Integrative Analyses of Biomarkers and Pathways for Osteosarcopenia

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Research Article

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Abstract

Osteosarcopenia is a geriatric syndrome coexistence of osteoporosis and sarcopenia. However, the molecular mechanism underlying osteosarcopenia have not been fully elucidated. Differentially expressed genes (DEGs) for osteoporosis and sarcopenia were respectively identified by analyzing four expression datasets from the GEO. We extracted the gene expression datasets GSE56814 and GSE56815 for osteoporosis, GSE1428 and GSE8479 for sarcopenia. 133 co-expressed DEGs were included in osteoporosis and sarcopenia datasets. Furthermore, functional enrichment analyses and PPI network construction were performed to explore the potential biological function of the DEGs and identify hub genes. S100 protein binding (GO:0044548; p-value = $1.83\text{E-}06$) and regulation of mRNA metabolic process (GO:1903311; p-value = $2.30\text{E-}05$) were significantly enriched in gene ontology(GO) analysis. Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment analysis showed that salmonella infection (hsa05132; p-value = $1.05\text{E-}04$) and AMPK signaling pathway (hsa04152; p-value = $2.18\text{E-}03$) were significantly enriched. According to the results of PPI, we finally identified five most critical genes as the hub genes, including AKT1, ANXA2, VIM, S100A6 and S100A11. The integrated analysis will contribute to the understanding of comprehensive molecular changes in osteosarcopenia and the development of new target therapies.

Introduction

Population aging is accelerating rapidly worldwide. Sarcopenia and osteoporosis are two conditions that are associated with aging, with similar risk factors that include genetics, endocrine function, and mechanical factors ¹. Osteoporosis is a metabolic bone disorder that is characterized by low bone mass and micro-architectural deterioration of bone tissue which can lead to an increased risk of fracture ². Sarcopenia means loss of muscle either mass or function in the elderly, and can reduce mobility, diminish quality of life, and lead to fall-related injuries ^{3,4}. Usually, osteoporosis and sarcopenia occur in consort, it is recently referred as 'osteosarcopenia' ^{1,5}.

Bone and muscle closely interact with each other, not only anatomically, but also chemically and metabolically ^{6,7}. Additionally, specific pathophysiological finding, such as fat infiltration, is common to both diseases. It suggests that sarcopenia and osteoporosis are closely linked ⁸.

Despite sharing common risk factors and biological pathways, the inherent molecular mechanisms underlying the pathogenesis of osteosarcopenia have not been fully clarified. Further researches are needed to provide deeper understanding to explore more advantageous therapeutic targets.

In the past few years, microarray technology had been widely used for gene expression profiling in osteoporosis and sarcopenia patients ^{9,10}. However, there was no microarray study in osteosarcopenia patients. Thus, we conducted a gene expression meta-analysis between osteoporosis and sarcopenia using integrated bioinformatics methods. Additionally, based on the result of this analysis, gene

enrichment and pathway annotation analysis were performed and hub genes were also identified in the DEGs.

Results

Identification of DEGs

After standardization of the datasets (Supplementary Figure 1), there were 590 DEGs out of GSE56814, 2925 DEGs out of GSE56815. There were 1496 DEGs in datasets of GSE1428, and 4200 DEGs in datasets of GSE8479. The DEGs were shown in the volcano plots respectively (Figures 1). The co-expressed DEGs were detected using the Venn diagram online tool, including 133 DEGs in at least three datasets in osteoporosis and sarcopenia (Figure 2, Supplementary Table 1).

GO and KEGG pathway enrichment analyses

133 DEGs were analyzed and visualized in R software. According to the cut-off standard, there were 5, 13 and 4 functions enriched in for BP, CC and MF in GO analysis (Figure 3). And 19 pathways enriched in KEGG analysis (Figure 4). The most three significantly enriched for BP were regulation of mRNA metabolic process, mRNA stability and RNA stability. For CC in GO analysis, DEGs were significantly enriched in the vacuolar membrane and membrane coat. For MF, DEGs were significantly enriched in S100 protein binding, MHC class II protein binding and mRNA 3'-UTR AU-rich region binding. The result of KEGG pathway enrichment analysis indicated that DEGs were mainly enriched in salmonella infection, AMPK signaling pathway and dopaminergic synapse.

