4.28E-03	Inflammation
Fgf21	fibroblast growth factor 21	NM_020013	4.72	4.28E-03	Stress
Gbp10	predicted gene, EG634650; guanylate-binding protein 10;RIKEN cDNA 5830443L24 gene	NM_001039646	4.69	4.28E-03	Inflammation
Cxcl10	chemokine (C-X-C motif) ligand 10; similar to Small inducible cytokine B10 precursor (CXCL10) (Interferon- gamma-induced protein CRG-2) (Gamma-IP10) (IP-10) (C7)	NM_021274	4.51	4.28E-03	Inflammation
Gbp4	predicted gene, EG634650; guanylate-binding protein 4	NM_001256005	4.34	4.28E-03	Inflammation
Sftpd	surfactant associated protein D	NM_009160	4.11	4.28E-03	Inflammation
Gbp9	predicted gene, EG634650; guanylate-binding protein 9, cDNA sequence BC057170	NM_172777	4.10	4.28E-03	Inflammation
Sostdc1	sclerostin domain containing 1	NM_025312	3.86	4.28E-03	Stress
Gbp8	predicted gene, EG634650; guanylate-binding protein 10; RIKEN cDNA 5830443L24 gene	NM_029509	3.80	4.28E-03	Inflammation
Gsta4	glutathione S-transferase, alpha 4	NM_010357	3.67	1.77E-02	Stress
Matn2	matrilin 2	NM_016762	3.44	4.28E-03	Inflammation
Col5a2	collagen, type V, alpha 2	NM_007737	3.43	4.28E-03	Stress
Noxa1	NADPH oxidase activator 1	NM_001163626	3.41	4.24E-02	Stress
Gbp6	IFI16	NM_194336	3.39	4.28E-03	Inflammation
Nr0b2	nuclear receptor subfamily 0, group B, member 2 (SHP)	NM_011850	3.23	4.28E-03	Stress
Ngfr	nerve growth factor receptor (TNFR superfamily, member 16)	NM_033217	3.15	4.28E-03	Stress
Reg3a	regenerating islet-derived 3 alpha	NM_011259	3.13	4.28E-03	Inflammation
Klk1b8	kallikrein 1-related peptidase b8	NM_008457	3.12	1.54E-02	Inflammation
Atf3	activating transcription factor 3	NM_007498	3.07	7.26E-03	Inflammation
Gfra3	glial cell line derived neurotrophic factor family receptor alpha 3	NM_010280	3.01	4.28E-03	Inflammation
Zfp36	zinc finger protein 36	NM_011756	2.96	4.28E-03	Inflammation
Cish	cytokine inducible SH2-containing protein	NM_009895	2.90	4.28E-03	Inflammation
Csf2rb	colony stimulating factor 2 receptor, beta, low-affinity (granulocyte-macrophage)	NM_007780	2.89	4.28E-03	Inflammation

