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ORIGINAL RESEARCH

Association Between Proteasome 26S Subunit, Non-ATPase 3 Methylation and Insulin β Cell Apoptosis in Type 2 Diabetic Mellitus

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Background: The methylation of *PSMD3* and its influence on protein stability and degradation could play a crucial role in the pathogenesis of type 2 diabetes mellitus (T2DM), although the underlying molecular mechanisms are not yet fully understood. This study investigates the molecular and bioinformatic features of *PSMD3* methylation in T2DM.

Methods: Bioinformatics analyses were conducted on the T2DM database chip. A model of T2DM was established in rat RIN-m5F cells induced by high glucose (HG) concentration. The function of the *PSMD3* gene in T2DM was examined through its overexpression. Western blotting was used to detect the expression of *PSMD3* and USP14 proteins. Flow cytometry was used to detect cell apoptosis and proliferation.

Results: Methylation of *PSMD3* was upregulated in the T2DM tissue microarray data and associated with USP14. *PSMD3* overexpression reduced apoptosis and enhanced proliferation in HG-treated RIN-m5F cells. In HG-treated RIN-m5F cells, *PSMD3* was linked to USP14 inactivation.

Conclusion: *PSMD3* methylation might potentially influences cell apoptosis and proliferation in T2DM development which might be associated with activating USP14. This study offers an in-depth examination of *PSMD3* methylation's molecular and bioinformatic traits in T2DM, advancing our comprehension of the molecular mechanisms leading to T2DM.

Keywords: proteasome 26S subunit, non-ATPase 3, methylation, insulin β cell, apoptosis, type 2 diabetic mellitus

Introduction

Type 2 diabetes mellitus (T2DM) is a rapidly increasing global disease that presents significant challenges to healthcare systems worldwide. This complex metabolic disorder is characterized by insulin resistance and a progressive decline in functional pancreatic β-cell mass, primarily due to impaired insulin secretion and increased β-cell apoptosis. The pathogenesis of β-cell dysfunction involves intricate interactions between genetic predisposition and environmental factors. Epigenetic modifications, particularly DNA methylation, provide a crucial mechanistic link between environmental cues and gene expression regulation in T2DM. Hypermethylation in gene promoter regions is generally associated with transcriptional silencing, while hypomethylation can promote gene expression.[1] Notably, methylation states of genes critical for β-cell identity and survival are often dysregulated. For instance, methylation of the PDX-1 gene promoter impairs β-cell proliferation and function in T2DM models,[2] and abnormal methylation of the CDKN2A/B locus is strongly linked to β-cell dysfunction and diabetes risk.[3] Despite these advances, the specific epigenetic mechanisms governing β-cell loss, particularly through the regulation of key cellular processes like proteostasis, remain incompletely understood.

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The ubiquitin-proteasome system (UPS) is the primary pathway for controlled intracellular protein degradation.[4] The 26S proteasome, the catalytic core of the UPS, comprises a 20S core particle and regulatory 19S particles. *PSMD3* (Proteasome 26S Subunit, Non-ATPase 3) is a vital component of the 19S regulatory cap, playing a non-enzymatic but essential role in substrate recognition, deubiquitination, and translocation into the 20S core for degradation. PSMD3 dysregulation has been implicated in various pathologies.[5] Critically, genetic variants in *PSMD3* demonstrate significant associations with insulin resistance across diverse populations, suggesting a fundamental role in metabolic regulation.[6] However, the potential role of *PSMD3* methylation in T2DM pathogenesis, specifically concerning β-cell proliferation and survival, remains virtually unexplored.

Furthermore, the functional integrity of the proteasome is dynamically regulated by associated proteins, including deubiquitinating enzymes (DUBs). USP14 (Ubiquitin Specific Peptidase 14) is a major DUB reversibly associated with the 19S regulatory particle, alongside *PSMD*3.[7] Dysregulation of USP14 has been linked to metabolic stress responses.[8] Given the critical role of proteostasis in β-cell health and survival under diabetic stress conditions,[9] and the close physical and functional association between *PSMD3* and USP14 within the 19S regulatory particle, it is plausible that alterations in *PSMD3* (potentially driven by DNA methylation) could impact USP14 function, thereby disrupting proteasome activity and contributing to β-cell dysfunction and apoptosis in T2DM.

Therefore, this study aims to investigate the role of *PSMD3* methylation in regulating β-cell proliferation and apoptosis, and to explore its functional interplay with USP14 in the context of T2DM development. The expression of *PSMD3* methylation in T2DM was examined using bioinformatic analysis to gain a clearer understanding of its role. We investigated the link between *PSMD3* and the signaling pathway in high glucose-stimulated RIN-m5F cells to explore the molecular mechanisms of T2DM.

Materials and Methods

Bioinformatics Analysis

Data Download

We initially used the GEO database (GSE29226) based on 24 subcutaneous fat biopsies (three biological replicates and four technical replicates) from three T2DM patients and three non-diabetic patients[10] to forecast *PSMD3* mRNA expression and the association between its expression and T2DM through the limma package in R.[11] Methylation of *PSMD3* was simultaneously analyzed using another public data (GSE38291) based on 10 subcutaneous adipose tissue from five T2DM patients and five non-diabetic patients[12] through the ChAMP package in R[13] to investigate the correlation between its methylation levels and mRNA expression as well as T2DM.

