Identification and Characterization of The Gene Profile in Diabetes Induced Erectile Dysfunction Rat Model

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Abstract

Background: Genes are differentially expressed in diabetes induced erectile dysfunction (DED); however, there is still a lack of systematic and in-depth understanding of the cellular and molecular mechanisms of DED. This study aimed to systematically analyze molecular correlates leading to ED in diabetes by bioinformatics analysis, and provide new insights into the mechanism of DED.

Results: A total of 800 differentially expressed genes (DEGs, 407 upregulated and 393 downregulated) were identified in the diabetic-induced ED samples compared with the control group. The upregulated DEGs were mainly enriched in glucose and lipid metabolism-related pathways, and the downregulated DEGs were primarily enriched in tissue development and structure. The dysregulated extracellular matrix genes (especially related to collagen and elastin) may be closely related to the damage of erectile function of the corpus cavernosum. Sixteen hub genes (6 upregulated, 10 downregulated) and 24 modules were detected by hub gene and MCODE analysis. Protein phosphorylation might play an important role in DED. Furthermore, 20 TFs targeting DEGs were identified by ChEA3.

Conclusions: Our research comprehensively and systematically described the molecular characteristics of DED, and suggested that dysregulated extracellular matrix genes and protein phosphorylation may play critical roles in DED.

Background

Diabetes mellitus is a chronic, non-communicable disease accompanied by complications of multiple organs and systems [1]. In recent decades, the global incidence of adult diabetes has been increasing, and the growth rate has been accelerating. By 2040, diabetic patients aged 20–79 years were expected to increase to 642 million [2]. Diabetes will bring a series of health problems, including sexual dysfunction [3]. In male diabetic patients, sexual dysfunction is mainly manifested as erectile dysfunction (ED). The global prevalence of ED in male diabetes patients overall is 52.5% (Type I Diabetes: 37.5%, Type II Diabetes: 66.3%) [4]. In China, the prevalence of ED in men with type 2 diabetes is also as high as 64.2% [5].

The etiology of diabetic erectile dysfunction (DED) is extremely complicated and may be related to endothelial dysfunction, smooth muscle injury, vascular disease, neuropathy, abnormal hormone levels, inflammation, cavernous fibrosis [6]. For endocrinologists or urologists, DED has always been a huge challenge. Oral phosphodiesterase type-5 inhibitors (PDE5i) are the first-line treatment for ED, including DED. However, DED is often resistant to PDE5i treatment [7]. Intracavernous administration of vasoactive drugs, commonly used as a second-line medical treatment when PDE5i have failed, is often given up due to unavoidable complications, including penile pain, hydrophallus, priapism, fibrosis [7]. Eventually, the patients have to choose penile prosthesis implantation. Therefore, although medicine has made great progress in recent decades, DED is still difficult to treat medically [8]. In addition, research on the mechanism of DED is not uncommon in recent years [9–12], but there is a lack of systematic and profound understanding of the cellular and molecular mechanisms of DED.

Here, we systematically analyzed molecular correlates leading to ED in diabetes by bioinformatics analysis. The differentially expressed genes (DEGs) were identified in diabetes-induced ED rat model. We analyzed the function and interaction pathways of DEGs, and performed hub gene and module analysis. The conserved motifs of the promoter regions and their post-translational phosphorylation modification were studied. In addition, we looked for transcription factors (TFs) that might target DEGs. We present our findings in accordance with the STROBE reporting checklists.

Results

Identification and enrichment analysis of DEGs

Array analysis was based on > 1.5 (or < 0.67) fold change in expression and $P < 0.05$. According to these criteria, a total of 800 DEGs (407 upregulated and 393 downregulated) were detected (**Table S1**).

