Original Article

Exploring the role of glycolysis in the pathogenesis of erectile dysfunction in diabetes

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Background: Diabetes mellitus-related erectile dysfunction (DMED) is characterized by complicated pathogenesis and unsatisfactory therapeutic remedies. Glycolysis plays an essential role in diabetic complications and whether it is involved in the process of DMED has not been expounded. The aim of this study was to investigate the genetic profiling of glycolysis and explore potential mechanisms for DMED.

Methods: Glycolysis-related genes (GRGs) and gene expression matrix of DMED were obtained from the molecular signatures database and gene expression omnibus dataset. Differentially expressed analysis and support vector machine-recursive feature elimination (SVM-RFE) method were both used to obtain hub GRGs. Interactive network and functional enrichment analyses were performed to clarify the associated biological roles of these genes. The expression profile of hub GRGs was validated in cavernous endothelial cells, animals, and clinical patients. The subpopulation distribution of hub GRGs was further identified. In addition, a miRNA-GRGs network was constructed and expression patterns as well as molecular functions of relevant miRNAs were explored and validated. In addition, the relationship between hypoxia and DMED was also uncovered.

Results: Based on the combined analysis, 48 differentially expressed GRGs were obtained. Gene Ontology and Kyoto Encyclopedia of Genes and Genomes enrichment analyses revealed that these genes were significantly enriched in carbon metabolism and oxidoreductase activities. Then hub GRGs including down-regulated as well as up-regulated genes in DMED were identified ultimately. Among them, *ALDOC*, *ANGPTL4*, and *CITED2* were well-validated. In addition, 334 glycolysis-related miRNAs were verified and they were involved in endoplasmic reticulum membrane activity, smooth muscle cell differentiation and angiogenesis. After validation of miRNA signature in DMED patients, miR-222-5p, let-7e-5p, miR-184, and miR-122-3p were identified as the promising glycolysis-related miRNA biomarkers in DMED.

Conclusions: We clarified the expression signature of GRGs in DMED based on multi-omics analysis for the first time. It will be significantly important to reveal pathological mechanisms and promising treatments in DMED.

Keywords: Erectile dysfunction (ED); diabetes; glycolysis; bioinformatics

Submitted Jan 02, 2025. Accepted for publication Feb 27, 2025. Published online Mar 26, 2025.
doi: 10.21037/tau-2025-6
View this article at: https://dx.doi.org/10.21037/tau-2025-6

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Introduction

Erectile dysfunction (ED) is a kind of common sexual dysfunction, defined as the inability of the penis to achieve or maintain enough rigidity to perform satisfactory sexual intercourse, and troubles males for a long time (1). There will be more than 322 million males suffering from ED in 2025 (2). Penile erection is regarded as a vascular event under neuroregulation in essence and ED has a close relationship with diabetes (3). A significantly higher international index of erectile function-5 (IIEF-5) mean value in ED patients using glucagon-like peptide-1 receptor agonists was noted (4). Besides, in male patients with type 2 diabetes, health literacy, unrealistic optimism, and adherence to glycometabolic disease management were closely related to ED (5). The global diabetes prevalence in 20–79 years old is estimated to be 12.2% (783.2 million) in 2045 (6). As a common complication of diabetes, diabetes mellitus-related erectile dysfunction (DMED) is characterized by more intricate pathomechanism and unsatisfactory therapeutic remedies compared with ED without diabetes (7,8). Therefore, exploring the pathogenesis and new targets for treatment in DMED is of significant importance in the urology and endocrinology departments.

It is reported that glycolytic intermediates diverted into pathological pathways play an essential role in diabetic complications (9). The level of glycolysis is increased in nephropathy with diabetes and decreased in neuropathy, respectively (10-12). In fact, endothelial cells generate ATP primarily via glycolysis rather than oxidative phosphorylation (13). Impaired glycolysis manifests the glycometabolic disorder in diabetic hearts (14). During cardiomyopathy with diabetes, restoration of oxygen and nutrient delivery to the impaired issue is mediated by revascularization via endothelial cells, which is the compensatory measure for cellular adaptation in the impaired metabolic microenvironment (13). In other words, aberrant glycolysis is involved in the pathogenesis of diabetic complications. The corpus cavernosum is part of the systemic vasculature, and how glycolysis influences the process of DMED has not been studied yet.

In recent years, genetic alternations in the process of ED have been studied by bioinformatics like microarray and high-throughput sequencing technologies (15,16). These would help us identify the pathogenesis of ED at the molecular level and promote targeted therapy. However, the present transcriptome data of DMED are limited, and sole expression analysis is not persuasive enough to screen biomarkers in DMED. In the present study, we adopted support vector machine-recursive feature elimination (SVM-RFE) based on machine learning to process the gene profiling of DMED (17,18). The genetic patterns of DMED rats in the Gene Expression Omnibus (GEO) database were analyzed based on the combination of differentially expressed analysis and SVM-RFE algorithm to identify hub glycolysis-related genes (GRGs) in DMED and verified by the clinical specimen. Also, relevant miRNAs were selected and validated in humans. Our study is of great significance in clarifying the genetic landscape of glycolysis and finding promising treatments for DMED in further research. We present this article in accordance with the TRIPOD reporting checklist (available at https://tau.amegroups.com/article/view/10.21037/tau-2025-6/rc).

Highlight box

Key findings

- After multi-omics analysis, we clarified and validated the expression signature of glycolysis-related genes as well as associated miRNAs in diabetes mellitus-related erectile dysfunction (DMED) for the first time.

What is known and what is new?

- DMED is characterized by complicated pathogenesis and unsatisfactory therapeutic remedies. Glycolysis plays an essential role in diabetic complications.
- Glycolysis is vital in the process of DMED.

What is the implication, and what should change now?

- It will be significantly important to reveal pathological mechanisms and promising treatments in DMED.

Methods

Data source

DMED-related expression profiling was downloaded in the GEO (https://www.ncbi.nlm.nih.gov/geo/) database including GSE2457 (15), GSE146078 (19), GSE182053 (16) and GSE206528 (20). GSE2457 deposited Affymetrix data files of 10 DMED rats and corresponding control penile tissues. In the databases for validation, GSE146078 contained gene data of cavernous endothelial cells in high-glucose and normal-glucose conditions. Similarly, non-coding RNA profiling from blood samples of 20 ED patients with type 2 diabetes and controls was selected from GSE182053. ED was identified by IIEF-5 scores, and diagnostic criteria for type 2 diabetes was in line with WHO

diabetes diagnostic criteria in 1999. Furthermore, a single-cell transcriptome Atlas of the human corpus cavernosum from three males with normal erections and five organic ED patients was explored from GSE206528. Three normal tissue samples were obtained from the tumour margin of penile carcinoma resection. The five ED corpus cavernosum tissues were obtained from biopsy samples in artificial cavernous body implantation. ED was identified by the nocturnal penile tumescence and intracavernosal injection tests. Besides, the DMED patients were identified with type 1 diabetes with more than 10-year history. GRG sets were obtained in the Molecular Signatures Database (MSigDB, https://www.gsea-msigdb.org/gsea/msigdb/) with "glycolysis" and "glycolytic" as the keywords. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013).

Identification of differentially expressed GRGs

The transcriptome data in GSE2457 were preprocessed first. To be specific, the expression value of the genes corresponding to the multi-microarray probes was averaged and the defect value was removed. After normalization, calibrated gene files were used to obtain differentially expressed genes (DEGs) between control and DMED groups with the selected criteria of P value <0.05 by "limma" package (21) in R software (Version 4.2.1). Then, differentially expressed GRGs (DE-GRGs) were identified via the intersection between DEGs and GRG sets.

Functional annotation by functional enrichment analysis

Analysis of the function and associated molecular pathways of DE-GRGs was conducted by functional enrichment analysis, including the Gene Ontology (GO) and Kyoto Encyclopedia of Genes and Genomes (KEGG). First, the gene symbols were converted to gene ID via ID conversion tool in the Database for Annotation, Visualization and Integrated Discovery (DAVID) (https://david.ncifcrf.gov/conversion.jsp). Then, GO and KEGG enrichment terms were explored by R packages "clusterProfiler" (22) and "GOplot" (23). Adjusted P<0.05 and q<0.05 were set as statistical significance.

