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Expression of m^{6}A Methylation Regulator in Osteoarthritis and Its Prognostic Markers

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Abstract

Objective. Osteoarthritis (OA) is a multifactorial disorder, in which genetic factors are strongly associated with its development. However, the pathogenesis of OA is still unclear, and recently it has been observed that epigenetic modifications are also involved in the pathogenesis of OA. This study aims to study the potential role of m^{6}A-related genes in the occurrence and development of OA. *Design.* We downloaded the OA expression profile data (GSE55235) from the Gene Expression Omnibus database. First, function enrichment analysis of 17 representative m^{6}A methylation regulatory factors was performed using the DAVID database and Metascape online tool. Then, we analyzed the expression of 17 m^{6}A methylation regulatory factors in OA and the correlation between regulatory factors using Perl software. Finally, receiver operating characteristic (ROC) curve analysis and the area under the ROC curve were used to evaluate the diagnostic effectiveness of m^{6}A-related genes for discriminating patients with OA and healthy. *Results.* We first identified that 12 of the 17 genes were differentially expressed in OA. ALKBH1, EIF3, IGF2BP3, WTAP, and YTHDC1 were associated with early diagnosis and prognosis of OA. *Conclusions.* m^{6}A RNA methylation regulator factors are key players in the progression of OA and have potential role in the stratification of prognosis and the formulation of treatment strategies.

Keywords

osteoarthritis (OA), N6-methyladenosine (m^{6}A) modification, biomarkers, gene expression

Introduction

Osteoarthritis (OA) is the most common form of arthritis; the main characteristics are matrix degradation and collagen synthesis imbalance, cartilage cell metabolism disorder, inflammation activation, and cartilage aging.[1,2] According to statistics, the prevalence rate of OA in people over 60 years old can reach 60% to 65%, and with the aging of the population and the increase in obesity, its prevalence is increasing, which seriously threatens human health and quality of life.[3,4] There are many factors that affect the occurrence of OA, and its etiology and pathogenesis are unclear.

OA is a complex disorder, in which environmental and genetic factors are strongly related to its development. Genetic heterogeneity does not seem to explain all the characteristics of OA. Therefore, it is important to study the epigenetic factors and mechanisms related to OA progression and treatment response. N6-methyladenosine (m^{6}A) is a universal modification of RNA molecules in eukaryotes, which plays key roles in regulating fundamental biological processes such as gene expression control and regulation of mRNA stability and homeostasis.[5,6] Recent studies suggested that m^{6}A is involved in various aspects of RNA metabolism including pre-mRNA splicing, 3′-end processing, translation regulation, and mRNA decay processing.[7] Similar to DNA methylation, m^{6}A modification is reversible and catalyzed by corresponding enzymes, namely, "Adenosine methyltransferases (m^{6}A writers), RNA-binding proteins (m^{6}A readers) and Demethylases (m^{6}A erasers)." The m^{6}A RNA methylation regulator regulates

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gene expression in epigenetics, thereby playing role in regulating biological processes. Emerging evidence reported m^{6}A modification is involved in the progression of various human diseases, such as rheumatoid arthritis (RA),[8] cancer,[9] obesity,[10] and atherosclerosis (AS).[11] However, little is known about the role of m^{6}A in OA, and the expression and prognostic value of m^{6}A RNA methylation regulator factors have not been reported.

In the present study, we applied bioinformatics methods to analyze the expression patterns of m^{6}A methylation regulators in OA. Furthermore, the relationship between differential m^{6}A genes and the prognosis and early diagnosis of OA was analyzed. We found that the expressions of m^{6}A genes play key roles in the process of OA and firstly identified five genes as potential biomarkers.

Methods

Data sets

The expression profile data sets from the OA data sets (GSE55235) were collected from the Gene Expression Omnibus (GEO) databases, based on the inclusion and exclusion criteria of the GEO. Inclusion criteria include data sets involving human synovial tissue and expression profiling by array. R software was used in pre-treating and transforming gene.

Selection of m^{6}A RNA Methylation Regulator Factors

A list of 17 m^{6}A RNA methylation regulator factors were sorted from the published literature (shown in Table 1),[12-14] including m^{6}A writers (METTL3, RBM15, WTAP, and ZC3H13), m^{6}A readers (EIF3, HNRNPA2B1, HNRNPC, IGF2BP2, IGF2BP3, YTHDC1, YTHDC2, YTHDF1, YTHDF2, and YTHDF3), and m^{6}A erasers (FTO, ALKBH1, and ALKBH4). Then, we systematically compared their expression in OA.