Then, we used Metascape to perform annotation and enrichment analysis of these genes. **Figure 1** shows the Metascape enrichment analysis results as a clustered heatmap. In the top 100 enriched clusters (**Figure S1**), the clusters of upregulated genes and downregulated genes were mostly different, and only a small part of the results overlapped. When the results were limited to top 20 clusters, the overlap probability of enrichment results seemed to increase. However, the main functions of upregulated genes and downregulated genes were significantly different. The upregulated DEGs were enriched in glucose and lipid metabolism-related pathways: ‘fatty acid oxidation’, ‘monocarboxylic acid metabolic process’, ‘negative regulation of fatty acid metabolic process’ and ‘AMPK signaling pathway’. The downregulated DEGs were primarily enriched in tissue development and structure: ‘extracellular matrix organization’, ‘muscle contraction’, and ‘skeletal system development’. **Tables 1** showed the top 20 clusters with their representative enriched terms, the number of genes enriched in each term, and the percentage of genes that are found in the given ontology term.

We further built an enrichment network to demonstrate the relationships between the top 20 terms. In the network, each node represented an enriched cluster. The node was colored first by its cluster ID (**Figure 2A**) and then by its p-value (**Figure 2B**). From the network, we found that there were mainly 5 term groups formed: Group 1 - ‘extracellular matrix organization’ and ‘skeletal system development’; Group 2 - ‘response to peptide’, ‘cellular response to hormone stimulus’, and ‘response to fatty acid’; Group 3 - ‘monocarboxylic acid metabolic process’, ‘purine ribonucleotide metabolic process’, ‘negative regulation of fatty acid metabolic process’, and ‘fatty acid oxidation’; Group 4 - ‘regulation of protein secretion’; Group 5 - ‘regulation of ion transport’ and ‘muscle contraction’. In **Figure 2C**, network of enriched terms represented as pie charts, and the color of the pie charts represented the identities of the gene lists (upregulated or downregulated). It could be seen from this network that groups 1 and 5 (related to tissue development and structure) mainly represented downregulated DEGs pathways, and groups 3 and 4 (related to metabolism pathways) were mainly derived from upregulated DEGs. This was consistent with the results reflected in the clustered heatmap (**Figure 1**).

PPI network, hub gene and module analysis

STRING and Cytoscape tool were applied to detect the interactions of the DEGs and construct the PPI network (**Figure 3A**). Considering the large number of DEGs, we set the minimum required interaction score to 0.7 to avoid the network being too bloated. In the PPI network, the edge color reflected the combined score of the DEGs.

Then, the top 20 hub genes were detected by cytoHubba plug-in used 12 different algorithms (**Table 2**). Finally, 16 hub genes, detected by more than six algorithms, were selected to build the hub gene PPI network (**Figure 3B**). Most of the hub genes had a higher degree of connectivity. These genes were enriched in 'post-translational protein phosphorylation' and tissue development – related terms (for example, 'tissue remodeling', 'extracellular matrix organization', 'skeletal system development') (**Figure 3C**).

MCODE plug-in was used to identify significant modules in the DEGs PPI network, and a total of 24 modules were detected. We selected the top 5 modules to perform analysis using Metascape. Interestingly, we found that all 14 genes in module 1 belong to hub genes. Not surprisingly, module 1 also enriched in 'post-translational protein phosphorylation' and tissue development – related terms (**Figure 4A**). It was worth noting that the same 11 genes were enriched in 'post-translational protein phosphorylation' term: *Fstl3*, *C3*, *Apob*, *Men1*, *Fam20c*, *Mepe*, *Fbn1*, *Igfbp5*, *Tnc*, *Msln*, *Timp1*. Thirteen genes belonging to module 2 were enriched in 'protein digestion and absorption' and 'collagen metabolic remodeling' terms (**Figure 4B**). Module 3 (11 genes) enriched in protein ubiquitination process (**Figure 4C**). Genes belonging to module 4 regulated signaling pathways involving G proteins (**Figure 4D, S2**). Module 5 (9 genes) mainly enriched in 'glutathione metabolism' term (**Figure 4E, S3**).