Interactive network and functional analysis

DE-GRGs were processed in the Search Tool for the Retrieval of Interacting Genes (STRING) database (http://string-db.org) to explore the relationship among proteins of interest. Then a protein-protein interaction (PPI) network was reintroduced in Cytoscape (version 3.7.1, https://cytoscape.org/) for rearrangement. Furthermore, Metascape (http://metascape.org) was utilized to show the relationship of enriched biological terms, including biological processes, KEGG pathway, Reactome gene sets, and WikiPathways. Terms with the threshold of P<0.05, and enrichment factor >1.5 were regarded as statistical significance.

Feature selection of hub GRGs based on SVM-RFE method

SVM-RFE is a machine learning approach characterized by a sequence backward selection strategy and maximum interval criterion based on SVM (17,18). In this algorithm, gene expression was considered as the feature. The weight of each feature was calculated and features were ranked based on weights. After iteration, the optimal gene signature with the smallest error was selected as the final model (16). This method has a good learning ability in the case of small samples. In the present study, SVM-RFE was applied using R package "e1071" (24). Finally, the hub GRGs were obtained by the overlapping genes from SVM-RFE and DE-GRGs.

Construction and validation of miRNA-GRGs network

GRGs-associated miRNAs were predicted with the threshold of score more than 0.95 in miRWalk database (http://mirwalk.umm.uni-heidelberg.de/). In this database, gene set enrichment analysis (GSEA) was conducted to analyze the biological functions of hub GRGs using the GO gene sets from MSigDB with the threshold of adjusted P<0.001. Furthermore, a miRNA-mRNA regulatory network was constructed using Cytoscape software (Version 3.7.1). Similarly, the functional enrichment analysis including GO and KEGG terms of relevant miRNAs was conducted in the miRNA Enrichment Analysis and Annotation Tool (miEAA 2.0) database with the threshold of P<0.05 and minimum required hits per sub-category >2 (https://ccb-compute2.cs.uni-saarland.de/mieaa2/). Finally, the expression profiling of miRNAs was validated in clinical specimens of DMED patients.

External validation of hub GRGs and correlation analysis

To enhance authority and stringency, the expression profiling of hub GRGs was validated in cavernous endothelial cells

and clinical patients based on the Male Health Atlas database (http://www.malehealthatlas.cn/) (20) simultaneously. In the dataset of cavernous endothelial cells, DEGs were selected with the threshold of P value <0.05 by "edegR" package. In MHA, average and percent expression patterns of hub GRGs were explored among different diseases and cell types. Also, correlation analysis of hub GRGs to show their associations was carried out using Spearman's method. The results were visualized by the R package "corrplot".

Experimental validation of hub GRGs by animal samples

To experimentally validate the hub GRGs, the corpus cavernosum samples were harvested from control and DMED rats. For DMED rats, 8-week-old Sprague-Dawley rats from Hunan Slack Jingda Experimental Animal Co., Ltd. were injected with streptozotocin (60 mg/kg) intraperitoneally. The remaining rats served as the control group and were treated with citric acid buffer. Three days post-injection, rats with fasting blood glucose levels exceeding 16.7 mmol/L were considered diabetic. These diabetic rats were then fed under normal conditions for 8 weeks. At the study's conclusion, an apomorphine test was conducted to screen for DMED rats. Specifically, rats were administered apomorphine (100 μg/kg) and observed in a dark environment for 30 minutes, with penile erection recorded. Rats exhibiting negative results were assigned to the DMED group (7). Later, an electrostimulation test was employed to evaluate the erectile function of control and DMED rats (7). Experiments were performed under a project license (No. 2019006) granted by the institutional ethics board of Experimental Animal Administration Committee of Wuhan Servicebio Biotechnology, China, in compliance with Servicebio institutional guidelines for the care and use of animals.

For quantitative real-time polymerase chain reaction (qPCR), RNA was extracted from the rat corpus cavernosum using relevant reagents (G3013, Servicebio, Wuhan, China). Then cDNA was synthesized using the cDNA Synthesis SuperMix (G3330, Servicebio, Wuhan, China). The amplification of target genes was conducted with the Master Mix (G3320, Servicebio, Wuhan, China). The specific primer sequences for qPCR are detailed in Table S1.

Association between DMED and hypoxia

Hypoxia is one of the major features of glycolysis microenvironment (12). The set of hypoxia-associated genes was acquired in hallmark gene sets of MSigDB. Then the overlapping genes were exacted from DEGs in GSE2457 and hypoxia-associated gene sets. Different expressions of hypoxia-related genes were analyzed by the Wilcoxon test.

Statistical analysis

The analysis in this study was conducted using *t*-test and the results were visualized with GraphPad Prism version 8. The results of the data were presented as the mean ± standard deviation.

Results

Differentially expressed GRGs analysis

Figure 1 reveals the path of the present study. After calibration and normalization, a total of 1,570 DEGs between DMED and control groups were obtained and depicted as a heatmap (*Figure 2A*). After screening, five gene sets including 326 genes were selected as GRGs. Finally, 48 DE-GRGs were obtained based on the comparison of DEGs and GRGs (*Figure 2B*). Of them, 14 genes were up-regulated while 34 genes were down-regulated.

Functional enrichment analysis

The biological function of 48 DE-GRGs was annotated by GO and KEGG enrichment tools. The results showed that 24 GO terms and 28 KEGG terms were relevant to DE-GRGs. The top GO terms involved hexosyltransferase activity, oxidoreductase activity, NAD binding, and NAD(P)+ activities (*Figure 3A*,*3B* and Table S2). As for KEGG enrichment analysis, the significant KEGG terms included glycolysis, hypoxia-inducible factor (HIF)-1 signaling pathway, carbon metabolism, AMPK signaling pathway, and so on (*Figure 3C*,*3D* and Table S3). These findings indicated that the significant functions of DE-GRGs were focused on cellular metabolism in the glycolytic microenvironment.

Interactive network and functional analysis

To delineate the interplays among 48 DE-GRGs, a PPI network was constructed with an interaction score more than 0.7. The network contained 32 nodes and 67 interactions (*Figure 4A*). Furthermore, functional analysis revealed that these DE-GRGs could regulate