DEGs

To assess the impact of the *PSMD3* gene on diabetic nephropathy (DN), samples were categorized into high and low expression groups according to *PSMD3* gene expression levels. The limma package in R was utilized to analyze differentially expressed genes (DEGs). The differential methylation analysis was performed using the ChAMP R package. DEGs were identified with criteria of log fold change (logFc) > 2.0 and adjusted *P*-value < 0.05.

Gene-Set Enrichment Analysis (GSEA)

GO analysis is frequently employed in extensive functional enrichment research. GO analyses encompass biological processes, molecular functions, and cellular components. The KEGG database is extensively utilized for storing data on genes, biological pathways, diseases, and drugs.[14] The Clusterprofiler R package facilitated the analysis of GO and KEGG enrichment data associated with the Signature gene.[15] A threshold of FDR < 0.05 was considered statistically significant.

We conducted GSEA on the gene expression profiles of DN patients to examine variations in biological processes. GSEA assesses significant expression differences in specific genes and estimates pathway and biological process changes.[16] The c2.cp.kegg.v 6.2.- Symbols dataset was obtained from the MSigDB database for use in GSEA.A significance threshold was set at an adjusted *P*-value of less than 0.05. Genes associated with relevant pathways were

obtained from the GeneCard database.[17] Single-sample GSEA was used to calculate enrichment scores for samples across various pathways, and the correlation of *PSMD3* with different biological pathways was assessed.

Establishment of T2DM Model in HG-Treated RIN-m5F Cells

Cell Culture and Treatment

RIN-m5F cells were obtained from OriCell (Cyagen Biosciences, Guangzhou, China). RIN-m5F cells were cultured in RIN-m5F cell line complete medium (Cyagen Biosciences, Guangzhou, China) in an environment of 5% CO2 at 37°C. Cells at 80–90% confluence were digested with 0.25% trypsin-EDTA and either passaged at a 1:3 ratio or seeded into 6-well plates.

PSMD3 Overexpression and the USP14 Inhibitor

To explore the role of *PSMD3* in T2DM, the *PSMD3* gene was overexpressed. Constructs for *PSMD3* overexpression vectors were created, and all plasmid constructs underwent sequence verification.

RIN-m5F cells, at passages 3 to 10, were seeded into 6-well plates at a density of approximately 2×10^5 cells per well. Cells were categorized into six groups: control (normal glucose, 5.5 mmol/L D-glucose); high glucose (HG) exposure for 24 hours (25 mmol/L D-glucose);[18] plasmid vector transfection followed by 24-hour HG treatment; *PSMD3* plasmid transfection followed by 24-hour HG treatment; plasmid vector transfection with IU1 negative and 24-hour HG treatment; and *PSMD3* plasmid transfection with IU1 (T6107, Target Mol, Wellesley Hills, MA) and 24-hour HG treatment. Plasmid transfection was conducted using Lipofectamine 2000 (Invitrogen Corporation, Carlsbad, CA). Twenty-four hours post-transfection, the medium was replaced with HG Dulbecco's modified Eagle's medium (25 mmol/L D-glucose; Gibco, Grand Island, NY, USA) containing 10% fetal bovine serum.

Western Blotting

Cell pellets from each experimental group underwent three washes with ice-cold phosphate-buffered saline before being lysed in RIPA buffer (MCE, New Jersey, USA) containing protease inhibitors. The lysis procedure was carried out on ice for 30 min with periodic agitation. Lysates were obtained by scraping and then clarified through centrifugation at 12,000 × g for 15 minutes at 4°C. Supernatants containing protein were aliquoted and stored at −80°C for later analysis.

Proteins (50μg) were equally loaded, separated via SDS-PAGE, and transferred to 0.22μm polyvinylidene difluoride (PVDF) membranes using a GenScript semi-dry transfer system. Membranes were incubated in protein-free blocking buffer (*Epizyme*, Shanghai, China) for 10 minutes at room temperature. Immunodetection utilized primary antibodies: anti-*PSMD3* (1:2,000, 12,054-1-AP, ProteinTech, Wuhan, China), anti-USP14 (1:2,000, ProteinTech, Wuhan, China), and β-actin (1:1,000, ProteinTech, Wuhan, China) as a loading control. Membranes were incubated with primary antibodies overnight at 4°C, then washed three times with TBST (0.1% Tween-20; Solarbio Life Sciences, Beijing, China). Detection was performed using a horseradish-peroxidase-conjugated goat anti-rabbit secondary antibody (1:8,000; Jackson Immuno Research, West Grove, PA, USA) with a 1-hour incubation at room temperature. The PSMD3 band (61kDa), USP14 band (54kDa) and β-actin band (42kDa) were separated according to prestained SDS-PAGE protein marker. Following three more TBST washes, protein bands were visualized with an enhanced chemiluminescence detection system (GeneCopoeia, Rockville, USA) and normalized to β-actin. The Western blotting results were quantified through densitometric analysis using ImageJ software.

