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Fat Mass and Obesity-Associated Protein-Mediated Adipogenic Differentiation of Bone Marrow Mesenchymal Stem Cells Promotes Chemotherapy Resistance in Acute Myeloid Leukemia

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Background: Chemotherapy resistance and relapse in acute myeloid leukemia (AML) are associated with poor prognosis. Bone marrow mesenchymal stem cells (MSCs) show enhanced adipogenic differentiation in AML, which may contribute to chemoresistance; however, the underlying mechanisms remain incompletely understood.

Aims: To investigate how adipogenic differentiation of MSCs from chemotherapy-resistant AML patients (CR-AML-MSCs) promotes chemoresistance, focusing on the roles of the fat mass and obesity-associated protein (FTO) and the mTORC1 pathway.

Study Design: Experimental study.

Methods: This study compared bone marrow MSCs from chemotherapy-sensitive (CS) and CR AML patients. Adipogenic differentiation was assessed using Oil Red O staining. RNA sequencing, Quantitative real-time PCR (qPCR), and western blotting were employed to analyze expression of FTO, Raptor, and PPARγ. Global N6-methyladenosin (m6A) levels were measured, and co-culture models with AML cell lines (U937, HL-60, THP-1) were established to evaluate chemoresistance. Gain- and loss-of-function experiments for FTO were performed using lentiviral overexpression and shRNA knockdown. mTORC1 pathway involvement was tested using rapamycin. Statistical analyses included t-tests and ANOVA, with significance set at ($p < 0.05$). MSCs from CS and resistant AML patients were compared. Adipogenic differentiation was induced and assessed by Oil Red O staining. RNA sequencing, qPCR, and western blotting were used to analyze the expression of FTO, Raptor, and PPARγ. Global m^6A levels were measured, and co-culture models with AML cell lines (U937, HL-60, THP-1) were established to evaluate chemoresistance. Gain- and loss-of-function experiments for FTO were performed.

Results: CR-AML-MSCs exhibited significantly enhanced adipogenic differentiation ($p < 0.01$) and elevated PPARγ expression. Co-culture with adipocyte-differentiated CR-AML-MSCs markedly increased resistance to daunorubicin and cytarabine in AML cells ($p < 0.01$). RNA-Seq analysis revealed enrichment of the mTORC1 signaling pathway and upregulation of raptor. Inhibition of mTORC1 with rapamycin suppressed adipogenic differentiation. Total m^6A levels were reduced in CR-AML-MSCs, whereas FTO expression was increased. Overexpression of FTO further promoted adipogenesis, upregulated raptor and PPARγ, and enhanced chemoresistance, whereas FTO knockdown attenuated these effects.

Conclusion: FTO enhances adipogenic differentiation of CR-AML-MSCs through m^6A demethylation of raptor, leading to mTORC1 pathway activation and subsequent chemoresistance. These findings reveal a novel mechanism underlying AML chemoresistance and suggest potential therapeutic targets.

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INTRODUCTION

Acute myeloid leukemia (AML) is the most common hematological malignancy in adults.[1] Chemotherapy resistance and disease relapse frequently lead to poor treatment outcomes and reduced long-term survival.[2] Identifying key molecules involved in chemotherapy resistance, elucidating the underlying mechanisms, enhancing therapeutic efficacy, and improving prognostic prediction and management remain urgent challenges in AML treatment.

The bone marrow microenvironment is a dynamic and complex system.[3] Reciprocal interactions between AML cells and this microenvironment contributes to chemotherapy resistance[4], but the underlying mechanisms remain incompletely understood. Mesenchymal stem cells (MSCs), a major cellular component of the bone marrow microenvironment, have multilineage differentiation potential. The balance between adipogenic and osteogenic differentiation is tightly regulated to maintain bone marrow homeostasis.[5] Evidence indicates that MSCs can support the proliferation and survival of leukemia cells and increase their resistance to chemotherapeutic agents.[6] Furthermore, multiple studies have shown that bone marrow MSCs derived from AML patients (AML-MSCs) display growth deficiencies and impaired osteogenic potential, along with a markedly enhanced capacity for adipogenic differentiation.[7] These AML-MSCs promote leukemogenesis and disease progression by favoring adipogenic differentiation.[8] AML cells exploit the adipocyte-rich microenvironment to promote their proliferation and evade chemotherapy-induced cell death.[9] Our previous work showed that, compared with healthy controls, AML-MSCs generally display elevated adipogenic potential, which enhances chemotherapy resistance in AML cells.[10,11] However, substantial interindividual variability exists in the adipogenic differentiation capacity of AML-MSCs among patients. The differentiation fate of MSCs is influenced by multiple signaling pathways, including PI3K/AKT, mTOR, Notch, and Hedgehog.[12] This raises a critical question: what key factors drive the heterogeneity in adipogenic differentiation among bone marrow MSCs from different AML patients? To address this, we performed RNA sequencing and bioinformatics analyses on these cells.

Fat mass and obesity-associated protein (FTO) is closely linked to human weight gain and obesity.[13] Animal model studies have shown that FTO deficiency prevents obesity and causes growth retardation[14], whereas FTO overexpression increases food intake and induces obesity.[15] In humans, loss-of-function mutations in FTO can also result in severe growth retardation and multiple malformations, leading to premature death.[16] Identified as the first identified N6-methyladenosine (m^6A) demethylase regulating target mRNA[17], FTO participates in the splicing of adipogenic regulatory factors and thus plays a key role in adipogenesis.[18] However, no studies have yet reported how FTO-mediated m^6A modification regulates the adipogenic differentiation of bone marrow MSCs, alters the bone marrow microenvironment, and affects the chemosensitivity of AML cells.

