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Transcription factor FOS promotes ferroptosis and inflammation in *S. aureus*- infected osteomyelitis via EIF5A

Lei Gao[1], Zhipeng Tang[2], Zhijin Zhang[3], Dehua Wei[1] and Jiangning Wang[1*]

Abstract

Background Osteomyelitis (OM) is a bone disease that can leave people disabled. Eukaryotic translation initiation factor (EIF5A) is involved in cell proliferation, apoptosis, differentiation, and inflammation, but the role of EIF5A in staphylococcus aureus (*S. aureus*)-infected OM remains unclear.

Methods The mRNA and proteins were detected by qRT-PCR and western blot. Cell viability was examined by CCK8 assay. The reactive oxygen species (ROS), malondialdehyde (MDA), ferrous iron (Fe^{2+}), and glutathione (GSH) levels were analyzed using the ROS, MDA, GSH, and Fe^{2+} detection kits. The levels of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and Interleukin-6 (IL-6) were examined using Enzyme-linked immunosorbent (ELISA) kits. The binding between FOS and promoter of EIF5A was detected by chromatin immunoprecipitation (CHIP) assay. The interaction between EIF5A and Fos proto-oncogene (FOS) was detected by dual-luciferase reporter assay. The diagnostic values of EIF5A and FOS were analyzed with blood of *S. aureus*-infected OM patients and healthy volunteers by ROC curve.

Results The EIF5A was up-regulated in *S. aureus*-infected OM. EIF5A knockdown promoted cell viability in *S. aureus*-infected MG-63 cells and reduced ROS, MDA, and Fe^{2+} levels, and increased GSH levels. Meanwhile, silencing EIF5A could increase expression of glutathione peroxidase 4 (GPX4), and ferritin heavy chain1 (FTH1) and reduce acyl-CoA synthetase long-chain family member 4 (ACSL4) expression, and silencing EIF5A could reduce immune factors (TNF-α, IL-1β, and IL-6) levels. FOS could bind to EIF5A. Silencing FOS promoted cell viability, and increased GSH levels in *S. aureus*-infected MG-63 cells, but reduced ROS, MDA, and Fe^{2+} levels. Meanwhile, promoted GPX4 and FTH1 expression, inhibited ACSL4 expression, and reduced immune factor levels in *S. aureus*-infected MG-63 cells. Interestingly, EIF5A overexpression could weaken the actions. FOS promotes ferroptosis and inflammation via EIF5A in *S. aureus*-infected MG-63 cells. Besides, the EIF5A and FOS might be potential molecular diagnostic markers in the progression of OM.

Conclusion FOS promotes ferroptosis and inflammation via EIF5A in *S. aureus*-infected OM. This study is first to report the role of FOS and EIF5A in *S. aureus*-infected OM, but we found that there are still some limitations in our work, such as not covering all possible types of infection, which is the focus of future research.

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Highlights

- EIF5A is up-regulated in *S. aureus*-infected OM.
- EIF5A promotes ferroptosis inflammation of *S. aureus*-infected MG-63 cells.
- FOS can target EIF5A.
- FOS promotes ferroptosis and inflammation in *S. aureus*-infected MG-63 cells via EIF5A.
- FOS promotes ferroptosis and inflammation via EIF5A.

Keywords FOS, EIF5A, Staphylococcus aureus, Osteomyelitis

Introduction

The inflammation of the bone marrow and bones is known as osteomyelitis (OM), and its main cause of infection is the invasion of bacterial pathogens into the skeleton [1]. The OM is divided into acute and chronic diseases and has a high morbidity and recurrence rate [2]. Staphylococcus aureus (*S. aureus*) is the main pathogen causing OM [3]. *S. aureus* can invade and persist in osteoblasts, and it has widespread antimicrobial resistance, making successful treatment of osteomyelitis difficult [4, 5]. At present, the therapy of OM is a challenge. It is still necessary to research the mechanism of OM.

Eukaryotic translation initiation factor (EIF5A) is a polyamine-dependent protein and highly conserved protein from archaea to yeast and human [6]. EIF5A is a transcription factor and has two EIF5A isoforms (EIF5A1 and EIF5A2). EIF5A1 and EIF5A2 are 84% indentical [7]. EIF5A1 is related to human diseases such as viral infection, diabetes and several human cancer types [8]. Maier et al. exhibited that in mice, the unique hypusine modification of EIF5A facilitated islet β cell dysfunction and inflammation [9]. Yang et al. reported that overexpression of miR-221-3p inhibited medulloblastoma cell proliferation and promoted cell cycle arrest and apoptosis via targeting the gene of EIF5A2 [10]. Blocking EIF5A hypusination limits the growth of colorectal cancer by inhibiting MYC elongation [11]. S-adenosylmethionine decarboxylase proenzyme promotes aggressiveness of breast cancer through the spermidine-EIF5A hypothesis-transcription factor axis [12]. Spermine prevents acute kidney injury by modulating the activation of inflammatory vesicle in macrophage NLR family pyrin domain containing 3 and mitochondrial respiration in pathways associated with EIF5A oxidation [13]. The EIF5A is correlated with cancer and inflammation. Besides, EIF5A plays a role that is still unclear in OM.

Activator protein 1 transcription factor subunit (FOS), a proto-oncogene, is a protein-coding gene and belongs to the family of FOS genes, meanwhile, it encodes the proto-oncoprotein FOS (C-FOS) [14, 15]. Bakiri et al. discovered that C-FOS affected bile acid metabolism and cholesterol, as well as, led to inflammation and damage of DNA, thereby, facilitating liver cancer [16]. The microRNA-181α could suppress oxidized-LDL-stimulated inflammation response of immune via targeting C-FOS in dendritic cell [17]. FOS is important in the inflammation and the role of FOS has not been established in *S. aureus*-infected OM development.

In this work, the purpose is to reveal the function of EIF5A and FOS in *S. aureus*-infected OM. This study may help explore new molecular mechanisms of action for *S. aureus*-infected OM development.