Annexin V/7-Aminoactinomycin D (7-AAD) Staining for Assessing Apoptosis

A dual-staining approach utilizing annexin V and 7-AAD was used (BD PharMingen, San Diego, CA, USA). Cell suspensions were labeled with 5 μL annexin V and 10 μL 7-AAD, then incubated for 15 minutes at room temperature in the dark. Fluorescence analysis was subsequently performed using a CytoFlex flow cytometer (Beckman Coulter Inc., Brea, CA, USA) within a 2 h window post-staining to ensure optimal signal detection.

This method leveraged the specific binding properties of annexin V to externalized phosphatidylserine on the cell membrane (a hallmark of early apoptotic cells), while 7-AAD served as a nuclear stain that permeated compromised membranes in late-stage apoptotic and necrotic populations. These markers facilitated the distinction of viable, early apoptotic, and late apoptotic/necrotic cell populations via flow cytometry.

PI Staining to Assess the Cell Cycle

Propidium (PI), a fluorescent nucleic acid stain, exhibits enhanced emission properties upon intercalation into double-stranded DNA, with its fluorescence intensity directly correlating with cellular DNA content. For cell cycle analysis, cellular suspensions were processed according to the standardized protocol (Absin, Shanghai, China). Cell pellets were fixed in 75% ethanol at 4°C for 12–16 hours. Post-fixation, cells were permeabilized and stained with a PI solution containing RNase A for specific DNA labeling. The staining reaction was carried out at ambient temperature under light-protected conditions for 30 min. The fluorescence intensity distribution was measured using a CytoFlex flow cytometer (Beckman Coulter Inc., Brea, CA, USA). DNA histograms were analyzed to ascertain the relative proportions of cells in the G0/G1, S, and G2/M cell cycle phases.

Statistical Analysis

Statistical data analysis was conducted using R software (version 4.0.2). An independent samples Student's *t*-test was conducted to assess the statistical significance of variable distributions and compare continuous variables between two groups. The Mann–Whitney *U*-test, also known as the Wilcoxon rank-sum test, was employed to assess differences in variables that do not follow a normal distribution. Pearson's correlation analyses were employed to calculate correlation coefficients between genes. All *P* values were two-tailed, with significance set at $P < 0.05$.

Results

Analysis of PSMD3 Expression and DNA Methylation in Patients with Type 2 Diabetes Mellitus (T2DM)

The mRNA levels of *PSMD3* were significantly downregulated in patients with T2DM (*t*-test, P=0.002) (Figure 1A). Conversely, DNA methylation of *PSMD3* was markedly upregulated in T2DM patients (*t*-test, P=0.0462) (Figure 1B). These findings suggested that expression of *PSMD3* was suppressed in T2DM patients, potentially due to epigenetic regulation, such as DNA methylation. The increased methylation of *PSMD3* DNA may have contributed to the observed reduction in its mRNA level, indicating a possible mechanism for the dysregulation of *PSMD3* function. This epigenetic alteration could be implicated in the pathophysiology of T2DM, highlighting the role of *PSMD3* in the disease process.

Correlation Between PSMD3 Expression and Signaling Pathways

Patients with T2DM were categorized into high and low *PSMD3* expression groups for GSEA analysis (Figure 1C and D). GSEA identified significant enrichment of apoptosis and cell cycle KEGG signaling pathways in groups categorized by *PSMD3* expression levels. These findings suggested that *PSMD3* played a regulatory role in apoptosis and cell cycle processes in T2DM, highlighting its potential involvement in the molecular mechanisms underlying the disease. The key enriched genes identified by GSEA are shown in Figure 2A and B, respectively. These genes were associated with the significantly enriched KEGG pathways, namely apoptosis and cell cycle, which were highlighted in the GSEA results. As the GSEA was performed by comparing *PSMD3*-high vs *PSMD3*-low expression groups, these enriched genes might represent molecular signatures co-varying with *PSMD3* expression. This co-expression pattern might reveal potential interacting partners or co-regulators that may operate within the same biological module.

PSMD3 Promotes Apoptosis of T2DM Cells and Is Associated with USP14 Expression

Figure 3A and B verifies the expression of *PSMD3* and USP14 in HG-treated RIN-m5F cells. RIN-m5F cells were transfected with a *PSMD3* overexpression plasmid. Original images for Figure 3A could be found from supplement. In HG-treated RIN-m5F cells, *PSMD3* was associated with USP14 inactivation. HG treatment significantly reduced the expression of *PSMD3* and USP14 in RIN-m5F cells (*t*-test, P<0.01). We employed flow cytometry to assess the impact of *PSMD3* overexpression on cell proliferation and apoptosis by analyzing the cell cycle and apoptosis in RIN-m5F cells (Figure 4A). Overexpression of *PSMD3* enhanced apoptosis (*t*-test, P<0.01), which was further increased by the USP14 inhibitor IU1 (*t*-test, P<0.01) (Figure 4B). *PSMD3* overexpression led to more cells in the G2 phase, enhanced proliferation, and reduced apoptosis (Figure 4C and D).

