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ORIGINAL RESEARCH

Comprehensive Analysis of the Role of Metabolic Features in Osteoporosis: A Multi-Omics Analysis

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Purpose: This study aims to comprehensively explore the metabolic features related to the pathogenesis of osteoporosis (OP) through multi-omics analysis strategy.

Patients and Methods: Gene expression profiles of OP patients (GSE56815) were downloaded from GEO, and metabolism-related genes (MRGs) were extracted. Plasma samples from 45 OP patients and 18 healthy controls (CON) were collected for metabolomics. We predicted miRNA and transcription factors (TFs) regulating the expression of MRGs on public databases ENCORI and JASPAR, and analyzed the expression levels of target miRNAs using miRNA sequencing of femoral tissues from 7 samples (OP:CON=4:3). Three machine learning algorithms were used to evaluate the diagnostic potential of metabolic signatures for OP.

Results: A total of 402 significantly differentially expressed MRGs (DEMRGs) were identified in the transcriptome, and these DEMRGs were enriched in 11 metabolic pathways ($P<0.05$). Metabolomics identified 119 differential plasma metabolites, enriched in 5 metabolic pathways ($P<0.05$). Purine metabolism, Tryptophan metabolism, and Tyrosine metabolism were identified as key metabolic pathways and were significantly enriched in DEMRGs. Femoral miRNA sequencing found 124 differentially expressed miRNAs, with 23 regulating key metabolic pathway gene expression ($P<0.05$). Additionally, 13 differentially expressed TFs were predicted to regulate the expression levels of these 23 miRNAs. Finally, three MRGs and one plasma metabolite were selected based on the machine learning algorithm, with AUC of 0.782, 0.714, 0.772 and 0.836, respectively. The diagnostic performance of these metabolic features was better than that of traditional bone metabolism biochemical markers.

Conclusion: This multi-omics study comprehensively explores the metabolic landscape in OP progression, highlighting the central role of metabolic features in the disease. The constructed multi-omics regulatory network aids in understanding the molecular mechanisms of metabolic features in OP progression.

Keywords: osteoporosis, metabolic features, multi-omics analysis, machine learning, biomarkers

Introduction

Osteoporosis (OP) is a prevalent chronic skeletal disease characterized by reduced bone mineral density and increased susceptibility to fractures.[1] The pathogenesis of OP is multifaceted, involving complex interactions between genetic factors, hormonal changes, and lifestyle behaviors.[2] With the aging of the global population, the prevalence of OP is on the rise, and it is projected to further increase in the future, imposing a significant burden on healthcare systems.[3] The risk of fragility fractures is a major clinical manifestation of OP, with fracture risks of 46.4% in women and 22.4% in men.[4] These fractures markedly impact patients' quality of life and also elevate the risk of mortality.[5] Recent studies have emphasized the role of altered metabolites in the pathogenesis of OP, with metabolic dysregulation being considered a factor affecting skeletal health.[6] Therefore, OP is also regarded as a metabolic disease. However, a comprehensive profile of the metabolic landscape in OP remains to be fully elucidated, indicating a need for further research in this area.[7]

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Metabolomics has gained popularity in the study of diseases, including OP, as it can reveal dynamic changes in an organism's current phenotype. Numerous studies have demonstrated that various metabolites play a significant role in the pathogenesis of OP, encompassing amino acid and lipid metabolites.[8,9] Previous studies have confirmed that there were significant differences in amino acid metabolism between osteoporosis patients and healthy controls, including kynurenine, arginine, citrulline and methionine, which were expected to be potential biomarkers for the diagnosis of OP.[10] Moreover, combining metabolites with traditional bone turnover markers enhances OP diagnostic performance compared to using bone turnover markers alone.[11] The biomarkers identified by metabolomics also have great potential in the early diagnosis of OP and monitoring the therapeutic effect.[8] Studies indicate that certain metabolites can serve as potential predictors of fragility fractures in OP patients.[12] Notably, metabolomics can aid in the development of therapies for OP, offering valuable insights into the side effects, efficacy, and dose response relationships of novel drugs.[13] This supports the exploration of new therapeutic strategies for OP. Some studies have reported alterations in multiple metabolites, including lipid and amino acid metabolism, in OP. These findings indicate metabolic pathway dysregulation in OP, which contributes to disease development via osteoblast or osteoclast dysfunction.[6,14–16] The free fatty acid receptor G protein coupled receptor 40 inhibits osteoclast differentiation, thereby preventing bone loss.[17] Short chain fatty acids are associated with protecting bone mass by inhibiting osteoclast differentiation and bone resorption both in vitro and in vivo.[15] Although studies have shown an association between OP and metabolic features (metabolites, metabolism-related genes (MRGs), and metabolic pathways), the mechanisms by which these metabolic features participate in disease progression have not been fully explored.

In recent years, the role of microRNAs (miRNA) in the pathogenesis of OP has been extensively studied. Studies have demonstrated that miRNAs were involved in the differentiation of bone cells by regulating key modulators of bone metabolism and signal transduction pathways.[18] miRNAs can also target genes that promote bone regeneration and treat OP.[19] The expression of miRNAs themselves is regulated by transcription factors (TFs), which were also involved in the generation of osteoclasts and the regulation of bone homeostasis, providing a basis for the early diagnosis and targeted treatment of OP.[20,21] The emergence of multi-omics joint analysis aids in understanding molecular changes and interactions in diseases, which is key to promoting precision treatment of diseases.[22] Regrettably, no multi-omics joint studies have comprehensively reported the core role of metabolic features in OP. In this study, we integrated miRNA sequencing, transcriptomics, and metabolomics to comprehensively analyze the role of MRGs, metabolites, and metabolic pathways in the progression of OP. We also mapped the regulatory network from transcription factors, miRNAs, MRGs to metabolites during the occurrence and development of the disease. Finally, we used three machine learning algorithms to explore the potential of metabolic features in the diagnosis and differentiation of OP, hoping to identify new biomarkers for the disease.