Materials and methods

Bioinformatics

The OM-related datasets (GSE6269) were obtained from the Comprehensive Gene Expression (GEO) databases, and the GSE6269 was based on the GPL9 platform. Peripheral blood samples were analyzed from 11 participants, including 5 patients with *S. aureus* who developed OM (osteomyelitis group) and 6 unrelated healthy controls (normal group). The R language LIMMA software package was used to process and filter the raw microarray data of the dataset. $P < 0.05$ and $|\log FC| > 1$ were considered significant.

We downloaded the transcription factors (TFs) from the hTFtarget online databases (https://guolab.wchscu.cn/hTFtarget/#!/tf). Subsequently, the Venn diagram was used to show overlapping differently expressed genes in GSE6269 and transcription factors of bone marrow.

The FOS expression in peripheral blood samples with OM was analyzed using the GEO database (GSE6269). The JASPAR database (https://jaspar.elixir.no) was used to predict the binding sites of FOS on the EIF5A promoter.

Samples collection

Blood samples were obtained from healthy volunteers ($n = 30$) and *S. aureus*- infected OM patients ($n = 30$). The informed consent form had been completed by every participant. The experiment in this study was granted approval by the Ethics Committee of Capital Medical University Affiliated Beijing Shijitan Hospital.

Cell culture

The human MG-63 osteoblast-like cells (MG-63) were obtained from Yaji Biological (Shanghai, China) and cultivated into Dulbecco's modified Eagle medium (Biosharp,

Beijing, China) containing 10% fetal bovine serum (FBS) (Solarbio, Beijing, China).

Osteoblast infection and cell transfection

S. aureus was obtained from a Chemical biology test (Shanghai, China). The *S. aureus* were grown in brain heart infusion broth for 12 h at 37℃. In the present study, the cells of MG-63 were infected with *S. aureus* at an optimal concentration of 100:1.

The MG-63 cells were cultivated into 6-well cell culture plate. After cell fusion, the MG-63 cells were infected with *S. aureus*. In short, bacterial suspensions were prepared without penicillin/streptomycin, and the bacterial suspension was added to the MG-63 cell culture, the culture bottle or culture plate was gently shaken to make the bacteria evenly distributed, and then the culture was continued in the incubator at 37℃ and 5% CO_2 for 2 h.

The lentivirus including negative control of short hairpin (sh) RNA (sh-NC), shRNA targeting EIF5A and FOS, pcDNA, and EIF5A plasmids was obtained from GenePharma (Shanghai, China). The *S. aureus*-infected MG-63 cells were transferred with the lentivirus vector or plasmids using Lipofectamine™ 2000 (Thermo Fisher Scientific, Waltham, MA, USA).

Quantitative real-time polymerase chain reaction (qRT-PCR)

Total RNA was isolated and extracted from cells or serum samples by TriQuick Reagen (Solarbio) and the quality of RNA was detected. The cDNA was synthesized using the SuperScript® VILO™ cDNA Synthesis Kit (Invitrogen, Carlsbad, CA, USA). qRT-PCR was carried out by SYBR Green qRT-PCR Kit (Thermo Fisher Scientific) and the primers sequence used were as follows: EIF5A, forward 5′-GCGTTCGCGCGAGTTGG-3′ and reverse 5′-CCAACCAGATGGACCTTGGC-3′; FOS, forward 5′-CAGACTACGAGGCGTCATCC-3′ and reverse 5′-TCTGCGGGTGAGTGGTAGTA-3′; GAPDH, forward 5′-AAAGCCTGCCGGTGACTAAC-3′ and reverse 5′-TTCCCGTTCTCAGCCTTGAC-3′.

Western blot assay

RIPA lysis and extraction buffer (Solarbio) was used to isolate and extract the total protein. The total protein concentration was examined by BCA protein assay kit (Abcam, Cambridge, MA, USA). To separate proteins, 10% sodium dodecyl sulfate-polyacrylamide gel electrophoresis (Vazyme, Nanjing, China) was used. Next, the proteins were transferred onto the polyvinylidene fluoride (PVDF) membrane (Vazyme). The PVDF membranes were incubated with antibodies against EIF5A (ab32407, 1:1000, Abcam), glutathione peroxidase 4 (GPX4) (67763-1-Ig, 1:5000, Proteintech, Chicago, IL, USA), acyl-CoA synthetase long-chain family member 4 (ACSL4) (22401-1-AP, 1:6000, Proteintech), ferritin heavy chain1 (FTH1) (11682-1-AP, 1:1000, Proteintech), β-actin (ab8227, 1:5000, Abcam), FOS (ab222699, 1:1000, Abcam) for 12 h at 4℃. The PVDF membranes (Vazyme) were incubated with the corresponding secondary antibody (Proteintech) for 2 h at 37℃. Finally, the protein was examined using the ECL plus hypersensitive luminescent liquid (Solarbio).

Cell counting Kit-8 assay (CCK8)

The different treatment cells were cultured into the 96-well cell culture plates. The cells were maintained at 37℃ in a 5% CO_2 incubator. Then 10 μL CCK8 solutions were added to the 96-well cell culture plates and were maintained in an incubator with 5% CO_2 at 37℃ for 2 h. Finally, the Varioskan LUX multimode reader (Thermo Fisher Scientific) was used to examine the optical density of 96-well cell culture plates.

Reactive oxygen species assay (ROS)

The levels of ROS were examined using the reactive oxygen species detection kit (Beyotime, Shanghai, China) and the procession experiment followed the manufacturer's recommendation. In brief, cells were collected and incubated with fluorescent probe 2,7-dichlorofluorescein diacetate solution (Beyotime) at 37℃ for 20 min. Ultimately, the cell was observed under the fluorescence microscope.

Measurement of glutathione (GSH)

The intracellular levels of GSH were detected by the lipid oxidation detection kit (Beyotime). Briefly, the cells with different treatments were washed three times and then were lysed. After centrifugation, the supernatants were collected for analysis of GSH content following the instructions of the lipid oxidation detection kit.

Detection of malondialdehyde (MDA) and ferrous iron (Fe^{2+})

The different treatment cells were cultured into the 6-well cell culture plates. Then MDA and Fe^{2+} contents were examined using the MDA detection kit (Beyotime) and iron assay kit (Elabscience, Wuhan, China) following the manufacturer's recommendation.