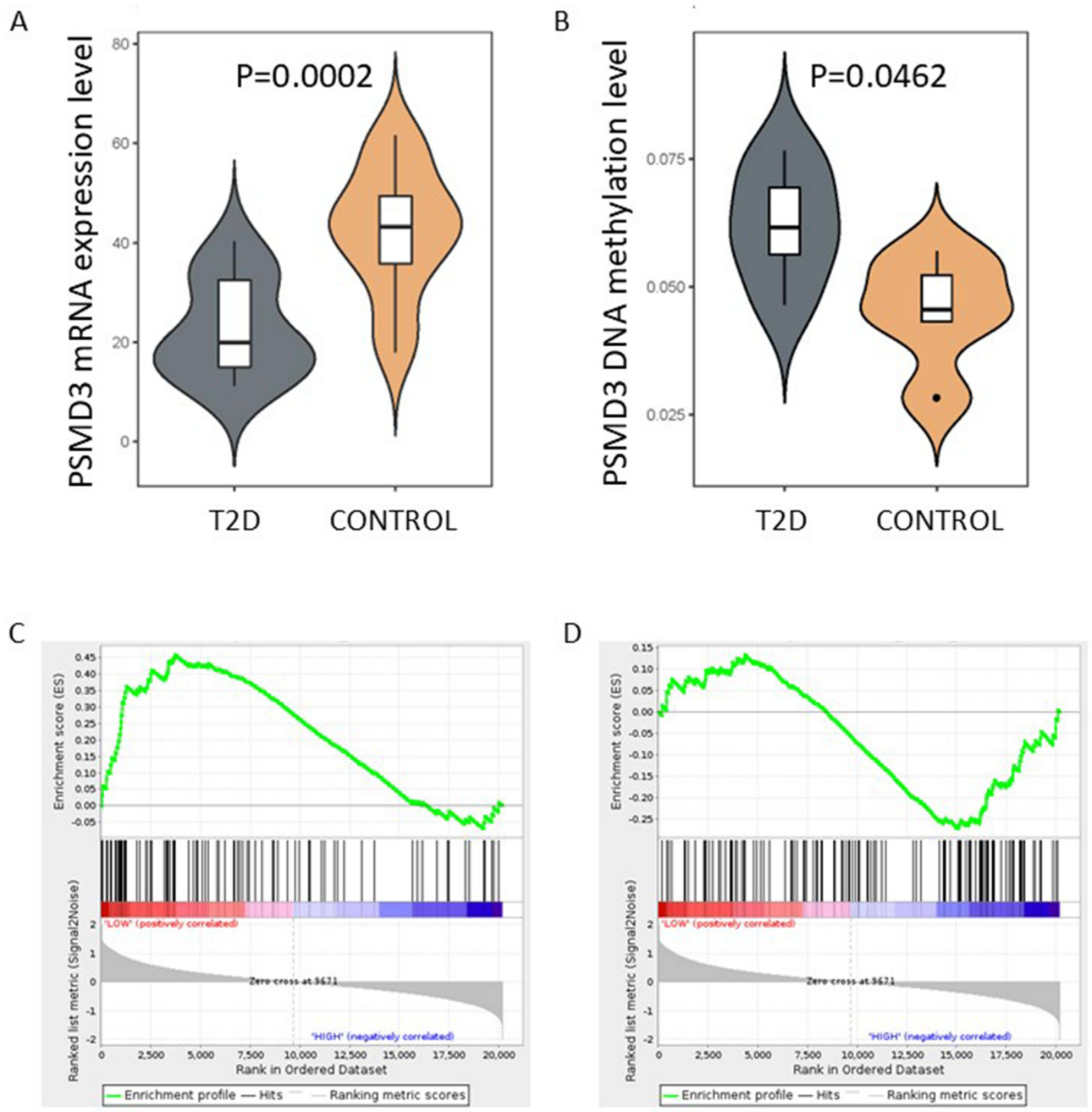

Figure 1 The mRNA expression and DNA methylation of *PSMD3* in T2DM patients, and GSEA of KEGG terms with *PSMD3*. (**A**) The mRNA expression of *PSMD3* in T2DM patients (*t*-test,). (**B**) DNA methylation of *PSMD3* in T2DM patients (*t*-test,). (**C**) GSEA of the apoptosis signaling pathway. (**D**) GSEA of the cell cycle signaling pathway.