To further investigate the differences in adipogenic differentiation of MSCs from chemotherapy-resistant AML patients (CR-AML-MSCs), this study used RNA-Seq combined with bioinformatics analysis to explore the underlying molecular mechanisms, which were subsequently validated through *in vitro* experiments. Based on these findings, we discuss the role of FTO as an m^6A demethylase in mediating the mTORC1 signaling pathway to regulate adipogenic differentiation of bone marrow CR-AML-MSCs. These insights provide a novel perspective for the diagnosis and treatment of AML.

MATERIALS AND METHODS

Bone marrow MSCs

Clinical bone marrow specimens were obtained from Department of Hematology, Union Hospital, Fujian Medical University. All patients were diagnosed with non-APL type AML, and diagnostic classification followed the 5th Edition of the World Health Organization Classification of Tumors of Haematopoietic and Lymphoid Tissues: Myeloid Neoplasms and Acute Leukemia.[19] This study adhered to the regulations of the Declaration of Helsinki (2013) regarding human experiments and was approved by the Ethics Review Committee of Fujian Medical University (approval number: 2024-121, date: 06.03.2024). Privacy rights were protected, and informed consent was obtained for all participants. A total of 20 bone marrow samples were collected, comprising 10 from CR-AML cases and 10 chemotherapy-sensitive (CS)-AML cases. Bone marrow mononuclear cells were isolated within 2 hours of collection using Ficoll-Paque density gradient centrifugation (TBDscience). The cells were cultured in Dulbecco's Modified Eagle medium (DMEM; low glucose; Gibco) supplemented with 20% fetal bovine serum (FBS; Gibco) and 1% penicillin/streptomycin (Gibco) at 37 °C in a 5% CO_2 atmosphere. The medium was replaced every 3 days to remove non-adherent cells. Once 80-90% confluent, adherent cells were passaged at a 1:2 ratio using 0.25% trypsin-EDTA (Gibco). MSCs at passages 3-5 were used for experiments. Identification was performed by flow cytometry using the following antibodies: anti-CD73-PE (17-0739-42, eBioscience), anti-CD90-PE (14-0909-82, eBioscience), anti-CD105-PE (17-1057-42, eBioscience), anti-CD34-PE (14-0341-82, eBioscience), and anti-CD45-PE (14-0451-82, eBioscience). Cells positive for CD73, CD90, and CD105 (> 95%) and negative for CD34 and CD45 (< 5%) were characterized as MSCs. For adipogenic differentiation, MSCs were induced with a commercial adipogenic induction medium (Cyagen Biosciences) for 14 days, with medium refreshed every 3 days. Differentiation was confirmed by Oil Red O staining (Solarbio) of lipid droplets. Osteogenic differentiation was induced using osteogenic induction medium (Cyagen Biosciences) for 21 days and verified by Alizarin Red S staining. Clinical information of AML patients is provided in Supplementary Table 1.

Main reagents

DMEM medium (low glucose), FBS, and penicillin-streptomycin were purchased from Gibco. TRIzol reagent, cDNA Synthesis Kit, and Lipofectamine 3000 were purchased from Invitrogen. Ficoll peripheral blood lymphocyte separation solution was obtained from TBD Company. The adipogenic induction differentiation kit for adult bone marrow MSCs was purchased from Cyagen Biosciences. The Oil Red O staining kit was purchased from Solarbio. The EpiQuik m^6A RNA methylation quantification kit was purchased from EpigenTek.

Rapamycin was purchased from MCE. SYBR Green PCR Master Mix was purchased from Roche. The SDS-PAGE gel preparation kit was obtained from Beijing Dingguo. Monoclonal antibodies against FTO, raptor, and PPARγ were purchased from Cell Signaling Technology. Monoclonal antibodies against GAPDH and β-actin were purchased from Beyotime Institute of Biotechnology. Horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG were purchased from Shanghai Beyotime. The BeyoECL Star kit was purchased from Beyotime. Lentiviral plasmids and packaging plasmids were preserved in the laboratory. All other biochemical reagents were analytical grade, either imported or domestic. All primers and recombinant plasmids were designed and synthesized by Sangon Biotech (Shanghai) Co., Ltd.

Main methods

Quantitative real-time PCR (qPCR): Total RNA was extracted using TRIzol reagent, and cDNA was synthesized according to the instructions of the cDNA Synthesis Kit. Gene expression levels were measured using SYBR Green PCR Master Mix on an ABI 7500 real-time qPCR system. GAPDH served as an internal reference, and relative mRNA expression was calculated using the $2^{-\Delta\Delta Ct}$ method. Each assay was performed in technical triplicates for each biological replicate. The primer sequences used for qPCR are provided in Supplementary Table 2.

Western blotting (WB): Proteins were analyzed by WB using the following primary antibodies: rabbit anti-FTO monoclonal antibody (1:1000, ab126605, Abcam), rabbit anti-raptor monoclonal antibody (1:1000, ab40768, Abcam), rabbit anti-PPARγ monoclonal antibody (1:1000, ab178860, Abcam), mouse β-actin monoclonal antibody (1:1000, 4967, CST), and mouse anti-GAPDH monoclonal antibody (1:1000, 2118, CST). Secondary antibodies included horseradish peroxidase-conjugated goat anti-mouse or anti-rabbit IgG (1:5000, IH-0031/IH-0011, Dingguo). Chemiluminescence signals were detected using the BeyoECL Star kit and imaged on a ChemiDoc Touch system (Bio-Rad). Band intensities were quantified using ImageJ software. GAPDH or β-actin served as loading controls. Each assay was performed in technical triplicates for each biological replicate.