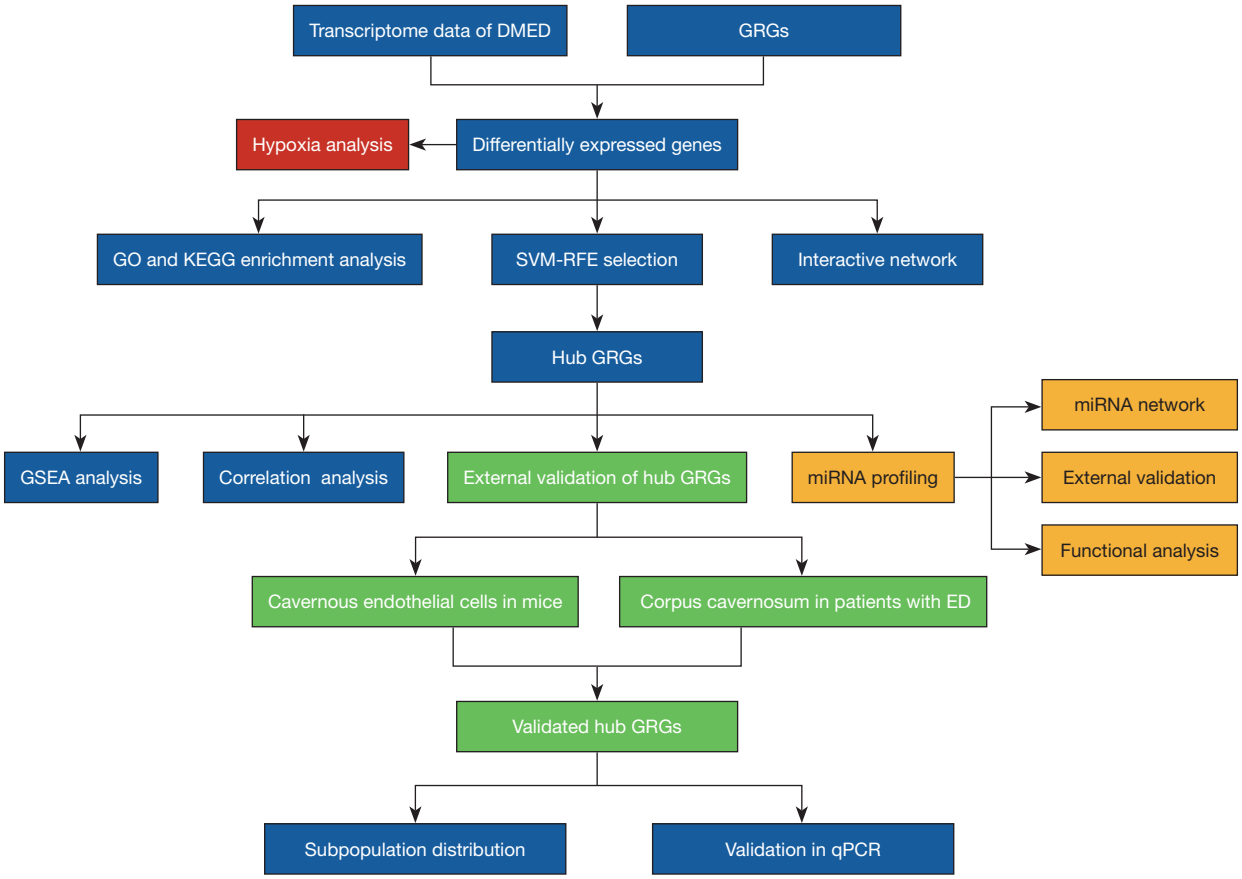

Figure 1 The flow path of the study. ED, erectile dysfunction; DMED, diabetes mellitus-related erectile dysfunction; miRNA, microRNA; SVM-RFE, support vector machine-recursive feature elimination; GRGs, glycolysis-related genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; GSEA, gene set enrichment analysis; qPCR, quantitative PCR; PCR, polymerase chain reaction.

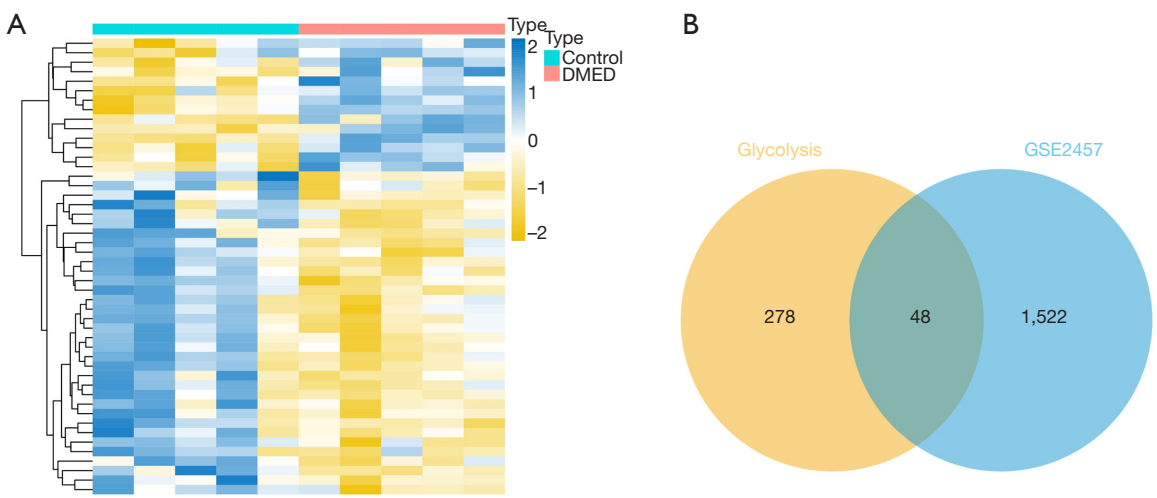

Figure 2 Differentially expressed GRGs analysis. (A) The heatmap of significant GRGs between DMED and control groups. (B) Intersection of DEGs of GSE2457 and GRGs. DEGs, differentially expressed genes; DMED, diabetes mellitus-related erectile dysfunction; GRGs, glycolysis-related genes.

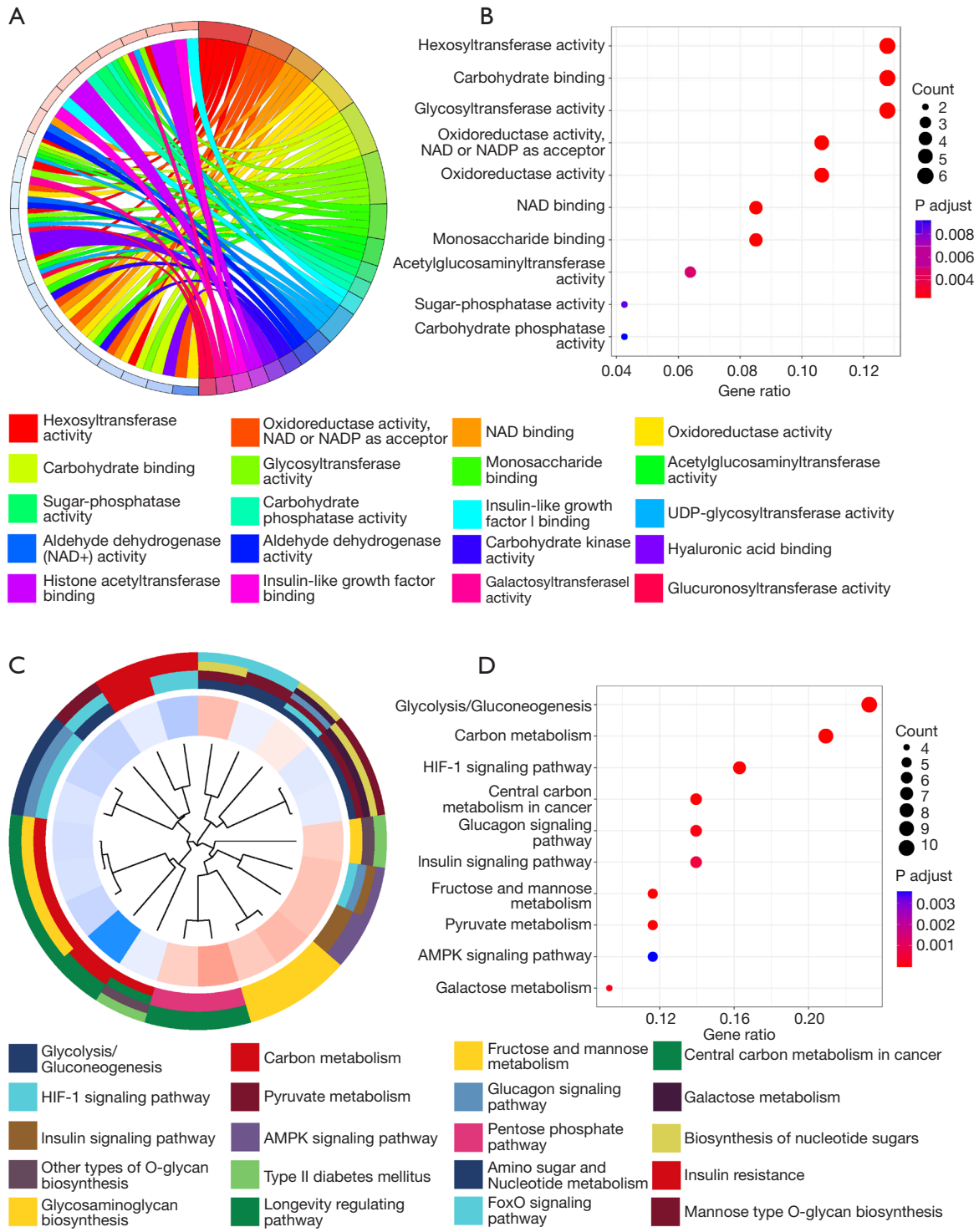

Figure 3 Functional enrichment analysis of differentially expressed GRGs. (A) GO circle map. (B) The dot plot of significant GO terms. (C) KEGG circle map. (D) The dot plot of significant KEGG terms. For A and C, the inner part represents significantly enriched GO and KEGG terms of GRGs. The outer part represents the gene symbols of GRGs, and the color varying from blue to red shows the expression changes from down-regulation to up-regulation. NAD, nicotinamide adenine dinucleotide; NADP, nicotinamide adenine dinucleotide phosphate; HIF, hypoxia-inducible factor; KEGG, Kyoto Encyclopedia of Genes and Genomes; GO, Gene Ontology; GRGs, glycolysis-related genes; UDP, uridine diphosphate.

