

Identification of the genetic central dogma in osteogenic differentiation of MSCs by osteoinductive medium from transcriptional data sets

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Abstract

Background: The genetic central dogma (GCD) has been demonstrated its essential function in many biological processes and diseases. However, its roles in the process of osteogenic differentiation of mesenchymal stem cells (MSCs) remain unclear.

Methods: In this project, we analyzed an online database of osteogenic differentiation of MSCs after 14 days and 28 days by osteoinductive medium (GSE83770). The differentially expressed genes were screened by GEO2R, with further conducting of KEGG pathways using DAVID. In addition, protein–protein interactions of the enriched pathways were performed using STRING with marked hub genes measured by the CytoHubba. Hub genes were verified by quantitative reverse-transcription polymerase chain reaction.

Results: Results showed that six pathways related to GCD, including DNA replication, Aminoacyl-tRNA biosynthesis, Mismatch repair, Ribosome, Spliceosome, and RNA degradation pathways enriched in the early stage (14 days vs. undifferentiated MSCs) of osteogenesis. The Lysosome pathway was highly enriched in the late stage (28 vs. 14 days) of osteogenesis, and Ribosome pathway plays a key role throughout the entire process (28 days vs. undifferentiated MSCs) of osteogenesis.

Conclusion: Both DNA replication and protein translation were functionally worked in the early stage of osteogenesis, whereas the Lysosome pathway was the only GCD-related one in the late stage of osteogenesis. The GCD-related Ribosome pathway occupied the entire process of osteogenesis.

KEYWORDS

genetic central dogma, lysosome, mesenchymal stem cells, osteogenic differentiation, ribosome

Highlights

- We identified six pathways associated with genetic central dogma (DNA replication, Aminoacyl-tRNA biosynthesis, Mismatch repair, Ribosome, Spliceosome, and RNA degradation pathways), which participated in the early stage of osteogenesis (0–14 days).
- The Lysosome pathway plays a crucial role in the late stage of osteogenesis (14–28 days), whereas the Ribosome pathway participates in the

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entire process (28 days vs. undifferentiated mesenchymal stem cells) of osteogenesis.

1 | INTRODUCTION

For decades, mesenchymal stem cells (MSCs) have been demonstrated their multiple differentiation capacities into bone tissue, cartilage tissue, and their clinical treatment potential on a variety of disease such as cerebral infarction.¹⁻³ In the case of osteogenic differentiation of MSCs, there are numerous methods and specific ways that can successfully induce MSCs differentiating into osteoblastic lineage, including osteoinduction media, pharmaceuticals, growth factors, genes, biomaterials, even mechanical stress stimulations, when used singly or combined together.⁴⁻⁷ Yet, there is still a lack of knowledge for understanding the mechanism of how MSCs differentiate into osteogenic lineage cells under these massive conditions.

The genetic central dogma (GCD), otherwise known as central dogma of molecular biology, which detailed the genetic information from nucleic acid downstream to protein production, was first proposed by Francis Crick⁸ on 1958 and restated on 1970.⁹ Traditional knowledge of the GCD includes the replication of deoxyribonucleic acid (DNA), transcription, and translation processes.⁹ To date, the concepts of splicing and ribonucleic acid (RNA) editing consummate the GCD; meanwhile, entire information on DNA-RNA-protein processes is included.^{10,11} GCD makes different contributions to many physical and pathological processes, including oncogenesis,¹² mechanical stress on human cells,¹³ osteoarthritis,¹⁴ and so on. However, the roles of GCD in the differentiation of stem cells remain unknown. Recently, high-throughput technologies and gene editing have been used for investigating the contributions of GCD in various fields including the cutting-edge stem cell factory.^{15,16} Through the high-throughput methods, we could calculate and measure the specific pathways and molecular function of GCD participating in a specific biological process or disease.¹⁵ Nevertheless, no studies have reported the function of GCD in the process of osteogenic differentiation of MSCs.

Osteoinduction medium is one of the above convincing approaches in the application of inducing MSCs into osteogenesis. Scientists have screened a majority of chemical molecules for constructing conditioned medium for osteoinduction, of which β -glycerol phosphate, L-ascorbic acid-2 phosphate, dexamethasone, and sodium pyruvate are the key components.^{17,18} Therefore, we investigated the role of GCD in osteogenic differentiation of murine MSCs by the established osteoinductive medium through a high-throughput

sequencing technology in this study, which would give new insights into the roles of genetic central dogma on osteogenic of MSCs.

2 | MATERIALS AND METHODS

2.1 | Data acquisition

Gene expression files were obtained from the Series GSE83770 (<https://www.ncbi.nlm.nih.gov/geo/query/acc.cgi?acc=GSE83770>) in Gene Expression Omnibus using platform GPL16570, Affymetrix Mouse Gene 2.0 ST Array. A total of three groups were involved, including MSCs without osteoinduction (GSM2218686, GSM2218687, GSM2218688, termed control, 0 day), MSCs after osteoinduction of 14 days (GSM2218689, GSM2218690, GSM2218691, termed 14 days), and MSCs after osteoinduction of 28 days (GSM2218692, GSM2218693, GSM2218694, termed 28 days). Each group contained three independent samples.