Identification of conserved motifs in promoter site of genes and prediction of the phosphorylation sites of protein

Through hub gene and module analysis, we found that the same 11 genes were enriched in 'post-translational protein phosphorylation' pathway. We speculated that this pathway may play an important role in DED, and these genes may have similarities in regulatory patterns. Therefore, we first used GLAM2 tool to identify common conserved motifs in the upstream of these genes. The consensus sequence AAA (G/C) AAA was observed in the promoter site of most studied genes (**Figure 5**). Then we explored whether the proteins transcribed by these genes have abundant phosphorylation sites. According to the prediction results of NetPhos 3.1 Server, most genes, especially *Tnc*, *Fbn1*, *C3* and *Apob*, have abundant phosphorylation sites (**Figure 6**).

Prediction of TFs associated with DEGs

TFs are important regulators of gene expression. Through the ChEA3 tool, we found 20 TFs that target upregulated genes or downregulated genes (**Figures 7A, 8A**). The results showed that TFs related to upregulated genes were mainly concentrated in 'adipose tissue' (**Figure 7B**), 'thyroid hormone generation', and 'positive regulation of wound healing' (**Figure 7C**). And TFs related to downregulated genes were mainly concentrated in 'muscle' (**Figure 8B**) and 'regulation of skeletal muscle tissue development' (**Figure 8C**).

Discussion

Penile erection, a complex neurovascular event, is the result of the joint action of nerves, blood vessels, and smooth muscle [13]. The main pathophysiologies of ED are vascular insufficiency and neuropathy. Since diabetes, a metabolic disorder characterized by hyperglycemia, can cause neurological and vascular disease, men with diabetes are at risk of ED. In fact, 52.5% of patients suffer from ED after the diagnosis of diabetes. Type 2 diabetes (T2DM) accounts for 90%~95% [14] of diabetes cases, and the incidence of ED in male patients with type 2 diabetes exceeds 60%. Unfortunately, the existing treatments for ED are not very effective for DED. Considering the huge number of diabetic patients, DED places a huge burden on individuals, families, and society. The difficulty of DED treatment is that the pathogenesis is complicated and unclear. Hence, identifying underlying cellular and molecular mechanisms in DED is important for improving diagnostic and therapeutic strategy.

Although previous studies [15–17] have reported changes in the gene expression profile of DED cavernous tissues, the analysis methods of these studies are relatively simple. In this study, we systematically analyzed molecular correlates leading to DED by bioinformatics analysis. Through the GEO2R tool, 800 DGEs were identified. Changes in the expression of a large number of genes also indicate the complexity of the cause of DED. To further explore the relationships between them, we performed enrichment analysis, built a PPI network, did hub gene and module analysis, identified conserved motifs in promoter site, and predicted the phosphorylation sites. Finally, we predicted TFs associated with DEGs.

The results demonstrated that upregulated genes were mainly involved in lipid metabolism-related pathways. This was consistent with the fact that DED patients suffer from diabetes. For example, Mitochondrial 3-hydroxy-3-methylglutaryl-CoA synthase (*Hmgcs2*), which catalyzes the first reaction of ketogenesis [18], was the one with the highest fold change (31.40) in upregulated DEGs. Studies have shown that *Hmgcs2* was closely related to diabetes [19, 20], and inhibiting *Hmgcs2* could alleviate DED [17]. Ceruloplasmin (*Cp*) increased in diabetes [15, 21], and inhibited the activation of endothelial nitric oxide synthase [22]. The downregulated DEGs were primarily enriched in tissue development and structure: 'extracellular matrix organization', 'muscle contraction', and 'skeletal system development'. 'Extracellular matrix organization' was the most statistically significant term for enrichment analysis, and 24 downregulated genes were enriched in this term. Collagen, elastin, and proteoglycan constitute the extracellular matrix of human and rat erectile tissues. The important pathological changes of ED are the apoptosis of cavernous smooth muscle cells, the change of the ratio of smooth muscle to collagen, and the atrophy of endothelial cells. Therefore, changes in the composition and structure of the extracellular matrix caused by diabetes may impair erectile function. We found that the downregulated genes included collagens type 1, 2, 3, 5, 6, 11, 14, 15. Collagen types 1, 2, 3, 5, and 11 were the fibril forming collagen [23]. Among them, collagen type 1 is the most abundant form of collagen in erectile tissues of humans and rats, and constitutes the framework of the cavernous matrix structure. Collagen 1 is usually formed by two identical $\alpha 1(I)$ -chains (*Col1a1*) and one $\alpha 2(I)$ -chain (*Col1a2*), and provides tensile stiffness in most organs. In our analysis, both *Col1a1* and *Col1a2* have decreased expression in DED, which would inevitably affect the erectile function of the penis.

