More potential risk factors for implant in patients with type 2 diabetes were detected by proteomics in addition to hyperglycemia.

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Research

Keywords: Type 2 diabetes, dental implants, bone marrow mesenchymal stem cells, osseointegration, proteomics

Posted Date: March 11th, 2020

DOI: https://doi.org/10.21203/rs.3.rs-16788/v1

Abstract

Background: It is commonly accepted glycemic control can decrease the negative effects of implant in type 2 diabetes mellitus (T2DM) patients. Whether the remaining pathological changes besides hyperglycemia caused by T2DM will affect implant-bone integration during the healing period has remained unclear. This study aims to determine whether other risk factors besides hyperglycemia lead to failed osseointegration in T2DM patients during healing period.

Methods: First, we compared the success rate between T2DM patients and non-T2DM patients during the healing period at our center. Bone marrow mesenchymal stem cells (BMSCs) were cultured from all enrolled subjects. Then, proteomics was used to detect differentially expressed proteins (DEPs) among the DM failure (DM-F), DM success (DM-S) and control (Con) groups. Additionally, the relationship between expression of the nine target DEPs and glucose concentration in media was investigated.

Results: Significantly higher failure rates in T2DM patients were found. Fifty-two DEPs were found in DM-F group compared with DM-S group. Seventy-three DEPs were found in DM-F group compared with the Con group. Forty-three DEPs were found in DM-S group compared with Con group. Four target DEPs was influenced by glucose, while the other five expressed the same in different glucose concentration media. DEPs in DM-F group may affect the biological function and regulatory potential of BMSCs through gene ontology annotation and functional enrichment analysis.

Conclusions: DEPs in DM-F group can be potential risk factors and intervention targets for dental implant in T2DM patients. More potential risk factors affect implant-bone integration besides hyperglycemia.

Background:

Diabetes mellitus is a group of metabolic diseases characterized by hyperglycemia[1]. Diabetes is recognized as a risk factor for implant therapy. However, common opinion holds that well-controlled type 2 diabetes mellitus (T2DM) should be excluded from risk factors[2 – 4]. Such opinion implies that hyperglycemia plays a key role in failed implantation in diabetics. Bone marrow mesenchymal stem cells (BMSCs) are multilineage differentiation potential cells that can be induced into osteoblasts and other stem cells. BMSCs play a substantial role in the process of osseointegration around implants. BMSCs migrate to the sites of titanium implants and differentiate into osteoblasts[5]. However, the proliferation, differentiation, migration and mineralization ability of BMSCs are impaired by high-glucose media[6 – 7]. Thus, glycemic control is an important intervention to reduce the adverse effects of implant in clinic. Some scholars have even considered that satisfactory glycemic control could eliminate the adverse effect on implant success rate in T2DM patients[8]. Unfortunately, few studies of the relationship between glycemic control and implant success in T2DM patients have been conducted. Thus, the question arises: does glycemic control reduce or eliminate the adverse effect of T2DM on implant success? In other words, are there factors other than blood glucose that affect implant-bone integration in T2DM patients?

To answer these questions, we carried out a clinical study to determine the implant success rate in the healing period of well-controlled T2DM patients and non-T2DM patients at our center and used proteomics to detect protein expression profiles in diabetics with failed osseointegration, T2DM patients with successful osseointegration, and non-T2DM patients with successful osseointegration. The differentially expressed proteins (DEPs) were then identified. The relationship between the expression of partial DEPs and glucose concentration in culture media were studied. Gene ontology (GO) annotation and functional enrichment analysis were conducted to determine the impaired biological functions of BMSCs derived from T2DM patients with failed osseointegration.

Methods

Subject enrollment and ethics statement

The present study was approved by the Ethics Committee of Beijing Stomatological Hospital, School of Stomatology, Capital Medical University (Approval No.: CMUSH-IRB-KJ-PJ-2018-08) with informed patient consent. All subjects were recruited by one surgeon in the department of Dental Implant Center. All patients with controlled T2DM (HbA1c < 8%) and ready for implant surgery in the past three years were enrolled as study subjects (T2DM group). Subjects in the non-T2DM group were enrolled at a ratio of 2:1 compared to the T2DM group (non-T2DM group), and all basic information was matched to avoid the influence of other elements, including age, sex, general health condition, DM type, implant system, and implant position. Implant sites were left to heal for at least three months after tooth extraction. Subjects with implants inserted with guided bone regeneration were excluded from the study. Patients with contraindications for implant surgery, such as cardiovascular disorders, renal diseases or uncontrolled periodontitis were excluded. The STROBE (Strengthening the Reporting of Observational studies in Epidemiology) guidelines were followed to ensure the rigor of our study (Appendix Table 1).

Table 1
General characteristics and implant failure rate of the T2DM group and the non-T2DM group.