Bioinformatic Analysis

The OA data sets were converted into expression estimates. Then the background correction, quartile data normalization and probe summarization were performed by the Robust Multi-array average algorithm in the R(v3.6.0) affy package.

The differential expression of m^{6}A-related genes was analyzed using limma method. Two-sided Wilcoxon rank-sum test was used to evaluate the gene expression difference between two groups. Heat maps of differential expression for m^{6}A-related genes were generated using heatmap package, and Corrplot package was used to analyze the correlation between m^{6}A-related genes themselves in R. Functional enrichment was performed using the Sangerbox tools (http://www.sangerbox.com/tool).

To determine the prognostic value of m^{6}A RNA methylation regulators, we performed receiver operating characteristic (ROC) analysis of the expression of m^{6}A RNA methylation regulators in GEO database, and calculated the area under the curve (AUC), as well as the corresponding sensitivity, specificity. $P < 0.05$ is considered statistically significant, and all statistics were performed with SPSS 22.0 software.

Result

Functional Enrichment Analysis

We analyzed 17 m^{6}A-related genes by protein-protein interaction (PPI) analysis using Metascape and STRING online database, and the results are shown in Suppl. Figure S1, which suggests that there is a strong relationship between m^{6}A-related genes. Then GO enrichment analysis showed that they are mainly involved in the regulation of mRNA metabolic process, nuclear speck, and mRNA binding, which contain biological process (BP), cellular component (CC), and molecular function (MF), respectively (Table 2, Fig. 1).

The Expression of m^{6}A RNA Methylation Regulator Factors between Normal and OA Patients

The result of the expression of m^{6}A-related genes between the normal and OA patients is shown in the heat map (Fig. 2), which identified that 12 of the 17 genes were differentially expressed between normal and OA patients (Table 3). Then, quantitative analysis showed that in patients, the expression levels of ALKBH1 ($P < 0.0001$), EIF3 ($P = 0.0005$), FTO ($P = 0.0337$), HNRNPA2B1 ($P = 0.0118$), IGF2BP3 ($P = 0.0001$), RBM15 ($P = 0.0093$), WTAP1 ($P < 0.0001$), YTHDC1 ($P < 0.0001$), and YTHDF1 ($P = 0.0195$) are downregulated, while METTL3($P = 0.0167$) and IGF2BP2 ($P = 0.0418$) are upregulated (Fig. 3). Furthermore, to better understand the interactions of 17 m^{6}A RNA methylation regulators in OA, we further analyzed the correlations among these genes (Suppl. Fig. S2). Both many positive correlations correlation were observed, such as HNRNPA2B1 and EIF3, EIF3 and WTAP, YTHDC1 and IGF2BP3, and so on.

Diagnosis Value of m^{6}A RNA Methylation Regulators in OA

To explore the relationship between m^{6}A-related genes and early diagnosis of OA, we used ROC and the area under the curve to analyze and evaluate the diagnostic effectiveness of genes for discriminating patients with OA and normal

Table 1. m^{6}A-Related Genes.

Functions	Genes
Adenosine methyltransferases (m^{6}A writers)	METTL3, RBM15, WTAP, ZC3H13
RNA-binding proteins (m^{6}A readers)	EIF3, HNRNPA2B1, HNRNPC, IGF2BP2, IGF2BP3, YTHDC1, YTHDC2, YTHDF1, YTHDF2, YTHDF3
Demethylases (m^{6}A erasers)	FTO, ALKBH1, ALKBH4

Table 2. The Top 10 GO Terms of m^{6}A.