In short, the cells with different treatments were lysed using the RIPA lysis (Solarbio). After lysed, the samples were centrifuged for 10 min (10000 g). The MDA test solution was mixed with supernatants and was heated at 100℃ for 15 min. The supernatants were collected and added to the 96-well plates. Finally, the MDA levels were examined using a microplate reader.

The cells with different treatments were lysed and then centrifuged. After centrifugation, the supernatants were collected and reacted with the reagent containing iron

reductase for 40 min. The microplate reader was carried out to analyze the Fe^{2+} levels.

Enzyme-linked immunosorbent assay (ELISA)

ELISA kits (Boster Biological Technology, Wuhan, China) were used to examine the levels of tumor necrosis factor-alpha (TNF-α), interleukin-1β (IL-1β), and Interleukin-6 (IL-6). Shortly, the supernatants of different treatment cells were obtained, and then the supernatants were added to the 96-well plates coated with specific antibodies for incubation. After incubation, the plates were washed thoroughly with the provided washing solution to remove unbound components. Following the detection reagents were added in turn according to the instructions. Finally, the levels of inflammatory factors were analyzed using the microscope.

Chromatin immunoprecipitation assay (CHIP)

CHIP assay was conducted using the CHIP kit (Beyotime) according to the manufacturer's protocol. Briefly, the cells were fixed with formaldehyde (1%; Solarbio) at 37℃ for 10 min, and then the Glycine Solution (10 ×) was reacted for 5 min at 37℃. Subsequently, the medium containing formaldehyde and glycine was removed and the cells were washed with pre-cooled PBS containing 1mM PMSF. The cells were collected by centrifugation and then cells were lysed by SDS Lysis Buffer containing 1mM PMSF. Following that, the samples were sheared by ultrasonic. The diluted supernatant was incubated with anti-FOS (ab222699, 1:30, Abcam) or IgG at 4℃ for 12 h. The Protein A + G Agarose/Salmon Sperm DNA was performed to obtain the antibody-antigen-DNA complex. The antibody-antigen-DNA complex was washed with the reagent according to the instructions. The DNA was collected and quantified using PCR.

Dual-luciferase reporter assay

EIF5A reporter plasmids of the wild-type (WT) and mutant-type (MUT) were cloned into the pmirGLO (Promega Corporation, Madison, WI, USA). The binding site of FOS in the EIF5A promoter was mutated using site-directed mutagenesis (Stratagene, La Jolla, CA, USA). Thereafter, the cells were co-transfected with sh-FOS or sh-NC together with WT-EIF5A or MUT-EIF5A. Finally, the cells were collected. Afterward, the dual-luciferase reporter assay system (Solarbio) was used to examine the samples.

Receiver operating characteristic (ROC) curve analysis of EIF5A and FOS

EIF5A and FOS genes were analyzed using ROC curves to distinguish OM from healthy subjects. Defining the area under ROC curve > 0.5 has a diagnostic value.

Statistical analysis

GraphPad Prism version 7.0 (GraphPad Software, Boston, MA, USA) was used to analyze the experimental data. The experimental results of this study were presented as the mean ± standard deviation. The differences between two groups, three or more groups were analyzed using the Student's *t-test* and one-way analysis of variance, respectively. $P < 0.05$ represented the statistical significance.

Results

EIF5A is up-regulated in *S. aureus*-infected OM

As showed in Fig. 1A, the expression of EIF5A was up-regulated in OM by the Comprehensive Gene Expression GEO database. The RNA sequencing data of OM (GSE6269) was downloaded from the GEO database, and it was found that EIF5A was up-regulated in the blood of patients with *S. aureus*-infected OM (Fig. 1B). The mRNA and protein of EIF5A were up-regulated compared to MG-63 cells of healthy humans (Control group) (Fig. 1C, D). Taken together, EIF5A is related to *S. aureus*-infected OM.

Silencing EIF5A inhibits ferroptosis and inflammation in *S. aureus*-infected MG-63 cells

The western blot result found that the expression of EIF5A was up-regulated compared with the Control group, but knockdown of EIF5A could reverse the effect (Fig. 2A). The ability of cell viability was induced in *S. aureus*-infected MG-63 cells (Fig. 2B), however, silencing EIF5A would recede the role (Fig. 2B). In *S. aureus*-infected MG-63 cells, the ROS levels were increased, whereas, silencing EIF5A could weaken the action (Fig. 2C). The levels of MDA, and Fe^{2+} were elevated, still, the GSH levels were decreased compared to Control group. Besides, the knockdown of EIF5A could undermine these actions in *S. aureus*-infected MG-63 cells (Fig. 2D-F). The GPX4, ACSL4, and FTH1 were ferroptosis-related proteins. The expression of GPX4 and FTH1 were down-regulated but the expression of ACSL4 was up-regulated in *S. aureus*-infected MG-63 cells (Fig. 2G). Meanwhile, EIF5A knockdown would return these effects (Fig. 2G). It was suggested that the knockdown of EIF5A inhibited the ferroptosis of *S. aureus*-infected MG-63 cells. The levels of TNF-α, IL-1β, and IL-6 were increased in *S. aureus*-infected MG-63 cells, while knockdown of EIF5A could weaken the action (Fig. 2H-J). The data proved that knockdown EIF5A was related to inflammation in *S. aureus*-infected MG-63 cells. Consequently, silencing EIF5A inhibited the ferroptosis and inflammation of *S. aureus*-infected MG-63 cells.

