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PLAGL1 Is Identified as a Potential Diagnostic Marker for Co-Occurrence with Osteoporosis and Multiple Myeloma

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Abstract

Background: Osteoporosis (OP) is a common clinical manifestation of multiple myeloma (MM). The aim of this study was to investigate the possible molecular pathways and shared genes in the co-occurrence of OP and MM. **Methods:** The Gene Expression Omnibus database was used to retrieve gene expression information. Use WGCNA and differential analysis to screen out Hub genes. The GENEMANIA was used to build protein-protein interaction (PPI) networks. Enrichment analyses were performed to explore the functions. Validation datasets were selected to verify the diagnostic marker reliability of PLAGL1. The immune microenvironment of diseases was analyzed by immune infiltration analyses. **Results:** We confirmed a hub gene called PLAGL1, which is significantly under-expressed in both OP and MM. We found hub genes were associated with glucose and energy metabolism. Subsequently, the reliability of PLAGL1 for diagnosing OP and MM was verified using ROC curves, with all areas under the curve > 0.75. Moreover, PLAGL1 regulates t lymphocytes and may participate in the occurrence of OP in MM through immune pathways. **Conclusions:** PLAGL1 is a hub gene for the co-occurrence of OP and MM. It can regulate T-lymphocyte involvement in disease development. PLAGL1 may be a novel diagnostic marker for the co-occurrence of OP and MM.

Keywords

Osteoporosis, Multiple Myeloma, PLAGL1, Immunity, Weighted Gene Co-Expression Network Analysis

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#These authors made equal contributions to this work.

1. Introduction

Multiple myeloma (MM) is a malignant disease of plasma cells in which tumor cells arise from plasma cells in the bone marrow. MM is closely associated with monoclonal proliferation, the production of monoclonal antibodies, and end-stage organ injury [1]. According to recent data, there were 0.9% of all cancer diagnoses in 2018 and an estimated 160000 instances of MM worldwide. There were approximately 90,000 men and 70,000 women among those Multiple myeloma Patients, which corresponds to an age-standardized incidence of 2.1/100,000 and 1.4/100,000, respectively. The cumulative risk of being diagnosed from birth to 74 is 0.24% among men and 0.17% among women, making the disease about 1.5× more likely in men [2]. Its incidence is increasing in developed countries. From 1990 to 2016, the global incidence of MM increased by 126% [3]. It is the second most common hematologic malignancy, affecting mainly older male patients. CRAB symptoms, which include increased calcium ions, renal failure, anemia, and bone disease, are common in MM patients. When MM is suspected, blood and urine electrophoresis should be conducted to test for tumor-secreted monoclonal light chains. Blood IgG, IgM, and IgA levels can be used to determine the subtype of light chain generated. To detect osteolytic lesions, imaging techniques such as CT (without contrast owing to renal impairment), MRI, and PET scans are employed. A skeletal examination is utilized instead of imaging if the patient is unable to undergo imaging. At the time of diagnosis, 80% of patients have a skeletal lesion, fracture, or bone loss [4] [5] [6].

Bone damage is one of the characteristic manifestations of MM, which is present in up to 75% of patients with a primary diagnosis of MM [7]. Bone damage includes osteolytic damage, pathological fractures, and osteoporosis [8]. Osteoporosis (OP) is a key process in the reduction of bone mass and can often result in fractures [9]. China has an estimated 60.2 million OP patients, with the incidence of OP reaching 6.46% in elderly males and 29.13% in elderly females [10]. In the UK, the prevalence of osteoporosis in people over 50 years old is 6.8% in men and 21.8% in women [11]. In conclusion, the prevalence of OP is high in both Asia and Europe. Typical symptoms of osteoporosis are weakness, generalized bone pain, spinal deformities and even vertebral fractures.

The interaction between osteoclasts, osteoblasts, adipocytes and osteocytes results in increased bone destruction and decreased bone remodeling leading to osteoporosis. Among the molecular mechanisms of osteoporosis, the Osteoclasts are induced to bone resorption by secreting protein hydrolases (cathepsin K, MMP-9) and hydrochloric acid [12] [13] [14]. Some cytokines such as IL-1, IL-6 and tumor necrosis factor-α (TNF-α) and $\alpha V\beta 3$ integrin can regulate osteoclast differentiation [15] [16]. Runt-related transcription factor 2 (Runx2), osterix (Osx), β-catenin, activating transcription factor 4 (Atf4), and activator protein 1 (AP-1) family play a key role in osteoblast turnover [17] [18] [19]. Similarly,

ibroblast growth factors (FGFs), transforming growth factor-β1 (TGF-β1), IGF-1, Notch, and PTH have also been shown to promote osteogenic differentiation [20] [21] [22]. Osteoblasts can regulate bone formation by secreting substances, such as sclerostin and DKK1, that inhibit osteoblast function [23] [24]. Osteoblast osteocytes secrete RANKL and M-CSF, or nitric oxide and OPG to inhibit osteoclast function [25] [26]. Adipocyte differentiation while competing with osteoblasts, and expression of RANKL by mature adipocytes can promote osteoclastic differentiation [27] [28].

MM is one of the many causes of osteoporosis. Specifically, most MM patients develop osteolytic lesions and OP [29]. An imbalance between bone degradation and reconstruction is behind the pathogenesis of osteolytic lesions and progressive bone loss in MM. This imbalance is related to the activation of osteoclasts, including an increase in their apoptosis and the simultaneous suppression of osteoblasts. The mechanism of bone destruction in myeloma can be summarized as osteoblast promotion and osteoclast activation. The molecular mechanisms involved include the RANKL/RANK/OPG axis, Notch signaling, the Wnt/β-Catenin signaling pathways, and signaling molecules such as DKK-1, sclerostin, osteopontin, activin A, chemokines, and interleukins [30].

MM cells can secrete NF-κ B ligand (RANKL), interleukin (IL)-1, IL-6, and chemokine C-C motif ligand 3 (CCL3) to promote osteoclast proliferation. Osteoblast inhibitors dickkopf-1 (DKK1) and sclerostin can also be secreted to inhibit osteoblast proliferation [8] [31] [32] [33] [34]. In addition, MM and BMSCs cells can also secrete vascular endothelial growth factor (VEGF), insulin-like growth factor (IGF-1), TGF-β, angiopoietin-1 (Ang-1), platelet-derived growth factor (PDGF), and basic-fibroblast growth factor (bFGF) to promote angiogenesis, increase osteoclastic differentiation, and promote tumor cell proliferation [35] [36] [37]. What is more, activin A was found in MM patients with advanced bone disease, and a correlation was found between elevated activin A levels and lysis bone lesions [38]. Deregulation of the bone compartment can create a suitable microenvironment for MM cell proliferation and differentiation [39]. Bone marrow stromal cells (BMSCs) can promote MM cell growth and increased osteolytic bone lesions within the bone marrow through adhesion molecules [40].

These studies are all about osteolytic destruction, but the genetic and molecular mechanisms of osteoporosis are not clear. The purpose of this study is to detect whether the early causes of osteoporosis are associated with myeloma and to treat multiple myeloma at an early stage. In this study, a search of gene expression data from MM and OP was performed using the Gene Expression Omnibus (GEO) database, which identified several differentially expressed genes that strongly overlapped between these conditions. These genes may be potential diagnostic markers for the complication of osteoporosis and multiple myeloma.

We used a public database to analyze the potential pathogenesis of MM and OP. A flowchart of this study is shown in **Figure 1**.

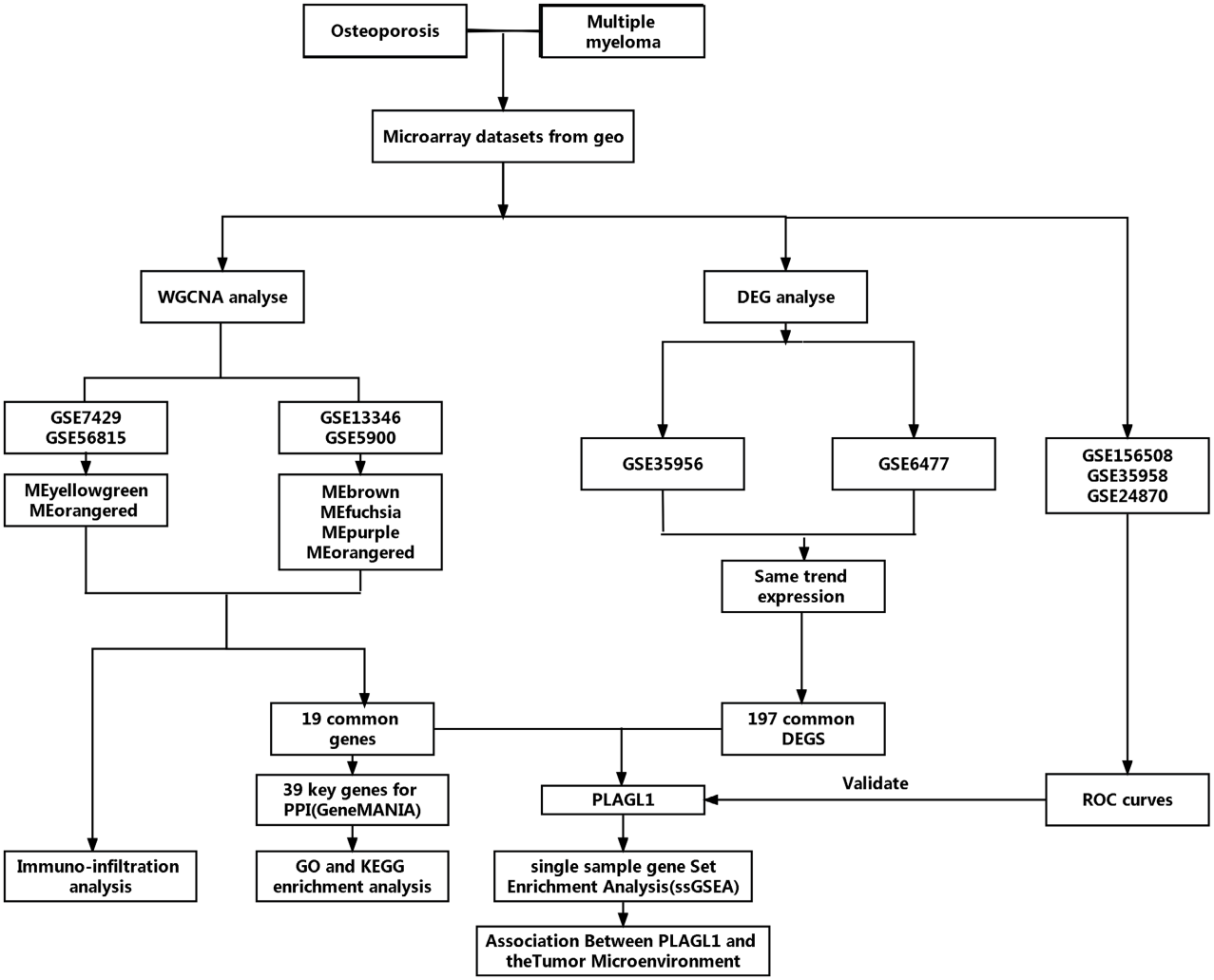


Figure 1. Research design flow chart.

2. Research Methodology

2.1. Data Sources

In this study, we obtained data from the Gene Expression Omnibus (GEO). Multiple myeloma (MM) and osteoporosis (OP) were keywords for the related gene expression data set. The selection criteria for the datasets were as follows. The data we selected included both the disease and normal groups. Each group should contain a test sample of adults with MM who had not received radiation therapy. These datasets should further contain raw analytical data. Based on the above criteria, The GSE133346, GSE5900, GSE7429, GSE56815, GSE6477, and GSE35956 datasets were finally incorporated as training datasets, and the GSE24870, GSE156508, and GSE35958 were incorporated as validation datasets. The details of these datasets are summarized in Table 1. GSE7429 data show the gene expression of circulating B cells in the blood of 10 high-density and 10 low-density subjects. GSE56815 data show the gene expression of circulating monocytes from 40 hip high density subjects and 40 hip low density subjects.

Table 1. Information on the GEO datasets for OP/MM patients.

ID	GSE number	Platform	Samples	Disease	Dataset
1	GSE7429	GPL96-57554	10 patients and 10 controls	OP	Training
2	GSE56815	GPL96-57554	40 patients and 40 controls	OP	Training
3	GSE133346	GPL570-55999	12 patients and 12 controls	MM	Training
4	GSE5900	GPL570-55999	56 patients and 22 controls	MM	Training
5	GSE35956	GPL570-55999	5 patients and 5 controls	OP	Training
6	GSE6477	GPL96-57554	147 patients and 15 controls	MM	Training
7	GSE24870	GPL570-55999	7 patients and 5 controls	MM	Validation
8	GSE156508	GPL96-57554	6 patients and 6 controls	OP	Validation
9	GSE35958	GPL570	6 patients and 4 controls	OP	Validation

OP, Osteoporosis; MM, Multiple myeloma; GEO, Gene Expression Omnibus.