Category	Term	Functional Enrichment	*p* Value	Count
BP	GO:1903311	regulation of mRNA metabolic process	3.73E-17	11
	GO:0043488	regulation of mRNA stability	3.89E-11	7
	GO:0043487	regulation of RNA stability	4.85E-11	7
	GO:0061013	regulation of mRNA catabolic process	9.02E-11	7
	GO:0006417	regulation of translation	5.98E-10	8
	GO:0009451	RNA modification	6.04E-10	6
	GO:0034248	regulation of cellular amide metabolic process	1.67E-09	8
	GO:0006446	regulation of translational initiation	3.09E-09	5
	GO:0045948	positive regulation of translational initiation	5.31E-09	4
	GO:0006402	mRNA catabolic process	7.91E-09	7
CC	GO:0016607	nuclear speck	5.04E-07	6
	GO:0034708	methyltransferase complex	8.02E-07	4
	GO:0071013	catalytic step 2 spliceosome	2.20E-03	2
	GO:0005681	spliceosomal complex	8.09E-03	2
	GO:0035770	ribonucleoprotein granule	1.10E-02	2
	GO:0005697	telomerase holoenzyme complex	1.62E-02	1
	GO:0031965	nuclear membrane	2.36E-02	2
	GO:0005635	nuclear envelope	4.92E-02	2
	GO:0000932	P-body	5.26E-02	1
	GO:0015030	Cajal body	6.24E-02	1
MF	GO:0003730	mRNA 3'-UTR binding	3.90E-07	4
	GO:0003729	mRNA binding	1.54E-06	6
	GO:0032451	demethylase activity	3.35E-06	3
	GO:0016706	oxidoreductase activity	6.65E-06	3
	GO:0051213	dioxygenase activity	6.48E-05	3
	GO:0140098	catalytic activity, acting on RNA	9.53E-05	4
	GO:0008198	ferrous iron binding	1.26E-04	2
	GO:0048027	mRNA 5'-UTR binding	2.08E-04	2
	GO:0043022	ribosome binding	8.79E-04	2
	GO:0045182	translation regulator activity	9.17E-04	2

BP = biological process; CC = cellular component; MF = molecular function.

samples. The results presented that ALKBH1, EIF3, IGF2BP3, WTAP, and YTHDC1 genes were associated with OA, which could serve as valuable biomarkers. Among them, the AUC was 0.96 (95% confidence interval [CI], 0.8747-1.045, $P < 0.0001$) for ALKBH1, 0.9100 (95% CI, 0.7854-1.035, $P = 0.0005$) for EIF3, 1 (95% CI, 1.000-1.000, $P < 0.0001$) for IGF2BP3, 1 (95% CI, 1.000-1.000, $P < 0.0001$) for WTAP, and 1 (95% CI, 1.000-1.000, $P < 0.0001$) for YTHDC1, respectively, suggesting that five differential genes have diagnostic value in OA (Fig. 4).

Discussion

OA is a chronic degenerative osteoarthropathy, many studies have explored the pathogenesis of OA, but the mechanisms of different epigenetic alterations are not fully understood, so its etiology remains unclear. m^{6}A modification is a type of epigenetic modification, which is necessary for the biogenesis and function of RNA and is involved in the occurrence and development of many diseases. In this study, we used the gene expression profile data set in the