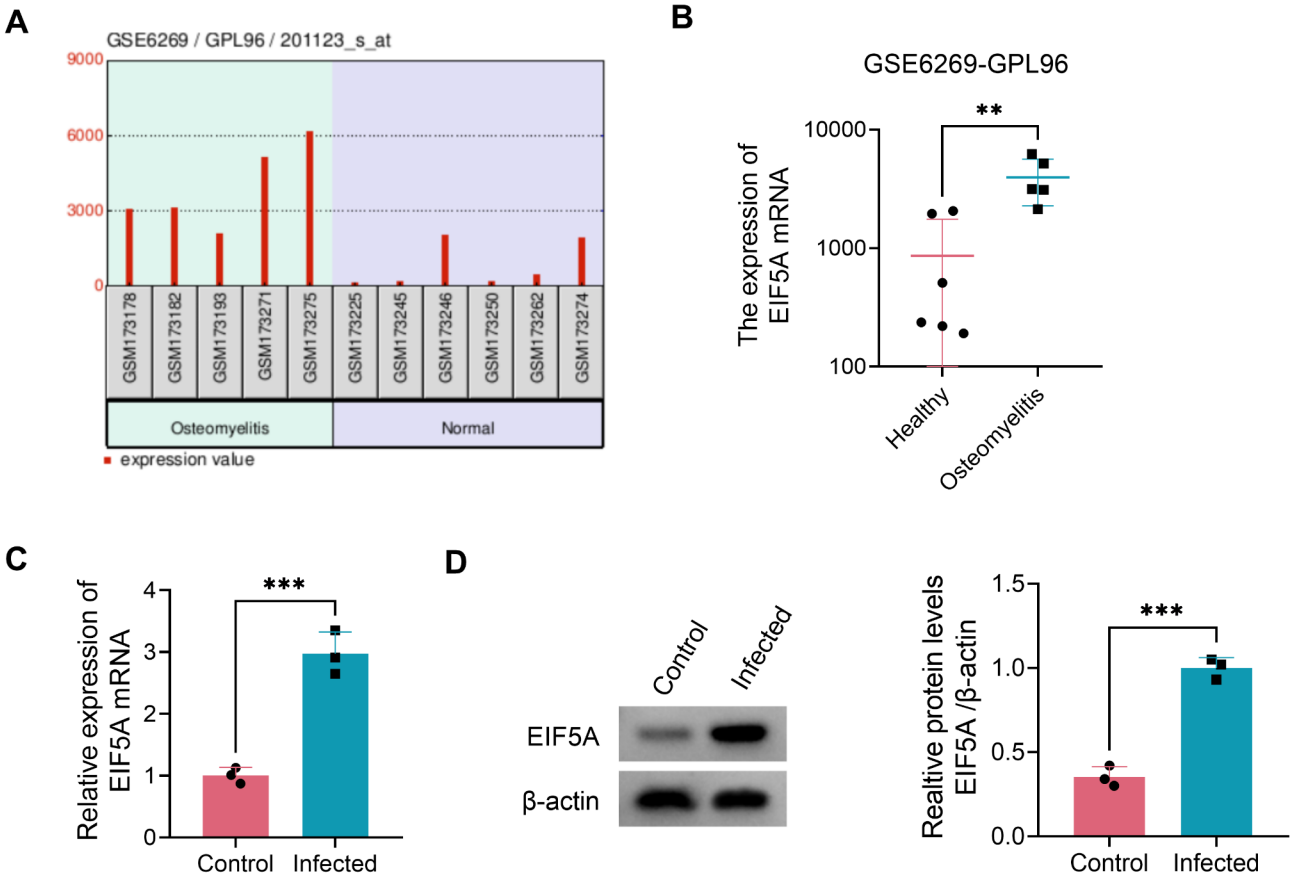

Fig. 1 EIF5A is up-regulated in *S. aureus*-infected OM and in vitro. (**A**) The expression of EIF5A was analyzed in OM on the GEO database (**B**) The expression of EIF5A was analyzed in the blood of patients with OM caused by normal and *S. aureus* infection by GEO database (**C**-**D**) EIF5A expression of the blood of patients with OM caused by *S. aureus* infection or healthy human osteoplast were detected using qRT-PCR and western blot. ** $P < 0.01$, *** $P < 0.001$

FOS can bind to EIF5A

As showed in Fig. 3A, the Venn diagram showed that there are four common differently expressed genes in OM (GSE6269) and transcription factors of bone marrow (https://guolab.wchscu.cn/hTFtarget/#!/tf). The qRT-PCR showed that silencing FOS could significantly inhibit the transcriptional factor EIF5A expression (Fig. 3B). The expression of FOS was up-regulated in OM in the GEO database (Fig. 3C, D). The mRNA and protein expression of FOS was up-regulated in *S. aureus*-infected MG-63 cells (Fig. 3E, F). The binding sites of FOS on the EIF5A promoter were predicted via the JASPAR database (Fig. 3G). The FOS in site 1 was notably enriched group compared with IgG group, but the FOS enrichment in site 2 did not alter compared with the IgG group (Fig. 3H). These results demonstrated that the binding site 1, but not site 2 in the EIF5A promoter could be bound by FOS. Besides, the luciferase activity in 293 T with WT-EIF51 and sh-FOS co-transfection was decreased when compared to 293 T co-transfected with WT-EIF51 and sh-NC, while the luciferase activity did not change in 293 T with MUT-EIF51 and sh-FOS co-transfection compared to the control group (Fig. 3I). It is suggested that EIF5A could bind with FOS. Overall, FOS could bind to EIF5A.

FOS promotes ferroptosis and inflammation of *S. aureus*-infected MG-63 via EIF5A

As showed in Fig. 4A, the expression of EIF5A was down-regulated by sh-FOS in *S. aureus*-infected MG-63 cells, interestingly, overexpression of EIF5A returned the effect (Fig. 4A). In *S. aureus*-infected MG-63 cells, knockdown of FOS could increase cell viability, whereas, EIF5A overexpression could weaken the effect (Fig. 4B). Silencing FOS could reduce the ROS and MDA levels in *S. aureus*-infected MG-63 cells, up-regulated EIF5A could abate effect (Fig. 4C, D). The GSH level was increased by sh-FOS in *S. aureus*-infected MG-63 cells, miraculously, EIF5A overexpression could weaken the action (Fig. 4E). In *S. aureus*-infected MG-63 cells, FOS knockdown could reduce Fe^{2+} levels, up-regulated EIF5A could weaken the action (Fig. 4F). Silencing FOS inhibited expression of ACSL4 and promoted GPX4, FTH1 expression in *S. aureus*-infected MG-63 cells (Fig. 4G), but after overexpression EIF5A, these effects were weakened (Fig. 4G). FOS knockdown could reduce the levels