GSE133346 data are the gene expression of Adipose Stromal Cells (ASC) from 12 healthy donors and 12 multiple myeloma patients. GSE5900 data are the gene expression of human mesenchymal stem cells (hMSC) from 22 healthy donors and 56 myeloma patients. GSE35956 data show gene expression of human mesenchymal stem cells from 5 subjects with high bone density and 5 subjects with low bone density. GSE6477 data are the gene expression of bone marrow plasma cells from 15 healthy donors and 147 multiple myeloma patients. GSE24870 are the gene expression of hematopoietic stem cells, common myeloid progenitors, granulocyte/monocyte progenitors, and megakaryocyte/erythroid progenitors from 5 healthy donors and 7 myeloma patients. GSE156508 data are the gene expression of primary osteoblasts from 6 osteoarthritis donors and 6 osteoporotic fracture patients. GSE35958 data are the gene expression of human mesenchymal stem cells from 4 healthy donors and 6 patients with osteoporosis. Batch corrections can significantly reduce differences between datasets (**Figure 2**).

2.2. Weighted Gene Coexpression Network Analysis (WGCNA)

We identified the coexpression network and coexpression modules between MM and OP using WGCNA-related R packages. First, using the flash tool in R, we analyzed the samples by hierarchical clustering and detected and eliminated abnormal samples. Second, based on the scale threshold topology criterion, we set the "soft" threshold power of WGCNA using the "picksoft threshold" algorithm (β). We chose $R^2 = 0.88$ and the soft-threshold $\beta = 3$ in MM and chose $R^2 = 0.86$ and the soft-threshold $\beta = 6$ in OP. Third, we established a topological superposition matrix (TOM) based on an adjacency matrix. Fourth, we detected gene modules using the dynamic tree-cut algorithm. Fifth, we calculated gene significance and module dependence, and associated the modules with particular clinical features. The other parameters both in OP and MM were the following:

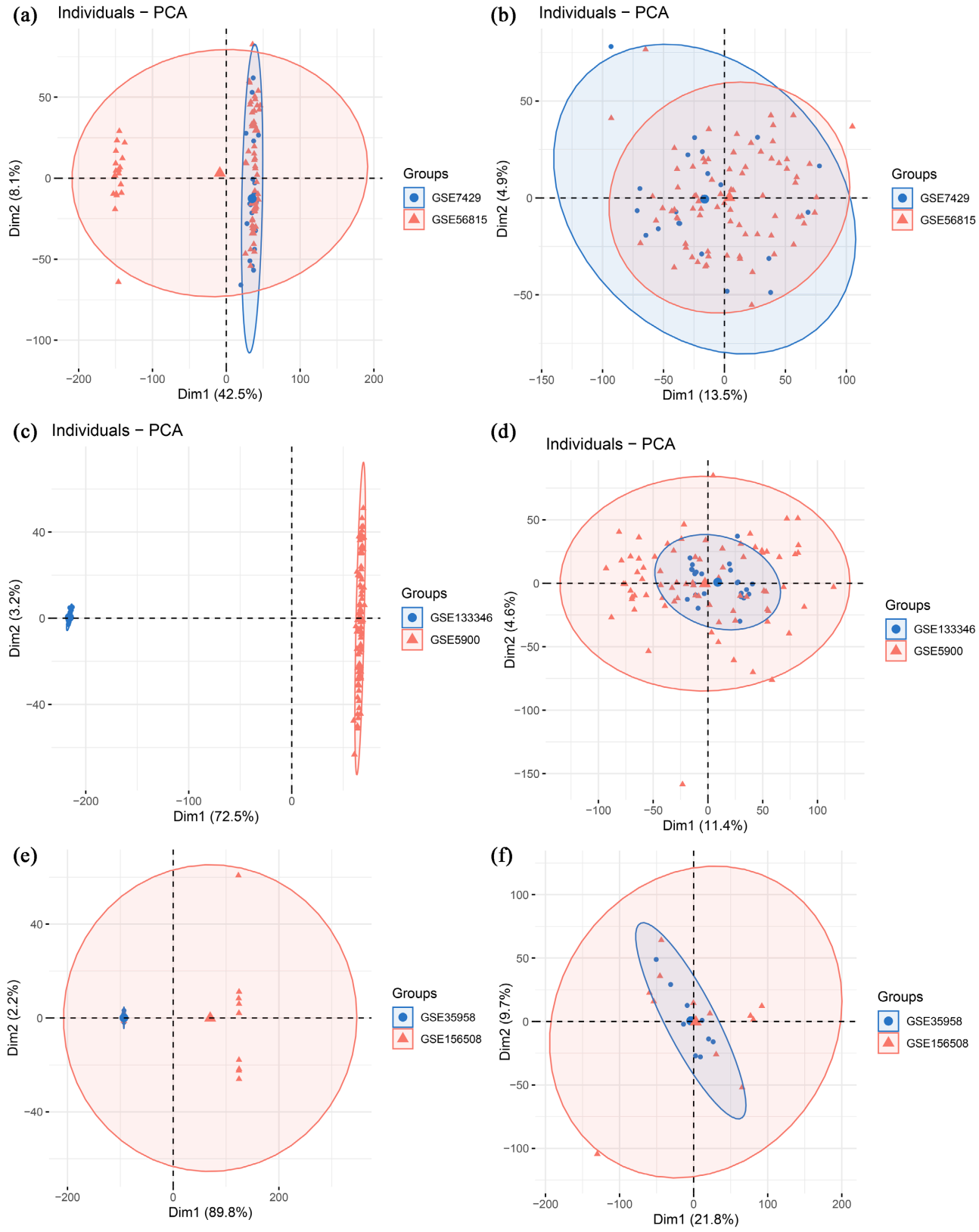


Figure 2. Batch correction. ((a), (c), (e)) principal components analyses (PCA) of OP ((a), (e)) and MM (C) prior to batch correction. ((b), (d), (f)) PCA of OP (b) and MM ((d), (f)) after batch correction.

minModuleSize = 60, mergeCutHeight = 0.25 and deepSplit = 2, geneSigFilter = 0.5, moduleSigFilter = 0.8.

2.3. Identification of Common Gene

The online program venny2.1.0 (https://bioinfogp.cnb.csic.es/tools/venny/index.html) was used to identify the genes overlapping between OP- and MM-related preservation modules using Venn diagrams.

2.4. Analyses of the Functional Enrichment of Genes

Gene expression data can be annotated by enrichment analyses of Gene Ontology (GO) terms and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways. "ClusterProfiler" package in R software was used for functional enrichment analysis. We studied the vital biological functions associated with the genes expressed in OP and MM. Analyses of the KEGG and GO pathways particularly associated with the expressed genes revealed the primary signaling pathways of OP and MM. $p < 0.05$ was considered to reflect enrichment of the genes/pathways. Bar graphs were drawn using the ggplot2 package in R.

2.5. Gene Set Enrichment Analyses

Using the median level of PLAGL1 expression, we classified GSE6477 samples into high- and low-expressing groups. We then applied GSEA, a feature-typology-based technique that computes genomic enrichment and identifies diverse pathways that may be relevant to biological functions [41]. GSEA was conducted by using the clusterProfiler package in R and hallmark signatures (h.all.v7.2.symbols.gmt) from MsigDB [42] [43]. Results were considered significant when normalized p value < 0.05 and FDR < 0.25. We show only the top 5 sorted pathways, sorted from smallest to largest according to P-value.

2.6. Protein-Protein Interaction Network Construction

GeneMANIA (http://www.genemania.org) can be utilized for protein–protein interaction (PPI) networks, which provide possibilities for predicting gene functions and identifying genes with closely linked effects. Using 19 common genes between OP and MM as input data, this study used GeneMANIA technique for PPI of common genes. The species setting of GeneMANIA is Homo sapiens.

2.7. Immune Infiltration Analyses

We evaluated the tumor immune microenvironment (TIME) in OP and MM by immune infiltration analyses via a single-sample gene enrichment assay (ssGSEA) using the R package "GSVA" [44]. Gene sequence analyses (GSVA) is a tool for calculating enrichment scores [45]. Next, we compared the differences in immune function between the disease and control groups in OP and MM. PLAGL1 may be involved in immune cell infiltration, which we investigated using

Spearman's correlation analyses.