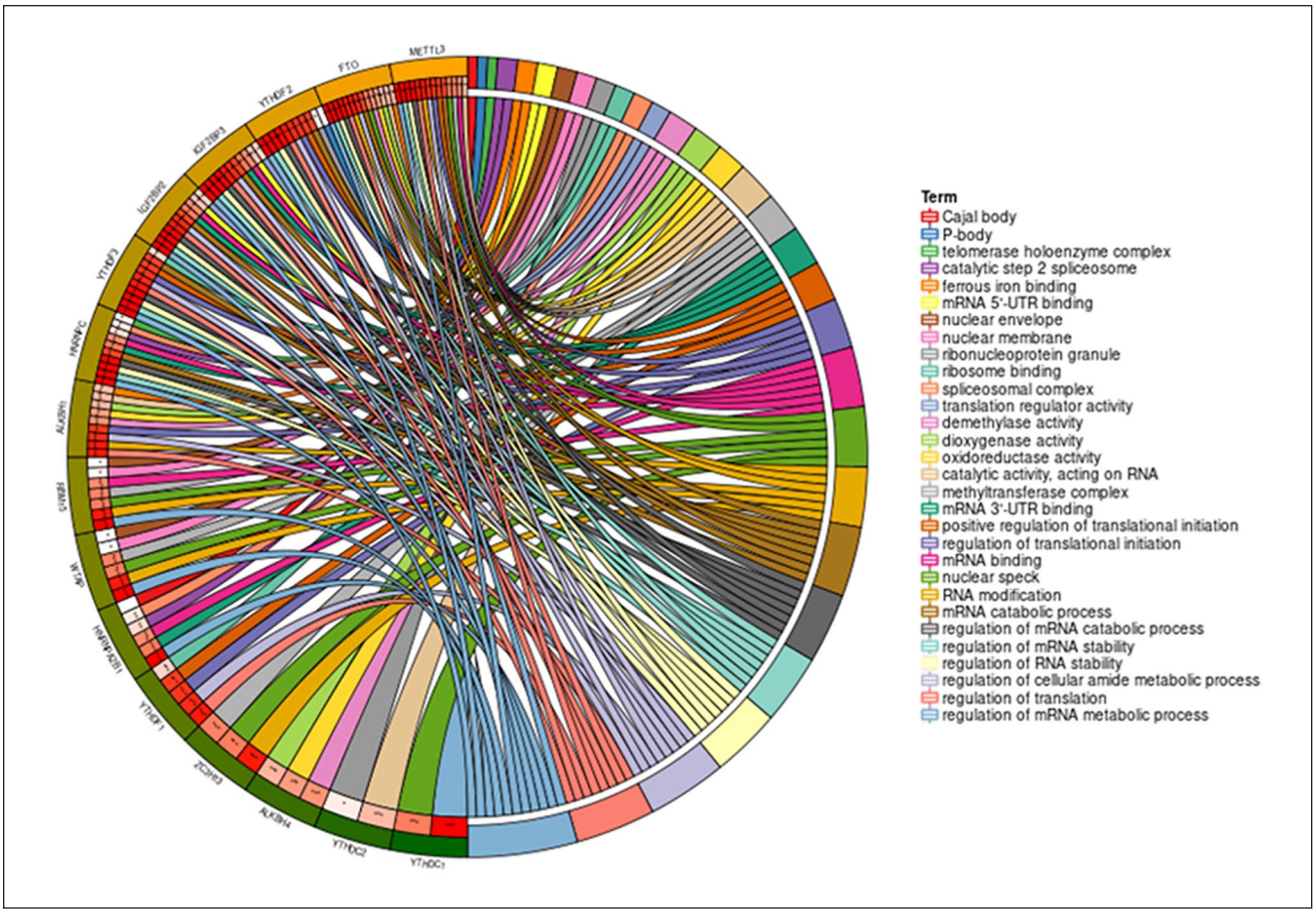

Figure 1. The top 10 GO terms of m^{6}A.

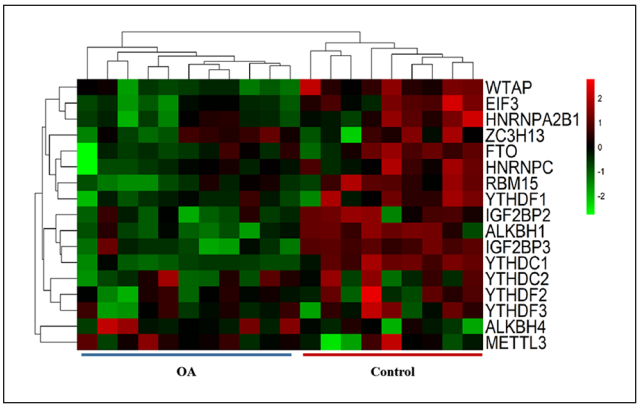

Figure 2. Heat maps of m^{6}A-related genes. The column of the heat map represents samples and the row represents m^{6}A-related genes. Red represents upregulated genes and green represents downregulated genes. OA = osteoarthritis.

GEO database to conduct bioinformatics analysis to identify the m^{6}A differential genes involved in the pathogenesis of OA. A total of 17 genes were analyzed, including 2

Table 3. The Relative Expression of m^{6}A-Related Genes.

Gene	logFC	*P* Value
ALKBH1	–0.540299322	5.53E-06*
ALKBH4	0.102425541	2.97E-01
EIF3	–0.357514354	1.38E-04*
FTO	–0.28622283	2.65E-02*
HNRNPA2B1	–0.488873319	7.83E-03*
HNRNPC	–0.630204106	1.78E-02*
IGF2BP2	0.628202099	5.41E-04*
IGF2BP3	–0.131735601	1.42E-06*
METTL3	0.191577743	1.60E-02*
RBM15	–0.561227978	5.93E-03*
WTAP	–0.8330836	5.18E-06*
YTHDC1	–0.850317454	5.16E-08*
YTHDC2	–0.040232578	5.95E-01
YTHDF1	–0.27786291	1.53E-02*
YTHDF2	–0.150316781	4.11E-01
YTHDF3	–0.075833352	6.87E-01
ZC3H13	–0.029087682	7.59E-01

*$P < 0.05$.