Construction of recombinant plasmids, generation of lentiviral particles, and infection: The full-length CDS of the human *FTO* gene (NM_001080432.3) was cloned into the pLJM1-EGFP mammalian expression vector. Short hairpin RNA (shRNA) oligonucleotides targeting specific regions of human FTO mRNA were designed and synthesized by Sangon Biotech. The sequences were as follows:

- shRNA-FTO-F: 5'-CCGGCGGTTCACAACCTCGGTTTAGCTCGAGCTAAACCGAGGTTGTGAACCGTTTTTG-3'
- shRNA-FTO-R: 5'-AATTCAAAAACGGTTCACAACCTCGGTTTAGCTCGAGCTAAACCGAGGTTGTGAACCG-3'
- shRNA-NC-F: 5'-CGGGCCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGGTTTTTG-3'
- shRNA-NC-R: 5'-AATTCAAAAACCTAAGGTTAAGTCGCCCTCGCTCGAGCGAGGGCGACTTAACCTTAGG-3'

The recombinant vectors were co-transfected with the viral packaging plasmids psPAX2 and PMD2.G into 293T cells to produce lentiviral particles. These viral particles were subsequently used to infect AML-MSCs, and infection efficiency was verified by WB and qPCR.

Co-cultivation and chemoresistance assays: Differentiated adipocytes were washed twice with standard growth medium and maintained in the same medium. To assess the paracrine effect of adipogenically differentiated MSCs on AML chemoresistance, AML cell lines (U937, HL-60, and THP-1; 5.0×10^5 cells/well; 6-well plates) were cultured in conditioned medium collected from adipocyte cultures. For direct co-culture, AML cells were seeded into transwell inserts (0.4 μm pore size; Corning) placed above adherent differentiated adipocytes, allowing exchange of soluble factors without direct cell–cell contact. After 24 hours of co-culture or conditioned medium exposure, chemotherapy sensitivity of AML cells was assessed using the Cell Counting Kit-8 (CCK-8) assay as previously described.[10] Briefly, AML cells were seeded into 96-well plates and treated with daunorubicin (100 ng/mL), cytarabine (10 μM), or PBS (vehicle control). The cell growth inhibition rate (%) was calculated using the following formula: $[1-(OD_{\text{experimental group}}-OD_{\text{blank control}})]/(OD_{\text{control group}}-OD_{\text{blank control}}) \times 100\%$. Each assay was performed in technical triplicates per biological replicate.

RNA m^{6}A quantitation: Total RNA was isolated using TRIzol according to the manufacturer's instructions. RNA quality was assessed using a NanoDrop spectrophotometer. m^{6}A quantification in total RNA was performed using the EpiQuik m^{6}A RNA Methylation Quantification Kit. Briefly, 200 ng of RNA was coated onto each well, followed by sequential addition of capture and detection antibodies at appropriate concentrations, as instructed by the manufacturer. m^{6}A levels were determined by measuring absorbance at 450 nm to generate a standard curve. Each assay was performed in technical triplicates for each biological replicate.

RNA sequencing and bioinformatics analysis: Total RNA was extracted from MSCs using TRIzol reagent (Invitrogen), and RNA integrity was confirmed with an Agilent 2100 Bioanalyzer (RIN > 8.0). Libraries were prepared and sequenced on an Illumina NovaSeq 6000 platform to generate 150-bp paired-end reads. Raw reads were assessed with FastQC and processed with Trimmomatic to remove adapters and low-quality bases. Clean reads were aligned to the human reference genome (GRCh38) using STAR aligner, and gene expression levels were quantified with featureCounts. Differential expression analysis was performed using the DESeq2 package in R, with significantly differentially expressed genes (DEGs) defined as $|\log_2\text{FoldChange}|>1$ and adjusted $p < 0.05$ (FDR, Benjamini-Hochberg correction). Functional enrichment analyses of Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways and Gene Ontology terms were conducted using the clusterProfiler package.

Statistical analysis

All experiments were conducted with at least three independent biological replicates (i.e., MSCs from different patients or independent lentiviral transductions), with each assay measured in technical triplicates unless otherwise specified. Data are presented

as means ± standard deviation of three independent experiments. Statistical analyses were performed using GraphPad Prism version 8.0. Comparisons between two groups were evaluated using Student's t-tests, while comparisons among three or more groups were assessed using one-way ANOVA followed by Tukey's post hoc multiple comparisons test. A p -value < 0.05 was considered statistically significant. Because of relatively small sample sizes in certain subgroups, multiple comparison corrections (e.g., Bonferroni or FDR) were not applied; therefore, the results should be interpreted with caution, and validation in larger cohorts is recommended.

RESULTS

Adipogenic differentiation of CR-AML-MSCs is significantly enhanced and promotes AML Chemoresistance

During AML clinical chemotherapy, significant differences were observed in the adipogenic differentiation ability of bone marrow CS-AML-MSCs and CR-AML-MSCs. Compared with CS-AML-MSCs, the adipogenic differentiation ability of CR-AML-MSCs was significantly enhanced (Figures 1a and 1b; fold change = 4.33, $p < 0.01$), with significantly increased mRNA expression (Figure 1c; fold change = 17.67, $p < 0.05$) and protein levels of the adipogenic marker PPARγ (Figure 1d; fold change = 3.02, $p < 0.05$). Chemotherapy resistance of AML cells co-cultured with adipogenically differentiated CR-AML-MSCs was also significantly enhanced (Figure 1e). This acquired resistance occurred both in direct co-culture and when AML