Methods and Materials

Study Population Characteristics

We enrolled 18 healthy volunteers and 45 OP patients hospitalized at Dazhou Central Hospital. All OP patients met the diagnostic criteria, with T-scores for both total hip and lumbar spine (L1-L4) bone mineral density (BMD) less than −2.5.[23] Exclusion criteria included: 1. History of thyroid or parathyroid diseases; 2. Long-term use of corticosteroids; 3. Presence of malignant tumors; 4. Prior regular anti-OP treatment; 5. Use of lipid-lowering drugs or medications that could affect lipid levels. Clinical information was collected from all participants, including age, gender, and body mass index (BMI). We also collected data on biochemical markers of bone metabolism in the traditional sense of OP, It includes N-terminal mid-fragment of osteocalcin (N-MID), β-C-Terminal telopeptide of type I collagen (β-CTX), type 1 N-terminal propeptide (TP1NP), parathyroid hormone (PTH), and 25-hydroxyvitamin D (25(OH)VD). These biomarkers collectively assess bone turnover imbalance (formation vs resorption), hormonal dysregulation, and nutritional deficiencies, all central to OP pathogenesis. Detailed information is presented in Table S1.

Sample Collection

Fasting peripheral venous blood (5 mL) was collected from all subjects on the second day after admission in EDTA anticoagulant tubes and immediately transported to the laboratory for standard preprocessing. Plasma samples of approximately 1.5 mL were retained and immediately stored at −80°C for non-targeted metabolomics analysis. Additionally, femoral tissue samples from 4 OP patients and 3 healthy controls (CON, from accidental trauma, only for miRNA sequencing) were collected for miRNA sequencing.

Non-Targeted Metabolomics Analysis

Plasma samples from subjects were analyzed by a bio-company (MagiBio, China) using ultra-performance liquid chromatography-tandem mass spectrometry for non-targeted metabolomics analysis. The detailed process, as described previously,[24] included metabolite extraction, mass spectrometry analysis, data searching, and annotation. Metabolites with missing values greater than 20% within each group and relative standard deviations greater than 30% were removed from the raw data. Additionally, missing values were imputed with the minimum value, and metabolite expression data were log10-transformed for preprocessing. Differential analysis of plasma metabolites and Kyoto Encyclopedia of Genes and Genomes (KEGG) pathway enrichment were both completed on the Majorbio cloud platform (www.majorbio.com).

Femoral Tissue miRNA Sequencing

Briefly, an appropriate amount of femoral tissue was thoroughly ground and then QIAzol Lysis Reagent (Qiagen, Germany) was added for RNA extraction. A high-throughput sequencing platform was used to sequence small RNA fragments of 16–35nt, and a QIAseq miRNA Library Kit (Qiagen, Germany) was employed to construct the library. The specific process included total RNA extraction, adapter ligation, reverse transcription to synthesize cDNA, and library enrichment and purification. The final purified library was sequenced on the Illumina NovaSeq 6000 platform. Raw sequencing data were filtered using the Fastx-Toolkit (http://hannonlab.cshl.edu/fastx_toolkit/) to obtain high-quality sequencing data (clean data). Based on the characteristics of miRNAs, reads of lengths 18–32nt were selected as useful reads and aligned with the reference genome to identify known or novel miRNAs. Known miRNA identification was performed by alignment with the miRBase 22.0 database (http://www.mirbase.org/). The software miRDeep2 (https://www.mdc-berlin.de/content/mirdeep2-documentation) was used for the prediction of novel miRNAs.

Differential Expression Analysis of miRNAs

Expression levels of miRNAs were quantified using software, and TPM (Transcripts Per Million) was utilized for normalization of expression levels. Differential expression analysis was conducted using the Limma software, defining $p < 0.05$ and $|\log2FC| \geqq 1$ as significantly differentially expressed miRNAs.

Extraction and Analysis of MRGs

We downloaded gene expression data (Expression Profile by array) GSE56815 from the Gene Expression Omnibus (GEO) database (https://www.ncbi.nlm.nih.gov/geo/), which includes 40 samples with low BMD and 40 with high BMD.[25] Based on the reference gene set "C2.cp.kegg.v7.5.1.symbols.gmt" from the MSigDB database and 2752 previously identified MRGs, we extracted the expression data of OP MRGs from GSE56815.[26] Differential expression gene analysis was conducted using the "limma" R package, defining genes with $P<0.05$ as differentially expressed metabolic-related genes (DEMRGs). The "Cluster Profiler" R package was used for functional enrichment analysis of DEMRGs in terms of Gene Ontology (GO) and KEGG pathways.

Prediction of miRNAs and TFs

The prediction of upstream regulatory mirnas of MRGs was made via the online website ENCORI (http://starbase.sysu.edu.cn/), which includes seven software tools for predicting miRNAs (PITA, RNA22, miRmap, microT, miRanda, PicTar, and TargetScan). miRNAs predicted by two or more software tools were retained. The targeting relationship between transcription factors and miRNAs was predicted using the JASPAR database (https://jaspar.elixir.no/). The

transcriptional regulatory region was defined as 5000 upstream to 1000 downstream of the miRNA TSS. The significance threshold for prediction results was set at $P<0.0005$.