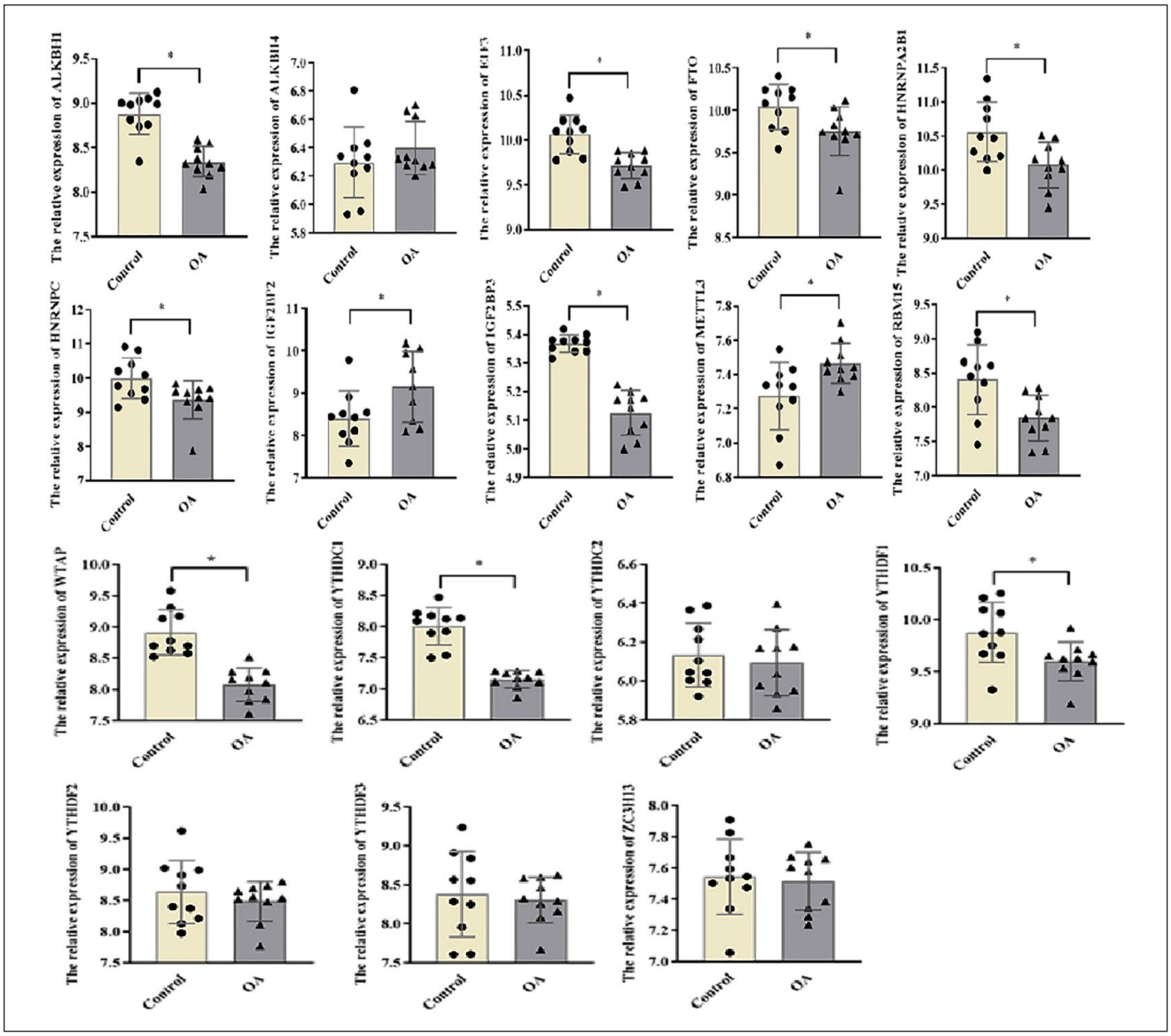

Figure 3. The expression of m^{6}A-related genes. OA = osteoarthritis.

upregulated and 10 downregulated genes. In the prognostic analysis, it was found that the gene expression levels of 5 genes are closely related to the prognosis and early diagnosis of OA. This should provide a comprehensive environment for OA, which helps to develop its diagnosis and treatment.