Discussion

Type 2 Diabetes Mellitus (T2DM) is a chronic metabolic disorder marked by insulin resistance and pancreatic β-cell dysfunction, leading to persistent hyperglycemia and subsequent microvascular and macrovascular complications, including cerebral infarction and diabetic retinopathy. The etiology of T2DM is complex and involves genetic, environmental, and lifestyle factors. The relationship between T2DM and DNA methylation has been one of the hot topics in recent years. Methylation, a crucial epigenetic modification, potentially plays a significant role in the development of T2DM.The study found that some single nucleotide polymorphisms and methylation sites of the KCNQ1 gene were significantly associated with the occurrence of T2DM.[19] A study identified a link between methylation levels in the

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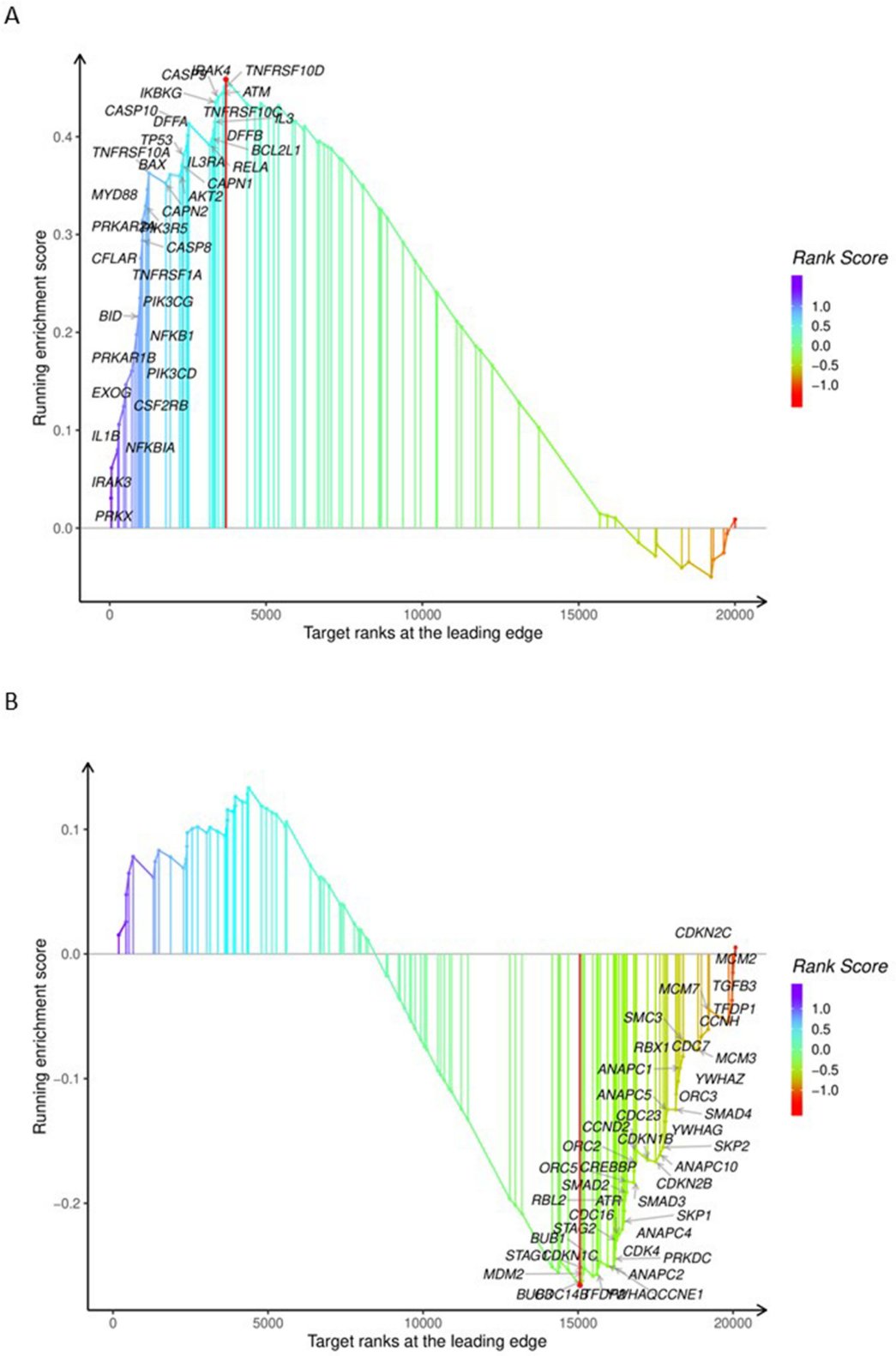

Figure 2 Gene Set Enrichment Analysis (GSEA) for varying *PSMD3* expression levels in Type 2 Diabetes Mellitus (T2DM) patients. (**A**) Key enriched genes identified from GSEA of the apoptosis signaling pathway. (**B**) Key enriched genes identified from GSEA of the cell cycle signaling pathway.