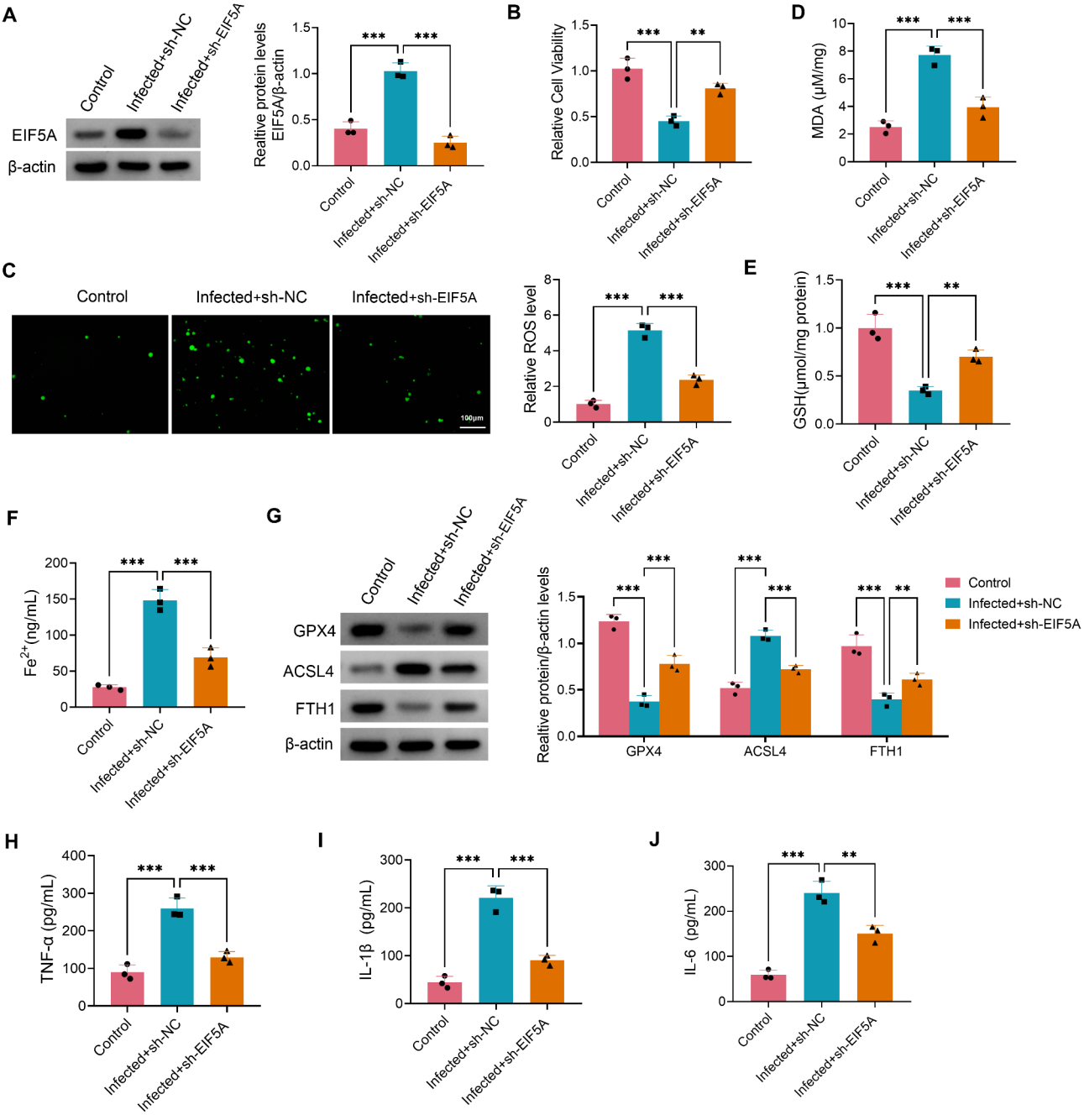

Fig. 2 Silencing EIF5A inhibits ferroptosis, inflammation in *S. aureus*-infected MG-63. The experiment was divided into three groups according to different treatments and named Control (MG-63 cell), Infected + sh-NC (MG-63 transfected with lentivirus sh-NC after *S. aureus* infection), and Infected + sh-EIF5A (MG-63 transfected with lentivirus sh-EIF5A after *S. aureus* infection). (**A**) The protein of EIF5A was examined by western blot (**B**) The cell viability was detected by CCK8 assay (**C**) The levels of ROS were detected (**D**-**F**) The levels of MDA, GSH and Fe^{2+} were examined (**G**) The proteins of iron death-related GPX4, ACSL4 and FTH1 were examined by western blot (**H**-**J**) The TNF-α、IL-1β、IL-6 levels were detected by ELISA kit. ** $P < 0.01$, *** $P < 0.001$

of TNF-α, IL-1β and IL-6 in *S. aureus*-infected MG-63 cells, overexpression of EIF5A could weaken the actions (Fig. 4H-J). To sum up, silencing FOS inhibited the ferroptosis and inflammation of *S. aureus*-infected MG-63 cells via EIF5A.

EIF5A and FOS are the potential prognostic biomarkers in OM

The mRNA expression of EIF5A and FOS was up-regulated in the blood of OM (Fig. 5A, B). EIF5A was positively correlated with FOS mRNA expression (Fig. 5C). Finally, we demonstrated the potential of EIF5A and FOS as a diagnostic biomarker for OM using ROC curve