PPI network analysis and hub gene selection

Based on the STRING database, the PPI network of DEGs were gathered as a cluster consisting of 133 nodes and 116 edges (Figure 5). MCODE plug-in of Cytoscape software was applied to identify the most significant module that was comprised of 5 nodes (MCODE score ≥ 2). We finally defined the five hub genes, including AKT1, ANXA2, VIM, S100A6 and S100A11.

Discussion

Osteosarcopenia is a recent terminology, which means a person under osteoporosis and sarcopenia at the same time. Some professors also called it as sarco-osteoporosis¹¹. Previous studies demonstrated that sarcopenia patients had a higher risk of having coexisting osteoporosis compared with non-sarcopenia individuals¹². Some other studies showed that one with both osteoporosis and sarcopenia has a higher risk of fall and fracture than those with osteoporosis or sarcopenia alone^{11,13}. Osteosarcopenia is recognized as a complex disease, not only associated with age, but also with endocrine disorders, malnutrition, obesity and use of corticosteroids¹⁴. Identifying the susceptibility gene of osteosarcopenia could help us to deeper understand this disease and to find potential treatments.

Our study was the first to systematically search and incorporate the microarrays on osteosarcopenia in GEO. In the present study, we included two microarrays in osteoporosis and sarcopenia respectively, compared gene expression profiles between old and young person, identified the co-expressed DEGs in the two diseases. Furthermore, functional and pathway enrichment analyses were performed to understand the potential biological function of the DEGs, and PPI network construction were performed to identify interactions between DEGs.

133 DEGs were filtered out across multiple datasets. Among these DEGs, DUSP1 and SARAF got significant difference in all the four datasets. AKT1, ANXA2, VIM, S100A6 and S100A11 were identified as the hub genes under PPI network analysis. The AKT1 gene could be transcribed to produce a protein called AKT1 kinase, which plays a critical role in many signaling pathways, helps regulate cell growth and division, and also helps control apoptosis. Several researches have reported that AKT1 could control osteoblast differentiation and development ¹⁵, and also could influence metabolic and differentiation of muscle ¹⁶. It was also be validated in our study, that AKT1 was high expressed in osteoporosis patients while low expressed in sarcopenia patients.

ANXA2 encodes a member of a calcium-dependent phospholipid-binding protein family, which play a role in the regulation of cellular growth and signal transduction pathways. Previous studies have shown that elevated ANXA2 protein expression level could stimulate peripheral blood monocytes (PBM) differentiate into higher number of osteoclasts and resorb bone at higher rates to decreasing BMD ¹⁷. In our study, ANXA2 was low expression in osteoporosis datasets. In sarcopenia datasets, the ANXA2 was low expression in GSE1428, while high expression in GSE8479. It is consistent with previous studies that ANXA2 is helpful to repair muscle cell membrane and also could mediate the occurrence of muscle inflammation ¹⁸⁻²⁰.

VIM gene could encode a type III intermediate filament protein, which maintaining the shape of cell and integrity of the cytoplasm, and stabilizing interaction of cytoskeleton. Previous study has report that VIM has a key role in regulating inflammation in macrophages ²¹. And increased expression of VIM could be considered as a reliable marker of muscle regeneration ^{22,23}. The expression of VIM in sarcopenia datasets was difference in our study, that maybe the result of different micro-environment. VIM gene was up-regulated in osteoporosis patients compared with contral group. The regulatory mechanism of VIM in osteoporosis still need to be further studied.

S100A6 and S100A11 are members of the S100 family. Several studies demonstrated S100 proteins may participate in functional inhibition of osteoblast progenitor cells, inflammatory, and combined with ANXA1 to participate in membrane repair ^{18,24-26}. In our study, S100A6 and S100A11 were up-regulated in osteoporosis patients, but different expressed in sarcopenia datasets. This result showed that S100 protein has various regulatory functions in muscle tissue under different situation.