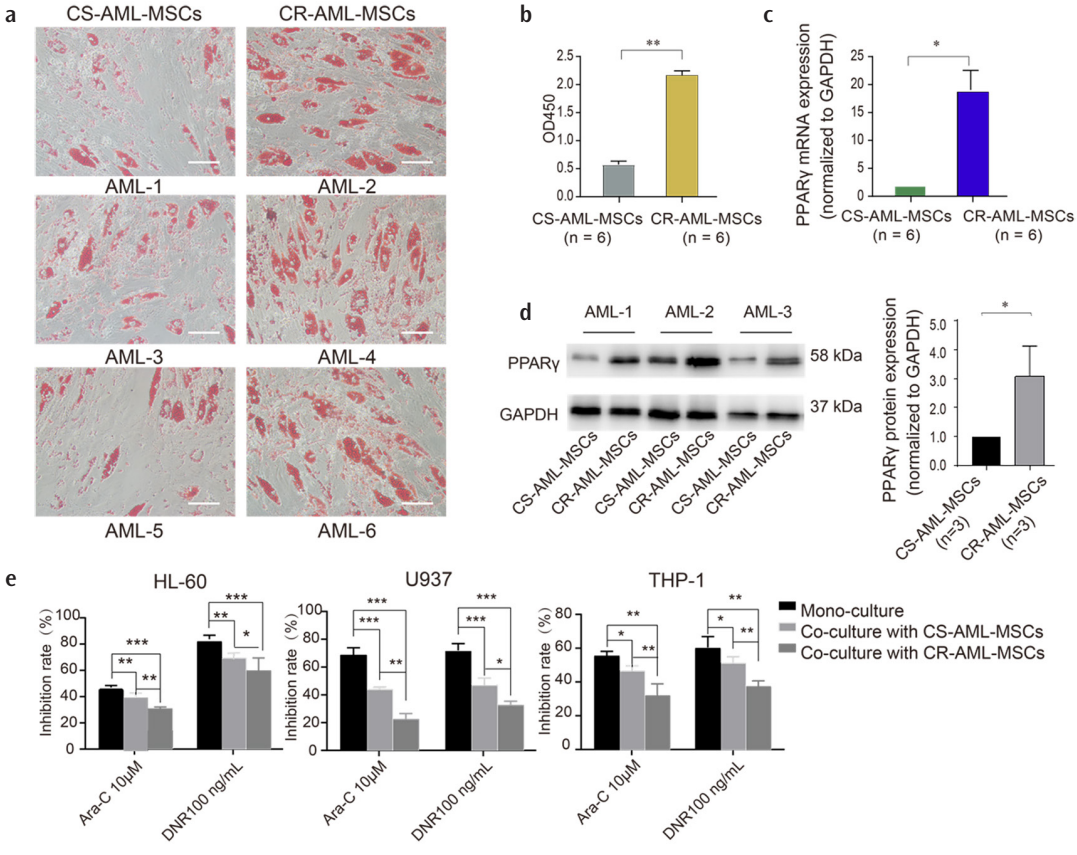

FIG. 1. Differences in adipogenic differentiation and changes in chemotherapy sensitivity of CS/CR-AML-MSC. Microscopic observation of CS/CR-AML-MSCs after adipogenic differentiation and Oil Red O staining; (b) Absorbance value at 450 nm detected by microplate reader after Oil Red O staining and isopropanol lipid dissolution treatment of CS/CR-AML-MSCs after adipogenic differentiation; (c) qPCR detection of the expression level of *PPARγ* mRNA in CS/CR-AML-MSCs; (d) WB detection of the expression level of PPARγ protein in CS/CR-AML-MSCs; (e) Co-culture of adipogenic differentiated CS/CR-AML-MSCs with AML cells (HL-60/U937/THP-1) for 48 h, separation of AML cells, and detection of changes in chemotherapy sensitivity of AML cells; CS-AML-MSCs: Bone marrow MSCs from chemotherapy-sensitive AML patients; CR-AML-MSCs: Bone marrow MSCs from chemotherapy-resistant AML patients; OD450: Absorbance (OD) value at 450 nm detected by microplate reader after isopropanol lipid dissolution treatment of MSCs after adipogenic differentiation, indicating the degree of adipogenic differentiation (the same below); Monoculture: AML cells cultured alone in suspension; co-culture with CS-AML-MSCs: AML cells co-cultured with CS-AML-MSCs; co-culture with CR-AML-MSCs: AML cells co-cultured with CR-AML-MSCs; Ara-C, cytarabine; DNR, daunorubicin; $*p < 0.05$, $**p < 0.01$, $***p < 0.001$, NS, no significant difference (the same below). WB, western blotting.

cells were exposed to conditioned medium from adipogenically differentiated CR-AML-MSCs, indicating that the chemoprotective effect is mediated primarily by soluble factors secreted by adipocytes rather than by intrinsic properties of the AML cells. However, no significant differences were found between the two groups in terms of cell morphology, surface marker antigens, proliferation activity, or osteogenic differentiation ability. These results indicate that enhanced adipogenic differentiation of MSCs in the bone marrow microenvironment of CR-AML promotes chemotherapy resistance.

The mTORC1 signaling pathway is a key regulator of adipogenic differentiation in CR-AML MSCs

To investigate the molecular mechanism underlying adipogenic differentiation of bone marrow CR-AML-MSCs, we performed RNA-Seq on three independent samples each of CS-AML-MSCs and CR-AML-MSCs. DEGs were identified using DESeq2 with a threshold of $|\log_2 FC| > 1$ and FDR-adjusted $p < 0.05$. The results were as follows: the heat map revealed significant differences in gene expression between the two groups (Figure 2a). Compared with CS-AML-MSCs, volcano plot analysis showed that CR-AML-MSCs had 265 upregulated genes and 387 downregulated genes (Figure 2b), among which adipogenic-related genes such as *PPARγ* were significantly upregulated (Figure 2c), consistent with the *in vitro* adipogenic differentiation results. KEGG analysis indicated that the DEGs were enriched in the mTORC1 signaling pathway (Figure 2d). Meanwhile, GSEA analysis indicated that DEGs were enriched in the mTORC1 signaling pathway, with higher expression in CR-AML-MSCs than in CS-AML-MSCs (Figure 2e). To further clarify the role of the mTORC1 signaling pathway, we measured the expression of raptor, a key component of mTORC1, in clinical specimens. The results showed that raptor expression was significantly higher in CR-AML-MSCs (Figure 2f). Inhibition of the mTORC1 signaling pathway with rapamycin significantly suppressed the adipogenic differentiation of MSCs (Figures 2g and 2h). These findings suggest that the mTORC1 signaling pathway plays a key role in the adipogenic differentiation of bone marrow MSCs from CR-AML.