Machine Learning-Based Selection of Metabolic Features Biomarkers

In this study, we employed three machine learning algorithms—Least Absolute Shrinkage and Selection Operator (LASSO), Random Forest (RF), and Support Vector Machine (SVM)—to comprehensively explore metabolic feature biomarkers from MRGs and plasma metabolites. We performed feature selection using LASSO regression, which was implemented via the R package "glmnet" with a penalty parameter. The regularization parameter (λ) was optimized through 10-fold cross-validation, with the λ value corresponding to the minimum mean squared error being selected. The RF is an ensemble classifier composed of multiple decision trees that can efficiently and rapidly identify the most important biomarkers for sample classification. Our RF model was configured with 500 trees, each with a maximum depth of 10, and feature selection was performed using the square root rule. During the model building process, validation was performed using the areas under the curve (AUC) value, and the number of important features was determined under the condition of maximum AUC. This part of the analysis was completed on the Majorbio cloud platform (www.majorbio.com). SVM is also an effective feature selection method. We completed the analysis using the R packages "e1071", "kernlab", and "caret". The SVM model was validated through 10-fold cross-validation, and the number of features was determined based on the minimum cross-validation error.

Statistical Analysis

Data statistical analysis was performed using SPSS Statistics (v25.0.0.0), and R software (v4.1.1) and GraphPad Prism (v8.0) (GraphPad Software, Inc., CA, USA) were used for graphing. Cytoscape (V3.9.1) was employed for the construction of regulatory network graphs. Continuous variable data that were normally distributed were analyzed using *t*-tests, while those that were not normally distributed were analyzed using rank sum tests. Correlation analysis was conducted using Spearman correlation. All results with $P<0.05$ were considered to have statistically significant differences.

Results

Comprehensive Analysis of OP Metabolic Features

We filtered transcriptome data and identified a total of 2068 MRGs. Among these, 402 DEMRGs were detected, including 122 down-regulated and 280 up-regulated genes (Figure 1A, $P<0.05$). Figure 1B displayed the top 10 significantly enriched pathways in three categories of GO enrichment analysis for DEMRGs. Three metabolic process pathways were identified in biological process (BP), including purine-containing compound metabolic process, purine nucleotide metabolic process, and alcohol metabolic process (Figure 1B). Similarly, KEGG enrichment analysis was performed on DEMRGs, resulting in the identification of 95 pathways. Figure 1C shows the top 30 significantly enriched pathways, including 11 metabolic pathways such as Carbon metabolism, Purine metabolism, and Tryptophan metabolism (Figure 1C).

To confirm that transcriptional changes in the Op population resulted in metabolic level changes, we conducted a non-targeted plasma metabolite assay for 63 samples. A volcano plot revealed a total of 119 differential plasma metabolites, including 69 up-regulated and 50 down-regulated metabolites (Figure 1D, $P<0.05$). Through annotation and classification using the Human Metabolome Database (HMDB), the most prevalent differential plasma metabolites were found to be Organic acids and derivatives and Lipids and lipid-like molecules (Supplementary Figure 1). The two types of metabolites are confirmed to regulate bone metabolism through multiple mechanisms, including promoting the proliferation of bone marrow mesenchymal stem cells and the differentiation of osteoblasts, as well as regulating the fluidity of bone cell membranes and signaling pathways.[27,28] KEGG enrichment analysis of these differential plasma metabolites revealed five significantly enriched metabolic pathways: Tryptophan metabolism, Glycerophospholipid metabolism, Lysine degradation, Purine metabolism, and Tyrosine metabolism (Figure 1E, $P<0.05$). Notably, three metabolic pathways—Tryptophan metabolism, Purine metabolism, and Tyrosine metabolism—were significantly enriched at the