Gene Symbol	Full Name	RefSeq ID	Fold Change	FDR-adjusted p-value	Annotation
Il1rn	interleukin 1 receptor antagonist	NM_001039701	2.87	4.28E-03	Inflammation
Ifit3	interferon-induced protein with tetratricopeptide repeats 3	NM_010501	2.77	4.28E-03	Inflammation
Lck	lymphocyte protein tyrosine kinase	NM_001162433	2.74	3.85E-02	Inflammation
Gbp5	guanylate binding protein 5	NM_153564	2.58	4.28E-03	Inflammation
Ifit1	interferon-induced protein with tetratricopeptide repeats 1	NM_008331	2.58	4.28E-03	Inflammation
Nr5a2	nuclear receptor subfamily 5, group A, member 2	NM_030676	2.45	4.28E-03	Inflammation
Il22ra1	interleukin 22 receptor, alpha 1	NM_178257	2.40	4.28E-03	Inflammation
Crispld2	cysteine-rich secretory protein LCCL domain containing 2	NM_030209	2.38	4.28E-03	Inflammation
Tnfrsf11b	tumor necrosis factor receptor superfamily, member 11b (osteoprotegerin)	NM_008764	2.37	4.28E-03	Inflammation
Klk1b11	kallikrein 1-related peptidase b11	NM_010640	2.37	7.26E-03	Stress
Iigp1	interferon inducible GTPase 1; interferon-inducible GTPase- like	NM_001146275	2.36	4.28E-03	Inflammation
Rsad2	radical S-adenosyl methionine domain containing 2	NM_021384	2.34	4.28E-03	Inflammation
Tnip3	TNFAIP3 interacting protein 3	NM_001001495	2.32	2.90E-02	Inflammation
C4b	complement component 4B (Childo blood group)	NM_009780	2.25	4.28E-03	Inflammation
C3	complement component 3; similar to complement component C3 prepropeptide, last	NM_009778	2.18	4.28E-03	Inflammation
C2cd4a	family with sequence similarity 148, member A	NM_001163143	2.17	4.28E-03	Inflammation
Xaf1	XIAP associated factor 1	NM_001037713	2.16	4.38E-02	Inflammation
Anxa1	annexin A1	NM_010730	2.14	2.90E-02	Inflammation
Sntb1	syntrophin, basic 1	NM_016667	2.13	7.26E-03	Inflammation
Chac1	ChaC, cation transport regulator-like 1 (E. coli)	NM_026929	2.13	1.54E-02	Stress
Oasl2	2'-5' oligoadenylate synthetase-like 2	NM_011854	2.08	7.26E-03	Inflammation
Fgfr2	fibroblast growth factor receptor 2	NM_010207	2.07	4.85E-02	Inflammation
Parp14	poly (ADP-ribose) polymerase family, member 14	NM_001039530	2.05	4.28E-03	Inflammation
Tap1	transporter 1, ATP-binding cassette, sub-family B (MDR/TAP)	NM_001161730	2.05	4.28E-03	Inflammation
Socs2	suppressor of cytokine signaling 2; predicted gene 8000	NM_007706	2.03	4.28E-03	Inflammation
Sgk1	serum/glucocorticoid regulated kinase 1	NM_011361	2.03	4.28E-03	Stress
F13a1	coagulation factor XIII, A1 subunit	NM_001166391	0.16	4.28E-03	Inflammation
Dapl1	death associated protein-like 1	NM_029723	0.32	4.28E-03	Stress
Mlf1	myeloid leukemia factor 1	NM_001039543	0.34	4.28E-03	Inflammation
Lyve1	lymphatic vessel endothelial hyaluronan receptor 1	NM_053247	0.36	4.28E-03	Inflammation
Ccdc8	coiled-coil domain containing 8	NM_001101535	0.42	2.57E-02	Stress

Gene Symbol	Full Name	RefSeq ID	Fold Change	FDR-adjusted p-value	Annotation
Gimap6	GTPase, IMAP family member 6	NM_153175	0.42	4.28E-03	Inflammation
Il10	interleukin 10	NM_010548	0.43	4.85E-02	Inflammation
Capsl	calcyphosine-like	NM_029341	0.45	4.28E-03	Inflammation
Prdm1	PR domain containing 1, with ZNF domain	NM_007548	0.46	1.77E-02	Inflammation
Sele	selectin, endothelial cell	NM_011345	0.46	3.85E-02	Inflammation
Il1b	interleukin 1 beta	NM_008361	0.48	4.28E-03	Inflammation
Mum1l1	melanoma associated antigen (mutated) 1-like 1	NM_001164631	0.50	4.28E-03	Inflammation
Defb1	defensin beta 1	NM_007843	0.50	4.28E-03	Inflammation

*Abbreviations: DEG, differentially expressed genes; FDR, false discovery rate.

Table 2

Single-gene Analysis

The table highlights 36 significantly DEGs which involve ion channel, glucose metabolism, cAMP-PKA signaling, membrane polarization, and exocytosis.