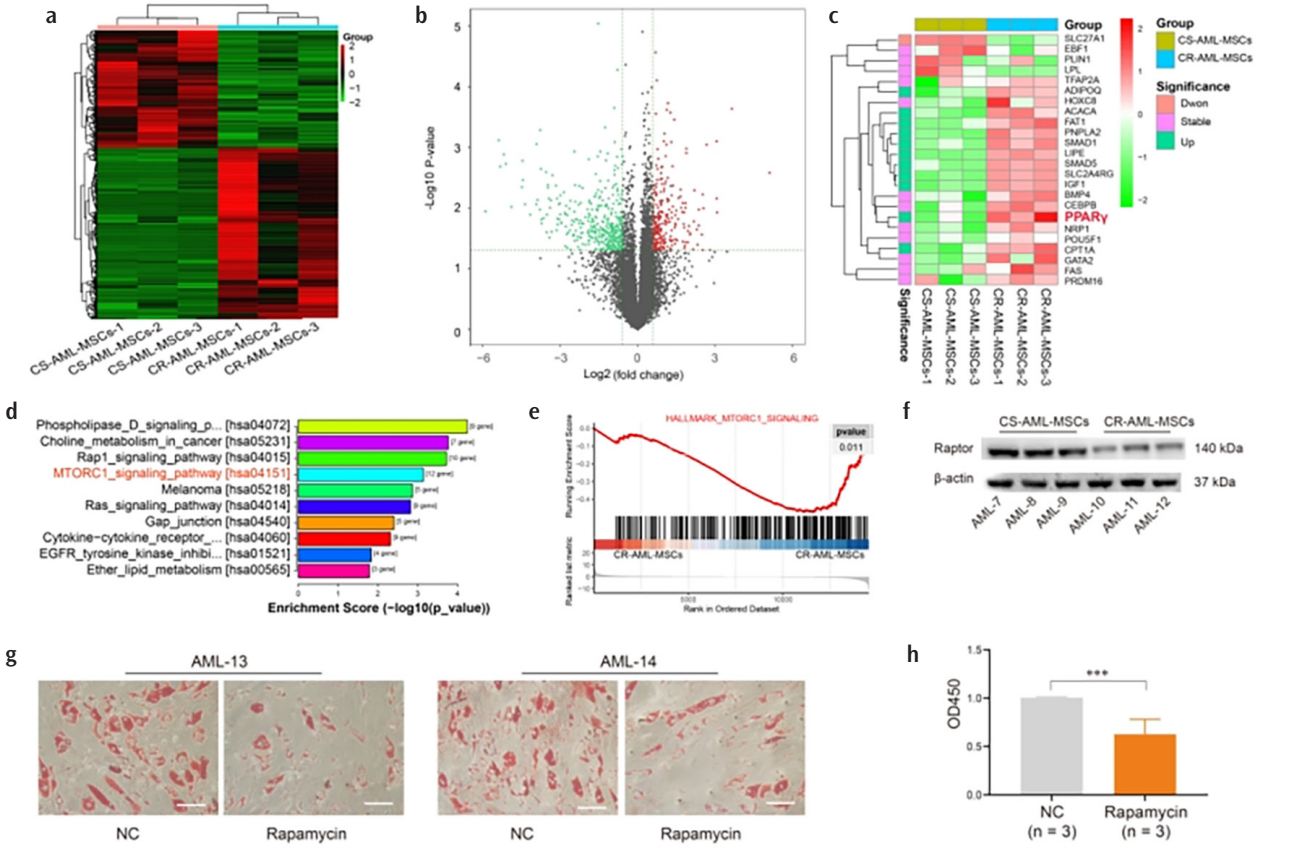

FIG. 2. RNA sequencing analysis of CS/CR-AML-MSCs. (a) Heatmap of differentially expressed mRNAs in CS/CR-AML-MSCs; (b) Volcano plot showing differentially expressed mRNAs in CS/CR-AML-MSCs; (c) Heatmap of differentially expressed mRNAs related to adipogenesis in CS/CR-AML-MSCs; (d) KEGG analysis of differentially expressed genes in CS/CR-AML-MSCs; (e) GSEA analysis of mTORC1 signaling pathway in CS/CR-AML-MSCs. (f) WB detection of raptor protein expression in bone marrow MSCs from 6 AML patients with chemotherapy sensitivity and resistance. (g) Oil Red O staining of AML-MSCs treated with Rapamycin and induced for adipogenic differentiation, observed under a microscope. (h) Oil Red O staining of AML-MSCs treated with Rapamycin and induced for adipogenic differentiation, followed by isopropanol treatment at room temperature to dissolve lipids, and absorbance at 450 nm was measured by a microplate reader.