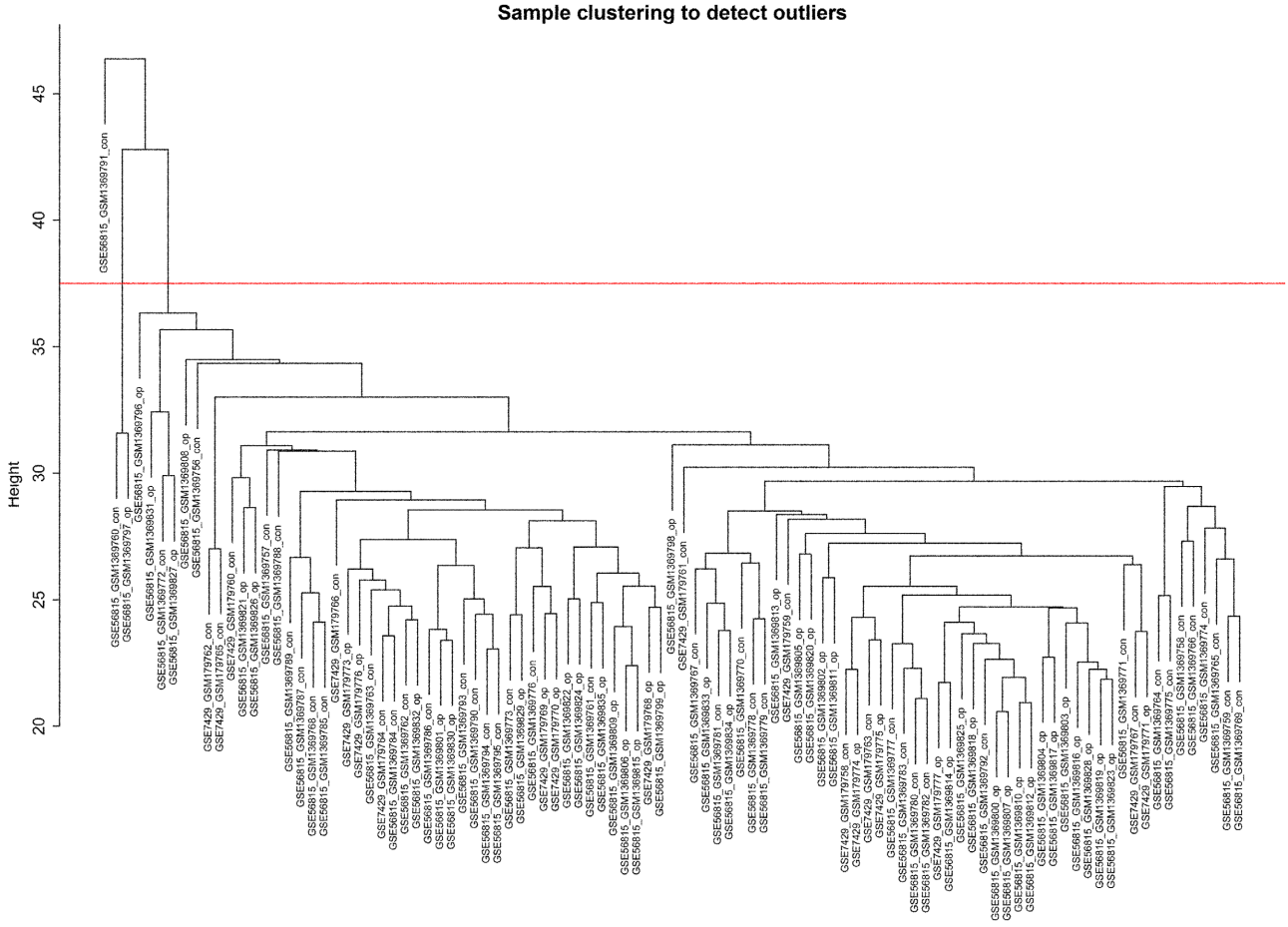
3. Results

3.1. Data Processing

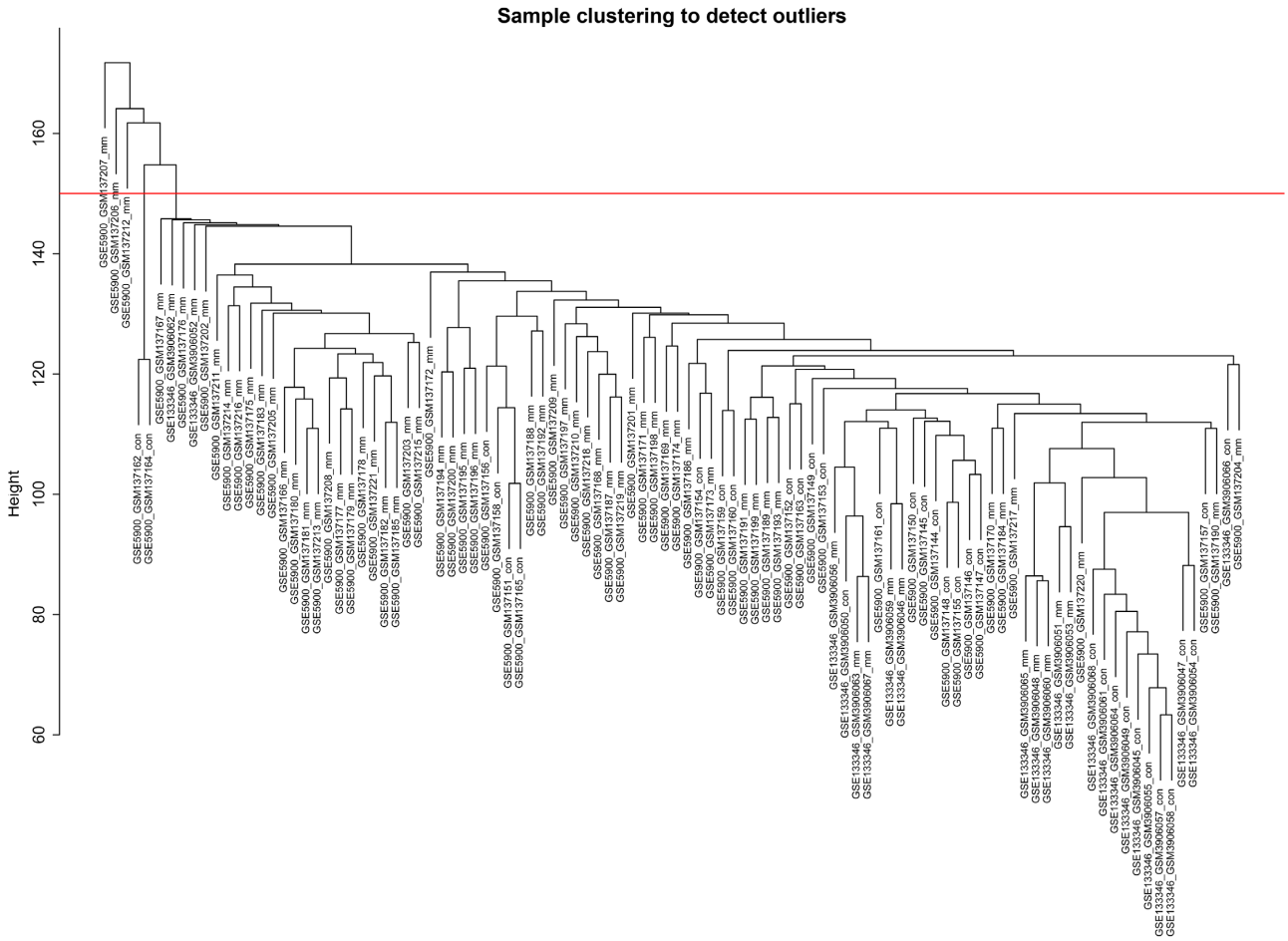
Next, we clustered the OP samples to determine if there were significant outliers and excised them at a resection height of 37.5 (**Figure 3(a)**). Similarly, the MM samples were clustered to determine if there were significant outliers and excised them at a resection height of 150 (**Figure 3(b)**).

3.2. Modules of Genes Coexpressed in OP and MM

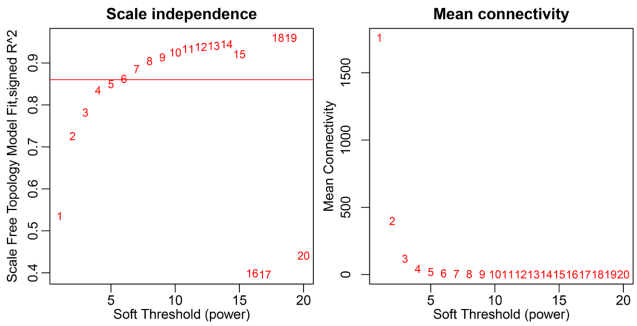
Using WGCNA, we determined the modules of genes coexpressed in the MM and OP datasets. Initially, two training datasets, GSE133346 and GSE5900, were pooled, and batch effects were eliminated for the WGCNA of MM later. The two training datasets GSE7429 and GSE56815 were pooled, batch effects were eliminated for the WGCNA of OP later, and samples from patients from both datasets were grouped into two clusters with no detectable outliers: a disease group (MM or OP) and a healthy group. Before calculating the adjacency, we determined the soft threshold power and raised the coexpression similarity. We



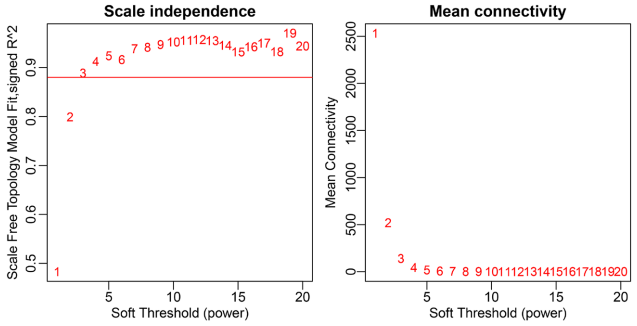
(a)


Sample clustering to detect outliers
Height
60
80
100
120
140
160

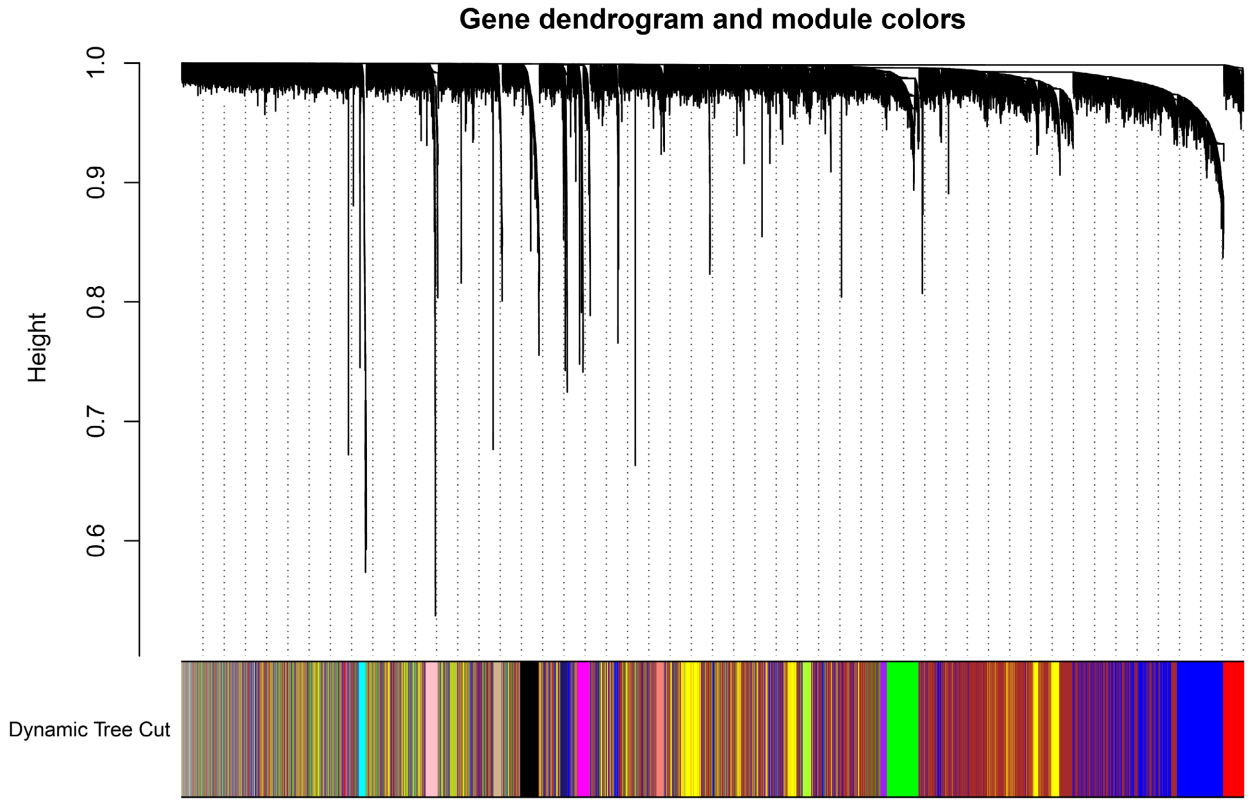
(b)


Scale independence
Scale Free Topology Model Fit,signed R^2
Soft Threshold (power)
Mean connectivity
Mean Connectivity
Soft Threshold (power)

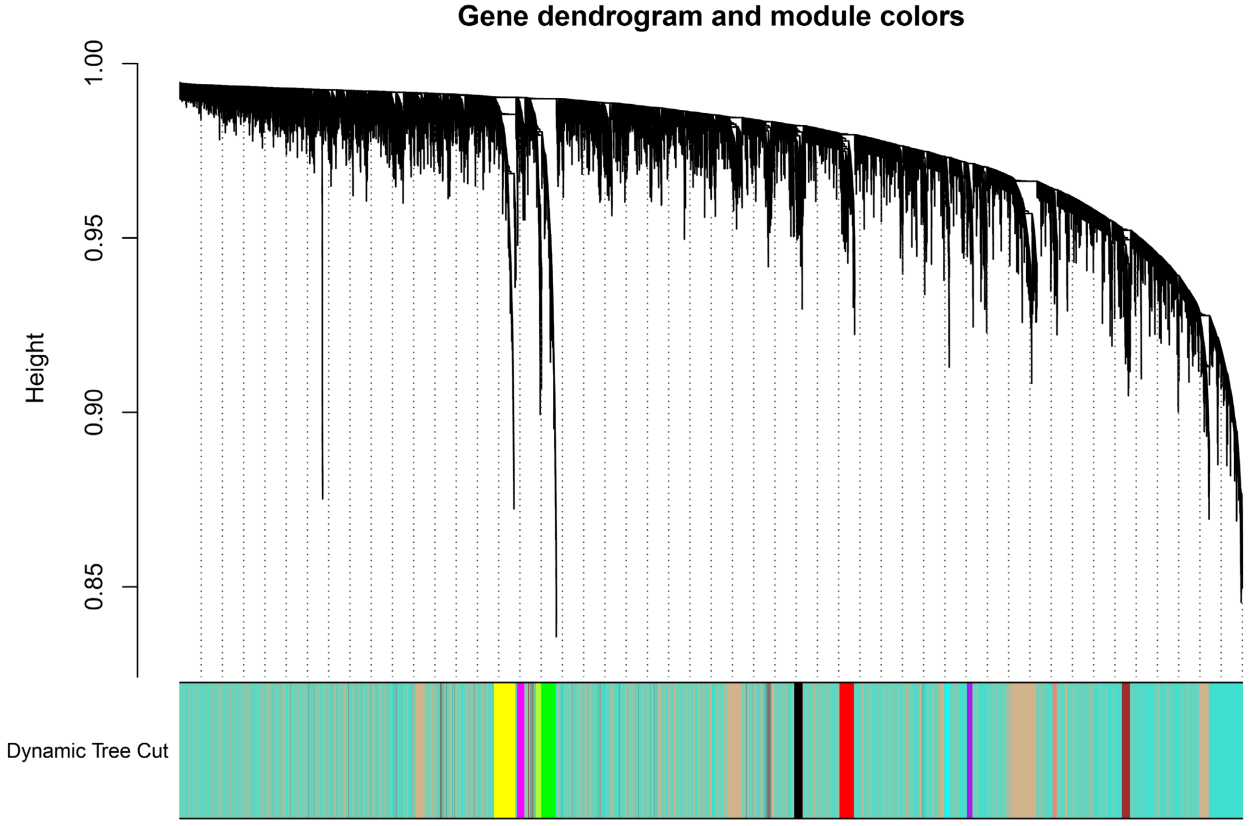
(c)


Scale independence
Scale Free Topology Model Fit,signed R^2
Soft Threshold (power)
Mean connectivity
Mean Connectivity
Soft Threshold (power)

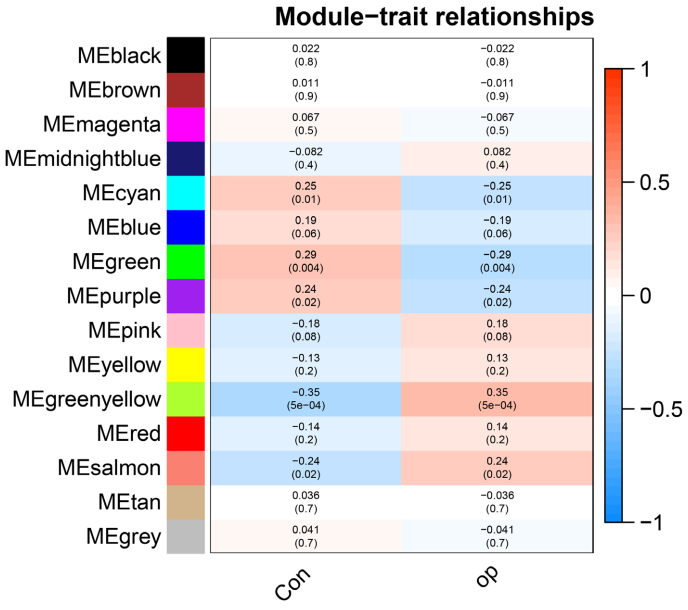
(d)