Parameters	T2DM group	Non-T2DM group	P value
Individuals (numbers)	38	76	–
Implants (numbers)	130	133	–
Gender(male/ female)	29/9	58/18	1.0
Mean age (years)	58.5 ± 9.26	56.3 ± 9.32	0.252
Duration of T2DM (years)	8.33 ± 5.03	–	–
Failed individuals(numbers)	9	1	–
Failed implants (numbers)	14	1	–
Implant failure rate (%)	10.77	0.75	0.000**
** denotes p-value < 0.01			

Implant placement and bone chip harvest and cell culture

All implant surgeries in the present study were performed by one surgeon. Implants were inserted with the method as previously described[9]. Bone chips were harvested during implant socket preparation and were reserved in phosphate-buffered saline (PBS, Gibco, USA) with antibiotics (10,000 U/ml penicillin, 10 mg/ml streptomycin, Gibco, USA). After being centrifuged at 1100 rpm, the bone chips were resuspended in mesenchymal stem cell medium (MSCM, ScienCell, USA), seeded in dishes and then cultured in a humidified, 5% CO2 cell incubator at 37 °C without movement for seven days. A 70-μm pore-size strainer (Falcon, BD Labware, USA) was used to obtain single-cell suspensions, which were then cultured in MSCM. The medium was replaced every three days. The fourth-passage BSCMs were used for subsequent experiments.

Clinical assessments

The healing periods for the upper and lower jaw were six months and three months, respectively. Before implant loading, failed implants, defined as implants with clinical mobility and peri-implant radiolucency, were recorded to determine the failure rate and to perform whole-cell proteomic analysis.

Grouping for whole-cell proteomic analysis

The isolated BMSCs were divided into three groups: BMSCs derived from the T2DM patients with failed implant (DM-F group), BMSCs derived from T2DM patients with successful implant (DM-S group), and BMSCs derived from the non-T2DM patients with successful implant (Con group). Five subjects from the DM-F group were selected randomly for proteomic analysis with each from the DM-S and Con groups,

respectively, were also selected for proteomics and were matched to the five subjects selected from the DM-F group according to all the basic information mentioned above.

Whole-cell quantitative proteomic analysis

Tandem mass tagging (TMT)-based proteomic analysis was performed as previously described[10]. Proteins from 15 BMSC samples from the DM-F (five samples), DM-S (five samples) and Con (five samples) groups were extracted and digested enzymatically. The peptides obtained were desalted, vacuum-dried, reconstituted and processed according to the TMT kit manufacturer's protocol. The tryptic peptides were first separated and then combined by high-pH reverse-phase high-performance liquid chromatography (HPLC). Then, the peptides were subjected to a nanospray ionization (NSI) source, followed by tandem mass spectrometry (MS/MS) in an Orbitrap FusionTM Lumos (Thermo) system linked online to the ultra-performance liquid chromatography (UPLC) system.

A MaxQuant search engine (v.1.5.2.8) was used to analyze the resulting MS/MS data. The false-discovery rate (FDR) was adjusted to < 1%. GO annotation and functional enrichment analysis were conducted and the proteins were divided into three categories, including biological process, cellular component and molecular function. For each category, a two-tailed Fisher's exact test was used to determine the enrichment of the differentially expressed proteins (DEPs) against all identified proteins. GO results with a corrected p-value < 0.05 were considered significant. The proteomics data have been deposited to the ProteomeXchange Consortium with the dataset identifier PXD016489.

Western Blot analysis

RIPA buffer (Sigma, USA) was used to lyse cells. A 15% SDS polyacrylamide gel was used to isolate the samples, which were then transferred to polyvinylidene difluoride (PVDF) membranes (Bio-Rad, USA) using a semidry transfer apparatus (Bio-Rad, USA). The membranes were blocked using 5% dehydrated milk for 1 h and then incubated with primary antibodies overnight. Horseradish peroxidase-conjugated anti-rabbit IgG (Abcam, UK) were utilized to detect the immune compounds and samples viewed with SuperSignal reagents (Bio-Rad, USA). The information for primary antibodies, including apolipoprotein (APOE), fibulin-1 (FBLN1), glutamine synthetase (GLUL), integrin alpha-10 (ITGA10), matrix metalloproteinase 2 (MMP2), transgelin (TAGLN), insulin-like growth factor-binding protein 2 (IGFBP2), leptin receptor (LEPR), and atrial natriuretic peptide receptor 3 (NPR3), is shown in Appendix Table 2.

Table 2
Top 10 of DEPs in DM-F, DM-S and Con group.