Furthermore, genes associated with elastic fibers (Lox [24], Fbn1 [25], and so on) were also decreased. Different from other studies, we also built an enrichment network to demonstrate the relationships between the enriched terms. Terms with similar functions were also gathered: groups 1 and 5 - related to tissue development and structure, groups 3 and 4 - related to metabolism pathways. These all shown close molecular connections in DEGs.

In the PPI network, we found that there were rich interactions between the most DEGs. Sixteen hub genes (6 upregulated, 10 downregulated) were selected by 12 algorithms in cytoHubba plug-in. Interesting, these genes were enriched in 'post-translational protein phosphorylation' and terms regarding tissue development, not lipid metabolism-related pathways.

MCODE can find and extract the closely related regions in the PPI network, and a total of 24 modules were detected. We selected the top 5 modules for further analysis. It is worth noting that the genes in module 1 (14 genes) all belong to hub genes. The results of module 1 enrichment analysis were also similar to that of hub genes. The role of these genes in DED deserves further study. In addition, we found that 'post-translational protein phosphorylation' was the most statistically significant term for enrichment analysis in module 1 and hub genes, and the same 11 genes were enriched in this term. To verify whether these genes have similar regulatory patterns, we used GLAM2 tool to identify common conserved motifs in the upstream of them. In this study, AAA - rich conserved motifs was detected in the promoter site of these genes affecting the expression patterns by activating and suppressing the transcription of studied genes. We also found that the proteins transcribed by these genes have abundant phosphorylation sites. Protein phosphorylation is one of the most common and important post-translational modifications, and it is an important cellular regulatory mechanism [26]. Abnormal protein phosphorylation can be found in many diseases, and the strategy of activating or inhibiting this process to treat diseases is theoretically feasible. There are relatively few studies on protein phosphorylation in the occurrence and progression of ED. Module 2 mainly enriched signatures regarding collagen metabolism. From the perspective of enrichment analysis, module 1 and module 2 represented downregulated genes. It is very interesting that both the enrichment analysis of hub genes and modules lacks terms regarding glucose and lipid metabolism. Perhaps genes related to glucose and lipid metabolism do not play a key role in penile lesions, and just reflected the body's metabolic disorders. Because despite good control of blood glucose level, ED is still getting worse in some DED patients [27].

TFs are a group of proteins that ensure that the target genes are expressed at a specific time and space with a specific intensity. They are important regulators of gene expression. We predicted TFs associated with DEGs used the ChEA3 tool. By analyzing the tissue specificity and function of these TFs, we found that TFs targeting upregulated genes were closely related to glucose and lipid metabolism, and TFs targeting downregulated genes were more related to tissue development. These were consistent with the enrichment results of DEGs. Studies have reported that some of these TFs, (for example, *Foxa1* [28], *ELF3* [29], *Tbx15* [30], *Twist1*, *Twist2* [31]) were involved in diabetes or muscle development. But the role of these TFs in DED has not been studied.