Gene dendrogram and module colors
Height
1.0
0.9
0.8
0.7
0.6
Dynamic Tree Cut

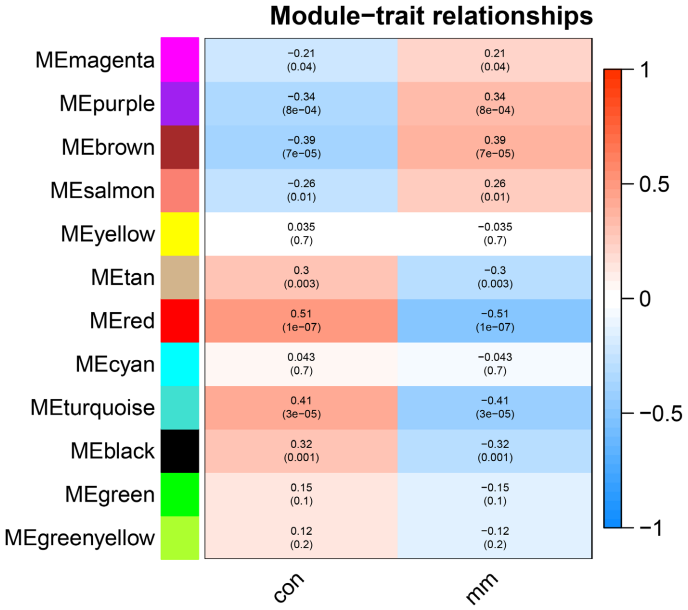
(e)


Gene dendrogram and module colors
Height
1.00
0.95
0.90
0.85
Dynamic Tree Cut

(f)



(g)



(h)

Figure 3. The WGCNA module is linked to clinical features. (a) A sample clustering dendrogram with tree leaves corresponding to individual samples in OP. (b) A sample clustering dendrogram with tree leaves corresponding to individual samples in MM. (c) Network topology for different soft threshold powers in OP. (d) Network topology for different soft threshold powers in osteoporosis in MM. ((e), (f)) A cluster dendrogram of genes coexpressed in OP (e) and MM (f). ((g), (h)) A heatmap of the correlation between module eigengenes and disease status in OP (g) and MM (h). OP, osteoporosis; CON, control; MM, multiple myeloma

utilized the WGCNA function pickSoftThreshold to investigate the network topology. Because of the scale independence of 0.86 (**Figure 3(c)**) and the relatively high average connection, a soft threshold capacity of 6 was set for the OP group for future study. Similarly, for the MM group, a soft threshold capacity of 3 was set because the scale independence reached 0.88 (**Figure 3(d)**) and the av-

erage connection was quite high. We generated gene networks and identified modules using the WGCNA R software's one-step network-building feature. The minimum module size was set to 60 for cluster splitting, and deepSplit was set to 2 (which indicates medium sensitivity).

3.3. Modules of Coexpression between OP and MM

WGCNA established 15 modules in the OP group dataset and 12 in the MM group dataset, with each color representing a different module. We calculated Spearman's correlation coefficient. Then, we created a heatmap of the modular attribute relationship to evaluate the relationship between each module and the disease. In this analysis, the yellow-green and orange modules are positively correlated with OP (**Figure 3(g)**; yellow-green modules: r = 0.35, P = 5e−04, genes = 135; orange-red module: r = 0.24, p = 0.02, genes = 109). Meanwhile, brown, red-purple, purple, and orange modules are positively correlated with MM. The brown, red-purple, purple, and orange-red modules are positively correlated with MM (**Figure 3(h)**; brown module: r = 0.39, P = 7e−05, genes = 410; red-purple module: r = 0.21, p = 0.04, genes = 136; purple module: r = 0.34, P = 8e−04, genes = 107; orange-red module: r = 0.26, p = 0.01, genes = 82).

3.4. Common Genes Signatures in OP and MM

The module positively correlated with OP and MM consists of 19 common genes, which are referred to as gene set 1 (GS1) (**Figure 4**). These genes are strongly related to the pathogenesis of OP and MM.

3.5. Genes Signatures in OP and MM

Using the GeneMANIA database (http://www.genemania.org/), we obtained a PPI network of the genes shared between OP and MM. In addition, 20 genes were discovered to be tightly connected to the common genes (**Figure 5(a)**). So we got 39 genes. To explore the pathways associated with these genes derived from the PPI network, we performed GO enrichment analyses with GlueGo, which identified various important biological processes, including aerobic electron

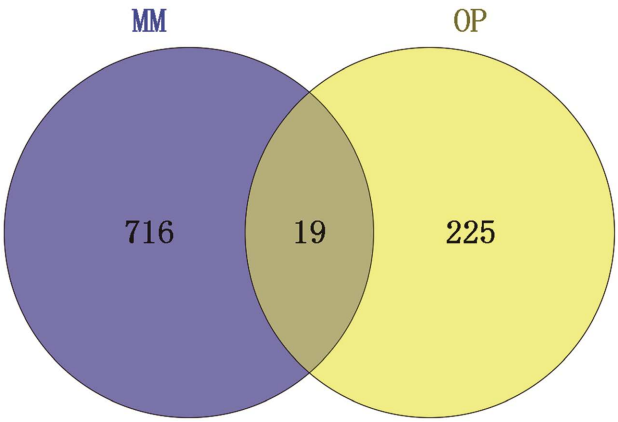
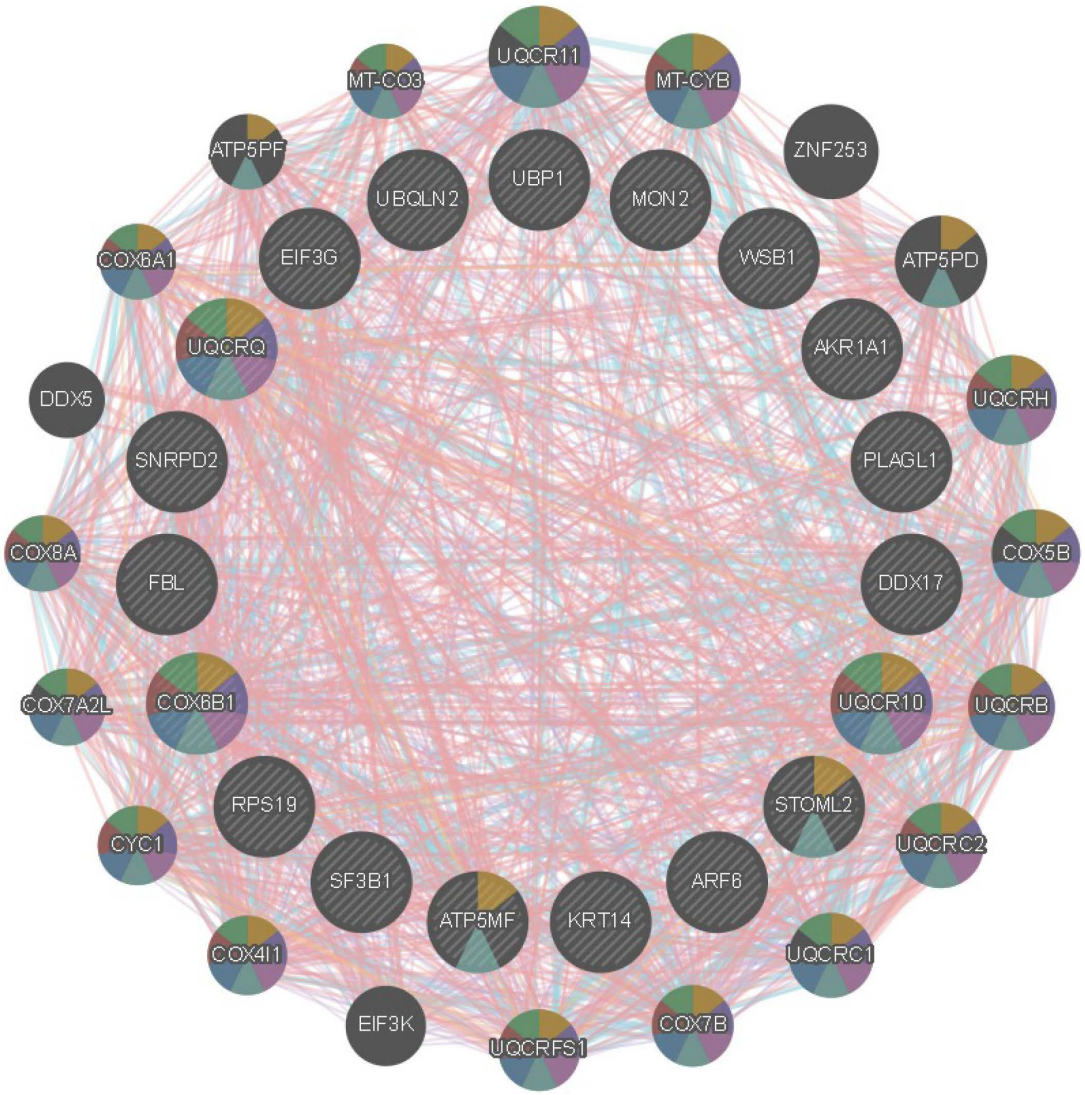


Figure 4. Common genes of OP and MM.

GeneMANIA report

Created on : 26 February 2023 12:23:19
Last database update : 13 August 2021 00:00:00
Application version : 3.6.0



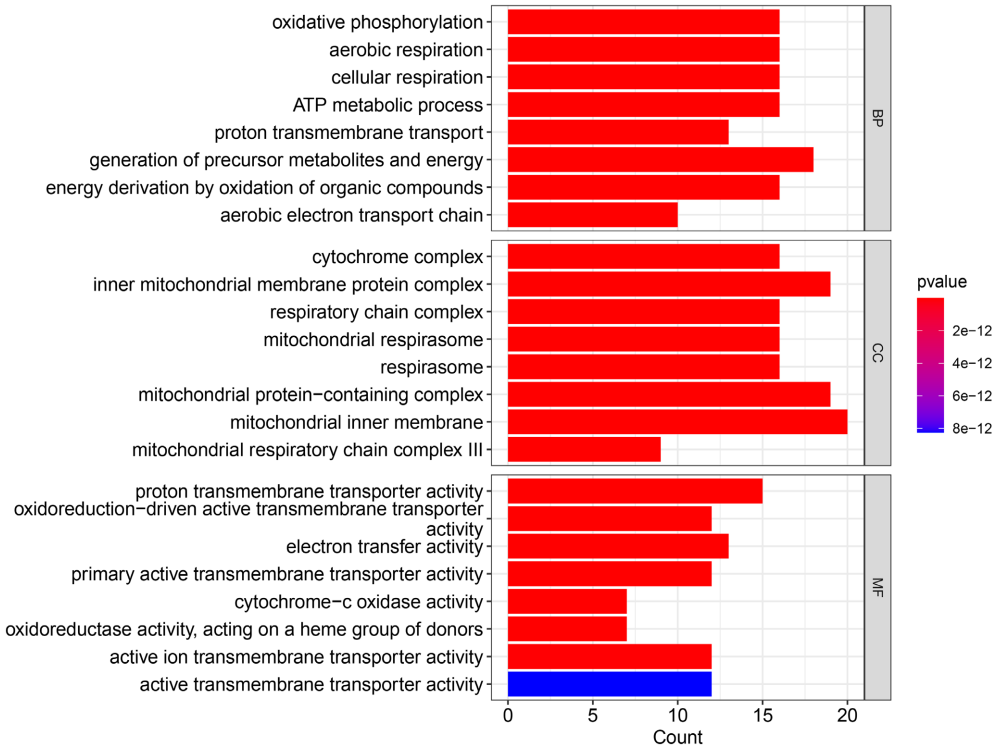
Networks

- Co-expression
- Physical Interactions
- Pathway
- Co-localization
- Predicted

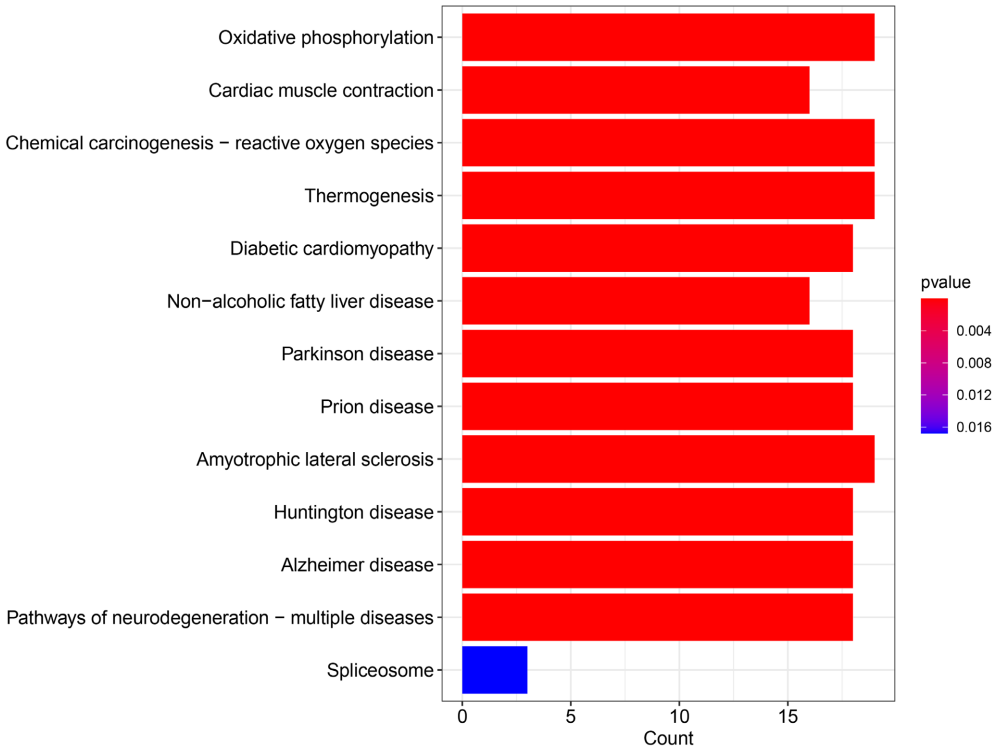
Functions

- oxidative phosphorylation
- mitochondrial ATP synthesis coupled electron transport
- ATP synthesis coupled electron transport
- ATP metabolic process
- respiratory electron transport chain
- cytochrome complex
- electron transport chain