Further functional enrichment analyses demonstrated that DEGs in osteosarcopenia were significantly enriched in regulation of mRNA metabolic process and stability. Previous studies showed that some

hormone, such as growth hormone/insulin-like growth factor-1 (GH/IGF1), play a key role in the development of osteosarcopenia ²⁷. Besides the AMPK signaling pathway, a pathway that affects the metabolism of various substances, the DEGs were significantly enriched in the salmonella infection with KEGG analyses. Several studies have confirmed that some pro-inflammatory cytokines could promote bone resorption, and influence the development of sarcopenia through the activation of ubiquitin-protease pathway ^{28–30}.

However, there was a limitation in our study that the four datasets were included from two diseases. Until now, there have been no genetic sequencing studies in osteosarcopenia patients. Further researches are needed to verify the changes of gene expression in osteosarcopenia patients in the future.

Conclusions

In summary, the study provided deeper insight to the comprehensive molecular changes in the pathogenesis of osteosarcopenia, and identified several potential therapeutic targets, including AKT1, ANXA2, VIM, S100A6 and S100A11. Through GO and KEGG pathway analysis, we found that the DEGs were mainly enriched in regulation of mRNA metabolic process, regulation of mRNA stability and S100 protein binding, and were involved in salmonella infection and AMPK signaling pathway. The results could help to anticipate adverse health outcomes and development of new target therapies.

Materials And Methods

Microarray Data

We selected GEO (<http://www.ncbi.nlm.nih.gov/geo>), which is a publicly available database of gene/microarray profiles for our study ³¹. The search strategy ("osteoporosis, postmenopausal" [MeSH Terms] OR "osteoporosis" [MeSH Terms] OR osteoporosis [All Fields]) AND "Homo sapiens" [porgn] AND "gse" [Filter] was adopted for osteoporosis patients. And the search strategy (sarcopenia [All Fields] AND "Homo sapiens" [porgn] AND "gse" [Filter]) was adopted for sarcopenia patients. All the included datasets contain normal tissue as controls. We extracted the gene expression datasets GSE56814 and GSE56815 for osteoporosis, GSE1428 and GSE8479 for sarcopenia (Table 1). The tissues of osteoporosis datasets are blood monocytes, which are progenitors of osteoclasts and produce important factors to bone metabolism. The tissues of sarcopenia datasets are vastus lateralis muscle, which can directly demonstrate the gene expression of skeletal muscles.

Table 1
Characteristics of the included microarray datasets.

GSE ID	Participants	Tissue	Analysis type	Platform
GSE56814	16 postmenopausal females with low BMD and 16 premenopausal females with high BMD	blood monocytes	Array	GPL5175
GSE56815	20 postmenopausal females with low BMD and 20 premenopausal females with high BMD	blood monocytes	Array	GPL96
GSE1428	12 old males and 10 young males	muscle	Array	GPL96
GSE8479	25 old males and 26 young males	muscle	Array	GPL2700

The platform for GSE56814 is GPL5175 [HuEx-1_0-st] Affymetrix Human Exon 1.0 ST Array [transcript (gene) version], which include 16 postmenopausal females with low BMD and 16 premenopausal females with high BMD. The platform for GSE56815 is GPL96 [HG-U133A] Affymetrix Human Genome U133A Array, which include 20 postmenopausal females with low BMD and 20 premenopausal females with high BMD. Additionally, the platform for GSE1428 is also GPL96, which include older (n = 12) and younger (n = 10) male. And the platform for GSE8479 is GPL2700, Sentrix HumanRef-8 Expression BeadChip, which included older (n = 25) and younger (n = 26) adults. Series matrix files and related annotation document of GSE56814, GSE56815, GSE1428 and GSE8479 were downloaded.

Identification of differentially expressed genes

The corresponding annotation document was used to map the microarray probes to gene symbols. The mean value was adopted when multiple probes mapped to the same symbol. All the expression microarray datasets were first standardized. And the DEGs were determined between osteoporosis or sarcopenia tissues and normal tissues in each microarray by using the limma V3.46.0 (linear models for microarray data) package of the R software program (version 4.0.3)³². The $|\log_2 \text{fold change (FC)}| > 0.1$ and $p\text{-value} < 0.05$ were regarded as the cut-off criteria to determine DEGs. Online tool Calculate and draw custom Venn diagram (<http://bioinformatics.psb.ugent.be/webtools/Venn/>) was applied to detect common DEGs among the 4 datasets. DEGs in any three of the four datasets were considered to the common DEGs.