Upregulation of the m⁶A demethylase FTO in CR-AML-MSCs

Based on previous research, m⁶A epigenetic modification and its key enzymes play important roles in stem cell differentiation. Our experimental results confirmed that the total m⁶A level in bone marrow CR-AML-MSCs was lower than in CS-AML-MSCs (Figure 3a; fold change = 0.663, $p < 0.001$). Analysis of m⁶A-related enzymes (methyltransferases METTL3, METTL14, WTAP; demethylases FTO, ALKBH5) revealed that FTO expression was significantly increased in CR-AML-MSCs (Figure 3b). Additionally, compared with CS-AML-MSCs, the adipogenic differentiation ability of CR-AML-MSCs was notably enhanced (Figures 3c and 3d; fold change = 4.681, $p < 0.01$), along with significantly increased FTO protein expression (Figure 3e, fold change = 3.535, $p < 0.05$). These findings suggest that the enhanced adipogenic differentiation of CR-AML-MSCs may be closely associated with increased FTO expression.

FTO enhances differentiation of AML-MSCs

To investigate the regulatory effect of FTO on the adipogenic differentiation of AML-MSCs, we assessed adipogenic differentiation following FTO overexpression or knockdown and measured the expression of related genes. Overexpression of FTO in AML-MSCs significantly promoted adipogenic differentiation (Figures 4a-c), whereas knockdown produced the opposite effect (Figures 4d-f). These results indicate that modulation of FTO expression correlates with changes in the adipogenic differentiation ability of AML-MSCs and that the microenvironment created by FTO-overexpressing MSCs is associated with a significant increase in chemotherapy resistance of AML cells in co-culture (Figure 4g). Moreover, FTO overexpression enhanced the expression of mTORC1 (raptor) and PPARγ protein (Figure 4b), while knockdown led to the opposite effects (Figure 4e). Collectively, these findings suggest that the m⁶A demethylase FTO may promote adipogenic differentiation of AML-MSCs through regulation of mTORC1 and PPARγ.

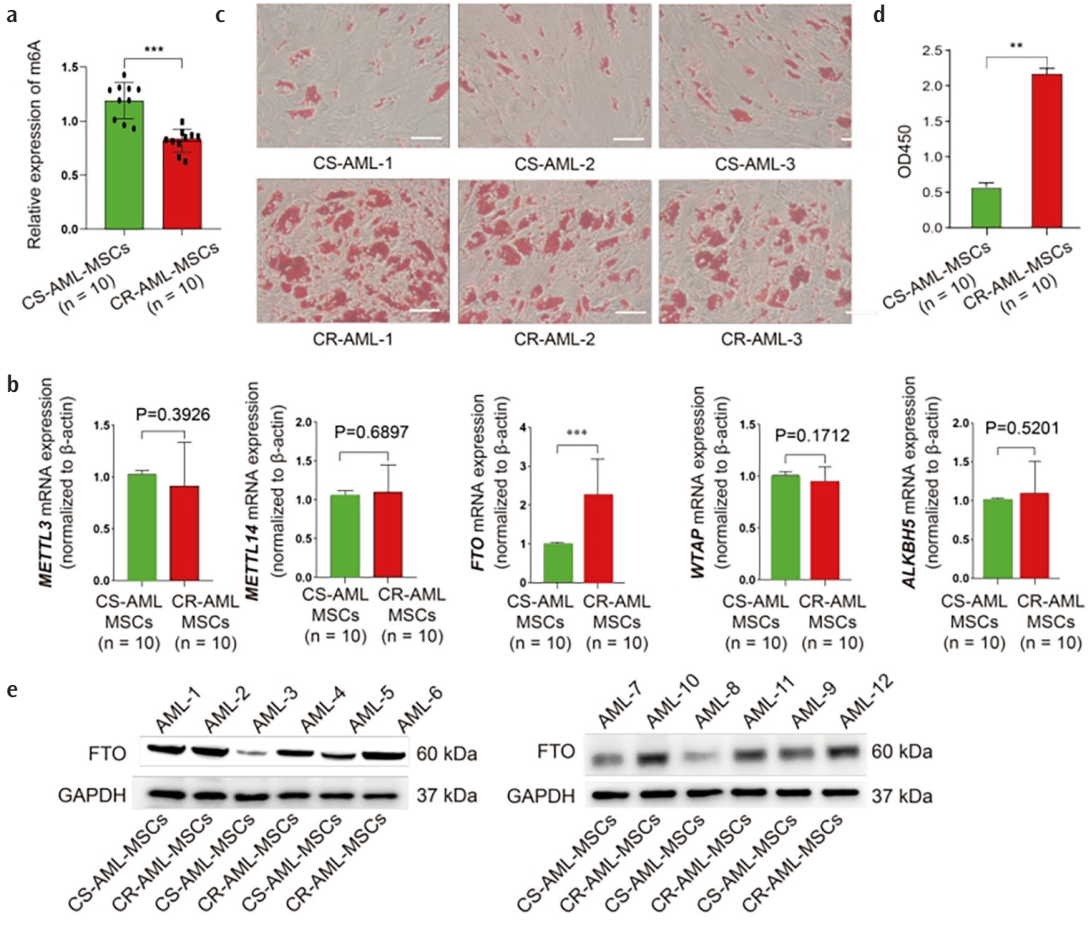

FIG. 3. Detection of FTO expression and adipogenic differentiation level in CS/CR-AML-MSCs. (a) EpiQuik m⁶A RNA methylation quantification of total RNA in CS/CR-AML-MSCs; (b) qPCR detection of mRNA expression of *METTL3*, *METTL14*, *WTAP*, *FTO*, and *ALKBH5* in CS/CR-AML-MSCs; (c) Differences in adipogenic differentiation ability of CS/CR-AML-MSCs; (d) The results of Oil Red O staining and absorbance at 450 nm after isopropanol treatment at room temperature. (e) WB detection of FTO protein expression in CS/CR-AML-MSCs. WB, western blotting; FTO, fat mass and obesity-associated protein.