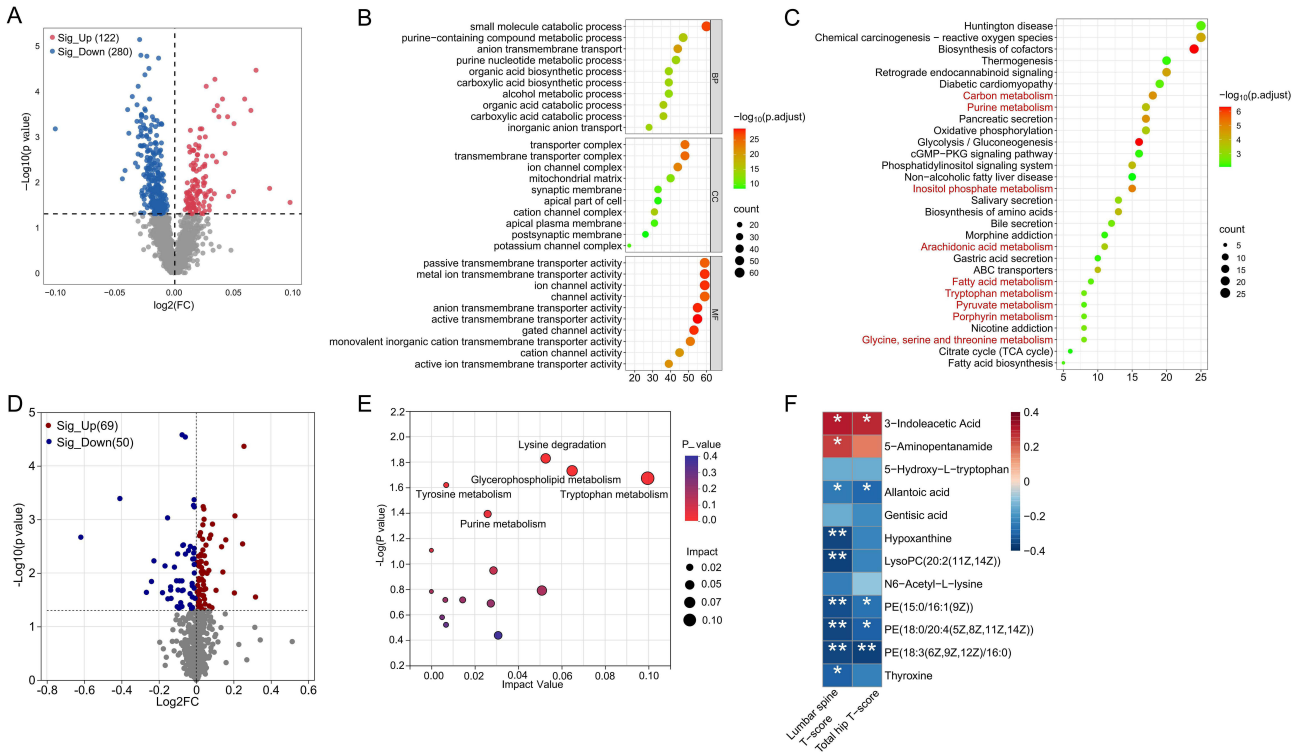

Figure 1 Comprehensive analysis of metabolic characteristics in the OP population. (**A**) Volcano plot of differentially expressed MRGs. Red indicated significantly up-regulated genes, and blue indicated significantly down-regulated genes. (**B**) GO enrichment analysis of differentially expressed MRGs. (**C**) The top 30 KEGG significantly enriched pathways. Metabolic pathways were highlighted in red font. (**D**) Volcano plot of differentially expressed plasma metabolites. Red represented significantly up-regulated metabolites, and blue represented significantly down-regulated metabolites. (**E**) KEGG enrichment analysis of significantly differential plasma metabolites. (**F**) Spearman correlation heatmap of metabolites from the 5 significantly enriched metabolic pathways with BMD T-scores. The color bar indicates the magnitude of the correlation, with red representing positive correlation and blue representing negative correlation. *P<0.05, **P<0.01.

Abbreviations: OP, osteoporosis; MRGs, metabolic-related genes; GO, Gene Ontology; KEGG, Kyoto Encyclopedia of Genes and Genomes; Sig_Up, significantly up-regulated; Sig_Down, significantly down-regulated.

transcriptional level and were defined as key metabolic pathways. Additionally, plasma metabolites enriched in these five metabolic pathways showed significant correlations with T-scores of the total hip and lumbar spine (L1-L4) (Figure 1F). 3-Indoleacetic Acid shows a significant positive correlation with T-scores, and its expression is significantly reduced in OP, indicating that its downregulation may promote bone loss and lead to OP.[29] The above results indicated that metabolic features in OP had changed, especially the three key metabolic pathways and their metabolic features, which were associated with the occurrence and development of OP.

Identification of miRNAs and TFs Regulating MRGs

Subsequently, we identified miRNAs and TFs that regulate MRGs associated with the occurrence and development of OP. Initially, differential expression analysis revealed a total of 124 differentially expressed miRNAs, including 57 up-regulated and 67 down-regulated miRNAs (Figure 2A, |Log2FC|≥1, P<0.05). Secondly, miRNAs related to metabolic features were screened. As shown in (Figure 2B), a total of 225 miRNAs were predicted from 30 MRGs associated with three key metabolic pathways. Among these, 23 miRNAs exhibited significantly different expression levels, including 10 up-regulated and 13 down-regulated miRNAs (Figure 2C and Table 1). Concurrently, using the JASPAR database, multiple TFs were predicted for differentially expressed miRNAs, Thirteen TFs showed significant differences in OP (Figure 2D, FDR<0.05).

Construction of the TFs-miRNA-MRGs-Metabolite Regulatory Network

In summary, we identified alterations in three key metabolic pathways in the occurrence and development of OP at both the transcriptional and metabolomic levels. Plasma metabolites in these pathways correlated with T-scores and exhibited