Functional and Pathway Enrichment Analyses

Gene ontology (GO) analysis is used extensively to identify the characteristic biological attributes of genes, gene products, and sequences, including biological process (BP), cell components (CC) and molecular function (MF)³³. Kyoto Encyclopedia of Genes and Genomes (KEGG) analysis provides a comprehensive set of bio-interpretation of genomic sequences and protein interaction network information³⁴.

In this study, GO analysis and KEGG pathway enrichment analysis of DEGs were automatically completed and visualized by the clusterProfiler V3.18.0³⁵, org.Hs.eg.db V3.10.0, and Goplot V1.0.2 package³⁶ in the R software statistical analysis platform. P.adjust < 0.1 and q-value < 0.2 were regarded as the cut-off criteria in GO analysis, while p-value < 0.05 was regarded as the cut-off criteria in KEGG analysis.

Protein-Protein Interaction (PPI) Network Analysis

We searched the STRING database (<http://www.string-db.org/>)³⁷ to identify and predict interactions between DEGs, and construct the PPI network with the cut-off standard as a confidence > 0.4. Cytoscape software V3.7.0³⁸ was used to visualize the PPI network for DEGs. And use the MCODE (Molecular Complex Detection) V1.6.1, one of plug-in of Cytoscape, to identify significant modulators (MCODE score ≥ 2).

Declarations

Author Contributions

Yang Li: Analysis, Paper writing

Jianmin Sun, Guodong Wang: Reserach design, Paper editing.

Conflicts of Interest

No potential conflicts of interest were reported by any of the authors in the conduct of this work.

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References

1. Clynes, M. A., Gregson, C. L., Bruyère, O., Cooper, C. & Dennison, E. M. Osteosarcopenia: Where Osteoporosis and Sarcopenia Collide. *Rheumatology*. **60**, 529-537. doi:10.1093/rheumatology/keaa755 (2021)
2. Eastell, R. et al. Postmenopausal Osteoporosis. *Nature Reviews Disease Primers*. **2**, doi:10.1038/nrdp.2016.69 (2016)
3. Cruz-Jentoft, A. J. et al. Sarcopenia: European Consensus On Definition and Diagnosis: Report of the European Working Group On Sarcopenia in Older People. *Age Ageing*. **39**, 412-423. doi:10.1093/ageing/afq034 (2010)
4. Cruz-Jentoft, A. J. et al. Sarcopenia: Revised European Consensus On Definition and Diagnosis. *Age Ageing*. **48**, 16-31. doi:10.1093/ageing/afy169 (2019)