Expression	DM-F/DM-S		DM-F/Con		DM-S/Con	
	Gene name	Fold change	Gene name	Fold change	Gene name	Fold change
Up- regulated	IFIT1	3.243	IGFBP2	3.034	VCAM1	3.93
	IFIT3	2.417	IFIT1	2.784	APOE	2.43
	MX1	2.411	MX1	2.455	CRISPLD2	2.15
	ERAP2	1.775	IFIT3	2.349	TNFRSF19	1.841
	OAS2	1.692	BCAR3	2.252	NPR3	1.736
	TAGLN	1.619	TNS3	1.885	SORBS2	1.729
	MSRB2	1.614	OAS2	1.846	LEPR	1.704
	DCBLD2	1.496	RALGAPA1	1.811	MMP2	1.682
	KANK1	1.45	RAB27B	1.79	RAB27B	1.658
	ALDH1B1	1.446	TAGLN	1.786	C10orf54	1.65
Down-regulated	APOE	0.478	GLUL	0.605	MASTL	0.643
	TMEM119	0.565	PLCB4	0.656	GSTT2B	0.669
	MMP2	0.571	FBLN1	0.676	BCAT1	0.738
	FBLN1	0.579	PDGFRA	0.697	MICAL1	0.755
	OR10G3	0.605	CCDC61	0.7	FMNL2	0.757
	CRISPLD2	0.611	FADS2	0.705	IGF2BP3	0.762
	PHF12	0.633	NDRG1	0.736	MOCS3	0.765
	CENPV	0.638	BCAT1	0.739	–	–
	ITGA10	0.655	FGFR1OP	0.741	–	–
	COL16A1	0.672	WNT5A	0.742	–	–

Statistical analysis

SPSS version 21.0 was used for statistical analysis. Data are presented as the mean ± SD, and Student's t test was used to determine significant differences. Significant differences in the failure rate and sex ratio were determined by a Chi-square test. A p-value < 0.05 was considered significant.

Results

Significantly higher implant failure rates in the T2DM group than in the non-T2DM group

114 patients (38 in the T2DM group and 76 in the non-T2DM group) with 263 implants (130 in the T2DM group and 133 in the non-T2DM group) were enrolled in the present study. In the T2DM group, 29 individuals were male, and nine individuals were female. The mean age of subjects was 58.5 years, ranging from 34 to 76 years. The mean duration of diabetes was 8.3 years, ranging from one to twenty years. Fourteen implants (nine individuals) failed in this group, yielding an implant failure rate of 10.77%. In the non-T2DM group, 58 individuals were male, and 18 individuals were female. The mean age of subjects was 56.3 years, ranging from 36 to 77 years. No significant difference was found between the T2DM and non-T2DM groups in sex or age, indicating that after matching, the basic conditions in the two groups were similar. However, only one implant (one individual) failed in the non-T2DM group, yielding an implant failure rate of 0.75%, compared to 10.77% in the T2DM group (Table 1). Compared to the non-T2DM group, the implant failure rate was significantly higher in the T2DM group.

Identification of DEPs among the DM-F, DM-S and Con groups

To further explore the probable key factors affecting the osseointegration of implants in T2DM patients, a whole-cell proteomic study was conducted to identify the protein expression profile of the three groups, i.e., the DM-F, DM-S, and Con groups. A total of 15 individuals from the three groups (five per group) were included in the analysis, and the DEPs were determined according to the fold change and p-value. Proteins with a fold change > 1.3 or < 1/1.3 and a p-value < 0.05 were considered DEPs. Among the 6987 identified proteins, 52 proteins were differently expressed, with 22 proteins upregulated and 30 proteins downregulated in the DM-F group compared to the DM-S group (DM-F/DM-S). Moreover, 73 proteins were differentially expressed, including 57 upregulated proteins and 16 downregulated proteins in the DM-F group compared to the Con group (DM-F/Con). In addition, 43 proteins were differentially expressed, with 36 proteins upregulated and seven proteins downregulated in the DM-S group compared to the Con group (DM-S/Con). The top 10 DEPs in each group are shown in Table 2.

To confirm the reliability of proteomic results, nine proteins were chosen from Table 2 to detect protein expression by Western blot as target DEPs, including apolipoprotein (APOE), fibulin-1 (FBLN1), glutamine synthetase (GLUL), integrin alpha-10 (ITGA10), matrix metalloproteinase 2 (MMP2), transgelin (TAGLN), insulin-like growth factor-binding protein 2 (IGFBP2), leptin receptor (LEPR), atrial natriuretic peptide receptor 3 (NPR3). The functions of the nine proteins were reported in association with cell proliferation, migration and osteogenesis[11 – 19]. Western blot results showed that all DEPs had the same expression tendency as the proteomics results in Table 2, except for MMP2 (Fig. 1A).

The relationship between the expression of target DEPs and glucose concentration

To determine whether the expression of DEPs was associated with glucose concentration, the fourth-passage BMSCs from the Con group were cultured in media with different glucose concentrations, including normal glucose concentration (1000 mg/L, NG group), high glucose concentration (4500 mg/L, HG(+) group), and ultra-high glucose concentration (9000 mg/L, HG(++) group) (DMEM, HyClone, USA) for seven days. The expression of GLUL is decreased by high glucose concentration, while the expression of LEPR, MMP2, and NPR3 are increased with ultra-high glucose concentration. The expression of APOE, FBLN1, IGFBP2, ITGA10 and TAGLN are independent of the medium's glucose concentration (Fig. 1B).