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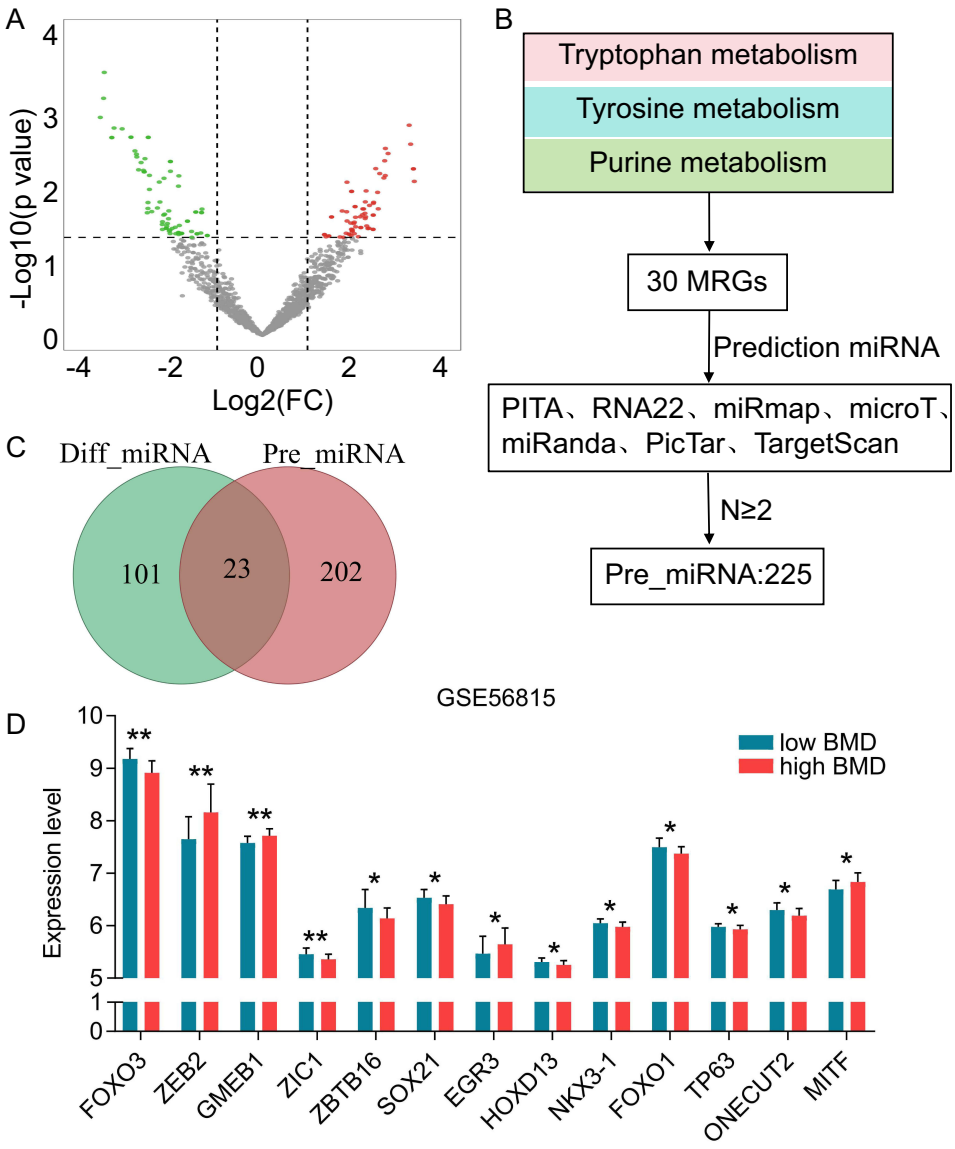

Figure 2 Identification of miRNAs and TFs associated with OP metabolic pathways. (**A**) Volcano plot of differentially expressed miRNAs in bone tissue. miRNAs with *P*<0.05 and |log2(FC)|>1 were defined as significantly differential miRNAs. Red represented significantly up-regulated miRNAs, and green represented significantly down-regulated miRNAs. (**B**) Flowchart illustrating the miRNA prediction process. miRNAs predicted by at least two out of seven databases were retained. (**C**) VENN diagram showing the overlap of 23 miRNAs between significantly differential miRNAs and predicted miRNAs. (**D**) Identification of upstream TFs of miRNAs in the GSE56815 dataset. **FDR*<0.05, ***FDR*<0.01.

Abbreviations: TFs, transcription factors; OP, osteoporosis; MRGs, metabolic-related genes; Diff_miRNA, differential miRNAs; Per_miRNA, prediction miRNA.

significant intercorrelations (Supplementary Figure 2A). Additionally, MRGs involved in the biotransformation of metabolites showed significantly differential expression levels, and interconnections among these MRGs were identified (Supplementary Figure 2B). More importantly, miRNAs and TFs that regulate the expression levels of MRGs exhibited differential expression during the progression of OP. Based on these findings, we concluded that a regulatory network composed of TFs, miRNAs, MRGs, and metabolites collectively promoted the occurrence and development of OP (Figure 3).

Identification of Metabolic Feature Biomarkers

Ultimately, we employed machine learning algorithms to maximize the potential of metabolic features in disease diagnosis. Specifically, we selected three machine learning algorithms—LASSO (Figure 4A), RF (Figure 4B), and SVM (Figure 4C)—to comprehensively screen for biomarkers among MRGs. The three algorithms identified 39, 40, and

Table 1 There Were 23 Significantly Differentially Expressed miRNAs

miRNA Name	Log2FC(OP/CON)	*P* value	Regulate
miR-150-5p	−3.051	<0.001	Down
miR-513b-5p	−2.908	0.002	Down
miR-483-3p	−2.526	0.002	Down
miR-141-3p	−3.332	0.002	Down
miR-29b-3p	−2.035	0.005	Down
miR-187-3p	−2.615	0.007	Down
miR-584-5p	1.977	0.012	Up
miR-369-3p	2.053	0.020	Up
miR-195-5p	−1.474	0.023	Down
miR-146a-5p	−1.346	0.023	Down
miR-512-3p	2.455	0.025	Up
miR-455-5p	1.532	0.027	Up
miR-335-5p	−1.657	0.030	Down
miR-665	2.180	0.037	Up
miR-138-5p	−2.128	0.038	Down
miR-1323	2.452	0.039	Up
miR-3619-5p	1.990	0.041	Up
miR-488-3p	−1.785	0.044	Down
miR-29c-3p	−1.446	0.045	Down
miR-519d-3p	1.919	0.045	Up
miR-19a-3p	1.376	0.046	Up
miR-361-3p	−1.218	0.047	Down
miR-212-3p	1.471	0.048	Up

Abbreviations: FC, Folding change; OP, Osteoporosis; CON, controls.