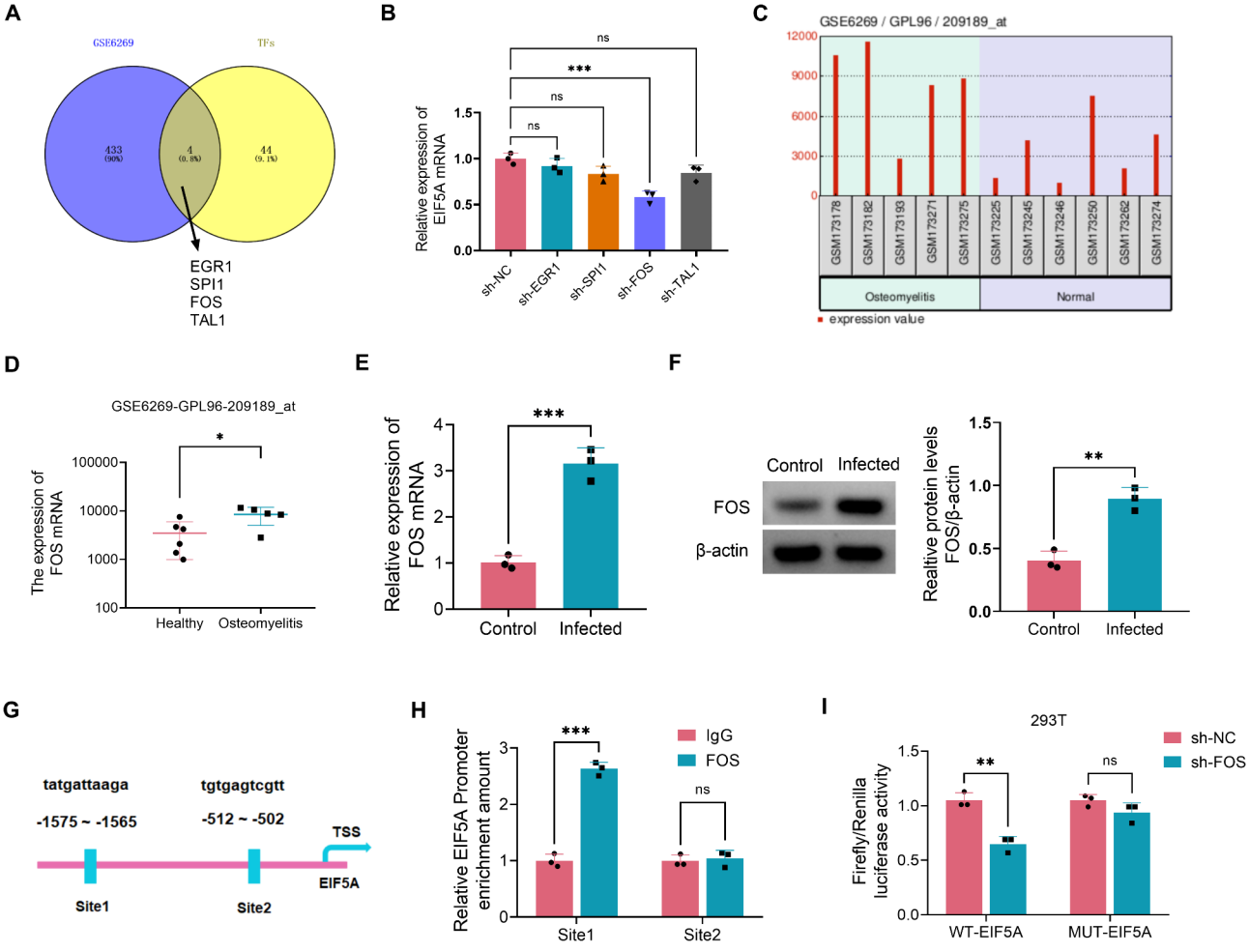

Fig. 3 FOS can bind to EIF5A. (**A**) Venn diagram was used to analyze the differently expressed genes in OM and transcription factors in bone marrow based on GEO and hTFtarget database (**B**) The common differently expressed genes in OM and transcription factors in bone marrow were screened by the GEO and hTFtarget databases and examined by qRT-PCR. $P < 0.05$ and logFC up-regulates TOP20 were considered significant (**C**) The expression of FOS was analyzed in *S. aureus* infection leading to OM in GEO database (**D**) The expression of FOS analyzed in *S. aureus*-infected leading to OM in GEO database (**E**) The mRNA of FOS was examined in the MG-63 cells or *S. aureus*-infected MG-63 cells by qRT-PCR (**F**) The protein of FOS was examined in the MG-63 or *S. aureus*-infected MG-63 cells by western blot (**G**) The EIF5A initiation sites were predicted in JASPAR database (**H**) The promoter of EIF5A was enrichment (**I**) Dual-luciferase reporter assay was used to analyze the interacted with EIF5A and FOS. * $P < 0.05$, ** $P < 0.01$, *** $P < 0.001$

analysis. The ROC curve showed that EIF5A and FOS had potential as a diagnostic biomarker for patients, and area under the curve (AUC) values scores were 0.8920 and 0.8112 respectively (Fig. 5D, E). Overall, EIF5A and FOS might be potential molecular diagnostic markers for OM.

Discussion

OM is one of the diseases of challenge in orthopedics and the aggressiveness and dysregulation of the host immune response drive the inflammatory destruction of bone and bone marrow tissue [18]. *S. aureus* can invade human cortical bone and lead to intracellular infection of osteoblasts, which may lead to long-term infection that is difficult to eliminate [19]. Yang et al. reported that the immunological component of lung adenocarcinoma was negatively linked with increased expression of EIF5A in immune cells [20]. In this study, EIF5A was up-regulated in *S. aureus*-infected OM via GEO databases. The mRNA and protein of EIF5A were up-regulated in *S. aureus*-infected MG-63 cells and the result is consistent with the data analysis. EIF5A was related to *S. aureus*-infected OM.

EIF5A is related to pathological processes such as apoptosis, proliferation, and inflammation [21]. Cyclic RNA protein tyrosine kinase 2 promotes ATP efflux mediated by the miRNA-766/eukaryotic initiation factor 5 A axis, which exacerbates inflammation and pyroptosis in the tissue of septic lung [22]. Tan et al. demonstrated that EIF5A played an important role in production of IFNγ and cell cycle regulation in primary CD8 T lymphocytes [23]. The ferroptosis is related to iron or ROS-related