2.2 | Data sample preparing of the sequencing

According to He et al.,¹⁸ primary MSCs were obtained from mice bone marrow, cultured in α -MEM media without ascorbic acid (Invitrogen, A10490-01) containing 10% MSC-qualified fetal bovine serum (FBS) (Invitrogen, 12763025). The osteoinduction media was based on Dulbecco's modified Eagle's medium (DMEM) containing 10% FBS, 0.2 mmol/L L-ascorbic acid-2 phosphate, 0.1 μ mol/L dexamethasone, 1 mmol/L sodium pyruvate, and 10 mmol/L β -glycerophosphate as previously reported.¹⁸ All media were changed every 3-4 days. All RNAs were extracted according to the manufacturer's instructions and then qualified for Affymetrix sequencing. According to He et al., the total RNA of all the cells was obtained using Trizol (Invitrogen, 15596026) as indicated by the manufacturer. To evaluate the integrity and concentration of RNA, an Agilent 2100 Bioanalyzer and the Nano Eukaryotic RNA chip were used. All RNA samples had an average RNA Integrity Number (RIN) of 9.9. Hybridization (3.0 μ g of RNA at 100 ng/ μ l), wash, and stain were carried out using standard Affymetrix protocols. An Affymetrix 3000 7G scanner with Affymetrix GeneChip[®] Command Console[®] Software (AGCC) was applied for scanning and analyzing the samples.

TABLE 1 Primers in qRT-PCR

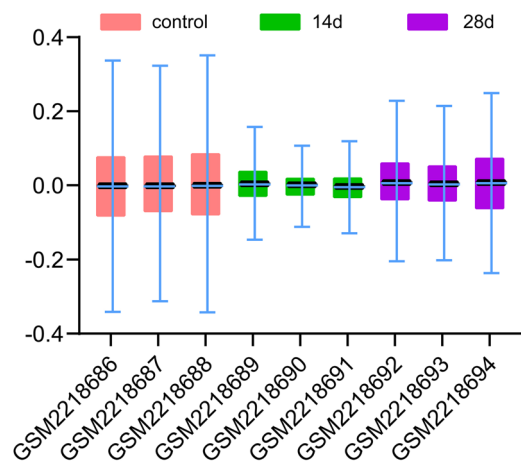
Gene	Forward primer	Reverse primer
<i>Bmp2</i>	5'-GGCCGAAGGTGGATTCTCC-3'	5'-GTCGGGTGTGTTATTGACATACA-3'
<i>Exo1</i>	5'-ATGCTACGTTCTACCGGCTTC-3'	5'-TCTGGGTTGTACGGGTCATAG-3'
<i>Dcp2</i>	5'-GAAAGAGACAATGCGATCCGA-3'	5'-CGCACTGAGGTAATCCTGGTG-3'
<i>Rpl22</i>	5'-CAGACGCTGCGGGTTTTAC-3'	5'-TGGACATGCCTCGTATCATCTTT-3'
<i>Ap1b1</i>	5'-ACTATGGGCTGCATCCGAGT-3'	5'-GAGGTCTTTAAGCGTGTCCAG-3'
<i>Snrnp40</i>	5'-ATCGAGCAGCAGAAGCGTAAG-3'	5'-GCAGCATGATTGGAGCTTGAAG-3'
<i>Rpa2</i>	5'-GAGTCCGAGCCCAGCATATTG-3'	5'-CCTGTGAAATCTCGACATCTCCA-3'
<i>Sars</i>	5'-CGGGTGGATAAAGGAGGGGA-3'	5'-TGCCCCGAAATCTGCATCGTC-3'
<i>Rps26</i>	5'-CATGACCCGCCTGCCAAAT-3'	5'-TCTCCCGTACTGTCGGTAG-3'
<i>Gapdh</i>	5'-AATGGATTGGACGCATTGGT-3'	5'-TTTGCCTGGTACGTGTTGAT-3'

2.3 | Data analyzing

According to He et al.,¹⁸ an Agilent's GeneSpring GX software was used to select the Affymetrix Exon Expression. The RMA-16 method was performed to normalize the data. Filtration was selected from 20% to 100% expression using one-way analysis of variance (ANOVA) followed by post hoc tests using the Student–Newman–Keuls method. A Benjamini–Hochberg FDR method was used to corrected the p value. Differentially expressed genes (DEGs) were determined using the online software GEO2R as indicated previously.¹⁹ DEGs selecting criteria was set at $p < 0.05$ and $|\log_{2}FC| > 1$. KEGG pathway analysis based on DEGs was analyzed using a website source named DAVID (version 6.7, <https://david.ncifcrf.gov/>).²⁰ The Enrichment score of pathway in this study was set as, $-\log_{10}(p \text{ value})$. Protein–protein interaction (PPI) was analyzed using another online database named STRING (version 11.0).^{21,22} The top 10 hub genes were obtained using the feature called CytoHubba in the software Cytoscape (version 3.7.1) under MCC mode.²³

2.4 | Cell culture

Mouse bone marrow MSCs were provided by Hangzhou Bio-science Ltd. (<http://www.bio-science.top/>) from OriCell® (Mcyagen Biosciences Inc., MUBMX-01001). Undifferentiated MSCs were cultured in α -MEM media without ascorbic acid (Invitrogen, A10490-01) containing 10% MSC-qualified FBS (Invitrogen, 12763025). The osteoinduction media was based on DMEM (Gibco, C11885500BT) containing 10% FBS (Ephraim, 34080619), 0.2 mmol/L L-ascorbic (Sigma, A4403), 0.1 μ mol/L dexamethasone (Sigma, G9422), 1 mmol/L sodium pyruvate (Sigma, P2256), and 10 mmol/L β -glycerophosphate (Sigma, D4902).