Although we got meaningful results, there are several noteworthy limitations in our study. First, sequencing data comes from rat samples. Although some genes are conserved between species, species differences are a key issue that cannot be ignored. In the future, the findings of this study should be verified in human tissues. Cavernous tissue from men undergoing penile surgery or brain-dead male organ donors is an ideal solution [32]. Second, the valuable genes discovered in this study need to be experimentally verified to explore their mechanism in DED. Third, this study only involves changes in mRNA expression, lack of the data of non-coding RNA (miRNA, lncRNA, and circRNA). Non-coding RNA plays an important role in the occurrence and development of diabetes and ED.[9, 33–35] We can reveal molecular mechanisms of DED more deeply by constructing potential lncRNA-miRNA-mRNA regulatory network.

Conclusions

In this study, we successfully defined molecular signatures in DED. We found that 800 DEGs (407 upregulated and 393 downregulated) in the diabetic-induced ED samples compared with the control group. The upregulated DEGs were enriched in glucose and lipid metabolism-related pathways, and the downregulated DEGs were primarily enriched in tissue development and structure. The dysregulated extracellular matrix genes (especially related to collagen and elastin) may be closely related to the damage of erectile function of the corpus cavernosum. Through hub gene and module analysis, we found some genes that may play a key role in the development of DED, the role of protein phosphorylation in DED needs to be taken seriously.

Methods

Data collection

We used "diabetes" and "erectile dysfunction" to search the gene expression datasets in the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>). Considering ethical principles, it is extremely difficult to obtain penile tissue samples from DED patients. Finally, GSE2457 was selected for the next analysis [15]. In this study, researchers performed microarray experiments on the cavernous tissue of the diabetes-induced ED rat model.

Identification of differentially expressed genes

The GEO2R tool (<https://www.ncbi.nlm.nih.gov/geo/geo2r/>) was used to extract differentially expressed genes (DEGs). $P < 0.05$ and fold change > 1.5 (or < 0.67) were chosen as the cutoff criteria.

Gene annotation and enrichment analysis of DEGs

Metascape [36] (<http://metascape.org>), a web-based tool, can provide automated gene list annotation and enrichment analysis. Enrichment analysis was based on the following ontology sources: gene ontology (GO) processes, KEGG pathways, canonical pathways, Reactome gene sets, PaGenBase and CORUM. In addition, we used this tool to draw the heatmap and network of the enriched terms.

PPI network, hub gene and module analysis

Search Tool for the Retrieval of Interacting Genes [37] (STRING, <https://string-db.org/>) was applied to detect the interactions of the DEGs and construct the PPI network. Cytoscape software [38] was used to optimize the PPI network. Two Cytoscape software plug-ins, cytoHubba and Molecular Complex Detection (MCODE), were used to identify hub genes and screen modules of the PPI network.

Identification of conserved motifs in promoter site

Ensembl [39] (<https://ensembl.org/>) was used to obtain the promoter site (2000 bp upstream of transcription start site). Gapped Local Alignment of Motifs [40] (GLAM2) was used to discover common conserved motifs in the gene of interest.

Prediction of the phosphorylation sites

The protein sequence was retrieved from the Protein (<https://www.ncbi.nlm.nih.gov/protein>). NetPhos 3.1 Server [41] (<http://www.cbs.dtu.dk/services/NetPhos/>) was used to predict serine, threonine and tyrosine phosphorylation sites in selected proteins.

Prediction of TFs associated with DEGs

ChEA3 (<https://maayanlab.cloud/chea3/>) is an online tool that can be used for transcription factor enrichment analysis by orthogonal omics integration [42]. We used this tool to find TFs targeting DEGs and annotate these TFs.

Statistical analysis

All the statistical analyses were conducted with SPSS version 20.0 (SPSS Inc., Chicago, IL, USA) and GraphPad Prism version 8.0 (GraphPad Software, San Diego, CA, USA). $P < 0.05$ indicated statistical significance.