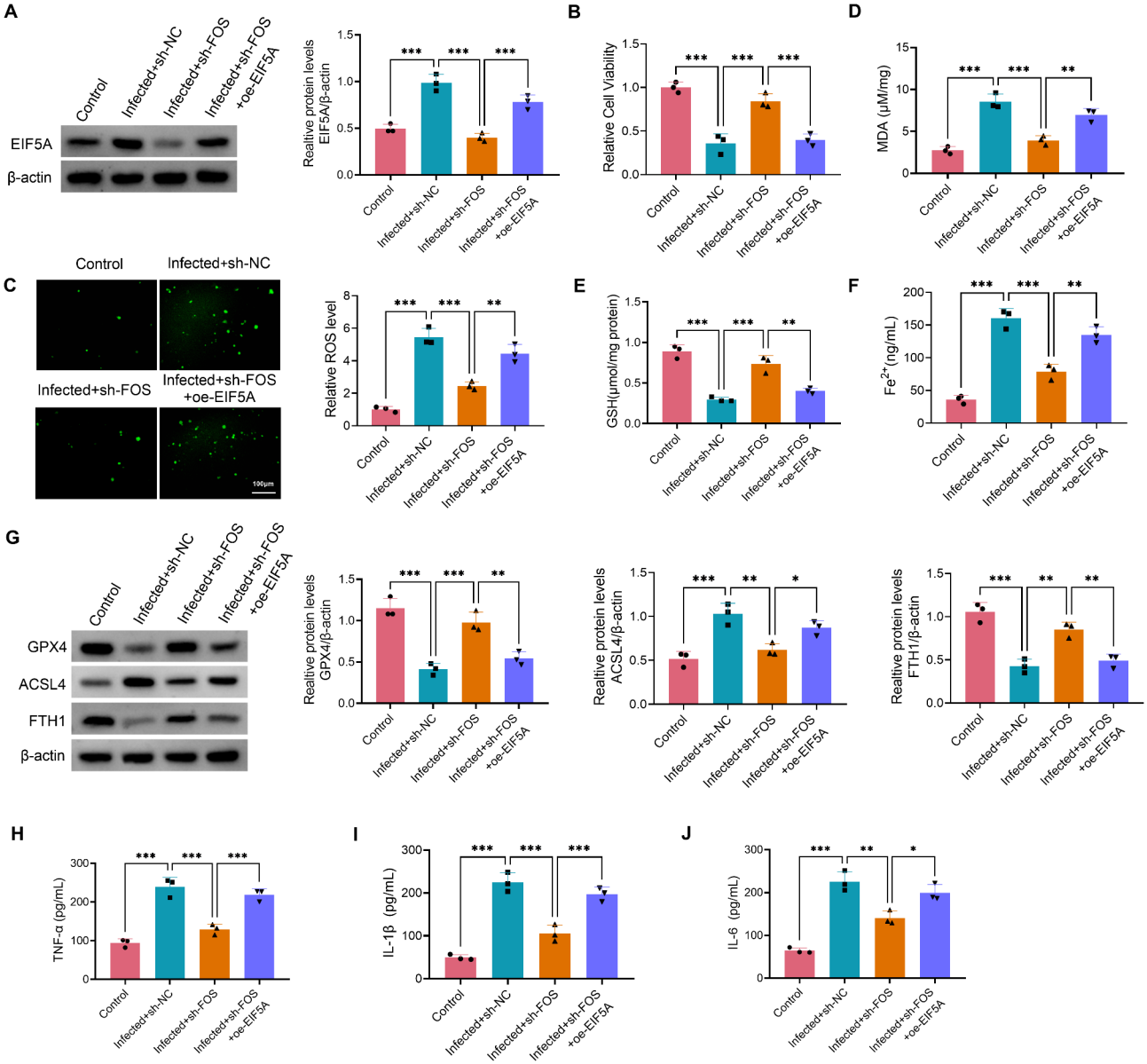

Fig. 4 FOS promotes ferroptosis, inflammation of *S. aureus*-infected MG-63 via EIF5A. The experiment was divided into four groups named Control (MG-63), Infected + sh-NC (MG-63 transfected with lentivirus sh-NC after *S. aureus* infection) Infected + sh-FOS (MG-63 transfected with lentivirus sh-FOS after *S. aureus*-infected), and Infected + sh-FOS + oe-EIF5A (MG-63 transfected with lentivirus sh-FOS and oe-EIF5A after *S. aureus*-infected) (**A**) The protein of EIF5A was detected by western blot (**B**) The cell viability was examined by CCK8 assay (**C**) The levels of ROS were detected (**D**-**F**) The levels of MDA, GSH and Fe^{2+} were examined (**G**) The proteins of iron death-related GPX4, ACSL4 and FTH1 were examined by western blot (**H**-**J**) The TNF-α、IL-1β、IL-6 levels were detected by ELISA kit. * $P<0.05$, ** $P<0.01$, *** $P<0.001$

diseases such as infection and inflammatory diseases [24, 25]. The ferroptosis is characterized by iron-dependent accumulation of lipid peroxides [26]. GPX4 is an important enzyme that regulates ferroptosis by removing lipid peroxides [27]. ACSL4 belongs to the long-chain family of acyl-CoA synthetase proteins and has an important role in ferroptosis [28]. FTH1 could resist ferroptosis via regulating lipid peroxidation and iron metabolism [29]. In this study, when *S. aureus*-infected MG-63, EIF5A knockdown could promote viability, inhibit the ROS, MDA, and Fe^{2+} levels, as well as, increase the level of GSH. Interestingly, silencing EIF5A could facilitate the expression of GPX4 and FTH1 and suppress the ASCL4 expression, at the same time, it reduced the levels of TNF-α, IL-1β, and IL-6. EIF5A promoted the ferroptosis and the results of this study are consistent with those previously reported.

The mechanism of EIF5A was explored in *S. aureus*-infected MG-63 cells. This study found the interaction between transcriptional factor FOS and EIF5A in *S.*