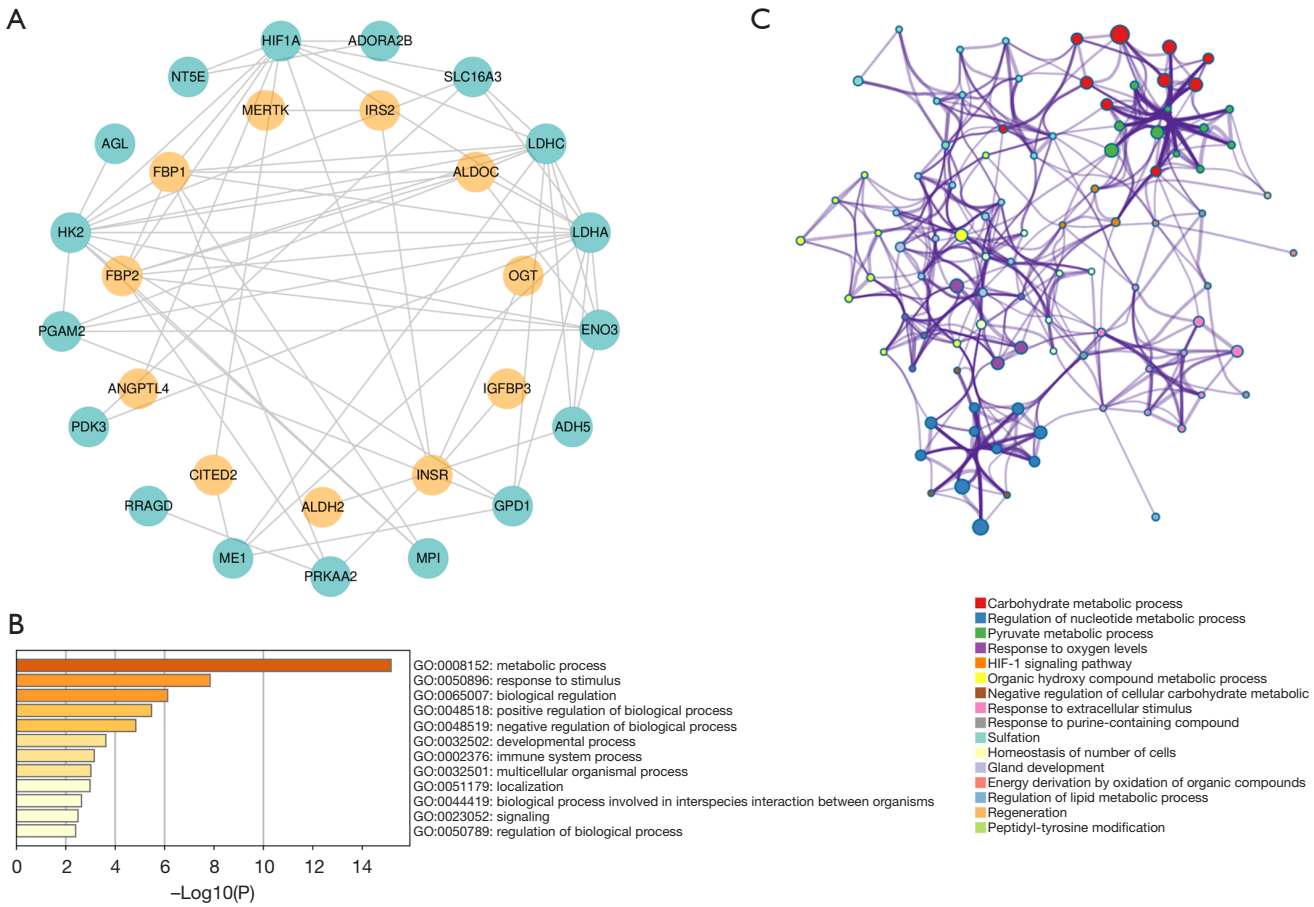

Figure 4 PPI network construction and module analysis of significant GRGs. (A) PPI network of significant GRGs. (B) GO enrichment analysis of significant GRGs in the present network. (C) Pathway and process enrichment analysis of significant GRGs in the present network. For (A), yellow labels represent up-regulated GRGs and green labels for down-regulated GRGs. For (C), each node represents an enriched term and is colored by cluster ID. PPI, protein-protein interaction; GRGs, glycolysis-related genes; GO, Gene Ontology.

many biological processes, including the immune system process, carbohydrate, pyruvate, and lipid metabolic processes (*Figure 4B*). In enriched pathways, these genes were associated with HIF-1 signaling pathway, cellular homeostasis, and energy derivation by oxidation of organic compounds (*Figure 4C*).

Identification of hub GRGs via SVM-RFE method

By using the SVM-RFE algorithm, a subset of 13 feature-corresponding genes among GRGs in GSE2457 were identified with the smallest error (*Figure 5A*). Based on differentially expressed analysis, the overlapping genes among SVM-RFE and DE-GRGs were ultimately selected as hub GRGs (*Figure 5B*). Of them, *SLC37A4*, *COL5A1*, *GALK1*, *RBCK1* and *HIF1A* were down-regulated while *ZBTB7A*, *MERTK*, *ALDH2*, *ANGPTL4*, *CITED2*, and *ALDOC* were up-regulated in the DMED group compared with the control group (*Figure 5C*). The results of GSEA in the miRWalk database indicated that hub GRGs were involved in the regulation of cellular response to hypoxia, glycolytic process, apoptotic process, NF-κB transcription factor activity, and so on (*Table 1*).

Construction of miRNA-GRGs network and miRNA functional enrichment analysis

Based on the expression and functional enrichment analysis of hub GRGs, we explored the expression profile of the associated miRNAs in DMED. And 334 miRNAs related