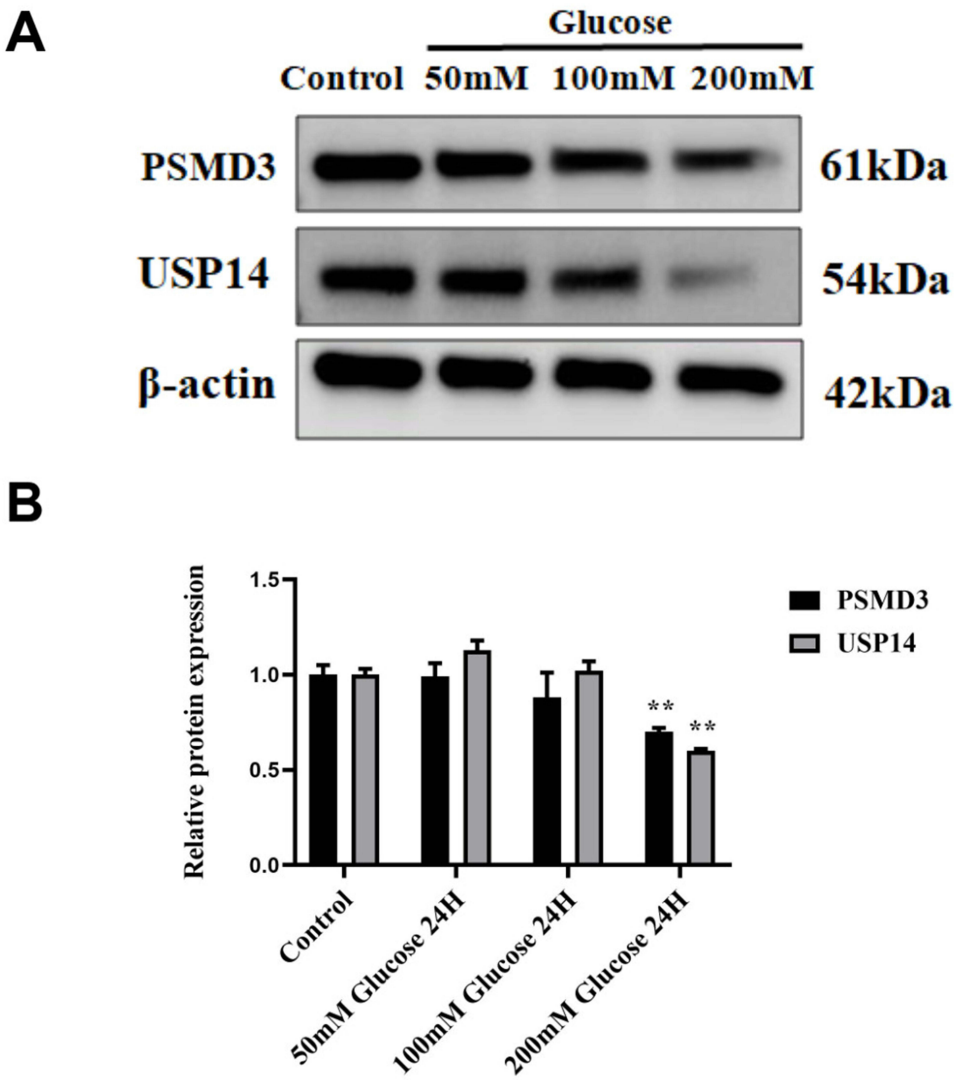

Figure 3 The expression of *PSMD3* and USP14 in HG-treated RIN-m5F cells. (**A**) In RIN-m5F cells exposed to high glucose for 24 hours, *PSMD3* was associated with the inactivation of USP14. HG treatment significantly reduced the expression of *PSMD3* and USP14 in RIN-m5F cells. (**B**) Densitometry was used to quantify protein expression. Mean ± SD; n = 3; ** *t*-test, P < 0.01 versus the control group. Western blot analysis was conducted for *PSMD3* (61 kDa), USP14 (54 kDa), and β-actin (42 kDa).

promoter regions of the NLRP3, AIM2, and ASC genes and type 2 diabetes mellitus (T2DM) along with its vascular complications. Reduced methylation in these genes may elevate the risk of T2DM and its complications.[20] However, hypermethylation in Cg12869254 and cg04026387 may complement the known risk factors that contribute to the pathogenesis of diabetic retinopathy.[21] In our study, the mRNA levels of *PSMD3* were significantly downregulated in patients with T2DM. Conversely, the DNA methylation levels of *PSMD3* were markedly upregulated in T2DM patients. HG treatment led to reduced *PSMD3* expression in RIN-m5F cells.

PSMD3 is essential for protein degradation and signal transduction, influencing various biological processes. *PSMD3* is associated with the occurrence and development of various cancers. In chronic myeloid leukemia cells resistant to tyrosine kinase inhibitors, *PSMD3* expression is elevated. Reducing *PSMD3* expression decreases cell survival and enhances apoptosis.[22] *PSMD3* facilitates tumor progression in lung adenocarcinoma by modulating the TGF-β/SMAD signaling pathway. The *PSMD3* gene is located in the 17q12–17q21.1 chromosomal region, which is associated with susceptibility to adult asthma. Single nucleotide polymorphisms in this region are significantly linked to the development of asthma, especially when in the combination of *PSMD3*, CSF3, and MED24 genes.[23] Certain polymorphisms in the *PSMD3* gene can influence high-density lipoprotein cholesterol