5. Fagundes Belchior, G., Kirk, B., Pereira Da Silva, E. A. & Duque, G. Osteosarcopenia: Beyond Age-Related Muscle and Bone Loss. *Eur. Geriatr. Med.* **11**, 715-724. doi:10.1007/s41999-020-00355-6 (2020)
6. Laurent, M. R. et al. Muscle-Bone Interactions: From Experimental Models to the Clinic? A Critical Update. *Mol. Cell. Endocrinol.* **432**, 14-36. doi:10.1016/j.mce.2015.10.017 (2016)
7. De Rui, M. et al. Dietary Strategies for Mitigating Osteosarcopenia in Older Adults: A Narrative Review. *Aging Clin. Exp. Res.* **31**, 897-903. doi:10.1007/s40520-019-01130-9 (2019)
8. Hirschfeld, H. P., Kinsella, R. & Duque, G. Osteosarcopenia: Where Bone, Muscle, and Fat Collide. *Osteoporosis Int.* **28**, 2781-2790. doi:10.1007/s00198-017-4151-8 (2017)
9. Zhou, Y. et al. Transcriptomic Data Identified Key Transcription Factors for Osteoporosis in Caucasian Women. *Calcified Tissue Int.* **103**, 581-588. doi:10.1007/s00223-018-0457-6 (2018)
10. Giresi, P. G. et al. Identification of a Molecular Signature of Sarcopenia. *Physiol. Genomics.* **21**, 253-263. doi:10.1152/physiolgenomics.00249.2004 (2005)
11. Wang, Y. et al. Sarco-Osteoporosis: Prevalence and Association with Frailty in Chinese Community-Dwelling Older Adults. *Int. J. Endocrinol.* **2015**, 1-8. doi:10.1155/2015/482940 (2015)
12. Locquet, M. et al. Bone Health Assessment in Older People with Or without Muscle Health Impairment. *Osteoporosis Int.* **29**, 1057-1067. doi:10.1007/s00198-018-4384-1 (2018)
13. Huo, Y. R. et al. Phenotype of Osteosarcopenia in Older Individuals with a History of Falling. *J. Am. Med. Dir. Assoc.* **16**, 290-295. doi:10.1016/j.jamda.2014.10.018 (2015)
14. Kawao, N. & Kaji, H. Interactions Between Muscle Tissues and Bone Metabolism. *J. Cell. Biochem.* **116**, 687-695. doi:10.1002/jcb.25040 (2015)
15. Mukherjee, A. & Rotwein, P. Selective Signaling by Akt1 Controls Osteoblast Differentiation and Osteoblast-Mediated Osteoclast Development. *Mol. Cell. Biol.* **32**, 490-500. doi:10.1128/MCB.06361-11 (2011)
16. Rotwein, P. & Wilson, E. M. Distinct Actions of Akt1 and Akt2 in Skeletal Muscle Differentiation. *J. Cell. Physiol.* **219**, 503-511. doi:10.1002/jcp.21692 (2009)
17. Deng, F. et al. Peripheral Blood Monocyte-Expressed Anxa2 Gene is Involved in Pathogenesis of Osteoporosis in Humans. *Mol. Cell. Proteomics.* **10**, M111-M11700. doi:10.1074/mcp.M111.011700 (2011)
18. Koerdt, S. N. & Gerke, V. Annexin a2 is Involved in Ca²⁺-Dependent Plasma Membrane Repair in Primary Human Endothelial Cells. *Biochimica et Biophysica Acta (BBA) - Molecular Cell Research.* **1864**, 1046-1053. doi:10.1016/j.bbamcr.2016.12.007 (2017)
19. Defour, A. et al. Annexin a2 Links Poor Myofiber Repair with Inflammation and Adipogenic Replacement of the Injured Muscle. *Hum. Mol. Genet.* **26**, 1979-1991. doi:10.1093/hmg/ddx065 (2017)
20. Bittel, D. C. et al. Annexin a2 Mediates Dysferlin Accumulation and Muscle Cell Membrane Repair. *Cells.* **9**, 1919. doi:10.3390/cells9091919 (2020)