DISCUSSION

AML, the most common hematological malignancy in adults, is characterized by poor chemotherapy efficacy and high relapse rates due to drug resistance and disease recurrence.[20] Overcoming these challenges remains a critical clinical priority. This study examined the relationship between adipogenic differentiation of bone marrow CR-AML-MSCs and further elucidated the underlying molecular mechanisms. The findings provide insights of both theoretical and clinical significance.

We found that AML patients with enhanced MSC adipogenic differentiation exhibited significantly higher chemotherapy resistance. These results highlight the pivotal role of the bone marrow microenvironment, particularly adipogenically differentiated MSCs, in driving chemoresistance in AML. Notably, previously CS-AML cells acquired resistance after exposure to factors secreted by adipocytes derived from CR-AML-MSCs. This paracrine mechanism-rather than the selection of intrinsically resistant AML clones-underscores the critical role of the microenvironment in treatment failure. These findings align with emerging evidence that adipocytes within the leukemic niche secrete protective factors (e.g., fatty acids, cytokines) that attenuate the cytotoxic effects of chemotherapy on AML cells.[21] Collectively, our results establish a strong link between the adipogenic differentiation potential of MSCs and chemotherapy resistance in AML, providing important mechanistic insights into disease progression and therapeutic failure.

Further investigation revealed the critical involvement of the mTORC1 signaling pathway in the adipogenic differentiation of CR-AML-MSCs. RNA sequencing and bioinformatics analysis demonstrated that DEGs between CS-AML-MSCs and CR-AML-MSCs were enriched in the mTORC1 signaling pathway, as shown by both KEGG and GSEA.[22] Moreover, mTORC1 signaling was significantly activated in CR-AML-MSCs, with expression of raptor-a key component of the mTORC1 complex-markedly upregulated compared with that in CS-AML-MSCs. Treatment with the mTORC1 inhibitor rapamycin significantly suppressed adipogenic differentiation in CR-AML-

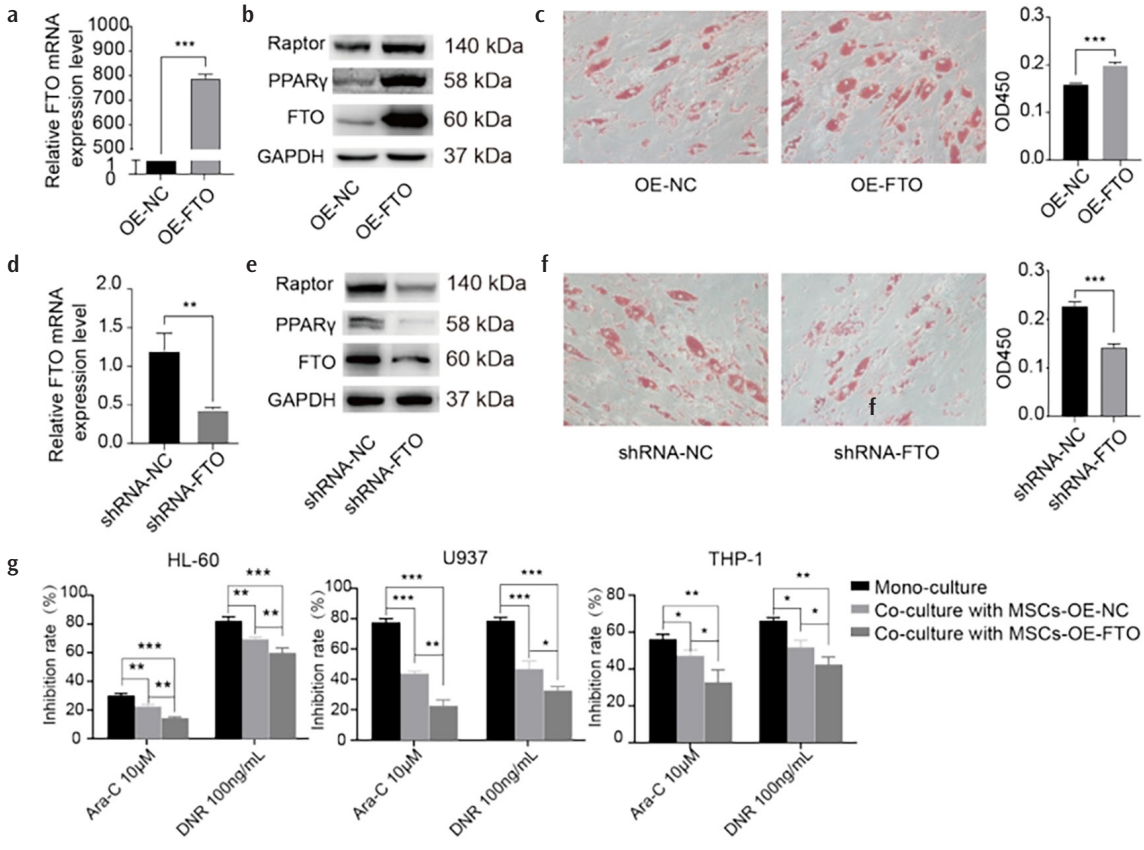

FIG. 4. Detection of the effect of FTO on adipogenic differentiation of MSCs and AML chemosensitivity. (a) qPCR detection of *FTO* mRNA expression level in MSCs; (b) WB detection of FTO, Raptor and PPARγ protein expression levels in MSCs; (c) Oil Red O staining results and absorbance value at 450 nm after dissolving fat with isopropanol at room temperature after FTO overexpression-induced adipogenic differentiation; (d) qPCR detection of *FTO* mRNA expression level in MSCs; (e) WB detection of FTO, Raptor and PPARγ protein expression levels in MSCs; (f) Oil Red O staining results and absorbance value at 450 nm after dissolving lipids with isopropanol at room temperature after FTO knockdown-induced adipogenic differentiation; (g) Chemosensitivity test results of AML cells (HL-60/U937/THP-1) after co-culture with MSCs overexpressing FTO for 48h; OE-NC, overexpression empty vector control group; OE-FTO, overexpression FTO; shRNA-NC, knockdown empty vector control group; shRNA-FTO, knockdown FTO; WB, western blotting; FTO, fat mass and obesity-associated protein.