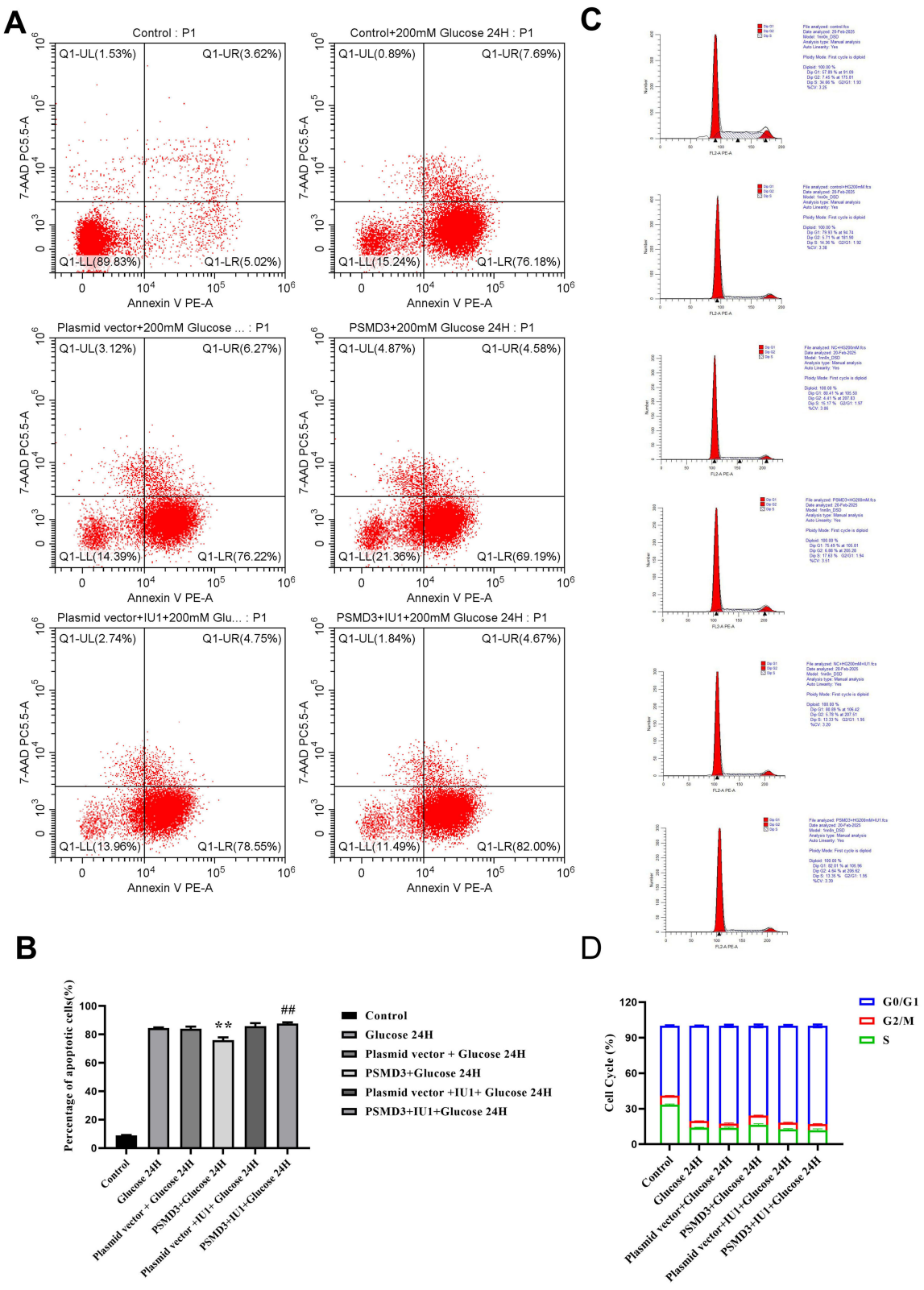

Figure 4 The impact of *PSMD3* overexpression on cell proliferation and apoptosis by analyzing the cell cycle and apoptosis in RIN-m5F cells. *PSMD3* overexpression led to more cells in the G2 phase, enhanced proliferation, and reduced apoptosis. Overexpression of *PSMD3* enhanced apoptosis, which was further increased by the USP14 inhibitor IU1. (**A**) Flow cytometry results of apoptosis detection. Apoptotic cell percentages were assessed using annexin V/7-AAD staining. (**B**) Statistical results of percentage in apoptosis cells. (**C**) Flow cytometry results of cell cycle detection. (**D**) Statistical results of percentage in cell cycle. Mean ± SD; n = 3; ** *t*-test, $P < 0.01$ compared to the HG-treated group for 24 h; ## *t*-test, $P < 0.01$ compared to the *PSMD3* overexpression group treated with HG for 24 h.

levels and elevate the risk of both macrovascular and microvascular complications.[24] *PSMD3* is associated with insulin signaling, and its genetic variations may influence the development of insulin resistance.[6]

After incubating RIN-m5F cells with HG for 24 hours, there was an increase in apoptotic cells compared to the control group. *PSMD3* overexpression enhanced cell proliferation and reduced apoptosis. In HG-treated RIN-m5F cells, *PSMD3* was associated with USP14 inactivation.