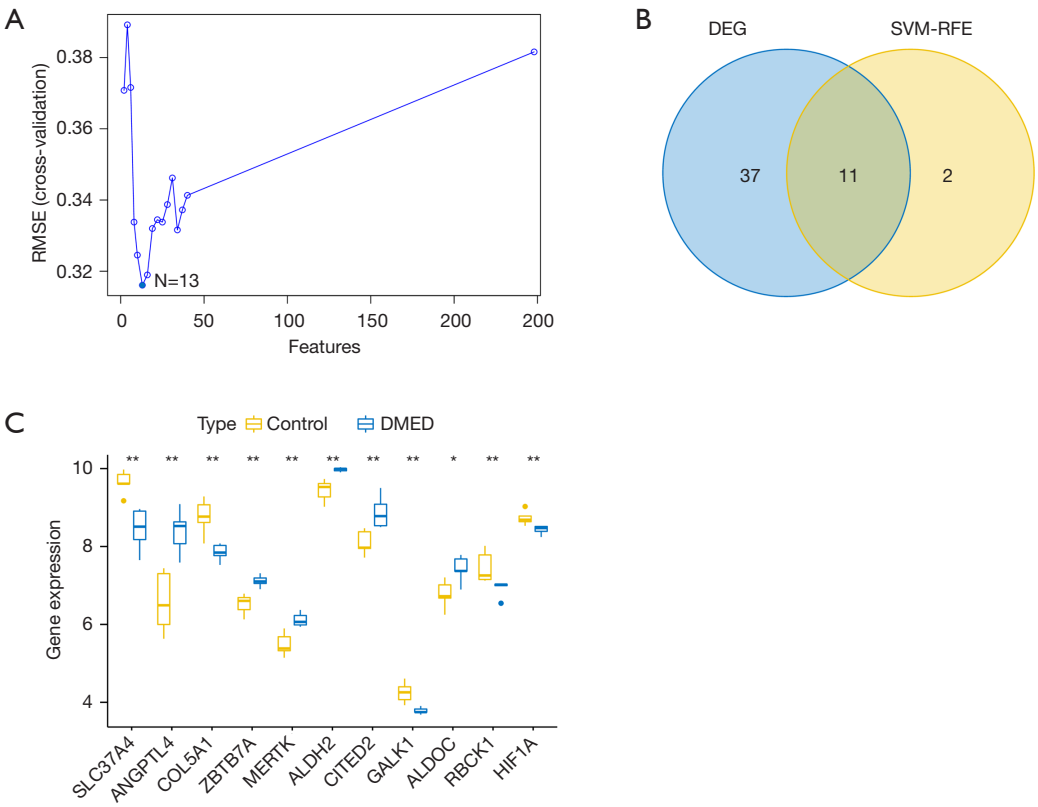

Figure 5 Acquisition and gene expression of hub GRGs. (A) Candidate genes identified by SVM-RFE. (B) The Venn diagram showing the hub GRGs extracted via SVM-RFE and differentially expressed analysis. (C) The boxplot of hub GRGs in DMED and control groups. Statistical analysis was performed with the Wilcoxon test. *, P<0.05; **, P<0.01. DEG, differentially expressed gene; DMED, diabetes mellitus-related erectile dysfunction; GRGs, glycolysis-related genes; RMSE, root mean-square error; SVM-RFE, support vector machine-recursive feature elimination.

Table 1 GSEA results of hub GRGs in miRWalk database

Class	ID	Description
Molecular function	GO:0004332	Fructose-bisphosphate_aldolase_activity
Molecular function	GO:0035035	Histone_acetyltransferase_binding
Molecular function	GO:0003700	DNA-binding_transcription_factor_activity
Biological process	GO:0001666	Response_to_hypoxia
Biological process	GO:0006110	Regulation_of_glycolytic_process
Biological process	GO:0010629	Negative_regulation_of_gene_expression
Biological process	GO:0051092	Positive_regulation_of_NF kappaB_transcription_factor_activity
Biological process	GO:0071456	Cellular_response_to_hypoxia
Biological process	GO:0010628	Positive_regulation_of_gene_expression
Biological process	GO:0043066	Negative_regulation_of_apoptotic_process

GSEA, Gene set enrichment analysis; GRGs, glycolysis-related genes; GO, Gene Ontology.

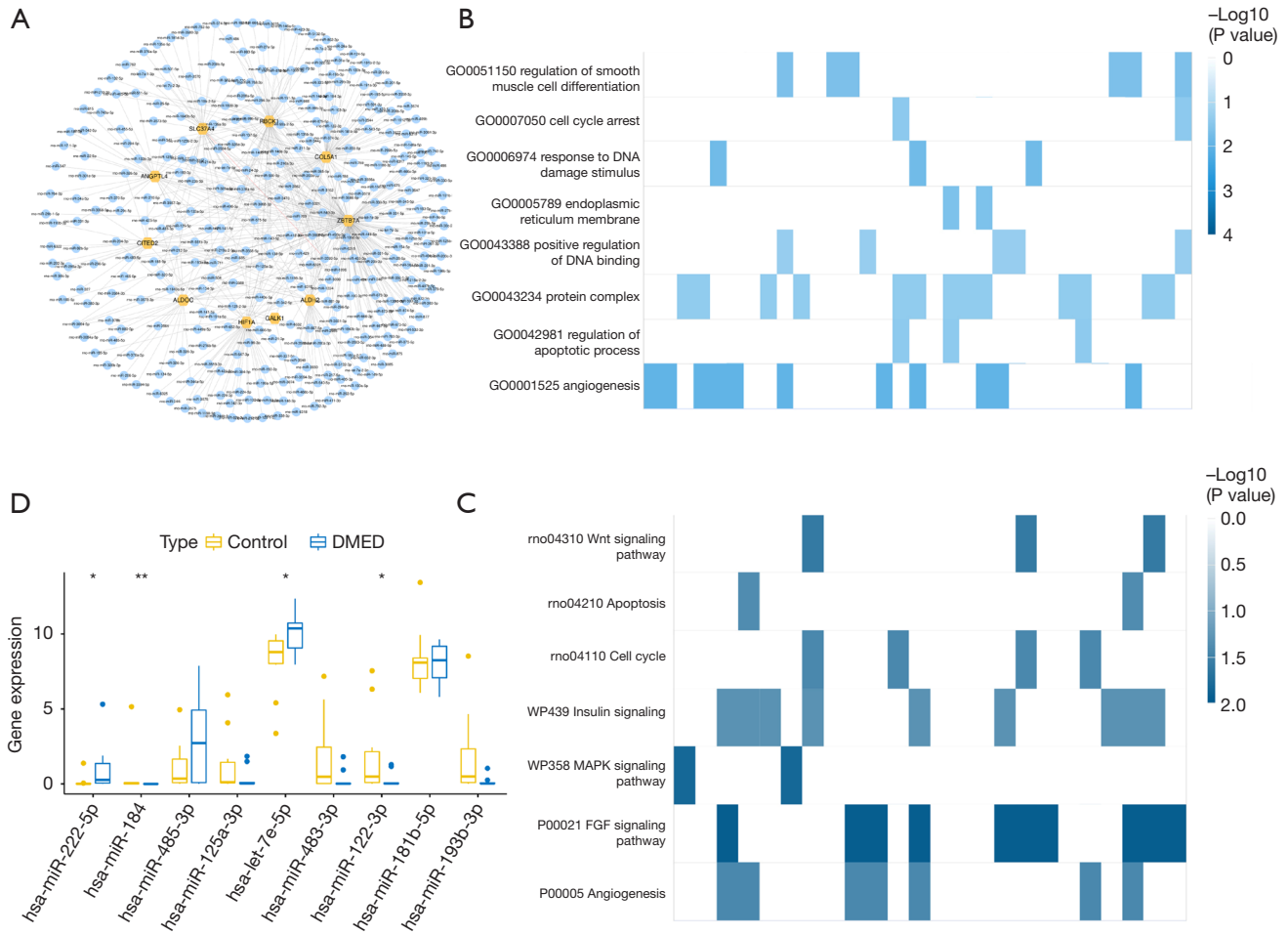

Figure 6 Construction of miRNA-GRGs network and miRNA functional enrichment analysis. (A) The miRNA-hub GRGs network. (B) GO enrichment plot of relevant miRNAs. (C) KEGG enrichment plot of relevant miRNAs. (D) The boxplot of miRNAs in DMED and control groups after clinical validation. For (A), yellow circles represent hub GRGs and blue circles represent relevant miRNAs. For (B,C), blue bars represent different miRNAs. For (D), statistical analysis was performed with the Wilcoxon test. *, P<0.05; **, P<0.01. GRGs, glycolysis-related genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; DMED, diabetes mellitus-related erectile dysfunction.

to hub GRGs were predicted in the miRWalk database. Then a miRNA-hub GRGs network comprising 334 interactions was constructed (*Figure 6A*). Furthermore, the functional enrichment analysis showed that these miRNAs participated in the regulation of smooth muscle cell differentiation, endoplasmic reticulum membrane activity, apoptotic process, and angiogenesis (*Figure 6B*). Similarly, the corresponding KEGG terms included Wnt signaling pathway, apoptosis, cell cycle, MAPK signaling pathway, and so on (*Figure 6C*). Finally, miRNA expression was validated in clinical samples of DMED patients. It showed that 9 miRNAs existed in differentially expressed miRNAs of clinical specimens (*Figure 6D*). After statistical analysis, miR-222-5p and let-7e-5p had significantly higher expressions while miR-184 and miR-122-3p had significantly lower expressions in the DMED group compared with the control group (P<0.05).