GO annotation of DEPs among the DM-F, DM-S, and Con groups

To characterize the function of the DEPs in the three groups, a bioinformatic analysis based on GO which are categorized as biological process, molecular function and cellular component was carried out.

In the biological process category, DEPs among the three groups mostly showed enrichment in cellular processes, single-organism processes, and biological regulation, reaching more than 35% (Fig. 2A). In the molecular function category, approximately 70% of DEPs showed enrichment in binding and catalytic activity in all three groups (Fig. 2B). The cellular component enrichment revealed that over 65% of the DEPs were cell-, organelle- and membrane-associated proteins (Fig. 2C).

GO functional enrichment of DEPs among the DM-F, DM-S, and Con groups

To further illuminate the specific functional classifications of DEPs, a functional enrichment analysis was conducted. In the biological process category, the upregulated DM-F/DM-S DEPs were obviously enriched in defense responses to viruses, cellular response to type I interferon, and the type I interferon signaling pathway. The molecular function enrichment terms included oxidoreductase activity, acting on a sulfur group of donors, glutathione disulfide oxidoreductase activity, and disulfide oxidoreductase activity, among other terms. The cellular component category showed enrichment in ruffle membrane, leading edge membrane, and mitochondrion, among other terms (Fig. 3A, left). In addition, the downregulated DM-F/DM-S DEPs were categorized. In the biological process category, DEPs showed enrichment primarily in the regulation of osteoblast proliferation, reproductive processes, reproduction, extracellular structure organization, extracellular matrix organization, and positive regulation of osteoblast proliferation, among other terms. In the molecular function category, DEPs showed enrichment in glycosaminoglycan binding, oxidoreductase activity, actin, heparin binding and integrin binding. In the cellular component category, DEPs showed enrichment in the extracellular matrix, among other terms (Fig. 3A, right).

In the biological process category, the upregulated DM-F/Con DEPs were enriched in their defense response to viruses, response to viruses, and defense response to other organisms, among other terms. Regarding molecular function, DEPs were enriched in heparin binding, sulfur compound binding, and glycosaminoglycan binding, among other terms. The cellular component showed enrichment in the membrane, plasma membrane region, and proteinaceous extracellular matrix (Fig. 3B, left). However, the downregulated DM-F/Con DEPs showed enrichment in the positive regulation of macrophage activation, positive regulation of fibroblast proliferation, and positive regulation of cell migration. Regarding molecular function, DEPs showed enrichment in fibrinogen binding, fibronectin binding, and identical protein binding, among other terms. Regarding the cellular component, DEPs showed enrichment in the extracellular matrix, centrosome, and proteinaceous extracellular matrix (Fig. 3B, right).

Regarding the biological process category, the upregulated DM-S/Con DEPs showed enrichment in plasma lipoprotein particle remodeling, macromolecular complex remodeling, and protein-lipid complex remodeling. Regarding molecular function, the DEPs showed enrichment in sulfur compound binding, heparin binding, and glycosaminoglycan binding, among other terms. Regarding cellular component, DEPs showed enrichment in the plasma membrane, cell periphery, and membrane (Fig. 3C, left). Moreover, the downregulated DM-S/Con DEPs showed enrichment in the organonitrogen compound biosynthetic process, mitotic cell cycle phase transition, and cell cycle phase transition, among other terms. Regarding molecular function, DEPs showed enrichment in Ras GTPase binding, small GTPase binding, and transferase activity, among other terms (Fig. 3C, right).

Discussion

Diabetes mellitus is recognized as a risk factor for implant surgery. Despite this risk, with the continuous improvement of implants, especially implant surface technology, the implant success rate has increased, including in T2DM patients[20]. These results mean that most scholars believe that patients with well-controlled T2DM do not need to be excluded from implant therapy. Hyperglycemia is the main characteristic of diabetes[21]. Thus, some scholars have even considered that satisfactory glycemic control could eliminate the adverse effect on the success rate of implantation in T2DM patients. However, little evidence to date has revealed the exact relationship between implant success rate during the healing period in T2DM patients and glycemic control. A review of previous clinical studies revealed that most follow-up durations were longer than 6 months[22 – 23]. Long-term observations may obscure the negative influence of well-controlled T2DM on bone-implant integration during the healing period because additional factors (prosthodontics, peri-implantitis etc.) can induce implant failure after loading. SomeT2DM patients with high risk may be overlooked in clinical treatment due to the consensus that well-controlled T2DM is excluded from risk factors. In this study, the clinical results showed significant differences between well-controlled T2DM patients and non-T2DM patients in implant failure rate (10.77% vs 0.75%). This result indicates that implantation failure rate in T2DM patients during the healing period was still higher than that in non-T2DM patients, even for patients with satisfactory glycemic control. Thus, we propose a hypothesis that hyperglycemia is not the sole risk factor for implant

surgery in T2DM patients. Other risk factors for osseointegration during the healing period must be explored in further studies.