USP14 is a deubiquitinase integral to numerous signaling pathways, affecting protein degradation and stability through its regulation of deubiquitination, and thus contributing to diverse biological processes. USP14 promotes the growth and metastasis of liver cancer cells by interacting with the AKT and epithelial–mesenchymal transition signaling pathways.[25] USP14 suppresses brain metastasis in non-small cell lung cancer by regulating the PI3K/AKT/mTOR signaling pathway.[26] In the nervous system, USP14 influences learning and memory processes by regulating long-chain memory formation.[27] Dysregulation of USP14 has been linked to metabolic stress responses.[8] USP14 influences oxidative stress and inflammation in diabetic retinopathy via the NF-κB signaling pathway.[28] USP14 is also a major DUB reversibly associated with the 19S regulatory particle, alongside *PSMD*3.[7]

PSMD3 and USP14 are both important proteins associated with protein degradation and cell signaling. In this study, *PSMD3* potentially affects cell cycle and apoptosis pathways through its interaction with USP14, contributing to the pathology of T2DM. Apoptosis is a crucial mechanism in the pathological process of diabetes. The hyperglycemic environment in diabetic patients can induce activation of apoptosis signaling pathways, leading to apoptosis and loss of function of pancreatic β cells.[29] Oxidative stress is one of the primary factors inducing apoptosis, directly causing cell death by activating apoptosis-related proteins such as caspase-3 and BAX.[30,31] Besides, *PSMD3* and USP14 are crucial deubiquitinating enzymes involved in maintaining protein homeostasis and cellular signaling. USP14 is overexpressed in non-small cell lung cancer, especially in adenocarcinoma cells. USP14 overexpression correlates with reduced patient survival and enhances tumor cell proliferation by increasing β-catenin levels. Downregulation of USP14 leads to cell cycle arrest, which may be related to the degradation of β-catenin.[32] *PSMD3* promotes lung cancer cell proliferation, migration, and invasion.[33] The functions of *PSMD3* and USP14 in tumors may affect tumor cell proliferation and apoptosis by regulating protein homeostasis and signaling pathways.

The regulatory relationship between *PSMD3* and USP14 is primarily indirect and structural, mediated through the 19S proteasome complex. *PSMD3* is essential for building a functional Lid complex.[34] A stable Lid is a prerequisite for Rpn1 to adopt its correct conformation and effectively recruit and position USP14.[35] Dysfunction or depletion of *PSMD3* likely impairs USP14's association and/or activity by destabilizing its essential platform (the Lid/Rpn1 complex).[36] Both molecules influence the proteasome's ability to degrade proteins targeted by USP14.[37] *PSMD3* dysfunction broadly impairs proteasome function, while USP14 activity selectively regulates the degradation of specific ubiquitinated substrates (including apoptotic regulators). Their combined dysfunction could synergistically disrupt protein homeostasis and apoptotic signaling.[38] To date, there is no strong evidence for direct physical interaction or specific enzymatic regulation between *PSMD3* and USP14. The regulation appears contextual within the proteasome holoenzyme. However, comparable outcomes were observed in RIN-m5F cells treated with HG in this study. *PSMD3* overexpression led to an increase in G2/M phase cells, enhanced proliferation, and reduced apoptosis. The overexpression of *PSMD3* enhanced apoptosis when treated with the USP14 inhibitor IU1.These findings suggest that *PSMD3* might serve as a therapeutic target by influencing apoptosis and proliferation in T2DM. However, how *PSMD3* and USP14 regulate and promote apoptosis remains to be further studied.

While our findings offered insights into the molecular and bioinformatic aspects of *PSMD3* methylation and enhanced our understanding of the molecular mechanisms underlying T2DM, certain limitations remained. Initially, due to small-sample subcutaneous adipose tissue bioinformatics results and experimental results of insulin β cell, various clinical factors need assessment to elucidate the regulatory mechanism of *PSMD3* methylation in T2DM. Second, even though significant associations were found, independent cohort validation from other public databases is needed. Third, these results should be verified by in vivo experimental studies (such as animal models) to explore how *PSMD3* methylation and USP14 regulate and participate in precise signaling pathways and interaction mechanisms.

Conclusion

This study is the first to offer a comprehensive analysis of the molecular and bioinformatic features of *PSMD3* methylation in T2DM. Using GEO database data, we assessed *PSMD3* expression in T2DM and found that functional enrichment analysis revealed significantly reduced *PSMD3* methylation levels in T2DM patients. In HG-treated RIN-m5F cells, *PSMD3* overexpression decreased apoptosis and enhanced cell proliferation, effects that were counteracted by the USP14 inhibitor IU1. In summary, our integrated analysis suggests that *PSMD3* methylation might potentially influences cell apoptosis and proliferation in T2DM development which might be associated with activating USP14. While this correlation requires mechanistic validation, it highlights a novel targetable pathway in diabetes pathophysiology.

Data Sharing Statement

The data that support the findings of this study are openly available in GEO database at https://www.ncbi.nlm.nih.gov/geo/, reference number GSE29226 and GSE38291.

Ethics Approval and Consent to Participate

The human transcriptome data of this study were derived from GEO database (No. GSE29226). The methylation levels were derived from public data (GSE38291). The Ethics Review Committee of Guangdong Provincial People's Hospital approved the use of public databases for this study (No. GDERC: KY-Q-2021-244-01).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors confirm that they have no conflict of interest.

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