MSCs.[23] Collectively, these findings suggest that the mTORC1 pathway plays a pivotal role in regulating adipogenic differentiation of bone marrow CR-AML-MSCs and may represent a promising molecular target for elucidating AML chemoresistance mechanisms.

When examining the factors underlying the alteration of the mTORC1 signaling pathway, we found that expression of the m^{6}A demethylase FTO in bone marrow MSCs from CR-AML patients was significantly increased, thereby regulating adipogenic differentiation. Compared with CS-AML-MSCs, CR-AML-MSCs exhibited lower m^{6}A levels in total RNA, while the mRNA and protein expression levels of FTO were significantly elevated. As a protein closely associated with human weight gain and obesity, FTO plays a key role in adipogenesis and has important functions in various tumors. To our knowledge, this study is the first to demonstrate that FTO-mediated m^{6}A modification regulates the adipogenic differentiation of bone marrow MSCs, alters the bone marrow microenvironment, and consequently affects the chemotherapy sensitivity of AML cells. Manipulating FTO expression confirmed that changes in its levels were correlated with the adipogenic differentiation ability of human bone marrow MSCs, and overexpression of FTO in MSCs was associated with a marked increase in the chemotherapy resistance of AML cells in the co-culture system. At the same time, increased expression of raptor, a key component of mTORC1, and a significant elevation of PPARγ protein were observed. On this basis, we speculated that high FTO expression in bone marrow CR-AML-MSCs modifies raptor through m^{6}A demethylation, activates the mTORC1 signaling pathway, and enhances the adipogenic differentiation of MSCs, thereby contributing to chemotherapy resistance in AML cells. These findings provide a new theoretical foundation and potential targets for the clinical prediction, diagnosis, and treatment of AML chemotherapy resistance. Furthermore, our findings align with recent studies highlighting the oncogenic role of FTO in AML. For example, Zhang et al.[24] demonstrated that FTO mediates chemotherapy resistance and relapse in AML through m^{6}A demethylation of key transcripts, reinforcing its importance in AML pathogenesis and treatment resistance. Similarly, Su et al.[25] reported that FTO inhibition sensitizes AML cells to tyrosine kinase inhibitors by regulating the stability of MYC and MYB mRNAs, underscoring its therapeutic potential. In addition, Li et al.[26] showed that the m^{6}A writer METTL3 modulates the adipogenic differentiation of MSCs via m^{6}A modification on JAK1, thereby influencing the tumor microenvironment. While these studies focused primarily on leukemic cells or other writers, our work extends this concept to the bone marrow stromal microenvironment, specifically FTO-mediated adipogenic differentiation of MSCs, revealing a novel stromal mechanism of chemoresistance.

Furthermore, we acknowledge that clinical heterogeneity, including variations in baseline white blood cell (WBC) counts among the AML patients in this study, may have influenced the bone marrow microenvironment and could represent a potential confounding factor. Although our results demonstrate a clear association between MSC adipogenic potential and chemoresistance, the impact of systemic factors linked to elevated WBC counts, such as inflammation or cytokine release, cannot be completely excluded.[27] Future studies with larger patient cohorts, stratified by specific clinical parameters such as WBC count, will be important to further validate the independence and relative contribution of the FTO-mTORC1-adipogenesis axis described here.[28] Nonetheless, certain limitations remain. For instance, the number of clinical cases in this study was relatively small and may not fully represent the clinical situation. Given the exploratory nature of this study and the challenges for obtaining samples from CR-AML patients, the sample sizes for RNA sequencing ($n = 3$ per group) and certain validation experiments ($n = 6$ per group) were determined based on feasibility and preliminary effect sizes. Despite these modest numbers, consistent and statistically significant effects were observed across multiple assays. Future studies with larger cohorts will be needed to further validate these findings. Additionally, although this study elucidated the mechanism by which FTO, as an m^{6}A demethylase, modifies raptor to activate the mTORC1 signaling pathway-thereby promoting adipogenic differentiation of MSCs and contributing to AML chemotherapy resistance-the specific role of m^{6}A reader proteins in this process remains unclear and warrants further investigation. Moreover, this study mainly examined the relationship between adipogenic differentiation of MSCs and AML chemotherapy resistance, with limited investigation into the synergistic effects of other components in the bone marrow microenvironment. Given the rapid advancement of targeted therapies for AML, our findings suggests that targeting the FTO-mTORC1-adipogenic axis in the bone marrow microenvironment may represent a promising strategy to overcome chemotherapy resistance. Future studies should evaluate the efficacy of FTO inhibitors or mTORC1 blockers in combination with conventional chemotherapeutics, both in preclinical models and clinical settings, to translate these findings into improved outcomes for patients with AML. Further research will also be required to expand understanding of the bone marrow microenvironment and the mechanisms underlying AML chemotherapy resistance, thereby providing an experimental foundation for targeted interventions, improving diagnosis and treatment, and enhancing the predictive value of clinical laboratory tests for disease prognosis.

Ethics Committee Approval: This study adhered to the regulations of the Declaration of Helsinki (2013) regarding human experiments and was approved by the Ethics Review Committee of Fujian Medical University (approval number: 2024-121, date: 06.03.2024).

Informed Consent: The privacy rights of human subjects have been observed and that informed consent was obtained for experimentation with human subjects.

Data Sharing Statement: The datasets analyzed during the current study are available from the corresponding author upon reasonable request.

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