**FIGURE 1** Boxplot for normalization before differentially expressed genes selecting.

All media were changed every 3 to 4 days. All cells were cultured in six-well cell plates.

2.5 | Test of quantitative reverse-transcription polymerase chain reaction (qRT-PCR)

Osteogenic gene (*Bmp2*) and hub genes (*Rpa2*, *Sars*, *Exo1*, *Rps26*, *Snrnp40*, *Dcp2*, *Ap1b1*, and *Rpl22*) were analyzed by qRT-PCR when MSCs were cultured with osteoinduction media 14 and 28 days, respectively. Briefly, 1 ml of Trizol (Invitrogen, 15596018) was added to each well of the cell plate and kept for 10 min at room temperature for cell lysis. Lysates were transferred into a 1.5 ml tube, and then to it, 200 μ l chloroform (Sino-pharm, 10006818) was added and mixed. After reaction

at room temperature for 2 min, tubes were centrifuged at 4°C/10,142g for 10 min. The supernatant was transferred to a new 1.5 ml tube, mixed with 500 µl isopropanol (Sinopharm, 40064360), and rested for 15 min. After centrifuging at 4°C/10,142g for 15 min, the supernatant was discarded and washed with 1 ml of 75% ethanol. Then centrifuged at 4°C/10,142g for 5 min, discarded the supernatant and added 1 ml of 100% ethanol (Beyotime Biotechnology, 10009218) for wash. Followed with centrifugation at 4°C/10,142g for 5 min, discarded the supernatant and dry at room temperature for 10 min. Finally, 40 µl DEPC (Beyotime Biotechnology, R0021) was added to dissolve the RNA.

Reverse transcription reaction was performed at 42°C for 30 min, then 85°C for 10 min with a total of 20 µl reaction system containing RNase Free ddH₂O, 10 µl RNA, 1 µl RT Primer mix, 1 µl PrimeScript® RT Enzyme Mix 1 and 4 µl 5×PrimeScript Buffer 2 (TIANGEN BIOTECH). For PCR reaction, a 20 µl standard solution containing 1 µl complementary DNA, 1 µl forward primer (10 µmol/L), 1 µl reverse primer (10 µmol/L), 10 µl 2×SYBR Green PCR Master Mix (Lifeint, A4004M), and 7 µl ddH₂O was used. All samples were submitted to MX3000P (Stratagene, AgilentTechnologies) followed the instruction: denaturation at 95°C for 3 min then 40 cycles reaction by 95°C, 12 s; 62°C, 40 s. Undifferentiated MSCs (0 day) were used as control. Results were analyzed using 2^{-ΔΔC_t} methods with GAPDH as an internal reference. All tests were repeated three times and primers are listed in Table 1.

2.6 | Statistical analysis

PCR results were presented as mean ± standard deviation (SD) and analyzed using one way-ANOVA by GraphPad Prisma. Statistical difference threshold was set as $p < 0.05$.

3 | RESULTS

3.1 | Genetic central dogma in the early period (0–14 days) of osteoinduction

As shown in Figure 1, all data were normalized before DEGs were selected. There were 361 upregulated genes and 3346 downregulated genes in the MSCs after 14 days of osteoinduction as compared with the control group (Figure 2A). The top 10 pathways were Cell cycle (gene count: 55, p value: 4.57E-14), DNA replication (DNA replication gene count: 24, p value: 1.08E-11), Aminoacyl-tRNA biosynthesis (gene count: 23, p value: 1.37E-08), Mismatch repair (gene count: 15, p value: 2.47E-07), Ribosome (gene count:

33, p value: 6.46E-07), p53 signaling pathway (gene count: 27, p value: 2.64E-06), Spliceosome (gene count: 39, p value: 5.57E-06), RNA degradation (gene count: 24, p value: 7.13E-06), Homologous recombination (gene count: 15, p value: 7.42E-06) and Focal adhesion (gene count: 53, p value: 1.76E-05) rank by the enrichment score (Figure 2B). And PPI networks of those related with genetic central dogma (Table 2), including DNA replication (Figure 2C), Aminoacyl-tRNA biosynthesis (Figure 2D), Mismatch repair (Figure 2E), Ribosome (Figure 2F), Spliceosome (Figure 2G), and RNA degradation (Figure 2H) were further submitted for hub gene screening. The top 1 hub genes in the DNA replication (Figure 3A), Aminoacyl-tRNA biosynthesis (Figure 3B), Mismatch repair (Figure 3C), Ribosome (Figure 3D), Spliceosome (Figure 3E), and RNA degradation (Figure 3F) were *Rpa2*, *Sars*, *Exo1*, *Rps26*, *Snrnp40*, and *Dcp2*, respectively.