Proteomics is often used to find biomarkers for early diagnosis of tumors and target drugs[24 – 25]. In the present study, proteomic results showed a certain number of proteins to be differently expressed in the three groups. The results indicated that failure in the DM group was not an accident and that DEPs in the DM-F group may cause failed osseointegration during the healing period. Therefore, could hyperglycemia cause the abnormal expression of these DEPs?

To explore the relationship between hyperglycemia and DEP expression, BMSCs from the Con group were cultured in different glucose concentration media. Nine proteins were selected from DEPs, which related to cell proliferation, migration and osteogenesis. Western blot results suggested that the expression of GLUL, LEPR, MMP2 and NPR3 were changed with the glucose concentration, whereas the expression of other five DEPs was unrelated. This result indicated that a high-glucose microenvironment was not the only reason for DEPs to arise, even if it was the major reason. Combined with proteomics results, we conclude that hyperglycemia is not the single risk factor for implant surgery in T2DM patients. More studies on the function of DEPs in T2DM patients with failure implantation during the healing period should be carried out. Some DEPs could be indicators to assess the failure risk of implantation before surgery in well-controlled T2DM patients in clinic. Moreover, some DEPs can be potential intervention targets besides glycemic control to increase implantation success.

DEPs may change the biological behavior of BMSCs, thereby affecting implant-bone integration during the healing period. To find further information, GO classification and enrichment were used to determine the biological functions of BMSCs affected by the DEPs from the DM-F/DM-S group. Noteworthy enrichment in downregulated proteins that might inhibit bone-implant integration was found. Regarding biological processes, the downregulated DEPs principally showed enrichment in osteoblast proliferation. Thus, the decreased expression of proteins, which regulate osteoblast proliferation in diabetics, might consequently damage the ability of bone formation. Integrin binding, extracellular matrix and extracellular matrix organization were also major enrichment terms revealed by the GO function. Integrin is a receptor that is associated with the ligands in the extracellular matrix and plays an indispensable role in cell migration[26]. In the bone repair process, BMSCs first migrate to the injury site to participate in bone formation. The decreased integrin binding ability might also be a reason for weak bone formation in T2DM patients. The extracellular matrix is a cell microenvironment, and thus, alterations in the extracellular matrix could also impair bone formation, which must be further investigated.

Conclusions

The clinical portion of the present study indicated that the success rate in well-controlled T2DM patients was still lower than for non-T2DM patients, meaning that T2DM is still a risk factor for implant therapy. The molecular changes of BMSCs in T2DM patients contribute to failed osseointegration, which arose not only due to hyperglycemia. Glycemic control cannot eliminate the negative effect completely, even

though it helps increase the success rate of implantation in T2DM patients to some extent. DEPs in DM-F can be indicators or intervention targets for implant therapy in T2DM patients. All results remind us that it is time to reappraise the position of hyperglycemia in implantation failure with T2DM patients, and more potential risk factors at the molecular level should be paid more attention in the future.

Abbreviations

T2DM: type 2 diabetes mellitus; BMSCs: Bone marrow mesenchymal stem cells; DEPs: differentially expressed proteins; GO: Gene ontology; HbA1c: Hemoglobin A1c; STROBE: Strengthening the Reporting of Observational studies in Epidemiology; PBS: phosphate-buffered saline; MSCM: mesenchymal stem cell medium; TMT: Tandem mass tagging; HPLC: high-performance liquid chromatography;NSI: nanospray ionization;FDR: false-discovery rate; APOE: apolipoprotein; FBLN1: fibulin-1; GLUL: glutamine synthetase; ITGA10: integrin alpha-10; MMP2: matrix metalloproteinase 2; TAGLN: transgelin IGFBP2: insulin-like growth factor-binding protein 2; LEPR: leptin receptor; NPR3: atrial natriuretic peptide receptor 3.

Declarations

Acknowledgments

We appreciate American Journal Experts (AJE) for their professional English editing.

Authors' Contribution Statement:

D.T. contributed to clinical data collection and analysis, financial support and drafting the manuscript. E.W. contributed to cells culture, proteomic analysis and drafting the manuscript. Y. X. contributed to Western Blot and preliminary literature data collecting and drafting the manuscript. C.L. contributed to technical consulting and preliminary literature data collecting. C.L. contributed to financial support, data collection and analysis. X.L. contributed to conception and experimental design, technical expertise, manuscript revision and manuscript approval. J.L contributed to conception and experimental design, technical expertise, manuscript revision, manuscript approval, financial support and all implant surgeries. All authors have read and approved the final manuscript.