40 MRGs, respectively, with three MRGs (GLT8D2, MAN2A1, and TRPM6) shared across all methods (Figure 4D). The AUC of GLT8D2, MAN2A1, and TRPM6 for diagnosing OP was 0.782, 0.714, and 0.772, respectively, while the AUC of the combined diagnosis model reached 0.875 (Figure 4E). Using the same approach, we analyzed 821 plasma metabolites. The three machine learning algorithms selected 12, 9, and 13 plasma metabolites, respectively; however, only one plasma metabolite, N-Acetyl-S-(N -methylcarbamoyl)-cysteine, was shared among them, with an AUC of 0.836 for diagnosing OP (Figure 4F, Supplementary Figure 3A–D). Notably, the AUC values of the metabolic feature biomarkers we identified exceeded those of traditional biochemical markers of bone metabolism (N-MID, β-CTX, TP1NP, and PTH) (Supplementary Figure 3E). These results collectively indicate that the metabolic features identified in this study have significant potential as biomarkers for the differential diagnosis of OP.

Discussion

In this study, we conducted an integrated multi-omics analysis by combining miRNA sequencing, transcriptomics, and metabolomics, emphasizing the changes in metabolic features during the progression of OP. Our findings highlighted the core role of three key metabolic pathways—Tryptophan metabolism, Purine metabolism, and Tyrosine metabolism—across different dimensions from the microscopic to the macroscopic levels in the disease. Additionally, by integrating miRNA and TF analysis, we constructed a regulatory network involving key metabolic pathways, TFs, MRGs, and metabolites in the occurrence and development of OP. Finally, based on three machine learning algorithms, we comprehensively screened for three MRGs and one plasma metabolite, and these metabolic features demonstrated superior performance in the diagnosis and differentiation of OP, serving as promising candidate biomarkers.

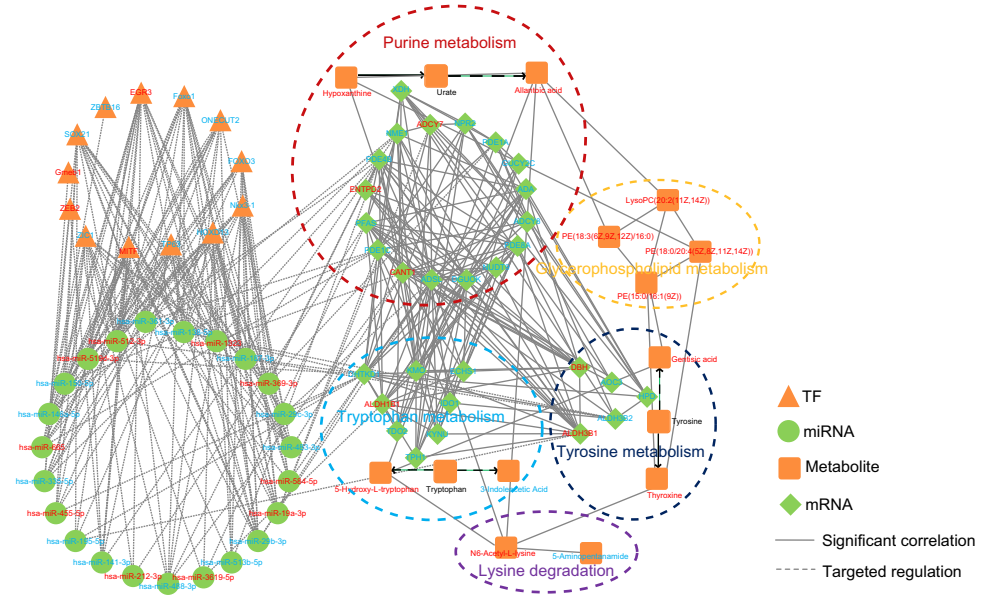

Figure 3 Construction of the TF-miRNA-MRGs-metabolite network. Target regulatory relationships between TFs, miRNAs, and mRNAs were established based on database predictions. mRNAs and metabolites were co-involved in the alteration of metabolic pathways, and mRNAs and metabolites enriched in different metabolic pathways show significant correlations. Triangles represented TFs, circles represented metabolic mRNAs, squares represented plasma metabolites, and diamonds represented miRNAs. Solid lines indicated significant correlations (Spearman correlation), and dashed lines indicated target regulatory relationships. Red font indicated up-regulated expression, and blue font indicated down-regulated expression.
Abbreviations: MRGs, metabolic-related genes; TF, transcription factor.