3.2 | Genetic central dogma in the late period (14–28 days) of osteoinduction

To further screen the vital genes of the central dogma during the 14–28 days of osteoinduction, we also compared the difference between the two time points. There were 1238 upregulated genes and 523 downregulated genes in 28 days as compared to 14 days (Figure 4A). Ranked by the enrichment score, the forefront pathways (Figure 4B) in this period were Lysosome (gene count: 23, p value: 6.68E-06), Glutathione metabolism (gene count: 10, p value: 0.005905), Arrhythmogenic right ventricular cardiomyopathy (ARVC) (gene count: 11, p value: 0.0231048), Ether lipid metabolism (gene count: 7, p value: 0.024163003), Renin-angiotensin system (gene count: 5, p value: 0.026422467), Valine, leucine, and isoleucine degradation (gene count: 8, p value: 0.028247944), Butanoate metabolism (gene count: 7, p value: 0.031030679), Propanoate metabolism (gene count: 6, p value: 0.042773684), ECM-receptor interaction (gene count: 11, p value: 0.042859343), and Axon guidance (gene count: 15, p value: 0.045881878). Among these above pathways, only lysosome was highly associated with the central dogma; therefore, we investigated the PPI network of Lysosome (Figure 4C) and its hub genes (Figure 4D). As shown in Figure 4D, the peak hub gene in lysosome was *Ap1b1*.

3.3 | Genetic central dogma in the whole period (0–28 days) of osteoinduction

To fully understand the roles of central dogma in the whole period of osteoinduction, we compared the profiles of 28 days to the undifferentiated MSCs. As shown in

TABLE 2 Six KEGG pathways related to genetic central dogma enriched in early osteoinduction (0-14 days)

Pathways	Count	<i>p</i> Value	Genes	Fold enrichment	FDR
mmu03030:DNA replication	24	1.08E-11	POLA1, POLA2, MCM3, MCM4, RNASEH2B, MCM5, MCM6, POLD3, RPA1, PRIM1, RFC5, RPA2, DNA2, RFC3, RFC4, MCM7, POLE2, RFC1, RFC2, POLD1, PRIM2, POLD2, PCNA, FEN1	4.585814	1.33E-08
mmu00970:Aminoacyl-tRNA biosynthesis	23	1.37E-08	YARS, CARS, NARS, SARS, GARS, EPRS, WARS2, DARS2, VARS, KARS, SARS2, IARS, WARS, TARS, RARS, FARSB, LARS, HARS, MARS2, FARSA, YARS2, TARSL2, MARS	3.662282	1.69E-05
mmu03430:Mismatch repair	15	2.47E-07	EXO1, MSH6, MSH3, POLD3, RFC5, RPA1, RPA2, RFC3, RFC4, RFC1, RFC2, POLD1, POLD2, PCNA, PMS2	4.559758	3.03E-04
mmu03010:Ribosome	33	6.46E-07	RPL13, GM12191, RPL15, RPS15A, RPL37, GM11362, RPL39, GM5093, RPS26, MRPL13, RPL30, RPS28, RPL6, RPL31, FAU, RPL5, RPL4, RPL12, RSL24D1, RPS23, RPS27A, RPS24, RPL27, RPS9, RPL24, RPL23A, RPS4X, GM5978, RPL29, GM8841, RPS19, GM7429, RPL23, RPL21, RPL37A	2.479689	7.94E-04
mmu03040:Spliceosome	39	5.57E-06	CHERP, NHP2L1, LSM6, PPIL1, SNRPD3, TRA2A, LSM7, WBP11, BC005561, NAA38, SF3B3, TCERG1, HNRNPK, DDX46, RBM8A, DHX15, U2AF1, LSM4, LSM3, LSM2, SNRNP70, RBM25, RBM22, SNRPA1, MAGOH, PRPF3, RBMX, SF3A1, HNRNPA1, PRPF4, HNRNPU, EIF4A3, AQR, SNRNP200, SNRPB, SNRNP40, PRPF38B, PUF60, SNRPG	2.103372	0.006854
mmu03018:RNA degradation	24	7.13E-06	EXOSC8, LSM6, CNOT10, LSM7, TTC37, PNPT1, CNOT3, PAPD7, EXOSC2, CNOT2, SKIV2L2, CNOT1, EXOSC1, NAA38, CNOT6, CNOT4, DIS3, DCP2, RQCD1, LSM4, LSM3, HSPD1, LSM2, HSPA9	2.675058	0.00876

Figure 5A, there were 701 upregulated genes and 1451 downregulated genes after MSCs induced by the osteoinduction media for 28 days. And the Ribosome (gene count: 33, *p* value: 1.29E-14) ranked first in the top 10 pathways (Figure 5B), followed by Huntington's disease (gene count: 23, *p* value: 0.019935961), acute myeloid leukemia (gene count: 10, *p* value: 0.026257964), Gap junction (gene count: 13, *p* value: 0.027814528), prostate cancer (gene count: 13, *p* value: 0.037969666), arginine, and proline metabolism (gene count: 9, *p* value: 0.04442331), Prion diseases (gene count: 7, *p* value: 0.045441397), TGF- β signaling pathway (gene count: 12, *p* value: 0.063013376), neurotrophin signaling pathway (gene count: 16, *p* value: 0.065945215), and circadian rhythm (gene count: 4, *p* value: 0.069967399). Among these, only the ribosome pathway is related to genetic central dogma, and its PPI is shown in Figure 5C. The 10 hub genes in the ribosome pathway are shown in Figure 5D, and the top 1 is *Rpl22*.