Correlation analysis and external validation of hub GRGs

The correlation analysis showed that half of the expression correlation coefficients were more than 0.7, which revealed a collaborative effect among hub GRGs during the glycolytic process of DMED (*Figure 7A*). Furthermore,

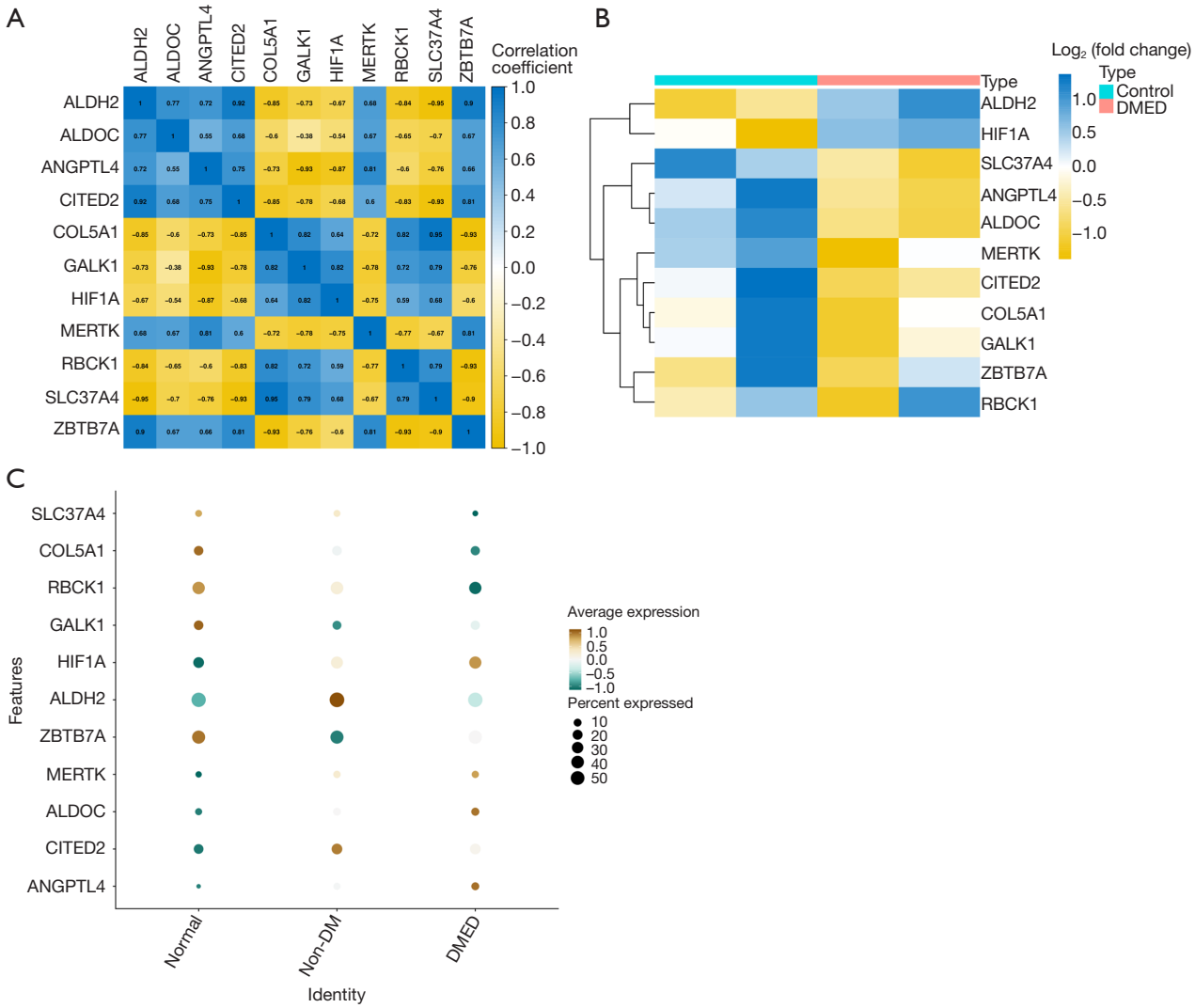

Figure 7 Correlation analysis and external validation of hub GRGs. (A) Correlation analysis of hub GRGs. (B) The profiling of hub GRGs in cavernous endothelial cells. (C) The profiling of hub GRGs in clinical specimen. GRGs, glycolysis-related genes; ED, erectile dysfunction; DM, diabetes mellitus; DMED, diabetes mellitus-related erectile dysfunction.

the expression pattern of hub GRGs was validated in cavernous endothelial cells (*Figure 7B*) and clinical specimens of ED patients (*Figure 7C*) simultaneously. After performing a differentially expressed analysis, overlapped hub GRGs in the two gene sets were screened. Finally, *ALDOC*, *ANGPTL4*, and *CITED2* were validated well. They may serve as key genes in the glycolytic process of DMED. What's more, the expression profiles of *ALDOC*, *ANGPTL4*, and *CITED2* were validated in DMED rats and controls by qPCR. In Figure S1, the results of qPCR suggested that notable increases were observed in the expressions of *ALDOC* and *ANGPTL4* in the *DMED* group compared with the control group ($P<0.05$), indicating the critical roles of *ALDOC* and *ANGPTL4* during the glycolytic process of DMED.

Subpopulation distribution of validated hub genes

Subpopulation distribution in specific tissues is vital for performing the functions of genes. Firstly, different types of cells were identified in the human corpus cavernosum including endothelial cells, fibroblasts, pericytes, smooth muscle cells, Schwann cells, macrophages, and T cells (*Figure 8A,8B*). ANGPTL4 was predominately localized