Abbreviations

DED diabetes induced erectile dysfunction

DEGs differentially expressed genes

PPI protein–protein interaction

TFs transcription factors

ED erectile dysfunction

PDE5i phosphodiesterase type-5 inhibitors

GEO Gene Expression Omnibus

GO gene ontology

T2DM Type 2 diabetes

Declarations

Ethics approval and consent to participate: Not applicable.

Consent for publication: Not applicable.

Availability of data and material: The data can be found in the Gene Expression Omnibus (GEO) database (<https://www.ncbi.nlm.nih.gov/geo/>, GSE2457).

Competing interests: The authors declare that they have no competing interests.

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Tables

Table 1 Top 20 clusters with their representative enriched terms

Gene list	Terms	Category	Description	Count	%	Log10(P)	Log10(q)
R-RNO-1474244	Reactome Gene Sets	Extracellular matrix organization	24	6.78	-11.15	-6.90
GO:0032787	GO Biological Processes	monocarboxylic acid metabolic process	61	8.43	-10.44	-6.16
R-RNO-397014	Reactome Gene Sets	Muscle contraction	20	5.65	-10.23	-6.90
GO:1901652	GO Biological Processes	response to peptide	63	8.70	-9.72	-5.74
GO:0043269	GO Biological Processes	regulation of ion transport	68	9.39	-9.36	-5.74
GO:0019395	GO Biological Processes	fatty acid oxidation	16	4.31	-9.27	-6.06
GO:0048871	GO Biological Processes	multicellular organismal homeostasis	53	7.32	-9.25	-5.74
GO:0009611	GO Biological Processes	response to wounding	61	8.43	-9.05	-5.73
GO:0050708	GO Biological Processes	regulation of protein secretion	28	7.55	-7.56	-4.84
GO:0032870	GO Biological Processes	cellular response to hormone stimulus	64	8.84	-7.51	-4.65
GO:0009150	GO Biological Processes	purine ribonucleotide metabolic process	45	6.22	-7.47	-4.63
GO:0002931	GO Biological Processes	response to ischemia	15	2.07	-7.31	-4.52
ko04152	KEGG Pathway	AMPK signaling pathway	20	2.76	-7.02	-4.33
GO:0001501	GO Biological Processes	skeletal system development	30	8.47	-6.70	-4.12
GO:0045922	GO Biological Processes	negative regulation of fatty acid metabolic process	8	2.16	-6.63	-4.10
GO:0010038	GO Biological Processes	response to metal ion	45	6.22	-6.34	-3.84
GO:0070848	GO Biological Processes	response to growth factor	60	8.29	-5.97	-3.54
GO:0032526	GO Biological Processes	response to retinoic acid	14	3.95	-5.92	-3.54
GO:0070542	GO Biological Processes	response to fatty acid	19	2.62	-5.87	-3.47
GO:0001659	GO Biological Processes	temperature homeostasis	23	3.18	-5.78	-3.39

Abbreviations: upregulated genes; downregulated genes; GO, gene ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; AMPK, Adenosine 5'-monophosphate-activated protein kinase