3.4 | PCR results

As shown in Figure 6, *Bmp2* was increased from 0 to 28 days after osteoinduction ($p < 0.05$), which indicated the success of osteoinduction. *Exo1* (hub gene in Mismatch repair) was also increased, whereas

Snrnp40 (hub gene in Spliceosome) was decreased ($p < 0.05$) from 0 to 28 days of osteoinduction of MSCs. *Dcp2* (hub gene in RNA degradation), *Rpl22* (hub gene in Ribosome), and *Ap1b1* (hub gene in Lysosome) were only increased after 28 days of osteoinduction ($p < 0.05$). On contrast, *Rpa2* (hub gene in DNA replication) was only decreased after 28 days of osteoinduction ($p < 0.05$).

4 | DISCUSSION

The GCD guides us through the rules of how genetic materials become functional proteins and the interactions among them.⁹ Detailly, the pathways such as DNA replication, Mismatch repair, Ribosome, Spliceosome, Aminoacyl-tRNA biosynthesis, RNA transport, RNA degradation, and messenger RNA surveillance pathway are associated to GCD due to their participations in DNA-RNA-protein processes.^{11-14,24,25} For example, the disfunction of ribosome and associated pathways, that is, the RNA transcription and protein translation, attributes to osteoarthritis.¹⁴ In human cells, mechanical stress influences the rheology through the pathways related to DNA replication and repair.¹³ Thus, this central

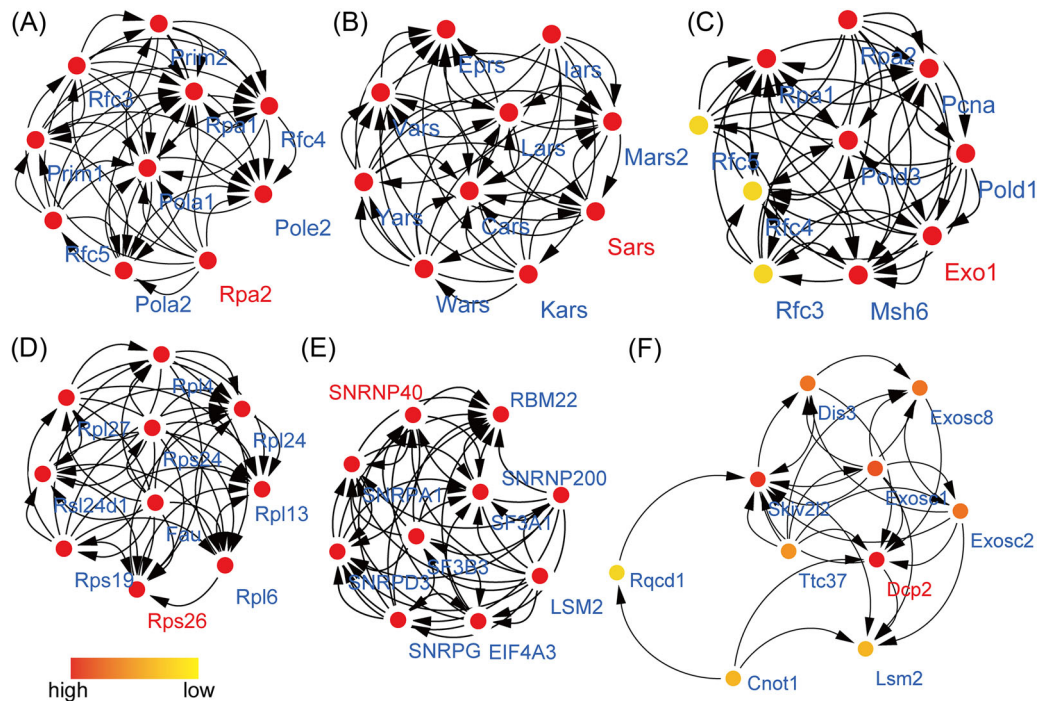


FIGURE 3 Hub genes are involved in genetic central dogma in the early period of osteoinduction (14 days vs. control). (A) DNA replication, (B) Aminoacyl-tRNA biosynthesis, (C) Mismatch repair, (D) Ribosome, (E) Spliceosome, (F) RNA degradation pathways. Red genes are the top 1 hub genes in each pathway

dogma is certainly work in organic species and participates in massive biological processes without doubt.¹²⁻¹⁴ However, its certain roles in osteogenic differentiation have not been well described due to the limitation of technology for the past. Recently, the prosperous of high-throughput technology open a new era for understanding the activities and functions of GCD in various biological processes¹⁵ as well as its role in osteogenesis.