(a)



(b)



(c)

Figure 5. PPI network and enrichment analyses of genes shared between OP and MM. (a) A characterized gene coexpression network from the GeneMANIA database. (b) Gene Ontology analyses of shared genes to identify the key biological processes, cellular components, and molecular functions in which they are involved. (c) The top 15 most significantly enriched KEGG pathways. PPI, protein-protein interaction; OP, osteoporosis; MM, multiple myeloma.

transport chain, energy derivation by oxidation of organic compounds, generation of precursor metabolites and energy, proton transmembrane transport, ATP metabolic process, aerobic respiration, oxidative phosphorylation, and cellular respiration. Meanwhile, mitochondrial inner membrane, mitochondrial protein-containing complex, respirasome, mitochondrial respirasome, respiratory chain complex, inner mitochondrial membrane protein complex, and cytochrome complex were the primary cellular components. Proton transmembrane transporter activity was the main molecular function (**Figure 5(b)**). KEGG enrichment analyses of critical targets revealed 15 items, which were particularly associated with oxidative phosphorylation, chemical carcinogenesis-reactive oxygen species, thermogenesis, and amyotrophic lateral sclerosis (**Figure 5(c)**).

3.6. Analyses of Differentially Expressed genes and the Identification of Hub Gene in OP and MM

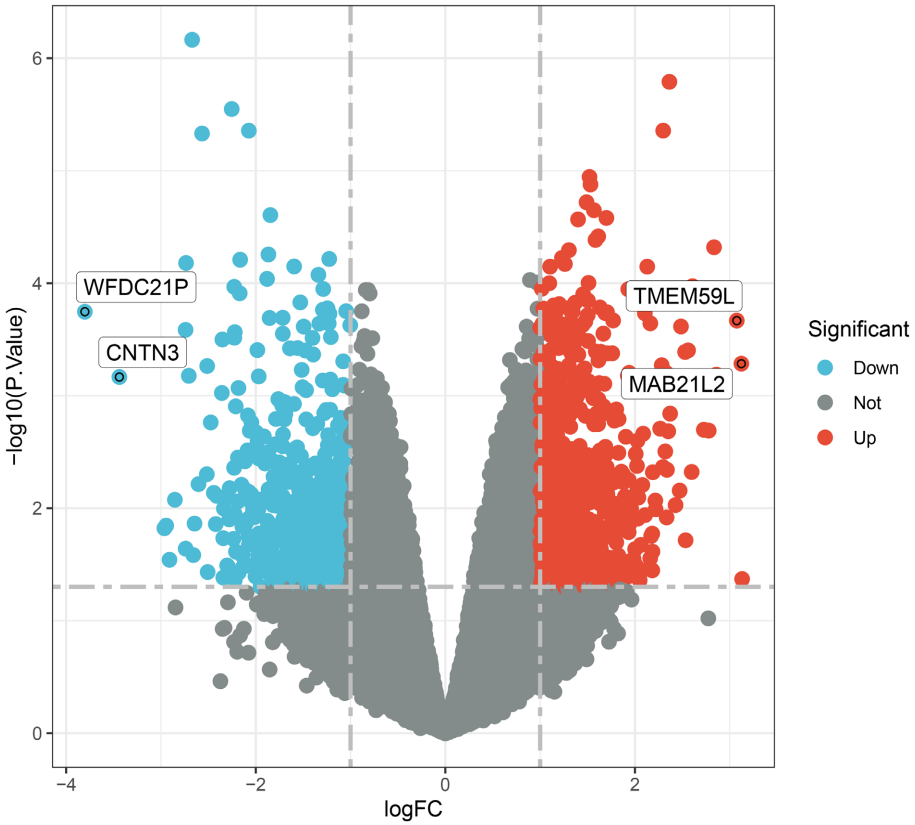
Using the GSE35956 and GSE6477 datasets, we performed an analysis of the differentially expressed genes. For GSE35956, 2789 differentially expressed genes (DEGs), comprising 1457 upregulated genes and 1331 downregulated genes in OP, were discovered. For GSE6477, 2762 DEGs, comprising 1440 upregulated genes and 1322 downregulated genes in MM, were found. Volcano plots of the differentially expressed genes were created for the two diseases (**Figure 6(a)** and **Figure 6(b)**). After selecting 50 genes with the most differential expression (up and downregulated), we created a heatmap of the differentially expressed genes for the two diseases (**Figure 6(c)** and **Figure 6(d)**). Overall, 112 genes were upregulated in GSE359562 and GSE6477, whereas 85 genes were downregulated, as defined by gene set 2 (GS2). Intriguingly, one of the hub gene overlapping between GS1 and GS2 was PLAGL1, whose downregulation of expression may be essential for the development of OP and MM (**Figure 6(e)**). It is thus highly likely that PLAGL1 is a joint target for OP and MM.

3.7. GSEA Identifies a Signaling Pathway in Which PLAGL1 Is Involved

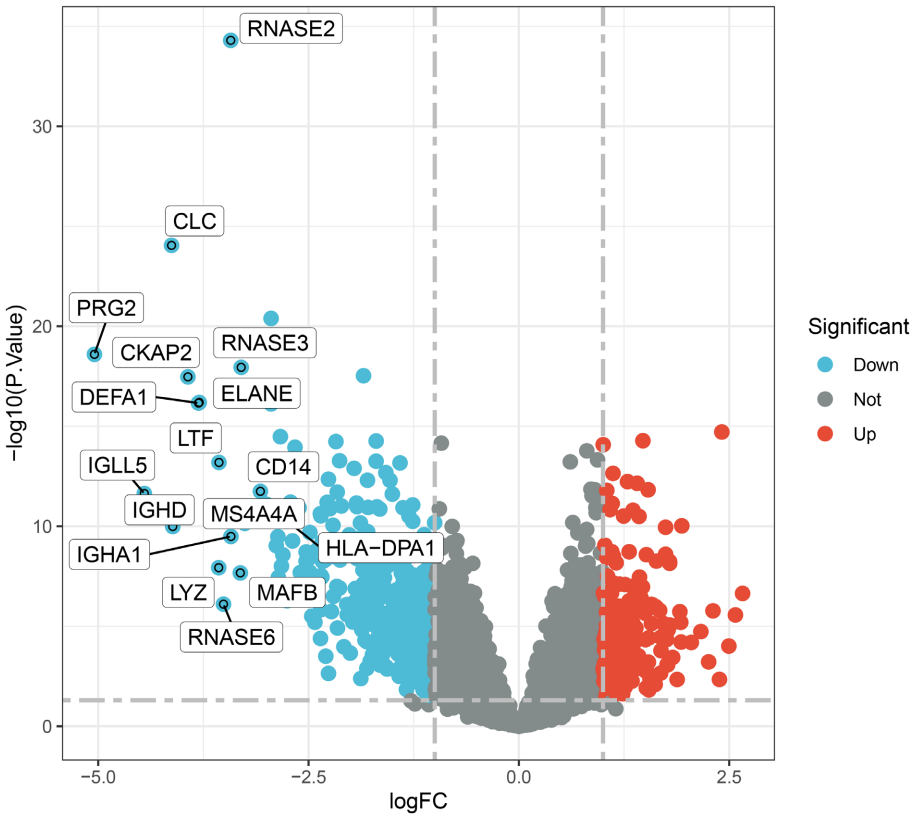
Gene expression analyses (GSEA) revealed that PLAGL1 expression was downregulated in association with glycolysis, glycogenesis, and oxidative phosphorylation (**Figure 6(f)**).

3.8. Validation of Joint Targets

GSE35958 and GSE156508 were combined by removing batch effects and later used as the validation set for the OP. Validation was performed in GSE27870 for MM from GEO. As compared to normal tissues, the findings demonstrated that PLAGL1 was significantly downregulated in both OP and MM (**Figures 7(a)-(d)**). In order to evaluate the efficacy of the diagnostic features, receiver operating characteristic (ROC) curves were created in these datasets using the pROC tool in the R programming language (**Figures 7(e)-(h)**). For OP, GSE35956 were selected as the training dataset to create the ROC curve for PLAGL1 with an AUC


WFDC21P
CNTN3
TMEM59L
MAB21L2
−log10(P.Value)
logFC
Significant
Down
Not
Up

(a)


RNASE2
CLC
PRG2
CKAP2
RNASE3
DEFA1
ELANE
LTF
CD14
IGLL5
IGHD
MS4A4A
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IGHA1
LYZ
MAFB
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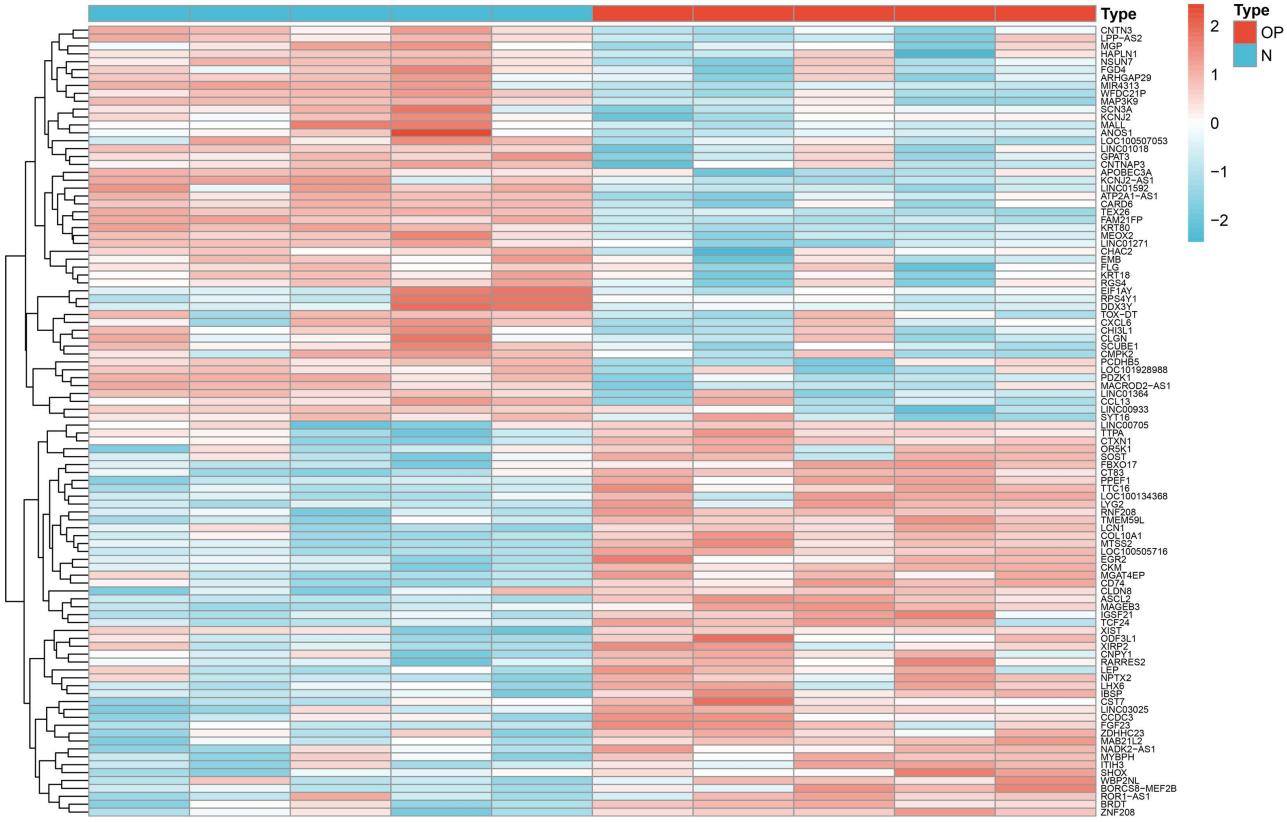
Up