Table 2 Top 20 hub genes detected using the 12 algorithms in cytoHubba plug-in

Rank	algorithms											
	MCC	DNMC	MNC	Degree	EPC	BottleNeck	EcCentricity	Closeness	Radiality	Betweenness	Stress	
1	C3	Fam20c	Apob	Fn1	Col1a1	Fn1	Fbxo32	Fn1	Fn1	Fn1	Fn1	
2	Fn1	Mepe	Fn1	Apob	Fn1	Apob	Anapc7	Apob	Apob	Hras	Apob	
3	Fbn1	Fstl3	Fbn1	C3	Msln	Hras	Kctd6	Fbn1	Fstl3	Fbxo32	Fbxo32	
4	Timp1	Igfbp5	Col1a1	Fbn1	Fbn1	Fbxo32	Fbxo2	Cp	Cp	Apob	Foxo3	
5	Msln	Men1	Col3a1	Col1a1	Col5a1	Mstn	Ubac1	C3	Fbn1	Foxo3	Hras	
6	Apob	Tnc	Col1a2	Timp1	Apob	Fstl3	Asb2	Timp1	Timp1	C3	Fasn	
7	Cp	C3	Timp1	Col3a1	Cp	Calm1	Rnf213	Msln	Msln	Cat	Cops5	
8	Men1	Col15a1	Msln	Col1a2	C3	Cp	Rbbp6	Fstl3	C3	Mstn	Me1	
9	Vcan	Col14a1	Col5a2	Cp	Col3a1	Ins2	Ln timer	Men1	Men1	Calm1	Cat	
10	Fstl3	Anapc7	Col5a1	Msln	Timp1	Srebf1	Fbxo21	Hras	Ctnnb1	Fstl3	Ldhc	
11	Fam20c	Ubac1	Col2a1	Hras	Tnc	Ceacam1	Rnf7	Vcan	Vcan	Psmb8	Mstn	
12	Mepe	Rnf213	Cp	Fbxo32	Col1a2	C3	Ankrd9	Fam20c	Fam20c	Cops5	Cp	
13	Igfbp5	Rbbp6	Vcan	Men1	Mepe	Foxo3	Cops5	Mepe	Mepe	Ctnnb1	Fstl3	
14	Tnc	Ln timer	Fam20c	Col5a2	Igfbp5	Fbn1	Myh1	Igfbp5	Igfbp5	Ceacam1	C3	
15	Col1a1	Col4a3	Mepe	Col5a1	Col5a2	Cat	Foxo3	Tnc	Tnc	Cp	Xab2	
16	Col3a1	Col11a1	Fstl3	Col2a1	Col2a1	Pak3	Ring1	Ceacam1	Hras	Fasn	Psmb8	
17	Col1a2	Col5a3	Sparc	Ceacam1	Sparc	Cops5	Mstn	Col1a1	Cat	Anapc7	Aldoc	
18	Col5a2	Cp	Igfbp5	Fstl3	Men1	Arfgap1	Prkaa2	Sparc	Ceacam1	Me1	Fbn1	
19	Col5a1	Vcan	Men1	Vcan	Fam20c	Mapk6	Tnnt2	Col1a2	Foxo3	Espl1	Hnrmpa2b1	
20	Col2a1	Serpinh1	Tnc	Calm1	Fstl3	Fasn	Casq1	Col3a1	Mstn	Ldhc	Calm1	

Figures

Figure 1

Heatmap of enriched terms across input gene lists.

Figure 2

Visualizations of functional enrichment and interactome analysis results of DEGs. Network of enriched terms: (A) colored by enriched terms and 5 term groups formed, (B) colored by *P* value, (C) color-coded based on the identities of the gene lists.

Abbreviations: DEGs, differentially expressed genes; GO, gene ontology; AMPK, Adenosine 5'-monophosphate-activated protein kinase.

Figure 3

(A) PPI network of DEGs. The edge color reflected the combined score of the genes (green→red = low score→high score). (B) PPI network of 16 hub genes, and enrichment analysis of these genes. Abbreviations: DEGs, differentially expressed genes; GO, gene ontology; PPI, protein – protein interaction.

The conserved motifs in the promoter sequence of selected genes.

Figure 6

The prediction of phosphorylation sites of proteins transcribed by selected genes.

Figure 7

The prediction of TFs related to upregulated genes. (A) top 10 TFs targeting upregulated genes; (B) the tissue locations of predicted TFs; (C) the biological functions of predicted TFs. Abbreviations: TFs, transcriptional factors.

Figure 8

The prediction of TFs related to downregulated genes. (A) top 10 TFs targeting downregulated genes; (B) the tissue locations of predicted TFs; (C) the biological functions of predicted TFs. Abbreviations: TFs, transcriptional factors.

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