Funding

This work was supported by grants from the National Natural Science Foundation of China (81371115), Scientific Research Nurturing Foundation of Beijing Stomatological Hospital, School of Stomatology, Capital Medical University (PYZ2018040), Discipline Construction Foundation (17-09-06), and Beijing Stomatological Hospital, School of Stomatology, Capital Medical University Discipline Construction Foundation (18-09-17).

Availability of data and materials

The datasets used or analyzed during the current study are available from the corresponding author on reasonable request.

Ethics approval and consent to participate

The present clinical study was approved by the Ethics Committee of Beijing Stomatological Hospital, School of Stomatology, Capital Medical University (Approval No.: CMUSH-IRB-KJ-PJ-2018-08).

Consent for publication

All the authors agree to publish the research.

Competing interests

The authors declare that they have no competing interests.

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Figures

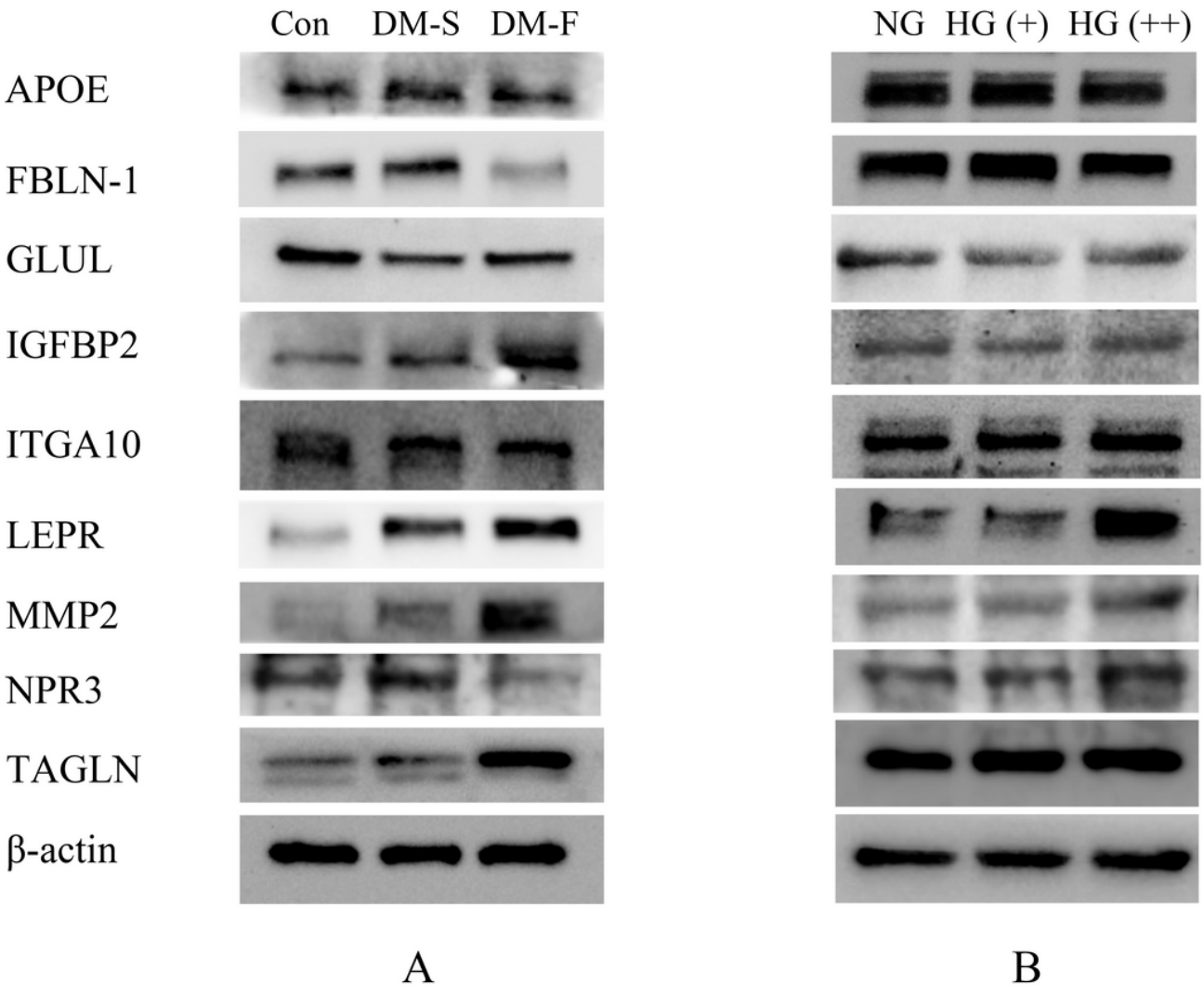

Figure 1

Verification of proteomics study and high glucose among the nine target DEPs .The expression tendency of APOE, FBLN-1, GLUL, IGFBP2, ITGA10, LEPR, MMP2, NPR3, TAGLN and β-actin were analyzed by Western blot. The left panel showed APOE expressed