Gene Symbol	Full Name	RefSeq ID	Fold Change	FDR- adjusted p-value	Annotation
Sntg2	syntrophin, gamma 2	NM_172951	9.10	4.28E-03	Ion Channel
Duox2	dual oxidase 2	NM_177610	8.29	4.28E-03	Metabolism
Hs3st1	heparan sulfate (glucosamine) 3-O-sulfotransferase 1	NM_010474	6.28	4.28E-03	Metabolism
Syt10	synaptotagmin X	NM_018803	6.13	4.28E-03	Exocytosis
Car2	carbonic anhydrase 2	NM_009801	5.92	4.28E-03	Glucose metabolism
Cftr	cystic fibrosis transmembrane conductance regulator homolog	NM_021050	5.42	4.28E-03	Ion Channel
Fmo1	flavin containing monooxygenase 1	NM_010231	5.41	4.28E-03	Metabolism
Rasgrp1	RAS guanyl releasing protein 1	NM_011246	5.28	4.28E-03	Ras-related protein/ GTPase
Gpr26	G protein-coupled receptor 26	NM_173410	4.87	1.54E-02	cAMP-PKA signaling
Kcnj5	potassium inwardly-rectifying channel, subfamily J, member 5	NM_010605	4.62	4.28E-03	Membrane polarization
Rph3a	rabphilin 3A	NM_011286	4.48	4.28E-03	Exocytosis
Hcn1	hyperpolarization-activated, cyclic nucleotide-gated K+ 1	NM_010408	4.12	4.28E-03	Ion Channel
Slc39a4	solute carrier family 39 (zinc transporter), member 4	NM_028064	4.12	3.08E-02	Metabolism
Rasl11b	RAS-like, family 11, member B	NM_026878	3.86	3.21E-02	Metabolism
Grem2	gremlin 2 homolog, cysteine knot superfamily (Xenopus laevis)	NM_011825	3.72	4.28E-03	Metabolism
Lrrc55	leucine rich repeat containing 55	NM_001033346	3.46	4.28E-03	Membrane polarization
Tph2	tryptophan hydroxylase 2	NM_173391	3.45	4.28E-03	Metabolism
Kcnk10	IFI16	NM_029911	3.27	4.28E-03	Membrane polarization
Hk2	hexokinase 2	NM_013820	3.21	4.28E-03	Glucose metabolism
Tdh	L-threonine dehydrogenase; predicted gene 13929	NM_021480	2.91	4.28E-03	Metabolism
Tph1	tryptophan hydroxylase 1	NM_009414	2.87	4.28E-03	Metabolism
Kctd14	potassium channel tetramerisation domain containing 14	NM_001010826	2.81	4.28E-03	cAMP-PKA signaling
Klf15	Kruppel-like factor 15	NM_023184	2.73	4.28E-03	Metabolism
Gpr126	G protein-coupled receptor 126	NM_001002268	2.67	4.28E-03	cAMP-PKA signaling
Enpp2	ectonucleotide pyrophosphatase/phosphodiesterase 2	NM_001136077	2.65	4.28E-03	cAMP-PKA signaling
Rerg	RAS-like, estrogen-regulated, growth-inhibitor	NM_181988	2.61	4.28E-03	Ras-related protein/ GTPase