21. Håversen, L. et al. Vimentin Deficiency in Macrophages Induces Increased Oxidative Stress and Vascular Inflammation but Attenuates Atherosclerosis in Mice. *Sci. Rep.-Uk.* **8**, doi:10.1038/s41598-018-34659-2 (2018)
22. Soglia, F. et al. Distribution and Expression of Vimentin and Desmin in Broiler Pectoralis Major Affected by the Growth-Related Muscular Abnormalities. *Frontiers in Physiology.* **10**, doi:10.3389/fphys.2019.01581 (2020)
23. Bornemann, A. & Schmalbruch, H. Desmin and Vimentin in Regenerating Muscles. *Muscle Nerve.* **15**, 14-20. doi:10.1002/mus.880150104 (1992)
24. Hong, D. et al. Quantitative Proteomic Analysis of Dexamethasone-Induced Effects On Osteoblast Differentiation, Proliferation, and Apoptosis in Mc3T3-E1 Cells Using Silac. *Osteoporosis Int.* **22**, 2175-2186. doi:10.1007/s00198-010-1434-8 (2011)
25. Andrés Cerezo, L. et al. Pro-Inflammatory S100a11 is Elevated in Inflammatory Myopathies and Reflects Disease Activity and Extramuscular Manifestations in Myositis. *Cytokine.* **116**, 13-20. doi:10.1016/j.cyto.2018.12.023 (2019)
26. Tsoporis, J. N., Izhar, S. & Parker, T. G. Expression of S100a6 in Cardiac Myocytes Limits Apoptosis Induced by Tumor Necrosis Factor- α . *J. Biol. Chem.* **283**, 30174-30183. doi:10.1074/jbc.M805318200 (2008)
27. Girgis, C. M., Mokbel, N. & DiGirolamo, D. J. Therapies for Musculoskeletal Disease: Can we Treat Two Birds with One Stone? *Current Osteoporosis Reports.* **12**, 142-153. doi:10.1007/s11914-014-0204-5 (2014)
28. Curtis, E., Litwic, A., Cooper, C. & Dennison, E. Determinants of Muscle and Bone Aging. *J. Cell. Physiol.* **230**, 2618-2625. doi:10.1002/jcp.25001 (2015)
29. Ding, C., Parameswaran, V., Udayan, R., Burgess, J. & Jones, G. Circulating Levels of Inflammatory Markers Predict Change in Bone Mineral Density and Resorption in Older Adults: A Longitudinal Study. *The Journal of Clinical Endocrinology & Metabolism.* **93**, 1952-1958. doi:10.1210/jc.2007-2325 (2008)
30. McLean, R. R. Proinflammatory Cytokines and Osteoporosis. *Curr Osteoporos Rep.* **7**, 134-139. doi:10.1007/s11914-009-0023-2 (2009)
31. Barrett, T. et al. Ncbi Geo: Archive for Functional Genomics Data Sets—Update. *Nucleic Acids Res.* **41**, D991-D995. doi:10.1093/nar/gks1193 (2012)
32. Ritchie, M. E. et al. Limma Powers Differential Expression Analyses for Rna-Sequencing and Microarray Studies. *Nucleic Acids Res.* **43**, e47. doi:10.1093/nar/gkv007 (2015)
33. Consortium, G. O. The Gene Ontology (Go) Project in 2006. *Nucleic Acids Res.* **34**, D322-D326. doi:10.1093/nar/gkj021 (2006)
34. Kanehisa, M. & Goto, S. Kegg: Kyoto Encyclopedia of Genes and Genomes. *Nucleic Acids Res.* **28**, 27-30. doi:10.1093/nar/28.1.27 (2000)
35. Yu, G., Wang, L., Han, Y. & He, Q. Clusterprofiler: An R Package for Comparing Biological Themes Among Gene Clusters. *OMICS: A Journal of Integrative Biology.* **16**, 284-287.

doi:10.1089/omi.2011.0118 (2012)

36. Walter, W., Sanchez-Cabo, F. & Ricote, M. Gplot: An R Package for Visually Combining Expression Data with Functional Analysis. *Bioinformatics*. **31**, 2912-2914. doi:10.1093/bioinformatics/btv300 (2015)
37. Szklarczyk, D. et al. String V10: Protein–Protein Interaction Networks, Integrated Over the Tree of Life. *Nucleic Acids Res.* **43**, D447-D452. doi:10.1093/nar/gku1003 (2015)
38. Kohl, M., Wiese, S. & Warscheid, B. Cytoscape: Software for Visualization and Analysis of Biological Networks. *Methods Mol Biol.* **696**, 291-303. doi:10.1007/978-1-60761-987-1_18 (2011)

Figures

Figure 1

Volcano plots of the four microarray datasets. Red points represented up-regulated genes, while blue points represented down-regulated genes. Gray points represented genes with no significant difference.

Figure 2

Venn diagram of common differentially expressed genes from the four datasets.

Figure 3

Gene Ontology (GO) enrichment analysis of differentially expressed genes (DEGs). (A) Bar chart shows GO enrichment significance items of DEGs in three functional groups: biological processes (BP), and cell composition (CC), molecular function (MF). (B) Bubble chart shows GO enrichment significance items of DEGs in three functional groups. (C) Network diagram shows the relationship between the DEGs and GO terms.

Figure 4

Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway analysis of differentially expressed genes (DEGs). (A) Bar chart shows enrichment of DEGs in signalling pathways. (B) Bubble chart shows enrichment of DEGs in signalling pathways. (C) Network diagram shows the relationship between the DEGs and KEGG pathways.


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graph TD
    S100A6((S100A6)) --- S100A11((S100A11))
    S100A6 --- ANXA2((ANXA2))
    S100A11 --- ANXA2
    ANXA2 --- VIM((VIM))
    ANXA2 --- AKT1((AKT1))
    VIM --- AKT1
  
```

Protein-protein interaction (PPI) network. (A) PPI network of differentially expressed genes (DEGs). (B) subnetwork of top five hub genes from the PPI network.

Supplementary Files

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