(b)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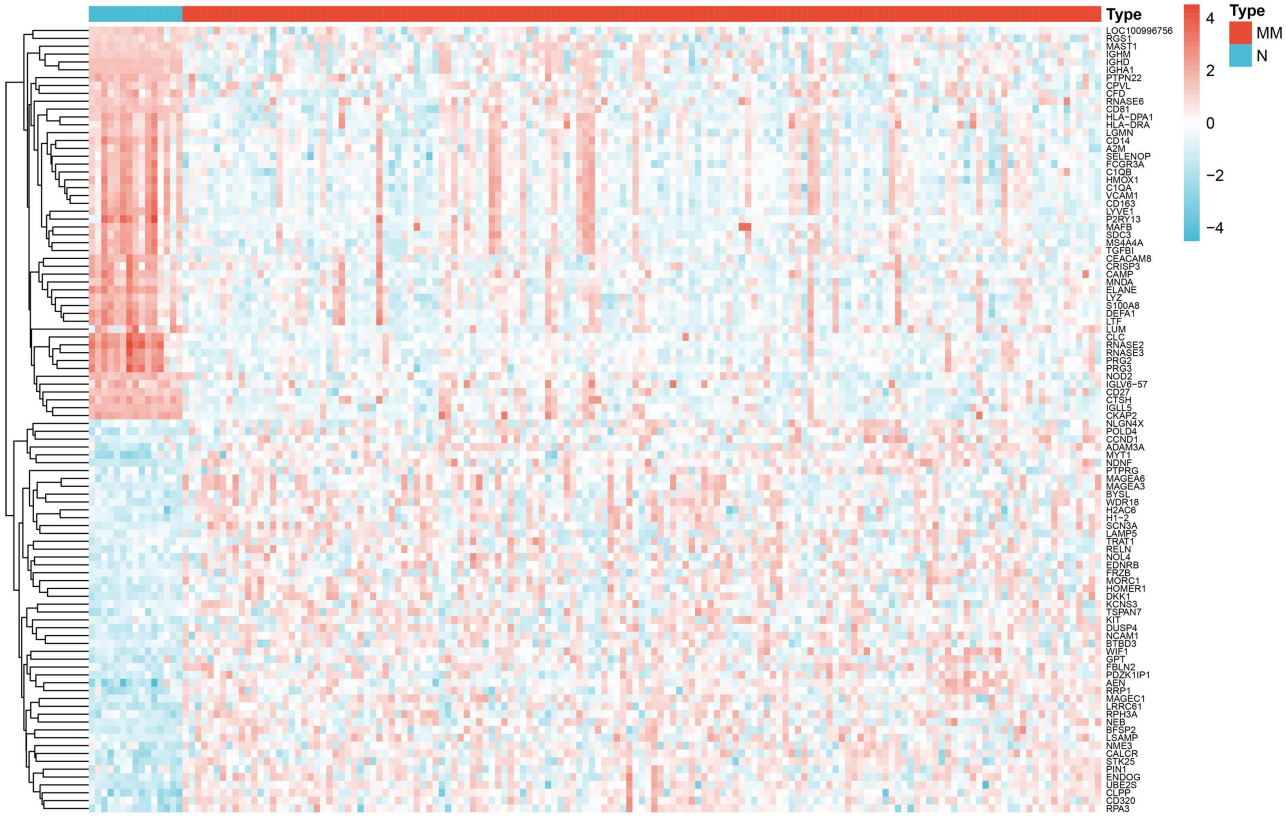
(c)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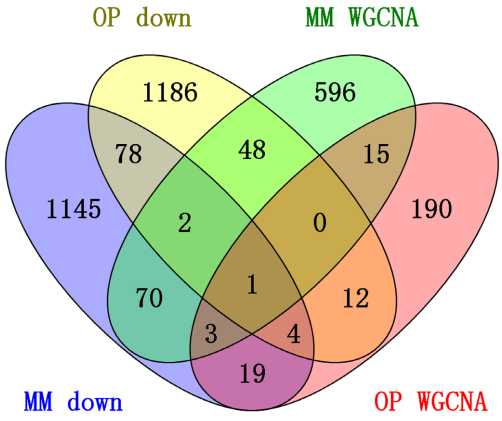
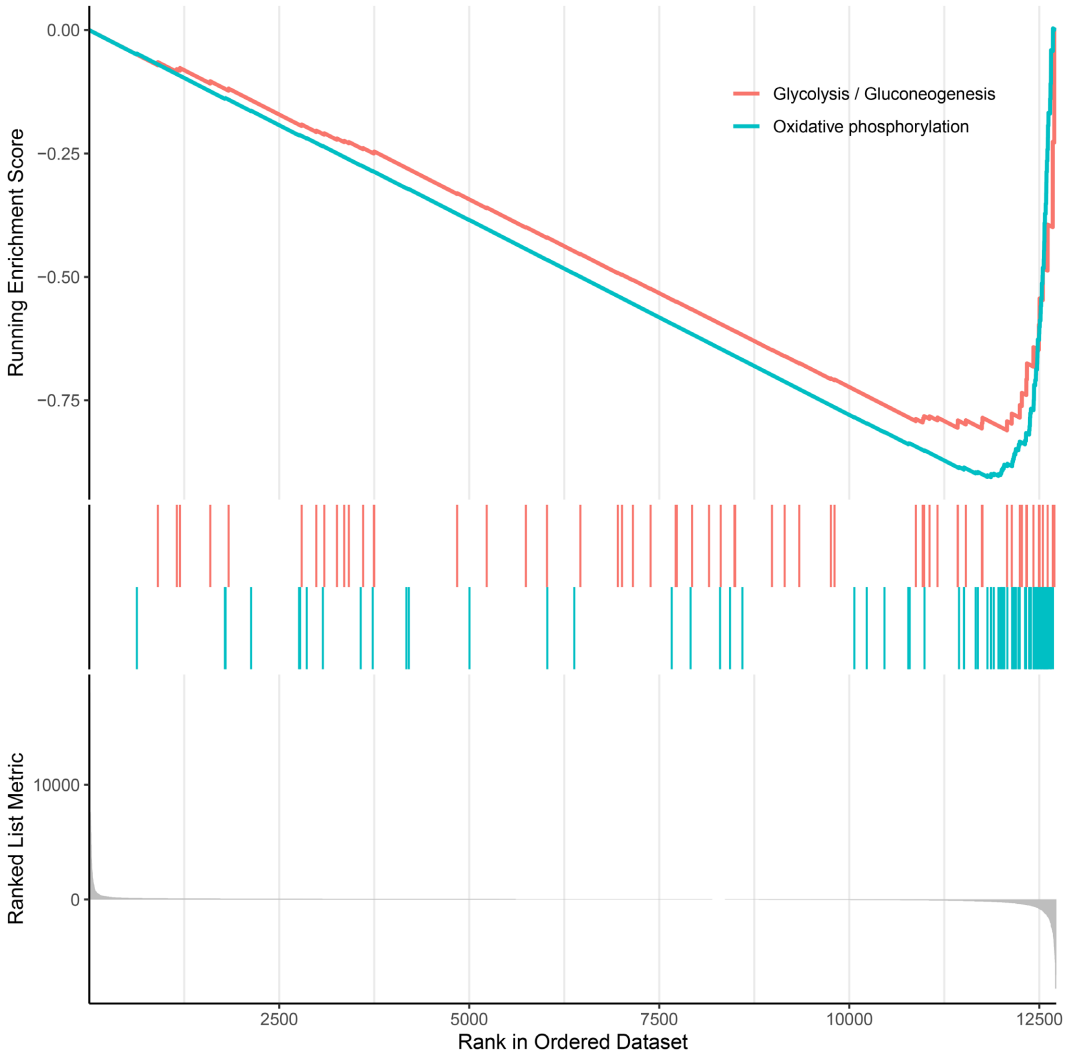
(d)



(e)



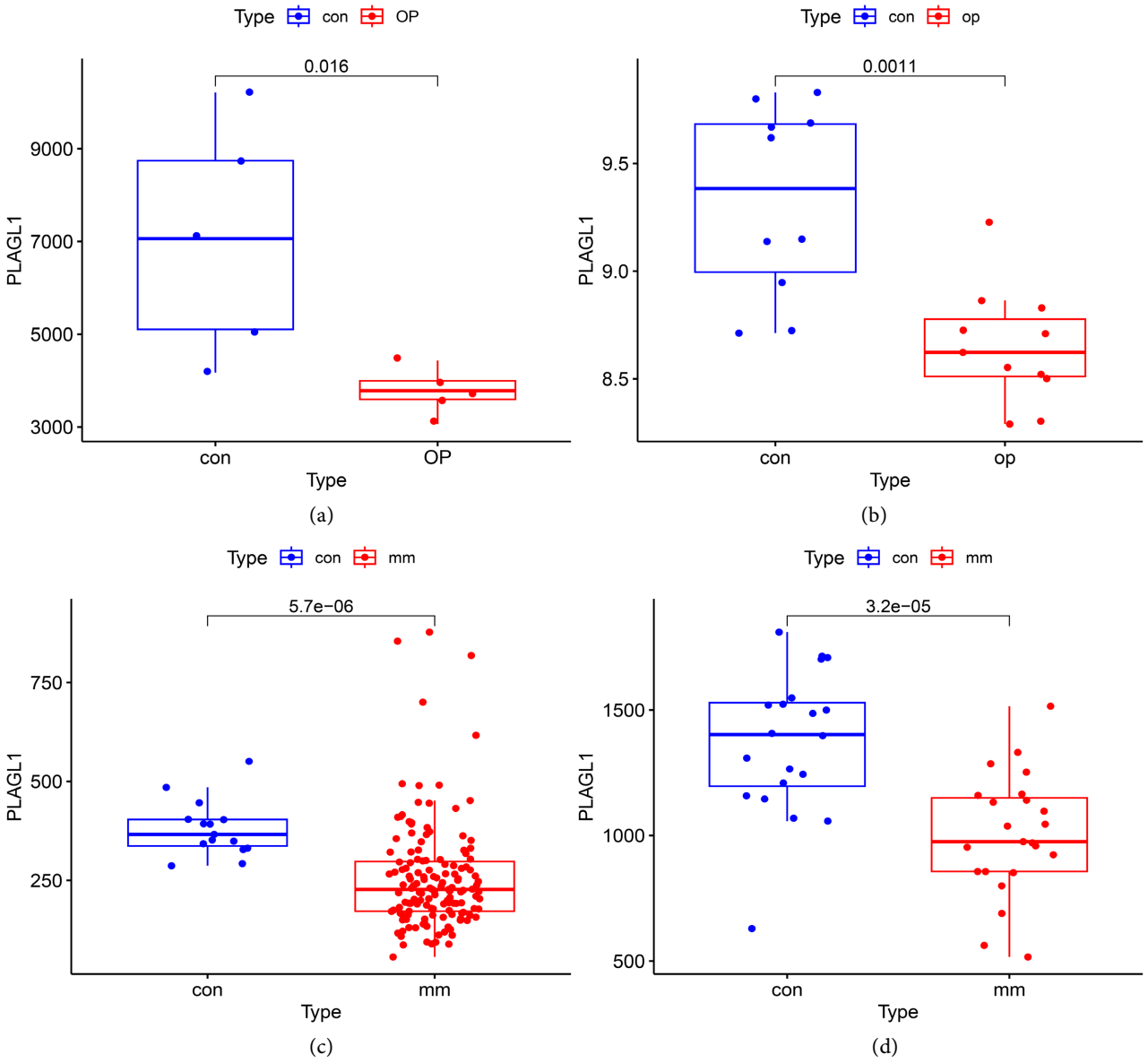
(f)

Figure 6. Identification of overlapping genes and GSEA of PLAGL1. (a) A DEG volcano plot between control and OP samples. (b) A DEG volcano plot between control and MM samples. ((c), (d)) Heatmap of genes differentially expressed in OP (c) and MM (d). (e) The Venn diagram shows the overlap of DEGs and genes shared between OP and MM. (f) Single-gene enrichment analyses of MM patients with high PLAGL1 expression followed by a demonstration of the top 10 enriched pathways. GSEA, Gene Set Enrichment Analyses; OP, osteoporosis; MM, multiple myeloma.

of 0.960 (Figure 7(e)), and GSE156508 and GSE35958 were selected as the validation dataset to create the ROC curve for PLAGL1 with an AUC of 0.778 (Figure 7(f)). For MM, the GSE6477 datasets were selected as the training dataset to create a receiver operating characteristic (ROC) curve for PLAGL1, with an AUC of 0.856 (Figure 7(g)), and the GSE27870 datasets were selected as the validation dataset to create a receiver operating characteristic (ROC) curve for PLAGL1, with an AUC of 0.852 (Figure 7(h)). The AUC values for PLAGL1 were greater than 0.75 in all datasets, so PLAGL1 had an excellent prognostic capacity for the diagnosis of MM and OP.