Gene Symbol	Full Name	RefSeq ID	Fold Change	FDR- adjusted p-value	Annotation
Pde7b	phosphodiesterase 7B	NM_013875	2.60	4.28E-03	cAMP-PKA signaling
Fam13a	family with sequence similarity 13, member A	NM_153574	2.59	7.26E-03	Ras-related protein/ GTPase
Cckar	cholecystokinin A receptor	NM_009827	2.55	3.98E-02	cAMP-PKA signaling
Pfkfb3	6-phosphofructo-2-kinase/fructose-2,6-biphosphatase 3	NM_001177752.1	2.54	4.28E-03	Glucose metabolism
UGT1A1	UDP glucuronosyltransferase 1 family, polypeptide A1	NM_201645	2.48	4.28E-03	Metabolism
Chst2	carbohydrate sulfotransferase 2	NM_018763	2.36	1.54E-02	Metabolism
Tmc5	transmembrane channel-like gene family 5	NM_028930	2.29	4.28E-03	Ion Channel
Adm	adrenomedullin	NM_009627	2.27	3.98E-02	Secreted insulinotropic factor
Cachd1	cache domain containing 1; similar to Cache domain containing 1	NM_198037	2.20	7.26E-03	Ion Channel
Gnb1l	guanine nucleotide binding protein (G protein), beta polypeptide 1- like	NM_023120	2.18	2.22E-02	Ras-related protein/ GTPase
B3galt1	UDP-Gal:betaGlcNAc beta 1,3-galactosyltransferase, polypeptide 1	NM_020283	2.18	2.78E-02	Metabolism
Kcnq1	potassium voltage-gated channel, subfamily Q, member 1; similar to Potassium voltage-gated channel, subfamily Q, member 1	NM_008434	2.17	4.28E-03	Membrane polarization
Bmp7	bone morphogenetic protein 7	NM_007557	2.15	4.28E-03	Secreted insulinotropic factor
Hkdc1	hexokinase domain containing 1	NM_145419	2.11	2.90E-02	Glucose metabolism
Dhrs3	dehydrogenase/reductase (SDR family) member 3	NM_011303	2.07	7.26E-03	Metabolism
Ptger3	prostaglandin E receptor 3 (subtype EP3)	NM_011196	2.07	1.54E-02	cAMP-PKA signaling
Clic6	chloride intracellular channel 6	NM_172469	2.06	4.53E-02	Ion Channel
Rasd2	RASD family, member 2	NM_029182	2.21	4.28E-03	Ras-related protein/ GTPase
Ppargc1a	peroxisome proliferative activated receptor, gamma, coactivator 1 alpha	NM_008904	0.28	4.28E-03	Glucose metabolism
Trpc4	transient receptor potential cation channel, subfamily C, member 4	NM_001253682	0.31	4.28E-03	Ion Channel
Phactr3	phosphatase and actin regulator 3 (Scapinin)	NM_001007154	0.38	1.77E-02	Glucose metabolism
Rasgrf2	RAS protein-specific guanine nucleotide-releasing factor 2	NM_009027	0.38	4.28E-03	Ras-related protein/ GTPase
Rgs7bp	regulator of G-protein signalling 7 binding protein	NM_029879	0.41	4.28E-03	Ras-related protein/ GTPase
Ppp1r3c	protein phosphatase 1, regulatory (inhibitor) subunit 3C	NM_016854	0.42	3.42E-02	Metabolism
Ust	uronyl-2-sulfotransferase	NM_177387	0.43	1.28E-02	Metabolism
Vsnl1	visinin-like 1	NM_012038	0.43	1.77E-02	Ion Channel
Dse	dermatan sulfate epimerase	NM_172508	0.45	7.26E-03	Metabolism
Gpr161	G protein-coupled receptor 161	NM_001081126	0.46	3.42E-02	cAMP-PKA signaling
Kctd12	potassium channel tetramerisation domain containing 12	NM_177715	0.47	4.28E-03	cAMP-PKA signaling
Hapln1	hyaluronan and proteoglycan link protein 1	NM_013500	0.49	1.28E-02	Metabolism