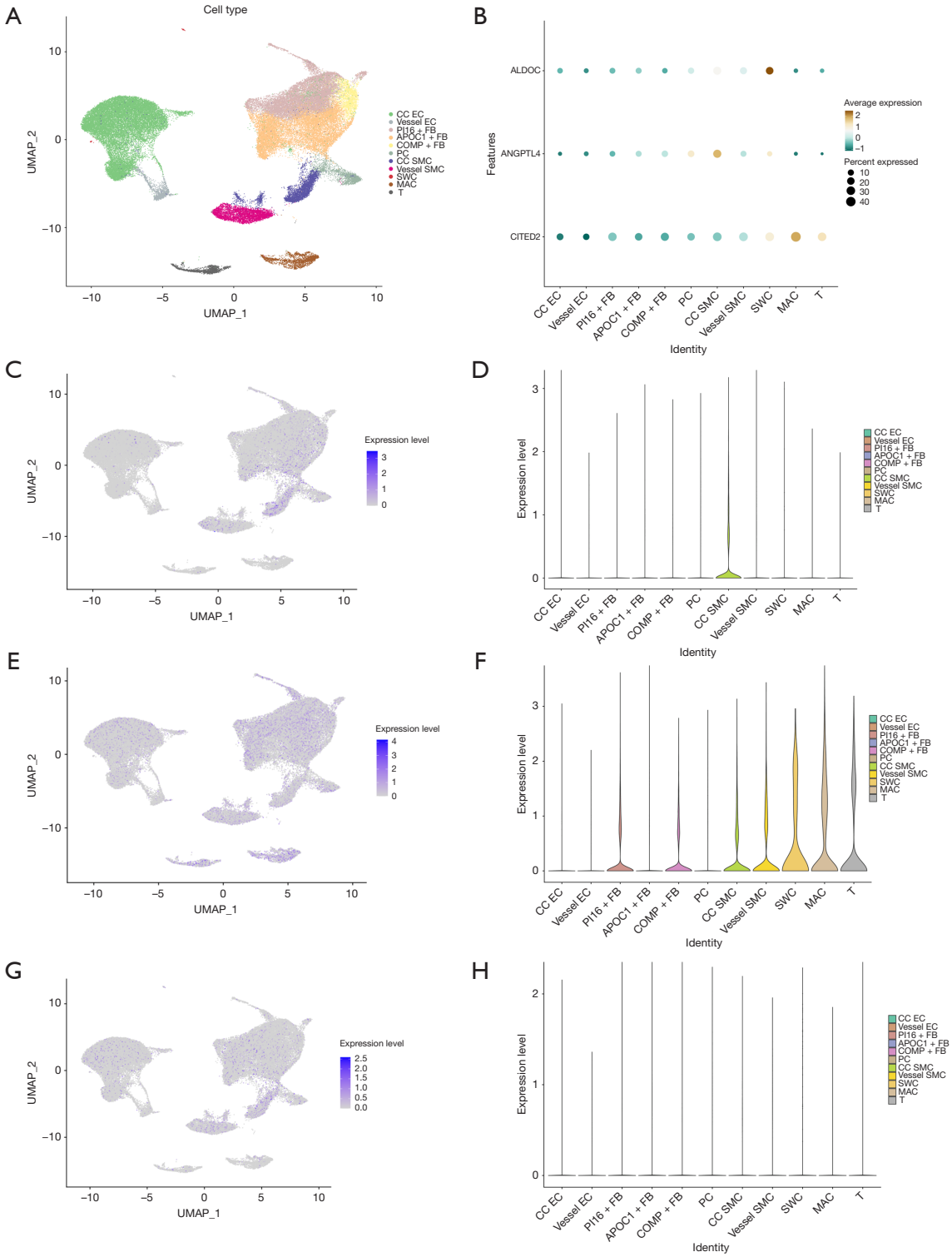

Figure 8 Subpopulation distribution of validated hub genes. (A) UMAP visualization of subpopulations clustering in human corpus cavernosum. (B) Expression distribution of genes in ED patients grouped by cell types. (C,D) The subpopulation distribution of ANGPTL4. (E,F) The subpopulation distribution of CITED2. (G,H) The subpopulation distribution of ALDOC. CC, corpus cavernosum; COMP, cartilage oligomeric matrix protein; EC, endothelial cell; ED, erectile dysfunction; FB, fibroblast; MAC, macrophage; PC, pericyte; SMC, smooth muscle cell; SWC, Schwann cell; T, T cell; UMAP, uniform manifold approximation and projection.

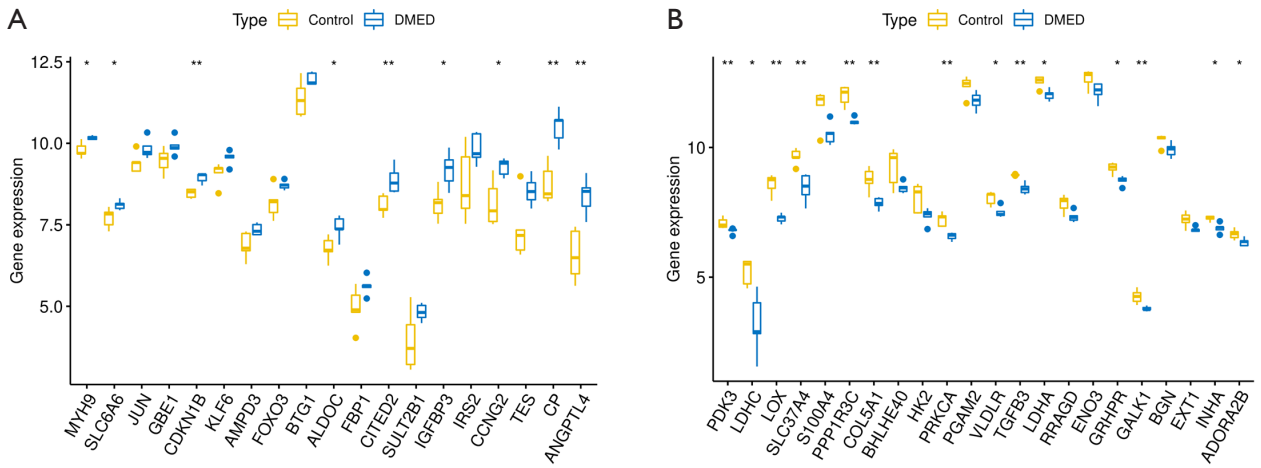

Figure 9 Associations between DMED and hypoxia. (A) The boxplot of up-regulated hypoxia-related genes in DMED and control groups. (B) The boxplot of down-regulated hypoxia-related genes in DMED and control groups. Statistical analysis was performed with the Wilcoxon test. *, P<0.05; **, P<0.01. DMED, diabetes mellitus-related erectile dysfunction.

in corpus cavernosum smooth muscle cells (*Figure 8C,8D*). CITED2 was focused on PI16 and COMP-positive fibroblasts, corpus cavernosum and vessel smooth muscle cells, Schwann cells, macrophage as well as T cells (*Figure 8E,8F*). While ALDOC showed the least distribution, which was enriched in Schwann cells (*Figure 8B,8G,8H*).

Association between DMED and hypoxia

Functional enrichment analysis as above noted that glycolysis had a close relationship with hypoxia. The hypoxia-associated gene set including 200 genes was acquired in MSigDB. Then 170 genes were used to conduct differentially expressed analysis in DMED, and finally, 41 differentially expressed hypoxia-associated genes were obtained. After statistical analysis, a total of 9 and 14 hypoxia-associated genes were up-regulated and down-regulated in the DMED group compared with the control group, respectively (P<0.05, *Figure 9*).

Discussion

Nowadays, the pathogenesis and treatment for DMED are the pressing concerns in andrology. Compared with ED arising from other organic causes, the manifestations of DMED appear earlier and more severe and affect 35–90% of men with diabetes (7). Glycolysis plays an important role in the occurrence of diabetic complications. In our study, we investigated the relationship between glycolysis and DMED based on machine learning and bioinformatic methods for the first time and identified key genes related to glycolysis and associated miRNA profiles. It would be significant for us to explore genetic alternations and promote effective treatments for DMED.

Based on the integrated analysis, we obtained 14 up-regulated and 34 down-regulated GRGs which were differentially expressed between the DMED and control groups. It seemed that gene inhibition played a predominant role in the occurrence of DMED. Similarly, 529 genes including 206 up-regulated and 323 down-regulated genes were differentially expressed in the cavernosum of rats with diabetes (15). More than half of the DEGs were down-regulated in mouse cavernous endothelial cells of high-glucose condition compared with normal-glucose condition. Subsequent functional enrichment analysis showed that DE-GRGs were involved in hexosyltransferase activity, glycosyltransferase activity, and carbon metabolism. After all, hexosyltransferase and glycosyltransferase are important glycolytic enzymes in glycogen metabolism (25). Besides, DE-GRGs took part in oxidoreductase activity as well as NAD(P)+ activity and were associated with HIF-1 and AMPK signaling pathways. Oxidoreductase activity is a part of the glycolytic process and it also plays a vital role in the pathogenesis of DMED (26). Oxidoreductase dysfunction could trigger excessive accumulation of reactive oxygen species (ROS), which would have a detrimental effect on endothelium, vascular smooth cells and nerves in the corpus cavernosum with diabetes (27,28). Therein, nicotinamide

adenine dinucleotide phosphate (NADPH) enzymes are the major sources of ROS (29), and NADPH oxidase inhibitors have been noted to be effective in improving erectile function in DMED (30). Besides, DE-GRGs in DMED also had a relationship with HIF-1 signaling pathway. HIF-1, known as hypoxia-inducible factor-1, is a master driver of glucose metabolism. HIF-1-alpha (HIF-1α) could induce an increased expression of glycolytic enzymes (31,32). Also, the differentially expressed analysis of hypoxia-associated genes in DMED was conducted in this article, and 23 genes were identified after statistical analysis. Fanfulla *et al.* (33) reported that the incidence of ED was higher in males with chronic hypoxia. And hypoxia could disturb erectile function by interfering with endothelial function (34). It seems that the interaction between glycolysis and hypoxia affects erectile function in diabetes to a large extent which needs to be explored in depth further.