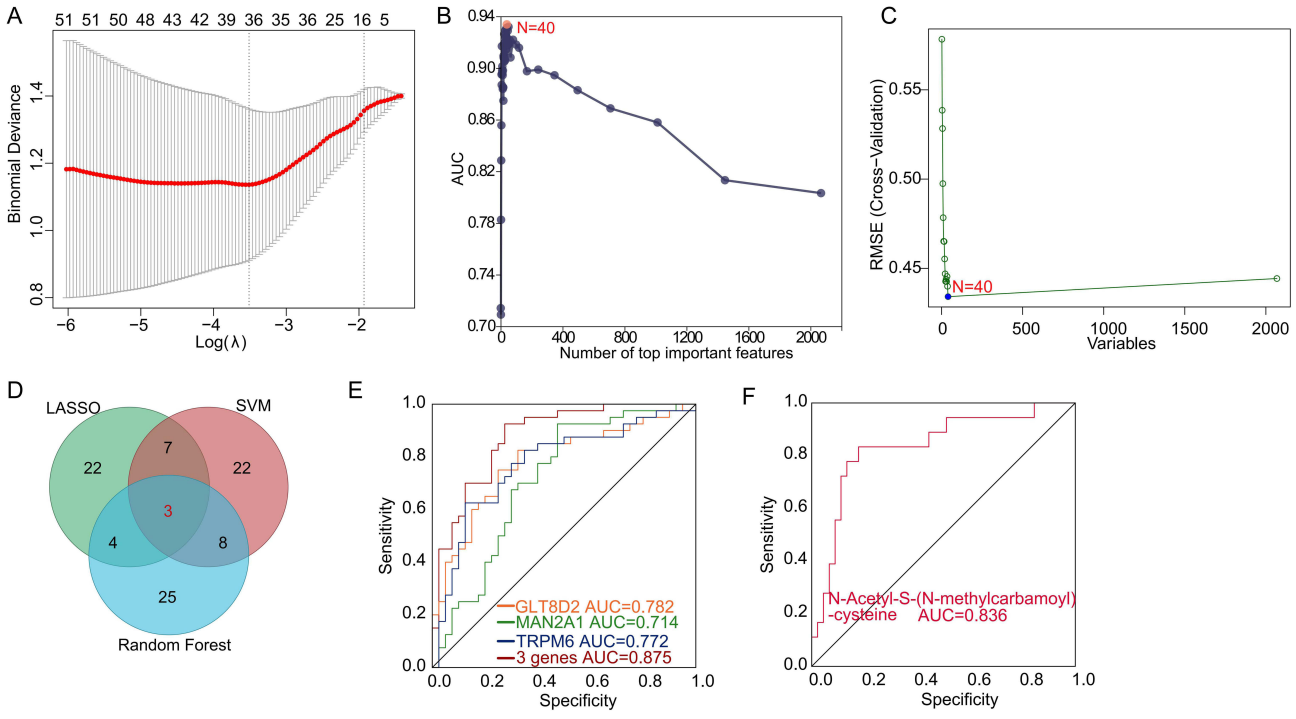

Figure 4 Machine learning screening for metabolic features biomarkers. (**A**) LASSO logistic regression coefficient penalty plot. (**B**) Model validation using AUC values with RF. (**C**) SVM cross-validation. (**D**) Three machine learning algorithms share 3 MRGs. (**E**) ROC analysis of MRGs. (**F**) ROC analysis of the plasma metabolite N-Acetyl-S-(N-methylcarbamoyl)-cysteine shared by three machine learning algorithms. *FDR<0.05, **FDR<0.01.
Abbreviations: LASSO, Least Absolute Shrinkage and Selection Operator; RF, Random Forest; SVM, Support Vector Machine; ROC, receiver operator characteristic curve; MRGs, metabolic-related genes; ROC, receiver operator characteristic curve;AUC, areas under the curve.

OP, a skeletal disease marked by reduced bone strength and heightened fracture risk, presents with increased bone turnover and decreased bone mass.[30] Factors like aging, estrogen decline in women, and low BMI can impact bone health and contribute to OP. Additionally, nutritional status, medication use, and comorbidities can influence bone metabolism via distinct mechanisms, stimulating osteoclast activity and accelerating bone loss.[31,32] Prior studies have established the critical role of the Wnt/β-catenin and Notch signaling pathways in bone formation regulation.[33] Abnormalities in Wnt/β-catenin signaling can lead to insufficient bone formation and are closely linked to OP development. Metabolomics research on OP has underscored the significance of metabolite alterations in disease progression. Multiple studies have confirmed that amino acid, lipid, energy, and glucose metabolism are dysregulated in OP populations. These metabolic pathway disruptions contribute to disease progression by affecting bone cell biological functions.[34–37]

Previous studies have observed Purine metabolism disorders in OP.[38] These disorders can lead to excessive release of uric acid into the bloodstream, which has a dual impact on OP. The level of uric acid in the physiological range has a protective effect on bone metabolism and was positively correlated with BMD.[39] However, a large amount of uric acid accumulation causes hyperuricemia, which could become a risk factor for OP through the production of a large number of reactive oxygen species (ROS) and the induction of vitamin D deficiency and hyperparathyroidism.[40,41] In this study, the Purine metabolic pathway was significantly enriched, and two metabolites within the pathway, Hypoxanthine and Allantoic acid, showed significant negative correlations with BMD T-scores. Hypoxanthine produces the end metabolite uric acid through xanthine dehydrogenase (XDH). Long-term hyperuricemia can induce the occurrence of Gout, which was significantly associated with OP.[42,43] Similarly, changes in the levels of metabolites in the Tryptophan metabolic pathway have been observed in bone metabolic diseases. Tryptophan metabolites such as serotonin, melatonin, and kynurenine were all considered to be associated with OP.[44] The kynurenine pathway of tryptophan degradation is activated during the process of osteoblast generation and may be related to the occurrence of bone diseases.[45] Our study found that 3-Indoleacetic Acid on the Tryptophan metabolic pathway was reduced in OP and had a significant positive correlation with T-scores. The latest study shows that supplementation of the tryptophan metabolite 3-Indoleacetic Acid significantly improves bone loss by restoring the intestinal barrier.[29] Therefore, exploring changes in metabolites during the progression of OP helps to fully understand the pathogenesis of the disease.