Functional enrichment analysis revealed that there is a strong relationship between m^{6}A-related genes. GO enrichment analysis revealed a number of biological processes and pathways related to the occurrence and development of OA, such as mRNA metabolic process regulation, nuclear speck and mRNA binding, all of which are also closely related to its function. We revealed that m^{6}A RNA methylation regulators are involved in many biological processes and signal transduction pathways, indicating their important role in the initiation and development of OA, which is consistent with previous research that m^{6}A modification is closely related to bone and joint diseases such as RA.[15]

We identified that 12 of the 17 genes were differentially expressed in OA, including 2 upregulated genes and 10 downregulated genes. Methyltransferase 3 (METTL3) is the key enzyme for m^{6}A methylation modification.[16] Studies have reported that the methylation mediated by METTL3 is tissue and cell specific.[16,17] METTL3 was highly expressed in osteoarthritis and can promote experimental osteoarthritis by regulating the inflammatory response and apoptosis of chondrocytes, and can attenuate lipopolysaccharide-induced inflammation in macrophages through NF-κB,[18,19] which

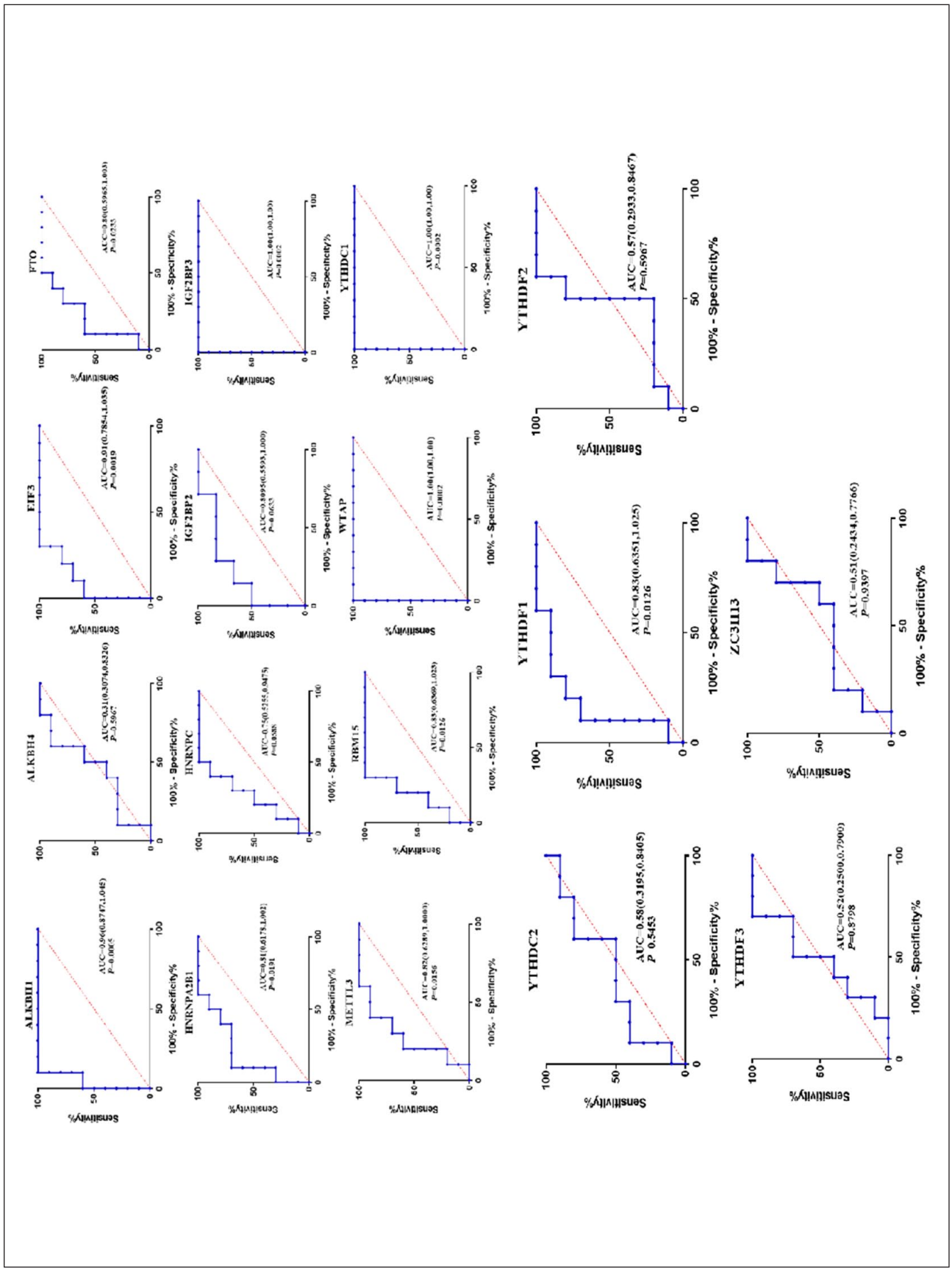

Figure 4. ROC curves of m^{6}A RNA methylation regulators for OA diagnosis. ROC = receiver operating characteristic; OA = osteoarthritis.