Table 3

Significantly Enriched KEGG Pathways.*

Pathway	Pathway ID	P Value	# Genes
Cytokine-cytokine receptor interaction	Kegg:04060	5.78E-06	11
Jak-STAT signaling pathway	Kegg:04630	2.68E-04	7
Tryptophan metabolism	Kegg:00380	4.48E-04	4
Butirosin and neomycin biosynthesis	Kegg:00524	6.47E-04	2
Glycosphingolipid biosynthesis - lacto and neolacto series	Kegg:00601	1.07E-03	3
Cell adhesion molecules (CAMs)	Kegg:04514	1.15E-03	6
Leishmaniasis	Kegg:05140	1.85E-03	4
African trypanosomiasis	Kegg:05143	2.01E-03	3
Gastric acid secretion	Kegg:04971	2.57E-03	4
Osteoclast differentiation	Kegg:04380	2.63E-03	5
Bile secretion	Kegg:04976	2.70E-03	4
Complement and coagulation cascades	Kegg:04610	3.46E-03	4
Fructose and mannose metabolism	Kegg:00051	3.63E-03	3
Insulin signaling pathway	Kegg:04910	5.02E-03	5
Hepatitis C	Kegg:05160	5.18E-03	5
Malaria	Kegg:05144	5.49E-03	3
MAPK signaling pathway	Kegg:04010	5.96E-03	7
Starch and sucrose metabolism	Kegg:00500	6.22E-03	3
Glycosphingolipid biosynthesis - globo series	Kegg:00603	6.44E-03	2
Glycosaminoglycan biosynthesis - keratan sulfate	Kegg:00533	6.44E-03	2
Type II diabetes mellitus	Kegg:04930	7.84E-03	3
Staphylococcus aureus infection	Kegg:05150	7.84E-03	3
Pancreatic secretion	Kegg:04972	9.40E-03	4

*Abbreviations: KEGG, Kyoto Encyclopedia of Genes and Genomes.

Table 4

GO Analysis*

A total of 43, 17, and 23 BP, CC and MF categories were detected significantly enriched (FDR<0.05) by GeneTrail and GeneCoDis3. Among them, representative GO categories include (i) BP: “inflammatory response”, “ion transport” and “insluin secretion” (ii) CC: “extracellular region”, “extracellular space” and “integral to membrane”, and (iii) MF: “GTPase activity”, “calcium ion binding”, and “GTP binding”, respectively.

GO Category	GO ID	GO Name	GeneTrail # Genes	GeneTrail FDR	GeneCoDis3 # Genes	GeneCoDis3 FDR
BP	GO:0006950	response to stress	35	2.09E-05	5	3.92E-02
BP	GO:0005975	carbohydrate metabolic process	16	4.50E-04	6	0.0380599
BP	GO:0030073	insulin secretion	7	7.59E-04	4	1.04E-02
BP	GO:0006954	inflammatory response	12	8.04E-04	10	5.56E-04
BP	GO:0006811	ion transport	21	8.98E-04	15	4.80E-03
BP	GO:0040008	regulation of growth	11	4.32E-03	4	2.82E-02
BP	GO:0007155	cell adhesion	16	5.15E-03	11	2.65E-02
BP	GO:0009968	negative regulation of signal transduction	5	1.06E-02	4	1.88E-02
BP	GO:0006915	apoptosis	18	2.33E-02	12	2.28E-02
BP	GO:0032870	cellular response to hormone stimulus	4	0.0467718	2	0.0285125
CC	GO:0016021	integral to membrane	81	1.62E-03	79	2.19E-06
CC	GO:0008076	voltage-gated potassium channel complex	4	1.65E-02	4	1.72E-02
CC	GO:0005576	extracellular region	51	2.81E-10	44	1.75E-12
CC	GO:0005887	integral to plasma membrane	11	4.91E-02	13	1.53E-03
MF	GO:0003924	GTPase activity	8	1.10E-03	8	4.48E-03
MF	GO:0005249	voltage-gated potassium channel activity	5	1.13E-02	4	4.13E-02
MF	GO:0005215	transporter activity	24	1.15E-03	7	2.24E-02
MF	GO:0005216	ion channel activity	10	1.44E-02	9	2.26E-02
MF	GO:0005509	calcium ion binding	13	6.47E-03	17	2.04E-04
MF	GO:0004396	hexokinase activity	2	9.44E+01	2	2.14E-02
MF	GO:0005525	GTP binding	10	9.44E-03	10	1.69E-02
MF	GO:0005267	potassium channel activity	6	9.64E-03	4	4.44E-02

*Abbreviations: BP, biological process; CC, cellular component; FDR, false discovery rate; GO, gene ontology; MF, molecular function. Selection criteria: # genes in GO category ≥2 and FDR < 0.05 for both programs. GO terms are first sorted by “GO Category” in ascending order, then by “GeneTrail FDR” in ascending order, and then by “GeneCoDis3 FDR” in ascending order.