MiRNAs are small molecules found in higher eukaryotes. Studies have shown that they can inhibit protein synthesis by targeting mRNA expression levels or inducing the formation of RNA silencing complexes.[46] Additionally, miRNAs play a crucial role in cell proliferation, differentiation, and the maintenance of bone homeostasis regulation, and have been identified as significant pathological factors in OP.[47,48] In this study, we identified 124 differentially expressed miRNAs, 23 of which were associated with metabolic pathways involved in the progression of OP. miR-150-5p has been shown to activate the Wnt/β-catenin pathway by down-regulating MMP14 expression, thereby inducing osteoblast proliferation and maturation.[49] Notably, miR-150-5p was predicted to regulate the Purine metabolism pathway by targeting the gene PDE1C, and its expression level was significantly reduced in OP, consistent with previous research findings.[49] Interestingly, the expression level of the target gene PDE1C was also significantly decreased. PDE1C encodes phosphodiesterase 1C, an enzyme hydrolyzing cyclic nucleotides (cAMP/cGMP) to regulate downstream signaling, including the Wnt/β-catenin pathway.[50] Thus, the dysregulation of miR-150-5p expression may contribute to disease progression by reducing PDE1C expression levels and disrupting the Purine metabolism pathway. miR-512-3p is upregulated in OP and inhibits osteoblast differentiation by targeting DYRK2, a kinase involved in the Hippo signaling pathway. Overexpression of miR-512-3p suppresses DYRK2, disrupting Hippo-mediated transcriptional activation of pro-osteogenic genes.[51] Moreover, miRNA expression levels are regulated by TFs, which participate in the pathogenesis of OP by regulating osteoclast differentiation.[52] The main function of MITF in the TF-miRNA-MRGs-metabolite regulatory network we constructed was to control osteoclast differentiation, but this was also controlled by other TFs.[53]

Omics technologies have undoubtedly contributed to exploring the molecular characteristics of disease progression from various dimensions. The integrated application of multi-omics has enhanced the understanding of molecular networks/pathways in disease development.[54] Several studies have employed multi-omics approaches, combining transcriptomics, methylomics, and metabolomics, to identify the interactions and causal mechanisms of molecular characteristics in OP.[55] Multi-omics joint analysis has also been used to explore biomarkers for disease and mechanisms

related to therapeutic treatments.[56,57] However, the application of multi-omics joint analysis in OP is still insufficient, particularly in exploring specific molecular characteristics in disease progression. This study was the first to integrate miRNA sequencing, transcriptomics, and metabolomics to comprehensively analyze changes in metabolic features in OP and construct a regulatory network of metabolic pathways associated with disease development. Notably, this study also explores the potential of metabolic features in disease diagnosis. The metabolic features screened by the machine learning were superior to biochemical markers of bone metabolism and other molecular characteristics in disease diagnosis, holding promise as new biomarkers.[58,59]

This study has several limitations. Firstly, this study included less omics data, especially miRNA data. The small sample size may lead to insufficient statistical power and affect the stability and reproducibility of the results. Importantly, a small sample size may underestimate the differential expression of certain metabolites or genes, leading to potential omission of information. Future studies will incorporate a larger number of analytical samples to uncover more latent disease characteristics. Secondly, the omics samples were obtained from different individuals. Given inter-individual differences and varying disease progression, the levels of metabolic signatures can vary among patients. Thus, omics data from different individuals may not fully capture the molecular regulatory mechanisms of metabolic signatures in disease progression. Nevertheless, the metabolic signatures we identified were linked to OP progression and were verified through existing literature, which helps ensure our results are reliable. Finally, metabolic features identified by machine learning have better diagnostic performance than traditional biochemical markers of bone metabolism, which will help to improve the ability of early diagnosis of OP in clinical practice. However, its real transformation into a clinical diagnostic tool still needs to face more adjustments, including large cohorts, multi-stage clinical and multi-center validation to determine its robustness. Similarly, machine learning algorithms can be used to identify the metabolic characteristics during OP drug treatment, which can help clinicians to effectively monitor the treatment effect of OP, and even develop personalized treatment strategies. Our subsequent research will construct a multi-omics diagnostic model based on multi-omics features from the same individuals, which may significantly enhance the diagnostic performance of the model.

Conclusion

In summary, our combined multi-omics analysis of miRNA sequencing, transcriptomics, and metabolomics highlights the metabolic features, especially the central role of three key metabolic pathways in OP. The constructed transcription TF-miRNA-MRGs-metabolite regulatory network aids in understanding the molecular regulatory mechanisms of metabolic characteristics in disease progression. Finally, metabolic features comprehensively screened based on machine learning algorithm have better diagnostic performance than biochemical markers of bone metabolism, which has the potential to assist clinical practice to improve the early diagnosis of OP.

Data Sharing Statement

The original contributions presented in the study are included in the article/Supplementary Material. Further inquiries can be directed to the corresponding author.

Ethical Statement

The study was approved by the Ethics Committee of Dazhou Central Hospital (approval number: irb00000001-17001). All participants provided written informed consent for their involvement in the study. The research involving human participants was performed in accordance with the Declaration of Helsinki.

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Disclosure

All the authors declare no conflict of interest in this work.

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