lower in DM-F than DM-S and higer in DM-S than Con; FBLN-1 expressed lower in DM-F than DM-S and lower in lower in DM-F than Con; GLUL expressed lower in DM-F than Con; IGFBP2 expressed higher in DM-F than Con; ITGA10 expressed lower in DM-F than DM-S; LEPR expressed higher in DM-S than Con; MMP2 expressed higher in DM-F than DM-S and higher in DM-S than Con; TAGLN expressed higher in DM-F than DM-S; NPR3 expressed higher in DM-S than Con. All expression tendencies were as same as the results in Table 2 except MMP2 in DM-F/DM-S. The right panel showed that the expression tendencies of all target DEPs were indepedent with glucose except GLUL, LERP ,MMP2 and NPR3. GLUL expressed lower in HG(+) than NG and HG(++); LEPR, MMP2 and NPR3 expressed higher in HG(++) than in HG(+) and NG.

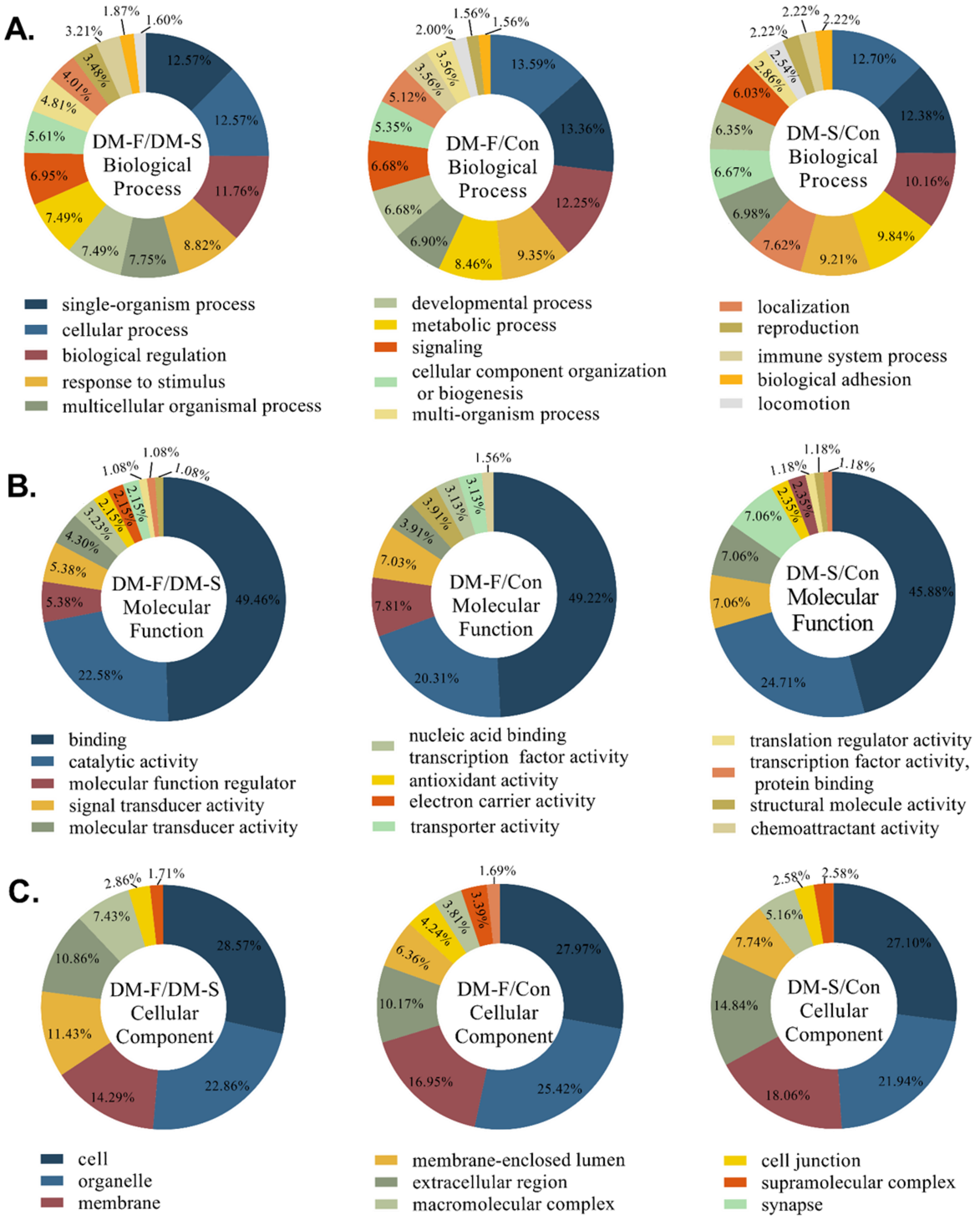

Figure 2

GO Annotation of DEPs in whole-cell proteomic analysis based on biological process, molecular function, and cellular component. (A): Biological process annotation of DEPs between three groups DM-F/DM-S (left), DM-F/Con (middle), and DM-S/Con (right); (B): Molecular function annotation of DEPs between three groups DM-F/DM-S (left), DM-F/Con (middle), and DM-S/Con (right); (C): Cellular component annotation of DEPs between three groups DM-F/DM-S (left), DM-F/Con (middle), and DM-S/Con (right).