In this study, we found different GCD-related pathways participated in the different periods of osteogenic differentiation from MSCs *in vitro*. To our knowledge, the first 14 days of osteogenic differentiation were determined as the early period in the osteogenesis process since the activity of alkaline phosphatase is still increasing gradually with the deposition of extracellular matrix and calcium nodules.^{26,27} During this early period of osteogenesis (0-14 days), our results showed some pathways associated with GCD, including DNA replication, Aminoacyl-tRNA biosynthesis, Mismatch repair, Ribosome, Spliceosome, and RNA degradation pathways, were enriched. Most of these pathways are either related to the DNA packaging (a general initiation of GCD), or related to the term named translation, which represents the process form RNA to proteins.²⁸ Moreover, the enrichment scores of DNA replication and Mismatch repair were higher than those of Ribosome, Spliceosome, and RNA

degradation pathways, indicating that the DNA replication process was flourisher than the translation process during the early period of osteogenic differentiation. Therefore, at the beginning of the transformation of MSCs into osteogenic lineage cells, the intracellular activity of gene level is higher than the protein level. The MSCs may endeavor their selves package their genomic materials together and translate into proteins to excrete alkaline phosphatase and extracellular matrix at the early stage of osteogenesis.

Since the deposition of extracellular matrix and calcium nodules are peaked and will not statistically aggrandize from 21 to 28 days, osteoinduction at 28 days is considered as the end of osteogenic differentiation.²⁶ From 14-28 days of osteoinduction, we only found one pathway related to the genetic central dogma, which was the Lysosome pathway. The function of lysosome has drawn a lot of respect since the conception of autophagy was carried out.²⁹ And the role of autophagy in GCD has been investigated to identify the associating noncoding RNAs.³⁰ Therefore, the Lysosome pathway is highly GCD-related. Also, the lysosome is not only the organelle that help cell recycle its intracellular waste disposal but also the degrade the extracellular materials by endocytosis.³¹ Moreover, lysosome also develops a unique marker, mannose-6-phosphate (M6P), which assists proteins in the trans-Golgi network.³² Thus, the MSCs might obtain small molecules form the osteoinductive media

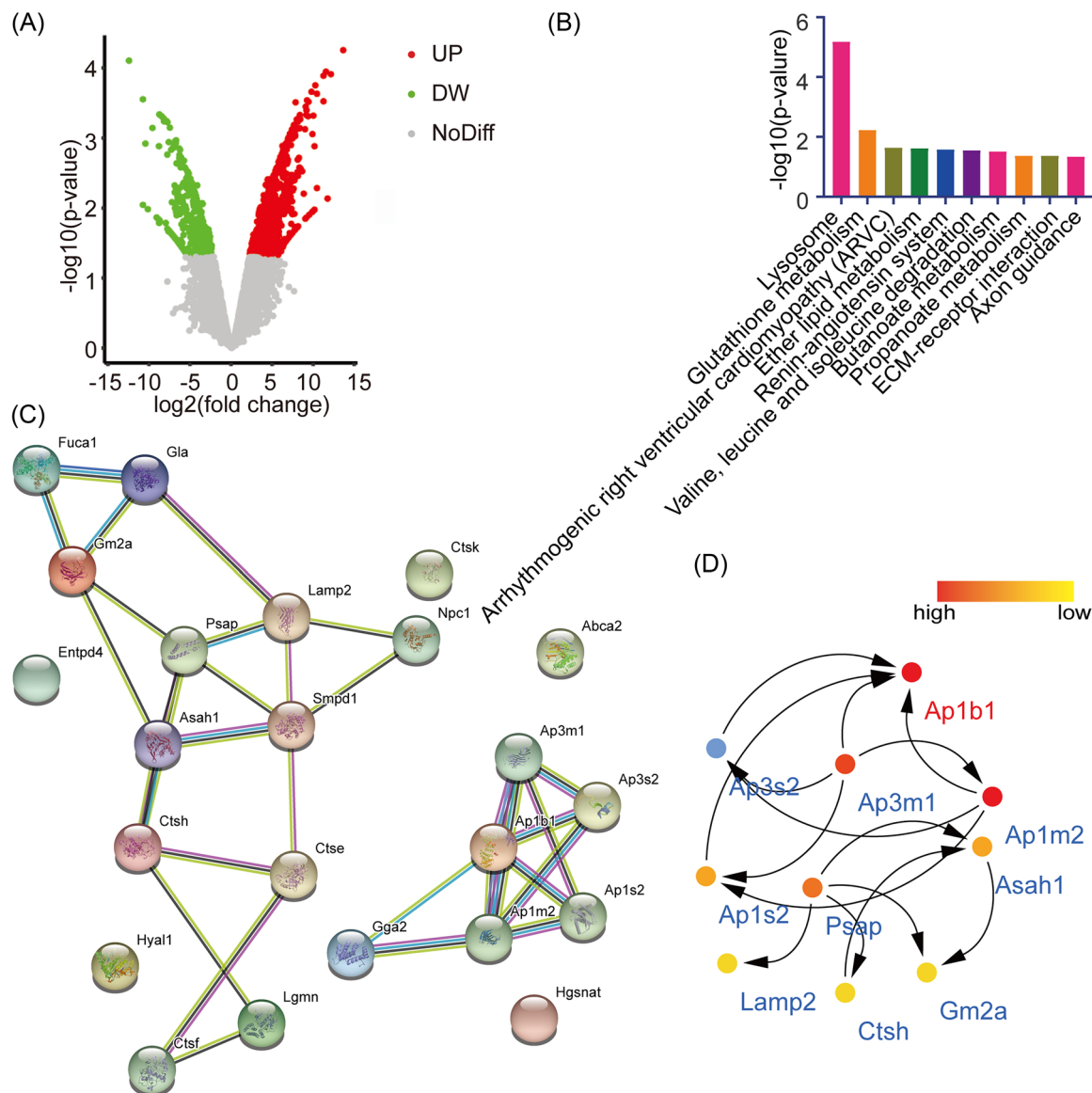


FIGURE 4 Genetic central dogma in the late period of osteoinduction (28 vs. 14 days). (A) Volcano plot. (B) Top 10 KEGG pathways ranked by the enrichment score. (C) PPI of lysosome. (D) Top 10 hub genes involved in Lysosome. Red gene is the top 1 hub gene. DW, downregulated gene; Nodiff, no difference gene; UP, upregulated gene