3.9. Association between PLAGL1 and the Tumor Microenvironment

The ssGSEA algorithm was used to provide an overview of the infiltration of



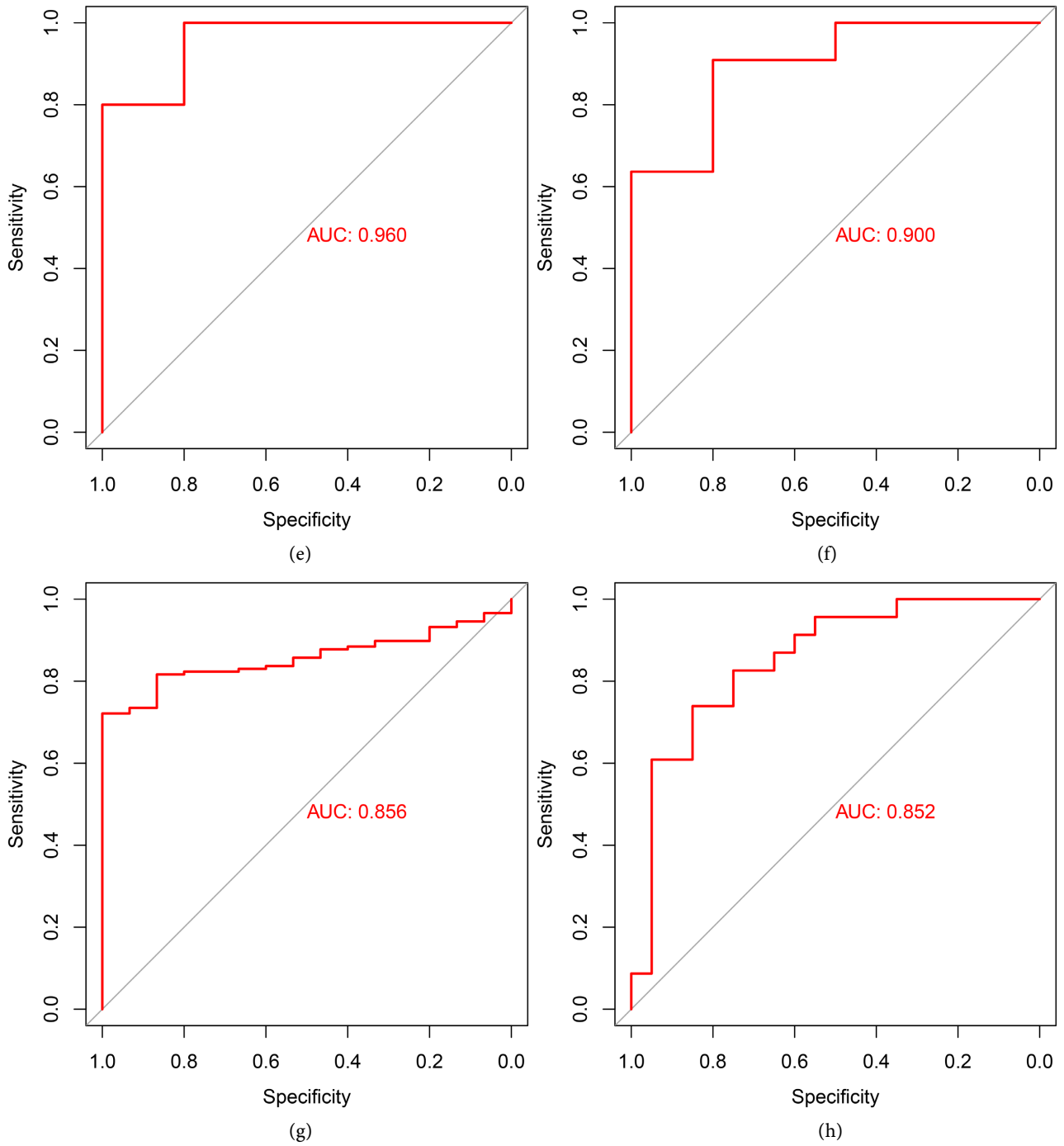


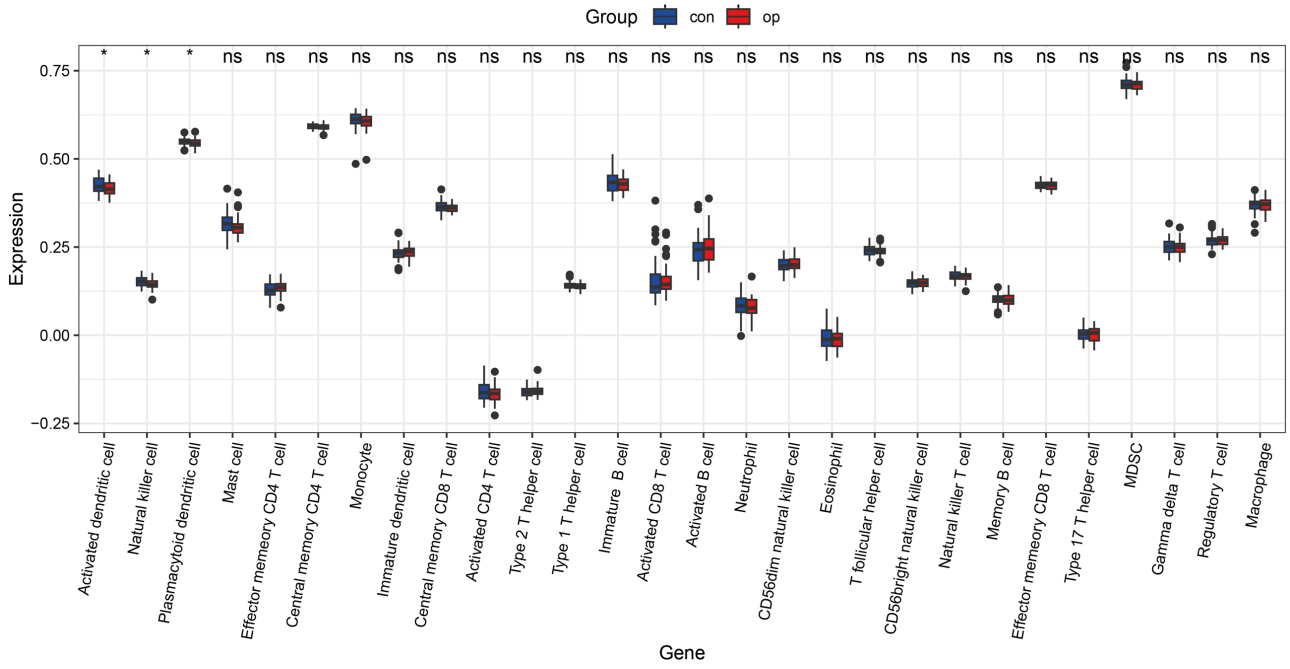
Figure 7. Validation of hub genes. ((a), (c)) The box plot of hub gene expression in the training set indicates that the OP (a) or MM (c) group has lower expression than the control group. (b) In GSE35958 and GSE156508 for OP, PLAGL1 was verified. The boxplots demonstrate that OP samples have lower hub gene expression levels. (d) In GSE27870 for MM, PLAGL1 was verified. The boxplots demonstrate that MM samples have lower hub gene expression levels. The diagnostic efficacy of the hub genes in OP ((e), (f)) or MM ((g), (h)) diagnosis was assessed using ROC curves. ROC: receiver operating characteristic; OP, osteoporosis; MM, multiple myeloma.

immune cells in OP and MM. Wilcoxon's test showed four immune subgroups that were significantly altered between the OP and normal samples, including activated dendritic cells, spontaneously destroyed cells, and plasmacytoid den-

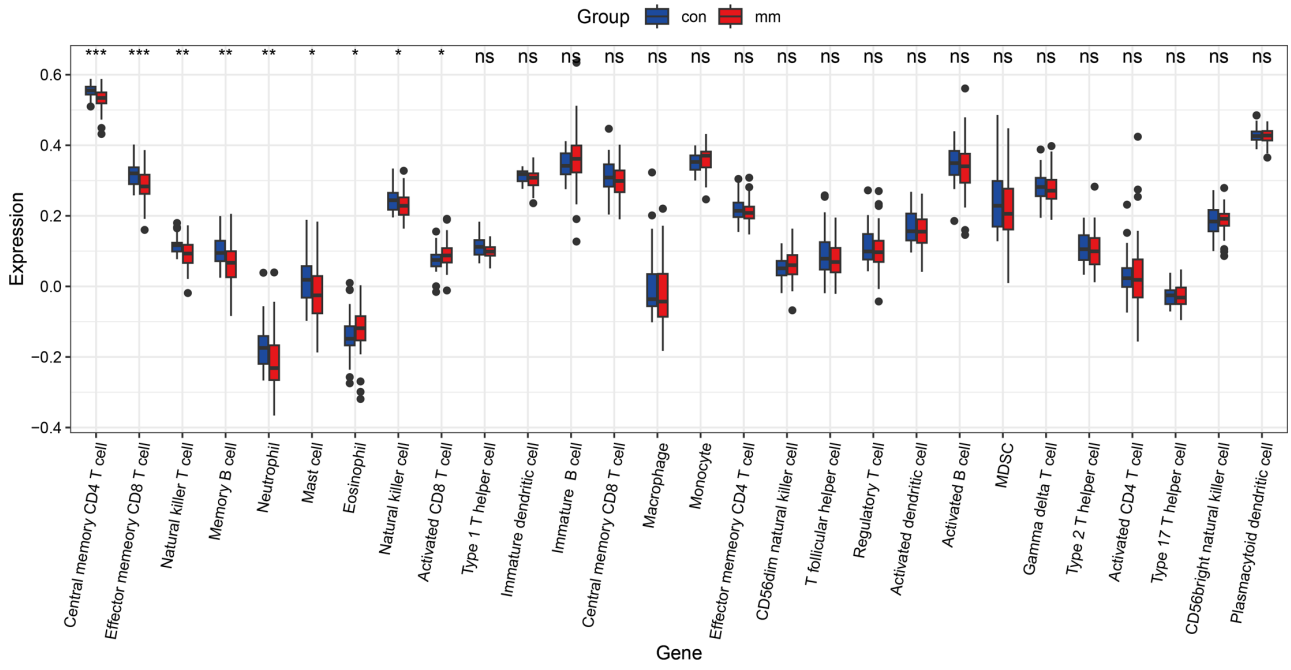
dritic cells (Figure 8(a)).

In addition, nine significantly altered immune subgroups were identified in the comparison between the MM and normal samples, including central memory CD4 T cells, affected memory CD8 T cells, natural T cells, memory B cells, neutrophils, hypertrophies, eosinophils, natural killer cells, and activated CD8 T lymphocytes (Figure 8(b)).

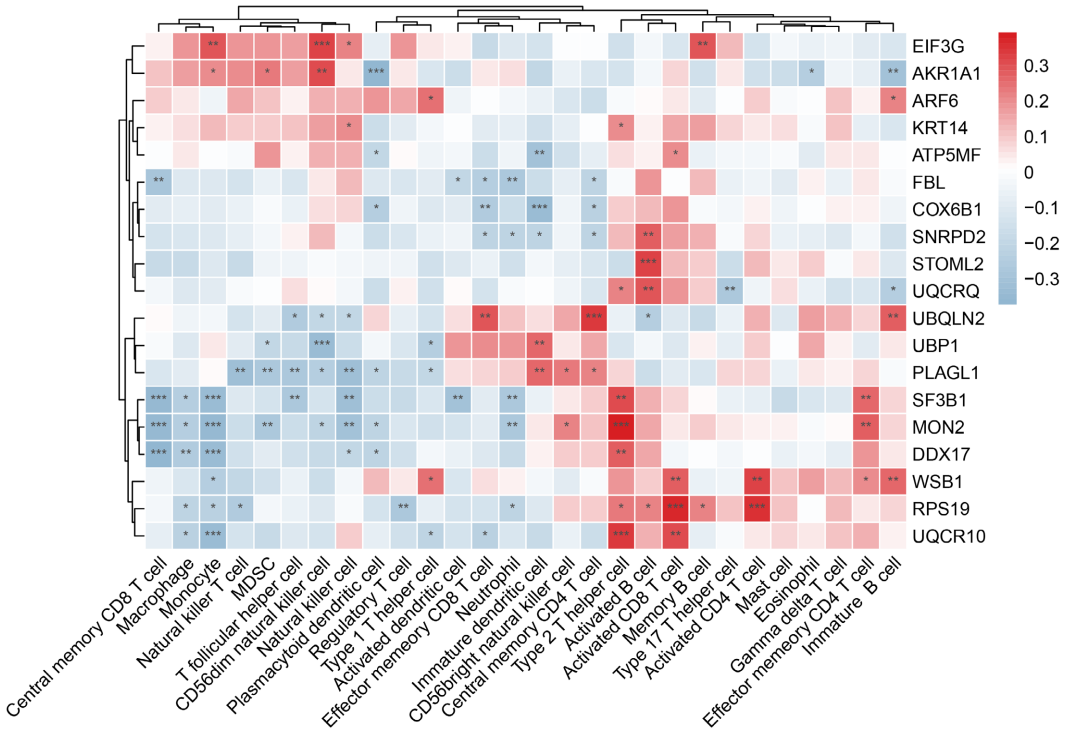
The composition of natural killer cells was significantly altered in both OP