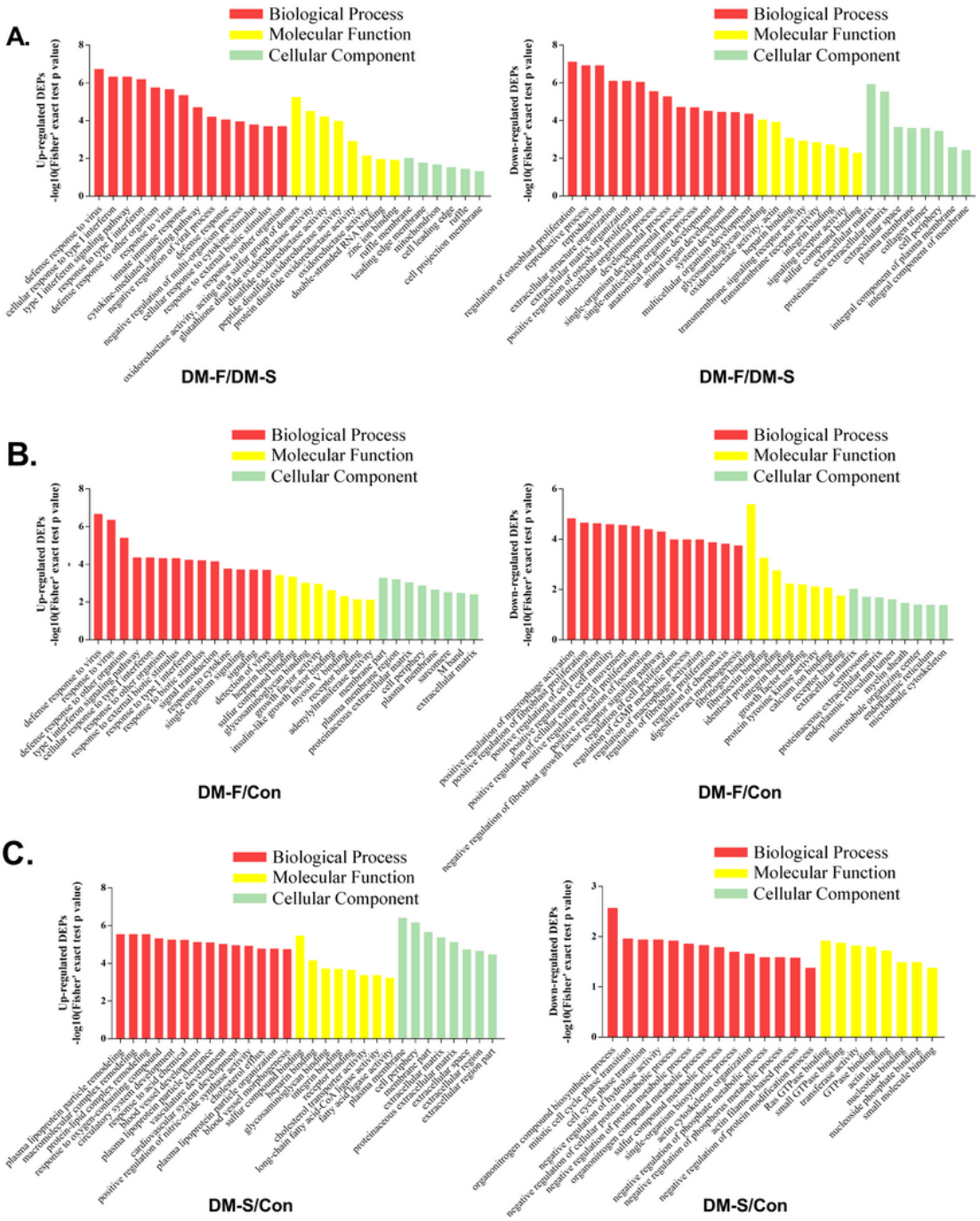

Figure 3

GO enrichment of DEPs in whole-cell proteomic analysis. Enrichment analysis was performed based on biological process (red bar), molecular function (yellow bar) and cellular component (green bar) categories. (A): Go enrichment analysis of up-regulated (left) and down-regulated (right) DEPs between DM-F and DM-S group; (B): Go enrichment analysis of up-regulated (left) and down-regulated (right) DEPs

between DM-F and Control group; (C): Go enrichment analysis of up-regulated (left) and down-regulated (right) DEPs between DM-S and Conl group.

Supplementary Files

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- SupplementaryFile.doc