to help themselves differentiate into osteoblastic lineage cells through Lysosome pathway in the late period of osteogenic differentiation. Or the MSCs produced waste particles that harmful to osteogenic differentiation and activated the Lysosome pathway to recycle them during the late period. Intriguingly, ECM-receptor pathway was also enriched in this period (14–28 days) in our results. As we all known, ECM-receptor pathway plays a pivotal role in the osteogenesis and extracellular matrix deposition,³³ which maybe endocytosed by the MSCs again through Lysosome pathway then contributes to a cascade

osteoinduction for MSCs. And the late period of osteogenesis is more catabolism than anabolism since Lysosome pathway was activated.

During the whole period of osteoinduction (0–28 days), there was only one pathway enriched related to GCD, which was the Ribosome pathway. Ribosome is the organelle works as a biological machine for protein synthesis in a cell.³⁴ Moreover, many non-coding regions in transcriptomes are occupied by ribosome and thus influence GCD.³⁵ Our results showed that the Ribosome pathway was high enriched throughout the entire process of osteogenic

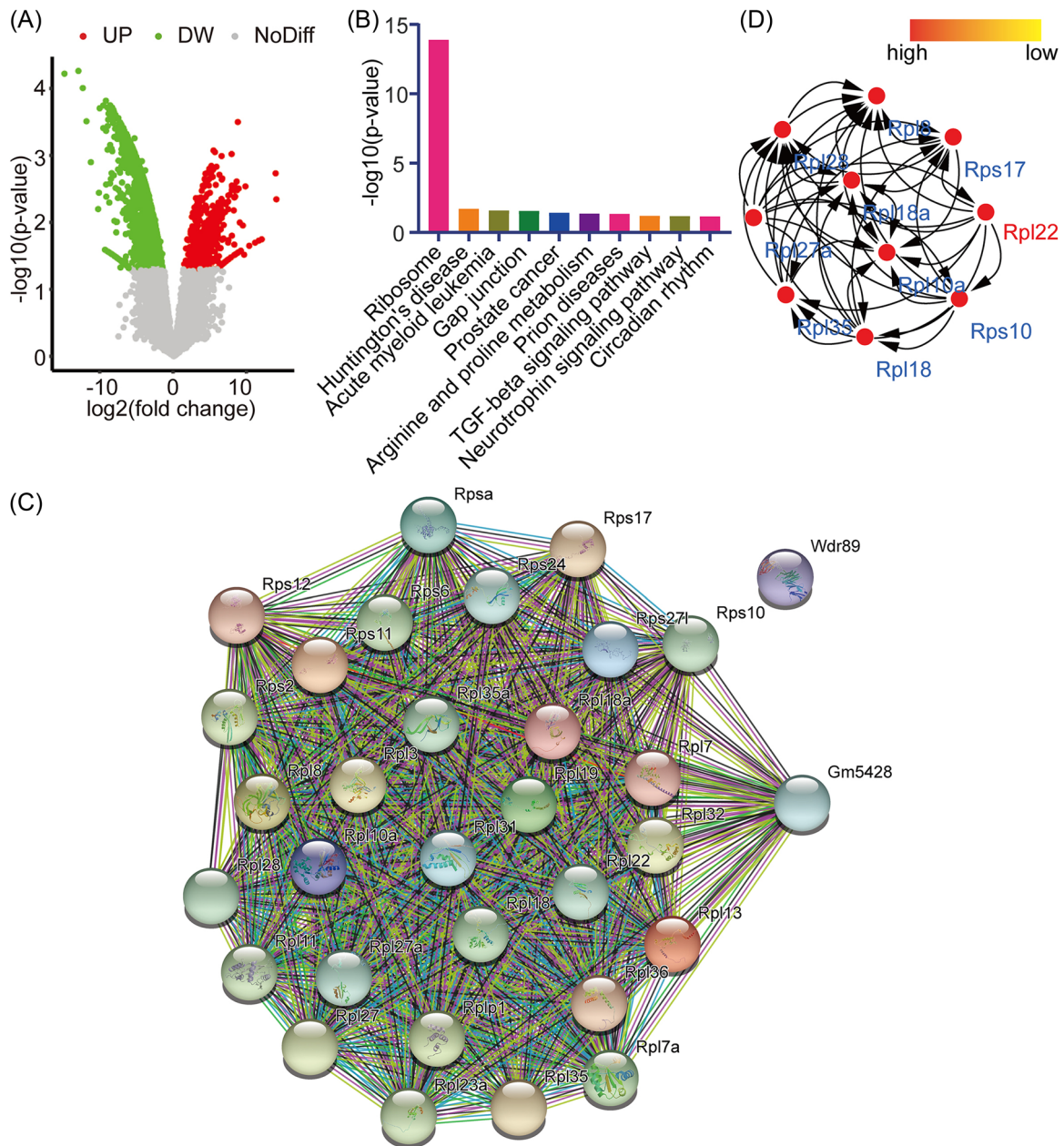


FIGURE 5 Genetic central dogma in the whole osteoinduction (28 days vs. control). (A) Volcano plot; (B) Top 10 KEGG pathways ranked by the enrichment score. (C) PPI of Ribosome. (D) Top 10 hub genes involved in Ribosome. Red gene is the top 1 hub gene. DW, downregulated gene; Nodiff, no difference gene; UP, upregulated gene

differentiation of MSCs, revealed that there were massive proteins produced in the whole period. Therefore, the whole period of osteogenesis is more like an anabolism course than catabolism course.