was consistent with our results. The major function of insulin-like growth factor 2 mRNA-binding protein 2 (IGF2BP2) is to regulate cell metabolism; IGF2BP2 was highly expressed in pancreatic cancer, and upregulation of IGF2BP2 was associated with poor prognosis of pancreatic cancer patients.[20] FTO (fat mass and obesity-related protein) is a well-known eraser enzyme that is involved in mediating methylation reversal and is related to inflammation. Relevant studies have shown that decreased expression of FTO in peripheral blood is a risk factor for RA, and the expression of FTO may be used as an indicator of activity and inflammation.[8,21] Heterogeneous nuclear ribonucleoprotein C (HNRNPC), a member of the nuclear protein family, has evidence that HNRNPC participates in the p53 regulatory network by interacting with long non-coding RNA (lncRNA).[22] Furthermore, we analyzed the correlations among these genes, and many positive correlations correlation were observed, such as HNRNPA2B1 and EIF3, EIF3 and WTAP, YTHDC1 and IGF2BP3, and so on. Studies found that m^6A-related genes were highly correlated in pancreatic cancer, cervical squamous cell carcinoma, and endometrial adenocarcinoma,[23,24] which is consistent with this study.

Furthermore, we analyzed the factors related to the early diagnosis and prognosis of OA. ROC analysis showed that the area under the ROC curve of ALKBH1, EIF3, IGF2BP3, WTAP, and YTHDC1 genes was more than 0.9, which were associated with the prognosis of OA. ALKBH1, a member of ALKB family, is a 2-ketoglutarate and Fe^{2+} dependent hydroxylase. ALKBH1 is indispensable for the osteogenic differentiation of human mesenchymal stem cells (MSCs) and indicates that DNA N6-mA modifications are a new mechanism for the epigenetic regulation of stem cell differentiation.[25] Other studies have shown that ALKBH1 interacts with the core transcriptional pluripotency network of embryonic stem cells and participates in the regulation of pluripotency and differentiation.[26]

Eukaryotic translation initiation factor 3 (EIF3) is a multi-subunit complex, which plays a key role in translation initiation. Recent studies have shown that EIF3b silencing can inhibit the growth of osteosarcoma cells and induce apoptosis.[27] IEIF3b can inhibit cell proliferation and migration and promotes apoptosis by downregulating Wnt signaling pathway in acute myeloid leukemia.[28] Studies have shown that the osteosarcoma cells with DNA methyltransferase inhibitors or histone deacetylase inhibitors treated could upregulate the expression of insulin-like growth factor2 mRNA-binding protein3 (IGF2BP3). In addition, IGF2BP3 activity can be influenced by mTORC, which is major downstream effectors of phosphoinositide 3 kinase (PI3K) and mitogen-activated protein kinase (MAPK) pathway.[29] YTHDC1, a N6-methyladenosine-binding protein, which is adjacent to nuclear speckles, regulated mRNA splicing by recruiting splicing factors to the targeted mRNA.[30]

Wilms tumor 1-associated protein (WTAP) is a putative splicing regulator. Study showed that Smad2/3 interacted with METTL3-METTL14-WTAP, and Smad2/3 could act as a hub for coordinating proteins that play roles in mRNA modification, apoptosis, DNA repair, and transcriptional regulation.[31]

In conclusion, this study first demonstrates the critical role of ALKBH1, EIF3, IGF2BP3, WTAP, and YTHDC1 in OA which provides novel insights into recognizing the pathogenesis of OA and a promising biomarker for OA. However, little is known about the function and molecular mechanism of these key m^6A methylation genes in OA. Therefore, functional experiments are needed to further explore its potential role and mechanism.

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Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

Ethical Approval

GEO belongs to public databases, and the patients involved in the database have obtained ethical approval. User can download relevant data for free for research and public relevant articles. Our study is based on open source data, so there are no ethical and other conflict.

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