To screen hub GRGs, we adopted the SVM-RFE approach. It shows advantages in small-sample studies, which would get a high error rate in other bioinformatics methods. Therefore, it is suitable for the analysis of ED. Finally, we obtained 11 hub GRGs including *SLC37A4*, *COL5A1*, *GALK1*, *RBCK1* and *HIF1A*, which were down-regulated in the DMED group. SLC37A4 functions in encoding glucose 6-phosphate translocase, and its mutation would cause glycogen storage disease type 1b, which could lead to arteriovenous malformation (35,36). A previous study reported that COL5A1 could be modulated by hemoglobin A1c (HbA1c), and interaction between COL5A1 rs59126004 and HbA1c had a relationship with the risk of retinopathy with diabetes (37). Besides, COL5A1 has been identified as a promising lipid-species–associated loci linked to cardiovascular diseases (38). Sun *et al.* (39) unveiled that astaxanthin could reduce oxidative stress induced by diabetes via reacting with free radicals with the help of COL5A1. We speculated that COL5A1 may be associated with DMED to some extent. GALK1 is a major enzyme for the metabolism of galactose and protein glycosylation (40). Wang *et al.* (41) found that GALK1 may serve as a hub gene in the process of acute aortic dissection. Besides, it also belongs to the hypoxia-related gene set in MSigDB (42). RBCK1 is a component of the linear ubiquitin chain assembly complex (43), and the latter could participate in the activation of the canonical NF-κB pathway, which plays a key role in inflammatory responses (44). It may be involved in the microenvironment disturbance of DMED.

Herein, *ZBTB7A*, *MERTK*, *ALDH2*, *ANGPTL4*, *CITED2* and *ALDOC* were up-regulated in DMED. ZBTB7A was reported to bind to the promoter and regulate the transcription of critical glycolytic genes (45). And the loss-of-function mutation of ZBTB7A could induce elevated glycolysis and proliferation in cancers (46). MERTK is a member of receptor tyrosine kinases, and is involved in promoting apoptotic cell clearance through efferocytosis and macrophage polarization in the tumor microenvironment (47). ALDH2 belongs to the aldehyde dehydrogenase family and is associated with the oxidative pathway. Its dysregulation is relevant to oxidative stress-induced diseases (48,49). He *et al.* (50) uncovered that ALDH2/SIRT1 deficiency resulted in prominent oxidative stress and retinopathy with diabetes progression. Besides, ALDH2 was found to protect against heart stress-induced ROS accumulation and preserve the viability of endothelial cells (51). Therefore, we infer that ALDH2 has a close relationship with DMED. ANGPTL4 functions as regulating glucose homeostasis and lipid metabolism, and it also serves as an apoptosis survival factor in vascular endothelial cells. It is highly expressed in endothelial cells, especially in hypoxia conditions, which regulates nitric oxide production, vascular inflammation, and vascular permeability (52). It is worthwhile to explore the role of ANGPTL4 in DMED. As for CITED2, it could inhibit the transactivation of HIF1α-induced genes by competing with the binding of HIF-1α (53). These strengthen the interaction between hypoxia and glycolysis. ALDOC is one of aldolase family genes, which is abundant in the central nervous system. It correlates with neuronal damage (54). As a target of NRF2, ALDOC is involved in NRF2-dependent cytoprotective process, characterized by NRF2-ALDOC-autopagy axis in the process of stroke (55).

We validated 11 hub GRGs in cavernous endothelial cells and clinical specimen of ED patients simultaneously. Finally, the expression profiles of *ALDOC*, *ANGPTL4* and *CITED2* showed significant results in the glycolytic process of DMED. The discrepancy between rats and patients could be explained by the heterogeneity of species and small sample size. Considering that miRNA activity might be a key reason for modulation of the glycolytic process, miRNAs related to hub GRGs were obtained and these miRNAs had a close relationship with the pathogenesis of DMED. Finally, miR-222-5p, let-7e-5p, miR-184, and miR-122-3p were screened as promising glycolysis-related miRNA biomarkers in DMED after validation in clinical specimens. Liu *et al.* (56) reported that miR-222-5p accelerated the dysfunction of human

vascular smooth muscle cells and promoted pathological changes of coronary atherosclerosis. It could regulate the differentiation process from mesenchymal stem cells to smooth muscle cells for vascular tissue grafts (57), which may guide the effect of stem cell therapy for DMED. Let-7e-5p could be modulated by METTL3 to regulate angiogenesis in endothelial cells (58). It has been proven as one of the key influencing factors in DMED (16). MiR-184 was reported to have a tight correlation with oxidative stress. It attenuated hypoxia and oxidative stress-related injury by suppressing DNA damage in age-related macular degeneration (59). Besides, it markedly inhibited oxidative stress and inflammation to prevent cardiomyocyte apoptosis through the regulation of FBXO28 (60). With respect to miR-122-3p, previous studies revealed that it was involved in liver diseases (61,62). Whether it takes part in the process of DMED needs to be expounded further.

It is the first time to investigate the relationship between glycolysis and DMED based on machine learning, and key genes related to glycolysis as well as associated miRNA profiles were identified and validated. The study is of great significance in clarifying the genetic landscape of glycolysis and finding promising treatments for DMED in further research. However, the limitations of this study must be elucidated. First, due to limited data in DMED, the sample size of rats and clinical patients in our research is relatively small, and there is not enough data verification. A larger cohort and prospective studies are needed to show the good universality of hub GRGs and miRNAs. Second, the study related to ED was conducted based on diabetes, restricting the generalization of other types of organic ED. Whether these findings are associated with other types of ED will be mined further. In addition, though qPCR was employed, the validation was not conclusive, and more molecular and animal experiments are needed. Also, the specific mechanisms of hub GRGs were not well explored by molecular experiments, which will be our future research direction.

Conclusions

In conclusion, we revealed the gene profiling and biological functions of GRGs in DMED via differentially expressed analysis and machine learning methods. Based on a multi-omics analysis, a hub gene signature, and associated miRNAs were identified and validated ultimately. These results broaden the understanding of glycolytic changes and guide the promising treatments in DMED.

Acknowledgments

The authors would like to thank the associated databases for the availability of the data.

Footnote

Reporting Checklist: The authors have completed the TRIPOD reporting checklist. Available at https://tau.amegroups.com/article/view/10.21037/tau-2025-6/rc

Data Sharing Statement: Available at https://tau.amegroups.com/article/view/10.21037/tau-2025-6/dss

Peer Review File: Available at https://tau.amegroups.com/article/view/10.21037/tau-2025-6/prf

Funding: This study was supported by the grant from the National Natural Science Foundation of China (No. 82201775) and the Medical Science and Technology Research-related joint construction project of Henan Province (No. LHGJ20220343).

Conflicts of Interest: All authors have completed the ICMJE uniform disclosure form (available at https://tau.amegroups.com/article/view/10.21037/tau-2025-6/coif). The authors have no conflicts of interest to declare.

Ethical Statement: The authors are accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved. The study was conducted in accordance with the Declaration of Helsinki (as revised in 2013). Experiments were performed under a project license (No. 2019006) granted by institutional ethics board of Experimental Animal Administration Committee of Wuhan Servicebio Biotechnology, China, in compliance with Servicebio institutional guidelines for the care and use of animals.

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Cite this article as: Deng W, Cao H, Sun T, Yuan P. Exploring the role of glycolysis in the pathogenesis of erectile dysfunction in diabetes. Transl Androl Urol 2025;14(3):791-807. doi: 10.21037/tau-2025-6