In conclusion, in the present investigation, we found that both DNA replication and translation played important roles in the early period (0–14 days)

of osteogenic differentiation of MSCs by the osteogenic media. Lysosome pathway was the GCD-related one in the process of the late stage (14–28 days) of osteogenesis. Ribosome pathway participates in the entire process (0–28 days) of osteogenesis. Our study may give new insights into the roles of genetic central dogma in the process of osteogenic differentiation of MSCs.

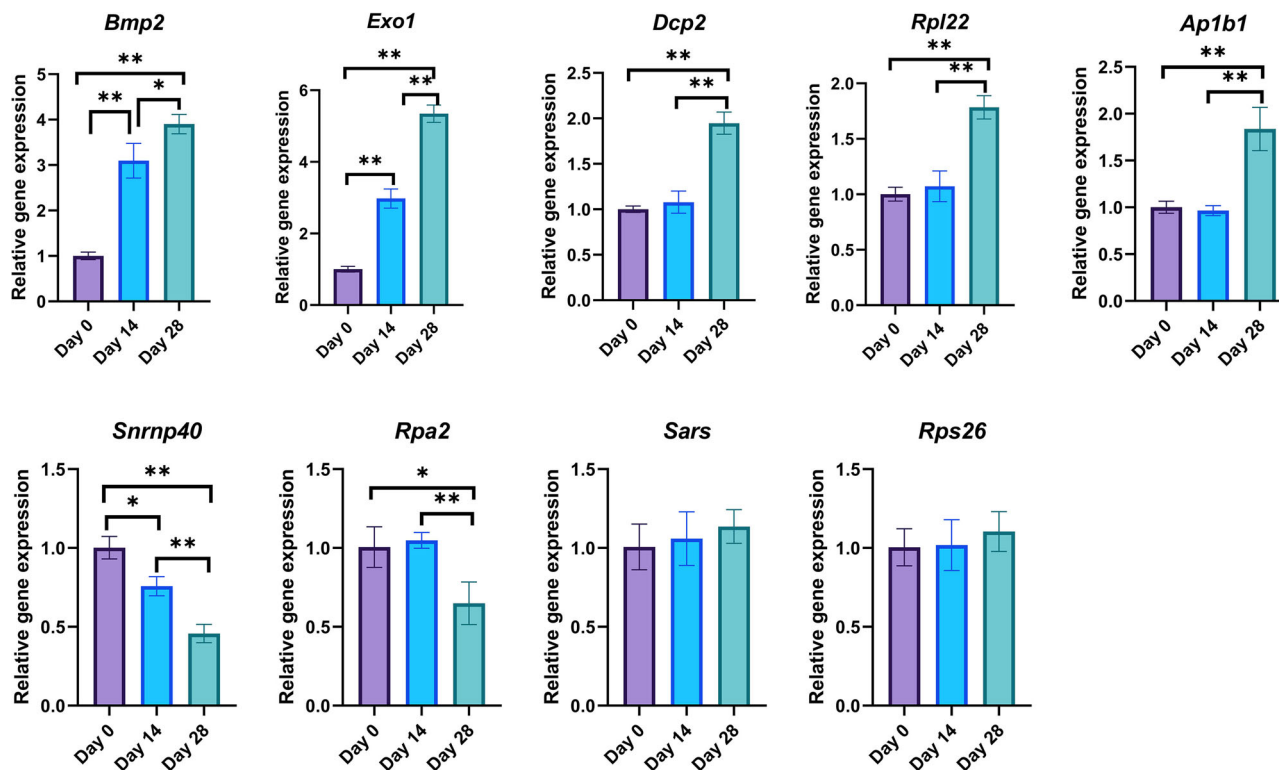


FIGURE 6 Polymerase chain reaction results of osteogenic gene (*Bmp2*) and hub genes (*Rpa2*, *Sars*, *Exo1*, *Rps26*, *Snrnp40*, *Dcp2*, *Ap1b1*, and *Rpl22*) in genetic central dogma associated with osteogenesis. * $p < 0.05$, ** $p < 0.01$

AUTHOR CONTRIBUTION

Tong-Meng Jiang contributed to the study design, tests, data analysis, and manuscript writing.

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CONFLICT OF INTEREST

The author declares no conflict of interest.

DATA AVAILABILITY STATEMENT

Gene Series GSE83770 are obtained from Gene Expression Omnibus (<https://www.ncbi.nlm.nih.gov/geo>). Other data are available on request to the corresponding author.

ETHICS STATEMENT

Not applicable.

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