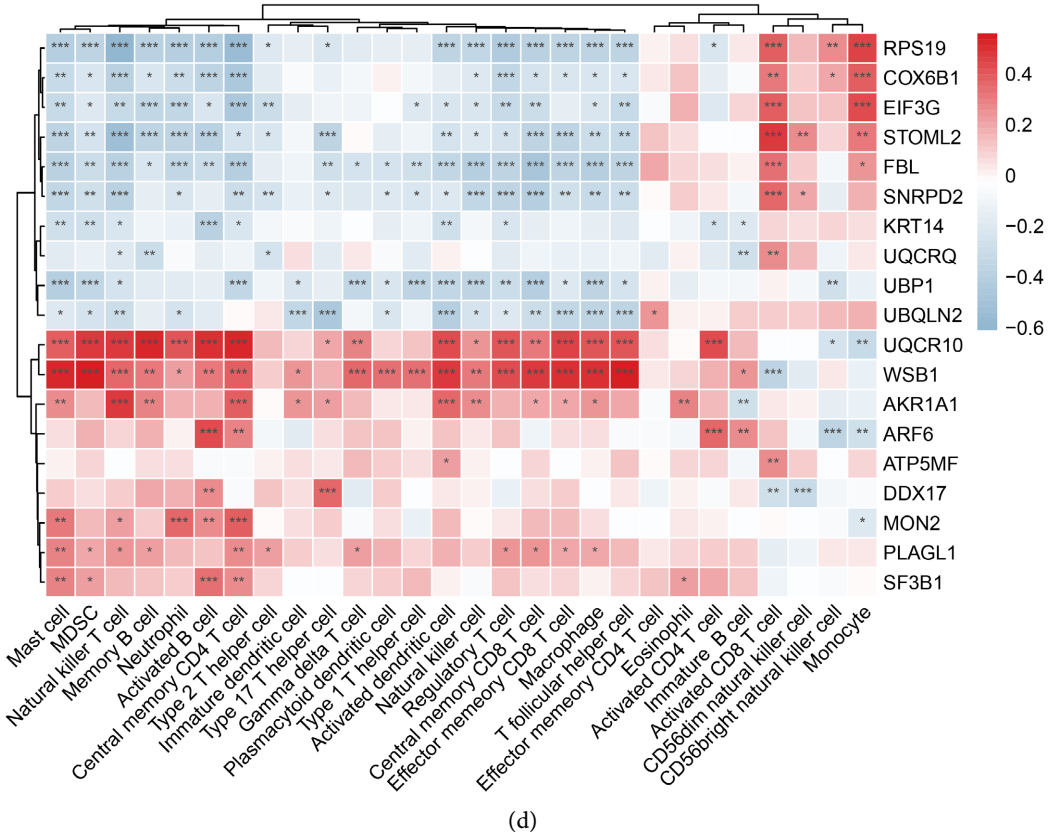
(a)



(b)



(c)



(d)

Figure 8. Analyses of the immunological microenvironment in OP and MM. ((a), (b)) Different immune cell subtypes are associated with the establishment of OP (a) and MM (b). ((c), (d)) Heatmap of immune cells associated with key genes in OP (c) and MM (d).

and MM. In addition, we found that three immune cell subpopulations (immature dendritic cells, CD56bright natural killer cells, and central memory CD4 T cells) were positively correlated with PLAGL1 in OP, whereas natural killer T cells, myeloid-derived suppressor cells (MDSCs), follicular helper T cells, CD56dim NK cells, natural killer cells, plasmacytoid dendritic cells, and type 1 T helper cells were negatively correlated with PLAGL1 (**Figure 8(c)**).

PLAGL1 was positively correlated with mast cells, MDSCs, natural killer T cells, memory B cells, central memory CD4 T cells, type 2 T helper cells, T cells, regulatory T cells, central memory CD8 T cells, effector memory CD8 T cells, and macrophages in the MM samples (**Figure 8(d)**).

The results indicated that PLAGL1 may be involved in the immune processes associated with natural killer cells in OP.

Our study also showed that PLAGL1 may be jointly involved in immune processes regulating central memory T cells in both OP and MM.

4. Discussion

The effects of blood disorders on bones are caused by circulatory factors, such as cytokines. For example, MM is associated with osteolytic changes and progressive bone loss due to uneven remodeling of bone. The mechanism behind this involves the promotion of osteoclasts and the inhibition of osteoblasts [46]. Moreover, tumor cells secrete different cytokines that induce the proliferation and activity of osteoclasts, and can also secrete interleukin (IL)-1 to suppress the formation of bone cells in MM. There are other related molecular mechanisms which reported such as RANKL/RANK/OPG axis, Wnt/β-linked protein signaling pathway and signaling molecules such as DKK-1, sclerostin, bone bridge protein, chemokines [8] [31] [32] [33] [34]. These findings explain the molecular mechanism between MM and osteolytic destruction. However, few studies have focused on the common pathogenetic mechanism between OP and MM at the genetic level.

Information on gene expression derived from global gene expression data deepens our understanding of the etiology of OP and MM and how these conditions interact. The results of analyzing these enriched genes in OP and MM in public disease databases by GO enrichment analyses reveal their particular associations with ribonucleoprotein complex biogenesis, oxidative phosphorylation, aerobic respiration, cellular respiration, ATP metabolic processes, organic compounds, and ATP metabolic pathways. KEGG enrichment analyses reveal that the key targets are particularly associated with biological processes such as oxidative phosphorylation, chemical carcinogenesis-reactive oxygen species, thermogenesis, and amyotrophic lateral sclerosis. GSEA suggests that PLAGL1 may be involved in glucose and energy metabolism. These results suggest both are closely related to energy production, probably because of the large amount of energy required for tumor growth and bone destruction activities.

OP is often associated with systemic energy metabolism and glycolipid meta-

bolism disorders [47] [48] [49] [50]. Osteoblasts need to consume large amounts of ATP to synthesize new collagen to complete the modeling and remodeling of bone [51] [52]. Excessive bone resorption by osteoclasts is also a process that requires large amounts of adenosine triphosphate (ATP) produced by glycolysis and oxidative phosphorylation [53]. The decrease in energy supply for osteogenic activity and the increase in energy supply for osteoclastic activity lead to an imbalance in energy supply between osteoblasts and osteoclasts as the underlying cause of osteoporosis [54].

A close relationship between oxidative phosphorylation and MM has also been identified. For example, it is concluded that FoxM1 promotes glycolysis and energy production in myeloma cells. FOXM1 is an active regulator of myeloma metabolism and were involved in a bioenergetic pathway and oxidative phosphorylation (OXPHOS) that significantly affect glycolysis [55]. Meanwhile, PGC-1a-mediated overexpression of OXPHOS provides a structural basis for enhancing OXPHOS in MM cells. Sr18292, an inhibitor of PGC-1*α*, has a promising anti-myeloma effect and provides a potentially effective approach for treating myeloma. Sr18292 demonstrates actual antitumor activity against MM by suppressing oxidative phosphorylation [56]. Both OP and MM in these studies are closely associated with energy metabolic pathways, respectively, which is consistent with the results derived from our study. Therefore, the common mechanism behind the pathogenesis of MM and OP may be caused by the regulation of their shared genes and oxidative phosphorylation.

To further discover the hub gene involved in these diseases, an analysis of genes differentially expressed between MM and OP was performed. Intriguingly, we discovered PLAGL1 among the differentially expressed genes that overlapped between these conditions. PLAGL1 was likely involved in the development of MM and OP.

Polymorphic adenoma gene 1 (PLAGL1, Zac1, or lot1) can encode a homonymous zinc finger protein to regulate the cell cycle and apoptosis. PLAGL1 is expressed in several types of tissues in embryos and adults. The PLAGL1 gene maps to chromosome 6q24 [57] [58] [59]. It has been confirmed that PLAGL1 is a tumor repressor gene that is found in lots of cancers, such as diffuse large B-cell lymphoma, lung tumorr, gastric tumor, colorectal tumor, breast tumor, ovarian tumor, and prostate tumor [60] [61] [62] [63] [64]. Specifically, tumor progression is associated with reduced or absent expression of PLAGL1 (Zac1) in these conditions, which has been confirmed as a novel prognostic marker for cervical cancer [65]. The p53 and p21 genes are crucial for cell division and differentiation to produce different cell groups [66] [67]. However, the mechanism of action of PLAGL1 in MM has yet to be reported and warrants attention. Reduced PLAGL1 expression was found to be associated with glycolysis, gluconeogenesis, and phosphorylation, suggesting that this reduction may be associated with energy metabolism and the rapid growth of cancer cells. These results indicate that PLAGL1 may be a key factor in the signaling pathways associated with

OP and MM.

The expression of PLAGL1 in MM was found to be positively correlated with significant infiltration of immune cells in patients in this study, including mast cells, MDSCs, natural killer T cells, memory B cells, central memory CD4 T cells, type 2 T helper cells, GAMMA delta T cells, regulatory T cells, central memory CD8 T cells, effector memory CD8 T cells, and macrophages. The expression of PLAGL1 in OP was strongly associated with immature dendritic cells, CD56bright natural killer cells, CD4 T cells with central memory, and PLAGL1. PLAGL1 regulates T-cell immune activity and may promote the development of OP with MM.

Therefore, we speculate that PLAGL1 can regulate immune cells to participate in developing OP and MM. Immune surveillance is the process by which the immune system detects and eliminates malignancies. Immunity is intimately related to carcinogenesis and progression. Recent research has demonstrated that the tumor immune microenvironment (TIME) is crucial for carcinogenesis and cancer progression [68].

T lymphocytes eliminate MM by identifying tumor-specific or tumor-associated antigens. This process occurs directly in the killing of tumor cells by CD8 T cells or indirectly in the activation of NK cells or macrophages (e.g., IFN) following cytokine release [69]. It has been concluded that cloning expanded T cells, primarily CD8 cells (93%), improves the survival of patients with MM [70]. What is more, it has been shown that T cells also express TNF-α to promote osteoblast apoptosis and stimulate osteoclastogenesis, leading to fracture osteoporosis. In conclusion, T cells are strongly associated with multiple myeloma and OP, which is the same as our findings [71].

Plasma cells originate from B-cell differentiation and are involved in the development of MM. Large populations of B cells directly or indirectly reduce the differentiation of malignant plasma cells. The reduction of malignant plasma cells may also promote the proliferation of normal B lymphocytes. B cells may be a good predictor of MM patient prognosis [72].

MDSCs are a heterogeneous population of immature bone marrow cells [73]. Studies have shown that M-MDSCs are significantly increased in patients with mild MM compared with the level in healthy donors [74]. More importantly, the M-MDSC count is significantly associated with disease activity and tumor progression [75].

Tregs are a T-cell subgroup that controls autoimmune responsiveness in the body and can secrete immunosuppressive cytokines. Functionally, Tregs were reported to exhibit inhibitory effects regardless of the state of MM disease [76].

Because macrophages are capable of cosecreting angiogenic factors that promote MM-associated neovascularization, they are a potential therapeutic target for cancer [77].

Dendritic cells (DC) are also intimately involved in the process of osteoporosis. It has been concluded that DCs can express more cytokines to drive bone

loss due to inflammation [78].

NK cells are capable of promoting osteoporosis, and it has been shown that NK cells are capable of promoting bone loss in the presence of IL-15 [79].

In conclusion, it can be stated that PLAGL1, a gene shared by OP and MM, plays an essential function in TIME.

PLAGL1 expresses a transcription factor that suppresses proliferation and thus inhibits the growth of cancer cells [80] [81] [82] [83]. PLAGL1 cosuppresses or activates the transcription of nuclear hormone receptors [84]. Interestingly, mice lacking PLAGL1 showed a delay in growth and changes in bone formation [85]. Therefore, lowering PLAGL1 may cause osteoporosis.

This research has several limitations, which should be mentioned here. First, there is a need for more clinical data in databases. Second, contaminated tissues may have skewed the WGCNA findings. Third, further *in vitro* investigations are required to obtain a deeper understanding of the mechanisms shared by OP and MM, including complex ribonucleoprotein synthesis, oxidative phosphorylation, aerobic respiration, cellular respiration, and ATP metabolic activities. Moreover, there is inadequate evidence that PLAGL1 is a good predictor of OP and MM co-occurrence, so additional clinical trials are required to verify this point. Nevertheless, the present study shows that PLAGL1 has diagnostic and predictive promise for patients with OP and MM and is also an immune-related biomarker.

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Author Contributions

All authors contributed to the study's conception and design. Wencong Zhang: conceptualization, writing, software; Jiani Mo: conceptualization, writing, visualization; Aiguo Li: supervision, funding acquisition, writing—review & editing, Corresponding author. All authors have read and approved the final submitted manuscript.

Data Availability Agreement

The datasets presented in this study can be found in online repositories.

Informed Consent

The authors of this work use an open bioinformatics database as its foundation, and they legally procure all pertinent information. So Research Ethics Committee has confirmed that no ethical approval is required.

Conflicts of Interest

The authors declare no conflicts of interest regarding the publication of this paper.

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