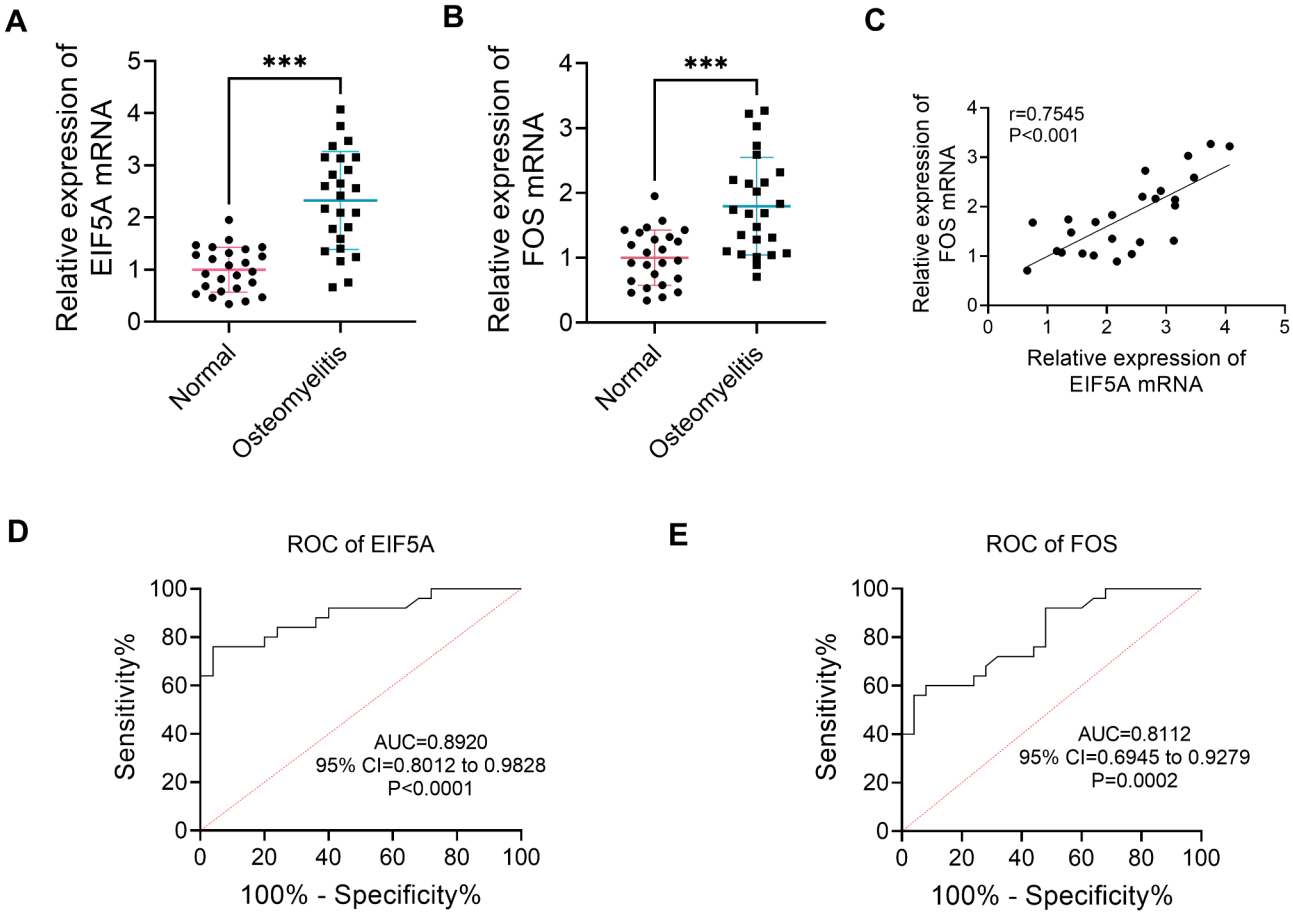

Fig. 5 EIF5A and FOS are the potential prognostic biomarkers in OM. The blood was obtained from the healthy volunteers and patients of OM ($n=30$) (**A**) The EIF5A expression was examined by qRT-PCR (**B**) The FOS expression was detected by qRT-PCR (**C**) The correlation was analyzed between EIF5A and FOS mRNA expression (**D**-**E**) ROC curve analysis evaluated the diagnostic potential of EIF5A and FOS in OM patients. *** $P<0.001$. *** $P<0.001$

aureus-infected OM based on GEO and hTFtarget database. The expression of FOS was increased in the OM and *S. aureus*-infected MG-63 cells, and the dual-luciferase reporter assay demonstrated an interaction with EIF5A and FOS.

FOS has long been considered to be involved in the pathogenesis of bone tumors, and recurrent rearrangement of FOS and its collateral FOSB occurs in benign bone tumors, osteoblastoma, and osteoid osteoma [30]. Yang et al. reported that N-acetyltransferase 10 enhances osteoclast generation in an inflammatory bone loss via catalyzing FOS mRNA N4-acetylcysteine modification and up-regulating mitogen-activated protein kinase signaling pathway [31]. The osteoarthritis was alleviated by targeting the aging-related genes Mitogen-Activated Protein Kinase 12 and FOS [32]. In *S. aureus*-infected MG-63, FOS knockdown could promote the viability, and GSH expression, and reduce ROS, MDA and Fe^{2+} levels. Besides, silencing FOS could inhibit the expression of ASCL4 and promote the expression of GPX4 and FTH1, as well as inhibit TNF-α, IL-1β and IL-6 expression. Up-regulated EIF5A would weaken these actions. Therefore, silencing FOS inhibited the ferroptosis and inflammation of *S. aureus*-infected MG-63 via EIF5A.

Taken together, the expression of EIF5A was up-regulated in the blood of patients with *S. aureus*-infected OM, and FOS promoted ferroptosis and inflammation in *S. aureus*-infected OM via EIF5A *in vitro.* Besides, EIF5A and FOS might be potential molecular diagnostic markers for OM. This study would pave the way for the treatment of *S. aureus*-infected OM. Finnaly, this finding is the first to report the role of FOS and EIF5A in OM, which will contribute to a deeper and more comprehensive understanding of the occurrence and development of OM. However, there are still some limitations in this study, such as insufficient sample size and not covering all possible blood cell populations or infection types, which can be improved in future studies.

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s13018-025-05815-y.

Supplementary Material 1

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None.

Author contributions
Conceptualization and Methodology: Zhipeng Tang and Zhijin Zhang; Formal analysis and Data curation: Dehua Wei and Jiangning Wang; Validation and Investigation: Lei Gao and Zhipeng Tang; Writing - original draft preparation and Writing - review and editing: Lei Gao, Zhipeng Tang and Zhijin Zhang; Approval of final manuscript: all authors.

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Data availability
No datasets were generated or analysed during the current study.

Declarations

Ethics approval and consent to participate
The present study was approved by the ethical review committee of the Capital Medical University Affiliated Beijing Shijitan Hospital. Written informed consent was obtained from all enrolled patients.

Consent for publication
Patients agree to participate in this work.

Competing interests
The authors declare no competing interests.

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